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Association of Serum Paraoxonase/Arylesterase Activity with All-Cause Mortality in Maintenance Hemodialysis Patients

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Context: In end-stage renal disease (ESRD), serum high-density lipoprotein cholesterol (HDL-C) level is not an accurate predictor of mortality, partly because it does not necessarily correlate with indices of HDL function. Paraoxonase (PON) is a major enzyme constituent of HDL, and key component of HDL antioxidant activity. Apolipoprotein (Apo) A-I is the core HDL structural protein which plays a major role in various aspects of HDL function.
Objective: We sought to examine PON activity and Apo A-I levels in ESRD patients versus healthy controls.
Design and Setting: PON/Arylesterase activity was measured in 499 maintenance hemodialysis (MHD) patients and 24 healthy controls with similar distributions of age, sex and race/ethnicity. Serum acrolein-modified Apo A-I was measured in 30 MHD patients and 10 healthy controls.
Main Outcome Measure(s): Multilevel Cox models were used to assess associations between PON activity, Apo A-I and HDL-C levels with 12-month all-cause mortality.
Results: PON activity was significantly lower in MHD patients versus controls. Furthermore, acrolein-modified Apo A-I levels were higher in MHD patients versus controls. In fully adjusted models, high PON activity was associated with lower 12-month mortality, whereas no difference of mortality risk was observed across HDL-C levels. The combination of high PON and low Apo A-I compared to low PON and low Apo A-I was associated with lower mortality risk.

Conclusions: In MHD patients, PON activity had a stronger association with 12-month mortality than HDL-C. Future studies are needed to examine the role of these markers as potential diagnostic and therapeutic tools in ESRD.

Paraoxonase Activity and Mortality in Hemodialysis.

Introduction

End stage renal disease (ESRD) is associated with a significantly higher risk of all-cause and cardiovascular (CV) mortality (1). Nearly half of all deaths in ESRD are attributed to CV disease (2). While increasing serum concentrations of high density lipoprotein (HDL) cholesterol (HDL-C) are associated with reduced risk of all-cause and CV mortality in the general population (3, 4), in certain subsets of patients, including those with ESRD being treated with hemodialysis, elevated HDL-C levels are paradoxically associated with worse outcomes (5-7).

It is well-known that HDL can play an important role in prevention of atherosclerosis and CV disease via mechanisms such as reverse cholesterol transport as well as anti-oxidant, anti-apoptotic, vasoprotective and anti-inflammatory properties (8-11). Apolipoprotein A-I (Apo A-I) is the major apolipoprotein component of HDL, and also plays a key role in these functions (12). However, there is accumulating evidence that under certain conditions, including those associated with inflammation such as ESRD, HDL becomes dysfunctional and loses these protective characteristics (13). Oxidant/chemical modification of Apo A-I or enrichment of HDL with pro inflammatory proteins (such as serum amyloid A [SAA]) can lead to this significant impairment of HDL function and potentially result in a deleterious pro-inflammatory HDL, which can be associated with worse outcomes (14-16).

More recently, there has been increasing interest in determining whether measures of HDL function can be better predictors of HDL-related outcomes. However, determining which index of function to measure can be difficult, given (17-19) the complexities involved in isolation of the HDL particle and the assays utilized to evaluate its function (17). Therefore, serum measurements which are related to HDL function and that can be easily adopted in the laboratory setting would be of significant value. Paraoxonase (PON) is a major antioxidant enzyme which is mostly associated with the HDL particle in serum and is thought to play a significant role in HDL-mediated antioxidant activity. (20, 21). Therefore, increasing serum PON activity can be associated with improved HDL function and several studies have found low PON activity to be a predictor of atherosclerosis and CV disease (22-24). In ESRD patients, serum PON activity has been consistently shown to be decreased compared to healthy controls (25, 26), especially in those on long-term hemodialysis therapy (27). Furthermore, recent studies have found that low serum and HDL PON function is associated with significantly worse outcomes in patients with advanced (pre-dialysis) chronic kidney disease (CKD) (28, 29).

In light of these observations, we sought to determine the relationship between serum PON activity alone as well as in consideration of serum HDL-C and Apo A-I levels with ESRD-related mortality. Given the previous studies indicating increased oxidative modification of Apo A-I in MHD patients, we also determined and compared the concentration of acrolein-modified Apo A-I in a subgroup of ESRD patients and healthy controls (30-32).

Materials and Methods

Study Population

In this study, a cohort of healthy controls (n=24) was recruited from the University of California (UC), Irvine Institute for Clinical and Translational Science (ICTS). Eligible control subjects

were at least 18 years old, not diagnosed with hypertension, diabetes or other major cardiovascular comorbidities, and not taking medications. We then identified a subset of 500 maintenance hemodialysis (MHD) patients enrolled in the prospective Malnutrition, Diet, and Racial Disparities in Chronic Kidney Disease (MADRAD) study (trial registry: ClinicalTrials.gov; study number: NCT01415570) over the period of June 2014 and May 2017 with a similar distribution of age, sex, and race/ethnicity to that of the healthy control cohort. In brief, MADRAD is a prospective cohort study that examines the differences in dietary factors and nutritional status across racial/ethnic groups of MHD patients recruited from large dialysis outpatient (LDO) facilities in the Los Angeles-Orange County, California areas. A detailed description of the MADRAD study design and enrollment criteria have been previously reported (33). From the MADRAD MHD cohort, one patient was excluded on the basis of not having a serum paraoxonase (PON) measurement.

Clinical Characteristics of MHD Patients

Baseline data on patient demographic and clinical characteristics and medication use were collected at study entry by MADRAD study coordinators. A combination of patient self-reports and ICD-9 codes from LDO records were used to identify the presence of diabetes as a pre-existing condition. During regular hemodialysis sessions, body composition surrogates were measured by MADRAD study coordinators. Additional details about the collection of body anthropometric data have been previously described (33). Information on body mass index (BMI) (determined from post-dialysis weight) was obtained from LDO electronic records and MADRAD study coordinators. We used the nearest body anthropometric data and BMI values collected within 90 days before or after the measurement of serum PON activity. Given that data on residual kidney function (RKF) were unavailable, we used patient self-reported urine output and frequency (as measured by a validated questionnaire) closest to PON measurement as an estimate of RKF. We defined dialysis vintage for MHD patients as the time interval between the dates of serum PON activity measurement and first hemodialysis treatment.

Medication Ascertainment

Information on medication use was obtained from LDO records and entries by MADRAD study coordinators. Given limited data on medication use for MHD patients in our study cohort, we defined medication ever-use as having received a prescription within 1 year before or after the measurement of serum PON activity.

Serum Samples and Laboratory Tests

Serum samples in MHD patients were collected pre-dialysis during routine HD sessions, coinciding chronologically with blood tests performed at the LDOs, and frozen at -80°C until analysis. Laboratory tests obtained from MHD patients at the LDOs were drawn using standardized techniques and measured using automated and standardized methods at a central laboratory in Deland, Florida, typically within 24 hours. Serum from healthy controls was purchased from Innovative Research (Novi, MI, USA) and obtained via the assistance of UC Irvine ICTS. In all analyses, we used laboratory values measured closest to the time of serum PON activity measurement.

This study was approved by the Institutional Review Committees of the Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, CA, and the UC Irvine Medical Center, Orange, CA (MADRAD study Institutional Review Board Protocol # 2012-9045).

Laboratory Measures

Serum Apo A-I was checked using Apolipoprotein A-I Human SimpleStep ELISA Kit purchased from Abcam (Cambridge, MA, USA). Serum PON activity was measured using an arylesterase/paraoxonase assay kit purchased from ZeptoMetrix, Inc. (Buffalo, NY, USA). Serum concentrations of interleukin (IL)-6 were determined using ELISA assay kits from R&D systems (Minneapolis, MN, USA) and Affymetrix ThermoFisher Scientific. All measurements were performed according to the manufacturer's specifications and provided protocols.

Acrolein-Modified Apo A-I

In additional analyses, we randomly selected 30 non-smoking MHD patients and 10 healthy controls from the study cohort. Only non-smokers were selected (given that smoking can increase serum acrolein levels) via measurement of serum cotinine levels using a cotinine ELISA kit purchased from MyBioSource, Inc. (San Diego, CA, USA) following the manufacturer's protocol. Serum acrolein-modified apo A-I adduct was measured using a sandwich ELISA assay using the ELISA kit purchased from Abcam, commercial antibody (GeneTex) against acrolein and acrolein-modified Apo A-I as a positive control (obtained from V.N.'s laboratory). Serum (100 µl) was inoculated in duplicate manner in the wells precoated with anti-human Apo A-I and incubated for 2.5 hours at room temperature with gentle shaking. After incubation, the wells were decanted and washed 4 times. 100 µl acrolein antibody (GeneTex, Irvine, CA, USA) was added to each well and incubated for 2 hours. After 4 times wash, 100 µl anti-rabbit IgG horseradish peroxidase preadsorbed (Abcam, Cambridge, MA, USA) was added to each well and incubated for 1 hour. After 4 times wash, we added TMB ELISA substrate and stop solution (Abcam, Cambridge, MA, USA) and performed coloriometric detection at 450 nm in a microplate reader. Measurement was corrected by both negative controls and blanks.

Exposure and Outcome Ascertainment

The main exposure of interest was serum PON activity, which we categorized into quartiles (<44.9, 44.9-<76.0, 76.0-<104.4 and ≥104.4 kU/L). Other exposures of interest included HDL-C (n=498) and Apo A-I (n=493), which were divided into quartiles as follows: <29, 29-<39, 39-<50 and ≥50 mg/dL, and <79.7, 79.7-<101.3, 101.3-<126.3 and ≥126.3 mg/dL, respectively.

We also estimated rank scores of PON, HDL-C and Apo A-I, separately based on their distribution in our cohort. We defined a low and high threshold for scores as <50th and ≥50th percentiles, respectively. We then created a 2x2 matrix of high and low categories for exposure groups based on PON with HDL-C (PON/HDL-C) or PON with Apo A-I levels (PON/Apo A-I).

The primary endpoint was 12-month all-cause mortality. Follow-up began at the date of measured serum PON activity to death, transplantation, loss-to-follow-up, end of study period (April 7, 2018) or 12-month follow-up, whichever occurred first. Information on mortality and censored events were collected every six months by MADRAD study coordinators and reviewed by MADRAD study nephrologists (C.M.R. and K.K.-Z.).

Statistical Analysis

Values are reported as mean (± standard deviation, SD) or median (interquartile range, IQR) for continuous variables, and as percentages for categorical variables. Parametric and non-parametric tests for trend were used, as appropriate. Baseline patient characteristics of demographics, comorbidities and laboratory tests were compared between included and excluded patients. Using the Shapiro-Wilk test, we determined that PON activity was non-normally distributed. We therefore used the non-parametric Wilcoxon-Mann-Whitney test to compare PON activity in MHD patients and healthy controls. Spearman's rank correlations were used to assess the relationship between PON and laboratory, clinical and body anthropometric data.

Extreme outliers <0.5th or >99.5th percentile were removed and replaced with lower or upper threshold values, respectively, for laboratory tests, time on hemodialysis, Kt/V, BMI and body anthropometric measurements.

Cox proportional hazards models were used to assess the association between exposure groups and 12-month all-cause mortality with hierarchical adjustment for covariates in the following three models: (i) Model 1—unadjusted; (ii) Model 2—adjusted for case-mix variables (age, sex, race and ethnicity); and (iii) Model 3–adjusted for covariates in Model 2 plus diabetes and dialysis vintage.

Additionally, we conducted subgroup analyses examining the association of high (≥76) versus low (<76 kU/L, reference) PON and 12-month all-cause mortality in the fully adjusted model. We tested for potential effect modification by gender and race (White or non-White and Black or non-Black) on the PON-mortality relationship by using the Wald test and including interaction terms for PON-gender and PON-race, respectively, in separate Cox models.

To identify other possible confounders on the PON quartile-mortality association, we also conducted sensitivity analyses of expanded models comprised of covariates in Model 3 plus either of the following: albumin, BMI, albumin and BMI, hypertension, myocardial infarction, other cardiovascular disease, cerebrovascular accident, heart failure, polycystic kidney disease, peripheral vascular disease and current smoking status.

All patients had complete data on all covariates used in the primary analysis. In sensitivity analyses, we used imputation by mean for missing data (7% and 16% for BMI and albumin, respectively). A two-sided p-value <0.05 was considered significant for all analyses in this study. All statistical analyses were conducted with SAS, version 9.4 (SAS Institute Inc., Cary, North Carolina).

Results

MHD Patient Characteristics

There were 499 patients included in our analytical cohort (comparisons between selected patients and 662 excluded patients are summarized in **Supplemental Table 1 (34)**). Baseline characteristics of demographic, clinical and medication use data for the 499 MHD patients according to quartiles of PON levels are shown in **Table 1**. The mean (±SD) age of the cohort was 55±15 years with 44% female, 34% Black, and 49% Hispanic patients, and 53% had diabetes. Baseline values of laboratory tests for the cohort are reported in **Table 2**. Patients in the highest PON quartile were more likely to have higher albumin and serum Apo A-I levels versus patients in the lowest PON quartile. Additionally, as previously shown, serum PON activity was significantly lower in MHD patients than in healthy controls (mean±SD, 77.2±35.8 kU/L and 135.2±38.7 kU/L, respectively, p<0.0001, **Figure 1**).

Spearman correlation coefficients between serum PON activity and laboratory data are presented in **Table 3**. Serum PON positively correlated with levels of albumin (rho=0.13, p=0.009), Apo A-I (rho=0.20, p<0.0001) and BMI (rho=0.11, p=0.02, but negatively correlated with IL-6 (rho= -0.13, p=0.01) after adjustment for case-mix, diabetes and dialysis vintage covariates.

Associations of PON Activity, Serum HDL-C and Apo A-I with 12-Month All-cause Mortality

Among 499 patients, there were 61 deaths during a total 12 months follow-up of 459 patient-years, and the incidence rate of 12-month mortality was 13.3 [95% Confidence Interval (CI), 10.0, 16.6] per 100 person-years.

Patients in the highest serum PON quartile (≥104.4 kU/L) had the lowest risk of 12-month all-cause mortality compared with patients in the first quartile (<44.9 kU/L, reference) across all levels of adjustment, with an adjusted hazard ratio (aHR) of 0.26 (95% CI, 0.11, 0.59) in Model 3 **(Figure 2A and Supplemental Table 2A (34))**.

In analyses evaluating the association between HDL-C quartiles and 12-month all-cause mortality, no significant association was observed **(Figure 2B and Supplemental Table 2B (34)).**

The lowest serum Apo A-I quartile (<79.7 mg/dL) was associated with 3.3-, 4.3- and 4.2-fold higher risks of 12-month all-cause mortality in Models 1, 2 and 3, respectively, when compared to the third quartile (101.3-<126.3 mg/dL, reference) **(Figure 2C and Supplemental Table 2C (34)).** (Results of the Apo A-I-mortality associations using Quartile 1 (<79.7 mg/dL) as the reference category are presented in **Supplemental Figure 1 (34)**.)

In analyses using rank scores, compared with a reference of low PON/low HDL-C, we observed that high PON/low HDL-C was significantly associated with lower 12-month all-cause mortality risk across all adjustment models (aHRs [95% CI] of 0.26 [0.09, 0.80], and 0.24 [0.08, 0.74] for Models 2 and 3, respectively; **Figure 3B-C and Supplemental Table 3A (34)**). However, there were no differences in mortality risk for either high PON/high HDL-C or low PON/high HDL-C versus low PON/low HDL-C. Compared to low PON/low Apo A-I, all other PON/Apo A-I rank score combinations had lower 12-month all-cause mortality risk in all adjustment models **(Figure 3D-F and Supplemental Table 3B (34)).**

In subgroup analyses, we analyzed the association between high PON (≥76 versus <76 kU/L) and 12-month mortality risk according to gender (male, female), and race (White, non-White, Black and non-Black). A significantly lower mortality risk was observed for high PON across all subgroups, except in Black patients which also had a lower mortality risk but did not reach statistical significance **(Supplemental Figure 2 (34))**. Furthermore, we did not find a significant interaction between gender and PON ($P_{interaction}$=0.68) or between race and PON ($P_{interaction}$= 0.36 for Black and 0.63 for White) for the association with 12-month all-cause mortality.

As sensitivity analyses, adjustment for additional covariates in expanded models did not change the association between higher PON and lower 12-month mortality risk **(Supplemental Table 4 (34))**.

Serum Concentration of Acrolein-Modified Apo A-I

Of note, in a subset of 10 healthy controls and 30 MHD patients with negative serum cotinine tests, we found that the MHD patients had significantly higher (+60%) levels of acrolein-modified Apo A-I adduct as compared to healthy controls (p=0.047) **(Supplemental Figure 3 (34))**.

Discussion

ESRD is associated with abnormal HDL metabolism and function with evidence indicating that in subgroups of patients HDL can become proinflammatory in nature (35-37). Furthermore, it has been postulated that this HDL dysfunction may underlie the paradoxical lack of relationship observed between higher concentrations of serum HDL-C levels and mortality in MHD patients (5). In this study, we found that while HDL-C concentration was not significantly associated with 12-month all-cause mortality risk, higher PON was associated with lower mortality. Compared to patients with low PON/low Apo A-I, all other combinations of PON and Apo A-I in the 2x2 analysis had a lower risk of death. Similarly, patients with high PON/low HDL-C also had a significantly lower risk of death versus the reference group of patients with low PON/low

HDL-C. Lastly, we did not find a significant correlation between serum PON activity and HDL-C concentrations, but did find a modest correlation between serum PON activity and serum Apo A-I levels. The latter findings highlight the important point that serum HDL-C levels are not a good marker for indices of HDL function.

While decreased serum and HDL activity of PON has been extensively studied and reported in MHD patients, our study is the first to examine the role of serum PON activity in ESRD-related mortality (28, 38, 39). We did not find serum HDL-C concentrations to be associated with any change in outcomes in this small cohort whereas in the past we had found a U-shaped relationship between HDL-C and mortality. This is most likely due to the small number of patients in this investigation compared with previous studies. In our study, however, high PON combined with either low or high Apo A-I or low HDL-C was associated with lower mortality risk possibly indicating the potential for a strong role of PON in predicting mortality outcomes in ESRD patients.

A previous study reported that increased oxidized Apo A-I levels in hemodialysis patients is associated with worse CV outcomes (16). We also found that acrolein modified/content of Apo A-I is significantly increased in patients with ESRD on MHD when compared to healthy controls. This is consistent with previous reports which demonstrated that serum protein acrolein adducts are increased in ESRD patients (40). Increased Acrolein-content/modification of Apo A-I impairs ATP-binding cassette transporter A1 (ABCA1)-mediated cholesterol efflux and HDL function (41, 42). It is also important to note that free serum acrolein levels have been found to be inversely correlated with serum PON activity in ESRD patients (43), and increased acrolein-modified Apo A-I in MHD patients may be contributing to reduced PON activity and impaired HDL antioxidant activity in this patient population. Therefore, our findings provide another potential mechanism by which Apo A-I may become dysfunctional in ESRD setting. Further studies investigating the association of acrolein-modified Apo A-I with mortality in ESRD patients are needed.

Several limitations need to be mentioned. Firstly, this is an observational study, and future mechanistic studies are needed to confirm our hypotheses. In addition, the relatively small sample size of our cohort limits our ability to adjust for all possible confounders. Therefore, future large validation studies are needed to further elucidate the relationship between serum PON activity, HDL-C, Apo A-I levels and outcomes in ESRD. Moreover, in this study we did not have access to genetic information, and hence, could not assess PON genetic variants which may play a role in the activity of this enzyme and its association with outcomes. We were also unable to consider high-sensitivity C-reactive protein (hs-CRP) as a covariate in the adjusted models because this inflammatory marker was not measured regularly in MHD patients. However, additional adjustment for albumin as a marker of nutritional status and inflammation did not alter observed associations. Future investigations will be needed to evaluate the role of acrolein-modified Apo A-I in reduced PON activity and the association of increased Apo A-I levels and mortality.

In conclusion, we found a stronger relationship between serum PON activity as measured by its arylesterase function with 12-month all-cause mortality, compared to exposure measurements of serum HDL-C and Apo A-I. Furthermore, lower serum Apo A-I levels were associated with a significantly higher risk of death at 12 months and acrolein content/modified Apo A-I was increased in patients on MHD. Further studies are needed to determine the potential utility of these markers as diagnostic and therapeutic tools in the ESRD population.

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Potential Conflicts of Interest
HM has received grant funding from the NIH, VA ORD, Amgen and Novartis, and VN from the NIH.
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Figure 1. Distribution of serum PON activity in controls and MHD patients. Serum PON activity in healthy control subjects (n=24) and MHD patients (n=499) are shown. Abbreviations: PON, paraoxonase; and MHD, maintenance hemodialysis. * p<0.0001.

Figure 2. 12-month all-cause mortality and PON, HDL-C and Apo A-I quartiles. Associations between (A) PON quartiles, (B) HDL-C quartiles, and (C) serum Apo A-I quartiles and 12-month all-cause mortality with adjustments for covariates in the following models: (i) Model 1 (unadjusted); (ii) Model 2 (case-mix variables: age, sex, race and ethnicity); and (iii) Model 3 (Model 2 covariates plus diabetes and dialysis vintage). For visual purposes, the plots have log-transformed y-axes. Abbreviations: PON, paraoxonase; HDL-C, high-density lipoprotein cholesterol; Apo A-I, apolipoprotein A-I; and HR, hazard ratio.

Figure 3. 12-month all-cause mortality with rank scores of PON and HDL-C, and PON and Apo A-I. Associations between (3A-C) low or high PON and low or high HDL-C rank scores, and (3D-F) low or high PON and low or high Apo A-I with 12-month all-cause mortality with adjustment for covariates in the following models: (3A, 3D) Model 1 (unadjusted); (3B, 3E) Model 2 (case-mix variables: age, sex, race and ethnicity); and (3C, 3F) Model 3 (Model 2 covariates plus diabetes, and hemodialysis vintage). * p<0.05. Abbreviations: PON, paraoxonase; HDL-C, high-density lipoprotein cholesterol; Apo A-I, apolipoprotein A-I; HR, hazard ratio; and Ref., reference.

Table 1. Baseline patient characteristics of 499 maintenance hemodialysis patients according to serum PON activity quartiles.

Variables	Total	Serum PON Activity (kU/L)				P for trend
		<44.9	44.9-<76.0	76.0-<104.4	≥104.4	
N (%)	499	122 (24)	126 (25)	128 (26)	123 (25)	
Age (years)	55±15	56±15	55±16	54±14	55±13	0.45
Female (%)	44	35	47	44	50	0.05
Race (%)						
Caucasian	56	53	44	63	63	0.01
Black	34	33	39	31	33	0.65
Asian	7	7	12	5	4	0.17
Other	3	7	6	0.78	0	0.0002
Hispanic ethnicity (%)	49	47	39	55	55	0.04
Body mass index (kg/m^2)	27.8±6.5	27.8±6.8	27.1±6.4	27.6±5.9	28.9±7.0	0.18
Current smoking status (%)	11	3	8	17	16	0.0001
Vintage (%)						
12 months	11	18	15	6	5	0.0001
12-<36 months	27	34	29	27	19	0.006
36-<72 months	29	20	21	33	41	<0.0001
≥72 months	33	28	35	34	35	0.28
Comorbidities (%)						

Hypertension	28	25	25	30	32	0.14
Diabetes	53	50	52	55	54	0.41
Dyslipidemia	18	12	19	19	24	0.03
Cardiovascular disease	19	20	17	20	19	0.96
Myocardial infarction	7	7	6	10	7	0.72
Cerebrovascular incident	0.20	0	0	0	0.81	0.18
Heart failure	11	13	11	11	8	0.23
Polycystic kidney disease	0.40	0	0	0.78	0.81	0.20
Peripheral vascular disease	3	2	2	3	7	0.02
Medication use (%)						
Statin	23	24	27	23	20	0.34
ACEI/ARBs	38	37	34	44	37	0.56
Urine output (%)	73	69	74	77	71	0.66
Urination frequency (%)						0.66
More than once a day	66	64	66	69	64	
Approximately once a day	25	27	26	24	23	
Every 2 to 3 days	10	8	9	8	13	

Values are reported as mean (± standard deviation, SD) for continuous variables, and as percentages for categorical variables. Abbreviations: ACEI, angiotensin-converting-enzyme inhibitor; ARBs, angiotensin II receptor blockers; and PON, paraoxonase.
Percentages may not add up to 100 due to rounding.

Table 2. Baseline characteristics of laboratory data in 499 maintenance hemodialysis patients according to serum PON activity quartiles.

Variables	**Total**	**Serum PON Activity (kU/L)**				**P for trend**
		<44.9	**44.9-<76.0**	**76.0-<104.4**	**≥104.4**	
Albumin (g/dL)	4.0±0.3	3.9±0.4	4.0±0.3	4.0±0.3	4.0±0.3	0.01
Hemoglobin (g/dL)	10.7±1.1	10.7±1.1	10.6±0.9	10.6±1.0	10.8±1.2	0.50
Phosphorus (mg/dL)	5.2±1.5	5.1±1.4	5.1±1.6	5.5±1.6	5.1±1.3	0.70
HDL-C (mg/dL)	41.3±16.3	39.6±13.2	43.8±16.3	40.0±16.9	41.6±18.2	0.75
IL-6 (pg/mL)	2 (1, 4)	2 (1, 5)	3 (1, 5)	2 (1, 4)	2 (1, 4)	0.12
Apo A-I (mg/dL)	105.7±35.3	92.7±27.9	110.5±33.1	106.6±39.0	112.9±36.8	<0.0001

Values are reported as mean (± standard deviation, SD) or median (interquartile range, IQR) for continuous variables, where appropriate. Abbreviations: Apo A-I, apolipoprotein A-I; HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6; and PON, paraoxonase.

Table 3. Correlations of serum PON activity with laboratory, clinical and body anthropometric data in 499 maintenance hemodialysis patients.

Variables	**Unadjusted**		**Adjusted**	
	Rho	**p-value**	**Rho**	**p-value**
Laboratory tests				
Albumin (g/dL)	0.12	0.01	0.13	0.009
Hemoglobin (g/dL)	0.02	0.68	0.03	0.60
Phosphorus (mg/dL)	0.04	0.46	0.01	0.76
HDL-C (mg/dL)	-0.02	0.61	-0.03	0.45
IL-6 (pg/mL)	-0.11	0.05	-0.13	0.01
Apo A-I (mg/dL)	0.20	<0.0001	0.20	<0.0001
Time on hemodialysis (min.)	-0.005	0.91	0.03	0.52
Kt/V	0.03	0.60	-0.07	0.15
Body mass index (kg/m^2)	0.10	0.03	0.11	0.02
Body anthropometric measurements				
Biceps average (mm)	0.12	0.03	0.09	0.11
Triceps average (mm)	0.14	0.009	0.11	0.05
Mid-arm muscle circumference (cm)	-0.04	0.44	0.04	0.46
Mid-arm circumference (cm)	0.07	0.23	0.12	0.03
Near-infrared body fat (%)	0.11	0.04	0.06	0.28

Spearman correlation coefficients (Rho) are shown for all variables. Correlation coefficients were adjusted for covariates in the following models: (i) unadjusted; and (ii) adjusted (age, sex, race, ethnicity, diabetes and dialysis

vintage). Abbreviations: Apo A-I, apolipoprotein A-I; HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6; and PON, paraoxonase.