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ORIGINAL ARTICLE

Relationship between renalase and N-terminal pro-B-type Natriuretic Peptide (NT pro-BNP) in haemodialysis patients

Marcin Dziedzic[1], Beata Petkowicz[2], Anna Bednarek-Skublewska[3], Janusz Solski[1], Agnieszka Buczaj[4], Piotr Choina[5]

[1] *Department of Laboratory Diagnostic, Medical University of Lublin, Poland*
[2] *Oral Medicine Independent Unit, Medical University of Lublin, Poland*
[3] *Department of Nephrology, Medical University of Lublin, Poland*
[4] *Institute of Rural Health, Lublin, Poland*
[5] *Department of Public Health, Institute of Rural Health, Lublin, Poland*

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Abstract

Introduction. Our knowledge in the field of cause of deaths in dialysis patients is rapidly expanding, yet we still do not fully understand how renalase regulates the processes of cardiovascular disease developing in end-stage renal disease. Increased sympathetic nerve activity observed in chronic kidney diseases due to raised catecholamines in plasma results from the absence of renalase. Renalase synthesized and secreted by the kidneys participate in the regulation of sympathetic tone and blood pressure. A family of natriuretic peptides has been identified – NT pro-BNP – which seems to be the best predictor of clinical outcome and marker of extracellular fluid overload, as well as predicting mortality, irrespective of renal function.

Objective. The aim of the presented study was to investigate renalase concentration and investigate associations between NT-proBNP, as well as analyzed parameters in haemodialysis patients.

Materials and method. The study was conducted among residents of the municipality and neighbouring villages in the province of Lublin, central-eastern Poland. 49 male subjects on haemodialysis, aged 65.3 ± 14.2 years, median time on haemodialysis: 37.5 months, were included. All study subjects underwent haemodialysis 3 times a week. The mean concentration of renalase in the entire study population was 126.59 ± 32.63 ng/mL. The circulating levels of NT-proBNP was 813.64 ± 706.96 pg/mL. A significant inverse correlation was found between NT-proBNP and renalase plasma levels ($R = -0.3$, $P = 0.03$).

Conclusions. Inverse correlation between NT-proBNP and renalase plasma levels in haemodialysis patients were due to impaired kidney function, accompanied by increased sympathetic nerve activity, which have an impact on the development of hypertension and cardiovascular complications.

Key words

Renalase, N-terminal pro-B-type natriuretic peptide (NT-proBNP), haemodialysis, cardiovascular disease

INTRODUCTION

Our knowledge in the field of the cause of deaths in dialysis patients are rapidly expanding, yet we still do not fully understand the problem of high mortality in patients on dialysis. The major cause of death in haemodialysis (HD) patients is cardiovascular disease (CVD), which constitute 53% of causes of death in Poland [1]. In addition to traditional risk factors for CVD, such as hypertension – diabetes and lipid disorders are also responsible. Other determinants are, e.g. anaemia, volume overload of the circulatory system associated not only with renal failure (RF), but also the presence of arteriovenous anastomosis and rapid changes in blood volume during HD, chronic systemic inflammation, accumulation of uraemic toxins and disorders of calcium and phosphate. Most patients starting dialysis are hypertensive, suggesting that blood pressure (BP) control is an important target for reducing cardiovascular mortality [2]. Catecholamines (CA) are able to regulate many biological processes and are closely related to regulating blood pressure. Increased sympathetic nerve activity observed in chronic kidney diseases, is due to raised CA in plasma resulting from inhibition of nitric oxide, followed by increased angiotensin II and the absence of renalase, as well as reduced CA clearance in the kidneys. Moreover, raised sympathetic activity is now recognized as an important mechanism involved in cardiovascular complication in humans [3]. Importantly, CVD disease is a major complication in patients with chronic kidney disease (CKD) and has a main impact on morbidity and life expectancy of adults. Myocardial disease in CKD patients, as part of the more generalized cardiovascular disorder, is manifested as left ventricular hypertrophy (LVH), diastolic dysfunction and, to a lesser extent, systolic dysfunction [4].

There are multiple factors involved in the control of BP and CVD developing in ESRD. Renalase is a protein made of 342 amino acids. Recently discovered as a new renal hormone, renalase – flavin adenine dinucleotide (FAD)-dependent amine oxidase [5], is secreted by the kidneys and metabolizes circulating CA [6], as well as perhaps playing a role in the regulation of sympathetic tone and blood pressure. It has a significant haemodynamic effect; therefore, it is likely to participate in the regulation of

Address for correspondence: Marcin Dziedzic, Department of Laboratory Diagnostic, Medical University of Lublin, Chodźki 1, Lublin, Poland
e-mail: marcin8303@interia.eu

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cardiovascular function, although its exact mechanism remains unclear. Current studies indicate that the plasma renalase levels are decreased in patients with CKD and ESRD. This is particularly significant as the abnormal regulation of catecholamine metabolism contributes to the pathogenesis of LVH, ventricular arrhythmia, myocardial ischemia (MI) and myocardial necrosis [7]. Growing evidence suggests that renalase may contribute to the development of cardiac dysfunction.

B-type natriuretic peptides (BNP) belong to the natriuretic peptide family, which is comprised of 4 peptide hormones. BNP is secreted predominantly by cardiomyocytes from the left ventricle. The hormone BNP is cleaved from its prohormone proBNP, leaving a biologically-inactive fragment – N-terminal (NT)-proBNP. Both BNP and N-terminal pro-B-type natriuretic peptide (NT-proBNP) are regarded as markers of myocardial stress, and considered to be good diagnostic and prognostic markers in patients with heart failure [8,9]. In the family of natriuretic peptides, NT-proBNP seems to be the best predictor of clinical outcome and the best marker of extracellular fluid overload. NT-proBNP has become an important tool for diagnosing left ventricular dysfunction and predicting mortality, irrespective of renal function in adults. However, in patients with CKD, their use is confounded by concomitant volume overload and reduced renal excretion [10]. Nevertheless, NT-proBNP is a part of the routine for cardiovascular risk assessment in HD patients.

Taking all these observations together, the aim of the presented study was to investigate renalase concentration and explore associations with NT-proBNP, as well as analyze parameters in haemodialysis patients.

MATERIALS AND METHOD

The study was conducted among residents of the municipality and neighbouring villages in the province of Lublin, central-eastern Poland. 49 male subjects on haemodialysis, aged 65.3 ± 14.2 years, median time on HD: 37.5 months, were included. ESRD results: diabetic nephropathy (14 patients), glomerulonephritis (12 patients), hypertensive nephropathy (9 patients), connective tissue disease (5 patients), and polycystic kidney disease (3 patients). In 6 patients, the underlying cause remained undetected. In 28 patients, concomitant ischemic heart disease were diagnosed, while 21 patients were diagnosed with heart failure and qualified as NYHA II (6 patients) and III (15 patients) functional class (New York Heart Association). The study protocol was approved by the local Ethics Committee at the Medical University in Lublin. Written informed consent was obtained from each patient qualified to participate in the study. All patients underwent a clinical examination at the Department of Nephrology of the Medical University in Lublin.

All study subjects underwent HD 3 times a week. Each procedure lasted from 3.5 – 4.5 hours. Low-flux dialyzers were used; bicarbonate dialysate contained a calcium concentration of 1.5 mmol/ml. A dialyzer blood flow rate ranged between 230 – 400 ml/min, while the dialysate flow rate was 500 ml/min.

Blood samples were obtained from the venous part of the vascular access prior to HD. Standard phlebotomy techniques were used to obtain samples. The samples were collected in chilled tubes (EDTA) at 4 °C. Plasma was separated and stored at -80 °C until the assay. In the plasma, concentration of interleukin 6 (IL-6), high-sensitivity C-reactive protein (hs-CRP), NT-proBNP, albumin, and renalase were measured prior HD. Similarly, haematology testing was carried out with the blood. Moreover, several scores and parameters were calculated for each patient: comorbidity score (CS) according to the scale published by Charlson et al. [11]. HD adequacy was expressed by Kt/V parameter [12], normalized protein catabolic rate [13], body mass index (BMI), and mean arterial pressure (MAP). MAP was calculated on the basis of blood pressure measurement taken prior to HD in a horizontal position. The following formula was used: MAP = diastolic pressure + ⅓ (systolic pressure-diastolic pressure). The following biochemical parameters were measured using an enzyme-linked immunosorbent assay (ELISA): interleukin 6 (IL-6) and renalase (USCN Life Science, Inc.). NT-proBNP was measured by an electrochemiluminescence immunoassay using a Cobas 6000 system with e601 module (Roche Diagnostics, Mannheim, Germany).

Statistical analysis. All values were expressed as the mean and standard deviations. Distributions of the analyzed variables were tested using the Shapiro-Wilk test. Statistical relationship between the 2 variables was investigated using Spearman`s correlation coefficient R. In all tests, P-value <0.05 was considered statistically significant for differences or correlations. All statistical analyses were conducted using Statistica 10.0 software.

RESULTS

The main demographic and clinical characteristics of the patients included in the study are detailed in Table 1. The mean concentration of renalase in the entire study population was 126.59 ± 32.63 ng/mL. Correlations between renalase and different parameters are presented in Table 2. As a result

Table 1. Demographic and biochemical characteristics of patients on long-term dialysis

Parameter	Mean ± Standard deviation	Median	Range
age [years]	65.3 ± 14.2	68	45 – 92
time on HD [months]	40 ± 43.2	32.5	22 – 394
Kt/V	1.56 ± 0.23	1.62	1.01 – 1.88
BMI [kg/m^2]	25.28 ± 4.27	23.9	20.01 – 32.9
nPCR [g/kg/day]	1.03 ± 0.24	1.08	0.68 – 1.49
MAP [mm/Hg]	85.27 ± 11.79	83.00	70.0 – 106.0
NT-proBNP [pg/mL]	813.74 ± 706.96	765.91	64.97 – 3189.49
IL-6 [pg/mL]	5.33 ± 2.2	5.85	1.82 – 9.57
hs-CRP [mg/l]	6.74±7.25	4.1	0.38 – 23.0
haemoglobin [g/dL]	10.36 ± 1.39	10.5	7.5 – 13.8
albumin [g/dl]	3.76 ± 0.26	3.8	3.3 – 4.3
renalase [ng/mL]	126.59 ± 32.63	127.96	30.86 – 214.07
cholesterol [mg/dl]	208.16 ± 43.85	199.0	138.0 – 288.0
triglycerides [mg/dl]	139.27 ± 44.18	132.0	51.0 – 234.0
LDL-cholesterol [mg/dl]	125.65 ± 37.21	110.0	76.0 – 204.0
HDL-cholesterol [mg/dl]	49.88 ± 12.06	50.0	25.0 – 73.0
Comorbidity score	5.61 ± 2.11	5.5	2.0 – 11.0

Table 2. Correlations between renalase and analyzed parameters

Parameter	Spearman R	P
MAP	0.43	0.04
Kt/V	–0.31	0.19
Time on HD	–0.18	0.04
IL-6	0.49	0.04
Albumin	0.69	0.008
hs-CRP	0.12	0.61
NT-proBNP	-0.3	0.03

of the conducted study, a significant inverse correlation was found between NT-proBNP and renalase plasma levels (R = -0.3; P = 0.03 (Fig. 1). Moreover, a positive correlation was noted between renalase and MAP (R = 0.43, P = 0.04), IL-6 (R = 0.49, P = 0.04), and albumin (R = 0.69; P = 0.008).

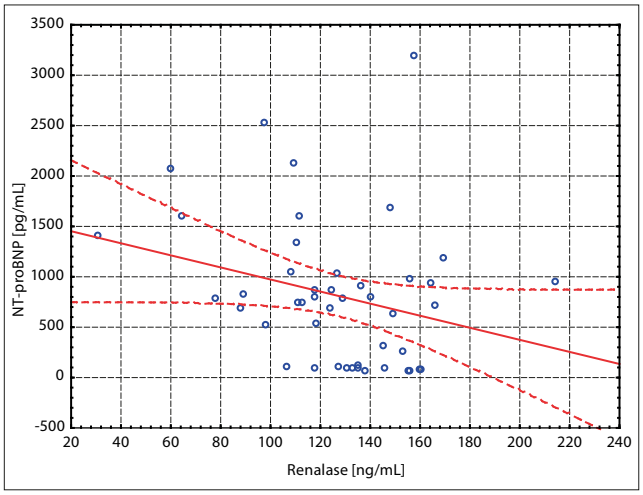

Figure 1. Correlation between levels of renalase and NT-proBNP. Solid Line – Regresion Line; Dotted lines – 95% confidence intervals

In the presented study, the duration of long-term HD treatment did not influence the concentration of renalase (P = 0.46). Furthermore, no correlation could be found between renalase and Kt/V (P = 0.19), or any of the tested parameters. The circulating level of NT-proBNP was 813.64 ± 706.96 pg/mL. NT-proBNP correlations were found to be statistically significant (Tab. 3). NT-proBNP was inversely related to haemoglobin (R = – 0.38; P = 0.0001), and albumin (R = – 0.22; P = 0.03). NT-proBNP levels positively correlated with time on dialysis (R = 0.31; P = 0.002), MAP (R = 0.2; P = 0.04), IL-6 (R = 0.44; P = 0.001). However, the NT-proBNP did not correlate with Kt/V (P = 0.36) and hs-CRP (P = 0.53).

Table 3. Correlations between NT-proBNP and analyzed parameters

Parameter	**Spearman R**	**P**
MAP	0.2	0.04
Kt/V	0.09	0.36
Haemoglobin	–0.38	0.0001
Albumin	–0.22	0.03
IL-6	0.44	0.0001
Time on HD	0.31	0.002
hs-CRP	0.15	0.53
Renalase	–0.3	0.03

DISCUSSION

Patients with CKD represent a population not only at risk for progression to ESRD, but also at even greater risk for CVD. In addition to traditional risk factors, patients with CKD may have other risk factors for in increase cardiovascular risk, such as inflammation, oxidative stress, anaemia, metabolic disorders, calcium-phosphorous disorders, hypervolaemia, and structural and functional abnormalities of the heart, which may help to explain the high cardiovascular morbidity and mortality. Recently, it has been suggested that renalase is a novel, soluble monoamine oxidase that regulates cardiac function and blood pressure [14]. It might contribute to the pathophysiology of human heart failure via the increased CA with impaired signaling pathway, and focused to be related to heightened cardiovascular risk in HD patients. A recent study by Wu et al. [15] provides information that cardiac renalase deficiency may contribute to the increased susceptibility to myocardial injury due to ischemia and rhythm disturbances frequently found in CKD.

In the presented study, in HD patients it was noticed that the plasma concentration of renalase was more markedly reduced than the mean plasma renalase (386.0 ± 7.3 ng/mL) control group in the study presented by Zbroch and Małyszko [16]. Thus, it is possible that lower plasma renalase levels lead to the heightened sympathetic tone observed in ESRD patients, and that renalase administration may decrease the incidence of cardiovascular complication and improve patients survival. Renalase degrades circulating CA, causing a significant fall in blood pressure. In the presented study, it was observed that renalase expression correlated with MAP, suggesting that renalase plays a role in the development of hypertension, and thus directly or indirectly contributing to renal injury. The study also showed that there were elevated levels of cardiac biomarker NT-proBNP in the studied patients, with a significant inverse correlation to renalase levels in plasma. Focusing on these the negative correlations, the outcome of the current study supports the hypothesis renalase could play a role in heightened cardiovascular risk. This finding can be explained by the fact that the NT-proBNP are hormones released in response to volume expansion, increased wall stretch, and high ventricular end diastolic pressure, as well as high filling pressure.

NT-proBNP is released from myocytes in response to ventricular wall stench and wall tension. The presented study shows that there were elevated levels of cardiac biomarker NT-proBNP in the studied patients, with a significant an inverse correlation with renalase levels in plasma. NT-proBNP is released from myocytes in response to ventricular wall stench and wall tension. The diagnostics and prognostic role of NT-proBNP have been well established in a variety of cardiac diseases. Most investigators have excluded CKD patients from their study because of potentially elevated levels of the peptide. Thus, measurement of NT-proBNP in HD patients may by challenging because their concentration can be substantially modified by the HD session. The presented study demonstrated that in haemodialysis patients the levels of NT-proBNP were increased in HD patients due to impaired glomerular filtration rate (GFR). These results were in agreement with Austin et al. [17] and Spanaus et al.[18] who stated that the level of NT-proBNP was significantly increased with more deterioration in kidney function. In addition, the degree of change in NT-proBNP is also dependent on the left

ventricular ejection fraction, which may reflect an increased volume overload of the heart as a consequence of volume expression due to restricted GFR.

It is interesting to note that in research by Tagore et al. [19], the NT-proBNP levels were significantly affected by hypertension and the haemoglobin level. Uraemic toxins, hypothyroidism, hypersplenism and ongoing infection can reduce the erythrocyte life span, leading to contributing to renal anaemia. Anaemia has been shown to be significant associated with left ventricular hypertrophy in dialysis patients. Therefore, anaemia can be an important novel risk factor in CKD patients. The present findings are in agreement with Saha [20], Afshar [21] and Ijoma et al. [22], who stated a high prevalence of anaemia in CKD patients. In agreement with data from medical literature, that albuminuria in CKD may represent diffuse endothelial damage, and hence is considered as novel cardiac risk factor, it can therefore be considered as cardiac biomarker in CKD patients [23, 24]. In the presented study, the majority of patients had hypoalbumenaemia. Hypoalbuminaemia may be due to albuminuria and/or nutritional deficiency which may lead to malnutrition, infection and atherosclerosis, and predispose to cardiovascular events in CKD. Moreover, inflammation in ESRD patients is a multi-factorial problem. Both dialysis-related and dialysis-independent factors may promote inflammation by stimulating the synthesis and/or release of several pro-inflammatory cytokines, such as CRP, IL-1, IL-6, and Tumour Necrosis Factor (TNF-α); [25]. Circulating levels of NT-proBNP were directly and significantly related to IL-6, indicating that inflammation and endothelial dysfunction are parallel processes in ESRD.

CONCLUSIONS

In conclusion, the results of the presented study demonstrate an inverse correlation between NT-proBNP and renalase plasma levels in haemodialysis patients. Indeed, elevated renalase levels in HD patients may be due to impaired kidney function, and may have an impact on the development of hypertension and cardiovascular complications. It is hypothesised that abnormalities in the renalase pathway and high concentration of CA with NT-proBNP in blood contribute to the heightened cardiovascular risk observed in HD patients. Therefore, it can be stated that apart from having an action on physiological blood pressure regulation, increasing evidence suggests a role for renalase in the development of pathophysiological states of the cardiovascular system in renal failure. It is worth pointing out that renalase may provide the missing link between hypertension and cardiovascular disease in haemodialysis patients. Further studies are needed to prove or disprove the possible role of renalase in the pathogenesis of hypertension in haemodialysis patients.

REFERENCES

1. Rutkowski B, et al. Report On The Renal Replacement Therapy In Poland – 2007 Gdańsk 2009. p.1–122 http://www.nephro-quest.org (access: 17.03. 2014).
2. Agarwal R, Nissenson AR, Batlle D, Coyne DW, Trout JR, Warnock DG. Prevalence, treatment, and control of hypertension in chronic hemodialysis patients in the United States. Am J Med. 2003; 115(4): 291–297.
3. Sarnak MJ, Levey AS. Epidemiology, diagnosis, and management of cardiac disease in chronic renal disease. J Thromb Thrombolysis. 2000; 10(2): 169–180.
4. Schärer K, Schmidt KG, Soergel M. Cardiac function and structure in patients with chronic renal failure. Pediatr Nephrol. 1999; 13(9): 951–965.
5. Xu J, Li G, Wang P, Velazquez H, Yao X, Li Y, et al. Renalase is a novel, soluble monoamine oxidase that regulates cardiac function and blood pressure. J Clin Invest. 2005;115(5): 1275–1280.
6. Li G, Xu J, Wang P, Velazquez H, Li Y, Wu Y, Desir GV. Catecholamines regulate the activity, secretion, and synthesis of renalase. Circulation. 2008; 117(10): 1277–1282.
7. Xu J, Desir GV. Renalase, a new renal hormone: its role in health and disease. Curr Opin Nephrol Hypertens. 2007; 16(4): 373–378.
8. Rinat C, Becker-Cohen R, Nir A, Feinstein S, et al. B-type natriuretic peptides are reliable markers of cardiac strain in CKD pediatric patients. Pediatr Nephrol. 2012; 27(4): 617–625.
9. Bettencourt P, Azevedo A, Pimenta J, Friões F, Ferreira S, Ferreira A. N-terminal-pro-brain natriuretic peptide predicts outcome after hospital discharge in heart failure patients. Circulation. 2004; 110(15): 2168–2174.
10. David S, Kümpers P, Seidler V, Biertz F, Haller H, Fliser D. Diagnostic value of N-terminal pro-B-type natriuretic peptide (NT-proBNP) for left ventricular dysfunction in patients with chronic kidney disease stage 5 on haemodialysis. Nephrol Dial Transplant. 2008; 23(4): 1370–1377.
11. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987; 40(5): 373–383.
12. Daugirdas JT. Second generation logarithmic estimates of single-pool variable volume Kt/V: an analysis of error. J Am Soc Nephrol. 1994; 4(5): 1205–1213.
13. Kopple JD; National Kidney Foundation K/DOQI Work Group. The National Kidney Foundation K/DOQI clinical practice guidelines for dietary protein intake for chronic dialysis patients. Am J Kidney Dis. 2001; 38: 68–73.
14. Xu J, Li G, Wang P, Velazquez H, Yao X, Li Y, et al. Renalase is a novel, soluble monoamine oxidase that regulates cardiac function and blood pressure. J Clin Invest. 2005; 115(5): 1275–1280.
15. Wu Y, Xu J, Velazquez H, Wang P, Li G, Liu D, Sampaio-Maia B, et al. Renalase deficiency aggravates ischemic myocardial damage. Kidney Int. 2011; 79(8): 853–860.
16. Zbroch E, Malyszko J, Malyszko JS, Koc-Zorawska E, Mysliwiec M. Renalase, a novel enzyme involved in blood pressure regulation, is related to kidney function but not to blood pressure in hemodialysis patients. Kidney Blood Press Res. 2012; 35(6): 395–399.
17. Austin WJ, Bhalla V, Hernandez-Arce I, Isakson SR, Beede J, et al. Correlation and prognostic utility of B-type natriuretic peptide and its amino-terminal fragment in patients with chronic kidney disease. Am J Clin Pathol. 2006;126(4): 506–512.
18. Spanaus KS, Kronenberg F, Ritz E, Schlapbach R, et al. B-type natriuretic peptide concentrations predict the progression of nondiabetic chronic kidney disease: the Mild-to-Moderate Kidney Disease Study. Clin Chem. 2007; 53(7): 1264–1272.
19. Tagore R, Ling LH, Yang H, et al. Natriuretic peptides in chronic kidney disease. Clin J Am Soc Nephrol. 2008; 3(6): 1644–1651.
20. Saha M, Faroque MO, Alam KS, Alam MM, Ahmed S. Chronic kidney disease specific cardiovascular risk factors among non dialytic patients with chronic kidney disease stage-V--an experience of a specialized hospital. Bangladesh Med Res Counc Bull. 2012; 38(1): 18–22.
21. Afshar R, Sanavi S, Salimi J, Ahmadzadeh M. Hematological profile of chronic kidney disease (CKD) patients in Iran, in pre-dialysis stages and after initiation of hemodialysis. Saudi J Kidney Dis Transpl. 2010; 21(2): 368–371.
22. Ijoma C, Ulasi I, Ijoma U, Ifebunandu N. High prevalence of anemia in predialysis patients in Enugu, Nigeria. Nephrology Reviews, 2010; 2e14: 61–65.
23. Effat A.E. Tony, Mohamed M.A. Ashmawy, Soheir M, et al. Prognostic Values of N-Terminal-Pro Brain Naturetic Peptide and Myocardial Perfusion Single Photon Emission as diagnostic tools for Asymptomatic Cardiac Events in Chronic kidney Disease. Journal of American Science 2012; 8(12): 1037–1056.
24. Vanzetto G, Jacon P, Calizzano A, Neuder Y, Faure P, Fagret D, Machecourt J. N-terminal pro-brain natriuretic peptide predicts myocardial ischemia and is related to postischemic left-ventricular dysfunction in patients with stable coronary artery disease. J Nucl Cardiol. 2007; 14(6): 835–842.
25. Tripepi G, Mattace Raso F, Sijbrands E, Seck MS, et al. Inflammation and asymmetric dimethylarginine for predicting death and cardiovascular events in ESRD patients. Clin J Am Soc Nephrol. 2011; 6(7): 1714–1721.