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HDL-inflammatory index correlates with poor outcome in hemodialysis patients

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Oxidative stress and cardiovascular disease are risk factor of patients with chronic kidney disease (CKD) on maintenance hemodialysis. We used the fluorescence of low-density lipoprotein as an index of its proinflammatory potential to examine any role that high-density lipoprotein (HDL) might have in promoting this effect. The total body fat of the patients was measured by means of near-infrared interactance and their quality of life by means of SF36 questionnaires. In 189 randomly selected patients, followed for 30 months, HDL was found to be significantly anti-inflammatory but with a large standard deviation. Fully 17% of the patients had a decidedly proinflammatory index along with inferior SF36 scores. The patients were divided into 10% increments of total body fat percentages up to 40%. HDL was found to be progressively proinflammatory the higher the body fat content. Patients with a higher HDL proinflammatory index had a higher 30-month adjusted hazard ratio for death than those whose HDL were seen to be anti-inflammatory. Our findings suggest an important role of inflammatory HDL in patients with CKD leading to poor outcome.

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KEYWORDS: HDL; LDL; chronic kidney disease; dialysis; inflammation; cardiovascular disease; hemodialysis

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Individuals with chronic kidney disease (CKD) have a very high mortality rate and a high burden of cardiovascular disease.[1] At least one of every five CKD patients requiring maintenance dialysis treatment, currently $\sim$400 000 individuals in the United States, dies every year.[2] This results in a 5-year survival rate of $<$35%, worse than the survival rate of many malignant diseases.[2] Half of all deaths are attributed to cardiovascular disease.[2] However, such traditional risk factors as hypercholesterolemia, hypertension, and obesity do not appear to be related to cardiovascular death risk in dialysis patients. Indeed, a low, rather than a high, serum cholesterol or low-density lipoprotein (LDL) level is associated with poor survival in these individuals.[3] A recent randomized trial, Die Deutsche Diabetes-Dialyse (4D) Study, did not show a significant reduction in mortality with cholesterol lowering by atorvastatin in diabetic dialysis patients.[4]

Most observational studies indicate that malnutrition, inflammation, and/or oxidative stress, together known as the 'malnutrition–inflammation complex (or cachexia) syndrome' (MICS) may be a much stronger predictor of death risk in dialysis patients than traditional cardiovascular risk factors.[5,6] Lipoprotein oxidation appears to play a central role in atherogenesis.[7–10] The oxidation hypothesis of atherogenesis pertains to specific proinflammatory oxidized phospholipids.[7,11–13] The oxidized phospholipids are largely generated by potent oxidants produced by the lipoxygenase and myeloperoxidase pathways.[7,14] Recent evidence suggests that the function of high-density lipoprotein (HDL) can be dramatically altered by these oxidants and the oxidized lipids formed by their action.[7,10,15–17]

A measure of HDL inflammatory/anti-inflammatory properties has been shown to distinguish patients with atherosclerotic cardiovascular disease (or metabolic equivalents such as long-standing diabetes) from healthy control subjects better than HDL-cholesterol levels.[18] To our knowledge, these properties of HDL have neither yet been measured in individuals with CKD undergoing maintenance hemodialysis (MHD) treatment, nor has its association with body composition or markers of MICS or its association with clinical outcomes including quality of life and mortality been examined in the CKD population. We report here that the

inflammatory/anti-inflammatory properties of HDL identified a cohort of MHD patients who were at exceptionally high risk for death in the 30 months following the measurement.

RESULTS

HDL-inflammatory index (HII) was assessed in all 189 randomly selected serum samples (Figure 1) and averaged 0.501 ± 0.574 (mean $\pm$ s.d., median: 0.289). In patients with a previous history of cardiovascular disease ($n = 122$), the averaged HII (0.563 ± 0.458) was 0.178 higher than those without cardiovascular disease history ($P = 0.02$). Thirty-three patients (17%) had an HII > 1.0. Table 1 shows the relevant demographic, clinical, and laboratory measures in the two categories of MHD patients (HII < 1.0 versus $\geqslant$1.0). The MHD patients with HII $\geqslant$ 1 had significantly higher comorbidities according to the Charlson score and worse self-reported quality of life, but higher body mass index, larger total body fat, and thicker upper arm skinfolds. Among laboratory measures, serum albumin levels, one of the strongest predictors of survival in dialysis patients,[19] were not significantly different between the two HII groups. Similarly, serum lipoprotein levels, including HDL-cholesterol, were similar in the two groups, suggesting that HII is independent of the serum HDL-cholesterol level. Among inflammatory markers, serum C-reactive protein (CRP) was slightly higher in the HII $\geqslant$ 1 group, but interleukin (IL)-6 and tumor necrosis factor-α were similar in the two groups of patients. Predialysis serum creatinine,[20] an indicator of muscle mass and probably meat intake, was lower in subjects with HII $\geqslant$ 1.

Table 2 shows the association between SF36 scores and HII. All eight scales and the two main dimensions (physical and mental health) of the SF36 had inverse correlations with HII, that is, better self-reported quality of life with lower HII values, although the correlation coefficients were small. The reported quality of life was consistently worse among those who had HII $\geqslant$ 1; the difference between the two HII groups was more prominent for the scales Physical Function and Role Emotional.

To further examine the nature of the association between the measured body fat and HII, the near infrared (NIR) measured total body fat categories were divided into five *a priori* selected categories of <10, $\geqslant$40 and three 10% groups in-between. As shown in Table 3, both the absolute HII level and the proportion of patients with an HII $\geqslant$ 1 were progressively greater in MHD patients with a larger percent of body fat. To examine if the association between the total body fat and HII was due to other confounding demographic or clinical variables, logistic regression analyses were conducted. Table 4 and Figure 2 show the odds ratios of HII $\geqslant$ 1 in each of the body fat categories. Comparing the 10–19.9% body fat group, the odds of an HII $\geqslant$ 1 was 4–10 times higher in MHD patients with larger total body fat percent, and these associations were independent of other covariates.

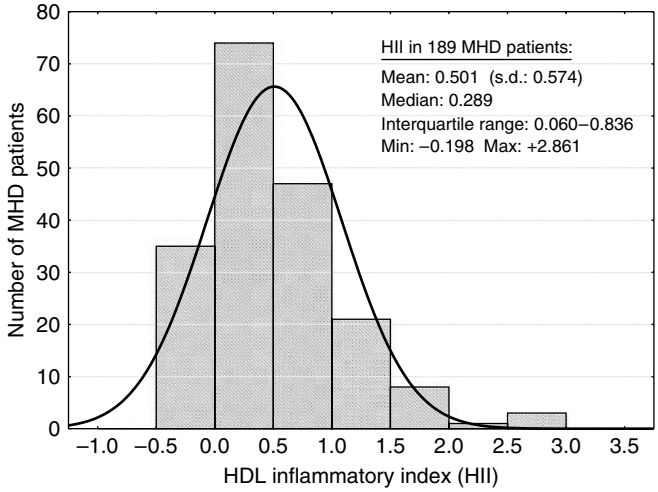

Figure 1 | Distribution of the measured 'HDL-inflammatory index' (HII) in 189 MHD patients.

Table 1 | Relevant demographic, clinical, and laboratory values according to the HII cutoff level of 1.0 in 189 MHD patients at baseline

	HII < 1	HII $\geqslant$ 1	*P*-values
Number of MHD patients	156 (83%)	33 (17%)	
Gender (% women)	42	61	0.05
Diabetes mellitus (%)	50	67	0.08
Race (% Blacks)	28	23	0.3
Ethnicity (% Hispanics)	51	58	0.3
Age (years)	54 ± 1	56 ± 2	0.5
Dialysis vintage (months)	38 ± 34	32 ± 23	0.4
Charlson comorbidity score	1.9 ± 1.4	2.7 ± 1.5	0.003
SF36 quality of life score	57 ± 26	47 ± 17	0.02
Body composition			
NIR measured body fat (%)	24.4 ± 10.6	31.6 ± 9.2	<0.001
Body mass index (kg/m^2)	25.2 ± 5.5	28.1 ± 7.9	0.02
Triceps skinfold (mm)	15.9 ± 10.0	20.5 ± 10.5	0.02
Biceps skinfold (mm)	10.0 ± 7.9	13.1 ± 8.6	0.05
Dialysis dose (K_t/V)	1.60 ± 0.27	1.72 ± 0.28	0.02
Protein intake (g/kg/day)	1.07 ± 0.24	1.09 ± 0.23	0.6
Serum concentrations			
HII	0.297 ± 0.328	1.455 ± 0.524	<0.001
Albumin (g/dl)	3.92 ± 0.39	3.83 ± 0.35	0.17
Ferritin (ng/ml)	634 ± 389	777 ± 697	0.05
Iron saturation (%)	34 ± 12	40 ± 15	0.01
Creatinine (mg/dl)	11.3 ± 3.3	8.9 ± 2.8	<0.001
Total cholesterol (mg/dl)	147 ± 39	146 ± 28	0.9
LDL (mg/dl)	81 ± 31	77 ± 20	0.5
HDL (mg/dl)	38 ± 14	36 ± 16	0.5
Triglyceride (mg/dl)	129 ± 113	131 ± 55	0.9
Total homocysteine (μmol/l)	27 ± 12	25 ± 12	0.4
CRP (mg/l)	5.2 ± 6.7	6.7 ± 6.2	0.03*
IL-6 (pg/ml)	18 ± 61	13 ± 27	0.7*
TNF-α (pg/ml)	9.3 ± 11.9	7.1 ± 4.7	0.3*

CRP, C-reactive protein; HDL, high-density lipoprotein; HII, HDL-inflammatory index; IL-6, interleukin-6; LDL, low-density lipoprotein; MHD, maintenance hemodialysis; NIR, near infrared; SF36, short form quality of life score with 36 questions; TNF, tumor necrosis factor.
P-value pertains to *t*-test.
**P*-values for CRP, IL-6, and TNF-α are based on the logarithmic values of these measures.

Table 2 | SF36 quality of life score and its association with HII

	Correlation with HII levels	HII < 1 (*n*=156)	HII ⩾ 1 (*n*=33)	*P*-values
SF36 total score	−0.28 (*P* < 0.001)	**57** ± 26	**47** ± 17	0.02
SF36 dimensions (2)				
SF-36 mental health	−0.26 (*P* < 0.001)	**59** ± 25	**50** ± 16	0.03
SF-36 physical health	−0.24 (*P* < 0.001)	**51** ± 27	**41** ± 18	0.03
SF36 scales (8)				
Body pain	−0.14 (*P*=0.06)	**59** ± 29	**55** ± 26	0.3
General health	−0.18 (*P*=0.02)	**44** ± 22	**40** ± 21	0.16
Mental health	−0.11 (*P*=0.16)	**67** ± 21	**63** ± 18	0.17
Physical function	−0.28 (*P* < 0.001)	**54** ± 33	**38** ± 28	0.008
Role emotional	−0.20 (*P*=0.01)	**64** ± 84	**38** ± 44	0.04
Role physical	−0.21 (*P*=0.006)	**49** ± 84	**24** ± 37	0.06
Functionality	−0.16 (*P*=0.04)	**69** ± 31	**67** ± 23	0.4
Vitality	−0.14 (*P*=0.07)	**49** ± 24	**42** ± 21	0.06

HII, high-density lipoprotein inflammatory index; SF36, short form quality of life score with 36 questions.
The first column shows the correlation coefficients between the two main dimensions and eight scales of the health-related quality of life and HII in 189 MHD patients. The following columns compare SF36 scores in patients with HII < 1 versus ⩾1 (*P*-value is based on the *t*-test).

Table 3 | The association between total body fat, measured by the NIR interactance, and the serum HII concentration

NIR body fat (%)	Number of MHD patients in each category	HII (mean ± s.d.)	Number of MHD patients with HII ⩾ 1
< 10	14 (7%)	**0.099** ± 0.234	0 (0%)
10–19.9	52 (28%)	**0.405** ± 0.515	3 (6%)
20–29.9	51 (27%)	**0.531** ± 0.485	10 (20%)
30–39.9	51 (27%)	**0.584** ± 0.617	13 (25%)
⩾40	21 (11%)	**0.738** ± 0.816	7 (35%)
All patients	189 (100%)	**0.501** ± 0.574	33 (17%)

HII, high-density lipoprotein inflammatory index; MHD, maintenance hemodialysis; NIR, near infrared.
Body fat is categorized into five *a priori* categories based on increments of 10%.

Table 4 | Odds ratio of HII ⩾ 1 based on the body fat categories in the cohort in 189 MHD patients of the NIED Study

NIR body fat (%)	Unadjusted	Case-mix adjusted[a]	Case-mix and MICS adjusted[b]
< 10[b]	⩽1.0	⩽1.0	⩽1.0
10–19.9 (reference group)	1.0	1.0	1.0
20–29.9	4.0 (1.1–15.4) *P*=0.04	4.7 (1.1–19.5) *P*=0.03	4.8 (1.2–20.0) *P*=0.03
30–39.9	5.6 (1.5–21.0) *P*=0.01	6.5 (1.3–31.8) *P*=0.02	6.1 (1.2–30.2) *P*=0.03
⩾40	8.8 (2.0–38.8) *P*=0.004	10.6 (1.7–64.6) *P*=0.01	9.7 (1.6–59.4) *P*=0.02

CRP, C-reactive protein; HII, high-density lipoprotein inflammatory index; IL-6, interleukin-6; MHD, maintenance hemodialysis; MICS, malnutrition–inflammation–cachexia syndrome; NIED, Nutritional and Inflammatory Evaluation in Dialysis Study; NIR, near infrared.
[a]Case-mix models are adjusted for age, gender, race and ethnicity, diabetes, Charlson comorbidity scale, and dialysis vintage. Case-mix and MICS-adjusted models are also controlled for serum albumin, CRP, and IL-6.
[b]None of the MHD patients with a total body fat < 10% had HII ⩾ 1; hence, a mathematic OR cannot be calculated, because the logarithm of OR is minus infinity for this group.

During the 30 months of prospective longitudinal observation (1 July 2002 through 31 December 2004), 46 patients died, 22 underwent renal transplantation, and 4 were lost to follow-up. The remaining 117 subjects were confirmed alive as of early January 2005. Among patients with HII ⩾ 1, 35 subjects (22%) died versus 11 subjects (33%) in those with HII < 1. To examine whether HII ⩾ 1 at the baseline of the cohort was associated with a higher death rate, Kaplan–Meier plots were constructed. Figure 3 shows that by the end of the 30 months of observation, the MHD patients with an HII ⩾ 1 had worse survival, although this difference was not statistically significant. The survival difference between the two HII groups was more prominent and statically significant after adjusting for case-mix covariates ($P = 0.03$; Figure 3, lower panel). Table 5 compares the hazard ratios of HII ⩾ 1 with serum albumin < 3.8 g/dl, CRP ⩾ 10 mg/l, and IL-6 ⩾ 10 pg/ml. In the fully adjusted model, HII ⩾ 1 was associated with 2.47-fold higher risk of death compared to HII < 1 (95% confidence interval: 1.14–5.34, $P = 0.02$). Among other nutritional and inflammatory measures, hypoalbuminemia was the only statistically significant predictor of death (Table 5). Inclusion of serum creatinine,

a possible muscle mass indicator, in the fully adjusted Cox model resulted in similar death hazard ratio estimates for HII ⩾ 1 (data not shown).

DISCUSSION

Seventeen percent of 189 MHD patients at the start of a 30-month period of observation had an HII ⩾ 1. HDL-cholesterol and other lipoprotein levels in the patients with HII ⩾ 1 were similar from those with HII < 1. All scales of SF36 health-related quality of life were reported worse in subjects with HII ⩾ 1. Body composition had a bearing on the HII, in that HII was progressively higher across the larger total body fat categories, and this association was independent of demographic and clinical characteristics or the severity of comorbid conditions. In this prospective study, the MHD patients with HII ⩾ 1 had approximately a 2.5-fold higher adjusted death risk independent of other mortality predictors, including body fat or hypoalbuminemia. These findings may have important clinical implications for the management of inflammation and oxidative stress and subsequent atherosclerotic cardiovascular disease in MHD patients.

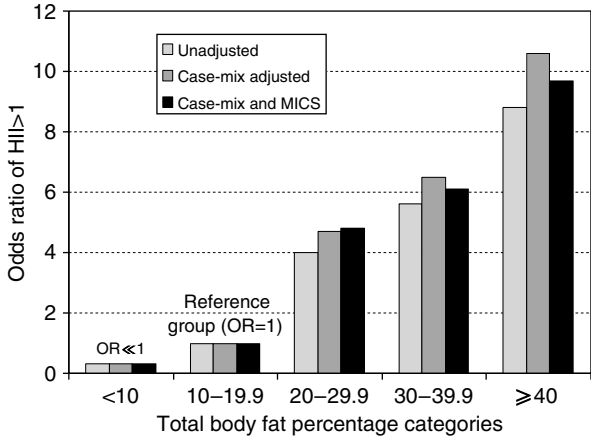

Figure 2 | Odds ratio of HII ⩾ 1 in 189 MHD patients among the *a priori* selected categories of total body fat percentage based on fat percentage increments of 10%. Case-mix models are adjusted for age, gender, race and ethnicity, diabetes, Charlson comorbidity scale, and dialysis vintage. Case-mix and MICS-adjusted models are also controlled for serum albumin, CRP, and IL-6.

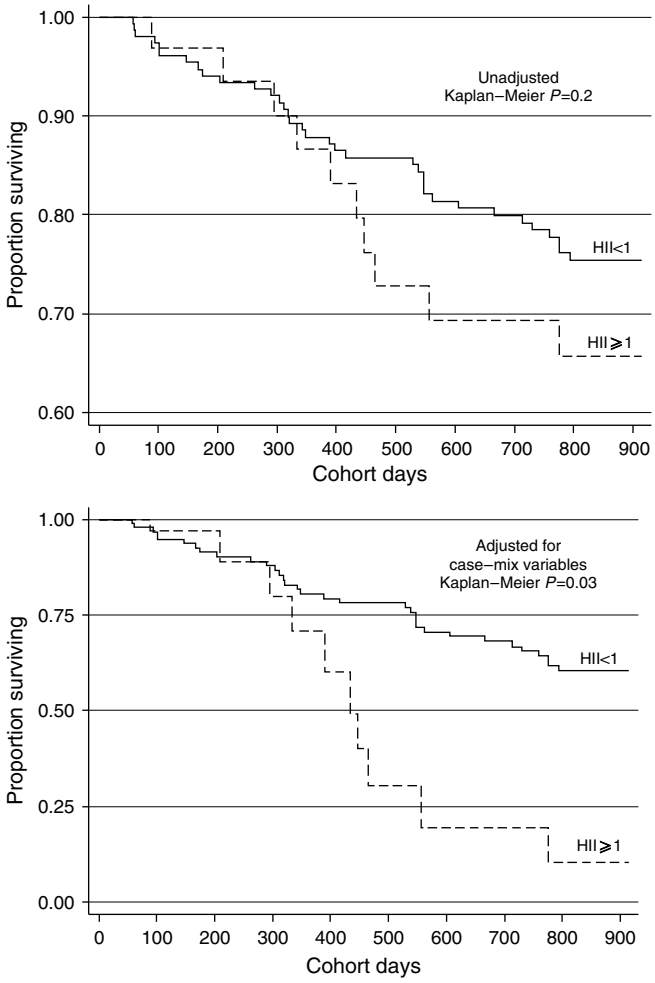

Figure 3 | Kaplan–Meier proportion of surviving MHD patients after 30 months of observation using an HII cutoff level of 1 in the cohort of 189 MHD patients of the NIED Study. Upper panel: unadjusted survival. Lower panel: adjusted for case-mix variables, including age, race and ethnicity (Blacks, Asians, and Hispanics), diabetes mellitus, Charlson comorbidity score, dialysis vintage, dialysis dose (K_t/V), and body fat percentage according to the NIR measurements.

Table 5 | Hazard ratio of 30-month death according to the HII cutoff level of 1 in 189 MHD patients of the NIED Study

	3-year hazard ratio of death in 189 MHD patients		
	Unadjusted	Case-mix[a]	Case-mix and MICS (full model)[b]
HII ⩾ 1	1.61 (0.82–3.18)	**2.37** (1.10–5.12)	**2.47** (1.14–5.34)
	P=0.16	*P*=0.03	*P*=0.02
Albumin < 3.8 g/dl	**3.41** (1.89–6.19)	**2.49** (1.26–4.09)	**2.39** (1.18–4.87)
	P < 0.001	*P*=0.01	*P*=0.02
CRP ⩾ 10 mg/l	1.39 (0.67–2.87)	1.02 (0.48–1.23)	0.81 (0.37–1.77)
	P=0.4	*P*=0.9	*P*=0.6
IL-6 ⩾ 10 pg/ml	**1.76** (1.00–3.14)	1.52 (0.81–2.83)	1.30 (0.68–2.46)
	P=0.05	*P*=0.19	*P*=0.4

CRP, C-reactive protein; HII, high-density lipoprotein inflammatory index; IL-6, interleukin-6; MHD, maintenance hemodialysis; MICS, malnutrition–inflammation–cachexia syndrome; NIED, Nutritional and Inflammatory Evaluation in Dialysis Study.
[a]Case-mix model is adjusted for age, race and ethnicity (Blacks, Asians, and Hispanics), diabetes mellitus, Charlson comorbidity score, dialysis vintage, dialysis dose (K_t/V), and body fat percentage according to the NIR measurements.
[b]Case-mix and MICS model (the full model) also includes serum albumin (< 3.8 versus ⩾ 3.8), CRP (⩾ 10 versus less), and IL-6 (⩾ 10 versus less).

Inflammation and oxidative stress are likely contributors to morbidity, lower health-related quality of life, and mortality in MHD patients.[21] Although in the general population higher HDL is associated with better survival, a recent study did not find any association between HDL and survival in MHD patients.[22] Qualitative abnormalities of HDL function in MHD patients were reported in an *in vitro* study by Morena *et al.*[23] including the reduction of HDL protective capacity against oxidative stress because of impairment of LDL oxidation prevention and impairment of reverse cholesterol transport by HDL in these patients.

Several studies have indicated an association between low serum total cholesterol and poor survival of dialysis patients.[22,24,25] An observational study by Liu *et al.*[26] showed that MICS may be the cause of the inverse association between cholesterol and mortality in these patients. In the 4D Study,[4] 1255 diabetic dialysis patients were randomized to receive either atorvastatin (20 mg/day) or placebo for 5 years. The study was recently reported to be negative[4] with a nonsignificant 8% reduction of the primary composite end points achieved by treatment with the cholesterol/LDL-lowering agent. This was in distinct contrast to the recently published CARDS trial (Collaborative Atorvastatin Diabetes Study)[27] in type II diabetic patients who had not yet developed significant kidney disease. Hence, the association between traditional risk factors and survival in CKD patients is likely confounded by factors related to CKD and MHD.

Protein-energy malnutrition and inflammation, independently or together as in MICS, are common occurrences in CKD patients.[5,28] MICS is associated with poor clinical conditions and worse outcomes.[29,30] The confounding effect of MICS on associations between traditional risk factors such as obesity and hypercholesterolemia and clinical outcome is so strong that it even reverses these associations. Hence, a low, rather than a high, body mass index or serum cholesterol level is associated with mortality in MHD patients.[24,31] This phenomenon has been termed reverse epidemiology[3] and is also observed in individuals with chronic heart failure.[32] In our study in MHD patients, high HII values were associated with a higher body fat percentage and body mass index, indicating a 'paradox within the paradox'.[33] In this subset of MHD patients with high HII, the positive association between higher body fat mass and better survival in dialysis patients was not observed, rather the reverse was true.[34] The increased HII appeared to reverse the benefit of higher body fat mass seen in a general MHD population. It should be noted that in the high HII group serum creatinine, a surrogate of muscle mass linked to greater survival in dialysis patients,[35] was significantly lower, whereas diabetes and female gender were more prevalent. However, the mortality predictability of HII $\geqslant 1$ was independent of the said covariates.

A potential limitation of this study is a selection bias during enrollment. However, as the mortality in our cohort was less than the base population, it might be argued that a selection bias with such a direction generally would lead to a bias toward the null, so without this bias, our positive results may have been even stronger. In our cohort, CRP was not associated with mortality, and the mortality predictability of IL-6 lost statistical significance after multivariate adjustment. However, serum albumin remained a strong predictor of survival. Another potential limitation is that the HII measurement methodology and criteria, including using the cutoff point of 1.0, were originally developed and tested in patient populations without CKD.[18,36] However, we have shown that using the same criteria HII has significant associations with relevant clinical measures and outcomes in MHD patients. Our and similar data[18,36] indicate that the composition of HDL may be different in patients with increased HII. Future studies are to examine the HDL composition in this regard. Finally, in this study we did not include an *a priori* selected non-dialysis (control) group. However, two previous studies[18,36] included separate control groups, in whom HII averaged 0.66[18] and 0.68.[36]

The strengths of this study include the sample size, which was moderately large, the comprehensive clinical and laboratory evaluations, and the inclusion of many individuals with diabetes mellitus. Unlike previous cohorts that have been studied, ours has been extensively characterized for markers of inflammation and nutritional status, including direct total body fat measurements. The availability of these measures allowed us to demonstrate that HII was able to predict mortality risk independent of influences from other known inflammatory markers or comorbid states in this group of MHD patients. Another strength of this cohort is that the subjects were selected randomly without having any previous knowledge of their oxidative stress or inflammation status. Finally, the very same blood specimens that were utilized to measure markers of MICS and cytokines were also used for the HII measurements.

In conclusion, we found that serum HII correlated with several surrogates of body composition and poor quality of life in MHD patients. Moreover, HII $\geqslant 1$ was independently associated with increased death risk. Understanding the role of HDL inflammatory properties in the CKD population may lead to new diagnostic and therapeutic approaches for the 20 million individuals with CKD.

MATERIALS AND METHODS

Patient population

Subjects participating in the Nutritional and Inflammatory Evaluation in Dialysis (NIED) Study originated from a pool of approximately 1300 MHD outpatients in eight DaVita Inc., chronic dialysis facilities in the South Bay Los Angeles area (see NIED Study website at http://www.NIEDStudy.org for more details, as well as previous publications[14,37,38]). Inclusion criteria were outpatients who had been undergoing MHD for at least 8 weeks, were 18 years or older and who signed a local Institutional Review Board-approved consent form. Patients with an anticipated life expectancy of less than 6 months (e.g., due to a metastatic malignancy or advanced HIV disease) were excluded. From July through December 2002, blood samples were obtained from 189 MHD patients from eight DaVita dialysis clinics had given informed written consent to

participate in the NIED Study and whose sera were adequate for measurement of HDL inflammatory/anti-inflammatory properties.

The medical chart of each MHD patient was thoroughly reviewed by a collaborating physician, and data pertaining to underlying kidney disease, cardiovascular history, and other comorbid conditions were extracted. A modified version of the Charlson comorbidity index, that is, without the age and kidney disease components, was used to assess the severity of comorbidity.[39,40] The 189 MHD patients were followed for up to 30 months, that is, until 31 December 2004.

SF36 health-related quality of life scores

The SF36, assessed before blood measurements, is a short form health-related quality of life scoring system with 36 items, including eight independent scales. It is a well-documented, self-administered questionnaire and has been widely used and validated in MHD patients.[41,42] The eight scales of SF36 are summarized into two dimensions: 'physical health' and 'mental health'.

Anthropometric measures

Body weight assessment and anthropometric measurements were performed while patients were undergoing a hemodialysis treatment or within 5–20 min after termination of the treatment. Biceps skinfold and triceps skinfold thicknesses were measured with a conventional skinfold caliper using standard techniques as described elsewhere.[43,44]

Near-infrared interactance

To measure the percentage of body fat and estimate fat-free body mass, NIR interactance technology was utilized at the same time as the anthropometric measurements.[45,46] A commercial NIR interactance sensor with a coefficient of variation of 0.5% for total body fat measurement (portable Futrex 6100®, Gaithersburg, MD, USA, http://www.futrex.com) was used. NIR measurements were performed by placing a Futrex sensor on the non-access upper arm for several seconds, after entering the required data (date of birth, gender, weight, and height) from each patient. NIR measurements of body fat have been shown to correlate significantly with other nutritional measures in MHD patients.[46]

Laboratory tests

Predialysis blood samples and postdialysis serum urea nitrogen were obtained on a mid-week day and coincided chronologically with the drawing of quarterly blood tests in the DaVita facilities. The single-pool K_t/V was used to represent the weekly dialysis dose. All routine laboratory measurements were performed by DaVita® Laboratories (Deland, FL, USA) using automated methods.

Serum high-sensitivity CRP was measured by a turbidometric immunoassay in which a serum sample is mixed with latex beads coated with anti-human CRP antibodies forming an insoluble aggregate (mg/l, normal range: <3.0 mg/l; WPCI, Osaka, Japan).[47,48] IL-6 and tumor necrosis factor-α were measured using immunoassay kits based on a solid phase sandwich enzyme-linked immunosorbent assay using recombinant human IL-6 and tumor necrosis factor-α (pg/ml, normal range: <9.9 pg/ml (IL-6) and <4.7 pg/ml (tumor necrosis factor-α); R&D Systems, Minneapolis, MN, USA).[49–51] CRP and the cytokines were measured in the General Clinical Research Center Laboratories of Harbor-UCLA Medical Center. Plasma total homocysteine concentrations were determined by high-performance liquid chromatography at Harbor-UCLA Clinical Laboratories.

Measurement of HII

In addition to its role in reverse cholesterol transport (that is, from peripheral tissues to the liver for excretion in the bile), normal HDL has anti-oxidant and anti-inflammatory properties. In the presence of excessive metabolic and oxidative stress, HDL loses its protective capacity and can become proinflammatory. To determine HDL inflammatory properties, sera for assay were sucrose cryopreserved without EDTA, and HDL-containing supernatants were prepared by removal of the apolipoprotein B-containing proteins by a modification of previously described methods[18,52] as recently performed by McMahon *et al.*[36] The HDL containing supernatants were prepared using HDL Magnetic Bead Reagent (catalog no. 5030, Polymedcoa). Two hundred microliters of sera were incubated with 40 μl of the Magnetic Bead Reagent at room temperature for 5 min. The mixture was placed on the magnetic particle concentrator for 3 min and the HDL-containing supernatant was removed. The fluorescence of LDL was determined in the absence or presence of the test HDL by a minor modification of the procedures described previously.[18,52] Briefly, dichlorofluorescein diacetate (DCFH-DA) was dissolved in fresh methanol and incubated at room temperature and protected from light for 30 min, resulting in the release of DCFH. Ten microliters containing 0.5 μg of normal human LDL-cholesterol was added to wells with or without the HDL-containing supernatants (90 μl containing 0.45 μg of HDL-cholesterol) in round-bottom, black polypropylene microtiter plates and mixed. The plates were then incubated at 37°C on a rotator for 1 h. DCFH solution (10 μl containing 2 μg DCFH) was then added to each well including wells without lipoproteins, mixed, and incubated with rotation at 37°C for 2 h while being protected from light. Fluorescence was determined with a plate reader. Fluorescence intensity was determined with a Farrand (Valhalla, NY, USA) system 3 scanning spectrofluorometer set at an excitation wavelength of 485 nm and an emission wavelength of 530 nm.[52] A sensitivity level of 0.1 and slit widths of 2.5 and 10 nm were used for excitation and emission, respectively.[52] The fluorescence values from wells without lipoproteins were subtracted from the values of the wells with lipoproteins.

Values in the absence of HDL were normalized to 1.0. The values obtained in the presence of the test HDL were divided by the value obtained in the absence of HDL to yield the HII. Values >1.0 after the addition of the test HDL indicated proinflammatory HDL; values <1.0 indicated anti-inflammatory HDL.[18,52] In some cases, the values for fluorescence in wells containing LDL together with the test HDL were less than the fluorescence values of wells containing only DCFH. In these instances, the HII values were not only <1.0, they were negative values.

Statistical methods

Conventional *t*-tests were used to detect significant differences among HII levels, that is, HII <1 versus HII $\geqslant 1$. χ^2 and rank tests were used for categorical variables. Pearson's correlation coefficient (r) was used for analyses of associations. Multivariate regression analyses and analysis of covariance were performed to obtain adjusted *P*-values controlled for case-mix and comorbidity covariates. Logistic regression models were fitted to construct odds ratio of HII >1 within the body fat percentage categories controlling for confounding covariates. To calculate the relative risks of death, we obtained hazard ratios using Cox proportional hazard models after controlling for the above-mentioned covariates. Plots of log (−log (survival rate)) against log (survival time) were performed to establish the validity of the proportionality assumption. Kaplan–Meier analyses were utilized to assess the differences in surviving

proportions between the two HII categories. Case-mix and comorbidity covariates included gender, age, race and ethnicity (Hispanics, Blacks, Asians, and others), diabetes mellitus, Charlson comorbidity scale, and dialysis vintage (number of months on MHD treatment); and laboratory surrogates of MICS in fully adjusted Cox models included serum CRP, IL-6, and albumin concentrations. Fiducial limits are given as mean $\pm$ s.d. or median and interquartile range. Risk ratios include 95% confidence interval levels. A $P<0.05$ or a 95% confidence interval that did not span 1.0 was considered to be statistically significant. A *P*-value between 0.05 and 0.20 is also listed with two decimals to identify potential type II errors. Descriptive and multivariate statistics were carried out with the statistical software 'Stata version 9.0' (Stata Corporation, College Station, TX, USA).

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Contribution of authors

Dr Kalantar-Zadeh contributed to the design, conduct and analysis of the study, PI of the grants, and writing of the paper. Drs Kopple and Fogelman contributed to the analysis and interpretation of the data, reviewing, amending, and approving of the paper. Dr Navab and Ms Kamranpour contributed to the design and conduct of the study, conduct and analysis of the study, reviewing and approving the paper.

Conflict of interest

MN and AMF are principals in Bruin Pharma and AMF is an officer in Bruin Pharma.

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