ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LXIII, N 1, 37 SECTIO D 2008

[1]Department of Nephrology, [2]Department of Vascular Surgery and Angiology
[3]Interfaculty Chair and Department of Laboratory Diagnostics, Medical University of Lublin

ANNA BEDNAREK-SKUBLEWSKA[1],
IWONA BARANOWICZ-GĄSZCZYK[1], STANISŁAW PRZYWARA[2],
DARIUSZ DUMA[3], ANDRZEJ KSIĄŻEK[1]

Serum procalcitonin concentration in patients on chronic hemodialysis with inflammation of native arterio-venous fistula

Infection is one of the leading causes of vascular access failure in patients treated with hemodialysis (HD) (1). At the beginning of this process the symptoms may be unspecific and similar to other non-infectious processes (stenosis, thrombosis, hematoma). Likewise C-reactive protein (CRP) is the most current and frequently used marker of infection and when combined with white blood cell (WBC) count is useful in diagnosis and therapeutic strategy improvement. But these parameters may be affected by uremia, silent underlying disease or by HD *per se* (2). The concentrations of these parameters may be useful indicators for inflammation in patients with renal disease, but have low specificity for the diagnosis of bacterial infection.

Procalcitonin (PCT), 116 amino-acids prohormon of calcitonin is a new marker of acute bacterial or fungal infection, physiologically synthesized by the thyroid C cells (2). PCT levels are not significantly affected by the loss of renal function. The usefulness of PCT as a diagnostic parameter of infection and sepsis was demonstrated in various clinical studies (3–8). PCT is as well routinely measured to differentiate autoimmune disorders from infection. But in the opinion of Dahaba et al. (9) higher plasma of PCT of not dialyzed, uremic and nonseptic patients (pts) indicates that uremia *per se* and not the dialysis process is the origin of this rise.

The early diagnosis of native arteriovenous fistula (AVF) infection is crucial for an appropriate course of the HD treatment. In the literature there is no information regarding the analysis of serum PCT concentration (conc.) in the early phase of this clinical setting. The interesting theme is to detect if measurement of the serum PCT level may be useful in early detection of occult AVF infection, before the development of clinical symptoms.

The aim of the present study was to compare the usefulness of the measurements of serum PCT and CRP levels in differentiating a suspicion of infectious deterioration of AVF in chronic HD patients from other processes.

MATERIAL AND METHODS

The study was conducted in 33 patients with deterioration of native AVF, mean age – 62 years (range 24–89), mean duration of HD treatment – 33 months (range 1-193). The cause of the end stage renal failure in that group was: glomerulonephritis in 10 cases, diabetic nephropathy in 8 cases, interstitial nephritis in 5 cases, hypertension nephropathy in 4 cases, polycystic renal disease in 3

cases, connective tissue diseases in 3 cases. The serum levels the following parameters: PCT, CRP, transferrin, hemoglobin, iron (Fe), hemoglobin, WBC and urea were measured at the beginning of AVF deterioration, when the infectious process was suspected (Group Ia; G Ia), and repeated when time of observation and the treatment was completed (Group Ib; G Ib). The symptoms of suspected infection included the following signs: fever, swelling of arm, redness of skin, tenderness and pain during needle insertion. At the beginning of treatment the blood cultures were obtained from all patients. The ultrasonographic evaluation of AVF was not performed. During the time of the observation and treatment there was no necessity for surgical intervention regarding vascular access, but the needle insertion was changed in 20 cases and in 13 patients the temporal central catheter was implanted.

Besides, serum levels of CRP and PCT were measured in 42 stable HD patients, mean age 57 years (Group II; G II) and in 12 healthy volunteers, mean age 39 years (Control Group, CG). Serum PCT was determined using the LUMItest® PCT kit, Brahms, Berlin, Germany. The inter-assay coefficients of variation were 10% and 5.1 % at the PCT concentrations of 1.5 and 45.6 ng/ml (n=25) (10). The detection limit of the assay was 0.1 ng/ml and PCT levels of the healthy subjects were usually <0.1 ng/ml. Serum CRP was determined using the ARCHITECT® c800 System, Abbott, USA. Other parameters were determined using standard autoanalyser. Kt/V was calculated by the Daugirdas method (11) nPCR was calculated according to K/DOQI Guidelines (12).

Statistical analysis. The results were expressed as the arithmetic mean ± standard deviation (SD). To compare the groups, the U-test of Mann-Whitney was used because of the diagonal distribution of the parameters tested. Nonparametric r-Spearman test was used to define correlation. The diagonal distribution was ascertained and all the nonparametric tests were used, so these parameters were not log-transformed, $p<0.05$ was considered to be statistically significant. The area under the receiver operating characteristic curve (ROC) for PCT was assessed.

RESULTS

Biochemical characteristics of the studied HD patients and CG were presented in Table 1. The mean serum conc. of PCT was significantly higher in G Ia when compared to: CG ($p<0.0001$), G Ib ($p<0.0002$), and in G II ($p<0.007$). The mean serum conc. of PCT was significantly higher in G Ib and in G II than in CG ($p<0.0005$). The mean plasma CRP level was significantly higher in G Ia than in: CG ($p<0.0001$), G Ib ($p<0,006$), and in G II ($p<0.0005$). The mean serum conc. of CRP was significantly higher in G Ib than in CG ($p<0,001$). We did not find any significant differences in mean serum CRP conc. between G Ib and G II, but mean CRP conc. were significantly higher in G II than in CG ($p<0.0005$). The significant positive correlations (cor.) were found in G Ia between: PCT and CRP ($r=0.822$, $p<0.001$) and PCT and WBC ($r=0.474$, $p<0.005$) and negative cor. between PCT and Fe ($r= -0.537$, $p<0.006$). In G Ib positive correlation was found between PCT and CRP ($r=0.533$, $p<0.003$). The cut off value for plasma PCT conc. in pts with and without AVF infection was 1.2 ng/ml. The sensitivity of PCT for detection of AVF infection in HD patients was 66.7% and specificity 85.3%.

DISCUSSION

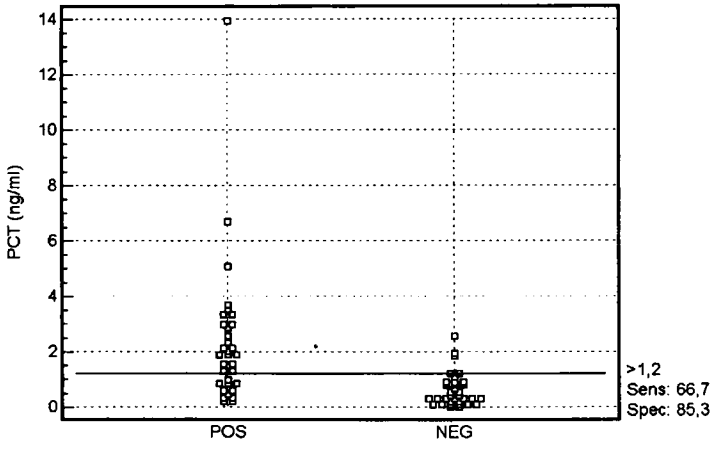
PCT is a co-factor capable to modulate various effects during endotoxin shock. The PCT concentration remains low when an infection does not lead to a systemic inflammatory response (3). It takes only around 4 hours to detect PCT in plasma after induction and serum half-life is 25–30 hrs.

Table 1. Biochemical characteristics of patients with arteriovenous fistula infection, stable hemodialysis patients with good function of vascular access and control group

Parameter	G Ia n=33	G Ib N= 33	G II n= 42	C G n=12
PCT (ng/ml)	2.5 ± 2.3	0.8 ± 0.7/***	0.9± 0.6/**/*o	0.2 ± 0.1 */*****/
CRP (mg/l)	33.1 ± 25.2	16.2 ±13.2 /°°°	13.1 ± 3.1 /°°/*°	4.0 ± 1.1 °/°°°°
Transferrin (mg/dl)	172.4 ± 63.0	177.3 ± 49.5	185.0 ± 23.2	N D
Fe (µg/dl)	48.7 ± 20.6*	62.6 ± 18.3	78.4 ±11.2	N D
Albumin (g/l)	31.3 ± 4.0*	33.3 ± 4.0 °°°°	41.1 ± 8.0	N D
Hb (g/dl)	9.3 ± 1.0*	9.9 ±1.0°°°°	11.3 ± 1.1	N D
WBC (G/l)	9.0 ± 2.3	8.9 ± 1.7	6.9 ± 1.7	N D
nPCR (g/kg/day)	1.1 ± 0.2	1.2 ± 0.3	1.0 ± 0.3	N D
Kt/V	1.2 ± 0.7	1.2 ± 0.8	1.2 ± 0.6	N D

* p< 0.0001 (G Ia vs CG) ° p< 0.0001 (G Ia vs CG)
** p< 0.007 (G Ia vs G II) °° p<0.0005 (G I a vs G II)
*** p< 0.0002 (G Ia vs G Ib) °°° p<0.006 (G Ia vs G Ib)
***** p< 0.0005 (G Ib vs CG) °°°° p<0.001 (G Ib vs CG)
*°p < 0.0005 (G II vs CG)

Fig. 1. Serum PCT concentration in HD patients with a-v infection and in patients with/without infection. The cut-off value for PCT is 1.2 ng/ml with the Sens (sensitivity) of 66.7%, and Spec (specificity) of 85.3%



PCT – procalcitonin, CRP – C-reactive protein, Hb – hemoglobin, WBC – white blood cell, nPCR – normalized protein catabolic rate, Kt/V – index of hemodialysis, Fe – iron, G Ia –hemodialysis patients with arteriovenous fistula infection, G Ib – hemodialysis patients after completion of infection treatment, G II – stable hemodialysis patients, CG – control group, N D – not done

HD would alter serum PCT concentrations independently of presence, nature or activity of infection. The HD procedure *per se* is as well partly responsible for an inflammatory reaction in uremic pts (2). Bacterial contamination during the extracorporeal circulation and bioincompatibility explain only a very small part of high prevalence of inflammation as defined by increase in CRP and PCT levels in patients treated with HD. Level et al. (13) found in HD patients with acute infection

a positive correlation between PCT and CRP. They reported that PCT was more closely related to the presence of infection that CRP, which is more representative for a chronic inflammatory status. We observed positive relations between CRP and PCT in patients at the onset of infection suspicion of AVF as well as when the treatment was completed, but after this action the correlation was not so high. Serial PCT measurements can be used to monitor disease activity in patients with sepsis and systemic inflammation. Meisner et al. (14) found that the absolute PCT concentration level more closely reflects the severity of infection and potential life threatening complications than other parameters like cytokines and CRP. Herget-Rosenthal et al. (15) suggested that serum PCT concentrations exceeding 1.5 ng/ml accurately indicated HD patients with severe infections and sepsis and discriminated these patients well from the ones without infection. We observed at the beginning of deterioration of AVF by probably acute infection that 60% of patients had PCT concentration higher than 1.5 ng/ml, but when the treatment was completed only 15% of patients had PCT level higher than normal. Likewise 16% of stable HD patients had PCT level exceeding the normal values. We suspect in that situation that the persistent high concentrations of PCT would be connected with persistent inflammation process caused by HD treatment or the underlying disease.

Albumin is negative acute phase reactant and many proinflammatory substances, elevated in HD patients, have influence on albumin synthesis. Negative correlation in HD patients between PCT and albumin was found by Sitter et al. (16). They concluded that elevated CRP, but not raised PCT was associated with increased mortality. Interesting associations between PCT and some parameters of nutritional status were found by Odamaki et al. (17). They investigated the factors that may affect liver albumin synthesis and indicate that PCT acts against the suppression of hepatic albumin synthesis caused by proinflammatory cytokines, which suggests the potential role of PCT in preventing hypoalbuminaemia in HD patients. In our study we did not observe any relations between PCT and albumin or nPCR.

Infectious and inflammatory disease commonly results in reduced serum iron (hypoferraemia). It is generally thought that inflammation alters macrophage iron homeostasis, resulting in increased iron retention and reduced iron release. Low baseline serum iron indicators are associated with increased mortality and hospitalization in HD patients independent of hemoglobin level, EPO and iron doses, indicators of nutrition and inflammation conditions (18). We found only in patients at the beginning of observation, when the suspicion of infection has taken place, a significant negative correlation between PCT and Fe.

Our data indicate that in patients with deterioration of AVF PCT is a rather early marker of inflammatory response and in this clinical setting this parameter was no more specific than CRP. We could not confirm that the abnormal function of AVF was connected with the acute bacterial infection. Before antibiotic treatment, which was applied, the blood cultures were obtained from all patients and in all cases the results were negative.

CONCLUSIONS

We observed that thanks to PCT and CRP measurements in patients with deterioration of AVF we could only monitor the activity of inflammation process. The elevated PCT concentration was found as well in stable uremic patients without any signs of acute infection.

REFERENCES

1. Hakim R., Himmelfarb I.: Hemodialysis access failure. A call to action. Kidney Int., 54, 1029, 1998.
2. Pereira J. G.: Implications of cytokine measurements during *in vitro* and clinical hemodialysis. Nephrol. Dial. Transplant., 10, 18, 1995.
3. Meisner M.: Pathobiochemistry and clinical use of procalcitonin. Clin. Chimica Acta, 323, 17, 2002.
4. Brunkhorts F. M., Heinz U., Forycki Z. F.: Kinetics of procalcitonin in iatrogenic sepsis. Intensive Care Med., 24, 888, 1998.
5. De Werre I. et al.: Cytokines, nitite/nitrate, soluble tumor necrosis factor receptors, and procalcitonin concentrations: comparisons in patients with sepsis shock, cardiogenic shock, and bacterial pneumonia. Crit. Care Med., 25, 607, 1997.
6. Assicot M et al.: High serum procalcitonin concentrations in patients with sepsis and infection. Lancet, 343, 515, 1996.
7. Bernard L., Ferrere F., Cassassus P.: Procalcitonin as an early marker of bacterial infection in severely neutropenic febrile adults. Clin. Infect. Dis., 27, 914, 1998.
8. Al.-Nawas B., Krmmer I., Shah P. M.: Procalcitonin in diagnosis of severe infections. Eur. J. Med. Res., 1, 331, 1996.
9. Dahaba A. A., Rehak P. H., List W. F.: Procalcitonin and C-reactive protein plasma concentrations in nonseptic uremic patients undergoing hemodialysis. Intensive Care Med., 29, 579, 2003.
10. Whang K. T. et al.: Serum calcitonin precursors in sepsis and systematic inflammation. J. Clin. Endocrinol. Metab., 83, 3296, 1998.
11. NKF-K/DOQI clinical practice guidelines for hemodialysis adequacy: update 2000. Am. J. Kidney Dis., 37, 7, 2001.
12. K/DOQI, National Kidney Foundation: Clinical practice guidelines for nutrition in chronic renal failure. Am. J. Kidney Dis., 35, 1, 2000.
13. Level C. et al.: Procalcitonin: a new marker of inflammation in haemodialysis patients? Nephrol. Dial. Transplant., 16, 980, 2001.
14. Meisner M. et al.: Comparison of procalcitonin (PCT) and C-reactive protein (CRP) plasma concentrations at different scores during the course of sepsis and MODS. Crit. Care, 3, 45, 1999.
15. Herget-Rosenthal S. et al.: Procalcitonin for accurate detection of infection in haemodialysis. Nephrol. Dial. Transplant., 16, 975, 2001.
16. Sitter T. et al.: Differential diagnosis of bacterial infection and inflammatory response in using procalcitonin. J. Nephrol., 15, 297, 2002.
17. Odamaki M. et al.: Counter-regulatory effects of procalcitonin and indoxyl sulphate on net albumin secretion by cultured rat hepatocytes. Nephrol. Dial. Transplant., 19, 797, 2004.
18. Kalantara-Zadeh K. et al.: A low serum level is a predictor of poor outcome in hemodialysis patients. Am. J. Kidney Dis., 43, 671, 2004.

SUMMARY

The early diagnosis in patient with end stage renal diseases receiving chronic hemodialysis (HD) of dysfunction the arterio-venous fistula which could be caused by acute bacterial infection remains difficult. The applied laboratory parameters may be affected by the underlying diseases, uremia or renal replacement therapy. Procalcitonin (PCT), the precursor of calcitonin as a new consecutive marker has been reported to increase in patients during sepsis and bacterial or fungal infection. The aim of the present study was to compare the usefulness of the measurements of serum PCT and C-reaction protein (CRP) levels in differentiating a suspicion of infectious deterioration of native arterio-venous fistula (AVF) in 33 hemodialysed (HD) patients (pts) (G Ia) from other inflammatory processes. The measurements of these parameters were also done in 42 stable HD pts (G II) and in 12 healthy volunteers as a control group (CG). The mean serum concentration (conc.) of PCT and CRP were significantly higher in G I when compared to: CG ($p<0.0001$) and in G II ($p<0.007$). The cut off value for plasma PCT conc. in pts with and without suspicion of infectious deterioration AVF was 1.2 ng/ml. The sensitivity of PCT for detection of this process in HD pts was 66.7% and specificity was 85.3%. Our data indicate that in studied group of pts PCT is a rather marker of inflammatory response and in this clinical setting no more specific than CRP. We could not confirm that the abnormal function of AVF was connected with the acute bacterial infection.

Stężenie prokalcytoniny u chorych przewlekle hemodializowanych ze stanem zapalnym dostępu naczyniowego wykonanego z własnych naczyń

Zdiagnozowanie ostrego zakażenia bakteryjnego u chorych ze schyłkową chorobą nerek leczonych przewlekle hemodializą (HD) napotyka na trudności ze względu na niespecyficzność oznaczanych rutynowo parametrów biochemicznych, których synteza ulega także zwiększeniu w przebiegu zastosowanej terapii nerkozastępczej, mocznicy jak i pierwotnej choroby nerek. Wykorzystanie do oznaczeń nowego białka diagnostycznego – prokalcytoniny (PCT), specyficznego dla ostrego zakażenia oraz sepsy, pozwala na wczesne rozpoznanie rozwijającej się infekcji oraz zastosowanie prawidłowej terapii. W pracy analizowano zachowanie się stężeń PCT oraz białka C-reaktywnego (CRP) w surowicy 33 chorych leczonych przewlekle HD (G I), z podejrzeniem zakażenia bakteryjnego jako możliwej przyczyny dysfunkcji zespolenia tętniczo-żylnego wykonanego z własnych naczyń. Oznaczenia PCT oraz CRP wykonane były również u 42 stabilnych hemodializowanych chorych (GII) oraz w grupie kontrolnej osób zdrowych (GK). Przeprowadzone badania nie pozwoliły na rozpoznanie ostrego zakażenia bakteryjnego jako przyczyny występującej dysfunkcji dostępu naczyniowego. W obu badanych grupach chorych przewlekle hemodializowanych stężenia PCT oraz CRP były znamiennie podwyższone w porównaniu z grupą kontrolną. Występujący wzrost istotny stężenia PCT u chorych z dysfunkcją dostępu naczyniowego mógł być spowodowany zapaleniem o wieloczynnikowej etiologii.