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Original Article

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Interleukin-6 is a stronger predictor of total and cardiovascular mortality than C-reactive protein in haemodialysis patients

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Abstract

Background. Despite the well known association between interleukin-6 (IL-6) and cardiovascular mortality, no study has so far verified whether IL-6 adds prognostic information to that provided by C-reactive protein (CRP).

Methods. A cohort of 218 haemodialysis patients from four different dialytic centres was followed-up retrospectively. Plasma IL-6 and CRP concentrations were determined. Full information on co-morbidities was available in 162 patients.

Results. With respect to the lowest quartile (<3.6 pg/ml for IL-6, and <2.2 mg/l for CRP), the crude relative risk (RR) of death from all causes of the upper quartile (>13.9 pg/ml for IL-6, and >12.8 mg/l for CRP) was 5.20 (95% confidence interval 2.06–13.011) for IL-6 and 3.16 (1.41–7.12) for CRP. When both variables were included, the estimates were 4.10 (1.30–12.96) for IL-6 and 1.29 (0.47–3.57) for CRP. As to continuous variables, the relationship between both variables and mortality tended to level off for the highest values, but became fairly linear after log transformation of the variables. For one unit SD of the log (variable), the RR was 2.09 (1.52–2.88) for IL-6 and 1.66 (1.23–2.24) for CRP. When they were included in the same model, the estimates were 1.90 (1.18–2.82) for IL-6 and 1.16 (0.81–1.66) for CRP.

Conclusions. IL-6 has a stronger predictive value than CRP for cardiovascular mortality and provides independent prognostic information, while conveying most of that provided by CRP.

Keywords: cardiovascular mortality; C-reactive protein; haemodialysis; interleukin-6; outcome

Introduction

Epidemiological studies in the general population have shown that even minor elevations of C-reactive protein (CRP), an acute-phase reactant which markedly increases during an inflammatory response [1], predict the development of coronary heart disease and cardiac failure [2–4]. CRP may directly promote the development of atherosclerosis, through complement activation, tissue damage and activation of endothelial cells [5]. Recent studies performed in end-stage renal disease (ESRD) patients have shown that CRP is a strong predictor of cardiovascular death [6,7]. Recently, it has been shown that other proinflammatory cytokines such as interleukin-6 (IL-6) may exert a direct inflammatory effect on the heart and peripheral circulation [8]. Few studies have investigated the role of plasma IL-6 as an outcome predictor in ESRD patients [9,10].

In the present study, we investigated the joint predictive power of CRP and IL-6, in order to ascertain what is the prognostic information that each index carries independently of the other. To this aim, IL-6 and CRP plasma levels were measured in a cohort of ESRD patients from different centres over a 4-year follow-up. Main outcomes were cardiovascular and total mortality.

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Subjects and methods

Study population

Between February and May 1998, 218 haemodialysis patients from four different haemodialysis centres (Internal Medicine Department at Pisa $n=25$; Renal Unit at Pisa $n=33$; Renal Unit at Massa $n=75$; and G. Maranon at Madrid $n=85$) provided blood samples for the measurement of CRP and IL-6. No selection was made and the patients could be considered representative of the dialysis population in the region. Patients were not included in the study if they had clinical signs of infection (such as fever and/or leukocytosis with or without a documented focus of infection), amyloidosis, non-skin cancer or liver disease.

Data collection

At the end of December 2002 using databases available at each centre, we followed-up patients until transplantation or death. For those who died, we recorded the cause of death. The latter was obtained from the patients' record. We assessed cardiovascular disease (as documented by serial 12-lead electrocardiogram evidence or Q-wave infarction and appropriate myocardial enzyme elevations; coronary revascularization including coronary artery bypass surgery or percutaneous transluminal coronary angioplasty; typical history of angina with abnormal coronarography), stroke or cerebrovascular disease (non-haemorrhagic strokes confirmed by neurological examination findings consistent with new onset focal neurological deficits, with or without computed tomography or magnetic resonance imaging evidence of cerebral infarction); symptomatic extracranial artery stenosis resulting in carotid endarteriectomy; abdominal aortic or lower extremity arterial disease, abdominal aortic repair; lower extremity revascularization via bypass surgery or angioplasty; lower extremity amputation; new onset of intermittent claudication confirmed by Doppler or arteriography findings. Diabetes was defined by the use of insulin or oral hypoglycaemic agents. Two physicians independent of the study were responsible for the clinical ascertainment. This analysis was performed without knowledge of baseline characteristics. Furthermore, data regarding smoking status, body mass index (BMI), blood pressure, use of antihypertensive medications, calcium, phosphate, serum intact parathyroid hormone (PTH) levels, albumin, total serum cholesterol, haemoglobin, use of epoetin, Kt/V and type of membranes used were recorded.

Among 218 patients, 35 had neither IL-6 or CRP values recorded. Thus 183 patients were eligible for the present study. In addition, 21 patients were excluded from the main analysis because of missing values in the database. Overall, 162 patients could be included in the primary analysis.

Laboratory measurements

Blood samples were drawn from the artero-venous fistula before commencing dialysis. Plasma collected using heparin as anticoagulant was separated <30 min after drawing and stored at −80°C in different aliquots until analysis. CRP was measured by a modification of the laser nephelometric technique [high sensitivity (hs) CRP assay, Behring Diagnostics, GmbH, Rarburg, Germany]. The CRP assay was standardized according to the World Health Organization First International Reference Standard and had a sensitivity of 0.1 μg/ml, with a standard reference range of between 0.1 and 0.4 mg/l. In order to evaluate the intra-patient variability of CRP levels, plasma samples from five clinically stable patients were obtained three times a week for 2 weeks. IL-6 was measured by a quantitative sandwich enzyme immunoassay technique (RD Systems, Minneapolis, MN). In order to evaluate the intra-patient variability of CRP and IL-6 levels, plasma samples from five clinically stable haemodialysis patients were taken before each session (three times a week) for 3 months. Samples were assayed in duplicate with a coefficient of variation <5% for CRP and <6.2% for IL-6. Samples were assayed in duplicate and the intra- and inter-assay coefficient of variation was <4%. Serum albumin was measured with a nephelometric technique (Dade Behring Marburg GmbH) with an intra- and inter-assay variability of 4.3 and 4.4%, respectively. Standard laboratory techniques were used for the determination of cholesterol, triglycerides, haematocrit, white blood cells, calcium and phosphate.

Statistical analysis

We used the Pearson's correlation coefficient after log transformation of the variables to explore the relationship between IL-6 and CRP, and the Mann–Whitney test to compare IL-6 and CRP levels in patients with or without history of cardiovascular disease. Since our primary aim was to examine the relationship of IL-6 and CRP levels to cardiovascular mortality, we censored the follow-up for death due to non-cardiovascular causes. We used the Kaplan–Meier method to estimate the crude probability of cardiovascular mortality associated with quartiles of IL-6, and Cox regression analysis to examine the adjusted relationship between IL-6 and CRP and cardiovascular mortality. This was performed first by examining IL-6 or CRP in separate models, then including both in the same model in order to isolate the predictive value of each indicator independently of the other. The relationship of IL-6 and CRP to mortality was examined either as categorical variables (quartiles) or as continuous variables. Categorical variables were tested for trend by scoring each quartile by its median value, entering the score as a continuous term in the regression model, and testing its statistical significance. As to continuous variables, we carefully explored the shape of the relationship with log(hazard ratio) of death, since the lack of linearity with CRP or IL-6 values might influence the strength of either statistical linear relationship. To this aim, we used a generalized additive Cox model [11] using the gam program of Stata [12] which is an interface between Stata and the FORTRAN program originally written by Hastie and Tibshirani and available on the Internet from http://lib.stat.cmu.edu/general. To test the departure from the proportional assumption, we used the procedure suggested by Grambsch and Therneau [13,14]. Finally, we searched in order to exclude whether some observations might have exerted undue influence on estimated parameters, by plotting calculated scaled score residuals after each regression model [14,15]. These residuals provide the comparison between the estimated relative risk (RR) obtained from the full data with that obtained by fitting the model after each observation had been removed. To

obtain an immediate visual appreciation of the predictive value expressed by each of the adjusted hazard ratios for the Cox estimates of IL-6 and CRP, we plotted covariate-adjusted survival probabilities [14,15], i.e. the probability of survival associated with a given value of IL-6 or CRP. These survival probabilities were estimated from the Cox model which included IL-6 and CRP as continuous variables. All analyses were repeated after adjustment for confounding factors (age, gender, diabetes, dialysis duration transformed by polynomials, time × Kt/V interaction, dialyser type, hypertension, anaemia and albuminaemia). All reported *P*-values refer to the likelihood ratio test. All analyses were performed using Stata SE 8.0 (Stata Corporation, College Station, TX).

Results

Description of the population

Characteristics of the study population are reported in Table 1. Half of the population had a history of cardiovascular disease; 16% were diabetics. Median CRP was 5.7 mg/l, therefore nearly half of the patients had CRP values within the range of standard 'normal limits'. However, CRP varied widely, the 2.5 and 97.5 percentiles being 0.3 and 81.0 mg/l, respectively. Interestingly, the scale in pg/ml of IL-6 proved to be similar to that in mg/l of CRP, which made the interpretation of IL-6 values easier. With regard to the other indexes of risk for death, such as age, dialysis duration, Kt/V, albumin, haemoglobin and blood pressure, figures were similar to those commonly found in ESRD patients.

Table 1. Characteristics of the study population

Number	162
Age (years)	61.9 (14.2)
Males	110 (67.9)
Diabetes	26 (16.0)
BMI (kg/m^2)[a]	24.9 (3.8)
History of acute myocardial infarction	19 (11.7)
History of stroke	12 (7.4)
History of peripheral artery disease	52 (32.3)
History of cardiovascular disease[b]	78 (48.1)
Dialysis duration (months)	16 (0.5–247)
Systolic blood pressure (mmHg)	140.6 (20.7)
Diastolic blood pressure (mmHg)	79.1 (9.9)
Hypertensive treatment	70 (43.2)
Kt/V	1.26 (0.21)
Type of dialysis treatment	
Bicarbonate HD	90 (55.6)
HDF	70 (43.2)
HF and PFD	2 (1.2)
Type of membrane	
Modified cellulose	120 (74.1)
Synthetic	42 (25.9)
CRP (mg/l)	5.7 (0.3–81.0)
IL-6 (pg/ml)	6.2 (1.2–51.2)
Haemoglobin (g/dl)	11.1 (1.7)
Use of epoetin	123 (75.9)
Albumin (g/dl)	3.94 (0.46)
$Ca \times P$[a] (mg^2/dl^2)	43.9 (15.2)
PTH (pg/ml)	135.5 (7.9–1088.8)
Total serum cholesterol (mg/dl)	180.1 (39.6)
Serum triglyceride (mg/dl)[a]	159.2 (90.2)
Positive test for anti-HCV Ig	25 (15.4)

Continuous variables are reported as means (SD) or median (95% central range). Categorical variable are reported as number (percentage).
[a]Available only in a subgroup of patients.
[b]History of cardiovascular disease means any of the following: myocardial infarction, angina, stroke, transient ischaemic attack or peripheral artery disease.

Description of IL-6 and CRP

As expected, IL-6 and CRP levels were highly correlated ($r = 0.63$, $P < 0.001$). The correlation coefficient was 0.55 ($P < 0.001$) and 0.68 ($P < 0.001$) in patients without and with history of cardiovascular disease, respectively. Both CRP and IL-6 levels were higher in patients with history of cardiovascular disease (Figure 1). After a median follow-up of 3.4 years, 44 had died from cardiovascular causes. Another 37 patients had died from other causes.

Incidence of cardiovascular events and deaths

As shown in Figure 2, the probability of death increased significantly in the upper quartiles of IL-6 and CRP levels (test for trend: $P < 0.001$ and $P = 0.001$, respectively). Results from the adjusted analysis of quartiles are reported in Table 2. In the crude analyses, the trend across quartiles in the RR of cardiovascular death was rather similar for IL-6 and CRP, although it was somewhat steeper for IL-6. However, RR estimates changed substantially when each index was adjusted for the other. In fact, whereas IL-6 kept most of its relationship to cardiovascular mortality, the trend for CRP became nearly flat. Analyses performed after adjusting for potential confounders yielded similar results.

When IL-6 and CRP levels were analysed as continuous (numerical) values, it appeared that the relationship to mortality tended to level off for the highest values (not shown). However, the relationship became fairly linear for both IL-6 and CRP after log transformation of the variables (Figure 3).

Relationship to cardiovascular deaths

The crude RR of cardiovascular death for the increase of 1 SD unit was 2.09 (95% confidence interval 1.52–2.88) for log(IL-6) and 1.66 (1.23–2.24) for log(CRP). When each variable was adjusted for the other, the RR became 1.90 (0.18–2.82) for log(IL-6) and 1.16 (0.81–1.66) for log(CRP).

Figure 4 presents an easy way to interpret these last two RR estimates. This figure shows that hypothetical patients showing the same CRP levels but different IL-6 levels would present sharp differences in prognosis. In contrast, patients showing the same IL-6 levels would present a similar prognosis, even if they had different CRP levels.

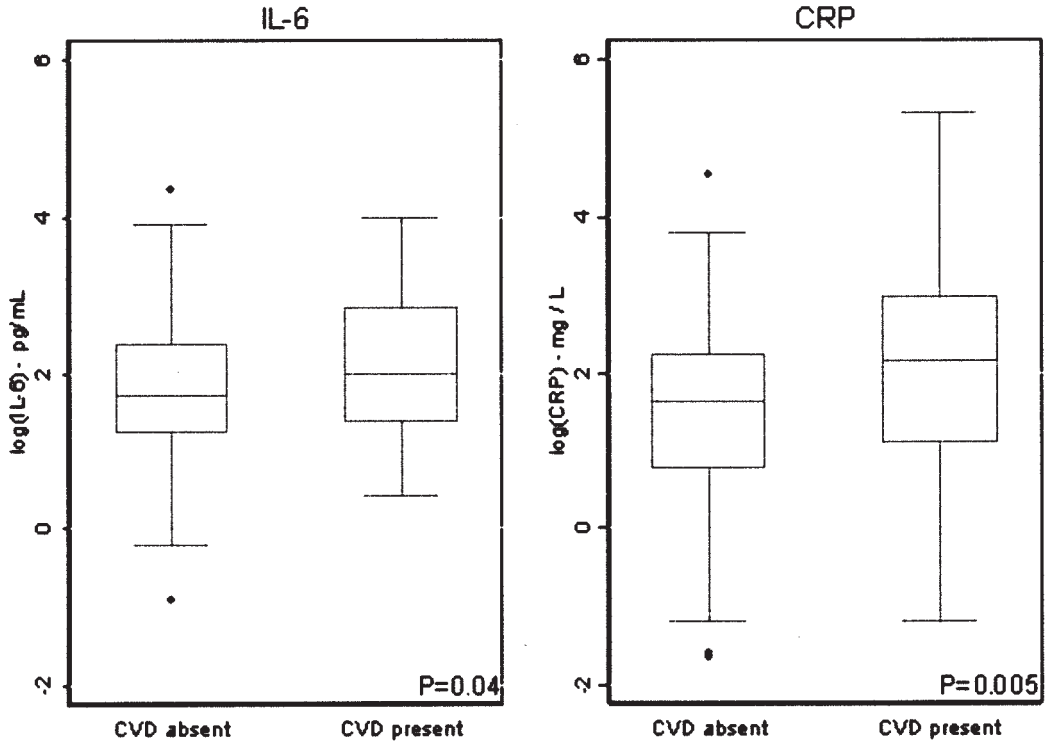

Fig. 1. Levels of log(IL-6) (left panel) and log(CRP) (right panel) according to the presence of cardiovascular disease at the time of IL-6 and CRP measurement. *P*-values refer to the Mann–Whitney test.

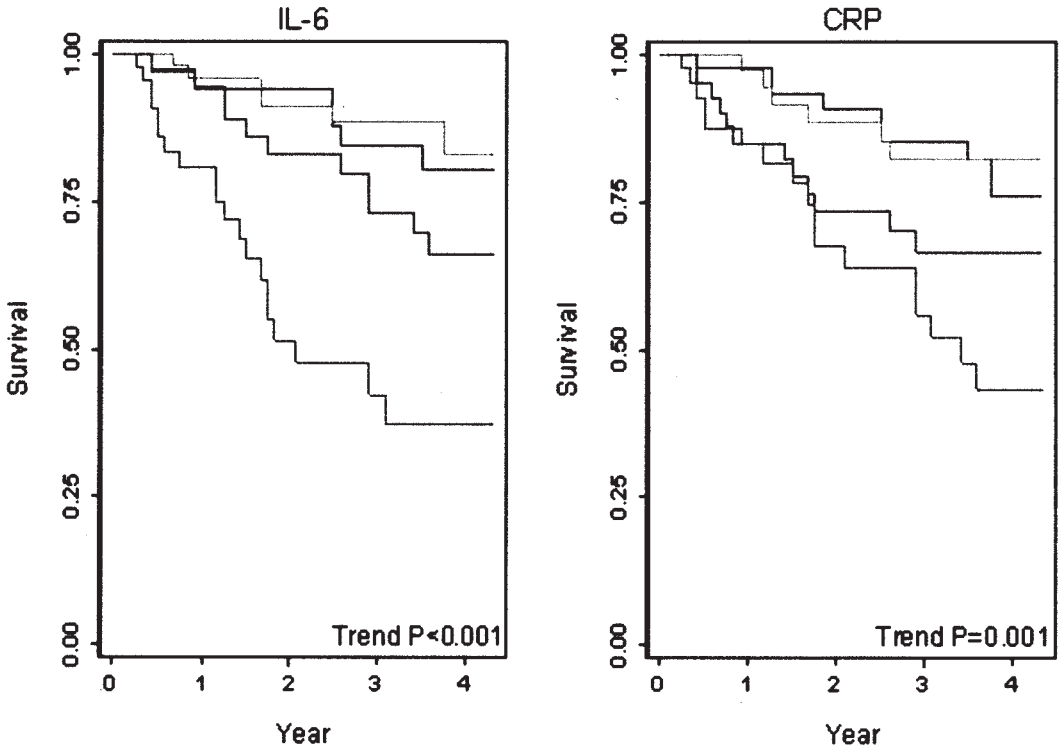

Fig. 2. Kaplan–Meier survival probability according to IL-6 and CRP quartiles. *P*-values report the test for trend.

Table 2. Relative risks (95% CI) for cardiovascular death according to quartiles of IL-6 and CRP

	Quartiles			*P* for trend
	2	3	4	
Crude				
IL-6	0.84 (0.28–2.49)	1.83 (0.68–4.95)	5.20 (2.06–13.11)	<0.001
CRP	0.82 (0.29–229)	1.89 (0.79–4.78)	3.16 (1.41–7.12)	0.001
IL-6 adjusted for CRP	0.80 (0.25–2.54)	1.63 (0.54–4.98)	4.10 (1.30–12.96)	<0.001
CRP adjusted for IL-6	0.85 (0.29–2.52)	1.31 (0.50–3.46)	1.29 (0.47–3.57)	0.49
Adjusted				
IL-6	0.96 (0.32–3.03)	1.58 (0.55–4.58)	6.21 (2.19–17.56)	<0.001
CRP	0.69 (0.24–1.99)	1.65 (0.65–4.19)	4.04 (1.68–9.69)	<0.001
IL-6 adjusted for CRP	1.20 (0.34–4.28)	1.41 (0.40–4.92)	4.26 (1.12–16.19)	0.005
CRP adjusted for IL-6	0.61 (0.18–1.99)	0.99 (0.33–2.96)	1.94 (0.62–6.08)	0.07

[a]Adjusted for age, gender, diabetes, dialysis duration, albumin levels, haemoglobin levels, use of epoetin, blood pressure, use of antihypertensive medications, type of membrane (cellulosic or otherwise), and Kt/V × time interaction.
Quartiles of CRP are the <2.2, 2.2–5.7, 5.8–12.8 and >12.8 mg/l.
Quartiles of IL-6 are the <3.6, 3.6–6.2, 6.3–13.9 and >13.9 pg/ml.

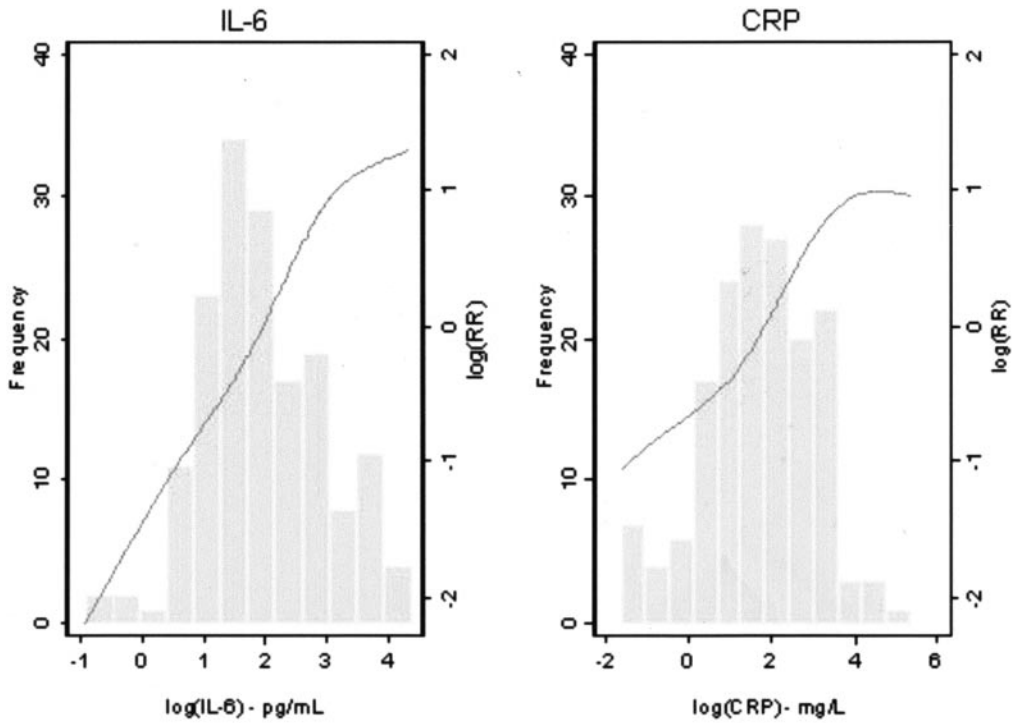

Fig. 3. Shape of the relationship (line) between log(IL-6) (left panel) and log(CRP) (right panel) to cardiovascular mortality, estimated using the generalized additive Cox model. The histogram on the background of each figure shows the frequency of each value of IL-6 and CRP in the study population. The deflections at the upper extreme end of each curve are estimated from sparse data. Within the range which encompasses most of the values of IL-6 and CRP, the relationship is fairly linear for both.

Table 3 reports on the RRs of cardiovascular death after stratification for history of cardiovascular disease at the time of IL-6 and CRP measurement. The RRs for either IL-6 or CRP did not differ statistically in patients with and without history of overt cardiovascular disease, albeit that they tended to be higher in the latter category. After adjustment for history of cardiovascular disease, the RRs shown above became

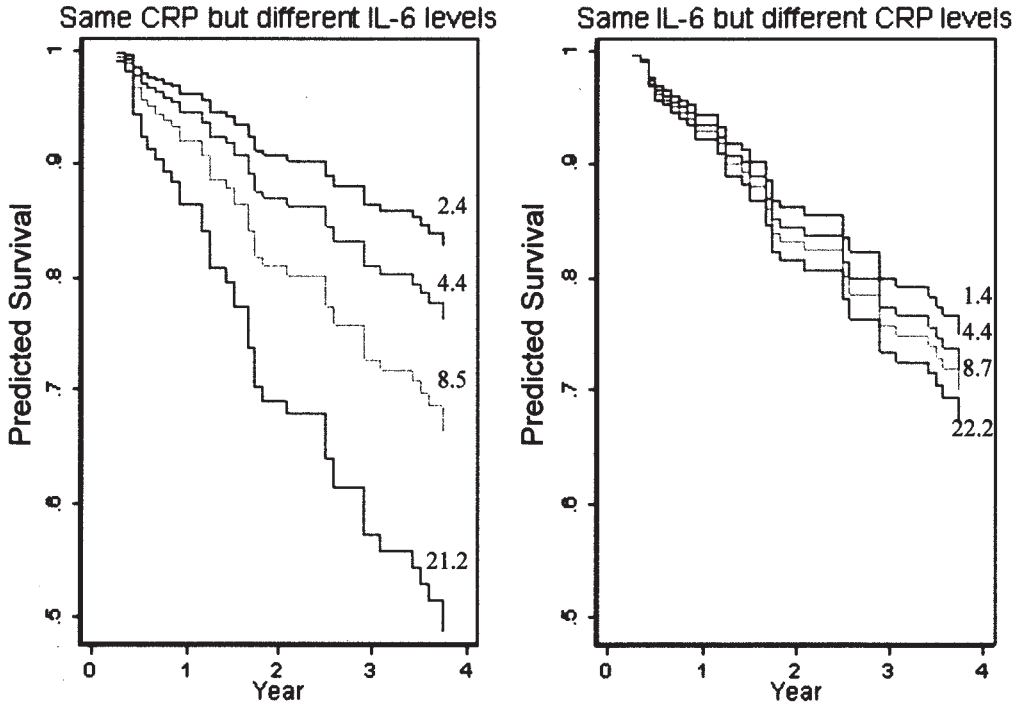

Fig. 4. Survival curves estimated from the Cox model which includes IL-6 and CRP as continuous variables. The left panel shows the predicted survival probability among patients with the indicated IL-6 values (pg/ml) of 2.4, 4.4, 8.5 and 21.2 (i.e. the median value of each IL-6 quartile) and all with a CRP value of 5.8 mg/l (i.e. the median CRP value in the study population). The right panel shows the predicted survival probability among patients with the indicated CRP values (mg/l) of 1.4, 4.4, 8.7 and 22.2 (i.e. the median value of each CRP quartile) and all with a IL-6 value of 6.3 pg/ml (i.e. the median value in the study population).

Table 3. Relative risks (95% CI) for an increased SD unit of log(IL-6) and log(CRP) in patients with or without history of cardiovascular disease

	CVD present	CVD absent	*P* for different RR
IL-6 adjusted for CRP	3.01 (1.24–7.29)	1.88 (1.14–3.08)	0.22
CRP adjusted for IL-6	1.13 (0.47–2.68)	0.97 (0.63–1.50)	0.49

CVD = cardiovascular disease (i.e. history of myocardial infarction, angina, stroke, transient ischaemic attack or peripheral artery disease)
Patients are classified according to CVD at the time of IL-6 and CRP measurement.

2.05 (1.33–3.16) for log(IL-6) and 0.96 (0.66–1.41) for log(CRP). Additional adjustment for other potential confounding factors did not modify the results substantially (not shown).

Discussion

The present study shows that plasma IL-6 rather than CRP better predicts outcome in ERSD patients. To our knowledge, this is the first study investigating the independent prognostic value of IL-6 over CRP. Various possible explanations may underline the advantage of IL-6 over CRP as an outcome predictor. One possibility is that, being located upstream in the cascade of events which lead to the synthesis of many acute-phase reactants, IL-6 is a better marker of the inflammatory burden affecting the development of cardiovascular disease. Another possibility is that levels of IL-6 vary less than those of CRP, leading to a more accurate classification of patients at risk when one single sample is taken. Finally, the toxic effects of IL-6 on the heart and peripheral vasculature might be stronger than those of CRP [8]. In our view, the present study still has some important implications. First, it gives further support to the hypothesis about the role of inflammatory mediators in the genesis of cardiovascular disease in dialysis patients [16–18]. Secondly, it provides evidence supporting the use of IL-6 in addition to, or even in place of, CRP for the identification of patients at risk.

We admit several limitations in this study. First, the study was retrospective as we were not able to gather data regarding some traditional coronary risk factors, such as smoking status, high-density lipoprotein (HDL), BMI, calcium × phosphorus product and homocysteine levels. Thus, our analysis leaves unanswered the question of whether IL-6 adds prognostic value to that provided by these risk factors. However, in the subset of patients in which smoking status, BMI and calcium × phosphorus product were available, the

analyses yielded similar results. Secondly, we did not collect information regarding subclinical cardiovascular disease using, for instance, echocardiogram, ECG or ultrasound of the carotids at the time when blood samples were taken. Consequently, we were not able to determine whether IL-6 reflected existing diseases, which might easily be recognized using other standard diagnostic tools or, rather, truly predicted the incidence of future disease. However, the finding that the prognostic value of IL-6 is similar in patients with and without overt coronary artery disease may indirectly suggest that IL-6 might be a valuable pre-clinical tool for the prediction of total and cardiovascular death. Thirdly, a possible bias by pre-selection of the patients with respect to the predictive value of IL-6 needs to be pointed out. Finally, we did not investigate whether the prognostic advantage of IL-6 over CRP was due to a reduced measurement intra- or inter-patient variability.

Our findings need to be confirmed by further studies. Those studies should consider the role of IL-6 (and, possibly, of other proinflammatory cytokines) as outcome predictor in patients in whom the presence of subclinical cardiovascular disease is evaluated at the time the sample is taken. All traditional risk factors for death should be measured accurately. Clinical events should be identified prospectively and, whenever possible, IL-6 levels should be measured repeatedly during the course of follow-up.

Conflict of interest statement. None declared.

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