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Procalcitonin as a Diagnostic Aid in Diabetic Foot Infections

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Uzun, G., Solmazgul, E., Curuksulu, H., Turhan, V., Ardic, N., Top, C., Yildiz, S. and Cimsit, M. *Procalcitonin as a Diagnostic Aid in Diabetic Foot Infections*. Tohoku J. Exp. Med., 2007, **213** (4), 305-312 —— The diagnosis of diabetic foot infection (DFI) is usually a challenge to the clinician. Procalcitonin (PCT), a 116-amino acid propeptide of calcitonin, is a new marker of bacterial infections and sepsis. We evaluated the serum value of PCT as a marker of bacterial infection in diabetic patients with foot ulcers. Forty-nine diabetic patients with foot ulcers were consecutively enrolled into the study. DFI was diagnosed clinically by the presence of purulent secretions or at least two of the symptoms of inflammation including redness, warmth, swelling, and pain. According to these criteria, DFI was determined in 27 patients (DFI group) and not detected in 22 patients (NDFI group). The blood samples were taken for biochemical analysis on admission. PCT, white blood cell count (WBC) and erythrocyte sedimentation rate (ESR), but not C-reactive protein (CRP), was found significantly higher in DFI group compared with NDFI group. The best cut-off value, sensitivity and specificity were 0.08 ng/ml, 77% and 100% for PCT, 32.1 mg/dl, 29% and 100% for CRP, 8.6 10^9/L, 70% and 72% for WBC and 40.5 mm/h, 77% and 77% for ESR, respectively. The area under the receiver operating characteristic curve for infection identification was greatest for PCT (0.859; $p < 0.001$), followed by WBC (0.785; $p = 0.001$), ESR (0.752; $p = 0.003$), and finally CRP (0.625; $p = 0.137$). These results suggest that PCT may be a useful diagnostic marker for DFI. Additional research is needed to better define the role of PCT in DFI. ———— procalcitonin; infection marker; C-reactive protein; erythrocyte sedimentation rate; white blood cell count

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Diabetic foot is one of the most important problems of the 220-million global diabetic population, with 2.0% of these developing diabetic foot ulcers every year (Ramsey et al. 1999). Foot ulcers are responsible for 85% of lower extremity amputations in diabetic patients (Pecoraro et al. 1990). Despite a multidisciplinary approach, patients with diabetic foot ulcers are exposed to high morbidity and mortality (Reiber et al. 1995; Mayfield et al. 2004).

Diabetic foot ulcers are frequently infected (Singh et al. 2005). Such ulcers take longer to heal and are more likely to lead to amputation compared to non-infected ulcers (Oyibo et al. 2001). Therefore, determining the presence of infection occupies an important place in diabetic foot management (Lipsky et al. 2004). However, prediction of infection and determining the status of infection and the degree of penetration (abscess and osteomyelitis) in diabetic foot ulcers is frequently difficult for inexperienced clinicians (Lipsky et al. 2004). There may even be no erythema, warmth, pain or tenderness in diabetic foot infections (DFIs) (Williams et al. 2004). Furthermore, clinical, hematological and bacteriological markers of infection may all mislead the physician in DFIs (Williams et al. 2004). Up to 50% of patients with a deep foot infection will not have leukocytosis (Eneroth et al. 1999). Since DFI can progress rapidly and cause extremity loss it must be identified at an early stage and treated without delay.

In recent studies procalcitonin (PCT), a 116-amino acid peptide, has been proposed as a useful marker of bacterial infection. PCT was discovered 25 years ago as a precursor peptide of the hormone calcitonin, and is normally produced in the C cells of the thyroid gland (Muller and Becker 2001). PCT is stimulated by severe bacterial infections, but remains fairly low in viral infections and non-specific inflammatory diseases (Snider et al. 1997). Serum PCT concentration has been studied in many infectious diseases in both pediatric and adult populations (Hatherill et al. 1999; Moulin et al. 2001; Luzzani et al. 2003; Balci et al. 2003; Castelli et al. 2004; Giamarellou et al. 2004). However, the diagnostic role of PCT in DFI has not been evaluated previously.

In this study, we determined serum PCT levels in diabetic patients with foot ulcers. In addition, we evaluated the use of PCT as an infection marker in the diagnosis of DFI and compared it with other more commonly used markers, including erythrocyte sedimentation rate (ESR), white blood cell count (WBC) and C-reactive protein (CRP).

Methods

Patients

A total of 49 diabetic patients admitted to the Gulhane Military Medical Academy, Haydarpasa Training Hospital, Hyperbaric Oxygen Therapy Unit between January 2005 and June 2005 with a foot ulcer, were consecutively enrolled. An additional group of healthy controls ($n = 22$) was also included. Those with other infectious diseases such as sepsis, meningitis, inflammatory intestinal disease and pneumonia, patients who had undergone surgery in the previous six weeks, patients with hematological malignancy known to raise PCT levels, and patients receiving systemic immunosuppressive treatment were excluded from the study. The study was approved by Gulhane Military Medical Academy Ethical Committee, and informed consent was obtained from each patient before enrolment.

Patients were evaluated for DFI by a medical team, which included an infectious diseases expert, a microbiology expert and an internal diseases expert. These physicians were blinded to the biochemical analysis. DFI diagnosis was performed according to Infectious Diseases Society of America guidelines (Lipsky et al. 2004). DFI was diagnosed clinically by the presence of purulent secretions or at least 2 of the symptoms of inflammation including redness, warmth, swelling or induration, and pain or tenderness (Lipsky et al. 2004).

The Wagner system (Wagner 1981) was used for the classification of DFI. Wound depth (superficial or deep), localization (toe, metatarsal or mid foot/heel) and the presence of purulent secretion were noted. During initial presentation patients were asked whether or not they used antibiotics. Culture specimens from foot lesions for microbiological analysis were obtained using deep tissue sampling. Osteomyelitis was evaluated using the probe to bone test (Grayson et al. 1995), plain X-rays and magnetic resonance imaging (MRI), where necessary. Blood samples were taken for the measurement of PCT, WBC, ESR, CRP, plasma glucose and hemoglobin A1c level.

Laboratory analysis

The blood taken for the analysis of PCT level was centrifuged for 20 min at 3,000 rpm after being kept at room temperature for 30 min, and specimens was kept at –80°C in a deep freeze until the end of the study. The PCT concentration in serum was measured with a fully automatic kryptor device using a B.R.A.H.M.S procalcitonin kit (B.R.A.H.M.S Diagnostics, Berlin, Germany). The functional detection limits for the PCT assay were 0.06 and 5,000 ng/ml (0.06 to 50 ng/ml directly and up to 5,000 ng/ml after sample dilution). The analysis was performed according to the manufacturer's recommendations. CRP, WBC and ESR were measured by the hospital biochemistry laboratory. All tests were performed in a blinded manner.

Statistical analysis

Data were presented as mean ± standard deviation (S.D.). The Kolmogorov–Smirnov test was used to assess sample distributions. One-way ANOVA or the Kruskal-Wallis test with Bonferroni adjustment was used for the comparison of multiple groups. The *t*-test or Mann-Whitney's U-test was used for the comparison of two independent samples. The receiver operating characteristic (ROC) curve and the respective areas under the ROC curve were calculated for all parameters. The best cut-off value was determined using Youden's index, J = specificity + sensitivity – 1. The highest J value is the best cut-off value for that parameter. Sensitivity, specificity, and the positive and negative predictive values of parameters were determined by using these best cut-off values. A $p < 0.05$ was regarded as statistically significant. Statistical analyses were performed using SPSS for Windows 11.0 (SPSS Inc., Chicago, IL, USA).

Results

DFI was determined in 27 (DFI group) of the 49 diabetic patients with foot ulcers. The remaining patients without DFI were assigned to NDFI group ($n = 22$). The demographic characteristics of the control, DFI and NDFI groups are summarized in Table 1. There was no statistically significant difference between the three groups in terms of age or gender ($p > 0.05$). Plasma glucose and hemoglobin A1c levels in diabetic patients were, however, significantly higher than those in healthy controls ($p < 0.05$).

Wound characteristics of patients are presented in Table 2. Probe to bone test was positive for 11 of 49 patients. Eight of these 11 patients were from the DFI group and three from the NDFIgroup. Osteomyelitis was determined using MRI in seven of these 11 patients.

Reproduction occurred in wound cultures taken on admission in 29 patients, and no reproduction in the other 20. Twenty of the patients with reproduction in wound culture were from the DFI group and nine from the NDFI group. The positive culture results in NDFI group were evaluated as colonization. A total of 11 different bacterial species were isolated from the wound cultures. Of all the isolates, 67% were Gram-positive and 33% were Gram-negative bacteria. *Staphylococcus* spp. and *Escherichia coli* were the most frequently observed Gram-positive and Gram-negative bacteria, respectively. All bacteria

Table 1. Characteristics of the control subjects and diabetic patients.

	Control group	NDFI group	DFI group
n	22	22	27
Age (yrs)	62.9 ± 5.7	63.6 ± 10.5	65.4 ± 12.1
Diabetic age (yrs)	-	12.2 ± 6.5	12.6 ± 8.7
Sex (male/female)	14/8	11/11	19/8
Plasma glucose (mg/dl)	90.2 ±13.1	170.1 ± 66.4*	169.2 ± 87.4*
Hemoglobin A1c level (%)	5.2 ± 0.6	9.1 ± 1.8*	8.9 ± 2.1*

Diabetic foot infection (DFI) was diagnosed clinically by the presence of purulent secretions or at least 2 of the symptoms of inflammation including redness, warmth, swelling or induration, and pain or tenderness (Lipsky et al. 2004).

Data are mean ± S.D. *n*, number of patients. DFI group: diabetic patients with infected foot ulcers. NDFI group: diabetic patients with non-infected foot ulcers; * $p < 0.05$ difference from control group.

Table 2. Wound characteristics of diabetic patients with foot ulcers.

	DFI group *n* (%)	NDFI group *n* (%)	Total *n* (%)
Patients	27 (55)	22 (45)	49 (100)
Wound localization			
Toe	9 (33)	8 (36)	20 (35)
Metatarsals	11 (41)	7 (32)	17 (37)
Mid foot and heel	7 (26)	7 (32)	12 (28)
Wound characteristics			
Superficial ulcer	0 (0)	5 (23)	5 (10)
Deep ulcer	27 (100)	17 (77)	44 (90)
Osteomyelitis[1]	7 (26)	0 (0)	7 (14)
Purulent secretion	20 (74)	0 (0)	20 (41)
Probe to bone test	8 (30)	3 (13)	11 (22)
Antibiotic use on admission	17 (63)	11 (50)	28 (57)
Wound culture positive	20 (74)	9 (41)	29 (59)
Fever (mean ± s.d.)	36.3 ± 0.3	36.6 ± 0.5	-

Diabetic foot infection (DFI) was diagnosed clinically by the presence of purulent secretions or at least 2 of the symptoms of inflammation including redness, warmth, swelling or induration, and pain or tenderness (Lipsky et al. 2004).

n, number of patients. DFI group: diabetic patients with infected foot ulcers. NDFI group: diabetic patients with non-infected foot ulcers.

[1]Osteomyelitis was determined with magnetic resonance imaging in 7 of 11 patients with positive probe to bone test.

isolated from wound cultures were listed in Table 3.

Twenty eight patients were receiving antibiotics on admission. Of these patients, 11 patients were assigned to the NDFI group and antibiotic therapy was stopped in these patients. Seventeen of these patients were assigned to the DFI group. Ten of the 21 patients not using antibiotics on admission were placed in the DFI group and 11 in the NDFI group. Empirical antibiotic therapy was initiated in patients diagnosed with DFI until the wound culture results were obtained, after which antibiotics appropriate to those results were administered.

Laboratory parameters of all groups are presented in Table 4. The PCT levels in the DFI group were significantly higher than those in the NDFI ($p < 0.001$) and control groups ($p < 0.001$). Although CRP levels in the DFI group were higher than in the NDFI group, the difference was not statistically significant ($p = 0.801$). However, CRP levels in the NDFI and DFI groups were significantly higher than those in the control group ($p < 0.05$ and $p < 0.001$, respectively). WBC in the DFI group was significantly higher than in the NDFI ($p = 0.001$) and control groups ($p < 0.001$). ESR levels in the DFI group were significantly higher than in the NDFI ($p = 0.001$) and control groups ($p < 0.001$). In addition, ESR levels in the NDFI group were significantly higher than those in the control group ($p < 0.05$).

The ROC curve predicting the presence of bacterial infection in diabetic patients was calculated (Fig. 1). The area under the ROC curve for infection identification was greatest for PCT (0.859; $p < 0.001$), followed by WBC (0.785; $p = 0.001$), ESR (0.752; $p = 0.003$) and finally CRP (0.625; $p = 0.137$). The best cut-off value was 0.08 ng/ml for PCT, 32.1 mg/dl for CRP, 8,6 10^9/L for WBC and 40.5 mm/h for ESR. Optimum sensitivity, specificity, and positive and negative predictive values are presented in Table 5.

TABLE 3. All bacterial isolates obtained from wound cultures of diabetic patients with foot ulcers.

	DFI (*n*)	NDFI (*n*)
Total gram (+) organisms	12	7
Staphylococcus aureus	5	3
Coagulase negative staphylococci	3	4
Streptococci	2	-
Enterococcus	1	-
Corynebacterium spp.	1	-
Total gram (–) organisms	8	2
Escherichia coli	2	1
Pseudomonas aeruginosa	2	-
Enterobacter spp.	1	1
Acinetobacter spp.	1	-
Proteus mirabilis	1	-
Klebsiella pneumoniae	1	-

Diabetic foot infection (DFI) was diagnosed clinically by the presence of purulent secretions or at least 2 of the symptoms of inflammation including redness, warmth, swelling or induration, and pain or tenderness (Lipsky et al. 2004).

n, number of patients. DFI group: diabetic patients with infected foot ulcers, NDFI group: diabetic patients with non-infected foot ulcers.

TABLE 4. Infection markers in the control group and diabetic patients.

Groups	PCT (ng/ml)	CRP (mg/dl)	WBC (10^9/L)	ESR (mm/h)
Control group ($n = 22$)	< 0.06[1]	4.4 ± 3.2	6.4 ± 1.5	14.7 ± 6.4
NDFI group ($n = 22$)	0.06 ± 0.006	11.0 ± 7.9 *	8.0 ± 1.8	31.4 ± 22.7 *
DFI group ($n = 27$)	0.18 ± 0.17 *,#	49.4 ± 74.4 *	11.4 ± 5.2 *,#	62.7 ± 30.5 *,#

Diabetic foot infection (DFI) was diagnosed clinically by the presence of purulent secretions or at least 2 of the symptoms of inflammation including redness, warmth, swelling or induration, and pain or tenderness (Lipsky et al. 2004).

Data are mean ± S.D. PCT, Procalcitonin; CRP, C-reactive protein; WBC, white blood cell count; ESR, Erythrocyte sedimentation rate. DFI group: diabetic patients with infected foot ulcers. NDFI group: diabetic patients with non-infected foot ulcers. $^{*}p < 0.05$ difference from control group. $^{\#}p < 0.05$ difference from NDFI group. [1]All values were below the functional sensitivity of PCT assay.

DISCUSSION

Foot infections are one of the serious consequences of diabetes mellitus. Diagnosis of foot infection is mainly based on clinical findings (Lipsky et al. 2004). However, the classical signs of infection may be absent in diabetic patients (Williams et al. 2004). When clinical signs are misleading, laboratory tests assist with infection diagnosis.

Sensitivity is the proportion of true positives that are correctly identified by the test. Specificity is the proportion of true negatives that are correctly identified by the test.

In the present study, we evaluated the various biochemical infection markers including PCT, WBC, ESR and CRP with respect to their diagnostic accuracy in DFI. Our results demonstrated that PCT, WBC and ESR, but not CRP, are valuable as an infection marker for diabetic patients

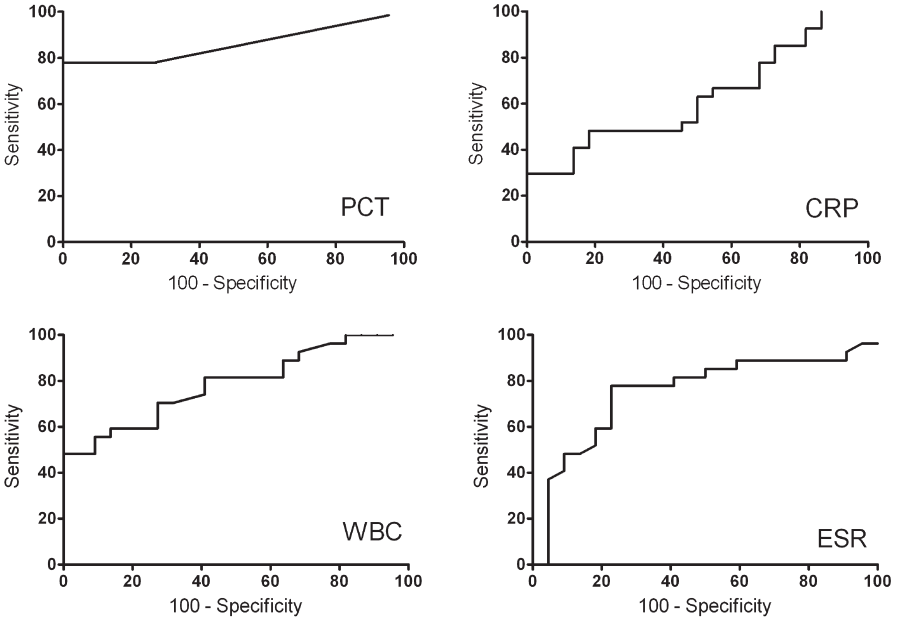

Fig. 1. Receiver operating characteristic curves of procalcitonin (PCT), C-reactive protein (CRP), white blood cell count (WBC) and erythrocyte sedimentation rate (ESR) in the diagnosis of diabetic foot infection. The area under the curve was 0.859 for PCT, 0.625 for CRP, 0.785 for WBC and 0.752 for ESR.

Table 5. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (PPV) of infection markers for diabetic foot infection.[1]

Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PCT > 0.06 ng/ml	78	73	78	73
PCT ≧ 0.08 ng/ml	**77**	**100**	**100**	**78**
PCT ≧ 0.1 ng/ml	59	100	100	67
CRP ≧ 32.1 mg/dl	**29**	**100**	**100**	**53**
CRP ≧ 20 mg/dl	41	82	73	53
WBC ≧ 8.6 10^9/L	**70**	**72**	**76**	**66**
WBC ≧ 10 10^9/L	36	95	89	60
ESR ≧ 40.5 mm/h	**77**	**77**	**80**	**74**
ESR ≧ 70 mm/h	44	91	86	57

PCT, procalcitonin; CRP, C-reactive protein; WBC, white blood cell count; ESR, erythrocyte sedimentation rate. [1]Optimum diagnostic cut-off values derived from the receiver operating characteristic curve are shown in bold.

with foot ulcers. PCT has been shown to be superior to other infection markers in the diagnosis of both systemic and localized bacterial infections, as well as of sepsis populations (Hatherill et al. 1999; Moulin et al. 2001; Luzzani et al. 2003; Balci et al. 2003; Castelli et al. 2004; Giamarellou et al. 2004). However, the diagnostic performance of PCT in DFI has not been elucidated previously. Serum PCT levels are variable and depend on the site and extent of the infection. As confirmed in our study PCT levels are usually below 0.05 ng/ml in healthy subjects (Muller and Becker 2001). As expected, PCT levels in patients with localized infections are usually lower compared to those with systemic bacterial infections (Christ-Crain and Muller 2005). In this study, PCT levels were below the lower detection limit of PCT assay (0.06 ng/ml) in the healthy controls, were ranged between 0.06 and 0.07 ng/ml in patients with non-infected diabetic foot ulcers, and 0.06 and 0.83 ng/ml in patients with DFI. Previous studies used a cut-off level of 0.5 ng/ml for PCT in localized bacterial infections. In our study, with a cut-off level of 0.5 ng/ml, sensitivity will be very low (4%), in contrast to the presence of high specificity (100%). We determined a best cut-off value of 0.08 ng/ml for PCT with a sensitivity and specificity of 77% and 100%, respectively. Although the PCT demonstrated the best performance in predicting DFI compared to the other infection markers, the fairly low sensitivity is an important limitation of PCT as an infection marker in DFI, because, the objective in an infection marker is high sensitivity, otherwise patients with true infection who need antibiotic treatment may be missed. In patients with clinical signs of diabetic foot infection and after exclusion of coinfection, a PCT value of > 0.08 is in favour of a true infection, but a value < 0.08 does not exclude an infection. PCT levels were found below the functional detection limit of PCT assay used in this study in 72% of patients in the NDFI group (16/22) and in 22% of patients in the DFI group (6/27). Furthermore, the best cut off value detected for PCT in our study is below the detection limit of some commercially available PCT assays (Christ-Crain and Muller 2005). Sensitive PCT assays should therefore be used in mild to moderate localized bacterial infections.

CRP, an acute-phase protein, increases during inflammatory processes occurring in the body. It is also detected in high concentrations in cases in which there are no bacterial infections. CRP levels are higher in diabetic patients compared with healthy subjects (King et al. 2003). In addition, Upchurch et al. (1997) showed that CRP increases significantly in diabetic patients with foot ulcers compared to those without foot ulcers. We determined a significant difference between the CRP levels of diabetic patients and healthy controls. In contrast to previous findings, comparison of CRP levels in patients with and without DFI revealed no statistically significant differences. Furthermore, the area under the ROC curve for CRP was smaller than those for other markers, indicating that CRP has the lowest diagnostic accuracy.

WBC is a universally accepted marker of infection; however, nearly 50% of patients with DFI do not have leukocytosis. In this study, WBC and ESR exhibited moderate sensitivity and specificity for DFI. Kaleta et al. (2001) showed that an ESR greater than 70 mm/h has a sensitivity of 89.5% and a specificity of 100% for osteomyelitis in diabetic patients. We determined osteomyelitis in seven patients in the DFI group. Using a cut-off level of 70 mm/h, a sensitivity of 71% and specificity of 65% were obtained.

The low number of patients is a limitation in our study. This study now needs to be repeated with a large patient series. Another limitation is the gold standard used in this study. Diagnosis of infection was based on the criteria including only clinical evaluation of the diabetic ulcers. Additionally, microbiological techniques and probe to bone test were insufficient in supporting the diagnostic process. However, the criteria employed in this study were drawn from the generally accepted guide for the management of diabetic foot infections (Lipsky et al. 2004) and patients were examined by a team of diabetic foot experts. An intervention trial using specific cutoff values needs to be performed in order to solve this problem.

In conclusion, in this group of patients PCT, WBC and ESR, but not CRP, were determined to be useful parameters in DFI. PCT has superior specificity but comparable sensitivity to WBC and ESR in the diagnosis of DFI. In addition, since PCT may not rise sufficiently in localized infections, care must be taken when deciding not to use antibiotics. This study warrants further research in order to evaluate the diagnostic role of PCT in patients with DFI.

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