Chapter 31

Diagnosis of Pulmonary Infections

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Introduction

Two types of pulmonary infections are important in critical care medicine: infections which *cause* severe alterations of gas exchange and respiratory failure and infections which arise as *complications* of another critical illness. The former are generally community-acquired infections, while the latter obviously arise within the hospital, usually the intensive care unit (ICU). Organisms responsible for these two types of infection differ and different diagnostic approaches may be required. This chapter will focus on methods for ascertaining the correct diagnosis in seriously ill patients suspected of having either type of respiratory infection.

Infections Causing Respiratory Failure

Previously Normal Hosts

Severe pneumonia is one of the most common causes of respiratory failure in previously normal people. Certain epidemiological features may provide important clues to the diagnosis. The most common viral cause of respiratory failure is influenza. This infection occurs in epidemic form and has an incubation period of only a few days. Thus, the patient has usually been exposed to others with acute respiratory symptoms, and the presence of influenza in the community is well known or at least suspected. Peak occurrence is during the winter months in the northern hemisphere. Severe influenza pneumonia is usually an explosive illness, with the patient seeking care within 48 h after the onset of illness; the chief complaint is usually dyspnea, not fever, cough, or other symptoms. Chest radiographs reveal bilateral infiltrates of varying intensity. Severely impaired gas exchange may occur in influenza without extensive infiltrates due to widespread bronchiolitis, but this is much more common in patients with underlying chronic bronchitis than in normal persons.

Other viruses may produce a similar clinical picture. Adenovirus in particular causes a clinical syndrome which may be difficult to distinguish from influenza; exudative pharyngitis is common with adenovirus infections, but not influenza. Varicella pneumonia is always accompanied by the characteristic rash and can be a devastatingly severe pneumonia in adults.

Mycoplasma pneumoniae is a common cause of pneumonia in children and healthy adults but only rarely causes respiratory failure. The illness typically begins with pharyngitis followed by tracheobronchitis, with pneumonia developing after 5–7 days. Bullous myringitis is present in some patients. A clinically similar, usually mild form of pneumonia is caused by *Chlamydia*, including the TWAR strain of *C. psittaci*. Patients should always be questioned about exposure to insects, birds, or animals. Psittacosis, Q-fever, rickettsial infections, and even histoplasmosis can present in this fashion. Outdoor activities, such as hunting, camping, and hiking, can be accompanied by insect bites or other exposure to infectious agents. Clinical suspicions must be tempered by considerations of the prevalence of certain agents in the region, the time of year, and the type of exposure. The importance of thinking about these agents is

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Table 31.1. Historical clues

Community outbreaks
Animal/insect exposures
HIV risk factors
Prodromal/concurrent symptoms
Underlying diseases therapy

that the history may provide important clues to the etiology (Table 31.1); without that guidance, specific diagnostic studies will not be undertaken.

Bacterial infections may also produce respiratory failure in previously normal people, particularly when occurring as complications of a preceding viral infection. Pneumococcal pneumonia is occasionally overwhelmingly severe in an apparently normal host, although this presentation is more likely in patients with certain predispositions such as asplenism, sickle cell anemia, or hypogammaglobulinemia. Pneumonia due to *Streptococcus pyogenes* is uncommon but can be rapidly progressive and severe. *Staphylococcus aureus* also produces a rapidly progressive, severe form of pneumonia that is often accompanied by respiratory failure. Staphylococcal disease associated with intravenous drug abuse, endocarditis, or infected intravascular catheters appears radiographically as multiple nodular densities which increase rapidly in size and cavitate. Lobar pneumonia due to *Staphylococcus* is uncommon as a community-acquired infection except as a complication of influenza. Pneumonias due to these "pyogenic cocci" are associated with high fever, chills, and marked left shift of circulating neutrophils; the absolute blood leukocyte count may be high, low, or normal. Pleural effusions are common with each of these infections. In contrast, effusion is rare with the non-bacterial agents.

Legionella pneumonia typically begins with prodromal symptoms of weakness, myalgia, and headache. Fever and chills associated with a non-productive cough appear shortly thereafter, often followed by nausea, vomiting, and diarrhea. Mental confusion, hyponatremia, and hypophosphatemia in association with pneumonia and diarrhea complete a clinical picture which is highly suggestive of this infection.

Hosts with Underlying Disease

Patients with underlying cardiopulmonary disease may develop respiratory failure with infections caused by any of the above organisms and often do so in the presence of what appears to be a relatively mild infection. This is particularly well documented in the case of influenza, in which the bronchial involvement which always accompanies influenza may worsen lung function and precipitate respiratory failure, even in the absence of new infiltrates. However, relatively mild pneumonias caused by the pneumococcus or any other agent may cause respiratory failure in patients with underlying disease.

Underlying diseases associated with immunosuppression open a veritable Pandora's box of infectious agents. In patients with acquired immune deficiency syndrome (AIDS), severe community-acquired pneumonias are usually caused by *Pneumocystis carinii* or cytomegalovirus (CMV); mycobacterial and fungal infections are less likely to cause respiratory failure. These organisms also produce infections in patients receiving immunosuppressive therapy, although bacterial agents are actually more common in these patients. It is clear that life-threatening pulmonary infection may be the initial clinical manifestation of AIDS and that risk factors for this disease are not always identified quickly. A careful review of the patient's history with respect to administration of blood or blood products, sexual practices, and use of intravenous drugs is important. It follows that in any patient with severe pneumonia, underlying immunosuppression needs to be considered and appropriate studies, with appropriate precautions, need to be undertaken.

General Considerations

As illustrated above, the history can provide critically important clues to the etiology of severe pneumonia in a patient presenting with respiratory failure (Table 31.1). Often one of the difficult aspects clinically is to distinguish between a truly acute process in a previously normal person and an acute exacerbation in a person who has had a slowly progressive but subclinical problem for some period of time. It is not rare in our experience for a lung biopsy obtained early in the course of a supposedly acute illness to show extensive fibrosis, indicative of a chronic underlying disease of which the patient and the family were unaware. Unfortu-

nately, previous radiographs and other potentially valuable information are usually not available but should be sought whenever possible.

The general physical examination uncommonly provides information that is diagnostically useful but should be performed with care in all cases. Lymphadenopathy may be the clue to human immuno deficiency virus (HIV) infection. Oral candidiasis may be an important clue to both the presence of HIV infection and a tip-off that the pulmonary process is caused by *P. carinii.* Infected cutaneous lesions or tender, erythematous venipuncture sites suggest infection with *S. aureus*. Insect bites may be present in sites that are inaccessible to inspection by the patient and should be searched for in people with outdoor exposures.

Pleural effusions should be aspirated for diagnostic tests, as a general rule. Pleural effusions associated with respiratory infections may be sterile exudates, so-called parapneumonic effusions, but provide a specific diagnosis if organisms are seen or recovered in culture. In all patients, the fluid should be examined by Gram's stain and cultured for both aerobic and anaerobic bacteria. Examination of the fluid for mycobacteria and fungi is appropriate in many, if not all, patients if Gram's stains are negative. Immunodiagnostic techniques have been infrequently applied to pleural fluid, but fluid can be frozen for later study if the initial smears and cultures are unrevealing. Demonstration of the capsular antigens of the pneumococcus, *Hemophilus influenzae*, or *Neisseria meningitidis* by immunological means has essentially the same significance as recovery of the organism in culture. These approaches may be particularly helpful in making a specific diagnosis in patients who have received prior antibiotics. In our experience, immunodiagnostic techniques applied to pleural fluid have not been highly rewarding but are probably worth pursuing in settings where bacterial infection is considered most likely and other studies have failed to indentify an agent.

Similarly, blood cultures should always be obtained in patients with severe pneumonia. While the yield of positive cultures remains disappointingly small, approximately 25%–30%, a positive culture provides the basis for a firm diagnosis. Cultures should be obtained prior to the initiation of antimicrobial therapy, using two different venipunctures if possible. Cultures drawn through central lines are frequently contaminated and therefore difficult to interpret.

Collection of Respiratory Secretions for Microbiological Study

It is important that physicians caring for patients with serious illnesses understand the principles underlying the sampling of respiratory secretions for microbiological study. Oropharyngeal secretions are massively contaminated by both aerobic and anaerobic bacteria. The exact composition of the oropharyngeal flora differs among normal people, but potentially pathogenic bacteria are either transiently or persistently present in most. Aerobic organisms which commonly produce pneumonia but are regularly found in respiratory secretions of normal people include *S. aureus*, *S. pyogenes*, *H. influenzae*, and *Branhamella catarrhalis*. Colonization of the respiratory tract by Gram-negative bacilli is rare among healthy people but occurs in most seriously ill patients. Thus, in the latter patients, respiratory secretions often contain *Escherichia coli, Pseudomonas aeruginosa*, *Proteus*, *Serratia* or other Gram-negative bacilli. The clinician must always be aware of the potential for false positive cultures from the respiratory tract, i.e., cultures that reveal the presence of pathogenic bacteria which are not, in fact, responsible for the patient's symptoms.

At the same time, respiratory secretions do not always reflect the bacteriology of the distal lung, i.e., cultures of secretions have the additional problem of false negativity, or failing to demonstrate organisms which are, in fact, responsible for the patient's symptoms. The frequency of false negativity varies with the organism involved as well as for a variety of factors. In some studies as many as 50% of patients with bacteremic pneumococcal pneumonia failed to have pneumococci cultured from sputum.

These considerations lay the groundwork for the clinician who is considering the significance of smear and culture results obtained on respiratory secretions. The presence of an organism does not mean that it is responsible for the clinical infection, and, conversely, failure to find a suspected agent may not mean that it is not present.

Expectorated Sputum

Analysis of expectorated sputum is plagued with each of the foregoing problems. Sputum arises from all areas of the tracheobronchial tree, not necessarily in the region of pneumonia. It has been kept warm and moist in the tracheobronchial tree for some period of time

prior to expectoration, allowing bacterial multiplication to occur. During expectoration, it passes through the heavily contaminated oropharynx and acquires the bacterial flora resident in that area. Finally, the specimen itself is markedly non-homogeneous, and different findings may result from analysis of one region versus another. Despite these shortcomings, sputum analysis has the advantages of being non-invasive and readily available. Analysis of sputum should begin with instruction of the patient as to what is being sought – a specimen from deep within the chest. Some authorities recommend brushing the patient's teeth or at least rinsing the mouth with saline prior to specimen collection to diminish bacterial contamination. A deep cough is encouraged and the sputum raised is expectorated directly into a sterile container. Pooling sputum over a period of time is not advised since further bacterial overgrowth will occur. The specimen should be taken promptly to the laboratory, where a purulent portion is selected for microscopic study. If the specimen contains fewer than 25 squamous epithelial cells per low power field, it is reasonable to conclude that it represents sputum from the lower airways, regardless of the number of neutrophils. If the specimen contains more than 25 squamous epithelial cells, it should be discarded and another obtained. The types and relative numbers of bacterial forms present, along with the number of polymorphonuclear leukocytes (PMNs) and macrophages should then be assessed and a portion of the specimens should then be cultured. The information thus obtained may be sufficient in many clinical situations. If the patient appears to have staphylococcal sepsis clinically and radiographically and the sputum reveals numerous plump Gram-positive cocci in clusters, further studies would be unnecessary, except, of course, for blood cultures. In other words, if the sputum reveals a clear pattern that is consistent with other aspects of the patient's illness, the clinician may reasonably decide not to proceed with other techniques. On the other hand, if the sputum findings are non-specific or are not consistent with the patient's illness, additional studies should be considered.

Various techniques have been proposed to improve the accuracy of sputum analysis, including quantitative cultures of sputum homogenates and repetitively rinsing of sputum specimens to remove surface contaminants. It is doubtful that these techniques have much merit in the evaluation of community-acquired pneumonias. Care in the collection of the specimen is much more important than time-consuming and expensive processing in the laboratory.

Transtracheal Aspiration

Sputum induction, performed by having the patient inhale an ultrasonically generated aerosol of 3% saline, may be useful in diagnosing *P. carinii* infections in patients with AIDS. The sensitivity of this procedure has been reported to exceed 50% in this setting, but falsely negative specimens are frequent as well. Since *P. carinii* pneumonia with or without respiratory failure is commonly the initial manifestation of AIDS, examination of induced sputum is worth considering before proceeding to invasive procedures if the patient has risk factors for HIV infection. Most clinicians have found sputum induction to be of limited value in other settings, but some reports suggest that it may be useful in diagnosing infections, especially *P. carinii*, in immunosuppressed states other than AIDS. It is probably true that the yield from sputum induction varies directly with the care and effort invested in both performing the procedure and in examining its product. Since a positive result usually obviates the need for more invasive procedures, hospitals that care for immunosuppressed patients should develop expertise with this technique. The technique of transtracheal aspiration was a useful tool in its time. Using this approach, investigators defined the clinical spectrum of anaerobic pleuropulmonary infections and confirmed the sterility of the lower airways in previously healthy people with non-bacterial lung infections. Neither of these useful findings warrants continued routine use of the procedure. The presence of PMNs in the absence of bacteria in tracheal secretions obtained from a young person with a diffuse pneumonia is strong evidence of a non-bacterial pneumonia. But such would have been strongly suspected on the clinical presentation alone. In elderly patients with underlying disease, aspiration of oropharyngeal contents occurs with regularity and the findings on transtracheal aspiration closely mirror expectorated sputum; in these patients, the procedure is an added risk with little benefit. In our view, transtracheal aspiration is rarely, if ever, indicated.

Certain conditions are absolute contraindications to the technique, including uncorrected bleeding diatheses, an uncooperative patient, and the presence of a small trachea, as found in

small children. The major complication is bleeding, and if the patient's ability to expectorate blood from the trachea is in doubt, the procedure should not be performed. Subcutaneous and/or mediastinal emphysema may occur, and perforation of the esophagus has been reported.

Transthoracic Needle Aspiration of the Lung

Transthoracic needle aspiration of the lung has the major advantage of directly attacking the problem. Unfortunately, the results of this procedure in the hands of most investigators have been disappointing. The rate of false negative samples is in the range of 30% due to several factors. One is the obvious potential sampling error because it is usually performed as a bedside technique without fluoroscopic guidance. However, even in the presence of large consolidated pneumonias false negative aspirations are fairly common, perhaps reflecting the interior milieu of the lesion. Since the amount of material obtained is small, it is possible that important portions of it are lost during processing, thus leading to negative results. One study found that directly plating the specimen at the bedside significantly improved the yield of positive cultures in experimental infections [12].

Complications are primarily pneumothorax and bleeding. The former occurs in 20%–30% of patients and is clinically significant in about one-half of these patients. Pneumothorax virtually always occurs in patients receiving mechanical ventilation, and the technique should not be used in such patients unless a chest tube is in place beforehand. Bleeding is occasionally massive and, rarely, fatal; uncorrected bleeding diatheses are contraindications to the procedure. Transthoracic aspiration is an occasionally useful technique which is limited primarily by the small size of the sample obtained and the frequency of complications in seriously ill patients.

Bronchoscopic Procedures

The advent of the fiberoptic bronchoscope dramatically altered the accessibility of the lower respiratory tract. A variety of techniques have been devised to obtain samples via the bronchoscope. The instrument affords a convenient and safe method of bypassing the contaminated oropharynx and of delivering a sampling device into the area of involvement, thus overcoming two of the major drawbacks to other techniques. In addition, samples of substantial size can be obtained, at least with some sampling techniques.

Simple aspiration of secretions from the lower airways has been found by some investigators to be of as much value as any of the more complicated approaches to be described. These studies are difficult to evaluate since the findings are likely to be both disease-specific and technique-specific to some extent. This is to say that, with proper patient selection and meticulous technique, a relatively non-specific sampling technique can be made to appear rather selective, whereas in practice it turns out not to be so. It seems likely that direct aspiration of the lower airways via the bronchoscope would be only slightly more selective than analysis of expectorated sputum and similar to transtracheal aspiration. Studies in experimental animals tend to support that view.

Four techniques have been used to sample peripheral lung units through a bronchoscope: the protected specimen brush (PSB), aspiration through a needle attached to a long catheter, transbronchial forceps biopsy, and lavage. Aspiration through a needle passed through a bronchoscope has little to recommend it except avoidance of barotrauma; the specimen collected is still minute and highly selective, thereby risking false negatives. When directly compared with other techniques, it has not proved to be worthwhile [11].

The PSB technique has been studied extensively [18,20]. Most investigators have used the device in a similar fashion. The bronchoscope is placed in a subsegmental bronchus of interest and the brush passed into it within two telescoped catheters. The outer catheter is plugged by a small biodegradable plug, which prevents contamination of the inner catheter; the brush is kept well within the inner catheter until use. After the bronchus has been entered, the outer catheter is advanced beyond the bronchoscope and several centimeters into the airway. The inner catheter is then advanced, dislodging the plug and entering a distal portion of the airway which has not been contaminated by the procedure. At this point the brush is advanced and the specimen collected. The brush and catheters are then withdrawn in reverse order and removed from the bronchoscope. In some laboratories the catheters are then severed and the brush removed in retrograde fashion from the catheters in order to minimize possible contact with the tip of the catheters which have contacted secretions. In most reported studies the brush has been placed in 1 ml of sterile saline,

shaken vigorously, and quantitative cultures performed on the fluid.

The only study in which protected brush samples have been directly compared with cultures of lung tissue in humans found that brush cultures yielded more than 10^3 organisms per milliliter in patients whose lung tissue showed the pathological findings of pneumonia; cultures of this tissue revealed more than 10^4 organisms per gram of tissue [4]. Very similar bacterial concentrations have been reported from comparable samples in non-human primates undergoing prolonged ventilatory support for experimental respiratory failure [8]. These findings suggest that recovery of bacteria in concentrations of 10^3/ml or more from the brush is "significant," whereas recovery of fewer organisms is not.

Early studies showed that the brush picked up approximately 0.001 ml during sampling. The presence of 10^3 organisms per milliliter in the final solution following dilution in 1.0 ml would indicate that the initial concentration in secretions was at least 10^6/ml. This value is consistent with the observation that pneumonias are typically associated with bacterial concentrations exceeding 10^6/ml in sputum. However, the bacterial population is not evenly distributed throughout the non-homogeneous secretions in the lower airways, and a very real possibility for sampling errors exists when small samples are obtained. Further, recent observations suggest that multiple species of bacteria are commonly isolated from the periphery of the lung in patients with either community-acquired or nosocomial pneumonia. No one species need be present in very high concentration; rather, it appears that the aggregate bacterial population is important. These considerations serve to add caution to the interpretation of quantitative cultures of brush specimens.

Bronchoalveolar lavage is generally performed by wedging the bronchoscope in a subsegmental bronchus and instilling 20–50 ml aliquots of sterile saline to a total volume of 100–150 ml. Smaller volumes are usually used when lavage is performed in intubated patients with respiratory failure. Lavage samples a wider area but is more likely to be contaminated. However, the large volume of sample recovered allows a variety of diagnostic procedures to be performed, including special stains, cultures, and cytological examinations [15,17].

It is probable that selective brushing and lavage are additive diagnostic procedures for diagnosing pulmonary infections. Lavage is the safer technique, having virtually no complications other than those associated with bronchoscopy. Brushing causes bleeding in a small percentage of patients and can cause pneumothorax if the brush is advanced too far without guidance. Lavage can be used in anyone undergoing bronchoscopy, while brushing should not be performed in patients with uncorrected coagulopathies.

Transbronchial lung biopsy can be performed blindly at the bedside. Several recent studies have found that fluoroscopic guidance did not improve the yield of specific diagnoses, although personal experience suggests that better (more peripheral) specimens are obtained with such help. In general, larger forceps yield larger specimens of lung tissue and fewer non-specific diagnoses. Compared with open thoracotomy biopsy, the fragments of lung recovered by transbronchial lung biopsy are minute. Despite this, the reported yield for infectious processes is very good, e.g., nearly 90% in immunocompromised patients with diffuse pneumonia [19]. In the management of patients with acute, diffuse pulmonary infiltrates leading to respiratory failure, a lung biopsy showing only infectious pneumonitis can be both reassuring and diagnostic. In the case of some organisms, especially common saprophytic fungi such as Aspergillus and Candida, demonstration of tissue invasion is necessary to establish infection; isolation of the organisms from secretions alone is insufficient. Unfortunately, transbronchial biopsy specimens often contain pathological changes which are less diagnostic in this setting. The most common finding is diffuse alveolar damage (DAD), a non-specific manifestation of tissue injury which accompanies most viral and some bacterial pneumonias, as well as most causes of adult respiratory distress syndrome (ARDS). With good luck, the biopsy may contain areas of necrotizing pneumonia, suggesting that bacterial pneumonia is responsible for the process. Similarly, the presence of granulomata or typical viral inclusions in the tissue limits the differential diagnosis substantially.

The biopsy specimen may be important in excluding other diagnostic possibilities, although the sampling error inherent in the technique limits its usefulness in this regard. The pulmonary toxicity of some chemotherapeutic agents may be manifested as widespread epithelial cell changes in the lung; the presence of such changes in a transbronchial biopsy establishes that diagnosis, while the absence of this finding does not exclude drug toxicity. Pulmonary

hemorrhage due to coagulopathies associated with a variety of underlying diseases can mimic infection and can be diagnosed by biopsy. Lymphangitic spread of malignancy can occasionally present as rapidly worsening respiratory failure with fever and is usually found by transbronchial biopsy.

In summary, bronchoscopic techniques provide a powerful armamentarium for the clinician faced with a patient in respiratory failure suspected of having an acute, severe, pulmonary infection. The bronchoscope can be passed through an endotracheal tube or transnasally. For the assessment of infections, at least lung lavage and transbronchial biopsies should be performed. Whether selective brushing with a protected brush provides sufficient additional information to warrant the expense and slight added risk is less clear.

Diagnostic Approaches

A prompt and thorough evaluation of the specimens obtained requires close cooperation between the clinician and the laboratory. Specimens will be subjected to microscopic study, culture, and perhaps other techniques.

At the time of bronchoscopy at least four specimens should be obtained and placed in appropriate fixative, usually 10% neutral buffered formalin, for light microscopic study. The number of specimens submitted for histopathological studies should be recorded on the surgical pathology slip so that the pathologist can verify that all specimens are recovered from the fixative fluid. Specimens for fungal, mycobacterial, or viral cultures, if needed, should be transported in sterile saline to the appropriate laboratories as soon as possible. Additional biopsy specimens should be frozen for later studies, if possible.

Paraffin blocks that contain bronchoscopic specimens are cut at three step levels. Usually 20–30 sections (mounted several per slide) are cut for hematoxylin and eosin (H & E) staining. Then a few unstained slides are taken for special stains, followed by another series of sections for H & E, etc., until at least two-thirds to three-quarters of the tissue block is sectioned. Special stains for bacteria (Brown & Hopps or Brown & Brenn tissue Gram stains), fungi (Gomori's methenamine-silver; GMS), protozoa (GMS and/or Giemsa) and acid-fast bacilli (Kinyoun's, Ziehl–Neelson, Fite–Faraco) should be utilized as indicated. For *Legionella*, either a Steiner & Steiner or a modified Dieterle stain can be used.

Lavage fluid should be cultured on multiple media. The latter should contain, at a minimum, media for aerobic bacteria, mycobacteria, fungi, and, in most circumstances, *Legionella*. Whether cultures for viruses and other nonbacterial agents are performed depends on local expertise and interest. In general, cultures remain the most sensitive technique available for most infectious agents but are expensive and require experience. Aliquots of the lavage fluid should be centrifuged for cytological preparations. Smears of the latter should be stained with Gram's, GMS and/or Giemsa, and for acid-fast bacilli, as well as for cytology.

The field of "rapid diagnosis" using monoclonal antibodies and DNA hybridization has expanded at an incredible pace. However, the usefulness of many of these new tests remains uncertain in clinical medicine. These tests are, by design, highly specific. This specificity is advantageous in some ways, it can be a major disadvantage when faced with a clinical situation in which screening for a variety of agents would be more appropriate. The role of the clinician is to narrow the range of diagnostic possibilities as much as possible. Although monoclonal antibodies have been prepared against antigens of many bacteria, few of these have proven useful as diagnostic reagents as yet. Identification of bacterial antigens in blood, urine, sputum, lavage fluid, or tissue is certainly possible with any of a number of techniques, but the advantages of these approaches over culture for diagnosis are uncertain and unproven [13]. An enzyme-linked immunoadsorbent assay (ELISA) procedure for the detection of lipid A in lavage fluid has been tested in rats with *Pseudomonas aeruginosa* pneumonia and found to be both specific and sensitive for infection [3]. This type of approach, using an antigen common to a number of organisms, may have diagnostic utility. Immunological techniques should be particularly helpful in the diagnosis of agents which are difficult or impossible to culture or which require lengthy periods of incubation. For example, direct immunofluorescence (IF) techniques are routinely used to detect Legionella in respiratory secretions but remain less sensitive than culture [9]. Direct IF examination of sputum for *Pneumocystis carinii* in AIDS patients increases the accuracy of diagnosis over other staining techniques [10]. In situ hybridization techniques for mycobacteria are being actively investigated. Commercial probes for *Mycobacterium tuberculosis*, *M. avium* or *M. intracellulare* have shown

high specificity in the clinical setting [16]. The procedure can be done in 2 h, a real asset when a patient is severely ill. A recently developed probe to *Mycoplasma pneumoniae* is now commercially available and reportedly has high sensitivity and specificity.

Formalin-fixed, paraffin-embedded tissue specimens can be used for many immunocytochemical and in situ hybridization techniques, making these tests very valuable tools for surgical pathologists. DNA probes are available for adenovirus 5, cytomegalovirus (CMV), herpes simplex I and II, *Chlamydia trachomatis*, and Epstein–Barr virus. Antisera for CMV and herpes simplex 1 and 2 are available which can be used on paraffin-embedded lung specimens.

There have been significant advances in the identification of infectious agents in the diagnostic virology laboratory. One promising technique involves testing of lavage fluid or tissue specimen with an antiviral mouse monoclonal antibody pool which includes antibodies for Influenza A and B, Parainfluenza 1,2,3, respiratory syncytial virus (RSV), and adenovirus. If any positivity by IF is seen in the "pooled" specimen, individual tests with each of the antibodies can then be performed and confirmed by an additional IF procedure. This technique takes less than 24 h, compared with several days to weeks for viral cultures.

The vial "shell spin" has allowed for the more rapid diagnosis of CMV in patients with AIDS and organ transplants and in babies with congenital CMV. This procedure utilizes a vial that contains a coverslip with MRC-5 (embryonic lung cell line) cells. Filtrate from a lavage or tissue specimen is centrifuged onto the coverslip for 1 h, followed by an incubation of 16 h. The coverslip is then stained with an IF antibody technique and read, allowing a diagnosis of CMV to be made in 24 h rather than waiting 21 days for a positive culture. Although the in situ hybridization method for CMV could yield a more rapid diagnosis, in reality slides for the in situ hybridization technique are "bunched" in the laboratory to conserve time and expense thus results tend to be delayed.

Application

While it is true that any or all of the above procedures can be done, the question which must be answered is which, if any, should be performed in an individual patient. The minimum evaluation should include the history, physical examination, chest radiograph, and staining of expectorated sputum, if present. Blood cultures should be obtained and pleural fluid aspirated, if present. Obviously, other tests may also be advisable for patient care. At this time, the first major decision point is reached.

There is at least suggestive evidence that delaying the initiation of antimicrobial therapy is harmful. In our view, treatment should be started following the above procedures. The decision to proceed with additional diagnostic procedures depends upon the level of certainty of diagnosis based on the available data and the presumed likelihood of serious adverse effects which might be prevented if the correct diagnosis is not established promptly. In no small part the latter depends upon the severity of the acute illness and the presence or absence of underlying diseases. For most patients, we recommend initiating broad empiric therapy after the initial assessment, reserving invasive procedures for those who fail to improve. Factors which predict adverse outcomes in community-acquired pneumonias include advanced age, diastolic hypotension (<60 mmHg), leukocytosis >30 000/mm^3, leukopenia (>4000/mm^3), increased blood urea nitrogen, and severe hypoxemia [7]. The presence of one or more of these factors indicates increased importance for an accurate diagnosis and in such patients invasive procedures should be performed early. We favor a bronchoscopic approach, usually following endotracheal intubation, with performance of lavage and transbronchial biopsy.

Infections in Patients Hospitalized with Serious Illness

Nosocomial pneumonias, surgical wound infections, and urinary tract infections are the three most common hospital-acquired infections. Unfortunately, the clinical criteria by which pneumonias are diagnosed are much less precise than that for the other infections. The incidence of nosocomial pneumonia in intubated intensive care patients based on clinical criteria of a new fever, infiltrates, leukocytosis, and purulent secretions has been consistently found to be in the range of 20%–40% of patients, with no evidence of change over the past 20 years. Other studies have found that 72% of patients dying of respiratory failure have pneumonia

at autopsy. Obviously, the frequency of pneumonia varies with the criteria used for diagnosis as well as with the patient population under study.

We have used the presence of an intense neutrophil infiltrate centered on airspaces surrounding terminal and respiratory bronchioles to identify bacterial pneumonias [1]. In some patients the process is focal and this geographic localization is readily apparent. In others, the process is more extensive and may spread across many distal lung units; lung tissue necrosis may or may not be present. The appearance of this lesion requires the presence of about 10^4 bacteria per gram of lung tissue in experimental animals. Limited data suggest that a similar number is required in humans [4]. It is apparent, if over 70% of patients who die in respiratory failure have pneumonia by these histological criteria but only 20%–40% of such patients are thought to have pneumonia clinically, that not all histological pneumonias are clinically significant.

Clinical criteria for the presence of nosocomial pneumonia are probably reasonably accurate in many groups of patients. However, in patients with ARDS, and presumably other forms of pulmonary edema, they do not perform well. In a direct comparison of clinical findings with the histology of the lungs at necropsy among ARDS patients who died during ventilatory support, clinical diagnoses as to the presence or absence of pneumonia were correct in only 70% of patients. The major problems encountered were the presence of fever, leukocytosis, and pathogenic bacteria in secretions in most patients, whether or not pneumonia was present [1].

Bacterial infection in polytrauma patients is often associated with the development of impaired function of multiple organs, including the lungs. This association of bacterial infection with the syndrome of "multiple organ failure" (MOF) has been noted in other clinical settings as well. Of particular significance is the finding that unsuspected nosocomial bacterial pneumonia may be the only site of infection found at necropsy in such patients. This observation indicates that a major clue to the presence of pneumonia may be a deterioration of function of the lungs or other organs which cannot be explained by other factors.

Gram-negative bacilli are isolated from the secretions of about 70% of patients with nosocomial pneumonias. However, for the reasons discussed earlier, the recovery of these, or of any other organism from secretions, does not necessarily indicate the presence of pneumonia. In immunocompetent patients, respiratory infection is accompanied by an influx of neutrophils into the airspaces and patients with pneumonia might be expected to have numerous PMNs in secretions. An increase in the number of PMNs over time, as estimated from serial smears of tracheal aspirates or sputum, appears to be useful in identifying the development of pneumonia, although a single observation at one point in time is less helpful. Similarly, an increase in the bacterial population of secretions over time may serve to identify the onset of infection in a given patient, but comparisons of cultures obtained at a single point in time in groups of infected and uninfected patients have not shown differences. The presence of elastin fibers in sputum or tracheal aspirates may serve as a valuable marker of Gram-negative, necrotizing infection. This finding is present in only about one-half of patients with nosocomial pneumonia but it is highly specific [14].

Invasive sampling techniques have been applied in ARDS patients in the ICU setting by many investigators. Transthoracic needle aspiration has essentially no role in ventilator patients. In a very interesting French study, the PSB technique was shown to predict reliably the presence of pneumonia (diagnosed histologically) if the concentration of bacteria recovered by the brush exceeded 10^3/ml. However, this finding held only if the patient was not receiving antibiotics. In the presence of antibiotics, most samples containing more than 10^3 organisms per milliliter were not associated with pneumonia, i.e., antibiotic therapy appeared to produce false positive culture results. Many factors could explain this puzzling finding, but the most likely would be that areas of pneumonia were in fact present but were missed by the histological sampling procedure. If this is the case, it would support the argument that the PSB technique may be too selective in a setting in which multiple areas of pneumonia may be separated by areas of either normal lung or lung involved with other pathological processes.

Bronchoalveolar lavage samples a wider region of lung, although by including more proximal airways there is a greater risk of contamination. False negative samples are less likely with this technique than with the brush. There is a general relationship between the level of bacterial contamination and the histological findings in lung tissue, although the best method for expressing these bacterial counts is uncer-

tain. The simplest approach is to enumerate the total organisms present on quantitative cultures and report them in terms of "colony-forming units" (CFU) per milliliter. The next level of complexity might be to enumerate separately the different species present and report CFU/ml values for each; implicit in this approach is the notion that the presence of a given species will be significant at some concentration. Calculating mean values appears to have little merit since bacterial concentrations tend to vary by enormous amounts and the arithmetic mean is heavily weighted by the largest number.

We have used the "bacterial index" to express the level of bacterial contamination in lavage specimens [8]. With this approach, concentrations of individual species are converted to logarithms and added together (in effect multiplying them). The sum is the bacterial index. This technique allows all species present to contribute to the final total. If anything, this technique may give too much weight to organisms which are present in low concentrations. In experimental animals undergoing prolonged ventilatory support, a bacterial index of greater than 5.0 was present in all animals with pneumonia which were receiving either no antibiotics or only intravenous antibiotics. In animals which received large doses of antibiotics topically into the airways, the bacterial index tended to be suppressed. This finding does not pose a major problem since the drugs are not used in this fashion clinically. A bacterial index greater than 5.0 was found in several lavages from lung lobes which did not contain pneumonia at necropsy; moderate or severe pneumonia was present in another lobe in each of these animals.

Immunological techniques aimed at improving the ability to detect infectious agents in lung tissue or respiratory secretions appear to have little role. Organisms which are difficult to culture are not a major problem in this setting, although hospital outbreaks with organisms such as Legionella or influenza can occur. Rather, the major clinical problem remains to distinguish airway colonization with pathogenic organisms from distal lung infection. At the present time, the most reliable indicator of infection is the presence of large numbers of bacteria in specimens collected from the periphery of the lung by lavage or brush. Evidence of tissue damage such as the presence of elastin fibers in secretions provides strong supporting evidence of invasive infection. Worsening function of the lungs or other remote organs should raise suspicion of lung infection in seriously ill patients in whom another cause is not apparent [2]. While the great majority of patients with nosocomial pneumonias will manifest fever, leukocytosis, purulent secretions, and new or progressing lung infiltrates, a prospective study found that only 31% of ventilator patients with these findings had pneumonia by bacteriological criteria [6]. In that study, recovery of $\geq 10^3$ CFU/ml with the PSB technique was used to define the presence of pneumonia. Clinical criteria alone were not useful in predicting the presence of pneumonia. These findings indicate that patients receiving mechanical ventilation who demonstrate clinical findings suggesting pneumonia should undergo a bronchoscopic evaluation and selective sampling. This approach will avoid unnecessary administration of antimicrobial agents with the attendant cost and risk. While animal studies suggest that lavage is a suitable technique, recent work in intubated humans suggests that the optimal technique is a combination of lavage to obtain material for immediate microscopic examination and PSB for quantitative culture [5].

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