p-Cresyl Sulfate and Indoxyl Sulfate in Hemodialysis Patients

Björn K. I. Meijers,* Henriette De Loor,* Bert Bammens,* Kristin Verbeke,† Yves Vanrenterghem,* and Pieter Evenepoel*

**Department of Medicine, Division of Nephrology and †Laboratory for Gastro-Intestinal Research and Leuven Food Science and Nutrition Research Centre, University Hospital Leuven, Leuven, Belgium*

Background and objectives: Indoxyl sulfate and *p*-cresyl sulfate are important representatives of the protein-bound uremic retention solutes. Serum levels of *p*-cresyl sulfate and indoxyl sulfate are linked to cardiovascular outcomes and chronic kidney disease progression, respectively. They share important features such as the albumin-binding site, low dialytic clearance, and both originate from protein fermentation. Whether serum concentrations are related is, however, not known.

Design, setting, participants, & measurements: In an observational study in 75 maintenance hemodialysis patients, we studied agreement between indoxyl sulfate and *p*-cresyl sulfate serum concentrations, dialytic reduction rates, and dialytic clearances. Concentrations were determined by HPLC. Dialytic clearances were determined from total spent dialysate collections. *In vitro* spiking experiments were performed to explore protein binding characteristics.

Results: Indoxyl sulfate and *p*-cresyl sulfate total serum concentrations were not related ($r = 0.02$, $P = 0.9$), whereas free serum concentrations were only moderately related ($r = 0.53$, $P < 0.001$). Indoxyl sulfate and *p*-cresyl sulfate share the same albumin binding site, for which they are competitive binding inhibitors. Intriguingly, indoxyl sulfate and *p*-cresyl sulfate reduction rates ($r = 0.91$, $P < 0.001$) and dialytic clearances ($r = 0.97$, $P < 0.001$) correlated tightly.

Conclusions: Indoxyl sulfate and *p*-cresyl sulfate serum concentrations are not associated, suggesting different metabolic pathways. Indoxyl sulfate and *p*-cresyl sulfate are both valid markers to monitor behavior of protein-bound solutes during dialysis. Finally, they are competitive binding inhibitors for the same albumin binding site.

Clin J Am Soc Nephrol 4: 1932–1938, 2009. doi: 10.2215/CJN.02940509

Protein-bound uremic retention solutes are implicated in the uremic syndrome and might be a missing link to explain the persistently high mortality rates in chronic kidney disease (CKD) (1–3). Efforts are mounting to reduce serum concentrations, either by reducing intestinal uptake (4) or by improving blood clearances (5,6).

Indoxyl sulfate and *p*-cresyl sulfate are perhaps the most widely used marker molecules to study the behavior of the protein-bound uremic retention solutes during hemodialysis, hemodiafiltration, peritoneal dialysis, and experimental adsorption-based blood purification devices (5–10). In addition, both indoxyl sulfate and *p*-cresyl sulfate are thought to contribute directly to uremic syndrome (1).

Indoxyl sulfate is considered a key player in the progression of CKD (11–13). A large multicenter trial is ongoing to study whether reduction of indoxyl sulfate serum concentrations will slow down CKD progression (Evaluating Prevention of Progression In Chronic Kidney Disease [EPPIC-1/2; www.clinicaltrials.gov NCT00500682/NCT00501046]). Furthermore, *in vitro* and animal data suggest that indoxyl sulfate contributes to CKD-associated bone mineral disease (14,15).

p-Cresyl sulfate, indirectly quantified as *p*-cresol, is associated with overall mortality and cardiovascular disease in hemodialysis (HD) patients (16,17). Preliminary findings suggested that *p*-cresyl sulfate contributes directly to endothelial dysfunction (18). Moreover, *p*-cresyl sulfate activates leukocyte free radical production (19), linking *p*-cresyl sulfate to inflammation.

Although frequently approached as individual uremic retention solutes, they share common ground. First, *p*-cresyl sulfate and indoxyl sulfate both originate from bacterial protein fermentation in the large intestine. Colonic microbiota degrade tryptophan to indole. Further hydroxylation results in 3-hydroxy-indole, the majority of which is sulfonated to produce indoxyl sulfate (20,21). In parallel, fermentation of tyrosine results in *p*-cresol. Recently, we reported on sulfate conjugation of *p*-cresol in CKD (8,22). Second, most *p*-cresyl sulfate and indoxyl sulfate circulates noncovalently bound to albumin, presumably at Sudlow site II. Ligands of this albumin binding site are aromatic and are either neutral or bear a negative charge located peripherally on the molecule (23,24). Finally, blood clearances of *p*-cresyl sulfate and indoxyl sulfate by dialysis are limited (9). Meyer *et al.* (25) elegantly elaborated a mathematical model describing the behavior of protein bound solutes during hemodialysis. In this model, clearances are directly associated with free fractions.

Received May 5, 2009. Accepted August 28, 2009.

Published online ahead of print. Publication date available at www.cjasn.org.

Correspondence: Dr. P. Evenepoel, Dienst nefrologie, Universitair Ziekenhuis Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium. Phone: 32-16-344591; Fax: 32-16-344599; E-mail: pieter.evenepoel@uz.kuleuven.ac.be

ISSN: 1555-9041/412–1932

Although indoxyl sulfate and *p*-cresyl sulfate are related with respect to colonic protein fermentation, albumin binding, and theoretically, dialytic clearances, it is unclear to what extent blood concentrations are related. Tight agreement would alleviate the need to measure both. Furthermore, close agreement would provide alternative explanations for observed associations with clinical endpoints.

The aims of this study were to investigate (1) whether serum concentrations of indoxyl sulfate and *p*-cresyl sulfate are related, (2) to what extent their dialytic clearances are related, and (3) whether indoxyl sulfate and *p*-cresyl sulfate albumin binding is related in an unselected cohort of HD patients.

Materials and Methods

Study Population

Patients, treated with maintenance HD for at least 3 mo at the nephrology department of the University Hospital Gasthuisberg, Leuven, Belgium, were enrolled in this study. Eligible patients were 18 yr or older and able to give written informed consent. The study was performed according to the World Medical Association Declaration of Helsinki and approved by the local ethics committee. All patients provided written informed consent before enrollment.

Sample Collection

Blood was sampled at the start and at the end of a midweek dialysis. The slow flow/stop pump technique was used. Total spent dialysate was collected during the midweek dialysis session in 300-L polystyrene vessels. To calculate dialytic solute removals, spent dialysate collections were weighed, vigorously stirred, and sampled.

HPLC

p-Cresyl sulfate and indoxyl sulfate were quantified as described previously (26), using an HPLC (HPLC Alliance 2695; Waters, Zellik, Belgium), coupled to a Waters 2475 fluorescence detector. Free *p*-cresyl sulfate and indoxyl sulfate concentrations were measured in serum ultrafiltered at 37°C using 30,000-D molecular cut-off filters (Centifree UF devices; Amicon, Beverly, MA). Ultrafiltrates (600 μl) were concentrated using a vacuum concentrator (Christ rotary vacuum concentrator 2-18 and cool trap 2-50; Qlab, Vilvoorde, Belgium) at 30°C overnight. Dried ultrafiltrates were dissolved in 200 μl PBS by sonification for 30 min. Limits of quantification of total serum concentrations were 3.2 μM for indoxyl sulfate and 1.8 μM for *p*-cresyl sulfate. Limits of quantification of free serum concentrations were 1.1 and 0.6 μM, respectively. Recovery, tested in HD patients, was 102% for indoxyl sulfate and 105% for *p*-cresyl sulfate. Total, within-run, between-run, and between-day imprecision for indoxyl sulfate and *p*-cresyl sulfate were <6%. Intraindividual variation of indoxyl sulfate and *p*-cresyl sulfate serum concentrations was found to be limited. Over a 1-mo period, we observed modest variability in indoxyl sulfate (median, 7.2; interquartile range, 5.6 to 16.0 coefficient of variation [CV%]) and *p*-cresyl sulfate (median, 10.2; IQR, 7.6 to 14.0 CV%) serum concentrations ($n = 10$, 2-wk sampling interval).

Spiking Experiments

Serum from 20 HD patients was collected at the start of the HD session. Total and free indoxyl sulfate and *p*-cresyl sulfate serum concentrations were determined on aliquots before spiking and after spiking with indoxyl sulfate at a final concentration of 100 μM or *p*-cresyl sulfate at a final concentration of 166 μM. Concentrations were chosen to represent mean total serum concentrations in HD patients of indoxyl sulfate and *p*-cresyl sulfate, respectively. Samples were incubated in a 37°C water bath for 30 min.

Calculations

According to the law of mass action, binding can be expressed as

$$C_t = C_b + C_f$$

where C_t, C_b, and C_f represent the total, the bound, and free concentration of a given molecule.

The free fraction f is defined by the ratio between the unbound (free) concentration and the total concentration of a given ligand. Assuming a single binding site with a binding constant K_A, this relation can be expressed as (24)

$$f = \frac{C_f}{C_s} = \frac{1}{1 + (C_{alb} - C_t + C_f) \cdot K_A}$$

Dialytic solute removals (DSRs) of solute x were calculated as

$$DSR_x = V_d \cdot C_{d,x}$$

where V_d is the spent dialysate volume and $C_{d,x}$ is the dialytic solute concentration. Given that dialysate relative density is near 1, dialysate weight is taken as spent dialysate volume. Dialytic solute clearances ($K_{d,x}$) were calculated by

$$K_{d,x} = \frac{DSR_x}{t \cdot \xi_x}$$

where t is the actual dialysis time (min) and ξ_x is the logarithmic mean serum concentration

$$\xi_x = \frac{C_{x,pre} - C_{x,post}}{Ln(C_{x,pre}) - Ln(C_{x,post})}$$

Statistics

Continuous variables are expressed as mean ± SD for normally distributed variables or median (interquartile range) otherwise. Normality was tested according to Shapiro-Wilk. Correlations were tested according to Spearman. A two-sided $P < 0.05$ was considered statistically significant.

Results

Study Population

Between February and June 2006, 75 maintenance HD patients (Figure 1), followed at the nephrology department of the University Hospital Gasthuisberg, Leuven, Belgium, provided informed

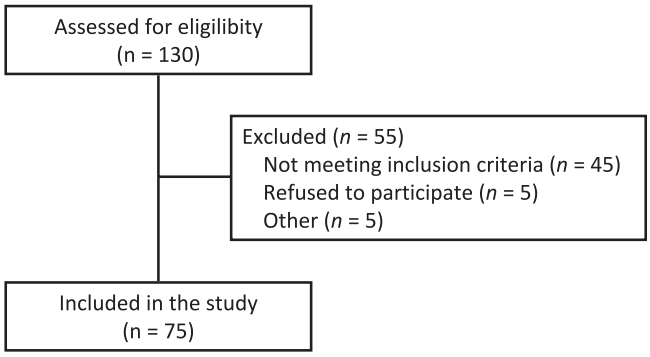

Figure 1. Flow chart showing patient screening and inclusion.

consent and were included in the study. Table 1 represents the demographic and baseline characteristics of the study population.

Serum Concentrations of Indoxyl Sulfate and p-*Cresyl Sulfate*

Associations between indoxyl sulfate and *p*-cresyl sulfate serum concentrations were analyzed using Spearman rank correlation analysis (Table 2). Total serum concentrations of indoxyl sulfate and *p*-cresyl sulfate were not correlated ($P = 0.9$). Free serum concentrations of indoxyl sulfate and *p*-cresyl sulfate showed a moderate direct association ($r = 0.53$, $P < 0.001$), whereas indoxyl sulfate and *p*-cresyl sulfate free fractions (*f*) were tightly correlated ($r = 0.87$, $P < 0.001$; Figure 2). On average, free fractions of indoxyl sulfate were 1.53 ± 0.20-fold higher than free fractions of *p*-cresyl sulfate.

Dialytic Kinetics of Indoxyl Sulfate and p-*Cresyl Sulfate*

To study whether indoxyl sulfate and *p*-cresyl sulfate behave similar during dialysis, we first compared reduction rates (RRs) during hemodialysis. Indoxyl sulfate and *p*-cresyl sulfate RR correlated tightly ($r = 0.91$, $P < 0.001$). As expected, we also observed a good correlation between urea and creatinine RR ($r = 0.89$, $P < 0.001$). However, urea RRs were only moderately correlated with indoxyl sulfate RRs ($r = 0.48$, $P < 0.001$) and with *p*-cresyl sulfate RRs ($r = 0.45$, $P < 0.001$; Figure 3).

Second, we compared dialytic clearances of indoxyl sulfate, *p*-cresyl sulfate, urea, and creatinine. Indoxyl sulfate and *p*-cresyl sulfate clearances correlated tightly ($r = 0.97$, $P < 0.001$). On average, dialytic clearances of indoxyl sulfate were 33 ± 11% higher than dialytic clearances of *p*-cresyl sulfate. As expected, we also observed a good correlation between urea and creatinine dialytic clearances ($r = 0.73$, $P < 0.001$). However, dialytic clearances of indoxyl sulfate and *p*-cresyl sulfate were not significantly correlated with dialytic urea clearances ($P = 0.2$ for both; Figure 4).

Albumin Binding of Indoxyl Sulfate and p-*Cresyl Sulfate*

To determine whether indoxyl sulfate and *p*-cresyl sulfate compete for their albumin binding site, we performed *in vitro* spiking experiments on serum from 20 HD patients. Addition of *p*-cresyl sulfate significantly increased free serum concentrations and free fractions of both *p*-cresyl sulfate and indoxyl sulfate (Table 3). Also, addition of indoxyl sulfate significantly increased free serum concentrations and free fractions of both *p*-cresyl sulfate and indoxyl sulfate ($P < 0.001$ for all; Table 2).

Changes in the free fraction of *p*-cresyl sulfate and indoxyl sulfate of individual patients correlated well ($r = 0.85$, $P < 0.001$) after spiking with indoxyl sulfate and after spiking with *p*-cresyl sulfate ($r = 0.79$, $P < 0.001$), despite opposite changes of total serum concentrations (Table 3). The observed small reductions in total *p*-cresyl sulfate and indoxyl sulfate total serum concentrations when spiked with indoxyl sulfate and *p*-cresyl sulfate, respectively, can be attributed to dilution.

Discussion

The main finding of this study in 75 maintenance HD patients is that, despite similar protein binding and blood clearances, total indoxyl sulfate and *p*-cresyl sulfate serum concentrations are not related.

Serum concentrations of indoxyl sulfate and *p*-cresyl sulfate are the result of generation from bacterial protein fermentation in the large intestine, metabolism including "detoxification" by sulfate conjugation and elimination. Residual renal function contributes little to total solute removal of protein-bound uremic retention solutes in HD patients (10). In this study, indoxyl sulfate and

Table 1. Demographics, dialysis treatment, and biochemical variables

Variable	
Age (yr) (median, minimum − maximum)	75 (30–87)
Sex (man/woman) [*n* (%)]	40/35 (54/46)
Vintage (mo)	20 (9–31.5)
$Q_{b,effective}$ (ml/min)	304.0 (40.1)
Q_d (ml/min)	505.6 (22.7)
Unipuncture/bipuncture [*n* (%)]	8/67 (11–89)
Residual renal function (yes *versus* no) (%)	39/36 (52/48)
Urea (mg/dl)	119.3 (26.9)
Creatinine (mg/dl)	6.9 (2.1)
Albumin (mg/L)	39.5 (3.5)
Total *p*-cresyl sulfate (μM)	183.6 (114.4–305.3)
Free *p*-cresyl sulfate (μM)	10.3 (5.6–19.9)
Free fraction *p*-cresyl sulfate	0.054 (0.042–0.070)
Total indoxyl sulfate (μM)	104.7 (67.2–134.6)
Free indoxyl sulfate (μM)	9.1 (5.4–12.5)
Free fraction indoxyl sulfate	0.081 (0.068–0.106)

Data are expressed as mean (SD) or median (25–75 percentile) as appropriate. To convert creatinine in mg/dl to μM, multiply with 88.4.

$Q_{b,effective}$, effective blood flow; Q_d, dialysate flow.

Table 2. Spearman rank correlations between uremic retention solutes

	Urea	Creatinine	IS, Total	IS, Free	IS, *f*	*p*CS, Total	*p*CS, Free	*p*CS, *f*
Urea	1.00	—	—	—	—	—	—	—
Creatinine	$r = 0.37$, $P = 0.002$	1.00	—	—	—	—	—	—
Indoxyl sulfate, total	$r = 0.30$, $P = 0.01$	$r = 0.47$, $P < 0.001$	1.00	—	—	—	—	—
Indoxyl sulfate, free	$r = 0.38$, $P = 0.001$	$r = 0.37$, $P = 0.001$	$r = 0.81$, $P < 0.001$	1.00	—	—	—	—
Indoxyl sulfate, *f*	$r = 0.33$, $P = 0.005$	$r = 0.13$, $P = 0.3$	$r = 0.3$, $P = 0.02$	$r = 0.73$, $P < 0.001$	1.00			—
p-cresyl sulfate, total	$r = 0.21$, $P = 0.08$	$r = 0.21$, $P = 0.08$	$r = 0.02$, $P = 0.9$	$r = 0.30$, $P = 0.01$	$r = 0.56$, $P = < 0.001$	1.00	—	—
p-cresyl sulfate, free	$r = 0.28$, $P = 0.02$	$r = 0.21$, $P = 0.08$	$r = 0.15$, $P = 0.20$	$r = 0.54$, $P < 0.001$	$r = 0.76$, $P < 0.001$	$r = 0.93$, $P < 0.001$	1.00	—
p-cresyl sulfate, *f*	$r = 0.38$, $P = 0.001$	$r = 0.27$, $P = 0.03$	$r = 0.44$, $P < 0.001$	$r = 0.54$, $P < 0.001$	$r = 0.92$, $P < 0.001$	$r = 0.62$, $P < 0.001$	$r = 0.84$, $P < 0.001$	1.00

IS, indoxyl sulfate; *p*CS, *p*-cresyl sulfate; *f*, free fraction.

Figure 2. Agreement between (A) total serum concentrations, (B) free serum concentrations, and (C) free fractions of *p*-cresyl sulfate and indoxyl sulfate in a cohort of 75 hemodialysis patients. Spearman correlation coefficients are reported.

p-cresyl sulfate dialytic clearances are tightly correlated and were almost equal within individual patients. These findings indicate that the generation of indole and *p*-cresol is unrelated, despite the fact that they both originate from bacterial protein fermentation. Intervention studies also suggest that different metabolic pathways are involved. Oral intake of Lebenin (a preparation of anti-

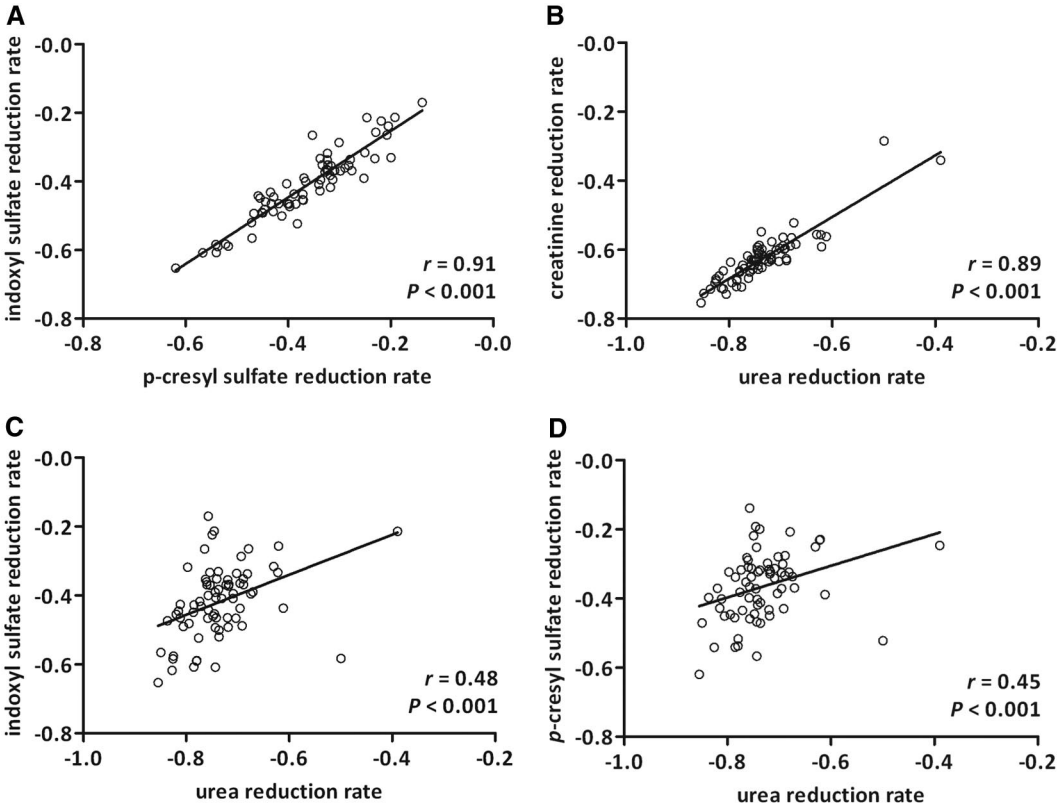

Figure 3. Agreement between reduction rates of (A) *p*-cresyl sulfate and indoxyl sulfate, (B) urea and creatinine, (C) urea and indoxyl sulfate, and (D) urea and *p*-cresyl sulfate in a cohort of 75 hemodialysis patients. Spearman correlation coefficients are reported.

biotic-resistant lactic acid bacteria) reduced serum concentrations of indoxyl sulfate but not of *p*-cresyl sulfate in HD patients (27). *Vice versa*, we recently observed that intake of the prebiotic oligofructose-enriched inulin significantly reduced *p*-cresol generation and *p*-cresyl sulfate serum concentrations, whereas indoxyl sulfate serum concentrations were not systematically reduced, nor was the indole generation rate (clinicaltrials.gov NCT00695513) (28). This allows for targeted therapies specifically reducing intestinal generation and adsorption of either *p*-cresol or indole.

Intriguingly, indoxyl sulfate and *p*-cresyl sulfate free fractions are tightly correlated ($r = 0.87$, $P < 0.001$). In the absence of a direct association of total serum concentrations, this strongly suggests interrelated protein binding. Albumin bears specific binding sites for anionic, neutral, and cationic ligands (24). Using competitive binding experiments with selective probes, indoxyl sulfate was shown to bind to Sudlow site II (29,30). We recently showed that *p*-cresyl sulfate binds to Sudlow site II as well (26). Although it is generally accepted that competition by uremic retention solutes is responsible for impaired adsorptive capacity of serum proteins including albumin in renal insufficiency, reported by Breyer and Radcliff more than half a century ago (31), little is known about competition between different uremic retention solutes for specific albumin binding sites. In this study, we showed that changes in total indoxyl sulfate concentrations affect free *p*-cresyl sulfate concentrations and *vice versa* through competitive binding inhibition.

Meyer *et al.* (25) modeled that dialytic clearances of protein-bound uremic retention solutes are directly proportional to free fractions. According to their model, one would expect good agreement in blood clearances. Indeed, we observed tight correlations between indoxyl sulfate and *p*-cresyl sulfate dialytic clearances ($r = 0.97$, $P < 0.001$) and RRs ($r = 0.91$, $P < 0.001$). In contrast, we observed no significant correlations between either *p*-cresyl sulfate or indoxyl sulfate dialytic clearances and urea dialytic clearances.

Our findings have several clinical implications. Total indoxyl sulfate and *p*-cresyl sulfate serum concentrations are not associated and free concentrations are only modestly associated. As a consequence, observed associations between total and, to a lesser extent, free solute concentrations of either one and clinical endpoints cannot be attributed to known physiologic effects of the other. In other words, *p*-cresyl sulfate and indoxyl sulfate are not interchangeable risk markers.

Intriguingly, indoxyl sulfate and *p*-cresyl sulfate free fractions are tightly correlated and, secondary to this, dialytic clearances of indoxyl sulfate and *p*-cresyl sulfate are also closely related. The implication is that either indoxyl sulfate or *p*-cresyl sulfate can be used as marker molecule to study the dialytic behavior of the Sudlow site II protein-bound uremic retention solutes during dialysis.

Finally, because we observed competitive albumin binding, therapies targeted at reducing indoxyl sulfate serum concentrations, *e.g.*, AST-120 (Kremezin®; Kureha Chemical Industry,

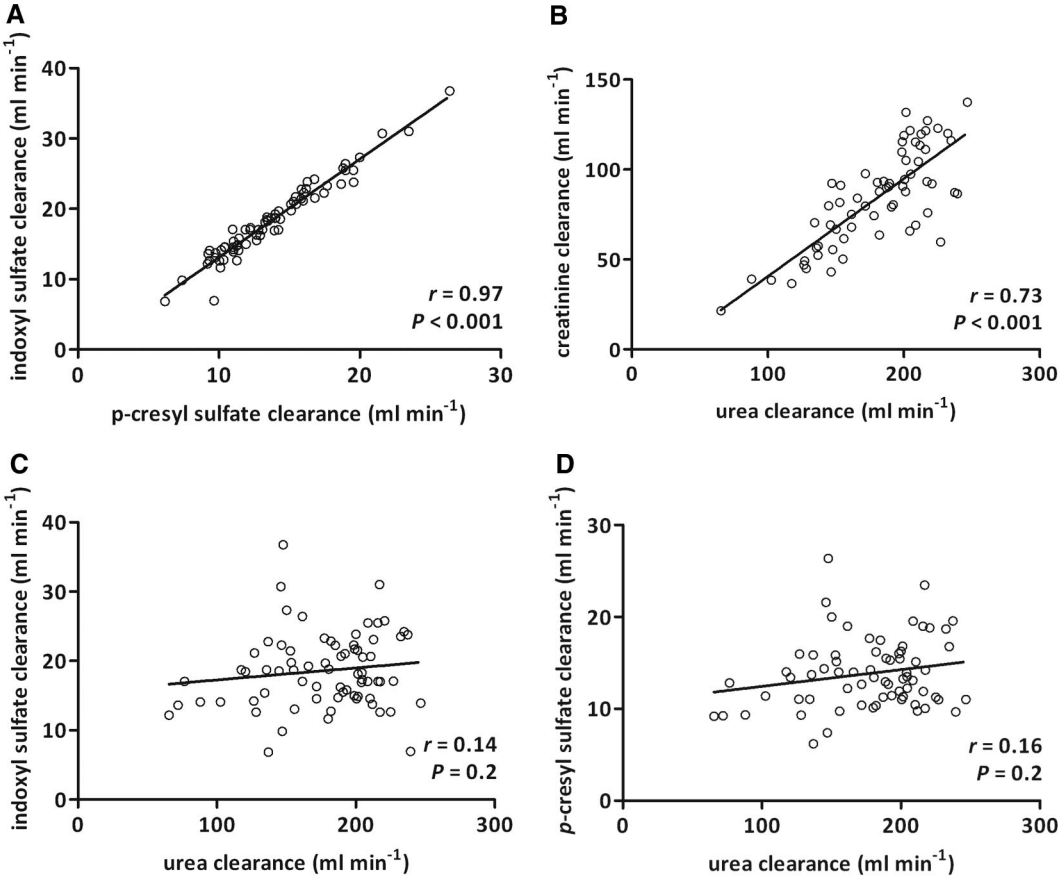

Figure 4. Agreement between dialytic clearances of (A) *p*-cresyl sulfate and indoxyl sulfate, (B) urea and creatinine, (C) urea and indoxyl sulfate, and (D) urea and *p*-cresyl sulfate in a cohort of 75 hemodialysis patients. Spearman correlation coefficients are reported.

Table 3. Albumin binding of indoxyl sulfate and *p*-cresyl sulfate

Variable	Baseline Concentration	Spiking *p*-Cresyl Sulfate		Spiking Indoxyl Sulfate	
		Concentration	Change (%)	Concentration	Change (%)
p-Cresyl sulfate					
Total (μM)	189.0 (130.6)	354.3 (123.4)	88.5 (54.1)[a]	186.0 (125.0)	−4.9 (4.2)[a]
Free (μM)	11.6 (13.4)	30.1 (24.4)	164.2 (109.4)[a]	14.6 (17.2)	24.7 (15.3)[a]
Free fraction (%)	6.7 (4.0)	8.6 (4.9)	37.1 (20.3)[a]	8.6 (6.2)[a]	27.3 (15.9)[a]
Indoxyl sulfate					
Total (μM)	114.3 (76.3)	110.7 (73.7)	−3.1 (1.6)[a]	210.4 (66.4)	89.4 (125.4)[a]
Free (μM)	12.8 (13.8)	16.8 (17.2)	28.5 (12.2)[a]	28.3 (18.4)	134.4 (144.0)[a]
Free fraction (%)	9.3 (4.7)	12.1 (6.1)	34.3 (13.0)[a]	11.4 (6.2)	20.9 (7.5)[a]

Data are expressed as median (interquartile range).
[a]$P < 0.001$ *versus* baseline.

Tokyo, Japan) (4), are expected to reduce free *p*-cresyl sulfate serum concentrations, even if total *p*-cresyl sulfate concentrations are not affected.

In conclusion, indoxyl sulfate and *p*-cresyl sulfate are interchangeable marker molecules to monitor behavior of protein-bound uremic retention solutes during dialysis and are competitive binding inhibitors for the same albumin binding site. The absence of an association between total serum concentrations refutes the thesis that *p*-cresyl sulfate and indoxyl sulfate are interchangeable risk markers.

Acknowledgments

We thank M. Dekens, E. Vanhalewyck, and A. Van Esch for excellent technical assistance and T. W. Meyer for critical appraisal of the manuscript.

Disclosures

None.

References

1. Meyer TW, Hostetter TH: Uremia. *N Engl J Med* 357: 1316–1325, 2007
2. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu Cy: Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 351: 1296–1305. 2004
3. Depner T, Himmelfarb J: Uremic retention solutes: The free and the bound. *J Am Soc Nephrol* 18: 675–676, 2007
4. Schulman G, Agarwal R, Acharya M, Berl T, Blumenthal S, Kopyt N: A multicenter, randomized, double-blind, placebo-controlled, dose-ranging study of AST-120 (Kremezin) in patients with moderate to severe CKD. *Am J Kidney Dis* 47: 565–577, 2006
5. Meyer TW, Peattie JW, Miller JD, Dinh DC, Recht NS, Walther JL, Hostetter TH: Increasing the clearance of protein-bound solutes by addition of a sorbent to the dialysate. *J Am Soc Nephrol* 18: 868–874, 2007
6. Meijers BKI, Weber V, Bammens B, Dehaen W, Verbeke K, Falkenhagen D, Evenepoel P: Removal of the uremic retention solute p-cresol using fractionated plasma separation and adsorption. *Artif Organs* 32: 214–219, 2008
7. Luo FJ, Patel KP, Marquez IO, Plummer NS, Hostetter TH, Meyer TW: Effect of increasing dialyzer mass transfer area coefficient and dialysate flow on clearance of protein-bound solutes: A pilot crossover trial. *Am J Kidney Dis* 43: 1042–1049, 2009
8. Martinez AW, Recht NS, Hostetter TH, Meyer TW: Removal of p-cresol sulfate by hemodialysis. *J Am Soc Nephrol* 16: 3430–3436, 2005
9. Bammens B, Evenepoel P, Verbeke K, Vanrenterghem Y: Removal of the protein-bound solute p-cresol by convective transport: A randomized crossover study. *Am J Kidney Dis* 44: 278–285, 2004
10. Evenepoel P, Bammens B, Verbeke K, Vanrenterghem Y: Superior dialytic clearance of [beta]2-microglobulin and p-cresol by high-flux hemodialysis as compared to peritoneal dialysis. *Kidney Int* 70: 794–799, 2006
11. Miyazaki T, Ise M, Hirata M, Endo K, Ito Y, Seo H, Niwa T: Indoxyl sulfate stimulates renal synthesis of transforming growth factor-beta 1 and progression of renal failure. *Kidney Int* 52: S211–S214, 1997
12. Niwa T, Nomura T, Sugiyama S, Miyazaki T, Tsukushi S, Tsutsui S: The protein metabolite hypothesis, a model for the progression of renal failure: an oral adsorbent lowers indoxyl sulfate levels in undialyzed uremic patients. *Kidney Int* 52: S23–S28, 1997
13. Niwa T: Organic acids and the uremic syndrome: protein metabolite hypothesis in the progression of chronic renal failure. *Semin Nephrol* 3: 167–182, 1996
14. Adijiang A, Goto S, Uramoto S, Nishijima F, Niwa T: Indoxyl sulphate promotes aortic calcification with expression of osteoblast-specific proteins in hypertensive rats. *Nephrol Dial Transplant* 23: 1892–1901, 2008
15. Nii-Kono T, Iwasaki Y, Uchida M, Fujieda A, Hosokawa A, Motojima M, Yamato H, Kurokawa K, Fukagawa M: Indoxyl sulfate induces skeletal resistance to parathyroid hormone in cultured osteoblastic cells. *Kidney Int* 71: 738–743, 2007
16. Bammens B, Evenepoel P, Keuleers H, Verbeke K, Vanrenterghem Y: Free serum concentrations of the protein-bound retention solute p-cresol predict mortality in hemodialysis patients. *Kidney Int* 69: 1081–1087, 2006
17. Meijers BKI, Bammens B, De Moor B, Verbeke K, Vanrenterghem Y, Evenepoel P: Free p-cresol is associated with cardiovascular disease in hemodialysis patients. *Kidney Int* 73: 1173–1180, 2008
18. Meijers BK, Van Kerckhoven S, Verbeke K, Dehaen W, Vanrenterghem Y, Hoylaerts MF, Evenepoel P: The uremic retention solute p-cresyl sulfate and markers of endothelial damage. *Am J Kidney Dis* 2009, in press
19. Schepers E, Meert N, Glorieux G, Goeman J, Van der Eycken J, Vanholder R: P-cresylsulphate, the main in vivo metabolite of p-cresol, activates leucocyte free radical production. *Nephrol Dial Transplant* 22: 592–596, 2007
20. Stanfel LA, Gulyassy PF, Jarrard EA: Determination of indoxyl sulfate in plasma of patients with renal failure by use of ion-pairing liquid chromatography. *Clin Chem* 32: 938–942, 1986
21. Niwa T, Takeda N, Tatematsu A, Maeda K: Accumulation of indoxyl sulfate, an inhibitor of drug-binding, in uremic serum as demonstrated by internal-surface reversed-phase liquid chromatography. *Clin Chem* 34: 2264–2267, 1988
22. de Loor H, Bammens B, Evenepoel P, De Preter V, Verbeke K: Gas chromatographic-mass spectrometric analysis for measurement of p-cresol and its conjugated metabolites in uremic and normal serum. *Clin Chem* 51: 1535–1538, 2005
23. Sudlow G, Birkett D, Wade D: The characterization of two specific drug binding sites on human serum albumin. *Mol Pharmacol* 11: 824–832, 1975
24. Meijers BKI, Bammens B, Verbeke K, Evenepoel P: A review of albumin binding in CKD. *Am J Kidney Dis* 51: 839–850, 2008
25. Meyer TW, Leeper EC, Bartlett DW, Depner TA, Lit YZ, Robertson CR, Hostetter TH: Increasing dialysate flow and dialyzer mass transfer area coefficient to increase the clearance of protein-bound solutes. *J Am Soc Nephrol* 15: 1927–1935, 2004
26. de Loor H, Meijers BK, Meyer TW, Bammens B, Verbeke K, Dehaen W, Evenepoel P: Sodium octanoate to reverse indoxyl sulfate and p-cresyl sulfate albumin binding in uremic and normal serum during sample preparation followed by fluorescence liquid chromatography. *J Chromatogr A* 1216: 4684–4688, 2009
27. Hida M, Aiba Y, Sawamura S, Suzuki N, Satoh T, Koga Y: Inhibition of the accumulation of uremic toxins in the blood and their precursors in the feces after oral administration of Lebenin, a lactic acid bacteria preparation, to uremic patients undergoing hemodialysis. *Nephron* 74: 349–355, 1996
28. Meijers BK, De Preter V, Verbeke K, Vanrenterghem Y, Evenepoel P: p-Cresyl sulfate serum concentrations in haemodialysis patients are reduced by the prebiotic oligofructose-enriched inulin. *Nephrol Dial Transplant* 2009, in press
29. Sakai T, Yamasaki K, Sako T, Kragh-Hansen U, Suenaga A, Otagiri M: Interaction mechanism between indoxyl sulfate, a typical uremic toxin bound to site II, and ligands bound to site I of human serum albumin. *Pharm Res* 18: 520–524, 2001
30. Sakai T, Takadate A, Otagiri M: Characterisation of binding site of uremic toxins on human serum albumin. *Biol Pharm Bull* 18: 1755–1761, 1995
31. Breyer B, Radcliff F: The adsorptive capacity of serum proteins in renal insufficiency. *Austral J Exp Biol* 32: 411–420, 1954