Procalcitonin for Triage of Patients with Respiratory Tract Symptoms: A Case Study in the Trial Design Process for Approval of a New Diagnostic Test for Lower Respiratory Tract Infections

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Symptoms of cough, fever, chest pain, and shortness of breath are common reasons that patients seek medical care, and they can be due to a variety of medical conditions, including lower respiratory tract infection (LRTI). Only a small proportion of these patients will actually have a bacterial etiology, but many will receive antibiotic treatment because physicians cannot readily determine the etiology at the time of presentation. Current diagnostic methodologies are not sensitive or specific enough to reliably distinguish bacterial from viral or noninfectious etiologies. Procalcitonin (PCT) is a marker of host response. PCT serum levels are elevated in patients with bacterial infection, compared with levels in those with viral infections or other inflammatory pulmonary conditions. Studies have suggested that the determination of PCT levels can identify a subset of patients with LRTI symptoms who can safely avoid antibiotic treatment. As with any new test, clinical trials are necessary to demonstrate the safety and efficacy of the test to obtain U.S. Food and Drug Administration clearance. However, in the absence of standard reference methods for comparison that are reliably sensitive and specific, meeting the regulatory requirements for proof of safety and efficacy is a major challenge. Additional challenges include the choice of study design, the definition and determination of end points, and the justification of statistical analysis.

LRTIs are infections of the airway (e.g., bronchitis, tracheitis, and tracheobronchitis) or lung parenchyma (e.g., pneumonia) caused by a pathogen (bacteria, viruses, or, rarely, fungi). Symptoms may include cough, fever, purulent sputum, chest pain, and shortness of breath. These clinical symptoms are not specific for pulmonary infection, and patients with other conditions, such as pulmonary embolus, tumor, allergic pneumonitis, and congestive heart failure, may present with several of these complaints. It is difficult to consistently and conclusively establish the presence of a pulmonary infection and to determine the precise etiology of the infecting agent. Pneumonia is a serious, potentially life-threatening illness. Establishing a diagnosis and ascertaining the need for antibiotics in a timely fashion improves the prognosis for a successful clinical outcome [1], and published guidelines [2] recommend initiation of antibiotic treatment as quickly as possible. Acute bronchitis is usually of viral etiology and is seldom life threatening [3]. It is often difficult to distinguish early pneumonia from acute bronchitis, leading physicians to prescribe antibiotics more often than is necessary. Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation that is not fully reversible and is usually progressive. Typically, patients with COPD experience episodic acute worsening of their chronic symptoms. These acute exacerbations of COPD (AECOPD) may be triggered by infectious (e.g., bacteria or viruses) or noninfectious causes (e.g., exposure to air pollution or pollen). Some patients with AECOPD may benefit from a course of antibiotics [4], but at the time of presentation, it is difficult to determine which patients truly require antibiotics.
Physicians know that most nonpneumonia LRTIs are of viral origin and that antibiotics are unnecessary in such cases. They recognize that overuse of antibiotics leads to the emergence of resistant strains and the potential for antibiotic adverse effects. They know that unnecessary antibiotic prescriptions add cost to the health care system. They have read the guidelines on appropriate treatment of LRTIs. They want to do what is best for their patients. However, they continue to prescribe antibiotics for the majority of patients with LRTI, because the actual clinical syndrome (bronchitis versus pneumonia), etiology (bacterial versus viral versus noninfectious) and disease severity are difficult to distinguish with traditional clinical and laboratory means. To appropriately limit the prescription of antibiotics, physicians need better tools to assist them in making the diagnosis of nonbacterial LRTI.

Procalcitonin (PCT) is a marker of host response, and PCT serum levels are elevated in patients with bacterial infection, compared with levels in those with viral infections or other inflammatory pulmonary conditions [5–7]. Hence, the use of PCT could potentially assist physicians in identifying patients at initial clinical presentation who do not require antibiotics. Müller and colleagues conceived and successfully validated the use of a PCT algorithm to guide antibiotic therapy in patients with LRTI using 4 PCT cut-off ranges. They studied >2500 patients in 5 intervention trials: ProRESP (n = 243) [8], ProCAP (n = 302) [9], ProCOLD (n = 226) [10], ProDOC (n = 458) [11], and ProHOSP (n = 1359) [12]. In these trials, patients presenting to the emergency department with signs and symptoms of LRTI were randomized to PCT-guided antibiotic prescription versus standard prescribing practice. In the PCT-guided group, treating physicians were encouraged to give antibiotics to patients with PCT levels in the high ranges and were discouraged from giving antibiotics to patients with PCT levels in the low ranges. All patients received non-antibiotic therapy (eg, steroids and bronchodilators) as appropriate. There was no requirement for extensive diagnostic testing to prove the presence or absence of a bacterial pathogen; it was assumed that recovery without antibiotic therapy equated with the absence of bacterial infection. These published study results suggest that a strategy using PCT guidance can safely limit antibiotic use in a subset of patients with signs and symptoms of LRTI.

A commercial test for PCT has been available in Europe since 1996 and in the United States since 2006. The first commercially available test, B-R-A-H-M-S PCT LIA (BRAHMS) was a manual test with limited sensitivity in the lower range that did not gain widespread use in the United States. In 2007, bioMérieux (Marcy, France) launched the VIDAS B-R-A-H-M-S PCT test, a more sensitive second-generation test, on an automated platform. The test was cleared by the FDA through the 510(k) process for use in conjunction with other laboratory findings and clinical assessments to aid in the risk assessment of critically ill patients on their first day of intensive care unit (ICU) admission for progression to severe sepsis and septic shock [13].

Recognizing both a medical need and a commercial opportunity, bioMérieux decided to pursue a claim for the VIDAS B.R.A.H.M.S. PCT test as an aid in the identification of a subset of patients with LRTI who do not require antibiotic therapy (ie, antibacterial agents).

Development of Trial Design

PCT-Guided Algorithm (Based on the ProRESP Trial)

The ProRESP study [8] was a randomized interventional trial that enrolled 243 subjects presenting to the emergency department of a Swiss hospital with signs and symptoms of LRTI. The subjects were randomized to receive antibiotics either on the basis of current guidelines (Standard group) or on the basis of an algorithm incorporating the PCT value (PCT group). Overall, the number of patients who received antibiotic therapy in the PCT group decreased by 47%, compared with the standard group. The decrease in antibiotic prescription rate was minimal for patients who received a diagnosis of CAP (10%) and was much larger in patients with AECOPD (56%) and acute bronchitis (73%). Withholding antibiotic treatment in the PCT-guided group had no discernable negative impact on disease recovery, but the trial was insufficiently powered to conclusively demonstrate this end point.

Building on the work of Müller and colleagues, the VIDAS PCT-LRTI protocol was initially designed as a larger (n = 1200) version of the ProRESP trial. It was planned as a multicenter, prospective, randomized, controlled trial to be conducted in 16–20 hospital emergency departments throughout the United States. Patients presenting with signs and symptoms of LRTI would be randomized to standard care versus PCT-guided antibiotic prescription utilizing the same algorithm and cut-off points as in the Müller studies. End points would be the number of patients receiving antibiotics and clinical outcome of LRTI. During initial discussion with the FDA, it was made clear that a dichotomous cut-off value was preferred over multiple cut-off values, as utilized by Müller and colleagues. Therefore, it was agreed that a single cut-off value would be used to separate subjects into high PCT level and low PCT level categories. In discussions with potential investigators, it became apparent that physicians were very likely to prescribe antibiotics regardless of PCT level for subjects with community-acquired pneumonia (CAP), evidence of severe illness (eg, persistent hypotension, inadequate oxygenation, and need for ICU admission), or factors correlated with high risk for poor outcome (eg, Global Initiative for Chronic Obstructive Lung Disease class IV). Therefore, it was decided to exclude these patients and limit enrollment to those patients with mild to moderate AECOPD and acute bronchitis. The initial study design proposed to the FDA is represented in Figure 1.
Of key importance in the trial design is that subjects would be enrolled soon after their presentation to the emergency department, prior to their diagnostic work up. This allowed for minimal disruption in emergency department workflow and prevented substantial delay in patient treatment and disposition. However, it meant that many subjects would not meet the criteria necessary to continue in the trial (ie, mild to moderate AECOPD or acute bronchitis), thus raising the target enrollment number. Similar to the Müller studies, the proposed protocol did not require definitive proof of the presence or absence of a bacterial pathogen as the etiology for the LRTI; an absence of serious bacterial infection was assumed if the patient recovered without antibiotics.

**FDA Critique of Proposed PCT-Guided Algorithm Design**

When undertaking a program to obtain clearance for a diagnostic test, it is customary and advisable to seek input from the FDA as to the acceptability of the trial design. During discussions, the FDA concluded that the proposed PCT-guided algorithm trial design was not acceptable. There were 5 main concerns expressed by the FDA, as follows:

1. The end point of reduction of antibiotic use would address a medical need but was deemed by the FDA as inadequate to establish a claim. The FDA required a demonstration of the specific value of the test result to an individual patient and a correlation of the test result with a clinical condition or outcome. As an example, the FDA suggested demonstrating that a PCT of a certain level correlated with a positive bacterial culture result.
2. The initial enrollment of all patients presenting to the emergency department with signs and symptoms of acute mild to moderate LRTI was intended to mirror everyday practice and minimize disruption to emergency department workflow. However, the FDA concluded that patients with AECOPD and patients with acute bronchitis differ substantially and therefore cannot be combined into a single population (ie, non-CAP LRTI). The FDA required that the trial be limited to a single population or that the trial be sized such that adequate numbers of both types of subjects would be enrolled to meet the statistical criteria.
3. The protocol specified that a blinded evaluator would perform all post-enrollment evaluations. The FDA required that the study be truly blinded. Because the study algorithm required that the PCT value be used to determine whether a subject received antibiotics, it was not possible to blind physicians at the time of subject enrollment to either the PCT value or prescription of antibiotics. Blinding patients would only be possible if a matching placebo were used.
4. The FDA prefers the use of a Patient Reported Outcome (PRO) instrument, rather than physician assessment, for determination of clinical outcome [14]. For the proposed VIDAS PCT-LRTI trial, the FDA required the use of a PRO that was validated and approved by the FDA, even though no such PRO exists. The FDA suggested the use of the EXAcerbations of Chronic pulmonary disease Tool–Patient Reported Outcome (EXACT-PRO) [15], even though this instrument was still in validation and had not been officially approved by the FDA. Development of the EXACT-PRO was funded by a consortium of industry and academia and would be available to bioMérieux for a fee. No PRO is available for acute bronchitis, so one would have to be developed to include this population in the trial.
5. The FDA requested a superiority trial. Noninferiority trials are accepted by the FDA only when there is adequate evidence of a defined effect size for the control treatment so that
the proposed equivalence margin (delta) can be supported. [16] To utilize a noninferiority trial design, a comprehensive synthesis of the evidence that supports the effect size of the active control and the proposed equivalence margin, including a summary of historical data derived from placebo-controlled trials, must be presented.

Proposed Observational Trial Design
Given the challenges inherent in the FDA comments and requirements, alternative designs were considered. Building on the FDA suggestion of a trial correlating PCT level with a positive bacterial culture result, one proposal involved conducting a multicenter, observational study of emergency department patients with CAP, AECOPD, and acute bronchitis. Extensive microbiological studies would be performed, including multiplex polymerase chain reaction (PCR) for respiratory viruses, urine antigen for *Streptococcus pneumoniae* and *Legionella pneumophila*, sputum gram stain and culture (if a specimen was available), and blood culture (only for patients with CAP). The end point could be the correlation of low PCT levels with documentation of the presence of a respiratory virus and absence of a bacterial pathogen. Alternatively, the end point could be the correlation of high PCT levels with documentation of the presence of a bacterial pathogen.

It was recognized that the interpretation of the results from this study would be problematic. Blood cultures are positive in only ~10% of patients with CAP and are positive much less frequently in patients with AECOPD and almost never in patients with acute bronchitis. Bacterial cultures of sputum specimens may grow a respiratory pathogen, but the sensitivity and specificity of sputum culture to determine the need for antibiotics in patients with LRTI (especially AECOPD) are often poor. There are multiple factors affecting the utility of sputum cultures, ranging from the quality of the specimen to the interpretation of the results [17, 18]. Patients are often unable to provide a good-quality sputum specimen, and the culture is reported as “normal oral flora” despite the presence in the pulmonary tree of pathogenic bacteria responsible for symptoms. Conversely, potentially pathogenic organisms may be isolated from a sputum specimen but not represent the true cause of the patient’s symptoms because of contamination of the specimen from oropharyngeal flora (*S. pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* are all potential respiratory tract pathogens that can also colonize the upper airway in the absence of disease). It is possible to establish the presence of a respiratory virus by culture or PCR, but these techniques do not test for all possible respiratory viruses nor are they 100% sensitive. The presence of a respiratory virus does not exclude the possibility of a secondary or co-existing bacterial infection. Perhaps only patients who are “well characterized” (ie, with definite bacterial or definite viral infection) should be included for analysis, but if so, who would make the determination of microbial etiology and on the basis of what criteria? Could results be generalized to the remaining patients, who comprise the vast majority? Would such a trial provide adequate data to convince physicians of the utility of the PCT test? This trial design was abandoned.

Placebo-Controlled Antibiotic Trial in Patients With High PCT Levels and Low PCT Levels (Based on Marker by Treatment Interaction)
The FDA proposed limiting the trial to a single patient population and utilizing a marker by treatment interaction design, as described by Sargent et al. [19]. This design assumes that the marker (PCT) divides the population (patients with AECOPD) into 2 groups (those with high PCT levels and those with low PCT levels). Subjects in each marker group are then randomly assigned to 2 different treatments to determine whether one treatment is superior to the other, separately within each marker group. The bioMérieux interpretation of this trial design is represented in Figure 2. The population of patients with AECOPD was selected because of the availability of the Patient Reported Outcome instrument (EXACT-PRO) that was undergoing FDA review. There is no validated and FDA-approved PRO for acute bronchitis, so this population was dropped. Both the FDA and the medical community strongly urged testing for common respiratory tract viruses, so multiplex PCR for the detection of viral nucleic acid was included.

Changing to the marker by treatment design in the AECOPD population resolved the first 4 of the 5 FDA concerns about the originally proposed PCT-guided algorithm design (as listed above) but left unresolved the issue of the statistical analysis based on a noninferiority trial with the required selection and justification of an equivalence margin (delta). The hypothesis...
that the subjects with low PCT levels do not need antibiotics and that therefore the receipt of antibiotic does not impact the recovery from the exacerbation or the time to return to pre-exacerbation baseline implies that the clinical outcome between the 2 low PCT level groups (antibiotics and placebo) should be the same. It would not be expected for the placebo treated subjects with low PCT levels to have a better clinical outcome of their AECOPD, although they should have a lower incidence of antibiotic-related adverse events. If the hypothesis is wrong and these PCT-low subjects do require antibiotics, then the results of the placebo group should be substantially worse than those of the antibiotics group. Given this hypothesis, an appropriate trial design is noninferiority, because achieving superiority would be highly unlikely. Currently, for pharmaceutical trials, the FDA does not accept noninferiority trials in AECOPD [16], because the data to adequately support the selection of an appropriate delta is deemed inadequate.

It is necessary to prospectively define the delta because it has a substantial impact on sample size. As the delta decreases, the sample size increases. For any given delta, as the recovery rate decreases, the sample size increases. The delta does not define the expected difference but, rather, defines the difference in outcome (eg, recovery rate) that is possible to exclude with the size of the enrolled population sample. The predefined delta should be sufficiently small to exclude any decrease in outcome that would be clinically significant to patients and clinicians. However, the ability to perform a trial in AECOPD with an equivalence margin <10% is severely limited because of the impact on the sample size. The sample size for the number of necessary evaluable subjects quadruples as the delta decreases from 10% to 5%. Many anti-infective drugs have been approved using a noninferiority design with deltas of ≥10%.

The marker by treatment interaction trial design introduced a new difficulty; the requirement that half of the subjects with high PCT levels be randomized to placebo. The FDA was adamant that, to adequately evaluate the utility and performance of the test, data from placebo-treated subjects with high PCT levels were necessary. Many investigators expressed doubts that the design would obtain institutional review board approval at their institution. Only patients with mild to moderate disease would be enrolled, and few were anticipated to require hospitalization. Thus, in the majority of cases, there would be limited opportunity to adequately observe a subject’s course. Combined with the lack of sensitive and specific measures to reliably identify early clinical failure that could indicate the need to “rescue” subjects, most investigators considered the risk to randomized subjects with high PCT levels to be too great.

The trial based on the marker by treatment design would be more costly to perform and potentially of less commercial value for bioMérieux. The requirements to utilize a PRO, provide antibiotics and placebo to ensure blinding, and perform multiplex viral PCR for all subjects substantially increase the complexity, start-up time, and cost of the proposed trial. Changing from the PCT-guided algorithm design that has been successful in 5 published studies to the marker by treatment design when the performance of PCT level has not been previously tested in this population increases the uncertainty of a successful outcome. The PCT-guided algorithm commands a higher commercial value because of the inclusion of multiple subsets of patients with LRTI and the demonstration of a valuable outcome (reduction in the use of antibiotics). Currently, discussion continues in an effort to design a trial that meets regulatory and commercial requirements to demonstrate the utility of PCT level in identifying patients with signs and symptoms of LRTI who can safely avoid antibiotic therapy.

What can be learned from the bioMérieux experience of protocol development for the VIDAS B.R.A.H.M.S. PCT LRTI trial to facilitate other new diagnostic tests for patients with symptoms of LRTI?

- Carefully consider how interpretation of reference methods with low sensitivity and/or specificity will impact development of new tests.
- Develop consensus in the medical community around what is necessary to establish the utility of diagnostic tests and, at the same time, to ensure patient safety. Use this consensus to drive the development and improvement of regulatory guidance documents. The industry relies on these guidance documents to plan the research and development of new technologies.
- Develop an understanding of the inherent differences between in vitro diagnostic tests and other devices and therapeutic agents. Use this understanding to revise or develop new regulations for diagnostic tests.
- Carefully consider requirements that add substantial time and expense, including the use of PRO instruments, extremely large sample sizes, and extensive microbiological testing.

**CONCLUSIONS**

LRTIs drive a substantial portion of unnecessary antibiotic use, in large part because the etiology is not apparent at the time of clinical presentation. Given the current state of diagnostic technology, it is difficult to prove the presence of a bacterial infection. It is even harder to conclusively prove the absence of a bacterial pathogen. It is this difficulty in establishing a definitive diagnosis that drives the need for biomarker tests, such as one based on PCT level. However, it is challenging to obtain regulatory clearance for these tests because of the lack of reliably sensitive and specific standard methods for comparison. The assumption that, if a patient with LRTI symptoms recovers...
without antibiotics, then he did not need them (and therefore did not likely have a bacterial infection) may convince physicians, but it is unlikely to be acceptable to regulators.

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