An exploratory study of the relationship between systemic microcirculatory function and small solute transport in incident peritoneal dialysis patients

Jennifer Williams[1] , Mark Gilchrist[1], William David Strain[1],
Donald Fraser[2] and Angela Shore[1]

Abstract

Background: The peritoneal capillary endothelium is widely considered to be the most influential structure in dictating the rate of small solute transport (SST) during peritoneal dialysis (PD). PD patients are at significant risk of systemic microcirculatory dysfunction. The relationship between peritoneal and systemic microcirculations in patients new to PD has not been well studied. We hypothesised that for patients on PD for less than 6 months, dysfunction in the systemic microcirculation would be reflected in the rate of SST.

Methods: We recruited 29 patients to a cross-sectional, observational study. Rate of SST was measured using a standard peritoneal equilibration test. Laser Doppler Flowmetry was used to measure response to physical and pharmacological challenge (post-occlusive hyperaemic response and iontophoretic application of vasodilators) in the cutaneous microcirculation. Sidestream Darkfield imaging was used to assess sublingual microvascular density, flow and endothelial barrier properties.

Results: We found no moderate or strong correlations between any of the measures of systemic microcirculatory function and rate of SST or albumin clearance. There was however a significant correlation between dialysate interleukin-6 concentrations and both SST ($r_s = 0.758$ $p \leq 0.0001$) and albumin clearance ($r_s = 0.53$, $p = 0.01$).

Conclusions: In this study, systemic microvascular dysfunction did not significantly influence the rate of SST even early in patients PD careers. In conclusion, this study demonstrates that intraperitoneal factors particularly inflammation have a far greater impact on rate of SST than systemic factors.

Keywords

Peritoneal dialysis, peritoneal transport, systemic microcirculation

Introduction

The primary function of the microcirculation, which encompasses arterioles, capillaries and venules, is to allow exchange of nutrients and gas transfer across their semi-permeable vascular wall. A monolayer of endothelial cells forms the blood interface throughout the cardiovascular system. This layer performs multiple functions integral to the proper functioning of the microvascular network including regulation of vascular permeability, inhibition of platelet aggregation and regulation of vascular tone.[1] Vascular endothelial cells are covered on their luminal surface by the glycocalyx, a negatively charged layer of glycoproteins. The glycocalyx exerts several important vasculoprotective effects including inhibiting coagulation

and leucocyte adhesion[2] and mediating shear stress-induced nitric oxide release,[3] but it also plays a significant role in vascular permeability.[4]

The peritoneal capillary endothelium is widely considered to be the most influential structure in dictating the rate

[1] Diabetes and Vascular Medicine Research Centre, NIHR Exeter Clinical Research Facility, University of Exeter College of Medicine and Health, UK
[2] Wales Kidney Research Unit, Cardiff University, UK

Corresponding author:
Angela Shore, Diabetes and Vascular Medicine Research Centre, NIHR Exeter Clinical Research Facility, University of Exeter College of Medicine and Health, Barrack Road, Exeter, EX2 5DW, UK.
Email: a.c.shore@exeter.ac.uk

of small solute transport (SST) across the peritoneum during peritoneal dialysis (PD).[5] The capillary wall as the major barrier to solute transport forms the basis for the most widely applied and best understood model of peritoneal transport – Rippe's three pore model.[6] More recently, this model has been extended by others to propose a role for the endothelial glycocalyx.[7]

All dialysis patients including those on PD have multiple risk factors for microcirculatory dysfunction. Experimentally, uraemia has been associated with endothelial cell activation,[8] impaired endothelial repair[9] and oxidative stress.[10] Additionally, within kidney cohorts, there is a high prevalence of comorbidities such as diabetes and coronary artery disease, which have independent detrimental effects on the systemic microcirculation.[11,12] Recent years have seen the development of several techniques to non-invasively study, *in vivo*, the structure and function of the microcirculation.[13,14] In kidney cohorts, these techniques have been used to demonstrate reduced capillary density in the skin and under the tongue,[15–17] reductions in the vasodilatory response of the skin microcirculation to physical and chemical stimuli[18,19] and impairment of glycocalyx barrier properties.[20]

The relationship between the systemic and peritoneal microcirculations is likely to be complex, wherein both are influenced by demographic and biochemical factors including age, sex, co-morbidity and inflammation. In addition, with time on PD, the peritoneal microcirculation is damaged through exposure to non-physiological dialysis solutions in a way that the systemic microcirculation is not. It remains unclear how the systemic microcirculation relates to the peritoneal microcirculation in patients who have recently commenced PD.

We conducted a cross-sectional, observational study in patients who had been receiving PD for less than 6 months, comparing rate of SST at first test of peritoneal permeability with non-invasive tests of the structure and function of the cutaneous and sublingual microcirculations, to test the hypothesis that changes in the systemic microcirculation such as loss of glycocalyx integrity, alterations in small vessel density and impairments in reactive vasodilation would be reflected in the rate of SST.

Methods

Subjects

Participants were recruited from patients attending the Royal Devon and Exeter Hospital and University Hospital Wales, Cardiff, for their dialysis care between December 2016 and November 2019. Patients were eligible to take part if they were aged over 18 years, had been receiving PD for less than 6 months and underwent a standard test of peritoneal permeability within the first 6 months of treatment. Potential participants were excluded if they were prescribed a calcineurin inhibitor, had a history of

Raynauds disease or experienced a major vascular event in the preceding 3 months, all of which are known to alter microvascular function.

Peritoneal permeability test

All participants underwent a test of peritoneal permeability as part of their routine clinical care. Participants recruited at the Royal Devon and Exeter site underwent a standard peritoneal equilibration test (PET) as originally described by Twardowski.[21] Participants recruited at the Cardiff site underwent a 'fast PET', whereby dialysate samples were taken for analysis only at the end of the 4th hour and not at time 0 or 2 h. All other aspects of the PET were as for the standard PET. The primary outcome variable was dialysate to plasma ratio of creatinine at 4 h (D/P$_{cr}$4 H). Excellent agreement between D/P$_{Cr}$4 H for standard PET and fast PET has been shown ($r = 0.77$; $p = 0.0001$).[22] Differences in laboratory creatinine assays are known to influence results,[23] therefore samples taken during the Cardiff PETs were frozen and subsequently reanalysed in the Royal Devon and Exeter clinical biochemistry department (Creatinine Jaffe Gen.2 by Cobas). Manufacturers of this assay state that no interference is expected at glucose concentrations <120 mmol/L. None of the 4-h dialysate samples (used for our primary outcome measure) had glucose concentrations in excess of this level; therefore, creatinine concentrations were not corrected for glucose concentration.

Biochemical analyses

Albumin was measured in blood and the 4-h dialysate samples (Roche immunotrubidimetric microalbumin assay), expressed as milligram per litre and used to calculate peritoneal albumin clearance using the following formula;

$$\text{Peritoneal albumin clearance (mL}/4 - \text{h dwell)}$$
$$= \text{dialysate albumin (mg/L)}/\text{plasma albumin (mg/L)}$$

Interleukin-6 (IL-6) was measured in blood and dialysate samples (taken from the overnight dwell prior to the PET); these were processed at the immunoassay biomarker core laboratory, University of Dundee, using IL-6 Meso Scale Discovery V-PLEX assay.

Study protocol

All participants attended for a single study visit during which all the microvascular tests were performed. Participants attended in the morning, having fasted orally overnight for at least 8 h. Participants attended 'dry' (without any dialysate in situ), their overnight dialysis regime the night before was conducted as per their individual prescription. Information was recorded based on demographic details, past medical history, current medications and dialysis regime. To reduce the influence of food intake on microvascular parameters, participants were given a
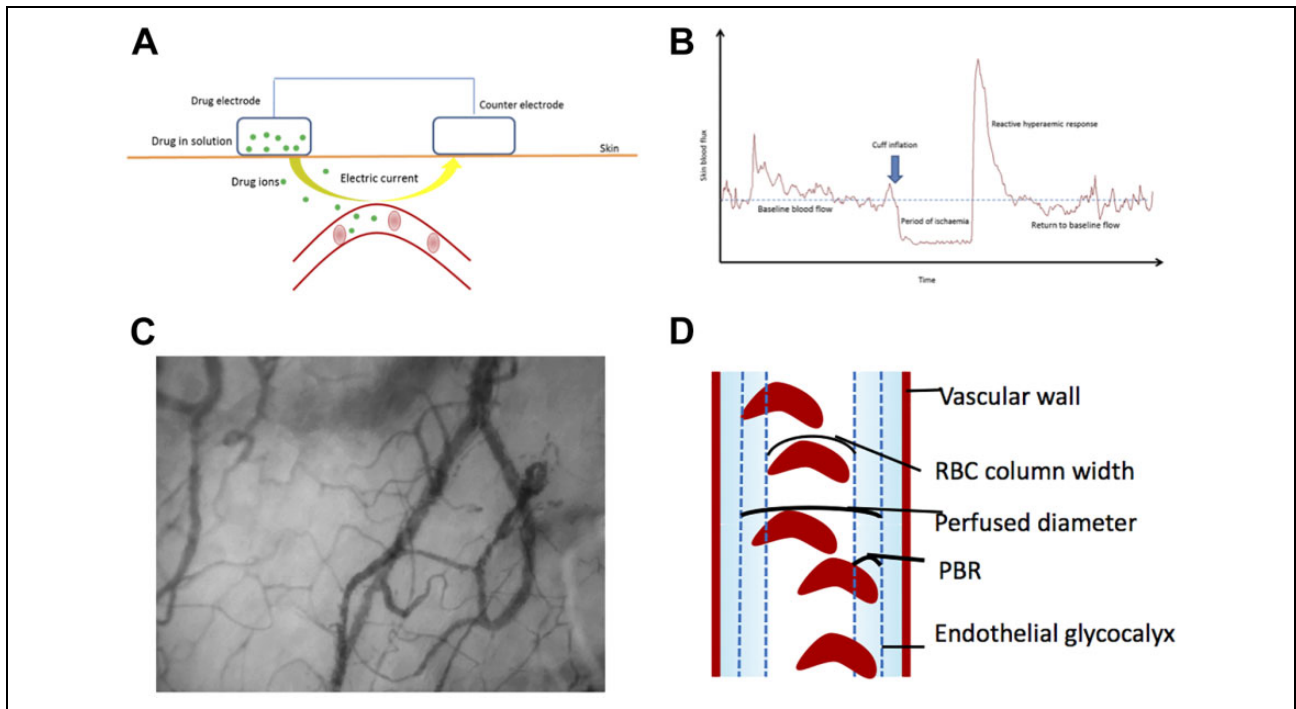
Figure 1. Techniques used to study the cutaneous and sublingual microcirculations (modified from Williams et al.[14], with permission). (A) Schematic representation of iontophoretic delivery of vasoactive substances. Vasodilation in response to the delivery of vasoactive substances is measured using Laser Doppler Perfusion Imaging producing a 2D perfusion map, brighter colours indicate greater flux. (B) Representative laser Doppler trace obtained before, during and after a brief period of arterial occlusion. A reactive hyperaemia can be induced by a brief period of arterial occlusion using a cuff placed around the upper arm or leg. This response takes the form of a post-ischaemic flow initially many times faster than normal followed by exponential decay to baseline. The response is measured using a single-point laser doppler flowmeter. (C) Example of sublingual microcirculation as visualised using sidestream dark field imaging. A handheld camera illuminates the tissue using green light-emitting diodes, the scattered light is within the wavelength of the haemoglobin absorption spectrum (530 nm) and is therefore absorbed by haemoglobin contained in RBCs. Reflected light is captured on a charge coupled camera. RBCs are visualised as black or grey bodies on a white background. Penetration of the light is up to 3 mm consequently superficial capillaries and venules that contain RBCs are visualised. (D) Schematic depicting the principles used by Glycocheck software to measure glycocalyx permeability. Dotted lines represent the edge of the cell permeable and impermeable areas. The PBR for vessels between 5 μm and 25 μm is calculated as perfused diameter − median RBC column width/2 (as the PBR is present on both sides of the RBC column). PBR: perfused boundary region; 2D: two dimensional; RBC: red blood cell.

standardised meal of two pieces of brown toast with butter and a glass of water. All tests were conducted in a temperature-controlled room (23 ± 1°C) in which participants had acclimatised for 30 min.

Microvascular tests

Using these techniques, impairments of microcirculatory structure and function have been demonstrated in other kidney cohorts compared with healthy controls.[17,20,24] Significant correlations between these and other established parameters of altered microcirculation have also been demonstrated.[25–28]

All microvascular tests were conducted and analysed by a single operator. Reproducibility data, as determined by multiple measurements on different days in the same individual, were acquired for all techniques, coefficients of variation fell within acceptable ranges in the published literature (Supplemental Data 1).

Cutaneous microcirculatory function

To facilitate temperature regulation, human skin has a high vasodilatory reserve. Relative changes in skin blood flow provoked by physical and pharmacological stimuli can be easily and non-invasively measured using laser Doppler techniques.[29] We performed two tests of cutaneous microcirculatory function using Laser Doppler Flowmetry. The flexor aspect of the right arm was used for both tests unless the participant had an arterio-venous fistula on that arm, when the flexor aspect of the left arm was used.

Iontophoretic application of vasodilators

Iontophoresis allows minimally invasive trans-dermal delivery of charged molecules to a localised area of cutaneous microcirculation[30] (Figure 1(A)). Endothelium dependant and endothelium independent vasodilation were assessed using iontophoretic application of acetylcholine (ACh) and sodium nitroprusside (SNP), respectively. These

were individually applied using a separate Perspex direct electrode chamber, attached by an adhesive disk to separate sites on the flexor aspect of the forearm. ACh (1% w/v Miochol-E, Novartis, Camberley, UK) and ACh vehicle solution (3% w/v mannitol in water) were delivered using an anodal current of 0.1 mA for 5 × 20 second bursts with 60 s between currents; SNP (0.25% w/v, 25 mg/mL Hospira Pty Ltd., Melbourne, Australia) and SNP vehicle solution (0.45% w/v saline, Baxter, UK) were delivered using a single cathodal current of 0.2 mA applied for 60 s. Flux was recorded, using a solid-state Laser Doppler Perfusion Imager, Moor LDI (Moor Instruments, Axminster, Devon), immediately prior to current application and at 60 s intervals for 5 min for both ACh and SNP and was analysed using MoorLDI software V 5.3. Current was delivered by a battery powered iontophoresis controller (Moor iontophoresis controller, MIC2, Moor Instruments).

For each of the test substances, the primary outcome measures were peak vasodilatory response during the monitoring period in arbitrary units (AUs) and total vasodilatory response (area under the curve) adjusted for baseline.

Post-occlusive reactive hyperaemia

The hyperaemic response following the release of a brief period of arterial occlusion can also be captured using Laser Doppler Flowmetry[31] (Figure 1(B)). This post-occlusive hyperaemic response is a global assessment of cutaneous microvascular function.

Flux was captured using a DRT4 laser Doppler perfusion monitor (Moor Instruments), attached to the flexor aspect of the forearm using an adhesive disk. Baseline flux was recorded for 10 min. A multi-outlet blood pressure cuff attached to a pressure reservoir placed around the upper arm was rapidly inflated to 200 mmHg; this pressure was maintained for exactly 4 min. Complete arterial occlusion was ensured by monitoring the flux trace. After 4 min, the cuff was rapidly deflated. Flux was monitored for a further 10 min or until it returned to baseline, whichever occurred sooner.

Offline analysis of the flux traces was performed. The primary outcome measures were absolute peak flux (Moorsoft DRT4 version 2.0) and area under the reactivity curve, that is, the total hyperaemic response (MoorVMS-PC version 3.1 software).

Sublingual microcirculation

Sidestream Darkfield imaging – capillaroscopy. Sidestream Darkfield (SDF) imaging allows direct visualisation of flow in microvascular beds with a mucosal covering and is most commonly used to study the sublingual circulation[32] (Figure 1(C)).

Participants were studied in the supine position. Images were acquired using a handheld SDF device (Microscan, Microvision Medical, Amsterdam, the Netherlands), with a 5× objective lens and a numerical aperture of 0.16. A single

use plastic cap was applied to the tip of the device which was then placed sublingually, and illumination levels and focus adjusted to achieve optimal images. Video sequences of 20 s duration were recorded using AVA 3.1 software (Academic Medical Centre, University of Amsterdam, the Netherlands). Sufficient recordings were made such that the operator was confident of having three recordings that could be reliably analysed offline as per European Society of Intensive Care Medicine recommendations.[33] Analysis was performed using the semi-automated AVA 3.1 software, as previously described in detail.[34] All vessels were identified manually and segmented from background tissue by drawing the vessel centreline and lumen boundaries, then allocated to one of three groups: small (<20 μm), medium (20–50 μm) or large (50–100 μm). Full examination was only made of small vessels as these are the most physiologically important for oxygen and nutrient exchange. Flow in each vessel was semi-quantified by the person analysing the record as continuous, sluggish (flow for 50–100% of the recording), intermittent (flow for <50% of the recording) or no flow. The AVA software uses these data to calculate the following outcome variables: total vessel density (mm/mm^2), perfused vessel density (mm/mm^2) and proportion of perfused vessels (%).

Sidestream dark field imaging – glycocalyx barrier properties. SDF images also enable an assessment of the endothelial glycocalyx.[35] Images were acquired using a handheld SDF video microscope (KK technology, Honiton, Devon, UK), connected to a laptop running Glycocheck measurement software (Glycocheck BV, Maastricht, the Netherlands). Analysis is fully automated and has previously been described in detail.[20] In short, it is not possible to visualise the glycocalyx directly using SDF; the parameters produced by the Glycocheck software are based on the principle that an intact endothelial glycocalyx has a cell permeable and a cell-impermeable layer. The outer edges of the microvascular lumen (from the surface of the endothelial cell to the most luminal aspect of the proposed cell-impermeable layer) are relatively devoid of red blood cells (RBCs). Damage to the endothelial surface layer means RBCs have greater access to the usually cell-impermeable region and consequently deviate further from their central region of flow (Figure 1(D)). The primary outcome variable generated by Glycocheck is the perfused boundary region (PBR) for vessels measuring 5–25 μm. The PBR reflects the degree to which RBCs can deviate from their median column width and penetrate the usually cell-impermeable area, larger PBRs represent greater lateral movement and imply greater penetration into the glycocalyx.

Ethics

The study was approved by East-Midlands Leicester Central Research Ethics Committee (REC ref 16/EM/0395) and conducted per the Declaration of Helsinki. All participants gave their written informed consent.

Table 1. Characteristics of study participants in the analysis.[a]

Patients (*n*)	29	Previous HD (*n* (%))	8 (27.6)
Age (years)	68 [58–77]	AVF (*n* (%))	2 (6.9)
Male (*n* (%))	19 (65.5)	Serum	
Caucasian (*n* (%))	26 (89.7)	Haemoglobin (g/L)	116.5 [108.5–127.5]
Systolic bp (mmHg)	140 ± 21	Calcium (mmol/L)	2.35 ± 0.15
BMI	27.6 ± 5.7	Phosphate (mmol/L)	1.48 ± 0.22
Davies Comorbidity Index		PTH (pmol/L)	19.35 [11.2–42.9]
Low/intermediate/high (%)	34.5/62.1/3.4	CRP (mg/L)	6 [2–10]
Diabetes (*n* (%))	9 (31)	Interleukin-6 (pg/mL)	1.48 [0.72–2.69]
Medications (*n* (%))		Albumin (g/L)	37.48 ± 5
RAAS blockers	11 (37.9)	Dialysate	
Beta blockers	13 (44.8)	Interleukin-6 (pg/mL)	31.25 [16.68–71.54]
Active vitamin D	18 (62)	Albumin (mg/L)	404.3 [321.2–510]
EPO	18 (62)	Residual CrCl (L/week)	62.68 [47.79–97.18]
Phosphate binder	12 (41.4)	D/P_{Cr} at 4 h	0.83 [0.71–0.93]
CAPD (*n* (%))	13 (44.8)	D/D_0 glucose at 4 h	0.25 [0.23–0.34]
Time on PD (week)	15.4 ± 5	Peritoneal albumin clearance (mL/4 h dwell)	20.4 [15.6–25]

BMI: body mass index; RAAS: renin angiotensin aldosterone system; EPO: erythropoietin; CAPD: continuous ambulatory peritoneal dialysis; AVF: arteriovenous fistula; PTH: parathyroid hormone; CRP: C reactive protein; CrCl: creatinine clearance; D/P_{Cr}: dialysate to plasma ratio of creatinine; $D/D_{0\ glucose}$: ratio of dialysate glucose compared with time 0.
[a]Data are presented as mean ± SD or median (Inter-quartile range).

Statistics

Statistical analysis was performed using StataSE-16 (Stata Corporation, Texas, USA). Graphical analysis was performed using GraphPad Prism 8 software. All variables were tested for normality by Shaprio-Wilks test. For normally distributed data correlations, between variables were assessed using Pearson's correlation coefficient. For nonnormally distributed data correlations, between variables were assessed using Spearman's rank correlation coefficient. A result was considered statistically significant if $p \leq 0.05$.

Results

All eligible patients were approached during the recruitment period. A total of 37 participants were recruited across the two sites. Eight participants withdrew or were withdrawn prior to microvascular investigations. Twenty-nine participants were included in the final analysis (see Supplementary Figure 1 for flow-chart summarising recruitment). Further recruitment was not possible within the time frame of the study. Demographic data for the cohort are detailed in Table 1. Primary diagnoses of kidney failure were hypertensive nephropathy 27.6%, glomerular nephropathy 17.2%, small vessel vasculitis 10.3%, polycystic kidney disease 10.3%, small kidneys/unknown cause 10.3%, diabetic nephropathy 6.9%, reflux nephropathy 6.9% and other 10.3%.

SST as defined by dialysate to plasma ratio of creatinine at 4 h ($D/P_{Cr}4$ H) was not normally distributed and therefore all analyses using this variable were done using nonparametric tests. The results of the microvascular tests in this cohort are summarised in Supplementary Table 1.

Relationship between SST and the systemic microcirculation

Correlations between SST and all the microvascular parameters measured are summarised in Table 2.

Cutaneous microcirculation

Twenty-three participants had adequate data for analysis of response to iontophoretic application of ACh and SNP. There was no significant relationship between $D/P_{Cr}4$ H and either the peak (ACh $p = 0.779$, SNP $p = 0.66$) or total response to ACh or SNP (ACh $p = 0.782$, SNP $p = 0.643$).

Twenty-five participants had adequate data for analysis of post-occlusive hyperaemic curves. There was no significant relationship between $D/P_{Cr}4$ H and the absolute peak of the hyperaemic curve ($p = 0.448$) or the total response represented by the area under the response curve ($p = 0.728$) (Figure 2(A)).

Sublingual microcirculation

Twenty-five participants had adequate data for sublingual capillaroscopy. No significant relationship was seen between $D/P_{Cr}4$ H and any of the parameters for the smallest vessels (<20 μm) (Figure 2(B)). No significant correlations were found when analysis was extended to include all vessel sizes (up to 100 μm), data not shown.

Twenty-six participants had complete Glycocheck data. No significant relationship was seen between $D/P_{Cr}4$ H and PBR in vessels measuring 5–25 μm.

Participants were grouped into quartiles according to $D/P_{Cr}4$ H. There was no statistically significant difference between participants in the top and bottom quartiles for any

Table 2. Summary of correlations between small solute transport (D/P$_{Cr}$4 H) and systemic microcirculatory parameters.[a]

Cutaneous parameters			Sublingual parameters		
	r_s	p value		r_s	p value
Post-occlusive reactive hyperaemia			SideStream Darkfield capillaroscopy – vessels <20 μm		
Peak response (AU)	0.159	0.448	TVD (mm/mm^2)	0.154	0.461
Total response (AUC)	0.073	0.728	PVD (mm/mm^2)	0.07	0.749
			PPV (%)	−0.147	0.483
Iontophoretic application of vasodilators			Glycocheck – vessels 5–25 μm		
Peak response to ACh (AU)	0.0619	0.779	PBR (μm)	−0.015	0.944
Total response to ACh (AUC)	0.06	0.782			
Peak response to SNP (AU)	0.09	0.66			
Total response to SNP (AUC)	0.099	0.643			

AU: arbitrary units; AUC: area under the curve; ACh: acetyl choline; SNP: sodium nitroprusside; TVD: total vessel density; PVD: perfused vessel density; PPV: proportion of perfused vessels; PBR: perfused boundary region.
[a]Data are presented at Spearmans r (r_s) for the relationship with D/P$_{Cr}$4 H (dialysate to plasma ratio of creatinine at 4 h).
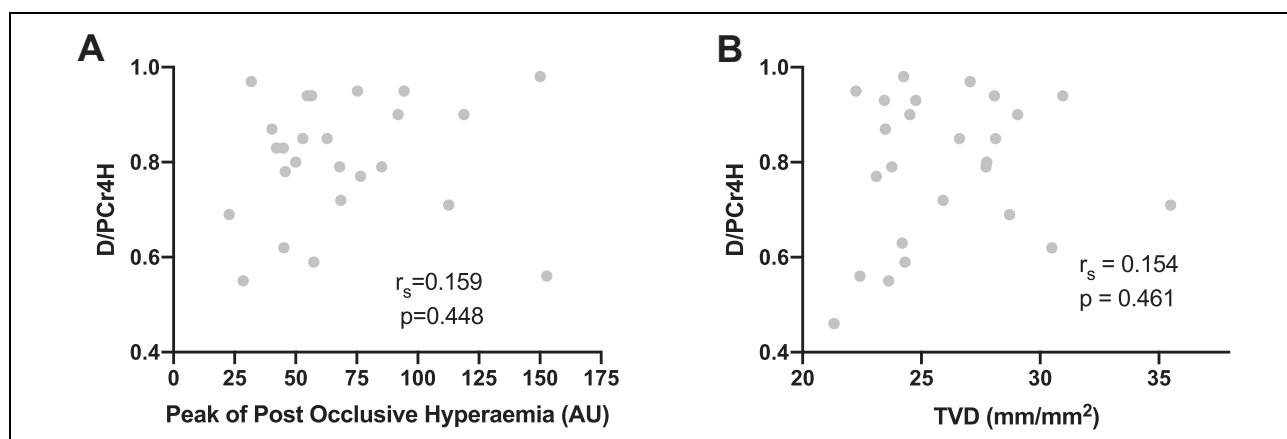
Figure 2. Graphs showing the relationship between SST (D/P$_{Cr}$4 H) and systemic microvascular structure and function. (A) No relationship between SST and peak post-occlusive hyperaemic response measured on the forearm. (B) No relationship between SST and sublingual TVD. $p \geq 0.05$ for both correlations. D/P$_{Cr}$4 H: dialysate to plasma ratio of creatinine after 4 h; AU: arbitrary units; TVD: total vessel density; SST: small solute transport.

of the microvascular parameters. This study included a high number of fast transporters (D/P$_{Cr}$4 H > 1 SD above the mean in Twardowskis's original study[21]); it has previously been suggested that patients in the highest transport category early in their PD careers are qualitatively different to the rest of the cohort.[36] Therefore, all correlation analyses were repeated excluding the top quartile of participants (D/P$_{Cr}$4 H > 0.93). This did not reveal any significant associations.

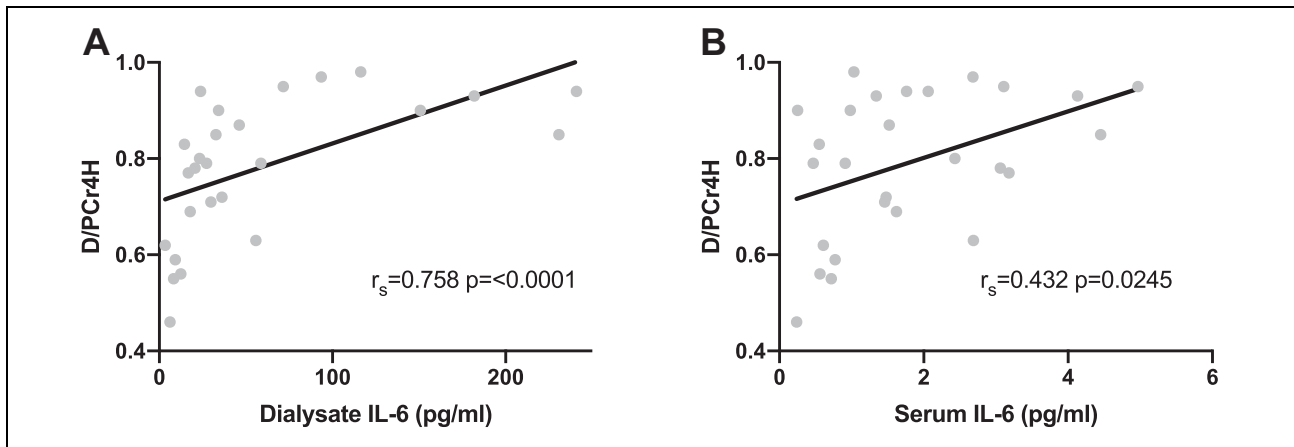### Relationship between inflammation, SST and the systemic microcirculation

There was a strong correlation between dialysate IL-6 and D/P$_{Cr}$4 H ($r_s = 0.758$; $p \leq 0.0001$) (Figure 3(A)). There was also a significant correlation between serum IL-6 and D/P$_{Cr}$4 H ($r_s = 0.432$; $p = 0.0245$) (Figure 3(B)) and serum IL-6 and dialysate IL-6 ($r_s = 0.513$; $p = 0.0087$). After adjustment for systemic and intraperitoneal inflammation,

in multiple regression analysis, the relationship between D/P$_{Cr}$4 H and serum IL-6 was no longer significant ($p = 0.28$), although the relationship between D/P$_{Cr}$4 H and dialysate IL-6 remained ($p = 0.019$). There were no significant correlations between dialysate IL-6 or serum IL-6 and any of the measures of systemic microvascular structure or function.

Relationship between transport of larger molecules and the systemic microcirculation

Peritoneal albumin clearance during the 4-h dwell was calculated as outlined above. There were no significant correlations between 4-h peritoneal albumin clearance and any of the measures of cutaneous or sublingual microvascular structure and function (Supplemental Data).

There was a significant correlation between D/P$_{Cr}$4 H and 4-h peritoneal albumin clearance ($r_s = 0.51$,

Figure 3. Graphs showing the relationship between small solute transport (D/P$_{Cr}$4 H) and (A) intraperitoneal inflammation and (B) systemic inflammation. D/PCr4 H: dialysate to plasma ratio of creatinine at 4 h; IL-6: interleukin-6.

$p = 0.009$). There was a significant correlation between peritoneal albumin clearance and dialysate IL-6 concentrations ($r_s = 0.53$, $p = 0.01$). There was also a significant correlation between peritoneal albumin clearance and serum IL-6 ($r_s = 0.61$, $p = 0.0018$) (Supplemental Figure 2).

Discussion

This is the first study to investigate the relationship between systemic microvascular structure and function and peritoneal SST in a cohort of patients exclusively with a duration of PD less than 6 months, thus examining the relationship between the systemic and peritoneal microcirculations and not the effects on peritoneal microvessels of chronic exposure to dialysate solutions.

We found no moderate or strong relationship between endothelial function, microvascular responsiveness, the glycocalyx (endothelial cell surface layer) or microvascular density as assessed in the cutaneous or sublingual microcirculations and rate of SST. Previous studies of the relationship between peritoneal SST and systemic vascular function have also reported no correlation with sublingual endothelial glycocalyx properties[36] or conduit vessel endothelial function measured using flow mediated dilatation of the brachial artery.[37,38] However, these studies examined patients well established on PD (mean duration of PD 42 months) in contrast to those in the present study.

The rate of SST across the peritoneum is widely thought to be primarily dictated by factors pertaining to the peritoneal microvascular capillary wall. If changes demonstrated in the systemic microcirculation of dialysis patients[14] such as changes in vessel density and glycocalyx barrier properties were reflected in the peritoneal microcirculation, this could in theory account for some of the variability in rate of SST observed in patients new to PD. We did not observe any moderate or strong correlations between SST and any of the measures of microvascular structure or function in

either of two separate vascular beds. Albumin has a much larger molecular radius than creatinine, consequently much of it's transport is predicted to be via the large pore pathway. Pore-matrix theory[7] would seem to dictate that loss of glycocalyx may play a greater role in the transport of solutes larger than creatinine, due to increased permeability of small pores or creation of more large pores. As predicted by traditional pore theory, we found a strong correlation between SST and albumin clearance but no relationship between peritoneal albumin clearance and any aspect of cutaneous or sublingual microvascular function.

We found a strong relationship between intraperitoneal inflammation and transport of creatinine and albumin. There is increasing evidence for a significant role for intraperitoneal inflammation in the rate of transport of small solutes and larger solutes such as albumin.[23,39,40] The Global fluid investigators reported that dialysate IL-6 concentration was the most significant predictor of SST not only in prevalent but also incident patients, a relationship that was independent of all patient-related factors and dialysis prescription.[23] As reported previously,[23] we have demonstrated that although there is an association between intraperitoneal and systemic inflammatory levels, inflammation within the peritoneum is many times higher than in the systemic circulation and exerts a greater influence on SST.

This study has a number of strengths. It is the first to prospectively study *in vivo* the relationship between the systemic microcirculation and peritoneal SST in incident patients. We present a well described cohort of patients who underwent a range of tests of microvascular structure and function across two vascular beds contemporaneously to their first test of peritoneal permeability.

This study also has several limitations. This is a relatively small study comprising 29 individuals; this number of participants would allow detection of a correlation coefficient of 0.5 or greater. Therefore, it is possible that a correlation smaller than this exists that this study was

unable to detect. All *in vivo* studies of the microcirculation have to contend with significant inter and intra subject temporal and spatial heterogeneity.[31] Despite measures to reduce this including a standardised pre-test meal, peritoneal fasting and a single investigator for all tests, the inherent heterogeneity of the microcirculation in this small cohort may have further reduced our ability to detect small correlations.

Although systemic microvascular dysfunction is likely to have implications for long-term cardiovascular health, in this study, it did not significantly influence the rate of SST even early in patients PD careers. This study adds to an increasing body of evidence that intraperitoneal factors particularly intraperitoneal inflammation have a far greater impact on rate of SST than systemic factors.

Authors' note

Acknowledgements

Author contributions

All authors made substantial contributions to the design of the study. JW was responsible for all data collection and analysis. All authors contributed to interpretation of the data. The first draft of the manuscript was written by JW. All authors contributed to revisions of the manuscript and approved the final submitted draft.

Data availability statement

The data supporting this publication are currently available by contacting Dr J Williams (j.k.williams@exeter.ac.uk). Data will be made publicly available from the University of Exeter's institutional repository.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

ORCID iD

Jennifer Williams https://orcid.org/0000-0002-2779-9620

Supplemental material

Supplemental material for this article is available online

References

1. Levick JR. *Cardiovascular physiology*. 5th ed. Great Britain: Hodder Arnold, 2010.

2. McDonald KK, Cooper S, Danielzak L, et al. Glycocalyx degradation induces a proinflammatory phenotype and increased leukocyte adhesion in cultured endothelial cells under flow. *PLoS ONE* 2016; 11: e0167576.

3. Zeng Y, Zhang XF, Fu BM, et al. The role of endothelial surface glycocalyx in mechanosensing and transduction. *Adv Exp Med Biol* 2018; 1097: 1–27.

4. Huxley VH and Williams DA. Role of a glycocalyx on coronary arteriole permeability to proteins: evidence from enzyme treatments. *Am J Physiol Heart Circ Physiol* 2000; 278: H1177–1185. Research Support, U.S. Gov't, P.H.S.

5. Rippe B, Rosengren BI and Venturoli D. The peritoneal microcirculation in peritoneal dialysis. *Microcirculation* 2001; 8: 303–320. Research Support, Non-U.S. Gov't Review.

6. Rippe B. A three-pore model of peritoneal transport. *Perit Dial Int* 1993; 13(Suppl 2): S35–38. Research Support, Non-U.S. Gov't.

7. Flessner MF. Endothelial glycocalyx and the peritoneal barrier. *Perit Dial Int* 2008; 28: 6–12.

8. Fasoli G, Esposito C, Cornacchia F, et al. Uremic serum induces proatherogenic changes in human endothelial cells. *J Nephrol* 2006; 19: 599–604.

9. de Groot K, Bahlmann FH, Sowa J, et al. Uremia causes endothelial progenitor cell deficiency. *Kidney Int* 2004; 66: 641–646.

10. Dou L, Jourde-Chiche N, Faure V, et al. The uremic solute indoxyl sulfate induces oxidative stress in endothelial cells. *J Thromb Haemost* 2007; 5: 1302–1308.

11. Fuchs D, Dupon PP, Schaap LA, et al. The association between diabetes and dermal microvascular dysfunction non-invasively assessed by laser Doppler with local thermal hyperemia: a systematic review with meta-analysis. *Cardiovasc Diabetol* 2017; 16: 11.

12. Cekic EG, Basaran O, Filiz Basaran N, et al. Cutaneous microvascular reactivity and aortic elasticity in coronary artery disease: comparison of the laser Doppler flowmetry and echocardiography. *Microvasc Res* 2017; 109: 19–25.

13. Houben A, Martens RJH and Stehouwer CDA. Assessing microvascular function in humans from a chronic disease perspective. *J Am Soc Nephrol JASN* 2017; 28: 3461–3472.

14. Williams J, Gilchrist M, Strain D, et al. The systemic microcirculation in dialysis populations. *Microcirculation* 2020; 27: e12613.

15. Thang OH, Serne EH, Grooteman MP, et al. Capillary rarefaction in advanced chronic kidney disease is associated with high phosphorus and bicarbonate levels. *Nephrol Dial Transplant* 2011; 26: 3529–3536.

16. Nissel R, Fischer DC, Puhlmann A, et al. Short-term growth hormone treatment and microcirculation: effects in patients with chronic kidney disease. *Microvasc Res* 2009; 78: 246–252.

17. Yeh YC, Chao A, Lee CY, et al. An observational study of microcirculation in dialysis patients and kidney transplant recipients. *Eur J Clin Invest* 2017; 47: 630–637.

18. Wilkinson SP, Spence VA and Stewart WK. Arterial stiffening and reduced cutaneous hyperaemic response in patients with end-stage renal failure. *Nephron* 1989; 52: 149–153.

19. Stewart J, Kohen A, Brouder D, et al. Noninvasive interrogation of microvasculature for signs of endothelial dysfunction in patients with chronic renal failure. *Am J Physiol Heart Circ Physiol* 2004; 287: H2687–2696.

20. Vlahu CA, Lemkes BA, Struijk DG, et al. Damage of the endothelial glycocalyx in dialysis patients. *J Am Soc Nephrol* 2012; 23: 1900–1908. Research Support, Non-U.S. Gov't.

21. Twardowski ZJNK, Khanna R, Prowant B, et al. Peritoneal equilibration test. *Perit Dial Bull* 1987; 7: 138–147.

22. Kazancioglu R, Topcuoglu D, Kiricimli N, et al. Comparison of fast peritoneal equilibrium test with the standard method: a pilot study. *J Ren Care* 2012; 38: 29–33.

23. Lambie M, Chess J, Donovan KL, et al. Independent effects of systemic and peritoneal inflammation on peritoneal dialysis survival. *J Am Soc Nephrol* 2013; 24: 2071–2080. Research Support, Non-U.S. Gov't.

24. Farkas K, Nemcsik J, Kolossvary E, et al. Impairment of skin microvascular reactivity in hypertension and uraemia. *Nephrol Dial Transplant* 2005; 20: 1821–1827.

25. Khan F, Patterson D, Belch JJ, et al. Relationship between peripheral and coronary function using laser Doppler imaging and transthoracic echocardiography. *Clin Sci (Lond)* 2008; 115: 295–300.

26. Seliger SL, Salimi S, Pierre V, et al. Microvascular endothelial dysfunction is associated with albuminuria and CKD in older adults. *BMC Nephrol* 2016; 17: 82.

27. Nieuwdorp M, Mooij HL, Kroon J, et al. Endothelial glycocalyx damage coincides with microalbuminuria in type 1 diabetes. *Diabetes* 2006; 55: 1127–1132. Research Support, Non-U.S. Gov't.

28. Casanova F, Wood AR, Yaghootkar H, et al. A mendelian randomization study provides evidence that adiposity and dyslipidemia lead to lower urinary albumin-to-creatinine ratio, a marker of microvascular function. *Diabetes* 2020; 69: 1072–1082.

29. Cracowski JL and Roustit M. Current methods to assess human cutaneous blood flow: an updated focus on laser-based-techniques. *Microcirculation* 2016; 23: 337–344.

30. Kalia YN, Naik A, Garrison J, et al. Iontophoretic drug delivery. *Adv Drug Deliv Rev* 2004; 56: 619–658.

31. Cracowski JL, Minson CT, Salvat-Melis M, et al. Methodological issues in the assessment of skin microvascular endothelial function in humans. *Trends Pharmacol Sci* 2006; 27: 503–508.

32. Goedhart PT, Khalilzada M, Bezemer R, et al. Sidestream Dark Field (SDF) imaging: a novel stroboscopic LED ring-based imaging modality for clinical assessment of the microcirculation. *Opt Express* 2007; 15: 15101–15114.

33. Ince C, Boerma EC, Cecconi M, et al. Second consensus on the assessment of sublingual microcirculation in critically ill patients: results from a task force of the European Society of Intensive Care Medicine. *Intensive Care Med* 2018; 44: 281–299.

34. Hubble SM, Kyte HL, Gooding K, et al. Variability in sublingual microvessel density and flow measurements in healthy volunteers. *Microcirculation* 2009; 16: 183–191.

35. Nieuwdorp M, Meuwese MC, Mooij HL, et al. Measuring endothelial glycocalyx dimensions in humans: a potential novel tool to monitor vascular vulnerability. *J Appl Physiol* 2008; 104: 845–852. Evaluation Studies Research Support, Non-U.S. Gov't.

36. Vlahu CA, Lopes Barreto D, Struijk DG, et al. Is the systemic microvascular endothelial glycocalyx in peritoneal dialysis patients related to peritoneal transport? *Nephron Clin Pract* 2014; 128: 159–165.

37. Han SH, Lee SC, Kang EW, et al. Reduced residual renal function is associated with endothelial dysfunction in patients receiving peritoneal dialysis. *Perit Dial Int* 2012; 32: 149–158.

38. Figueiredo AE, Pinheiro da Costa BE, Conti A, et al. Peritoneal transport function and endothelium-dependent vasodilation. *Perit Dial Int* 2007; 27: 203–205.

39. Shi Y, Yan H, Yuan J, et al. Different patterns of inflammatory and angiogenic factors are associated with peritoneal small solute transport and peritoneal protein clearance in peritoneal dialysis patients. *BMC Nephrol* 2018; 19: 119.

40. Sawai A, Ito Y, Mizuno M, et al. Peritoneal macrophage infiltration is correlated with baseline peritoneal solute transport rate in peritoneal dialysis patients. *Nephrol Dial Transplant* 2011; 26: 2322–2332.