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Free *p*-cresylsulphate is a predictor of mortality in patients at different stages of chronic kidney disease

Sophie Liabeuf[1,2], Daniela V. Barreto[1,2,*], Fellype C. Barreto[1,2,*], Natalie Meert[4], Griet Glorieux[4], Eva Schepers[4], Mohammed Temmar[2], Gabriel Choukroun[1,3], Raymond Vanholder[4], Ziad A. Massy[1,2,3] and on behalf of the European Uraemic Toxin Work Group (EUTox)

[1]INSERM ERI-12 (EA 4292) and the Clinical Research Centre—Division of Clinical Pharmacology, Amiens University Hospital, Amiens, France, [2]Clinical Research Centre—Division of Clinical Pharmacology, Amiens University Hospital, Amiens, France, and the Jules Verne University of Picardy, Amiens, France, [3]Division of Nephrology, Amiens University Hospital, Amiens, France and [4]Nephrology–Dialysis–Transplantation Department, Department of Internal Medicine, University Hospital, Gent, Belgium

Correspondence and offprint requests to: Z.A. Massy; E-mail: massy@u-picardie.fr
*The second two authors contributed equally to this article.

Abstract

Background. Uraemic toxins are considered to be emerging mortality risk factors in chronic kidney disease (CKD) patients. *p*-Cresol (a prototype protein-bound uraemic retention solute) has been shown to exert toxic effects *in vitro*. Recently, it has been demonstrated that *p*-cresol is present in plasma as its sulphate conjugate, *p*-cresylsulphate. The present study evaluated the distribution of free and total *p*-

cresylsulphate and sought to determine whether these parameters were associated with vascular calcification, arterial stiffness and mortality risk in a cohort of CKD patients.

Methods. One hundred and thirty-nine patients (mean ± SD age: 67 ± 12; males: 60%) at different stages of CKD (8% at Stage 2, 26.5% at Stage 3, 26.5% at Stage 4, 7% at Stage 5 and 32% at Stage 5D) were enrolled in this study.

Results. Baseline total and free *p*-cresylsulphate presented an inverse relationship with renal function and were significantly associated with vascular calcification. During the study period (mean follow-up period: 779 ± 185 days), 38 patients died [including 22 from cardiovascular (CV) causes]. In crude survival analyses, free (but not total) *p*-cresylsulphate was shown to be a predictor of overall and CV death. Higher free *p*-cresylsulphate levels (>0.051 mg/100 mL; median) were associated with mortality independently of well-known predictors of survival such as age, vascular calcification, anaemia and inflammation.

Conclusions. Serum levels of free and total *p*-cresylsulphate (the main *in vivo* circulating metabolites of *p*-cresol) were elevated in later CKD stages. However, only free *p*-cresylsulphate seems to be a predictor of survival in CKD.

Keywords: cardiovascular disease; chronic kidney disease; mortality; *p*-cresylsulphate; Uraemic toxins

Introduction

Chronic kidney disease (CKD) patients have a markedly higher risk of overall and cardiovascular (CV) mortality than the general population [1,2]. However, the high prevalence of traditional CV risk factors does not fully explain this augmented CV risk [3].

Uraemic syndrome is attributed to the progressive retention of a large number of compounds which, under normal conditions, are excreted by the healthy kidneys [4,5]. These compounds are called Uraemic retention solutes or when they interact negatively with biological functions, Uraemic toxins. Recently, it has been suggested that these Uraemic toxins may play a role in the genesis of vascular disease in a CKD setting [6]. *p*-Cresol (a volatile phenol with a molecular weight of 108.1 Da) is the prototype member of a larger group of protein-bound Uraemic toxins and is present in plasma largely in the form of its sulphate conjugate, *p*-cresylsulphate. *p*-Cresol emanates from metabolism of the amino acids tyrosine and phenylalanine by the intestinal flora. These amino acids are generated from nutritional proteins and are metabolized into 4-hydroxyphenylacetic acid, which is then decarboxylated to *p*-cresol [7]. During its passage through the intestinal mucosa, a cytosolic sulfotransferase has the potential to metabolize *p*-cresol into *p*-cresylsulphate [8]. Notably, since the protein binding of *p*-cresylsulphate is approximately 90%, it is difficult to remove by dialysis [9,10].

p-Cresol is known to affect the inflammatory response by decreasing the reaction of activated polymorphonuclear leukocytes [11] and the endothelial cell response to inflammatory cytokines *in vitro* [12]. In Uraemic patients, serum levels of *p*-cresol are elevated by a factor of around ten, and those of the free, non-protein-bound *p*-cresol are increased even more substantially [10]. This is why the impact of free serum *p*-cresol concentrations on various outcomes has been studied in distinct cohorts of haemodialysis patients; this parameter has been shown to be associated with the rate of hospitalization for infectious diseases [13], the occurrence of CV disease [14] and mortality [15].

In contrast, unconjugated *p*-cresol cannot be detected in normal or uraemic human plasma [9,16]. Indeed, most of the intestinally generated *p*-cresol appears *in vivo* in the circulation as *p*-cresylsulphate. Recently, Schepers *et al.* demonstrated that *p*-cresylsulphate has a pro-inflammatory effect (substantiated by increased oxidative burst activity in leukocytes) and might, therefore, contribute to the increased susceptibility to vascular damage in renal patients [17]. Effectively, it has been recently been demonstrated that *p*-cresylsulphate induces the detachment of endothelial microparticles even in the absence of overt endothelial damage in haemodialysis patients, suggesting that *p*-cresylsulphate may alter the endothelial function in this setting [18]. Thus, it makes sense to study *p*-cresylsulphate rather than *p*-cresol *per se*. Hence, in the present study, we evaluate the distribution of free and total *p*-cresylsulphate in a cohort of patients at different CKD stages. In addition, we sought to assess the link between *p*-cresylsulphate and all-cause mortality and the association between *p*-cresylsulphate levels and major CV surrogate markers (namely vascular calcification and stiffness) in the same cohort.

Materials and methods

Patient selection

Over an 18-month period (from January 2006 to June 2007), a total of 150 Caucasian prevalent CKD patients were recruited from the Nephrology Department's outpatient clinic at Amiens University Hospital. All patients gave their informed, written consent. The study was approved by the local Investigational Review Board and performed in accordance with the ethical principles of the Declaration of Helsinki.

Included patients had to be over the age of 40, with a confirmed diagnosis of CKD (defined as being on haemodialysis or having two previous, estimated creatinine clearances—calculated according to the Cockcroft and Gault formula—with an interval of 3 to 6 months and values <90 ml/min/1.73 m^2). Stage 5D CKD patients had been on chronic haemodialysis three times a week for at least 3 months. Exclusion criteria consisted of the presence of chronic inflammatory disease, atrial fibrillation, complete heart block, abdominal aorta aneurysm, the presence of an aortic and/or femoral artery prosthesis, primary hyperparathyroidism, kidney transplantation and any acute CV event in the 3 months prior to screening for inclusion. The 139 patients who met all inclusion criteria and had available serum *p*-cresylsulphate assay results were included in the present analysis.

Study protocol

All patients were hospitalized for the day in order to perform laboratory blood tests, blood pressure measurements, a pulse wave velocity (PWV) determination, a lateral lumbar X-ray and multislice spiral computed tomography (CT) scanning. Hence, for a given patient, all examinations were performed between 9am and 2pm on the same day. Haemodialysis patients were seen on a dialysis-free day, or if this was not possible, the morning before the dialysis session. A patient interview focused on comorbidities and the personal disease history (especially any previous vascular events). The patients' medical files were reviewed in order to identify and record any concomitant medications. For descriptive pur-

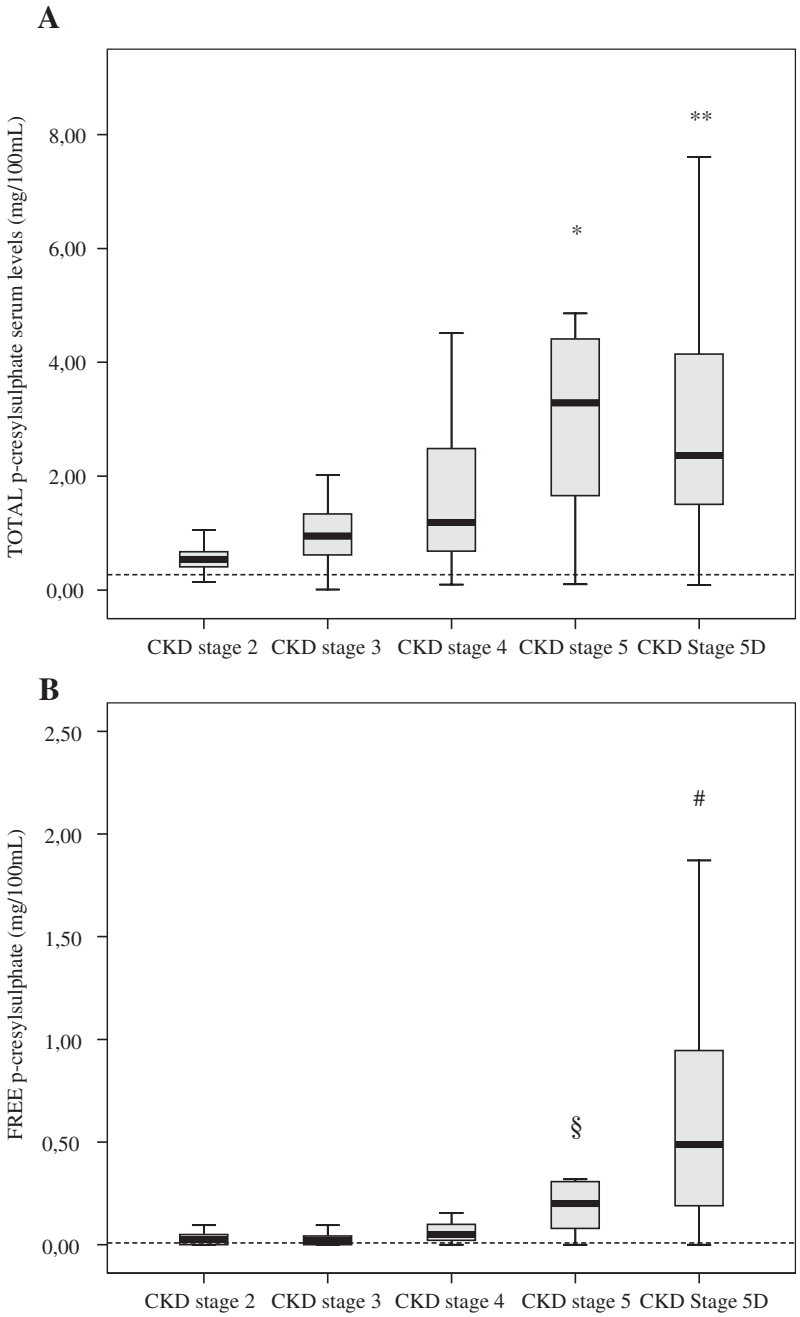


Fig. 1. (**A**) serum levels of total *p*-cresylsulphate as a function of CKD stage.*$P < 0.05$ vs CKD Stage 2; **$P < 0.05$ vs CKD Stages 2 and 3; the **dotted line** indicates the reference value for healthy subjects (0.272 ± 0.148 mg/100 mL). (**B**) Serum levels of free *p*-cresylsulphate as a function of CKD stage. §$P < 0.05$ vs CKD Stages 2 and 3; #$P < 0.005$ vs CKD Stages 2, 3 and 4; the **dotted line** indicates the reference value for healthy subjects (0.008 ± 0.009 mg/100 mL).

poses, patients who reported current or past use of insulin and/or orally administered hypoglycaemic drugs were considered to be diabetics. Previous CV disease was defined as a history of any of the following events: myocardial infarction, stroke, heart failure, angina pectoris or surgical procedures for angina or coronary/peripheral artery disease (including percutaneous transluminal angioplasty).

Laboratory tests

Blood samples were collected in the morning, before the other investigations were undertaken. Selected assays were performed after the samples had been frozen and stored at −80°C. Serum calcium, phosphate, albumin, cholesterol, haemoglobin, creatinine (Scr) and C-reactive protein (CRP) levels were assayed in an on-site biochemistry laboratory using standard auto-analyzer techniques (the Modular IIP® system, Roche Diagnostics, Basel, Switzerland). Serum intact parathyroid hormone (iPTH 1–84) was determined in a chemiluminometric immunoassay (Liaison N-tact PTH CLIA®, Diasorin, Stillwater, USA). To establish the concentration of *p*-cresylsulphate, serum samples were deproteinized by heat denaturation and then analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC) [19]. The serum concentrations were then determined by fluorescence detection (excitation 265 nm; emission 290 nm) [19]. The same method was used for total and free *p*-cresylsulphate determinations, exception that serum samples were ultrafiltered through a

Centrifree (Millipore) prior to the deproteinization for the latter compound. Reference values for healthy subjects were 0.275 ± 0.160 and 0.008 ± 0.009 mg/100 mL for total *p*-cresylsulphate and free *p*-cresylsulphate, respectively. Serum cystatin C (CysC) levels were determined by immunonephelometry (N Latex Cystatin C®, Dade Behring, Marburg, Germany). In order to describe the true glomerular filtration rate (GFR) as closely as possible, the estimated GFR combining Scr and CysC measurements (CKD-epi) was calculated for all non-dialyzed patients according to the following, recently published 'CKD-epi' equation [20]: $177.6 \times Scr^{-0.65} \times CysC^{-0.57} \times age^{-0.20} \times$ (0.82 if female). For descriptive purposes, patients were then classified into CKD stages, according to the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines [21].

PWV evaluation

Carotid–femoral PWV was determined automatically with a dedicated, validated device (Complior Colson, Createch Industrie, Massy, France), as previously described [22]. PWV was evaluated by a trained physician with two pressure probes. Simultaneously recorded pulse waveforms were obtained transcutaneously over the common carotid artery and the femoral artery in the groin. PWV was calculated as the distance between recording sites measured over the body's surface (L), divided by the time interval (t) between the feet of the flow waves (PWV = L/t); this result was averaged over 10 cardiac cycles [23]. This automatic method has been validated previously and has an intra-observer repeatability coefficient of 0.93 and an interobserver reproducibility coefficient of 0.89 [22,23].

Abdominal aorta imaging with plain radiography

A technique similar to that described by Kauppila *et al.* [24] was used to obtain images of the lower abdominal aorta and generate an aortic calcification score. All X-rays were reviewed by two independent investigators, and a consensus on the interpretation was reached in all cases.

Multislice spiral CT scan

In order to quantify the presence and extent of aortic calcifications, each patient underwent a multislice spiral CT scan. All examinations were performed with a 64-detector CT scanner (Lightspeed VCT®, GE Healthcare, Milwaukee, WI, USA).

The volume acquisition started at the aortic hiatus of the diaphragm and ended at the third lumbar vertebra. The scanning parameters were as follows: collimation: 64 × 0.625 mm; slice thickness: 0.625 mm; pitch: 1; gantry rotation speed: 0.5 s/rotation; tube voltage: 120 kV; tube current: 300 mA.

The volume acquisition was analyzed with commercially available software (Volume Viewer® software, GE Healthcare, Milwaukee, USA). The abdominal aorta was segmented manually. In order to reduce errors due to noise, a threshold of 160 UH was applied. The total calcification volume was calculated as the sum of all voxels in the remaining volume. The abdominal aorta calcification score was calculated as follows: [(total calcification volume) / (aorta wall surface area) * 100)].

Survival

Death records were established prospectively by considering all patients included at least 20 months before the study end date (1 March 2009). Each medical chart was reviewed, and the cause of death was assigned by a physician on the basis of all the available clinical information. For out-of-hospital deaths, the patient's general practitioner was interviewed to obtain pertinent information on the cause. CV mortality was defined as any death directly related to CV system dysfunction (stroke, myocardial infarction, congestive heart failure or sudden death).

Statistical analyses

Data are expressed as the mean ± SD, median and range or frequency, as appropriate. *p*-Cresylsulphate % binding was calculated as: [(total *p*-cresylsulphate − free *p*-cresylsulphate) / total *p*-cresylsulphate] * 100. Since we demonstrated that only free *p*-cresylsulphate significantly influences outcomes (overall and CV mortality) in the study population, patients were stratified according to the median serum free *p*-cresylsulphate level (i.e. serum free *p*-cresylsulphate ≤0.051 mg/100 ml vs serum free *p*-cresylsulphate >0.051 mg/100 ml) for descriptive and analytical purposes. Intergroup comparisons were performed using a χ^2 test for categorical variables and the Student's t test or the Mann–Whitney test for continuous variables. Pearson's correlation coefficient or Spearman's rank correlation was used to assess the relationships between serum *p*-cresylsulphate levels and selected clinical or biochemical variables. Linear regression analyses were performed to assess the relationship between serum *p*-cresylsulphate levels and vascular measurements. For variables with a non-Gaussian distribution, log-normalized values were considered in tests that assumed normally distributed variables. A Kaplan–Meier actuarial curve was used to estimate overall and CV mortality. The log–rank test was used to compare the survival curves. Univariate and multivariate analyses of mortality were performed by using a Cox proportional hazards model of death as a function of *p*-cresylsulphate levels [either categorized by the median level (0.051 mg/100 ml for the entire cohort and 0.038 mg/100 ml for the predialysis pop-

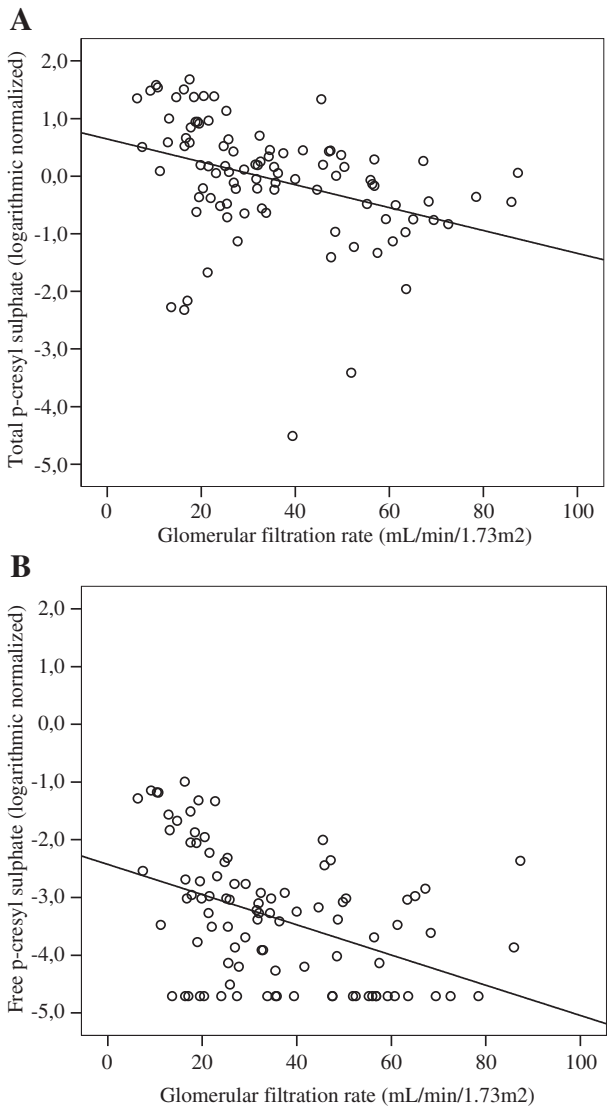


Fig. 2. Linear regression curves. (**A**) The relationship between log-normalized total *p*-cresylsulphate serum levels and the GFR for patients at CKD Stages 2 to 5; $r^2 = 0.129$, $P < 0.0001$ ($n = 96$). (**B**) The relationship between log-normalized free *p*-cresylsulphate serum levels and the GFR for patients at CKD Stages 2 to 5 ($n = 96$); $r^2 = 0.200$, $P < 0.0001$.

Table 1. Clinical and demographic characteristics of the study population

Free *p*-cresylsulphate	All *n* = 139	≤0.051 mg/100 mL *n* = 70	>0.051 mg/100 mL *n* = 69	*P*
Age, years	67 ± 12	67 ± 12	66 ± 13	0.467
Male gender, *n* (%)	84 (60)	42 (50)	42 (50)	0.917
Body mass index (kg/m^2)	28 ± 6	29 ± 7	27 ± 6	0.014
Diabetes mellitus, *n* (%)	59 (42)	32 (46)	27 (39)	0.432
Smoking habit, *n* (%)	56 (41)	29 (42)	27 (40)	0.782
Presence of CVD, *n* (%)	43 (31)	16 (23)	27 (39)	0.040
Systolic arterial pressure, mmHg	153 ± 26	151 ± 25	156 ± 28	0.300
Diastolic arterial pressure, mmHg	81 ± 12	82 ± 10	81 ± 13	0.578
CKD stage, *n* (%)				<0.0001
2	12 (8)	10 (14)	2 (3)	
3	37 (26.5)	32 (46)	5 (7)	
4	37 (26.5)	20 (29)	17 (24)	
5	10 (7)	2 (3)	8 (11.5)	
5D	43 (32)	6 (8.5)	37 (53.5)	
Aortic calcification score on CT, %; (median)	3.04 ± 3.0 (1.9)	2.3 ± 2.5 (1.5)	3.6 ± 3.2 (2.8)	0.010
Aortic calcification score on X-ray, scale 0–24; (median)	6.3 ± 6.6 (4.5)	4.32 ± 5.7 (2.0)	7.74 ± 6.8 (6.0)	0.002
PWV, m/s	14.8 ± 3.8	14.19 ± 3.5	14.9 ± 4	0.274

Data are expressed as means ± SD, or for binary variables, number (frequency) CVD: cardiovascular disease; CKD: chronic kidney disease; PWV: pulse wave velocity.

Table 2. Biochemical characteristics of the study population

Free *p*-cresylsulphate	All *n* = 139	≤0.051 mg/100 mL *n* = 70	>0.051 mg/100 mL *n* = 69	*P* value
Calcium, mMol/L	2.30 ± 0.18	2.30 ± 0.14	2.30 ± 0.22	0.772
Phosphate, mMol/L	1.30 ± 0.46	1.19 ± 0.38	1.40 ± 0.50	0.006
Calcium * phosphate (mMol/L)^2	2.95 ± 0.99	2.72 ± 0.83	3.18 ± 1.08	0.006
iPTH, pg/mL	137 ± 138 (85)	97 ± 88 (67)	178 ± 165 (122)	<0.0001
Albumin, g/L	38 ± 6	38 ± 7	37 ± 6	0.219
CRP, mg/L	10.7 ± 23 (3.5)	8.7 ± 16 (2.7)	14 ± 30 (4.0)	0.433
Haemoglobin, g/L	12 ± 1.7	12.4 ± 1.6	11.8 ± 1.8	0.059
GFR-epi[a], mL/min/1.73 m^2	35 ± 19	40 ± 18	25 ± 17	<0.0001
Total cholesterol, mMol/L	4.9 ± 1.2	4.9 ± 1.1	4.8 ± 1.1	0.356
LDL cholesterol, mMol/L	2.7 ± 0.9	2.7 ± 0.8	2.6 ± 0.9	0.667
Triglycerides, mMol/L	2.0 ± 1.3	2.0 ± 1.5	2.0 ± 1.2	0.980
Free *p*-cresylsulphate, mg/100 mL	0.26 ± 0.51 (0.05)	0.019 ± 0.018 (0.017)	0.5 ± 0.63 (0.26)	N/A
Total *p*-cresylsulphate, mg/100 mL	1.89 ± 1.73 (1.28)	0.75 ± 0.47 (0.67)	3.06 ± 1.78 (2.57)	<0.0001
p-Cresylsulphate % binding	91.4 ± 11 (95.8)	97.1 ± 4.0 (97.5)	85.5 ± 12.6 (90.2)	<0.009

Data are expressed as means ± SD and (median) for variables with a non-Gaussian distribution. LDL: low density lipoprotein, N/A: not applicable.
[a]Calculated for patients at CKD Stages 2 to 5 (*n* = 96).

ulation) or as a continuous variable]. In the multivariate analysis, the predefined models included all variables significantly associated with death in univariate analyses. Due to the study's small sample size, an additional Cox regression analysis was performed and included a propensity score adjustment; this considers each individual's probability of exposure to measured confounding variables (i.e. haemoglobin levels, aortic calcification score and CKD stage), as detailed elsewhere [25]. A *P* value ≤0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS (SPSS Inc, Chicago, IL), version 13.0 for Windows (Microsoft Corp, Redmond, WA).

Results

Figure 1 illustrates the distribution of total and free *p*-cresylsulphate levels by CKD stage. Serum levels of both total and free *p*-cresylsulphate were significantly higher in the later CKD stages (5 and 5D). When considering non-dialyzed patients only (*n* = 96; age = 67 ± 12 years; body mass index (BMI) = 29 ± 6 kg/m^2; male gender: 61.5%; presence of cardiovascular disease (CVD): 29%; diabetes mellitus: 48%; smoking habits: 41%), we observed a significant, inverse association between both total and free *p*-cresylsulphate and the glomerular filtration rate, as illustrated in Figure 2.

Tables 1 and 2 depict the demographic, clinical and biochemical characteristics of the 139 analyzed patients. The univariate correlations between log-normalized serum total and free *p*-cresylsulphate levels and the clinical and biochemical characteristics of the study population are shown

Table 3. Correlations between log-normalized serum free and total *p*-cresylsulphate and selected clinical and biochemical characteristics

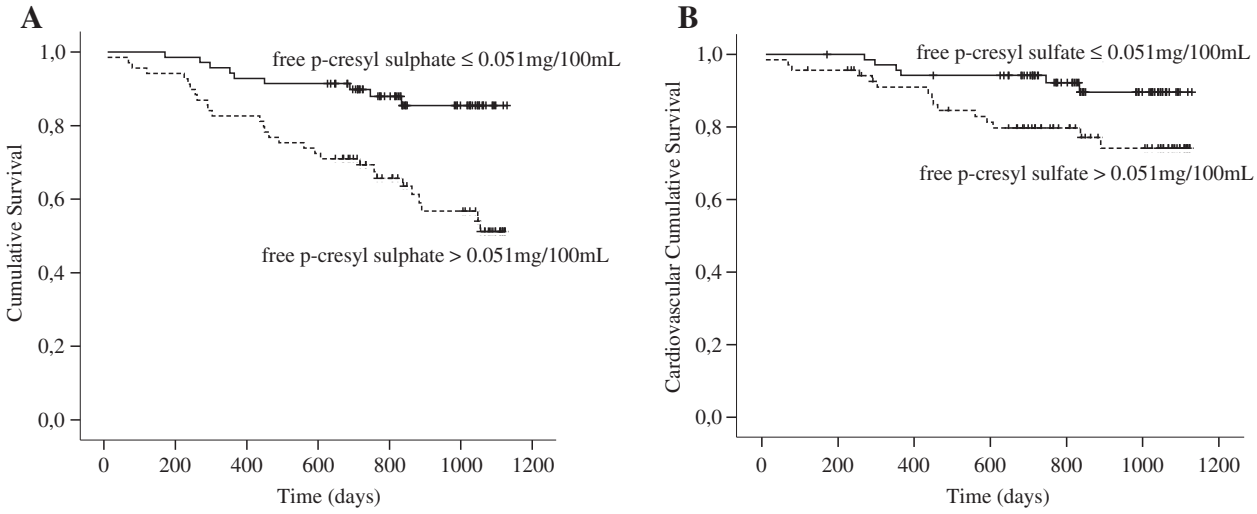
	Ln free *p*-cresylsulphate		Ln total *p*-cresylsulphate	
	r	*P*	*r*	*P*
Age	−0.034	0.688	0.158	0.06
Systolic arterial pressure	0.088	0.304	0.123	0.147
BMI	−0.227	0.007	−0.107	0.207
Albumin	−0.156	0.066	0.105	0.216
Ln CRP	0.095	0.268	−0.134	0.115
LDL cholesterol	−0.058	0.506	−0.076	0.386
Triglycerides	0.043	0.623	0.011	0.896
Haemoglobin	−0.224	0.008	−0.098	0.250
Calcium	−0.066	0.442	0.037	0.666
Phosphate	0.270	0.001	0.136	0.110
Calcium * phosphate	0.255	0.002	0.148	0.08
iPTH	0.257	0.002	0.172	0.043
Ln total *p*-cresylsulphate	0.773	<0.0001		

LDL: low density lipoprotein; I: intact; PTH: parathyroid hormone; PWV: pulse wave velocity; *r:* correlation coefficient.

in Table 3. There was an inverse correlation between free *p*-cresylsulphate serum levels on one hand and haemoglobin and BMI on the other, whereas a positive correlation was observed between the free *p*-cresylsulphate serum levels and phosphate, calcium phosphate product and iPTH. Total *p*-cresylsulphate levels were positively and significantly correlated with free *p*-cresylsulphate levels, as well as with age and the iPTH. Further multivariate linear regression analyses indicated that the BMI ($P = 0.002$) and CKD stage ($P < 0.0001$) were independently associated with free *p*-cresylsulphate levels, whereas the only independent variable associated with total *p*-cresyl levels was the CKD stage ($P < 0.0001$).

Considering the vascular measurements, there was a positive, linear association between the aortic calcification score on one hand and the log-normalized serum concentrations of both free *p*-cresylsulphate ($r^2 = 0.061$, $P = 0.005$ and $r^2 = 0.108$, $P < 0.0001$, for the CT and X-ray aortic calcification scores, respectively) and total *p*-cresylsulphate ($r^2 = 0.089$, $P = 0.001$ and $r^2 = 0.092$, $P = 0.001$, for the CT and X-ray aortic calcification scores, respectively) on the other. No association was found between PWV and serum free or total *p*-cresylsulphate.

During the study period (mean follow-up period: 779 ± 185 days; median: 815; range: 10–1129), 38 patients died (22 from CV causes, 8 from infectious disease and 8 from other causes). In crude analysis (Figure 3), a free *p*-cresylsulphate level >0.051 mg/100 mL was a significant predictor of overall and CV death (log–rank comparison between curves: $P < 0.0001$ and $P = 0.023$, respectively). Characteristics from Tables 1 and 2 were analyzed according to the median free *p*-cresylsulphate level (free *p*-cresylsulphate ≤0.051 mg/100 ml vs free *p*-cresylsulphate >0.051 mg/100 ml). The patients with higher free *p*-cresylsulphate se-



Number of patients at risk

	Days of follow-up					
	0	200	400	600	800	1000
Free p-cresylsulphate ≤ 0.051 mg/100 mL	70	69	65	64	42	22
Free p-cresylsulphate > 0.051 mg/100 mL	69	65	57	50	34	25

Fig. 3. (**A**) Kaplan–Meier estimates of overall motality as a function of free *p*-cresylsulphate levels relative to the median; $P < 0.0001$ in the log–rank comparison between curves. (**B**) Kaplan–Meier estimates of cardiovascular mortality as a function of free *p*-cresylsulphate levels relative to the median; $P = 0.023$ in the log–rank comparison between curves.

Table 4. Univariate Cox proportional hazard ratio (HR) analysis for association of baseline variables with all-cause mortality (n = 139)

	Unit of increase	HR (95% CI)	*P*
Age (years)	1 year	1.044 (1.013–1.076)	0.005
Male gender	Male vs female	1.201 (0.621–2.321)	0.587
Body mass index	1 kg/m^2	0.980 (0.929–1.033)	0.447
Diabetes mellitus	Present vs absent	0.752 (0.389–1.455)	0.398
Smoking habit	Present vs absent	1.822 (0.947–3.506)	0.073
Presence of CVD	Present vs absent	1.049 (0.536–2.052)	0.888
Dialysis	Present vs absent	2.495 (1.311–4.746)	0.005
Systolic arterial pressure	1 mmHg	1.009 (0.997–1.021)	0.144
Diastolic arterial pressure	1 mmHg	1.000 (0.974–1.027)	0.999
Calcium	1 mMol/L	0.656 (0.125–3.442)	0.618
Phosphate	1 mMol/L	1.508 (0.827–2.751)	0.180
iPTH	1 pg/mL	0.999 (0.996–1.001)	0.416
Albumin	1 g/L	0.945 (0.901–0.992)	0.022
Ln CRP	1 unit	1.313 (1.074–1.606)	0.008
Haemoglobin	1 g/L	0.681 (0.559–0.829)	< 0.0001
GFR-epi[a]	1 mL/min/1.73 m^2	0.970 (0.939–1.002)	0.063
LDL cholesterol	1 mMol/L	1.135 (0.804–1.602)	0.472
Triglycerides	1 mMol/L	0.963 (0.740–1.254)	0.782
Ln total *p*-cresylsulphate	1 unit	1.190 (0.868–1.632)	0.280
Aortic calcification score on CT	1%	1.243 (1.128–1.369)	< 0.0001
Aortic calcification score on X-ray	1 unit	1.062 (1.015–1.112)	0.01
PWV (m/s)	1 m/s	1.041 (0.964–1.124)	0.310

[a]Calculated for patients at CKD Stages 2 to 5 (n = 96).
CVD, cardiovascular disease; PTH, parathyroid hormone; GFR: glomerular filtration rate; LDL, low density lipoprotein; PWV: pulse wave velocity.

Table 5. Univariate and multivariate Cox regression analysis of risk factors at baseline for all-cause mortality, with free *p*-cresylsulphate entered as either a categorical variable[a] (free *p*-cresylsulphate >0.05 mg/100 mL vs free *p*-cresylsulphate ≤0.05) or a continuous variable[b]

Models of patient survival (event n = 38)	RR	95% CI	*P*
Unadjusted			
Free *p*-cresylsulphate[a]	3.567	1.686–7.546	0.001
Ln free *p*-cresylsulphate[b]	1.282	1.061–1.550	0.010
Model 1[c]			
Free *p*-cresylsulphate[a]	4.675	1.940–11.264	0.001
Ln free *p*-cresylsulphate[b]	1.329	1.063–1.663	0.013

RR: risk ratio, CI: confidence interval.
[c]Model 1 was adjusted for age (in 1-year increments), haemoglobin (in 1 g/L increments), ln CRP and aortic calcification score on CT (in 1% increments).
P values are stated for the trend across categories.

rum levels had a lower BMI and were also more likely to have a history of CV events. Concerning vascular parameters, patients with free *p*-cresylsulphate >0.051 mg/100 mL had a significantly higher aortic calcification score, whereas the groups did not differ significantly in terms of PWV. Regarding biochemical parameters, patients with a higher free *p*-cresylsulphate had higher phosphate, iPTH and total *p*-cresylsulphate levels and a lower GFR-epi and *p*-cresylsulphate % binding.

In a univariate Cox regression analysis, age, dialysis status, albumin, haemoglobin, CRP and the aortic calcification score were also significantly associated with the risk of death (Table 4). Strikingly, total *p*-cresylsulphate levels were not associated with the risk of death.

Table 5 shows the predictive power of serum free *p*-cresylsulphate levels for death in unadjusted models or models adjusted for multiple covariates. After adjustment for age, haemoglobin, CRP and the aortic calcification score, higher serum levels of free *p*-cresylsulphate still had a significant effect on the risk of death. In fact, for each 0.1 mg/100 mL increment in the free *p*-cresylsulphate serum level, there is a significant 5 % increase in the risk of death (data not shown). A supplementary Cox regression model (including age, CRP and the calculated propensity score (by quartiles), in order to better adjust for confounders and as detailed in the methodology section) confirmed that patients with free *p*-cresylsulphate levels above the median were at an increased risk of death (RR = 4.292; P = 0.007). These results were confirmed when the crude analysis was restricted to CKD pre-dialysis patients [n = 96, deaths = 18, P = 0.018 in the log–rank comparison between curves, for free *p*-cresylsulphate level >0.038 mg/100 mL (median) vs lower level].

Discussion

This study examines the distribution of both total and free *p*-cresylsulphate (the main *in vivo* circulating form of *p*-cresol) at different stages of CKD and the association between the compounds and surrogate markers of CV disease and mortality. Our study demonstrated that both total and free *p*-cresylsulphate levels were higher in later CKD stages. However, only free *p*-cresylsulphate was associated with total and CV mortality. Indeed, we demonstrated that higher free *p*-cresylsulphate concentrations (>0.051 mg/100 mL) were associated with mortality independently of well-known predictors of survival, such as age, vascular

calcification, anaemia and inflammation. These results were similar when the analyses were restricted to pre-dialysis CKD patients. Interestingly, our results concerning the association between serum *p*-cresylsulphate levels and mortality concur with previous reports on *p*-cresol, suggesting that the directly measured concentrations of *p*-cresylsulphate have a similar association with outcome parameters to that seen with previously measured *p*-cresol concentrations (which result from hydrolysis during acid deproteinization). Given that (i) unconjugated *p*-cresol is not detectable in normal and uraemic human plasma [6,13] and that (ii) *p*-cresol circulates as *p*-cresylsulphate [6], our findings support the notion that the previously identified relationship between *p*-cresol and clinical outcomes may be linked to *p*-cresylsulphate, rather than to *p*-cresol *per se*.

Moreover, *p*-cresylsulphate association with aortic calcification suggests that this toxin may have a role in the development of Uraemic-related CV disorders. Although *p*-cresol can affect endothelial barrier function [26], proliferation and wound repair [27], it remains to be seen whether *p*-cresylsulphate has a harmful effect on vascular cells *in vitro*. However, even though *p*-cresol and its sulphate conjugate potentially exert direct effects on the CV system, experimental data seems to suggest that their effects may not be comparable. Effectively, it has been recently demonstrated that *p*-cresylsulphate induces the detachment of endothelial microparticles even in the absence of overt endothelial damage in haemodialysis patients, suggesting that *p*-cresylsulphate may alter the endothelial function in this setting [18]. Additionally, Schepers *et al.* observed that *p*-cresylsulphate (but not *p*-cresol) has a pro-inflammatory effect on non-stimulated leukocytes *in vitro* [10]—so that *p*-cresylsulphate might contribute to the propensity to vascular damage seen in renal patients. However, this latter hypothesis was not confirmed by our *in vivo* results, since CRP values were not different between CKD patients with free *p*-cresylsulphate $\leq$0.051 mg/100 ml and those with >0.051 mg/100 ml, although there was a non-significant trend for a higher CRP at higher free *p*-cresylsulphate values. These findings might be due to a lack of power and/or a local effect of free *p*-cresylsulphate not translated into systemic inflammation, suggesting that the issue needs further investigation. Since free serum levels of *p*-cresylsulphate seem to increase mortality in CKD patients, it will be interesting to evaluate whether interventional reduction of *p*-cresylsulphate is associated with a decrease in mortality. In healthy subjects, it has been shown that *p*-cresol serum concentrations can effectively be reduced by acarbose, a glucosidase inhibitor commonly used as an oral hypoglycaemic agent [28]. The effect of this drug in CKD patients is not known.

Although most of the emphasis on the reduction of protein-bound compounds with the oral sorbent AST-120 (Kremezin^{R}) has been placed on indoxysulphate [29], early studies by Niwa *et al.* showed also a decrease in levels of phenolic compounds, such as *p*-cresol [30]. It is noteworthy that at least two recent studies have shown a beneficial effect with AST-120, compared with placebo [31,32]. Recently, it has been also shown that the prebiotic oligofrutose–inulin, a fermentable carbohydrate, significantly decreases *p*-cresylsulphate generation rates and its serum concentrations in haemodialysis patients [33].

Limitations of the present study include the relatively small size of our cohort. Moreover, one can speculate that the influence of *p*-cresylsulphate concentration on overall and CV mortality among CKD patients is more likely to be a group effect for protein-bound solutes as a whole. Furthermore, evidence of increased risk should be reproduced in multiple groups of patients and in a wide range of clinical settings; validation in several studies increases confidence that the initial reports were not spurious [34]. The major strengths of this study include the enrolment of patients at different stages of CKD and the method (with non-acid deproteinization) used to measure *p*-cresylsulphate; this avoids the decomposition of the conjugate which occurs when acid deproteinization is used.

To conclude, in the present study, free and total *p*-cresylsulphate (the main *in vivo* circulating metabolite of *p*-cresol) have been evaluated in patients at different CKD stages. Our data underline the importance of free (but not total) *p*-cresylsulphate as a predictor of survival. In order to evaluate the utility of measuring *p*-cresylsulphate in routine clinical practice, these results must be confirmed in larger clinical studies.

Conflict of interest statement. None declared.

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The association of renal impairment with all-cause and cardiovascular disease mortality

Dorothea Nitsch[1], Debbie A. Lawlor[2], Rita Patel[2], Claire Carson[1] and Shah Ebrahim[1]

[1]Non-Communicable Disease Epidemiology Unit, Department of Epidemiology and Population Health, London School of Hygiene & Tropical Medicine, London, UK and [2]Department of Social Medicine, University of Bristol, Bristol, UK

Correspondence and offprint requests to: Dorothea Nitsch; E-mail: dorothea.nitsch@lshtm.ac.uk

Abstract

Background. The prognostic value of reduced glomerular filtration rate (GFR) was examined in a community-based cohort of British women.

Methods. Serum creatinine measurements were available for 90% (n = 3851) of a representative random sample of 4286 women aged 60–79 years. GFR was estimated using the Modification of Diet in Renal Disease equation. Hazard ratios (HR) were calculated using Cox regression with outcomes of all-cause and cardiovascular disease (CVD) mortality.

Results. Eight hundred and thirty-two women (21.6%) had a GFR <60 ml/min/1.73 m^2. Over a median follow-up of 5.6 years, there were 318 deaths (100 CVD deaths). Women with GFR <60 ml/min/1.73 m^2 compared to all others showed only a borderline increased risk of all-cause mortality [HR 1.35 (95% confidence intervals: 0.99, 1.85)] and CVD mortality [1.34 (0.97, 1.85)]. Adjustment for