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Research Article

EVALUATION OF SERUM PROCALCITONIN AND IT'S ASSOCIATION WITH ETIOLOGY OF INFECTIONS IN PATIENTS WITH SYSTEMIC INFLAMMATORY RESPONSE SYNDROME/SEPSIS

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A B S T R A C T

Objective: To correlate Procalcitonin (PCT) levels with infectious etiologies in patients with systemic inflammatory response syndrome (SIRS)/ sepsis.
Introduction: Sepsis is defined as SIRS in the presence of an underlying infectious process, and is associated with high rates of morbidity and mortality particularly when initial therapy is delayed. 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 other infections and canbe of importance in guiding antimicrobial therapy.
Methods: Total numbers of 200 cases of SIRS/ sepsis admitted in medical ICUs were included in the study. PCT levels and cultures/serology were done. Correlations of PCT values with culture results were analyzed. Infective foci and clinical diagnosis were also compared in moderate SIRS, severe sepsis and septic shock.
Results: Out of total 200 cases, 182 had PCT value ≥0.5 ng/ml whereas 18 had PCT value of <0.5 ng/ml. Infective foci were seen in 74.7% (136/182) of patients with PCT levels of ≥0.5ng/ml.
Conclusion: PCT as a biological marker appears to have a significant value inidentifying or ruling out an infection. PCT may be of value in distinguishing Gramnegative from Gram positive and fungal infections.

INTRODUCTION

Sepsis is a critical condition often caused by bacterial infection and associated with death and mortality. The word, sepsis has a complete association with SIRS concept. To define SIRS, following criteria are available i.e. Temperature more than 38˚C or less than36˚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). Adapted from American college of chest physicians / Society of Critical Care Medicine. Sepsis is defined as the presence of two SIRS criteria in association with clinical evidence of infection. Severe sepsis is the presence of sepsis with organdys function, and septic shock is defined as sepsis with hypotension.[1] The definite diagnosis of sepsis is a positive blood culture and this test is time consuming, so other biochemical parameters have been introduced. Clinical history and routine laboratory investigation (WBC count, thrombocyte count, coagulation studies, ESR etc) aid in diagnosis of SIRS/ sepsis.[2]

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There are a number of limitations to the use of conventional diagnostic markers for patients with clinical suspicion of infection. As a consequence, unnecessary and prolonged exposure to antimicrobial agents adversely affects patient outcomes, while inappropriate antibiotic therapy increases antibiotic resistance.[3]
PCT is a prohormone of calcitonin synthesized by C cells of thyroid gland. It is encoded by the CALC 1 gene located on the short arm of the chromosome 11.[4,5]

During microbial infections there is increased CALC-1 gene expression in various extra thyroid tissues and cells including kidneys, pancreas, liver, leucocytes, and adipose tissue with concomitant release of PCT throughout the body.(6)

The normal physiological level of PCT in serum is less than 0.1ng/mL which can increase several folds in bacterial infections.(7) IFN ɣ released in response to viral infections can cause a down regulation of PCT. This makes PCT more specific marker for bacterial infection.(8)

METHODS

This was a prospective observational clinical study done in a tertiary care hospital of North India. A total number of 200 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. [1] Patients below the age of 18 years; with any malignancy or cardiogenic illness were excluded from the study.

Procalcitonin Assay

The PCT levels were measured using an automated system based on electrochemiluminescence (ECL) technique (Roche diagnostics Cobase 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).[9]

Blood and body fluid culture

Blood and body fluids samples were collected taking all aseptic precautions and were 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. [10]

RESULTS

During the study period, a total of 200 patients satisfying the criteria of SIRS/ sepsis admitted to the ICU were included in the study. Highest percentage of patients belonged to 56-65 years age group (24%) followed by 46-55 years age group (20.5%). Males (60.5%) outnumbered the females (39.5%) in the study. Out of 200 cases of SIRS/ Sepsis, 182 (91%) had PCT values ≥ 0.5 ng/ml. Out of 182 patients with PCT values ≥ 0.5 ng/ml, 63 (31.5%) patients had PCT values ≥10 ng/ml indicative of septic shock; 73 (36.5%) patients had PCT values in the range of ≥2-<10 ng/ml suggestive of severe sepsis and 46 (23%) patients had PCT values between ≥0.5-<2 ng/ml indicating moderate SIRS. Only 18 (9%) of the patients had normal PCT values i.e. <0.5 ng/ml suggestive of no bacterial infection.

Table 1 Correlation of PCT with culture/serology in SIRS/ sepsis cases

(n=200)

	Culture/serology positive (n=147)	Culture/serology negative (n=53)
PCT (+) (≥0.5 ng/ml) (n=182)	136 (74.7%)	46 (25.3%)
PCT(-) (<0.5 ng/ml) (n=18)	11 (61.1%)	7 (38.9%)
Total (n=200)	147 (73.5%)	53 (26.5%)

Sensitivity; specificity, positive predictive value and negative predictive value of PCT were 92.5%, 13.2%, 74.7% and 38.8% respectively. The mean PCT values of 147 cases were 17.01± 27.51 ng/ml and 53 cases were 9.62 ± 19.94 ng/ml. The mean PCT values of 136 cases (18.36± 28.18 ng/ml) were more than that of 46 cases (11.05± 16.66 ng/ml). The mean PCT values of 11 cases (0.30 ± 0.13 ng/ml) were higher than the 7 cases (0.23 ± 0.08 ng/ml).

Out of 46 (≥0.5-<2 ng/ml), 73 (≥2- <10 ng/ml) and 63 (≥10 ng/ml) patients; 28 (60.8%), 60 (82.2%) and 48 (76.2%) patients had laboratory confirmed infections whereas 18 (39.2%), 13 (17.8%) and 15 (23.8%) patients in the above mentioned PCT ranges did not have any laboratory confirmed infections. The correlation of PCT with infections was statistically significant (p value <0.05)

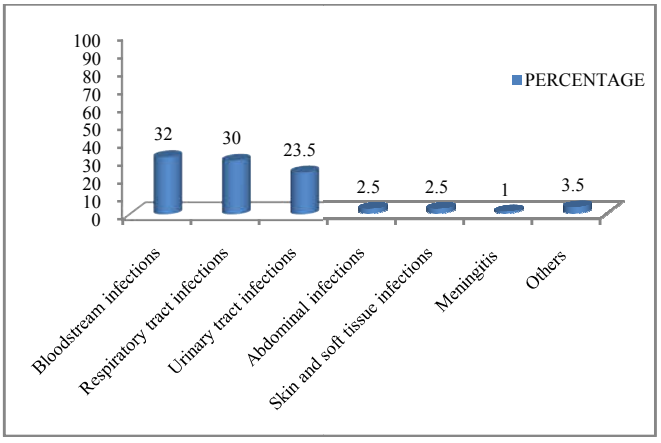

Figure 1 Infections in patients with SIRS/ sepsis (n=200)

Bloodstream infections (BSI) were seen in maximum number of patients (32%), followed by respiratory tract infections (RTI) (30%), urinary tract infections (UTI)(23.5%),abdominal infections (2.5%), skin and soft tissue infections (2.5%), meningitis (1%) and other infections (3.5%).

Table 2 Correlation of PCT values with bloodstream infections in SIRS/ sepsis cases (n=200)

	Blood culture positive	Blood culture negative
PCT positive (≥0.5 ng/ml)	60 (30%)	122 (61%)
PCT negative (<0.5 ng/ml)	4 (2%)	14 (7%)

The sensitivity, specificity, positive predictive value, negative predictive values and accuracy of PCT were 93.75%, 10.29%, 32.97%, 77.78% and 37% respectively with 95% confidence interval.

Table 3 Correlation of PCT values with urinary tract infections in SIRS/ Sepsis cases (n= 172)

	Culture positive (n=47)	Culture negative (n=125)
PCT positive (≥0.5 ng/ml) (n=156)	45(26.2%)	111(64.5%)
PCT negative (<0.5 ng/ml) (n=16)	2(1.2%)	14(8.1%)

The sensitivity, specificity, positive predictive value, negative predictive values and accuracy of PCT were 95.74%, 11.20%, 28.85%, 87.50% and 34.30% respectively with 95% confidence interval.

Table 4 Correlation of PCT values with respiratory tract infections in SIRS/ Sepsis cases (n= 109)

	Culture positive (n=61)	Culture negative (n=48)
PCT positive (≥0.5 ng/ml) (n=105)	61 (56%)	44 (40.4%)
PCT negative (<0.5 ng/ml) (n=4)	0	4 (3.6%)

The sensitivity, specificity, positive predictive value, negative predictive values and accuracy of PCT were 100%, 8.3%, 58.10%, 100% and 59.63% respectively with 95% confidence interval.

Table 5 PCT values in BSI, UTI and RTI

Organisms	BSI Total	Mean (range)	UTI Total	Mean (range)	RTI Total	Mean (range)
Klebsiellapneumoniae	13	15.68 (2.61-87.46)	4	15.74 (1.18-44.89)	20	21.25 (1.18-100)
Escherichia coli	13	40.21 (0.18-100)	10	4.14 (2.23-14.83)	5	31.75 (0.88-100)
Acinetobacterbaumannii	4	9.50 (1.35-22.42)	2	14.21 (4.05-24.36)	8	5.94 (1.3-22.42)
Enterobacter cloacae	3	18.52 (4.56-38.04)	-	-	3	18.52 (4.56-38.04)
Pseudomonas aeruginosa	2	27.77 (1.97-53.57)	-	-	2	55.5 (53.57-57.53)
Staphylococcus aureus	4	5.13 (0.57-8.69)	-	-	1	7.23
Enterococcus faecium	4	2.50 (0.18-5.95)	2	1.6 (1.29-1.91)	-	-
Staphylococcus epidermidis	8	9.98 (0.95-100)	-	-	-	-
Candida albicans	6	24.63 (1.82-57.53)	8	23.34 (0.96-84.27)	-	-
Candida tropicalis	5	2.47 (0.46-5.08)	13	4.14 (0.46-14.83)	-	-

Table 6 Mean PCT values in various infections

	Mean ± SD PCT values (ng/ml)		
Bacterial infections	BSI	UTI	RTI
• Gram negative	27.10 ± 32.22	10.76 ± 11.66	20.23 ± 30.06
• Gram positive	12.52 ± 24.31	1.60 ± 0.44	4.48 ± 3.88
Fungal infections	14.56± 20.52	17.78 ± 30.30	4.06 ± 4.83

The mean PCT values were higher in Gram negative BSI, UTI and RTI as compared to Gram positive and fungal infections.

Receiver operating characteristic (ROC) curve of different cut-offs of PCT in differentiating Enterobacteriaceae from nonfermentative Gram negatives (AUC 0.555, 95% CI 0.390-0.720). Sensitivity and specificity of different cut-off values are reported. ROC analysis suggests that although PCT values >3.05 ng/ml do not discriminate between the two groups of pathogens, values ≤ 3.05 ng/ml are indicative of low probability of infection by Enterobacteriaceae.

PCT cut-off value(ng/ml)	Sensitivity	Specificity
0.53	0.98	0.00
1.93	0.93	0.17
2.45	0.88	0.28
3.05	0.81	0.33
4.27	0.75	0.44
5.28	0.65	0.44
6.35	0.60	0.56
8.55	0.53	0.61
10.71	0.46	0.61
25.17	0.23	0.78
59.51	0.14	0.89
93.73	0.11	0.89

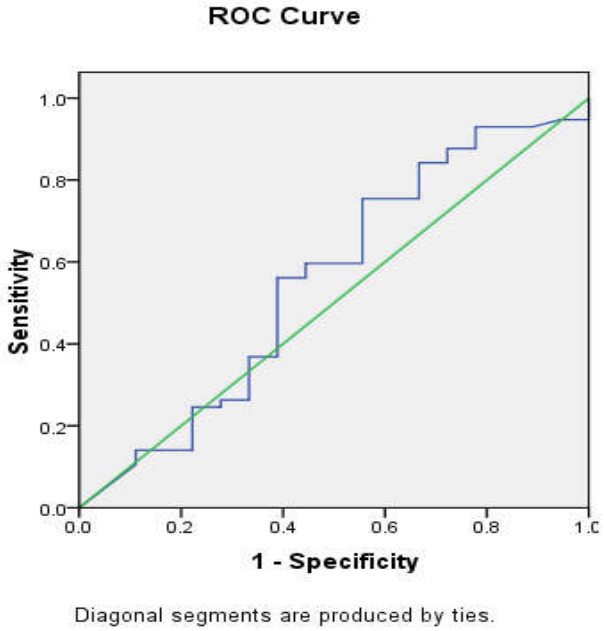

Figure 2 Enterobacteriaceae versus nonfermentative Gram negatives

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. [4, 5] Biomarkers to diagnose sepsis may allow early intervention which, although primarily supportive, can reduce the risk of death. It has become the most 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.[11] In our study, 200 patients satisfying the criteria of SIRS/sepsis (ACCP) were included and 182 (91%) patients had PCT value of ≥0.5 ng/ml. Infective foci were seen in 74.7% (136/182) of patients with PCT levels of ≥0.5ng/ml as demonstrated by positive cultures (bacterial and fungal) or serological evidence. 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.[12] In

another study, PCT proved to be an excellent indicator of sepsis with sensitivity of 94%.[13] A large number of observational studies have investigated the diagnostic potential of PCT in different clinical situation and different types of infections. In the present study the most common infections were the BSI (32%), followed by RTI (30%), UTI (23.5%), skin and soft tissue infections (2.5%), abdominal infections (2.5%), meningitis (1%) and others (3.5%). Min-Yi Huang *et al* also demonstrated that most common infection was BSI (75.0%), followed by RTI (57.1%), UTI (41.1%), abdominal infections (16.1%), andskin and soft tissue infections (14.3%).[14] For the diagnosis of bloodstream infections and bacteremia, studies found a high diagnostic performance of PCT.[15-17]In the study by Schuetz *et al*, the cut-off of 0.1 ng/dl for PCT had sensitivity and specificity, 100% and 80% on the day of blood culture collection.[15] In the present study out of total 200 patients, 64 patients were blood culture positive. Sixty 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 (93.6%, 60/64). 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.[18] 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 %.[19]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 than with Gram positives, with a specificity of 82.5%. A cut-off of 3.1 ng/ml may help in excluding an infection caused by Enterobacteriaceae but not by non-fermentative Gram-negatives, with a sensitivity of 90.1%.[20] A study published in 2015 by Guo *et al* also had similar findings. It showed that cutoff value of 3.39 ng/ml for PCT helped in predicting Gram-Negative BSI with a sensitivity of 80%, and specificity of 71%.[21] In the present study, Gram-negative BSI were predominant over the Gram positive (Gram-negative in 57.8 % of cases and Gram-positive in 25% of cases). Fungal infection (Candida) was isolated in only 17.2% of individuals. Most commonly isolated Gram negative organisms from BSI were *Klebsiellapneumoniae* (14), followed by *Escherichia coli* (13) and *Acinetobacterbaumannii* (5). Higher average values of PCT were observed in Gram negative septicemia (27.10 ± 32.22 ng/ml) as compared to Gram positive septicemia (12.52 ± 24.31 ng/ml) or fungal (Candida spp.) septicemia (14.56± 20.52ng/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.[19]PCT can be used as a marker for diagnosing urinary tract infections. In the present study the sensitivity of PCT as a marker for UTI was found to be 95.74%.

The sensitivity of PCT in diagnosing UTI was 90.1% in a study done by Bharath MS *et al* Mean value of PCT in upper urinary tract infection was 3.56 ng/ml and lower urinary tract infection was 0.98ng/ml.[22] The sensitivity of PCT in detecting pyelonephritis was 90.47%.ThePCT levels in pyelonephritis was found to be 3.90± 3.51 ng/ml.[23] There is limited available data regarding the mean PCT values found in Gram negative and Gram positive isolates from UTI. The mean values of Gram negatives were 10.76 ± 11.66ng/ml, which were higher than Gram positives (1.60 ± 0.44ng/ml) and fungal infections (17.78 ± 30.30ng/ml).

There is diagnostic value of PCT in respiratory tract infections. The sensitivity of PCT in diagnosing RTI was 94.1 %.[3] In the present study the sensitivity of PCT in diagnosis of RTI at a cut-off 0.5 ng/ml was 100%. The mean PCT values of Gram negatives were 20.23 ± 30.06ng/ml. Gram-negative bacteremia induces a greater inflammatory response than Gram positive bacteremia may help explain the higher PCT levels in Gram-negative bacteremia.

The present study showed receiver operating characteristic (ROC) curve of different cut-offs of PCT in differentiating Enterobacteriaceae from nonfermentative Gram negatives (AUC 0.555, 95% CI 0.390-0.720). In the study done by Leli *et al* it was seen ROC curve of different cut-offs of PCT in differentiating Enterobacteriaceae from nonfermentative Gram negatives (AUC 0.691, 95% CI 0.593-0.789, $P< 0.0001$).[20]

CONCLUSIONS

To conclude 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.

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