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Soluble CD14 Levels, Interleukin-6, and Mortality Among Prevalent Hemodialysis Patients

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Abstract

Background: CD14 is a pattern-recognition receptor that plays a central immunomodulatory role in pro-inflammatory signaling in response to a variety of ligands, including endotoxin. CD14 protein is present in two forms soluble (sCD14) and membrane-bound. Hereby we studied the implications of elevated sCD14 in hemodialysis patients. We hypothesized that sCD14 elevation may link to cytokine activation and protein-energy wasting, predisposing to increased mortality risk.

Study Design: Prospective observational study of prevalent hemodialysis patients.

Setting & Participants: 211 prevalent hemodialysis patients, median age of 66 years, 29 months of vintage dialysis time followed-up for mortality for a median of 31 months.

Predictors: Tertiles of baseline circulating sCD14 corresponding to <2.84, 2.85-3.62, and >3.63 µg/mL, respectively.

Outcome: The major outcome of interest was all-cause mortality.

Measurements: sCD14 and endotoxin, together with other markers of inflammation and protein-energy wasting.

Results: The median value of sCD14 was 3.2 µg/mL (25th to 75th percentile, 2.7 to 3.9). sCD14 correlated positively with C-reactive protein, interleukin-6, endotoxin and long pentraxin-3, while negatively with serum albumin, muscle mass and handgrip strength. Patients with elevated sCD14 had lower body mass index and increased prevalence of muscle atrophy. Patients within the highest sCD14 tertile had a crude mortality Hazard ratio of 1.94 (95% CI 1.13-3.32), that persisted after

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adjustment for multiple confounders (Hazard ratio 3.11 [95% CI 1.49-6.46]). Among patients with persistent inflammation, the presence of concurrent elevation of sCD14 levels gradually increased the mortality risk, but this effect was less than multiplicative and failed to show a statistical interaction.

Limitations: Those inherent to an observational study.

Conclusions: sCD14 is associated with inflammation and protein-energy wasting in hemodialysis patients. It is a strong and independent predictor of mortality that warrants further assessment in the clinical setting regarding its usefulness as a complementary prognosticator to other general inflammatory markers.

Keywords

Endotoxin; IL-6; protein-energy wasting; CRP; hemodialysis; pentraxin-3

INTRODUCTION

While the mechanisms explaining the elevated mortality risk of end-stage renal disease (ESRD) patients are not yet fully elucidated, both protein-energy wasting (PEW)¹ and inflammation² strongly augment the hazards of death. Inflammation has been proposed as the central integrating factor linking PEW and cardiovascular disease (CVD) in ESRD, and although the etiology of unprovoked inflammation in the uremic *milieu* is essentially unknown, both dialysis-related and dialysis-unrelated factors are likely to contribute.^{3;4} One potentially important, yet scarcely explored source of inflammation in ESRD is subclinical endotoxemia, as transmembrane passage of lipopolysaccharide (LPS) fragments may constitute an important cause of immune activation in dialysis patients.^{5;6} CD14 is a pattern-recognition receptor that plays a central immunomodulatory role in pro-inflammatory signaling in response to a variety of ligands, including endotoxin and other bacterial products from both gram negative as well as gram positive bacteria.⁷ An LPS concentration as low as 0.01 ng/ml induces upregulation of CD14 expression⁸ stimulating the activation of cytokines, myokines and adipokines.^{9;10} CD14 protein is present in two forms soluble (sCD14) and membrane-bound (mCD14). While mCD14 binds LPS and induces the release of pro-inflammatory cytokines and reactive oxygen species,¹¹ sCD14 increases in response to LPS challenge and is derived both from secretion of CD14 and enzymatically cleavage.⁷

In individuals without kidney disease, elevated sCD14 has been related to aortic stiffness and metabolic disorders, including hypertriglyceridemia, insulin resistance and activation of the inflammatory cascade.^{12;13} In hemodialysis (HD) patients, increased CD14 expression and increased sCD14 serum levels have been reported.⁸ Heine et al.¹⁴ recently showed that the number of CD14++ monocytes was predictive of cardiovascular events and death in a dialysis population. However, the phenotype associated with elevated sCD14 and its links to endotoxin in dialysis patients have not been well explored. In this study, we hypothesized that elevated sCD14 predisposes to increased mortality risk. We therefore examined the association between plasma sCD14 concentrations with other inflammatory and PEW markers as well as its implications on outcome in a carefully phenotyped cohort of prevalent HD patients.

METHODS

Patients and experimental design

This study includes prevalent patients undergoing HD at five dialysis units at Stockholm and one at Uppsala, Sweden. In all participating dialysis units, and at time of blood extraction, water conductivity was <1 µS/cm, number of viable microorganisms <100/ml and endotoxin concentration <0.25 IU/ml. As per hospital protocols, dialysis filters were not re-used. This is

a *post hoc* analysis from a cross-sectional study that originally aimed at investigating the variability of inflammatory markers in HD patients.¹⁵ Patient recruitment and baseline sampling took place between October 2003 through September 2004. From 254 patients invited to participate, several exclusions were made due to unwillingness to participate (n=6) and HIV (n=1). Once the study was finalized further exclusions were made due to insufficient clinical information (n=18) and sudden death (n=1). Thus, 228 patients were included in the study and further followed for assessment of overall and cardiovascular mortality. From this material, sCD14 and endotoxin could be determined only in 211 due to not enough plasma available in sixteen of the patients and sCD14 values outside the calibration range in one further patient. A comparison between the included and excluded patients did not reveal any major population difference with regards to age, sex distribution, dialysis vintage, or body weight (not shown). Survival was determined from the day of examination and until 10th March 2007, with a mean follow-up period of 31 (21 to 37) months. There was no loss of follow-up of any patient. The study protocols were approved by the Ethics Committee of Karolinska Institutet and Uppsala University. Signed informed consent was obtained from all patients.

Laboratory analyses

Blood samples were collected before the HD session after the longest interdialytic period. Plasma and serum were separated and kept frozen at -70° C if not analyzed immediately. Serum concentrations of IL-6 were quantified by immunometric assays on an Immulite Analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, CA) according to the instructions of the manufacturers. Plasma PTX3 concentration was measured by using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Perseus Proteomics Inc., Japan). high sensitivity C-reactive protein (hsCRP), fibrinogen, S-albumin, S-creatinine, total cholesterol, triglycerides, hemoglobin concentrations as well as the percentage of hypochromic red blood cells (RBC) were analyzed using certified methods at the Department of Laboratory Medicine in Karolinska University Hospital or Uppsala Academic Hospital. We used quantitative chromogenic Limulus Amebocyte Lysate (LAL) test for endotoxins in plasma (both free and protein-bound forms) using the commercially available kit (QCL-1000, Cambrex bioscience Inc, Walkersville, MD) following manufacturers instructions. The lower limit of detection is 0.01 EU/mL and the coefficient of variation was 3-9%. Concentration of sCD14 was measured using commercially available ELISA kit (sCD14 Quantikine ELISA Kit, R&D Systems, MN, USA). The inter- and intra-assay coefficient of variations are <7.5 and <6.5% respectively. All samples were measured in triplicates and the mean value was used.

Nutritional status and anthropometric evaluation

Body mass index (BMI) and anthropometric and dynamometric measurements were determined on a dialysis day. Height was obtained from the patient's chart. Fat mass and lean body mass were assessed according to Durnin et al¹⁶ by using the four skinfold thicknesses (biceps, triceps, subscapular, and suprailiac), measured with a conventional skinfold caliper (Cambridge Scientific Instruments, Cambridge, MD). Mid-arm circumference (MAC) was measured with a plastic tape; Mid-arm muscle circumference (MAMC) was calculated by using the following formula: $MAC - \pi \times \text{triceps skinfold thickness}$.¹⁶ Handgrip strength was measured in both the dominant and non-dominant hands by using a Harpenden Handgrip Dynamometer (Yamar, Jackson, MI). Each measurement was repeated three times for each arm, and the highest value for each arm was noted. For our analysis, we used the arm contralateral to the fistula/graft arm. Subjective Global Assessment (SGA) was used to evaluate the overall PEW.¹⁷ PEW was defined, for the purpose of this study, as $SGA > 1$. This assessment was performed either at the time of or within one week of blood sample collection. Estimation of the presence of muscle atrophy is a part of the SGA questionnaire, which has recently been validated as a useful muscle assessment in dialysis patients.¹⁸

Statistical analyses

All variables were expressed as mean $\pm$ SD or as median (25th to 75th percentile), unless otherwise indicated. Statistical significance was set at the level of $p < 0.05$. Differences among more than two groups were analyzed by analysis of variance (ANOVA) using one-way ANOVA or Kruskal-Wallis test, as appropriate. Spearman's rank correlation (ρ) was used to determine correlations of sCD14 with other variables. Multinomial logistic regression analyses were used to study variables associated with sCD14 in this patient population. Selection of the confounders in this model was done on the basis of sCD14 pathophysiology, and all covariates satisfied the proportional odds assumption. Restricted cubic splines (RCS) were utilized to evaluate the nonlinear relationships between sCD14 and outcome.¹⁹ We chose four knots at quantiles, which has been suggested to offer adequate fit of the model and is a good compromise between flexibility and loss of precision caused by overfitting a small sample.²⁰ Thereafter, the univariate and multivariate Cox regression analysis based on tertile distribution were presented as hazard ratio (HR; 95% confidence intervals [CI]). In order to avoid the possibility that patients within dialysis centers may have correlated time to event, we adjusted for center as a fixed effect in the Cox models. All covariates satisfied the proportional hazards assumption. Because it has been suggested that persistent inflammation may influence effects of elevated CD14++CD16+ monocytes on adverse outcomes,¹⁴ the statistical interaction between sCD14 and IL-6 was tested by the inclusion of the product term of these two variables. Statistical computations were carried out using SAS, version 9.1.3 (SAS Institute, Cary, NC). As P values are not adjusted for multiple testing, they have to be considered as descriptive.

RESULTS

Patient characteristics

Patients received HD three times a week (4–5 h per session) using bicarbonate dialysate. Most of the patients had polyamide membranes (59%) followed by polysulfone (35%). Regarding vascular access, 58% had an AV fistula, while 22 and 20% had grafts and central dialysis catheters, respectively. Further baseline and prescription data is described in Table 1. Most patients were on antihypertensive medications (β -blockers; $n=104$ [49%], calcium channel blockers; $n=51$ [21%], and angiotensin converting enzyme inhibitors (ACE inhibitors)/angiotensin receptor blockers (ARB); $n=67$ [31%]), as well as other commonly used drugs in ESRD (such as phosphate and potassium binders) and vitamin B, C, and D supplementation. Sixty four patients (30%) were on lipid-lowering medication (statins). One hundred and ninety six patients (93%) were receiving erythropoiesis stimulating agents (ESA) at time of evaluation. Weekly doses of darbepoetin in μg were converted to international units of epoetin by multiplying with the conversion factor of 200. The median ESA dose was equivalent to 10,000 (6,000–14,750) U/week, which was normalized for body weight and presented as U/Kg/week.

Plasma sCD14 and endotoxin levels

The median values of sCD14 and endotoxin were 3.2 (2.7 to 3.9) $\mu\text{g/mL}$ and 0.65 (0.43–1.16) EU/mL, respectively. sCD14 levels were significantly higher in women compared to men (3.5 [2.8–4.0] vs 3.1 [2.7–3.7] $\mu\text{g/mL}$; $p=0.02$). Plasma sCD14 levels were not elevated in patients with diabetes, or among patients with clinical history of previous cardiovascular events (data not shown). Current smokers presented higher ($P=0.03$) sCD14 values (3.4 [3.0–4.1] $\mu\text{g/mL}$, $n=41$), than former (3.0 [2.6–3.6] $\mu\text{g/mL}$, $n=94$) and non-smokers (3.2 [2.5–4.0] $\mu\text{g/mL}$, $n=68$). Patients on statins (3.0 vs 3.3 $\mu\text{g/mL}$; $n=64/147$; $P=0.03$), ACE inhibitors or ARB (2.9 vs 3.4 $\mu\text{g/mL}$; $n=67/143$; $P=0.003$), β -blockers (3.0 vs 3.4 $\mu\text{g/mL}$; $n=104/107$; $P=0.02$) or acetylsalicylic acid derivatives (3.0 vs 3.3 $\mu\text{g/mL}$; $n=63/148$; $P=0.05$) had significantly lower median sCD14 levels. No difference was found in endotoxin levels with regards to sex, smoking status, different comorbidities and medication prescriptions.

Variables related to sCD14 and endotoxin levels in HD patients

As expected, in univariate analysis, sCD14 levels were positively related to inflammatory markers, such as hsCRP ($\rho=0.30$, $p<0.001$), IL-6 ($\rho=0.26$, $p<0.001$), PTX3 ($\rho=0.24$, $p<0.001$) and fibrinogen, as well as blood lipids (cholesterol and triglycerides), ESA dose and the percentage of hypochromic RBCs. There was also a positive correlation with endotoxin values ($\rho=0.21$, $p<0.001$), but not with white blood cell or lymphocyte count (not shown). At the same time, sCD14 showed negative associations with salbumin levels ($\rho=-0.20$, $p<0.01$) and surrogates of muscle mass (lean body mass [LBM; $\rho=-0.19$, $p<0.01$], mid arm muscle circumference [MAMC; $\rho=-0.20$, $p<0.01$] and handgrip strength [$\rho=-0.19$, $p<0.01$]) but not with fat body mass or creatinine. Because of the observed association between muscle strength and sCD14, patients were divided according to the subjective grading of muscle atrophy (included in the SGA. Patients with no signs of muscle atrophy had lower median sCD14 level (3.0 [2.5-3.7]) $\mu\text{g/mL}$, $n=131$) than those with mild (3.3 [2.8-3.8]) $\mu\text{g/mL}$, $n=49$) or severe (3.9 [2.1-4.7]) $\mu\text{g/mL}$, $n=25$) signs of muscle atrophy ($p=0.002$). Finally, we performed a multinomial logistic regression analysis searching for predictors of sCD14 levels. Sex, IL-6 and endotoxin levels were related to higher odds of having elevated plasma sCD14 concentration (Table 2). Whereas endotoxin values were related to age ($\rho=-0.14$; $P<0.05$), ESA dose ($\rho=-0.13$; $P<0.05$), hemoglobin ($\rho=0.18$; $P<0.01$) and triglyceride values ($\rho=0.24$; $P<0.001$), no significant association was found to inflammatory markers.

Tertiles of sCD14 distribution—The study participants were divided into tertiles of sCD14 distribution (Table 1). Across increasing sCD14 tertiles, there was a trend towards increased proportion of women. Also, patients within the higher tertile of sCD14 distribution were more often inflamed and had higher endotoxin values. They also presented a lower BMI (attributed to decreased LBM rather than a decline in fat body mass) and lower handgrip strength and MAMC values. Finally, across increasing sCD14 tertiles, incrementally higher EPO doses and percentage of hypochromic RBCs were observed.

Impact of sCD14 on survival and relative risks

During the follow up period 78 patients died. Age- and sex- adjusted spline curves showed that only the higher sCD14 concentrations have impact on mortality (Figure 1). Thus, we used the strategy of tertile distribution to distinguish the group of patients within the upper side of the hyperbole. Compared with the lower sCD14 tertile, patients in the top tertile of distribution remained at higher risk of death during the follow-up period even after various adjustments (Table 3).

Cross-classification IL-6 and sCD14

The prognostic value of sCD14 levels was independent of inflammation (Table 2). However, as it has been suggested that the effect of elevated CD14++CD16+ monocyte levels on death counts is magnified in the presence of a persistently inflamed environment¹⁴, we tried to confirm this postulate by studying the prognostic use of sCD14 in the presence or absence of concomitant elevated IL-6 levels. For this purpose, different groups with high and low concentration values were established according to the median value of sCD14 and IL-6 and cross-classified. The proportion of patients dying during the follow-up period rose across incremental groups of sCD14 for any group of IL-6 levels (Table 4). Whereas inflamed groups were associated with a worse survival, a moderate gain in prognostication and a higher death count was observed when both elevated IL-6 and elevated sCD14 were present. However, statistical interaction analysis failed to show a departure from multiplicity of effects (crude HR for the product term sCD14*IL-6 was 0.89 [95% CI 0.62-1.29]; $p=0.6$).

DISCUSSION

We have shown that sCD14 level is positively associated with surrogate markers of inflammation, such as hsCRP, IL-6 and PTX3, in a carefully phenotyped cohort of prevalent HD patients being followed for a median period of 41 months. Furthermore, patients with higher sCD14 had lower BMI, decreased muscle strength and overt evidence of muscle atrophy. Finally, survival analysis showed that patients within the highest tertile of sCD14 distribution had the worst survival, irrespective of multiple confounders. Altogether, the present study evidences a detrimental linkage between sCD14 levels and markers of inflammation, PEW and mortality in hemodialysis patients.

Lipid A (endotoxin), the hydrophobic anchor of LPS, is a glucosamine-based phospholipid that makes up the outer monolayer of the outer membranes of most Gram-negative bacteria.⁵ The concentration of endotoxin observed in our patients is comparable to that reported previously in HD²¹ and peritoneal dialysis (PD).²² Combined use of bicarbonate buffered dialysate and highly permeable dialyzers HD membranes could potentially increase the risk of reverse transfer of dialysate contaminants, including endotoxin fragments in to the blood compartment.^{8;23} Endotoxin level has been shown to be positively associated with atherosclerosis in the general population²⁴ as well as ESRD patients.²² However, we did not observe a significant association between plasma endotoxin level and markers of inflammation, body composition or mortality. This may be, in part due to the characteristics of our study design and the dialysis procedure in our centers, but also to the fact that endotoxin mediates the clinical response through interaction with various specific receptors such as sCD14, lipoprotein binding protein and bactericidal/permeability-increasing protein.^{25;26} Also, our analysis measured both free and protein-bound endotoxin. In parallel, we could also observe in both univariate and multivariate analysis that endotoxin was significantly but weakly associated to sCD14. This may be due to the complex endotoxin-sCD14 interaction. Although sCD14 receptor mediates the inflammatory response of endotoxin, the same receptor also promotes its clearance and neutralization.^{27;28} Furthermore, low concentrations of endotoxin act through mCD14, high concentrations of endotoxin do it through sCD14.⁷ Thus, it is possible as well that by measuring solely sCD14 may provide only an incomplete view of the effects of this molecule. In our results, different medication prescriptions, such as statins, were associated with lower sCD14 levels, which are in accordance with previous reports showing that statin use leads to reduced sCD14 expression, linking the pleiotropic anti-inflammatory effects of statins to its capacity to attenuate LPS responsiveness.²⁹ Finally, immune responsiveness has been reported to be greater in women than men,³⁰ and we indeed found that sCD14 was significantly higher in the women of our study. In contrast, El Temple et al³¹ showed that although the percentage of cells expressing intracellular TLR-4 protein in response to LPS challenge was higher in male vs female healthy individuals, no gender difference in CD14 expression was observed.

Not surprisingly, we observed an association between sCD14 and markers of inflammation, such as hsCRP, IL-6 and PTX3. The latter association with PTX3 deserves some attention, as pentraxins are a family of multimeric pattern-recognition which are divided into two groups based on the primary structure of the subunit as short pentraxins and long pentraxins. CRP and serum amyloid P-component are classic short pentraxins, whereas the prototype of the long pentraxin family is PTX3.³² PTX3 is expressed in a variety of cell types in response to inflammatory cytokines and TLR engagement. Elevated PTX3 levels in ESRD patients are related to CVD, PEW and increased mortality,³³ being closely related to the development of albuminuria and endothelial dysfunction.³⁴ Nonetheless, since sCD14 was not related to white blood cell or lymphocyte count in our study, we cannot directly extrapolate our findings with those of CD14++ activated monocytes¹⁴.

Another interesting finding in our study is the association of sCD14 with markers of muscle mass and PEW, indicating cross-talk between inflammatory molecules and nutritional status in dialysis patients. Indeed, sCD14 has been previously related to insulin resistance,¹³ muscle wasting,³⁵ atherosclerosis¹² and acute myocardial infarction in the general population.³⁶ PEW and inflammation are common phenomena that usually occur concurrently in maintenance dialysis patients.³⁷ PEW is associated with abnormal cardiac geometry,³⁸ atherosclerosis,² and increased mortality in patients with ESRD.^{38;39} Many structural and functional alterations in skeletal muscles contribute to limited work capacity in ESRD.⁴⁰ Patients with higher sCD14 had evidence of lower BMI, muscle atrophy and lower handgrip strength in our study. While Fernández-Real et al.¹³ did not observe an association between sCD14 and BMI in 123 healthy well-nourished and non-inflamed individuals with normal renal function, these results are not fully comparable with ours, since cytokine retention in dialysis patients may substantially exaggerate the links between persistent inflammation and PEW.¹ In fact, we could previously reported that cytokine expression is increased in skeletal muscle post-HD and is related to muscle protein breakdown and acute phase protein synthesis.^{41;42} As exposure to LPS induces cytokine expression in the skeletal muscle in a dose dependent manner,⁹ this may establish, altogether, a link between endotoxin-sCD14 activation and PEW through increased muscle atrophy.

The present study shows that sCD14 independently associates with the patient's mortality. Consequently, we could observe that, in agreement with a previous study using CD14++CD16+ monocytes¹⁴, only the highest sCD14 tertile was persistently associated with increased mortality, irrespective of both traditional and non-traditional risk confounders. Thus, our results are in accordance with those of Ulrich *et al*,⁴³ who showed that activated CD14++CD16+ monocytes presented increased ACE expression, evidencing the prominent role of these proinflammatory cells in atherogenesis. As Heine *et al*¹⁴ observed a bigger death count across different CRP and CD14++CD16+ monocyte groups, they hypothesized that the effect of CD14 activation on mortality was magnified in the presence of concomitant elevation of CRP levels. In agreement with this observation, we found a moderate gain in prognostication when both IL-6 and sCD14 were included in the model. However, as this effect failed to show a departure from multiplicity of effects, a statistical interaction could not be demonstrated. As we cannot exclude insufficient power to demonstrate these interaction effects, this should be re-assessed in larger materials.

When interpreting our findings, the following limitations should be taken into consideration: First, our cross-sectional design precludes from causality. Second, the classification of CVD only included patients with clinically significant vascular disease, which may underestimate the true prevalence of CVD. Third, the prevalent nature of our cohort may represent a selection of patients who have survived from CVD or survived despite presence of factors potentially contributing to increased cardiovascular risk. Fourth, we lack information regarding volume status, which may influence our findings. Finally, we based our determinations on single measurements of inflammatory markers that are subjected to certain variability over time.⁴⁴

To summarize, sCD14 is positively associated with various markers of inflammation but negatively related to BMI, lean body mass and muscle strength. At the same time, sCD14 emerged as an independent predictor of mortality. Further studies need to examine the relationship between circulating endotoxin concentrations and sCD14 levels in the inflamed uremic milieu and their effect on vascular health and outcome measures. This may lead to better prognostication and better risk stratification in dialysis patients.

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Figure 1.

Spline curve showing the log-transformed hazard ratios and 95% confidence intervals (dashed line) for all-cause mortality associated with sCD14 values in 211 prevalent hemodialysis patients. The model is plotted as restricted cubic splines with 4 knots and adjusted for age and sex. P for linearity = 0.4.

Table 1

Patient characteristics and biochemical profile of all patients and according to tertiles of sCD14 distribution.

	All patients N=211	Low tertile <2.84 ug/mL N=70	Middle tertile 2.85-3.62 ug/mL N=70	High tertile >3.63 ug/mL N=71	P-value
Age, years	65 (50-74)	63 (48-76)	67 (49-75)	64 (51-73)	0.3
Vintage, months	29 (15-57)	26 (14-51)	31 (12-57)	31 (18-70)	0.7
Sex (% men)	56	64	60	45	0.06
PEW (%)	47	44	46	51	0.7
Diabetes mellitus (%)	23	23	26	21	0.8
CVD (%)	63	61	64	62	0.9
hsCRP (mg/L)	6.5 (2.5-21.0)	4.9 (1.5-11.3)	6.0 (2.5-21.0)	10.0 (4.2-26.0)	0.001
Interleukin-6 (pg/mL)	8.5 (4.9-15.0)	6.2 (4.1-10.8)	8.6 (5.6-15.0)	10.1 (6.3-21.6)	0.001
Pentraxin-3 (ng/mL)	10.3 (7.0-17.0)	2.4 (2.1-2.7)	3.2 (3.0-3.4)	4.2 (3.9-4.8)	<0.001
Fibrinogen (mg/dL)	420 (340-525)	380 (330-470)	445 (368-525)	425 (360-560)	0.04
Albumin (g/dL)	3.5 ± 0.5	3.5 ± 0.4	3.4 ± 0.4	3.3 ± 0.5	0.01
Endotoxin (EU/mL)	0.65 (0.43-1.16)	0.42 (0.34-0.87)	0.71 (0.47-0.95)	0.80 (0.51-2.65)	<0.001
Body mass index (kg/m ²)	24.3 ± 5.1	24.2 ± 5.1	25.5 ± 5.3	23.3 ± 8.0	0.01
Lean Body Mass (kg)	48.1 ± 10.4	49.3 ± 10.4	49.8 ± 11.1	45.2 ± 9.3	0.02
Fat Body Mass (kg)	22.6 ± 8.4	22.4 ± 8.2	23.9 ± 7.9	21.3 ± 8.9	0.08
MAMC (cm)	24.2 ± 3.6	24.4 ± 3.6	24.8 ± 3.2	23.2 ± 3.7	0.008
Handgrip strength (%)	62.1 ± 22.6	61.1 ± 24.0	52.2 ± 23.0	48.4 ± 22.0	0.02
ESA (U/Kg/week)	134 (82-210)	120 (74-181)	116 (74-187)	180 (115-255)	0.001
Hemoglobin (g/dL)	11.9 ± 1.3	11.9 ± 1.2	11.9 ± 1.3	1.17 ± 1.4	0.3
Hypochromic RBCs (%)	1.3 (0.7-3.4)	1.0 (0.4-1.6)	1.1 (0.5-3.7)	2.5 (0.9-3.9)	0.003

hsCRP=high sensitive C-Reactive protein, MAMC= mid arm muscle circumference, ESA= Erythropoiesis stimulating agents, RBCs=Red blood cells. Ns, not significant. Note= Conversion factors for units: serum albumin in g/dL to g/L, ×10.

Table 2

Odds ratios and 95% CI for tertiles of sCD14 distribution in a multinomial logistic regression including 211 prevalent HD patients.

	Odds ratio (95% CI)	P value
IL-6, middle vs low tertile	1.97 (0.99-3.93)	0.05
IL-6, high vs low tertile	4.74 (2.30-9.77)	<0.001
Sex, women vs men	0.35 (0.19-0.62)	<0.001
Age, 45-65 vs <45 years	0.79 (0.33- 1.89)	0.6
Age, ≥ 65 vs <45 years	0.65 (0.27-1.53)	0.3
Endotoxin, middle vs low tertile	2.37 (1.22-4.63)	0.01
Endotoxin, high vs low tertile	5.35 (2.67-10.74)	<0.001

The pseudo r^2 for this model equals 0.20. The independent variable was sCD14 levels divided into ordered tertiles of distribution, choosing the lowest sCD14 tertile as the reference. IL-6 tertiles were defined as follows: Low tertile ≤ 6.03 pg/mL; middle tertile 6.03 to 11.35 pg/mL, high tertile ≥ 11.36 pg/mL. Endotoxin tertiles were defined as follows: Low tertile ≤ 0.48 EU/mL; middle tertile 0.48 to 0.88 EU/mL; high tertile ≥ 0.89 EU/mL.

Table 3

Cox regression analysis for all-cause mortality.

	Middle sCD14 tertile	P value	High sCD14 tertile	P value
Unadjusted	0.98 (0.54-1.81)	0.9	1.94 (1.13-3.32)	0.01
Adjusted				
Model 1	0.98 (0.51-1.83)	0.9	3.05 (1.56-5.89)	<0.001
Model 2	0.91 (0.46-1.84)	0.8	3.11 (1.49-6.46)	0.002

Indicated are hazard ratios, their 95% confidence interval and the level of significance. The low sCD14 tertile was considered as the reference. Model 1 includes tertiles of sCD14 distribution following adjustment for age, sex, smoking habits, the prevalence of cardiovascular disease and diabetes and dialysis centre; model 2 further adjusted for medication impacting sCD14 levels (statins, ACEI/ARB, β -blockers and acetylsalicylic acid) as well as for vintage, IL-6 and PEW (SGA>1).

Table 4

Crude all-cause mortality risk according to median values of sCD14 and IL-6 cross-combined.

	Deaths, n (%)	HR (95% CI)	P-Value
Low IL-6, Low sCD14	13 (21%)	1.00	
Low IL-6, High sCD14	11 (25%)	1.25 (0.56-2.79)	0.6
High IL-6, Low sCD14	18 (40%)	2.36(1.15-4.82)	0.01
High IL-6, High sCD14	36 (58%)	3.71(1.96-7.01)	<0.001

Groups are created according to the median value of IL-6 (8.5 pg/mL) and sCD14 (3.2 µg/mL) in the population of study.