New Diagnostic Tests for Pneumonia: What is Their Role in Clinical Practice?

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The utility of diagnostic studies to determine the etiologic agents of community-acquired pneumonia (CAP) has been controversial in part because of the lack of rapid, accurate, easily performed, and cost-effective methods, which might allow results for most patients at the initial point of service (ie, the initial evaluation by a clinician in an office or acute care setting). Of note, except for the introduction of the urinary antigen tests for *Streptococcus pneumoniae* and *Legionella,* there has been slow advancement in diagnostic methods for determining the etiologic agent of CAP over the past century. For the most part, we have relied on techniques used since the time of Koch and Pasteur—standard microbiological stain and culture methods.

Recently, advancements in molecular testing methods have brought forth new potentials for diagnosis, and more rapid identification of pathogens is possible. In addition, recent studies have suggested that procalcitonin levels help to distinguish between bacterial and viral pneumonia, reduce antibacterial use, and can predict severity of CAP. This article explores new diagnostic tests (immunochromatographic antigen tests and molecular tests) and procalcitonin, and assesses their clinical utility.

PRESENT PROBLEMS WITH ETIOLOGIC DIAGNOSIS OF PNEUMONIA

Identification of the etiology of CAP in recent years has been minimal. From the results of most randomized clinical trials, the cause of CAP for patients admitted to a hospital is determined in approximately 20% to 40% of cases, and in observational studies has ranged from less than 10% for outpatients to approximately 20% for inpatients.[1] At present, several issues contribute to the difficulty with establishing an etiologic diagnosis in pneumonia, including (1) problems with currently available diagnostic tests, (2) recent trend toward empirical therapy without an emphasis on establishing an etiologic diagnosis, and (3) delays because of outsourcing of laboratory tests.

Traditional culture methods for detection of respiratory tract pathogens can be slow, are often insensitive, may not distinguish infection from colonization, and may be influenced by previous antibiotic therapy. Results of diagnostic laboratory tests may require several days (culture identification, serologic tests, in vitro susceptibility results), but timely administration of antibiotics is the pivotal factor in the recovery from severe infection, so empiric therapy has become the recognized

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and recommended practice. For culture of sputum, only a minority of patients expectorate adequate specimens, and if sputum is obtained, there is always the problem distinguishing colonization from true pathogen.

Over the past several decades, broad-spectrum empiric therapy had become the predominant approach to the management of CAP. Most studies found that once empiric therapy was given, the results of diagnostic laboratory tests did not affect management[2–6] because the spectrum of the empiric agents was so broad that regardless of whether or not the pathogen was identified, the clinical response was uniform and often was evident by the time the laboratory results were available to the physician. As a result, microbiological testing, including sputum cultures, Gram stains, and even blood cultures became deemphasized. According to the most recent Infectious Diseases Society of America–American Thoracic Society guideline, microbiological tests are presently universally recommended for high-risk patients intensive care unit (ICU) admitted to the hospital.[7] Although numerous reasons have been proposed for a decrease in microbiology testing, the most powerful influence by far has been the rise of empiricism.[8] With widespread use of broad-spectrum empiric therapy, antimicrobial resistance has increased.[8]

In addition, there has been a trend toward a significant decline in the role of the microbiology laboratory in the hospital setting.[9] In many hospitals, microbiology specimens are outsourced to other health care facilities or private laboratories, which may lead to delays in turnaround times, decreased communication for results, and loss of specimen viability. In addition, the Clinical Laboratory Improvement Amendments of 1988, which required that staff have credentials to interpret Gram stains, virtually eliminated house staff and attending laboratories located on the ward.[9]

A NEW ERA OF DIAGNOSTICS FOR MICROBIAL PATHOGENS

Recently there has been a rapid increase in technology for innovative molecular tests, most significantly associated with the use of nucleic acid amplification tests (NAATs), such as polymerase chain reaction (PCR). These new tests are becoming available with marked expansion of diagnostic capability for infectious diseases. Newer tests that may allow more rapid etiologic diagnosis include the newer generation of immunochromatographic urinary antigen tests as well as NAATs.

Urinary Antigen Tests

Immunochromatographic (ICT) tests that detect soluble pneumococcal antigen or Legionella antigen in urine have been an important advance in the diagnostic assessments of these 2 pathogens. These tests are much less influenced by prior antibiotic therapy than sputum or blood culture. The ease of performing the ICT card-type urine test makes it ideal for use in emergency departments, long-term care facilities, and even physician offices (although presently they are not waived by the Food and Drug Administration [FDA] for nonlaboratory, office use).

Pneumococcal urinary antigen

The ICT urinary antigen test is particularly attractive for detecting pneumococcal pneumonia when cultures cannot be obtained in a timely fashion or when antibiotic therapy has already been initiated. In serial specimens from known bacteremic cases, the pneumococcal urinary antigen detected by ICT assay was still positive in 83% of cases after 3 days of therapy.[10] This form of urinary antigen testing has the principal additional advantages of rapidity (about 15 minutes), simplicity, and reasonable specificity in adults. Studies in adults have shown a sensitivity of 50% to 80% and specificity exceeding 90%.[11–13] In one study, the use of the ICT pneumococcal urinary antigen test increased the yield of etiologic diagnosis of patients admitted for CAP from 39.1% to 53.1%.[11] Of 269 patients in this study who had no defined etiology using conventional methods, 69 (25.7%) had a positive pneumococcal urinary antigen test. The immunochromatography assay is also highly accurate in diagnosing pneumococcal meningitis (95% sensitivity with cerebrospinal fluid, 57% sensitivity with urine, and 100% specificity).[14]

The disadvantages of urinary antigen testing include the cost and the lack of an organism isolate for in vitro susceptibility tests. Notably, immunochromatography is not suitable for evaluation of therapeutic effect, because positive test results are obtained for several weeks to months after recovery. Moreover, the immunochromatographic assays are nonspecific for pneumococcal infections in children, particularly the very young, as nasopharyngeal carriage of S pneumoniae can cause false-positive results.[12] In one study, the presence of azotemia was an independent factor associated with a higher rate of a positive test for patients with bacteremia.[13] The investigators suggested this may have been because of increased concentration of urine for these patients, as most of the patients had reversible

impaired renal function most likely caused by dehydration. Supporting the theory of the effect of concentrated urine, Gutierrez and colleagues[11] reported increased test sensitivity after urine concentration by centrifugation, and a study conducted by the manufacturer found that the test's ability to detect pneumococcal antigen decreased with serial dilution.[15] Thus, patients may be more likely to test positive after urine sample concentration or before intravenous fluid resuscitation. Of note, *pneumococcal* vaccine may cause false-positive results in urine in the Binax NOW *Streptococcus pneumoniae* test in the 48 hours following vaccination.[15]

Legionella urinary antigen

Presently the urinary antigen is the most used test in North America for detection of *Legionella*.[8] Although the test can reliably detect only one species, *Legionella pneumophila*, and only one serogroup, serogroup 1, it has significant advantages over previous "standard" tests (direct fluorescent antibody testing, serology, and culture), including its relatively low cost and rapid performance. Direct fluorescent antibody stains require substantial expertise for interpretation, and selection of reagents is critical. Culture on selective media detects all but very rare strains but is technically more demanding and requires 3 to 7 days.[16] Accurate interpretation of serologic tests requires comparison of acute and convalescent specimens, which is not relevant for clinical management. The *Legionella* urinary antigen test is 70% sensitive and greater than 90% specific for infections caused by *L pneumophila* serogroup 1 and should particularly be useful in the United States and Europe, as approximately 85% of community-acquired isolates are serogroup 1.[17,18] It may be less sensitive for nosocomial cases because of frequent involvement of serogroups other than serogroup 1. Urine is usually positive for antigen on day 1 of illness and continues to be positive for weeks.[19,20]

A recent meta-analysis by Shimada and colleagues[21] summarized the performance characteristics of the *Legionella* urinary antigen as having very good specificity but lower sensitivity for *L pneumophila* serogroup 1; thus, it is better for ruling in than ruling out disease. A positive urinary antigen test result, in the appropriate clinical setting, virtually rules in legionellosis, but a negative urinary antigen test result does not rule out the presence of disease, as 26% of patients with confirmed legionellosis have a negative urinary antigen test result. One potential "unintended" adverse consequence of the availability of the *Legionella* urinary antigen test is decreased use of *Legionella* culture.

All too often, clinicians order a urine antigen test without submitting or requesting a sputum culture. Both the urine antigen test and the *Legionella* culture should be performed for maximal effectiveness, especially if non–serogroup 1 *L pneumophila* is a consideration.

Nucleic Acid Amplification Tests

The development of NAATs has been a major advance in the understanding of respiratory infections.[8] PCR and related methodologies have revolutionized the field of molecular biology, and automated instrumentation has now been introduced successfully to the clinical laboratory setting. Molecular-based tests have moved from the research bench to the clinical diagnostic laboratory and now are becoming commercially available. Clinical application of these methods as comprehensive and rapid techniques may improve our ability to quickly and efficiently identify etiologic organisms associated with CAP. They may eventually have the potential to be point-of-care tests and allow pathogen-directed therapy at the time of initial administration of antimicrobial agents.

PCR directly detects microbial nucleic acid in clinical samples. The basic steps of PCR include DNA extraction from either a cultured pathogen or from a patient specimen sample and amplification of an established target gene.[22,23] Enzymes are used to copy this DNA via multiple rounds of replication, resulting in exponential amplification of the target sequence of interest. The PCR products can then be identified by gel electrophoresis and DNA sequencing.

Initially, PCR methods had several limitations, which included:

Requirement of adequate sample to detect DNA

Presence of PCR inhibitors in samples that can lead to false-negative results

Contamination, which can lead to false-positive results

Differentiation of colonization from true pathogens (eg, identification of *S pneumoniae* in a respiratory specimen; quantifying organisms may be helpful in this regard)

Equipment expense and requirement for trained personnel.

Lack of standardization of test methods (many hospital laboratories have their own methods that have not been validated in independent studies)

Only a few methods are presently approved by the FDA.

Many of these limitations have been addressed with advancements in methodology (see later in

this article) or are expected to be resolved as technology improves. It is anticipated that molecular tests will be more available in the near future.

An important advance in NAAT technology has been the development of quantitative, real-time PCR.[24] With this method, amplification and detection of the DNA sequence occurs in a single tube, thus simplifying the procedure, as gel electrophoresis sequencing is not needed. The reaction is performed with fluorescent-labeled DNA probes, which allow the number of gene copies to be determined. This increases the speed and efficiency of testing and reduces the risks of operator error and cross contamination. This process can be performed with faster turnaround times, allowing results to be used in a more prominent role in direct patient management. Another advancement has been the development of multiplex PCR systems, in which multiple DNA targets are assessed in one reaction without increasing the required amount of technician time.[22,23] Some commercially available assays can measure more than a dozen respiratory pathogens. These assays may also have the ability to recognize potential dual or triple infections in the same patient. Several commercial assays, which are based on automated extraction instruments, are available (but few are FDA approved at the time of this writing) and these vary according to methodology. Specifications for commercially available real-time PCR (including gene targets) are beyond the scope of this article, but readers are referred to other reviews for greater details.[22–24] Presently FDA-approved tests are listed in **Table 1**.

Although PCR methods have been developed for several pneumonia pathogens, the clinical utility of these tests varies.[25–29] There are several advantages of PCR testing methods as compared with standard microbiological culture methods in the detection of pneumonia pathogens (**Box 1**). PCR is a potentially attractive diagnostic tool for rapid diagnosis because it does not rely on bacterial growth or the viability of the organism. Many pathogens, including *Chlamydophila pneumoniae, Mycoplasma pneumoniae*, and respiratory viruses, can be difficult to culture because of special growth requirements and slow growth. The time required for a final result is often too long to be clinically useful in the acute management of a patient. Real-time PCR has been shown to be as effective as culture methods for detecting these pathogens.[24]

PCR for specific pathogens–bacteria
Streptococcus pneumoniae, the most common pathogen associated with CAP, is easily detected by PCR in respiratory specimens. PCR techniques based on amplification of the pneumolysin or autolysin genes are applicable for the diagnosis of pneumonia, otitis media, and meningitis. Autolysin and pneumolysin PCR using sputum have shown a high sensitivity (more than 80%) but a low specificity (30%–40%).[30,31] Interpretation of sputum PCR is limited by the difficulty in differentiating between pneumococcal colonization and true infection. On the other hand, in pleural fluid, PCR detects the pneumolysin gene with a sensitivity of 78% and a specificity of 93%.[32] One approach that may help to resolve the problem of colonization versus infection is quantification of the target organism by real-time PCR. Yang and colleagues[33] evaluated the utility of a real-time pneumolysin gene PCR test using sputum samples from patients admitted with CAP. Of 129 patients, 23% had *S pneumoniae* isolated from blood or sputum. The sensitivity and specificity using real-time quantitative PCR were 90% and 80%, respectively. Of note, PCR of blood samples from patients with *S pneumoniae* infection does not appear to be useful. One study compared the ICT *S pneumoniae* urine antigen test to PCR of blood in patients with bacteremic pneumococcal infections.[34] The urinary antigen test was positive in 51 of 58 bacteremic pneumococcal cases (sensitivity, 88%; 95% confidence interval [CI], 77% to 95%), whereas PCR was positive in only 31 cases (sensitivity, 53.5%; 95% CI, 40% to 67%; $P<.0001$), and all of these had detectable urinary antigens. Both tests gave positive results in 2 of 51 control patients (referred to as other-organism septicemia), giving a specificity of 96% (95% CI, 86.5% to 99.5%). In 77 patients with nonbacteremic CAP, urinary antigen was detected significantly more often (in 21 patients [27%]) than a positive result by the PCR protocol (6 [8%]) ($P<.002$).[34] A recent meta-analysis concluded that currently available PCR methods using blood samples for the diagnosis of invasive pneumococcal diseases lack the sensitivity and specificity necessary for clinical practice.[35] To date there are no FDA-approved PCR tests for *S pneumoniae*.

There are several commercially available and/or institutionally developed NAATs for the atypical pathogens.[26,36–41] However, none of these are officially FDA approved or available at the present time in the United States. Despite this, PCR is increasingly being recognized as a method of choice for detection of *M pneumoniae* and *C pneumoniae*. For both of these pathogens, diagnosis has usually relied on serology, which, as indicated previously, is usually not useful to the clinician during acute medical management. One large study examined the use of real-time PCR to detect *M pneumoniae* in children with CAP and found that

Table 1
FDA-cleared/approved molecular tests for respiratory pathogens

Bacteria	Manufacturer	Test Name	Method
Francisella tularensis	Idaho Technology, Inc Salt Lake City, UT	Joint Biologic Agent Identification and Diagnostic System Tularemia Detection kit	Real-time PCR
Mycobacterium tuberculosis	Gen-Probe, Inc San Diego, CA	AMPLIFIED Mycobacterium tuberculosis Direct Test	Transcription-mediated amplification
Virus			
Adenovirus	Gen-Probe, Inc (Prodesse)	ProAdeno + Assay	Multiplex Real-time RT-PCR
Avian Flu	Centers for Disease Control and Prevention	Influenza A/H5	Real-time RT-PCR
Influenza virus panel	Centers for Disease Control and Prevention	Human Influenza virus Real-time RT-PCR Detection and Characterization Panel	Real-time RT-PCR
	Focus Diagnostics, Cypress, CA	Simplexa Influenza test	Real-time RT-PCR
Respiratory virus panel	Luminex Molecular Diagnostics, Toronto, Canada	xTAG Respiratory Viral Panel(Luminex LX 100/200) [includes Influenza A/H1, A/H3, A/2009 H1N1; Influenza B; Adenovirus; RSV A&B; Metapneumovirus, Parainfluenza 1,2,3; Rhinovirus]	RT-PCR
	Nanosphere, Inc Northbrook, IL	Verigene Respiratory Virus Nucleic Acid test and Verigen Respiratory Virus Test	Multiplex Gold Nanoparticle Probes
	Gen-Probe, Inc (Prodesse)	ProFlu + Assay (Influenza A/B, RSV); ProFast + Assay (Seasonal A/H1, A/H3, 2009 H1N1; ProParaFlu + Assay (+Parainfluenza virus)	Multiplex Real-time RT-PCR

Since the submission of this paper, the U.S. Food and Drug Administration has issued a clearance for the FilmArray instrument and the FilmArray Respiratory Panel from Idaho Technologies. The FilmArray Respiratory Panel (RP) is a multiplexed nucleic acid test designed for the simultaneous detection of 15 respiratory viruses in 1 hour (http://www.idahotech.com/pdfs/mediakit/PRESS%20RELEASE%20-%20FA_FDA.pdf).

Abbreviation: RT-PCR, Reverse transcriptase-polymerase chain reaction.

Data from FDA Office of In vitro Diagnostic Evaluation and Safety. Available at: www.fdagov/MedicalDevices/ProceduresandMedicalProcedures/DeviceApprovalsandClearance. Accessed December 16, 2010.

PCR detected more cases than standard diagnostic techniques, which included culture.[42] Of significance, PCR results were available within 2 hours—a major improvement over the 2 to 6 weeks usually required for serologic diagnosis. For *C pneumoniae*, culture on cell lines has traditionally been considered as a gold standard for diagnosis. However, cell cultivation is technically complex, and is associated with limited viability and slow growth such that it is restricted to specialized laboratories and is, therefore, not often used. For these reasons, PCR has become an option for diagnosis. There are numerous assays described (again non-FDA approved) but with various results of test performances and significant interlaboratory discordance of detection rates.[26,36,37]

For tuberculosis (TB), molecular techniques have been valuable.[27] Because the organism can require 3 to 8 weeks to grow in culture, molecular techniques can be useful, allowing appropriate isolation, treatment, and disease control. FDA-approved PCR assays are available, which are

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Box 1

Advantages of molecular techniques compared with conventional diagnostic techniques

Advantages

Rapid

Greater sensitivity

Possibility to identify drug resistance

Ability to identify specific clones for epidemiologic assessment

Possibility to test for multiple pathogens simultaneously

Less affected by prior antimicrobial therapy

Able to detect organisms unable to be cultured

Data from Chan YR, Morris A. Molecular diagnostic methods in pneumonia. Curr Opin Infect Dis 2007;20:157–64.

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most useful in patients with positive acid-fast smears. A positive PCR in a smear-positive patient is extremely likely to signal TB. Conversely, a negative PCR in a smear-positive patient likely signals infection with another species. Importantly, a negative PCR in a smear-negative patient does not rule out TB.

PCR for viruses

Perhaps the area where PCR can have the greatest impact on pathogen detection has been for respiratory viruses.[43–47] The gold standard for viral identification has been conventional cell culture. However, even in specialized laboratories many viruses cannot be readily cultivated. Thus, many cases of viral illness go undetected and the exact incidence of viruses in CAP has remained uncertain. PCR offers the potential to significantly improve viral detection. For many respiratory viruses, PCR is now the most sensitive diagnostic approach. Most clinical microbiology laboratories use reverse transcriptase PCR (RT-PCR) assays to detect RNA viruses from clinical specimens.[22] This technique is very sensitive and can detect transcript from a single cell. The method uses a reverse transcriptase enzyme to synthesize a complementary strand of DNA from an RNA template. The resulting complementary DNA is then used as the template in a PCR assay.

PCR was vital for epidemiology during the recent influenza H1N1 pandemic because commercially available rapid influenza detection tests (RIDTs) were found to be relatively insensitive (sensitivity ranging from 10%–70% depending in part on the method used).[48] Several recent studies have demonstrated that when

PCR methods are used for viral detection, there is a high frequency of viral identification from patients with lower respiratory tract infection. In a prospective study during a 12-month period (2004–2005) of adult patients admitted for CAP, etiology was assessed using molecular methods (PCR for viruses, *Legionella*, *Mycoplasma*, *Mycobacterium tuberculosis*, and *S pneumoniae*; urinary antigen assay for *S pneumoniae* and *Legionella pneumophila*, serogroup 1) in addition to conventional studies (blood, respiratory culture, serology) for 184 patients.[49] A microbial etiology could be identified for 67% of all the patients. However, in 38 patients for whom all diagnostic methods were applied, a pathogen was identified for 89% of cases. The most frequently detected pathogens were *S pneumoniae* and respiratory viruses (**Table 2**). Another study using NAATs for the identification of respiratory viruses in adult patients with CAP evaluated 183 adult patients with CAP, 450 control subjects, and 201 patients with nonpneumonic lower respiratory tract infection.[47] At least one respiratory virus was identified in 58 patients with CAP (31.7%) compared with 32 (7.1%) in control subjects and 104 (51.7%) in patients with nonpneumonic lower respiratory tract infections (*P*<.01 and *P*<.01, respectively) (**Table 3**). Of interest, the proportion of viruses identified in healthy subjects was not zero and this should be considered when interpreting corresponding proportions among patients.

Thus, by supplementing traditional diagnostic methods with new PCR-based techniques, it is now apparent that viruses are becoming increasingly recognized as important causes of CAP in adults, but in standard practice, except for influenza virus, respiratory viruses are not often identified. However, as stated in a recent editorial commentary by Niederman, "This may change once these new diagnostic tools become more widely available, especially if they help us define an etiologic role of these pathogens and if they encourage the development of new and effective antiviral therapies."[50]

UTILITY OF NEW METHODS FOR DIAGNOSIS

There are good reasons for establishing an etiologic diagnosis of CAP: (1) to permit optimal antibiotic selection of agents against a specific pathogen and limit the consequences of antibiotic misuse; (2) to identify pathogens of potential epidemiologic significance such as *Legionella* and TB; (3) to reduce overuse of broad-spectrum antimicrobials, which hopefully will reduce selection pressure antimicrobial resistance; and (4) to potentially reduce

Table 2
Etiology of community-acquired pneumonia using molecular and conventional diagnostic methods

Pathogen	No. (%) N = 184	Blood Culture	Respiratory Culture	Urinary Antigen	PCR	Serology
Streptococcus pneumoniae	70 (38)	27	17	16	10	—
Mycoplasma pneumoniae	15 (8)	—	—	—	8	7
Haemophilus influenzae	9 (5)	—	7	—	—	—
Moraxella catarrhalis	7 (4)	—	7	—	—	—
Staphylococcus aureus	4 (2)	2	2	—	—	—
Legionella pneumophila	3 (1)	—	1	2	—	—
Streptococcus milleri group	1 (0.5)	1	—	—	—	—
Nocardia sp	1 (0.5)	—	1	—	—	—
Fusobacterium necrophorum	1 (0.5)	1	—	—	—	—
Mycobacterium tuberculosis	2 (1)	—	2	—	—	—
Viruses	54 (29)	—	8	—	26	20
Influenza virus	14 (8)	—	3	—	4	7
Rhinovirus	12 (7)	—	—	—	12	—
RSV	7 (4)	—	1	—	1	5
Parainfluenza virus	7 (4)	—	1	—	1	5
Coronavirus	4 (2)	—	—	—	4	—
Metapneumovirus	3 (2)	—	1	—	3	—
Adenovirus	3 (2)	—	—	—	—	3
HSV 1	2 (1)	—	2	—	—	—
Enterovirus	1 (0.5)	—	—	—	1	—

Abbreviations: HSV, Herpes simplex virus; PCR, polymerase chain reaction; RSV, respiratory syncytial virus.
Data from Ref.[49]

Table 3
Respiratory viruses associated with community-acquired pneumonia in adults

Virus	CAP (n = 183)	Controls (n = 450)	NPLRTI (n = 201)
Coronavirus	24 (13.1)	17 (3.8)	21 (10.4)
RSV	13 (7.1)	4 (0.2)	7 (3.5)
Rhinovirus	9 (4.9)	9 (2.0)	15 (7.5)
Influenza A	8 (4.4)	2 (0.4)	62 (30.8)
Influenza B	0	0	1 (0.5)
Adenovirus	3 (1.6)	0	0
Human metapneumovirus	2 (1.1)	0	0
Parainfluenza virus (2 or 3)	0	0	3 (1.5)
TOTAL			
Viruses	59 (32.2)	32 (7.1)	110 (54.7)
Positive subjects	58 (31.7)	32 (7.1)	104 (51.7)

Abbreviations: CAP, community-acquired pneumonia; NPLRTI, nonpneumonic lower respiratory tract infection; RSV, respiratory syncytial virus.
Data from Lieberman D, Shimoni A, Shemer-Avni Y, et al. Respiratory viruses in adults with community-acquired pneumonia. Chest 2010;138:811–6.

adverse events. Thus, diagnostic testing to determine pneumonia etiology can have an essential role for patient care by ensuring appropriate and effective therapy for an individual. It can also play a vital role in disease surveillance and in defining etiologic spectrum and epidemiology characteristics of pneumonia cases and deaths. With the development of rapid antigen and molecular testing methods, the clinical laboratory is no longer reliant solely on traditional culture methods for detection of pathogens in clinical specimens and more rapid etiologic diagnoses may be achievable. However, the clinical impact of the use of molecular tests and the potential for point-of-care diagnosis remains to be clearly defined.

Clinical Impact of Pneumococcal Urinary Antigen Testing

A few studies have evaluated the clinical utility of urinary antigen testing for S pneumoniae in patients with CAP. Guchev and colleagues[51] prospectively assigned patients with mild pneumonia to 2 groups. Those with positive urinary antigen test results were treated using pneumococcal-directed therapy with amoxicillin. Those with negative urinary antigen test results were treated with clarithromycin, based on perceived likelihood of infection by atypical pathogens. Of 219 evaluable patients, 22% had a positive urinary antigen test result. There was no difference in the clinical outcomes of the 2 groups. Notably, 47 (62%) of 71 patients in whom an atypical pathogen was identified were in the urinary antigen–negative arm, whereas 24 (38%) were in the urinary antigen–positive arm, indicating that they had S pneumoniae in association with an atypical pathogen. However, since the atypical pathogens were determined by serologic methods, these latter cases might have represented a primary atypical infection followed by secondary infection by S pneumoniae. In such cases, it is probable that the clinical manifestations of infection that were treated were attributable to S pneumoniae, and this may explain the good response to amoxicillin alone. The investigators concluded that the urinary antigen test allowed them to administer targeted therapy with a penicillin-class antibiotic rather than a broader-spectrum agent, and added that such narrow-spectrum therapy can be more cost effective and can allow broad-spectrum agents, such as macrolides or fluoroquinolones, to be reserved for patients whose urinary antigen test result is negative. Potential cost reductions are likely to be influenced by price differences between the targeted and broad-spectrum agents and by the proportion of positive test results. Also, it should be noted that at the time of this study in Russia, there was little beta-lactam or macrolide resistance of S pneumoniae. In addition, the study was performed in military trainees who were young (mean age, 19 years) and generally healthy. The investigators suggested that additional trials are needed for other clinical settings.

In another study, Stralin and Holmberg[52] evaluated the urinary antigen test in 215 hospitalized patients with CAP, all of whom received initial beta-lactam monotherapy. The median age was 74 years, and approximately 45% of patients had a pneumonia severity index of class IV or V. Thus, these patients were more severely ill than those in the previously described study. Thirty-eight patients had a positive urinary antigen result for S pneumoniae, and 92% of these had a successful outcome; 114 had a negative urinary antigen result, and 78% of these had a successful outcome. There were no patients with a positive PCR sputum test result for an atypical pathogen in the urinary antigen–positive group, whereas 6 patients had a positive PCR result for Mycoplasma spp or Chlamydophila spp in the urinary antigen negative group. The investigators suggested that a positive urinary antigen test result supports treatment with narrow-spectrum beta-lactam antibiotics and that additional coverage for atypical pathogens is needed more frequently in patients with negative test results.

A more recent study purported to show no significant clinical benefit for "targeted treatment" based on the pneumococcal urinary antigen.[53] For a 2-year period (2006–2008), patients admitted for CAP to a hospital in Spain were randomly assigned to receive either empirical antimicrobial therapy according to international guidelines or to receive targeted treatment based on the urinary antigen test: 177 patients were randomized (89/88 for each arm); most cases of CAP were Pneumonia Severity Class IV–V. According to the investigators, targeted therapy was associated with a nonsignificant, slightly higher overall cost (primarily because of the cost of the antigen test), reduction in adverse events, and lower exposure to broad-spectrum antimicrobials. The investigators observed no significant clinical differences in outcomes (such as mortality, clinical relapse, or length of stay in the hospital). In fact, they observed more relapses in the targeted arm. The problem with this study, however, is that the investigators did not "target" therapy until 2 to 6 days after initial intravenous broad-spectrum therapy was initiated. Thus, this study really did not assess the potential for point-of-care decision. Indeed, the investigators

acknowledge in their discussion that if there had been earlier introduction of targeted therapy, there may have been an economic benefit, and they indicate that targeted therapy has the potential to lead to *less resistance*.

Clinical Impact of Molecular Diagnostic Testing

Rapid identification of viral and bacterial pathogens is now possible with the use of PCR methods. An open randomized clinical trial was conducted to evaluate the clinical impact of PCR use for detection of etiologic pathogens in patients hospitalized with lower respiratory tract infections.[54] Between November 2002 and March 2004, 107 patients were included (55 had CAP, 22 had exacerbation of chronic obstructive pulmonary disease, and 30 had "other respiratory infections"). Patients were randomized to an intervention group, whereby results of PCR analyses (respiratory viruses and atypical pathogens) were reported (≤48 hours), or a control group, which relied on conventional diagnostic tests (although PCR was run but not reported). Real-time PCR increased the diagnostic yield from 21% to 43% of patients compared with conventional tests and this was primarily because of an increase in the detection of respiratory viruses. This led to cessation of antibiotic treatment for 6 (11%) patients, but overall antibiotic use was comparable in the intervention groups and the control groups. Use of PCR was associated with an increase in cost (because of the cost of the PCR test). Clinical outcomes (mortality, length of therapy) were not significantly different. As pointed out in an accompanying editorial, the lack of a change in antibiotic use was not unexpected, given the study design.[55] Most additional diagnoses in the intervention group were of viral pathogens. Many clinicians would not be brave enough to stop antibacterial agents solely on the basis of discovery of a viral pathogen, especially given the possibility of bacterial co-pathogens in adults. Furthermore, the results were not available at the point of initial antimicrobial decision when directed therapy might be most effective. As the investigators indicate, "to mimic real-life situations, decisions regarding treatment changes after results of PCR analysis were left at the discretion of the physician." Thus, there was no standardized approach using this information by the clinician. One wonders if there had been an educational process to provide strategies for directed therapy there might have been a different result. Certainly with the present influence of antimicrobial stewardship programs, the knowledge of earlier diagnosis might be better

suited for directed point-of-care therapy. As stated by the investigators in the discussion, "real-time PCR might have been more cost-effective if clinicians would have been less reluctant to change clinical management on the basis of test results. Studies with protocol-based and more-rigorous patient management are needed to address this issue." Such a protocol is now under way, funded by the National Institutes of Health.[56]

PROCALCITONIN

Biologic markers have been used in an attempt to distinguish between bacterial and nonbacterial causes of pneumonia. The most promising marker is procalcitonin (PCT). PCT is a peptide precursor of calcitonin that is released by parenchymal cells in response to bacterial toxins and certain bacterial-specific proinflammatory mediators (ie, interleukin [IL]-1b, tumor necrosis factor-α, and IL-6), leading to elevated serum levels in patients with bacterial infections.[57,58] PCT shows a prompt increase upon initial infection within 6 to 12 hours and reduces rapidly when the bacterial infection is controlled by the host immune system and antimicrobial therapy. In contrast, PCT is downregulated in patients with viral infections because of release by cytokines typically associated with viral infections (interferon-γ). The 2 most commonly available tests are the Kryptor assay and the (Lumiphore; Brahms Aktiengesellschaft, Hennigsdorf, Germany) assay; the former is preferred because of higher sensitivity.[57]

PCT has been studied prospectively to facilitate the decision of whether to use antibacterial agents in patients with pneumonia. Using diagnosis-specific clinical algorithms, highly sensitive PCT measurements have been shown to markedly reduce the overuse of antimicrobial therapy without increasing risk to patients in 11 randomized clinical trials including more than 3500 patients.[58] These studies have been performed mostly in European countries and primarily in primary care or emergency department settings. In 2 trials, clinicians were strongly recommended not to prescribe antibacterials in patients with a PCT level lower than 0.1 μg/L, but were encouraged to use antibacterials in patients with levels higher than 0.25 μg/L.[59,60] Subsequent analysis suggested the correct decision in 83%.[60]

Several trials have shown that using PCT results to help determine whether antibiotics are necessary results in lower rates of antibiotic exposure.[59–61] A large (1359 patients), randomized noninferiority trial compared guideline-directed usual care with use of

a rapid PCT assay to guide antibiotic use in a group of patients with lower respiratory symptoms presenting to an emergency room in Switzerland.[61] Patients were randomized to administration of antimicrobials based on a PCT algorithm with predefined cutoff ranges for initiating or stopping antimicrobials (PCT group; **Table 4**) or according to standard guidelines (control group). Use of PCT testing among the 150 patients with acute bronchitis halved the percentage of patients who received antibiotic therapy (50.0% for usual care vs 23.2% for PCT-guided treatment) with no difference in rates of adverse outcomes. Antimicrobial prescribing rates in patients with CAP (n = 925) remained appropriately high at 91%. In addition, the mean duration of antimicrobials in the PCT groups was lower (7 days) than in the control groups (10 days). Furthermore, the adverse effect rate for antimicrobials was also lower in the PCT group (23.5%) versus the control group (33.1%; 95% CI, −15.4 to −3.8). In another study, PCT testing led to a 72% decrease in antibiotic use in primary care for patients presenting with a variety of respiratory infections, including acute bronchitis, with no difference in ongoing symptoms or relapse at 28 days between groups.[62] Findings were similar in another randomized noninferiority trial of 550 patients with acute respiratory symptoms presenting to primary care.[63] There was no difference in the number of days with health impairment after day 14, comparing those who were assigned to PCT testing and controls, but there was a 42% decrease in antibiotic prescriptions in the intervention group.

Other studies have shown that PCT levels correlate with the severity of pneumonia.[64,65] In one study, PCT levels increased over time in nonsurvivors but decreased in survivors.[65] However, the prognostic value of PCT levels to predict mortality and other adverse events in CAP remains undefined. In a large prospective randomized clinical trial, Schuetz and colleagues[66] assessed the performance of PCT stratified into 4 predefined procalcitonin tiers (<0.10, 0.10–0.25, >0.25–0.50, >0.50 μg/L) and stratified by Pneumonia Severity Index (PSI) and CURB-65 (confusion, urea nitrogen, respiratory rate, blood pressure, 65 years and older) score to predict all-cause mortality and adverse events within 30 days of follow-up in patients with CAP. Initial PCT levels only moderately predicted mortality; however, PCT was helpful during follow-up and for prediction of adverse events and, thereby, improved the PSI and CURB-65 scores.

Clinical Impact of Procalcitonin

There is accumulating evidence that PCT testing can be useful in helping to identify patients with acute respiratory infection who do not warrant antibacterial therapy. If a practitioner has the capability of obtaining the results of a valid test in a timely manner at the point of care, the result can be useful for assessment of patients presenting with manifestations of acute respiratory infection, including pneumonia. PCT-guided initiation and termination of antibiotic therapy is a novel

Table 4
Use of procalcitonin for antimicrobial stewardship for respiratory tract infections based on PCT level

PCT <0.1 μg/L	Bacterial infection very unlikely	NO antimicrobials	Consider repeat in 6–24 hours; reassess based on clinical status and new result
PCT 0.1–0.25 μg/L	Bacterial infection unlikely	NO antimicrobials	Use of antimicrobials should be considered despite low PCT level if: Respiratory or hemodynamic instability; Life-threatening condition; Need for ICU admission; Evidence of empyema; Positive microbiological test (eg, pneumococcal or *Legionella* urinary antigen)
PCT >0.25–0.5 μg/L	Bacterial infection likely	YES antimicrobials	Consider clinical course and repeat PCT at days 3, 5, 7:
PCT >0.5 μg/L	Bacterial infection very likely	YES antimicrobials	Stop antimicrobial using the cutoffs above If peak PCT was very high, consider stopping antimicrobials when 80%–90% decrease If PCT remains high, consider treatment failure

Abbreviations: ICU, intensive care unit; PCT, procalcitonin assay.

approach to reduce antibiotic overuse and guide duration of therapy. This is essential to decrease the risk of side effects and emerging bacterial multidrug resistance. Interpretation of PCT levels must always account for the clinical setting and knowledge about assay characteristics. When PCT is used to guide diagnostic and therapeutic decisions in patients with CAP, the functional assay sensitivity and cutoff ranges need to be considered. The most sensitive assay, and the one with which most of the data are derived, is the Kryptor assay, from which results can be obtained within 1 hour.

Recommendations for antimicrobial stewardship have used specific PCT cutoffs (see **Table 4**). These specify 1 of 4 recommendations, ranging from "strongly discourage" and "discourage" to "recommend" and "strongly recommend," respectively.[58] The utility of this approach has been validated in multiple randomized controlled trials, as indicated previously. Thus, using the Kryptor method, initiation or continuation of antimicrobials is discouraged (<0.10 and <0.25, respectively) or encouraged (>0.50 or 0.25, respectively) (see **Table 4**). In case antimicrobials are initially withheld, clinical reevaluation and repeat PCT are recommended after 6 to 24 hours; if PCT has increased, a decision to initiate antimicrobials can be appropriate at that time. As with any guideline and to ensure patient safety, specific "overruling" criteria have been established such that the PCT-based recommendation should be bypassed based on associated factors and clinical judgment (see **Table 4**).

SUMMARY

Over the past decade, diagnostic tests for detection of respiratory pathogens are rapidly evolving. Immunochromatographic-based urinary antigen tests are rapid, simple-to-perform assays that can be easily developed as point-of-care patient tests. Further development is dependent on defining new antigens that can be readily detected. Molecular diagnostic techniques are becoming increasingly popular in clinical microbiology laboratories. Many of these combine sensitivity, specify, and rapid turnaround time to allow timely patient care. With the development of these methods, the clinical microbiology laboratory is no longer reliant solely on the conventional culture methods for detection of pathogens. Molecular methods have created new opportunities for the clinical microbiology laboratory to affect patient care in the areas of initial diagnosis and therapy. Over time, the methods have become more automated and the potential for clinical utility

increased. In addition to providing excellent new tools for diagnosis, molecular tests will also serve useful roles in infection control and public health.[55]

A critical issue regarding the clinical utility of the molecular tests will be the turnaround time. If we are to be able to use these tests for point-of-care diagnosis, it will be optimal to have results within 1 or 2 hours. However, even if the turnaround time is longer, they can still be useful for more appropriate antimicrobial therapy by allowing earlier pathogen-directed or discontinuation of therapy. Moreover, there will be the question of 24/7 availability. Will smaller community hospitals be able to rationalize the added cost of equipment and personnel to run these tests in a timely manner? In addition, the issue of laboratory reimbursement needs to be addressed, as the current reimbursement by CPT code does not adequately cover the cost of many molecular tests (of course if the test results can result in downstream lower total cost of care by shortening illness and length of stay, these costs can be justified).

As more molecular tests become available, more studies will be necessary to evaluate the real clinical value: Can use of these tests result in real improvement in patient care and outcomes, be cost effective, and reduce adverse events and antimicrobial resistance? Until results of such studies are available, the controversy concerning targeted therapy and empirical therapy will remain.

Finally, many studies (mostly from Europe) show that PCT levels help to distinguish between bacterial and viral pneumonia, reduce antibacterial use, predict severity based on the magnitude of the result, and may predict survival. As with the molecular tests, the timing of availability of results will determine to a great extent the utility as to a point-of-care test. Ongoing studies will further substantiate the utility of PCT.

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