

## Commentary

### **Biomarkers for the diagnosis of bacterial infections: in pursuit of the ‘Holy Grail’**

Fever is one of the most frequent causes for hospitalization in developing countries. While several aetiological causes result in a febrile illness, bacterial infections constitute an important “curable” cause of fever. Systemic bacterial infection, bacterial sepsis and related syndromes are life-threatening illnesses that need early initiation of appropriate antimicrobial therapy. In a systematic review and meta-analysis<sup>1</sup> (22 eligible studies, 58296 patients), 2051 (13.5%) of 15166 adults and 3527 (8.2%) of 43130 children were found to have community acquired bloodstream infections in Africa. In another systematic review<sup>2</sup> (17 eligible studies, 40644 patients), blood stream infections were evident in 1784 (12%) of 14386 adults and 1722 (7%) of 26258 children in South and South-East Asia. In spite of availability of reliable diagnostic methods for detecting bacterial infections, these are not widely available or accessible in routine practice in developing countries and confirmation of diagnosis of bacterial infection is done mainly in referral hospitals or research facilities.

An ideal biomarker for bacterial infections should facilitate early rapid diagnosis, predict the course and prognosis of the disease and guide therapeutic decisions (*e.g.* antibiotic stewardship). Leucocyte count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), soluble triggering receptor expressed on myeloid cells 1 (sTREM-1), pro-adrenomedullin (ProADM), serum procalcitonin (PCT), mid-regional pro-atrial natriuretic peptide (ANP), pancreatic stone protein (PSP)/regenerating protein (reg), interleukin-6 (IL-6), IL-8, IL-27, soluble urokinase-type plasminogen activator receptor (suPAR) among others, have been studied as potential biomarkers to facilitate diagnosis and aid prognostication in bacterial sepsis<sup>3-6</sup>.

The study by Qu *et al*<sup>7</sup> featured in this issue, is an attempt in the quest for defining the diagnostic value of

PCT, CRP, IL-6 and serum amyloid A (SAA) for bacterial infection in febrile patients. The authors prospectively studied 326 adult patients admitted with fever in an infectious diseases department and measured serum PCT, CRP, IL-6 and SAA, within 24 h of admission, in these patients. Following in-hospital diagnostic work-up, the patients were categorized as having bacteraemia group (n=58, 17.8%; group 1), bacterial infection with negative blood culture (n=218, 66.9%; group 2) and non-bacterial infection group (n=50, 15.3%; group 3). PCT at a cut-off value of 0.26 ng/ml was found to have a sensitivity and specificity of 64.5 and 84 per cent, respectively for detecting bacterial infection in febrile patients. In this study<sup>7</sup>, PCT with an area under the curve of 0.804, was found to be superior to CRP ( $P < 0.05$ ), IL-6 ( $P < 0.001$ ) and SAA ( $P < 0.01$ ) in the early identification of bacterial infection. PCT, a 116 amino acid polypeptide precursor of the hormone calcitonin, belongs to the class of molecules, called “hormokines” that can exhibit either classical hormonal expression, or upon stimulation due to inflammation manifest more cytokine-like behaviour<sup>3,6,8</sup>. PCT, following translation from calcitonin-messenger RNA (mRNA), is enzymatically cleaved into smaller peptides, to yield the 32-amino acid mature calcitonin. In microbial infections and in various forms of inflammation, circulating level of PCT has been found to increase several-fold. Microbial infection is thought to result in ubiquitous increase of first calcitonin (CALC-I) gene-expression and a constitutive release of PCT from all parenchymal tissues and differentiated cell types in the body<sup>9</sup>. PCT peptide release from parenchymal cells (including liver, lung, kidney, adipocytes and muscle) in comparison to circulating cells (*e.g.* leucocytes), suggests a tissue based, rather than a leucocyte based mechanism of host defense<sup>3,6,8</sup>. Hence, PCT has been assessed as a diagnostic marker for bacterial infection in febrile neutropenic patients also<sup>10</sup>.

The inflammatory release of PCT is thought to be induced either directly via microbial toxins (e.g. endotoxin) or indirectly via a humoral or cell-mediated host immune response [e.g. IL-1 $\beta$ , tumour necrosis factor-alpha (TNF- $\alpha$ ), IL-6]. PCT levels rarely increase in response to viral infections and this lack of viral response is thought to be due to the virus-stimulated synthesis of  $\alpha$ -interferon by macrophages, which, in turn, inhibits TNF synthesis<sup>3,6,8</sup>. So, PCT has also been used to distinguish bacterial infections from viral infections. PCT with its wide biological range, short time of induction (2-4 h) following a bacterial stimulus, and long half-life (22-26 h) has been found to be a useful biomarker for identification of bacterial infection and antibiotic stewardship<sup>3,11</sup>. Some studies<sup>12,13</sup> have suggested that elevated PCT levels are useful in predicting bacteraemia in febrile patients. Two meta-analyses published in 2006<sup>14</sup> and 2007<sup>15</sup> had yielded conflicting results regarding the diagnostic utility of PCT. In the meta-analysis<sup>14</sup> published in 2006 (33 studies, 3,943 patients) that included predominantly surgery or trauma patients, PCT was found to be a useful diagnostic marker for sepsis, severe sepsis, or septic shock, in critically ill patients and was found to be superior to CRP. In the subsequent systematic review and meta-analysis<sup>15</sup> that included 18 studies [14 phase 2 studies (group 1), 1602 patients; and 4 phase 3 studies (group 2), 495 patients], the authors report that PCT cannot reliably differentiate sepsis from other non-infectious causes of systemic inflammatory response syndrome in critically ill adult patients. In this meta-analysis<sup>15</sup>, studies where sites of infection typical in sepsis (e.g. abdominal sepsis, pancreatitis, or meningitis) were clearly evident and studies that assessed the ability of procalcitonin to diagnose septic shock were excluded. Therefore, selection bias and other methodological issues appear to be the reasons for the differences in these results. Further, in a more recent meta-analysis<sup>16</sup>, (30 studies, 3244 patients) that assessed the accuracy and clinical value of PCT for diagnosis of sepsis in critically ill patients, bivariate analysis yielded a mean sensitivity of 0.77 [95% confidence intervals (CI) 0.72-0.81] and specificity of 0.79 (95% CI 0.74-0.84) and an area under the curve (AUC) of 0.85 (95% CI 0.81-0.88) suggesting PCT as a useful biomarker.

In the study by Qu *et al*<sup>7</sup>, median PCT levels were observed to be lower in patients with Gram-positive compared to Gram-negative bacterial infections [0.53 (0.18-2.90) vs 2.13 (0.46-10.12),  $P < 0.01$ ]. Further research with adequately powered studies is required to delineate this issue in greater detail.

Overall, PCT appears to hold promise as a useful biomarker for bacterial infection in patients presenting with fever. While interpreting test results of biomarkers for bacterial infections, sepsis and related syndromes as “rule-in” or “rule-out” tests, issues concerning false-positive and false-negative results must be kept in mind. Several common causes of falsely elevated serum PCT levels in the absence of bacterial infection, such as, acute respiratory distress syndrome (ARDS), severe complicated falciparum malaria, trauma, chemical pneumonitis, among others should be carefully considered<sup>3,8,11</sup>. It has also been reported that low PCT levels at presentation are useful in excluding bacterial infection as an aetiological cause<sup>17</sup>. Caution should be exercised in excluding bacterial infections based on a low PCT level because low levels of PCT are often seen early in the course of infection; in subacute bacterial endocarditis with bacteraemia; and in localized infections. Therefore, if the clinical evaluation suggests a possible diagnosis of bacterial sepsis, but serum PCT levels are not elevated at the time of initial presentation, patients should still be treated for sepsis initially. Clinical monitoring over the next 48 h with serial PCT measurements can help in clarifying whether the initial diagnosis of bacterial sepsis is correct and antibiotics can be discontinued early if sepsis is excluded and PCT remains low<sup>18,19</sup>.

As on today, PCT is an expensive test as compared to other biomarkers (such as, CRP) and this may be a limiting factor to its wider use in developing countries. Cost-effectiveness analysis takes into account a variety of factors including cost of the PCT assay, the frequency of PCT measurement, and the cost and duration of antibiotic therapy among others<sup>4</sup>. This issue merits further study in the Indian context.

**Alladi Mohan\* & J. Harikrishna**

Department of Medicine  
Sri Venkateswara Institute of Medical Sciences  
Tirupati 517 507, Andhra Pradesh, India

\*For correspondence:  
alladimohan@svims.gov.in

## References

1. Reddy EA, Shaw AV, Crump JA. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infect Dis* 2010; 10 : 417-32.
2. Deen J, von Seidlein L, Andersen F, Elle N, White NJ, Lubell Y. Community-acquired bacterial bloodstream infections in developing countries in south and southeast Asia: a systematic review. *Lancet Infect Dis* 2012; 12 : 480-7.

3. Christ-Crain M, Müller B. Procalcitonin in bacterial infections--hype, hope, more or less? *Swiss Med Wkly* 2005; *135* : 451-60.
4. Nelson GE, Mave V, Gupta A. Biomarkers for sepsis: a review with special attention to India. *Biomed Res Int* 2014; *2014* : 264351.
5. Su L, Han B, Liu C, Liang L, Jiang Z, Deng J, *et al.* Value of soluble TREM-1, procalcitonin, and C-reactive protein serum levels as biomarkers for detecting bacteremia among sepsis patients with new fever in intensive care units: a prospective cohort study. *BMC Infect Dis* 2012; *12* : 157.
6. Hatzistilianou M. Diagnostic and prognostic role of procalcitonin in infections. *Scientific World J* 2010; *10* : 1941-6.
7. Qu J, Lü X, Liu Y, Wang X. Evaluation of procalcitonin, C-reactive protein, interleukin-6 & serum amyloid A as diagnostic biomarkers of bacterial infection in febrile patients. *Indian J Med Res* 2015; *141* : 315-21.
8. Lee H. Procalcitonin as a biomarker of infectious diseases. *Korean J Intern Med* 2013; *28* : 285-91.
9. Müller B, White JC, Nylén ES, Snider RH, Becker KL, Habener JF. Ubiquitous expression of the calcitonin-*r* gene in multiple tissues in response to sepsis. *J Clin Endocrinol Metab* 2001; *86* : 396-404.
10. Meidani M, Khorvash F, Abolghasemi H, Jamali B. Procalcitonin and quantitative C-reactive protein role in the early diagnosis of sepsis in patients with febrile neutropenia. *South Asian J Cancer* 2013; *2* : 216-9.
11. Limper M, de Kruif MD, Duits AJ, Brandjes DP, van Gorp EC. The diagnostic role of procalcitonin and other biomarkers in discriminating infectious from non-infectious fever. *J Infect* 2010; *60* : 409-16.
12. Kim MH, Lim G, Kang SY, Lee WI, Suh JT, Lee HJ. Utility of procalcitonin as an early diagnostic marker of bacteremia in patients with acute fever. *Yonsei Med J* 2011; *52* : 276-81.
13. Delèveaux I, André M, Colombier M, Albuisson E, Meylheuc F, Bègue RJ, *et al.* Can procalcitonin measurement help in differentiating between bacterial infection and other kinds of inflammatory processes? *Ann Rheum Dis* 2003; *62* : 337-40.
14. Uzzan B, Cohen R, Nicolas P, Cucherat M, Perret GY. Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: a systematic review and meta-analysis. *Crit Care Med* 2006; *34* : 1996-2003.
15. Tang BM, Eslick GD, Craig JC, McLean AS. Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis. *Lancet Infect Dis* 2007; *7* : 210-7.
16. Wacker C, Prkno A, Brunkhorst FM, Schlattmann P. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. *Lancet Infect Dis* 2013; *13* : 426-35.
17. Chirouze C, Schuhmacher H, Rabaud C, Gil H, Khayat N, Estavoyer JM, *et al.* Low serum procalcitonin level accurately predicts the absence of bacteremia in adult patients with acute fever. *Clin Infect Dis* 2002; *35* : 156-61.
18. Meisner M. Update on procalcitonin measurements. *Ann Lab Med* 2014; *34* : 263-73.
19. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, *et al.* Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* 2013; *39* : 165-228.