Biological Markers to Determine Eligibility in Trials for Community-Acquired Pneumonia: A Focus on Procalcitonin

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Clinical features such as cough, sputum production, fever, and the presence of a new lung infiltrate seen on radiograph are not specific to respiratory tract infection, nor do they define the need for antibiotic therapy. Therefore, investigators have looked for biological markers that can supplement clinical information to determine whether the etiology of the infection is more likely bacterial, needing antibiotic therapy, or viral. There are studies of a number of biological markers in serum and bronchoalveolar lavage fluid, including cytokines, acute-phase reactants, and immunoglobulins. The 2 most promising markers in serum are C-reactive protein and procalcitonin (PCT). PCT is a hormokine, produced primarily by parenchymal cells in response to microbial toxins and in response to certain host inflammatory mediators (interleukin-1β, tumor necrosis factor–α, and interleukin-6). Because PCT is down-regulated in the presence of viral infection, PCT seems most promising for defining the need for antibiotic therapy among patients with radiographic evidence of pneumonia. Studies using the highly sensitive Kryptor assay have shown that PCT guidance can lead to the safe withholding of antibiotics among patients with low PCT levels (<0.25 μg/L) and no clinical signs of severe illness. In addition, serial measurements of PCT have been reported to correlate with clinical response to therapy and may be able to guide short durations of therapy. In the future design of trials for community-acquired pneumonia, we may want to exclude patients with low PCT levels, because they are unlikely to benefit from antibiotic therapy. On the other hand, inclusion of patients with low PCT values creates heterogeneity in the study population and confounds the interpretation of clinical trial end points.

WHY BIOLOGICAL MARKERS MAY BE USEFUL FOR PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA (CAP)

The presence of CAP and its severity, as well as the response to therapy, are traditionally determined by clinical and radiographic assessment—that is, the presence of symptoms such as cough, sputum production, fever, and dyspnea in a patient with a new lung infiltrate seen on radiograph. These clinical features are not always specific to respiratory infection and also do not distinguish bacterial from viral infections. Thus, the use of clinical parameters to define the need for antibiotic therapy for CAP can be misleading because the illness may be diagnosed as a bacterial infection in some patients with noninfectious or viral respiratory disease. In addition, with this approach, CAP can be diagnosed late in the course of illness for patients who initially have negative or subtle clinical and radiographic findings. Clinical features depend on the host response to infection, which varies with the identity of the specific organism, inoculum size, the receipt of previous antibiotic therapy, the status of individual patient host response (often defined by genetic polymorphisms in immune reactivity), and the presence of concomitant illnesses (such as acute lung injury).

For many of these reasons, investigators have ex-
explored a variety of biological markers, which can be objectively quantitated in serum or bronchoalveolar lavage fluid, with the goal of identifying the presence of bacterial respiratory infection [1]. Investigators have measured proinflammatory cytokines, such as TNF-α, IL-1, and IL-6; factors in the coagulation cascade (plasminogen activator inhibitor 1); and anti-inflammatory cytokines, such as IL-1 receptor antagonist and IL-10. Currently, the most promising studies are those of serum measurements of the acute-phase reactants C-reactive protein (CRP) and procalcitonin (PCT) [2–5]. In addition, studies of bronchoalveolar lavage fluid levels of the soluble triggering receptor expressed on myeloid cells (sTREM), a member of the immunoglobulin superfamily, have provided interesting data, but bronchoalveolar lavage fluid analysis is not a methodology that can be widely applied to outpatients and moderately ill inpatients [6]. This review focuses on recent studies of sTREM, CRP, and PCT as biological markers.

STUDIES OF BIOLOGICAL MARKERS FOR CAP

sTREM. A group of French investigators performed a prospective study of patients receiving mechanical ventilation with CAP and ventilator-associated pneumonia, to evaluate the ability of sTREM in bronchoalveolar lavage fluid to define the presence of pneumonia [6]. This molecule is a member of the immunoglobulin superfamily, and its soluble form is shed from the membrane surface of phagocytes, into bronchoalveolar secretions, with levels up-regulated by the presence of microbial products. The study included 148 patients receiving mechanical ventilation, all of whom had suspected pneumonia. sTREM was measured using a rapid immunoblot method, and the findings were correlated with quantitative cultures and the clinical pulmonary infection score, which were used to diagnose pneumonia. Of the patients studied, 38 had CAP, 48 had ventilator-associated pneumonia, and 64 had not had pneumonia. Multiple logistic regression analysis demonstrated that elevated sTREM levels in bronchoalveolar lavage fluid were better predictors of bacterial and fungal pneumonia (OR, 41.5) than were clinical pulmonary infection score >6 (OR, 3.0) or bronchoalveolar lavage fluid levels of TNF-α and IL-1 [6]. However, there was some overlap in sTREM levels between patients with pneumonia and those without pneumonia. Although these findings are provocative, widespread use of this test is unlikely because of the need to sample bronchoalveolar lavage fluid. In addition, all the patients studied were severely ill, and the value of this marker for patients with mild or early disease was not determined.

CRP. CRP is an acute-phase reactant. The use of serum CRP levels in patients with suspected CAP as a predictor of the need for antibiotic therapy has had only limited success. In one study performed in a region in Spain, serum CRP levels were measured in early CAP, during a nearly 2-year period, for all individuals aged >14 years with radiographic evidence of CAP [2]. Patients residing in nursing homes were excluded, and 201 patients with CAP were compared with 84 healthy control individuals matched by age, sex, and municipality. In addition, there were 25 patients with suspected CAP that was later ruled out. The median CRP level was 110.7 mg/L among the patients with CAP, compared with 1.9 mg/L among the control individuals and 31.9 mg/L among those with suspected CAP. An etiology for CAP was identified for 90 (44.8%) of the 201 patients, and CRP levels varied by pathogen: statistically significantly higher levels were seen for pneumococcus (166.0 mg/L) and Legionella pneumophila (178.0 mg/L) than for other organisms. Lower levels were seen for viral infection. In addition, patients admitted to the hospital had higher levels than did outpatients [2].

In another study involving patients with cough, CRP level was found to be a useful predictor of the presence of pneumonia [3]. In this study, 168 patients with cough of duration <3 weeks were evaluated using a fingerstick measurement of CRP. Only 12% of all patients had radiographic evidence of CAP, but they had a higher median CRP level than did the other patients (60 mg/L vs. 9 mg/L; P < .0001). Of the 20 patients with CAP, 12 were admitted to the hospital, but CRP level was not useful in helping to guide the decision of whether to admit them. Overall, a CRP level of ≥40 mg/L had a sensitivity of 70% and a specificity of 90% for the diagnosis of CAP, but the diagnostic ability improved with the addition of clinical variables such as tachypnea and decreased breath sounds. The authors suggested that, on the basis of their data, patients with very high levels of CRP (>100 mg/L) were likely to have CAP and might benefit from empirical antibiotic therapy, if the diagnosis was otherwise in doubt.

In another study involving 364 patients with respiratory infections who were seen by general practitioners, 48 had pneumonia. An elevated CRP level had a sensitivity of 73% and a specificity of 65% for the diagnosis of CAP, with a positive predictive value of 24% and a negative predictive value of 94% [7]. To date, there have been no prospective intervention studies using CRP level as a tool to guide decisions about antibiotic use.

PCT

Overview of PCT biology. The PCT peptide is the precursor of calcitonin. PCT is one of a group of calcitonin gene–related products produced by cells containing a common ancestral gene. PCT has been termed a “hormokine,” because it can be either hormonally expressed by neuroendocrine cells (such as the thyroid and K-cells of the lung) or released in a cytokine-like manner by a variety of parenchymal cells, including liver, kidney, and monocytes, but not by leukocytes [1]. PCT levels are constitutively released by parenchymal cells in response to
microbial toxins and to certain host proinflammatory mediators (IL-1β, TNF-α, and IL-6). PCT levels are attenuated by the cytokines released in response to a viral infection, thus helping to distinguish bacterial infections from viral illness. When levels increase in response to a bacterial infection, it is from a parenchymal source, because there is little intracellular storage of PCT. After a septic stimulus, parenchymal cell PCT protein production can be detected after 10 h, whereas mRNA is detectible after 6 h. Levels can stay elevated for at least 24 h, and often longer, when produced by parenymal cells, whereas monocyte levels are only transiently elevated. In sepsis, protein production can be detected after 10 h, whereas mRNA storage of PCT. After a septic stimulus, parenchymal cell PCT from a parenchymal source, because there is little intracellular.

Measurement of PCT levels. When used to guide the diagnosis of infection and the prescription of antibiotics, the precise PCT assay that is chosen has great importance. There are 2 commercially available assays: the LUMI test and the more sensitive Kryptor assay [1]. The LUMI test detects markedly elevated PCT levels with a luminometer but is relatively insensitive, with a lower limit of detection of 0.3–0.5 µg/L. A number of studies used the LUMI test and had less reliable results than those of the studies conducted by Swiss investigators who used the Kryptor assay [4, 5]. The Kryptor assay is based on a sheep polyclonal anti-calcitonin antibody, and it is very sensitive, with a lower detection limit of 0.06 µg/L. Results can be obtained within 1 h from 20–50 µL of plasma. The Kryptor assay is reported to be helpful in prospective intervention studies of lower respiratory tract infections to aid in antimicrobial stewardship. These studies that used the Kryptor assay have distinguished bacterial pneumonia from viral and noninfectious causes of lung infiltrates and have led to the use of fewer antibiotics among patients with suspected pneumonia [4, 5]. In addition, serial PCT assay measurements are reported to have successfully guided the duration of CAP therapy [8]. PCT has also been reported to be an accurate prognostic marker for severe CAP [9].

Clinical studies using PCT measurement. In a study published in 2004, Christ-Crain et al. [4] showed the utility of PCT measurement among patients with suspected lower respiratory tract infection. The study involved 243 emergency department patients with lower respiratory tract infection in a cluster-randomized, prospective, single-blinded (the investigator was given PCT data) intervention. A total of 119 patients were randomized to receive treatment with antibiotics according to clinical judgment, whereas 124 patients were given PCT-guided therapy. This meant that, by a protocol based on PCT levels, the use of antibiotics was more or less discouraged (PCT level, <0.1 µg/L or <0.25 µg/L, respectively) or more or less encouraged (PCT level, ≥0.5 µg/L or ≥0.25 µg/L, respectively). Reevaluation was possible after 6–24 h for both groups, and the investigator could use clinical judgment to override the therapy suggested by PCT levels. The primary end point was the use of antibiotics. Of the patients studied, 87 had CAP, 60 had acute exacerbations of chronic bronchitis, 59 had acute bronchitis, 13 had asthma, and 24 had other respiratory tract infections. Of the 175 patients tested for viral infection, 141 had serological evidence of recent infection. The outcomes for both groups were similar, but the group given PCT-guided therapy used statistically significantly fewer antibiotics than did the group given standard care (44% vs. 83% received antibiotics; P < .001) and had a shorter mean duration of therapy (10.9 vs. 12.8 days; P = .03). Of patients with radiographic evidence of CAP, all 45 in the standard care group received antibiotics, whereas antibiotic treatment was withheld for 4 of 42 in the PCT-guided therapy group. One of these patients had bronchoalveolar lavage culture results positive for pneumococcus but still recovered uneventfully without antibiotic therapy. Although PCT led to the identification of relatively few patients with CAP who could avoid antibiotic therapy, the impact on the patients with acute and chronic bronchitis was more dramatic.

On the basis of these findings, the same investigative group designed a prospective intervention study exclusively involving patients with radiographic evidence of CAP (the Procalcitonin-guided Reduction of the Duration of Antibiotic Therapy in Community-acquired Pneumonia [ProCAP] study) [5]. A total of 302 patients were randomized to receive standard care or PCT-guided therapy (151 in each group). In the group receiving PCT-guided therapy, antibiotic treatment was strongly discouraged for those with PCT levels <0.1 µg/L, was discouraged for those with PCT levels <0.25 µg/L, was encouraged for those with PCT levels >0.25 µg/L, and was strongly encouraged for those with PCT levels >0.5 µg/L. Physician judgment was the final factor in the decision on antibiotic therapy, and the physician could override the PCT-guided recommendations. Reevaluation of clinical status and PCT levels was recommended after 6–24 h for those from whom antibiotics were withheld. PCT levels were also used to guide duration of therapy, with use of the same cutoff levels above but on the basis of PCT measurements taken on days 4, 6, and 8 of hospitalization. For patients with very high initial PCT levels (>10 µg/L), discontinuation of antibiotic treatment was encouraged when levels decreased to <10% of the initial value, instead of to <0.25 µg/L. Among the group receiving PCT-guided therapy, 28% had initial PCT levels <0.25 µg/L, but antibiotics were withheld for only 15% of the patients; in addition, 8% of patients in this group did not have antibiotic treatment stopped according to the recommendations of the algorithm. However, the PCT-guided therapy group received significantly fewer antibiotics (85% vs. 99% received antibiotic treatment at admission; P < .001) and had a 55% shorter duration of therapy (median, 5 vs. 12 days; P < .001) than did the standard care group. The
reduction in duration of therapy applied to patients in all risk groups, as defined by the pneumonia severity index (PSI). Outcomes were similar for both groups, with an overall success rate of 83%. There were no differences between the groups in duration of hospitalization, quality-of-life score, need for admission to the intensive care unit (ICU), or other complications, including pneumonia-related mortality. On the basis of the findings of this study, the investigators concluded that PCT measurement was more useful than were clinical parameters in guiding the prescription of antibiotics to patients with radiographic evidence of CAP.

In a subsequent analysis, the investigators combined patients from the 2 previous studies, and, of the 545 patients studied, 373 had radiographic evidence of CAP. Of these patients, 20 ultimately received diagnoses of noninfectious disease, and another 24 recovered without antibiotic therapy [10]. To evaluate the discriminatory value of PCT measurement, compared with clinical features and highly sensitive CRP measurement, the investigators first compared how accurately each measure could predict the presence of an abnormal finding on chest radiograph (which was present for 373 of the 545 patients evaluated). For this end point, PCT and highly sensitive CRP measurements were similarly accurate, with both being more useful than clinical signs such as fever, leukocyte count, abnormal finding on lung examination, sputum production, cough, and dyspnea ($P < .001$). A clinical model alone had an area under the curve (of sensitivity vs. 1 − specificity) of 0.79 to predict an abnormal finding on radiograph, compared with 0.88 when PCT measurement was added to the clinical assessment and 0.92 when both PCT and highly sensitive CRP measurements were added. When the 44 patients who received either a noninfectious diagnosis or no therapy with antibiotics were excluded from the group of 373 patients with radiographic evidence of CAP, to define the ability to predict the need for antibiotic therapy, the diagnostic accuracy of PCT measurement was greater than that of highly sensitive CRP measurement ($P < .001$) and that of clinical parameters ($P < .001$) such as leukocyte count and fever. PCT measurement was also more accurate than highly sensitive CRP measurement or clinical features in predicting the presence of bacteremia ($P < .01$) and the severity of pneumonia, as defined by the PSI. Thus, either PCT or highly sensitive CRP measurement could be used for symptomatic patients with lower respiratory tract infection, in conjunction with clinical features, to improve the ability to predict the presence of an abnormal finding on chest radiograph, but PCT measurement was best for determining whether patients with an abnormal finding on radiograph would benefit from antibiotic therapy.

A few caveats are in order. Most of the data for the Swiss studies were collected at a single center and thus may not be widely applicable. In addition, the standard care group did not have strict enforcement of antibiotic treatment guidelines, and length of stay was never a target of the trials, only the use of antibiotics was. For these reasons, a prospective trial is being planned. The ProHOSP study plans to enroll 1002 emergency department patients with lower respiratory tract infections from 6 Swiss centers [11]. Patients will be randomized by center and by type of respiratory tract infection, with management guided by PCT levels or by clinical treatment guidelines. On the basis of presenting symptoms, clinical treatment guidelines will specify which patients with bronchitis can safely have antibiotic treatment withheld. For patients whose therapy is guided by PCT levels, clinicians can override the recommendation to withhold antibiotics if the patients have respiratory or hemodynamic instability, severe comorbidity, extrapulmonary infection, immune suppression, or need of ICU admission. The protocol primary end point is treatment failure at 30 days, and secondary end points are antibiotic exposure, rate of hospitalization, cost-effectiveness, and time to clinical stability. This type of study should address many of the uncertainties about PCT-guided therapy for CAP and other lower respiratory tract infections.

Although the Swiss studies have shown the value of PCT levels for prediction of the need for antibiotic therapy among patients with radiographic evidence of CAP, there is still some question about whether PCT measurement can recognize pneumonia caused by atypical pathogens and whether PCT levels can distinguish these types of infection from bacterial infection. A small study evaluated 30 patients with CAP, 20 with bacterial pneumonia, and 10 with infection involving Chlamydia pneumoniae, Mycoplasma pneumoniae, or Legionella pneumophila [12]. Unlike the Swiss studies, this study used the less sensitive LUMI assay for PCT, rather than the Kryptor assay. PCT levels were higher for bacterial pneumonia than for infection with atypical pathogens (7.64 vs. 0.8 μg/L; $P = .03$). Although PCT levels could distinguish the different types of pneumonia, clinical parameters and CRP levels were not able to differentiate bacterial CAP from CAP caused by atypical pathogens.

**PCT and CAP severity.** Some investigators report that PCT levels correlate with severity of illness and prognosis in CAP [8, 9, 13]. In a study involving 185 patients (144 inpatients and 44 outpatients) who had PCT levels measured within 24 h after the diagnosis of CAP, higher levels correlated with PSI (PCT levels were higher for PSI risk classes III–V than for risk classes I and II), the development of complications (PCT levels were higher for those with empyema, those receiving mechanical ventilation, and those with septic shock), and mortality [8]. Interestingly, PCT levels were higher for patients with a low mortality risk (PSI risk class, I–II) and CAP with a bacterial etiology than for those with a low mortality risk and no bacterial infection, but similar findings were not found for those in
higher PSI risk classes. This may mean that low PCT levels in outpatients could indicate that it is safe to withhold antibiotic therapy. This study accounted for confounding conditions by calculating PSI for all patients. In another recent study, PCT was measured by the Kryptor assay at first presentation in 1508 patients with CAP [14]. With increasing severity of illness, as measured by the CRB-65 criteria (confusion, respiratory rate >30 breaths/min, low systolic or diastolic blood pressure, and age ≥65 years), the PCT level also increased. In a multivariate analysis, only PCT level and CRB-65 score were independent predictors of mortality within 28 days, but the PCT level added value to the information provided by the CRB-65 score [14].

Serial measurements of PCT are reported to define prognosis for patients with severe CAP. In one study involving 110 patients who had only 1 measurement taken within 48 h after ICU admission, levels of PCT were higher in those with positive bacteriological results than in those who had negative results and were higher in those with complications (septic shock and organ dysfunction) or who died than in those without complications who survived [13]. Bolstered by these findings, the same investigative group collected serial PCT levels in 100 ICU patients with CAP on days 1 and 3 [9]. Not only did nonsurvivors have significantly higher PCT levels on day 1 than did survivors, but, with serial measurement, survivors had a decrease in PCT levels by day 3, whereas nonsurvivors had an increase. Numerous clinical parameters were also measured, as well as serial levels of CRP, but, in the multivariate prediction of mortality, the relevant factors were need for mechanical ventilation (OR, 9.9), multilobar infiltrates (OR, 5.6), increasing PCT levels (OR, 4.5), and worsening of a multiorgan failure score. In addition, a low PCT level at day 3 was associated with a low mortality rate. Serial measurements of CRP did not have any predictive value in this study.

**HOW BIOLOGICAL MARKERS CAN BE INCORPORATED INTO CAP TRIAL DESIGN**

From the available data, PCT seems to be the most promising biomarker for defining the need for antibiotic treatment among patients with radiographic evidence of CAP. PCT level is more valuable than other markers, such as CRP level. The basis for this value is the ability of PCT measurement to differentiate bacterial CAP (and probably CAP caused by atypical pathogens) from viral pneumonia. Because the most promising data are from studies that used the highly sensitive Kryptor assay, which has not been widely used by multiple investigators, the PCT findings need validation by other studies in a wide variety of clinical situations. In addition, high PCT levels may identify patients with a worse prognosis and a greater severity of illness, as reflected by the PSI score. Thus, if outcomes in a trial of treatment for CAP are examined to see the differences between 2 drugs, inclusion of only patients with high PCT levels should enrich the study population with patients with bacterial infection who could benefit from antimicrobial therapy and thus increase the validity of the measured end points. In addition, serial measurements of PCT have prognostic value and might serve as a surrogate marker for rate of clinical response to therapy.

On the basis of these findings, it seems possible to design CAP trials that include the measurement of PCT, along with clinical parameters, in the inclusion criteria and in definitions of outcomes for therapy. For all trials, it appears desirable to exclude patients with radiographic evidence of CAP who have a low PCT level (cutoff level, <0.1 or <0.25 μg/L), unless there are clinical signs of severe illness. Patients with low PCT levels are unlikely to benefit from antibiotic therapy, compared with no therapy. Inclusion of such patients in a clinical trial of a new antibiotic could result in no observed differences in outcome between the new drug and either a placebo or a highly active comparator. In addition, these patients could dilute any beneficial effect that occurred in other patient subsets. In trials involving outpatients with CAP, all patients should meet clinical and radiographic inclusion criteria and have a PCT level >0.25 μg/L, because these patients are likely to benefit from antibiotic therapy. Because therapy is likely to have value for this population, it seems unwise to conduct a placebo-controlled trial involving these patients. A superiority trial is difficult to conduct, and outcomes are unlikely to differ between 2 effective treatments for outpatients who generally have low mortality and few other complications. However, if such a trial were contemplated, inclusion of only those patients with the highest PCT values (>0.5 μg/L) would be best, because they have the greatest risk of a poor outcome, and differences between therapy groups, if they exist, might be observed. Lastly, the rate of decrease from an elevated PCT level at baseline during therapy could serve as a surrogate end point of treatment outcome.

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