International Journal of Research in Health Sciences
ISSN: 2321-7251
Available online at: http://www.ijrhs.org/
Original Article

Procalcitonin as a biomarker of infection in patients with systemic inflammatory response syndrome/ sepsis

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Received: 01-07-2017 / Revised Accepted: 28-08-2017 / Published: 29-09-2017

ABSTRACT

Procalcitonin (PCT) is currently the most studied infection biomarker and its blood levels seem to mirror the severity of illness and outcome. PCT may help in discriminating bacterial from viral infections and true bacteremia from contaminated blood cultures. Correlations of PCT values with blood culture results were analyzed. Infective foci and clinical diagnosis were also compared in moderate SIRS, severe sepsis and septic shock. Out of total 100 cases, 87 had PCT value >=0.5 ng/ml whereas 13 had PCT value of <0.5 ng/ml. Infective foci were seen in 85% (74/87) of patients with PCT levels of >=0.5ng/ml. Blood culture positivity in moderate SIRS, severe sepsis and septic shock were 31.8%, 30.7% and 57.6 % respectively. PCT as a biological marker appears to have a significant value in identifying or ruling out an infection. It may be of value to distinguish Gram negative from Gram positive and fungal infections.

Key words: Biomarker, Procalcitonin, Infection, Septicemia, Blood culture

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How to Cite this Article: Gupta Divyani, Chhina Deepinder, Gupta Rama and Dhooria HS. Procalcitonin as a biomarker of infection in patients with systemic inflammatory response syndrome/ sepsis. Int J Res Health Sci 2017; 5(4): 6-12.

INTRODUCTION

Sepsis is among the most common causes of death in hospitalized patients. Despite improvements in antimicrobial therapy, it is still associated with high mortality.[1] Timely diagnosis and treatment is highly important in reducing morbidity and mortality associated with sepsis. 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) have been studied as potential biomarkers to facilitate diagnosis and prognostic aid in bacterial sepsis.[2-6] PCT is a prohormone of calcitonin and is synthesized by the C cells in the thyroid gland. It is produced ubiquitously in response to endotoxin or mediators released in response to bacterial infections.[2] PCT has good sensitivity and specificity for predicting systemic bacterial inflammation and can distinguish between viral and bacterial infections.[2,7-8] Gram-positive or Gram-negative bacteria or fungi are known to activate different Toll-like receptor (TLR) signaling pathways, resulting in production of different pro-inflammatory cytokines that ultimately stimulate PCT release in varying proportion.[9] PCT may assist in decisions about the initiation and/or duration of antibiotic therapy (antibiotic stewardship). [10-11] Limited literature is available on the utility of PCT in differentiating Gram-negative, Gram-positive, or fungal bacteremia's. [12-13] The aim of the present study was to investigate PCT as an early surrogate marker for prediction of bacterial sepsis using blood culture as gold standard, and its ability to discriminate between bacterial (Gram positive vs Gram negative) or fungal etiology in patients with SIRS/Sepsis.

METHODS

This was a prospective observational clinical study done in a tertiary care hospital of North India. A total number of 100 cases admitted in medical ICU's, satisfying two or more criteria's of SIRS/sepsis i.e. Temperature more than 38˚C or less than 36˚C, heart rate more than 90 beats/ minute, respiratory rate more than 20 times/ minute or PaCO2 less than 32mm Hg, WBC more than 12,000 cells/µL or less than 4,000 cells/µL (1992 ACCP/SCCM Sepsis definitions) were included. [14] Patients below the age of 18 years; with any malignancy or cardiogenic illness were excluded from the study.

Procalcitonin Assay: The procalcitonin levels were measured using an automated system based on electro-chemiluminescence (ECL) technique (Roche diagnostics Cobas e 411 analyzer).Interpretation of PCT concentrations for diagnosis of sepsis was: -0.05 - < 0.5ng/ml - no bacterial infection; ≥0.5 - < 2 ng/ml - local infection, moderate SIRS, severe trauma, surgery, cardiogenic shock ;≥ 2.0 - <10 ng/ml - severe SIRS (sepsis and organ dysfunction ; ≥ 10 ng/ml - severe bacterial sepsis/ septic shock(sepsis and hypotension)[15].

Blood and body fluid culture: Blood and body fluids samples were collected taking all aseptic precautions andwere inoculated into blood culture bottles. The bottles were incubated in the BacT/Alert or BACTEC blood culture system till they were flagged positive or maximum for a period of 7 days. Gram's stained smears from the positive culture bottles were prepared. Simultaneously subcultures from positive bottles were done on blood agar and MacConkey's agar plates. The plates were incubated at 37°C for 18-24 hours. Growth was identified and antimicrobial sensitivity testing was done in VITEK 2 system. For each patient, only one bloodstream infection episode and, for each episode, only the first samples were considered. Coagulase- negative staphylococci and other skin commensals were considered contaminants when isolated from only one blood culture.

Other specimens (apart from blood and body fluids samples) were inoculated on blood agar and MacConkey's agar and incubated for 24-48 hours. The organisms were identified as per the standard protocols. [16]

RESULTS

During the study period, a total of 100 patients satisfying the criteria of SIRS/ sepsis admitted to the ICU were included in the study. The mean age of the patients was 52.29± 15.87 years, with 77% male and 34% female patients. The average length of ICU stay was 12.31 ±6.86 days. A mortality/DAMA of 45.4% (10/22), 28.2% (11/39) and 80.7% (21/26) were observed in patients with moderate SIRS, severe sepsis and septic shock respectively.

Out of total 100 cases, 87 had PCT value >=0.5 ng/ml whereas 13 had PCT value of <0.5 ng/ml. Hence, PCT positivity of 87% was observed in patients with SIRS/sepsis. Out of these 87 patients with PCT levels >=0.5 ng/ml; 22 patients had PCT value in range of >=0.5 - <2 ng/ml, 39 (>=2->10 ng/ml) and 26 patients with (>=10ng/ml). Hence on the basis of PCT values patients with moderate

SIRS, severe sepsis and septic shock were 22%, 39% and 26% respectively. (Table 1)

Infective foci were seen in 85.1% (74/87) of patients with PCT levels of >=0.5ng/ml as demonstrated by positive cultures (bacterial and fungal) or serological evidence. Bloodstream infections (39.1%) were identified as the commonest infection in patients with positive PCT (>=0.5 ng/ml) in the medical ICU's. The other infections were the lower respiratory tract infections, urinary tract infections, upper respiratory tract infections, abdominal infections, skin and soft tissue infections and others (13.7%, 9.2%, 4.6%, 3.4%, 1.1% and 13.7% respectively). Other infections include Aspergillosis, Leptospirosis, Scrub typhus, Cryptococcal meningitis etc. Amongst the positive PCT (>=0.5 ng/ml), no site of infection was found in 14.9% of patients. (Table 2)

Blood culture was positive in 31.8%, 30.7% and 57.6 % patients with moderate SIRS (PCT- >=0.5-<2 ng/ml), severe sepsis (PCT- 2-<10 ng/ml) and septic shock (PCT- >=10 ng.ml), respectively. (Table 1). Table 3 demonstrates various organisms isolated from blood culture of all the patients with PCT >=0.5 ng/ml. Gram-negative bloodstream infections (BSI) were predominant over the Gram positive (Gram-negative in 64.7% of cases and Gram-positive in 26.4% of cases). Fungal infection (Candida) was isolated in only 8,8% of individuals. Most commonly isolated Gram negative organisms from BSI were *E. coli* (9), followed by *Klebsiella spp.* (8), and *Pseudomonas aeruginosa* (3). Higher values of PCT were observed in Gram negative septicemia (average mean- 26.9 ng/ml) as compared to Gram positive septicemia (12.43 ng/ml) or fungal (*Candida* spp.) septicemia (2.14 ng/ml). In bloodstream infections by Gram-negatives, PCT mean value corresponding to *Enterobacteriaceae* was higher than that found for non-fermentative bacteria.

Out of the 13 patients with PCT value <0.5 ng/ml, four of the patients had confirmed viral etiology. Only one patient with viral etiology had PCT value >=0.5 ng/ml (Table 1). Therefore, it seems PCT has limited role in the diagnosis of viral infection.

DISCUSSION

Sepsis and its complications have a significant and increasing impact on health sector, and are one of the leading causes of mortality. The incidence of sepsis is increasing in all areas of the world. [17] Biomarkers to diagnose sepsis may allow early intervention which, although primarily supportive, can reduce the risk of death. Markers of the hyper-inflammatory phase of sepsis, such as pro-inflammatory cytokines and chemokines; proteins such as CRP and PCT which are synthesized in response to infection and inflammation; and markers of neutrophil and monocyte activation, markers of end organ damage like lactate are studied.[18] PCT is produced ubiquitously in response to endotoxin or to mediators released in response to bacterial infections.[2] It has become the mostly widely used biomarker in the management of sepsis. The ambiguous conclusions of different studies regarding the diagnostic accuracy of PCT and CRP are mainly due to the lack of a gold standard for infection, the propagation and misuse of an insensitive assay in the wrong clinical setting (e.g. early infection or immune-compromised patients), and the negligence of the fact that, as for all hormones, different cut-off levels have to be used according to the clinical questions asked. But the definition of infection is a methodological limitation in all similar studies. [19] In our study, 100 patients satisfying the criteria of SIRS/sepsis (ACCP) were included and 87 patients had PCT value of >=0.5 ng/ml. Infective foci were seen in 85.1% (74/87) of patients with PCT levels of >=0.5ng/ml as demonstrated by positive cultures (bacterial and fungal) or serological evidence. The findings of a significantly higher PCT levels in patients with SIRS/sepsis in the present study is consistent with previous studies. Sinha *et al* studied a group of 40 patients and found a statistically significant correlation between the presence of sepsis and a PCT levels. The study concluded that PCT levels above 2 ng/ml are effective markers of sepsis. [20] In another study, PCT proved to be an excellent indicator of sepsis with sensitivity of 94 %. [21]

A large number of observational studies have investigated the diagnostic potential of PCT in different clinical situation and different types and sites of infections. In this study the most common site of infection was the bloodstream infections (39%), followed by LRTI (13.7%), UTI (9.2%), URTI (4.6%), skin and soft tissue infections (1.1%), abdominal infections (3.4%) and others (13.7%) in patients with PCT value >=0.5 ng/ml. Min-Yi Huang *et al* also demonstrated most common site of infection being the bloodstream infections.[22]

For the diagnosis of bloodstream infections and bacteremia, studies found a high diagnostic performance of PCT.[23-25] In the present study out of total 100 patients, 36 patients were blood culture positive. Thirty-four patients had PCT value >=0.5 ng/ml. Therefore, at a cut-off of 0.5 ng/ml PCT had a very high sensitivity for the diagnosis of bacteremia (94.4%, 34/36). However, in the present study the patients with single isolation of Coagulase negative staphylococci were excluded,

hence the discriminatory ability of PCT between blood contaminants (CONS) and true pathogen (CONS) cannot be commented.

The ability of PCT to discriminate infections by Gram positive or Gram-negative organisms has been recently described in various studies. Charles *et al*., in a retrospective study on 97 bacteremia episodes, found that serum PCT levels were markedly greater for Gram-negatives than for Gram positives.[13] In another study 166 patients were evaluated and it was found that PCT cut-off of 15 ng/mL can discriminate between sepsis caused by Gram-negative and Gram-positives bacteria/ fungi, with a specificity of 87.8 %.[12] Leli *et al* found that in patients with suspected sepsis, the PCT cut-off value of 10.8 ng/mL could be of help in predicting an infection caused by Gram-negatives, with a specificity of 82.5%. A cut-off of 3.1ng/ml may help in excluding an infection caused by *Enterobacteriaceae* but not by non-fermentative Gram-negatives, with a sensitivity of 90.1%. [26] A study published in 2015 by Guo *et al* also had similar findings. [27] In the present study, Gram-negative bloodstream infections (BSI) were predominant over the Gram positive (Gram-negative in 64.7% of cases and Gram-positive in 26.4% of cases). Fungal infection (Candida) was isolated in only 8.8% of individuals.

Most commonly isolated Gram negative organisms from BSI were *E. coli* (9), followed by *Klebsiella spp.* (8), and *Pseudomonas aeruginosa* (3). Higher average values of PCT were observed in gram negative septicemia (26.9 ng/ml) as compared to gram positive septicemia (12.43 ng/ml) or fungal (*Candida* spp.) septicemia (2.14 ng/ml). The finding of a significantly higher PCT level in Gram-negative BSI than in Gram-positive BSI and fungal BSI is consistent with previous reports. [12,28]

Although the mechanism underlying PCT production in response to different bacterial pathogens is not completely clear, it could possibly be explained by the different interaction of Gram-positive or Gram-negative bacteria with host's cells, involving lipo-teichoic acids or LPS, respectively, and different pathogen-associated molecular patterns (PAMPs), engaging different TLRs, expressed on human cells.[9] In particular, Gram-positive bacteria activate the TLR2 pathway [29-30], whereas Gram-negative bacteria the TLR4 pathway [31], resulting in different production of inflammatory cytokines, such as interleukin-1β, interleukin-6 (IL6), and tumor necrosis factor-α, that ultimately stimulate ubiquitous transcription of calcitonin-mRNA and release of PCT from multiple tissues throughout the body.[32-33] Probably, Gram-negative bacteremia induces a greater inflammatory response than Gram positive bacteremia may help explain the higher PCT levels in Gram-negative bacteremia.

IFN Ɣ released in response to viral infections can cause a down regulation of PCT. This makes PCT more specific marker for bacterial infection. [34] In our study 13 patients with PCT value (<0.5 ng/ml), four of the patients had confirmed viral etiology. Only one patient with viral etiology had PCT value >=0.5 ng/ml. Therefore, it seems PCT has limited role in the diagnosis of viral infection supporting the above mentioned evidence.

Clinically once the diagnosis of sepsis is made, the prediction of survival is important for the risk stratification of the patients and in indicating the potential success or failure of treatment. We evaluated the predictive value of PCT for survival of patients with PCT value >=0.5 - <2 ng/ml (moderate SIRS), >=2 - <10 ng/ml (severe sepsis), and >=10 ng/ml (septic shock). The mortality is significantly higher in patients with PCT (>=10 ng/ml) which were 21 patients i.e. in patients classified as septic shock on the basis of PCT value as compared to moderate SIRS and severe sepsis. Severe sepsis with septic shock is a major cause of morbidity and mortality in the ICU's. Mortality increases with the severity of sepsis. In-hospital mortality rates for severe sepsis and septic shock are high, ranging between 18% and 50%. [35-37] The mortality because of severe sepsis and septic shock has been found to be 22.9%. [20] In the present study, a mortality of 45.4% (10/22), 28.2% (11/39) and 80.7% (21/26) were observed in patients with moderate SIRS, severe sepsis and septic shock respectively.

Biomarkers have been effective at reducing mortality; existing supportive measures alone will probably not be enough to finally bring sepsis under control. Since most of the new innovative approaches to treating sepsis target specific biomarkers, more robust ways to measure them will help support the success of these new modes of treatment. Although, measurement of biomarkers at a single time point may be of limited value because of the large variability of biomarker secretion at different times during the progression of critical illness and needs to be further evaluated.

Conclusions

PCT as a biological marker appears to have a significant value in identifying or ruling out an infection and assessing severity of the disease primarily for triaging decisions. Significantly higher values of PCT were observed in Gram negative as compared to Gram positive septicemia, hence PCT is valuable to distinguish Gram negative from Gram positive infections, however cut off needs to be determined. PCT has no role in the diagnosis of viral infection, though the number

of patients with viral etiology were very few in the present study, therefore no definite conclusion can be made in this regard.

Conflict of interest: The authors declare that no conflict of interests existed in the organization, results, presentation and the finance of the research article.

Table 1: Correlation of PCT with etiology of infections

Procalcitonin (PCT)	**>=0.5-<2 ng/ml**	**>=2-<10 ng/ml**	**>=10 ng/ml**	**Total >=0.5 ng/ml**	**<0.5ng/ml**
	Moderate SIRS	**Severe Sepsis**	**Septic shock**		**No SIRS/Sepsis**
Total number of patients (100)	22 (22%)	39(39%)	26 (26%)	**87 (87%)**	13 (13%)
Infective focus (viral/fungal/bacterial)	20	28	26	**74**	6
Bacterial focus (except BSI)	6	14	11	**31**	-
Bloodstream infections	7 (31.8%)	12 (30.7%)	15 (57.6%)	**34**	2
Viral etiology	1	-	-	**1**	4
Fungal etiology (except Candidemia)	6 (Aspergillus-5; Cryptococcus-1)	2 (Aspergillus)	-	**8**	-
Mortality (Death/DAMA)	10 (45.4%)	11 (28.2%)	21(80.7%)	**42**	

Table 2: Infective foci/original site of infection in patients with PCT value >=0.5 ng/ml (n= 87)

	NUMBER OF PATIENTS	**%**
INFECTED FOCI OBSERVED	**74**	**85.1**
• Bloodstream infections	**34**	**39.1**
• Lower respiratory tract infections	**12**	**13.7**
❖ Pneumonia, empyema, lung abscess	11	12.6
❖ Nocardiosis	1	1.1
• Urinary tract infections	**8**	**9.2**
• Upper respiratory tract infections	**4**	**4.6**
• Abdominal infection	**3**	**3.4**
❖ Biliary tract infections and cholecystitis	1	1.1
❖ Appendicitis	2	2.3
• Skin and soft tissue infections	**1**	**1.1**
• Others	**12**	**13.7**
❖ Leptospirosis	1	1.1
❖ Scrub typhus	2	2.3
❖ Aspergillosis	7	8.0
❖ Cryptococcosis	1	1.1
❖ Herpes simplex viral infection	1	1.1
NO INFECTED FOCI	13	14.9

Table 3: Average PCT Values corresponding to pathogens isolated from patients with bacterial septicemia

Pathogen	No of patients	Average PCT Values (Range) (ng/ml)
Gram Negatives	**22 (64.7%)**	**26.9 (1.97-100)**
Escherichia coli	9	37.11(5.47-100)
Klebsiella Species	8	22.12(3.76-87.46)
Acinetobacter species	1	18.45
Pseudomonas Aeruginosa	3	19.6(1.97-53.57)
Enterobacter cloacae	1	3.64
Gram Positives	**9 (26.4%)**	**12.43(1.79-23.1)**
Staphylococcus aureus	2	18.95(4.84-14.11)
Enterococcus faecium	3	7.59(1.91-12.12)
CONS	4	12.81(1.79-23.1)
***Candida* species**	**3 (8.8%)**	**2.14(1.82-3.83)**
C.albicans	2	1.29 (0.77 -1.82)
C.tropicalis	1	3.83

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