Procalcitonin as a Biomarker in Respiratory Tract Infection

David N. Gilbert

Infectious Diseases and Earle A Chiles Research Institute, Providence Portland Medical Center and Oregon Health and Sciences University, Portland, Oregon

Serum procalcitonin (PCT) levels rapidly increase in patients with invasive bacterial disease. PCT levels increase faster than do C-reactive protein levels. Furthermore, a rapid decrease in the PCT level is supporting evidence that the source of the bacterial infection is responding to clinical management. In patients with community-acquired bacterial pneumonia, sequential PCT levels are useful as a guide to shorter courses of antimicrobial therapy. With use of emerging multiplex real–time polymerase chain reaction platforms for the detection of viral and bacterial respiratory pathogens, it should be possible to critically assess whether an elevated serum PCT level is a valid biomarker of invasive bacterial infection.

Serum procalcitonin (PCT) levels are of interest as a biomarker in patients with respiratory tract infections for several reasons. Serum PCT levels are elevated in patients with bacterial pneumonia and septic shock [1, 2]. To the contrary, data suggest that serum PCT levels are not elevated in patients with viral respiratory tract infection unless there is a superimposed or coincident bacterial infection [3]. Therefore, serum PCT levels potentially can assist in clinical decisions regarding whether patients with respiratory tract infection would benefit from empirical antibiotic therapy.

Furthermore, in patients with an initial elevation of PCT levels due to bacterial infection, subsequent sequential PCT levels can be used to assess the effectiveness and duration of antibiotic therapy. To understand these clinical data, it is necessary to assess the current understanding of PCT biology.

Correspondence: David N. Gilbert, MD, Providence Portland Medical Center, 5050 NE Hoyt, Ste 540, Portland, OR 97213 (david.gilbert@providence.org).

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What Is PCT?

Role of PCT in Innate Immunity

The hormone calcitonin is synthesized in the C cells of the thyroid gland in response to hypercalcemia or constitutively in patients with medullary thyroid cancer [3]. Calcitonin has 33 amino acids and is part of the larger 116–amino acid prohormone PCT [3]. The serum of normal persons contains intact PCT, calcitonin, an aminoterminus 57–amino acid sequence (NProCT), a 21–amino acid carboxyterminus peptide (CCP-I or katacalcin), and the CCP-I attached to calicitonin [2–4]. Which of these molecules are detected by commercial PCT assays is discussed below.

A series of clinical observations documented elevated serum PCT levels in patients with septic shock due to bacteremia [3, 4]. Subsequent investigations indentified PCT as part of the complex pro-inflammatory response of the innate immune system. In vitro stimulation of macrophages with either bacteria or endotoxin results in rapid synthesis and release of tumor necrosis factor, (TNF), interleukin (IL)–1, and IL-6 [4]. Within 4 h, the synthesis of PCT is detectable. In a hamster model of Escherichia coli peritonitis, transcription and translation of PCT synthesis was demonstrated in virtually all organs tested and in macrophages [5]. In vitro, endotoxin stimulated adipocytes to synthesize PCT in the absence of macrophages, but synthesis was augmented if

adipocytes were incubated with endotoxin-sensitized macrophages [6]. Albeit intriguing, the data are incomplete. In the absence of TNF or other macrophage-derived proinflammatory cytokines, what is the PCT response of tissues to the presence of bacterial or fungal infection.

Whether PCT is helpful or detrimental to the host is uncertain. Exogenous PCT given to healthy animals produces no recognizable ill effects, whereas exogenous PCT given to septic animals increases mortality. Administration of specific PCT antibody to septic animals is associated with a reduced mortality rate [2, 7]. It is tempting to speculate that PCT assists the host by recognition of invasion by bacteria.

PCT differs from other biomarkers of invasion by bacterial pathogens. Serum PCT serum levels are detectable as early as 3–4 h after invasion, which is much earlier than the increase in the C-reactive protein level or erythrocyte sedimentation rate [4]. Thus far, elevated PCT levels have not been noted for other inflammatory conditions, such as inflammatory bowel disease, temporal giant cell arteritis, polyarteritis nodosa, systemic lupus erythematosis, gout, and Still disease [8–11]. Available data indicate that PCT levels are not influenced by therapy with glucocorticoids or nonsteroidal anti-inflammatory agents [12, 13]. PCT levels do not increase or increase only modestly in patients with infection due to respiratory viruses [14, 15].

In vitro, IL-1β induces PCT secretion by cultured adipocytes [6]. The PCT secretion is nearly completely blocked if interferon (IFN)–γ is added to the medium. Serum IFN-γ levels increase in response to a variety of viral respiratory tract infections [16]. Thus, the absence of an increase in serum PCT levels in patients with viral respiratory tract infection may be due to inhibition of PCT synthesis by IFN-γ.

Serum PCT Assays

The first commercial PCT assay was an immunometric assay (LUMI Test; Brahms), which measures levels of PCT plus the combination of calcitonin and calcitonin-carboxyl-peptide- I [3]. The functional lower limit of sensitivity is ~0.5 ng/mL. In patients with documented pure viral infection, the serum PCT may increase to no more than 0.1 ng/ml. Conversely, in response to bacterial pneumonia, the PCT level may increase to no more than 0.25 ng/mL. Therefore, the LUMI test is suboptimal as a method of distinguishing a virus-induced increase in the PCT level to 0.1 ng/mL and a bacteria-induced increase to ⩾0.25 ng/mL. The US Food and Drug Administration–approved second-generation assay is described as a time-resolved cryptate emission (Kryptor; Brahms) immunoassay that has a functional lower limit of detection of 0.05 ng/mL [17]. The antibody used also recognizes both PCT and CCP-I. With use of a research assay that detects only the PCT molecule in the absence of infection, normal human serum PCT levels were measured at 0.033 ± 0.003 ng/mL (mean +/− 2 SD) [3]. In short, none of the currently commercially available PCT assays measure the PCT level alone, although such an assay is in development [18].

PCT and Viral RTIs

Unfortunately, most studies have used the less sensitive LUMI test; thus, the results are difficult to interpret. There is one pertinent published study of children and a published abstract in adults that used the more sensitive Kryptor assay [14, 15].

The pediatric study involved 1154 children seen for bronchitis and pneumonia in a German emergency department during the period August 2004–October, 2006 [14]. In 327 children (age, 2 months to 17 years), both serum PCT levels and nasopharyngeal swab reverse-transcription polymerase chain reaction (RT-PCR) assays for 13 respiratory viruses and 4 "atypical" bacterial pathogens were performed. A potential viral pathogen was identified in 149 of 225 patients with a serum PCT level ⩽0.1 ng/mL. A respiratory viral pathogen was identified in 64 of 102 patients with a serum PCT level ⩾0.25 ng/mL. The problem is that no bacterial cultures of sputum or blood samples were performed for either group. Without culture data for bacteria, it is not possible to determine how many of the patients with higher PCT levels had both viral and bacterial pathogens present. For example, 3 patients with high PCT levels had positive blood culture results.

The adult study focused on hospitalized patients with lower respiratory tract infections over 4 winter seasons from 1999 to 2003 [15]. Data from RT-PCR for 7 common respiratory viruses and sputum plus blood cultures were reported for 63 patients. Only a respiratory virus was found in 34 patients, whose mean PCT levels (±2 standard deviations [SDs]) were 0.7 ± 2.3 ng/mL. The higher-than-expected result is confounded by 2 patients who had blood cultures positive for *Staphylococcus epidermidis* that may represent invasive pathogens or contaminants. In 23 patients with a virus detected by a positive PCR result and bacteria detected by a positive culture result, the mean PCT level (±2 SDs) was 2 ± 5 ng/mL (mean +/− 2 SD). In 9 bacteremic patients without evidence of viral infection, the mean PCT level (±2 SDs) was 19 ± 26 ng/mL. In short, there are limited surveillance data supporting the statement that the PCT level does not increase in patients with infection due to only respiratory viruses.

In contrast, there are substantive data that human infection with rhinovirus, respiratory syncitial virus (RSV), influenza, adenovirus, and metapneumovirus stimulate a robust cytokine response that includes gamma interferon [16, 19]. Furthermore, the magnitude of the IFN-γ response varies with the type of inciting virus (eg, IFN-γ levels are higher in nasopharyngeal secretions obtained from patients with influenza than in RSV-infected patients [16]. In addition, a deficiency in the receptor for IFN-γ is reported to increase the severity of respiratory viral infection [20]. It is tempting to speculate that the magnitude of the IFN-γ response correlates with the degree of suppression of

the PCT response. More studies are needed; for example, PCT levels should be measured in animals without the ability to make IFN-γ that are infected with influenza virus. In addition, the normal PCT response in patients or animals with dual infection with pathogenic viruses and bacteria is unknown.

PCT Guidance of Antibiotic Therapy

Several studies have evaluated the role of elevated serum PCT levels as a way of increasing the probability of a bacterial etiology of respiratory tract infections in adults (Table 1) [21–26]. The studies are investigator-initiated, noninferiority, and randomized, controlled trials with nearly identical trial designs. After a clinical diagnosis, a sensitive serum PCT assay was performed, with results available around-the-clock within 1 h. Patients were randomized to a control group or a PCT-guidance group. Physicians caring for control group patients were not made aware of PCT results and treated patients in accordance with local guidelines.

Physicians in the PCT-guidance group were informed of the PCT level results along with interpretative guidance: (1) for subjects with PCT levels <0.1 ng/mL, antibiotic was use strongly discouraged; (2) for subjects with PCT levels of 0.1–0.25 ng/mL, antibiotic use discouraged; and (3) for subjects with PCT levels >0.25 ng/mL, antibiotic use was strongly encouraged.

If antibiotic therapy was not started, a PCT level was again determined in 6–24 h for verification and to insure that a rising level was not missed. For hospitalized patients randomized to the PCT group, PCT levels were repeatedly determined after 3, 5, and 7 days of antibiotic therapy, and interpretative advice was the same as in the initial report. If the initial PCT level was >10 ng/mL, discontinuation of antibiotic was "recommended" after an 80% decrease from the baseline level and "strongly recommended" after a 90% decrease from the initial value. In both control and PCT-guided groups, the choice of antibiotic(s) was at the discretion of the treating physician.

As summarized in Table 1, six studies have been published, with the largest randomizing >1300 patients. With the exception of 1 Danish multiple-hospital study, all the studies were conducted in Switzerland. Patients with a variety of upper and lower respiratory tract infections were randomized, but the majority of patients had either acute exacerbations of chronic obstructive pulmonary disease (or community-acquired pneumonia).

Routine sputum or throat cultures were performed in some but not all studies. Virologic diagnosis was performed using antibody titers in only 1 of the 6 studies. In short, there was no systematic attempt to correlate PCT levels with the microbial etiology of the patient's respiratory tract infection. If the PCT level was low and no antibiotic was given, resolution of the patient's infection was accepted as tacit evidence that the microbial etiology was most likely viral. Alternatively, if the baseline PCT was high and the patient did well with antibiotic therapy, the microbial etiology of the infection was interpreted as most likely bacterial or a mixed viral-bacterial infection. Finally, a high initial PCT level may decrease with resolution of a bacterial infection even if the etiologic bacteria is resistant to the antibiotic administered.

There were 3 consistent findings in the 6 studies. The level of treatment "clinical success" was high, with no detectable failures as a consequence of following the PCT "guidance" recommendations. With 1 exception, there was only a modest

Table 1. Randomized Controlled Trials That Used Procalcitonin (PCT) Serum Levels to Guide Antibiotic Therapy in Adult Patients With Respiratory Tract Infections

			No. of evaluable patients		Percentage of patients who started antibiotic therapy		Duration of antibiotic therapy, mean days	
Reference	Clinical syndrome(s)	Study site (location)	Control group	PCT group	Control group	PCT group	Control group	PCT group
[21]	Pneumonia, AECOPD, acute bronchitis	Emergency department (Basel, Switzerland)	119	124	77.3	44.4	12.8	10.9
[22]	Community-acquired pneumonia	Emergency department (Basel, Switzerland)	151	151	99	85	12	5
[23]	AECOPD	Emergency department (Basel, Switzerland)	106	102	72	40	…	…
[24]	Rhinosinusitis, tonsillitis, pharyngitis, acute otitis media, tracheobronchitis, AECOPD, community-acquired pneumonia	General practices at multiple outpatient facilities (Switzerland)	226	232	97	25	7.1	6.2
[25]	Community-acquired pneumonia	Hospitals (Denmark)	107	103	79	85	6.8	5.1
[26]	Acute bronchitis, AECOPD, community-acquired pneumonia	Emergency departments at 6 hospitals (Switzerland)	688	671	87.9	75.4	3.8	3.2

NOTE. AECOPD, acute exacerbation of chronic obstructive pulmonary disease.

reduction in the number of patients who initially received empirical antibiotic therapy in the PCT-guidance group. In the 5 studies in which it was measured, patients whose physicians were provided sequential PCT levels as guidance for duration of therapy received substantially shorter courses of antibiotic therapy, compared with patients whose physicians did not receive this information.

Low PCT Levels in Patients With Empyema

Two reports have evaluated PCT levels in patients with empyema. In the first study of 18 patients with culture-positive empyema, the sensitivity and specificity of serum PCT level were 76% and 81%, respectively, using a cutoff value of 0.19 ng/mL [27]. The sensitivity and specificity for pleural fluid PCT levels were only 67% and 77%, respectively. The levels were not analyzed on the basis of type of infecting bacteria [27]. The latter poor results were confirmed in 30 patients with empyema and pleural fluid PCT levels that ranged from 0.06 to 0.63 ng/mL (positive likelihood ratio, 1.6 [1.1–2.3 95% confidence interval]) [28]. No bacteriology data were provided.

There is no ready explanation for the low pleural fluid and serum PCT levels in patients with culture-positive empyema. One could speculate that either there is an absence of activated TNF-producing macrophages and/or that the cells of the pleura lack the necessary genes to synthesize PCT [6].

The Respiratory Tract and PCT Levels: Unanswered Questions

There is clear potential for PCT serum levels to serve as a biomarker of host invasion by bacteria. However, clinical confidence in PCT levels would be bolstered significantly with more data in several areas.

There is a need for better understanding of how PCT fits in the innate immune response. What happens to the host response to infection, bacterial and viral, in the absence of PCT genes? What happens in the absence of IFN-γ genes? If virus-induced IFN-γ turns off transcription and translation of PCT, is the inhibition blocked or reversed by concomitant bacterial infection? We know that the IFN-γ response varies among different viruses [16]. There is a scarcity of data that compares the potency of various bacterial and viral respiratory pathogens as stimulators of PCT synthesis. Is E. coli more potent than *Staphylococcus aureus*? These and related questions could be addressed in studies conducted in vitro, in animal models, and in clinical trials. In future clinical assessments of PCT levels in patients with respiratory tract infections, there is a compelling need for aggressive efforts to establish the presence or absence of viral and bacterial pathogens so that the microbial etiology can be correlated with the PCT level.

Improvements in diagnostic methods should allow identification of candidate etiologic microorganisms in a higher percentage of patients with respiratory tract infection. As discussed elsewhere in this supplement, one multiplex PCR panel for respiratory viruses (xTag;, Luminex Molecular Diagnostics) has been approved by the US Food and Drug Administration, and several other multiplex platforms are in development [29, 30]. There is also promise for highly sensitive and quick detection of bacterial pathogens with multiplex PCRs. In one study, a multiplex PCR panel for the detection of *S. pneumonia, Legionella pneumophila, Haemophilus influenzae, Streptococcus pyogenes, Mycoplasma pneumoniae,* and *Chlamydohila pneumoniae* was compared with conventional cultures. The sensitivity and specificity varied from 93% to 100% [31]. Detection by PCR does not replace culture; culture is still necessary for determination of in vitro antibiotic susceptibility testing. Furthermore, as discussed elsewhere, detection by PCR does not necessarily define infection.

There is great promise for new molecular diagnostic methods that detect both viral and bacterial pathogens. However, analytical sensitivity requires validation in clinical studies [32].

PCT in Clinical Trials

It should be possible to use advanced molecular diagnostics to validate the ability of the serum PCT to separate viral from bacterial infection. Using mostly RT-PCR platforms for both bacterial and viral respiratory pathogens, Johansson et al [33] identified candidate etiologic organisms in 67% of 124 patients with community-acquired pneumonia. In 29 of 70 patients with *S. pneumoniae* infection, a viral copathogen was identified [33]. Assuming all the patients met clinical criteria for pneumonia, highly sensitive PCT level measurements for such patients would substantively improve knowledge regarding the ability of PCT to discriminate viral from bacterial infection. In addition, such analysis would clarify PCT responses in mixed viral and bacterial infections. Furthermore, such data would provide the justification for use of, or non use of, antibacterials in patients with respiratory tract infections.

If both a potential viral and a potential bacterial pathogen (eg, *S. pneumoniae* and rhinovirus) are identified, PCT levels could assist in the interpretation of the result. For example, a low PCT level would suggest the *S. pneumoniae* colonization and that rhinovirus was the relevant pathogen. A high PCT level would suggest bacterial invasion concomitant with either viral colonization or dual viral/bacterial infection. A more detailed discussion of interpretation of PCT levels with mixed infection is published elsewhere [34].

SUMMARY

The time-resolved amplified cryptate emission (Kryptor; Brahms) assay is sufficiently sensitive and specific to judge PCT levels in response to viral and bacterial infections of the respiratory tract. PCT serum levels have several advantages when compared with erythrocyte sedimentation rate and C-reactive protein level as a biomarker for invasive bacterial disease. Confidence in the use of PCT serum levels as part of the initial diagnosis of viral versus

bacterial respiratory tract infection will require additional clinical trials that focus on aggressive attempts to establish a viral and/or bacterial etiology. Similarly, validation of the use of sequential PCT levels to guide duration of antibacterial therapy would be bolstered by microbiologic or molecular diagnostic evidence of a bacterial etiology of the respiratory tract infection. In short, additional studies are needed to clarify the value of PCT levels in the management of respiratory tract infections.

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