SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

Changes of PON1 paraoxonase and lactonase activities in hemodialysis and renal transplant patients

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Introduction

Cardiovascular diseases are the major cause of morbidity and mortality in chronic kidney disease (CKD) patients. Previouly have been suggested that traditional risk factors alone might not explain the higher prevalence and incidence of cardiovascular diseases in hemodialyzed (HD) and renal transplant (TRX) patients. Non-traditional risk factors, such as inflammation, oxidative stress and malnutrition are gaining acceptance in CKD especially in HD patients.

Most of the HD patients have some degree of malnutrition; protein and energy depletion have been associated with increased morbidity and mortality in this population. Numerous studies have shown that markers of malnutrition and inflammation, such as low body mass index (BMI), elevated C-reactive protein (CRP) and increased plasma concentration of asymmetric dimethylarginine (ADMA), were strong independent predictors of cardiovascular mortality in CKD.

In the last decades, several studies have published this unexpected and paradoxical phenomenon in patients with chronic heart disease referred to as „reverse epidemiology" suggested that higher BMI is associated with improved survival in overweight and obese CKD patients on maintenance hemodialysis compared to the general population both CRP as a marker of inflammation and albumin as a marker of nutritional status have been shown to be important independent predictors of mortality. Therefore, it is conceivable that both malnutrition and inflammation – referred to as the malnutrition-inflammation complex syndrome - may be related to the reverse epidemiology.

Human serum paraoxonase (PON1) is the most potent high-density lipoprotein (HDL)-associated antioxidant enzyme which prevents low-density lipoprotein (LDL) from lipid peroxidation. PON1 is able to hydrolyze a number of substrates,

such as paraoxon and phenylacetate. Previous data, however, suggest that this enzyme can also hydrolyze various esters, thioesters, carbonates and thiolactones via its lactonase activity. A possible physiological substrate is homocysteine thiolactone, which is a known risk factor in atherosclerosis, because metabolic conversion of homocysteine to thiolactone and protein homocysteinylation by thiolactone may play a role in homocysteine-induced vascular damage. Previous studies have demonstrated decreased PON1 paraoxonase activity in HD and TRX patients; however lactonase activity has not been investigated yet.

Hyperhomocysteinemia and dyslipidemia are well known cardiovascular risk factors in hemodialyzed patients. In a previous study we found that homocysteine level correlated negatively with PON1 activity in hemodialyzed and renal transplant patients.

Both obesity and chronic inflammation enhance oxidative stress that leads to the oxidative modification of lipoprotein particles resulting in accelerated atherogenesis. In our previous work, we reported lower PON1 paraoxonase activity in HD patients. Previous studies have also demonstrated that PON1 paraoxonase activity was inversely associated with cardiovascular risk; serum PON1 paraoxonase activity was shown to correlate negatively with the degree of oxidative stress.

Adipose tissue possesses various functions as energy storage and secretion of a number of adipocytokines including leptin, adiponectin with potential endocrine functions. Adiponectin levels have been found to be decreased in obesity and increased during weight loss. It has been demonstrated that adiponectin has potential anti-inflammatory and anti-atherogenic properties due to its modulatory effect on endothelial adhesion molecules acting as protective factor

against atherogenesis. Serum leptin levels have been previously reported to be elevated in patients with chronic renal failure and to correlate with C-reactive protein levels suggesting that inflammation may be an important factor in the development of hyperleptinemia in CKD.

Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, is reported to increase in patients with CRF and was suggested to play a role in the pathogenesis of accelerated atherosclerosis. Various studies document that ADMA seems to be not only a cause of endothelial dysfunction, but also a predictor of the cardiovascular outcome in ESRD patients on hemodialysis. Other investigators have shown that the decrease in the ADMA level may contribute to improved vascular and endothelial functions after renal transplantation.

Study designs

The purpose of our study was to examine the changes in PON1 lactonase activity in HD and TRX subjects. We also assumed that if ADMA is a marker of oxidative stress, the lower PON1 paraoxonase activity may be associated with higher ADMA levels. Therefore, the aim of our study was to determine both the PON1 paraoxonase and lactonase activities in HD and TRX patients, and to clarify the relationship between PON1 lactonase activity and a set of cardiovascular risk factors.

Furthermore, we hypothesized that the changes in PON1 paraoxonase and lactonase activity in HD patients are associated with their nutritional status and may contribute to the increased risk of accelerated atherosclerosis in CKD. Therefore, the other aim of our study was to determine PON1 paraoxonase and lactonase activities, ADMA, adiponectin, leptin concentrations and to reveal the

relationship between paraoxonase activity and cardiovascular risk factors in malnourished and obese HD patients.

Patients and methods

Study population

In the first part of our study we included 78 kidney transplant patients, 108 patients treated with chronic hemodialysis and 63 healthy controls. Transplanted patients received combined immunosuppressive therapy (cyclosporine or tacrolimus, azathioprine or mycophenolate mofetil, methylprednisolone). Patients with renal insufficiency had 4 hour sessions of hemodialysis therapy three times per week. The mean dialysis time was 49±28 months. In the second part we compared 114 patients receiving hemodialysis three times weekly between 2005 and 2009. According to their BMI, patients were divided into three goups: malnourised (BMI<20 kg/m^2), normal and overweight (20 kg/m^2≤BMI≤30 kg/m^2) and obese (BMI>30 kg/m^2) groups. We excluded patients with alcoholism, liver disease, elevated liver enzymes, recent myocardial infarct (38,2% of hemodialyzed and 25,3% of transplanted patients have a previous history of angina pectoris or ischaemic heart disease), endocrine diseases (thyroid and parathyroid diseases, pituitary and adrenal gland disorders, etc.), pregnancy, lactation, patients on lipid-lowering therapy and smokers.

Blood sampling

After 12 hours of fasting, 10 ml venous blood sample was taken between 7.30 and 8.00 in the morning before dialysis. Lipid parameters, homocysteine and cystatin C concentrations were determined in fresh sera. The sera for enzyme

activity measurements and for ELISA determinations were kept at – 70^{o}C before analysis.

Measurement of homocysteine and lipid parameters

Fasting plasma total Hcy concentrations were determined by enzyme-linked immunoassay and automated fluorescence polarization analyzer (FPIA, IMX System, Abbott Diagnostics, Rome, Italy).

Plasma cystatin C measurements were performed by latex enhanced reagent (N Latex Cystatin C, Dade Behring, Deerfield, IL, USA) using a Behring BN ProSpec analyzer (Dade Behring) and calibrators from Dade Behring. The method was a fully automated particle-enhanced nephelometric immunoassay.

Serum cholesterol and triglyceride levels were measured by using enzymatic, colorimetric tests (GPO-PAP, Modular P-800 Analyzer, Roche/Hitachi), while high-density lipoprotein cholesterol (HDL-C) was assessed by a homogenous, enzymatic, colorimetric assay (Roche HDL-C plus 3rd generation). LDL-cholesterol was measured by homogenous, enzymatic, colorimetric assay (Roche LDL-C plus 2rd generation, Basel, Switzerland).

Analysis of PON1 paraoxonase activity

PON1 paraoxonase activity was measured by using paraoxon (O,O-diethyl-O-p-nitrophenylphosphate, Sigma) as substrate, and the generation of 4-nitrophenol was measured spectrophotometrically. 50 µl serum was dissolved in 1 ml Tris/HCl buffer (100 mmol/l, pH=8.0) containing 2 mmol/l CaCl2 and 5.5 mmol/l paraoxon. We measured the absorbance at 412 nm (25 oC), using a Hewlett-Packard 8453 UV-visible spectrophotometer. Enzyme activity was calculated using the molar extinction coefficient 17100 M-1cm-1. One unit of paraoxonase activity is defined

as 1 nmol of 4-nitrophenol formed per minute under the assay conditions mentioned above.

Analysis of PON1 arylesterase activity

Arylesterase activity was measured spectrophotometrically. The assay contained 1 mM phenylacetate in 20 mM Tris/HCl pH 8.0. The reaction was started by the addition of the serum and the increase in absorbance was read at 270 nm. Blanks were included to correct the spontaneous hydrolysis of phenylacetate. Enzyme activity was calculated using a molar extinction coefficient of 1310 M−1cm−1. 1 unit (U) is defined as 1 µmol phenylacetate hydrolyzed per minute.

Analysis of PON1 lactonase activity

PON1 lactonase activity was measured by a commercially available assay kit (Alfresa Auto HTLase; Alfresa Pharma Corporation, Japan). This kit utilizes gamma-thiobutyrolactone as substrate and Ellman's procedure to monitor the accumulation of free sulfhydryl groups via coupling with 5,5-dithiobis(2-nitrobenzoic acid).

Paraoxonase Genotyping

PON1–55 and PON1–192 polymorphisms were determined using Light Cycler real-time technology based on fluorescence resonance energy transfer combined with melting point analysis.

ADMA measurement

ADMA concentrations in serum of hemodialyzed patients were measured with commercially available ELISA kit (ADMA- ELISA, DLD Diagnostika GmbH, Hamburg,

Germany). ADMA concentrations in samples were measured by a competitive enzyme immunoassay.

Adiponectin and Leptin Measurement

Total adiponectin and leptin concentrations in serum of hemodialyzed patients were measured with commercially available ELISA kits (Human Total Adiponectin/Acrp30 Quantikine and Human Leptin Quantikine Immunoassays, R&D Systems, Minneapolis, USA; ADMA- ELISA, DLD Diagnostika GmbH, Hamburg, Germany).

Statistical methods

SAS for Windows 6.12 (SAS Institute Inc.) computer program was used for the statistical analysis. Normality of data distribution was tested by Kolmogorov-Smirnov test. One-way analysis of variance was used to compare different groups of HD patients. Data were expressed as means ± SD in case of normal distribution, and medians and quartiles in case of non-normal distribution. Comparisons between groups were performed by unpaired t-test analysis of variance (ANOVA with Tukey post test). In case of PON1 paraoxonase and lactonase activity we used median test and due to multiple testing Bonferroni-Holm correction was performed. Relationships between parameters were assessed by Pearson correlation analysis. We carried out multiple regression analysis (backward-stepwise method) to test which of the variables predicted best paraxonase activity. PON1-192 and PON1-55 genotype distribution for 3 groups with different body weights were analysed by chi-square test.

Results

Decreased paraoxonase 1 (PON1) lactonase activity in hemodialyzed and renal transplanted patients

Triglyceride and total cholesterol levels were higher and HDL-C levels were significantly lower in HD patients compared to TRX patients, whereas total and LDL-C did not differ between the two groups. Serum glucose, hsCRP, cystatin C homocysteine and creatinine were significantly higher in HD patients compared to the TRX group. We found significantly higher ADMA levels in HD patients compared to the TRX population. Significantly higher levels of creatinine, hsCRP, glucose, homocysteine, cholesterol, triglyceride and ADMA levels were found in both patient groups compared to controls.

PON1 lactonase and paraoxonase activities in HD and TRX patients were significantly lower compared to controls. We measured significantly lower paraoxonase and lactonase activities in HD patients compared to TRX patients. There were no significant differences between the allelic frequencies and genotype distributions in the HD and TRX patients.

We found a significant positive correlation between paraxonase and lactonase activities in HD patients ($r=0.3086$, $p<0.05$). A similar, but statistically not significant trend was observed in TRX patients ($r=0.1136$, $p=0.41$). Significant positive correlation was found between lactonase and paraoxonase activities both in control patients ($r=0.6111$, $p<0.0001$) and in the whole study population ($r=0.5761$, $p<0.0001$). ADMA levels showed a significant negative correlation with lactonase activity in both patient groups (HD: $r=-0.2799$, $p<0.05$; TRX: $r=-0.2899$, $p<0.05$) and in the whole study population ($r=-0.5003$, $p<0.0001$).

In the whole study population there were significant negative correlations between lactonase activity and BMI (r=-0.2819, p<0.01), age (r=-0.414, p<0.001), homocysteine concentrations (r=-0.3421, p<0.001) and hsCRP levels (r=-0.2431, p<0.001).

To test whether the associations lactonase activity with homocysteine levels, paraoxonase activity and ADMA levels existing in the univariate analysis were independent of age, BMI, hsCRP and HDL-C parameters, we carried out multiple regression analysis. Homocysteine levels and paraoxonase activity were independent predictors of lactonase activity adjusting age, BMI, hsCRP, ADMA and HDL-C to the model.

Effect of nutritional status on human paraoxonase-1 activity in patients with chronic kidney disease

No significant differences were observed in serum HDL-cholesterol concentrations, glucose and CRP levels in each groups. Malnourished subjects had significantly lower triglyceride levels and significantly lower mean total and LDL-cholesterol concentrations than the normal weight and obese patients. Creatinine concentrations were similar in the three groups of patients, and plasma levels of albumin were also similar in malnourished, obese and normal weight subjects. Leptin levels were significantly higher ($p \leq 0.001$) and PON1 paraoxonase activities were significantly lower ($p=0.019$) in obese patients compared to the malnourished group. There was a significant correlation between PON1 activity and the BMI ($p \leq 0.05$). Lactonase activity was higher in malnourished patients compared to both of the obese and normal weight groups but this difference was not statistically significant.

PON1 paraoxonase activity negatively correlated with CRP level in all HD patients ($r=-0.344$, $p<0.01$) and in malnourished subjects ($r=-0.519$, $p<0.01$). We could not find any significant correlations between serum PON1 paraoxonase activity and CRP levels in obese and normal-weight HD patients. We found a significant positive correlation between BMI and CRP levels in all HD patients ($r=0.586$, $p<0.01$, in malnourished, $r=0.402$, $p<0.01$ and in obese subjects, $r=0.485$, $p<0.01$, respectively). Furthermore, we found a significant inverse correlation of PON1 paraoxonase activity and BMI in all patients on dialysis ($r=-0.314$, $p<0.05$) and in the whole patient population ($r=-0.303$, $p<0.05$). We found a positive correlation between adiponectin and PON1 lactonase activity ($r=0.303$, $p<0.05$) and a significant negative correlation between PON1 lactonase activity and CRP levels ($r=-0.318$, $p<0.05$) in all HD patients. There were no significant differences between the allelic frequencies and genotype distributions in the HD and TRX patients.

To test whether the association between PON1 paraoxonase activity and CRP levels in the univariate analysis was independent of age, BMI and other parameters, we carried out multiple regression analysis. Lactonase activity, CRP level and leptin concentration were independent predictors of PON1 paraoxonase activity adjusting age, BMI, ADMA and adiponectin to the model.

Discussion

Human paraoxonase (PON) gene family consists of three members: PON1, PON2 and PON3. PON1 possesses paraoxonase, arylesterase, and lactonase activities; PON2 and PON3 have lactonase activity but practically no paraoxonase or arylesterase activity. Human PON1 is present in serum and is associated with HDL. PON3 levels compared to PON1 levels are very low and PON2 is not present in serum at all. Therefore, the lactonase assay predominantly reflects the lactonase activity of PON1. Some investigators have suggested that homocysteine thiolactonase may be a potent marker for atherosclerotic risk in diabetes mellitus. Our previous research has shown that PON1 genotype, total homocysteine and total cholesterol are the major determinants of PON1 activity in healthy subject and patients with CRF. Furthermore, we could not find difference in PON1 geno- and phenotypes between healthy subject and hemodialyzed or renal transplant patients. In accordance with our previous results we could not find any significant difference in PON1 genotypes between HD and TRX patients.

Although several authors have demonstrated a decrease in PON1 paraoxonase activity in hemodialysis patients there was no previous study focusing on the changes of lactonase activity in ESRD. Our results support the initial hypothesis that a decrease in paraoxonase and lactonase activities and thus the reduction of its antiatherogenic effects may contribute to the accelerated atherogenesis in ESRD, especially in patients on dialysis, while paraoxonase and lactonase activities seem to be partly restored after renal transplantation. We have found increased lactonase and paraoxonase activities associated with higher HDL levels in transplant recipients compared to dialyzed patients suggesting a better antioxidant status after kidney transplantation. Paraoxonase and lactonase

activities normalized to HDL were not significantly different between HD and TRX patients, indicating that the mechanism underlying HDL elevation may result in synergistic PON1 elevation in the plasma contributing to the increased paraoxonase and lactonase activities.

The chronic hemodialysis patients with impaired renal function had significantly higher levels of homocysteine and cystatin C. Recent findings suggested that cystatin C, a low molecular weight basic protein, the product of a housekeeping gene produced by all nuclear cells at a close to constant rate, is a more sensitive marker of GFR than serum creatinine in adults including patients with chronic renal failure. We also found elevated homocysteine levels in dialyzed patients, and we found similar results in the transplanted group. The level of cystatin C was lower in transplanted patients compared to the dialyzed patients but the difference was not significant.

Several vasoactive substances have been implicated in the pathogenesis various kidney diseases leading to uremic angiopathy. One of the major vasoactive mediators is nitric oxide (NO) produced by the enzyme NO synthase from the amino acid precursor L-arginine. Asymmetric dimethylarginine (ADMA) may behave as a competitive inhibitor of NO synthase and was suggested to play a role in lipid peroxidation and vascular superoxide production. Moreover ADMA and oxidative stress may contribute to the progression of renal disease and urinary excretion of ADMA shows inverse correlation with decreasing creatinine clearance. Elevated plasma levels of ADMA were observed in CRF due to both reduced renal excretion and reduced catabolism, and several studies have demonstrated that ADMA may be one of the so-called “uremic toxins” in hemodialyzed patients. Previous studies have shown that ADMA is a marker of oxidative stress in kidney diseases and elevated level of this molecule is an

important risk factor for cardiovascular disease in chronic renal failure. Our results are consistent with this notion; we found considerably higher ADMA levels in patients on dialysis than in renal-transplant recipients.

We have hypothesized that there would be a correlation between elevated ADMA levels and serum paraoxonase/lactonase activities, and also a potential association between ADMA and cystatin C levels in patients with ESRD. A significant inverse correlation was found between ADMA concentration and lactonase activity, and a similar, but not significant trend was detected between ADMA level and paraoxonase activity in both patients groups. Our findings may indicate a close association between oxidative stress, increased endothelial ADMA concentrations and a decreased antioxidant activity in response to increased production of ROS in the pathogenesis of atherosclerosis in chronic renal failure. Based on these data, and in agreement with many experts in the field, we assume that the elevated ADMA levels and oxidative stress may be responsible for endothelial dysfunction in ESRD patients. On the other hand, we did not find a significant correlation between ADMA and cystatin C levels, which suggest a complex mechanism of metabolism, overall regulation and clearance of ADMA. This result indicates that ADMA concentrations were not closely associated with renal clearance deterioration in our patients.

Hyperhomocysteinaemia exists both in hemodialyzed and in renal transplant patients. In our study there was significant difference in homocysteine levels between the two groups, and the homocysteine concentration was 2-fold higher than the upper limit of the normal range in both patient groups. Previous study has revealed that elevated Hcy levels correlated with a lower PON1 activity in patients with coronary artery disease, and their coexistence were significantly associated with the severity of cardiovascular disease. Other investigators have

shown that high homocysteine concentrations and decrease of HTLase activities are independent risk factors for coronary artery disease, moreover, plasma homocysteine levels correlated negatively with HTLase activities. Similar to these previous findings we found significant negative correlation between PON1 lactonase activity and homocysteine levels.

Although our work focused on several different variables (age, BMI, HDL-C , hsCRP and ADMA levels) to establish their relationship to lactonase activity, based on the result of multiple regression analysis, the homocysteine levels and the PON1 paraoxonase activity were the only independent predictors of lactonase activity.

Our previous studies have shown that paraoxonase and lactonase activities of PON1 enzyme are significantly decreased in chronic renal failure. In the present work, we found higher PON1 paraoxonase activities in malnourished HD patients compared to obese patients, and similar changes in lactonase activities; however, it did not prove to be statistically significant, We detected a positive correlation between adiponectin and PON1 lactonase activity and a significant negative correlation between lactonase activity and CRP levels in all HD patients. This result suggests an impaired antioxidant status in CKD patients with higher BMI and supports the initial hypothesis that the decrease in paraoxonase and lactonase activities through the reduction of their antiatherogenic effects may contribute to accelerated atherogenesis in CKD, especially in obese hemodialyzed patients.

Previous studies have demonstrated a direct relationship between BMI and CKD risk and have shown a correlation between BMI and the increasing prevalence of chronic renal failure in overweight and obese patients. In our study, a moderate

increase in serum creatinine level was found parallel with BMI. It has been previously suggested that this increase may be linked to the progression of CKD in HD patients; however we could not demonstrate a significant difference between the obese and malnourished groups. Moreover, there was no connection between BMI and serum albumin levels in HD patients supporting the findings of other studies where albumin was an unreliable marker of the nutritional status in CKD and elderly patients.

The role of leptin in kidney function has not been completely defined so far. It is thought to be a potential salt-regulating factor and may function pathophysiologically as a common link to obesity and hypertension. It has been also recognized that hyperleptinemia was linked to renal structural changes associated with obesity. Although leptin is partly cleared by the kidneys and patients with either kidney disease or HD have been demonstrated to have higher leptin levels, our previous research showed that hyperleptinemia was not responsible for decreased paraoxonase activity in HD patients. Therefore, hyperleptinaemia may be an independent predictor for the progression of renal diseases and for the increased risk of cardiovascular diseases in HD patients with higher BMI.

Oxidative stress and inflammation have been implicated in albuminuria and renal dysfunction in uremic patients. It has been previously shown that low adiponectin levels are associated with inflammation and atherosclerosis in CKD; however, we could not find any relationship between adiponectin and PON1 paraoxonase activity in the present study.

This result suggests that PON1 paraoxonase activity may be a reliable indicator regarding the progression of renal failure in malnourished patients compared

with the obese HD group. Investigating the relationship of different variables (age, BMI, HDL-C, CRP and ADMA levels) to paraoxonase activity in multiple regression analysis, only PON1 lactonase activity, leptin and CRP levels proved to be independent predictors of PON1 paraoxonase activity. Several authors have demonstrated that higher BMI is associated with improved survival in overweight and obese CKD patients on maintenance hemodialysis compared to the general population. „Reverse epidemiology" in CKD is one of the most discussed and controversial topic regarding the mortality of HD patients. Reverse epidemiology suggests some beneficial effects of higher BMI in patients with CKD. Various studies have concluded that the presence of the malnutrition-inflammation complex syndrome may also explain the existence of reverse epidemiology in HD patients. It seems that higher BMI itself is not beneficial but is a marker of lower catabolic rate. Thus, higher BMI and better prognosis are two independent consequences of a better metabolic status. In general, malnourished HD patients have worse prognosis than those with adequate body weight, whereas PON1 was higher in malnourished patients. These data indicate that PON1 is not involved in the mechanism of "reverse epidemiology", and that protective effect of PON1 is either lost in malnourished patients or is outweighed by other detrimental mechanisms in this group.

Summary

Our current study is the first account of the alteration of lactonase activity in ESRD patients. Moreover, we showed in hemodialyzed and renal transplant patients that PON1 lactonase activity correlates with paraoxonase activity, homocysteine levels and serum ADMA levels. We conclude that hyperhomocysteinemia, elevated ADMA levels, reduced PON1 activity and decreased ability to hydrolyze homocysteine-thiolactone may lead to an increase

in N-homocysteinylated and oxidatively modified protein levels, which might contribute to the accelerated atherosclerosis in uremic and renal transplanted patients.

Our results show significantly lower activities of the antiatherogenic PON1 in obese HD patients compared to malnourished subjects. Despite our findings regarding the reverse epidemiology for the mortality of HD patients, further studies are needed to reveal the real effects of nutritional state on atherosclerosis in obese and malnourished CKD patients. There is growing evidence in the literature to support our initial hypothesis that the antioxidant properties of PON-1 enzyme are closely associated with PON1 paraoxonase activity and not with lactonase activity. Therefore, in the present study we have primarily investigated the relationship between PON1 paraoxonase activity and the antioxidant status in chronic kidney disease depending on nutritional status. Our goal was to evaluate the alteration of PON1 paraoxonase and lactonase activities and their correlations with nutrition levels in malnourished, normal-weight and obese hemodialyzed patients. Our result suggests that PON1 paraoxonase activity may be a reliable marker regarding the progression of renal failure in malnourished subjects compared with the obese hemodialyzed patients. To our best knowledge, there is no specific substance or enzyme, which can determine the lactonase activity of PON1 enzyme. Otherwise, we have shown in our previous study that the PON1 lactonase activity was not independent of PON1 paraoxonase activity in patients with chronic kidney disease.

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List of publications related to the dissertation

1. **Sztanek, F.**, Seres, I., Harangi, M., Lőcsey, L., Padra, J.T., jr. Paragh, G., Asztalos, L., Paragh, G.: Decreased human paraoxonase-1 (PON1) lactonase activity in hemodialyzed and renal transplanted patients. A novel cardiovascular biomarker in end-stage renal disease. *Nephrol. Dial. Transplant.* 27 (7), 2866-2872, 2012.
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3. Varga, É., Seres, I., Harangi, M., Kárpáti, I., Koncsos, P., **Sztanek, F.**, Paragh, G.: Low high-density lipoprotein cholesterol is not responsible for decreased paraoxonase activity in chronic renal failure. *Kidney Blood Press. Res.* 35 (4), 265-272, 2012.
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