Circulating Endotoxemia: A Novel Factor in Systemic Inflammation and Cardiovascular Disease in Chronic Kidney Disease

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Summary
Background and objectives Translocated endotoxin derived from intestinal bacteria has a wide range of adverse effects on cardiovascular (CV) structure and function, driving systemic inflammation, atherosclerosis and oxidative stress. This study’s aim was to investigate endotoxemia across the spectrum of chronic kidney disease (CKD).

Design, setting, participants, & measurements Circulating endotoxin was measured in 249 patients comprising CKD stage 3 to 5 and a comparator cohort of hypertensive patients without significant renal impairment. Patients underwent extended CV assessment, including pulse wave velocity and vascular calcification. Hemodialysis (HD) patients also received detailed echocardiographic-based intradialytic assessments. Patients were followed up for 1 year to assess survival.

Results Circulating endotoxemia was most notable in those with the highest CV disease burden (increasing with CKD stage), and a sharp increase was observed after initiation of HD. In HD patients, predialysis endotoxin correlated with dialysis-induced hemodynamic stress (ultrafiltration volume, relative hypotension), myocardial stunning, serum cardiac troponin T, and high-sensitivity C-reactive protein. Endotoxemia was associated with risk of mortality.

Conclusions CKD patients are characteristically exposed to significant endotoxemia. In particular, HD-induced systemic circulatory stress and recurrent regional ischemia may lead to increased endotoxin translocation from the gut. Resultant endotoxemia is associated with systemic inflammation, markers of malnutrition, cardiac injury, and reduced survival. This represents a crucial missing link in understanding the pathophysiology of the grossly elevated CV disease risk in CKD patients, highlighting the potential toxicity of conventional HD and providing a novel set of potential therapeutic strategies to reduce CV mortality in CKD patients.


Introduction
Systemic inflammation is well recognized to be associated with an increased CV disease risk in patients with and without chronic kidney disease (CKD) (1). However, the mechanisms linking the observed associations are still largely unelucilated. Bacterial endotoxin is a lipopolysaccharide (LPS) and the major glycolipid component of the outer membrane of gram-negative bacteria, which comprise 70% of the total bacteria in the healthy human gut.

Exposure to endotoxin, a profoundly proinflammatory stimulus, results in release of a wide variety of proinflammatory cytokines and binding via CD14 to systemic immune competent cells (2). It results in a broad range of negative cardiovascular (CV) effects including peripheral vasodilation and reduction in cardiac contractile performance (3).

Endotoxin (without sepsis) was initially proposed as a stimulus for immune activation in the proinflammatory state of congestive heart failure (4). Endotoxin is released by bacterial cell wall breakdown within and beyond the gut lumen, from effective host defense mechanisms and by autolysis. Endotoxin enters the circulation via bacterial translocation (passage of intact bacteria and macromolecules such as endotoxin across the intestinal barrier [5]), with bowel edema and hypoperfusion being the two main factors influencing bowel wall permeability in congestive heart failure (6).

Dialysis patients are characteristically volume overloaded. Hemodialysis (HD) in combination with ultrafiltration results in significant systemic hemodynamic perturbation and clinically significant reduction of regional perfusion in critical organs such as the heart (7,8). Such repeated ischemic injury to this vulnerable vascular bed results in acute cardiac in-
were run in duplicate and background subtracted. All samples from each patient to allow cross-sectional comparison of endotoxin levels. Serum lipopolysaccharide quantification was performed using a Limulus Amebocyte assay (Cambrex, Verviers, Belgium) as described previously (17). Carotid-femoral pulse wave velocity (PWV) was measured using a SphygmoCor (AtCor Medical Pty., Ltd., Australia). All PD, CKD stages 3 to 4 patients, and the non-CKD comparator group had VC and PWV assessed. Fifty-six percent of HD patients (68 of 120) were similarly assessed. All nondia-
yzed CKD stage 5 patients had PWV measured and 64% (16 of 25) had VC measured.

Effect of Dialysis on Endotoxemia
A group of 66 prevalent HD patients were recruited from a single hospital-based HD unit to undergo more detailed evaluation of factors associated with endotoxemia, including intradialytic BP and intradia-
lytic myocardial stunning, with blood samples (including endotoxin levels) pre- and postdialysis. Twelve pediatric HD patients were also studied. Patients were excluded if they had pre-existing severe systolic dysfunction (New York Heart Association class III to IV) or inadequate echocardiographic windows (one patient excluded). All studies were con-
ducted after the first 2-day interdialytic period. Adult patients were dialyzed using dual-pass water treat-
ment with undetectable levels of endotoxin. Patients were categorized as intradialytic hypotension prone if experiencing a systolic BP (SBP) <100 mmHg or a SBP fall of >40 mmHg.

Echocardiographic Assessment
As described previously, two-dimensional echocardiography was performed before commence-
ment (pre-HD), during HD at 2 and 4 hours, and 30 minutes into the recovery period (post-HD) to evaluate the presence and extent of HD-induced re-
gional wall motion abnormalities (19,20). Measurement of segmental fractional shortening was made subsequently (Echo-CMS; MEDIS, The Nether-
lands) with new regional wall motion abnormalities classified as segments showing a decline in percent segmental fractional shortening >20% from baseline. Regions with evidence of functional recovery in the postdialysis period were classed as stunned segments. Left atrial volume (LAV) was calculated by biplane disc method and indexed to height2.7 (LAVI).

Statistical Analyses and Sample Size Calculation
The primary endpoint was to detect a 50% difference in circulating endotoxin levels between pa-
tients receiving dialysis and those not. A sample size of at least 40 patients in each of the comparison groups was needed to detect this difference at 90% power. Final sample size was larger to allow for further investigation of factors relating to endotox-
emia and patient dropout. Group data are presented as mean ± SD unless
<table>
<thead>
<tr>
<th>Parameter</th>
<th>All Patients (n = 249)</th>
<th>Dialysis</th>
<th>CKD</th>
<th>Controls (n = 14)</th>
<th>P</th>
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<tr>
<td></td>
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<td>HD (n = 120)</td>
<td>PD (n = 25)</td>
<td>Stage 5 (n = 25)</td>
<td>Stage 4 (n = 49)</td>
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<td>Age (years; mean ± SD)</td>
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<td>61 ± 14</td>
<td>60 ± 14</td>
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<td>eGFR (ml/min; mean ± SD)</td>
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<td>Diabetes mellitus (n [%])</td>
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<td>39 (33%)</td>
<td>8 (31%)</td>
<td>7 (27%)</td>
<td>15 (31%)</td>
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<td>Previous CV comorbiditiesb (n [%])</td>
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<td>39 (33%)</td>
<td>11 (42%)</td>
<td>8 (31%)</td>
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<td>48 (19%)</td>
<td>4 (15%)</td>
<td>14 (8%)</td>
<td>4 (27%)</td>
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<td>25 (100%)</td>
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<td>4 (3%)</td>
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<td>diabetic nephropathy</td>
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<td>30 (25%)</td>
<td>8 (32%)</td>
<td>5 (20%)</td>
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<td>glomerular disease</td>
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<td>25 (21%)</td>
<td>4 (16%)</td>
<td>5 (20%)</td>
<td>8 (16%)</td>
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<td>8 (7%)</td>
<td>1 (4%)</td>
<td>2 (8%)</td>
<td>3 (6%)</td>
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<tr>
<td>urological</td>
<td>21 (9%)</td>
<td>12 (10%)</td>
<td>4 (16%)</td>
<td>2 (8%)</td>
<td>3 (6%)</td>
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<tr>
<td>renovascular</td>
<td>18 (8%)</td>
<td>8 (7%)</td>
<td>2 (8%)</td>
<td>1 (4%)</td>
<td>5 (10%)</td>
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<tr>
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<td>15 (12%)</td>
<td>4 (16%)</td>
<td>3 (12%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>unknown</td>
<td>54 (23%)</td>
<td>22 (18%)</td>
<td>2 (8%)</td>
<td>7 (28%)</td>
<td>12 (25%)</td>
</tr>
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<td>Albumin (g/L)</td>
<td>35 ± 5</td>
<td>35 ± 4</td>
<td>27 ± 4</td>
<td>35 ± 1</td>
<td>36 ± 3</td>
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<td>Phosphate (mmol/L)</td>
<td>1.52 ± 0.40</td>
<td>1.65 ± 0.46</td>
<td>1.57 ± 0.24</td>
<td>1.62 ± 0.29</td>
<td>1.33 ± 0.23</td>
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<td>Calcium (mmol/L)</td>
<td>2.42 ± 0.14</td>
<td>2.44 ± 0.13</td>
<td>2.51 ± 0.11</td>
<td>2.36 ± 0.14</td>
<td>2.33 ± 0.10</td>
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<td>PWV (m/s; mean ± SD)</td>
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<td>105 ± 3.2</td>
<td>92 ± 29</td>
<td>9.1 ± 3.1</td>
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<td>SFA CaSc (median [IQR])</td>
<td>–</td>
<td>142 (245 to 622)</td>
<td>29 (68 to 264)</td>
<td>12 (46 to 198)</td>
<td>2 (7 to 176)</td>
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</tbody>
</table>

*GFR, estimated GFR; APKD, adult polycystic kidney disease; SFA CaSc, superficial femoral artery calcification score. Results analyzed using one-way ANOVA and Kruskal–Wallis test where appropriate, with Tukey post-test for normally distributed data and χ² test for nonparametric data.

Kt/V in HD is per single session and in PD is weekly.

Defined as any previous description of ischemic heart disease, heart failure, cerebrovascular disease, or peripheral vascular disease recorded in the patient’s medical notes.

Albumin: comparison of HD with controls and CKD stage 3, P < 0.001; comparison of PD with all other groups, P < 0.001; comparison of CKD stage 5 with controls and CKD stage 3, P = 0.003.

Phosphate: comparison of HD with controls, CKD stages 3 and 4, P < 0.001, comparison of PD with controls, P = 0.007, with CKD stage 3, P = 0.016, comparison of CKD stage 5 with controls, P = 0.002, with CKD stage 4, P = 0.021, with CKD stage 3, P = 0.004.

Corrected calcium: comparison of HD with CKD stage 4, P < 0.001, with CKD stage 5, P < 0.023; comparison of PD with CKD stage 4 and 5, P < 0.001.

Sixty-eight of 120 HD patients underwent PWV measurement.

Sixty-eight of 120 HD patients and 56 of 74 CKD stages 4 and 5 patients had SFA calcium scoring performed.
Effect of HD on Endotoxemia

Eighteen patients (all with no previous HD use or vascular access other than native arteriovenous fistulas) commenced HD from the studied low-clearance population during the study period. Initial and second measurements of circulating endotoxin were taken no longer than 12 weeks before or after starting HD. All patients commenced HD electively with eGFRs of 10 to 15 ml/min. HD initiation was associated with a significant increase in circulating endotoxin level, rising from 0.13 ± 0.3 EU/ml to 0.34 ± 0.42 EU/ml (P = 0.002). Dialysis vintage in established HD patients did not correlate to endotoxin levels, suggesting that the increment postdialysis initiation is not related to loss of residual renal function.

Forty-one of 66 of established HD patients studied exhibited significant levels of dialysis-induced myocardial stunning. Twenty-one of 66 patients were defined as suffering significant intradialytic hypotension. Predialysis circulating endotoxin levels showed a statistically significant correlation with myocardial stunning severity (r = 0.44, P = 0.035) and maximum reduction in SBP and DBP during HD (r = 0.45, P = 0.032). Levels were significantly correlated with evidence of dialysis-related cardiac injury, with a significant correlation with predialysis cardiac troponin T (r = 0.34, P = 0.005) in the whole HD group.

There was little evidence of volume overload being significantly associated with the degree of endotoxemia. In addition to the lack of significant correlation of endotoxemia with N-terminal pro-brain natriuretic peptide (as a composite biochemical marker for volume overload), there was no positive correlation with LAVI (as an echocardiogram-based measure of volume overload). The degree of endotoxemia positively correlated with high-sensitivity C-reactive protein (hsCRP; r = 0.42, P = 0.047) but not with IL-6 levels (Figure 2a).

In patients dialyzing with polysulfone membranes, circulating mean endotoxin levels fell over the period of the HD treatment (0.68 ± 0.30 EU/ml versus 0.53 ± 0.30 EU/ml, P = 0.004). Post-HD circulating endotoxin levels were still significantly correlated with fluid removal (r = 0.24, P = 0.001) (Figure 2b). There was an increase in circulating endotoxin level during the HD session in 8 of the 12 pediatric patients who dialyzed using cellulose acetate-based membranes (0.72 ± 0.02 versus 0.86 ± 0.017, P = 0.054).

Survival was assessed in the HD patients because they had the largest range of circulating endotoxin levels and the highest number of deaths. Endotoxemia was strongly associated with an increased risk of death (P = 0.034) (Figure 3). This association disappeared when corrected for adverse dialysis-related CV factors (myocardial stunning, ultrafiltration volume, and intradialytic fall in BP).

Discussion

We have demonstrated that significant endotoxemia is common in patients with advanced CKD. Endotoxemia appears to be aggravated by initiation of dialysis.
and is higher in those HD patients with the greatest degree of dialysis-induced hemodynamic instability, who also exhibit high degrees of dialysis-induced myocardial stunning. Elevated levels of circulating endotoxin are significantly associated with reduced survival. The more severe degree of renal impairment is associated with significantly greater levels of circulating endotoxin. This has been previously reported in PD patients (21) but has otherwise been largely unappreciated (22,23). A follow-up PD study that focused on this highly selected patient cohort with limited access to HD did not find a negative effect of endotoxemia on survival (24). The biologic fate of plasma-free endotoxin is well known and relies on humoral inactivation and uptake into liver and mononuclear phagocyte cells (25–28) rather than renal clearance. Endotoxins are complex, amphiphilic macromolecules of up to 1000 kD in molecular weight, making free glomerular filtration unlikely, supported by research showing endotoxin is not present in sterile urine (29).
Figure 2. (a) In HD patients with intradialytic hypotension, predialysis endotoxin levels were significantly correlated with (i) inflammation; (ii) the number of myocardial stunned segments; (iii) intradialytic hypotension; and (iv) predialysis cardiac troponin T, a marker of myocardial damage. (b) Postdialysis endotoxin levels were significantly correlated with ultrafiltration volume.
The levels seen in patients receiving dialysis are extremely high, comparable with those reported in severe liver disease (30), gut irradiation (31), and severe decompensated heart failure (4). Significant heart failure (New York Heart Association class III to IV) was an exclusion criterion and, in the HD patients, endotoxin levels did not correlate with markers of volume overload. The greater CV disease burden characteristic of patients with more severe CKD may be a critical factor predisposing to demand ischemia in the gut. The relative contribution of the factors likely to influence endotoxin translocation (intestinal bacterial load, membrane permeability, gut edema, and ischemic intestinal injury) may vary across the spectrum of CKD.

In CKD patients, low serum albumin appears to clearly correlate with the degree of endotoxemia. This is in keeping with the only previous report (in PD patients) (21). There was a lack of association with hsCRP in the patient group as a whole, in contrast to the association in HD patients (with a generally higher degree of systemic inflammation). This may be attributable to the narrow range of hsCRP values or differences in immunoactivity. Tachyphylactic response to endotoxin has been previously described. There was no observed association with VC or markers of arterial stiffness.

The complex biology resulting in systemic endotoxemia in dialysis patients requires further elucidation because dialysis modality may contribute to endotoxin translocation by different underlying mechanisms. We postulate venous congestion and edema are dominant in PD, in contrast to recurrent regional ischemia in HD. However, further assessment of these putative mechanisms is required.

HD itself appears to be responsible for increasing exposure to translocated intestinal endotoxin, as evidenced by a large difference between patients with very severe CKD stage 5 but not yet started on dialysis and those receiving dialysis. Predialysis CKD stage 5 patients are very similar for demographic factors and comorbidities when compared to patients established on HD. After commencing HD, patients swiftly demonstrated a marked increase in endotoxemia, potentially resulting from dialysis-induced splanchnic hypoperfusion.

In the HD patients who underwent echocardiography, left ventricular ejection fraction was relatively well preserved with no relationship between degree of endotoxemia and markers of volume overload (N-terminal pro-brain natriuretic peptide, LAVI). Children (with low prevalence of CV comorbidities and little evidence of increased left ventricular mass) exhibited the highest levels.

In contrast, there was direct evidence of the severity of the hemodynamic insult being related to the severity of the endotoxemic state. In patients who were unstable during HD, there was a direct correlation between magnitude of the fall in SBP and DBP with level of circulating endotoxin. HD is well described as being capable of inducing recurrent cardiac ischemic injury, associated with reduced segmental myocardial perfusion (8). Circulating endotoxin levels were highly correlated with cardiac troponin T as a biochemical marker of cardiac injury. The enteric circulation is exposed to a very similar set of predisposing factors to demand ischemia, including large-vessel atheroma, microcirculatory disturbances, increasing shear stress with ultrafiltration, and a significant demand on overall cardiac output (32). There was a significant correlation between severity of HD-induced cardiac stunning and endotoxin level. It is therefore possible that the observed changes in cardiac function might be a result of, or aggravated by, the direct myocardial effects of endotoxin (3).

The time course and interaction of CV, hemodynamic, and enteric responses when patients are repeatedly exposed to HD-induced systemic circulatory stress require further study to elucidate events in the potentially self-propagating cycle of regional ischemia, increased gut permeability, inflammation, and cardiac dysfunction.

We did not observe an increase in circulating levels of endotoxin during HD therapies, although post-HD levels of endotoxin still significantly correlated with ultrafiltration volume. Translocation may occur predominantly in the postdialytic period, which we did not have access to samples from. Other possibilities are that there was sequestration of endotoxin during the HD treatment as an effect of monocyte activation, which is commonly seen during extracorporeal circulation, or by direct adsorption onto the dialysis membrane. The polysulfone material used in most of these treatments is well described as having a potent ability to adsorb endotoxin and a wide variety of other circulating substances (33), deriving its high biocompatibility status from the ability to buffer complement and other factors within the reactive cascade. Cellulose acetate does not share the same potent binding characteristics as polysulfone (34) and was associated with an increase in circulating endotoxin over a dialysis session. Heparin (used to maintain the extracor-
poreal circuit) is also associated with a dose-dependent inhibition of the endotoxin assay.

In conclusion, this is the first study to systematically examine endotoxia across the spectrum of CKD. The key findings of endotoxia potentially driving interlinked malnutrition, inflammation, and CV disease in CKD patients represent an important advance in understanding the pathophysiology of grossly elevated CV mortality rates in this population. We have identified the role HD plays inducing systemic circulatory stress, predisposing to reduction of intestinal perfusion and exposure to sustained significant endotoxia (with resultant increase in risk of mortality). Not only do these insights highlight further the potential toxicity of conventional dialysis, but they also provide a justification for a frame shift in the search for potential therapeutic targets to reduce CV attrition. These might include a focus on dialysis-related interventions to reduce circulatory stress, reduction of gut venous congestion/edema, and reduction/sequestration of the intestinal reservoir of bacterially derived endotoxin.

Acknowledgments

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- C.W.M.—study conception and design; data acquisition, analysis, and interpretation; manuscript drafting and revision; and final responsibility for the analyses and manuscript content.
- C.C.S., K.B.L., P.K.T.L.—endotoxin assay, data collection, and manuscript preparation.

Disclosures

None.

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