# Free *p*-cresol is associated with cardiovascular disease in hemodialysis patients

BKI Meijers[1,4], B Bammens[1,4], B De Moor[2], K Verbeke[3], Y Vanrenterghem[1] and P Evenepoel[1]

*[1]Division of Nephrology, Department of Medicine, University Hospital Leuven, Leuven, Belgium; [2]Division of Nephrology, Department of Medicine, Virga Jesse Hospital, Hasselt, Belgium and [3]Division of Gastroenterology, Laboratory of Digestion and Adsorption, University Hospital Leuven, Leuven, Belgium*

**Cardiovascular disease (CVD) is highly prevalent in chronic kidney disease, suggesting that molecules retained in uremia might contribute to this increased risk. We explored the relationship between *p*-cresol, a protein-bound uremic retention solute, and CVD by comparing the strength of this relationship relative to traditional and novel cardiovascular risk factors. Univariate Cox proportional hazard analysis showed that the free serum *p*-cresol concentration was significantly associated with CVD when the primary end point was the time to the first cardiovascular event. In multivariate analysis, free *p*-cresol was significantly associated with CVD in non-diabetics. In diabetic patients, however, a significant relationship between *p*-cresol and cardiovascular events could not be demonstrated despite their having significantly higher *p*-cresol levels. Our study shows that free *p*-cresol is a novel cardiovascular risk factor in non-diabetic hemodialysis patients.**

*Kidney International* (2008) **73,** 1174–1180; doi:10.1038/ki.2008.31; published online 27 February 2008

KEYWORDS: cardiovascular disease; diabetes; hemodialysis; uremia; risk factors; *p*-cresol

**Correspondence:** *P Evenepoel, Dienst Nefrologie, Universitair Ziekenhuis Gasthuisberg, Herestraat 49, Leuven 3000, Belgium.*
*E-mail: pieter.evenepoel@uz.kuleuven.ac.be*

[4]These authors contributed equally to this work

**Received 10 August 2007; revised 21 November 2007; accepted 18 December 2007; published online 27 February 2008**

Mounting data point to the lethal synergy between chronic kidney disease (CKD) and cardiovascular disease (CVD).[1–5] Traditional cardiovascular risk factors are insufficient to predict true cardiovascular risk in patients with CKD.[6,7] Novel risk factors include the C-reactive protein (CRP) as a marker of inflammation, a disturbed mineral metabolism, albumin, and anemia.[8–12] Uremic retention solutes have been implicated in the pathogenesis of accelerated atherosclerosis as well.[13–17]

In recent years, the focus of the nephrology community is shifting toward protein-bound uremic retention solutes.[18] The HEMO and adequacy of dialysis Mexico studies[19,20] failed to show an improvement of patient outcome by increasing the removal of water soluble solutes above the current standards of care. The clinical importance of protein-bound uremic retention solutes is underscored by a recent observational study in hemodialysis patients in which we demonstrated that *p*-cresol, a prototypic uremic protein-bound retention solute, is independently associated with overall mortality.[21]

*In vitro* evidence suggests a deleterious effect of *p*-cresol on the endothelium.[22–24] The association between the protein-bound uremic retention solute *p*-cresol and CVD *in vivo* has not been investigated. The aims of this *post hoc* analysis of the *p*-cresol mortality study[21] were first to explore the relationship between *p*-cresol and CVD and second to compare the strength of this relationship relative to traditional and novel cardiovascular risk factors.

## RESULTS

### Study population

One hundred and seventy-five patients were included in the final analysis. Table 1 represents the demographic and baseline characteristics of the study population.

### Relation between *p*-cresol and other parameters

The concentration of unbound *p*-cresol correlated significantly with age ($R=0.26$, $P=0.0004$), total calcium concentration ($R=0.21$, $P=0.004$), dialysis vintage ($R=0.23$, $P=0.002$), urea ($R=0.38$, $P<0.001$), single pool $Kt/V$ (sp$Kt/V$) ($R=0.19$, $P=0.017$), and nPNA (normalized protein nitrogen appearance) ($R=0.41$, $P<0.001$) but not

**Table 1 | Main demographic, clinical, and biochemical characteristics of study population**

| | |
|---|---|
| Age (years, median (range)) | 64.7 (26–89) |
| Sex (male/female (%)) | 108/67 (62/38) |
| Dialysis vintage (months, median (range)) | 29.8 (2.3–158.1) |
| sp*Kt/V* | 1.64 (0.53) |
| r*Kt/V* | 0.06 (0.13) |
| nPNA (g $kg^{-1}$ $day^{-1}$) | 0.93 (0.26) |
| BMI (kg $m^{-2}$) | 23.9 (4.9) |
| Blood pressure (systolic/diastolic (mm Hg)) | 143 (19)/71 (11) |
| Diabetes (yes/no (%)) | 52/123 (29.7/70.3) |
| Current smoker (yes/no/unknown (%)) | 31/129/15 (18/74/8) |
| Cholesterol (mg per 100 ml)[a] | 166.9 (32.0) |
| LDL (mg per 100 ml)[a] | 92.8 (26.9) |
| Albumin (mg $l^{-1}$) | 36.4 (3.9) |
| PTH (ng $l^{-1}$) | 126.4 (177.5) |
| Calcium (mg per 100 ml) | 9.5 (0.8) |
| Phosphate (mg per 100 ml) | 4.8 (1.6) |
| Calcium × phosphate ($mg^2$ per 100 $ml^{-2}$) | 45.9 (15.7) |
| Urea (mg per 100 ml) | 142.4 (39.7) |
| $\beta_2$-Microglobulin (mg $l^{-1}$) | 27.7 (10.6) |
| CRP (mg $l^{-1}$) | 21.8 (34.4) |
| Total *p*-cresol (mg $l^{-1}$) | 19.0 (11.9) |
| Free *p*-cresol (mg $l^{-1}$) | 2.59 (2.25) |

BMI, body mass index; CRP, C-reactive protein; LDL, low-density lipoprotein; nPNA, normalized protein nitrogen appearance; PTH, parathyroid hormone; r*Kt/V*, residual *Kt/V*; sp*Kt/V*, single pool *Kt/V*.
Data are expressed as mean (s.d.), unless otherwise stated.
[a]One patient missing baseline data.

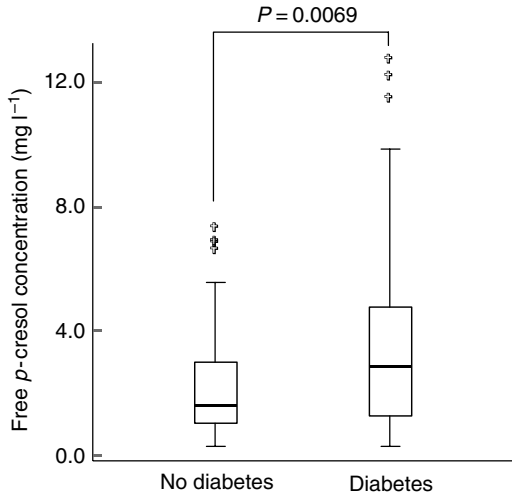


**Figure 1 | Box plot of free *p*-cresol concentrations in patients with and without diabetes.**

with smoking habit, body mass index, cholesterol concentration, CRP, albumin concentration, or the calcium phosphorus product. Patients with diabetes had significantly higher free *p*-cresol concentrations (analysis of variance (ANOVA), $P = 0.0069$) (Figure 1) as well as significantly higher total *p*-cresol concentrations (ANOVA, $P = 0.019$). Patients treated by hemodialysis had significantly higher free *p*-cresol concentrations (ANOVA, $P = 0.0008$) as well as significantly higher total *p*-cresol concentrations (ANOVA, $P = 0.023$) than those treated by hemodiafiltration.

### Event analysis

After a mean follow-up of 56.2 months (s.d. 5.5, median 60.1, minimum–maximum 48.1–60.1 months), 78 patients reached the combined primary end point of a new cardiovascular event or death due to CVD. In univariate analysis, age, diabetes, CRP, albumin, systolic blood pressure, and free *p*-cresol were significantly ($P < 0.05$) associated with the primary end point (Table 2). Figure 2 shows the Kaplan–Meier curves of patients with free *p*-cresol concentrations above and below the median (1.97 mg $l^{-1}$). More patients had new fatal or non-fatal cardiovascular events in the group with high free *p*-cresol concentrations (log rank, $P = 0.022$).

A multivariate model was constructed to compare the observed association between *p*-cresol and CVD with a set of novel and traditional cardiovascular risk factors. All cardiovascular risk factors significant at the $P \leqslant 0.2$ level on univariate analysis (age, diabetes, treatment modality, systolic blood pressure, albumin, calcium, CRP, and free *p*-cresol) were included. In this model, age, diabetes, and CRP were found to be independently associated with CVD (Table 3).

As patients with diabetes have a significantly higher concentration of *p*-cresol, we performed grouped multivariate analyses of the patients with and without diabetes. In the group of non-diabetics, *p*-cresol as a continuous variable surpassed blood pressure, albumin, treatment modality, and calcium and was the last variable to be eliminated from the model. As a binary variable, besides age and CRP, *p*-cresol is the only other variable to remain in the model. Figure 2b shows the Kaplan–Meier estimate of patients without diabetes (log rank, $P = 0.019$).

Residual renal function (RRF) was not significantly associated with CVD in univariate analysis (Table 2). When RRF was forced into multivariate analysis, the observed relationship between *p*-cresol and CVD remained identical (data not shown).

## DISCUSSION

The main finding of this study was that higher free *p*-cresol concentrations are associated with CVD in hemodialysis patients. This association persisted after adjustment for several covariates, including age and CRP, but was lost after adjustment for diabetes.

Cardiovascular mortality in CKD patients treated by hemodialysis is more than fivefold higher than in the general population, even after stratification for age, sex, race, and the presence of diabetes. Traditional cardiovascular risk factors are insufficient to accurately predict cardiovascular risk in patients with CKD.[7] Survival bias may disrupt the relationship between traditional risk factors and CVD.[25] Besides the traditional risk factors, an ever expanding list of novel cardiovascular risk factors has been proposed to contribute to the cardiovascular burden in CKD.[7–12] This is the first *in vivo* study to investigate the relationship between protein-bound uremic retention solutes, of which *p*-cresol is a prototypic representative, and CVD. In this study population, the strength of the association between *p*-cresol and CVD

**Table 2 | Univariate Cox proportional HR analysis for association of baseline values with time to first cardiovascular event**

| Variable | Unit of increase | HR | *P*-value |
|---|---|---|---|
| Sex | Male vs female | 0.90 (0.57–1.40) | NS |
| Age (years) | 1 year | 1.04 (1.02–1.06) | <0.001 |
| Diabetes | Present vs absent | 2.27 (1.44–3.58) | 0.0004 |
| Smoking | Yes vs no | 1.08 (0.57–2.00) | NS |
| Dialysis vintage | 1 month | 1.00 (1.00–1.01) | NS |
| Treatment modality | HDF vs HD | 0.68 (0.42–1.10) | 0.12 |
| sp*Kt/V* | 1 U | 0.96 (0.62–1.48) | NS |
| r*Kt/V* | Present vs absent | 0.99 (0.64–1.55) | NS |
| | 1 U | 0.31 (0.03–2.98) | NS |
| nPNA | $1\,g^{-1}\,kg^{-1}\,day^{-1}$ | 1.29 (0.80–2.10) | NS |
| Systolic BP | 1 mm Hg | 1.01 (1.00–1.03) | 0.028 |
| Cholesterol | 1 mg per 100 ml | 1.00 (0.99–1.01) | NS |
| | ⩾190 vs <190 mg per 100 ml | 1.12 (0.69–1.82) | NS |
| CRP | $10\,mg\,l^{-1}$ | 1.10 (1.04–1.16) | 0.0004 |
| Albumin | $1\,g\,l^{-1}$ | 0.92 (0.86–0.98) | 0.02 |
| | ⩾35 vs <35 $g\,l^{-1}$ | 0.68 (0.44–1.08) | 0.10 |
| Hemoglobin | 1 g per 100 ml | 0.91 (0.77–1.07) | NS |
| Calcium | 1 mg per 100 ml | 0.83 (0.64–1.07) | 0.15 |
| Phosphate | 1 mg per 100 ml | 1.03 (0.89–1.19) | NS |
| Calcium × phosphate | $1\,mg^2$ per $100\,ml^{-2}$ | 1.00 (0.99–1.02) | NS |
| | ⩾55 vs <55 $mg^2$ per $100\,ml^{-2}$ | 0.91 (0.53–1.56) | NS |
| PTH | $1\,ng\,l^{-1}$ | 1.00 (1.00–1.00) | NS |
| Free *p*-cresol | $1\,mg\,l^{-1}$ | 1.10 (0.98–1.19) | 0.10 |
| | ⩾1.97 vs <1.97 $mg\,l^{-1}$ | 1.69 (1.07–2.67) | 0.024 |
| Urea | 1 mg per 100 ml | 1.00 (1.00–1.01) | NS |
| $\beta_2$-Microglobulin | $1\,mg\,l^{-1}$ | 1.05 (0.84–1.31) | NS |

BP, blood pressure; CRP, c-reactive protein; HD, hemodialysis; HDF, hemodiafiltration; HR, hazard ratio; LDL, low-density lipoprotein; nPNA, normalized protein nitrogen appearance; NS, not significant; PTH, parathyroid hormone; r*Kt/V*, residual *Kt/V*; sp*Kt/V*, single pool *Kt/V*.
*P*-values above 0.2 are reported as NS.

surpassed several accepted novel risk factors, including markers of the calcium phosphate metabolism, albumin, and hemoglobin.

We selected an overall model including the most relevant traditional and novel risk factors based on univariate analysis. In this model, *p*-cresol was not significantly associated with the primary end point. Most possibly, adjustment for potent modifiers of cardiovascular risk, including age and diabetes, obscures less potent but still relevant cardiovascular risk factors, especially in smaller cohorts.[26,27] As diabetic patients have significantly higher *p*-cresol concentrations, we performed grouped Cox proportional hazard analyses of patients with and without diabetes. In non-diabetics, *p*-cresol was significantly associated with CVD ($P = 0.023$), whereas treatment modality, blood pressure, calcium, and albumin were not associated.

Although not associated with the primary end point in univariate analysis, RRF was considered a potential confounder. Indeed, RRF was found to be an important predictor of outcome in hemodialysis patients.[28] We have previously shown that especially in peritoneal dialysis patients, RRF contributes importantly to the total clearance of *p*-cresol.[29] In multivariate analysis, the observed relationship between *p*-cresol and CVD remained identical when RRF was taken into account. A potential explanation is that the contribution of RRF to *p*-cresol clearance in hemodialysis patients is limited.[30]

The causes underlying the observed association between *p*-cresol and CVD are not fully elucidated. As shown before, *p*-cresol can be considered a representative of a larger group of protein-bound uremic retention solutes.[31] Several molecules of this group have been shown to have deleterious effects on the endothelium *in vitro*.[23,32–34] In addition, *p*-cresol and/or its sulfate conjugate potentially exert direct effects on the cardiovascular system. Faure *et al.*[24] have shown that *p*-cresol induces shedding of endothelial microparticles *in vitro*, which in turn are associated with endothelial dysfunction in patients with end-stage renal failure.[35] Moreover, *p*-cresol inhibits endothelial cell proliferation and alters endothelial barrier function.[22,23] Although these *in vitro* studies clearly demonstrated the toxic potential of *p*-cresol, several groups independently have demonstrated that *in vivo* *p*-cresol largely circulates in the form of its conjugate *p*-cresyl sulfate.[36–38] The effects of *p*-cresol and *p*-cresyl sulfate may well be not alike or even be completely different. This is underscored by the observation that *p*-cresyl sulfate, but not *p*-cresol, has a proinflammatory effect on leukocytes *in vitro*.[39] This undermines the traditional view that sulfate conjugation is a mere detoxification step. In this study, *p*-cresol, including conjugated metabolites, was measured.

Several conditions should ideally be met before any factor can be considered as a novel cardiovascular risk factor.[7] With respect to *p*-cresol, most criteria seem to be fulfilled. First, several mechanisms point to a deleterious effect of either

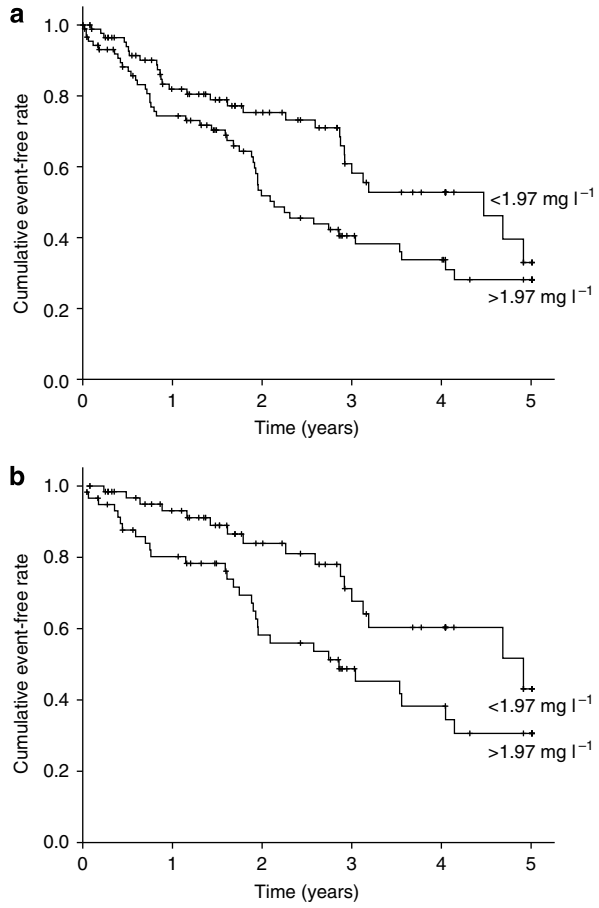


**Figure 2 | Kaplan-Meier curves of time to first cardiovascular event.** (**a**) Kaplan–Meier curve of time to first lethal or non-lethal cardiovascular event (all patients). Patients with high ($\geqslant 1.97\ mg\ l^{-1}$, $n=88$) free *p*-cresol concentrations are compared to low ($<1.97\ mg\ l^{-1}$, $n=87$) free *p*-cresol concentrations. Log rank $P=0.022$. (**b**) Kaplan–Meier curve of time to first lethal or non-lethal cardiovascular event (non-diabetics, $n=123$). Log rank $P=0.019$.

*p*-cresol or *p*-cresyl sulfate on the endothelium (see above). Second, the concentration of both total and free *p*-cresol increases with severity of kidney disease (unpublished data). Third, this study demonstrates, for the first time, an association between *p*-cresol and CVD. However, the most important piece of the puzzle is still missing. Indeed, as for most novel risk factors, a demonstration that treatment of the risk factor decreases CVD outcomes is completely lacking. Treatment to reduce *p*-cresol is not feasible at present. One strategy is to add adsorbents, such as charcoal, to the dialysate. Using this approach, Meyer *et al.*[40] were able to improve the clearance of various protein-bound retention solutes *ex vivo*. Mathematically, the maximal effect of adsorbent containing dialysate is equivalent to an unlimited increase in dialysate flow. We have recently studied the *p*-cresol clearance capacity of Fractionated Plasma Separation and Adsorption (FPSA), another adsorption-based system. The *in vivo* *p*-cresyl sulfate percent reduction rate was nearly two times as high as compared to a matched high-flux hemodialysis session (unpublished data). However, non-selective adsorption of coagulation factors induced major coagulation disturbances,[41] precluding clinical application.

It may be worth mentioning that the relationship of free *p*-cresol with overall mortality, as it was found in the original 34-month follow-up of this cohort,[21] was weakened with extended follow-up ($P=0.022$; $P=0.064$; $P=0.085$ at 3, 4, and 5 years, respectively; data not shown). This is most likely explained by the loss of discriminative power (type II error), and further research on a larger patient cohort is needed to clarify this issue. From a methodological point of view, one might question the validity of survival statistics based on a single baseline measurement of the *p*-cresol level. Indeed, considerable bias could be expected if intraindividual variability over time was large. However, by the measurement of three predialysis *p*-cresol serum levels in seven patients (a) during 1 week and (b) 12 weeks apart, the predialysis *p*-cresol serum level was found to be acceptably stable (coefficient of variation (a) 12.1% (s.d. 2.3, median 10.5, minimum–maximum 2.0–19.4); (b) 12.6% (s.d. 2.3, median 9.6, minimum–maximum 6.1–21.3)).[21]

An important new finding is that diabetic patients have higher concentrations of both free ($P=0.0069$) and total *p*-cresol ($P=0.019$) concentrations. Whether this is related to particular dietary habits or is reflecting decreased well-being of diabetic patients is not clear. Some evidence suggests altered colonic protein fermentation, the primary source of *p*-cresol, in diabetic patients. However, these findings are not consistent.[42]

In conclusion, *p*-cresol is a novel candidate cardiovascular risk factor in hemodialysis patients. This association is more important in lower risk groups, such as non-diabetics. Whether *p*-cresol is a modifiable cardiovascular risk factor in hemodialysis patients remains to be proven.

## MATERIALS AND METHODS

### Patients

One hundred and seventy-five patients with stage V CKD were enrolled in the original cohort. The study design has been described previously.[21] Briefly, patients on maintenance hemodialysis were enrolled in two Belgian hemodialysis centers (University Hospital Gasthuisberg, Leuven, and Virga Jesseziekenhuis, Hasselt) at two inclusion dates (January 2002 and January 2003). The study was performed according to the Declaration of Helsinki Principles and approved by the ethics committees of the University Hospital Leuven, Leuven, and the Virga Jesse Hospital, Hasselt. Informed consent was obtained from all patients.

### Baseline evaluation

At inclusion, blood was taken immediately before the midweek hemodialysis session for the measurement of hemoglobin (g per 100 ml), albumin (g per 100 ml), calcium (mg per 100 ml), phosphate (mg per 100 ml), $\beta_2$-microglobulin ($mg\ l^{-1}$), total and free *p*-cresol ($mg\ l^{-1}$). To level out day-to-day variations in the concentration of CRP ($mg\ l^{-1}$), concentrations were averaged from 45 days before to 45 days after the inclusion date (mean 3.4 measurements). To level out day-to-day variations in the measured blood pressure, mean systolic and diastolic blood pressure measured

**Table 3 | Multivariate Cox proportional HR analysis of time to first cardiovascular event**

| *p*-Cresol | 1 mg l$^{-1}$ increase | | ⩾1.97 vs <1.97 mg l$^{-1}$ | | |
|---|---|---|---|---|---|
| Variable | HR | *P*-value | Variable | HR | *P*-value |
| *All patients* | | | | | |
| Age (years) | 1.04 (1.02–1.06) | <0.0001 | Age | 1.04 (1.02–1.06) | 0.0003 |
| CRP | 1.10 (1.03–1.17) | 0.003 | CRP | 1.11 (1.04–1.17) | 0.001 |
| Diabetes | 2.35 (1.47–3.74) | 0.0003 | Diabetes | 2.28 (1.43–3.63) | 0.0005 |
| Albumin | | 0.14 | *p*-Cresol | | 0.085 |
| | | | Albumin | | 0.13 |
| BP | | | BP | | |
| Modality | | NS | Calcium | | NS |
| *p*-Cresol | | | Modality | | |
| Calcium | | | | | |
| *Non-diabetics* | | | | | |
| CRP | 1.13 (1.06–1.21) | 0.001 | CRP | 1.13 (1.06–1.20) | <0.001 |
| Age (years) | 1.03 (1.00–1.06) | 0.013 | Age | 1.03 (1.01–1.05) | 0.009 |
| *p*-Cresol | | 0.19 | *p*-Cresol | 2.04 (1.10–3.79) | 0.023 |
| Calcium | | | Calcium | | |
| Albumin | | NS | Albumin | | NS |
| BP | | | BP | | |
| Modality | | | Modality | | |

BP, systolic blood pressure; CRP, C-reactive protein; HR, hazard ratio; NS, not significant.
Multivariate Cox proportional HR analysis, using backward elimination at $P \geqslant 0.2$ (step 1), followed by backward elimination at $P \geqslant 0.05$ (step 2).

before start of the midweek hemodialysis session between 45 days before and 45 days after the baseline inclusion date was calculated. Cholesterol concentrations (mg per 100 ml) were not systematically collected at baseline. The mean of all concentrations measured between 180 days before and 180 days after the baseline inclusion date was calculated. On average, 2.9 measurements were used to calculate the cholesterol concentration in 174 patients.

Residual renal function was estimated from an interdialytic urine collection and expressed as weekly *Kt*/*V* of urea nitrogen (r*Kt*/*V* (residual *Kt*/*V*)). Measurements were performed as part of the baseline evaluation. Clearance (ml min$^{-1}$) was calculated from the collected volume of urea nitrogen concentration in urine and in serum. Values were recalculated to weekly clearances (l week$^{-1}$) by multiplication with 10.08. The urea distribution volume was calculated according to the Watson formula.

Albumin was measured using the bromcresol green method. Hemoglobin, calcium, phosphate, $\beta_2$-microglobulin, CRP, and cholesterol were all measured using standard laboratory techniques. Total and free (not bound to proteins) *p*-cresol serum concentrations were measured using GC-MS (gas chromatography mass spectrometry) as described previously. In short, the serum-binding proteins were heat- and acid-denatured. Subsequently, *p*-cresol was extracted in ethyl acetate and injected on the GC-MS (Trace GC-MS; Thermo Finnigan, San Jose, CA, USA). 2.6-Dimethyl-phenol was used as the internal standard. Free *p*-cresol concentrations were measured in serum ultrafiltered at room temperature using 30 000 Da molecular cutoff filters (Centrifree UF devices; Amicon, Beverly, MA, USA). We and others have recently demonstrated that *in vivo* *p*-cresol is almost entirely sulfated.[36–38] Both *p*-cresol and its conjugated metabolites were measured by our analytical technique.[37] Intra- and interassay coefficients of variation were 3.3 and 5.3%, respectively. Values below the limit of quantification (0.3 mg l$^{-1}$) were treated as 0.3 mg l$^{-1}$ for statistical analysis.

## End point evaluation

Follow-up was extended until 31 January 2007. Events, including cardiovascular events and cause of death, were identified by case note review. Events were reviewed by one physician (BKIM), blinded for the measured *p*-cresol concentrations. The participating hemodialysis units both used the same electronic medical database, in which all events were prospectively registered. To control the accuracy of these records, all cardiology, vascular surgery, and neurology case notes were reviewed as well. Finally, whenever there was any doubt about a given event, the coding physician discussed this with the treating physician of the particular hospital.

The primary end point (first cardiovascular event) was a composite of death from cardiac causes, non-fatal myocardial infarction, myocardial ischemia, ischemic stroke, or new peripheral vascular disease, whichever occurred first. Only one event per subject was included in the analysis.

After a review of the available information, the cause of death was classified as cardiovascular, infectious, malignant, or other. Cardiovascular deaths included fatal myocardial infarction, sudden death, and death due to congestive heart failure. Cases of unobserved sudden death were considered cardiovascular death only when other potential causes could be excluded. Otherwise, they were classified as other cause of death.

Non-lethal cardiovascular events, including myocardial infarction, were diagnosed on the basis of the elevated levels of cardiac enzymes and/or typical electrocardiography changes, myocardial ischemia with typical electrocardiography changes without elevated cardiac enzymes, and coronary intervention (thrombolysis, percutaneous coronary intervention, or coronary artery bypass grafting). Ischemic stroke was defined as a neurological deficit lasting more than 24 h. Hemorrhagic stroke was excluded from the primary end point. Peripheral vascular disease included ischemic pain in the lower limbs, with abnormal ankle brachial pressure index or radiological evidence of peripheral vascular disease, ischemic

necrotic lesions, or surgical arterial intervention (excluding arteriovenous conduits).

During the study period, 43 patients received a kidney transplant. Five patients had a cardiovascular event before transplantation, and the remaining 38 patients were censored. During the study period, none of the patients were transferred to peritoneal dialysis. Two patients were lost to follow-up owing to transfer to a different center. One patient was censored. A third patient was lost to follow-up but had already been censored after receiving a renal allograft. During the study period, 59 patients were classified as non-cardiovascular deaths, 33 of which were censored.

### Statistical analysis

Continuous variables are expressed as mean (s.d.) for normally distributed variables or median (minimum–maximum) for not normally distributed variables. The association between the free concentration of *p*-cresol and other variables was analyzed using a Spearman rank correlation matrix (continuous variables) and ANOVA (dichotomous variables).

Time to first cardiovascular event analysis was performed using Cox proportional hazards analysis. The relative risk of a new cardiovascular event was expressed as a hazard ratio. The Kaplan–Meier method was used to estimate cumulative incidence of the primary end point. To compare the strength of the association between *p*-cresol and the primary end point relative to other frequently used cardiovascular risk factors, *p*-cresol was tested in multivariate analysis, including all risk factors significant at the $P \leqslant 0.2$ level on univariate analysis (age, diabetes, treatment modality, systolic blood pressure, albumin, calcium, CRP, and free *p*-cresol). In the first step, the most representative variables were selected using backward elimination ($P \leqslant 0.20$). The selected subset of variables was then evaluated using backward elimination ($P \leqslant 0.05$) to define the best model to explain the outcome variable. A grouped analysis of diabetics and non-diabetics was performed using the same set of variables. For statistics, SAS (version 9.1, SAS Institute, Cary, NC, USA) and SPSS (version 15.0, Chicago, IL, USA) software packages have been used.

### ACKNOWLEDGMENTS

We thank all the patients involved in this study. K Claes, T Dejagere, H Keuleers, D Kuypers, K Stas, and J Vanwalleghem are acknowledged for their valuable contributions to the study. We thank M Dekens, H De Loor, and A Van Esch for excellent technical assistance. Part of this work was presented at the 40th Annual American Society of Nephrology Conference, San Francisco, CA, USA, 31 October 2007–5 November 2007. B Meijers (Grant no. 1.1.382.06N) and P Evenepoel (Grant no. G.0408.06) are supported by the Research Foundation—Flanders (FWO-Vlaanderen).

### REFERENCES

1. Sarnak M, Levey A. Cardiovascular disease and chronic renal disease: a new paradigm. *Am J Kidney Dis* 2000; **35**: s117–s131.
2. Foley R, Parfrey P, Sarnak M. The clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis* 1998; **32**: S112–S119.
3. Schiffrin EL, Lipman ML, Mann JFE. Chronic kidney disease: effects on the cardiovascular system. *Circulation* 2007; **116**: 85–97.
4. Foley RN, Wang C, Collins AJ. Cardiovascular risk factor profiles and kidney function stage in the US general population: the III NHANES study. *Mayo Clin Proc* 2005; **80**: 1270–1277.
5. Go AS, Chertow GM, Fan D *et al.* Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; **351**: 1296–1305.
6. Sarnak M, Coronado B, Greene T. Cardiovascular disease risk factors in chronic renal insufficiency. *Clin Nephrol* 2002; **57**: 327–335.
7. Sarnak MJ, Levey AS, Schoolwerth AC *et al.* Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation* 2003; **108**: 2154–2169.
8. Zoccali C. Traditional and emerging cardiovascular and renal risk factors: an epidemiologic perspective. *Kidney Int* 2006; **70**: 26–33.
9. Stuveling EM, Hillege HL, Bakker SJ *et al.* C-reactive protein is associated with renal function abnormalities in a non-diabetic population. *Kidney Int* 2003; **63**: 654–661.
10. Block G, Hulbert-Shearon T, Levin NW *et al.* Association of serum phosphorus and calcium × phosphate product with mortality risk in chronic hemodialysis patients: a national study. *Am J Kidney Dis* 1998; **31**: 607–617.
11. Obialo CI, Okonofua EC, Nzerue MC *et al.* Role of hypoalbuminemia and hypocholesterolemia as copredictors of mortality in acute renal failure. *Kidney Int* 1999; **56**: 1058–1063.
12. Lowrie EG, Lew NL. Death risk in hemodialysis patients: the predictive value of commonly measured variables and an evaluation of death rate differences between facilities. *Am J Kidney Dis* 1990; **15**: 458–482.
13. Vanholder R, De Smet R, Glorieux G *et al.* Review on uremic toxins: classification, concentration, and interindividual variability. *Kidney Int* 2003; **63**: 1934–1943.
14. Vanholder R, De Smet R, Lameire N. Protein-bound uremic solutes: the forgotten toxins. *Kidney Int* 2001; **59**: S266–S270.
15. Zoccali C, Bode-Böger SM, Mallamaci F *et al.* Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. *Lancet* 2001; **358**: 2113–2117.
16. Fliser D, Kronenberg F, Kielstein JT *et al.* Asymmetric dimethylarginine and progression of chronic kidney disease: the mild to moderate kidney disease study. *J Am Soc Nephrol* 2005; **16**: 2456–2461.
17. Ravani P, Tripepi G, Malberti F *et al.* Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. *J Am Soc Nephrol* 2005; **16**: 2449–2455.
18. Depner T, Himmelfarb J. Uremic retention solutes: the free and the bound. *J Am Soc Nephrol* 2007; **18**: 675–676.
19. Eknoyan G, Beck GJ, Cheung AK *et al.* Effect of dialysis dose and membrane flux in maintenance hemodialysis. *N Engl J Med* 2002; **347**: 2010–2019.
20. Paniagua R, Amato D, Vonesh E *et al.* Effects of increased peritoneal clearances on mortality rates in peritoneal dialysis: ADEMEX, a prospective, randomized, controlled trial. *J Am Soc Nephrol* 2002; **13**: 1307–1320.
21. Bammens B, Evenepoel P, Keuleers H *et al.* Free serum concentrations of the protein-bound retention solute *p*-cresol predict mortality in hemodialysis patients. *Kidney Int* 2006; **69**: 1081–1087.
22. Cerini C, Dou L, Anfosso F *et al. P*-cresol, an uremic retention solute, alters the endothelial barrier function *in vitro*. *Thromb Haemost* 2004; **92**: 140–150.
23. Dou L, Bertrand E, Cerini C *et al.* The uremic solutes *p*-cresol and indoxyl sulfate inhibit endothelial proliferation and wound repair. *Kidney Int* 2004; **65**: 442–451.
24. Faure V, Dou L, Sabatier F *et al.* Elevation of circulating endothelial microparticles in patients with chronic renal failure. *J Thromb Haemost* 2006; **3**: 566–573.
25. Kalantar-Zadeh K, Abbott KC, Salahudeen AK *et al.* Survival advantages of obesity in dialysis patients. *Am J Clin Nutr* 2005; **81**: 543–554.
26. Koenig W. Cardiovascular biomarkers: added value with an integrated approach? *Circulation* 2007; **116**: 3–5.
27. Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. *Circulation* 2007; **115**: 928–935.
28. Shemin D, Bostom A, Laliberty P *et al.* Residual renal function and mortality risk in hemodialysis patients. *Am J Kidney Dis* 2001; **38**: 85–90.
29. Bammens B, Evenepoel P, Verbeke K *et al.* Time profiles of peritoneal and renal clearances of different uremic solutes in incident peritoneal dialysis patients. *Am J Kidney Dis* 2005; **46**: 512–519.
30. Evenepoel P, Bammens B, Verbeke K *et al.* Superior dialytic clearance of [beta]2-microglobulin and *p*-cresol by high-flux hemodialysis as compared to peritoneal dialysis. *Kidney Int* 2006; **70**: 794–799.
31. De Smet R, Van Kaer J, Van Vlem B *et al.* Toxicity of free *p*-cresol: a prospective and cross-sectional analysis. *Clin Chem* 2003; **49**: 470–478.
32. Kato A, Odamaki M, Hishida A. Association between blood indoxyl sulfate and carbonyl stress marker in hemodialysis patients (abstract). *Clin Nephrol* 2003; **60**: 161–167.

33. Yamamoto H, Tsuruoka S, Ioka T *et al.* Indoxyl sulfate stimulates proliferation of rat vascular smooth muscle cells. *Kidney Int* 2006; **69**: 1780–1785.
34. Jankowski J, van der Giet M, Jankowski V *et al.* Increased plasma phenylacetic acid in patients with end-stage renal failure inhibits iNOS expression. *J Clin Invest* 2003; **112**: 256–264.
35. Amabile N, Guerin AP, Leroyer A *et al.* Circulating endothelial microparticles are associated with vascular dysfunction in patients with end-stage renal failure. *J Am Soc Nephrol* 2005; **16**: 3381–3388.
36. Martinez AW, Recht NS, Hostetter TH. Removal of *p*-cresol sulfate by hemodialysis. *J Am Soc Nephrol* 2005; **16**: 3430–3436.
37. de Loor H, Bammens B, Evenepoel P *et al.* Gas chromatographic-mass spectrometric analysis for measurement of *p*-cresol and its conjugated metabolites in uremic and normal serum. *Clin Chem* 2005; **51**: 1535–1538.
38. Meijers BKI, Loor Hd, Verbeke K *et al. p*-Cresol for better or worse: but what are we measuring? *Kidney Int* 2006; **70**: 232.
39. Schepers E, Meert N, Glorieux G *et al.* P-cresylsulphate, the main *in vivo* metabolite of *p*-cresol, activates leucocyte free radical production. *Nephrol Dial Transplant* 2007; **22**: 592–596.
40. Meyer TW, Peattie JW, Miller JD *et al.* Increasing the clearance of protein-bound solutes by addition of a sorbent to the dialysate. *J Am Soc Nephrol* 2007; **18**: 867–874.
41. Meijers BKI, Verhamme P, Nevens F *et al.* Major coagulation disturbances during fractionated plasma separation and adsorption. *Am J Transplant* 2007; **7**: 2195–2199.
42. Todesco T, Zamboni M, Armellini F *et al.* Plasma acetate levels in a group of obese diabetic, obese normoglycemic and control subjects and their relationships with other blood parameters. *Am J Gastroenterol* 1993; **88**: 751–755.