The Systemic Microcirculation In Dialysis Populations

Jennifer Williams [1,2], Mark Gilchrist [1,2], David Strain [1,2], Donald Fraser [3] and Angela Shore [1,2]

1 Diabetes and Vascular Medicine Research Centre, Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, UK.

2 NIHR Exeter Clinical Research Facility, Royal Devon and Exeter Foundation NHS Trust, Exeter UK

3 Wales Kidney Research Unit, Cardiff University, Cardiff, United Kingdom.

Running Title: The Systemic Microcirculation In Dialysis Populations

Corresponding Author

Dr Jennifer Williams

Diabetes and Vascular Medicine Research Centre

NIHR Exeter Clinical Research Facility

Barrack Road

Exeter

UK

EX2 5DW

j.k.williams@exeter.ac.uk

Funding Information Jennifer Williams is funded by a Kidney Research UK Training Fellowship TF_013_20151127

Abstract

In a rapidly expanding population of patients with chronic kidney disease, including 2 million people requiring renal replacement therapy, cardiovascular mortality is 15 times greater than the general population. In addition to traditional cardiovascular risk factors, more poorly defined risks related to uraemia and it's treatments appear to contribute to this exaggerated risk. In this context, the microcirculation may play an important early role in cardiovascular disease associated with chronic kidney disease. Experimentally the uraemic environment and dialysis have been linked to multiple pathways causing microvascular dysfunction. Coronary microvascular dysfunction is reflected in remote and more easily studied vascular beds such as the skin. There is increasing evidence for a correlation between systemic microvascular dysfunction and adverse cardiovascular outcomes. Systemic microcirculatory changes have not been extensively investigated across the spectrum of chronic kidney disease. Recent advances in non-invasive techniques studying the microcirculation *in vivo* in man are increasing the data available particularly in patients on haemodialysis. Here, we review current knowledge of the systemic microcirculation in dialysis populations, explore whether non-invasive techniques to study its function could be used to detect early stage cardiovascular disease, address challenges faced in studying this patient cohort and identify potential future avenues for research.

Keywords

Systemic microcirculation, dialysis, in vivo techniques, cardiovascular disease

List of Abbreviations

ACh acetylcholine

eGFR estimated glomerular filtration rate

NO nitric oxide

RBC red blood cell

SDF side-stream darkfield

SNP sodium nitroprusside

Introduction

Systemic microvascular dysfunction has been associated with increased cardiovascular morbidity [1, 2] and mortality[3, 4]. This association is potentially being driven by shared underlying pathological events instrumental in both macro and microvascular disease. Persistent changes in vascular tone lead to structural remodelling. Repeated activation of the vascular endothelium by pro-atherogenic insults results in an imbalance in the production of vasoactive substances, inflammation and a pro-thrombotic state[5]. In combination, these changes compromise the structural and functional ability of the microcirculation to compensate for fluctuating demands.

It is in the coronary circulation, where up to 80% of overall resistance resides in the microvessels[6], that dysfunction has been most convincingly linked to clinically relevant outcomes. The presence of coronary microvascular and endothelial dysfunction not only predicts subsequent cardiovascular events [7-9], but themselves constitute the first stage of atherosclerotic coronary artery disease[10, 11]. Up to 40% of patients referred for angiography following 'typical cardiac chest pain' are found to have normal epicardial coronary arteries[12]. These patients can be assumed to have a combination of functional and structural coronary microvascular disease contributing to abnormal myocardial perfusion.

This impairment of coronary microvascular structure and function is reflected in concurrent changes in remote and more easily studied vascular beds. For example significant reductions in dermal capillary numbers have been demonstrated in patients with 'typical cardiac chest pain' despite normal coronary arteries[13]. Peripheral microvascular dysfunction has therefore been used as a surrogate for dysfunction of the coronary microcirculation.

There are a growing number of novel methods being utilised to study the structure and function of the systemic microcirculation *in vivo* in multiple patient cohorts, including those with chronic kidney disease[14].

Why study the microcirculation in end stage renal disease?

The global prevalence of chronic renal disease is upwards of 13%[15] and more than 2 million people worldwide are dependent on renal replacement therapies[16]. In this population, rates of cardiovascular mortality are 15 times those of the general population[17]. Although traditional cardiovascular risk factors are prevalent within the dialysis population their presence alone does not fully account for this exaggerated risk[18]. In this context, systemic microcirculatory dysfunction may be a significant contributor to cardiovascular burden.

Patients with end stage renal disease who are on dialysis are at significant risk for systemic microvascular dysfunction. Uraemia is associated with endothelial cell activation[19], impaired endothelial repair[20], oxidative stress[21] and impaired nitric oxide bioavailability. Additionally, these patients exist in a state of chronic inflammation[22]. Levels of inflammatory mediators such as Interleukin-6 and tumour necrosis factor are strongly correlated with eGFR[23], both are associated with endothelial dysfunction[24, 25]. Other dialysis specific risk factors include repeated myocardial stunning and haemodynamic perturbation of vascular beds[26] during haemodialysis and exposure to non-physiological dialysis fluids in peritoneal dialysis[26].

Multiple studies have demonstrated links between surrogate markers of endothelial dysfunction and chronic renal disease . These include; circulating endothelial surface layer components[27], markers of inflammation and amino acids released by the endothelial cells in response to damage [28, 29], peptides known to inhibit pro-atherogenic changes[30] and endothelial dysfunction as measured in larger vessels[31].

The systemic microcirculation in dialysis patients

Techniques that directly and non-invasively study *in vivo* alterations in microvascular structure [32, 33] and function [28, 34-36] are increasingly being used to expand our knowledge of the relationship between chronic renal disease and microcirculatory dysfunction. Perturbation of microvascular

function in patients with chronic renal disease has been reproducibly demonstrated in different vascular beds, including skin[28, 36, 37], sublingual[27, 32, 38] and coronary[39].

Cutaneous Microcirculation

The cutaneous microcirculation, the most easily accessible vascular bed, has been of interest in patients on dialysis since histological alterations were first demonstrated in these patients in the 1980s. Skin biopsies from haemodialysis patients without known macrovascular disease or diabetes demonstrated thickening of the basement membrane, endothelial activation and chronic inflammatory cell infiltrates in cutaneous capillaries [40]. The extent of these changes correlated with the length of time these patients had been on haemodialysis[41]. *In vivo* the nail-fold capillary bed is easily visualised microscopically. Morphological changes here have also been correlated with duration of dialysis [42]. Reduction in capillary numbers is important as it reduces the surface area available for exchange, jeopardising tissue health. Capillary rarefaction has been demonstrated in the nail-fold capillaries of paediatric haemodialysis patients [33] compared with healthy, 'height-age' matched controls. The paediatric population is interesting to study with regards the microcirculation as unlike their adult counterparts, they often have a single renal limited pathology. This helps to differentiate microcirculatory pathology attributable to uraemia and it's treatments from that attributable to other systemic pathologies for example diabetes. This finding has been replicated in adult haemodialysis cohorts[43, 44] well matched for age, blood pressure and BMI with healthy controls.

Due to its role in temperature regulation, human skin has a high vasodilatory reserve and can change its flow more than a hundred-fold in response to metabolic, thermal and pharmacologic stimuli. Relative changes in skin blood flow can be easily and non-invasively measured using laser Doppler based techniques[45] (Figure 1). Even in the resting state oscillations in microvascular flow are modulated by multiple physiological factors. Spectral analysis can be used to sub-divide laser Doppler acquired recordings according to their frequency into those representing; endothelial

activity, sympathetic activity, vascular myogenic activity, respiratory activity and heart activity [14]. Reports of baseline skin blood flow in dialysis patients did not initially seem to be significantly different to healthy controls[28, 34, 46-48]. However, when examined in more detail subtle differences were apparent. Although the averaged flux was not different, 'hot spots' or distinct spots of high perfusion were reduced and significant impairments were noted in the frequency domains corresponding to endothelial, sympathetic and cardiac activity in dialysis patients compared with controls[36].

Maximal vasodilation of skin blood vessels can be achieved by localised heating to between 42-44°C [49]. A reactive hyperaemia can also be provoked by a brief period of arterial occlusion[50] (Figure 2). Impairments in the maximal vasodilatory response to heating[36, 46] and maximal post-occlusive flow[28, 36] have been reported in haemodialysis patients compared with healthy controls.

In their study of 63 haemodialysis patients and 33 healthy controls, Stewart and colleagues[36] reported a delay in the maximal vasodilatory response to heating in the dialysis patients compared with controls. However, they were only able to demonstrate a significant reduction in the size of the maximal post-occlusive flow compared with controls in those haemodialysis patients with known diabetes and cardiovascular disease, not the cohort as a whole. A smaller study (16 haemodialysis patients versus 16 controls)[28], wherein all participants were 'free of concomitant diseases causing alterations in endothelium-dependant vasomotion', did report a reduction in maximal post-occlusive flow in the dialysis cohort compared with controls. As would be expected, their dialysis group was significantly more hypertensive than their healthy controls and given that even borderline hypertension effects the microcirculation this may have contributed to the microvascular dysfunction observed.

More direct interrogation of this apparent reduction in microvascular function can be achieved by combining laser Doppler measurements with iontophoretic application of vasoactive substances [51] (Figure 3) to investigate which discreet areas of microvascular function are impaired. Impairments of

both endothelial-dependant and -independent responses have been demonstrated in haemodialysis patients compared with both age, sex and BMI matched healthy controls[28, 34] and pre-dialysis chronic renal disease patients with comparable cardiovascular burden[52].

Sublingual Microcirculation

More recently SDF imaging has allowed for direct visualisation of flow in other vascular beds with a mucosal covering. The most commonly studied is the sublingual bed [53, 54] (Figure 4 a + b). To date the only publish study using SDF to examine chronic changes in sublingual vessel density and flow in dialysis patients[32], reported a reduction in total and perfused vessel density plus increased vessel flow heterogeneity compared with controls. This was particularly pronounced in the very small vessels (diameter less than 20μm)[32].

Assessment of the sublingual circulation also provides an opportunity to non-invasively assess another component of the vascular system, the glycocalyx. The glycocalyx covers the luminal surface of endothelial cells. It is a negatively charged network of proteoglycans, glycosaminoglycans and plasma constituents, which acts as an interface between the blood and the vascular wall. The glycocalyx has important regulatory and protective roles including, regulating vascular wall permeability, mechano-transduction, and inhibiting leucocyte adhesion. It is susceptible to damage from oxidative stress, which may arise from inflammation, ischaemia, hyperglycaemia, or other causes[55]. Due to its delicate nature, study of the glycocalyx is challenging. Historical approaches have included measurement of total volume using tracers, and measuring shed glycocalyx components in plasma. SDF-acquired images (Figure 4b) can now be combined with Glycocheck© software to analyse spatial and temporal variations in erythrocyte column width within the microvasculature[56]. When the cell-impermeable glycocalyx is damaged, circulating red cells can travel closer to the endothelium. Using this approach, loss of glycocalyx barrier properties has been demonstrated in a mixed cohort of haemodialysis and peritoneal dialysis patients [27], and has been

found to associate with diminished eGFR and with increased circulating levels of shed endothelial surface layer components syndecan-1 and thrombomodulin [38].

Coronary Microcirculation

The ability of the coronary microcirculation to adapt to changing demands is vital. Coronary flow reserve is the maximum flow resulting from stress vasodilatation of coronary arteries and the coronary microcirculation, measured using positron emission tomography or magnetic resonance imaging. In this context, 90% of myocardial blood flow takes place through vessels with diameter less than 150μm, which penetrate the walls of the myocardium [57]. Coronary flow reserve is therefore a test of both endothelial dysfunction and coronary microvascular reserve. It is expressed as the ratio of hyperaemic to basal diastolic peak velocities, with a value above two considered normal. Low coronary flow reserve indicates a reduced ability to appropriately increase flow in response to increased oxygen demand. Coronary flow reserve has been found to be significantly lower in dialysis patients compared with healthy controls who were well matched for age, sex, BMI and blood pressure [39, 58]. In patients with angiographically normal coronary arteries, 50% of the dialysis cohort were found to have coronary flow reserve less than 2 compared with only 5% in the control group of non-dialysis patients [39].

Potential confounding factors

Caution must be exercised in attributing all the alterations observed in the above studies to renal failure and its treatments. Many patients with end stage kidney disease have co-morbid illnesses which may also affect the systemic microcirculation, most notably hypertension and diabetes mellitus. Several of the studies discussed above exclude from their control group of 'healthy volunteers' those with these conditions but hypertension, diabetes and other co-morbidities are present in a large proportion of the dialysis group[32, 36]. In these studies, measured differences

between groups are likely to represent the combined effects of chronic uraemia, dialysis and other co-morbidities.

Even in otherwise well matched cohorts, dialysis patients frequently have increased systolic blood pressure compared with their control counterparts[32, 33]. Therefore, in addition to their dialysis patients and healthy controls, Farkas and colleagues studied a third group of age-matched patients with essential hypertension[28]. They were able to demonstrate a significant reduction in both endothelium-dependant and independent vasodilatation in their dialysis patients compared with controls and those with hypertension.

Are microcirculatory changes associated with clinical outcomes?

A link between microvascular dysfunction and adverse cardiovascular outcomes has been demonstrated in other populations [3, 4, 59, 60]. Vascular dysfunction in the skin has been demonstrated to correlate with coronary disease[61] and be an independent marker for cardiovascular disease in patients with Type 2 diabetes[62]. As these techniques become better understood and increasingly used in renal cohorts, interest has turned to how they may be used as biomarkers to identify high risk patients and facilitate intervention at an earlier stage.

Coronary microvascular rarefaction has been postulated as contributory to sudden cardiac death in the dialysis population[63]. In a cohort study of nearly 4000 individuals encompassing the whole spectrum of chronic renal disease , coronary flow reserve was shown to be strongly associated with cardiovascular death independent of chronic renal disease stage[64]. Adjusting for coronary flow reserve in chronic renal disease 4,5 and dialysis-dependant groups attenuated their risk of cardiovascular death by 10%, supporting the concept that coronary microvascular dysfunction may underlie some of the increased mortality associated with chronic renal disease.

In separate multi-variate regression analyses, microvascular impairment as measured by forearm post-ischaemic vasodilatation [65] and coronary flow reserve [63] were found to be independently associated with all-cause mortality in haemodialysis patients.

Microvascular dysfunction of the coronary and peripheral circulations have also been correlated with outcome measures known to have negative prognostic implications such as hypoalbuminaemia[66, 67] and right ventricular dysfunction[68].

Chronic renal disease mineral bone disease can cause large vessel calcification, a strong predictor of cardiovascular death in haemodialysis patients[69]. There is some evidence for an association between large vessel calification and microvascular dysfunction in haemodialysis patients, those with femoral artery calcification exhibited lower maximal vasodilatory responses to ACh and SNP than both controls and haemodialysis patients without large vessel calcification [34]. There is also increasing evidence of a relationship between markers of worsening chronic renal disease mineral bone disease and microvascular abnormalities in the absence of large vessel calcification. Dermal capillary rarefaction and impaired coronary flow reserve have been associated with increasing levels of both iPTH[33] and phosphorous [43, 64] in chronic renal disease cohorts. Even in cohorts with normal renal function serum phosphate concentrations have been negatively correlated with postocclusive capillary recruitment[70] and endothelial dysfunction in larger vessels[71].

Patients at risk of other non- cardiovascular disease outcomes which significantly impact on morbidity and quality of life, such as wound healing have also been identified using these techniques. Those patients with lower skin blood flow both before and during haemodialysis, as measured by laser Doppler, have been shown to be at greater risk of developing wounds and skin defects[66]. All patients in this study who later went on to develop a skin defect had evidence of intradialytic ‘critical perfusion’ at the microvascular level in at least one measured area, although none exhibited intra-dialytic hypotension.

What are the effects of chronic dialysis?

Cardiovascular risk increases as patients progress through the stages of chronic renal disease (classified as stages 1 to 5 with progressive falls in glomerular filtration rate and increasing albuminuria) and with time on dialysis[72]. Is microvascular impairment similarly related to stage of chronic renal disease and time on dialysis?

It has been found that even the creation of an arteriovenous fistula in preparation for haemodialysis may have systemic microvascular effects. In pre-dialysis patients, successful formation of an arteriovenous fistula led to a reduction in endothelial dependant vasodilation in the fistula arm. Following fistula creation, these patients also exhibited a reduction in non-endothelium dependant vasodilation in the contralateral arm, indicating that localised changes to the structure of the macrocirculation can lead to widespread changes in the microcirculation. This was in contrast to those patients who had primary arteriovenous fistula failure, who exhibited no recordable local or systemic changes[73].

Cross-sectional studies also provide evidence for a relationship between stage of kidney disease and microcirculatory dysfunction. Plasma levels of shed glycocalyx components such as syndecan-1 and markers of endothelial activation such as angiopoietin-2 correlate inversely with eGFR [38]. Retinal microvessels also narrow progressively with each stage of chronic kidney disease[74]. Additionally, histopathological evidence of endothelial activation and infiltration by inflammatory cells in dermal capillaries [40, 41] and circulating levels of adhesion molecules such as sVCAM-1 correlate with duration of dialysis [75]. The potential effects of renal replacement therapy itself on the microcirculation remain less well defined. Using SDF technology, Dane and his colleagues were able to demonstrate impaired glycocalyx integrity associated with worsening eGFR. However, in their end stage renal disease group (n=23) no statistically significant difference was seen between the dialysis patients (n=9) and patients with end stage renal disease who were not on dialysis(n=14)[38]. Common to many

of the studies presented here small sample size may have contributed to the lack of statistically significant findings.

A large American cohort study found that although coronary microvascular function assessed by coronary flow reserve was 23% lower in dialysis patients compared with controls with preserved kidney function, this reduction occurred early in chronic kidney disease, with a nadir being reached in chronic renal disease stage 4[64]. The authors found no additional reductions in stage 5 or 5D. However, it is important to note that the chronic kidney disease stage 4 patients were on average 10 years older than the dialysis group and had a higher incidence of known ischaemic heart disease and oral nitrate use. It is possible in light of this that survivor bias has limited the apparent extent of microvascular dysfunction detected in the patients with chronic kidney disease stage 5 in this retrospective study. Some of these issues could be addressed by longitudinal studies directly investigating microvascular function in dialysis cohorts. INTHEMO is an ongoing two-year study primarily designed to assess the effects of haemodialysis intensity on micro and macrovascular parameters[76]. In a preliminary report, these investigators found no statistically significant change in glycocalyx parameters at 6 months follow-up compared with baseline. They did however note significant heterogeneity in the degree and direction of change of calculated glycocalyx properties at 6 months, and data at study completion is awaited. One important limitation of historical studies may be the effect of the haemodialysis procedure itself. The microcirculation is inherently dynamic, and as described below, timing of microvascular measurements with regards to the patients haemodynamic therapy itself may have significant impact on results. Standardisation of timing of measurements with respect to haemodialysis therapy is an important consideration for future studies.

What are the acute effects of dialysis?

Haemodialysis has been shown to cause varying degrees of macrohaemodynamic instability in patients often because of ultrafiltration of fluid, observed clinically as intradialytic hypotension.

Recurrent intradialytic hypotension is considered to have negative prognostic implications[77]. Studies of the sublingual microcirculation using SDF during a single haemodialysis session have demonstrated a reduction in microvascular flow and decrease in the proportion of the microcirculation that is perfused through the course of the treatment[78, 79]. This reduced flow in all microvessels has been attributed to a reduction in circulating volume secondary to ultrafiltration. In some studies, reduced microvascular perfusion has been demonstrated in patients undergoing isolated ultrafiltration but not in those undergoing haemodialysis with linear ultrafiltration [80]. This finding is supported by data showing the reduced flow may be partially corrected by a manoeuvre designed to increase central venous filling[78]. These microcirculatory changes were independent of macrohaemodynamic changes, for example blood pressure, implying an element of compensation by the microcirculation.

Decreased intradialytic perfusion has also been demonstrated in the peripheral circulation [66, 81]. However, it has been suggested that changes in perfusion here may be dependent on the patient's pre-dialysis volume status. Hypervolaemic patients who were ultrafiltrated to normovolaemia had improved skin perfusion[82], this was accompanied by a decrease in arterial and venous pressure and proposed to be as a result of decreased myogenic response as a local auto regulatory effect. Another potential mechanism could be interstitial fluid removal with reduced external compression of vessels.

Significant alterations in haemodynamics and shear stress result in stimuli noxious to the glycocalyx including oxidative stress[83] and inflammation[84]. An increase in plasma shed glycocalyx constituents has been demonstrated over the course of a 4 hour dialysis session. However, this was not accompanied by a deterioration in sublingual glycocalyx parameters, potentially reflecting differential responses to haemodialysis in different vascular beds [85]. Importantly, the reliability of plasma shed endothelial components as a marker of endothelial damage in patients with significant

renal impairment has been challenged, due to decreased renal excretion and unknown dialysis clearance[86].

It has been suggested that haemodialysis may not be entirely detrimental to the microcirculation. The process of haemodialysis results in the release of local vasodilatory substances [87] and removal of circulating inhibitors of endothelial function such as; asymmetrical dimethylarginine, an inhibitor of endothelial NO production[88, 89]. Improvements in retinal microvascular function during single haemodialysis sessions have been demonstrated in several studies [90, 91]. However, these potentially beneficial effects appear to be transient, returning to baseline within hours [88, 92]. This may however help to explain some of the heterogeneity in the literature and highlights the importance of timing of investigations with regards dialysis therapy when designing and evaluating data in studies of the microcirculation.

Is microcirculatory dysfunction modifiable?

As there is evidence of a relationship between microcirculatory function and eGFR[38, 41] it could be postulated that successful restoration of excretory function should improve microcirculatory parameters. Renal transplantation is the preferred mode of renal replacement therapy for all eligible patients as cardiovascular outcomes and quality of life are improved compared with dialysis.

Early skin biopsy studies indicated that 'uraemia associated microangiopathy' could be at least partially reversed by successful transplantation[93]. Using data and samples from a large biobank, a retrospective study of patients receiving their first renal transplant having previously been on dialysis, found that sVCAM-1 levels (a marker of endothelial injury) fell within 1 month of transplantation and continued to decline for at least 2 years[75] supporting an improvement in endothelial function with improvement in renal function.

Cross-sectional studies using SDF imaging in the sublingual circulation have demonstrated significant deterioration in glycocalyx and microvascular perfusion parameters in dialysis patients compared to

age matched healthy controls and renal transplant recipients[32, 38]. At a median of 5 years post-transplant, the glycocalyx parameters of patients with a stable functioning transplant were indistinguishable from the healthy controls[38]. Whilst microvascular flow was more heterogeneous in transplant recipients the total density of small vessels and the proportion that were perfused was not significantly worse than controls[32]. In the coronary microcirculation transplant recipients were found to have a significantly reduced coronary flow reserve compared with healthy controls (1.89 'v' 2.65), but better than a group of age matched haemodialysis patients (1.57) [58].

In those with a failing or failed transplant the relationship appears to be more complex. Transplant recipients with evidence of interstitial fibrosis and tubular atrophy had sublingual glycocalyx parameters comparable to haemodialysis patients despite their median eGFR of 22ml/min[38]. Furthermore, patients who return to dialysis after a failed transplant exhibited worse coronary microvascular function than dialysis (both haemodialysis and peritoneal dialysis) patients of similar vintage who have never been transplanted[67, 94]. The known association between inflammation and microvascular dysfunction[76] led the authors to speculate that inflammation associated with the failed allograft was partially responsible for the deterioration, in both these studies the failed transplant recipients had higher inflammatory markers than the transplant naïve group. This is an interesting hypothesis although the underlying pathology is likely to be multifaceted. While time on dialysis may have been similar between groups the patients with failed transplant are likely to have had a longer period with end stage renal failure, additionally they will have been exposed to immunosuppressant medications such as calcineurin inhibitors, with known vascular effects[95]. As discussed above, changes to the microcirculation occur throughout the stages of chronic renal disease and what is not clear from this study is how changes in the failing transplant group compare to patients with a native eGFR of 22ml/min.

Issues in the current literature

Comparison of studies in this area is impeded by methodological issues. By its nature the microcirculation exhibits significant temporal and spatial heterogeneity [81]. Consequently, most of the techniques outlined above have to contend with substantial intra-subject variability. Much of the literature reviewed here is cross-sectional therefore there will be significant variability in the outcome measures, reducing their ability to detect small differences between patient groups for example.

There are other experimental issues pertinent to studying a renal cohort. End stage renal disease is a phenotype, not a specific pathology and therefore renal cohorts are also heterogeneous. Secular, geographic, and ethnic variation impact prevalent primary and co-morbid pathologies, many of which have direct relevance to the microcirculation such as diabetes and hypertension. There is also high usage in this population of medications known to impact microvascular reactivity.

Studying patients undergoing an intermittent therapy, such as haemodialysis, presents its own challenges; as outlined above, timing of investigations is important, this varies both between and within studies [36]. Rapidly changing flow and haematocrit, changes in room and dialysate temperature, different compositions of dialysate and method of vascular access are all likely to affect the results of these non-invasive techniques. Perhaps more importantly, such a haemodynamic insult is likely to affect each vascular bed differently.

These inherent methodological issues are often compounded by small sample sizes.

Potential future work

The issues identified above mean several gaps remain in our knowledge with regards the state of the microcirculation as measured using these non-invasive techniques. What is required to adequately delineate the natural history of microvascular dysfunction in chronic kidney disease and dialysis are

large-scale, longitudinal studies in a variety of vascular beds with consensus on timing of investigations.

Along with the heterogeneous nature of a renal cohort there are also several treatment options available for renal replacement therapy. The two main forms of dialysis offered to patients, haemodialysis and peritoneal dialysis, are intrinsically different and likely to affect the systemic microcirculation in distinct ways. As a result of its acute haemodynamic effects and by virtue of the fact that they account for the large majority of the dialysis population, most microvascular work in dialysis patients has, to date, focused on haemodialysis. Studies investigating microcirculatory properties in peritoneal dialysis patients lag behind their haemodialysis contemporaries. When peritoneal dialysis patients are included in cohorts they are often analysed with the haemodialysis patients under the umbrella of 'dialysis requiring'. Attempts to analyse them as a sub-group are undermined by small numbers[27].

Peritoneal dialysis has been demonstrated to have cardiovascular effects[18] but they are both qualitatively and quantitatively different from those of haemodialysis. There are also other challenges unique to peritoneal dialysis which need examining, most notably the effect of absorbed glucose. There is a body of work examining the effects of peritoneal dialysis fluid variants on macrohaemodynamic measures such as blood pressure and cardiac output[87, 96, 97]. Similar work examining effects on the microcirculation could allow intervention at an earlier stage in the pathological process. The functionality of peritoneal dialysis is largely dependent on the structure and integrity of the peritoneal microcirculation. Are there insights to be gained from the systemic microcirculation that may increase understanding and aid preservation of the peritoneal circulation[42, 98]?

Despite these gaps in knowledge there is increasing evidence of microcirculatory dysfunction in dialysis cohorts that precedes large vessel disease and is associated with morbidity and mortality. This dysfunction appears to be the result of multiple insults including; uraemia and it's consequences

i.e. chronic renal disease, mineral bone disease; co-morbid pathologies such as hypertension and diabetes and renal replacement therapy itself. This should emphasise to the clinician the importance of primary preventative strategies already enshrined in clinical practice such as dialysis adequacy targets, stringent blood pressure control and correction of bone mineral abnormalities. Greater insights into the pathophysiology of microvascular dysfunction in these patients could advance clinical care of dialysis patients in several ways. It could improve our understanding of the potential benefits of commonly used medications such as ACE inhibitors, routinely used in proteinuric renal disease, there is evidence for a protective effect on systemic vascular endothelium in animal models of aging[99] and heart failure[100]. It could help us understand how best to administer renal replacement therapies for example the potential benefits of more 'extended' haemodialysis[76]. It could also aid development of more novel therapies aimed at protecting endothelial function such as eNOS transcriptase enhancers[101].

Conclusion

The importance of the microcirculation in systemic diseases is becoming increasingly apparent. Historically study of the microcirculation in patients with renal disease especially those on dialysis has lagged behind other chronic conditions. Difficulties in studying a heterogeneous patient group on intermittent therapies may have contributed to this disparity.

Studies have been small and largely cross-sectional. More traditional techniques for studying the microcirculation were often cumbersome and time consuming reducing their clinical utility. We are now gaining greater understanding of the role of newer, more patient friendly techniques such as SDF imaging which should allow expansion of participant numbers.

Reproducible differences in microvascular structure and abnormal function have been demonstrated in multiple vascular beds in dialysis patients compared with controls. The exact nature and chronology of these changes are yet to be fully defined.

As we anticipate an ever-expanding chronic renal disease population with its disproportionate cardiovascular burden, a greater understanding of this dysfunction becomes increasingly important. Large scale longitudinal studies are required to achieve this with the hope that the knowledge gained will guide future interventions to abrogate cardiovascular risk for these patients.

Acknowledgments

We would like to acknowledge the support of the NIHR Exeter Clinical Research Facility and Kidney Research UK grant number TF_013_20151127. All opinions reflected here are those of the authors and not those of NIHR or the UK Department of Health.

References

1. Wong TY, Klein R, Sharrett AR, Duncan BB, Couper DJ, Tielsch JM, Klein BE, Hubbard LD. Retinal arteriolar narrowing and risk of coronary heart disease in men and women. The Atherosclerosis Risk in Communities Study. *JAMA.* 2002;287(9):1153-1159.

2. Wong TY, Klein R, Couper DJ, Cooper LS, Shahar E, Hubbard LD, Wofford MR, Sharrett AR. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. *Lancet.* 2001;358(9288):1134-1140.

3. Mutlu U, Ikram MK, Wolters FJ, Hofman A, Klaver CC, Ikram MA. Retinal Microvasculature Is Associated With Long-Term Survival in the General Adult Dutch Population. *Hypertension.* 2016;67(2):281-287.

4. Liew G, Mitchell P, Rochtchina E, Wong TY, Hsu W, Lee ML, Wainwright A, Wang JJ. Fractal analysis of retinal microvasculature and coronary heart disease mortality. *European Heart Journal.* 2011;32(4):422-429.

5. Endemann DH, Schiffrin EL. Endothelial dysfunction. *J Am Soc Nephrol.* 2004;15(8):1983-1992.

6. Pries AR, Badimon L, Bugiardini R, Camici PG, Dorobantu M, Duncker DJ, Escaned J, Koller A, Piek JJ, de Wit C. Coronary vascular regulation, remodelling, and collateralization: mechanisms and clinical implications on behalf of the working group on coronary pathophysiology and microcirculation. *Eur Heart J.* 2015;36(45):3134-3146.

7. Britten MB, Zeiher AM, Schachinger V. Microvascular dysfunction in angiographically normal or mildly diseased coronary arteries predicts adverse cardiovascular long-term outcome. *Coron Artery Dis.* 2004;15(5):259-264.

8. Pepine CJ, Anderson RD, Sharaf BL, Reis SE, Smith KM, Handberg EM, Johnson BD, Sopko G, Bairey Merz CN. Coronary microvascular reactivity to adenosine predicts adverse outcome in women evaluated for suspected ischemia results from the National Heart, Lung and Blood Institute WISE (Women's Ischemia Syndrome Evaluation) study. *J Am Coll Cardiol.* 2010;55(25):2825-2832.

9. Halcox JP, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, Nour KR, Quyyumi AA. Prognostic value of coronary vascular endothelial dysfunction. *Circulation.* 2002;106(6):653-658.

10. Park KH, Sun T, Diez-Delhoyo F, Liu Z, Yang SW, Lennon RJ, Herrmann J, Gulati R, Rodriguez-Porcel M, Lerman LO, Lerman A. Association between coronary microvascular function and the vasa vasorum in patients with early coronary artery disease. *Atherosclerosis.* 2016;253:144-149.

11. Lerman A, Cannan CR, Higano SH, Nishimura RA, Holmes DR, Jr. Coronary vascular remodeling in association with endothelial dysfunction. *Am J Cardiol.* 1998;81(9):1105-1109.

12. Patel MR, Peterson ED, Dai D, Brennan JM, Redberg RF, Anderson HV, Brindis RG, Douglas PS. Low diagnostic yield of elective coronary angiography. *N Engl J Med.* 2010;362(10):886-895.

13. Antonios TF, Kaski JC, Hasan KM, Brown SJ, Singer DR. Rarefaction of skin capillaries in patients with anginal chest pain and normal coronary arteriograms. *Eur Heart J.* 2001;22(13):1144-1148.

14. Houben A, Martens RJH, Stehouwer CDA. Assessing Microvascular Function in Humans from a Chronic Disease Perspective. *J Am Soc Nephrol.* 2017;28(12):3461-3472.

15. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, Hobbs FD. Global Prevalence of Chronic Kidney Disease - A Systematic Review and Meta-Analysis. *PLoS One.* 2016;11(7):e0158765.

16. Couser WG, Remuzzi G, Mendis S, Tonelli M. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. *Kidney Int.* 2011;80(12):1258-1270.

17. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis.* 1998;32(5 Suppl 3):S112-119.

18. McIntyre CW. Hemodynamic effects of peritoneal dialysis. *Peritoneal Dialysis International.* 2011;31 Suppl 2:S73-76.

19. Fasoli G, Esposito C, Cornacchia F, De Mauri A, Grosjean F, Mangione F, Plati A, Villa L, Dal Canton A. Uremic serum induces proatherogenic changes in human endothelial cells. *J Nephrol.* 2006;19(5):599-604.

20. de Groot K, Bahlmann FH, Sowa J, Koenig J, Menne J, Haller H, Fliser D. Uremia causes endothelial progenitor cell deficiency. *Kidney Int.* 2004;66(2):641-646.

21. Dou L, Jourde-Chiche N, Faure V, Cerini C, Berland Y, Dignat-George F, Brunet P. The uremic solute indoxyl sulfate induces oxidative stress in endothelial cells. *J Thromb Haemost.* 2007;5(6):1302-1308.

22. Zoccali C, Vanholder R, Massy ZA, Ortiz A, Sarafidis P, Dekker FW, Fliser D, Fouque D, Heine GH, Jager KJ, Kanbay M, Mallamaci F, Parati G, Rossignol P, Wiecek A, London G. The systemic nature of CKD. *Nat Rev Nephrol.* 2017;13(6):344-358.

23. Gupta J, Mitra N, Kanetsky PA, Devaney J, Wing MR, Reilly M, Shah VO, Balakrishnan VS, Guzman NJ, Girndt M, Periera BG, Feldman HI, Kusek JW, Joffe MM, Raj DS, Investigators CS. Association between albuminuria, kidney function, and inflammatory biomarker profile in CKD in CRIC. *Clin J Am Soc Nephrol.* 2012;7(12):1938-1946.

24. Lee KW, Blann AD, Lip GY. Inter-relationships of indices of endothelial damage/dysfunction [circulating endothelial cells, von Willebrand factor and flow-mediated dilatation] to tissue factor and interleukin-6 in acute coronary syndromes. *Int J Cardiol.* 2006;111(2):302-308.

25. Piaserico S, Osto E, Famoso G, Zanetti I, Gregori D, Poretto A, Iliceto S, Peserico A, Tona F. Treatment with tumor necrosis factor inhibitors restores coronary microvascular function in young patients with severe psoriasis. *Atherosclerosis.* 2016;251:25-30.

26. Odudu A, McIntyre C. Influence of dialysis therapies in the development of cardiac disease in CKD. *J Ren Care.* 2010;36 Suppl 1:47-53.

27. Vlahu CA, Lemkes BA, Struijk DG, Koopman MG, Krediet RT, Vink H. Damage of the endothelial glycocalyx in dialysis patients. *Journal of the American Society of Nephrology.* 2012;23(11):1900-1908.

28. Farkas K, Nemcsik J, Kolossvary E, Jarai Z, Nadory E, Farsang C, Kiss I. Impairment of skin microvascular reactivity in hypertension and uraemia. *Nephrol Dial Transplant.* 2005;20(9):1821-1827.

29. Tripepi G, Mallamaci F, Zoccali C. Inflammation markers, adhesion molecules, and all-cause and cardiovascular mortality in patients with ESRD: searching for the best risk marker by multivariate modeling. *J Am Soc Nephrol.* 2005;16 Suppl 1:S83-88.

30. Caliskan Y, Gorgulu N, Yelken B, Yazici H, Oflaz H, Elitok A, Turkmen A, Bozfakioglu S, Sever MS. Plasma ghrelin levels are associated with coronary microvascular and endothelial dysfunction in peritoneal dialysis patients. *Ren Fail.* 2009;31(9):807-813.

31. Lee MJ, Han SH, Lee JE, Choi HY, Yoon CY, Kim EJ, Han JH, Han JS, Oh HJ, Park JT, Kang SW, Yoo TH. Endothelial dysfunction is associated with major adverse cardiovascular events in peritoneal dialysis patients. *Medicine.* 2014;93(11):e73.

32. Yeh YC, Chao A, Lee CY, Lee CT, Yeh CC, Liu CM, Tsai MK. An observational study of microcirculation in dialysis patients and kidney transplant recipients. *Eur J Clin Invest.* 2017;47(9):630-637.

33. Edwards-Richards A, DeFreitas M, Katsoufis CP, Seeherunvong W, Sasaki N, Freundlich M, Zilleruelo G, Abitbol CL. Capillary rarefaction: an early marker of microvascular disease in young hemodialysis patients. *Clin Kidney J.* 2014;7(6):569-574.

34. Sigrist MK, McIntyre CW. Vascular calcification is associated with impaired microcirculatory function in chronic haemodialysis patients. *Nephron Clin Pract.* 2008;108(2):c121-126.

35. Thang OH, Serne EH, Grooteman MP, Smulders YM, Ter Wee PM, Tangelder GJ, Nube MJ. Premature aging of the microcirculation in patients with advanced chronic kidney disease. *Nephron Extra.* 2012;2(1):283-292.

36. Stewart J, Kohen A, Brouder D, Rahim F, Adler S, Garrick R, Goligorsky MS. Noninvasive interrogation of microvasculature for signs of endothelial dysfunction in patients with chronic renal failure. *Am J Physiol Heart Circ Physiol.* 2004;287(6):H2687-2696.

37. Smogorzewski MJ. Skin Blood Flow and Vascular Endothelium Function in Uremia. *J Ren Nutr.* 2017;27(6):465-469.

38. Dane MJ, Khairoun M, Lee DH, van den Berg BM, Eskens BJ, Boels MG, van Teeffelen JW, Rops AL, van der Vlag J, van Zonneveld AJ, Reinders ME, Vink H, Rabelink TJ. Association of kidney function with changes in the endothelial surface layer. *Clin J Am Soc Nephrol.* 2014;9(4):698-704.

39. Tok D, Gullu H, Erdogan D, Topcu S, Ciftci O, Yildirim I, Muderrisoglu H. Impaired coronary flow reserve in hemodialysis patients: a transthoracic Doppler echocardiographic study. *Nephron Clin Pract.* 2005;101(4):c200-206.

40. Ichimaru K, Horie A. Microangiopathic changes of subepidermal capillaries in end-stage renal failure. *Nephron.* 1987;46(2):144-149.

41. Lundin AP, Fani K, Berlyne GM, Friedman EA. Dermal angiopathy in hemodialysis patients: the effect of time. *Kidney Int.* 1995;47(6):1775-1780.

42. Schumann L, Korten G, Holdt B, Holtz M. Microcirculation of the fingernail fold in CAPD patients: preliminary observations. *Perit Dial Int.* 1996;16(4):412-416.

43. Thang OH, Serne EH, Grooteman MP, Smulders YM, ter Wee PM, Tangelder GJ, Nube MJ. Capillary rarefaction in advanced chronic kidney disease is associated with high phosphorus and bicarbonate levels. *Nephrol Dial Transplant.* 2011;26(11):3529-3536.

44. Nissel R, Fischer DC, Puhlmann A, Holdt-Lehmann B, Mitzner A, Petzsch M, Korber T, Tiess M, Schmidt R, Haffner D. Short-term growth hormone treatment and microcirculation: effects in patients with chronic kidney disease. *Microvasc Res.* 2009;78(2):246-252.

45. Cracowski JL, Roustit M. Current Methods to Assess Human Cutaneous Blood Flow: An Updated Focus on Laser-Based-Techniques. *Microcirculation.* 2016;23(5):337-344.

46. Wilkinson SP, Spence VA, Stewart WK. Arterial stiffening and reduced cutaneous hyperaemic response in patients with end-stage renal failure. *Nephron.* 1989;52(2):149-153.

47. Rossi MC, A. Morelli, E. Tintori, G. Fabbri, A. Battini, S. Vagheggini, G. Barsotti, G. Laser Doppler Flowmeter Assessment of Skin Microcirculation in Uremic Patients on Hemodialysis Treatment. *Nephron.* 1996;73(4):544-548.

48. Rossi M, Cupisti A, Di Maria C, Galetta F, Barsotti G, Santoro G. Blunted post-ischemic increase of the endothelial skin blood flowmotion component as early sign of endothelial dysfunction in chronic kidney disease patients. *Microvasc Res.* 2008;75(3):315-322.

49. Gooding KM, Hannemann MM, Tooke JE, Clough GF, Shore AC. Maximum skin hyperaemia induced by local heating: possible mechanisms. *J Vasc Res.* 2006;43(3):270-277.

50. Cracowski JL, Minson CT, Salvat-Melis M, Halliwill JR. Methodological issues in the assessment of skin microvascular endothelial function in humans. *Trends Pharmacol Sci.* 2006;27(9):503-508.

51. Kalia YN, Naik A, Garrison J, Guy RH. Iontophoretic drug delivery. *Adv Drug Deliv Rev.* 2004;56(5):619-658.

52. Ryu JH, Yu M, Lee S, Ryu DR, Kim SJ, Kang DH, Choi KB. AST-120 Improves Microvascular Endothelial Dysfunction in End-Stage Renal Disease Patients Receiving Hemodialysis. *Yonsei Medical Journal.* 2016;57(4):942-949.

53. Hubble SM, Kyte HL, Gooding K, Shore AC. Variability in sublingual microvessel density and flow measurements in healthy volunteers. *Microcirculation.* 2009;16(2):183-191.

54. De Backer D, Hollenberg S, Boerma C, Goedhart P, Büchele G, Ospina-Tascon G, Dobbe I, Ince C. How to evaluate the microcirculation: report of a round table conference. *Critical Care.* 2007;11(5):R101.
55. Goligorsky MS. Vascular endothelium in diabetes. *Am J Physiol Renal Physiol.* 2017;312(2):F266-f275.
56. Nieuwdorp M, Meuwese MC, Mooij HL, Ince C, Broekhuizen LN, Kastelein JJ, Stroes ES, Vink H. Measuring endothelial glycocalyx dimensions in humans: a potential novel tool to monitor vascular vulnerability. *Journal of Applied Physiology.* 2008;104(3):845-852.
57. McIntyre CW, John SG, Jefferies HJ. Advances in the cardiovascular assessment of patients with chronic kidney disease. *NDT Plus.* 2008;1(6):383-391.
58. Caliskan Y, Oflaz H, Demirturk M, Yazici H, Turkmen A, Cimen A, Elitok A, Yildiz A. Coronary flow reserve dysfunction in hemodialysis and kidney transplant patients. *Clin Transplant.* 2008;22(6):785-793.
59. Shamim-Uzzaman QA, Pfenninger D, Kehrer C, Chakrabarti A, Kacirotti N, Rubenfire M, Brook R, Rajagopalan S. Altered cutaneous microvascular responses to reactive hyperaemia in coronary artery disease: a comparative study with conduit vessel responses. *Clin Sci (Lond).* 2002;103(3):267-273.
60. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Jr., Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation.* 2000;101(9):948-954.
61. RG IJ, de Jongh RT, Beijk MA, van Weissenbruch MM, Delemarre-van de Waal HA, Serne EH, Stehouwer CD. Individuals at increased coronary heart disease risk are characterized by an impaired microvascular function in skin. *Eur J Clin Invest.* 2003;33(7):536-542.
62. Yamamoto-Suganuma R, Aso Y. Relationship between post-occlusive forearm skin reactive hyperaemia and vascular disease in patients with Type 2 diabetes--a novel index for detecting micro- and macrovascular dysfunction using laser Doppler flowmetry. *Diabetic Medicine.* 2009;26(1):83-88.
63. Shah NR, Charytan DM, Murthy VL, Skali Lami H, Veeranna V, Cheezum MK, Taqueti VR, Kato T, Foster CR, Hainer J, Gaber M, Klein J, Dorbala S, Blankstein R, Di Carli MF. Prognostic Value of Coronary Flow Reserve in Patients with Dialysis-Dependent ESRD. *J Am Soc Nephrol.* 2016;27(6):1823-1829.
64. Charytan DM, Skali H, Shah NR, Veeranna V, Cheezum MK, Taqueti VR, Kato T, Bibbo CR, Hainer J, Dorbala S, Blankstein R, Di Carli MF. Coronary flow reserve is predictive of the risk of cardiovascular death regardless of chronic kidney disease stage. *Kidney Int.* 2017.
65. London GM, Pannier B, Agharazii M, Guerin AP, Verbeke FH, Marchais SJ. Forearm reactive hyperemia and mortality in end-stage renal disease. *Kidney International.* 2004;65(2):700-704.
66. Mistrík E, Dusilová-Sulková S, Bláha V, Sobotka L. Plasma albumin levels correlate with decreased microcirculation and the development of skin defects in hemodialyzed patients. *Nutrition.* 2010;26(9):880-885.
67. Gorgulu N, Yelken B, Caliskan Y, Elitok A, Cimen AO, Yazici H, Oflaz H, Golcuk E, Ekmekci A, Turkmen A, Yildiz A, Sever MS. Endothelial dysfunction in hemodialysis patients with failed renal transplants. *Clin Transplant.* 2010;24(5):678-684.
68. Dubin RF, Guajardo I, Ayer A, Mills C, Donovan C, Beussink L, Scherzer R, Ganz P, Shah SJ. Associations of Macro- and Microvascular Endothelial Dysfunction With Subclinical Ventricular Dysfunction in End-Stage Renal Disease. *Hypertension.* 2016;68(4):913-920.
69. Blacher J, Guerin AP, Pannier B, Marchais SJ, London GM. Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. *Hypertension.* 2001;38(4):938-942.

70. Ginsberg C, Houben A, Malhotra R, Berendschot T, Dagnelie PC, Kooman JP, Webers CA, Stehouwer CDA, Ix JH. Serum Phosphate and Microvascular Function in a Population-Based Cohort. *Clin J Am Soc Nephrol.* 2019;14(11):1626-1633.

71. Shuto E, Taketani Y, Tanaka R, Harada N, Isshiki M, Sato M, Nashiki K, Amo K, Yamamoto H, Higashi Y, Nakaya Y, Takeda E. Dietary phosphorus acutely impairs endothelial function. *J Am Soc Nephrol.* 2009;20(7):1504-1512.

72. Webster AC, Nagler EV, Morton RL, Masson P. Chronic Kidney Disease. *Lancet.* 2017;389(10075):1238-1252.

73. Korsheed S, Crowley LE, Fluck RJ, McIntyre CW. Creation of an arteriovenous fistula is associated with significant acute local and systemic changes in microvascular function. *Nephron Clin Pract.* 2013;123(3-4):173-179.

74. Ooi QL, Tow FKN-FH, Deva R, Alias MA, Kawasaki R, Wong TY, Mohamad N, Colville D, Hutchinson A, Savige J. The Microvasculature in Chronic Kidney Disease. *Clinical Journal of the American Society of Nephrology.* 2011;6(8):1872-1878.

75. Bueti J, Rush D, Tangri N, Mandelzweig K, Xu Y, Hiebert B, Sood MM, Komenda P, Nickerson P, Rigatto C. Effect of time on dialysis and renal transplantation on endothelial function: a longitudinal analysis. *Transplantation.* 2014;98(10):1060-1068.

76. Mitsides N, Cornelis T, Broers NJH, Diederen NMP, Brenchley P, Heitink-Ter Braak N, van der Sande FM, Schalkwijk CG, Kooman JP, Mitra S. Inflammatory and Angiogenic Factors Linked to Longitudinal Microvascular Changes in Hemodialysis Patients Irrespective of Treatment Dose Intensity. *Kidney Blood Press Res.* 2017;42(5):905-918.

77. Sands JJ, Usvyat LA, Sullivan T, Segal JH, Zabetakis P, Kotanko P, Maddux FW, Diaz-Buxo JA. Intradialytic hypotension: frequency, sources of variation and correlation with clinical outcome. *Hemodial Int.* 2014;18(2):415-422.

78. Bemelmans RH, Boerma EC, Barendregt J, Ince C, Rommes JH, Spronk PE. Changes in the volume status of haemodialysis patients are reflected in sublingual microvascular perfusion. *Nephrol Dial Transplant.* 2009;24(11):3487-3492.

79. Meinders AJ, Nieuwenhuis L, Ince C, Bos WJ, Elbers PW. Haemodialysis Impairs the Human Microcirculation Independent from Macrohemodynamic Parameters. *Blood Purif.* 2015;40(1):38-44.

80. Veenstra G, Pranskunas A, Skarupskiene I, Pilvinis V, Hemmelder MH, Ince C, Boerma EC. Ultrafiltration rate is an important determinant of microcirculatory alterations during chronic renal replacement therapy. *BMC Nephrol.* 2017;18(1):71.

81. Mistrik E, Dusilova Sulkova S, Blaha V, Kalousova M, Knizek J, Moucka P, Herout V, Kadlec M, Stilec R, Sobotka L. Evaluation of skin microcirculation during hemodialysis. *Ren Fail.* 2010;32(1):21-26.

82. Leunissen KM, van den Berg BW, Cheriex EC, Slaaf DW, Reneman RS, van Hooff JP. Influence of fluid removal during haemodialysis on macro- and skin microcirculation. Haemodynamic pathophysiologic study of fluid removal during haemodialysis. *Nephron.* 1990;54(2):162-168.

83. Miyazaki H, Matsuoka H, Itabe H, Usui M, Ueda S, Okuda S, Imaizumi T. Hemodialysis impairs endothelial function via oxidative stress: effects of vitamin E-coated dialyzer. *Circulation.* 2000;101(9):1002-1006.

84. Zhou J, Li YS, Chien S. Shear stress-initiated signaling and its regulation of endothelial function. *Arterioscler Thromb Vasc Biol.* 2014;34(10):2191-2198.

85. Cornelis T, Broers NJ, Titulaer DC, Henskens YM, van Oerle R, van der Sande FM, Spronk HM, Vink H, Leunissen KM, ten Cate H, Kooman JP. Effects of ultrapure hemodialysis and low molecular weight heparin on the endothelial surface layer. *Blood Purif.* 2014;38(3-4):203-210.

86. Vlahu CA, Krediet RT. Can Plasma Hyaluronan and Hyaluronidase Be Used As Markers of the Endothelial Glycocalyx State in Patients with Kidney Disease? *Advances in Peritoneal Dialysis.* 2015;31:3-6.

87. Selby NM, Fialova J, Burton JO, McIntyre CW. The haemodynamic and metabolic effects of hypertonic-glucose and amino-acid-based peritoneal dialysis fluids. *Nephrology Dialysis Transplantation.* 2007;22(3):870-879.
88. Cross JM, Donald A, Vallance PJ, Deanfield JE, Woolfson RG, MacAllister RJ. Dialysis improves endothelial function in humans. *Nephrol Dial Transplant.* 2001;16(9):1823-1829.
89. Errakonda PR, Paladugu R, Bitla AR, Musturu SM, Lakshman J, Pemmaraju SR, Vishnubhotla S. Effect of a single hemodialysis session on endothelial dysfunction. *J Nephrol.* 2011;24(1):83-90.
90. Nagaoka T, Takeyama Y, Kanagawa S, Sakagami K, Mori F, Yoshida A. Effect of haemodialysis on retinal circulation in patients with end stage renal disease. *Br J Ophthalmol.* 2004;88(8):1026-1029.
91. Tosun O, Davutluoglu B, Arda K, Boran M, Yarangumeli A, Kurt A, Ozkan D. Determination of the effect of a single hemodialysis session on retrobulbar blood hemodynamics by color Doppler ultrasonography. *Acta Radiol.* 2007;48(7):763-767.
92. Tow FK, Ooi QL, Wong TY, Kawasaki R, Colville D, Mount P, Savige J. Microvascular dilatation after haemodialysis is determined by the volume of fluid removed and fall in mean arterial pressure. *Kidney Blood Press Res.* 2012;35(6):644-648.
93. Gilchrest BA, Rowe JW, Mihm MC, Jr. Clinical and histological skin changes in chronic renal failure: evidence for a dialysis-resistant, transplant-responsive microangiopathy. *Lancet.* 1980;2(8207):1271-1275.
94. Gorgulu N, Yelken B, Caliskan Y, Elitok A, Cimen O, Yazici H, Oflaz H, Turkmen A, Bozfakioglu S, Sever MS. Endothelial dysfunction in peritoneal dialysis patients with and without failed renal transplants. *Transplant Proc.* 2009;41(9):3647-3650.
95. Rodrigues-Diez R, Gonzalez-Guerrero C, Ocana-Salceda C, Rodrigues-Diez RR, Egido J, Ortiz A, Ruiz-Ortega M, Ramos AM. Calcineurin inhibitors cyclosporine A and tacrolimus induce vascular inflammation and endothelial activation through TLR4 signaling. *Sci Rep.* 2016;6:27915.
96. John SG, Selby NM, McIntyre CW. Effects of peritoneal dialysis fluid biocompatibility on baroreflex sensitivity. *Kidney Int Suppl.* 2008(108):S119-124.
97. Selby NM, Fonseca S, Hulme L, Fluck RJ, Taal MW, McIntyre CW. Hypertonic glucose-based peritoneal dialysate is associated with higher blood pressure and adverse haemodynamics as compared with icodextrin. *Nephrology Dialysis Transplantation.* 2005;20(9):1848-1853.
98. Vlahu CA, Lopes Barreto D, Struijk DG, Vink H, Krediet RT. Is the systemic microvascular endothelial glycocalyx in peritoneal dialysis patients related to peritoneal transport? *Nephron Clin Pract.* 2014;128(1-2):159-165.
99. Mukai Y, Shimokawa H, Higashi M, Morikawa K, Matoba T, Hiroki J, Kunihiro I, Talukder HM, Takeshita A. Inhibition of renin-angiotensin system ameliorates endothelial dysfunction associated with aging in rats. *Arterioscler Thromb Vasc Biol.* 2002;22(9):1445-1450.
100. Varin R, Mulder P, Tamion F, Richard V, Henry JP, Lallemand F, Lerebours G, Thuillez C. Improvement of endothelial function by chronic angiotensin-converting enzyme inhibition in heart failure : role of nitric oxide, prostanoids, oxidant stress, and bradykinin. *Circulation.* 2000;102(3):351-356.
101. Cheang WS, Wong WT, Tian XY, Yang Q, Lee HK, He GW, Yao X, Huang Y. Endothelial nitric oxide synthase enhancer reduces oxidative stress and restores endothelial function in db/db mice. *Cardiovasc Res.* 2011;92(2):267-275.

Figures

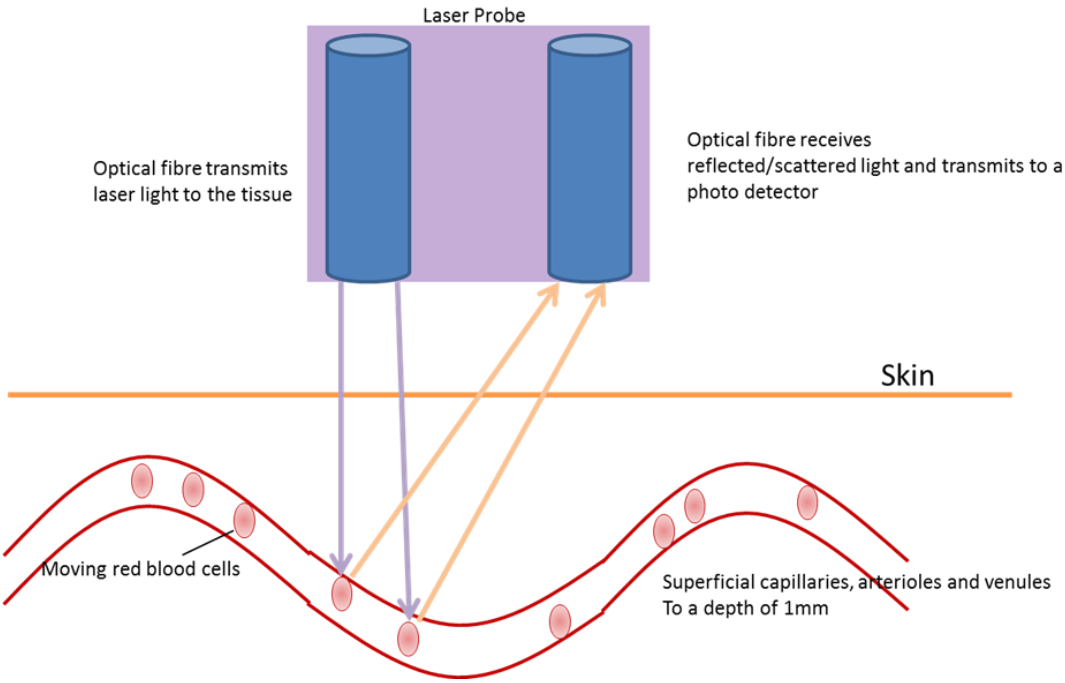

Figure 1 Schematic representation of the principles of Laser Doppler measured flux.

Laser Doppler technology measures blood flow in the microcirculation to a tissue depth of typically 1mm. Measurements are based on the Doppler principle whereby monochromatic light changes wavelength when it is reflected by moving objects, in this case red blood cells. The magnitude and frequency of the changes in wavelength are related to the number and velocity of the moving cells, termed red blood cell flux[47].

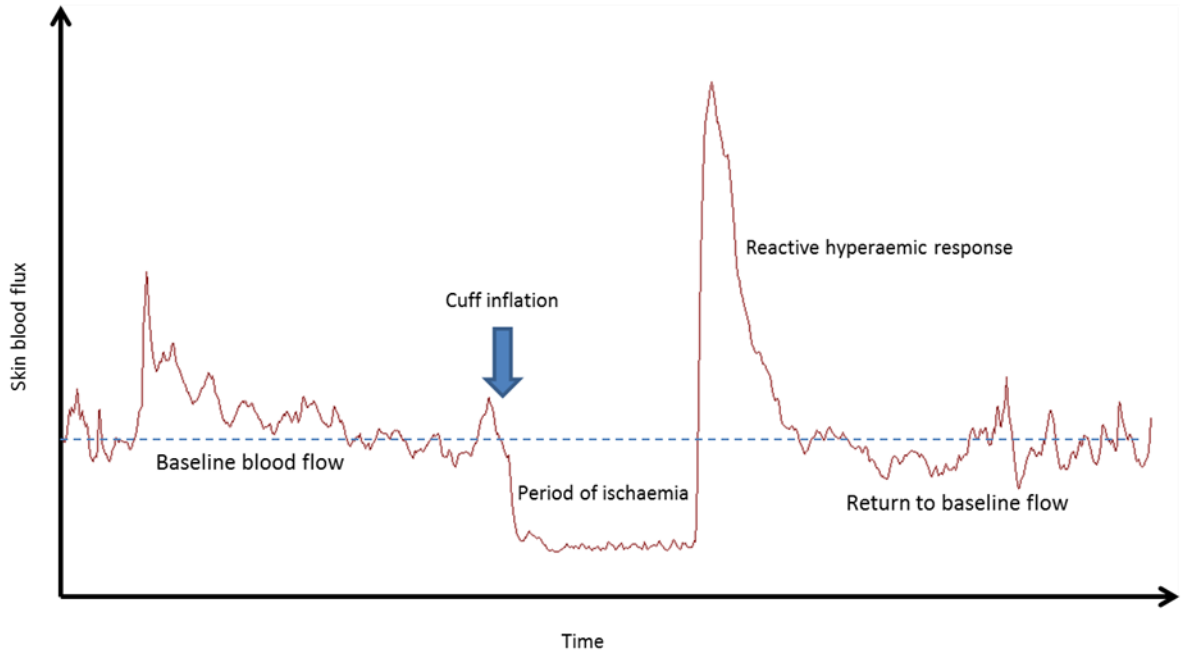

Figure 2 Representative laser Doppler trace obtained before, during and after a brief period of arterial occlusion.

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[54]. This is a complex response which remains incompletely understood however NO appears to play only a minor role.

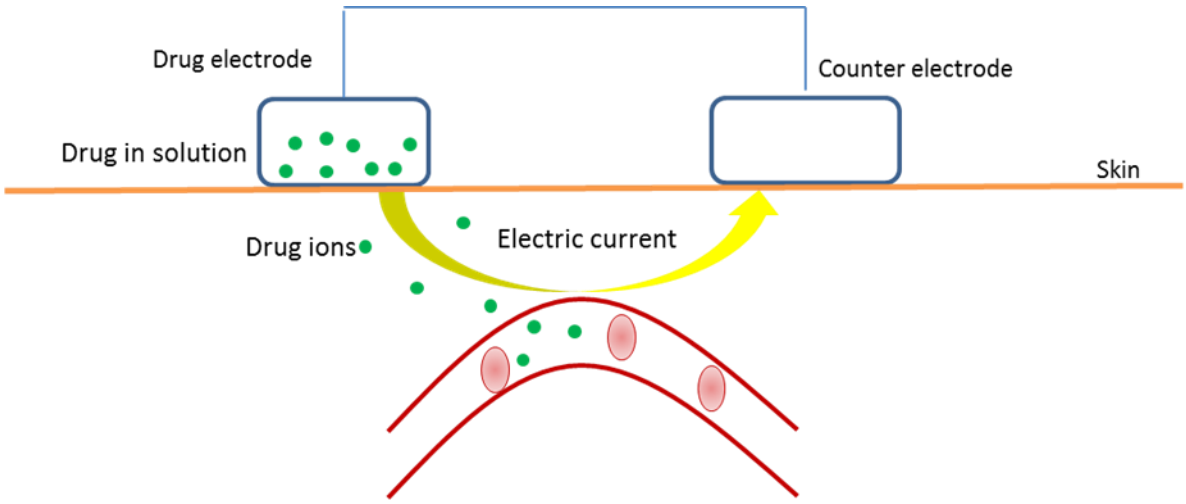

Figure 3 Schematic representation of iontophoretic delivery of vasoactive substances.

Iontophoresis delivers charged pharmacological agents in solution to a localised area of skin by applying an opposing electrical current. Laser Doppler technology in combination with iontophoretic application of vasoactive substances to the skin allows study of aspects of the vasodilatory capacity of dermal vessels. Traditionally ACh and SNP are used to provoke endothelium-dependant and endothelium-independent vasodilation respectively[51].

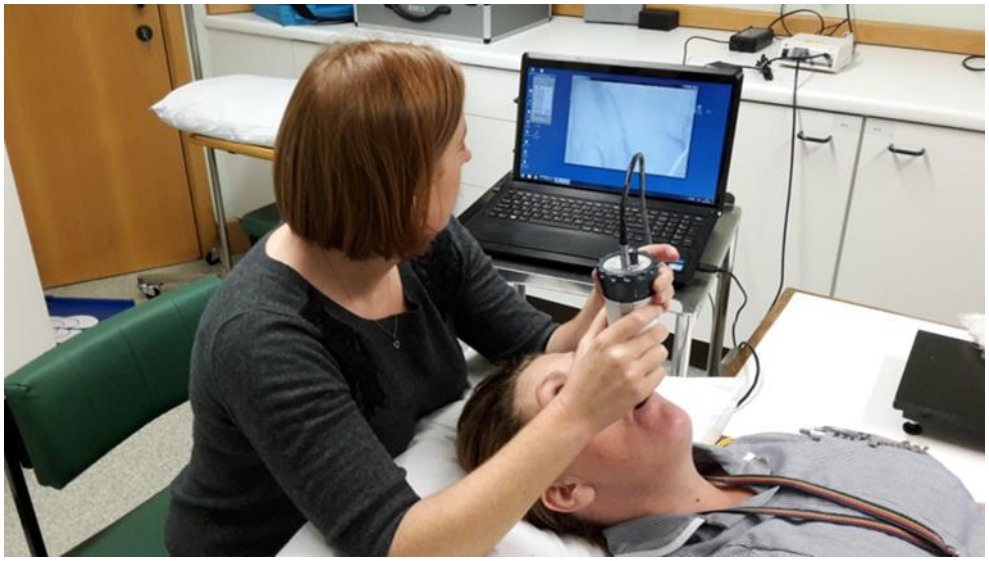

Figure 4a Acquisition of SDF images.

Hand held microscopes use side-stream dark field imaging to produce high-contrast real-time videos of the sublingual vessels

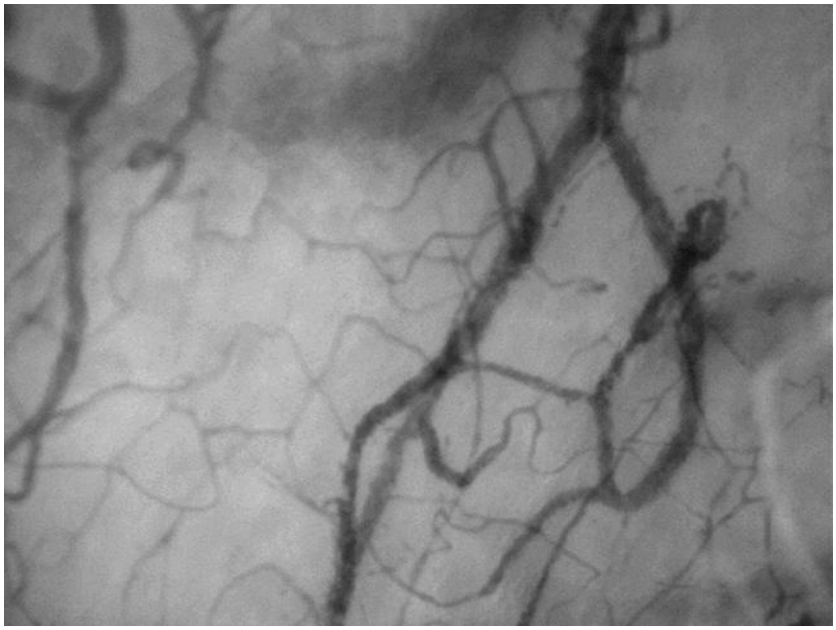

Figure 4b. Example of sublingual microcirculation as visualised using SDF imaging.

SDF is based on the principle that scattered green light is absorbed by haemoglobin in red blood cells, therefore any vessels containing RBCs can be visualised using this technique. These images can be used to assess; vessel density, perfusion indices and heterogeneity [45, 46].