Diagnosis of respiratory tract infection and the use of the laboratory

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RF GROSSMAN. Diagnosis of respiratory tract infection and the use of the laboratory. Can J Infect Dis 1994;5(Suppl C):34C-41C. Lower respiratory tract infections continue to be among the most common illnesses requiring medical attention with considerable morbidity and morality. Clinical features, including underlying conditions, presenting signs and symptoms, basic laboratory investigations and chest roentgenograms, are not sufficiently precise to infer an etiological agent. These investigations do permit an assessment of severity of illness and can assist in stratification of patients into high risk groups. Properly performed and interpreted Gram stain of sputum is still useful in the initial assessment of these patients, but sputum cultures are less helpful. Blood cultures should be drawn in patients ill enough to require hospitalization, but the yield is low. Pneumococcal antigen testing and serological studies do not add to the routine management of patients with pneumonia. In patients with nosocomial pneumonia, the diagnosis will be established by a synthesis of clinical, roentgenographic and simple laboratory results such as sputum analysis and blood culture. Invasive investigations should be reserved for critically ill patients.

Key Words: *Blood culture, Chest roentgenogram, Gram stain, Pneumonia diagnosis, Sputum culture*

Rôle du laboratoire dans le diagnostic des infections respiratoires

RÉSUMÉ : Les infections respiratoires inférieures se classent toujours parmi les affections qui nécessitent le plus fréquemment des soins médicaux et elles s'accompagnent d'un taux de morbidité et de mortalité considérable. Les caractéristiques cliniques, y compris les problèmes sous-jacents, les signes et les symptômes qui forment le tableau, les analyses de laboratoire de base et les radiographies pulmonaires, ne suffisent pas à identifier l'agent étiologique. Ces analyses permettent une évaluation de la gravité de la maladie et peuvent aider à classer les patients dans l'un ou l'autre des groupes à risque. Une coloration de Gram des expectorations bien effectuée et bien interprétée demeure utile lors de l'évaluation initiale de ces patients; les cultures d'expectorations le sont moins. Des hémocultures devraient être faites chez les patients suffisamment malades pour devoir être hospitalisés, mais les résultats sont peu satisfaisants. La recherche d'antigènes de pneumocoques et les épreuves sérologiques n'ajoutent pas au traitement de routine des patients atteints de pneumonie. Chez les patients qui souffrent de pneumonie nosocomiale, le diagnostic sera établi par une synthèse des résultats des examens cliniques, radiologiques et des résultats de laboratoire simples, comme l'analyse des expectorations et l'hémoculture. Les épreuves invasives doivent être réservées aux patients gravement malades.

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Upper and lower respiratory tract infections are among the most common illnesses requiring medical attention. In the mid-1980s the morbidity associated with upper respiratory tract infections in the United States required 75 million physician visits per year, almost 150 million days lost from work and more than $10 billion in costs for medical care (1). In the National Health Interview Survey of 1981 it was estimated that 3.3 million cases of pneumonia occurred in ambulatory children and adults for a rate of 1.5 episodes per 100 persons per year (2). In 1981 there were more than half a million admissions to hospital with community acquired pneumonia in the United States (3). The problem was considerably magnified in the elderly (over 65 years of age), among whom the admission rate to hospital was 11.5 per 1000 population compared with 1.0 per 1000 population in the 15 to 44 year age group. During the same period in the United Kingdom, respiratory tract infections accounted for over 15% of all consultations with general practioners. Three-quarters of these patients received antibiotics, leading to 25 million antibiotic prescriptions per year (4). In the out-patient setting, the mortality rate of pneumonia remains low – below 5% – but among those requiring hospitalization, the mortality rate approaches 25% (5,6).

Use of the clinical features of pneumonia to predict microbial etiology: Institution of early, specific antimicrobial therapy has been shown to reduce the morbidity and mortality associated with pneumonia and limit the associated toxicity of such therapy. While early diagnosis is optimal in the management of pneumonia, the etiology is not obtained in as many as 33 to 50% of all patients even if extensive diagnostic testing is used (7). Traditionally physicians have used the syndromic approach to make an etiological diagnosis of pneumonia (8). Patients are divided into those with a classical bacterial pneumonia syndrome and those with an atypical presentation. In the former, best exemplified by pneumococcal pneumonia, patients present with the acute onset of a high fever, cough productive of purulent sputum, pleuritic chest pain and have abnormal findings on physical examination of the chest. The roentgenographic abnormality found most commonly is lobar consolidation. In contrast, patients with the atypical pneumonia syndrome, as typified by infection with *Mycoplasma pneumoniae*, tend to have an illness with a more insidious onset, low-grade temperature, a nonproductive cough, and often the physical examination of the chest is normal. The roentgenographic features of atypical pneumonia are those of bronchopneumonia, or a diffuse interstitial pattern. Unfortunately this approach is limited since the clinical features of many infections overlap, with clinical symptoms being as much a reflection of the host, as of the pathogen. *Haemophilus influenzae*, *Staphylococcus aureus* and Gram-negative enteric bacteria have been implicated in clinical syndromes indistinguishable from that caused by *Streptococcus pneumoniae* (9). Agents such as *Chlamydia pneumoniae* and viruses have been noted to cause atypical pneumonia syndromes (10). The attribution of specific clinical features has been used particularly in patients infected with *Legionella* species. A gastrointestinal prodromal illness in association with hyponatremia has been reported with this infection (11,12). Fang and co-workers (7) in a prospective study could not identify this constellation of findings in patients infected with *Legionella* species any more frequently than with other respiratory pathogens. In general, in prospective studies, the clinical features of pneumonia cannot be sufficiently well defined to allow an accurate etiological diagnosis of pneumonia to be made (4,7,13).

The signs and symptoms of pneumonia are easily recognized but not unique to pneumonia. In a recent British Thoracic Society (5) survey of 453 patients, the most common symptoms were cough (88%), dyspnea (71%), sputum production (69%), chest pain (64%), hemoptysis (17%) and mental confusion (14%). Isaacs (14) could not distinguish viral from bacterial etiologies for childhood pneumonia using physical examination or roentgenographic techniques. Woodhead and Macfarlane (15) demonstrated that patients with community acquired pneumonia infected with *M pneumoniae* tended to have a mild illness, while other etiologies such as legionella and *S pneumoniae*-infected patients could not be reliably distinguished. Farr and coworkers (13) used a five-variable discriminate multivariate analysis to predict microbial etiology. Even with this elaborate model, the correct microbial etiology was predicted in only 42% of cases. Fang and coworkers (7) could not use the frequency of clinical findings to separate the various microbial etiologies and suggested the abandonment of the syndromic approach to the etiological diagnosis of pneumonia.

Epidemiology of community acquired pneumonia: While an early diagnosis is optimal in the management of community acquired pneumonia, the etiology of pneumonia is frequently not ascertained. No test is available that can identify all potential pathogens, and each diagnostic test is associated with limitations. Because of these limitations, most patients will be treated on an empirical basis. An etiological agent is not found in 30 to 50% of cases. Most studies clearly indicate a declining role for *S pneumoniae* and increasing importance of atypical pathogens (16-18). The most common pathogens in patients under the age of 65 years and without comorbid illnesses are *M pneumoniae*, *S pneumoniae*, respiratory tract viruses, *C pneumoniae* and *H influenzae*. The mortality of these patients is low and the majority can be treated as out-patients. Patients over the age of 65 years and with comorbid illnesses are likely to be infected with *S pneumoniae*, respiratory tract viruses, *H influenzae*, aerobic Gram-negative bacilli,

and *S aureus*. Less common pathogens include *Moraxella catarrhalis*, *Legionella* species, *Mycobacterium* species and endemic fungi.

Diagnostic studies in patients with community acquired pneumonia: A chest roentgenogram should be performed in all patients suspected of having pneumonia. An abnormal chest roentgenogram is the only way to confirm absolutely the diagnosis of pneumonia and at times may confirm the presence of coexisting conditions such as bronchial obstruction or pleural effusions. It may assist in the evaluation of severity of illness since multilobar infiltrates and rapid roentgenographic extension of disease is a harbinger of a complicated hospital course (19). Clinicians have used the chest roentgenogram to infer an etiological diagnosis of pneumonia, but this is fraught with hazard. While homogeneous alveolar infiltration is more common in bacterial etiologies, other findings such as atelectasis or pleural effusion can be seen in all etiologies (20). No one organism always produces the same roentgenographic abnormality, and similar roentgenographic patterns may be produced by different organisms. A panel of expert radiologists was better at diagnosing mycoplasma pneumonia than bacterial and viral pneumonia, but agreement on etiological diagnosis was present in only 29% of cases (21).

A properly performed Gram stain of expectorated sputum examined by an experienced observer may be useful in the initial evaluation of a patient with pneumonia (22,23). Direct staining of sputum may be diagnostic in infections caused by mycobacteria, endemic fungi, legionella and *Pneumocystis carinii*. However, false positive and false negative rates of 88% and 38%, respectively, have been reported (22,24). Failure to visualize a predominant organism on Gram stain despite the presence of many leukocytes should suggest the possibility of atypical pathogens (7). The results with expectorated sputum cultures are even worse, with fewer than 50% of samples processed by the usual clinical methods yielding reliable results (25). A properly obtained sputum sample with more than 25 neutrophils and fewer than five squamous epithelial cells per low power field on Gram stain may be helpful in identifying the likely etiological agent (26). Sputum culture and sensitivity may be useful if experience dictates that penicillin-resistant pneumococci are likely. Most patients are receiving antibiotics when first assessed, and the identification of a resistant pathogen may be helpful. Viral cultures are not useful in the initial evaluation of patients with community acquired pneumonia and should not be routinely performed (18). Blood cultures should be drawn in patients ill enough to require hospitalization but positive results are observed in only 15 to 25% of patients with pneumococcal pneumonia and less frequently with other pathogens (27).

Attempts have been made to improve the diagnostic yield in patients with suspected pneumococcal pneumonia. *S pneumoniae* elaborates a series of toxins (pneumolysin, purpura producing principle, neuraminidase, autolysins) and type- or species-specific surface markers (pneumococcal capsular polysaccharide antigens, pneumococcal C polysaccharide, M protein antigen, R protein antigen). Immunoassays have been developed to detect pneumococcal C polysaccharide (species-specific antigen) and pneumococcal capsular polysaccharide antigens (type-specific antigens). These antigens can be found in sputum, pleural fluid, serum and urine during acute pneumococcal pneumonia (28). Urine and serum antigen detection is specific but not sensitive for pneumococcal pneumonia. Frequencies of antigen detection ranging from 9% in serum to as high as 64% in urine have been reported (5,28-32). Detection of pneumococcal antigen in sputum is plagued by colonization of respiratory tract secretions with *S pneumoniae* in 35% of children, 20% of healthy adults and 40% of adults with chronic bronchitis (33,34).

Serological testing and cold agglutinin measurements are not helpful in the initial evaluation of patients with community acquired pneumonia. While their routine measurement is not useful, acute and convalescent serological testing may retrospectively confirm a suspected diagnosis and could be used for epidemiological purposes.

Routine hematology and biochemistry, while useful in predicting severity of illness, cannot separate etiological agents. Prospective studies indicate that patients with community acquired pneumonia of all etiologies will present with an average leukocyte count of 13,500±6.8 cells/mm^3 (7). In elderly patients with bacterial pneumonias, counts above 10,000 cells/mm^3 will occur 70% or more of the time (35). In half of the remaining patients a left shift in the differential is seen.

In a recent study, Woodhead and colleagues (36) noted that in 122 consecutive patients admitted to hospital with community acquired pneumonia, blood cultures were performed in 81% of cases, sputum was examined in 45% and serological studies were determined in 28%. Results of these investigations changed the initial therapeutic decision in only 8% of cases. They concluded that routine microbial investigation of all adults admitted to hospital with community acquired pneumonia was not necessary.

The technique of nucleic acid hybridization has developed to the point of being useful in clinical microbiology laboratories. These new methodologies offer rapid, sensitive and specific diagnostic results for *Mycoplasma* species, *Legionella* species, *Mycobacterium tuberculosis* and other mycobacteria species (37). For common bacterial pathogens such as *S pneumoniae* or *H influenzae*, difficulties similar to that seen with the detection of pneumococcal antigens can be anticipated.

More invasive procedures such as transtracheal aspiration, bronchoscopy, bronchoscopy with a protected brush catheter, bronchoalveolar lavage with or without

TABLE 1
Most frequently isolated nosocomial pathogens in the United States

Pathogen	Frequency (%)
Gram-positive cocci	
Staphylococcus aureus	12.9
Gram-negative bacilli	
Pseudomonas species	16.9
Klebsiella species	11.6
Enterobacter species	9.4
Escherichia coli	6.4
Serratia species	5.8
Proteus species	4.2

Data from reference 90

TABLE 2
Risk factors for nosocomial pneumonia

Endogenous factors	Exogenous factors
Host factors	Environmental factors
Advanced age	Seasonal trends
Male sex	Cross contamination
Chronic disease	Air/flow/water supply
Impaired immunity	Hospitalization
Malnutrition	Teaching hospital
Obesity	Intensive care unit
Life style factors	Medical/surgical wards
Smoking	Prolonged stay
Ethanol abuse	Therapeutic
Depressed consciousness	Sedatives/hypnotics
Aspiration	Immunosuppressives
Prior infection/antibiotics	Antacid $\pm$ H_2 blocker
Prior surgery	Invasive devices
Head and neck	Endotracheal tube
Thoracic	Tracheostomy tube
Abdominal	Nasogastric tube
	Intracranial pressure monitor

Adapted from reference 59

balloon protection, direct needle aspiration of lung or open lung biopsy should be reserved for the more critically ill patient not responding to usual empirical therapy.

Severe community acquired pneumonia: The mortality rate of community acquired pneumonia continues to be between 10 and 20%. Mortality is higher in patients developing acute respiratory failure and requiring mechanical ventilation. In six studies published between 1985 and 1991 of patients with community acquired pneumonia requiring intensive care unit (ICU) management, the mortality rate varied from 21 to 54% (6,19, 38-41). More than 50% of these patients required mechanical ventilation. The two most common organisms found in this clinical setting were *S pneumoniae* and *L pneumophila*. The factors associated with mortality included inadequate antibiotic therapy before ICU admission, mechanical ventilation requirement, use of positive end-expiratory pressure, FiO_2 greater than 0.6, coexistence of adult respiratory distress syndrome (ARDS), radiographic spread of pneumonia during the ICU admission, septic shock, bacteremia and *Pseudomonas aeruginosa* as the causative agent. Of all the factors listed above, radiographic spread of pneumonia and the presence of septic shock will accurately predict mortality in the majority of patients (19).

NOSOCOMIAL PNEUMONIA

Nosocomial pneumonia is defined as pneumonia occurring 48 h or more after admission to hospital. It occurs at a rate of 5 to 10 per 1000 discharges and is the second most common cause of hospital acquired infection, comprising 10 to 17% of all hospital acquired infections (42,43). It extends the length of hospitalization seven to nine days and costs in excess of $1 billion annually in the United States alone (43). The crude mortality is 28 to 37% in university teaching hospitals, and attributable mortality has been estimated to be 33% of the crude mortality (44-47).

Etiology and pathogenesis: With rapid changes in available technology, the etiological diagnosis of nosocomial pneumonia has evolved in the past few years. Most studies have reported that aerobic Gram-negative bacilli account for 60 to 80% of bacteria isolated and aerobic Gram-positive cocci, especially *S aureus*, for a further 20 to 30% (Table 1). *L pneumophila*, viruses and fungi may also be causative agents (11,48,49).

Many risk factors have been associated with nosocomial pneumonia (Table 2) (45,50). Endotracheal intubation and tracheostomy are associated with the highest rates of nosocomial pneumonia. The presence of endotracheal and nasogastric tubes alters natural host defenses and increases the rate of entry of microorganisms into the lung. Respiratory therapy such as mechanical ventilators, in-line medication nebulizers and mist tents have all been associated with increased rates of nosocomial pneumonia (51,52). Common to these identified risk factors is access to the lower respiratory tract by Gram-negative and other potential respiratory pathogens following initial colonization of the upper respiratory tract (53,54). Colonization occurs rapidly in critically ill populations. Attachment of Gram-negative rods by pili is an important step for colonization – a phenomenom that is inversely related to cell surface fibronectin (55,56). Once colonization of the upper respiratory tract has been established, aspiration of bacteria into the tracheobronchial tree from the oropharynx or stomach challenges the intrinsic respiratory defense mechanisms. Aspiration occurs commonly in healthy individuals and is more common in patients with altered consciousness, an inability to protect the upper airway, delayed gastric emptying or decreased gastrintestinal motility (57,58). Host factors such as the extremes of age, male sex, the presence of

TABLE 3
Sensitivity and specificity of diagnostic methods

Diagnostic method	Reference	Sensitivity	Specificity
Clinical/x-ray parameters	Andrews (61), Bell (62), Chastre (69), Fagon (63,67)	34–100	64–90
Blood culture	Fagon (67)	24	42
Sputum culture	Bartlett (25), Bryan (64)	80	45–90
Bronchial washing antibody-coated bacteria	Winterbauer (70)	54–73	98–100
Protected specimen brushing			
Gram stain	Higuchi (71)	30	100
QC	Higuchi (71), Chastre (69), Torres (72), Fagon (67), Pham (73), Baughman (74), Guerra (75), De Castro (76)	67–100	67–100
Nonbronchoscopic protected specimen brushing QC	Torres (72)	64	100
BAL			
Gram stain	Chastre (77), Pugin (78)	79–86	96–100
QC	Kahn (79), Torres (80)	77–88	100
Protected BAL QC	Torres (80)	84	100
Protected mini-BAL semi-QC	Rouby (81)	70	69
Nonbronchoscopic BAL bacterial culture	Piperno (82)	55	75

BAL Bronchoalveolar lavage; QC Quantitative culture

chronic disease, impaired immunity, malnutrition, obesity, smoking history and ethanol abuse have also been associated with nosocomial pneumonia (59).

Clinical diagnosis of nosocomial pneumonia: The diagnosis of nosocomial pneumonia is difficult because there is no gold standard. Most studies have used clinical criteria to establish a diagnosis and the Centers for Disease Control and Prevention has recently defined nosocomial pneumonia using clinical criteria (60). Their definition includes the presence of rales or dullness to percussion on physical examination of the chest, with chest roentgenographic evidence of a new or progressive infiltrate, consolidation, cavitation or pleural effusion with new purulent sputum, positive blood culture or isolation of an etiological agent obtained by transtracheal aspirate, bronchial brushing or biopsy. Andrews and colleagues (61) compared clinical criteria for the diagnosis of pneumonia to histological evidence in patients who died during treatment for ARDS. Pneumonia was present in 58% of patients but was unsuspected antemortem in a third. In a similar study by Bell and associates (62), 38% of cases of pneumonia were misdiagnosed. Fagon and coworkers (63) in a prospective study noted that an accurate diagnosis of nosocomial pneumonia in ventilated patients occurred in only 62% of cases. Antibiotics were prescribed for patients without pneumonia in 16% of cases. They concluded that the use of clinical criteria alone did not permit the accurate diagnosis of nosocomial pneumonia in ventilated patients and commonly led to inappropriate or inadequate antibiotic therapy.

Diagnostic studies in patients with nosocomial pneumonia: In a study of 172 patients with bacteremic nosocomial pneumonia, sputum cultures grew the relevant pathogen in only 49% of cases (64). Reliable results from sputum examination can be expected in fewer than half of cases mainly because of colonization of specimens by oropharyngeal or tracheal organisms (65). This is magnified in patients with tracheostomy or endotracheal intubation (61,66). Blood cultures are positive in patients with nosocomial pneumonia in fewer than 10% of cases (64). While the incidence of bacteremia in ventilator-associated pneumonia is 24%, an extrapulmonary source for the bacteremia is found in more than half the cases (67). The roentgenographic diagnosis of ventilator-associated pneumonia is very difficult. In patients without ARDS, the presence of an air bronchogram will correctly predict autopsy-proved pneumonia in only 64% of cases (68). In patients with ARDS, no roentgenographic sign can reliably predict the presence of pneumonia (68). Because of the inherent difficulties associated with making the clinical diagnosis of pneumonia, especially in the critically ill patient, other techniques have been introduced in order to improve diagnostic accuracy. Fibre-optic bronchoscopy by itself does not eliminate the problem since aspirates are contaminated as they pass through the suction channel. New techniques have been introduced to improve the sensitivity and specificity of the diagnosis of nosocomial pneumonia but none are perfect and all are invasive, which limits their utility (Table 3) (67,69-82).

Transtracheal aspiration: Transtracheal aspiration bypasses the upper airway and helps eliminate the problem of upper airway contamination. The test is invasive, and complications of the test include bleeding, puncture of the soft posterior tracheal wall, subcutaneous emphysema, pneumothorax and infection at the puncture site. Transtracheal aspirates were compared with direct needle aspirates of the lung in 25 patients with pneumonia (83). In five patients the tracheal aspi-

rate did not contain all the organisms found in the lung aspirate, and 12 potential pathogens isolated from the tracheal aspirate of 11 patients were not recovered from the corresponding lung aspirate. Because of the difficulties associated with this test, it has largely been abandoned.

Transthoracic lung puncture: This procedure has largely been avoided in patients on mechanical ventilators because of the risk of pneumothorax. However, Palmer and coworkers (84) found a sensitivity of 46% using a 20-gauge needle under fluoroscopic guidance while Torres and colleagues (85) using a 22-gauge needle without fluoroscopic guidance reported a sensitivity of 38% and a false positive rate of 8%. In the latter study, 7% of patients developed a pneumothorax.

Bronchoscopy: Bronchoscopy with bronchoalveolar lavage and transbronchial lung biopsy is useful in the management of immunocompromised patients because the detection of opportunistic pathogens is considered to be diagnostic (86). Unfortunately, contamination of the bronchoscopy with upper airway bacteria renders the results of routine bacterial culture of specimens obtained through the bronchoscope unreliable (87). A protected specimen brush technique has been introduced to avoid the difficulties associated with routine bronchoscopy (88). Using a cut-off of at least 10^3 colony forming units (CFU)/mL to distinguish between colonization and infection, a high rate of sensitivity and specificity has been reported so long as patients are not receiving antibiotics and are not being mechanically ventilated (67,69,71-76) (Table 3).

Bronchoalveolar lavage: Using a cut-off of 10^4 to 10^5 CFU/mL to distinguish colonization from infection, sensitivity and specificity rates as good as those obtained by protected specimen brush technique have been frequently reported (77-82) (Table 3). Bronchoalveolar lavage samples a far larger area of the lung than does protected specimen brush, thus decreasing sampling error.

Other investigations: The presence of elastin fibres by potassium hydroxide preparation appears to be specific for the presence of necrotizing pneumonia in patients without ARDS, but has a sensitivity of only 52% (89). In patients with ARDS, the test is unreliable. To date the antibody-coated bacteria test has not been shown to be consistently accurate, with sensitivities ranging between 48 and 73% and specificities between 50 and 100% (70,90).

In the majority of cases the diagnosis of nosocomial pneumonia will be established by a synthesis of information obtained from history including comorbid conditions and risk factors, physical examination and appropriate laboratory tests and/or procedures. A proper expectorated sputum sample for Gram stain and culture, blood cultures, blood count and differential and routine chest roentgenogram should be obtained. Bronchoscopic specimens or other special tests should be reserved for more critically ill patients such as those requiring ventilatory support.

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