Circulating Bacterial Fragments as Cardiovascular Risk Factors in CKD

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ABSTRACT

Cardiovascular disease (CVD) is a major cause of mortality and morbidity in patients with CKD. In the past decade, intestinal dysbiosis and altered gut epithelial barrier function are increasingly recognized in CKD. Uremic patients have slow intestinal transit time, impaired protein assimilation, and decreased consumption of dietary fiber. The use of multiple medications also may contribute to the proliferation of dysbiotic bacteria, which affect the barrier function of intestinal epithelium. In addition, fluid overload and uremic toxins per se directly reduce the gut barrier function. The major consequence of these alterations, the translocation of bacterial fragments from bowel lumen to systemic circulation, can lead to diverse biologic effects and probably represents an important nontraditional CVD risk factor in CKD. Among all bacterial fragments, endotoxin is the most well studied. Plasma endotoxin levels are markedly elevated in both patients with CKD and those on dialysis, and are associated with the systemic inflammatory state, accelerated atherosclerosis, and clinical CVD in patients on dialysis. Optimization of BP control and the use of ultrapure dialysate can reduce plasma endotoxin levels, with probable metabolic and cardiovascular benefits. The benefit of synbiotic therapy is not confirmed, although results from animal studies are impressive. The biologic effects and clinical relevance of other bacterial fragments, such as bacterial DNA fragments, are less well defined. Further studies are needed to delineate the pathogenic relation between circulating bacterial fragments and CVD, and to define the role of the plasma bacterial fragment level as a prognostic indicator of CKD.


The association between CKD and cardiovascular disease (CVD) has been noted for over 150 years.1 In patients with CKD, CVD is more frequent and more severe than in the general population, but is often not recognized and is undertreated.2 CVD accounts for 35% and 50% of all deaths among patients with stage 3 and 4 CKD, respectively.3,4 At the time of initiation of dialysis, 70% of patients with CKD have significant coronary atherosclerosis, 40% have symptomatic ischemic heart disease, 40% have heart failure, 20% have peripheral vascular disease, and 10% have had a previous stroke or transient ischemic attack.2

Cardiovascular risk factors in CKD are often classified as traditional or nontraditional. The increase in cardiovascular risk in CKD is partly because of the high prevalence of traditional cardiovascular risk factors, such as hypertension and diabetes.2 However, even after adjustment for traditional cardiovascular risk factors, the risk of cardiovascular mortality increases linearly once the eGFR falls below 75 ml/min per 1.73 m².3,4 Therefore, a number of kidney-specific nontraditional risk factors have been proposed,2 including volume overload, calcium phosphate imbalance,7 hyperhomocysteinemia,10,11 and the effect of other organic uremic toxins.12,13 In addition, dialysis-specific risk factors, such as dialysis membrane flux and biocompatibility, may also contribute.14

In the past decade, the clinical effect of the gut microbiome is increasingly recognized in various diseases.15,16 Symbiosis is usually defined, in the field of microbiome study, as the interaction between human host and microbiota in the context of maintaining homeostasis and beneficial effect to the human host, whereas dysbiosis is the term that describes microbial imbalance or maladaptation inside the body. In CKD, the symbiotic relationship between host and intestinal microbiome is disturbed because of the proliferation of dysbiotic bacteria.17,18 The causes of intestinal dysbiosis in CKD include slow intestinal transit time,19 impaired protein assimilation,20 decreased consumption of dietary fiber,21 iron therapy,22 and frequent
use of antibiotics. The results of gut dysbiosis are two-fold. First, fermentation of protein and amino acids by dysbiotic bacteria leads to the generation of toxic compounds such as ammonia, amines, thiol, phenols, and indoles, which trigger a systemic inflammatory state. Notably, trimethylamine N-oxide is a gut-derived amine oxide that has been implicated in the etiology of CVD in the normal population and in patients with CKD. Second, intestinal dysbiosis partly accounts for the impaired barrier function of the intestinal epithelium, resulting in the translocation of bacterial fragments through the bowel wall. In the past few years, numerous studies have shown that translocated bacterial fragments play important roles in the pathogenesis of uremic toxicity, inflammation, insulin resistance, protein-energy wasting, and progression of CKD, and are an important nontraditional cardiovascular risk factor in CKD.

**CIRCULATING BACTERIAL FRAGMENTS IN RENAL FAILURE**

**Intestinal Epithelial Barrier in Health and Diseases**

The intestinal epithelium is a single layer of columnar epithelial cells that separates the intestinal lumen from the underlying lamina propria. In good health, the intestinal barrier is highly effective, with the luminal side heavily colonized with gut bacteria and the basolateral side remaining sterile. Intestinal epithelial cells are bound together by tight junctions. Commensal gut microbes maintain functional integrity of gut by several mechanisms, including maintenance of tight junction protein structure, induction of epithelial heat-shock proteins, upregulation of mucin genes, competition with pathogenic bacteria for binding to intestinal epithelial cells, and secretion of antimicrobial peptides. Notably, commensal bacteria help maintain the intestinal epithelial barrier by suppressing intestinal inflammation via activation of the Toll-like receptor 2 with cell wall lipoteichoic acid. Toll-like receptor 2 stimulation preserves tight junction–associated barrier assembly against stress-induced damage through promotion of phosphatidylinositol 3-kinase/protein kinase B–mediated cell survival via myeloid differentiation factor 88. On the other hand, the gut innate immune system controls the overgrowth of pathobiotic bacteria inside the gut lumen.

**Bacterial translocation through the intestinal wall has long been recognized.** Exposure to toxins, drugs, pathogen, or local inflammation may affect the integrity of gut mucosa, leading to translocation of bacteria or their fragments into the systemic circulation. The classic scenario is the spontaneous bacterial peritonitis of liver cirrhosis. Most peritonitis episodes caused by *Enterobacteriaceae* species in patients on peritoneal dialysis (PD) are also caused by bacteria translocation.

Because intact bacteria can translocate through the intestinal barrier, it is logical to predict that nonviable but biologically active bacterial fragments could pass through even more readily. Notably, bacterial endotoxin, the cell wall component of Gram-negative bacteria, has been shown to be bioactive in the systemic circulation. An early study reported that plasma endotoxin levels were higher in patients with edematous heart failure than in patients without edematous heart failure and healthy volunteers. Short-term diuretic treatment led to a reduction in plasma endotoxin, although it did not affect other inflammatory cytokines. More recently, microbial translocation through the gut has been found to be a major cause of immune activation in patients with HIV type 1 (HIV-1). The plasma endotoxin level in patients with HIV-1 correlates with the degree of immune activation.

**Bacterial Fragments Present in the Systemic Circulation**

Among all possible bacterial fragments, bacterial endotoxin is most commonly measured in the systemic circulation, partly because the limulus amoebocyte lysate assay is readily available for quality assurance of the water supply in hemodialysis units. Other bacterial fragments that are identifiable in the systemic circulation include bacterial DNA fragments, peptidoglycan, and bacterial polysaccharide A.

**Mechanisms of Bacterial Fragment Translocation in CKD**

There are several causes of increased circulating levels of bacterial fragments in CKD patients, including gut dysmotility, bowel wall edema, overgrowth of pathobiotic bacteria, and loss of epithelial barrier integrity. Massive diffusion of urea into the gut and its conversion to ammonia and ammonium hydroxide plays a critical role. The metabolic changes in CKD alter the ecological balance in the gut, favoring the overgrowth of pathobiotic bacteria. Several metabolic changes in CKD may be responsible for inducing gut dysbiosis, including metabolic acidosis, organic uremic toxins, intestinal ischemia, volume overload, dietary changes, polymer phosphate binder, iron therapy, and frequent use of antibiotics. Excessive urease-producing bacteria and a decline in the production of short-chain fatty acids, which are the principal nutrients for colonic epithelial cells, also contribute to the leaky gut in cases of uremia. In addition, CKD is associated with prolonged gastric emptying, reduced fasting, and postprandial small bowel water content, indicating abnormal gastrointestinal motility and absorption. Reduced postprandial small bowel water content is correlated with plasma endotoxin level, suggesting that bowel wall edema leads to impaired gut barrier function. Taken together, the current evidence suggests that disruption of gut barrier function in CKD is the major mechanism that allows for the translocation of bacterial fragments to the systemic circulation.
per se contributes to endotoxiaemia. Circulating endotoxin level was observed to rise three-fold after dialysis was initiated.50 Recurrent hemodynamic stress and cardiac dysfunction, leading to altered intestinal perfusion and mucosal permeability, have been implicated in hemodialysis.50,51 In patients with AKI, hemodialysis and fluid removal result in a reduction of splanchic blood flow.52 In patients on chronic hemodialysis, the predialysis endotoxin level is correlated with dialysis-induced hemodynamic stress, myocardial stunning, serum cardiac troponin T, and C-reactive protein (CRP) levels.53

However, not all studies show that dialysis has these effects. Grant et al.53 found that superior mesenteric artery blood flow was similar between patients on PD and healthy controls. Although plasma endotoxin levels were significantly higher in the PD group, endotoxin levels did not correlate with baseline or postprandial superior mesenteric artery blood flow, body volume status, left ventricular mass index, or end-diastolic volume,53 suggesting that gut ischemia and edema were not directly related to the translocation of bacterial endotoxin in this setting.

**BACTERIAL ENDOTOXIN**

**Pathogenic Mechanisms**

Endotoxin is a phospholipid and a major component of the outer membranes of Gram-negative bacteria. It is partly transported into intestinal capillaries through a mechanism dependent on Toll-like receptor 4 (TLR4),54 and is then taken up by liver and mononuclear phagocytic cells.55 In healthy individuals, endotoxin can be detected in the systemic circulation at low concentrations (<200 pg/ml).56,57

Endotoxin translocation from the gut has long been suggested as a cause of systemic inflammation in CKD.17,58 Circulating endotoxin binds LPS-binding protein (LBP); this complex interacts with MD-2, which forms a complex with TLR4 and is anchored by CD14.59 TLR4 is a traditional pathogen-associated molecular pattern, the activation of which results in a downstream inflammatory cascade. Endotoxin concentration as low as 1 pg/ml can induce cellular activation and expression of CD14, which is a 55 kD glycosylphosphatidylinositol-anchored membrane protein (mCD14) that is also found as a soluble serum protein (sCD14). At low concentrations, LBP catalyzes the transfer of endotoxin to mCD14 on the immune cells, resulting in cytokine release.59 At higher concentrations, LBP transfers endotoxin to lipoproteins, which is eventually cleared from the circulation.

Endotoxin may trigger the initiation and progression of atherosclerosis by mediating endothelial cell injury, boosting monocyte recruitment, transforming macrophages to foam cells, and activating coagulant activity.50,60 Previous studies showed that sCD14 level is associated with the progression of renal function decline, CVD, and the mortality of patients with CKD.62–64 In addition, endotoxin contributes to the development of insulin resistance, obesity, and diabetes in mice.65 In patients on hemodialysis, predialysis plasma endotoxin levels are correlated with erythropoietin dosage and erythropoietin resistance index.66 Patients receiving conventional thrice weekly hemodialysis had higher predialysis serum endotoxin level than those receiving short daily hemodialysis or nocturnal hemodialysis, even after adjusting for age and diabetic status.57

**Relation with CVD in CKD**

Two studies have reported that plasma endotoxin level correlates with ultrafiltration rate in patients on hemodialysis67 and the drop in systolic BP during hemodialysis treatment.68 Plasma endotoxin level of patients on hemodialysis also correlates with cardiac troponin T and CRP levels.67 Another study showed graded increases in circulating endotoxin level with higher CKD stage.50 However, there was no association between circulating endotoxin level and vascular calcification, carotid–femoral pulse-wave velocity (PWV), or other factors relating to peripheral cardiovascular structure or function.50 Circulating endotoxin level was associated with an increased risk of mortality, but the association disappeared when corrected for cardiovascular risk factors,50 suggesting that the effect on mortality was mediated through its cardiovascular effects.

Patients on PD had higher plasma endotoxin level than predialysis patients with CKD, which was in turn higher than healthy controls.68 Plasma endotoxin level was significantly correlated with serum CRP and albumin levels.68 Patients with preexisting CVD had higher plasma endotoxin levels than those without CVD, and plasma endotoxin levels correlated with carotid intima media thickness.68 In another study on patients on PD, plasma endotoxin level correlated with the number of hospital admission and duration of hospitalization for cardiovascular reasons.69 In patients on hemodialysis, a cohort study found that plasma endotoxin level had a modest but significant correlation with serum CRP level and is an independent predictor of patient death within 3 years.70 In another study, plasma endotoxin level significantly correlated with serum soluble CD14 level, which is associated with inflammation and protein-energy wasting.65 Taken together, the results suggest that circulating endotoxin may contribute to the systemic inflammatory state, accelerated atherosclerosis, and clinical CVD in patients on dialysis.

However, the results of published studies are not always consistent. A study reported that high plasma endotoxin level was associated with better technique survival in patients on PD.71 The reason for this paradoxical observation is unknown, but could be because of the lack of a standardized method for measuring plasma endotoxin level, or the rapid clearance of endotoxin from the systemic circulation despite the systemic effect being sustained. In this regard, soluble CD14 could be a valuable marker of circulating endotoxin load.

**Interventional Studies**

Several strategies have been proposed for the manipulation of the intestinal
microbiome, including probiotics, prebiotics, synbiotics, oral adsorbents, and genetically engineered bacteria. However, few have examined the effect on plasma endotoxin level. Treating human epithelial cell with metabolites secreted by Bifidobacterium infantis causes an increase in tight junction proteins zonula occludens-1 and occludin and reduces claudin-2, suggesting that intestinal barrier function is restored. Similarly, probiotic bacteria enhance intestinal epithelial barrier function in murine models of colitis and in human Crohn disease. Administration of either pasteurized Akkermansia muciniphila or its outer membrane protein increases the expression of tight-junction proteins claudin 3 and occludin in murine intestine, alleviating endotoxia in mice fed a high-fat diet.

Strategies to restore the biochemical milieu of the gut may also be beneficial. For example, consumption of a diet supplemented with an indigestible, fermentable complex carbohydrate leads to significant improvements in gut microbiome and plasma endotoxin level. Rossi et al. noted that plasma endotoxin levels of patients with CKD was marginally lower during synbiotic therapy, but the difference was not statistically significant. In this study, synbiotic therapy also reduced serum indoxyl sulfate but not p-cresyl sulfate level.

Other human trials show reductions in endotoxin with established clinical treatments. For example, circulating endotoxin level significantly decreased with the introduction and optimization of antihypertensive therapy in both patients with and without CKD. The reduction in endotoxin level was greater in patients without CKD despite similar drug usage and BP. However, the reduction in endotoxin level did not correlate with the change in arterial PWV or hemodynamic status. In patients on hemodialysis, plasma endotoxin level fell by one third within 4 weeks of conversion to ultra-pure dialysate. In this study, the time-averaged plasma endotoxin level correlated with serum CRP level, carotid–femoral PWV, and malnutrition inflammation score. In essence, this study strongly supports the notion that circulating endotoxin has direct effects on systemic inflammatory state, and ultra-pure dialysate is effective in reducing circulating endotoxin in patients on hemodialysis. However, ultra-pure dialysate has already become standard practice in most developed countries. Because endotoxin level was not measured in the spent dialysate, it remains unclear whether the clearance of circulating endotoxin is increased or the gastrointestinal leak of endotoxin is reduced by the use of ultra-pure dialysate.

Taken together, there are few approaches that may effectively reduce plasma endotoxin level. Although some strategies report a significant reduction of plasma endotoxin level and associated cardiovascular or metabolic benefits, the magnitude of reduction in the endotoxin level is generally modest. More importantly, it remains uncertain whether the cardiovascular or metabolic benefits are actually causally linked to the reduction in endotoxia.

**BACTERIAL DNA FRAGMENTS**

Endotoxin may not be the only source of microbial inflammatory trigger. Other types of bacterial fragments are also present in the systemic circulation and probably contribute to the pathogenesis of systemic inflammation and CVD. Among all microbial components, a bacterial-derived DNA fragment is the most readily detectable. Most bacterial genomes contain the highly conserved 16S ribosomal RNA gene. DNA fragments of bacterial origin can be easily detected and discerned from human DNA. Plasma bacterial DNA level may be a superior marker of the circulating load of bacterial fragments because endotoxin is the cell wall component of Gram-negative bacteria, whereas bacterial DNA assay detects both Gram-positive and Gram-negative bacteria. Previous studies showed a significant but modest correlation between plasma bacterial DNA and endotoxin levels. Both bacterial DNA and endotoxin levels are correlated with serum CRP but not serum procalcitonin level, suggesting that bacterial fragments are related to systemic inflammation but not ongoing infection.

**Pathogenic Mechanisms**

Toll-like receptor 9 (TLR9) is a well-characterized sensor for bacterial DNA, although TLR9-independent DNA recognition mechanisms may also exist. TLR9 is another classic pathogen-associated molecular pattern, and its stimulation results in the activation of intracellular signaling pathways, such as the mitogen-activated protein kinase, PI3-kinase, and Jun N-terminal kinase pathways NF-κB and AP-1. In PMN, bacterial DNA has profound effect on cellular trafficking, induces chemokine expression, regulates expression of adhesion molecules, enhances phagocyte activity, and rescues PMN from constitutive apoptosis.  

Bacterial DNA fragments induce IL-6 in mononuclear cells and promote the survival of inflammatory cells in patients with CKD. The induction of systemic inflammation by bacterial DNA may aggravate atherosclerotic plaque instability and trigger cardiovascular events.

In addition to its proinflammatory properties, bacterial DNA may directly affect the cardiovascular system. For example, Paladugu et al. showed that bacterial DNA causes dose-dependent suppression of rat cardiac myocyte contraction in vitro. Further, the signaling pathway of direct myocardial depression is not well established.

**Observational Studies**

There are few published studies on the relationship between circulating bacterial DNA fragments and CVD. Patients on chronic dialysis have significantly higher plasma bacterial DNA levels than those with stage 1–2 CKD, marginally higher levels than those with stage 3–4 CKD, and similar levels to patients receiving hemodialysis or PD. Plasma bacterial DNA level of patients on incident PD was significantly correlated with serum CRP level, malnutrition inflammation score, and subjective global assessment score, but not Charlson Comorbidity Index score. In addition, plasma bacterial DNA levels predict cardiovascular events. In a prospective study of patients on
incident PD, plasma bacterial DNA level was an independent predictor of the composite cardiovascular end point in 24 months. Plasma bacterial DNA level also correlated with the number of hospital admissions and duration of hospitalization for cardiovascular reasons. In contrast, plasma endotoxin level had only a marginally significant effect in predicting cardiovascular events, and the correlation with hospitalization was less substantial. Furthermore, baseline plasma bacterial DNA level was significantly correlated with change in carotid–radial PWV in 12 months. In another study, plasma bacterial DNA quartile was associated with patient survival and peritonitis-free survival by univariate analysis, but the associations became insignificant after multivariate analysis to adjust for clinical confounding factors. Taken together, circulating bacterial DNA fragment may contribute to the hemodynamic changes and CVD of patients on PD, but the effect on patient survival is not confirmed. There are, however, no published data regarding patients on hemodialysis or predialysis with CKD.

Interventional Studies

Few studies examined the effect of intervention on serum bacterial DNA levels. In the study on ultrapure dialysate described previously, serum bacterial DNA fragment level (contrary to plasma endotoxin level) showed no significant change after ultrapure dialysate. Although the time-averaged serum bacterial DNA level correlated with malnutrition inflammation score and the subjective global assessment score, it did not correlate with serum CRP level or arterial PWV. The results of this study suggest that ultrapure dialysate has little effect on serum bacterial DNA fragment levels. To the best of our knowledge, there is no published study on the change in serum bacterial DNA fragment levels after the manipulation of intestinal microbiome.

OTHER BACTERIAL FRAGMENTS

In addition to endotoxin and bacterial DNA, other bacterial fragments could be found in the systemic circulation and may be of clinical relevance. For example, peptidoglycan, an essential component of the bacterial cell wall, stimulates the innate immune system via signaling through the pattern-recognition receptor nucleotide-binding oligomerization domain-containing protein 1 pathway. Polysaccharide A, produced by Bacteroides fragilis, induces the accumulation of forkhead box protein 3-positive regulatory T cells and production of IL10, which potentially modulate cellular immunity. The role of these and other bacterial fragments in CKD and their relation with CVD warrants further study.

CONCLUSIONS

Intestinal dysbiosis is increasingly recognized in CKD. Multiple mechanisms contribute to the proliferation of pathogens in the gut. Intestinal dysbiosis, fluid overload, and uremic toxins per se all impinge on the gut barrier function (Figure 1). As a result, translocation of bacterial fragments to the systemic circulation is enhanced, which leads to multiple biologic effects and represents an important non-traditional cardiovascular risk factor in CKD. Plasma endotoxin levels are markedly elevated in CKD, and are associated with the systemic inflammatory state, accelerated atherosclerosis, and clinical CVD in patients on dialysis. Several strategies have been tested to reduce plasma endotoxin levels, but the clinical benefit is still not established. Published data on the biologic effects and clinical relevance of other bacterial fragments, such as bacterial DNA, are promising but not confirmed. Further studies are also needed to delineate the pathogenic relation between circulating bacterial fragments and CVD, and to define the role of plasma bacterial fragment levels as a prognostic indicator of patients with CKD.

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