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Original Research

Biochemical Significant Role of Serum Procalcitonin and C-reactive protein Concentration in Diabetic Foot ulcer Infections

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ABSTRACT:
Aim: Serum inflammatory markers, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR),white blood cells (WBC), and procalcitonin (PCT), have been used for the diagnosis of footinfections in patients with diabetes. However, little is known about their changes during treatmentof patients with foot infections. Procalcitonin (PCT) has been recently accepted as a marker for diagnosing infection. The aim of the present study was to determine whether PCT levels are associated withinfection severity of diabetic foot ulcers and whether PCT levels would be helpful to differentiateinfected diabetic foot ulcer (IDFU) from IDFU associated with other infectious diseases(IDFU + O). **Methods:** This research was conducted in a Sardar Patel Medical College, Bikaner over the 2016 academic year. We prospectively included 95 diabetic patients hospitalized for IDFU. Infection severity of diabetic foot ulcers was graded according to the Infectious Diseases Society of America-International Working Group on the Diabetic Foot clinical classification of diabetic foot infection. Chest radiograph, urinalysis, urine microscopy, urine culture, and blood cultures (if fever was present) were performed for all patients to diagnose other infectious diseases. Laboratory parameters were measured from blood venous samples. Quantitative data from mid-year examination marks were analysed at the end of the academic year. **Results:** PCT (0.286, $P < 0.001$) and C-reactive protein (0.368, $P < 0.001$) levels were significantly associated with infection severity of diabetic foot ulcers. However, only PCT levels could differentiate patients with associated infectious diseases from patients with no concomitant infection (area under the receiver-operator characteristic curve 0.729, $P < 0.0001$; cut-off value 0.44; sensitivity 88.7; specificity 70.2). **Conclusion:** PCT and CRP levels positively correlated with infection severity of diabetic foot ulcers and PCT levels > 0.48 ng/mL in patients with IDFU may be associated with other systemic bacterial infection.
Keywords: Diabetic foot Ulcer, Infections, Procalcitonin,C-reactive protein.

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INTRODUCTION

Approximately 15–25% of diabetic patients have foot ulcersduring their lifetime [1]. Diabetic foot ulcers are frequentlyinfected [2]. Fifty-nine percent of diabetic foot amputationshave been attributed to infection and infected diabetic footulcer (IDFU) is a major causal factor for lower-limb amputation[3,4].Conventional laboratory markers, such as erythrocyte sedimentationrate (ESR), white blood cell count (WBC) and C-reactiveprotein (CRP), cannot differentiate between infectiousand non-infectious inflammation and are of limitedvalue in the diagnosis of diabetic foot infection [5–7]. SerumProcalcitonin (PCT) level is elevated in patients with systemicbacterial infections and, unlike other markers, it is usuallynot elevated in patients with inflammation due to viralinfection or non-infectious diseases. Thus, serum PCT hashigher diagnostic accuracy for the diagnosis of bacterial infectionthan standard biochemical parameters, such as the WBCcount and serum CRP levels [8–10]. Hence, there has been aninterest in investigating the

usefulness of PCT for the diagnosisof diabetic foot infection. It has been reported in the literaturethat PCT levels have higher efficiency in distinguishingIDFU from a non-infected diabetic foot ulcer, followed by CRP,WBC, and ESR levels, and that the combination of PCT andCRP measurements increase the accuracy of predicting diabeticfoot infection [11–13]. We postulated that PCT would beuseful to assess the infection severity in diabetic foot ulcersand other infectious diseases. Because diabetic foot infectionis progressive and associated with the potential risk of gangreneand limb amputation, diabetic foot infection has a high morbidity and mortality rate [11,14–16]. Therefore, promptand adequate diagnosis and treatment of diabetic foot infectionis critical to reduce the amputation and mortality rate.The aim of the present study was to determine whetherPCT levels are associated with infection severity of diabeticfoot ulcers and whether PCT levels are helpful in differentiatingIDFU from IDFU + O

MATERIALS AND METHODS

PATIENTS

This study was approved by the S.P.Medical college, InstitutionalResearch Board. Between june 2016 to july 2016, we prospectivelyincluded consecutive diabetic patients hospitalized forinfected diabetic foot ulcer. The same foot and ankle surgeonin our department examined all patients in order to gradeinfection severity, according to the Infectious Diseases Societyof America-International Working Group on the Diabetic Foot(IDSA-IWGDF) clinical classification of diabetic foot infection[17] and IDFU was diagnosed if the grade of infection was ≥ 2. Chest radiograph, urinalysis, urine microscopy, urine culture,and blood cultures (if fever was present) were performedon every patient to diagnose other infectious diseases, suchas sepsis, pneumonia, and urinary tract infection. Where anabnormal laboratory test result was obtained or other infectiousdiseases were clinically suspected, the patient wasreferred to the department of infectious diseases, in order toconfirm the diagnosis of concomitant infectious diseases.Inclusion criteria were as follows: infection grade ≥ 2 accordingto the IDSA-IWGDF criteria, no history of antimicrobialtreatment within the previous 6 months, and no history ofsurgery in the previous 6 weeks. The exclusion criteria weremalignancy, inflammatory disease, and immune suppressive treatment.

LABORATORY PARAMETERS

A venous blood sample was obtained from all patients onadmission, before the commencement of antimicrobial treatment,to measure the following: WBC and neutrophil count,ESR, CRP, and PCT. For analyzing the PCT levels, blood sampleswerecollected in serum separating tubes and centrifugedfor 20 min at 3500 rpm, after being maintained at roomtemperature for 20 min. PCT levels were measured using an electrochemiluminescent immunoassayanalyzer (Roche Diagnostics, Meylan, France), and the functional detection limit was 0.02 ng/mL. The Department of biochemistry, Clinical laboratory analyzed the PCT while WBC and differential bloodcounts, CRP, and ESR were analysed in pathology department..

STATISTICAL ANALYSIS

Statistical analyses were performed using the software packageSPSS for Windows version 16.0.0 (SPSS Inc., Chicago, Illinois).The Mann–Whitney U test or Kruskal-Wallis test wereused to compare the continuous variables. To assess the correlationbetween the grade of infection severity and laboratoryparameters, Spearman rho correlation coefficients werecalculated for patients with no associated infectious diseases,to avoid the effect of other causes of infection. Comparisonsof the correlation coefficients were performed with the Ztest, using the Fisher's Z transformation. A receiveroperatingcharacteristic (ROC) analysis and the area underthe ROC curve (AUC) were calculated to measure the accuracyof the laboratory parameter to distinguish patients with IDFUfrom patients with IDFU + O. The best cut-off value was calculated,and specificity and sensitivity of the laboratory parameterswere determined using the best cut-off value.Comparison of the ROC curves was performed to comparethe accuracies of laboratory markers for distinguishing thegrades of infection severity. A P value < 0.05 was consideredstatistically significant.

RESULTS

A total of 95 patients diagnosed with infected diabetic footulcer (grade _ 2, IDSA-IWGDF criteria) were included in thisstudy (mean age 62.6 years; range, 40–88 years, ±7.4 years).The distribution of infection according to severity, usingIDSA-IWGDF criteria, was as follows: grade 2 (24 patients,25.26%), grade 3 (59 patients, 62.10%), and grade 4 (12 patients,12.63%). Twelve patients (12.63%) had other infectious diseasesin addition to IDFU. Of these, 7 (7.36%) patients had pneumonia,3 (3.15%) patients had a urinary tract infection, and 2 (2.10%)patients had sepsis (Table 1).

Tables 1:- Demographics.

Age (mean ± SD years)	62.6 ± 7.9
Sex (n,%)	
Male	81 (85.26 %)

Female	14 (14.73 %)
Duration of DM[a] (mean ± SD years)	16.8 ± 5.2
Infection Severity grade[b] (n,%)	
2	24 (25.26 %)
3	59 (62.10 %)
4	12 (12.63 %)
Combined other infections (n,%)	
No	83 (87.36%)
Yes	12 (12.63 %)
Pneumonia	7 (7.36 %)
Urinary tract infection	3 (3.15 %)
Sepsis[c]	2 (2.10 %)

a. DM- Diabetes mellitus.
b. IDSA-IWGDF Clinical Classification of Diabetic Foot Infection.
c. One patient had Pneumonia, One patient had Urinary tract infection, One patient had Pneumonia.

Among the 2 patients diagnosedwith sepsis, one had pneumonia, one had urinary tract infection,and one had pneumonia and urinary tract infection.In patients without any other infectious diseases, the comparisonof laboratory parameters among the grades of infectionseverity of diabetic foot ulcers is shown in Table 2.

Table 2- Laboratory Parameters according to the infection grade in IDFU without any other infectious disease.

Parameters	Grade 2 (n=20)	Grade 2 (n=59)	Grade 2 (n=05)	P value
ESR (mm/h)	60.75±30.30	68.25±29.40	72.15±30.43	0.598
CRP (mg/L)	32.20±32.28	58.10±53.28	141.48±48.62	<0.001
PCT (ng/ml)	0.15±0.22	0.18±0.23	3.44±3.32	<0.001
WBC ($\times10^9$/L)	8.62±1.80	8.89±3.12	10.34±3.02	0.221
Neutrophils($\times10^9$/L)	6.64±2.10	5.84±2.94	7.68±3.10	0.102

Table 3- Laboratory parameters in IDFU[a] and IDFU[b]+O.

Parameters	IDFU (n=83)	IDFU+O (n=12)	P value
ESR (mm/h)	68.65±30.74	76.26±15.64	0.156
CRP (mg/L)	60.21±57.23	78.62±73.65	0.456
PCT (ng/ml)	0.58±1.58	1.02±1.22	<0.001
WBC ($\times10^9$/L)	8.62±3.20	9.10±3.63	0.419
Neutrophils($\times10^9$/L)	6.32±2.45	7.84±3.84	0.213

a. IDFU ,infected diabetic foot ulcer.
b. IDFU+O, infected diabetic foot ulcer associated with other infectious disease.

There were significant differences in the PCT and CRP levelsamong the infection grades ($P < 0.001$ for both). The correlationanalysis in patients with no other infectious diseasesdemonstrated that PCT (Spearman's q 0.338, $P < 0.001$) andCRP (Spearman's q 0.477, $P < 0.001$) positively correlated withthe grade of infection severity of diabetic foot ulcers.

DISCUSSION

The most important findings of the present study was thatPCT and CRP levels were significantly associated with anincreased IDFU infection grade and that PCT was a usefuldiagnostic marker to differentiate patients with IDFU frompatients with IDFU + O.Procalcitonin, the 166 amino acid precursor of calcitonin,is produced by the thyroid C cells [18]. Serum PCT concentrationis generally very low in healthy patients, but PCT productionis activated in all parenchymal tissues andconcentrations increase rapidly following bacterial infection[19,20]. Production of PCT is stimulated directly by bacterialendotoxins and lipopolysaccharides and indirectly by inflammatorymediators, such as tumor necrosis factor-alpha,interleukin-6, and interleukin-1 [21]. However, mediators ofviral infection, such as interferon-gamma, attenuate PCTlevels [22]. Therefore, PCT has recently been recognized asamore specific marker of bacterial infection [13]. A number ofstudies have been conducted to investigate the diagnosticaccuracy of PCT in differentiating between infected andnon-infected diabetic foot ulcers, but the results have notbeen consistent [11–13,23]. Two out of 4 studies showed thatPCTwas the most useful marker among conventional laboratorymarkers [11,13], while 1 study reported that CRP showedthe greatest sensitivity and specificity to distinguish IDFUfrom non-infected diabetic foot ulcers [12]. A further studyreported that ESR was the most sensitive and specific inflammatorymarker [23]. Three of these studies concluded that thecombination of PCT and CRP or

ESR was the most sensitive method to distinguish infected from non-infected diabeticfoot ulcers [12,13,23]. Studies have also evaluated the diagnosticvalue of PCT to distinguish osteomyelitis from soft tissueinfection in patients with diabetic foot infection [24,25]. Onestudy reported that PCT failed to identify patients with boneinfection [25], while another study suggested that PCT is usefulto distinguish osteomyelitis in infected foot ulcers [24].Reports indicate that PCT and CRP levels correlate with theseverity of infection. In children with liver disease, PCT andCRP correlated with infection severity [26]. A linear relationshipbetween PCTand CRP values and the severity of infectionhas been previously demonstrated by Hatherhill et al. in astudy involving 175 children admitted to the paediatric intensivecare unit [27]. A number of studies have demonstratedthat higher PCT levelswere present in patients with IDFU thanin patients with non-infected diabetic foot ulcer; however, thecorrelation between PCT levels and infection severity of diabeticfoot ulcers was not analyzed [11,13,23]. Our studyassessed the correlation between laboratory parameters andinfection severity of diabetic foot ulcers, and showed thatPCT and CRP levels positively correlated with infection severity.However, ROC analysis demonstrated that CRP was a use-intensive care unit of patients with diabetic foot ulcers [29].Therefore, it is important to be aware of major cardiac eventsand nosocomial infection when treating patients with IDFU.The present study sought to determine whether PCT is usefulto differentiate IDFU from IDFU + O and, to the best of ourknowledge, this has not been examined previously.CRP values have been shown to significantly increase inresponse to local infection, while local infection, without systemicmanifestations, only results in a limited increase in PCTlevels [28]. PCT levels are generally higher in patients withsevere and systemic infection [30]. A prospective study evaluatingthe predictive value of PCT levels to identify systemicinfection showed that, in multivariate analysis, the only variableassociated with systemic infection was the Procalcitonin level, while body temperature, WBC count, and CRP, were notassociated with systemic bacterial infection [31]. Furthermore,in the present study, only PCTwas found to have a diagnosticvalue to distinguish patients with IDFU from thosewith IDFU + O, such as systemic bacterial infection, includingpneumonia; urinary tract infection; and sepsis.There are some limitations to this study. First, we performeda chest radiograph, urinalysis, urine microscopy,urine cultures, and blood cultures (in the presence of fever)on admission to diagnose sepsis, pneumonia, and urinarytract infection. Therefore, infectious diseases on admission,other than those indicated above, may not have been diagnosed.However, during hospitalization no patientswere diagnosedwith infections other than sepsis, pneumonia, andurinary tract infection. Second, the grade of infection severityof diabetic foot ulcers was determined on the basis of clinicalexamination only, according to the IDSA-IWGDF clinical classification.Therefore, there may have been interobserver variabilityin grading infection severity. Finally, the reliability ofPCT levels remains controversial as these are subject tochanges, according to age, pathogen, and type of infection[23]. Different types of pathogens cause different types ofimmune response and therefore, result in a variable degreeof increase in PCT [18]. It has been noted that PCT levels aregreatly elevated in patients with infections associated withGram-negative bacteria, compared to Gram-positive bacteria[32]. Non-infectious conditions, such as stress response (i.e.,after surgery, trauma, shock, burns), Kawasaki disease, andadult onset Still's disease also can cause elevated PCT levels[18,33–35]. Even though PCT may incur extra costs in additionto the costs of conventional laboratory markers in patientswith IDFU, it has been demonstrated to be cost-effective ina hospital setting to guide antibiotic usage in septic patients,when decreased length of stay and quality-of-life-years areconsidered [36–38]. However, there are only a limited numberof theoretical studies investigating the impact of PCT on thecosts incurred by patients with systemic bacterial infections.Therefore, further studies are needed to evaluate the costeffectivenessof PCT in patients with IDFU.

CONCLUSION

Although PCT and CRP levels positively correlated with thegrade of infection severity of diabetic foot ulcers, only CRPwas useful as a laboratory parameter for distinguishing diabeticfoot infection grades 2 and 3. PCT levels were elevated(>0.59 ng/mL) where infected diabetic foot ulcer was associatedwith other systemic bacterial infection. Therefore,infected diabetic foot ulcers should be managed promptlyand we should consider the presence of other infectious diseases,in addition to diabetic foot infection, when PCT levelsare elevated.

CONFLICT OF INTEREST

All authors declare the have no conflict of interest.

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