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Evolution of protein-bound uremic toxins indoxyl sulphate and p-cresyl sulphate in acute kidney injury

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Abstract

Background There is a gradual increase in serum concentrations of protein-bound colon-derived uremic toxins indoxyl sulphate (IxS) and p-cresyl sulphate (pCS) as chronic kidney disease (CKD) progresses. In acute kidney injury (AKI), up till now, the retention pattern has not been studied.

Methods In this study, 194 adult patients admitted with sepsis to the intensive care unit were included. IxS, pCS and serum creatinine (sCrea) were quantified at inclusion ($D0$) and at day 4, unless follow-up ended earlier (D_{end}).

Results Serum levels of sCrea ($P<0.001$), IxS ($P<0.001$) and pCS ($P<0.05$) were higher in patients with AKI according to RIFLE classification at $D0$. In contrast with sCrea, IxS and pCS levels only increased from stage I (IxS) and F (pCS) on. When grouped according to evolution in RIFLE class from $D0$ to D_{end}, all solute concentrations were higher ($P<0.001$) in the group with unfavourable evolution. In this group, there was a marked rise in sCrea ($P<0.001$), a moderate one for pCS ($P<0.05$), but no change for IxS ($P=0.112$). There was a decrease ($P<0.001$) of all solute concentrations in the group with favourable evolution. Comparing AKI with CKD patients matched for sCrea, total levels of both IxS and pCS were higher ($P<0.01$) in patients with CKD.

Conclusions Although concentrations of IxS and pCS both tend to rise in sepsis patients with AKI, their evolution does not conform with that of sCrea. For the same level of sCrea, IxS and pCS concentrations are lower in AKI compared with CKD.

Keywords AKI · Sepsis · Uremic toxins · Indoxyl sulphate · p-Cresyl sulphate

Introduction

Uremic retention, partially responsible for the uremic syndrome [1], increases as kidney function declines [1]. Although a typical feature of both chronic kidney disease (CKD) and acute kidney injury (AKI) [1], retention patterns have rarely been studied in AKI [2].

Uremic solute retention affects a heterogeneous group of organic waste products [1, 3, 4]. Although disregarded in early years of uremic toxin research, protein-bound uremic retention molecules originating from gut microbial metabolism progressively attracted more attention [3, 4]. Indoxyl sulphate (IxS) and p-cresyl sulphate (pCS) are the two most investigated protein-bound colon-derived uremic solutes [5–7]. Experimental data suggest a relation between IxS and pCS levels and cardiovascular morbidity and mortality [5, 6, 8–11], skeletal toxicity [12] and renal function decline [5, 8, 11, 13].

Griet Glorieux and Raymond Vanholder: equally contributing senior authors.

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In CKD, gut dysbiosis favours the generation of p-cresol and indole, precursors of pCS and IxS [4]. There is a gradual increase in IxS and pCS levels as CKD stages worsen [11, 13]. Generation [14–16] and individual toxicity of both IxS and pCS have been the subject of several reviews [7, 17, 18]. Retention patterns might be different in AKI vs. CKD for several reasons: different kinetics and distribution, changed gut microbial metabolism or differences in renal handling of waste products, but data are currently lacking.

Therefore, we conducted the present study in patients hospitalised with sepsis at the intensive care unit (ICU), with and without AKI, and evaluated total concentrations of IxS and pCS and their relation to serum creatinine (sCrea). The relationship between evolution of sCrea, IxS and pCS in proportion to the kidney function deterioration was examined. Finally, levels of total uremic solute concentrations in AKI were compared with a cohort of CKD patients with similar sCrea levels.

Materials and methods

Consecutive adults admitted with sepsis between 12/01/2010 and 27/03/2011 to the ICU of the Ghent University Hospital (UZGent, Belgium), were eligible for inclusion. Sepsis, severe sepsis and septic shock were defined according to the ACCP/SCCM Consensus Conference guidelines [19] (study design before publication of the 2016 Sepsis-3 consensus definitions [20]). Exclusion criteria were (1) ICU stay < 24 h or withdrawal of therapy; (2) no indwelling bladder catheter; (3) chronic hemodialysis; (4) need for renal replacement therapy (RRT) upon admission; (5) age < 17 years; (6) history of transplantation; (7) postrenal AKI and (8) no central line or arterial catheter. Patients who developed sepsis during their ICU stay were excluded.

During the study period, 253 patients were considered, of whom 59 were excluded [18 no bladder catheter, 13 RRT need, 10 history of transplantation, 7 withdrawal of therapy, 5 chronic hemodialysis, 3 ICU discharge before blood sampling, 1 obstructive AKI, 1 no central line and 1 HIV positive patient (imposing ethical concerns for sample handling)], leaving 194 patients for analysis.

All patients were started or continued on antibiotics at ICU admission. Fluid and medical management and need of RRT decisions were done by intensive care physicians blinded to the study, according to local protocols. The study was approved by the ethical committee of the UZGent. Written informed consent was obtained from the patients or their next of kin.

Blood samples were collected at the moment of inclusion (*D*0), 4 h thereafter (*D*0T4) and daily at 6 AM for the next 4 days (*D*1 to *D*4). All patients admitted between 6 AM and 18 PM were included at the day of admission. Patients admitted after 18 PM were included the following day at 6 AM (as described previously [21]). Blood samples were analysed in batch for quantification of uremic solutes. This study used only the samples at inclusion, *D*0 and *D*4 unless follow-up ended before *D*4 because of death, start of RRT, discharge from the ICU or refusal of further blood sampling. In the latter cases, the results of the last sample collected before drop-out were used. Accordingly, we defined D_{end} as the final sample used, which was *D*4 ($n = 155$; 80%), *D*3 ($n = 10$; 5%), *D*2 ($n = 6$; 3%), *D*1 ($n = 14$; 7%); when these exceptions occurred in the first 24 h the sample collected 4 h after inclusion (*D*0T4) ($n = 9$; 5%) served as D_{end}.

After centrifugation, serum was aliquoted and frozen at − 80 °C until batch analysis. Samples from *D*0 and D_{end} were assessed for sCrea, total IxS and pCS levels. sCrea was measured in the on-site biochemistry laboratory using an enzymatic modified Jaffe method (Roche, Switzerland). For ultra-performance liquid chromatography quantification of total IxS and pCS, samples were prepared and analysed as described previously [22]. Uremic solute concentration at *D*0 was either not available or not quantifiable in five patients [sCrea ($n = 2$), IxS ($n = 1$) and both IxS and pCS ($n = 2$)], and at D_{end} in ten patients [sCrea, $n = 7$, or IxS and pCS, ($n = 3$)]. In three other patients (IxS at *D*0 $n = 1$; and pCS at D_{end} $n = 2$), a signal was retrieved that remained below limit of detection (LOD) and the LOD divided by the square root of 2 was used for statistical analysis [23].

The percentage evolution of solute levels was calculated as the % difference (Δ%) of medians at D_{end} and *D*0, divided by the median level at *D*0.

AKI and its severity were defined according to the RIFLE classification [24]. As baseline creatinine, the most recent value before admission was used ($n = 188$), or backward calculation [24] was applied if the latter was not available ($n = 6$). Urinary output was assessed at 6-h blocks [25]. AKI was defined according to RIFLE at *D*0.

In a sub-analysis, patients were grouped according to the evolution of AKI class from *D*0 to D_{end}. A *favourable* evolution was defined as a decrease of at least one RIFLE stage(s) from *D*0 to D_{end}, or when patients had no AKI for the full observation period. All other patients were defined as having an *unfavourable* evolution.

Finally, we compared the concentration of IxS and pCS in the patients with unfavourable renal evolution as defined above, with exclusion of patients with CKD eGFR < 60 mL/min/1.73 m^2 at admission. We matched these patients to CKD patients with a sCrea value in the same range (± 0.1 mg/dL). The CKD patients were part of a cohort of 95 CKD outpatients from the Nephrology Department of Amiens University Hospital (France) [11]. The patients were at different stages of CKD (including 11 in stage 2, 37 in stage 3, 37 in stage 4 and 10 in stage 5). Thirty-two matched pairs were obtained. The concentrations of IxS and pCS in

this population were determined by the same method and in the same laboratory as the AKI samples.

Statistical analysis

Results are reported as medians with 25th and 75th percentile, unless otherwise specified. Mann–Whitney *U* test was used to compare the medians of unpaired non-normally distributed continuous variables. Wilcoxon signed-rank test was used to compare the medians of paired non-normally distributed continuous variables. Dichotomous variables were compared using χ^2 analysis.

Because of the non-Gaussian distribution of sCrea, IxS and pCS, logarithmic normalised values were used to calculate correlations. For IxS and pCS, we used $\log(x+10^{-7}$ mg/dL) if no ULPC signal could be retrieved (IxS *D*0 $n=15$, IxS D_{end} $n=33$; pCS *D*0 $n=16$, pCS D_{end} $n=16$) to replace the zero value of which the logarithm is undefined. To compute whether the differences between two correlation coefficients were significant, we used Fisher *r*-to-*z* transformation.

In all tests, a P value <0.05 was considered significant. All statistical analyses were performed using SPSS software (SPSS Inc, Chicago, IL, USA), version 24.

Results

Patient characteristics and solute concentrations

In this study, 194 patients with sepsis, severe sepsis and septic shock were included. Table 1 shows their demographic, clinical and biochemical characteristics. 9 (5%), 63 (32%) and 122 (63%) had sepsis, severe sepsis and septic shock, respectively. Overall mortality at the ICU and at 3 months after inclusion was 23% and 31%. 26 patients (13%) needed RRT during their ICU stay. 22 patients (12%) had CKD stage 3 and 4 (CKD-EPI) at ICU admission.

As also shown in Table 1, patients with AKI according to RIFLE classification (Risk, Injury, Failure, Loss of kidney function and End-stage renal disease) [24] at *D*0 ($n=130$)

Table 1 Demographic, clinical and biological characteristics of the study population, classified according to RIFLE

	Total population	Non-AKI *D*0	AKI *D*0	*P* value AKI vs. non-AKI
N (%)	194 (100)	64 (33)	130 (67)	
Age [years, mean (SD)]	61.5 (15.0)	58.7 (15.7)	62.9 (14.4)	0.105
Gender (% male)	62.4	60.9	63.1	0.772
Sepsis severity [*n*(%)]				
Sepsis	9 (5)	2 (3)	7 (5)	
Severe sepsis	63 (32)	27 (42)	36 (28)	
Septic shock	122 (63)	35 (55)	87 (67)	
ICU mortality [*n*(%)]	45 (23)	14 (22)	31 (24)	0.760
90 days mortality [*n*(%)]	61 (31)	20 (31)	41 (32)	0.968
Highest CRP first day of admission (mg/L)	198.5 (113.8–312.3)	169.0 (110.0–255.8)	222.5 (123.5–328.5)	0.020
APACHE II first 24 h ICU	23 (17–27)	22 (16–26)	23 (19–28)	0.420
Vasopressor need first 24 h ICU [*n*(%)]	117 (60)	31 (48)	86 (66)	0.018
Positive fluid balance first 24 h ICU (L, mean/SD)	3.4 (1.5)	2.6 (2.0)	3.6 (2.4)	0.003
Use of diuretics first 24 h ICU [*n*(%)]	28 (14)	9 (14)	19 (15)	0.918
Need for ventilation during ICU stay [*n*(%)]	105 (54)	32 (50)	73 (56)	0.419
RRT during ICU stay [*n*(%)]	26 (13)	3 (5)	23 (18)	0.012
Historical baseline CKD (eGFR < 60 mL/min/1.73 m^2) [*n*(%)]	22 (12)	5 (8)	17 (13)	0.670
CKD 3A [*n*(%)]	15 (8)	3 (5)	12 (9)	
CKD 3B [*n*(%)]	5 (3)	2 (3)	3 (2)	
CKD 4 [*n*(%)]	2 (1)	0 (0)	2 (2)	
Historical baseline eGFR (mL/min/1.73 m^2)	79.6 (71.5–91.9)	81.9 (71.7–93.5)	79.1 (71.1–90.4)	0.328
Historical baseline sCrea (mg/dL)	0.89 (0.73–1.04)	0.90 (0.76–1.01)	0.88 (0.71–1.08)	0.834

Unless specified otherwise (brackets), data are expressed as median (25th and 75th percentile)

AKI: Acute Kidney Injury according to RIFLE on *D*0 (RIFLE R + I + F), non-AKI: RIFLE—on *D*0. RIFLE: Risk, Injury, Failure, Loss of kidney function, End-stage renal disease classification; *D*0: day of inclusion; APACHE II Acute physiology and chronic health evaluation II score, first day of admission; CKD: chronic kidney disease; eGFR: estimated glomerular filtration rate (according to the CKD-EPI equation); ICU: Intensive Care Unit; RRT: Renal Replacement Therapy; CRP: C-reactive protein; sCrea: serum creatinine

Table 2 Evolution of uremic solute concentration, at inclusion (*D*0) vs. end (day 4 or drop-out), grouped by RIFLE as defined at *D*0

RIFLE UO + C	*N* *D*0	sCrea *D*0 (mg/dL)	sCrea D_{end} (mg/dL)	*P*	Δ%	IxS *D*0 (µg/dL)	IxS D_{end} (µg/dL)	*P*	Δ%	pCS *D*0 (µg/dL)	pCS D_{end} (µg/dL)	*P*	Δ%
Non-AKI	64	0.73 (0.52–0.89)	0.64 (0.48–0.99)	0.201	− 12	25.8 (9.7–61.0)	18.5 (1.0–51.5)	0.444	− 28	151.7 (27.4–308.1)	91.2 (23.5–350.3)	0.222	− 40
AKI	130	1.20[aa] (0.79–2.12)	0.87[aa] (0.60–1.80)	< 0.001	− 28	64.0[aa] (25.2–180.2)	29.6 (4.1–90.8)	< 0.001	− 54	250.0[a] (64.3–593.3)	105.1 (27.1–340.5)	< 0.001	− 58
R	40	0.94[a] (0.66–1.20)	0.63 (0.46–0.87)	< 0.001	− 33	37.7 (23.1–90.8)	10.0 (0.0–37.9)	< 0.01	− 73	212.9 (61.8–579.3)	51.4 (16.6–231.8)	< 0.001	− 76
I	57	1.11[aa,b] (0.74–1.60)	0.91[aa,bb] (0.63–1.65)	0.158	− 18	50.0[a] (16.9–171.6)	29.7[b] (6.1–81.1)b	< 0.05	− 41	202.1 (55.4–591.0)	111.3 (27.5–303.2)	< 0.05	− 45
F	33	2.46[aa,bb,cc] (1.89–3.30)	2.16[aa,bb,cc] (0.85–3.09)	0.059	− 12	178.5[aa,bb,cc] (76.2–340.0)	79.8[aa,bb,cc] (23.4–269.3)	0.085	− 55	470.2[aa] (81.9–989.7)	193.6[a,bb] (54.5–653.9)	0.367	− 59

Data are expressed as median (25th and 75th percentile); *D*0: day of inclusion; D_{end}: day 4 or day before drop-out; Δ%: percentage difference of medians D_{end} vs. *D*0; IxS: total Indoxyl sulphate; pCS: total p-Cresyl sulphate; RIFLE: Risk, Injury, Failure, Loss of kidney function, End-stage renal disease classification; UO + C: Rifle based on both serum creatinine and urinary output criteria; RIFLE −: no acute kidney injury (AKI); RIFLE +: AKI RIFLE R + I + F

[a] $P < 0.05$ vs. RIFLE −

[aa] $P < 0.01$ vs. RIFLE −

[b] $P < 0.05$ vs. RIFLE R

[bb] $P < 0.01$ vs. RIFLE R

[c] $P < 0.05$ vs. RIFLE I

[cc] $P < 0.01$ vs. RIFLE I

Table 3 Correlations between log-normalised sCrea, IxS and pCS levels

	IxS		pCS	
	r	*P*	*r*	*P*
*D*0				
sCrea	0.526[a]	< 0.001	0.277	< 0.001
pCS	0.571[a]	< 0.001		
D_{end}				
sCrea	0.441	< 0.001	0.330	< 0.001
pCS	0.612[a,b]	< 0.001		
	ΔIxS		ΔpCS	
	r	*P*	*r*	*P*
$D0 - D_{end}$				
ΔsCrea	0.495	< 0.001	0.456	< 0.001
ΔpCS	0.541	< 0.001		

*D*0: day of inclusion; D_{end}: day 4 or day before drop-out; *r*: Spearman's rho; IxS: log-normalised total Indoxyl sulphate; pCS: log-normalised total p-Cresyl sulphate; Δ value log-normalised difference ($D_{end} - D0$)

[a] $P < 0.01$ vs. *r* (pCS − sCrea)

[b] $P < 0.05$ vs. *r* (IxS − sCrea)

had a comparable historical baseline serum creatinine and eGFR (estimated glomerular filtration rate) as patients without AKI ($n = 64$) (P = NS). Patients with AKI had higher C-reactive protein at admission ($P = 0.020$), more vasopressor need in the first 24 h ($P = 0.018$) and a more positive fluid balance ($P = 0.003$).

When considering the total study population, total solute concentrations were higher at *D*0 vs. D_{end}: sCrea: 0.96 [0.67–1.60] mg/dL vs. 0.76 [0.56–1.46] mg/dL; IxS: 47.9 [17.1–147.2] µg/dL vs. 25.0 [3.4–86.6] µg/dL; pCS: 194.6 [44.9–539.0] µg/dL vs. 98.8 [27.0–346.0] µg/dL, $P < 0.001$. The percentage decrease was more than twice as important for IxS (−48%) and pCS (−49%) than for sCrea (−21%).

Evolution of IxS and pCS vs. sCrea

Solute concentrations according to RIFLE at *D*0

In 33% of patients, no AKI was present at *D*0, while 21%, 29% and 17% had RIFLE R, I or F. Table 2 shows that at *D*0, serum levels of sCrea ($P < 0.01$), IxS ($P < 0.01$) and pCS ($P < 0.05$) were higher in the AKI compared to the non-AKI group. While in the non-AKI group levels remained unchanged over time, a decrease of all solutes was observed in the AKI group ($P < 0.001$). At D_{end}, only serum levels of sCrea ($P < 0.01$), but not those of IxS and pCS remained higher in AKI vs. non-AKI.

When subdividing AKI into R, I and F stages at *D*0, a stepwise increase in the levels of sCrea was observed at each stage compared to non-AKI. In contrast, IxS and pCS levels only increased from RIFLE stage I (IxS) and F (pCS) on. From *D*0 to D_{end}, levels of IxS and pCS decreased in patients with both RIFLE R and I, whereas for sCrea this was only the case in patients with RIFLE R ($P < 0.001$). For none of the measured uremic toxins, a significant change was observed in the patients with RIFLE F (P = NS).

For pCS as for IxS, the percentage decrease (Δ%), D_{end} vs. *D*0, was more substantial than that for sCrea.

To avoid bias, the nine patients whose D_{end} data had been collected at *D*0T4 were included in the analyses; however, exclusion did not alter the results (data not shown).

In summary, although uremic solute concentration tends to increase for all stages of AKI, this was observed in later RIFLE stages for pCS and IxS than for sCrea. In general, the relative concentration decrease of pCS and IxS at D_{end} vs. *D*0 tends to be more substantial than for sCrea.

Correlation analyses

Correlations between log-normalised sCrea, IxS and pCS levels are summarised in Table 3. Both at *D*0 and D_{end}, total IxS and pCS levels were positively correlated with each other and with sCrea levels. At *D*0 correlations for both IxS vs. sCrea and IxS vs. pCS were stronger than for pCS vs. sCrea. At D_{end} the correlation between IxS and pCS was stronger than for both pCS and IxS vs. sCrea.

Further analysis revealed a positive correlation between evolution from *D*0 to D_{end} (Δ-values) for all solutes, without any differences.

Table 4 Uremic solute concentration at inclusion (*D*0) and end (day 4 or drop-out), grouped by RIFLE evolution

	N	sCrea *D*0 (mg/dL)	sCrea D_{end} (mg/dL)	*P*	Δ%	IxS *D*0 (μg/dL)	IxS D_{end} (μg/dL)	*P*	Δ%	pCS *D*0 (μg/dL)	pCS D_{end} (μg/dL)	*P*	Δ%
RIFLE favourable	147	0.89 (0.62–1.29)	0.68 (0.53–0.92)	< 0.001	− 24	40.2 (17.2–106.9)	17.9 (0.5–40.8)	< 0.001	− 55	210.3 (55.8–527.4)	80.1 (21.0–255.7)	< 0.001	− 62
RIFLE unfavourable	46	1.43[a] (0.81–2.41)	2.05[a] (1.21–3.29)	< 0.001	43	107.9 (17.0–234.8)	113.9[a] (23.1–279.5)	0.112	6	176.7 (26.6–553.5)	226.2[a] (43.1–548.9)	< 0.05	28

Data are expressed as median (25th and 75th percentile). RIFLE favourable: reduction in 1 or > 1 RIFLE stage from *D*0 to D_{end}, or RIFLE − stays RIFLE −. RIFLE unfavourable: all the other patients. Δ%: percentage difference of medians D_{end} vs. *D*0

[a] $P < 0.001$ vs. RIFLE favourable

Relation to the evolution of kidney function

Patients with a *favourable* evolution of their RIFLE stages (76%) were compared to patients with a *unfavourable* evolution (Table 4). At *D*0, sCrea levels were higher in the group with an unfavourable evolution ($P < 0.001$), whereas there were no differences for IxS ($P = 0.05$) and pCS ($P =$ NS). At D_{end}, concentrations of all measured solutes were higher in the group with unfavourable evolution ($P < 0.001$).

Comparing D_{end} to *D*0, there was a decrease in concentrations of all measured solutes in the group with favourable evolution ($P < 0.001$), but percent wise this decline is more pronounced for IxS (− 55%) and pCS (− 64%) than for sCrea (− 24% decline).

In the group with worsening kidney function, there was a marked rise in sCrea ($P < 0.001$), a moderate one for pCS ($P < 0.05$) but no change for IxS ($P =$ NS).

As shown in Supplementary table 1, exclusion of patients with CKD at admission (eGFR < 60 mL/min/1.73 m^2) did not alter the results.

In summary, concentrations of measured uremic solutes were higher in patients with worsening evolution of RIFLE class. The deteriorating trend in the evolution of sCrea in this group was translated only partially in changes of pCS, whereas there were none for IxS.

Comparison of AKI and CKD patients

Finally, we compared intrinsic AKI defined as an unfavourable renal evolution on D_{end} with a cohort of CKD patients [11]. We excluded AKI patients with CKD at admission (eGFR < 60 mL/min/1.73 m^2). The populations were matched for kidney function based on sCrea ($P =$ NS). The populations were also comparable in age (AKI vs. CKD: mean age 64.6 ± 10.7 vs. 67.6 ± 12.7 years, $P =$ NS) and gender (69% vs. 50% male, $P =$ NS). Both IxS and pCS were markedly higher in CKD compared with AKI (Fig. 1; $P < 0.01$).

Discussion

This study assesses serum concentrations of IxS and pCS at baseline and their evolution over time in relation to serum creatinine, in patients with and without AKI admitted to the ICU with sepsis. We also compared IxS and pCS in intrinsic AKI vs. CKD in patients with similar sCrea. The most important findings are (1) in AKI, IxS and pCS, like sCrea, tend to increase as kidney function deteriorates (Table 2), as in CKD [8, 11, 13]; (2) if sCrea increases over time, there is no or an attenuated rise in IxS and pCS (Table 4); in case of a favourable evolution of sCrea, the decrease of IxS and pCS tends to be more important (Table 4); (3) for similar

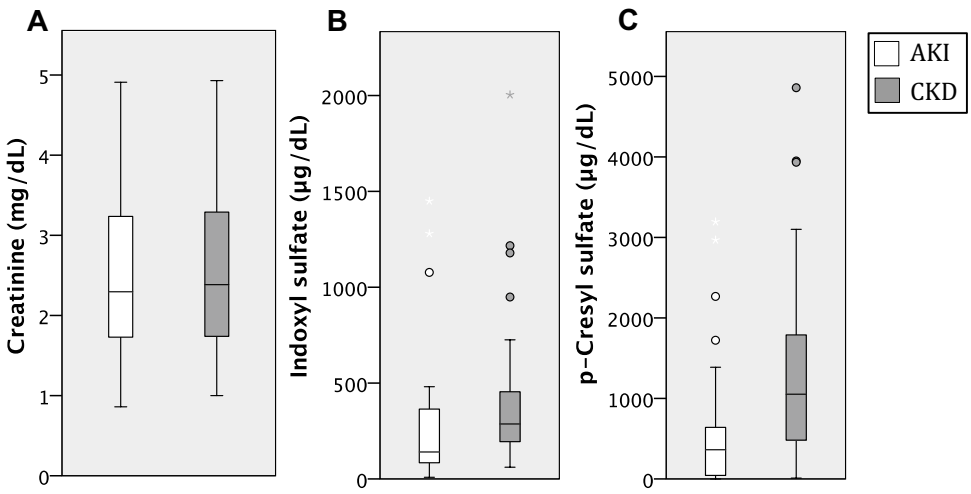

Fig. 1 Comparison of total IxS and pCS levels in AKI and CKD patients ($n=32$), matched for serum creatinine. Concentration of sCrea, IxS and pCS in patients with AKI according to RIFLE unfavourable evolution at D_{end} (with exclusion of CKD at admission) vs. a CKD population with comparable sCrea. Boxplot (median, IQR, range); $n=32$. **a** sCrea, $P=$NS CKD vs. AKI; **b** IxS, $P<0.01$ CKD vs. AKI; **c** pCS $P<0.001$ CKD vs. AKI. AKI: acute kidney injury, CKD: chronic kidney disease

sCrea values, total IxS and pCS serum levels are markedly lower in AKI, compared to CKD (twofold and threefold, respectively) (Fig. 1).

Although uremic retention is typical for both CKD and AKI [1], retention patterns have only rarely been studied in AKI [2], which is remarkable. Because of potential differences in underlying pathophysiology and metabolism, compared to CKD AKI is more abrupt, with a higher potential to affect global homeostasis [26].

According to our results, the relationship between the serum concentration of sCrea and IxS and pCS is not straightforward (Table 2; Fig. 2). Hence, in AKI changes in sCrea do not predict the evolution of other uremic solutes such as IxS and pCS. This discrepancy is reminiscent of the dissociation between eGFR (based on sCrea) and concentration of several protein-bound uremic toxins in CKD [27, 28]. In general, the evolution of serum concentrations of IxS and pCS from $D0$ to D_{end} tends to follow a lower trajectory than sCrea, unless at AKI stage F, where the lack of significance however might be attributed to the low patient number (Table 2).

These findings are attributable to several factors. All patients (AKI and non-AKI) were treated with antibiotics. The impact of antibiotics on the composition of the gut microbiota is known since long [29]; however, studies on their effect on uremic toxin generation/concentration are scarce. Nazzal et al. demonstrated that IxS and pCS decrease after a single administration of Vancomycin in haemodialysis patients [30]. In peritoneal dialysis patients, antibiotics decreased intestinal generation of pCS (preliminary [31]) [16, 31]. Loss of bacterial diversity and a decrease in urinary IxS after diverse antibiotics is reported in allogeneic stem cell transplants [32]. In our own experience, half of maintenance hemodialysis patients on antibiotics showed changes in serum concentration of a range of uremic solutes [33]. It can thus be presumed that antibiotics modify generation of uremic toxin precursors [16].

ICU patients with sepsis are usually severely ill, resulting in low food intake. As amino acids are building stones of bacterial generation of pCS and IxS precursors, also this may contribute to their lower concentration [14, 15, 17]. Changes in the microbiome of septic ICU patients could also be due to non-renal organ failure such as liver or gastrointestinal dysfunction [2].

One may wonder whether the retention solutes exert a biological effect in AKI, as acute rises in concentration result in shorter exposure times than in CKD. Most experimental studies show relevant biological changes in response to IxS or pCS within a few hours [34, 35]. Thus the biological effect of an increase in uremic solutes may start early in the evolution of AKI. We demonstrated that there is a more pronounced increase in uremic solute concentration in more advanced AKI stages. Some of the main toxic effects of IxS and pCS are vascular, promoting renal ischemia and renal tubular epithelial-to-mesenchymal transition [6, 36, 37], which are major mechanisms leading to renal fibrosis [36, 37]. IxS also increases renal oxidative stress, which induces abnormal oxygen consumption in renal tubules, aggravating hypoxia in the kidney [38], and seemingly interferes with endothelial function and recovery [2, 35]. All these elements can play an essential pathophysiological role in delaying recovery of kidney function and/or progression towards CKD, an evolution of which a substantial proportion of AKI patients are at risk, subsequently increasing the risk of cardiovascular events, hospitalisation and death [39, 40]. Uremic toxin retention is a very likely contributor to this evolution.

The use of sCrea for the comparison of kidney function between AKI and CKD might be skewed. In AKI, sCrea is lagging behind on GFR [41], in contrast to CKD where kidney function changes gradually. However, the differences in

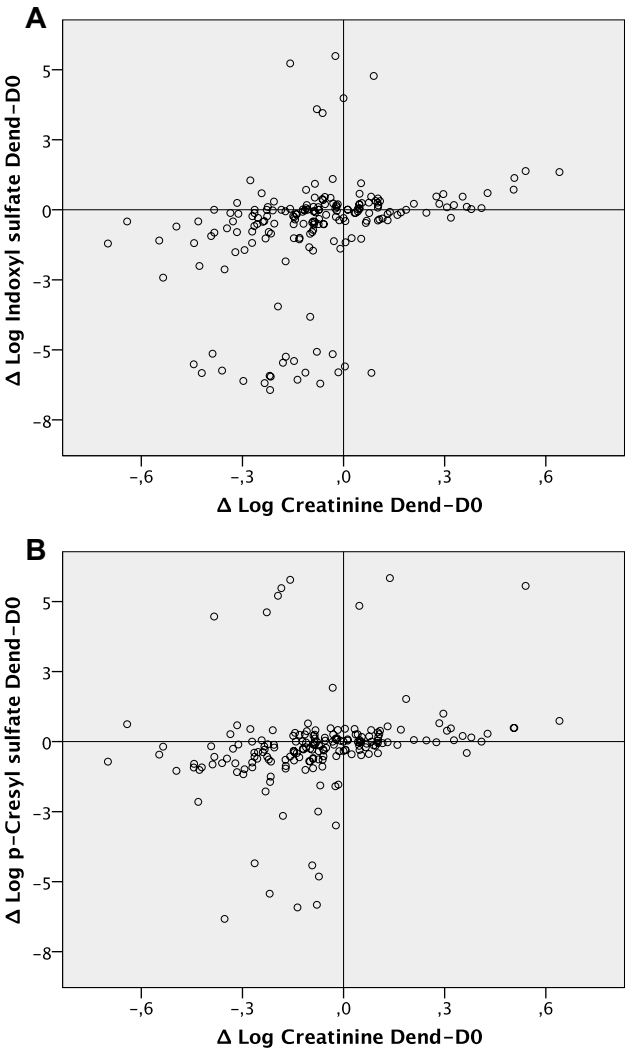

Fig. 2 Evolution in log-normalised uremic solute concentration from $D0$ to D_{end}. **a** Difference in log-normalised total indoxyl sulphate, on $D_{end} - D0$, vs. difference in log-normalised serum creatinine. **b** Difference in log-normalised total p-Cresyl sulphate, on $D_{end} - D0$, vs. difference in log-normalised serum creatinine. Reference lines showing delta values D_{end} vs. $D0 = 0$. $D0$: day of inclusion; D_{end}: day 4 or day before drop-out; Δ: value $D_{end} - D0$

IxS and pCS levels in AKI vs. CKD are striking. In addition, IxS and pCS likely are subjected to similar lag time effects as sCrea. Another factor causing a dissociation between sCrea and kidney function is fluid overload, decreasing sCrea irrespective of kidney function [42]. However, here also IxS and pCS should be influenced similarly.

Still other elements of AKI likely dissociate the handling of IxS and pCS vs. sCrea. Because of their protein binding, IxS and pCS rely primarily on proximal tubule secretion via organic anion transporters for their clearance [13, 43], rather than on GFR, in contrast to sCrea [13, 43, 44]. However, if lack of tubular secretion is added to fall in GFR to determine clearance, retention would be enhanced and concentrations higher in proportion to sCrea, not lower. A factor specific for sCrea is a decrease in concentration due to loss of muscle mass, which does not affect IxS and pCS. Sepsis is prone to substantial muscle loss [45], generating less creatinine [46].

This study has limitations. Conclusions cannot automatically be extrapolated to non-septic AKI. Enrollment started at admission to ICU, and information on events happening during pre-admission is missing.

Our study also has strengths. The cohort homogeneity, especially with regards to antibiotics, excluded this major confounder. A fraction of enrolled patients did not develop AKI, representing an internal control group without AKI, and the cohort was large enough to assess and compare different stages of AKI. Careful follow-up allowed assessment of the evolution of kidney function in all patients and the comparison of patients with and without deteriorating kidney function. SCrea prior to sepsis was known in 97% of patients, enabling RIFLE staging without extrapolated baseline sCrea [24], a potential source of error [47]. Urine output was included in the RIFLE definition, decreasing the risk for misclassification [48].

Patients with CKD at admission were not excluded in our primary analyses of the AKI population because we considered them to be an unmistakable part of the population at risk of AKI and even at higher risk than the population with normal kidney function, while their condition at onset serves as a valid control as much as in the patients with normal kidney function.

In conclusion, in a septic population, AKI is linked to higher IxS and pCS, especially in the more severe RIFLE classes. However the rise in IxS and pCS is more attenuated than that of sCrea, and, in some instances is even absent, so that sCrea in AKI represents an unreliable predictor of the evolution of IxS and pCS. We also demonstrated that total IxS and pCS are markedly lower in septic AKI, than in CKD.

Our data are suggestive of changes in the intestinal microbiome, linked to antibiotics and nutritional alterations. Yet, in a considerable number of patients, solute retention remains prominent enough to impact clinically relevant components of AKI, such as renal recovery. Further research needs to identify which solutes might impact patient outcomes in AKI, so that pharmacological or RRT can be targeted to neutralise those substances.

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Author contributions LV conducted the statistical analysis and wrote the first draft. JV included the patients, collected the samples and the demographic data. RV and GG designed the study, helped writing the draft and critically reviewed it. SL and ZAM collected and provided the samples of CKD patients. JV, WVB, ZAM and SL revised the paper.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

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