Malaysian J Pathol 2020; 42(1) : 77 – 84

ORIGINAL ARTICLE

Is Procalcitonin more superior to hs-CRP in the diagnosis of infection in diabetic foot ulcer?

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Abstract

Introduction: Procalcitonin (PCT) has recently emerged as a marker for diagnosing infection. This study aimed to compare the performance of PCT and other infection markers in diagnosing infected diabetic foot ulcer (IDFU). *Materials and Methods*: A total of 128 diabetic patients with foot ulcers were recruited and divided into two groups, consisting of 73 patients in the IDFU group and 55 in the non-infected diabetic foot ulcer (NIDFU). The severity of infection in IDFU patients was graded based on the Infectious Disease Society of America-International Working Group on the Diabetic Foot classification. Blood samples from all the patients were collected for measurement of PCT, high sensitivity C-reactive protein (hs-CRP) and white cell count (WBC). The area under the receiver operating curves (AUC) were then constructed and analysed. *Results*: PCT, hs-CRP and WBC levels were significantly higher in the IDFU group compared to NIDFU with hs-CRP demonstrated the highest AUC (0.91; $p < 0.001$) followed by PCT (0.814; $p < 0.001$) and lastly WBC (0.775; $p < 0.001$). The best cut off value, sensitivity and specificity for the presence of infection in diabetic foot, were 3.47 mg/dL, 80% and 89% for hs-CRP, 0.11 ng/ml, 70% and 87% for PCT and 11.8×10^9/L, 60% and 90% for WBC. All the infection markers showed significant positive correlations with infection severity of DFU. *Conclusion*: This study showed that hs-CRP is a more sensitive marker for diagnosing IDFU. Although PCT is useful in differentiating IDFU from NIDFU, the use of PCT is not necessary as it adds little value to the current practice.

Keywords: infected diabetic foot ulcer, procalcitonin, inflammatory markers

INTRODUCTION

Diabetic foot ulcer (DFU) is a known major complication of diabetes with a lifetime incidence of as high as 25%.[1] A recent study reported that 47.1% of diabetic patients attending diabetic foot clinic in a tertiary hospital were found to have foot ulcers.[2] DFUs are frequently infected due to impaired host defences in diabetic patients.[3] Approximately 85% of infected of individuals with diabetic foot ulcers required lower limb amputation with increased 5-year mortality rates.[4]

It is crucial to determine the presence of infection promptly in DFU to reduce the rate of amputation. According to the 2012 Clinical Practice Guidelines for the diagnosis and treatment of diabetic foot infections published by the Infectious Diseases Society of America, infection is defined by the presence of at least 2 classic symptoms or signs of inflammation (erythema, pain, warmth, tenderness, and induration) or presence of purulent secretion. However, the clinical diagnosis of infection in DFU is not always straightforward and could be challenging, especially in inexperienced clinicians.[5] The presence of peripheral neuropathy and vascular disease are common in diabetic patients and these may either diminish or mimic inflammatory findings and may mislead the physician.[6] In this condition, laboratory markers are important to aid in diagnosis.

Conventional markers such as erythrocyte sedimentation rate (ESR) and C-Reactive Protein (CRP) are increased in almost all inflammatory

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processes regardless of the presence of infection, while up to 50% of patients with deep foot infection do not have leukocytosis.[6] The dependence of laboratory isolation of microorganism from wound site for the diagnosis of infection is not feasible as all open wounds are colonised with microorganisms and normal bacterial flora, therefore, making the result unreliable particularly in swab specimens.[7]

Recently, Procalcitonin (PCT) was proposed as a useful marker in distinguishing bacterial infection. In the absence of infection, this 116-amino acid peptide is synthesised in the C cells of the thyroid gland as a prohormone of calcitonin. However, in the presence of bacterial infection, under the influence of inflammatory cytokines and bacterial endotoxin, PCT is produced in a number of tissues (lung, liver, kidney, adipose tissue) and secreted into the circulation causing the level to increase up to 1000 times more than the normal level.[8] Several studies have demonstrated the role of PCT in distinguishing infected from non-infected DFU.[9-10] However, there are limited data available to support that PCT has greater diagnostic accuracy than other markers in detecting infection in DFU.

In this study, we aimed to compare the diagnostic performance of serum PCT to other more commonly used markers like hs-CRP and WBC in diagnosing infected DFU. In addition, this study was to assess whether high level of PCT levels are associated with severe DFU infection.

MATERIALS AND METHODS

Study design

This was a cross-sectional study conducted between April 2016 and June 2017, after approval by the research and ethical review boards of UniversitiKebangsaan Malaysia Medical Centre. A total of 128 patients with DFU who attended Orthopaedic clinic or were admitted to Orthopaedic ward were recruited for the study. Patients with established bacterial infection such as sepsis, urinary tract infection, pneumonia, and meningitis, as well as patients with inflammatory diseases such as inflammatory bowel disease and rheumatoid disease were excluded from the study. In addition, patients with non-bacterial causes of raised PCT such as burns, medullary carcinoma of thyroid, malaria, patients who had any surgical procedure in the past six weeks, and patients receiving systemic immunosuppressive therapy were also excluded from the study.

Patients' wound was evaluated by the Orthopaedic team and the presence of infection was determined clinically based on 2012 Infectious Diseases Society of America Clinical Practice Guidelines for the diagnosis and treatment of Diabetic Foot infections by the presence of at least 2 classic symptoms or signs of inflammation (erythema, pain, warmth, tenderness, induration) or presence of purulent secretion.[11] Patients with the above signs were classified as infected diabetic foot ulcer (IDFU) whereas those who did not were classified as non-infected diabetic foot ulcer (NIDFU). For IDFU group, the severity of infection was graded according to the Infectious Diseases Society of America and International Working Group on the Diabetic Foot Classifications of Diabetic Foot Infection (IDSA-IWGDF) criteria summarised in Table 1.

Laboratory analysis

Blood samples were collected immediately after recruitment in Orthopaedic clinic or during admission to Orthopaedic ward for the measurement of PCT, hs-CRP and WBC. Specimens (swab/tissue) from ulcers were obtained for culture and sensitivity to determine the type of microorganism. Serum PCT levels were measured using Electrochemiluminescent Immunoassay (ELECSYS BRAHMS PCT) performed on COBAS e411 analyser (Roche Diagnostics). The analytical measuring range for PCT is from 0.02-100 ng/ml. The intra-assay coefficient of variation (CV) at low and high PCT level was 8.8% and 2.1% respectively. Hs-CRP was measured in this study using an immunoturbidimetric assay (CRP VARIO) that was performed by ARCHITECT analyser (Abbott Diagnostic, USA) with a detection limit of 0.01 mg/dL. The CV at low, medium and high concentrations were 4.6%, 1.6% and 0.8%, respectively. For each test run, quality control was performed. WBC level was measured in the Haematology laboratory.

Statistical analysis

The categorical data were presented in frequencies (n) and percentage (%). Continuous data were presented in median and inter-quartile as the assumption of the data was not normally distributed based on Kolmogorov- Smirnov/ Shapiro Wilk test. Comparison between the median of two groups was analysed by T-test for normally distributed variables and the Mann

TABLE 1: IDSA-IWGDF clinical classification of diabetic foot infection[11]

Clinical manifestations of infection	Infection severity	Grade
No purulent secretion or manifestations of inflammation	Uninfected	1
Presence of ≥ 2 manifestations of inflammation (induration, erythema, pain or tenderness, warmth, purulence). Involve only the skin and subcutaneous tissue (without involvement of deeper tissues and systemic signs). If erythema, must be >0.5 cm to ≤2 cm around the ulcer.	Mild	2
Infection (as above) with erythema >2cm or involving structures deeper than skin and subcutaneous tissues (e.g. abscess, osteomyelitis, septic arthritis, fasciitis) and no systemic signs.	Moderate	3
Infection (as above) with signs of SIRS manifested by ≥2 of the following; Temperature >38°C or < 36°C, Heart rate >80 beats/min, Respiratory rate >20 breaths/min or PaCO2 <32 mm Hg.	Severe	4

Whitney U-test for non-normal distribution of variables. For categorical variables, statistical differences between groups were assessed by Chi-square test. Spearman correlation coefficients were used to assess the correlation between the grade of infection and laboratory parameters.

Receiver Operating Characteristic (ROC) curve was constructed and the area under the ROC curve (AUC) was measured to evaluate the accuracy of PCT, hs-CRP and WBC in discriminating IDFU from NIDFU. The best cut-off value was calculated, and the specificity and sensitivity of the parameters were determined using the best cut off value. In all statistical analyses, $p<0.05$ was considered significant. All clinical and laboratory data were analysed using the Statistical Package for Social Sciences (SPSS) statistical software version 23.0.

RESULTS

Demographic data

A total of 128 patients comprising of Malays (64.8%), Indian (18.8%) and Chinese (16.4%) were included in the study. Fifty-five patients were classified as NIDFU (grade 1, IDSA-IWGDF criteria) and 73 patients as IDFU (grade ≥ 2, IDSA-IWGDF criteria). The demographic characteristics and co-morbidities for both

TABLE 2: Demographic profile and comorbidities of patients with diabetic foot ulcers

	NIDFU	IDFU	Statistical value	*p* value
Number of patients (n)	55	73		
Age, median (IQR)	61 (12)	61 (14)	2031[b]	0.910[b]
Sex				
Male	30 (54.5%)	52 (71.2%)		0.051[a]
Female	25 (45.5%)	21 (28.8%)		
HbA1c, median (IQR)	7.2 (4.5)	8.7 (3)	1370.5[b]	0.906[b]
Comorbidities				
Dyslipidaemia	20 (52.6%)	18 (47.4%)		0.151[a]
Hypertension	43 (45.7%)	51 (54.3%)		0.291[a]
IHD	5 (27.8%)	13 (72.2%)		0.16[a]
CKD	9 (33.3%)	18 (66.7%)		0.255[a]

[a]Pearson chi-square, [b]Mann Whitney U, p <0.05 is considered as statistically significant. IHD, Ischemic heart disease; CKD, Chronic kidney disease

TABLE 3: Wound characteristics and grading of patients with diabetic foot ulcers

	NIDFU	IDFU
Number of patients (n)	55	73
Grading		
1	55 (100%)	-
2	-	17 (23.3%)
3	-	36 (49.3%)
4	-	20 (27.4%0
Wound localisation		
Toe	14 (25.5%)	29 (39.7%)
Metatarsal	15 (27.3%)	18 (24.7%)
Midfoot/heel	22 (40%)	18 (24.7%)
Toe + metatarsal	3 (5.5%)	6 (8.2%)
Metatarsal + midfoot	1 (1.8%)	1 (1.4%)
Toe + midfoot	-	1 (1.4%)
Fever	0 (100%)	31 (42.5%)

groups are summarised in Table 2. There was no statistically significant difference between the two groups in terms of age (p = 0.910), gender (p = 0.051), HbA1c level (p = 0.906) and co-morbidities (p = 0.151 – 0.291).

Wound characteristics

Among IDFU patients, 17 ulcers (23.2%) were classified as grade 2, 36 ulcers (49.3%) as grade 3 and 20 ulcers (27.4%) as grade 4. The grading and location of the wound in IDFU patients are presented in Table 3. Thirty-one patients (42.5%) of IDFU group had fever more than 38°C at admission, while none of the NIDFU patients had fever at presentation.

Wound cultures were obtained in 53 of the 55 patients with NIDFU, and 46 of them revealed positive cultures. The positive cultures in NIDFU group were due to colonisation. A total of 12 different bacterial species were isolated from the wound cultures in IDFU patients with mixed growth as the most frequent results obtained. The types of all bacteria isolated from wound cultures are listed in Table 4.

TABLE 4: Bacteria isolated from wound cultures of diabetic patients with foot ulcers

	NIDFU (n=55)	IDFU (n=73)
No growth	7 (12.7%)	2 (2.7%)
Mixed growth	28 (50.9%)	34 (46.6%)
Candidiasis	-	1 (1.4%)
Gram positive organism		
Coagulase negative staphylococci	2 (3.6%)	1 (1.4%)
Streptococci	-	5 (6.8%)
Enterococcus	-	1 (1.4%)
Staphyloccocus aureus	2 (3.6%)	5 (6.8%)
Methicillin resistant *Staphylococcus aureus*	3 (5.5%)	2 (2.7%)
Diphteroids	1 (1.8%)	-
Gram negative organism		
Enterobacter spp.	4 (7.3%)	5 (6.8%)
Pseudomonas aeruginosa	6 (10.9%)	5 (6.8%)
Escherichia coli	-	4 (5.5%)
Klebsiella pneumonia	-	4 (5.5%)
Proteus mirabilis	-	4 (5.5%)
Not obtained for culture	2 (3.6%)	-

Laboratory findings

PCT, hs-CRP and WBC levels were significantly higher in the IDFU group compared to NIDFU with hs-CRP demonstrating the highest AUC (0.91; p <0.001) followed by PCT (0.814; p <0.001) and lastly WBC (0.775; p <0.001). The best cut off value, sensitivity and specificity for the presence of infection in diabetic foot, were 3.47 mg/dL, 80% and 89% for hs-CRP, 0.11 ng/ml, 70% and 87% for PCT and 11.8x10^9/L, 60% and 90% for WBC. Laboratory parameters of both groups are listed in Table 5. The levels of all infection markers were significantly higher in IDFU patients compared to NIDFU patients (p <0.001). Median PCT values were 0.27 ng/mL in IDFU patients and 0.05 ng/mL in NIDFU patients.

Analysis of correlation between infection markers and severity of infection among IDFU group demonstrated that all markers are positively correlated with the grade of infection of DFU (Table 6). The ROC curve analysis (Figure 1 and Table 7) for predicting the presence of infection demonstrated that the area under the curve (AUC) was greatest for hs-CRP (0.910; p <0.001) followed by PCT (0.814; p <0.001) and lastly WBC (0.775; p <0.001).Using these curves, the best cut off values for PCT was 0.11 ng/ml, 3.47 mg/dl for hs-CRP and 11.8 x 10^9/L for WBC. The sensitivity, specificity, negative predictive value and positive predictive value are presented in Table 8.

DISCUSSION

In this study, the performance of PCT, hs-CRP and WBC in diagnosing infected diabetic foot ulcer was evaluated. Our results showed that the serum concentrations of all three markers were significantly higher in IDFU compared to NIDFU group. This study also demonstrated that hs-CRP has the highest AUC and the greatest sensitivity and specificity in detecting infection in DFU. We also determined the correlation between infection markers and grading of IDFU based on IDSA-IWGDF criteria and found that PCT, hs-CRP and WBC were positively correlated to the severity of infection in patients with IDFU.

To date, there were six previously published studies which similarly evaluated the role of PCT in distinguishing IDFU from NIDFU. However, the outcomes of these studies were inconsistent. Two of the studies demonstrated that PCT was more efficient in detecting infection compared to other markers.[9,10] One study reported that

TABLE 5: Value of PCT, hs-CRP and WBC in NIDFU and IDFU groups

Parameters	NIDFU	IDFU	Statistical value	P value
PCT, ng/ml	0.05 (0.06)	0.27 (0.689)	3269[a]	p <0.001[a]
hs-CRP, mg/dl	1.09 (1.72)	11.62 (15.14)	3654[a]	p <0.001[a]
WBC, 10^9/L	9.3 (2.7)	12.8 (9.3)	3113[a]	p <0.001[a]

Values are expressed as median (IQR) [a] by Mann Whitney U test, p <0.05 is considered as statistically significant.

TABLE 6: Correlation of serum PCT, hs-CRP and WBC with infection

Parameters	Correlation coefficient (r)	r^2	P value
PCT, ng/ml	0.607	0.368	P<0.001
hs-CRP, mg/dl	0.420	0.176	P<0.001
WBC, 10^9/L	0.446	0.199	P<0.001

TABLE 7: ROC Analysis of PCT, hs-CRP and WBC

Parameters	AUC	95% CI	P value
PCT	0.814	0.740-0.888	0.001
hs-CRP	0.910	0.861-0.959	0.001
WBC	0.775	0.695-0.855	0.001

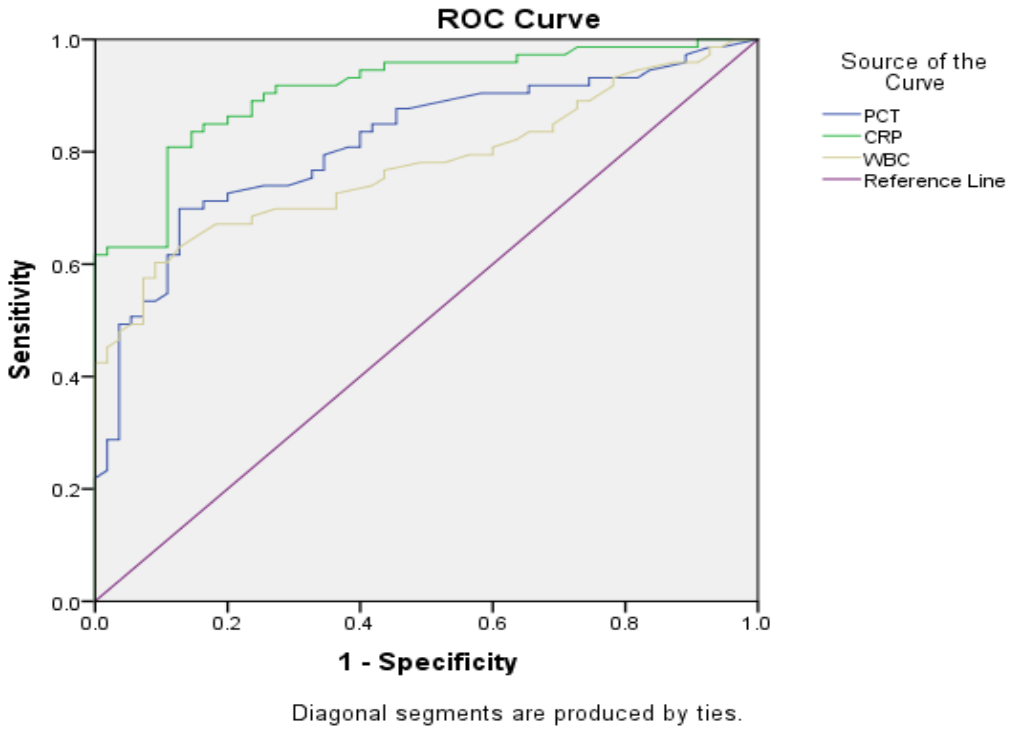

FIG. 1. Receiving Operating Characteristic (ROC) curve for PCT, hs-CRP and WBC for diagnosis of infected diabetic foot ulcer.

PCT was a useful marker only for distinguishing infection grade 3 and 4 and not for grade 2 and 3.[12] In contrast, one study concluded that serum PCT did not differ between IDFU and NIDFU groups. Other study reported that ESR was the most sensitive and specific marker.[13,14] Jeandrot *et al*.[15] reported that CRP was the single most informative parameter with the highest sensitivity and specificity compared to PCT, ESR and WBC which was similar with our findings.

CRP was found to be increased significantly in local infections compared to PCT, in which the latter was more sensitive for systemic infection.[16] Our study showed hs-CRP performed better especially in low-grade diabetic foot wounds. A study which evaluated the role of PCT in upper respiratory tract bacterial infection also demonstrated that CRP performed better than PCT.[17] However, other studies showed PCT had a more superior diagnostic accuracy in the diagnosis of sepsis than other markers.[9,10] The levels of PCT are variable and depends on the site, type and extent of the infection. Rothenburger *et al*.[18] showed there was no significant elevation of PCT level in post-cardiac surgery patients with local infection compared to patients with systemic infection.

Although PCT performance was not superior to other markers, our result showed PCT can still be helpful in diagnosing IDFU. At the cut off value of 0.11 ng/mL, PCT has a sensitivity of 70% and specificity of 87% in detecting IDFU. This study also demonstrated a significant positive correlation between PCT and the severity of infection.

WBC is the most basic and commonly used

TABLE 8: Sensitivity, specificity and predictive value of PCT, hs-CRP and WBC for diagnosis of diabetic foot infections

Cut off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PCT > 0.11 ng/ml	70	87	83	77
hs-CRP> 3.47 mg/dl	80	89	87	84
WBC> 11.8 x10^9/L	60	90	86	72

marker for infection. In this present study, as expected, WBC was significantly higher in IDFU than in NIDFU. However, the AUC for WBC in distinguishing IDFU from NIDFU was lower compared to PCT and hs-CRP. The low sensitivity (60%) of WBC in detecting IDFU may lead to failure to detect an infection. Our findings further ascertained the value of WBC in the diagnosis of IDFU by the majority of researchers.[12,14,15]

Microbiology culture of the wound from swab specimens is not recommended to be used as a marker of infection in DFU. Although few studies demonstrated high sensitivity of swab culture in the diagnosis of diabetic foot ulcer infection[19], IWDGF recommended that the use of wound culture is only to identify the causative organism and to determine its antibiotic sensitivity. This study showed 87% of swab culture from uninfected ulcers revealed positive findings due to coloniser. Both infected and non-infected ulcers had almost similar microbiology culture profile. Louie *et al*. showed that infection of diabetic foot was mainly influenced by wound care, diabetes control and vascular supply.[20]

In terms of cost, PCT is the most expensive test among other infection markers and the price is six times higher than hs-CRP. The time taken to analyse PCT is also longer (~20 mins) than hs-CRP (~5 mins). Due to the above disadvantages of PCT and lower diagnostic performance, PCT testing is not preferred over hs-CRP. However, further studies need to be done in order to assess the cost-effectiveness of PCT in guiding antibiotic therapy and its relation to antibiotic resistance in patients with IDFU. Some studies recommended the use of a combination of PCT and CRP to increase the detection rate of infection in DFU. [9,15] However, our study showed that hs-CRP alone is adequate. Furthermore, the use of multiple infection markers will result in a higher cost.

There were some limitations in this study. First, infection was diagnosed and graded based on clinical evaluation of the ulcers. Therefore, it was difficult to assess objectively due to inter-observer bias causing inconsistency. The low number of patients enrolled in this study is also one of the limitations. Our study also excluded all the patients with systemic disease and co-infection which may elevate serum infection markers. This exclusion might result in overestimation of PCT and hs-CRP specificity and it is likely that the performance of these markers may be lower during routine care.

CONCLUSION

All the infection markers are useful in distinguishing IDFU from NIDFU. Hs-CRP was found to be the single most effective marker and has a significant correlation with the grade of infection. PCT may not be the preferred choice as it adds little value to the current practice in diagnosing IDFU and is not cost-effective.

Acknowledgement: This study was supported by PPUKM Fundamental Grant FF-2016-078.

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