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CLINICAL RESEARCH

Exploring the Link Between Hepatic Perfusion and Endotoxemia in Hemodialysis

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Introduction: The liver receives gut-derived endotoxin via the portal vein, clearing it before it enters systemic circulation. Hemodialysis negatively impacts the perfusion and function of multiple organs systems. Dialysate cooling reduces hemodialysis-induced circulatory stress and protects organs from ischemic injury. This study examined how hemodialysis disrupts liver hemodynamics and function, its effect on endotoxemia, and the potential protective effect of dialysate cooling.

Methods: Fifteen patients were randomized to receive either standard (36.5°C dialysate temperature) or cooled (35.0°C) hemodialysis first in a two-visit crossover trial. We applied computed tomography (CT) liver perfusion imaging to patients before, 3 hours into and after each hemodialysis session. We measured hepatic perfusion and perfusion heterogeneity. Hepatic function was measured by indocyanine green (ICG) clearance. Endotoxin levels in blood throughout dialysis were also measured.

Results: During hemodialysis, overall liver perfusion did not significantly change, but portal vein perfusion trended towards increasing ($P = 0.14$) and perfusion heterogeneity significantly increased ($P = 0.038$). In addition, ICG clearance decreased significantly during hemodialysis ($P = 0.016$), and endotoxin levels trended towards increasing during hemodialysis ($P = 0.15$) and increased significantly after hemodialysis ($P = 0.037$). Applying dialysate cooling trended towards abrogating these changes but did not reach statistical significance compared to standard hemodialysis.

Conclusion: Hemodialysis redistributes liver perfusion, attenuates hepatic function, and results in endotoxemia. Higher endotoxin levels in end-stage renal disease (ESRD) patients may result from the combination of decreased hepatic clearance function and increasing fraction of liver perfusion coming from toxin-laden portal vein during hemodialysis. The protective potential of dialysate cooling should be explored further in future research studies.

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Hemodialysis (HD) induces circulatory stress which causes mesenteric ischemia, leading to disrupted gut mucosal structure and function.[1,2] The resulting increase in translocated endotoxin (gut-derived proinflammatory mediators) correlates with a multitude of hemodynamic and cardiovascular complications.[3] The liver normally receives endotoxin from the gut via portal vein blood[4] and under healthy conditions clears it before it reaches systemic circulation.[5] However, increased endotoxin has been found in HD patients compared to the general population and to early-stage chronic kidney disease patients,[3] suggesting that HD may disrupt liver hemodynamics and function, allowing more gut-derived products to reach the systemic circulation.

Multiple organs develop subclinical ischemia due to intradialytic circulatory stress.[6] Functional imaging studies in the heart,[7,8] brain,[9] and kidneys[10] have shown that these ischemic insults can be attenuated

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using dialysate cooling (DC),[11] providing intradialytic hemodynamic protection and minimization of chronic organ dysfunction. However, the liver's dual blood supply may allow it to potentially respond differently to HD-induced circulatory stress.[12] Previous work by our group and others has shown that liver hemodynamics and water content are not significantly affected by HD.[12-14] Even so, these studies have not assessed changes in the fractional supply (from portal vein or hepatic artery) and how it is related to endotoxemia in HD patients, or the effect of potentially protective interventions.

Therefore, we conducted an exploratory study of liver perfusion and excretory function using CT perfusion imaging to measure hepatic blood flow (derived separately from portal vein and hepatic artery) during HD. By assessing intradialytic hepatic perfusion and function, we aimed to test the hypothesis that HD disrupts liver hemodynamics and drives endotoxemia. In addition, we intended to explore whether DC ameliorates HD-induced changes in liver hemodynamics and limits systemic exposure to endotoxin.

METHODS

Patients

Patients from the London Health Sciences Centre Regional Renal Program (London, Ontario, Canada) were enrolled in the study after giving informed consent. Adult patients with HD vintage $\geq$3 months and low residual renal function ($<$250 ml/d to limit any potential effects of contrast-induced nephropathy) were eligible. Major exclusion criteria included the following: chronic liver or intestinal disease (excluding irritable bowel syndrome), previous liver transplant or resection, transjugular portosystemic shunt insertion, active infection/malignancy, current or planned pregnancy, breast feeding, uncontrolled diabetes mellitus (defined as recorded hypoglycemia during HD within the last 2 months), or known allergy to iodinated contrast agent.

Study Design

In this crossover study, patients underwent one standard (36.5°C) and one cooled (35.0°C) dialysate temperature HD session. Other than dialysate temperature, sessions were identical. Session order was randomly assigned, with patients acting as their own controls. The randomization list (in blocks of four) was generated by a London Health Sciences Centre Kidney Clinical Research Unit medical statistician and revealed to the investigator for allocation to the appropriate study group in the form of randomization envelopes. A washout period $\geq$7 days between sessions was scheduled to ensure no significant carryover effects.[15] During these sessions, we collected baseline characteristics and blood work, assessed hepatic function, and acquired CT liver perfusion imaging. This was a single-blinded study in which patients, HD unit staff, and the investigator were not blinded to the intervention; however, imaging analysis was performed with the operator blinded to allocation.

This study was approved by the University of Western Ontario Health Sciences Research Ethics Board and was conducted in compliance with approved protocols, Good Clinical Practice Guidelines, the Declaration of Helsinki, and all applicable regulatory requirements. The study was registered at ClinicalTrials.gov (NCT02997774).

CT Perfusion Imaging

CT liver perfusion imaging was performed on a GE Healthcare (Waukesha, WI) Revolution 256-slice CT scanner just before, 3 hours into (i.e., peak intradialytic stress), and 15 minutes post discontinuation of both HD sessions. Patients were moved to the CT bed for the intradialytic scan without interrupting HD treatment. Dynamic contrast-enhanced CT scanning of a 16-cm abdominal section was performed, without breath-hold, following iodinated contrast agent injection. Scan regions were optimized to include as much liver as possible and were divided into 32 slices of 5-mm thickness each. This region was scanned 42 times at 2.8-second intervals using 120 kV and 22.4 mAs for approximately 2 minutes. Iopamidol (Isovue 370, Bracco Imaging, Milan, Italy) at 1 ml/kg of pre-HD patient weight (up to maximum dose of 70 ml) was used as contrast agent in both HD sessions. Image noise was reduced using 100% ASIR (Adaptive Statistical Iterative Reconstruction, GE Healthcare) and liver motion from breathing between scans was minimized using nonrigid registration (GE Healthcare).

CT Perfusion 4D software (GE Healthcare) was used to analyze the registered images as follows: aortic and portal venous regions of interest (ROIs) were selected for generation of arterial and venous input functions, respectively. Next, model-based deconvolution[16] was used to compute liver perfusion and corresponding hepatic arterial fraction, yielding total liver, hepatic arterial, and portal venous perfusion maps. ROIs were then manually drawn over the liver in perfusion maps to encompass parenchymal areas. An ROI size-weighted average of the resulting perfusion values over the selected slices (i.e., slices containing contoured liver parenchyma) was performed to obtain mean perfusion values (in ml/min/100 g) for the whole liver.

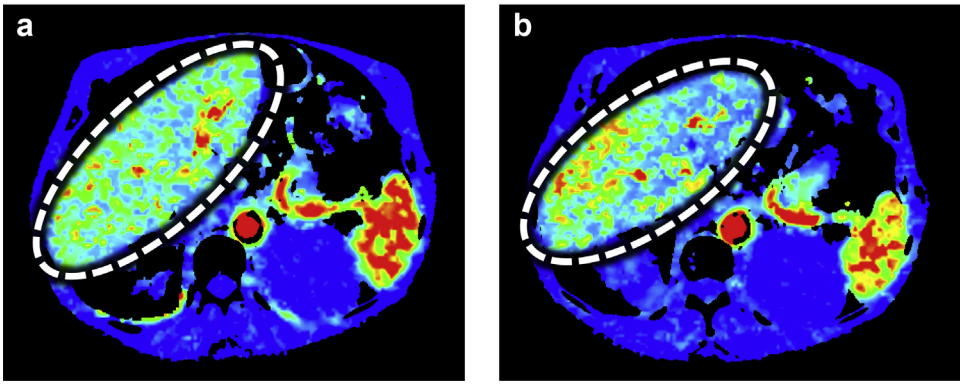

Figure 1. Insensitivity of global mean to changes in spatial perfusion heterogeneity visualized with hepatic perfusion maps. Total liver perfusion at baseline (a) and 3 hours into hemodialysis (b) for a patient. Liver parenchyma is outlined with dotted contours. Despite there being no measurable change in average liver perfusion between the two time-points, liver perfusion heterogeneity increased by approximately 25%.

Quantification of Perfusion Heterogeneity

Based on previous work,[12,13] we hypothesized that HD may cause heterogeneous redistribution of liver perfusion despite overall perfusion being maintained (Figure 1). Total liver perfusion heterogeneity was quantified using an in-house MATLAB (MathWorks, Natick, MA) program based on a previously developed algorithm.[17] For this work, the algorithm quantified the magnitude of perfusion gradation between all pixel-pair combinations of the liver in every slice.

The previously drawn liver parenchymal ROIs were used to segment the liver parenchyma for all relevant slices. The algorithm was then applied to all non-zero pixel-pairs within each slice's segmented region, yielding a single perfusion heterogeneity value for each region (i.e., for each slice). An ROI size-weighted average of the heterogeneity values over the selected slices was performed to obtain a mean perfusion heterogeneity value for the whole liver. A summary of this procedure is presented in Supplementary Figure S1.

Assessment of Hepatic Function

ICG is a synthetic dye solely taken up by hepatocytes and excreted in bile,[18] where ICG clearance from blood reflects excretory liver function. Pulsed-dye densitometry (DDG devices, Nihon Kohden, Japan) provides a real-time, noninvasive blood ICG concentration measurement using optical light at two wavelengths: 805 nm (maximal ICG absorption) and 890 nm (minimal absorption[19]). Detection of ICG is based on fractional changes in optical absorption between the two wavelengths, where heartbeat-induced blood vessel pulsations lead to optical path length changes.[20] Therefore, pulsed-dye densitometry measures ICG clearance from blood, reflecting excretory liver function. Although ICG has historically also been used to assess liver blood flow, this application carries methodological challenges[21,22] and was forgone in favor of using CT perfusion imaging.

Pulsed-dye densitometry was acquired for approximately 15 minutes following a single ICG bolus injection through a peripheral cannula by attaching a finger probe to the patient, measuring ICG concentration in blood over time with every heartbeat. These measurements were performed before and 3 hours into every HD session. A biexponential fit, based on an open two-compartment model of ICG uptake and excretion, was applied to the data to extrapolate past the 15-minute point and more accurately determine clearance.[23] The ICG clearance rate (ml/min) was calculated as the quotient of ICG dose (mg) and area under the ICG concentration versus time curve (mg·min/ml).

Quantification of Endotoxin Levels

Serum lipopolysaccharide endotoxin quantification was performed using a Limulus Amebocyte assay (Cambrex, Verviers, Belgium). Following collection in London, all serum samples were shipped to and assayed at the Prince of Wales Hospital in Hong Kong, and endotoxin quantification was performed as described previously[24] to ensure comparability with results generated from our previous studies of endotoxin (all of which were analyzed in the same lab). Briefly, samples were diluted to 20% with endotoxin-free water and heated to 70°C for 10 minutes to inactivate plasma proteins. The manufacturer's protocol was used to quantify serum lipopolysaccharide. Samples with lipopolysaccharide level below the detection limit of 0.01 EU/ml were taken as 0 EU/ml. Samples were run in duplicate and background noise was subtracted.

Statistical Analysis

Liver perfusion and function has been scarcely assessed previously in the context of HD and insufficient data exist to support performing a meaningful sample size calculation. This was an initial proof-of-principle study for hypothesis generation, with a sample size not powered for inferential statistical analysis. However,

the sample size is comparable with previously published norms[7,10,25,26] and recommendations,[27,28] and was chosen on a partly pragmatic basis.

All statistical analyses were performed using SPSS, version 25.0 (IBM, Chicago, IL). Repeated measure analysis of variance with Bonferroni-corrected *post hoc* Student *t* tests, and baseline-adjusted analysis of covariance, were used to detect differences between groups and subgroups. Pearson's product-moment correlation coefficient was used to determine associations between variables, and McNemar's test was used to detect differences between proportions. Two-tailed P values <0.05 were considered statistically significant. Results are presented as mean ± standard error of the mean, unless otherwise specified.

RESULTS

Clinical Characteristics of Study Population

Sixteen patients (10 males) aged 45 to 84 years were enrolled in this study. One patient was unable to return for the second session and was excluded from analysis (Supplementary Figure S2). Fifteen patients completed the study, and Table 1 presents the summary of patient baseline characteristics, including age, sex, HD treatment details, and comorbidities.

Hepatic Perfusion

Average baseline total liver perfusion was 82.7 ± 3.7 mL/min/100g, with an average hepatic arterial fraction of 22.6%. Average total liver, hepatic arterial and portal venous perfusion changed to 106.7% ± 5.4%, 101.3% ± 11.2%, and 111.1% ± 5.1% of baseline at peak HD stress, and 105.6% ± 3.7%, 102.7% ± 9.6%, and 109.1% ± 6.0% of baseline after HD, respectively. None of these changes were statistically significant, but portal vein perfusion showed the greatest trend towards changing during HD ($P = 0.14$) (Figure 2a). Perfusion heterogeneity increased by 12.5% ± 4.4% ($P = 0.038$) and 17.8% ± 3.7% ($P = 0.001$) with respect to baseline during and after HD, respectively (Figure 3a). There was an association between intradialytic changes in perfusion heterogeneity and total liver perfusion ($r = 0.70$, $P = 0.003$).

Table 1. Baseline Characteristics of Study Population (N = 15)

Characteristics	Median (Range)[a]
Age, yrs	63 (45–84)
Men	10 (67)
Dialysis vintage, yrs	3.0 (0.8–25.4)
Length of hemodialysis session (hours)	3.6 (3.1–4.2)
Ultrafiltration, ml/kg	23.3 (6.1–40.9)
Coronary artery disease	5 (33)
Congestive heart failure	3 (20)
Peripheral vascular disease	3 (20)
Diabetes	9 (60)
Hypertension	14 (93)

[a]Values shown are median (range) or n (%).

Hepatic Function

The ICG clearance rate decreased by 14.5% ± 5.3% ($P = 0.016$) with respect to baseline during HD (Figure 3a). Changes in ICG clearance rate correlated with changes in total liver perfusion ($r = 0.55$, $P = 0.034$) and showed an associative trend with changes in perfusion heterogeneity ($r = 0.49$, $P = 0.06$) during HD.

Endotoxin Levels

Average baseline endotoxin levels were 0.292 ± 0.0156 EU/ml and correlated with dialysis vintage ($r = 0.58$, $P = 0.024$). Endotoxin increased by 19% ± 9.1% ($P = 0.15$) and 28.4% ± 9.9% ($P = 0.037$) with respect to baseline during HD and after HD, respectively (Figure 3a). Increased post-HD endotoxin correlated with the presence of congestive heart failure ($r = 0.52$, $P = 0.046$) but not with ultrafiltration (UF) metrics (UF volume, mean, and maximum UF rates).

Effects of DC

In contrast to standard HD, changes from baseline in all hepatic perfusion, hepatic function, and endotoxin data during and after cooled HD were not statistically significant (Figures 2b and 3b). Although DC trended towards mitigating the hemodynamic and functional HD-induced changes that were observed for standard HD, session-specific baseline-adjusted analysis of covariance revealed that the changes in perfusion (total, hepatic arterial, and portal venous), perfusion heterogeneity, ICG clearance rate, and endotoxin level between dialysis treatments were not statistically significantly different.

Intradialytic Blood Pressure and Adverse Events

Three patients experienced intradialytic hypotension during both HD sessions, whereas 10 experienced a systolic blood pressure drop >20 mm Hg during standard HD compared with eight during cooled HD ($P = 0.69$). Intradialytic changes in blood pressure between dialysis treatments were not statistically significant according to session-specific baseline-adjusted analysis of covariance ($F(1,26) = 2.814$ and $P = 0.11$; $F(1,26) = 0.582$ and $P = 0.45$; $F(1,26) = 0.382$ and $P = 0.54$ for systolic blood pressure, diastolic blood pressure, and mean arterial pressure, respectively). During standard HD, increased endotoxin levels showed an associative trend with the maximum reduction in mean arterial pressure ($r = 0.47$, $P = 0.08$). In terms of adverse events from DC, only thermal symptoms were reported: six patients reported feeling cold or

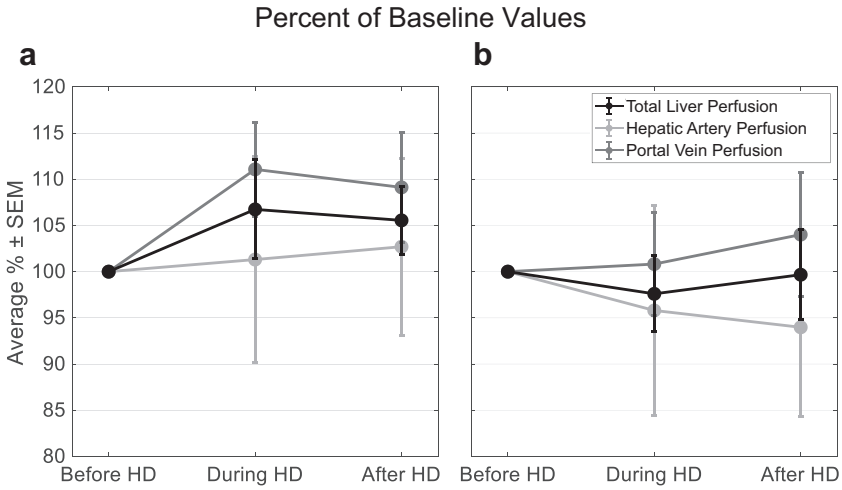

Figure 2. Changes in total liver, hepatic artery, and portal vein perfusion before, 3 hours into, and after hemodialysis (HD), with standard HD (a) and cooled HD (b). There were no significant changes in total liver, hepatic artery, and portal vein perfusion over the course of either standard or cooled HD. However, portal vein perfusion showed the greatest trend towards increasing during standard HD ($P = 0.14$). Results are given as average ± standard error of the mean (SEM).

experienced shivering during cooled HD, compared to two during standard HD ($P = 0.13$).

Additional exploratory results and discussion regarding liver perfusion heterogeneity can be found in the Supplementary Material, with findings presented in Supplementary Figure S3.

DISCUSSION

This is the first study to show that redistribution of liver perfusion and attenuation of hepatic function occurs contemporaneously during HD. In addition, this is the first time DC has been applied in the context of hepatic protection and endotoxemia mitigation. The important findings of this work may help to better understand how HD negatively affects the liver and results in the perpetuation of endotoxemia in maintenance HD patients, while also providing preliminary data to warrant further exploration of a potentially preventative intervention to limit systemic toxin exposure during HD and over the long-term.

Effects of HD on Hepatic Perfusion, Hepatic Function, and Endotoxemia

In this work, hepatic perfusion was measured using CT perfusion imaging, an approach which has been previously validated.[29] Overall liver perfusion did not significantly change during HD. Although there is a

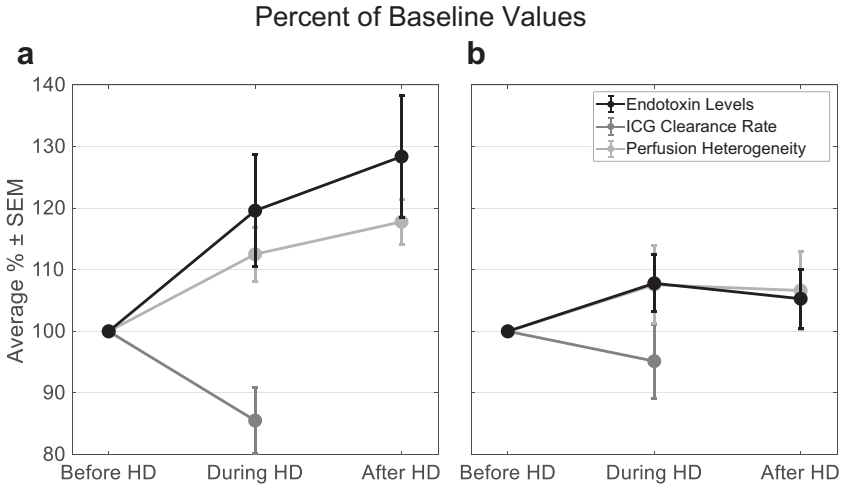

Figure 3. Changes in endotoxin levels, indocyanine green (ICG) clearance rate and perfusion heterogeneity before, 3 hours into and after hemodialysis (HD), with standard HD (a) and cooled HD (b). Endotoxin levels, ICG clearance rate and perfusion heterogeneity significantly changed over the course of standard HD but not cooled HD. a, For standard HD, the increase in endotoxin levels and perfusion heterogeneity, along with the decrease in ICG clearance rate, were statistically significant compared with pre-HD values ($P < 0.05$ for all). b, For cooled HD, there were smaller changes in all measurements (not statistically significant) compared with standard HD. Results are given as average ± standard error of the mean (SEM).

paucity of data regarding intradialytic liver perfusion measurements, our findings are consistent with prior work.[12,13] These findings likely resulted from the liver's dual blood supply system which may have protected it from subclinical perfusion shifts associated with reduction in hepatic arterial flow,[30] although further work is needed to elucidate details of this mechanism. In addition, intradialytic portal vein perfusion showed the greatest trend towards changing, increasing to 111% of baseline during HD. As portal venous blood is toxin laden,[4] this finding suggests that HD-induced circulatory stress may increase endotoxin influx from the gut to the liver following translocation, and may be responsible for the trend towards increasing endotoxin levels during HD.

Liver perfusion heterogeneity was also assessed in this study, as significant functional changes may occur in smaller segments; this signal would be lost if averaged over the entire liver volume (balanced by other areas shunting increased blood flow). There are various approaches to quantify medical image heterogeneity (i.e., texture analysis[31,32]), and although each analysis technique has its advantages and applications, we chose to implement the algorithm developed by Brooks and Grigsby[17] due to its intuitiveness and ease of implementation with respect to our data. This algorithm yields a single statistic per image, providing a simple, quantitative method of comparing images based on heterogeneity. Upon applying this algorithm, we observed a significant increase in hepatic perfusion heterogeneity during HD. Although never studied in the context of HD, liver perfusion heterogeneity has been assessed for various types of liver injury,[33–35] consistently showing a relationship with hepatic injury. In addition, changes in liver function (i.e., ICG clearance rate) were related to changes in hepatic perfusion and perfusion heterogeneity in this work.

Previous studies (including in dialysis patients[36,37]) have performed ICG-based measurements of hepatic function,[38] which in this work was assessed by optically measuring the rate of ICG clearance. Because clearance of ICG and endotoxin occur fully[18] and partially[39] within hepatocytes, respectively, and given that our patients had minimal residual renal function and a likely higher reliance on hepatic clearance, the ICG clearance rate therefore represents a suitable surrogate measure of hepatic endotoxin clearance.

Excretory liver function significantly declined during HD, as measured with pulsed-dye densitometry–based ICG clearance. Previous work has shown that decreased ICG clearance following hepatic injury is linked to increased production of reactive oxygen intermediates and neutrophil elastase,[40] and occurs contemporaneously with increased endothelin-1,[41] all of which mediate liver cell injury and/or dysfunction.[40,42,43] In addition, endotoxin itself has also been shown to induce oxidative stress[44,45] and attenuate ICG clearance.[46,47] Increased levels of these and other inflammatory mediators have been characterized in ESRD and HD patients,[3,44,45,48–51] suggesting that the liver is susceptible to recurrent HD-induced circulatory stress via inflammatory mediators which negatively affect hepatic function.

We have previously shown that renal perfusion significantly declines at peak dialytic stress,[10] representing repetitive, intradialytic episodes of ischemic acute kidney injury.[52] The potential negative relationship between kidney injury and the liver's clearance function has been previously discussed in the context of acute kidney injury[53,54] and ESRD,[13,55] and may be an important additional factor contributing to HD-induced hepatic dysfunction and increased endotoxemia.

In this work, endotoxin was quantified using a Limulus Amebocyte assay in a manner described previously.[24] Although the sensitivity of this technique has been criticized,[56] we have taken measures to establish internal and external consistency with regards to our results and help ensure that our findings are not artefactual. First, serum samples were collected before, during, and after HD, yielding three endotoxin measurements per patient per study visit. As these samples were stored, shipped, and analyzed identically to one another, any potential issues with endotoxin quantification methodology would affect all three samples equally (save for minor fluctuations). Therefore, although the absolute measures of endotoxin may not be completely accurate, the relative changes in endotoxin from baseline levels (which we present and focus on) still hold true and provide scientific value. Second, the endotoxin quantification methodology used in this work is the same as what was performed in our group's previous studies.[3,57–59] Given our familiarity and expertise with this methodology, along with the convincing, positive results it has enabled us to produce previously (e.g., endotoxin levels in HD patients correlate with negative clinical outcomes and reduced survival[3]), we were confident applying it to the current study as well.

The baseline endotoxin levels measured in this work (0.29 EU/ml) are consistent with findings in other studies of dialysis patients.[56,60] Endotoxin levels trended towards increasing during HD, and increased markedly from baseline after HD. This escalation has been attributed to increased endotoxin translocation from mesenteric injury and compromised gut mucosal permeability,[1,2] which is repeated during recurring dialysis sessions. This mechanism is reinforced by

the strong correlation observed between baseline endotoxin levels and dialysis vintage. In addition, other than an associative trend between increased endotoxin levels and maximum reduction in mean arterial pressure during HD, we did not find evidence of HD-induced circulatory stress (i.e., UF metrics and intradialytic hypotension) being linked to endotoxemia, differing from the results of other studies.[3,59] This suggests (i) that in our patients, endotoxemia was driven more by liver hemodynamic and functional changes than by direct effects of circulatory stress (i.e., UF metrics and intradialytic hypotension); and (ii) that more generally, it may be the combination of direct effects of circulatory stress together with changes in hepatic hemodynamics and function which contribute to increased endotoxin levels characteristically seen in HD patients.

Based on the results of this study (italicized items below), we propose the following pathway by which HD perpetuates endotoxemia in ESRD patients:

- Mesenteric ischemia (due to HD-induced circulatory stress) disrupts gut mucosal structure and function, and increases bowel wall permeability.
- Endotoxin more readily crosses intestinal barrier and translocates from gut to liver.
- *Endotoxin arrives via portal vein perfusion, which trends towards increasing during HD.*
- *Trend towards increased intradialytic endotoxin levels.*
- *Decreased ICG clearance rate during HD represents compromised hepatic function, likely due to increasing levels of endotoxin and other inflammatory mediators.*
- *Further increase in post-HD endotoxin levels likely results from the combination of more endotoxin arriving to the liver and lowered hepatic clearance function.*
- Recurrent cycles over many HD sessions lead to higher circulating endotoxin levels in ESRD patients.

In addition to the pathway proposed above, it is important to consider another physiological perspective that could lead to the perpetuation of endotoxemia by HD. ESRD patients characteristically show signs of gut mucosal ischemia (such as gastric intramucosal acidosis),[61] which, when compounded with intradialytic hemodynamic effects (such as HD-induced circulatory stress leading to reduced splanchnic blood volume),[62,63] results in mesenteric ischemia. It would then be expected that the contribution of the intestinal blood flow to total portal vein perfusion (sum of superior mesenteric venous perfusion and splenic venous perfusion) would be decreased. This suggests that the observed trend towards increasing portal vein perfusion during HD should be due to contributions from other splanchnic, nonintestinal sources of blood flow (e.g., spleen, stomach, pancreas, etc.) which are not toxin laden. To reconcile the above physiologic description with the findings of this work, two important points should be considered: (i) endotoxin can bypass the liver and can directly enter the systemic circulation via the peritoneal cavity in cases of prolonged intestinal wall injury that lead to increased bowel wall permeability,[5] and (ii) the spleen can contribute to increased portal vein blood flow (normal splenic perfusion of approximately 100 ml/min/100 g)[64] and hepatocyte injury (greater hepatotoxicity due to spleen-derived lipopolysaccharide-responsive macrophages).[65] Therefore, it may be worthwhile to focus future research efforts on measuring splenic perfusion during HD and assessing whether the spleen (along with other splanchnic organs like the liver) also plays an important role in the perpetuation of endotoxemia in ESRD patients.

Initial Description of DC Effects

With DC, intradialytic portal vein perfusion, intradialytic perfusion heterogeneity, intradialytic ICG clearance rate, and post-HD endotoxin levels all changed nonsignificantly relative to baseline levels. This suggests that DC did not negatively affect liver hemodynamics and function, or worsen endotoxemia, and may potentially help improve these metrics compared to standard HD. This is plausible because cooling potentiates better maintenance of organ perfusion due to peripheral vasoconstriction,[66] increased baroreflex sensitivity variability,[67] and reduced intradialytic hypotension.[67,68] Also, in the context of therapeutic hypothermia, cooling mitigates organ injury via several potential mechanisms of action (e.g., reducing inflammation, attenuating oxidative stress, and decreasing free radical production), showing effectiveness in multiple organs.[69,70] Therefore, it is reasonable to assume that DC can potentially maintain liver perfusion and mitigate hepatic injury, resulting in improved control of endotoxemia. However, the changes observed in this study during cooled HD were not statistically significantly different compared to changes during standard HD, and further work must show the protective potential of DC for preserving hepatic function and mitigating endotoxemia.

The DC results of this work coincide with findings of similarly designed studies assessing myocardial injury[7,8,25] and renal ischemia[10] during cooled HD. The effectiveness of DC was not universal in those studies (e.g., no difference in left ventricular ejection fraction between standard and cooled HD groups,[8,25] no difference between decreased kidney perfusion between standard and cooled HD groups[10]) or in our work. In addition, in our study 4 of 15 patients (27%) experienced cold-related symptoms (e.g., shivering and

feeling cold) during cooled HD only, which is consistent with the incidence of temperature-related symptoms reported in other studies.[7,71,72] These findings suggest that, although DC shows promise as an intradialytic intervention, combining cooling with other interventions (e.g., ischemic preconditioning[73,74]) and/or implementing other cooling techniques (e.g., body temperature-based DC[8,25]) may more effectively ameliorate HD-induced circulatory stress and cooling-related symptoms.

Study Limitations

There are several limitations associated with this early-phase study. First, endotoxin concentrations were not adjusted for intradialytic decreases in plasma water concentration.[75,76] However, the resultant overestimation is offset by underestimation due to endotoxin removal by HD,[77,78] suggesting that our conclusions would not change. Second, we measured a composite of free (more active) and bound (less active) endotoxin.[79,80] We previously used the same methodology to correlate total levels with important clinical effects[3] showing the value of composite endotoxin measurement. Third, this was a pilot experiment with a modest sample size of 15 patients, and generalization of findings should be withheld until a larger, randomized controlled trial is performed. However, this study incorporated detailed imaging measurements for assessment of both inter- and intrapatient variations. Fourth, only patients with low baseline renal function (urine output $<$250 ml/24 h) were assessed to minimize the risk of contrast-induced nephropathy damaging significant residual renal function, and this patient group may be predisposed to hepatic injury. However, this was a proof-of-principle study, and future experiments are required to examine the direct effects of standard and cooled HD upon endotoxemia and liver hemodynamics in individuals with higher residual renal function, and to longitudinally follow patients new to HD with respect to increasing endotoxin levels.

Conclusion

In summary, HD-induced circulatory stress resulted in redistribution of liver perfusion and attenuation of hepatic function. Endotoxin levels peaked after HD, and higher endotoxin levels in ESRD patients may result from the combination of two intradialytic effects: decreased hepatic clearance of endotoxin, and a trend towards increased toxin-laden portal vein perfusion to the liver. In addition, although the observed changes in endotoxin, hepatic perfusion, and function with DC did not reach statistical significance relative to standard HD, this intervention, which has already been applied in the protection of multiple organs from HD-induced injury, warrants further exploration for endotoxemia protection.

DISCLOSURES

TYL licenses the CT Perfusion software to GE Healthcare. The other authors of this article have no relevant conflicts of interest to disclose.

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AUTHOR CONTRIBUTIONS

EQ, TYL, and CWM designed the study; RM and EQ carried out experiments; RM, EQ, FL, and TYL analyzed the data; KBL, CCS, and PKTL performed endotoxin assays; RM created the figures; RM, EQ, TYL, and CWM drafted and revised the paper; all authors approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Theoretical basis of heterogeneity quantification algorithm and application of the algorithm in this study's workflow.

Figure S2. Consolidated Standards of Reporting Trials flow diagram.

Figure S3. Plots of relative change in endotoxin levels in ICG clearance rate.

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