Most exposed: the endothelium in chronic kidney disease

Marc Vila Cuenca¹, Peter L. Hordijk² and Marc G. Vervloet ¹

¹Department of Nephrology, Amsterdam Cardiovascular Sciences, VU University Medical Center, Amsterdam, The Netherlands and
²Department of Physiology, Amsterdam Cardiovascular Sciences, VU University Medical Center, Amsterdam, The Netherlands

Correspondence and offprint requests to: Marc G. Vervloet; E-mail: m.vervloet@vumc.nl

ABSTRACT

Accumulating evidence indicates that the pathological changes of the endothelium may contribute to the development of cardiovascular complications in chronic kidney disease (CKD). Non-traditional risk factors related to CKD are associated with the incidence of cardiovascular disease, but their role in uremic endothelial dysfunction has often been disregarded. In this context, soluble α-Klotho and vitamin D are of importance to maintain endothelial integrity, but their concentrations decline in CKD, thereby contributing to the dysfunction of the endothelial lining. These hormonal disturbances are accompanied by an increment of circulating fibroblast growth factor-23 and phosphate, both exacerbating endothelial toxicities. Furthermore, impaired renal function leads to an increment of inflammatory mediators, reactive oxygen species and uremic toxins that further aggravate the endothelial abnormalities and in turn also inhibit the regeneration of disrupted endothelial lining. Here, we highlight the distinct endothelial alterations mediated by the abovementioned non-traditional risk factors as demonstrated in experimental studies and connect these to pathological changes in CKD patients, which are driven by endothelial disturbances, other than atherosclerosis. In addition, we describe therapeutic strategies that may promote restoration of endothelial abnormalities by modulating imbalanced mineral homeostasis and attenuate the impact of uremic retention molecules, inflammatory mediators and reactive oxygen species. A clinical perspective on endothelial dysfunction in CKD may translate into reduced structural and functional abnormalities of the vessel wall in CKD, and ultimately improved cardiovascular disease.

52. Chen JF, Mandel EM, Thomson JM et al. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nat Genet 2006; 38: 228–233
53. Winbanks CE, Wang B, Beyer C et al. TGF-beta regulates miR-206 and miR-29 to control myogenic differentiation through regulation of HDAC4. J Biol Chem 2011; 286: 13805–13814
54. Dey BK, Gagan J, Dutta A. miR-206 and -486 induce myoblast differentiation by downregulating Pax7. Mol Cell Biol 2011; 31: 203–214
55. Kim HK, Lee YS, Sivaprasad U et al. Muscle-specific microRNA miR-206 promotes muscle differentiation. J Cell Biol 2006; 174: 677–687
56. Hitachi K, Nakatani M, Tsuchida K. Myostatin signaling regulates Akt activity via the regulation of miR-486 expression. Int J Biochem Cell Biol 2014; 47: 93–103
57. Panguluri SK, Bhatnagar S, Kumar A et al. Genomic profiling of messenger RNAs and microRNAs reveals potential mechanisms of TWEAK-induced skeletal muscle wasting in mice. PLoS One 2010; 5: e8760
58. Fuchs E, Poy MN, Yi R et al. A skin microRNA promotes differentiation by repressing ‘stemness’. Nature 2008; 452: 225–229
59. Dmitriev P, Barat A, Polesskaya A et al. Simultaneous miRNA and mRNA transcriptome profiling of human myoblasts reveals a novel set of myogenic differentiation-associated miRNAs and their target genes. BMC Genomics 2013; 14: 265
60. Goljanek-Whysall K, Sweetman D, Munsterberg AE. microRNAs in skeletal muscle differentiation and disease. Clin Sci 2012; 123: 611–625
61. Meyer SU, Thirion C, Polesskaya A et al. TNF-α and IGF1 modify the microRNA signature in skeletal muscle cell differentiation. Cell Commun Signal 2015; 13: 4
62. Motohashi N, Alexander MS, Shimizu-Motohashi Y et al. Regulation of IRS1/Akt insulin signaling by microRNA-128a during myogenesis. J Cell Sci 2013; 126: 2678–2691
63. Hu L, Klein JD, Hassounah F et al. Low-frequency electrical stimulation attenuates muscle atrophy in CKD–a potential treatment strategy. J Am Soc Nephrol 2015; 26: 626–635
64. Chen JF, Tao Y, Li J et al. microRNA-1 and microRNA-206 regulate skeletal muscle satellite cell proliferation and differentiation by repressing Pax7. J Cell Biol 2010; 190: 867–879
65. Walker SR, Gill K, Macdonald K et al. Association of frailty and physical function in patients with non-dialysis CKD: a systematic review. BMC Nephrol 2013; 14: 228
66. van Niehl G, Porto-Carreiro I, Simoes S et al. Exosomes: a common pathway for a specialized function. J Biochem 2006; 140: 13–21
67. Hudson MB, Woodward-Hobbs ME, Zheng B et al. miR-23a decreased during muscle atrophy by a mechanism that includes calcineurin signalling and exosome-mediated export. Am J Physiol Cell Physiol 2014; 15: 551–558
68. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov 2017; 16: 203–222

Received: 10.7.2019; Editorial decision: 27.8.2019
**INTRODUCTION**

Cardiovascular complications are more frequent and severe in patients with chronic kidney disease (CKD) as compared with the general population [1]. This complex association cannot be fully explained by the presence of traditional risk factors such as hypertension, hyperlipidaemia and diabetes. Alternatively, non-traditional risk factors related to a reduced kidney function provide some insights into the mechanisms of increased risk of cardiovascular events in CKD [1, 2]. These CKD-specific factors, besides proteinuria, include disturbed mineral metabolism and bone disease, inflammation, oxidative stress and the accumulation of uraemic toxins. Most of these factors are associated with reduced heart function, vascular stiffness and calcification, typically and most prominently of the medial layer. When compared with the role of the medial layer, attention to disturbed endothelial structure and function in CKD lags behind. The vascular endothelium constitutes a monolayer of endothelial cells, forming the inner lining of the entire circulatory system. The preservation of endothelial barrier function is crucial for the normal functioning of the vascular system and requires tightly regulated intercellular junctions and endothelial cell adhesion to the basement membrane. From this perspective, endothelial cell dysfunction (ECD) can be viewed as a compromised regulation of these vital properties and comprises structural changes in the actin cytoskeleton, reduced proliferative and migratory capacities, breakdown of endothelial cell-cell contacts and impairment of the barrier function. This progressive structural remodelling dampens the proper communication between endothelial cells and vascular smooth muscle cells (VSMCs), fundamental for vascular function, resulting in the earliest detectable changes of atherosclerosis [3]. As mentioned, CKD can also drive VSMC dysfunction or vessel structural alterations without disturbing the endothelial function [4]. However, non-atherosclerotic endothelial disturbances most likely exist as well in CKD, and are the focus of this review.

Despite strong suggestions that ECD may critically impact cardiovascular health [5], in the clinical setting of CKD, there is only limited information indicating whether ECD provides important prognostic information, or actually causes future cardiovascular complications [6]. However, the data that are available strongly suggest that, also in patients with CKD, ECD contributes to cardiovascular morbidity. In patients with CKD, impaired endothelial function has been associated with arterial thickness [6, 7], abnormal left ventricular structure and function [8], and importantly, excess of cardiovascular mortality in CKD [9]. However, while these few studies highlight the importance of vascular dysfunction as a marker for cardiovascular risk, the potential impact of ECD in the progression of cardiovascular complications remains to be elucidated. As a result of these limitations, there is insufficient knowledge supporting the concept of whether targeting vascular dysfunction and in particular ECD in CKD may beneficially impact cardiovascular disease and clinical outcome.

In view of these considerations, we aim to review available information on the morphological and functional abnormalities in the endothelial lining during CKD, and to evaluate how CKD-related, non-traditional risk factors critically impact endothelial integrity. Finally, we discuss some plausible therapeutic strategies aimed at targeting these CKD-associated disturbances, to possibly prevent progression of endothelial injury and thereby attenuate cardiovascular disease.

**ENDOTHELIAL DYSFUNCTION DURING CKD**

Dysfunctional endothelium in patients with CKD has been demonstrated in both large and small arteries [10, 11]. Patients with impaired renal function frequently display some common adverse endothelial characteristics that provide a better understanding of the impact of CKD on this cell type (Figure 1). In particular, impaired flow-mediated dilation (FMD), reflecting abnormal endothelium-dependent vasodilatory function, has been frequently reported in CKD patients, and its impairment is associated with the severity of renal damage [12, 13]. This non-invasive approach to assess endothelial function measures the ability of the artery to respond, by the release of the endothelium-derived relaxing factor nitric oxide (NO), to the 5-min occlusion of the branchial artery with a blood pressure cuff (reactive hyperaemia). Reduced NO bioavailability [14], however, is a critical feature and characteristic for patients with CKD [5, 15]. This abnormality is accompanied by a decreased expression or limited activation of the endothelial NO synthase due to the presence of renal disease-related toxins contributing to a reduced vasodilatory capacity [16]. Importantly, FMD provides crucial information about the vasodilatory status of the endothelium, but it is not a direct assessment of the production of vasoactive molecules. A more invasive approach can overcome this limitation through the infusion of acetylcholine, which dilates normal coronary arteries in the presence of intact endothelium by stimulating NO production. In the presence of ECD, however, acetylcholine may even induce vasoconstriction through a direct effect on the underlying VSMC. In this regard, the measurement of endothelial-dependent relaxation after acetylcholine stimulation in CKD animal models reflects a valuable approach to assess vascular function and test therapeutic strategies [17, 18].

Given the difficulties of assessing the structural changes of the vascular endothelium, the analysis of soluble factors is sometimes used as a non-invasive approach to explore the CKD-induced pathological consequences. During CKD, the endothelium loses its quiescent phenotype and becomes activated [5], which is exemplified by elevated levels of circulating cell adhesion molecules such as soluble Intercellular Adhesion Molecule 1 (sICAM-1), Vascular Cell Adhesion Molecule 1 (sVCAM-1), sE-selectin and platelet adhesion molecule von Willebrand factor (vWF) (as a first step of thrombus formation) in serum from patients with CKD [19, 20]. Interestingly, the presence of these ECD biomarkers has been associated with a defective FMD in CKD, which suggests that these endothelial structural changes may co-exist with an impaired endothelial function [20]. In addition, the analysis of circulating endothelial microparticles (EMPs), released into the extracellular space after endothelial injury, provides further clinical information of

**Keywords:** cardiovascular, CKD, endothelial dysfunction, mineral metabolism, uraemic toxins
the endothelial damage upon CKD [21]. Similarly, as the endothelial activation markers, EMPs levels are associated with loss of FMD and increased pulse wave velocity in patients with end-stage renal failure, reinforcing the hypothesis that endothelial damage results in both morphological and functional alterations [21]. Alternatively, patients with different degrees of impaired renal function also display increased levels of circulating endothelial cells (CECs) themselves [22]. This subpopulation of cells—originating from the blood vessel wall—is detached due to endothelial damage and detachment, and reflects ongoing injury. Finally, CKD reduces the number of circulating endothelial progenitor cells (EPCs), a bone marrow-derived CEC population that can be recruited to sites of endothelial injury and then mature, playing a major role in vascular repair to restore endothelial function [23, 24]. In this regard, CKD not only dampens the availability of circulating EPCs but also impacts on the normal functioning of EPCs, resulting in abnormal colony formation together with impaired adhesion and incorporation, further worsening the repair capacity of the vascular system [23, 25].

Mechanistically, the enhanced transcription of the above-mentioned adhesion molecules, vWf or EMPs is preceded by
activation of the nuclear factor-kappa B (NF-kappa B) signalling pathway [5]. In experimental studies, the most prominent changes observed in endothelial cells exposed to human uremic serum are suggested to be mediated by NF-kappa B signalling, substantiating its key role in the development of ECD during CKD [26, 27]. However, the harmful effects of uremic media are not limited to activation of the NF-kappa B pathway but extend to NF-kappa B-independent structural alterations such as lower expression of Vimentin and Annexin A2, which are both involved in cell-cell and cell-matrix interactions [28]. In line with this, it was shown that uremia modulates the expression of matrix metalloproteases in endothelial cells leading to a remodelling of the extra-cellular matrix, thereby promoting endothelial detachment from the basement membrane and its subsequent loss [29]. The importance of the loss of endothelial cell-cell interactions in CKD was also recently highlighted by our group where we confirmed that uremic plasma from pre-dialysis CKD patients was impairing the stability of the endothelial barrier function by reducing the vascular endothelial (VE)-cadherin adherens junctions on the cell surface [30]. Here, exposure to uremic media also resulted in the re-organization of the F-actin cytoskeleton towards increased stress fibers formation [30]. Similarly, Maciel et al. [31] recently confirmed that human renal arteries from CKD patients displayed reduced VE-cadherin and Zona occludens-1 (ZO-1) protein expression and that a uremic environment downregulated VE-cadherin and Vinculin in vitro [31]. These structural alterations make the endothelial barrier more susceptible to disruption upon electric wound or following exposure to the pro-permeability factor thrombin [30]. Finally, prolonged exposure to a uremic environment could affect the integrity of the vascular endothelium leading to enhanced permeability and endothelial cell detachment, as confirmed in a 3/4 nephrectomized rodent model [32].

Taken together, CKD-induced disturbances of the vascular endothelium are complex and involve a large number of mechanisms including impaired cell-cell and cell-matrix interaction, which contributes to detachment from the vessel wall, increased endothelial cell activation, lost vasodilating properties and limited repair capacity of damaged endothelial surfaces, all leading to loss of endothelial barrier function.

THE INFLUENCE OF SPECIFIC CKD-RELATED FACTORS ON ENDOTHELIAL HEALTH

Recently, many CKD-specific factors such as disturbed mineral metabolism, accumulation of uremic retention molecules, inflammation and oxidative stress have been identified as possibly being involved in ECD. Indeed, the vascular pathological features observed following exposure to these non-traditional risk factors in experimental uremic animal models or cell cultures resemble many clinical manifestations described in CKD patients, thus reinforcing that these specific factors may actually contribute to the pathogenesis of human ECD.

Disturbances in mineral metabolism

Compelling evidence suggests that the unavoidable progressive derangement in mineral homoeostasis due to progressive kidney failure may trigger or accelerate cardiovascular disease, at the level of both the medial layer and the intimal layer. Already in early CKD, the plasma concentrations of the kidney-derived protein alpha-Klotho decrease, while fibroblast growth factor-23 (FGF23) levels increase. The latter is probably responsible for decreased plasma 25 hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)2D] concentrations and all these factors, along with phosphate exposure, contribute to secondary hyperparathyroidism. The imbalance of each component worsens with advancing CKD and numerous studies established associations of these disturbances with cardiovascular calcification and heart disease. Experimental evidence, described below, also demonstrates that a disturbed mineral homoeostasis contributes to the development of a dysfunctional endothelium; however, this association is not well established in CKD patients, possibly due to a lack of clinically available tools to assess the endothelial function or structure.

alpha-Klotho. Originally identified as an anti-ageing protein, alpha-Klotho is now also recognized as a major player in mineral homoeostasis. Interestingly, clinical CKD shares many biochemical and histological features with the phenotype of alpha-Klotho-deficient mice, including its cardiovascular manifestations [33, 34]. The vascular abnormalities in alpha-Klotho mutant mice, such as impaired angiogenesis, insufficient endothelium-derived NO formation and reduced levels of circulating EPCs, may contribute to the development of ECD [34]. Membrane-bound alpha-Klotho is predominantly expressed in the distal tubule of the nephron. The mechanisms responsible for alpha-Klotho deficiency in CKD are not fully understood but are likely to be multifactorial [35]. Following tubular production and insertion in the plasma membrane, the ectodomain of membrane alpha-Klotho is cleaved from the cell surface by membrane-anchored proteases and released into the circulation, where it is suggested to be continuously required to maintain vascular health [36]. In this regard, one of the first vasculo-protective activities described for alpha-Klotho was its role in the maintenance of endothelial homoeostasis [33, 37]. Exposure of human umbilical vein endothelial cells to alpha-Klotho increased NO production and induced eNOS phosphorylation and inducible NOS expression [38]. Along the same line, alpha-Klotho has been shown to suppress the expression of the adhesion molecules ICAM and VCAM by the attenuation of NF-kappa B signalling pathway upon tumour necrosis factor-alpha (TNF-alpha) stimulation [39]. Another mechanism by which alpha-Klotho protects the endothelium was demonstrated by Kusaba et al., who showed that alpha-Klotho mediated the internalization of the transient receptor potential canonical 1 and vascular endothelial growth factor receptor 2 (VEGFR2) complex, thereby preventing hyperpermeability and endothelial apoptosis through an increase of calcium influx in endothelial cells incubated with VEGF [40]. Although extensive research has already provided much information on the beneficial effects of alpha-Klotho on endothelial damage, the relationship between alpha-Klotho and vascular dysfunction in patients with CKD remains poorly established. In CKD patients, lower alpha-Klotho levels were found to be an independent biomarker of arterial stiffness and defective FMD [41], and correlated with circulating von Willebrand factor levels [42]. However, while a deficiency of serum alpha-Klotho has been linked to cardiovascular complications

Endothelium in CKD

1481
in some studies [41, 43], this issue is still debated as Seiler et al. [2] found no relationship between soluble α-Klotho and cardiovascular outcomes in a cohort of CKD Stages 2–4 patients. Taken together, while experimental studies strongly suggest that α-Klotho preserves endothelial integrity in many different ways, there currently is no strong clinical evidence for a role of α-Klotho deficiency in CKD-mediated endothelial injury.

**Vitamin D.** Vitamin D deficiency, defined as serum 25(OH)D concentrations <20 ng/ml (50 nmol/L), is associated with both an increased prevalence and incidence of cardiovascular morbidity and mortality in CKD [44, 45]. In the kidney, 25(OH)D is converted by 1α-hydroxylase to its active form 1, 25(OH)2D to exert its effects on distant target tissue [46]. By binding the vitamin D receptor, 1,25(OH)2D activates both genomic and non-genomic pathways related to cellular proliferation and differentiation, and also on the endocrine and immune system [46]. As a consequence of CKD, there generally is a deficiency of 25(OH)D and a reduced production of active vitamin D, both contributing to reduced vitamin D actions on target tissues, including the vascular endothelium [45, 46]. Vitamin D deficiency is associated with decreased FMD in patients with CKD of different stages [47, 48]. In experimental models of CKD, active vitamin D analogues restored abnormal expression of aortic genes and improved endothelial function in a 5/6 nephrectomy rat model [18, 49]. Similarly, active vitamin D also protected against vascular leakage and endothelial cell detachment in *in vivo* models of CKD [32]. As a novel and potential protective mechanism, active vitamin D was shown to improve cell–cell interaction, disrupted after exposure to human uraemic plasma, leading to preservation of the endothelial barrier function [30]. In patients with CKD Stages 3 and 4, improvement of FMD by active vitamin D under low 25(OH)D circumstances has been reported [50]. Similar results were also observed in dialysis patients with vitamin D deficiency, where active vitamin D improved FMD of the brachial arteries [51–53]. In contrast, however, no effect of active vitamin D on brachial artery FMD or biomarkers of inflammation and oxidative stress was found in patients with advanced CKD and type 2 diabetes, and in the majority of clinical trials among diverse populations vitamin D administration has failed to show an improvement of endothelial function [54–57]. In addition, other clinical studies showed no significant effect of oral active vitamin D on left ventricular mass index in CKD patients (the PRIMO and OPERA trials) [58, 59] and no reduction of cardiovascular events in haemodialysis patients without secondary hyperparathyroidism (J-DAVID) [60]. These contradicting findings urge the search for better positioning the potential role of vitamin D administration in patients with CKD, especially in relation to disturbances in endothelial function and structure.

**Phosphate and FGF23.** CKD impairs phosphate balance, ultimately resulting in hyperphosphataemia [61]. In clinical studies, hyperphosphataemia and even high-normal serum phosphate concentrations represent one component of the increased risk of cardiovascular complications and mortality in both the general and CKD population [62, 63]. Recently, a number of studies suggested that phosphate may exert direct toxic effects on endothelial cells [64]. Specifically, *in vitro* experiments with endothelial cells demonstrated that high-phosphate concentration increases oxidative stress and decreases NO synthesis via inhibiting phosphorylation of eNOS [65]. This finding is in line with a clinical study in healthy subjects, which demonstrated that high dietary phosphate loading impaired flow-mediated vasodilation, indicating acute endothelial dysfunction [65]. In addition, exposure of endothelial cells to high-phosphate concentration also promoted the formation of EMPs with impaired capacity of angiogenesis [66, 67] and downregulated VE-cadherin and reduced ZO-1 protein levels, which are similar effects as found in endothelial cells exposed to uraemic media [31]. Importantly, in both healthy and CKD mice, it was reported that a high-phosphate diet promoted endothelial inflammation and dysfunction, and increased endothelial cell detachment [68].

To compensate for the decreased glomerular filtration of phosphate in the setting of CKD, FGF23, synthesized by osteocytes/osteoblasts, inhibits tubular reabsorption of phosphate, thereby restoring its net excretion. Besides phosphate exposure, other factors such as hyperparathyroidism and exogenous 1,25(OH)2D, calcium loading and inflammation also contribute to the elevation of plasma FGF23 concentration in CKD [69]. Although FGF23 may contribute to cardiovascular disease by the disturbance of mineral metabolism, FGF23 itself is independently associated with cardiovascular complications in different stages of CKD [70], and also with impaired vasoactivity and increased arterial stiffness in patients with impaired renal function [70]. Furthermore, experimental *ex vivo* data suggest that FGF23 can directly impair endothelium-dependent relaxation upon acetylcholine stimulation [71]. This effect appeared to be mediated by the reduction of NO bioavailability due to an accumulation of either asymmetrical dimethyl arginine (ADMA) [71] or superoxide levels [72]. Remarkably, the presence of a receptor for FGF23 is not firmly established on endothelial cells, and therefore the molecular mechanisms that underlie have remained obscure so far. Overall, further clinical studies are warranted to delineate the pathological mechanisms linking phosphate and FGF23 with endothelial cell abnormalities in CKD patients.

**Uraemic toxins**

Progression of CKD leads to the accumulation in blood and tissues of uraemic retention solutes [73]. As a result, the cardiovascular system is constantly exposed to the potentially toxic effects of a range of uraemic retention solutes inducing, among other complications, endothelial damage [74]. One well-characterized uraemic toxin is ADMA, which is known to exert a negative impact on endothelial cell stability in both *in vivo* and *in vitro* experimental models [74]. Indeed, ADMA is considered as a circulating endogenous inhibitor of eNOS [75], and its accumulation has been associated with ECD in patients with CKD [75, 76]. In CKD mice, increased serum concentration of ADMA caused attenuated endothelium-dependent vasodilation of aortic rings by inhibiting eNOS phosphorylation, by its property of being a competitor of l-arginine (the precursor of NO) as substrate for eNOS [77]. Furthermore, ADMA induces stress fibers and focal adhesion...
formation in a RhoA and Rho kinase-dependent pathway leading to a limited endothelial repair [78]. Importantly, ADMA also impairs the regeneration of injured endothelium by reducing the differentiation, mobilization and function of EPCs [79].

Formed by complex pathways, the covalently protein-bound toxins advanced glycation end products (AGEs) are the result of non-enzymatic glycation and oxidation of proteins, lipids and nuclear acids, and they accumulate in CKD [80]. In various cell types, AGEs exert diverse cellular responses via the multiligand cell-surface receptor for AGEs (RAGEs) [81]. The activation of RAGE in endothelial cells in vitro induced expression of adhesion molecules, increased endothelial permeability, impaired NO production and increased reactive oxygen species (ROS) formation [82]. Moreover, in patients with CKD, decreased endothelial reactivity has been correlated with increased circulating levels of AGEs [83]. Using an in vitro approach, this study demonstrated that AGEs isolated from serum of patients with CKD induced suppression of eNOS, and this effect was attenuated after RAGE blockade [83].

Recently, several studies demonstrated that other (non-covalently) protein-bound uraemic toxins such as p-cresyl sulphate (PCS) and indoxyl sulphate (IS) exert critical toxic effects on endothelial cells in CKD. In patients with CKD, PCS is the main circulating form of p-cresol and is independently associated with cardiovascular complications [84]. In addition, markers of endothelial damage such as EMPs are directly associated with free-serum p-cresol concentrations in haemodialysis patients [85]. The same study demonstrated in vitro that PCS induced a dose-dependent increase of shedding EMP, whereas this effect was prevented by inhibition of Rho kinase [85]. An in vitro study confirmed the role of the Rho-kinase pathway in PCS-mediated toxicity. Upon exposure to p-cresol, an increased endothelial permeability and barrier disruption were induced by alterations of VE-cadherin membrane distribution [86].

IS is another critical player in the development of vascular disease and is also associated independently with elevated mortality rate in patients with CKD [87]. IS is associated with worsened FMD and arterial stiffness in CKD patients [88]. This study also demonstrated that IS impairs the chemotactic motility and colony-forming ability of EPCs, suggesting that IS contributes to the pathogenesis of ECD by limiting the vascular repair capacity [88]. In addition, several in vitro studies showed that IS can directly disrupt the stability of the endothelial cells through other molecular pathways. Specifically, IS increased EMPs release and impaired endothelial wound healing capacity [89, 90]. Moreover, it promotes endothelial activation by ROS-induced activation of NF-κB. Similar to p-cresol, cell culture exposure to IS resulted in endothelial gap formation by VE-cadherin disassembly and stress fiber formation [91].

Overall, uraemic toxins may impact the vasculature by disrupting the integrity of the endothelial cell barrier, promoting endothelial activation and weakening its recovery capacity by impairing the EPCs function. Interestingly, as highlighted in Figure 2, the deleterious effects induced by uraemic toxins in experimental research share many characteristics with the endothelial abnormalities present in CKD patients or cell-based assays with endothelial cells exposed to uraemic media, suggesting that they are important mediators in the development of CKD-induced ECD in patients.

**Oxidative stress and inflammation**

Numerous studies have demonstrated that CKD is associated with increased oxidative stress and inflammation [92, 93]. Oxidative stress can be considered as accumulation of ROS in parallel with impaired or overwhelmed endogenous antioxidant mechanisms [94]. ROS are classically defined as partially reduced metabolites of oxygen that possess strong oxidizing capabilities [94]. The high production of ROS in CKD may contribute directly or indirectly to the pathogenesis of the cardiovascular disease by inducing endothelial injury [95]. Findings in animal models of chronic renal failure confirmed that enhanced generation of ROS leads to decreased NO bioavailability and impairment of the normal function of the endothelium [96]. Furthermore, increased levels of oxidative stress markers are associated with impaired endothelial function in CKD patients [97]. Moreover, chronic or prolonged ROS production is tightly connected to inflammatory processes [98], by activating transcription factors such as NF-κB, triggering a pro-inflammatory, pro-adhesion (of leucocytes) and pro-oxidant phenotype [98]. In addition, the activation of NF-κB pathway in endothelial cells is also triggered by inflammatory cytokines such as interleukin-6 and TNF-α [99]. These pro-inflammatory molecules are known to be elevated in patients with CKD and
cause ECD [8, 100]. Taken together, the development of a pro-inflammatory and pro-oxidative state during renal dysfunction is associated with oxidative stress, vascular NF-κB activation and inflammation, thus forming a vicious cycle amplifying ECD.

**THERAPEUTIC STRATEGIES TO PROTECT THE ENDOTHELIUM IN CKD**

Detailed knowledge of factors in CKD that induce ECD can pave the way to endothelial-protective therapeutic strategies, aiming to ameliorate cardiovascular disease in CKD. Based on the above, several options emerge and their clinical and experimental evidence are summarized in Table 1. The overarching approach might be the restoration of mineral metabolism network by correcting hormonal disturbances and counteracting the potential deleterious influence of uraemic toxins, inflammatory mediators and ROS. Recently, exogenous α-Klotho therapy has been shown to be effective in attenuating high-phosphate diet-induced renal and cardiac fibrosis and accelerated renal recovery after acute kidney injury in mice [101, 102]. Although the protective effects of exogenous α-Klotho administration in uraemia-mediated ECD in animal models remain to be investigated, *in vitro* data suggest that the endothelial-protective properties of α-Klotho are worthy of being tested *in vivo*. In this context, α-Klotho protein exerts protective effects by reducing the NF-κB translocation in cultured endothelial cells upon exposure to serum of Stage 5 CKD patients [103]. Moreover, exogenous α-Klotho attenuates *in vitro* the endothelial damage induced by the uraemic toxin IS and modulates the FGF23-mediated impaired NO synthesis and increased oxidative stress [104, 105].

As a potential option to restore impaired mineral balance and protect the endothelium, vitamin D replacement has raised great expectations to treat cardiovascular complications in CKD patients. However, as mentioned previously, data regarding the beneficial effects of vitamin D supplementation on cardiovascular disease including endothelial function are conflicting. In CKD animal models, active vitamin D treatment mitigates the impact of uraemia not only in endothelial function but also in structural alterations [18, 32, 49]. In randomized trials, several active vitamin D analogues lead to favourable changes on the vascular function in CKD patients of Stages 3–4 undergoing haemodialysis with or without vitamin D deficiency [50–53]; mean while, other studies reported no improvement in FMD with patients of advanced CKD [57, 106]. Overall, active vitamin D may potentially play different roles in protecting the vascular endothelium during CKD, but further studies are needed in this area.

Given its potential role in ECD, direct neutralization of the effects of phosphate and FGF23 may be another therapeutic option to protect the development of ECD. Options to accomplish the reduction of serum phosphate concentrations include treatment with phosphate binders. As an example, the phosphate-binder sevelamer hydrochloride was shown to ameliorate the phosphate-induced ECD in uraemic mice [68]. Furthermore, in hyperphosphataemic patients with Stage 4 CKD, sevelamer improved FMD, possibly mediated by parallel declines in FGF23 levels [107]. *In vitro*, sevelamer was effective also in protecting against endothelial activation upon uraemic media and AGEs exposure [108]. Thus, declining serum phosphorus concentrations might lead to better endothelial function and cardiovascular health in CKD patients. Alternatively, strategies to counteract high serum FGF23 concentrations such as the application of monoclonal antibodies has already been tested and shown to be effective for improving *ex vivo* vasodilator responses to acetylcholine in uraemic mice [71]. However, as demonstrated by Shalhoub et al. [109], the beneficial effects achieved by the neutralization of FGF23 signalling can be outbalanced by incrementing serum phosphate. Thus, inhibiting pathological FGF23-mediated pathways and lowering phosphate serum concentrations simultaneously may be a potential therapeutic strategy to reduce endothelial damage in CKD.

Because of their harmful effects on endothelial cells, reducing concentrations of uraemic toxins, ROS and inflammatory cytokines in CKD patients by dialysis may promote endothelial cell health [110, 111]. Indeed, ECD induced *in vitro* by serum from CKD patients led to remodelling of the extracellular matrix and this effect was mitigated in cells treated with serum from the same patients after haemodialysis therapy [29]. Unfortunately, most protein-bound uraemic retention molecules cannot be removed by dialysis. To overcome this limitation, as an absorbent of the uraemic toxin IS, AST-120 has shown to be effective to improve vascular relaxation in uraemic mice [112] and ameliorating the microvascular dysfunction in haemodialysis patients [113]. Other therapeutic approaches require components that may counterbalance the deleterious effects of oxidative stress, inflammation or toxicity, possibly by using anti-oxidants or inflammatory mediators [74]. Finally, patients with dialysis-dependent CKD following renal transplantation have improved endothelial function [114, 115].

---

**Table 1. Reported effective treatments against endothelial dysfunction in patients with CKD, in vivo CKD models and cell-based experiments**

| Treatment              | CKD patients | In vivo CKD | Cell-based assays exposed with |
|------------------------|--------------|-------------|--------------------------------|
|                        | Vascular function | Vascular function | Structural changes | Uraemic media | FGF23 | Phosphate | AGEs | IS |
| α-Klotho               |              |             |                               | 103          | 105    |          |       | 104 |
| Active vitamin D       | 50–53        | 18, 49      | 32                             |             |        |          |       |     |
| Sevelamer              | 107          | 68          |                                | 30           |        |          |       | 108 |
| Anti-FGF23             | 113          | 112         | 112                            | 108          | 68     | 108      |      |    |

Positive effects from the different treatments in the different conditions are highlighted in green.
These benefits also include the normalization of the functions of the EPCs, contributing to a better repair [114]. Overall, future studies should focus on the effective removal of these retention solutes in uremic patients in order to attenuate ECD and promote endothelial repair.

**CONCLUSIONS**

In patients with CKD, ongoing endothelial damage in the vascular system exists and is frequently overlooked. However, endothelial damage is thought to be a central driver of progressive cardiovascular complications. The pathogenesis of ECD in patients with renal dysfunction results from an imbalance between increased endothelial damage and impaired regeneration. In addition, limited vasoreactivity, in particular vasodilatory properties, exists. These processes may result from the progressive loss of the vasculoprotective factors vitamin D and α-Klotho together with an increment of ECD mediators such as FGF23, uraemic toxins, ROS and inflammatory cytokines. Therapeutic strategies aiming at a better endothelial health should be based on correcting the derangements of the mineral homeostasis, removing the retention solutes and limiting oxidative stress.

**CONFLICT OF INTEREST STATEMENT**

None declared.

**REFERENCES**

1. Fiser D, Wiecek A, Suleymanlar G et al. The dysfunctional endothelium in CKD and in cardiovascular disease: mapping the origin(s) of cardiovascular problems in CKD and of kidney disease in cardiovascular conditions for a research agenda. Kidney Int Suppl 2011; 1: 6–9
2. Seiler S, Rogacev IS, Roth HJ et al. Associations of FGF-23 and sKlotho with cardiovascular outcomes among patients with CKD stages 2–4. Clin J Am Soc Nephrol 2014; 9: 1049–1058
3. Gimbrone MA Jr, Garcia CG. Endothelial cell dysfunction and the pathology of atherosclerosis. Circ Res 2016; 112: 620–636
4. Iwamoto Y, Maruhashi T, Kajikawa M et al. Circulating endothelial cells: potential markers of the state of the endothelium in hemodialysis patients. Am J Kidney Dis 2003; 42: 704–712
5. Endemann DH, Schiffrin EL. Endothelial dysfunction. J Am Soc Nephrol 2004; 15: 1983–1992
6. Yilmaz MI, Stenvinkel P, Sonmez A et al. Vascular health, systemic inflammation and progressive reduction in kidney function; clinical determinants and impact on cardiovascular outcomes. Nephrol Dial Transplant 2011; 26: 3537–3543
7. Recio-Mayoral A, Banerjee D, Stretcher C et al. Endothelial dysfunction, inflammation and atherosclerosis in chronic kidney disease—a cross-sectional study of predialysis, dialysis and kidney-transplantation patients. Atherosclerosis 2011; 216: 446–451
8. Ioannou K, Stel VS, Dounouss E et al. Inflammation, endothelial dysfunction and increased left ventricular mass in chronic kidney disease (CKD) patients: a longitudinal study. PLoS One 2015; 10: e0134861
9. Stam F, van GC, Becker A et al. Endothelial dysfunction contributes to renal function-associated cardiovascular mortality in a population with mild renal insufficiency: the Hoorn study. J Am Soc Nephrol 2006; 17: 537–545
10. Verbeke FH, Pannier B, Guérin AP et al. Flow-mediated vasodilation in end-stage renal disease. Clin J Am Soc Nephrol 2011; 6: 2009–2015
11. Morris ST, McMurray JJ, Spiers A et al. Impaired endothelial function in isolated human uremic resistance arteries. Kidney Int 2001; 60: 1077–1082
12. Yilmaz MI, Saglam M, Caglar K et al. The determinants of endothelial dysfunction in CKD: oxidative stress and asymmetric dimethylarginine. Am J Kidney Dis 2006; 47: 42–50
13. Ghidoni L, Huang Y, Magagna A et al. Effect of acute blood pressure reduction on endothelial function in the brachial artery of patients with essential hypertension. J Hypertens 2001; 19: 547–551
14. Tousoulis D, Kampoli AM, Tentolouris C et al. The role of nitric oxide on endothelial function. Curr Vasc Pharmacol 2012; 10: 4–18
15. Baylis C. Nitric oxide deficiency in chronic kidney disease. Am J Physiol Renal Physiol 2008; 294: F1–F9
16. Oberg BP, McMenamin E, Lucas FL et al. Increased prevalence of oxidant stress and inflammation in patients to moderate to severe chronic kidney disease. Kidney Int 2004; 65: 1099–1106
17. Spradley FT, White JJ, Paulson WD et al. Differential regulation of nitric oxide synthase function in aorta and tail artery from 5/6 nephrectomized rats. Physiol Rep 2013; 1: e00145
18. Wu-Wong JR, Noonan W, Nakane M et al. Vitamin d receptor activation mitigates the impact of uremia on endothelial function in the 5/6 nephrectomized rats. Int J Endocrinol 2010; 2010: 625852
19. Stam F, van Guldener C, Schalkwijk CG et al. Impaired renal function is associated with markers of endothelial dysfunction and increased inflammatory activity. Nephrol Dial Transplant 2003; 18: 892–898
20. Chen J, Hamm LL, Mohler ER et al. Interrelationship of multiple endothelial dysfunction biomarkers with chronic kidney disease. PLoS One 2015; 10: e0132047
21. Amabile N, Guérin AP, Leroyer A et al. Circulating endothelial microparticles are associated with vascular dysfunction in patients with end-stage renal failure. J Am Soc Nephrol 2005; 16: 3381–3388
22. Koq M, Bihorac A, Segal MS. Circulating endothelial cells as potential markers of the state of the endothelium in hemodialysis patients. Am J Kidney Dis 2003; 42: 704–712
23. Jie KE, Zaikova MA, Begevoet MW et al. Progenitor cells and vascular function are impaired in patients with chronic kidney disease. Nephrol Dial Transplant 2010; 25: 1875–1882
24. Chen YT, Cheng BC, Ko SF et al. Value and level of circulating endothelial progenitor cells, angiogenesis factors and mononuclear cell apoptosis in patients with chronic kidney disease. Clin Exp Nephrol 2013; 17: 83–91
25. Mohandas R, Segal MS. Endothelial progenitor cells and endothelial vesicles - what is the significance for patients with chronic kidney disease? Blood Purif 2010; 29: 158–162
26. Serradell M, Diaz-Ricart M, Cases A et al. Uraemic medium accelerates proliferation but does not induce apoptosis of endothelial cells in culture. Nephrol Dial Transplant 2003; 18: 1079-1085
27. Serradell M, Diaz-Ricart M, Cases A et al. Uremic medium causes expression, redistribution and shedding of adhesion molecules in cultured endothelial cells. Haematologica 2002; 87: 1053–1061
28. Carbó C, Arderiu G, Escolar G et al. Differential expression of proteins from cultured endothelial cells exposed to uremic versus normal serum. Am J Kidney Dis 2008; 51: 603–612
29. Zafeiropoulou K, Bita T, Polykratis A et al. Expression of proteins that alter the biological actions of endothelial cells. PLoS One 2012; 7: e30975
30. Vila Cuenca M, van Bezu J, Beelen RHJ et al. Stabilization of cell-cell junctions by active vitamin D ameliorates uraemia-induced loss of human endothelial barrier function. Nephrol Dial Transplant 2018; 34: 252–264
31. Maciel RAP, Cunha RS, Busato V et al. Uremia impacts VE-cadherin and ZO-1 expression in human endothelial cell-to-cell junctions. Toxins 2018; 10: 404
32. Vila Cuenca M, Ferrantelli E, Meinsster E et al. Vitamin D attenuates endothelial dysfunction in uremic rats and maintains human endothelial stability. J Am Heart Assoc 2018; 7: e008776
33. Kuro-O M, Matsumura Y, Aizawa H et al. Mutation of the mouse Klotho gene leads to a syndrome resembling ageing. Nature 1997; 390: 45–51
34. Shimada T, Takeshita Y, Murohara T et al. Angiogenesis and vasculogenesis are impaired in the precocious-aging klotho mouse. Circulation 2004; 110: 1148–1155
35. Kato Y, Arakawa E, Kinoshita S et al. Establishment of the anti-Klotho monoclonal antibodies and detection of Klotho protein in kidneys. Biochem Biophys Res Commun 2000; 267: 597–602
36. Bloch L, Sineshchekova O, Reichenbach D et al. Klotho is a substrate for alpha- , beta- and gamma-secretase. FEBS Lett 2009; 583: 3221–3224
37. Saito Y, Yamagishi T, Nakamura T et al. Klotho protein protects against endothelial dysfunction. Biochem Biophys Res Commun 1998; 248: 324–329
38. Siu J, Okazaki H, Gross P et al. Direct, acute effects of Klotho and FGF23 on vascular smooth muscle and endothelium. PLoS One 2014; 9: e93423
39. Maekawa Y, Ishikawa K, Yasuda O et al. Klotho suppresses TNF-alpha-induced expression of adhesion molecules in the endothelium and attenuates NF-kappaB activation. Endocrine 2009; 35: 341–346
40. Kusaba T, Okigaki M, Matui A et al. Klotho is associated with VEGF receptor-2 and the transient receptor potential canonical-1 Ca2+ channel to maintain endothelial integrity. Proc Natl Acad Sci USA 2010; 107: 19308–19313
41. Kitagawa M, Sugiyama H, Morinaga H et al. A decreased level of serum soluble Klotho is an independent biomarker associated with arterial stiffness in patients with chronic kidney disease. PLoS One 2013; 8: e56995
42. Malyszko J, Koc-Zorawska E, Matuszkiewicz-Rowinska J et al. Vitamin D deficiency and chronic kidney disease. Ethn Dis 2019; 19: S5–811
43. Saito Y, Yamagishi T, Nakamura T et al. Klotho protein protects against endothelial dysfunction. Biochem Biophys Res Commun 1998; 248: 324–329
44. Williams S, Malatesta K, Norris K. Vitamin D and chronic kidney disease. Ethno Dis 2009; 19: 85–91
45. Bacchetta J, Pelletier S. Vitamin D deficiency is associated with mortality in maintenance dialysis: moving forward from epidemiology to clinical trials. Nephrol Dial Transplant 2018; 33: 1679–1682
46. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. Am J Physiol Renal Physiol 2005; 289: F8–F28
47. London GM, Guérin AP, Verbeke FH et al. Mineral metabolism and arterial functions in end-stage renal disease: potential role of 25-hydroxyvitamin D deficiency. J Am Soc Nephrol 2007; 18: 613–620
48. Chitalia N, Recio-Mayoral A, Kashi JC et al. Vitamin D deficiency and endothelial dysfunction in non-dialysis chronic kidney disease patients. Atherosclerosis 2012; 220: 265–268
49. Wu-Wong JR, Kawai M, Chen YW et al. Two novel vitamin D receptor modulators with similar structures exhibit different hypercalcemic effects in 5/6 nephrectomized uremic rats. Am J Nephrol 2013; 37: 310–319
50. Zoccali C, Curatola G, Panuccio V et al. Paricalcitol and endothelial function in chronic kidney disease trial. Hypertension 2014; 64: 1005–1011
51. Karakas Y, Sahin G, Urfalı FE et al. Effect of vitamin D supplementation on endothelial dysfunction in hemodialysis patients. Hemodial Int 2017; 21: 97–106
52. Levin A, Tang M, Perry T et al. Randomized controlled trial for the effect of vitamin D supplementation on vascular stiffness in CKD. Clin J Am Soc Nephrol 2017; 12: 1447–1460
53. Kumar V, Yadav AK, Lal A et al. A randomized trial of vitamin D supplementation on vascular function in CKD. J Am Soc Nephrol 2017; 28: 3100–3108
54. Tarcó O, Yavuz DG, Ozben B et al. Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. J Clin Endocrinol Metab 2009; 94: 4023–4030
55. Sokol SI, Srinivas V, Crandall JP et al. The effects of vitamin D repletion on endothelial function and inflammation in patients with coronary artery disease. Vasc Med 2012; 17: 394–404
56. Sugden JA, Davies JJ, Witham MD et al. Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels. Diabet Med 2008; 25: 320–325
57. Kendrick J, Andrews E, You Z et al. Cholecalciferol, calcitriol, and vascular function in CKD: a randomized, double-blind trial. Clin J Am Soc Nephrol 2017; 12: 1438–1446
58. Wang AY, Fang F, Chan J et al. Effect of paricalcitol on left ventricular mass and function in CKD—the OPERA trial. J Am Soc Nephrol 2014; 25: 175–186
59. Thadhani R, Appelbaum E, Pritchett Y et al. Vitamin D therapy and cardiac structure and function in patients with chronic kidney disease: the FRIMO randomized controlled trial. JAMA 2012; 307: 674–684
60. Shoji T, Inaba M, Fukagawa M et al. Effect of oral alfacalcidol on clinical outcomes in patients without secondary hyperparathyroidism receiving maintenance hemodialysis: The J-DAVID randomized clinical trial. JAMA 2018; 320: 2325–2334
61. Hruska KA, Mathew S, Lund R et al. Hyperphosphatemia of chronic kidney disease. Kidney Int 2008; 74: 148–157
62. Dhingra R, Sullivan LM, Fox CS et al. Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. Arch Intern Med 2007; 167: 879–885
63. Kestenbaum B, Sampson JN, Ruder KD et al. Serum phosphate levels and mortality risk among people with chronic kidney disease. J Am Soc Nephrol 2009; 16: 520–528
64. Burger D, Levin A. ‘Shedding’ light on mechanisms of hyperphosphatemic vascular dysfunction. Kidney Int 2013; 83: 187–189
65. Shuto E, Taketani Y, Tanaka R et al. Dietary phosphate acutely impairs endothelial function. J Am Soc Nephrol 2009; 20: 1504–1512
66. Di Marco GS, König M, Stock C et al. High phosphate directly affects endothelial function by downregulating annexin II. Kidney Int 2013; 83: 213–222
67. Abbassian N, Burton JO, Herbert KE et al. Hyperphosphatemia, phospho-protein phosphatases, and microparticle release in vascular endothelial cells. J Am Soc Nephrol 2015; 26: 2152–2162
68. Six I, Mainel J, Barreto FC et al. Effects of phosphate on vascular function under normal conditions and influence of the uraemic state. Cardiovasc Res 2012; 96: 130–139
69. Haussler MR, Whitfield GK, Kaneko I et al. The role of vitamin D in the FGF23, klotho, and phosphate bone-kidney endocrine axis. Rev Endocur Metab Disord 2012; 13: 57–69
70. Mirza MA, Larsson A, Lind L et al. Circulating fibroblast growth factor-23 is associated with vascular dysfunction in the community. Atherosclerosis 2009; 205: 385–390
71. Verkaik M, Juni RP, van Loon EPM et al. FGF23 impairs peripheral microvascular function in renal failure. Am J Physiol Heart Circ Physiol 2018; 315: H1414–H1424
72. Silwal N, Touchberry CD, Daniel DR et al. FGF23 directly impairs endothelium-dependent vasorelaxation by increasing superoxide levels and reducing nitric oxide bioavailability. Am J Physiol Endocrinol Metab 2014; 307: E426–E436
73. Vanholder R, De Smet R, Glorieux G et al. Klotho is associated with vascular dysfunction in the community. J Am Soc Nephrol 2005; 16: 520–528
74. Thanou TD, Vasa S, Potgieter B et al. Suppression of endothelial progenitor cells and reducing nitric oxide bioavailability. Circulation 2014; 130: 1934–1943
75. Jourde-Chiche N, Dou L, Cerini C et al. Vascular incompetence in dialysis patients—protein-bound uremic toxins and endothelial dysfunction. Semin Dial 2011; 24: 327–337
76. Vallance P, Leone A, Calver A et al. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet 1992; 339: 572–575
77. Böger RH, Bode-Böger SM, Szuba A et al. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation 1998; 98: 1842–1847
78. Kajimoto H, Kai H, Aoki H et al. Inhibition of eNOS phosphorylation mediates endothelial dysfunction in renal failure: new effect of asymmetric dimethylarginine. Kidney Int 2012; 81: 762–768
79. Vanholder R, De Smet R, Glorieux G et al. Review on uremic toxins: classification, concentration, and interindividual variability. Kidney Int 2003; 63: 1934–1943
80. Veleka M, Juni RP, van Loon EPM et al. FGF23 impairs peripheral microvascular function in renal failure. Am J Physiol Heart Circ Physiol 2018; 315: H1414–H1424
81. Thadhani R, Appelbaum E, Pritchett Y et al. Vitamin D therapy and cardiac structure and function in patients with chronic kidney disease: the FRIMO randomized controlled trial. JAMA 2012; 307: 674–684
82. Shoji T, Inaba M, Fukagawa M et al. Effect of oral alfacalcidol on clinical outcomes in patients without secondary hyperparathyroidism receiving maintenance hemodialysis: The J-DAVID randomized clinical trial. JAMA 2018; 320: 2325–2334
83. Linden E, Cai W, He JC et al. Endothelial dysfunction in patients with chronic kidney disease results from advanced glycation end products (AGE)-mediated inhibition of endothelial nitric oxide synthase through RAGE activation. Clin J Am Soc Nephrol 2008; 3: 691–698
84. Meijers BK, Bammens B, De Moor B et al. Free p-cresol is associated with cardiovascular disease in hemodialysis patients. Kidney Int 2008; 73: 1174–1180
85. Meijers BK, Van Kerckhoven S, Verbeke K et al. The uremic retention solute p-cresyl sulfate and markers of endothelial damage. Am J Kidney Dis 2009; 54: 891–901
86. Cerini C, Dou L, Anfosso F et al. P-cresol, a uremic retention solute, alters the endothelial barrier function in vitro. Thromb Haemost 2004; 92: 140–150
87. Barreto FC, Barreto DV, Liabeuf S et al. Serum indoxyl sulfate is associated with vascular disease and mortality in chronic kidney disease patients. Clin J Am Soc Nephrol 2009; 4: 1551–1558
88. Lin CJ, Wu CJ, Wu PC et al. Indoxyl sulfate impairs endothelial progenitor cells and might contribute to vascular dysfunction in patients with chronic kidney disease. Kidney Blood Press Res 2016; 41: 1025–1036
89. Faure V, Dou L, Sabatier F et al. Elevation of circulating endothelial microparticles in patients with chronic renal failure. J Thromb Haemost 2006; 4: 566–573
90. Dou L, Bertrand E, Cerini C et al. The uremic solute p-cresol and indoxyl sulfate inhibit endothelial proliferation and wound repair. Kidney Int 2004; 65: 442–451
91. Peng YS, Lin YT, Chen Y et al. Effects of indoxyl sulfate on adherens junctions of endothelial cells and the underlying signaling mechanism. J Cell Biochem 2012; 113: 1034–1043
92. Chung SH, Heimbürger O, Stenvinkel P et al. Association between inflammation and changes in residual renal function and peritoneal transport rate during the first year of dialysis. Nephrol Dial Transplant 2001; 16: 2240–2245
93. Douounsi E, Papavasiliou E, Makedou A et al. Oxidative stress is progressively enhanced with advancing stages of CKD. Am J Kidney Dis 2006; 48: 752–760
94. Galli F, Pinoddi M, Annetti C et al. Oxidative stress and reactive oxygen species. Contrib Nephrol 2005; 149: 240–260
95. Brown SA. Oxidative stress and chronic kidney disease. Vet Clin North Am Small Anim Pract 2008; 38: 157–166, vi
96. Hasdan G, Benchetrit S, Rashid G et al. Endothelial dysfunction and hypertension in 5/6 nephrectomized rats are mediated by vascular superoxide. Kidney Int 2002; 61: 586–590
97. Costa-Hong V, Bortolotto LA, Jorgetti V et al. Oxidative stress and endothelial dysfunction in chronic kidney disease. Arq Bras Cardiol 2009; 92: 381–386, 389–403, 413–418
98. Li N, Karin M. Is NF-kappaB the sensor of oxidative stress? FASEB J 1999; 13: 1137–1143
99. Donato AJ, Pierce GL, Lesniewski LA et al. Role of NFkappaB in age-related vascular endothelial dysfunction in humans. Aging (Albany NY) 2009; 1: 678–680
100. Landray MJ, Wheeler DC, Lip GY et al. Inflammation, endothelial dysfunction, and platelet activation in patients with chronic kidney disease: the chronic renal impairment in Birmingham (CRIB) study. Am J Kidney Dis 2004; 43: 244–253
101. Hu MC, Shi M, Gillings N et al. Recombinant Klotho may be prophylactic and therapeutic for acute to chronic kidney disease progression and uremic cardiomyopathy. Kidney Int 2017; 91: 1104–1114
102. Shi M, Flores B, Gillings N et al. Klotho mitigates progression of AKI to CKD through activation of autophagy. J Am Soc Nephrol 2016; 27: 2331–2345
103. Buendia P, Carracedo J, Soriano S et al. Klotho prevents NFXB translocation and protects endothelial cell from senescence induced by uremia. J Gerontol A Biol Sci Med Sci 2015; 70: 1198–1209
104. Yang K, Nie L, Huang Y et al. Amelioration of uremic toxin indoxyl sulfate-induced endothelial cell dysfunction by Klotho protein. Toxicol Lett 2012; 215: 77–83
105. Richter B, Haller J, Haffner D et al. Klotho modulates FGF23-mediated NO synthesis and oxidative stress in human coronary artery endothelial cells. Pflugers Arch 2016; 468: 1621–1635
106. The thi TK, Bajwa MA, Ghanim H et al. Effect of paricalcitol on endothelial function and inflammation in type 2 diabetes and chronic kidney disease. J Diabetes Complications 2015; 29: 433–437
107. Yilmaz M, Sonmez A, Saglam M et al. Comparison of calcium acetate and sevelamer on vascular function and fibroblast growth factor 23 in CKD patients: a randomized clinical trial. Am J Kidney Dis 2012; 59: 177–185
108. Gregorio PC, Favretto G, Sassi ki GL et al. Sevelamer reduces endothelial inflammatory response to advanced glycation end products. Clin Kidney J 2018; 11: 89–98
109. Shahroub V, Shatzen EM, Ward SC et al. FGF23 neutralization improves chronic kidney disease-associated hyperparathyroidism yet increases mortality. J Clin Invest 2012; 122: 2543–2553
110. Kuo HL, Chou CY, Liu YL et al. Reduction of pro-inflammatory cytokines through hemodialfiltration. Ren Fail 2008; 30: 796–800
111. Dhondt A, Vanholder R, Van Biesen W et al. The removal of uremic toxins. Kidney Int Suppl 2000; 76: S47–S59
112. Six I, Gross P, Remond MC et al. Deleterious vascular effects of indoxyl sulfate and reversal by oral adsorbent AST-120. Atherosclerosis 2015; 243: 248–256
113. Ryu JH, Yu M, Lee S et al. AST-120 improves microvascular endothelial dysfunction in end-stage renal disease patients receiving hemodialysis. Yonsei Med J 2016; 57: 942–949
114. Herbrig K, Gebler K, Oelschlaegel U et al. Kidney transplantation substantially improves endothelial progenitor cell dysfunction in patients with end-stage renal disease. Am J Transplant 2006; 6: 2922–2928
115. Covic A, Goldsmith DJ, Gusbeth-Tatomin P et al. Successful renal transplantation decreases aortic stiffness and increases vascular reactivity in dialysis patients. Transplantation 2003; 76: 1573–1577

Received: 28.12.2018; Editorial decision: 26.2.2019