ABSTRACT

The endothelial cell layer is responsible for molecular traffic between the blood and surrounding tissue, and endothelial integrity plays a pivotal role in many aspects of vascular function. Cardiovascular disease (CVD) is the main cause of death in patients with chronic kidney disease (CKD) and its incidence and severity increase in direct proportion with kidney function decline. Non-traditional risk factors for CVDs, including endothelial dysfunction (ED), are highly prevalent in this population and play an important role in cardiovascular (CV) events. ED is the first step in the development of atherosclerosis and its severity has prognostic value for CV events. Several risk markers have been associated with ED. Reduced bioavailability of nitric oxide plays a central role, linking kidney disease to ED, atherosclerosis, and CV events. Inflammation, loss of residual renal function, and insulin resistance are closely related to ED in CKD. ED may be followed by structural damage and remodelling that can precipitate both bleeding and thrombotic events. The endothelium plays a main role in vascular tone and metabolic pathways. ED is the first, yet potentially reversible step in the development of atherosclerosis and its severity has prognostic value for CV events. Therefore, evaluation of ED may have major clinical diagnostic and therapeutic implications. In patients with CKD, many risk factors are strongly interrelated and play a major role in the initiation and progression of vascular complications that lead to the high mortality rate due to CVD.

Keywords: Chronic kidney disease, endothelium, endothelial dysfunction.

INTRODUCTION

In patients with chronic kidney disease (CKD), the endothelium plays a pivotal role, not only with respect to their cardiovascular (CV) morbidity and mortality, but also with regard to progression of the disease. It has become clear from experimental studies that vascular rarefaction in the capillary system of the renal medulla as a result of endothelial damage is a central step towards tissue hypoxia and kidney damage. Renal insufficiency may be a constituent and/or cause of a systemic subclinical atherothrombotic process. Endothelial dysfunction (ED) is an early phenomenon that precedes structural changes and clinical manifestations of atherosclerosis, contributing to both plaque initiation and progression. The severity of ED has been shown to have prognostic value for CV events. Additionally, several risk markers have been associated with ED in CKD. Therefore, it is correct to consider ED as a heterogeneous syndrome, which can be focussed upon either as a local or a systemic condition as in CKD, with subclinical or clinical manifestations, being reversible or irreversible according to its severity, and with many related aetiological mechanisms. In CKD, ED is characterised by increased plasma concentrations of endothelium-derived molecules, an imbalance between circulating endothelial cells and bone-marrow endothelial progenitor cells (EPCs), or reduced endothelium-dependent vasodilatation. As renal function deteriorates, an atherogenic milieu is generated due to the accumulation of uraemic toxins with a direct impact on the endothelium and the vessel wall, contributing to oxidative stress (OS) and enhancing a subclinical inflammatory state. In CKD, these initial steps in ED may perpetuate if not identified at the early subclinical stages, leading to atherogenesis. Decreased endothelial function (EF) is thought to primarily...
Endothelin-1 (ET1) has two direct renal actions: causing renal vasoconstriction (via endothelin type A [ETA] receptors) and tubular sodium and water loss (via endothelin type B [ETB] receptors), which probably reflect separate sites of production in renal blood vessels and tubules. ETA antagonism alone, and/or combined ETA/ETB blockade, reduces CKD progression. Based on strong preclinical data, several clinical trials using ETA antagonists were conducted. Small trials involving acute intravenous endothelin receptor blockade suggest that ETA but not ETB blockade exerts protective renal and vascular effects in CKD patients. A large Phase III trial (A Study of Cardiovascular Events in Diabetes [ASCEND]) examined the effects of avosentan, an endothelin receptor antagonist, on renal disease progression in diabetic nephropathy.\textsuperscript{12} Proteinuria was reduced after 3-6 months of treatment. Several Phase II trials using avosentan at lower doses than in ASCEND, atrasentan, or sitaxsentan (the latter two being highly ETA-selective) showed reductions in proteinuria in addition to renin-angiotensin system blockade. Infrequent and clinically insignificant fluid retention was observed at the most effective doses. Additional trials using ETA blockers are ongoing or being planned in patients with diabetic nephropathy or focal segmental glomerulosclerosis.\textsuperscript{12,13} There is much optimism regarding their clinical benefit, although ‘hard’ renal outcomes analysing renal function remain to be determined. Finally, consideration needs to be given to conducting trials examining the effects of ETA receptor blockers in dialysis patients.

Additionally, albuminuria is a predictor of increased CV risk. Albuminuria is strongly associated with increased levels of pentraxin 3, an inflammatory mediator predominantly produced by endothelial cells, macrophages, and adipocytes in response to pro-inflammatory signals.\textsuperscript{14} This suggests that it may have an additional role in the atherogenic process common to inflammatory mediators.\textsuperscript{15} Because of its extrahepatic synthesis, pentraxin 3 levels are believed to be an independent indicator of disease activity occurring directly at sites of inflammation and linked to ED. Detached circulating endothelial cells may serve as potential markers of endothelial damage in CKD. In CKD there exists an impaired migratory activity and/or decreased numbers of these cells, which may have a role in neovascularisation of ischaemic tissue and the progression of atherosclerosis and cardiovascular disease (CVD).\textsuperscript{16} An imbalance between the numbers of circulating endothelial cells and EPCs seems to exist in CKD.\textsuperscript{15} This imbalance may contribute to the pathogenesis and progression of atherosclerosis.

The Endothelium and Oxidative Stress

Uraemia is a pro-oxidant state. Lipid peroxidation, oxidative modification of proteins and carbohydrates, and certain uraemic toxins themselves have been implicated in ED in CKD.\textsuperscript{17} Moreover, numerous defects in antioxidant systems lead to a decrease in the depuration of reactive oxygen species. OS appears to start early in CKD,\textsuperscript{18} but total antioxidant capacity has been observed to be exceeded only in end-stage renal disease (ESRD), suggesting that production of reactive oxygen species starts overcoming its clearance at the beginning of the decline in renal function. Reactive oxygen species react with and deactivate NO. Reduced bioavailability of NO as a resultant of ED enhances the development and maintenance of hypertension by augmenting systemic vascular resistance by increasing adrenergic tone, volume expansion and vascular smooth muscle cell proliferation, matrix accumulation, and vascular remodelling, which are inhibited by NO and promoted by free radicals.\textsuperscript{19} OS increases production of ET1 and the cytosolic concentration of calcium, thereby increasing vascular smooth muscle tone, systemic vascular resistance, arterial pressure, and accumulation of matrix proteins.\textsuperscript{20}

The Endothelium and Inflammation

The causes of the high prevalence of inflammation in ESRD are multifactorial and include decreased renal function, volume overload, comorbidities, intercurrent clinical events, and dialysis-associated factors.\textsuperscript{21} An acute-phase reaction may be a
direct cause of vascular injury. Pro-inflammatory cytokines play a central role in the genesis of both CVD and malnutrition in ESRD.\textsuperscript{22} Inflammation (which is interrelated to OS, ED, wasting, and insulin resistance) has been suggested to be a significant contributor to CVD in ESRD. It is difficult to define systemic inflammation in CKD patients because there is not a ‘gold standard’ inflammatory marker. Several different inflammatory biomarkers, such as high-sensitivity C-reactive protein (CRP), have been shown to independently predict mortality in these patients.\textsuperscript{21} Although CRP is the most frequently used in clinical trials, it is at the end of the inflammatory cascade and many early inflammatory processes are underdiagnosed. Therefore, several authors adopted the coincidental elevation of CRP and a pro-inflammatory cytokine (e.g. interleukin 6 (IL-6), matrix metallopeptidase 9, tumour necrosis factor alpha (TNF-\textalpha{})) plasma level as definition of inflammation. Elevated CRP relates to long-term prognosis in both patients with coronary artery disease and in apparently healthy men due to a blunted systemic endothelial vasodilator function. CRP has also been suggested to be a mediator of atherogenesis.\textsuperscript{23} Therefore, the identification of elevated CRP levels as a transient independent risk factor for ED might provide an important clue to link a systemic marker of inflammation to atherosclerotic disease progression.\textsuperscript{20} Chronic inflammation therefore emerged as a potential mediator between microalbuminuria and macrovascular disease. Pentraxin 3 is an inflammatory mediator produced by endothelial cells that may have a role in atherogenesis. In two cohorts: Stage 5 CKD and Type 2 diabetes with normal renal function, pentraxin 3 was found to be independently associated with proteinuria. Moreover, both pentraxin 3 and proteinuria were associated with ED in patients with Type 2 diabetes.\textsuperscript{22}

**The Endothelium and Uraemic Toxins**

During the development of the uraemic syndrome, loss of kidney function is accompanied by deteriorating organ function attributable to the accumulation of uraemic retention solutes. Compounds that exert an adverse biological impact are called uraemic toxins.\textsuperscript{24} Uraemic toxins provoke OS, inflammation, hypertrophy, constriction, and coagulation through various mechanisms.\textsuperscript{25} Uraemic toxins can activate the endothelium to produce the following effects: vasoconstriction (via ADMA, advanced glycation end products [AGEs], and homocysteine), inflammation (via indoxyl sulfate and AGEs), OS (via ADMA, AGEs, and homocysteine), or procoagulant activity. The procoagulant effect of endothelium is characterised by increased procoagulant factors (increased plasminogen activator inhibitor-1 and von Willebrand factor) and reduced anticoagulant factors. It follows then that a diverse group of toxins act on a variety of cell types to provoke OS, inflammation, vascular smooth vessel proliferation and constriction, ED, and coagulation, which account for some of the manifestations of the uraemic syndrome that include hypertension and accelerated atherosclerosis.\textsuperscript{25} Regulation of vascular tone is also impaired, with decreased endothelium-dependent vasodilatation. CKD also induces OS and inflammation in endothelial cells and production of reactive oxygen species in cultured endothelial cells by the protein-bound uraemic toxin indoxyl sulphate.

**The Endothelium and Haematological Alterations**

An important intermediary in the continued activation of endothelial cells is the interaction of the endothelium with platelets. The activation of glycoprotein IIb/IIla on the surface of platelets induces the expression of factors such as P-selectin that promote activation of the endothelium.\textsuperscript{26} EPCs contribute to the repair and structural maintenance of the vascular system. From Stage 1 CKD, a decrease in EPC number and function (hampered adherence, reduced endothelial outgrowth potential, and reduced antithrombogenic function) is observed. This alteration, which may hinder vascular repair and add to the CVD risk, becomes more significant with CKD progression.\textsuperscript{27}

Endothelial microparticles (EMPs) may be a reliable marker of subclinical atherosclerosis and arterial stiffness. In children on peritoneal dialysis, lower levels of EMPs and more favourable CV indices were observed when compared with patients on haemodialysis (HD). In these studies, blood pressure (BP) was the most important risk factor for atherosclerosis, and EMPs and BP the most important predictors for arterial stiffness.\textsuperscript{3}

Compared with erythropoietin-naïve patients, anaemic patients treated with erythropoietin display increased levels for IL-6 and IL-8, CRP, and TNF-\alpha{}. Long-term administration of erythropoietin has been associated with decreased levels of
TNF-α in dialysis patients, but also with enhanced inflammation and ischaemia-induced neovascularisation via increased mobilisation of EPCs.28 Furthermore, erythropoietin activates vascular smooth muscle cells, endothelium, and platelets, thus enhancing thrombogenicity and causing a loss of vasodilatory potential. In addition, this could be one explanation for the observation of an increased risk for all-cause and CV mortality in patients with higher haemoglobin levels targeted with higher doses of erythropoiesis-stimulating agents.29

The Endothelium and Protein-Energy Wasting

There has been an increase in understanding of the mechanisms causing syndromes of wasting, malnutrition, and inflammation, as well as their interrelationships in individuals with CKD. Approximately 18-75% of patients on chronic dialysis show evidence of wasting.16 This phenomenon has been referred to as uraemic malnutrition, cachexia, and malnutrition-inflammation atherosclerosis syndrome. In CKD, there are conditions resulting in loss of lean body mass not related to reduced nutrient intake and due to nonspecific inflammatory processes, intercurrent catabolic illnesses, nutrient losses into dialysate, and anaemia or its treatment.30 Among the number of disorders that can cause wasting in patients with CKD, inflammation, OS, acidaemia, nutrient losses into dialysate, anaemia, hyperparathyroidism, and retention of uraemic toxins interplay, which, as shown in previous sections, causes ED leading to atherogenesis and CVD. Elevated CV and haemodynamic markers of disease and endothelial stress, such as pro-brain natriuretic peptide or troponin T, are associated with wasting and inflammation in dialysis patients.31

The Endothelium and Vascular Calcification

Vascular calcification is responsible for the higher prevalence of CVD in dialysis patients. Consequently, its early detection is truly relevant in this population. Vascular calcification has been historically classified as i) intima calcification associated with atheroma plaques, or ii) medial calcification associated with CKD and disturbances in the metabolism of calcium, phosphorus, and vitamin D (VD). Age, dialysis, past medical history of CVD, atherosclerosis, and inflammation are variables significantly influencing calcification.32 Recently, the term ossification rather than calcification of both the arterial intima and media has been proposed for kidney patients.15 The presence of artery ossification is associated with functional estimates of arterial dysfunction, such as NO-dependent vasodilatation in dialysis patients and pulse-wave velocity.33 Vascular ossification should be considered as a CV risk marker and not an aetiologial factor of CVD in CKD.15 Serum levels of calcium and phosphorus have become established risk markers.34,35 Hyperparathyroidism has also been suggested as a risk factor for and a marker of vascular ossification,35 and VD deficiency is associated with increased early mortality. The best-studied inhibitor of vascular ossification is fetuin-A, the major carrier of calcium ions in the circulation. Only in CKD Stage 5 are low levels of fetuin-A associated with adverse CV outcomes.36 The ossification regulators matrix Gla-protein, bone morphogenic proteins, osteopontin, and osteoprotegerin promote an early and extensive vascular ossification process, and have been shown to interfere with EF by decreasing NO production and altering the normal anatomy of the vessel wall, rendering the endothelial surface prone to calcium deposition, prothrombotic events, and a more rigid vessel wall.37

Endothelium in Diabetics

Diabetic nephropathy is the leading cause of ESRD. Diabetes mellitus is characterised by generalised ED. However, recent data also emphasise the role of local renal ED in the pathogenesis of diabetic nephropathy. Hyperglycaemia triggers a complex network of signal-transduction molecules, transcription factors, and mediators that culminate in ED.38 In the glomerulus, vascular endothelial growth factor (VEGF)-A-induced neoangiogenesis may contribute to the initial hyperfiltration and microalbuminuria due to increased filtration area and immaturity of the neovessels, respectively. However, a subsequent decrease in the number of podocytes decreases VEGF production resulting in capillary rarefaction and decreased glomerular filtration rate (GFR). Decreased NO availability also plays a significant role in the development of advanced lesions of diabetic nephropathy through disruption of glomerular autoregulation, uncontrolled VEGF action, release of prothrombotic substances by endothelial cells, and angiotensin II-independent aldosterone production.39 In addition, disturbances in the endothelial glyocalyx contribute to decreased
permselectivity and microalbuminuria, whereas there is recent evidence that reduced glomerular fenestral endothelium leads to decreased GFR levels. Endothelial repair mechanisms are also impaired in diabetes since the number of circulating EPCs is decreased in diabetic patients with microalbuminuria. Finally, and in the context of elevated profibrotic cytokine transforming growth factor-β levels, endothelial cells also contribute to the detrimental process of fibrosis in advanced diabetic nephropathy through endothelial-to-mesenchymal transition.

THE ENDOTHELIUM AND RENAL REPLACEMENT THERAPY

The role of residual renal function (RRF) in the elimination of excess pro-inflammatory molecules has not generated much attention, and it seems that RRF protects dialysis patients from the excess of pro-inflammatory molecules. Effectively, the changes in endothelial damage and function markers caused by inflammation are more severe in those with less RRF. Volume overhydration is independently associated with worse ED in peritoneal dialysis (PD) patients. In addition, normalised extracellular water together with the product of phosphate times calcium and dialysis vintage were independent determinants of flow-mediated dilatation in PD patients, suggesting that ED might link volume overhydration and CV events in dialysis patients.

A number of studies have reported that PD is associated with lower levels of OS and inflammation when compared with HD. However, an association with vascular or myocardial structure and function could not be established. Some studies have shown that PD is associated with a lower mortality than HD in the first 1-2 years, but thereafter it may actually be higher in PD than in HD. In PD patients, inflammation induces ED as estimated by elevation of endothelial damage markers. In addition, pro-inflammatory cytokines are associated with elevations in pro-coagulable and pro-atherosclerotic mediators in plasma. PD patients without known atherosclerosis show ED and their advanced oxidation protein product (AOPP) levels independently predict EF level. Acute inflammation assessed by high-sensitivity CRP is associated with a temporary increase in peritoneal solute transport rate, as determined by the peritoneal equilibration test in PD patients. This may be caused by intraperitoneal inflammation through the IL-6 system or AOPP.

EF was shown to be better in transplanted patients than in dialysis patients. However, despite correction of uraemia after renal transplantation, substantial ED, structural alterations of the arterial wall, and disturbed mechanical vessel-wall properties persist. ED is more prominent among patients with failed transplants, which are usually complicated by inflammation, suggesting that the failed allograft may be responsible for this abnormality.

New Biomarkers of Endothelial Dysfunction

Alterations in mineral metabolism may play a major role in deranged EF in patients with ESRD because changes in 25-hydroxy (25-OH) VD, serum phosphate, and fibroblast growth factor 23 levels brought about by restored renal function after kidney transplantation correlate with improved endothelium-dependent vasodilatation. Cross-sectional studies associated 25-OH VD, 1,25-dihydroxy (1,25-[OH]₂) VD, and endothelium-dependent vasodilatation with Stage 3-4 CKD as well as those with ESRD, suggesting that VD insufficiency or deficiency may have adverse effects on the vascular system. Paricalcitol is a synthetic analogue of 1,25-(OH)₂ VD endowed with several actions impinging on vascular function. VD receptor activation by this compound ameliorates endothelium-dependent vasodilatation in subtotally nephrectomised rats, an effect which is completely independent of BP and parathormone levels.

As improvement in ED preludes regression of atherosclerosis in primates and signals a reduction in the risk of CV events, the findings in Zoccali’s study represent evidence that paricalcitol favourably affects a biological phenomenon of major relevance for atherosclerosis in humans, and add new experimental evidence in support of the hypothesis that VD may exert favourable effects on the CV system in patients with CKD.

Over the last decade the biological interference of uric acid with the CV system and the kidney has been intensively investigated, and several experimental studies in animal models and in vitro documented that hyperuricaemia may trigger hypertension and incite ED, vascular damage, and renal disease. A substantial proportion of epidemiological studies are compatible with the hypothesis that hyperuricaemia may be noxious
to both the CV system and the kidney. However, there are still no well-powered trials testing whether uric acid-lowering interventions may reduce BP or attenuate the risk of adverse CV and renal outcomes. Evidence still remains largely insufficient to recommend changes in the current policy of not prescribing uric acid-lowering drugs to individuals with asymptomatic hyperuricaemia.

CONCLUSION

The endothelium plays a major role in vascular tone and metabolic pathways. ED is the first, yet potentially reversible step in the development of atherosclerosis, and its severity has prognostic value for CV events. Therefore, evaluation of ED may have major clinical diagnostic and therapeutic implications. In patients with CKD, many risk factors are strongly interrelated and play a major role in the initiation and progression of vascular complications, leading to the high mortality rate attributable to CVD. Even initial CKD should result in intensive prevention of CV risk since risk of death due to CVD is much higher than that from ESRD.

REFERENCES


31. Trimarchi H et al. Elevated pro-brain natriuretic peptide, troponin T and malnutrition inflammatory score in...