https://doi.org/10.15218/zjms.2017.045

Measurements of serum high sensitivity-C reactive protein, and procalcitonin levels in type1 and 2 Diabetes complicated with diabetic foot syndrome

Received: 12/6/2016 Accepted: 30/10/2016

Zhian M.I. Dezayee* Marwan S.M. Al-Nimer**

Abstract

Background and objective: There is evidence that the procalcitonin levels are usually correlated with the high sensitivity C-reactive protein levels in inflammatory conditions. Therefore, this study aimed to assess the simultaneous changes of the procalcitonin and hs-CRP levels in the diabetic foot syndrome complicated type 1 and 2 diabetes.

Method: This observational study was carried in the Center of Diabetes Mellitus in Erbil, Iraq from 1st January to the 30th September 2015. A total number of 170 participants were enrolled in this study. They grouped into Group I (healthy subjects, n=30), Group II (type 1 diabetes with diabetic foot syndrome, n=70) and Group III (type 2 diabetes with diabetic foot syndrome, n=70). The anthropometric measurements, blood pressure, fasting serum glucose and lipid profile, and the inflammatory markers included high sensitivity C-reactive protein and procalcitonin were determined.

Results: Group III patients had a significant longer duration and score of diabetic foot syndrome, higher anthropometric measurements, higher blood pressure and fasting lipid profile levels compared with Group II. Serum procalcitonin and high sensitivity C-reactive protein levels were significantly higher in diabetic patients compared with Group I subjects. The serum levels of procalcitonin and high sensitivity C-reactive protein of Group III patients (1.11±0.47 ng/ml; 12.48±2.57 mg/L) were significantly higher than corresponding values of Group II patients (0.334±0.094 ng/ml; 5.73±0.89 mg/L). A non-significant correlation between procalcitonin with high sensitivity C-reactive protein in Group II and III was observed.

Conclusion: We conclude that the simultaneous measurements of high sensitivity C-reactive protein and procalcitonin as inflammatory biomarkers are not necessary because the correlation was not significant.

Keywords: Procalcitonin; High sensitivity C reactive protein; Diabetic foot syndrome.

Introduction

Procacitonin (PCT) is a peptide of 115 amino acids that produced by the C cells of the thyroid gland and other endocrine cells in the lung and intestine. It is one of the acute phase reactants that released in response to endotoxin or to the related cytokines that associated with bacterial infections.[1,2] It is a useful and accurate diagnostic marker of sepsis, and it's used to guide the antibacterial therapy in infections.[3,4] A diabetic foot syndrome (DFS) is a clinical condition characterized by ulceration, signs of inflammation and infections and in the presence or absence of gangrene. Wagner-Meggitt's classification was used for grading DFS.[5] This classification system has six grades (0-5) of lesions, the first four grades based on the physical depth of the lesion and the last two based on the gangrene extension. A strong correlations demonstrated between PCT levels and interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6.[6] In infected diabetic foot ulcer, the serum levels of PCT is significantly higher than patients without infection with a sensitivity and specificity of 70% and 74%,

* Department of Medical Microbiology, College of Health Sciences, Hawler Medical University, Erbil, Iraq.
** Department of Pharmacology, College of Pharmacy, Hawler Medical University, Erbil, Iraq.

https://doi.org/10.15218/zjms.2017.045

respectively, making this measurement as a useful diagnostic marker for existence of the bacterial infection when it combined with other markers like c-reactive protein.[7] The levels of PCT tended to decrease significantly during the early phase of healing in patients with diabetic foot syndrome (DFS) and osteomyelitis and this reduction run in parallel with a reduction in the IL-6.[8] In non-bacterial infection, PCT acts as pro inflammatory marker and it is considered as a good prognostic biomarker, for example a significant high serum level of PCT in patients with ischemic stroke at the time of admission predicts a long-term poor prognosis.[9] Moreover, PCT and hs-CRP are found as independent predictors of long-term mortality in patients with acute ischemic stroke and acute heart failure.[10,11] The rationale of this study is the changes in the PCT levels usually associated with changes in hs-CRP of whatever the pathological condition. Therefore, this study aimed to assess the simultaneous changes in the PCT and hs-CRP in DFS complicated type 1 and 2 diabetes.

Methods

This observational study was carried in the Center of Diabetes Mellitus in Erbil. The study was conducted according to the guidelines of the Declaration of Helsinki with approval from a local ethical review board. A consent form obtained from each patient before enrollment into the study. The entry criteria included DFS that complicated type 1 and 2 diabetes (T1D and T2D) patients treated with oral hypoglycemic agents and insulin according to the clinical status of the patient. The clinical examination of the patients as well as the grading of the diabetic foot manifestations were carried by the consultant endocrinologists at the diabetes center. The criteria of exclusion included a history of rheumatic conditions, hematological, neoplastic, renal, liver or thyroid diseases, or patients receiving treatment with anti-inflammatory drugs. The patients grouped according into:

Group I (n=30): Apparent healthy subjects served as a control
Group II (n=70): T1D with FDS
Group III (n=70): T2D with FDS

Demographic data, medical history and treatment were collected in the center. Modifiable risk factors, events or complications, and current therapy were recorded. A person who reported smoking on admission was defined as current smoker. Height, weight, waist circumference, hip circumference were measured, and body mass index (BMI) was calculated using Quetlete's equation.

Body mass index (kg/m^2) = weight (kg) / $height^2$(m)

Waist circumference (cm) and the waist to height (cm) ratio recorded.

The blood pressure was measured on sitting position and the mean of three readings was taken. The mean arterial blood pressure was determined using the following equation:

Mean arterial blood pressure (mmHg) = Diastolic + 1/3 pulse pressure

Pulse pressure is equal to systolic-diastolic blood pressure

Peripheral venous blood was drawn immediately after admission into plain test tubes, part of them were centrifuged at 2500 rpm for 10 min, and the sera were separated for determination of fasting serum glucose and lipid profile and the other uncentrifuged part was used for determination of HbA_{1c} (%) on the same day of collection. Part of sera were kept in deep freeze at -20°C for analysis of hs-CRP and precalcitonin. The determinants of lipid profile included fasting serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein-cholesterol (HDL-c). The low density lipoprotein-cholesterol (LDL-c) and very low density lipoprotein-cholesterol (VLDL-c) were determined by using the following equations:

VLDL-c = TG x 0.2

LDL-c = TC- (HDL-c + VLDL-c)[12]

Quantitative determination of serum high sensitivity C-reactive protein (hs-CRP) and

https://doi.org/10.15218/zjms.2017.045

PCT were carried on using the enzyme linked immunosorbent assay (ELISA) technique.

Statistical analysis

Data are expressed as number, percentages, and means ± SD. Unpaired difference between two means Student's t test, one way analysis of variance (ANOVA) *post hoc Tukey* (HSD) test and differences between percentages (whenever the data are presented as percentages) were used to look for the differences between the two groups and a simple (Pearson's) correlation test was used to detect the correlations between the inflammatory markers and other risk factors. For all tests, a two-tailed $P \leq 0.05$ was considered statistically significant. All data were analyzed using Excel 2003 program for Windows.

Results

Table 1 shows the characteristics of the participants. The number of males that presented with DFS is higher than the corresponding number of females with T1D and T2D. The mean age of T2D patients was significantly higher than the corresponding age of T1D patients or healthy subjects. There was no specific distribution of patients with FDS in respect to the residency. Group II showed significant longer duration of diabetes and higher glycemic indices compared with Group III.

Table 1: Characteristics of the study groups.

	Group I (n=30)	Group II (n=70)	Group III (n=70)	*P* value
Gender (Male : Female)	17: 13	54:16	50:20	
Age (Year)	52.4± 5.1	51.4±4.2	64.1±4.5	†0.363, ††< 0.001, †††< 0.001
Residency				†0.861, ††0.273, †††0.235
Urban	16(53.3)	36(51.4)	29(41.4)	
Rural	14(46.7)	34(48.6)	41(58.6)	†0.861, ††0.273, †††0.235
Duration of Diabetes (Year)	-	14.3±4.0	9.7±3.5	†††< 0.001
Fasting serum glucose (mmol/L)	4.58±0.23	17.4±2.6	12.5±1.3	†< 0.001, ††<0.001, †††<0.001
HbA_{1c} (%)	5.3±0.4	9.4±1.1	7.8±0.7	†< 0.001, ††<0.001, †††<0.001

The results are expressed as number (%) and mean ± SD. † Probability of the statistical difference between Group I andGroup II; †† Probability of the statistical difference between Group I and III, ††† between Group II and III using unpaired Student t test, difference between percentages test and ANOVA *post hoc*Tuckey (HSD) test.

https://doi.org/10.15218/zjms.2017.045

Table 2 shows that the mean duration of DFS among Group III was significantly shorter than the corresponding mean of Group II and a significant a higher grading score among Group III patients. Significant higher percentage of Group II patients presented with infected diabetic foot as compared with Group III patients who showed a significantly more loss of sensation and showed previous history of amputation in 50% compared with 32.9% in Group II (*P* = 0.038). Table 3 shows that systolic and pulse pressures among Group II patients were significantly higher than healthy subjects while the systolic, diastolic, pulse and mean arterial blood pressures were significantly higher in Group III patients compared with Group I and II.

Table 2: Clinical findings of diabetic foot lesions.

	Group II (n=70)	**Group III (n=70)**	***P value***
Duration (years)	3.1±1.7	2.3±1.3	<0.001
Side			
Right	33(47.1)	32(45.7)	0.865
Left	24(34.3)	26(37.1)	0.724
Bilateral	13(18.6)	12(17.1)	0.825
Clinical findings			
Ulceration	12(17.1)	17(24.3)	0.297
Infection	22(31.4)	11(15.7)	0.028
Gangrene	22(31.4)	32(45.7)	0.082
Ulceration plus infection	14(20)	10(14.3)	0.370
Pain	48(68.6)	38(54.3)	0.083
Loss of sensory sensation	22(31.4)	40(57.1)	0.002
Absence of pulsation at dorsalis paedis and/or tibi-alis posterior	22(31.4)	31(44.3)	0.117
Grading	3.1±1.1	3.44±0.97	0.013
Previous history of amputation	23(32.9)	35(50)	0.039

The results expressed as number (%) and mean ±SD. Probability of the statistical difference was calculated by using difference between percentages and unpaired Student (t) test.

Table 3: Blood pressure measurements.

Blood pressure (mmHg)	**Group I (n=30)**	**Group II (n=70)**	**Group III (n=70)**	***P* value**
Systolic	123.6±6.1	131.2±3.5	148.9±5.0	†<0.001, ††<0.001, †††<0.001
Diastolic	75.8±2.6	75.8±3.4	90.0±4.6	†0.988, ††<0.001, †††<0.001
Pulse	47.8±6.2	55.4±3.8	58.9±5.6	†<0.001, ††<0.001, †††<0.001
Mean	91.7±2.8	94.2±3.0	109.6±4.1	†<0.001, ††<0.001, †††<0.001

The results are expressed as mean ± SD. † Probability of the statistical difference between Group I and Group II; †† Probability of the statistical difference between Group I and III, ††† between Group II and III using unpaired Student t test, difference between percentages test and ANOVA *post hoc* Tuckey (HSD) test.

https://doi.org/10.15218/zjms.2017.045

Table 4 shows that the mean value of the BMI in Group II and III were in the range of obesity and there was insignificant difference between Group II and III. The mean value of waist circumference was significantly higher among Group III compared with Group II patients and this reflected on the significant high waist-height ratio in Group III patients. Table 5 shows that the fasting serum TC, TG, and atherogenic lipids levels were significantly higher in Group III patients compared with Group I patients. Also, the fasting serum HDL-c levels were significantly low compared with Group I and II. Serum hs-CRP levels in Group II (5.73±0.89 mg/L) and Group III(12.48±2.57 mg/L) were significantly (*P* <0.001) higher than Group I (Figure 1).

Table 5: Lipid profile measurements.

Lipid profile (mg/dl)	Group I (n=30)	Group II (n=70)	Group III (n=70)	*P* value
Total cholesterol	157.4±8.7	183.9±5.5	232.0±38.4	†<0.001, ††<0.001, †††<0.001
Total triglycerides	147.1±6.4	168.5±10.1	198.6±17.7	†<0.001, ††<0.001, †††<0.001
High density lipoprotein-cholesterol	53.7±3.5	52.0±3.7	46.1±2.5	†0.038, ††<0.001, †††<0.001
Low density lipoprotein-cholesterol	74.3±9.0	98.2±6.7	146.2±39.7	†<0.001, ††<0.001, †††<0.001
Very low density lipoprotein-cholesterol	29.4±1.3	33.7±2.0	39.7±3.5	†<0.001, ††<0.001, †††<0.001

The results expressed as mean ± SD. The results are expressed as mean ± SD. † Probability of the statistical difference between Group I and Group II; †† Probability of the statistical difference between Group I and III, ††† between Group II and III using ANOVA *post hoc*Tuckey (HSD) test.

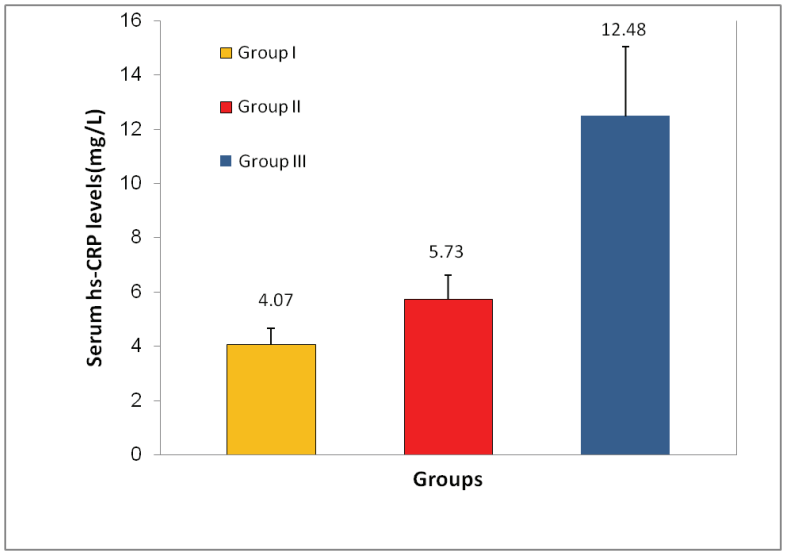

Figure 1: Significant (*P* < 0.001) high serum hs-CRP levels in Group II and Group III patients compared with Group I.

https://doi.org/10.15218/zjms.2017.045

Serum PCT levels in Group II (0.334±0.094 ng/ml) and Group III (1.11±0.47 ng/ml) were significantly (*P* <0.001) higher than Group I (Figure 2). In Group II, serum procalcitonin levels are positively and significantly correlated with HbA1c (%) and in Group III correlated significantly with fasting serum glucose (Table 6).

Table 6: correlation between cardio-metabolic risk factors and inflammatory marker with procalcitonin levels.

	Group II		Group III	
	Correlation factor (r)	*P* value	Correlation factor (r)	*P* value
Body mass index (kg/m^2)	-0.038	0.754	-0.020	0.869
Waist circumference (cm)	-0.052	0.669	0.005	0.967
Mean arterial blood pressure (mmHg)	0.000	1.000	0.030	0.805
Pulse pressure (mmHg)	+0.037	0.761	+0.193	0.109
High density lipoprotein-cholesterol (mg/dl)	-0.158	0.191	-0.027	0.824
Triglycerides(mg/dl)	-0.100	0.410	-0.097	0.424
Fasting glucose (mmol/L)	0.208	0.084	0.267	0.025
$HbA1_c$ (%)	0.336	0.004	-0.133	0.272
hs-C-reactive protein (mg/L)	0.037	0.761	0.038	0.754

The results are expressed as correlation factor (r). The *P* value represented the two tailed calculation of simple correlation test.

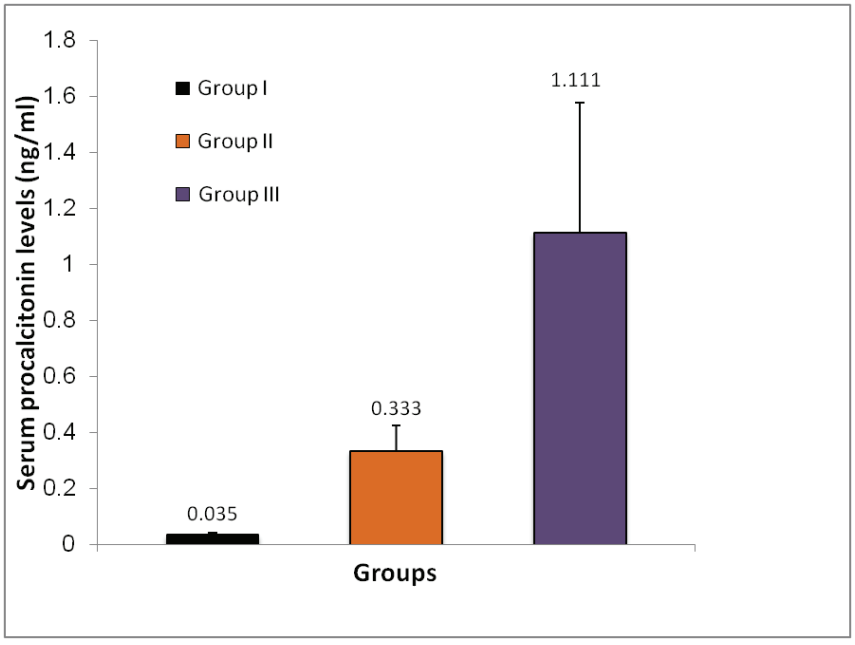

Figure 2: Significant (p < 0.001) high serumprocalcitonin levels in Group II and Group III patients compared with Group I.

https://doi.org/10.15218/zjms.2017.045

The serum levels of hs-CRP are insignificantly correlated with glycemic indices or lipid profile in Group II and III (Table 7). There was insignificant correlation between procalcitonin and hs-CRP in group II or Group III.

Discussion

The results of this study show that the serum levels of hs-CRP and PCT are significantly increased and attended higher levels among T2D patients compared with T1D. There is an insignificant correlation between serum levels of PCT with hs-CRP in both T1D and T2D. Fasting serum glucose and the glycosylated hemoglobin in T2D patients were significantly less thanT1D patients. Previous studies showed that the release of calcitonin from the adipose tissue did not trigger by the insulin like peptides which indicating no relation between glucose levels and PCT as shown in this study.[13]Therefore, the increased serum levels of PCT among T2D do not relate to the diabetic control. The increased levels of the PCT and hs-CRP are related to the grading of the DFS as this study showed that the significant high score of DFS grading among T2D compared with the T1D patients. Recent studies demonstrate that serum levels of PCT and hs-CRP are good biomarkers to distinct between infected and non-infected diabetic foot ulcer and they considered as a useful marker for diagnosis of DFS when the clinical signs are misleading.[7, 13] Radiological investigations including bones and joints X-rays do not carry based on the previous study that the changes in the serum levels of procalcitonin did not discriminate the bone infection.[14,15] Moreover, the cardio-metabolic risk factors; body mass index, waist circumference, high blood pressure and dyslipidemia were significantly found in T2D patients who had significant high PCT and hs-CRP levels compared with T1D. On the other hand, there were insignificant correlations were demonstrated between PCT or hs-CRP with cardio-metabolic risk factors except the fasting serum glucose leveland the glycosylated hemoglobin. Our findings are in agreement with other studies. Mica et al reported a significant lower incidence of sepsis in obese individuals and there

Table 7: Correlation (r) between cardio-metabolic risk factors and procalcitonin marker with hs-CRP levels.

	Group II		Group III	
	Correlation factor (r)	*P* value	Correlation factor (r)	*P* value
Body mass index (kg/m^2)	-0.139	0.251	0.002	0.986
Waist circumference (cm)	-0.057	0.639	0.066	0.587
Mean arterial blood pressure (mmHg)	-0.096	0.429	-0.039	0.748
Pulse pressure (mmHg)	0.065	0.593	-0.018	0.882
High density lipoprotein-cholesterol (mg/dl)	0.050	0.681	0.018	0.882
Triglycerides (mg/dl)	0.127	0.294	0.178	0.140
Fasting glucose (mmol/L)	0.002	0.986	0.084	0.489
HbA1c (%)	-0.048	0.693	-0.118	0.330
Procalcitonin (ng/ml)	0.037	0.761	0.038	0.754

The results are expressed as correlation factor (r). The *P* value represented the two tailed calculation of simple correlation test.

was insignificant differences in the PCT or hs-CRP between obese and non-obese individuals.[16] Abbasi et al., found that the normal range of plasma PCT levels was associated with obesity, insulin resistance and the features of the metabolic syndrome in the general population.[17]. In hypertensive patients managed with low dietary salts are more likely to have significant high PCT levels and this could explain our results that T2D patients have significant high PCT and blood pressure.[18] In severe sepsis, the lipoprotein levels are decreased by 50% and the PCT levels increased while this study shows that the picture of the lipoproteins levels is of metabolic derangement that found in diabetes mellitus.[19] Therefore, the infective theory of DFS plays a small role in the pathogenesis of DFS and the higher PCT levels are due to many causes other than sepsis. Limitations of the study are the radiological investigation of the foot bones and a microbiological study of tissue sloughing are not done. In conclusion that the simultaneous measurements of hs-CRP and PCT as inflammatory biomarkers are not necessary because there is non-significant correlation between them. The plasma levels of PCT are associated but not correlated with cardio-metabolic syndrome and such picture is significantly observed in T2D presented with DFS.

Conclusion

We conclude that the simultaneous measurements of hs-CRP and PCT as inflammatory biomarkers are not necessary because the correlation was not significant.

Conflicts of interest

The authors report no conflicts of interest.

References

1. Christ-Crain M, Muller B. Procalcitonin in bacterial infections--hype, hope, more or less? Swiss Med Wkly 2005; 135:451-60.
2. Uzun G, Solmazgul E, Curuksulu H, Turhan V, Ardic N, Top C, et al.Procalcitonin as a diagnostic aid in diabetic foot infections. Tohoku J Exp Med 2007; 213(4):305-12.
3. Meisner M. Update on procalcitonin measurements. Ann Lab Med 2014; 34(4):263-73.
4. Bouadma L, Luyt CE, Tubach F, Cracco C, Alvarez A, Schwebel C, et al. Use of procalcitonin to reduce patients' exposure to antibiotics in intensive care units (PRORATA trial): a multicenter randomised control trial. Lancet 2010; 375(9713):463-74.
5. James WB. Classification of foot lesion in diabetic patients. In: Bowker JH, Pfeifer MA, eds, Levin and O'Neal's The Diabetic Foot. 7th ed. Philadelphia: Mosby; 2008. P. 221-6.
6. Kocabaş E, Sarikçioğlu A, Aksaray N, Seydaoğlu G, Seyhun Y, Yaman A. Role of procalcitonin, C-reactive protein, interleukin-6, interleukin-8 and tumor necrosis factor-alpha in the diagnosis of neonatal sepsis.Turk J Pediatr 2007; 49(1):7-20.
7. JonaidiJafari N, SafaeeFirouzabadi M, Izadi M, SafaeeFirouzabadi MS, Saburi A. Can procalcitonin be an accurate diagnostic marker for the classification of diabetic foot ulcers? Int J Endocrinol Metab 2014; 12(1):e13376.
8. Altay FA, Sencan İ, Şentürk GÇ, Altay M, Güvenman S, Ünverdi S, et al. Does treatment affect the levels of serum interleukin-6, interleukin-8 and procalcitonin in diabetic foot infection? A pilot study. J Diabetes Complications 2012; 26(3):214-8.
9. Wang C, Gao L, Zhang ZG, Li YQ, Yang YL, Chang T, et al. Procalcitonin is a stronger predictor of long-term functional outcome and mortality than high-sensitivity C-reactive protein in patients with ischemic stroke. Mol Neurobiol 2016; 53(30):1509-17.
10. Li YM, Liu XY. Serum levels of procalcitonin and high sensitivity C-reactive protein are associated with long-term mortality in acute ischemic stroke. J Neurol Sci 2015; 352(1-2):68-73.
11. Villanueva MP, Mollar A, Palau P, Carratalá A, Núñez E, Santas E, et al. Procalcitonin and long-term prognosis after an admission for acute heart failure. Eur J Intern Med 2015; 26(1):42-8.
12. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18:499-502.
13. Boursier G, Avignon A, Kuster N, Boegner C, Leprieur E, Picandet M, et al. Procalcitonin, an Independent Marker of Abdominal Fat Accumulation in Obese Patients. Clin La 2016; 62:435-41.
14. Massara M, De Caridi G, Serra R, Barillà D, Cutrupi A, Volpe A, et al. The role of procalcitonin as a marker of diabetic foot ulcer infection. Int Wound J 2017; 14(1):31-4.
15. Mutluoğlu M, Uzun G, İpcioğlu OM, Sildiroglu O, Özcan Ö, Turhan V, et al. Can procalcitonin predict bone infection in people with diabetes with

infected foot ulcers? A pilot study.Diabetes Res Clin Pract 2011; 94(1):53-6.
16. Mica L, Vomela J, Keel M, Trentz O. The impact of body mass index on the development of systemic inflammatory response syndrome and sepsis in patients with polytrauma. Injury 2014; 45(1):253-8.
17. Abbasi A, Corpeleijn E, Postmus D, Gansevoort RT, de Jong PE, Gans RO, et al. Plasma procalcitonin is associated with obesity, insulin resistance, and the metabolic syndrome. J Clin Endocrinol Metab 2010; 95(9):E26-31.
18. Mallamaci F, Leonardis D, Pizzini P, Cutrupi S, Tripepi G, Zoccali C. Procalcitonin and the inflammatory response to salt in essential hypertension: a randomized cross-over clinical trial. J Hypertens 2013; 31(7):1424-30.
19. van Leeuwen HJ, Heezius EC, Dallinga GM, van Strijp JA, Verhoef J, van Kessel KP. Lipoprotein metabolism in patients with severe sepsis. Crit Care Med 2003; 31(5):1359-66.