Results of the HEMO Study suggest that p-cresol sulfate and indoxyl sulfate are not associated with cardiovascular outcomes

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Cardiovascular disease, the leading cause of mortality in hemodialysis patients, is not fully explained by traditional risk factors. To help define non-traditional risk factors, we determined the association of predialysis total p-cresol sulfate, indoxyl sulfate, phenylacetylglutamine, and hippurate with cardiac death, sudden cardiac death, and first cardiovascular event in the 1,273 participants of the HEMO Study. The results were adjusted for potential demographic, clinical, and laboratory confounders. The mean age of the patients was 58 years, 63% were Black and 42% were male. Overall, there was no association between the solutes and outcomes. However, in sub-group analyses, among patients with lower serum albumin (under 3.6 g/dl), a twofold higher p-cresol sulfate was significantly associated with a 12% higher risk of cardiac death (hazard ratio 1.12; 95% confidence interval, 0.98-1.27) and 22% higher risk of sudden cardiac death (1.22, 1.06-1.41). Similar trends were also noted with indoxyl sulfate. Trial interventions did not modify the association between these solutes and outcomes. Routine clinical and lab data explained less than 22% of the variability in solute levels. Thus, in prevalent hemodialysis patients participating in a large U.S. hemodialysis trial, uremic solutes p-cresol sulfate, indoxyl sulfate, hippurate, and phenylacetylglutamine were not associated with cardiovascular outcomes. However, there were trends of toxicity among patients with lower serum albumin.

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Cardiovascular disease morbidity and mortality in dialysis patients remain high and unexplained by traditional risk factors.[1] Despite the majority of US patients meeting target Kt/V_{UREA} goal, the median survival after starting dialysis is approximately 3.5 years and over half of all deaths are due to cardiovascular causes.[2] Uremic toxins, substances that are cleared by the kidney and retained in kidney failure are possible contributors to accelerated cardiovascular disease in dialysis patients. Identification of these toxins is essential to develop therapies, both dialytic and nondialytic, that can lower solute concentrations and hopefully improve survival of dialysis patients.[3]

P-cresol sulfate and indoxyl sulfate are among the most commonly studied uremic solutes. Substantial evidence has been accumulated that both these may cause vascular injury and have other toxic effects.[4] They share the property of generation by colon microbes followed by colonic absorption, conjugation, and clearance from the circulation by tubular secretion. Phenylacetylglutamine is also generated exclusively and hippurate partially through the action of colon microbes. Prior studies, including our work, also suggest that phenylacetylglutamine retention may also contribute to cardiovascular events in patients on hemodialysis[5] and also in patients with earlier stages of chronic kidney disease.[6] All these solutes share the property that they are cleared largely by secretion in the normal kidney. Because dialysis does not replicate secretory processes, their concentrations rise much higher relative to normal than concentrations of urea and creatinine in patients maintained on dialysis.[7]

We measured predialysis levels of p-cresol sulfate, indoxyl sulfate, hippurate, and phenylacetylglutamine in specimens of the Hemodialysis (HEMO) study, a US multicenter trial of hemodialysis dose and flux.[8] The goal of our study was to analyze the longitudinal association between these solutes and

physician-adjudicated cardiovascular outcomes in the HEMO study. The large sample size of the HEMO study, its national multicenter design, and inclusion of patients without significant residual kidney function provided us with a unique opportunity to examine the associations between these solutes and cardiovascular outcomes in hemodialysis patients.

RESULTS

Participant characteristics

Baseline characteristics of the 1273 participants included in this study are presented in Table 1.[9] Mean age of the participants was 57 years, 63% were black, and 57% were female. The participants included in this study were generally similar to the 1846 participants of the HEMO study (Supplementary Table S1), except for lower baseline cardiac disease (79% vs. 83%), fewer years of prior dialysis (3.5 vs. 4.4 years), higher residual urea clearance (0.3 vs. 0.2 ml/min per 35 l), and lower serum β2-microglobulin (36 vs. 38 mg/l).

Outcomes during follow-up

There were 221 cardiac deaths during 3282 person-years of follow-up (median, 2.3 years) with a crude cardiac death rate of 67 per 1000 person-years. The adjudicated causes of cardiac death included ischemic heart disease (62.3%), congestive heart failure (11.4%), arrhythmias and other conduction disorders (15.0%), and other heart diseases (11.4%). During follow-up, there were 127 sudden cardiac deaths (crude mortality rate, 39 per 1000 person-years), 641 cardiovascular events or any-cause deaths (crude event rate, 273 per 1000 person-years), and 563 any-cause deaths (crude mortality rate, 172 per 1000 person-years).

Association between solutes and outcomes

The association between the solutes and outcomes visualized using plots of age-, sex-, and race-adjusted mortality rates appeared linear and did not show a higher death rate with higher solute concentrations (Supplementary Figure S1). In unadjusted and sequentially adjusted Cox models (Table 2), there were no associations among p-cresol sulfate, indoxyl sulfate, hippurate, or phenylacetylglutamine and any of the outcomes. Table 3 presents the minimum hazard ratio (HR) that could be detected in this study with 90% power and alpha of 0.05, given the observed number of events and the correlation between solutes and other covariates. The study had at least 90% power to detect an HR for cardiac death of 1.19 for p-cresol sulfate, 1.29 for indoxyl sulfate, 1.19 for hippurate, and 1.26 for phenylacetylglutamine. Prespecified subgroup analyses are presented in Supplementary Tables S2 to S5. The results should be interpreted with caution due to multiple comparisons, and a *P*-value of 0.05/11 = 0.004 is suggested as a significant interaction between the groups. Using this threshold, among those with serum albumin below median (<3.6 g/dl), p-cresol sulfate was associated with higher risk of cardiac death (HR per 2-fold increase, 1.12; 95% confidence interval [CI], 0.98–1.27; *P*-interaction, <0.001) and sudden cardiac death (HR per 2-fold increase, 1.22; 95% CI, 1.06–1.41; *P*-interaction < 0.001). Similar trend was noted for indoxyl sulfate in patients with serum albumin <3.6 g/dl (HR for cardiac death per 2-fold increase, 1.09; 95% CI, 0.91–1.31;

Table 1 | Baseline characteristics of the 1273 Hemodialysis (HEMO) study participants

Characteristics	Results
Total P-cresol sulfate, mg/dl	
Mean	3.3 ± 1.7
Median	3.3 (2.2, 4.4)
Total indoxyl sulfate, mg/dl	
Mean	2.5 ± 1.2
Median	2.4 (1.7, 3.3)
Total hippurate, mg/dl	
Mean	5.4 ± 4.3
Median	4.5 (2.3, 7.4)
Total phenylacetylglutamine, mg/dl	
Mean	4.5 ± 2.8
Median	4.0 (2.5, 6.0)
Demographics	
Age, yr	57.5 ± 14.0
Sex, female	723 (56.8)
Race, black	799 (62.8)
Clinical characteristics	
Diabetes	574 (45.1)
Cardiac disease	1003 (78.8)
ICED score	2.0 ± 0.8
Gastrointestinal disease	477 (37.5)
Residual kidney urea clearance, ml/min per 35 l TBW	0.3 ± 0.5
Body mass index, kg/m^2[a]	25.7 ± 5.4
Body surface area, m^2[a]	1.8 ± 0.2
Dialysis characteristics	
Prior dialysis, yr	3.5 ± 4.1
Predialysis systolic blood pressure, mm Hg[a]	152.4 ± 25.8
Postdialysis weight, kg[a]	70.0 ± 15.3
Relative volume removed, %[a]	4.1 ± 1.7
Dose intervention	633 (49.7)
Flux intervention	632 (49.6)
Treatment time, min[a]	206.8 ± 28.3
Blood flow rate, ml/min[a]	343.0 ± 60.7
Dialysate flow rate, ml/min[a]	673.3 ± 129.8
Predialysis laboratory tests	
Blood urea nitrogen, mg/dl[a]	59.7 ± 18.8
Single-pool Kt/V_{UREA}[a]	1.5 ± 0.3
Serum albumin, g/dl[a]	3.6 ± 0.4
Serum β2-microglobulin, mg/l[a]	36.7 ± 14.2
Nutritional parameters	
Equilibrated nPCR, g/kg per day[a]	1.0 ± 0.3
Adjusted protein intake, g/kg per day (ABW)[b]	0.9 ± 0.3
Fat, %	35.5 ± 7.6
Carbohydrate, %	48.4 ± 9.3

ABW, adjusted body weight; ICED, Index of Coexistent Disease; IQR, interquartile range (25th, 75th percentiles); nPCR, normalized protein catabolic rate; SBW, standard body weight; TBW, total body water.
Data are presented as mean ± SD or median (IQR) for continuous variables and *n* (%) for categorical variables.
[a]Measured at the same time as solutes. Remaining variables are measured at baseline.
[b]If body weight was <90% or >120% of median SBW as determined from the National Health and Nutrition Examination Survey II (NHANES II) data then protein intakes were normalized to an ABW to standardize nutrient intake using the formula: ABW = ([Actual weight − SBW)] × 0.25) + SBW.[9]