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Permalink

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Journal

Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation, 22(3)

ISSN

1051-2276

Authors

Feroze, Usama
Kalantar-Zadeh, Kamyar
Sterling, Kevin A
et al.

Publication Date

2012-05-01

DOI

10.1053/j.jrn.2011.05.004

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Peer reviewed

Published in final edited form as:

J Ren Nutr. 2012 May ; 22(3): 317–326. doi:10.1053/j.jrn.2011.05.004.

Examining Associations of Circulating Endotoxin with Nutritional Status, Inflammation and Mortality in Hemodialysis Patients

Usama Feroze, MD^{1,2}, Kamyar Kalantar-Zadeh, MD, MPH, PhD^{1,2,3}, Kevin A Sterling, MD⁴, Miklos Z. Molnar, MD; PhD¹, Nazanin Noori, MD, PhD¹, Debbie Benner, RD⁶, Vhalab Shah, PhD⁵, Rama Dwivedi, PhD⁴, Kenneth Becker, MD⁶, Csaba P Kovessy, MD³, and Dominic S Raj, MD⁴

¹Harold Simmons Center for Chronic Disease Research and Epidemiology

²Division of Nephrology and Hypertension, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA 90502

³David Geffen School of Medicine at UCLA and the UCLA School of Public Health, Los Angeles, CA

⁴Division of Renal Diseases and Hypertension, George Washington University, Washington DC

⁵Department of Biochemistry, University of New Mexico Health Sciences Center, Albuquerque, NM

⁶Division of Endocrinology and Metabolism, George Washington University, Washington DC

⁵George Washington University School of Medicine, North Washington, DC.

Abstract

Objective—Lipopolysaccharide or endotoxin constitutes most part of the outer portion of the cell wall in the gram negative bacteria. Sub-clinical endotoxemia could contribute to increased inflammation and mortality in hemodialysis patients. Endotoxin level and clinical effect are determined by its soluble receptor sCD14 and high density lipoprotein. We examine the hypothesis that endotoxin level correlates with mortality.

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Correspondance/Reprint Request: Kamyar Kalantar-Zadeh, MD, MPH, PhD, Harold Simmons Center for Chronic Disease Research and Epidemiology, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, 1124 West Carson Street, C1-Annex, Torrance, CA 90502, USA, Phone: 310-222-3891, Fax: 310-782-1837, kamkal@ucla.edu, Dominic Raj, MD, George Washington University, 2121 I Street Northwest, Washington, DC, draj@mfa.gwu.edu.

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This study was presented in part as an abstract during the annual meeting of the *American Society of Nephrology*, Oct 27–30, 2009, San Diego, CA.

Relevant Potential Conflict of Interest

KKZ, CPK and/or JDK have received recent grants and/or honoraria from Genzyme, Inc, the manufacturer of Sevelamer (RenagelTM and RenvelaTM) and doxercalciferol (HectoralTM), Abbott laboratories, the manufacturer of Paricalcitol (ZemparTM) and calcitriol (CalcijexTM), Fresenius Medical Care, the distributor of calcium acetate (PhosLoTM), Shire Pharmaceuticals, the manufacturer of lanthanum carbonate (FosrenolTM), and/or Amgen, Inc, the manufacturer of Cinacalcet hydrochloride (SensiparTM or MimparaTM).

Methods—In this cohort study, endotoxin levels were measured in 306 long-term hemodialysis patients who were then followed for up to 42 months. Soluble CD14 and cytokines levels were also measured.

Results—The mean ($\pm$ SD) endotoxin level was 2.31 $\pm$ 3.10 EU/ml (min: 0.26 EU/ml, max: 22.94 EU/ml, inter-quartile range: 1.33EU/ml, median: 1.27EU/ml). Endotoxin correlated with C-reactive protein ($r = 0.11$, $p < 0.04$). On multivariate logistic regression analysis, high body mass index (BMI) and low HDL cholesterol levels were associated with higher endotoxemia (endotoxin below or above of median). In multivariable Cox regression analysis adjusted for case-mix and nutritional/inflammatory confounders, endotoxin levels in the 3rd quartile vs. 1st quartile was associated with a trend towards increased hazard ratio (HR) for death (HR 1.83, 95% confidence interval: 0.93–3.6, $p = 0.08$).

Conclusions—In this hemodialysis cohort, we found associations between endotoxemia and CRP, body composition and HDL. A moderately high endotoxin levels tended to correlate with increased mortality than the highest circulating endotoxin level. Additional studies are required to assess the effect of endotoxemia on mortality in dialysis population.

Keywords

Chronic Kidney Disease (CKD); hemodialysis; nutritional status; inflammation; endotoxin; cytokines

Introduction

End Stage Renal Disease (ESRD) patients have increased morbidity and mortality compared to the general population. Infection is the second most important cause of the increased mortality seen in these ESRD patients (1). More than 75% of deaths in these patients is as a result of septicemia (2). The incidence rate of bacterial infections in ESRD patients is one episode per 100 patient months (3, 4). These bacterial infections are often life threatening given the increased susceptibility of uremic patients to infection due to their immune dysfunction(5). While *Staphylococcus aureus* is the major pathogenic organism (4) responsible for infections in dialysis patients, it has been found that endotoxemia due to gram-negative organisms is also a potential source of inflammation in these ESRD patients. (6)

The Endotoxins (lipopolysaccharide in the outer wall of gram negative bacteria) can generate a complex host response through signaling pathways initiated after attachment of lipopolysaccharide (LPS) to the CD14 antigen on effector cells (7). Initiation of this complex response occurs after binding of the lipopolysaccharide to the lipopolysaccharide binding protein (LBP) through a lipid A moiety.(8).CD14 is then activated by the LPS-LBP complex which leads to the activation of the cellular immune complex (9). ESRD patients are exposed to higher levels of endotoxin due to: 1) bacteriolysis in patients suffering from gram-negative sepsis caused by bactericidal systemic antibiotics which release a high volume of endotoxin (10, 11), 2) entry of endotoxin through the intestinal mucosal epithelial by bacterial translocation (12),and 3) potential use of non-ultra pure dialysate for the dialysis (13). Nevertheless, a recent study found that endotoxemia is associated with better survival in peritoneal dialysis patients.(14)

Given that ESRD patients have higher baseline levels of inflammatory markers, (15) we hypothesized that there is a relationship between endotoxin levels and inflammation in these maintenance dialysis patients. In addition, we wanted to examine the impact of the proposed relationship on the nutritional status and mortality in these ESRD patients.

Methods

Patient Population

We studied a population of hemodialysis (HD) patients who were part of the Nutritional and Inflammatory Evaluation in Dialysis (NIED) study (16). The original NIED cohort consisted of more than 3000 MHD outpatients followed for 6 years in eight (8) DaVita maintenance dialysis clinics in Southern CA. (see the NIED study Website at www.Niedstudy.org for more details as well as previous publications (17–21)). To be included in the study, patients had to be at least 18 years old and on outpatient hemodialysis for at least 8 weeks. Patients were excluded if they had an acute infection or had a life expectancy of less than 6 months. The study was approved by the IRB and all subjects gave informed consent prior to being enrolled in the study. A total of 893 long term HD patients were randomly invited and agreed to participate in the NIED study. Out of these subjects, 310 also agreed to undergo an additional substudy including measurement of endotoxin, which led to 306 subjects with endotoxin data, since samples on 4 subjects had to be discarded for contamination. The medical record was thoroughly reviewed for each subject by a collaborating physician in the study. Information such as underlying kidney disease, cardiovascular disease history and other co-morbid illnesses was abstracted. A modified version of the Charlton Co-morbidity Index (i.e. excluding the age and kidney disease components) was used to assess severity of co-morbidities (22, 23). The 306 HD patients were followed for a total of 42 months (March 2004 - September 30, 2007).

Anthropometric and Body Composition Measures

Body weight and anthropometric measurements were performed while patients were on HD or within 5–20 minutes after termination of their hemodialysis treatment. Biceps and triceps skin fold thickness was measured by standard technique using the conventional skin fold caliper (24, 25).

Near Infrared Interactance

To estimate percentage body fat and fat free body mass, near infrared (NIR) interactance was measured at the same time as the anthropometric measurements (26). A commercial NIR interactance sensor with a coefficient of variation of 0.5% for total body fat measurement (portable Furtex 6100; Furtex, Inc, Rockville, VA; www.furtex.com) was used. NIR measurements were performed by placing a Furtex sensor on the upper arm (free of vascular access) for several seconds and entering the required data (data of birth, sex, weight, and height) for each patient. NIR measurements of body fat appear to correlate significantly with other nutritional measures in HD patients (27).

Endotoxin Measurement

We used quantitative Chromogenic Limulus Amebocyte Lysate (LAL) test for endotoxins in plasma (both free and protein-bound forms) using a commercially available kit (QCL-1000, Cambrex bioscience Inc, Walkersville, MD). The minimum detectable level of endotoxin is 0.1 EU/ml.

Other Laboratory Tests

Pre-dialysis and post-dialysis blood samples were obtained on a mid-week day that coincided with the day that the required quarterly blood tests were done at the DaVita dialysis facilities. Single pooled Kt/V was used to represent the weekly dialysis dose. All laboratory studies were performed by DaVita Laboratories (Deland, FL) using automated methods. Serum high sensitivity C-reactive protein (CRP) was measured using a turbidometric immunoassay (WPCI, Osaka, Japan; normal range <3.0mg/L) (28, 29).

Interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF- α) levels were measured with using immunoassay kits (R&D Systems, Minneapolis, Minn., USA; units: pg/ml; normal range: IL-6: < 9.9 pg/ml, TNF- α : < 4.7 pg/ml) (30, 31). The C-reactive protein (CRP), TNF- α and IL-6 levels were measured in the General Clinical Research Center Laboratories at Harbor UCLA. Serum Transthyretin (pre-albumin) was measured by immunoprecipitation and the plasma homocysteine concentration was measured by high performance liquid chromatography (HPLC) in the Harbor-UCLA Clinical Laboratories.

Statistical Methods

Pearson's correlation coefficient(r) was used for analyses of linear associations. Multivariate logistic regression analysis was performed to obtain adjusted p-values controlled for case-mix and other covariates. Death hazard ratios (HRs) were obtained using Cox proportional hazard models controlling for the relevant covariates.

We performed incremental levels of multivariate adjustment where: (A) Case-mix variables including age, gender, race (African-American), diabetes mellitus, and dialysis vintage were included. (B) Malnutrition-inflammation complex syndrome (MICS) variables included such as albumin, creatinine, hemoglobin, total iron binding capacity, normalized protein catabolic rate, white blood count and; normalized protein catabolic rate (nPCR) [also known as normalized protein nitrogen appearance (nPNA)]; and body mass index. (C) Additional adjustment was done for three inflammatory markers (CRP, IL-6, and TNF α) in a fully adjusted Cox regression model.

We expected significant confounding in the unadjusted models where relevant confounders such as age and gender were not taken into account. In fact, while the results from the adjusted models may have been over-adjusted (possibly due to inclusion of biological intermediates that are along the causal pathway from predictor to outcome variable), we make our inferences based on models adjusted for case-mix. Because of uncertainty regarding which final model is in fact the most parsimonious, we include 3 levels of adjustment in the presented data so that the full spectrum of results can be appreciated. The data analysis was done using STATA version 11.1 (STATA Corporation, College Station, TX).

Results

The mean ($\pm$ SD) endotoxin level was 2.31 ± 3.10 EU/ml (min: 0.26 EU/ml, max: 22.94 EU/ml, inter-quartile range: 1.33 EU/ml, median: 1.27 EU/ml). Baseline demographic, clinical, and laboratory values in the 306 MHD patients studied are shown in Table 1. The patients mean age ($\pm$ SD) was 55 ± 15 years; 48% of patients were women (n=149), 30% (n=92) were African-American and 57% were diabetic. The dialysis vintage was 50 ± 35 months (median + inter-quartile range: 45+ 44 months). Mean endotoxin level was 2.3 ± 3.1 EU/ml (median: 1.27 EU/ml). Figure 1 shows the distribution of endotoxin levels. After ranking subjects according to their serum endotoxin level, we categorized them into quartiles with 75–77 patients in each group. Table 1 lists relevant demographic, clinical and laboratory measures across quartiles of endotoxin levels. Older patients were more likely to be in the higher quartiles of endotoxin levels. No other significant trend was seen in the demographic, physical or biochemical variables as it relates to increasing quartile of endotoxin levels.

Factors Correlated with Endotoxin Level

Table 2 shows the unadjusted and adjusted correlations between Endotoxin levels and relevant nutritional, inflammatory and biochemical variables. There was a statistically significant correlation between endotoxin level and CRP level (p-value <0.05) after

adjustment for relevant covariates. This positive correlation was further supported by the scatter plot shown in figure 2 ($r=0.11$, $P<0.05$). No correlation was seen between the other markers of inflammation and Endotoxin level. We defined the endotoxin level is equal 5 $\mu\text{g}/\text{ml}$ when it was more than 5 $\mu\text{g}/\text{ml}$ in our further analyses. Table 3 shows the results of univariate (unadjusted) and multivariate logistic regression analysis of the association of the variables of interest and Endotoxin level. Body mass Index and HDL cholesterol level were associated with endotoxin levels after multivariate logistic regression analysis. There was a 5% increased odds of higher endotoxin levels for each $1\text{kg}/\text{m}^2$ increase in body mass index. There was a 3% decrease in endotoxin levels for each $1\text{mg}/\text{dl}$ decrease in HDL cholesterol level.

Serum Endotoxin and Survival

During the 42 months follow-up, 58(20%) subjects died, 33(11%) received a renal transplant and 26(8%) were lost to follow up. The hazard ratio for mortality is shown in Table 4. Hazard ratio for mortality was not significant across the quartiles of increasing endotoxin levels. However, there was a trend towards the 3rd quartile of endotoxin levels (1.28–2.23 EU/ml) being associated with an 83% increase in mortality [i.e. HR 1.83 (0.93, 3.60), p -value 0.08]. Cubic spline plots shown in Figure 3 further illustrate the nature of the relationships shown in Table 4.

Discussion

In 306 maintenance hemodialysis patients, we found that circulating endotoxin level was associated with higher CRP levels and BMI but lower HDL level. Whereas we did not find an incremental association between elevated circulating endotoxin levels and mortality in maintenance hemodialysis patients, we did find that moderately high (3rd quartile) but not the highest (4th quartile) circulating endotoxin levels (endotoxin greater than 1.265 EU/ml and less than 2.237 EU/ml) tended to be associated with increasing mortality (83% higher) compared to mortality in the lowest quartile of endotoxin levels. Though this trend was not statistically significant, it appeared robust when adjustment was made for case-mix and other nutritional and inflammatory measures, including serum IL-6 and TNF- α . A recent study by McIntyre et al has shown significant association between higher circulating level of endotoxin with higher mortality rate in hemodialysis patients (32).

CKD patients have higher prevalence of inflammation (33) which is an independent risk factor for cardiovascular events through promotion of atherosclerosis (34). Infection being the 2nd most common cause of death in hemodialysis patients (1), bacterial infections especially by gram negative bacteria serves as a major contributor (35). Endotoxin (Lipid A), a glucosamine based phospholipid, is the hydrophobic anchor of lipopolysaccharide and makes up the outer monolayer of the outer membranes of most gram-negative bacteria (36). As it is biologically active portion of lipopolysaccharide molecule (37), it contributes in the activation of the host immune cells like macrophages etc, and results in the release of inflammatory mediators(38). This activation of cascade occurs through the combination of lipopolysaccharide (LPS) with lipopolysaccharide binding protein (LBP) and then interaction of this complex with CD14 (cell surface antigen). CD14 has two forms namely soluble and myeloid, and these two forms interact with LPS-LBP complex through two different pathways as highlighted in Figure 4.

A possible explanation for the paradoxical effect of higher concentration of endotoxin on mortality can be explained through endotoxin action at receptor level shown in (Figure 4). Raj *et al* found that increased soluble CD14 level was associated with higher death risk in CKD patients (21). Endotoxin mediates its effect after binding with CD14 to specific receptors resulting in activation of a cascade of inflammatory cytokines(7). In lower

concentrations it activates the immune system to combat infection without causing overt damage while very high concentration suggests that they are not bound 50 pg/ml or greater were at increased risk for development of atherosclerosis (39). Lack of association with inflammatory cytokines apart from CRP, in our study, further supports this explanation. Indeed a recent study found that endotoxemia is associated with better survival in peritoneal dialysis patients.(14)

Another plausible explanation for this association is that in our study population, the mean level of endotoxin was 2.31 ± 3.10 EU/ml and sCD14 was 7.24 ± 2.45 ug/ml. sCD14 values across the quartiles of endotoxin level showed that group with higher mortality risk had higher sCD 14 as compared to the other two groups. Further analysis showed no significant correlation was found between the endotoxin and CD14. So this finding further suggests that, to manifest its effect, endotoxin requires a certain amount of sCD14 in the blood to show maximum activation of the inflammatory cascade Table 1.

Data also suggested that older patients had higher level of endotoxin and this increment had significant association (Table 1) but no significant correlation was found between endotoxin and age (Table 2). The only significant positive correlation that was found in this study is between CRP levels and endotoxin levels when adjusted for case-mix variables (Table 2). Szeto *et al* found a similar association between CRP and Serum endotoxin levels in peritoneal dialysis patients. Their study also found a negative correlation between serum albumin and serum endotoxin which we did not find in our study (6). Our findings are similar to those of Goncalves *et al* who reported that there were no association between endotoxin levels and circulating cytokines (40).

An inverse association was also found between HDL and endotoxin levels in after both unadjusted and adjusted linear regression analyses. This finding correlated with the fact that HDL levels decline more than any other lipoproteins in septic patients (41). LPS is detoxified in the circulation by incorporation into lipoproteins (LDL, VLDL, TGL and HDL)(42, 43).

Selection bias during study enrollment resulting in a younger maintenance HD cohort is one of the major limitations of this study. However, because mortality rate in the original NIED study cohort was lower than in the baseline dialysis population (16), it might be argued that the strength of the association seen is much lower than would be seen in a more randomly selected sample of dialysis patients. The strength of our study relates to the fact that participants were selected randomly without prior knowledge of their inflammation status. Further, we had a fairly large sample size with comprehensive clinical and laboratory evaluation. We were able to do body composition measurement, obtain detailed information on co-morbid illnesses and measure levels of pro-inflammatory cytokines.

Conclusion

In our study of 306 MHD patients who were followed for up to 3.5 years, increasing endotoxin levels was not associated with increased mortality. This possibly is due to complex interaction of endotoxin with its receptors and signaling cascade. Additional studies are necessary to assess the relationship between endotoxin concentration and other long-term outcomes in these maintenance hemodialysis patients.

Acknowledgments

The authors are thankful to dietitians and other teammates in DaVita Wild West and Gold Coast Divisions for supporting the study and the staff at Harbor-UCLA GCRC Core Laboratories for the management of blood samples and measuring inflammatory markers.

Funding Source

The study was supported by Dr. Kalantar-Zadeh's research grants from the National Institute of Diabetes, Digestive and Kidney Disease of the National Institute of Health (R01 DK078106, R21 DK078012, and K23 DK61162), a research grant from DaVita Clinical Research and a philanthropic grant from Mr. Harold Simmons. Dr. Raj is supported by the National Institute of Health Grants AG21560 and R01DK073665-01A1. Dr. Miklos Zsolt Molnar received grants from the National Research Fund (NKTH-OTKA-EU 7KP-HUMAN-MB08-A-81231), was also supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (2008–2011), and is recipient of the Hungarian Eötvös Scholarship (MÖB/66-2/2010).

References

1. Mailloux LU, Bellucci AG, Wilkes BM, Napolitano B, Mossey RT, Lesser M, Bluestone PA. Mortality in dialysis patients: analysis of the causes of death. *Am J Kidney Dis.* 1991; 18(3):326–335. [PubMed: 1882824]
2. Sarnak MJ, Jaber BL. Mortality caused by sepsis in patients with end-stage renal disease compared with the general population. *Kidney international.* 2000; 58(4):1758–1764. [PubMed: 11012910]
3. Kessler M, Hoen B, Mayeux D, Hestin D, Fontenaille C. Bacteremia in patients on chronic hemodialysis. A multicenter prospective survey. *Nephron.* 1993; 64(1):95–100. [PubMed: 8502343]
4. Hoen B, Paul-Dauphin A, Hestin D, Kessler M. EPIBACDIAL: a multicenter prospective study of risk factors for bacteremia in chronic hemodialysis patients. *J Am Soc Nephrol.* 1998; 9(5):869–876. [PubMed: 9596085]
5. Cohen G, Haag-Weber M, Horl WH. Immune dysfunction in uremia. *Kidney Int Suppl.* 1997; 62:S79–S82. [PubMed: 9350688]
6. Szeto CC, Kwan BC, Chow KM, Lai KB, Chung KY, Leung CB, Li PK. Endotoxemia is related to systemic inflammation and atherosclerosis in peritoneal dialysis patients. *Clin J Am Soc Nephrol.* 2008; 3(2):431–436. [PubMed: 18256376]
7. Wright, SD.; Ramos, RA.; Tobias, PS.; Ulevitch, RJ.; Mathison, JC. *Science.* Vol. 249. New York, NY: 1990. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein; p. 1431-1433.
8. Tobias PS, Soldau K, Ulevitch RJ. Identification of a lipid A binding site in the acute phase reactant lipopolysaccharide binding protein. *The Journal of biological chemistry.* 1989; 264(18):10867–10871. [PubMed: 2471708]
9. Tobias PS, Ulevitch RJ. Lipopolysaccharide-binding protein and CD14 in the lipopolysaccharide-dependent activation of cells. *Chest.* 1994; 105(Suppl)(3):48S–50S. [PubMed: 7510603]
10. Holzheimer RG. The significance of endotoxin release in experimental and clinical sepsis in surgical patients--evidence for antibiotic-induced endotoxin release? *Infection.* 1998; 26(2):77–84. [PubMed: 9561376]
11. Mock CN, Jurkovich GJ, Dries DJ, Maier RV. Clinical significance of antibiotic endotoxin-releasing properties in trauma patients. *Arch Surg.* 1995; 130(11):1234–1240. discussion 1240-1231. [PubMed: 7487468]
12. Van Leeuwen PA, Boermeester MA, Houdijk AP, Ferwerda CC, Cuesta MA, Meyer S, Wesdorp RI. Clinical significance of translocation. *Gut.* 1994; 35(Suppl)(1):S28–S34. [PubMed: 8125386]
13. Arizono K, Nomura K, Motoyama T, Matsushita Y, Matsuoka K, Miyazu R, Takeshita H, Fukui H. Use of ultrapure dialysate in reduction of chronic inflammation during hemodialysis. *Blood purification.* 2004; 22(Suppl 2):26–29. [PubMed: 15655319]
14. Szeto CC, Kwan BC, Chow KM, Lai KB, Pang WF, Chung KY, Leung CB, Li PK. Endotoxemia is associated with better clinical outcome in incident chinese peritoneal dialysis patients: a prospective cohort study. *Perit Dial Int.* 2010; 30(2):178–186. [PubMed: 20081047]
15. Stenvinkel P, Alvestrand A. Inflammation in end-stage renal disease: sources, consequences, and therapy. *Seminars in dialysis.* 2002; 15(5):329–337. [PubMed: 12358637]
16. Colman S, Bross R, Benner D, Chow J, Braglia A, Arzaghi J, Dennis J, Martinez L, Baldo DB, Agarwal V, Trundnowski T, Zitterkoph J, Martinez B, Khawar OS, Kalantar-Zadeh K. The Nutritional and Inflammatory Evaluation in Dialysis patients (NIED) study: overview of the NIED study and the role of dietitians. *J Ren Nutr.* 2005; 15(2):231–243. [PubMed: 15827897]

17. Shantouf RS, Budoff MJ, Ahmadi N, Ghaffari A, Flores F, Gopal A, Noori N, Jing J, Kovesdy CP, Kalantar-Zadeh K. Total and individual coronary artery calcium scores as independent predictors of mortality in hemodialysis patients. *Am J Nephrol.* 2010; 31(5):419–425. [PubMed: 20389057]
18. Noori N, Kopple JD, Kovesdy CP, Feroze U, Sim JJ, Murali SB, Luna A, Gomez M, Luna C, Bross R, Nissenson AR, Kalantar-Zadeh K. Mid-arm muscle circumference and quality of life and survival in maintenance hemodialysis patients. *Clin J Am Soc Nephrol.* 2010; 5(12):2258–2268. [PubMed: 20947789]
19. Noori N, Kalantar-Zadeh K, Kovesdy CP, Bross R, Benner D, Kopple JD. Association of dietary phosphorus intake and phosphorus to protein ratio with mortality in hemodialysis patients. *Clin J Am Soc Nephrol.* 2010; 5(4):683–692. [PubMed: 20185606]
20. Noori N, Kalantar-Zadeh K, Kovesdy CP, Murali SB, Bross R, Nissenson AR, Kopple JD. Dietary potassium intake and mortality in long-term hemodialysis patients. *Am J Kidney Dis.* 2010; 56(2):338–347. [PubMed: 20580474]
21. Raj DS, Shah VO, Rambod M, Kovesdy CP, Kalantar-Zadeh K. Association of soluble endotoxin receptor CD14 and mortality among patients undergoing hemodialysis. *Am J Kidney Dis.* 2009; 54(6):1062–1071. [PubMed: 19699018]
22. Mehrotra R, Kermah D, Fried L, Kalantar-Zadeh K, Khawar O, Norris K, Nissenson A. Chronic peritoneal dialysis in the United States: declining utilization despite improving outcomes. *J Am Soc Nephrol.* 2007; 18(10):2781–2788. [PubMed: 17804675]
23. Beddhu S, Bruns FJ, Saul M, Seddon P, Zeidel ML. A simple comorbidity scale predicts clinical outcomes and costs in dialysis patients. *The American journal of medicine.* 2000; 108(8):609–613. [PubMed: 10856407]
24. Nelson EE, Hong CD, Pesce AL, Peterson DW, Singh S, Pollak VE. Anthropometric norms for the dialysis population. *Am J Kidney Dis.* 1990; 16(1):32–37. [PubMed: 2195877]
25. Williams AJ, McArley A. Body composition, treatment time, and outcome in hemodialysis patients. *J Ren Nutr.* 1999; 9(3):157–162. [PubMed: 10431037]
26. Kalantar-Zadeh K, Dunne E, Nixon K, Kahn K, Lee GH, Kleiner M, Luft FC. Near infra-red interactance for nutritional assessment of dialysis patients. *Nephrol Dial Transplant.* 1999; 14(1):169–175. [PubMed: 10052499]
27. Kalantar-Zadeh K, Kuwae N, Wu DY, Shantouf RS, Fouque D, Anker SD, Block G, Kopple JD. Associations of body fat and its changes over time with quality of life and prospective mortality in hemodialysis patients. *The American journal of clinical nutrition.* 2006; 83(2):202–210. [PubMed: 16469976]
28. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *The New England journal of medicine.* 2002; 347(20):1557–1565. [PubMed: 12432042]
29. Erbagci AB, Tarakcioglu M, Aksoy M, Kocabas R, Nacak M, Aynacioglu AS, Sivrikoz C. Diagnostic value of CRP and Lp(a) in coronary heart disease. *Acta cardiologica.* 2002; 57(3):197–204. [PubMed: 12088177]
30. Pecoits-Filho R, Barany P, Lindholm B, Heimbürger O, Stenvinkel P. Interleukin-6 is an independent predictor of mortality in patients starting dialysis treatment. *Nephrol Dial Transplant.* 2002; 17(9):1684–1688. [PubMed: 12198224]
31. Beutler B, Cerami A. The biology of cachectin/TNF—a primary mediator of the host response. *Annual review of immunology.* 1989; 7:625–655.
32. McIntyre CW, Harrison LE, Eldehni MT, Jefferies HJ, Szeto CC, John SG, Sigrist MK, Burton JO, Hothi D, Korsheed S, Owen PJ, Lai KB, Li PK. Circulating Endotoxemia: A Novel Factor in Systemic Inflammation and Cardiovascular Disease in Chronic Kidney Disease. *Clin J Am Soc Nephrol.*
33. Panichi V, Migliori M, De Pietro S, Taccola D, Bianchi AM, Norpoth M, Metelli MR, Giovannini L, Tetta C, Palla R. C reactive protein in patients with chronic renal diseases. *Renal failure.* 2001; 23(3–4):551–562. [PubMed: 11499569]
34. Zimmermann J, Herrlinger S, Pruy A, Metzger T, Wanner C. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. *Kidney international.* 1999; 55(2):648–658. [PubMed: 9987089]

35. Chen CH, Hsu WH, Chen HJ, Chen W, Shih CM, Hsia TC, Tu CY. Different bacteriology and prognosis of thoracic empyemas between patients with chronic and end-stage renal disease. *Chest*. 2007; 132(2):532–539. [PubMed: 17699132]
36. Raetz CR, Whitfield C. Lipopolysaccharide endotoxins. *Annual review of biochemistry*. 2002; 71:635–700.
37. Rietschel ET, Kirikae T, Schade FU, Ulmer AJ, Holst O, Brade H, Schmidt G, Mamat U, Grimmecke HD, Kusumoto S, et al. The chemical structure of bacterial endotoxin in relation to bioactivity. *Immunobiology*. 1993; 187(3–5):169–190. [PubMed: 8330896]
38. Bosshart H, Heinzelmann M. Targeting bacterial endotoxin: two sides of a coin. *Annals of the New York Academy of Sciences*. 2007; 1096:1–17. [PubMed: 17405910]
39. Wiedermann CJ, Kiechl S, Dunzendorfer S, Schratzberger P, Egger G, Oberhollenzer F, Willeit J. Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck Study. *Journal of the American College of Cardiology*. 1999; 34(7): 1975–1981. [PubMed: 10588212]
40. Goncalves S, Pecoits-Filho R, Perreto S, Barberato SH, Stinghen AE, Lima EG, Fuerbringer R, Sauthier SM, Riella MC. Associations between renal function, volume status and endotoxaemia in chronic kidney disease patients. *Nephrol Dial Transplant*. 2006; 21(10):2788–2794. [PubMed: 16861246]
41. Gordon BR, Parker TS, Levine DM, Saal SD, Wang JC, Sloan BJ, Barie PS, Rubin AL. Low lipid concentrations in critical illness: implications for preventing and treating endotoxemia. *Critical care medicine*. 1996; 24(4):584–589. [PubMed: 8612407]
42. Levine DM, Parker TS, Donnelly TM, Walsh A, Rubin AL. In vivo protection against endotoxin by plasma high density lipoprotein. *Proceedings of the National Academy of Sciences of the United States of America*. 1993; 90(24):12040–12044. [PubMed: 8265667]
43. Feingold KR, Funk JL, Moser AH, Shigenaga JK, Rapp JH, Grunfeld C. Role for circulating lipoproteins in protection from endotoxin toxicity. *Infection and immunity*. 1995; 63(5):2041–2046. [PubMed: 7729918]

Figure 1a: Histogram of Serum Endotoxin Level **Figure 1b:** Histogram of variable Endotoxin (Endotoxin with every value above 5 is =5) **Figure 1.****a:** Histogram of Serum Endotoxin Level**b:** Histogram of variable Endotoxin (Endotoxin with every value above 5 is =5)

Figure 2. Scatter Plot, Regression Line, and 95% confidence intervals reflecting correlation between serum levels of endotoxin and value of serum CRP.

Unadjusted: **Full model** **Figure 3.**

Cubic Spline exhibiting the association between Serum Endotoxin (adjusted variable with $>5 = 5$) level and mortality level in 306 MHD patients.

LBP: Lipopolysaccharide binding Protein LPS: Lipopolysaccharide mCD14: membrane CD14 sCD14: soluble CD14

Figure 4.

Cellular mechanism of lipopolysaccharide action and activation of the cytokine system and interaction with lipoproteins.

Baseline Demographic, Clinical and Laboratory Values in Total and According to Quartiles of Endotoxin in 306 Maintenance Hemodialysis Patients.

Table 1

Variable	Quartile 1 N=77 (0.26-0.90)	Quartile 2 N=76 (0.91-1.26)	Quartile 3 N=77 (1.28-2.23)	Quartile 4 N=76 (>=2.24)	p-for- trend
Demographic					
Age(yr)	52.2±16.4	53.5±14.9	57.3±13.5	58.1±13.3	0.03
Women (%)	31(40%)	41(54%)	36(47%)	39(51%)	0.35
Race(% African American)	20(26%)	31(41%)	20(26%)	21(28%)	0.13
Diabetes Mellitus	42(55%)	37(49%)	46(60%)	49(65%)	0.20
Modified Charlson comorbidity Score	1.7±1.7	2.0±1.7	1.9±1.5	1.7±1.2	0.42
Crude Mortality Rate *	16(21%)	17(22%)	21(27%)	17(22%)	0.79
Primary Insurance(% Medicare)	25(40%)	37(60%)	32(52%)	35(57%)	0.14
Body Composition					
Body Mass Index(kg/m ²)	25.5±6.7	25.3±6.3	26.5±5.8	26.9±6.0	0.29
Triceps Skinfold (mm)	16.2±10.6	17.9±11.3	15.9±7.9	18.0± 10.1	0.48
Biceps Skinfold (mm)	8.9±7.9	9.6±8.0	8.7±5.6	9.7±7.0	0.81
Midarm Circumference (cm)	30.5±6.4	30.4±5.7	30.8 ±5.6	31.4±5.89	0.77
Hemodialysis Treatment					
Dialysis Vintage (mo)	43.0±28.6	59.0±38.4	49.8± 32.7	48.4± 38.5	0.04
Dialysis Dose(single-pool Kt/V)	1.75± 0.31	1.68 ±0.25	1.70 ±0.32	1.65±0.22	0.19
nPCR(g/kg/d)	1.13 ±0.28	1.03 ±0.19	1.06 ±0.23	1.11 ±0.27	0.08
Erythropoietin dose(1000 U/wk)	14.9±12.6	17.5±17.7	11.7± 96.5	13.1± 15.9	0.09
Biochemical Measurement					
Serum endotoxin	0.67±0.15	1.10±0.11	1.67±0.25	5.82±4.67	<0.01
soluble CD14 (ug/ml)	7.2±2.2	7.5±3.0	7.5±2.3	6.8±2.1	0.21
Ibumin(g/dl)	4.0± 0.4	4.0±0.40	4.0±0.3	3.9±0.3	0.27
Prealbumin (mg/dl)	29.7±9.2	28.9±8.3	30.0±9.1	28.5±7.4	0.71
creatinine(mg/dl)	10.4±3.0	10.1±2.7	9.3± 2.5	9.9±2.6	0.13
TIBC (mg/dl)	205.4±35.5	201.9±33.5	206.1± 36.8	209.6± 35.8	0.61
Calcium(mg/dl)	9.5±0.6	9.7±0.6	9.5±0.6	9.7±0.6	0.26

Variable	Quartile 1 N=77 (0.26-0.90)	Quartile 2 N=76 (0.91-1.26)	Quartile 3 N=77 (1.28-2.23)	Quartile 4 N=76 (>2.24)	p-for- trend
Phosphorus(mg/dl)	5.6±1.4	5.4±1.4	5.4±1.3	5.4±1.3	0.14
Alkaline Phos (u/l)	124.8±71.9	134.8±63.6	131.5±100	116.3 ±70.5	0.48
Ferritin (ng/ml)	656.7 ±427.6	634.6±350.6	612.4± 351.5	604.5± 383.5	0.84
Total Homocysteine(umol/l)	24.7±9.5	26.2±7.94	25.2± 6.6	26.5 ±8.2	0.54
LDL cholesterol(mg/dl)	74.7±27.9	80.8±36.1	80.9± 30.3	80.6± 35.0	0.62
HDL Cholesterol(mg/dl)	38.1±14.4	38.3±13.7	34.4 ±11.4	35.7± 11.4	0.22
Total Cholesterol(mg/dl)	152.0±31.2	166.9± 61.6	171.2± 57.8	149.8 ±34.9	0.55
Triglycerides(mg/dl)	134.3±88.5	161.2±154.1	155.4 ±88.6	160.9 ±155.6	0.56
C-reactive Protein(mg/dl)	4.2±5.3	5.1±5.6	5.2±6.0	5.4± 5.6	0.62
Interleukin 6(pg/ml)	12.4±19.1	10.5±10.2	9.1±11.1	10.2 ±12.4	0.52
Tumor Necrosis Factor(pg/ml)	2.5±0.9	2.6±1.3	2.5± 0.85	2.4±0.9	0.59
Blood Hemoglobin(g/dl)	12.0±0.9	12.0±0.9	12.3± 0.9	12.1±0.8	0.40
White blood Cells(103 cells/ul)	7.0±2.4	6.8±2.2	6.8±1.7	6.8±1.6	0.84

Note: Values expressed as mean SD or percentage. P values for dialysis dose (vintage),ferritin level, C-reactive protein level, interleukin 6 level, and tumor necrosis factor α level are based on the logarithmic values of these measures. Conversion factors for units: albumin in g/dl to $\mu\text{mol/L}$,x10;creatinine in mg/dl to $\mu\text{mol/L}$,x88.4;calcium in mg/dl to mmol/L,x0.2495;phosphorus in mg/dl to mmol/L,x0.3229; homocysteine in μmol to mg/L,x0.1352;total,low density lipoprotein, and high-density lipoprotein cholesterol in mg/dl to mmol/L,x0.2586;triglycerides in mg/dl to mmol/L,x0.01129;hemoglobin in g/dl to g/L,x10.Ferritin in ng/ml and $\mu\text{g/L}$ and white blood cell count in $10^3/\text{ul}$ and 109/l require no conversion.

Abbreviations: Kt/V, dialysis dose; nPCR, normalized Protein Catabolic rate

* Mortality pertains to a maximum of 33 months.

LDL: Low density lipoprotein

HDL: High Density Lipoprotein

TIBC: Total Iron Binding Capacity

Table 2

Bivariate (unadjusted) and partial (adjusted) correlation coefficients between soluble endotoxin and relevant variables in 306 maintenance hemodialysis patients.

Variable	Pearson Correlation Co-efficient	P	Adjusted Correlation Co-efficient*	P
Age	0.04	0.54	−0.05	0.44
Dialysis Vintage (log Scale)	−0.03	0.56	0.00	0.94
Body Mass Index	0.05	0.34	0.03	0.57
Normalized Protein	0.09	0.12	0.07	0.26
Catabolic Rate				
Calcium	0.02	0.79	0.04	0.55
Phosphorus	−0.06	0.32	0.05	0.39
Intact PTH (logScale)	0.03	0.61	0.05	0.35
Alkaline	0.04	0.54	0.06	0.32
Phosphatase				
Albumin	0.05	0.42	0.05	0.40
Pre-Albumin	−0.01	0.89	−0.01	0.87
TIBC	0.01	0.87	−0.03	0.62
Ferritin	0.05	0.37	0.05	0.41
Creatinin	−0.02	0.73	0.01	0.89
IL6(LogScale)	−0.04	0.46	−0.06	0.32
TNF(Log Scale)	−0.02	0.75	−0.01	0.88
CRP (log Scale)	0.07	0.2	0.11[#]	0.04
LDL	−0.02	0.74	0.00	0.99
HDL	−0.06	0.30	−0.06	0.30
Cholesterol	−0.05	0.70	−0.05	0.73
Triglycerides	−0.04	0.54	−0.05	0.47
Blood hemoglobin	−0.01	0.89	−0.02	0.68
White blood cells	−0.01	0.84	−0.04	0.45
Percentage	0.01	0.84	0.04	0.55
lymphocytes				
Zemplar	0.02	0.74	0.05	0.42
Ktv	−0.02	0.70	−0.03	0.66
Precalcitonin	−0.10	0.30	−0.15	0.15
CD14 (ug/ml)	−0.04	0.50	−0.00	0.98

* In adjusted analysis age, sex, diabetes, log Interleukin-6, log TNF alpha, log Vintage were included as covariates.

[#] Bold values have significant P-value.

Table 3

Linear logistic regression estimated odds ratios for endotoxemia (endotoxin level above vs. below of median) in 306 maintenance hemodialysis patients

Variable	Logistic Regression	P	Adjusted Logistic Regression [*]	P
Age(each 10 year increase in age)	1.02 (1.01–1.04)	0.004	1.02 (1.01–1.04)	0.10
Gender (women vs. men)	0.92 (0.59–1.45)	0.73	1.13 (0.68–1.88)	0.64
Dialysis Vintage(log Scale) (each 1 month unit increase)	0.93 (0.72–1.20)	0.59	1.12 (0.82–1.52)	0.45
Body Mass Index (each 1 kg/m ² increase)	1.04 (1.0–1.08)	0.07	1.05# (1.01–1.10)	0.04
Normalized Protein Catabolic Rate (each 1 g/kg/d unit increase)	1.03 (0.41–2.59)	0.95	1.06 (0.36–3.09)	0.92
Calcium (each 1 mg/dl unit increase)	1.05 (0.73–1.52)	0.79	1.40 (0.87–2.10)	0.18
Phosphorus (each 1 mg/dl unit increase)	0.90 (0.76–1.07)	0.25	0.95 (0.78–1.16)	0.63
Alkaline Phosphatase (each 1 mg/dl unit increase)	1.0 (1.0–1.0)	0.52	1.00 (1.00–1.01)	0.87
Albumin (each 1 mg/dl unit increase)	0.92 (0.48–1.74)	0.80	0.92 (0.40–2.08)	0.83
Pre-albumin (each 1 mg/dl unit increase)	1.0 (0.97–1.03)	0.96	1.00 (0.97–1.04)	0.78
TIBC (each 1 mg/dl unit increase)	1.0 (1.0–1.0)	0.30	1.0 (1.0–1.0)	0.55
Ferritin (each 1 mg/dl unit increase)	1.0 (1.0–1.0)	0.4	1.0 (1.0–1.0)	0.40
Creatinine (each 1 mg/dl unit increase)	0.92 (0.86–1.0)	0.06	0.92 (0.81–1.04)	0.17
IL6 (log Scale) (each 1 mg/dl unit increase)	0.88 (0.68–1.14)	0.35	0.71 (0.51–1.00)	0.05
TNF (log Scale) (each 1 mg/dl unit increase)	0.79 (0.42–1.47)	0.45	0.92 (0.46–1.85)	0.82
CRP (log Scale) (each 1 mg/dl unit increase)	1.07 (0.88–1.31)	0.5	1.12 (0.87–1.4)	0.39
LDL (each 1 mg/dl unit increase)	1.0 (1.0–1.01)	0.43	1.0 (1.0–1.0)	0.93
HDL (each 1 mg/dl unit increase)	0.98 (0.96–1.0)	0.05	0.97 (0.95–1.0)	0.02
Cholesterol (each 1 mg/dl unit increase)	1.0 (0.99–1.0)	0.98	1.0 (0.98–1.01)	0.66
Blood hemoglobin (each 1 mg/dl unit increase)	1.2 (0.94–1.61)	0.13	1.2 (0.92–1.65)	0.16

* In adjusted analysis age, gender, diabetes, albumin, body mass index, creatinine, hemoglobin, total ironbinding capacity, normalized protein catabolic rate and logarithm of 3 inflammatory markers. Log interleukin6, log tumor necrosis factor alpha, log C-reactive protein were included as covariates

Table 4

Hazard Ratio of 33 month mortality according to quartiles of Endotoxin in 306 maintenance hemodialysis patients.

Endotoxin Quartiles	Q1 (n=77) HR(%CI) (0.26–0.90)	Q2 (n=76) HR(%CI) (0.91–1.26)	Q3 (n=76) HR(%CI) (1.28–2.23)	Q4 (n=77) HR(%CI) (>=2.24)
Unadjusted	0.97 (0.49–1.92) P=0.09	1(Reference)	1.40 (0.75–2.7) P=0.28	0.89 (0.45–1.75) P=0.73
Case-mix *	1.16 (0.58–2.3) P=0.67	1(Reference)	1.60 (0.58–2.3) P=0.2	0.88 (0.44–1.76) P=0.73
Previous + MICS [#]	1.07 (0.51–2.22) P=0.85	1(Reference)	1.64 (0.84–3.2) P=0.14	0.82 (0.40–1.69) P=0.60
Previous + inflammation [†] (full mode)	1.07 (0.50–2.28) P=0.86	1(Reference)	1.83 (0.93–3.6) P=0.08	0.84 (0.40–1.75) P=0.64

Abbreviation: CI, confidence interval; HR, Hazard Ratio; MICs, Malnutrition-inflammation-cachexia syndrome.

* Case-Mix variables includes age, sex, race/ethnicity, diabetes, and log vintage

[#] MICS variables includes values for albumin, creatinine, hemoglobin, total iron-binding capacity, normalized protein catabolic rate, and body mass index.

[†] Full Model consists of case mix and MICS and logarithm of 3 inflammatory markers: C-reactive protein, interleukin 6, and tumor necrosis factor- α