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In Focus

Intestinal metabolites, chronic kidney disease and renal transplantation: Enigma Variations?

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In this edition of *Nephrology Dialysis Transplantation*, Poesen *et al.* [1] publish a study assessing during the first year after kidney transplantation the retention and generation pattern of solutes produced by the intestinal microbiota. Most if not all of these have been associated with cardiovascular morbidity or mortality. The results show a decrease in concentration, which is not unexpected. However, at several time points during the observation year, solute concentration was also markedly lower than in a non-transplant population with virtually identical glomerular filtration rates (GFRs). Almost simultaneously, Liabeuf *et al.* [2] published similar findings in the *Journal of Circulation*, be it restricted to a single solute, indoxyl sulphate. Starting from indirect extrapolations, one might develop several hypotheses for these intriguing findings, especially since the publication by Poesen *et al.* [1] also contains data on solute excretion and clearance by the kidneys. The results suggest that the observed changes are related to a mixture of mechanisms. The first involves changes in the composition of intestinal microbiota and/or their generation of the mother compounds of the solutes under study. Second, the activity of transporters that bring these metabolites into the general circulation via the intestinal wall and liver, to then excrete them from the body via the kidneys into the urine, may be modified as well. Third, there may be modifications in the conjugation processes that control the handling of these metabolites and the transformation of the mother compounds. One thing is almost certain: differences in GFR do not seem to play a major role. These changes may be due to immunosuppressive agents, antibiotics, other drugs, the transplantation procedure itself or changes in the concentration of uraemic toxins. Further studies are needed to clarify the mechanisms, which may open novel pathways to decrease the concentration of these solutes. In addition to these intriguing findings, the study by Liabeuf *et al.* [2] contains another remarkable piece of information, namely the lack of association between indoxyl sulfate concentrations and hard outcomes such as cardiovascular mortality, overall mortality or graft loss. This contrasts with multiple previous findings in the non-transplanted chronic kidney disease (CKD) population. It could be linked to statistical factors, but also to the fact that immunosuppression may annihilate a number of the pro-inflammatory biological effects of these uraemic toxins that are deleterious to the vessels. Analogous findings have been reported in other inflammatory disorders, such as rheumatoid arthritis. All these aspects together warrant further studies, of which the results may help to improve the outcomes of CKD patients ahead of or in addition to extracorporeal treatment.

> The fourteen Enigma Variations constitute an orchestral work, composed at the end of the 19th century by Edward Elgar. The name possibly refers to a currently unidentified melody that may be hidden throughout the work.

The concentrations of uraemic retention solutes generated by the intestinal microbiota (most of them but not all protein bound) have been assessed in diverse stages of CKD in haemodialysis and peritoneal dialysis. Data from renal transplant populations were, however, almost non-existent until recently, apart from a study on samples collected on average >5 years after transplantation, without comparison to a non-transplanted group with similar kidney function [3]. In this issue of *Nephrology Dialysis and Transplantation*, a study by Poesen *et al.* [1] contains a careful analysis of samples collected at fixed time points during the first year of transplantation. Almost

simultaneously, Liabeuf *et al.* [2] published a similar study in the *Journal of Circulation*. Although both studies are in several aspects confirmatory of each other, for some other points they are markedly different (Table 1), raising an interest to make comparisons. Indeed, the study by Poesen *et al.* [1] mainly focuses on the mechanisms that affect the concentration of solutes, whereas the one by Liabeuf *et al.* [2] assesses the association to hard outcomes. Consequently, both studies may serve as eye-openers to stimulate further exploration regarding the gut–kidney–heart axis.

The study by Poesen *et al.* [1] assesses in two relatively small but independent populations ($n = 51$ and $n = 65$) five different uraemic toxins (Table 1), of which the concentration, with the exception of trimethylamine-*N*-oxide (TMAO), appears to be lower after kidney transplantation than in a matched CKD population with similar estimated GFR (eGFR). It further shows that in spite of lower serum concentrations, renal clearances are also depressed versus non-transplants, with the exception of *p*-cresyl sulphate.

The study by Liabeuf *et al.* [2], which assesses a larger population ($n = 311$) and only indoxyl sulphate as a metabolite, also contains a comparison to a matched CKD population and comes to the same conclusion of relatively lower solute concentration after transplantation. In contrast to Poesen *et al.*'s [1] publication, this study does not assess renal excretion mechanisms, but rather the potential association of concentration to hard outcomes, i.e. mortality, cardiovascular events and graft loss.

Table 1. Comparison of study characteristics

	Poesen *et al.* [1]	Liabeuf *et al.* [3]
Number of patients	51 and 65[a]	311
Time points		
0 months	+	+
7 days	+	–
1 month	–	+
3 months	+	–
12 months	+	+
Validation in an independent population	+	–
Uraemic retention solutes		
p-Cresyl sulphate	+	–
p-Cresyl glucuronide	+	–
Indoxyl sulphate	+	+
Trimethylamide-*N*-oxide	+	–
Phenylacetylglutamine	+	–
Type of concentration		
Total	+	+
Free	–	+
Matched non-transplant population with similar CKD stage	+	+
Immunosuppression		
Steroids	± 100%[b]	+ (85–90%)
Mofetil mycophenolate	± 100%[b]	+ (75–80%)
Tacrolimus	± 100%[b]	± (15–25%)
Cyclosporine	–[b]	± (70–85%)
Sirolimus/everolimus	–[b]	+ (<5%)
Antimicrobial prophylaxis	+	+
Studies of urinary solute excretion	+	–
Studies of urinary solute clearance	+	–
Outcome analysis		
Overall mortality	–	+[c]
Cardiovascular events	–	+[c]
Graft survival	–	+[c]

[a]Two independent analyses.
[b]No exact percentages given in the text.
[c]No significant association found.

Both studies together, be it indirectly, point to profound changes in intestinal generation and handling of these solutes or their mother compounds in the aftermath of kidney transplantation. Two other possible mechanisms, a dissociation between eGFR and real GFR or a decrease in protein intake after transplantation, are virtually ruled out by Poesen *et al.*'s [1] findings, at least at Month 3, of similar creatinine clearances and urinary urea excretions among both groups. The reason (s) for these intriguing findings may give rise to a number of speculations (Figure 1).

Transplantation provokes many functional, biochemical and biological changes, according to the presently discussed data probably including a modification of the intestinal handling of digestive breakdown products. Although it has been shown that kidney transplantation has a profound impact on the composition of the intestinal microbiota [4, 5], to the best of our knowledge, direct proof that these changes also affect metabolite generation is lacking. However, in bone marrow transplantation [6, 7] and in non-transplant conditions of CKD [8, 9], changes in gut microbiome went together with changes in metabolite generation and/or excretion.

The administration of immunosuppressive drugs causes profound metabolic alterations that may affect the composition and function of intestinal microbiota [10], but direct studies in this regard on specific agents are extremely rare. In one such study, in a rodent model of inflammatory bowel disease, steroids were the origin of composition shifts in the intestinal microbiota [11]. Microbiota from donors pre-treated with those steroids had an anti-inflammatory effect [11]. Mofetil mycophenolate may cause a broad spectrum of morphological and functional intestinal changes, from overt colitis [12] to villous atrophy [13], that have the potential to modify the functional capacity of the intestinal wall, e.g. by diminishing absorption.

Of note, although both studies under discussion used corticosteroids and mofetil mycophenolate to an almost identical extent, there were marked discrepancies with regards to the third immunosuppressant (Table 1). In the study by Poesen *et al.* [1], virtually the entire group received tacrolimus, whereas the study by Liabeuf *et al.* [2] mainly applied cyclosporine (70–85% of patients) and also some mammalian target of rapamycin (mTOR) inhibitors (<5%). Hence, either it does not matter which third immunosuppressant (in essence which calcineurin inhibitor) is applied or calcineurin and mTOR inhibitors do not impact intestinal microbiota. This area certainly warrants further exploration.

Both study groups also received antimicrobial prophylaxis, essentially for *Pneumocystis jiroveci* infections by administration of co-trimoxazole, in the Poesen *et al.* [1] study for 3 months and in the Liabeuf *et al.* study [2] for 6 months. Undeniably, several antibiotics affect intestinal microbiota, among them co-trimoxazole [14–16], although the picture in most clinical situations is blurred by the intermittent or long-term use of other antimicrobials in addition to co-trimoxazole [6]. Renal transplants are prone to microbial infections as well

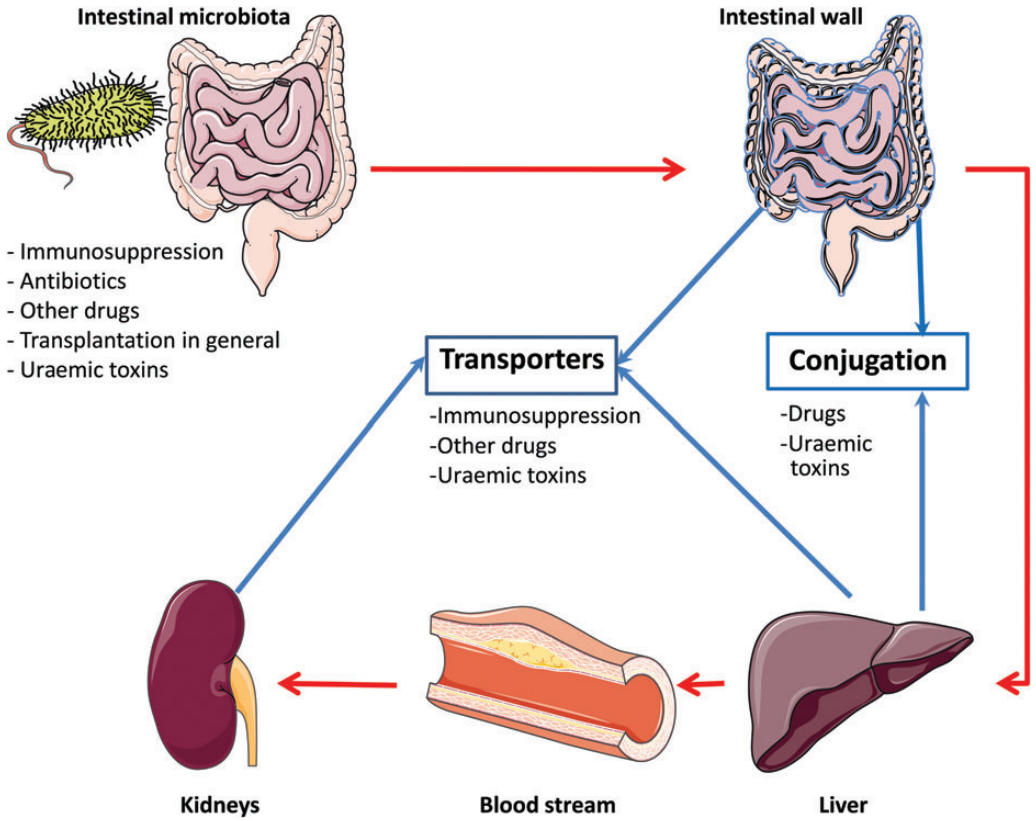

FIGURE 1: Flow chart of possible mechanisms involved in solute handling after renal transplantation. The red arrows indicate the flow of solutes through the body; the blue lines indicate the handling mechanisms that are likely to be involved for a given organ: conjugation: sulphatation, glucuronidation; transporters: ATP-binding cassette transporters, solute carrier transporters, organic anion transporters (peptides), organic cation transporters.

as non-microbial ones, especially in the first months [17], which in a substantial number of patients might necessitate antibiotic treatment at some time point. Although no statement in this regard is made, one may assume that in both studies discussed here [1, 2], patients with infections/antibiotic treatment were not excluded, which may thus have affected the results.

The final assessment in both studies occurred after 12 months [1, 2] (respectively, 9 and 6 months after the end of co-trimoxazole) and still showed a lower solute concentration. How long the effect of an antibiotic on microbiota may persist remains uncertain. In the paper by Holler *et al.* [6] in bone marrow transplants, urinary indoxyl sulphate started to rise immediately after arrest of antibiotics, but it returned to only ±30% of baseline over a period of 29–100 days. In a study on horses, microbiota composition had largely but not entirely recovered 25 days after end of treatment with co-trimoxazole alone [16]. Thus, the impact of antimicrobial prophylaxis on intestinal microbiota is worth further analysis.

Once the mother compounds of the solutes (e.g. p-cresol, indole) have been generated intestinally and absorbed, they are further metabolized by the gastrointestinal system via conjugation, e.g. by sulphotransferase (Figure 1). Some drugs may affect this conjugation process [18], although it is unclear whether this is also the case for those specific to transplantation. Also, uraemic retention solutes may affect conjugation processes [19], but this is essentially confined to inhibition at concentrations in the high range, hence the lower concentrations in the presently discussed studies should not have had a major impact on the production of conjugates.

Interestingly, in spite of lower serum concentrations of all solutes under study, their renal clearance was inhibited as well. This might be related to the transport systems in the tubules, and in view of the absence of differences in GFR, suggests a dissociation between glomerular and tubular function in these transplants. Indeed, most of the solutes studied are, due to their protein binding, to some extent dependent on tubular clearance [20, 21]. Also these pump systems may be modified by drug effects [22], and even moderate rejection may affect tubular functional status as well [23]. Furthermore, cyclosporine and tacrolimus in particular, but also other immunosuppressive agents, have been linked to transporter inhibition [22, 24, 25]. Alternatively, renal excretion was inhibited because less substrate was presented to the tubular pumps, but this should not have affected renal clearance, which was also suppressed.

Another enigmatic finding in the study by Poesen *et al.* [1] is the discrepancy in patterns followed by indoxyl sulphate and p-cresyl sulphate at Month 3. Indeed, whereas kidney clearance of indoxyl sulphate was lower than in non-transplant CKD, serum concentration was not different, but it was exactly the opposite for p-cresyl sulphate. However, dissociation between both molecules was observed for many other factors as well, e.g. protein binding pattern [26] or response to synbiotics [9] or prebiotics [27, 28]. This suggests that the handling of both

solutes, in spite of their structural similarities, might be different at least at some points.

Dissociation between transplantation and CKD that is attributable to intestinal changes is reminiscent of the dissociation of toxin concentration between peritoneal dialysis and haemodialysis, where better removal of the cresols with high-flux haemodialysis was not reflected in their serum concentration, which was lower with peritoneal dialysis [29, 30]. Here also, the difference was probably linked to as yet undefined intestinal or metabolic changes beyond the potential role of residual renal function. Thus it seems that affecting intestinal mechanisms of toxin generation, willingly or unwillingly, changes their plasma concentration and possibly also their toxicity.

The study by Liabeuf *et al.* [2] reveals another unexpected finding. It could not demonstrate an association between indoxyl sulphate concentration and hard outcomes, in contrast to the findings in non-transplanted CKD [31, 32]. Solute concentrations post-transplantation may have become too low, or the range of concentrations was too narrow, to allow detecting a relation with outcomes. However, pre-transplant indoxyl sulphate did not correlate to mortality [2]. One might consider a role for the anti-inflammatory effect of immunosuppression. Indeed, the vascular damage by indoxyl sulphate is mediated by the secondary messenger nuclear factor κB [33], whereas immunosuppression may inhibit this activity [34]. Likewise, anti-inflammatory manoeuvers also inhibited negative vascular effects of other pro-inflammatory conditions, e.g. rheumatoid disease [35]. In addition, indoxyl sulphate has been shown to stimulate smooth muscle cell proliferation via the mTOR pathway, and this effect is attenuated by sirolimus [36]. However, mTOR inhibitors were used in <5% of patients in the study of Liabeuf *et al.* [2] and not at all by Poesen *et al.* [1].

Unraveling the mechanisms and pathways involved in the above findings may help to develop future non-dialytic pathways of reducing the concentration of toxins [37] and their metabolic effects that are centred on inflammation, fibrosis, vascular damage and progression of kidney failure [38]. Beyond indoxyl sulphate, additional work is needed to assess the potential association of other uraemic toxins with cardiovascular risk and graft loss in kidney transplantation.

CONFLICT OF INTEREST STATEMENT

None declared.

(See related article by Poesen *et al.* The influence of renal transplantation on retained microbial–human co-metabolites. *Nephrol Dial Transplant* 2016; 31: 1721–1729)

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IN FOCUS

Should we aim for oral health to improve outcomes in chronic kidney disease?

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Chronic kidney disease (CKD) has well-documented effects on oral tissues including xerostomia, taste disturbance, tongue coating, increased dental calculus and mucosal inflammation [1]. In this issue of *NDT* Palmer *et al.* present the largest study to date on oral disease and oral health practices in the setting of CKD stage 5D, the ORALD study [2]. The study has been carried out in more than 4000 haemodialysis patients of European ancestry in 7 countries being treated in selected Diaverum dialysis centres. Most of the patients were examined in Argentina (41%), the other European countries' contributions from Hungary, Italy, France, Poland, Portugal and Spain were more balanced. All patients underwent a full oral examination according to the World Health Organization guidelines for oral health surveys. This approach guarantees the best practice when carrying out a multicentre trial. It was performed by a local dentist trained in periodontitis in each country according to the central study procedures. The mean age of the persons examined was 62 years with about 60% being male and a mean dialysis vintage of 60 months. Diabetes prevalence was 32%, and prior myocardial infarction and stroke were known in 13 and 10% of patients, respectively. Thus the examined population seems to be representative of a typical European haemodialysis cohort. The oral examination found 41% of patients to be affected by moderate to severe periodontitis, with an average of 22 decayed, missing or filled teeth in the dentate population, and 20% of patients to be edentolous. Because edentolous patients can no longer show signs of periodontitis, the proportion of people who are affected by periodontitis increases with decreasing numbers of missing teeth. Compared with a representative German general population sample [3], the proportion of haemodialysis patients who were affected by moderate to severe periodontitis was quite low (41% versus 73% to 88%, depending on age). This might be related to a higher proportion of haemodialysis patients being edentolous and the fact that periodontal disease varied markedly by country. In the present study this was independent of demographics, comorbidity and health practices, with edentulism and periodontitis being least prevalent in Argentina. Predictors of edentulousness were tobacco