ABSTRACT  This review will focus on the infectious etiologies and more common noninfectious causes of lower respiratory tract syndromes among major immunosuppressed populations. The changing epidemiology of infections in the era of highly active antiretroviral therapy (HAART) in the case of HIV-positive patients and the impacts of both newer immune-suppressant therapies and anti-infective prophylaxis for other immunocompromised hosts will be discussed, with emphasis on diagnostic approaches and practice algorithms.

LOWER RESPIRATORY TRACT INFECTIONS

Decades of advances in cancer treatments and transplantation immunology have expanded the population of severely immunocompromised patients. In addition, new therapies for the management of rheumatologic, autoimmune, and acquired immune diseases have reduced mortality among these patient groups. Pulmonary infections are the most common syndromes contributing to morbidity and mortality among immunocompromised patients (1–3). Virtually any potential pathogen can result in significant illness, and pulmonary infiltrates may be caused by a variety of noninfectious syndromes as well. Management of pulmonary syndromes in these vulnerable populations is a challenge for both clinicians and microbiologists, as prompt diagnosis can prevent irreversible pulmonary complications and/or allow withdrawal of potentially toxic empiric therapies. Diagnostic approaches should consider the tempo of the pulmonary process, the extent of immunosuppression, and the radiographic patterns. In addition, the likelihood of a specific infection may be affected by recently administered prophylaxis or empiric therapies.

This review will focus on the infectious etiologies and more common noninfectious causes of lower respiratory tract syndromes among major immunosuppressed populations. The changing epidemiology of infections in the era of highly active antiretroviral therapy (HAART) in the case of HIV-positive patients and the impacts of both newer immune-suppressant therapies and anti-infective prophylaxis for other immunocompromised hosts will be discussed. The article will emphasize diagnostic approaches and practice algorithms.

Pneumonia is defined as radiological evidence of new or increasing pulmonary infiltrate(s) plus one or more of the following: fever, hypothermia, cough with or without sputum production, tachypnea, dyspnea, hemoptysis, wheezing, physical findings such as rales, and hypoxemia.

Community-Acquired Pneumonia

The pathogen distribution in community-acquired pneumonia in immunocompromised hosts is generally the same as in “normal individuals.” Eighty-five percent of infections are caused by Streptococcus pneumoniae,
Haemophilus influenzae, and Moraxella catarrhalis (4). The remaining 15% are caused by Legionella spp., Mycoplasma pneumoniae, or Chlamydia pneumoniae. Legionella pneumophila is the most important atypical agent for patients with impaired T-lymphocyte function, and it may be either community or hospital acquired (4, 5). Both solid organ transplant (SOT) and human stem cell transplant (HSCT) patients are particularly at risk for Legionella pneumophila infections (6), whereas the risk in HIV patients is not substantially higher than what is seen in nonimmunocompromised patients (7). L. pneumophila and Legionella micdadei are the most common species, but other species cause infections in transplant patients, including Legionella bozemanae, Legionella birminghamensis, Legionella dumoffii, and Legionella cincinnatiensis (6). In addition, immunocompromised patients with legionellosis may have more severe presentations, characterized by rapidly expanding lesions and progression to pulmonary cavitation (5, 6). Pseudomonas and pneumonia caused by other Gram-negative bacilli are rare in normal hosts. P. aeruginosa may cause a bacteremic infection in patients with neutropenia. Klebsiella pneumoniae pneumonia has been reported most frequently in patients with alcoholic liver disease. Nontyphoidal Salmonella pneumonia is rare and is most frequently associated with patients with HIV infection (8), although it has also been described in patients with underlying conditions such as malignancy, diabetes mellitus, corticosteroid therapy, and alcohol abuse (8). In one retrospective study of 51 HIV-positive patients with Salmonella bacteremia, 8 patients (15.7%) met the criteria for definite or probable Salmonella pneumonia (8).

Patients with defects in cellular immunity are at risk for infections caused by intracellular pathogens. These include Mycobacterium species, most notably Mycobacterium tuberculosis, Mycobacterium avium-intracellulare complex (MAC), Nocardia sp., Toxoplasma gondii, Pneumocystis jirovecii, cytomegalovirus, Cryptococcus neoformans, and both opportunistic and pathogenic systemic molds.

The lung is usually the portal of entry for many fungal pathogens. Spores may remain dormant in the lung and present as clinical disease when the patient becomes immunosuppressed. Spores may also disseminate hematogenously. In a study comparing immunocompetent patients to immunocompromised patients with pulmonary cryptococcosis, immunocompromised patients had more extensive pulmonary abnormalities often characterized by cavities and higher serum cryptococcal antigen titers (9).

The discovery of several new respiratory viruses, namely human metapneumovirus and human coronaviruses, has focused attention on respiratory viruses and their importance in this vulnerable population. Some reports indicate that some immunocompromised patients may have nasopharyngeal colonization with human metapneumovirus (10), while others describe severe pneumonia associated with transplant rejection, respiratory failure, and death (11–13). The type of infection (upper respiratory tract infection versus pneumonia) is influenced by the degree of immunosuppression and the timing of infection in relationship to transplantation. Rapid diagnosis with prompt administration of antiviral therapy, such as in cases of respiratory syncytial virus (RSV) and influenza infection, has been shown to reduce severity of disease (12).

Nosocomial Pneumonia

Immunocompromised patients are particularly at risk for severe nosocomial infections related to a variety of factors. These include alterations in natural host defenses due to immunosuppressant therapy and prescribed antibiotics in the setting of increased exposure to nosocomial pathogens from considerable time spent in healthcare settings (14, 15). Transmission of multidrug-resistant, nonfermenting Gram-negative rods such as Pseudomonas spp. from waterborne environmental sources in healthcare facilities such as tap water and contaminated scopes, bypass machines, and dialysate fluids have been implicated with increasing frequency (14). Intubation predisposes patients to ventilator-associated pneumonia, and catheters predispose them to hematogenous involvement of the lung from pathogens such as S. aureus, aerobic Actinomycetes, and resistant Gram-negative bacilli. Gram-negative infections, often multidrug resistant, are occurring with increased frequency and present many therapeutic challenges. These include pathogens, such as extended spectrum β-lactamase-producing and carbapenemase-producing Enterobacteriaceae, Acinetobacter spp., and P. aeruginosa. Stenotrophomonas maltophilia, Burkholderia cepacia, and unusual nonfermenting Gram-negative bacilli such as Elizabethkingia meningoseptica (14–16) have also increased. S. maltophilia in particular has been reported to cause life-threatening hemorrhagic pneumonia in patients with hematological malignancies (17–19). These bacterial pathogens figure prominently among all immunocompromised hosts. Legionella, from contaminated water systems or construction, has been problematic for many institutions where the mode of transmission to patients is usually aspiration, but aerosolization or direct instillation into the
lung may also occur (6). In one multivariate analysis, nosocomial acquisition and the development of pulmonary complications (e.g., cavitation; see above discussion) were associated with increased mortality (20). The Centers for Disease Control and Prevention (CDC) recommends aggressive surveillance of water samples for centers that specialize in the care of immunocompromised patients (21). In the event of a single nosocomial laboratory-confirmed case, an epidemiologic and environmental investigation should occur (21). Several centers have discovered inapparent nosocomial transmission over an extended period of time on their transplant units after investigation of individual nosocomial cases (5).

Earlier in the AIDS epidemic, nosocomial transmission of tuberculosis, especially among AIDS patients, was reported from numerous centers (22–24). In many instances, the transmitted strains were multidrug resistant and healthcare workers also became ill (24). Numerous factors have been cited for nosocomial spread, but delay in recognition due to atypical presentations (and hence delay in treatment) as well as inadequate infection control are the major factors for nosocomial transmission (23). In developed countries, infection control practices and declining incidence of tuberculosis in this population due to HAART have reduced nosocomial transmission, but nosocomial acquisition is still a major problem in African countries. Clusters of Pneumocystis infection among hospitalized patients suggest the possibility of nosocomial transmission, and healthcare workers may serve as intermediate hosts (25).

Nosocomial spread of RSV has been reported as a significant problem on pediatric units for several decades. More recently, outbreaks of this virus on adult and pediatric transplant units have been the subject of several reports (12, 26). In a study in Canada from 2006 to 2012, 17% of influenza cases were healthcare-associated, with almost 40% acquired in acute care facilities (27). Likewise, other respiratory viruses such as parainfluenza 3 virus have been transmitted on oncology and transplant units (28). Prolonged viral shedding for up to 80 days among this patient population may contribute to nosocomial transmission (29). Because of the significant morbidity and mortality associated with these viruses among immunocompromised patients, especially HSCT recipients, strict adherence to published infection control measures (21, 30) and prompt treatment for those patients who become infected (12, 30) are mandatory strategies for hospitals with significant numbers of vulnerable populations.

In addition, oftentimes patients infected with RSV and other community respiratory viruses will have super-infections caused by bacterial or fungal pathogens (30). The frequency of opportunistic fungal infections caused by Aspergillus spp., Fusarium spp., and organisms belonging to the order Mucorales often increase in hospitals when construction is occurring.

HOST FACTORS AND SUBGROUPS

Pulmonary Infections Associated with Impaired Humoral Immunity

Patients with primary humoral immune disorders and acquired conditions such as multiple myeloma and chronic lymphocytic leukemia are most at risk for pneumonia caused by encapsulated bacteria, such as S. pneumoniae and H. influenzae. Prophylaxis for these infections has prevented the consequences of bacteremia such as disseminated intravascular coagulation. Other pathogens of importance to this group and to patients with combined immunodeficiency syndromes include P. jirovecii, herpesviruses, and community-associated respiratory viruses (31).

Pulmonary Infections Associated with Neutropenia

Neutropenia may be a consequence of therapy for hematologic and other malignancies or may represent an inherited or acquired immunological disorder. In general, regardless of the cause, bacterial infections are most common in the early phases of neutropenia, whereas prolonged and severe neutropenia is most often associated with fungal disease and other opportunistic infections, such as nocardiosis. Bacterial causes of pneumonia are often polymicrobial, with Gram-negative bacilli and S. aureus predominating (14, 32). In the setting of persistent neutropenia (>7 days) in patients on broad-spectrum therapy, superinfection with resistant Gram-negative bacilli such as Acinetobacter spp., Alcaligenes spp., Citrobacter spp., Enterobacter spp., P. aeruginosa, as well as other nonfermenters such as S. maltophilia and E. meningoseptica are a major concern (14). Many of these pathogens reach the lung via hematogenous spread. Fungal pathogens are also of concern in groups with prolonged neutropenia (32).

The frequency of aspergillosis is increasing among patients with hematologic malignancies. Aspergillus fumigatus is the most frequent pathogen, but other species, including Aspergillus flavus, Aspergillus nidulans, Aspergillus niger, Aspergillus glaucus, and Aspergillus
terreus, are also important. The latter species is resistant to amphotericin B. Identification to species level and differentiation of Aspergillus spp. from other hyaline molds is important in this patient population because of the variability in antifungal susceptibility. Infection is usually acquired by inhalation of spores, which increases in the setting of construction in proximity to the hospital (14, 32). Fusarium spp. and organisms belonging to the order Mucorales (e.g., Mucor spp. and Rhizopus spp.) are filamentous fungi that may mimic aspergillosis both clinically and radiographically. In the extensive series by Kontoyiannis et al (33), 71% of cancer patients who were infected with an organism from the Mucorales order were neutropenic. Trichosporon spp. are less commonly encountered (32). In the neutropenic patient with diffuse infiltrates, noninfectious causes are more likely, but in the appropriate clinical setting, pathogens such as P. jirovecii, M. pneumoniae, Strongyloides stercoralis, and herpesviruses and community respiratory viruses should be excluded (14, 32, 34).

Pulmonary Infections Associated with Defects in Cellular Immunity—Overview

Patients with impaired cellular immunity include ever-expanding numbers of individuals with lymphoproliferative disorders who are receiving chemotherapy with purine analogs, recipients of HSCT or SOT, and patients receiving corticosteroid therapy. Glucocorticoids are potent immunosuppressants and affect multiple cell lines—T and B cells, macrophages, granulocytes, and monocytes (35). The infections that develop in these patients are different from those seen in the neutropenic patient and patients with isolated humoral defects. In general, herpes viruses and respiratory viruses are significant causes of morbidity and mortality in this group of patients. Protozoan parasites, such as Toxoplasma gondii and Enterocytozoon bieneusi, and the helminth S. stercoralis are also important. Other pathogens that play a more prominent role in the patient with cell-mediated immune defects are P. jirovecii, mycobacteria, and opportunistic or systemic fungi. Detailed discussions of the specifics of the infections as they pertain to major vulnerable groups are described in detail below.

HIV/AIDS

The human immunodeficiency viruses cause significant impairment in lung host defenses. Several mechanisms have been elucidated and are reviewed by Beck (36). Several mechanisms of impairment are briefly described here. HIV decreases the numbers of cells that directly kill pathogens. In AIDS patients who are not on HAART, there is a decrease in CD4+ T cells and an increase in CD8+ T cells. The CD4/CD8 ratios may be lower in the lung than what is seen in the periphery. Production of T cells is also impaired. The increase in CD8+ T cells may cause an alveolitis (lymphoid interstitial pneumonia), the intensity of which correlates with viral load. HIV also causes qualitative defects in metabolic and secretory functions of effector cells. For example, HIV infection may impair phagocytosis of organisms that commonly cause infections in normal hosts. Neutrophil defense appears to be impaired. HIV infection also interferes with the capacity of circulating lymphocytes and other cells to migrate to the lung to kill pathogens in alveolar spaces. Coinfections may also contribute to immunological impairment. For example, it has been shown that patients with Pneumocystis pneumonia had a higher rate of bacterial pneumonia, and patients with a history of cytomegalovirus were at a higher risk of developing non-Hodgkin lymphoma (37, 38).

HAART has changed the spectrum and epidemiology of pulmonary disease in the HIV-positive patient. Access to combination antiretroviral therapy has extended longevity such that persons living with this chronic disease are developing comorbid conditions, some of which may be related directly to the therapy itself and others that are simply the common vulnerabilities associated with aging (39, 40). These include, but are not limited to, chronic obstructive lung disease (especially in smokers), lung cancer, asthma, and HIV-associated pulmonary hypertension (40). Also, despite treatment with HAART, residual inflammation and immunodeficiency persist (39, 40). Data from the CDC’s HIV Outpatient Study (HOPS), in which 7,300 patients have been followed longitudinally since 1993, show significant declines in overall hospitalizations for pulmonary disease and decreases in pulmonary morbidity and mortality since 1994 (41). Studies have shown changes in causes of pulmonary infections, namely a decline in infections due to P. jirovecii, P. aeruginosa, and opportunistic pathogens such as cytomegalovirus, Aspergillus spp., and Cryptococcus neoformans, among others (42–44).

Although several studies (37, 43) have demonstrated an overall reduction in the incidence of bacterial pneumonias in the HAART era, bacterial pneumonia continues to cause significant morbidity and mortality. HIV-infected patients continue to have greatly increased risk of acquiring bacterial pneumonia (especially pneumococcal disease) compared with persons without HIV.
infection (43). Factors significantly associated with bacterial pneumonia include injection drug use, lower CD4 cell counts, prior Pneumocystis infection, older age, smoking, and poor virological response to treatment (47, 45). The most important causes of bacterial pneumonia remain S. pneumoniae, H. influenzae, and S. aureus. P. aeruginosa has significantly declined since the pre-HAART era, and other pathogens such as Rhodococcus and Nocardia are rare (44). Tuberculosis remains an important disease even in the HAART era, and HIV infection is still the largest risk factor for development of tuberculosis worldwide. Multidrug-resistant tuberculosis is also more common in HIV-positive patients (46).

An interesting consequence of HAART has been the development of immune reconstitution syndrome. This is believed to be related to an enhanced T-cell-mediated immunopathological response to infection. The patients most likely to develop this syndrome are those who have CD4 counts <50 cells/μl and high viral loads (5.0 log_{10} copies) and who have a good response to treatment (41). Infectious diseases that appear prominently during immune reconstitution are candidiasis, cytomegalovirus infection, disseminated Mycobacterium avium-intracellulare complex infections, Pneumocystis pneumonia, varicella zoster, Kaposi’s sarcoma, and non-Hodgkin lymphoma (41). Antiretroviral therapy itself should also be considered when other etiologies of pulmonary infiltrates have been excluded. For example, abacavir has been associated with a hypersensitivity reaction in 3.7% of patients who use the drug (47). Although gastrointestinal symptoms are the most prominent manifestations, patients may have fever, pharyngitis, cough, tachypnea, and pulmonary infiltrates (47).

In summary, although most of the studies are observational, pulmonary infections in the HAART era have declined significantly, with most noticeable differences occurring in the rates of opportunistic pathogens. Bacterial pneumonia, tuberculosis, and noninfectious problems such as malignancies, chronic obstructive pulmonary disease, and drug reactions including pulmonary hypertension continue to be significant causes of pulmonary syndromes among this patient population.

**Solid Organ Transplant Patients**

Pneumonia is the most frequent infectious complication after SOT (15) and is highest in the period immediately following transplantation (the first 30 to 100 days) (5, 15, 48–50). The etiology of pneumonia among SOT recipients varies at different time points posttransplantation, but the spectrum of pathogens is similar across transplantation groups. During the first month after transplantation, >95% of infections are due to bacteria caused by nosocomial pathogens (5, 15, 35), and the infections are comparable to any postoperative patient. Reported Gram-negative organisms include Pseudomonas, Klebsiella, Escherichia, Legionella, Acinetobacter, Stenotrophomonas, Enterobacter, Serratia, Proteus, and Citrobacter spp. Notable Gram-positives include Staphylococcus, Corynebacterium, Enterococcus, and Streptococcus spp.; Nocardia and anaerobes are rare (35).

Community-acquired pneumonia tends to occur any time after transplantation, although it most often appears 6 months after transplantation. S. pneumoniae, H. influenzae, and Legionella spp. are the most commonly identified pathogens (5). Nontuberculous mycobacteria (NTM) are uncommon causes of pulmonary infection, except in the lung transplant recipient (5, 51). Species commonly recovered from SOT recipients include Mycobacterium avium-intracellulare complex, M. kansasi, M. fortuitum, M. chelonae, M. abscessus, and M. haemophilum (5, 51). M. tuberculosis is uncommon in developed countries (<2% of cases), but risk is 70-fold greater than for the general population (35). Infection with M. tuberculosis is very important in countries where tuberculosis is endemic (5, 52). Disseminated infection is higher in the transplant population than in the normal host. Finally, one must consider that in a significant percentage of cases an etiologic agent is never discovered (5).

In the period 1 to 6 months after SOT, infections caused by immunomodulating viruses such as cytomegalovirus, and those caused by opportunistic pathogens, such as P. jirovecii, Listeria monocytogenes, Aspergillus spp. (incidence 18 to 22%), Nocardia spp., and T. gondii, are the most prevalent. However, routine prophylaxis with trimethoprim-sulfamethoxazole has significantly reduced the incidence of P. jirovecii and T. gondii in SOT recipients (15, 48). Nocardia spp. cause infection in 0.7 to 3% of transplant recipients (53). Concomitant infection with cytomegalovirus, extreme immunosuppression, and hypogammaglobulinemia are important risk factors (5). Patients are at risk for dissemination from the lung to other foci, particularly the brain. Reactivation of latent infection present before transplantation may occur during this period (M. tuberculosis, Histoplasma capsulatum, and Coccidioides immitis). As mentioned above, the incidence of tuberculosis is low in developed countries, but in endemic areas, tuberculosis causes significant morbidity and mortality.

During the late period (>6 months posttransplant) populations can be divided into several groups whose
risk of infection varies. These include 1) patients with good results from transplantation (~2/3 of patients). In this group, the major risk is from community-associated bacterial pathogens (see above) and respiratory viruses; 2) 10 to 15% of patients who have chronic viral infections such as hepatitis viruses B and C; and 3) the remaining 5 to 10% who have poor allograft function, require chronic immunosuppression, and are at greatest risk for opportunistic pathogens such as C. neoformans, P. jiroveci, L. monocytogenes, and emerging filamentous fungi such as Trichoderma, Pseudallescheria boydii, Microascus, Penicillium spp., and microorganisms from the Mucorales group, especially Rhizopus and Absidia. Rare causes of infection among this group with poor graft function include Strongyloides and microsporidia (15, 35, 54, 55).

The incidence and timing of infections are affected by the type of organ transplanted, the degree of immunosuppression and the need for additional antirejection therapy, occurrence of problems during surgery, exposure to a donor pathogen, and a variety of environmental and epidemiological factors (55). Infections with hepatitis C, cytomegalovirus (CMV), Epstein-Barr virus (EBV), and HIV increase the likelihood of opportunistic infections. As mentioned above, the timing with which certain infections occur has historically been somewhat predictable. However, now prophylactic regimens make predictions unreliable. Figure 1 represents timelines pre- and postimplementation of standard prophylactic regimens among SOT recipients. The following paragraphs briefly discuss issues unique to specific transplanted populations.

**Lung transplantation**
The main obstacles to long-term success with lung transplantation are chronic rejection in the form of obliterative bronchiolitis and infections (56). CMV is the main contributing factor to obliterative bronchiolitis. Infectious complications are the most common cause of morbidity and mortality at all time points following lung transplantation and are two times more common among lung transplant patients compared to heart transplant recipients. Two-thirds of the infections involve the respiratory tract.

**FIGURE 1** A proposed infection timeline based on the use of common prophylaxis in solid organ transplant recipients. The dotted lines indicate onset of infection that would occur without prophylaxis. Solid lines indicate the most common times to onset of infection for each pathogen. On the x-axis, 0 indicates the time of transplantation. CAP, community acquired; CMV, Cytomegalovirus; EBV, Epstein-Barr virus; HSV, herpes simplex virus; Mtb, Mycobacterium tuberculosis. Modified with permission from reference 35.
There are several predisposing factors unique to lung transplantation. It is the only transplant continuously exposed to the environment. Denervation of the allograft leads to abnormal ciliary clearance, and diminished cough reflex and ischemia lead to anastomotic narrowing and interruption of lymphatic drainage (56). Finally, the native lung may harbor occult infection that reactivates after immunosuppression (15, 35, 56). Like other recipients of SOT, the donor lung may also transmit infections (35, 56).

The incidence of bacterial pneumonia ranges from 35 to 66%. Early in the posttransplant period, the causative microorganisms originate from the donor lung. However, the incidence of early episodes of bacterial pneumonia has decreased as a result of antimicrobial prophylaxis. Unfortunately, this has caused a shift in occurrence of bacterial pneumonia to later in the posttransplant period. The majority (75%) of infections are caused by *Pseudomonas* spp. and *Enterobacteriaceae*; *S. aureus*, enterococci, and *H. influenzae* are also important. Patients with cystic fibrosis are often colonized with *B. cepacia*, *S. maltophilia*, and *Alcaligenes xylooxidans*; *C. pneumoniae* infection has been associated with early graft rejection, bronchiolitis obliterans syndrome, and early mortality (57). *M. tuberculosis* may reactivate from remaining native lung (in cases of single organ transplantation) or may be occultly transmitted by the donor lung. In lung transplant recipients, infections with NTM may be common. In one large series, 8.8% of lung transplant recipients developed both pulmonary and extrapulmonary NTM infections (51). Many candidates for lung transplantation, such as patients with cystic fibrosis, are colonized with NTM pretransplantation, but it is unclear if this enhances the risk of posttransplantation infection (3).

Beyond the first month, viral pathogens are the most important group, representing the second most common cause of infections in lung transplant recipients (23 to 31% of all infections) (35). CMV disease is higher than in other solid-organ recipients; prophylaxis and monitoring are very important. Reactivation of herpes simplex virus (HSV) is usually prevented with antiviral prophylaxis, but severe pneumonia may occur in 10% of patients without prophylaxis (56). The incidence of EBV-related posttransplant lymphoproliferative disorders varies (2 to 33%) but is higher in EBV-negative recipients and patients transplanted because of cystic fibrosis (58). Infections caused by respiratory viruses range from asymptomatic illness to severe pneumonia; influenza A and B, RSV, and adenovirus lead to significant pneumonitis (59). Parainfluenza viruses, adenoviruses, and RSV have been linked directly to obliterative bronchiolitis in many patients (12, 35). Adenovirus infections are particularly severe in pediatric patients and are associated with high mortality and morbidity (59).

Fungal infections are also common. *Aspergillus* colonization is common in this group (60). In the review by Mehrad et al. (61), 26 to 29% of patients developed airway colonization, and in the Cahill study, 46% of 151 transplant recipients had airway colonization (60). Since colonization can lead to invasive disease, empiric therapy with itraconazole (200 mg bid for 6 months) in patients with airway colonization is indicated and has been shown to successfully eradicate the organism (60, 61). Semi-invasive forms can occur at anastomotic sites and in the large airways, the latter manifesting as tracheobronchitis (61). Isolated tracheobronchitis occurs in 4 to 5%, is usually asymptomatic, and is often found on surveillance bronchoscopy. Ulcerations may be extensive (61). However, tracheobronchitis can be associated with fever, wheezing, coughing, and hemoptysis. Invasive disease occurs in 5 to 8%, usually within the first year, and it presents in a manner similar to other immunocompromised hosts. Mortality is high at approximately 60% (61). The frequency of *P. jirovecii* varies; prevalence is high in patients not on prophylaxis. *T. gondii* occurs almost exclusively in heart–lung recipients.

**Heart transplantation**

The incidence of pneumonia has declined substantially over the past several decades, from 50 to 60% in the pre cyclosporine era to 14 to 21% in more recent evaluations (35, 62, 63). In several series, pneumonia is the most common pulmonary complication and the most frequent infectious complication postcardiac transplantation (62, 63). Pneumonia discovered in the first 6 months posttransplant are typically bacterial or fungal and nosocomial in origin, similar to other solid organ transplant populations (see above discussion). The incidence of CMV and *P. jirovecii* has declined due to preemptive screening for viremia in the case of CMV and prophylaxis for both pathogens. Prophylaxis for *P. jirovecii* with trimethoprim sulfamethoxazole likely accounts for the decline in the incidence of *Nocardia* pneumonia as well. Although *P. jirovecii* has declined in incidence, some centers are reporting late-onset cases (>6 months posttransplantation) and during periods of intensified immunosuppression, as in the case of treatment for acute rejection (64). During episodes of the latter, prophylaxis should be reinstituted. In the second 6 months posttransplantation, the etiology of
pneumonia is similar to that in other patients who
get community-acquired pneumonia (63). The highest
mortality is seen with Aspergillus and nosocomial
pneumonia—50 to 75% and 33%, respectively (62, 63).
Toxoplasmosis is highest among seronegative cardiac
transplant recipients who receive an organ from a sero-
positive patient and when prophylaxis has not been
administered (65).

Liver transplantation
The lung is the second most common site of infection
post-liver transplantation. Although the incidence of
bacterial pneumonia has declined to <10% (49), bacte-
ria remain the most common etiologic agents of pneu-
monitis following liver transplantation (48, 49, 66).

P. aeruginosa was the first or second most common
bacterial pathogen in three reported series (48, 49, 66).
Legionella spp. were important causes of community
and nosocomial pneumonia in the Pittsburgh series (48).
Bacterial infections occur within the first 6 months post-
transplantation but predominate in the first month, and
the majority of these are nosocomial (48). Risk factors
for bacterial pneumonia include older age, prolonged
mechanical ventilation, and receipt of more intraopera-
tive transfusions (49, 66). Significant risk factors asso-
ciated with increased risk for the development of
pneumonia caused by any etiology include recurrent
HCV hepatitis and severity of pretransplant disease (48).
Opportunistic pathogens occur most frequently after
the first month posttransplantation. CMV, P. jirovecii, and
Aspergillus spp. are the most commonly reported op-
portunistic pathogens (49), although, as noted above,
CMV and P. jirovecii have declined significantly in re-
cent series. Unfortunately, the contribution of fungi and
their associated mortality remain significant, especially
among those patients with risk factors (48).

Kidney transplantation
The incidence of pneumonia among renal transplant
recipients is the lowest of all SOT recipients. Most occur
in the first 12 months posttransplantation. In one large
study, the most common pathogens responsible for
pneumonia during this time period were bacterial and
mixed bacterial infections (S. aureus, S. pneumoniae,
and Gram-negative bacilli predominated), fungi (Crypt-
tococcus and Aspergillus spp.), and tuberculosis (52).
Similar to what has been described for other SOT, the
incidence of P. jirovecii and CMV has declined except
in those patients with prolonged immunosuppression or
in those patients noncompliant with prophylaxis (52).
Mortality rates have declined in most series (52).

Human Stem Cell Transplantation
Pulmonary infections are the most common infectious
cause of death in HSCT recipients. As is true for solid
organ transplant recipients, multiple factors other than
immunosuppressant therapy predispose to infection.
These factors include chemotherapy/radiation-induced
neutropenia, lung injury induced by conditioning regi-
mens, and rejection in the form of graft-versus-host
disease (GVHD). Autologous transplantation is associ-
ated with the least risk of infection. Higher rates of in-
fection are seen with allogeneic transplantation, with
matched unrelated-donor transplants having the greatest
risk of infections (1, 5, 67). Greater mismatch is associ-
ated with greater risk of GVHD, which results in im-
paired opsonization and reticuloendothelial function (5).
In addition, immunosuppressants used to treat the
GVHD result in further defects in cellular immunity (5,
67, 68). Serious infection occurs in the initial 2-year
period posttransplantation in 50% of uncomplicated
transplants with human leukocyte antigen-compatible
siblings and in 80 to 90% of matched unrelated donors
or histocompatible patients who develop GVHD (35).
The posttransplant period is usually divided into three
defined time periods (Fig. 2). Phase 1 is the first 30 days
after transplantation and includes the preengraftment
period; phase 2 (early postengraftment) occurs from
days 31 to 100; and phase 3 (late postengraftment) is
more than 100 days posttransplantation (68).

In the preengraftment period, patients are most at
risk for hospital-acquired bacterial infections since this
is the period of neutropenia and disruption of mucosal
barriers (5, 15, 68). The exact frequency of bacterial
pneumonia is unknown, largely because patients are
treated at the first sign of neutropenic fever with broad-
spectrum antimicrobial therapy. When an etiologic agent
is recovered, most often it is a resistant Gram-negative
bacterium such as P. aeruginosa or K. pneumoniae.
Legionella infections, occurring in past decades as noso-
comial clusters related to contaminated potable water
in hospitals, may be increasing in frequency, with a
shift to community acquisition as more patients are
managed in the outpatient community setting (69).
Both early and late infections have been reported. Infections
with nonpneumophilia species are more common in
transplant populations (69). Antibiotic prophylaxis has
reduced the occurrence of opportunistic pathogens such as
P. jirovecii, herpes viruses, and opportunistic fungi.
Despite this, Aspergillus remains an important patho-
gen and has a bimodal distribution (Fig. 2). The first
peak occurs around 16 d posttransplantation during
the neutropenic period, and the second peak is seen at
the end of phase 2 (15). Fever, dyspnea, dry cough, wheezing, pleuritic chest pain, and hemoptysis are clinical features consistent with invasive pulmonary aspergillosis. High-resolution computed tomography (HRCT) typically shows large (>10 mm) or small (<10 mm) nodules in almost all cases of pulmonary aspergillosis (70). HSV infections due to reactivation may be seen in patients who do not receive acyclovir prophylaxis. Most patients with HSV pneumonitis have obvious mucocutaneous infections in the form of either oropharyngeal or esophageal disease.

Noninfectious complications such as pulmonary edema and diffuse alveolar hemorrhage may also occur during this period and may be confused with infectious etiologies. The periengraftment respiratory distress syndrome is a noninfectious pulmonary complication typically occurring about 11 days posttransplantation and usually in patients with autologous or peripheral stem cell transplants (68). It is characterized by fever, erythematous rash, noncardiogenic pulmonary edema, and hypoxemia at the time of neutrophil recovery (68).

Postengraftment bacterial infections are less common unless the patient develops GVHD. Infections are more common in the early postengraftment period (phase 2, 30 to 100 days) and less common in the late postengraftment period. Focal infiltrates on chest radiograph are likely to be bacterial, whereas interstitial infiltrates are likely to be CMV or other viral infections (See Table 1).

Nocardia infections occur in the late postengraftment period and are seen almost exclusively in allogeneic bone marrow transplant recipients. Disease is more common in men, and predisposing factors include lymphopenia and higher prednisone doses (71). Frequently (67% in one study), Nocardia infections occur in patients coinfected with other opportunistic pathogens (71). Pulmonary infections with NTM are less commonly reported in HSCT recipients than in SOT (5, 68). Tuberculosis is also uncommon in nonendemic areas.
However, in patients with poor T-cell immunity who are from areas of high endemicity, unrecognized latent tuberculosis can result in serious reactivation disease (68, 72). During phase 2, viral and fungal infections predominate. CMV and respiratory viruses such as RSV, influenza and parainfluenza viruses are of most concern. CMV monitoring and prophylaxis with antiviral therapy has decreased the occurrence and has changed the usual onset of CMV disease from the first 100 d to beyond the first 100 d when prophylaxis is usually discontinued (72). Patients with GVHD or allogeneic transplants and patients whose conditioning regimens included total-body irradiation are at increased risk of CMV (68, 72). Clinically, fever, dry cough, dyspnea, and hypoxemia are seen in the setting of interstitial infiltrates on chest radiographs. A variety of abnormalities may be seen on high-resolution CT studies. Patchy ground-glass opacities are the most common features; however, patients with CMV pneumonitis may also have nodular infiltrates and air-space consolidation (70).

Respiratory viruses in HSCT may also be associated with severe morbidity and mortality. HSCT recipients are at greatest risk for severe infections. Patients infected preengraftment are most at risk for progression to pneumonia and subsequent death (73). Numerous transplant centers have reported the impact of RSV infections on morbidity and mortality in the HSCT population (12, 73, 74). While the majority of infections appear to involve the upper respiratory tract, severe lower respiratory tract disease has been described in 18 to 55% of patients (12, 73, 74). Risk factors for the development of lower respiratory tract disease include prior lung disease, and onset prior to HSCT (74). Lymphopenia and allogeneic transplantation also appear to be significant risk factors for the development of parainfluenza virus infections. Patients with upper respiratory tract infections usually survive, however, those who progress to pneumonia usually have a high mortality (12).

Adenoviruses cause a variety of syndromes in HSCT recipients and other immunocompromised patients. These include respiratory, gastrointestinal, genitourinary, and central nervous system manifestations. Diffuse disease have an extremely high mortality, approximately 73 to 80% (12).

TABLE 1 Radiographic appearances of pulmonary infiltrates in the immunocompromised host and the likely etiologic agents

<table>
<thead>
<tr>
<th>Nodular</th>
<th>Perihilar</th>
<th>Multifocal</th>
<th>Diffuse</th>
<th>Consolidation</th>
<th>Cavitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute infiltrates</td>
<td>Legionella spp.</td>
<td>CHF, P. jiroveci</td>
<td>Respiratory and herpes viruses, P. jiroveci, ARDS, alveolar hemorrhage</td>
<td>Bacterial pneumonia, including M. pneumoniae, pulmonary infarct, CHF, Aspergillus</td>
<td>P. aeruginosa, Klebsiella spp., S. aureus, Legionella spp.</td>
</tr>
<tr>
<td>Subacute/chronic infiltrates</td>
<td>Aspergillus, other opportunistic fungi. dimorphic fungi, Nocardia, Cryptococcus, Mycobacteria</td>
<td>RSV, CMV, Cryptococcus</td>
<td>Invasive fungi, CMV, HHV-6, miliary tuberculosis, drug-induced pneumonitis, radiation pneumonitis, leukaemaglutination reactions, lymphangitic spread of malignancies. BOOP</td>
<td>Aspergillus, Nocardia, Cryptococcus, carcinoma</td>
<td>Nocardia, Aspergillus, Anaerobic lung abscess M. tuberculosis, NTM</td>
</tr>
</tbody>
</table>

a Modified with permission from reference 4.
b ARDS, acute respiratory distress syndrome; BOOP, bronchiolitis obliterans organizing pneumonia; CHF, congestive heart failure; CMV, cytomegalovirus; HHV-6, human herpes virus-6; NTM, nontuberculous Mycobacteria; RSV, respiratory syncytial virus.
Multiplex molecular respiratory viral panel tests are capable of detecting not only the typical respiratory viruses but also less common viruses such as rhinoviruses and coronaviruses, human metapneumovirus, and bocavirus. All of these other viruses, with the possible exception of bocavirus, are unequivocally associated with upper respiratory tract infections that may progress to serious lower tract infections causing substantial morbidity and mortality among this patient population (74). These viruses are described in more detail in reference 74.

Several reports have emerged on the pathogenicity of human herpesvirus 6 (HHV-6) in this patient population. Fever, myelosuppression, delayed engraftment, rashes, and interstitial pneumonitis and encephalitis have been described (75, 76). There is some evidence demonstrating that severe interstitial pneumonia caused by HHV-6 occurs in the adult HSCT recipient. In one small retrospective study, HHV-6 was detected by PCR in patients with respiratory failure and lung infiltrates. One or more copathogens were detected in 25 of 29 patients. In the four patients in whom no other pathogen was detected, patients improved with antiviral therapy (75). Data are less compelling for pediatric patients (76).

During phases 2 and 3, approximately 10 to 17% of allogeneic bone marrow transplant (BMT) patients develop idiopathic pneumonia syndrome (1, 67, 68, 77). This syndrome is defined as widespread alveolar injury characterized by hypoxemia and bilateral pulmonary infiltrates in the absence of fluid overload, cardiac dysfunction, acute renal failure, or active lower respiratory tract infection (negative bronchoalveolar lavage, [BAL] and no response to antimicrobial therapy) (1, 68). The exact pathogenic mechanism is unclear, but this syndrome is seen most often in patients with risk factors such as high-dose total-body irradiation and GVHD (68). Often, the clinical course of idiopathic pneumonia syndrome is complicated by superinfection with viruses and fungi (1). Mortality is high (70 to 90%) (1, 67, 68, 77).

In phase 3 (>100 days posttransplantation), the development of chronic GVHD often dictates the type of pulmonary complication, and it is an independent risk factor for the development of late pneumonia (67, 68, 72). CMV and fungal infections may occur due to terminal prophylaxis and prolonged immunosuppressive therapy (5, 68, 72). Infection with other viruses such as varicella zoster virus, continued risk of infection from community-acquired respiratory virus pathogens, and EBV-related lymphoproliferative disease may emerge during phase 3. Most transplant centers report the elimination of P. jirovecii as a significant pathogen in this group due to prophylaxis.

When it does occur, P. jirovecii is seen almost exclusively in patients not taking trimethoprim sulfamethoxazole prophylaxis (67, 68, 72). Bacterial pneumonias with encapsulated bacteria (S. pneumoniae, H. influenzae) are common, and Gram-negative bacteria, they are the most frequently recovered organisms in situations where a pathogen is recovered (67, 68). Bronchiolitis obliterans is characterized by irreversible airflow obstruction on pulmonary function testing. Patients complain of dry cough, dyspnea, and wheezing. This syndrome is reported to occur most commonly in long-term survivors who have chronic GVHD (1, 68). The likely pathogenic mechanism is induction of bronchial epithelial injury by immunologic mechanisms (68). Superinfection with respiratory pathogens is high in patients with bronchiolitis obliterans (BO), as is mortality (68).

Other Vulnerable Populations

Collagen vascular diseases

Collagen vascular diseases include rheumatoid arthritis, systemic lupus erythematosus, Sjögren’s syndrome, polymyositis, dermatomyositis, systemic sclerosis and granulomatous vasculitic conditions. These conditions are associated with immune dysregulation. These diseases can be associated with mild to moderate infections, but the therapies used to treat them often result in significant immunosuppression and subsequent severe infections, as mentioned above (78). Treatments include corticosteroids; methotrexate, which induces neutropenia; cyclophosphamide, which induces lymphopenia and suppresses T and B lymphocyte activity; and the newest agents, called tumor necrosis factor alpha inhibitors (TNF-α). The latter selectively inhibit TNF, which subsequently downregulates other cytokines in the proinflammatory immune pathways. TNF is the cytokine most essential for formation and maintenance of granulomas.

Patients on antitumor necrosis factor agents and lymphocyte-depleting monoclonal antibodies

There is a vast array of biologic immune response modulators, and all of these agents have reported infectious diseases complications. This section will highlight the most common agents and their lower respiratory tract complications. There are several available anti-TNFα inhibitors which are commonly used to effectively treat a variety of conditions where tumor necrosis factor is believed to contribute to the pathophysiology of the dis-
ease, including inflammatory bowel disease, sarcoidosis and rheumatologic conditions such as rheumatoid arthritis, psoriatic arthritis, and some spondyloarthropathies. Infliximab (Remicade; Janssen Pharmaceuticals) is a partially humanized monoclonal antibody, and adalimumab (Humira; AbbVie Laboratories) and golimumab (Simponi; Centocor Inc.) are fully humanized monoclonal antibodies, while certolizumab (Cimzia; UCB Inc.) is a pegol-pegylated Fab fragment of human monoclonal antibody. Etanercept (Enbrel; Pfizer) is a dimeric soluble TNF-α receptor fusion protein, which acts as a decoy receptor for TNF-α (79).

A meta-analysis showed an increased risk for serious infection (odds ratio: 2.0; 95% confidence interval [CI]: 1.3 to 3.1) and malignancies (odds ratio: 3.3; 95% CI: 1.2 to 9.1) in patients treated with infliximab and adalimumab (80). Tuberculosis is the most frequently reported serious infection with a reversal of the normal pattern of disease, that is, more extrapulmonary disease (65%) versus pulmonary disease (81). An increase in granulomatous infections due to NTM and Nocardia spp., as well as an increase in pathogens responsible for lower respiratory tract infections, such as L. monocytogenes, Legionella spp., H. capsulatum, C. neoformans, Aspergillus spp., P. jirovecii, Candida spp., and C. immitis, have also been noted (79–81). The median time to onset of these infections was 40 days, suggesting reactivation of latent infection (81). In the same study by Wallis et al. (81), granulomatous infections were 3.25-fold greater among patients who received infliximab compared to those patients who received Etanercept.

Currently, there are several monoclonal antibodies that target T cells and B cells that are important in the management of hematologic malignancies and organ transplant rejection. Those agents, such as OKT3 (Orthoclone OKT3, Janssen-Cilag Inc.), rituximab (Rituxan, Biogen Idec Inc./Genentech Inc.), ofatumumab (Arzerra, Novartis Inc.), belimumab (BenLysta, GSK Inc.) and alemtuzumab (Campath, Lemtrada; Sanofi Inc.), cause profound lymphocyte depletion, resulting in significant increases in opportunistic infections among transplant patients treated for allograft rejection and patients with hematological malignancies. Up to 50% of patients treated with these agents will develop pneumonia caused by the pathogens highlighted above for the TNF-α inhibitors and especially with viral or fungal pathogens (82, 83). Many of the patients develop late infections (>200 days out from transplantation) during a period when both routine monitoring for diseases such as CMV and antibiotic prophylaxis have been discontinued. This has implications for diagnostic and empiric treatment algorithms (72, 83).

**Alcoholism**

Chronic alcoholism predisposes to a number of serious impairments of normal respiratory host defense mechanisms resulting in enhanced vulnerability to bacterial infections and M. tuberculosis (84). Both a decrease in saliva production and impairment in the normal acidic buffering capacity lead to gingival disease and consequent increased oral cavity colonization with anaerobes and Gram-negative bacteria such as K. pneumoniae (84). Altered mental status during periods of intoxication presents opportunities for aspiration of microbes into the lung. Once organisms reach the tracheobronchial tree, a combination of decreased ciliary activity and alcohol-induced impairments in innate and adaptive immunity prevent the host from resisting serious infections caused by organisms such as S. pneumoniae, S. aureus, L. pneumophila, K. pneumoniae, and M. tuberculosis (84). Complications such as bacteremia, abscess formation, and acute respiratory distress syndrome are a consequence of these bacterial infections in the alcoholic patient and result in extremely high mortality.

**Noninfectious pulmonary entities that may mimic pneumonia**

A comprehensive discussion of the noninfectious entities that may mimic infectious causes of pulmonary infiltrates is beyond the scope of this article. However, these entities should be considered in the differential diagnosis of pulmonary syndromes among certain vulnerable populations. While pulmonary complications caused by infections appear to be decreasing, the incidence of noninfectious pulmonary complications has remained the same (68). Noninfectious etiologies of pulmonary infiltrates account for 25 to 50% of abnormal radiographs (85). Some of these have been mentioned in the sections above and are incorporated into the discussion on diagnosis below, but a brief summary of the more common conditions is again mentioned here. Cardiac and noncardiac pulmonary edema is often seen in the second or third week after BMT and has also been reported to be a problem in renal and liver transplant recipients and patients with hematologic malignancy (85, 86). Diffuse alveolar hemorrhage is characterized by sudden onset of dyspnea, nonproductive cough, fever, and hypoxemia. Interstitial infiltrates on chest radiographs may be confused with opportunistic infections (68, 86). Bronchoscopy is required to differentiate hemorrhage from infection. Likewise, radiation pneumonitis, more commonly seen in the treatment of non-hematologic cancers, may also cause cough, fever, and abnormal chest radiographs. Pulmonary function tests

**Carroll and Adams**

[86]
showing a restrictive pattern and a reduced lung capacity along with characteristic computed tomography (CT) findings are helpful in elucidating the diagnosis in patients at risk (87). Drug reactions can take the form of hypersensitivity pneumonitis such as occurs with cytotoxic chemotherapy agents or interstitial pneumonitis and pulmonary vasculitis as has been reported with sirolimus, an agent frequently used in SOT patients (85, 88). Pulmonary embolic disease is often a problem in renal transplant patients, and veno-occlusive disease is a rare vascular complication following HSCT (68). In the latter circumstance, although suspected on the basis of clinical features, biopsy is often required to confirm the diagnosis (68). Primary malignancy and metastatic disease such as lymphangitic spread of carcinoma are important noninfectious causes of infiltrates in patients with AIDS (42).

**DIAGNOSTIC APPROACHES AND INTERPRETATION**

Patients with lower respiratory tract infections may present with a variety of clinical and radiographic manifestations which may be altered by the underlying immune defect. Some of the clinical manifestations of the various syndromes have been elaborated upon in the sections above. In general, fever, dyspnea, chest pain, cough, and hypoxemia even in the absence of other pulmonary symptoms should prompt immediate diagnostic evaluation. Empiric therapy can often be initially guided by the type of radiographic appearance (see Table 1), the pace of the disease and or infiltrates, knowledge of preexisting exposures and the status of the underlying immunodeficiency (such as CD4 count in patients with HIV disease), or immunocompromised state (elapsed time since transplant). Finally, it is imperative to keep in mind that both infectious and noninfectious etiologies can produce fever, leukocytosis, and pulmonary infiltrates.

**Radiography**

Plain chest radiographs are often inadequate in the diagnosis of lower respiratory tract infections in the immunocompromised host but may be helpful in the setting of new infiltrates. Radiographic findings may be divided into the general categories of consolidation, interstitial infiltrates, and nodular infiltrates (86, 89). Although findings may be nonspecific, certain patterns may be suggestive of particular categories of infectious diseases and/or noninfectious complications and can be helpful in triaging both empiric therapy and further diagnostic management (Table 1). If the pattern is one of diffuse interstitial infiltrates or peribronchial infiltrates, the infectious causes include viral pneumonia and *P. jirovecii* (86, 89, 90). Noninfectious entities to be entertained acutely include pulmonary edema, leukoagglutination reactions, engraftment reactions, and diffuse alveolar hemorrhage, whereas subacute presentation of diffuse interstitial infiltrates may be caused by radiation pneumonitis and drug-induced toxicities (86, 89). Focal air space opacities, especially if they are acute, are likely to be caused by bacteria, hemorrhage, or thromboembolic events; if subacute or chronic, resistant bacterial infections, fungi, *Nocardia*, and mycobacteria are more likely. Also to be considered are atypical *P. jirovecii* infections and bronchiolitis obliterans organizing pneumonia (89). The differential diagnosis of multifocal air space opacities includes bacteria, *P. jirovecii*, and fungi, such as *Cryptococcus* and *Aspergillus* (86, 89, 90). If the infiltrate has a nodular component, bacterial pathogens such as *S. aureus*, *Pseudomonas*, *Legionella*, and *Nocardia* should be sought, but in many cases, fungi or mycobacterial infections are responsible. Cavitary disease is most likely to be caused by fungi, *S. aureus*, *Klebsiella* spp., *P. aeruginosa*, *Legionella* spp., *Nocardia* spp., and *M. tuberculosis*.

Plain chest radiographs are usually followed quickly by CT images. High-resolution CT has replaced conventional CT imaging in many institutions. Oftentimes, patients who have normal-appearing plain chest radiographs will have abnormalities on HRCT, which is a highly sensitive technique. What follows is a brief description of the HRCT findings for some of the more common opportunistic pathogens. The most common CT findings with bacterial pneumonia are asymmetric areas of segmental or lobar (sometimes multilobar) consolidation. With some pathogens, such as *Nocardia* and even *Legionella*, observed nodular opacities may mimic those seen with fungal infections (15, 69, 86, 91). *P. jirovecii* causes symmetric, ground-glass opacities that are apically distributed and that spare the periphery; a negative HRCT essentially rules out *P. jirovecii* (90, 92).

Fungal infections may display a variety of different CT images depending upon the stage of infection and likely pathogen (93). Lung nodules greater than 1 cm and lung masses may progress to findings such as the “hypodense sign” (central hypodensity within the nodule or mass) or the “air crescent sign” (collection of air in a crescent shape that separates the wall of a cavity from the inner consolidation) (86, 91, 93). *Aspergillus* spp. characteristically produce both large and small nodules that are surrounded by a halo of ground-glass...
attenuation (halo sign) or plural wedge-shaped areas of consolidation which correspond to hemorrhagic infarcts (90, 91, 93). The reverse halo sign (a focal area of ground-glass opacities surrounded by a ring of consolidation) has also been seen in invasive pulmonary aspergillosis (90, 91, 92). Both the halo sign and reverse halo sign, while suggestive of aspergillosis, can also be seen in other conditions such as mucormycosis, Kaposis sarcoma, HSV, and CMV (90). Multifocal bilateral ground-glass opacities are the predominant abnormality seen in patients with CMV, although small nodules and focal areas of consolidation have also been described (90, 91, 93). Various combinations of nodules, ground-glass attenuation, and consolidation have been described with pneumonia caused by respiratory viruses such as RSV (90). In one retrospective study of RSV pneumonia in adults, areas of ground-glass consolidation were most common (64%), followed by consolidations and nodules in 56% and 55%, respectively (94).

In a study by Lee et al., CT findings in NTM infections in immunocompromised patients were compared with those in immunocompetent patients (95). In both groups, the most common CT findings were bronchiectasis and ill-defined nodules (95). Cavitation of the nodules and large opacities (>2 cm) with or without cavitation were more common in the immunocompromised groups (95).

Although some radiographic patterns are quite suggestive of particular pathogens (such as large nodules and the halo sign with aspergillosis), most findings are nonspecific and require confirmation by histopathology and directed microbiological studies.

Magnetic resonance imaging technology has advanced and has been used at some centers as a follow-up for pneumonia. However, some have found it to be less discriminating in characterizing internal features such as cavitation (91).

**Blood Cultures**
Any immunocompromised patient with fever should have blood cultures obtained. Adherence to current recommendations for specimen collection and appropriate volume are required to ensure adequate recovery and interpretation of results (96). Some recommend collection of 30 ml of blood per culture in this patient population (97). Blood cultures may be of limited value in patients on empiric antibiotics. However, in neutropenic patients and other populations at risk for bacterial pathogens, particularly *S. pneumoniae, S. aureus*, and *P. aeruginosa*, the blood cultures are often positive. In general, 15 to 30% of pneumonias in the immunocompromised patient are associated with positive blood cultures (97). In SOT recipients, bacteremia is common in the early or late postoperative period, occurring in 13 to 29% of heart transplant recipients (62) and in up to 25% of patients with lung transplants. *S. aureus, P. aeruginosa* and *Candida* spp. are the most common pathogens (56). Most yeasts, including *C. neoformans*, grow well in the nonradiometric continuous monitoring blood culture systems currently in use in clinical laboratories. When systemic or opportunistic fungi or mycobacterial infections are under consideration, the lysis centrifugation system, MycoF Lytic media (Becton Dickinson, Sparks, MD), or other media designed specifically to recover these organisms is recommended. Positive blood culture results may obviate the need for more invasive respiratory studies, particularly in the neutropenic patient and other immunocompromised hosts when bacterial etiologies are highest in the differential diagnosis.

**Urinary Antigen Studies**
A variety of commercially available assays that detect antigen in the urine of patients infected with specific pathogens are available and in many cases are useful adjuncts to more aggressive diagnostic techniques. Urinary antigen assays for the detection of *L. pneumophila* serogroup 1 have reported sensitivities that range from 70% to 90% and in general have high specificity (99 to 100%) (98). The variability in sensitivity may be a factor of the population studied, the timing of specimen collection in relationship to onset of disease, and whether specimens have been concentrated or not (98). An immunochromatographic assay (Binax NOW Legionella) provides results in 15 min. Sopena et al. (99) found that immunocompromised patients often excrete antigen for 60 days or longer and also take a longer time to defervesce. In geographic locations in which the predominant serogroup is not type 1 and/or the species of *Legionella* is other than *pneumophila*, culture methods or NAATS, if available, are required for adequate diagnosis.

The *S. pneumoniae* urinary antigen test is another immunochromatographic assay that is FDA cleared for use on urine and cerebrospinal fluid and has also been evaluated on positive blood cultures. To date, there have been no published studies that have evaluated this assay’s performance exclusively in immunocompromised patients. A recent meta-analysis comparing 27 studies was performed looking at the utility of the assay conducted at the time of hospital admission in diagnosing community-acquired pneumonia in adults. This study revealed a sensitivity of 74.0% and a specificity of 97.2% (100). The performance of the immuno-
chromatographic assay in this study was comparable to other published series (101–104). In Lasocki et al., the authors found that prior use of antibiotics did not have an impact on test performance (102). In general, this assay is more sensitive in patients who have bacteremic pneumonia compared to patients with positive sputum cultures. The specificity in adults is high, but false-positive results have been seen in children who have nasopharyngeal colonization in the absence of disease (105, 106).

Urine antigen tests also exist for the detection of histoplasmosis and blastomycosis (107–113). These tests are useful adjunctive diagnostic methods, particularly in patients with more extensive disease, and because urine samples are easy to obtain, these tests may often result in the initial diagnosis. However, neither test alone should be relied upon as definitive for the diagnosis. Ideally, cytology or histopathology of body fluids or tissues and culture methods should also be performed when the likelihood of infection with systemic mycoses is high. There are two FDA-cleared assays for the detection of Histoplasma antigen in urine and serum. The Histoplasma quantitative EIA is a sandwich enzyme immunoassay (MiraVista [MVista] Diagnostics, Indianapolis, IN) approved for use on serum, plasma, urine, cerebrospinal fluid (CSF), and bronchoalveolar lavage (BAL) as well as other body fluids (107). This assay requires the user to send specimens directly to MiraVista Diagnostics. The IMMY Histoplasma EIA (Immuno-Mycologics, Norman, OK) detects Histoplasma galactomannan and is a commercially available assay that can be purchased for use in hospital laboratories that prefer to perform on-site testing (109). There is more literature on the performance of the MiraVista Assay. In a large multicenter study performed on 218 patients with histoplasmosis and on 229 control patients, the second-generation MVista Histoplasma antigen assay was shown to be most sensitive in immunocompromised patients with severe disease (108). In that study, positive urine antigen results were reported in 91.8% of patients with disseminated disease, including 94.6% of patients with AIDS, 93.1% of patients with other immunocompromising conditions, and 73.3% of immunocompetent patients (108). Positivity rates were lower in patients with pulmonary disease (50% in immunocompromised patients and 41% in nonimmunocompromised patients) (108). Performance of the third-generation quantitative assay was further improved with 100% of AIDS patients with disseminated disease having a positive urine antigen test (107). A study performed by Theel et al. compared the IMMY GM assay to the MVista EIA on 150 urine samples (109). The authors used a modification of the manufacturer’s recommended cutoff values and were able to show an overall agreement between the two assays of 90%, with a positive agreement of 82.3% and a negative agreement of 100% (109). Of note, 8% of the samples yielded an indeterminate result (109). A recent meta-analysis that examined all literature between 1980 and 2014 reported an overall sensitivity for antigen detection in serum and urine of 81%, indicating that antigenuria and antigenemia have equivalent diagnostic value in diagnosing histoplasmosis (110). In addition to urine and serum, other specimen types that are amenable to testing include CSF and BAL specimens. Specificity is high at 99%, although cross-reactivity with endemic mycoses such as paracoccidioidomycosis, blastomycosis, African histoplasmosis, and Penicillium marneffei has been described (107).

A quantitative Blastomyces antigen enzyme immunoassay was developed by MiraVista laboratories in 2011. In an initial evaluation, antigenuria was detected in 80 of 89 (89.9%) otherwise-healthy patients with blastomycosis, half of whom had isolated pulmonary involvement and the other half having either extrapulmonary disease alone (19%) or combined pulmonary and extrapulmonary infection (29%) (111). Antigen concentrations were highest in patients with pulmonary manifestations and lowest in patients with extrapulmonary disease alone (111). Early studies report a sensitivity as high as 93% (112). In a recent study by Frost at the Marshfield Clinics in Wisconsin, an endemic area for blastomycosis, the performance of both the qualitative and quantitative assays was evaluated in a retrospective study spanning 11 years (113). All patients had confirmed culture or cytopathological diagnosis (113). Antigen testing was most sensitive in patients with isolated pulmonary disease (83%) and was lowest in patients with extrapulmonary disease alone (0%). The overall sensitivity of 76% was lower than in the Durkin study (113). The authors also evaluated the value of serial quantitative urine antigen concentrations to monitor treatment in 19 patients (113). Treatment failures were characterized by an increase in antigen levels, whereas decreasing levels correlated well with improvements in clinical symptoms (113). The immunoassay for Blastomyces dermatitidis does cross-react with Histoplasma antigen and is less useful in areas endemic for both in terms of distinguishing between the two diseases. As is true with the Histoplasma urinary antigen, a negative result is not definitive, and histopathology or cytology or culture are needed to exclude infection.
Tests on Other Nonrespiratory Sources

Patients with HIV, as well as patients following SOT, are particularly at risk for infections caused by C. neoformans, including pneumonia (52, 114). Diagnosis of pneumonia can be delayed because of nonspecific symptoms. Cryptococcal antigen tests performed on serum and CSF have been quite useful in rapidly diagnosing untreated patients with this infection. The performance of available commercial assays (latex agglutination and EIAs) has been reviewed among various populations (52, 114). Sensitivities of these tests have been shown to be higher among patients with HIV and disseminated infections compared to non-HIV patients, who are less likely to have dissemination (114). Depending upon the assay, Trichosporon spp. and interfering substances such as starch, disinfectants, and soap may affect specificity (114, 115). A recently available cryptococcal antigen lateral-flow assay (IMMY Inc., Norman, OK), which is an easy-to-use dipstick sandwich immunochromatographic immunoassay, has demonstrated equivalent, and in some reports superior, sensitivity compared to EIA and latex agglutination formats (114–116). Two recent studies have confirmed excellent sensitivity (97.6% to 100% in CSF and serum) of the LFA in patients without HIV and for both focal and disseminated infections (115, 116). In the study by Jimuang, the authors showed a lower limit of detection with the LFA compared to latex agglutination (115).

The performance of galactomannan and the 1,3 β-D-glucan tests, particularly when testing respiratory samples such as pleural fluid and bronchoalveolar lavage fluid, is summarized below in the section on bronchoscopy.

Serological Tests

In general, serological tests are likely to be limited when diagnosing infections in immunocompromised patients. Tests for measurement of antibody are more useful in prescreening patients likely to be at risk for reactivation of infection or in the case of SOT and HSCT recipients, who are at risk for new infection from a seropositive donor. Tests that measure antibody are not timely because of the delay in mounting an immunological response or, in the case of some patients, the inability to do so. Falsely negative serological tests have been reported in immunocompromised patients with Legionella infections (98), histoplasmosis (117) and toxoplasmosis (65). A single serologic test should not be relied upon to make a diagnosis of any of these pathogens. In the case of toxoplasmosis, serology is most useful pretransplantation and in monitoring the HIV-positive patient by predicting who is likely to recrudesce in the case of positive serology, and in counseling patients who are seronegative. Alternative methods of diagnosis are required.

Respiratory Specimens

A variety of respiratory specimen types can be collected in an attempt to diagnose respiratory diseases. Regardless of whether these are noninvasively obtained or require bronchoscopy or biopsy, serious consideration must be given to adequate specimen collection and to timely transport and coordination between the clinical microbiology laboratory, cytology, or surgical pathology laboratories and the clinician taking care of the patient. Development of diagnostic algorithms with consensus from pulmonologists, microbiologists, oncologists and infectious diseases specialists, among others, is important, particularly in centers with active transplantation programs.

Nasopharyngeal aspirates or washes are preferred to swabs for the initial diagnosis of respiratory virus infections, Bordetella pertussis (B. pertussis) and B. parapertussis, and the atypical pneumonia agents M. pneumoniae and C. pneumoniae. These specimen types are not acceptable for other types of analyses. In general, rapid antigen ELISA and immunochromatographic membrane tests, when positive for influenza or RSV, are useful for implementing treatment and infection control practices; however, if negative, then alternative testing using culture or NAATs should be performed. It is important to note that performance of these various antigen assays has not been well studied in immunocompromised patients. Also available are immunofluorescence methods for a broader range of pathogen detection such as adenovirus, parainfluenza viruses, RSV, influenza viruses and metapneumovirus. In general, immunofluorescence microscopy is more sensitive than rapid antigen detection testing. Although listed as an acceptable source in many product package inserts of several rapid diagnostic tests, throat swabs are less desirable samples for respiratory virus diagnosis (118). Multiplex NAATs are displacing insensitive antigen detection assays for respiratory virus diagnosis due to their enhanced sensitivity and ability to diagnose multiple pathogens simultaneously. In general, these assays are approved for testing on nasopharyngeal swabs and aspirates and BAL specimens (73).

Expectorated sputum, if available, may be useful for analysis in some immunocompromised patient populations, such as the HIV-infected patient. In HIV-infected patients with pulmonary infiltrates caused by bacterial
pneumonia, the yield of sputum culture can be as high as 65%, with a range of 35% to 60% (41). In other types of immunocompromised patients, expectorated sputum specimens are generally of low yield. Attempts should be made to minimize oropharyngeal contamination by not allowing the patient to ingest food 1 to 2 h prior to expectoration, by having the patient remove dentures, and by rinsing the mouth with water (119). Expectorated sputum should be transported to the laboratory as soon as possible after collection to minimize the loss of fastidious pathogens such as S. pneumoniae and to prevent overgrowth of normal microbiota or nonfastidious pathogens. Specimens that cannot be delivered and processed within 2 h of collection should be refrigerated (119). The standard criteria for sputum specimen acceptance (<10 epithelial cells/high-powered field and >25 polymorphonuclear leukocytes) are not valid for neutropenic patients. Early studies have shown decreased cellularity of pulmonary alveoli in neutropenic patients, including reductions in polymorphonuclear leukocytes, compared to patients without neutropenia (120). Sputum analysis is most useful when organisms that are not part of the normal microbiota, such as Legionella, mycobacteria, or systemic fungi, are recovered.

**Induced sputum**

When patients appear incapable of spontaneous expectoration, specimens are often obtained by induction. This procedure should be performed by a trained and experienced respiratory therapist. Patients must be able to cooperate with the procedure, which involves inhaling hypertonic saline (3%) through an ultrasonic nebulizer via a mouthpiece for up to 30 min. As with expectorated samples, induced sputa should likewise be collected in sterile containers and should be delivered promptly to the laboratory. The utility of this procedure for diagnosing bacterial pneumonia in either immunocompetent or immunocompromised patients has not been well studied, and most of the literature is old (41, 121, 122). In general, the available studies (121, 122) show no appreciable increase in bacterial pathogen recovery when compared to recovery by expectorated sputum, although stratification of patients by immunocompromised state was not performed. Sputum induction has been shown to be a reliable method in the diagnosis of tuberculosis regardless of the immune status of the host, particularly in patients who have difficulty expectorating spontaneously. One study examined the yield of repeated sputum induction in patients suspected of pulmonary tuberculosis who could not actively produce sputum (123). The authors reported that the yields for acid-fast smear and culture were 64% and 70%, respectively, for one sample and were 81% and 91%, respectively, for two samples and 91% and 99% for three induced samples (123). A prospective study performed among children in Africa in a high-HIV-prevalence area demonstrated the feasibility of using induced sputum in children less than 5 years of age (124). In that study, induced sputum was superior to gastric aspirates in the recovery of M. tuberculosis (124). In the recent systematic review by Hepple et al., sputum induction was found to be well tolerated by children and adults, with only mild adverse events reported, and diagnostic yield compared favorably between induced sputum and BAL (125). Likewise, among HIV-positive patients with tuberculosis and NTM infections, the systematic review by Oliveira et al. revealed good correlation for both smear and culture results between induced sputum and BAL (126). The authors commented that induced sputum is safer and less expensive, particularly in resource-limited countries, and the recommendation was to try initial sputum induction in this population in the patient unable to expectorate (126).

Likewise, sputum induction has been very useful in HIV-infected patients in the diagnosis of P. jirovecii (41, 123, 126). In the pre-HAART era, performance of direct fluorescent antibody (DFA) testing using monoclonal antibodies that detect both Pneumocystis cysts and trophozoites directly on induced sputum from susceptible HIV-positive patients significantly reduced the need to perform more invasive procedures (127). In this population, the organism burden was higher than in the non-AIDS patient (127, 128); therefore, this algorithm was more sensitive in the AIDS patient population (127, 128). More recently, however, Pneumocystis has significantly declined in the HIV patient and is diagnosed more often in the non-HIV immunocompromised patient in some centers (127). The quantity of P. jirovecii in these patients is low, reducing the yield from induced specimens. Molecular techniques performed upon oral washes (127, 129, 130), induced sputum (131) or more invasively obtained specimens are replacing DFA or less sensitive staining methods for diagnosing Pneumocystis in some centers. Assays that use multicopy gene targets such as mitochondrial rRNA or major surface glycoprotein genes have the highest sensitivity (127, 130). There are currently no standardized FDA-cleared molecular-based assays. The performance of several commercial NAATs in the diagnosis of P. jirovecii pneumonia (PPJ) in mostly BAL specimens from a variety of immunocompromised hosts has been recently evaluated in the literature (132, 133). In the study by Hauser et al.,
the MycAssay *Pneumocystis* assay (Myconostica Ltd., Manchester, United Kingdom) was evaluated in four centers in the U.S. and the U.K. Performance of the test was compared to clinical diagnoses, including DFA (132). The sensitivity, specificity, positive predictive value, and negative predictive value for PCR were 93%, 91%, 59%, and 99%, respectively (132). The comparable values for DFA in that study were 93%, 100%, 100% and 98% (132). In the Sasso study, the authors compared four real-time PCR assays, three commercial assays and one laboratory-developed test for the diagnosis of PJP in 148 patients with a variety of immunosuppressing conditions (133). There was excellent concordance among the four assays in patients with high probability of infection (133). The more sensitive assays detected patients with likely colonization (133). The utility and implications of a positive NAAT result in a patient without clinical disease need clarification, as such results reflect low-level infection or colonization (127, 128, 132, 133). Currently, it is recommended that NAAT results be interpreted in the context of clinical symptoms. Others have suggested quantitation of PCR results and/or using β-D-glucan in serum to assist with therapeutic decision-making (134).

**Invasive procedures**

When rapid and noninvasive tests are not revealing causative agent(s) within 24 to 48 h in the immunocompromised patient with a new pulmonary process, then quick progression to more definitive sampling of the lung is required. A suggested algorithm that includes empiric treatment and the use of noninvasive and invasive tests is depicted in **Fig. 3**. There are several diagnostic procedures that can be performed. These include fiber optic bronchoscopy with bronchoalveolar lavage, transbronchial biopsy, transthoracic needle aspiration, video-assisted minithoracotomy, and open lung biopsy. Each of these will be discussed below. In general, because they are more widely available, yield a greater volume of sample, and are somewhat safer than other methods, bronchoscopic techniques are usually performed first.

**FIGURE 3** Clinical approach to pulmonary infiltrates in immunocompromised patients. BAL, bronchoalveolar lavage; CMV, cytomegalovirus; CT, computed tomography; CXR, chest X-ray; MRSA, methicillin-resistant *Staphylococcus aureus*; PJP, *Pneumocystis jirovecii* pneumonia; SLB, surgical lung biopsy. Reprinted with permission from reference 15.
Fiber optic bronchoscopy
The proper procedure for selecting the type of bronchoscope and for performance of the procedure can be found in detail in standard pulmonary textbooks. It is very important that standardization for sampling the lung be agreed upon by microbiologists, pulmonologists, and those clinicians caring for patients. This is extremely important for ensuring quality of the sample and optimum yield. In this writer’s unpublished experience, procedures can vary tremendously depending upon the training and experience of the pulmonologist, even within the same institution. A variety of specimen types can be collected. A brief discussion of each is provided, with emphasis on bronchoalveolar lavage.

Bronchial washings and bronchial brushings. These specimens are usually obtained by installation of saline into a major airway through the bronchoscope channel, and aspiration back of the secretions. These secretions are not representative of processes in the alveoli or small airways. The best use of bronchial washings is in the diagnosis of strict pathogenic organisms such as M. tuberculosis and endemic fungi; these specimens are not appropriate for diagnosing bacterial pneumonia (135). In general, this is not the preferred procedure for diagnosing pneumonia in immunocompromised patients, but on occasion these may be the only samples available in unstable patients or when BAL return volume is inadequate (135) (see below).

Bronchial brushings are usually performed for obtaining cytologic samples for the diagnosis of malignancies and not for routine bacterial or other types of cultures. Because cells are obtained, cytopathic changes caused by infectious agents, such as the type of inflammatory cell and viral inclusion bodies, may be observed.

Protected specimen brush samples are collected using two telescoping catheters, the outer of which is occluded by a Carbowax plug that prevents secretions from entering the catheter during passage through the bronchoscope channel (135). After insertion of the bronchoscope, the device is passed through the bronchoscope channel, the inner catheter is advanced, and the Carbowax plug is expelled into the airway lumen where it is absorbed. The brush is advanced past the tip of the inner catheter where it has been protected from upper-airway secretions (135). Usually, the small-volume specimens are collected from the distal bronchioles. After the specimen is collected, the brush is retracted back into the inner catheter, which is retracted back into the outer catheter. After removal of the bronchoscope from the airway, the brush is carefully removed without contamination and placed into 1 ml of lactated Ringer’s solution or some other diluent, after which it is submitted to the microbiology laboratory as soon as possible. This procedure was designed for specific diagnosis of bacterial pneumonia by Gram stain and quantitative culture. The volume of sample is such that its use in recovery of a broad range of pathogens is not possible.

Bronchoalveolar lavage and transbronchial biopsy. In general, bronchoalveolar lavage is the preferred procedure for immunocompromised patients. In this procedure, the bronchoscope is carefully wedged into an airway lumen. In the patient with diffuse infiltrates, the scope is usually wedged in the right-middle lobe or lingula. When the infiltrate is focal, it is important to wedge the scope in the pulmonary segment corresponding to the radiographic abnormality. Once the scope is wedged, a large volume of 0.9% saline (100 to 300 ml) is injected in 3 to 4 aliquots of 30 to 60 ml each (135, 136). There are no data on the optimum volume to instill. In the pediatric patient, this amount is usually 1 ml/kg. The amount returned is variable and ranges from 40% to 60% of the instilled volume; a returned volume of 40 to 60 ml is ideal. The first returned aliquot is usually contaminated with oropharyngeal and upper-airway microbiota and should not be used for quantitative bacterial culture. It is usually discarded or used for detection of strict pulmonary pathogens such as Legionella, fungi, or mycobacteria (135). When performed properly, this procedure is estimated to sample about one million alveoli.

In some situations, BAL fluid alone may be inadequate in the diagnosis of certain conditions where tissue is required for histopathology to clarify positive cultures from fluid, or when noninfectious entities are being considered. In such cases, transbronchial biopsies (TBB) are often obtained at the same time that BAL is performed. In one study, the yield of BAL in immunocompromised patients increased from 40% to 70% when combined with TBB (137). TBB samples are obtained by passing a forceps through the working channel of the wedged bronchoscope under fluoroscopic guidance. Since the pieces of tissue obtained in this way may be quite small, several specimens are required. The number of biopsies correlates with diagnostic yield, and 5 to 7 biopsies strikes a balance between diagnostic yield and procedural complications such as pneumothorax and bleeding (136).

TBB (or other procedures to acquire lung tissue) are the preferred methods to diagnose acute rejection, CMV pneumonia, posttransplantation lymphoproliferative
disorder, *Candida* pneumonia and pneumonia caused by opportunistic fungi, and in some cases disease caused by NTM ([4], [56], [58], [136], [137]). Infiltrates caused by noninfectious etiologies, such as radiation and chemical pneumonitis, and neoplasia may also be best diagnosed by the inclusion of TBB ([138]).

**Specimen handling and analysis of bronchoscopically obtained specimens.** Bronchoscopic specimens should be transported to the laboratory in sterile leak-proof containers as soon as possible after collection. Since multiple aliquots have usually been obtained, the samples should be sent for cytologic, microbiologic, and chemical analyses. Microbiology laboratories need to work with transplant physicians, pulmonologists, infectious diseases physicians, and oncologists in developing standardized algorithms that include not only the timing of procedures, but the workup of BAL and TBB specimens once they reach the clinical laboratory. Since a broad range of pathogens are possible in most immunocompromised hosts and since more than one process or infection may be present, multiple procedures should routinely be performed. Recommendations for the minimum approach as well as some optional assays are listed in Table 2. The emphasis should be on rapid processing using tests with short turnaround such as direct microscopy, antigen detection, and NAATs when available. For bacterial diagnosis, quantitative cultures using either the calibrated loop method or dilutional method have been recommended (see reference 5). The diagnostic thresholds for significance of various quantities of bacteria are as follows. For protected specimens, quantities of bacteria that equal or exceed 10^3 CFU/ml should be identified and have susceptibility testing performed; for BAL samples, this number is 10^4 CFU/ml or greater. Direct stains at that are useful include Gram’s stain, Calcofluor white with KOH or other fungal stain, and acid-fast stains. Antigen detection methods for viruses, *Pneumocystis*, fungi (see below) and in selected cases *Legionella* are suggested. Culture for viruses, mycobacteria, *Nocardia* and fungi should be included routinely.

### Additional tests that are useful to perform on BAL

Galactomannan (GM) is a heat-stable heteropolysaccharide present in the cell wall of *Aspergillus* and *Penicillium* spp. Galactomannan is predominantly released by *Aspergillus* hyphae during growth, and to a lesser extent by conidia ([139]). The Platelia assay (Bio–Rad Laboratories, Hercules, CA), a double-direct sandwich ELISA, uses galactofuranose-specific rat monoclonal antibody EB-A2 for both capture and detection of GM ([114]). Performance of serum GM in the diagnosis of proven or probable invasive aspergillosis (using the European Organization for Research and Treatment of Cancer and the Mycoses Study Group consensus definitions) is reviewed in the reference by Lamoth et al. ([114]). In general, using a cutoff of ≥0.5 optical density index, the sensitivity ranges from 61% to 87%, with lower sensitivity observed in SOT recipients ([114]). Literature has emerged on the utility of testing BAL fluid and fluids other than serum ([7], [114], [139–143]). Two meta-analyses demonstrated higher sensitivity for BAL.

### Table 2 Recommended diagnostic studies to be performed on BAL, TBB, and SLB specimens

<table>
<thead>
<tr>
<th>Type of pathogen</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Cytospun Gram stain; quantitative bacterial culture; cytologic examination for intracellular pathogens, cell count and differential</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td>NAAT®; culture</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em></td>
<td>Culture on BCYE media; NAAT if available</td>
</tr>
<tr>
<td><em>Chlamydia pneumoniae</em></td>
<td>NAAT</td>
</tr>
<tr>
<td><em>Mycobacterium species</em></td>
<td>AFB smear; culture on selective media; NAAT for <em>M. tuberculosis</em></td>
</tr>
<tr>
<td>Fungi</td>
<td>Calcofluor white stain on BAL; PAS, silver stains of any obtained tissue; culture on selective media; galactomannan assay on BAL; 1-3-β-D-glucan on BAL; NAAT where available</td>
</tr>
<tr>
<td><em>Pneumocystis jirovecii</em></td>
<td>NAAT if available; DFA; cytologic stains such as PAP stain, silver stain; 1-3-β-D-glucan on BAL</td>
</tr>
<tr>
<td>Respiratory viruses</td>
<td>NAAT; combination antigen detection and shell vial culture</td>
</tr>
<tr>
<td>Herpesviruses</td>
<td>Combination antigen detection with shell vial culture; cytologic analysis and/or tissue for interpretation of CMV, HSV results; NAAT testing controversial</td>
</tr>
<tr>
<td>Noninfectious etiologies</td>
<td>Cytologic analysis (BAL)—cell count; examination for siderophages, gross hemorrhage, etc.; TBB, SLB tissue histopathology</td>
</tr>
</tbody>
</table>

^aRoutine testing in BAL algorithms probably not indicated unless these pathogens have been detected in the community. NAAT testing may be done from throat swabs.

^bNAAT, nucleic acid amplification tests; BAL, bronchoalveolar lavage; PAS, periodic acid Schiff; DFA, direct fluorescent antibody tests; CMV, cytomegalovirus; HSV, herpes simplex virus; TBB, transbronchial biopsy; SLB, surgical lung biopsy.
than what is reported when testing serum. In the review by Guo, the summary estimates of BAL-GM for proven or probable invasive aspergillosis revealed a sensitivity of 90% (95% CI: 0.79 to 0.96) and specificity of 94% (95% CI: 0.90 to 0.96) (142). The pooled sensitivity and specificity in the Zou study were 87% and 89%, respectively, in patients with proven or probable disease (143). In studies that compared both serum and BAL, BAL-GM was notably more sensitive (65% versus 85%, respectively) (143). These tests may be of maximum utility when combined with suggestive findings on high-resolution CT and/or in combination with PCR. Musher et al. (141) studied the utility of the Platelia assay and quantitative PCR (qPCR) performed on BAL fluid alone, in combination, and stratified by radiograph findings. In patients with nodular or focal infiltrates, the galactomannan assay had a sensitivity of 73 to 82% using an index for interpretation of 0.5 and 1.0, respectively. Sensitivity of qPCR was 73%. When both qPCR and GM EIA with index of 0.5 were used to diagnose invasive pulmonary aspergillosis in patients with nodular infiltrates, the overall sensitivity was 91% and in culture-positive patients this value was 100% (141). Of note, almost two-thirds of patients who were not diagnosed by culture (44% were culture-negative) had a positive GM or qPCR assay result (141).

1,3 β-D-Glucan is a component of the cell wall of most fungi, including the cyst form of Pneumocystis. There are four commercially available assays. Most experience in the U.S. is with the Fungitell assay (Associates of Cape Cod, East Falmouth, MA) (114). This assay can detect most pathogenic fungi, with the exception of the Mucorales and C. neoformans. The utility of testing serum for the diagnosis of invasive infections is reviewed in Lamoth et al. (114). A recent meta-analysis of serum 1,3 β-D-glucan found high diagnostic accuracy for PJP and moderate accuracy for invasive fungal infection (144). A more recent single-center study in HSCT recipients found the serum BDG test to have a very high negative predictive value (>99%) in that patient population for the diagnosis of invasive fungal infections (145). One of the strongest correlations of a positive serum test appears to be in HIV-related PJP (146, 147).

In the patient with PJP, the sensitivity when testing BAL samples has reportedly been high (148, 149) although not as reliable as serum in the study by Salerno (147). Testing of BAL samples has been less useful in the non-HIV-positive patient for the diagnosis of invasive fungal infections (148–150).

Availability of FDA-cleared NAATs has increased in the last decade, and they contribute to the diagnostic yield of bronchoscopic techniques; in the case of the respiratory viruses, they may prevent the need for more invasive tests. A comprehensive discussion is beyond the scope of this article. However, some summary statements are included here.

In general, for the diagnosis of bacterial pneumonia, advances in molecular testing have largely included single pathogen detection on specimens other than those from the lower respiratory tract. These include assays for the detection of S. aureus in nares swabs to identify patients at risk for development of more invasive infections including pneumonia. Some progress has been made in the development and approval of assays for the detection of B. pertussis, M. pneumoniae and other “atypical” pneumonia pathogens. In addition, there have been advances in the direct detection of M. tuberculosis (151). Broad-based bacterial respiratory panels that also include resistance markers for the diagnosis of pneumonia are in development, and one, the Curetis Unyvero assay, is currently in FDA clinical trials (152).

S. pneumoniae remains the most common cause of community-acquired pneumonia. Molecular assays amplifying the pneumolysin and/or autolysin genes have been applied in laboratory-developed assays for the diagnosis of pneumonia, otitis media and meningitis (153). This pathogen is included in the Unyvero assay and other broad-based assays that are in development (152, 154). PCR on sputum has demonstrated high sensitivity (>80%) but low specificity (30 to 40%) (153). The difficulty lies in distinguishing colonization from true infection. It is unclear at present if qPCR could be applied to improve the clinical specificity for lower respiratory tract specimens. In contrast to their performance on sputum, NAATs using pleural fluid have reasonable specificity (93%) while maintaining good sensitivity (153).

In contrast, given the suboptimal performance of serological testing and the difficulty in cultivating M. pneumoniae, NAATs are the preferred diagnostic method for detection of this organism (98, 151, 153, 155–157). Throat swabs and/or nasopharyngeal specimens are the specimens of choice. Several studies have demonstrated that PCR is more sensitive than conventional methods for detecting M. pneumoniae (98, 156, 157). Few data are available to assess the positive predictive value of NAATs for this infection. One study showed that 15% of patients who were positive by PCR continued to have M. pneumoniae DNA detected in their throats for 2 to 6 weeks following antibiotic therapy (156). There are two FDA-cleared platforms for the detection of M. pneumoniae: the Illumigene Mycoplasma DNA Amplification
Assay (Meridian BioScience Inc.), a loop-mediated isothermal amplification system, and the FilmArray Respiratory panel (bioMérieux Inc.), a multiplex PCR assay (155, 157). In the latter assay, *M. pneumoniae, C. pneumoniae* and *B. pertussis* are the three bacterial pathogens detected, along with 17 viruses (155). The performance of the Illumigene assay has been published, and the sensitivity and specificity were 100% and 99%, respectively, when testing archived culture-positive respiratory specimens that included a variety of sample types (157). There are no publications specifically evaluating the bacterial components of the FilmArray assay. *M. pneumoniae* can be detected by both the Unyvero assay and by PCR-ESI-MS assays that are in development (152, 154). Loens et al. reviewed other non-FDA-cleared assays available in the U.S. and other countries (155).

As mentioned above, *Legionella* spp. have emerged as opportunistic pathogens among immunocompromised patients, but especially in HSCT and SOT recipients. While *Legionella* urinary antigen testing and culture are excellent for detecting *L. pneumophila*, nonpneumophila species are often not detected by these methods (69). PCR detection of *Legionella* spp. using primers that amplify a variety of targets has been described. Various sample types have also been evaluated, including urine and blood. The positivity rate from these nonrespiratory sources has been variable (69, 98). One of the difficulties with PCR for *Legionella* spp. has been the presence of contaminating *Legionella* DNA in commercial extraction kits (158). At present, development of standardized protocols that address the optimum specimen types, gene targets, and extraction methods are needed. One recent study evaluated both induced sputum and throat swabs (159). In that study, a greater than 4-fold increase in *Legionella* cases was seen with PCR compared to culture (159). In addition, the authors emphasized that 40% of the patients were not able to expectorate a sputum sample. Sputum induction increased the case detection by 36% (159). Induced sputum had a higher yield than throat swabs (159). Standard approaches have been recommended for NAATs for *C. pneumoniae* detection (160). The FilmArray respiratory panel includes *C. pneumoniae*, but there is no literature that describes its performance specifically for that pathogen. PCR testing for *Pneumocystis* has been discussed above. The ideal specimens are BAL and induced sputa. PCR is more sensitive than cytological methods and/or DFA and, as mentioned, can be paired with 1,3 β-D-glucan testing on serum for an optimized approach for PJP diagnosis (132, 133).

Development of molecular methods for detection of respiratory viruses has led to the discovery of several new pathogens, such as human metapneumoviruses and additional coronaviruses, which at this time appear to be best detected by NAATs. Several studies have demonstrated that NAATs are more sensitive than the combination of culture, antigen detection and serological testing (10–13, 26, 161–163). There has been an explosion in the number and variety of FDA-cleared NAATs for respiratory virus detection. They range in complexity from the CLIA-waived Cobas Liat Influenza A/B (Roche Diagnostics, Indianapolis, IN) to the multiplex xTAG RVP (Luminex Molecular Diagnostics, Austin, TX) (10 viruses) and FilmArray Respiratory panel (17 viruses, 3 bacteria) tests (162–165). There are few comparative studies evaluating their performance. A recent study by Popowitch et al. compared four broad-based molecular panels; the overall sensitivities ranged from 84.4% to 98.3%, and specificities ranged from 99.2% to 100% (162). Sensitivity varied by the target, with influenza B and adenovirus detection demonstrating the greatest discrepancies (162). Ease of use and costs are also factors that need to be assessed when making decisions regarding implementation of these assays. Additional review of currently available and future molecular methods, including next-generation sequencing, is provided in the article by Somerville et al. (166).

Two FDA-cleared assays are available for detection of *M. tuberculosis*: the *Mycobacterium tuberculosis* direct test (Hologic Inc., San Diego, CA) and the Xpert MTB/RIF assay (Cepheid Diagnostics, Sunnyvale, CA) (166, 167). The performance characteristics of these tests and of other novel assays in the pipeline are discussed in the review by Pai (168).

**DIAGNOSTIC YIELD AND ASSAY INTERPRETATION**

Studies reporting upon the diagnostic utility of BAL and TBB provide conflicting and often controversial findings. In general, the reported diagnostic yield of BAL alone ranges from 31% to 80% in several retrospective (52, 64, 68, 120, 136, 169, 170) and prospective (137) studies across various immunocompromised host populations, but the majority report a diagnosis in roughly 60% of patients (52, 68, 120). Yield is higher when the patient has multifocal or diffuse pneumonia compared to a focal process (120). A recent systematic review and meta-analysis of 95 studies was performed to describe the diagnostic yield and complication rate of BAL and lung biopsy in patients with cancer and in HSCT recipients (170). Seventy-two studies in this review evaluated BAL procedures, and 31 evaluated lung biopsies (170).
In this review, lung biopsy and BAL had similar diagnostic yields, 54% and 53%, respectively (170). In addition, the data supported earlier studies that showed BAL was more likely to establish an infectious diagnosis compared to biopsy (49% versus 34%, P <0.001) whereas in contrast, noninfectious diagnosis was more common with lung biopsy (137, 170). Noninfectious etiologies that may be diagnosed routinely by BAL include alveolar hemorrhage, which is the presence of ≥20% hemosiderin-laden macrophages and/or progressive, diffusely bloody fluid, hypersensitivity pneumonitis, and pulmonary alveolar proteinosis (86, 136, 137).

Fewer studies have examined the impact of BAL on patient management. In a retrospective study of 71 BMT patients who underwent bronchoscopy and BAL, the bronchoscopic findings resulted in changes in therapy for 65% of patients in whom an organism was identified and in 22% of patients with completely negative results (171). A similar study performed in cardiac transplant patients found that changes in therapy occurred in 32% of patients (64). In the meta-analysis mentioned above, changes in management more often occurred following lung biopsy than with BAL (170).

In the study by Jain et al. (137), performance of TBB with BAL significantly increased the diagnostic yield. Also in that study, TBB provided the sole source of diagnosis in 15 of 17 patients with noninfectious etiologies (137); however, none of the above-mentioned conditions were included among those missed diagnoses. In lung transplant recipients, TBB helps establish or exclude acute allograft rejection as a cause of pulmonary infiltrates (15, 136). In a study in patients with hematological malignancies, TBB increased the diagnostic yield of noninfectious conditions such as neoplastic infiltrates and toxic pneumonitis (138). In contrast, other studies, primarily among HSCT patients, have not noted an improved diagnostic yield and do not recommend inclusion of TBB routinely (172). Yield may also be adversely affected by antimicrobial and antifungal treatments administered in the days prior to bronchoscopy. However, studies in neutropenic patients on broad-spectrum antibiotic or antifungal treatment have shown that BAL can yield important information such as recovery of organisms resistant to the empiric therapy at the time of the procedure (172).

“False-positive” BALs due to contaminating or colonizing organisms were seen most often with Candida spp., coagulase-negative staphylococci, and CMV. As mentioned elsewhere, in these instances, correlation with histopathology, cytology or other positive tests (e.g., simultaneously positive CMV antigenemia) is required. False-negative BALs are most frequently seen in patients with fungal pneumonia (120).

Complications of Bronchoscopic Techniques

The risk of complications during fiber optic bronchoscopy depends upon several important variables. These include the severity of the pulmonary process and the degree of hypoxemia, whether thrombocytopenia or a coagulopathy is present and whether the patient is intubated. The main complications are hypoxemia, bleeding, pneumothorax, cardiac compromise and rarely, transmission of infection from inadequately decontaminated bronchoscopes (136, 137, 170, 172). In a recent meta-analysis, brushings during BAL were associated with more complications than BAL without brushings (170). Children were more likely to have complications with biopsy procedures compared to adults (170). Patients with severe hypoxemia may require prophylactic intubation for successful and safe performance of the procedure. The addition of TBB does increase the complication rate.

Surgical Lung Biopsy

When BAL with or without TBB is unrevealing and the patient is not responding to empiric treatment, surgical lung biopsy (SLB) can be performed. In many cases, tissue can be obtained by video-assisted thoracic surgery, a minimally invasive procedure that uses a thoracoscope, camera, and trocars for obtaining the tissues without the need for opening the chest cavity, or by minithoracotomy, thereby reducing procedure-related morbidity and mortality (173). Tissues can be processed and analyzed following the same algorithms as for BAL samples (see Table 2), including special stains and interpretation by a surgical pathologist. Tissue specimens that are to be processed for fungal culture should not be ground up for processing, as this destroys the hyphal elements and may interfere with subsequent recovery in culture. Results from histopathology may frequently reveal an etiological agent or abnormal process before a pathogen is detected in the clinical microbiology laboratory. For example, on biopsy, a granulomatous process may be observed. In this case, special stains (e.g., Grocott’s methenamine silver stain) are utilized to identify various infectious etiologies (174).

The yield of surgical lung biopsy following a negative BAL has not been high in BMT patients (68). Studies performed among other patient populations have reported a change in therapy in a higher percentage of patients (approximately 57% in the study by White et al.) (175, 176), but no studies have demonstrated improved outcomes as a result of SLB (97). The decision
to perform SLB should be individualized and should include a realistic assessment of the likelihood of alterations in therapy and the risk involved in the procedure. If tissue is obtained, comprehensive analysis as listed in Table 2 should be performed.

Other Invasive Techniques
For focal lesions that are accessible, CT-guided needle biopsies have acceptable yield. Transthoracic needle aspiration is rarely performed because of the high incidence of pneumothorax and hemoptysis (97). At least one study compared the diagnostic yields from transthoracic needle aspiration, TBB and SLB in a small number of granulocytopenic patients with pulmonary infiltrates. The diagnostic yields were 30%, 59% and 94%, respectively (177). Regardless of the diagnostic method, recovered tissue should be processed for the pathogens listed in Table 2.

SUMMARY
Lower respiratory tract infections are the most common causes of morbidity and mortality among all groups of immunocompromised patients. In general, bacterial pathogens are the most frequent etiologic agents, but a vast array of opportunistic organisms may be responsible, depending upon the type, degree and duration of immunosuppression. Radiographic images, particularly those visualized by high-resolution CT scans, may be suggestive of a particular process, but there is enormous overlap. Therefore, diagnostic samples should be obtained as quickly as possible after new signs and symptoms appear. Fiber optic bronchoscopy and BAL with or without TBB is the mainstay of diagnosis. From the lavage sample, a broad range of tests are recommended. Rapid antigen detection tests and NAATs have emerged and currently complement existing culture-based methods. Some pathogens require histopathology to determine colonization versus true infection. When carefully performed early in the course, BAL can reveal an etiologic agent that results in a change in therapy in a substantial number of patients, leading to changes in empiric regimens in 30 to 40% of patients. When BAL is not revealing, SLB is usually the next step. The potential benefit provided by SLB must be weighed against the increased morbidity associated with this procedure.

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REFERENCES
Lower Respiratory Tract Infections


