



# Potential interactions between uraemic toxins and drugs: an application in kidney transplant recipients treated with calcineurin inhibitors

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## ABSTRACT

**Background.** The uraemic toxins that accumulate as renal function deteriorates can potentially affect drug pharmacokinetics. This study's objective was to determine whether plasma concentrations of certain uraemic toxins are correlated with blood concentrations of two immunosuppressants.

**Methods.** DRUGTOX was a cross-sectional study of 403 adult patients followed up after kidney transplantation and who had undergone therapeutic drug monitoring (TDM) of calcineurin inhibitors (tacrolimus or cyclosporin) between August 2019 and March 2020. For each patient, immunosuppressant trough concentrations ( $C_0$ ) were measured in whole blood samples and then normalized against the total daily dose ( $C_0:D$  ratio). The sample was assayed for five uraemic toxins [urea, trimethylamine N-oxide (TMAO), indole acetic acid (IAA), *p*-cresylsulphate (PCS) and indoxylsulphate (IxS)] using liquid chromatography–tandem mass spectrometry.

**Results.** The median age was 56 years [interquartile range (IQR) 48–66] and the median estimated glomerular filtration rate was 41 mL/min/1.73 m<sup>2</sup> (IQR 30–57). Age, sex, body mass index (BMI), urea, IxS and PCS were significantly associated with an increment in the tacrolimus  $C_0:D$  ratio. A multivariate analysis revealed an independent association with IxS [odds ratio 1.36 (95% confidence interval 1.00–1.85)] after adjustment for sex, age and BMI, whereas adjustment for age weakened the association for PCS and urea. In a univariate logistic analysis, age, sex, BMI and the TMAO level (but not PCS, IxS, IAA or urea) were significantly associated with an increment in the cyclosporine  $C_0:D$  ratio.

**Conclusions.** Even though TDM and dose adaptation of immunosuppressants keep levels within the therapeutic window,

increased exposure to tacrolimus (but not cyclosporine) is associated with an accumulation of PCS, IxS and urea.

**Keywords:** cyclosporine, kidney transplantation, tacrolimus, uraemic toxins

## INTRODUCTION

Kidney disease has a major effect on overall health, both as a direct cause of morbidity and mortality and as an important risk factor for cardiovascular disease [1]. Patients with chronic kidney disease (CKD) typically present several comorbidities and thus require polymedication [2–4]. CKD patients having undergone kidney transplantation have the highest drug burden and the most complex drug regimens [5]. Immunosuppressive agents increase the complexity of patient care due to their pharmacokinetic variability and their low therapeutic index, which prompt a need for therapeutic drug monitoring (TDM).

Drug management is particularly difficult in CKD patients. Kidney disease directly affects drug pharmacokinetics and, in parallel with the decrease in the glomerular filtration rate (GFR) and tubular secretion, leads to a decrease in renal drug clearance. Furthermore, CKD modifies the absorption, distribution, metabolism and non-renal clearance of drugs [6].

As CKD progresses, many molecules accumulate as a result of the kidneys' decreased excretory capacity, such as compounds called uraemic toxins. They are defined in particular by their concentration-dependent deleterious effects. These molecules are classified, according to their molecular weight, into small molecules such as urea and trimethylamine N-oxide (TMAO), medium molecules and molecules

## KEY LEARNING POINTS

### What is already known about this subject?

- In addition to affecting renal clearance of drugs, chronic kidney disease (CKD) changes the absorption, distribution, metabolism and non-renal clearance of drugs. As CKD progresses, the accumulation of uraemic toxins might contribute to changes in drug pharmacokinetics. Given the requirement for therapeutic drug monitoring (TDM) with most immunosuppressants, the latter are good models for understanding the potential impact of uraemic toxins on blood concentrations of drugs.

### What this study adds?

- In 403 kidney transplant recipients, the blood tacrolimus concentration was significantly associated with plasma urea, indoxylsulphate (IxS) and *p*-cresylsulphate (PCS) levels. IxS and PCS bind to albumin with high affinity and might directly compete for tacrolimus binding sites on the protein, whereas urea might carbamylate albumin and thus modify tacrolimus binding. The blood concentration of cyclosporine (which mainly binds to lipoprotein rather than albumin) was not associated with plasma IxS, PCS and urea levels.

### What impact this may have on practice or policy?

- Our findings have potential implications for other drugs that bind to albumin with high affinity (since an elevated free fraction might induce adverse drug reactions) and emphasize the need for more research on how to decrease uraemic toxin levels.

strongly bound to plasma proteins [such as *p*-cresylsulphate (PCS), indoxylsulphate (IxS) and indole acetic acid (IAA)]. All the aforementioned uraemic toxins accumulate when kidney function deteriorates and all have been linked to harmful effects on the cardiovascular system [7–11], bone and kidney in CKD patients [12, 13]. Uraemic toxins can also modify drug pharmacokinetics. For example, urea is able to carbamylate albumin [14, 15], which in turn alters the protein binding of many drugs. Several studies have shown that the interactions between uraemic toxins and organic anion transporters (OATs) 1/3 [16, 17] and other proteins [6, 18, 19] can affect renal or non-renal clearance of drugs and thus lead to toxin accumulation and toxicity. Among the drugs used in patients with CKD, immunosuppressants require TDM in order to maintain their concentration within the therapeutic range after kidney transplantation. The resulting dose adjustments are essential for preventing the onset of acute graft rejection and minimizing drug-related toxicity. The two calcineurin inhibitors tacrolimus and cyclosporine constitute the cornerstone of immunosuppressive treatment. They have similar pharmacological properties: low and variable bioavailability, major binding to plasma proteins, metabolism by cytochrome P450 3A4/5 and excretion in the bile [20]. The main difference between the two concerns the plasma protein to which the drug binds: albumin for tacrolimus and lipoprotein for cyclosporine. The impact of uraemic toxins on blood concentrations of these two immunosuppressants has not been comprehensively characterized [21]. Indeed, patients having undergone kidney transplantation could present a few years after transplantation with an elevation in uraemic toxin levels in line with estimated GFR (eGFR) decline.

Our starting hypothesis was that uraemic toxins influence the exposure of immunosuppressants. The study's objective was therefore to determine whether the accumulation of certain uraemic toxins was associated with differences in the blood concentrations of the immunosuppressants tacrolimus and cyclosporine.

## MATERIALS AND METHODS

The present results are reported according to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines [22].

### Study design and participants

DRUGTOX was a cross-sectional study of adult patients being monitored after kidney transplantation at Amiens University Medical Centre (Amiens, France) and who underwent calcineurin inhibitor TDM between 4 August 2019 and 11 March 2020. The main inclusion criteria were age  $\geq 18$  years, kidney transplantation 12 months previously, treatment with a calcineurin inhibitor (tacrolimus or cyclosporine) and available calcineurin inhibitor assay data. Patients with acute graft rejection and/or who refused to participate were not included in the study.

In line with the study's objective, we planned to include 200 patients with an  $eGFR \leq 40$  mL/min/1.73 m<sup>2</sup> (since these patients usually present high uraemic toxin levels) and 200 patients with an  $eGFR > 40$  mL/min/1.73 m<sup>2</sup> (since these patients usually present low uraemic toxin levels). First, all consecutive patients meeting the inclusion criteria were included in one eGFR category or the other. Second, the  $eGFR > 40$  mL/min/1.73 m<sup>2</sup> group was the first to attain 200 patients;

thenceforth, only consecutive patients with eGFR  $\leq 40$  mL/min/1.73 m<sup>2</sup> were included (up to a limit of 200). Each patient was included once only.

Blood samples were analysed routinely in the pharmacology department in order to determine tacrolimus or cyclosporine trough concentrations during the follow-up of kidney transplant patients. Next, for each patient, levels of uraemic toxins were measured (after centrifugation) in unused plasma from the same initial sample.

In line with French legislation on non-interventional studies, approval by an investigational review board was neither required nor sought. However, the study was registered with the French National Data Protection Commission (Commission Nationale de l'Informatique et des Libertés, Paris, France; registration PI2019\_843\_0060). Patients were provided with information about the study and were free to refuse to participate. Due to French regulations, data are not available to share.

### Study endpoints

The study's objective was to determine whether the accumulation of certain uraemic toxins was associated with tacrolimus or cyclosporine exposure [evaluated by the ratio of tacrolimus or cyclosporine trough concentration ( $C_0$ ) to the dose ( $D$ ) in milligrams per kilogram ( $C_0:D$ )] and uraemic toxin concentrations. Confounding factors that might have modified calcineurin inhibitor pharmacokinetics, and thus exposure, were included in the analysis.

Given that clinicians routinely adjust the tacrolimus or cyclosporine dose (to maintain concentrations within the therapeutic range), we analysed the  $C_0:D$  ratio rather than  $C_0$  alone.

### Collected data

The patients' sociodemographic (age and sex), anthropometric [body mass index (BMI)], clinical (blood pressure, aetiology of CKD, history of hypertension or liver disease and time since transplantation) and clinical biochemistry data [calcium, phosphate, glucose, uric acid, C-reactive protein (CRP), protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine] were extracted from the medical records. We used the Modification of Diet in Renal Disease equation to calculate the eGFR [23]. CKD stages were evaluated according to the Kidney Disease: Improving Global Outcomes classification [24].

Each prescribed drug was recorded and classified as a strong or weak inhibitor or inducer of P450 cytochrome 3A4 or 3A5 (CYP3A4/5), an inhibitor or inducer of glycoprotein P (P-gp) and/or a compound that bound strongly to plasma proteins (Supplementary data, Table S1). For each patient, the total set of prescribed drugs was used to construct the following variables: at least one strong CYP3A4/5 inhibitor, at least one weak CYP3A4/5 inhibitor, at least one strong CYP3A4/5 inducer, at least one weak CYP3A4/5 inducer, at least one P-gp inhibitor, at least one P-gp inducer and at least one drug with major plasma protein binding.

### Tacrolimus and cyclosporine assays

Calcineurin inhibitor concentrations were determined using an European Medicines Agency validated liquid chromatography (Shimadzu, Marne-la-Vallée, France)–tandem mass spectrometry (Api3200, Sciex, Les Ulis, France) method. A sample of 50  $\mu$ L of whole blood was deproteinized with 100  $\mu$ L of a zinc sulphate solution containing a mixture of deuterated internal standards. The compounds were separated on a Luna Phenyl-Hexyl column (5  $\mu$ m, 50  $\times$  2.0 mm, Phenomenex, Le Pecq, France). The 1202.8  $\rightarrow$  425.2 and 821.5  $\rightarrow$  768.4 transitions were used to quantify cyclosporine and tacrolimus, respectively, after positive-mode electrospray ionization.

### Uraemic toxin assays

Blood levels of uraemic toxins (IAA, IxS, PCS and TMAO) were determined using liquid chromatography (Shimadzu, Marne-la-Vallée, France)–tandem mass spectrometry (3200 QTRAP, Sciex, Les Ulis, France) [25]. Briefly, uraemic toxins were extracted from 50  $\mu$ L of plasma by adding 200  $\mu$ L of an ice-cold acetonitrile solution containing internal standards (<sup>13</sup>C<sub>6</sub>-IxS, d<sub>4</sub>-PCS and d<sub>9</sub>-TMAO). The compounds were separated on a pentafluorophenyl propyl column (5  $\mu$ m, 50  $\times$  2.1 mm, Restek, Lisses, France) using a gradient of acetonitrile with 0.1% formic acid and ultrapure water with 0.1% formic acid and a flow rate of 0.8 mL/min. Data were acquired in multiple reaction monitoring mode after negative-mode electrospray ionization (for IxS and PCS) or positive-mode electrospray ionization (for IAA and TMAO) [26].

The Atellica CH Urea Nitrogen Assay (Siemens, Munich, Germany) was used to quantify urea nitrogen in human plasma.

### Statistical analyses

Baseline characteristics were described for all participants and by subgroup according to the baseline eGFR ( $< 40$  or  $\geq 40$  mL/min/1.73 m<sup>2</sup>). The results were expressed as the median [interquartile range (IQR)] or the frequency (percentage). Student's *t*-test, Mann–Whitney test or chi-squared test were used for intergroup comparisons.

For each uraemic toxin, the plasma levels at each CKD stage were compared by Kruskal–Wallis test. We used logistic regression models to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) for uraemic toxins and other factors associated with the  $C_0:D$  ratio for tacrolimus and cyclosporine. First, univariate logistic analyses were performed for uraemic toxins and variables that might influence the  $C_0:D$  ratio for tacrolimus or cyclosporine. Variables with *P*-values  $> 0.10$  in the crude model were removed from the multivariate analyses. Due to the collinearity of the uraemic toxins tested, separate multivariate logistic regression models were built for each uraemic toxin with *P*  $> 0.10$  in the crude model. Confounders included age, sex and BMI. As all uraemic toxins have a high correlation with eGFR, they are considered as a proxy of eGFR. As eGFR is in the causal

pathway of the association between uraemic toxins and  $C_0:D$ , it is not a confounding factor and multivariate models were not adjusted for eGFR.

Statistical analyses were performed with SPSS for Windows version 18.0 (SPSS, Chicago, IL, USA) and R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

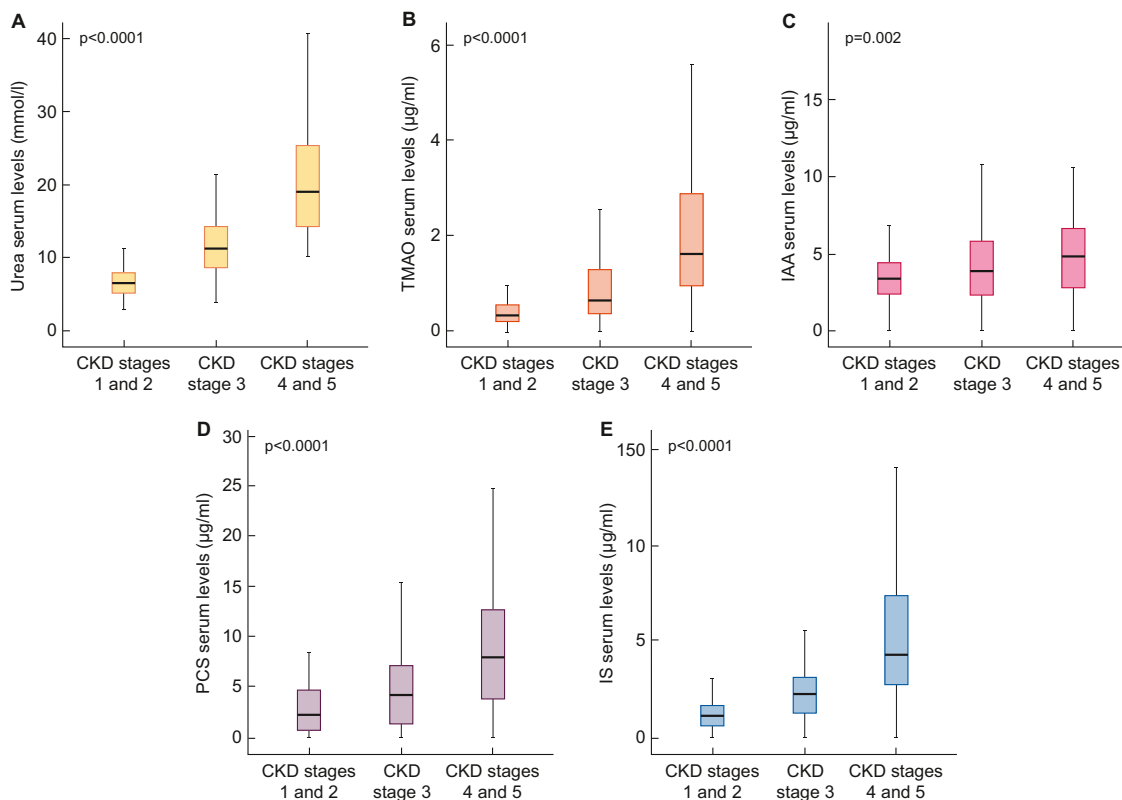
### Baseline characteristics

A total of 403 patients (250 men) were included in the study. The median age was 56 years (IQR 48–66). Hypertension was present in 97.2% of the study population and only one patient had liver failure. The time interval between kidney

**Table 1. Baseline characteristics of the study population**

Variables	All patients ( <i>N</i> = 403)	Baseline eGFR (MDRD equation; mL/min/1.73 m <sup>2</sup> )		P-value
		eGFR > 40 ( <i>n</i> = 203)	eGFR ≤ 40 ( <i>n</i> = 200)	
<b>Demographic characteristics</b>				
Males, <i>n</i> (%)	250 (62.0)	142 (69.9)	108 (54.0)	0.001
Age (years), median (IQR)	56.0 (48.0–66.0)	54.00 (44.5–64.0)	59.0 (50.8–90.1)	0.0001
BMI (kg/m <sup>2</sup> ), median (IQR)	26.5 (23.4–29.7)	26.3 (23.0–29.4)	26.5 (23.7–30.4)	0.153
<b>Clinical characteristics</b>				
CKD stage, <i>n</i> (%)				
1	13 (3.2)	13 (6.4)		–
2	68 (16.9)	68 (33.5)		–
3	228 (56.5)	122 (60.1)	106 (53.0)	–
4	84 (20.8)		84 (42.0)	–
5	10 (2.5)		10 (5.0)	–
Cause of CKD, <i>n</i> (%)				
Diabetes	20 (4.9)	12 (5.9)	8 (4.0)	0.840
Vascular disorder	26 (6.5)	10 (4.9)	16 (8.0)	
Chronic glomerulonephritis	38 (9.4)	16 (7.9)	22 (11.0)	
Polycystic kidney disease	63 (15.4)	31 (15.3)	31 (15.5)	
Interstitial nephritis	5 (1.2)	2 (1.0)	3 (1.5)	
Autoimmune disease	72 (17.9)	36 (17.7)	36 (18.0)	
Genetic cause	45 (11.2)	24 (11.8)	21 (10.5)	
Other causes	106 (26.3)	57 (28.1)	49 (24.5)	
Several causes	29 (7.2)	15 (7.4)	14 (7.0)	
Time since transplantation (months), median (IQR)	78.0 (40.0–158.5)	65.0 (32.5–138.0)	85.0 (48.0–179.2)	0.003
Transplantations, <i>n</i> (%)				
1	359 (89.1)	177 (87.2)	182 (91.0)	0.312
2	41 (10.2)	25 (12.3)	16 (8.0)	
3	2 (0.5)	1 (0.5)	1 (0.5)	
4	1 (0.2)	0 (0.0)	1 (0.5)	
SBP (mmHg), median (IQR)	142 (132–158)	140 (131–154)	144 (132–160)	0.160
DBP (mmHg), median (IQR)	80 (72–87)	80 (74–85)	80 (70.0–90)	0.720
PP (mmHg), median (IQR)	64 (54–75)	62 (53–70)	66 (54–79)	0.051
Hypertension, <i>n</i> (%)	392 (97)	195 (96)	196 (98)	0.381
Liver disease, <i>n</i> (%)	1 (0.3)	0 (0.0)	1 (0.5)	0.496
<b>Blood laboratory data, median (IQR)</b>				
Calcium (mmol/L)	2.41 (2.32–2.49)	2.43 (2.35–2.49)	2.39 (2.30–2.48)	0.034
Phosphate (mmol/L)	1.05 (0.91–1.20)	0.99 (0.87–1.09)	1.14 (0.99–1.30)	<0.001
Protein (g/L)	67 (64–70)	68 (65–71)	66 (63–69)	<0.001
Albumin (g/L)	38.9 (36.7–40.9)	39.4 (38.3–41.5)	37.5 (35.9–40.0)	0.009
Glucose (mmol/L)	5.4 (4.8–6.3)	5.3 (4.8–6.1)	5.5 (4.8–6.4)	0.583
AST (IU/L)	16 (13–21)	17.00 (13–22)	15 (12–19)	0.073
ALT (IU/L)	20 (15–27)	23 (16–29)	19 (14–24)	0.095
Creatinine (μmol/L)	146 (116–187)	116 (97–115)	187 (163–229)	<0.0001
eGFR (MDRD; mL/min/1.73 m <sup>2</sup> )	41 (30–57)	57 (49–65)	30 (23–35)	<0.0001
Uric acid (μmol/L)	471 (390–565)	446 (378–523)	509 (405–598)	0.0003
CRP (mg/L)	3.7 (0.6–9.0)	2.1 (0.6–5.5)	5.4 (0.6–13.5)	0.012
<b>Uraemic toxins, median (IQR)</b>				
Urea (mmol/L)	11.30 (8.20–15.65)	8.3 (6.45–9.80)	15.45 (12.07–20.60)	<0.0001
TMAO (μg/mL)	0.71 (0.35–1.54)	0.42 (0.24–0.93)	1.17 (0.58–1.98)	<0.0001
IAA (μg/mL)	0.39 (0.25–0.57)	0.35 (0.21–0.48)	0.45 (0.28–0.62)	<0.0001
PCS (μg/mL)	4.32 (1.52–7.76)	3.02 (1.10–5.65)	5.80 (2.88–10.63)	<0.0001
IxS (μg/mL)	2.22 (1.22–3.43)	1.39 (0.86–2.21)	3.11 (2.24–5.45)	<0.0001

DBP, diastolic blood pressure; PP, pulse pressure; SBP, systolic blood pressure; MDRD, Modification of Diet in Renal Disease.



**FIGURE 1:** Plasma levels of uraemic toxins by CKD stage: (A) urea, (B) TMAO, (C) IAA, (D) PCS and (E) IxS.

transplantation and study inclusion ranged from 1 to 40 years, with a median of 6.5 years. The median eGFR was 41 mL/min/1.73 m<sup>2</sup> (IQR 30–57) and the majority of the patients (56.5%) were at CKD Stage 3 (Table 1).

The group of patients with an eGFR <40 mL/min/1.73 m<sup>2</sup> was significantly older and had a higher median time since transplantation and a higher proportion of women when compared with the group with an eGFR >40 mL/min/1.73 m<sup>2</sup>. When considering the laboratory data, patients with an eGFR <40 mL/min/1.73 m<sup>2</sup> had higher plasma levels of phosphate, uric acid, CRP and uraemic toxins (Table 1).

### Uraemic toxin levels

The data on plasma urea, TMAO, IAA, PCS and IxS levels are summarized by CKD stage in Figure 1 and Supplementary data, Table S2. For all the uraemic toxins studied here, the level rose progressively with the CKD stage and peaked at CKD Stages 4 and 5.

### Immunosuppressant treatment

A total of 248 patients were being treated with tacrolimus. The median dose was 4 mg/day (IQR 3–6), which corresponded to a median dose of 0.06 mg/kg/day (IQR 0.04–0.08). The median blood tacrolimus concentration was 7.2 ng/mL (IQR 5.6–8.5) and the median C<sub>0</sub>:D ratio for tacrolimus was 123.7 (IQR 81.3–177.1) (Table 2).

Among the 155 patients being treated with cyclosporine, the median dose was 150 mg/day (IQR 120–175); this corresponded to a median dose of 1.82 mg/kg/day (IQR 1.5–2.25).

**Table 2. TDM of tacrolimus and cyclosporine and data on potential drug interactions**

Variables	Values
Characteristics of calcineurin inhibitors	
Cyclosporine (n = 155)	
Plasma concentration (ng/mL), median (IQR)	94.30 (74.20–123.00)
Dose (mg/day), median (IQR)	150.00 (120.00–175.00)
Dose (mg/kg/day), median (IQR)	1.82 (1.51–2.25)
Plasma concentration (ng/mL)/dose (mg/kg/day), median (IQR)	52.89 (36.42–69.25)
Tacrolimus (n = 248)	
Plasma concentration (ng/mL), median (IQR)	7.19 (5.65–8.51)
Dose (mg/day), median (IQR)	4.00 (3.00–6.00)
Dose (mg/kg/day), median (IQR)	0.06 (0.04–0.08)
Plasma concentration (ng/mL)/dose (mg/kg/day), median (IQR)	123.70 (81.32–177.05)
Concomitant treatments, n (%)	
At least one strong CYP3A4/5 inhibitor	14 (3.23)
At least one weak CYP3A4/5 inhibitor	5 (1.24)
At least one strong CYP3A4/5 inducer	6 (1.49)
At least one weak CYP3A4/5 inducer	3 (0.74)
At least one P-gp inhibitor	87 (21.34)
At least one P-gp inducer	5 (1.24)
At least one drug that binds strongly to plasma proteins	399 (98.76)

The median blood cyclosporine concentration was 94 ng/mL (IQR 74–123) and the median C<sub>0</sub>:D ratio for cyclosporine was 52.9 (IQR 36.4–69.3) (Table 2).



## Factors associated with the C<sub>0</sub>:D ratio for tacrolimus and/or cyclosporine

**Tacrolimus.** When we dichotomized the 248 tacrolimus-treated patients according to the median C<sub>0</sub>:D ratio, patients with an above-median ratio were significantly older, more likely to be male and had a higher BMI, higher glycaemia values, higher creatininaemia values and lower eGFR relative to patients with a below-median ratio. There were no differences in the use of drugs that interact with CYP3A4/5 or P-gp or that bind strongly to plasma proteins. Patients with an above-median tacrolimus C<sub>0</sub>:D ratio presented significantly higher plasma levels of urea, PCS and IxS (but not of TMAO or IAA) (Supplementary data, Table S3). Univariate logistic analyses gave the same results and thus highlighted the association between a high tacrolimus C<sub>0</sub>:D ratio and three uraemic toxins (namely urea, PCS and IxS) (Supplementary data, Table S4).

In a multivariate analysis, age, sex and BMI were independently associated with the tacrolimus C<sub>0</sub>:D ratio. The significant association between the tacrolimus C<sub>0</sub>:D ratio and plasma IxS levels [OR 1.36 (95% CI 1.00–1.85)] was independent of sex, BMI and age (Table 3, plasma IxS levels). The association between the tacrolimus C<sub>0</sub>:D ratio and plasma urea levels was still significant after adjustment for sex and BMI [OR 2.29 (95% CI 1.28–4.09)], whereas the association was no longer significant after full adjustment for sex, BMI and age [OR 1.71 (95% CI 0.93–3.16)] (Table 3, plasma urea levels). The association between the tacrolimus C<sub>0</sub>:D ratio and plasma PCS levels was still significant after adjustment for sex and BMI [OR 1.31 (95% CI 1.06–1.62)] but not after adjustment for sex, BMI and age [OR 1.22 (95% CI 0.99–1.51)] (Table 3, plasma PCS levels).

**Cyclosporine.** When we dichotomized the 155 patients treated with cyclosporine according to the median cyclosporine C<sub>0</sub>:D ratio, patients with an above-median ratio were significantly older, more likely to be female and had a higher BMI and higher glycaemia values. There were no differences with regard to the use of drugs that interacted with CYP3A4/5, P-gp or plasma proteins. Patients with an above-median cyclosporine C<sub>0</sub>:D ratio presented significantly higher plasma levels of TMAO but not of urea, PCS, IxS or IAA (Supplementary data, Table S5). A univariate logistic analysis gave the same results and again highlighted the association between a high cyclosporine C<sub>0</sub>:D ratio and the plasma TMAO level (Supplementary data, Table S6).

In a multivariate analysis, age, sex and BMI were independently associated with the cyclosporine C<sub>0</sub>:D ratio. However, the association between the cyclosporine C<sub>0</sub>:D ratio and the plasma TMAO level was no longer significant [OR 1.12 (95% CI 0.78–1.61)] after adjustment for sex, BMI and age (Table 4).

## DISCUSSION

In the present cross-sectional study of a cohort of kidney transplant recipients, we found that elevated plasma concentrations of certain uraemic toxins were associated with elevated blood concentrations of the calcineurin inhibitors tacrolimus and cyclosporine. However, the nature of the associations differed

**Table 3.** Multivariate logistic regression analysis of factors associated with the tacrolimus C<sub>0</sub>:D ratio and considered as a continuous [log-normalized (ln)] variable (n = 248)

Variables	OR (95% CI)	P-value
<b>Plasma IxS levels</b>		
Unadjusted		
ln IxS	1.41 (1.06–1.86)	0.018
Model 1, including ln IxS and sex		
ln IxS	1.43 (1.07–1.91)	0.015
Sex	1.83 (1.07–3.12)	0.027
Model 2, including ln IxS, sex and BMI		
ln IxS	1.44 (1.28–4.09)	0.017
Sex	1.85 (1.11–3.35)	0.029
BMI	1.12 (1.06–1.19)	<0.0001
Model 3, including ln IxS, sex, BMI and age		
ln IxS	1.36 (1.00–1.85)	0.047
Sex	2.03 (1.15–3.60)	0.015
BMI	1.11 (1.04–1.18)	0.001
Age	1.04 (1.02–1.06)	<0.0001
<b>Plasma urea levels</b>		
Unadjusted		
ln urea	2.08 (1.21–3.6)	0.008
Model 1, including ln urea, sex		
ln urea	2.24 (1.28–3.92)	0.005
Sex	1.89 (1.11–3.24)	0.019
Model 2, including ln urea, sex and BMI		
ln urea	2.29 (1.28–4.09)	0.005
Sex	1.93 (1.11–3.35)	0.020
BMI	1.12 (1.06–1.19)	<0.0001
Model 3, including ln urea, sex, BMI and age		
ln urea	1.71 (0.93–3.16)	0.085
Sex	2.04 (1.16–3.60)	0.014
BMI	1.11 (1.05–1.18)	0.001
Age	1.04 (1.01–1.06)	0.001
<b>Plasma PCS levels</b>		
Unadjusted		
ln PCS	1.27 (1.04–1.54)	0.018
Model 1 including ln PCS and sex		
ln PCS	1.27 (1.04–1.56)	0.017
Sex	1.88 (1.10–3.20)	0.021
Model 2 including ln PCS, sex and BMI		
ln PCS	1.31 (1.06–1.62)	0.011
Sex	1.93 (1.11–3.37)	0.020
BMI	1.13 (1.06–1.20)	<0.0001
Model 3 including ln PCS, sex, BMI and age		
ln PCS	1.22 (0.99–1.51)	0.067
Sex	2.07 (1.17–3.67)	0.012
BMI	1.11 (1.05–1.18)	0.001
Age	1.04 (1.02–1.06)	0.001

between tacrolimus and cyclosporine; indeed, both drugs have the same pharmacological target but bind strongly to different plasma proteins.

We first studied plasma levels of five notable uraemic toxins (IAA, IxS, PCS, TMAO and urea) in a cohort of patients in whom the time since transplantation ranged from 1 to 40 years. In fact, most studies of uraemic toxins

**Table 4. Multivariate logistic regression analysis of factors associated with the cyclosporine C<sub>0</sub>:D ratio, with plasma TMAO levels entered as a continuous [log-normalized (ln)] variable (n = 155)**

	OR (95% CI)	P-value
Unadjusted		
ln TMAO	1.37 (1.00–1.87)	0.046
Model 1, including		
ln TMAO and sex		
ln TMAO	1.39 (1.01–1.92)	0.042
Sex	0.42 (0.21–0.82)	0.012
Model 2, including ln TMAO, sex and BMI		
ln TMAO	1.26 (0.90–1.76)	0.182
Sex	0.40 (0.19–0.83)	0.014
BMI	1.18 (1.09–1.27)	<0.0001
Model 3, including ln TMAO, sex, BMI and age		
ln TMAO	1.12 (0.78–1.61)	0.540
Sex	0.33 (0.15–0.72)	0.005
BMI	1.20 (1.10–1.30)	<0.0001
Age	1.06 (1.02–1.09)	0.002

have focused on pre-dialysis and dialysis patients rather than kidney transplant recipients [27, 28]. Hence the present report extends the data on a panel of important uraemic toxins in a large number of patients at variable times since transplantation. We confirmed that uraemic toxin levels were significantly higher in patients with lower eGFRs than in patients with higher eGFRs [28]. However, the uraemic toxin levels were lower than those described in dialysis patients, reflecting the kidney's functional activity after transplantation. The broad range of uraemic toxin levels enabled us to evaluate the latter's association with the blood concentration of calcineurin inhibitors.

Many studies have demonstrated that CKD not only decreases renal drug clearance, but also modifies drug absorption, distribution and non-renal clearance [6]. Uraemic toxins accumulate as renal function deteriorates and have been linked to adverse outcomes [13, 29, 30]. These toxins might also affect drug pharmacokinetics [6]. As immunosuppressive drugs require TDM, kidney transplant recipients constitute a good model for studying the potential impact of uraemic toxins on blood levels of calcineurin inhibitors, especially since these drugs are also cleared through non-renal pathways.

In this study we found that elevated plasma levels of IxS and PCS (both of these toxins bind strongly to albumin) were associated with a higher tacrolimus C<sub>0</sub>:D ratio (98.8% of which is bound to albumin and alpha-1-acid glycoprotein *in vivo* [20]). These associations were independent of other factors that can modify immunosuppressant concentrations, such as the co-prescription of CYP3A4/5 or P-gp inducers or inhibitors, albuminaemia, liver disorders, sex, BMI and age for IxS. However, adjustment for age weakened the association with PCS. Indeed, IxS and PCS bind with high affinity to albumin, specifically with Sudlow site II [31], and might compete directly with other substrates for binding to albumin [31, 32]. Hence we hypothesize that IxS and PCS compete with tacrolimus and

lead to an increase in the C<sub>0</sub>:D ratio. However, it is hard to say whether the putative effect of IxS and PCS on the C<sub>0</sub>:D ratio is a specific effect or, in contrast, is indirectly linked to the deterioration in renal function. The mechanisms underlying uraemic toxin accumulation and the extent to which uraemic toxin accumulation may or not enhance the renal toxicity of tacrolimus require further study.

On the same lines, elevated plasma urea levels were independently associated with a higher tacrolimus C<sub>0</sub>:D ratio; however, adjustment for age weakened this association. Indeed, it is well known that age-related physiological changes induce changes in drug distribution, metabolism and elimination [33]. Furthermore, a chronic increase in the urea level is significantly associated with a higher mean protein carbamylation in animals [15] and humans [14]. Although carbamylation is a post-translational modification of some proteins, urea-derived isocyanic acid can react non-enzymatically with proteins and this induces structural and functional modifications. A high degree of albumin carbamylation might decrease the protein's binding of some drugs [34].

In contrast to results obtained in patients treated with tacrolimus, we failed to find an association between IxS, PCS and urea levels and the cyclosporine C<sub>0</sub>:D ratio. This lack of an association might be due to lower accumulation of uraemic toxins in patients treated with cyclosporine or to the fact that, unlike tacrolimus (which binds to albumin), cyclosporine binds strongly to high-density lipoprotein (HDL) and low-density lipoprotein [20]. We also found that elevated TMAO levels were associated with an elevated cyclosporine C<sub>0</sub>:D ratio. This association was no longer significant after adjustment for age, sex and BMI. TMAO is a small, water-soluble, gut-derived uraemic toxin produced by the oxidation of trimethylamine. Over the last decade, a growing body of pre-clinical and clinical evidence has identified TMAO as an important contributor to the pathogenesis of cardiovascular disease [35]. Indeed, elevated TMAO levels are associated with a greater incident risk of major cardiovascular adverse events [10, 36]. We are not aware of data linking TMAO to changes in drug pharmacokinetics/pharmacodynamics. However, pre-clinical data have shown an inverse association between TMAO and HDL, with a reduction in HDL-mediated reverse cholesterol transport as TMAO levels rise [37].

Maintaining levels of immunosuppressant within the therapeutic range is essential for preventing acute graft rejections while minimizing drug-related toxicity. In the present analysis, patients with a higher tacrolimus C<sub>0</sub>:D ratio presented higher glycaemia, blood pressure (BP) and creatinaemia values, all of which are well-known adverse drug reactions associated with calcineurin inhibitors. Even though TDM and dose adjustment can maintain tacrolimus levels within the therapeutic window, it appears that the accumulation of uraemic toxins (PCS, IxS and urea) can increase exposure to tacrolimus. This finding has potential implications for other drugs that bind strongly to albumin, since a larger free fraction might lead to unexpected exposures, which can alter their efficacy and/or tolerance. This finding emphasizes the need for more research on how to decrease uraemic toxin levels.

In the present report we focused on two drugs with similar pharmacokinetic and pharmacodynamic properties. Both drugs have low bioavailability, bind strongly to plasma proteins, are mainly metabolized by cytochrome P450 3A4/5 and are mainly excreted in the bile [20]. The main difference relates to the type of plasma protein to which they bind: albumin for tacrolimus and lipoproteins for cyclosporine. We hypothesize that our present results can be explained (at least in part) by this difference in binding. Furthermore, we suggest that some uraemic toxins might modify the tacrolimus  $C_0:D$  ratio by affecting its binding to albumin. Although protein binding is a major determinant of drug activity, the latter is influenced by many other factors. For example, uraemic toxins such as parathyroid hormone can induce the downregulation of cytochrome P450 or modify the activity of certain drug transporters [38, 39]. Machado *et al.* [21] recently suggested that IxS increases the expression and activity of the efflux transporter P-gp and that this effect depends on the aryl hydrocarbon receptor pathway. In addition, IxS and PCS are substrates of the membrane transporters OATs, which are expressed in the kidneys but also in other tissues, such as the liver and the small intestine. These transporters play a central role in the cell uptake and clearance of gut microbiota metabolites, including IxS and PCS [40]. Although tacrolimus does not appear to have an impact on the *in vitro* activity of OAT1 and OAT3 [41], an interaction between tacrolimus and the transporters involving uraemic toxins is not excluded and deserves further investigation. All of these factors may influence drug pharmacokinetics and blood concentration. Consequently it is very difficult to identify specific molecular mechanisms in patients.

Our study had some limitations. The cross-sectional design prevented us from evaluating possible changes over time in adverse drug reactions and outcomes related to concentrations of uraemic toxins and immunosuppressants. Even though we adjusted our multivariate analyses, it is possible that the association between uraemic toxins and the immunosuppressant concentration was due to other confounders. Likewise, we adjusted for potential drug interactions but cannot rule out potential interactions related to the patients' polypharmacy. Lastly, and as mentioned above, the large number of factors influencing drug pharmacokinetics means that it is difficult to prove the presence of an interaction between certain uraemic toxins and tacrolimus. However, this study also had strengths, such as its use of sensitive, validated assays of five uraemic toxins known to have harmful effects in kidney transplant recipients [25]. It is one of the first studies to have simultaneously evaluated blood uraemic toxin levels and drug concentrations in a clinical setting.

## CONCLUSION

In a cross-sectional study of 403 kidney transplant recipients, we observed an association between plasma levels of certain uraemic toxins and the blood levels of two calcineurin inhibitors. In particular, we observed a relationship between elevated plasma PCS, IxS and urea levels and elevated blood tacrolimus  $C_0:D$  ratios, suggesting a potential interaction through binding sites on albumin. The potential clinical impact of this

relationship must now be evaluated prospectively for tacrolimus and other drugs that bind strongly to albumin.

## SUPPLEMENTARY DATA

Supplementary data are available at [ndt online](http://ndt.online).

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## CONFLICT OF INTEREST STATEMENT

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(See related article by Koppe and Soulage. Protein-bound uremic toxins: putative modulators of calcineurin inhibitor exposure. *Nephrol Dial Transplant* 2022; 37: 2044–2047)

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