Gut-Derived Metabolites and Chronic Kidney Disease
The Forest (F)or the Trees?

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As CKD progresses, the capacity of the kidneys to eliminate metabolic waste is gradually lost, resulting in the retention of a host of compounds. Many of these solutes affect biologic functions and are likely contributing to the uremic syndrome, affecting not only morbidity and mortality but also patient-centered outcomes (fatigue, pruritus, sleep disorders) and quality of life (1). Our knowledge on the nature and action of the uremic compounds is still expanding—a progress at least partly attributable to the acquisitions of metabolomic analysis, which allows the blinded search for previously unknown molecules (2).

The intensive exploration of the role of the intestine in the generation of uremic toxins was set in motion only during the past decade (3). The chain of events at play generally begins with dietary protein degradation followed by the metabolic transformation (fermentation) of amino acids by the gut microbiota and their conjugation (mostly sulfatation and glucuronidation) in the intestinal wall or liver by phase 1 and phase 2 metabolizing enzymes. Conjugation is, essentially, intended to increase solubility and neutralize toxicity, but the latter effect may remain incomplete, as several end-products still exert toxicity (4).

In the search for intestinally generated uremic solutes, some proof-of-concept metabolomic studies identified a number of discriminators by comparing plasma or urine from either germfree rodents with control animals (5) or human subjects with and without a colon (6). Several identified molecules appeared to be known protein-bound uremic toxins, such as indoxyl sulfate, *p*-cresyl sulfate, and phenyl sulfate.

In this issue of the *Clinical Journal of the American Society of Nephrology*, Mair *et al*., in their study on patients on dialysis with and without a colon, used a slightly different approach than in their previous study (6), resulting in a considerable extension of our knowledge on uremic solutes of intestinal origin (7). A sophisticated analytic platform comparing urine of patients with normal kidney function with or without colon yielded 91 colon-derived molecules. This selection was then narrowed by comparing the plasma of controls with that of patients on maintenance hemodialysis, resulting in a definite list of 60 intestinally generated uremic solutes. By using a chemical reference library, Mair *et al.* (7) could then define the structure of 34 of these substances, among which 22 were classified as newly detected intestinally generated retention products. Many (but not all) of these solutes were protein bound and/or sulfates, glucuronides (as demonstrated by sample sulfatase treatment), or metabolites of phenyl acetic acid. For the majority of compounds of which it was possible to calculate kidney clearance, this parameter exceeded creatinine clearance. As the latter can be considered a proxy of GFR, this suggests substantial active kidney tubular secretion, and may cast some doubts on the value of GFR as an all-inclusive measure of kidney function.

The question is how this considerable extension of knowledge can be applied for the better to improve the quality and sustainability of the treatment and life quality of patients with kidney failure. One point is certain: the idea that the uremic syndrome is a simple condition, resulting from the activity of one or a few retention solutes, is further away than ever.

It seems tempting to embark upon a large experimental effort to unravel the toxicity of the newly detected solutes, but such an endeavor should not be underestimated. Next to the identification of the solutes *per se*, the uremic concentration needs to be determined which necessitates the availability of the pure compound (either purchased or synthesized) before the biochemical impact can be tested, preferably in several appropriate organ or cell systems (4). Such approach may be extremely time-consuming unless well designed, high-throughput systems can be applied. The number of solutes to be tested could, however, be reduced on the basis of data from large sample collections and big data analysis, taking into consideration hard outcomes as well as patient-centered end points. In addition, systems biology could be used to unravel interactions among metabolites, the most important pathways involved and the most important metabolites. Another option could be the selection of solutes with the most outspoken characteristics, *e.g.*, the largest concentration gap between normal and hemodialysis or between those with and without a colon, or those solutes with the highest tubular secretion.

Perhaps more importantly, the reported data may help guiding the development of novel therapeutic procedures. As the study by Mair *et al.* detected large clusters with common characteristics (sulfates and glucuronides; phenols, cresols, and indoles), these could be approached by specific sorption methods

aimed at capturing these structures. If such methods can be developed, they could also help to offer proof of concept regarding whether removing those solutes improves outcomes.

Another option suggested by this study is correction of the intestinal dysbiosis by modifying intestinal bacterial metabolism, for which several proof-of-concept studies are already available, applying different diets, probiotics, prebiotics, synbiotics, or antibiotics (8). However, it might be dangerous to jump to conclusions. When Mair *et al.* considered the urinary excretion ratio of patients without a colon versus controls per individual molecule, it almost always exceeded zero (7), suggesting extraintestinal sources. Likewise, even if one does not account for the five patients with an ileal pouch, which appears to preserve some intestinal microbial activity, more than 20 intestinally generated uremic solutes were found in the urine of some patients and seven were found in all patients without a colon.

Also for other reasons, targeting the colon as an isolated effort may offer an insufficient response to the uremic problem. A recent classification on the basis of preset criteria summing up 24 uremic solutes with the most important biochemical and clinical impact (9) contained only four of the solutes retrieved in the analysis of Mair *et al.* (7) (indoxyl sulfate, *p*-cresyl sulfate, trimethylamine-*N*-oxide, and indole acetic acid). This does not exclude yet unproven toxicity of some of the newly retrieved solutes by this study (7), but does suggest that a large number of solutes with toxic potential may not be entirely of intestinal origin. Of note, the aggregated results of the study by Mair *et al.* (7) also point to the role of an axis comprising the intestine, liver, plasma protein binding, and tubular excretion, rather than one of the intestine alone.

All these observations might translate into difficulties to sufficiently reduce concentration of the concerned solutes by applying intestinal methods only. Tackling the intestine may be one of the solutions, but it is not the only solution. Subsequently, research efforts should be continued and strengthened to develop also other alternative ways to enhance toxin removal, such as decreasing protein binding, sorption, preservation of kidney function, increasing tubular secretion, and applying various acquisitions at the intersection of kidney replacement therapy and regenerative medicine (bioartificial kidney) or regenerative medicine *per se* (stem cells, organoids, bioengineered kidneys). Stimulating research in these areas is an important task for the nephrological community and all those involved in health policy, as patients, their families, and health professionals are in need of innovations in a field that has stagnated for too long and has too high amounts of health expenditures in its current form. In this context, targeting the intestinal microbiome remains a cheap and noninvasive therapeutic option, with a potential outreach to a large number of patients.

In spite of the impressive efforts to accomplish this analysis and the vast amount of information offered, the study by Mair *et al.* (7) unavoidably raises some questions and concerns. Regardless of the comforting double-check of the reliability of the data provided for the most familiar uremic solutes, mass spectrometric analysis as used here remains semiquantitative, which may be a source of error, especially for calculated values such as clearances, ratios, or free fractions. Also, the use of spot urines sometimes necessitating drastic dilution is a potential source of error. Some seemingly obvious colon-derived candidate molecules, such as indoxyl glucuronide, hippuric acid, or phenyl acetic acid, that were traced by other metabolomic studies, sometimes by the same group (6), were absent in this analysis, which makes one suspect that either analytical bias or individual differences in nutritional status, metabolism, or genetics, may affect the findings. Other potentially skewing factors may be the overwhelming majority of patients with CKD and those on hemodialysis suffering from diabetes versus none in the control and colectomy groups, or the presence in the colectomy group of patients on immunosuppressive agents, as both may modify the intestinal microbiome (10,11).

In conclusion, this study contains convincing but not entirely unexpected evidence that colonic generation of uremic toxins concerns even more molecules than previously demonstrated. GFR is, in many cases, not representative of kidney clearance. The study also supports the concept of molecule families and the existence of a gut/liver/plasma protein binding/kidney tubular axis, the disruption of which by one or more therapeutic measures could bring down the concentration of those solutes. Research in these areas should be able to modify the current paradigm of kidney treatment to make it more sustainable. This includes the exploration of previously underexploited areas such as manipulation of intestinal function, and alternative extracorporeal removal strategies such as adsorption and regenerative medicine.

Disclosures

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See related article, "Characteristics of Colon-Derived Uremic Solutes," on pages 1398–1404.