Inflammation Markers, Adhesion Molecules, and All-Cause and Cardiovascular Mortality in Patients with ESRD: Searching for the Best Risk Marker by Multivariate Modeling

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Inflammation is a major risk factor for mortality and cardiovascular (CV) complications in patients with ESRD. The predictive value of C-reactive protein (CRP) of the main proinflammatory cytokines (IL-1β, IL-6, IL-18, and TNF-α) and of two adhesion molecules (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1) in 217 dialysis patients was compared. Serum IL-6 and CRP added significant prediction power to the multivariate Cox model of all-cause death, and the gain in the prediction power attributable to IL-6 was approximately two times higher than that of CRP. Patients in the third tertiles of serum IL-6 and CRP had a relative risk of all-cause mortality 2.5 and 1.8 times higher than those in the first corresponding tertiles, and there was no statistical difference between these two relative risks. The gain in prediction power associated with TNF-α, IL-β, IL-18, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 was of small degree (P = NS). Similarly, serum IL-6 added the highest prediction power to the CV death model, and the IL-6 attributable gain was approximately two times higher than that of serum CRP. However, the risk estimate for CV mortality of patients with high serum IL-6 did not differ significantly from that of patients with high serum CRP. IL-6 adds significantly greater predictive power for all-cause and CV death to statistical models based on traditional and nontraditional risk factors in ESRD patients. However, the risk estimate by CRP being reasonably close to that of IL-6, CRP may be a cheap alternative to IL-6 in clinical practice.

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ESRD is now considered a prototypical situation of chronic inflammatory state (1,2) and C-reactive protein (CRP), a nonspecific marker of inflammation, is regarded as a fundamental biomarker for cardiovascular (CV) risk stratification in these patients. The synthesis of CRP in the liver is induced by proinflammatory cytokines such as IL-1β, IL-6, and TNF-α (1–3). IL-6 is considered as the key factor in the acute-phase response (3–5), and it has been suggested that this cytokine plays a primary role in the pathogenesis of both malnutrition and atherosclerosis in the dialysis population (6). IL-18 is a novel proinflammatory cytokine that promotes atherosclerosis and plaque vulnerability in experimental models (7). The relationship between IL-18 and CV outcomes has been studied both in middle-aged healthy men (8) and in patients with ischemic heart disease (9), but the predictive value of this cytokine in patients with ESRD still remains to be tested.

Atherosclerosis is the main cause of morbidity and mortality in patients with ESRD, and there is consistent evidence that CRP and proinflammatory cytokines such as IL-1β, IL-6, and TNF-α are risk factors for atherosclerotic complications and predict death and adverse CV outcomes in these patients (6,10–17). Intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are two members of the Ig-like supergene family that are synthesized by endothelial cells. The expression of these molecules on the cell surface is upregulated by inflammatory processes, and these substances promote the adhesion of leukocytes to the endothelium, which is an early event in the pathogenesis of atherosclerosis (18). In ESRD, the shedding of ICAM-1 and VCAM-1 by the inflamed endothelium determines a marked increase in the serum level of these substances (19), which parallels that of serum CRP (17), and ICAM-1 predicts mortality in predialysis patients (20).

Although the prognostic value of inflammation in dialysis patients has been investigated extensively, no prospective cohort study has compared directly the predictive value of inflammatory markers in these patients. The issue is important because the identification of "the best predictor" is of obvious importance for risk stratification in ESRD. In the present study, we compared the prediction power for all-cause and CV mortality of CRP, of the inflammatory cytokines IL-1β, IL-6, IL-18, and TNF-α; and of adhesion molecules ICAM-1 and VCAM-1 in a large cohort of patients who were on chronic dialysis.

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Materials and Methods

Protocol

The protocol was in conformity to the ethical guidelines of our institutions, and informed consent was obtained from each participant. For hemodialysis patients, all studies were performed during a midweek nondialysis day and for chronic ambulatory peritoneal dialysis (CAPD) patients at empty abdomen, between 8:00 a.m. and 1:00 p.m.

Study Cohort

A total of 217 patients who had ESRD (120 men and 97 women) and had been on regular dialysis treatment for at least 6 mo (median duration of regular dialysis treatment, 43 mo; interquartile range, 20 to 101 mo), without history of congestive heart failure and without intercurrent inflammatory illnesses, were eligible for the study. The main demographic and clinical characteristics of the patients included in the study are detailed in Table 1. The prevalence of diabetes in this cohort was 15% (33 of 217 patients).

All patients were virtually anuric (24-h urine volume <200 ml/d). Hemodialysis patients were treated three times weekly with standard bicarbonate dialysis (138 mmol/L Na, 35 mmol/L HCO_3, 1.5 mmol/L K, 1.25 mmol/L Ca, 0.75 mmol/L Mg) with either cuprophan or semisynthetic membranes (dialysis filters surface area, 1.1 to 1.7 m^2). The average urea Kt/V in these patients was 1.22 ± 0.27. Patients who were on CAPD all were on a four-exchanges-per-day schedule with standard dialysis bags. The average weekly Kt/V in these patients was 1.69 ± 0.29. Eighty-six patients were habitual smokers (22 ± 17 cigarettes/d). A total of 110 patients were on treatment with erythropoietin. Ninety-seven patients were being treated with antihypertensive drugs (72 on monotherapy with angiotensin-converting enzyme inhibitors, AT-1 antagonists, calcium channel blockers, and α- and β-blockers and 25 on double or triple therapy with various combinations of these drugs).

Table 1. Somatometric, clinical, and biochemical parameters of the study population[a]

Age (yr)	60.7 ± 15.1
Duration of RDT (mo)	43 (20–101)
Male gender (n; %)	120 (55%)
Diabetic (n; %)	33 (15%)
Smokers (n; %)	86 (40%)
Patients on antihypertensive therapy (n; %)	97 (45%)
Patients with previous CV events (n; %)	107 (49%)
Systolic BP (mmHg)	132.6 ± 22.7
Diastolic BP (mmHg)	74.3 ± 12.0
Pulse pressure (mmHg)	58.2 ± 16.4
Heart rate (beats/min)	80.6 ± 11.2
Hemoglobin (g/L)	108.3 ± 19.3
Albumin (g/L)	40.1 ± 5.5
Cholesterol (mmol/L)	5.43 ± 1.42
Calcium × phosphate ($mmol^2/L^2$)	4.50 ± 1.15
Homocysteine (μmol/L)	28.1 (20.0–42.9)
ADMA (μmol/L)	2.98 (1.76–4.26)
CRP (mg/L)	7.5 (3.4–16.7)
TNF-α (pg/ml)	7.1 (4.0–10.6)
IL-1β (pg/ml)	0.39 (0.00–1.20)
IL-6 (pg/ml)	6.1 (3.0–10.3)
IL-18 (pg/ml)	563 (438–770)
ICAM-1 (pg/ml)	281 (238–341)
VCAM-1 (ng/ml)	1083 (854–1398)

[a]Data are expressed as mean ± SD, median (interquartile range), or percentage frequency, as appropriate. RDT, regular dialysis treatment; CV, cardiovascular; ADMA, asymmetric dimethylarginine; CRP, C-reactive protein; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule.

Follow-Up

After the initial assessment, patients were followed up for an average of 41 mo (range, 0.8 to 70.0 mo). During the follow-up, fatal CV events (myocardial infarction, electrocardiogram-documented arrhythmia, heart failure, stroke, and other thrombotic events except arteriovenous fistula thromboses) and death were recorded accurately. Each death was reviewed and assigned an underlying cause by a panel of five physicians. As a part of the review process, all available medical information about each death was collected. This information always included study and hospitalization records. In the case of an out-of-hospital death, family members were interviewed by telephone to ascertain better the circumstances surrounding death.

Laboratory Measurements

Blood sampling was performed after an overnight fast between 8:00 a.m. and 10:00 a.m. always during a midweek nondialysis day for hemodialysis patients and at empty abdomen for CAPD patients. After 20 to 30 min of quiet resting in semirecumbent position, samples were taken into chilled EDTA Vacutainer, placed immediately on ice, and centrifuged within 30 min at −4°C, and the plasma was stored at −80°C before assay. Serum lipids, albumin, calcium, phosphate, and hemoglobin measurements were made using standard methods in the routine clinical laboratory. The plasma concentrations of asymmetric dimethylarginine (ADMA) and homocysteine were determined as reported elsewhere (21,22). Serum CRP was measured by using a commercially available kit (Immunonephelometric method; lower limit of detection, 3.5 mg/L; Behring, Scoppito, L'Aquila, Italy). Serum levels of IL-6, IL-1β, IL-18, TNF-α, ICAM-1, and VCAM-1 were measured by ELISA with the use of Quantikine High Sensitivity kits (intra-assay coefficient of variation for these substances ranging from 1.6 to 10%; interassay coefficient of variation ranging from 3.3 to 10.2%; R&D Systems, Minneapolis, Minnesota).

Statistical Analyses

Data are reported as mean ± SD (normally distributed data), median, and interquartile range (nonnormally distributed data) or as percentage frequency, as appropriate. Interrelationships

between CRP, cytokines, and adhesion molecules were analyzed by Spearman rank correlation coefficients and *P* values.

The independent prognostic power for all-cause and CV mortality of each inflammation marker (CRP, IL-1β, IL-6, IL-18, and TNF-α) and adhesion molecules (ICAM-1 and VCAM-1) was analyzed by univariate and multivariate Cox's regression analysis. In this analysis, we considered a series of traditional risk factors (age, gender, smoking, diabetes, antihypertensive therapy, previous CV events, systolic BP, and cholesterol) that are peculiar to ESRD (duration of regular dialysis treatment, treatment modality, hemoglobin, albumin, and calcium phosphate product), as well as emerging risk factors (homocysteine and ADMA). To construct basic multivariate COX models for all-cause and CV mortality, we preliminarily identified a set of variables that were associated (with $P < 0.10$) with these outcomes at univariate COX regression analysis. These variables then were used to construct two basic models: The first for all-cause mortality and the second for CV mortality. The treatment modality (hemodialysis/CAPD) was always introduced into these Cox models. We then compared the prediction power attributable to each inflammation marker and adhesion molecule by using the −2 log likelihood (−2 Log L) test. This test compares different Cox models fitted to the same set of data and the smaller the −2 Log L value, the stronger the agreement between the model and the observed data (23). The difference between the −2 Log L of the models gives a statistical estimate as to which of them provides a better fit to the data. A 3.841 difference in −2 Log L coincides with a significance level of 0.05 in a χ^2 distribution with 1 degree of freedom and indicates a better prediction of risk estimate provided by the method leading to the lowest −2 Log L value. Hazard ratios and their 95% confidence intervals were calculated with the use of the estimated regression coefficients and their SE in the Cox regression analysis. All calculations were made using a standard statistical package (SPSS for Windows Version 9.0.1; Chicago, IL).

Results

Interrelationships between CRP, Cytokines, and Adhesion Molecules

As shown in the correlation matrix reported in Table 2, CRP was related directly to IL-6 and ICAM-1. Correlations of the same magnitude were also found between IL-6 and ICAM-1, TNF-α and ICAM-1, IL-18 and VCAM-1, and ICAM-1. TNF-α and VCAM-1 resulted to be weakly related to IL-1β and IL-6.

Inflammation Markers, Adhesion Molecules, and All-Cause Mortality Modeling

During the follow-up period (range, 0.8 to 70.0 mo), 112 patients died, 65 of them (58% of total deaths) of CV causes. On univariate Cox regression analysis, IL-6, CRP, and ICAM-1 were significantly associated with mortality (all $P \leq 0.004$), whereas TNF-α, IL-β, IL-18, and VCAM-1 failed to predict survival ($P = 0.13$ to 0.78). On multivariate analysis, including treatment modality as well as all univariate predictors of survival, IL-6 and CRP but not ICAM-1 added significant prediction power to the basic model, and the gain in prediction power attributable to IL-6 was approximately two times higher (9.9%) than that of CRP (4.6%; Figure 1). The gain in prediction power of the remaining inflammation markers and of adhesion molecules was of small degree and NS. In this analysis, patients in the third tertiles of IL-6 and CRP had a relative risk of all-cause mortality 2.5 and 1.8 times higher than patients in the first corresponding tertiles (Figure 1). On multivariate analysis, the hazard ratio of all-cause mortality for patients in the third tertile of TNF-α, IL-β, IL-18, ICAM-1, and VCAM-1 did not significantly differ from that of patients in the first corresponding tertile (Figure 1).

Inflammation Markers, Adhesion Molecules, and CV Mortality Modeling

On univariate Cox regression analysis, only IL-6 and CRP were significantly associated with incident CV mortality (both $P \leq 0.002$), whereas IL-β, IL-18, TNF-α, ICAM-1, and VCAM-1 were unrelated to this outcome ($P = 0.12$ to 0.91). On multivariate analysis, IL-6 added the highest prediction power to the basic model of CV death (7.7%), and the gain in prediction power was approximately two times higher than that of CRP (3.7%; Figure 2). The gain in prediction power for CV mortality associated with TNF-α, IL-β, IL-18, ICAM, and VCAM was of small degree and NS (Figure 2). Accordingly, patients in the third tertiles of IL-6 and CRP displayed higher relative risk for CV mortality when compared with those in the third tertiles of TNF-α, IL-1β, IL-18, ICAM-1, and VCAM-1 (Figure 2).

Table 2. Correlation matrix of CRP, IL-1β, IL-6, IL-18, TNF-α, ICAM-1, and VCAM-1[a]

	CRP	IL-1β	IL-6	IL-18	TNF-α	ICAM-1
CRP	—	—	—	—	—	—
IL-1β	−0.08 (0.21)	—	—	—	—	—
IL-6	0.39 (<0.001)	−0.02 (0.81)	—	—	—	—
IL-18	0.10 (0.18)	−0.004 (0.96)	0.06 (0.39)	—	—	—
TNF-α	0.09 (0.17)	0.16 (0.02)	0.14 (0.04)	−0.03 (0.67)	—	—
ICAM-1	0.30 (<0.001)	−0.09 (0.17)	0.38 (<0.001)	0.25 (<0.001)	0.29 (<0.001)	—
VCAM-1	0.02 (0.75)	−0.15 (0.02)	0.15 (0.02)	0.37 (<0.001)	−0.01 (0.84)	0.25 (<0.001)

[a]Data are Spearman rank correlation coefficients (ρ) and *P* values.

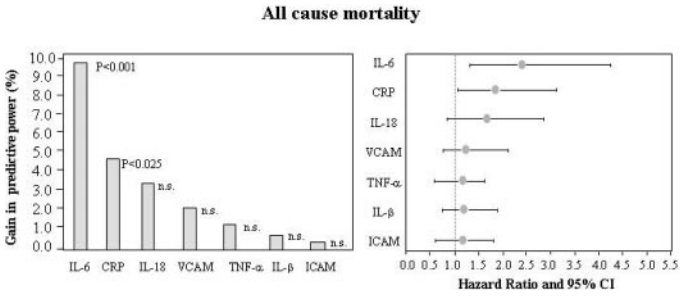

Figure 1. (Left) Gain in predictive value for all-cause mortality attributable to each inflammation marker. (Right) Hazard ratio (tertile III *versus* tertile I) and 95% confidence interval for all-cause mortality of each inflammation marker. Data were adjusted for all univariate predictors ($P < 0.10$) of all-cause death, namely age, gender, smoking, diabetes, systolic BP, previous cardiovascular events, albumin, and asymmetric dimethylarginine (ADMA). In this analysis, we also forced the treatment modality. See also Statistical Analyses section.

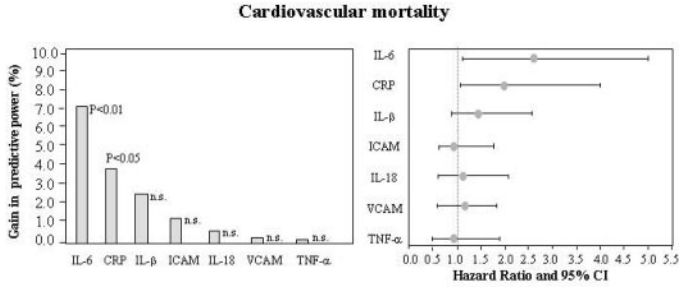

Figure 2. (Left) Gain in predictive value for cardiovascular mortality attributable to each inflammation marker. (Right) Hazard ratio (tertile III *versus* tertile I) and 95% confidence interval for cardiovascular mortality of each inflammation marker. Data were adjusted for all univariate predictors ($P < 0.10$) of cardiovascular death, namely age, gender, smoking, diabetes, systolic BP, previous cardiovascular events, and ADMA. In this analysis, we also forced the treatment modality. See also Statistical Analyses section.

Discussion

In this study, IL-6 and CRP were independently associated with all-cause and CV mortality and the prognostic value of these indicators resulted to be superior to that of IL-1β, IL-18, TNF-α, ICAM-1, and VCAM-1.

It is now well established that inflammation plays a primary role in arterial damage in dialysis patients. Although the precise mechanisms that are responsible for inflammation in ESRD are still unclear, low-grade infection, repeated exposure to dialysis filters, and auto-oxidation products are considered as likely inciting factors in these patients (1). Furthermore, a variety of traditional and nontraditional risk factors such as sympathetic hyperactivity, dyslipidemia, hyperphosphatemia/hyperparathyroidism, diabetes, and smoking may activate and/or amplify the inflammatory process in ESRD (1).

Inflammation and Outcomes in ESRD

CRP and the proinflammatory cytokines IL-1β, IL-6, and TNF-α are increased in dialysis patients (1,2,6,10–17), and these substances have been associated with mortality and CV complications in these patients (6,10–17). In the general population, IL-6 and CRP have emerged as the best predictors of CV risk among proinflammatory cytokines, but this issue has never been examined in ESRD. In our study, IL-6 and CRP were strongly interrelated, and both resulted to be predictors of all-cause and CV mortality largely superior to IL-1β, IL-18, TNF-α, ICAM-1, and VCAM-1 in statistical models that included traditional and nontraditional risk factors for these outcomes. Thus, the severity of inflammation as estimated by these markers adds significant prognostic information above and beyond traditional and nontraditional risk factors in dialysis patients. Two cohort studies in the elderly (24,25) recently emphasized the strong prediction power for CV events of CRP, IL-6, and TNF-α and pointed to the superiority of IL-6 over other markers. Similar findings were also reported in a recent study by Koukkunen *et al.* (26) in elderly patients with unstable angina. Partially in contrast with the above-mentioned studies, we found no independent association between TNF-α and all-cause and CV mortality. TNF-α may be a weaker indicator of CV risk in ESRD because also in a study by Kimmel *et al.* (27), this cytokine failed to predict survival in dialysis patients. An important, new, observation emerging from our analysis is that the gain in prediction power attributable to IL-6 is approximately two times higher than that of CRP (all-cause mortality, 9.9 *versus* 4.6%; CV death, 7.0 *versus* 3.7%). IL-6 has peculiar atherogenic properties, including effects on platelets, endothelium, and coagulation factors (28). Stenvinkel *et al.* (11) were the first to observe that IL-6 is a strong and independent predictor of the progression of carotid atherosclerosis and mortality (29) in patients with advanced chronic renal disease before commencing regular dialysis treatment. Our novel observation in dialysis patients reveals the superiority of IL-6 as a predictor of all-cause and CV mortality over others cytokines. Yet it should be pointed out that notwithstanding its strong link to mortality and CV outcomes, the relative risk estimate provided by IL-6 was of magnitude comparable to that of serum CRP.

IL-18 is a novel cytokine that exacerbates atherosclerosis in mice (7). We found that IL-18 was unrelated to all-cause and CV death on both univariate and multivariate analyses, and a similar lack of association was also true for IL-1β.

Adhesion molecules are upregulated in chronic inflammatory states (30), and this upregulation is currently considered as an expression of endothelial dysfunction. In our study, serum VCAM-1 was unrelated to outcomes, whereas serum ICAM-1 was related to all-cause mortality on univariate analysis but lost its predictive value after data adjustment for a series of potential confounders. In contrast with the present study, ICAM-1 was the strongest predictor of survival in a cohort of patients on conservative treatment (20). This apparent discrepancy most likely depends on differences in study population (chronic

renal failure *versus* dialysis patients) and on differences in risk modeling in the two studies.

In conclusion, IL-6 and CRP are independently associated with all-cause and CV mortality in dialysis patients, and the prediction value of these substances is higher than that attributable to other cytokines and adhesion molecules. Owing to its stronger link to outcomes, IL-6 seems to be the best option for risk stratification in dialysis patients, particularly in the context of clinical studies. However, being that the risk estimate by CRP for all-cause and CV death is reasonably close to that of IL-6, CRP may be considered as a cheap alternative to IL-6 in everyday clinical practice, a possibility that remains to be tested formally in a clinical trial.

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