Assessment of Endothelial and Microvascular Function in CKD: Older and Newer Techniques, Associated Risk Factors, and Relations with Outcomes

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Keywords

Chronic kidney disease · Microvascular function · Endothelial dysfunction · Flow-mediated dilatation · Near-infrared spectroscopy

Abstract

Background: Endothelium is the inner cellular lining of the vessels that modulates multiple biological processes including vasomotor tone, permeability, inflammatory responses, hemostasis, and angiogenesis. Endothelial dysfunction, the basis of atherosclerosis, is characterized by an imbalance between endothelium-derived relaxing factors and endothelium-derived contracting factors. **Summary:** Starting from the semi-invasive venous occlusion plethysmography, several functional techniques have been developed to evaluate microvascular function and subsequently used in patients with CKD. Flow-mediated dilatation of the forearm is considered to be the “gold standard,” while in the last years, novel, non-invasive methods such as laser speckle contrast imaging and near-infrared spectroscopy are scarcely used. Moreover, several circulating biomarkers of endothelial function have been used in studies in CKD patients. This review summarizes available functional methods and biochemical markers

for the assessment of endothelial and microvascular function in CKD and discusses existing evidence on their associations with comorbid conditions and outcomes in this population. **Key Messages:** Accumulated evidence suggests that endothelial dysfunction occurs early in CKD and is associated with target organ damage, progression of renal injury, cardiovascular events, and mortality. Novel methods evaluating microvascular function can offer a detailed, real-time assessment of underlying phenomena and should be increasingly used to shed more light on the role of endothelial dysfunction on cardiovascular and renal disease progression in CKD.

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Introduction

The endothelium constitutes the inner cellular lining of blood and lymphatic vessels; there are different kinds of endothelial cells which differ considerably from one another in structure and function [1]. The endothelium plays a central role in multiple physiologic functions, including regulation of vasomotor tone, vascular permeability, leukocyte trafficking, hemostasis, angiogenesis,

Table 1. Main advantages and limitations of the functional methods used to assess microvascular function

Method	Advantages	Limitations
VOP	High reproducibility and validity Assessment at rest and after local infusion of vasoactive agents	Semi-invasive Indirect measure of blood flow (not absolute numbers) Limited application in exercise Time consuming
FMD	Simple, inexpensive Strong correlation with coronary function Many outcome studies Best validated technique for assessment of conduit vessel function	High inter- and intraobserver variability Poor reproducibility if not performed in a standardized manner Requires adherence to strict scanning protocols Requires well-trained, experienced operators
LDF	Noninvasive, very simple Capable of measuring blood flow continuously during active exercise Coupling with other techniques	Relatively expensive equipment Requires mastered operator for high accuracy and low day-to-day variability Extremely sensitive to movement artifacts
Arterial glycocalyx (functional)	Tracer dilution method Direct measure of systemic glycocalyx volume	Tracer dilution method Invasive Time consuming
	OPS and SDF Simple, noninvasive Painless and rapid Are executed at bedside	OPS and SDF Indirect measures of glycocalyx
LSCI	Produces real-time image recording continuous changes in blood flow Noninvasive, simple	Relatively expensive equipment Requires mastered operator for high accuracy and low day-to-day variability Extremely sensitive to movement artifacts No recordings in absolute flow units
Nailfold capillaroscopy	Simple Noninvasive Video capillaroscopy: 3-phase evaluation	Video capillaroscopy requires skill and precision
NIRS	Noninvasive, portable Inexpensive Measures tissue oxygen consumption (muscle, cerebral, etc.) Recordings at rest and during exercise	Unknown contribution of myoglobin to the overall signals Impact of adipose tissue thickness

FMD, flow-mediated dilatation; VOP, venous occlusion plethysmography; LDF, laser Doppler flowmetry; LSCI, laser speckle contrast imaging; NIRS, near-infrared spectroscopy; OPS, orthogonal polarization spectral; SDF, sidestream dark field.

and innate and adaptive immunity [1]. Endothelial dysfunction is characterized by an imbalance between agents with vasodilating, antimitogenic, and antithrombogenic properties (endothelium-derived relaxing factors) and agents with vasoconstricting, prothrombotic, and proliferative properties (endothelium-derived contracting factors) [2]. Reduced nitric oxide (NO) bioavailability is a hallmark of endothelial dysfunction; NO plays a major role in endothelial dysfunction and arterial remodeling by several mechanisms [3].

Chronic kidney disease (CKD) is a major issue of public health with an estimated prevalence of 14.5% [4]. Patients with CKD have 2-fold higher mortality rate than those without CKD; cardiovascular disease is the main cause of death in this population [5]. The risk of cardiovascular events rises exponentially with the progression to end-stage kidney disease (ESKD) [6], so that more than the half of deaths in these patients is attributed to cardiovascular disease [7]. Many traditional and nontraditional risk factors are thought to have a role in cardiovascular

disease development in patients with CKD [8]. Among the latter, endothelial dysfunction occurs in the early stages of CKD, reflecting multifactorial endothelial injury from various factors including inflammation, hypertension, diabetes-associated factors, and uremic milieu [9]. Endothelial dysfunction is shown not only to contribute in the development of cardiovascular disease in this population [8] but also to CKD progression [10].

Starting from venous occlusion plethysmography (VOP), which was first described and used for many years as the “gold standard,” several methods have been developed to evaluate microvascular function and have also been used in patients with CKD [11]. Flow-mediated dilatation (FMD) of the forearm is considered now to be the “gold standard,” while in the last years, novel, noninvasive, and easily applicable methods such as near-infrared spectroscopy (NIRS) and laser speckle contrast imaging (LSCI) gain more and more ground. In this article, we present an overview of the currently used methods to assess microvascular and endothelial function in patients with CKD and discuss the existing evidence relevant to the associations of endothelial dysfunction with comorbid conditions and outcomes in this population with high burden of cardiovascular disease.

Assessment of Endothelial Dysfunction in CKD: Functional Methods

Venous Occlusion Plethysmography

VOP was the first semi-invasive technique used for the assessment of vascular function, but it is now rarely applied in clinical practice as it is semi-invasive and time consuming (Table 1) [12, 13]. The main principle of this method is simple; at a period when venous drainage from a tissue is obstructed without affecting arterial outflow, changes in tissue volume are proportional to arterial inflow rate [14]. VOP is based on the use of automatically calibrated mercury-in-silastic strain gauges, which are placed at a circular manner around the limb under examination [12, 15]. Typically, an inflation pressure of 40 mm Hg for 10-s intervals, followed by 5 s of deflation with the limb positioned at heart level, allows for venous emptying with minor changes in arterial inflow [12, 15]. Vessel responsiveness is evaluated by measurement of forearm volume changes in response to reactive hyperemia or infusion of vasoactive compounds (i.e., acetylcholine, nitroprusside, etc.) [12, 14]. Endothelium-dependent vasodilation on forearm blood flow (mL/min/100 mL tissue) is evaluated by infusion of endothelial agonists (i.e., ace-

tylcholine, bradykinin, etc.), whereas endothelium-independent vasodilation by the infusion of direct smooth muscle relaxing factors (i.e., nitrates) [14]. The main advantages and limitations of VOP and the other techniques discussed herein are presented in Table 1.

In previous studies in patients with CKD, Passauer et al. [16] showed that endothelium-dependent vasodilation assessed with VOP was reduced in hemodialysis patients compared to controls, but it was improved after renal transplantation [17]. This impaired endothelial function in ESKD was associated with traditional risk factors, such as increasing age and the presence of diabetes mellitus (DM) [18], but also with duration of ESKD, cardiac and arterial remodeling, and other nontraditional uremia-associated risk factors [19, 20]. In 44 patients with CKD stage 3–5, endothelial dysfunction assessed by VOP was correlated with oxidative stress but was independent from C-reactive protein (CRP) levels [21].

Furthermore, London et al. [22] studied 78 hemodialysis patients and found that decreased postocclusion forearm reactive hyperemia was associated with all-cause mortality, independently of the presence of LV hypertrophy (LVH) or arterial stiffness (Table 2). Perticone et al. [23] studied 500 treatment-naïve uncomplicated hypertensive patients in order to assess the role of endothelial dysfunction in the progression of CKD and demonstrated that acetylcholine-stimulated vasodilation and SBP were associated with estimated glomerular filtration rate (eGFR) loss after adjustment for other cardiovascular risk factors and antihypertensive treatment during a 92 ± 36 -month follow-up. Last but not least, long-term therapy with amlodipine and/or renin-angiotensin-system inhibitors significantly decreased forearm resistance measured by VOP compared to metoprolol treatment in patients with advanced CKD [24]. Similar results were observed in another randomized study using low-dose spironolactone versus placebo in hemodialysis patients without heart failure [25].

Flow-Mediated Dilatation

FMD is a well-validated and widely used noninvasive method for assessment of endothelium dysfunction in conduit arteries, such as the brachial, radial, and femoral artery [26, 27]. It is based on ultrasound imaging of the artery at rest and after reactive hyperemia, produced either after arterial occlusion by a suprasystolic cuff inflation (endothelium-dependent vasodilation) or after administration of an exogenous NO donor such as a sublingual nitroglycerin dose (endothelium-independent vasodilation) [28, 29]. Arterial diameter is recorded at

Table 2. Cohort studies using functional techniques to evaluate the associations of endothelial dysfunction with hard outcomes

Study	Method	N	Participants	Follow-up duration	Study variables	Outcome	Results
London et al. [22]	VOP	78	78 hemo-dialysis	60±27 months	VOP (FBF, FDR%) Office BP cIMT PWV LVMI	All-cause mortality	↓ FDR was independently associated with all-cause mortality (RR 0.69 for every 10% increase; 95% CI: 0.56–0.85) ↑ PWV was independently associated with all-cause mortality (RR 1.16 for 1 m/s increase; 95% CI: 1.04–1.29)
Yilmaz et al. [33]	FMD	304	304 CKD patients stage 1–5	41 (6–46) months	FMD cIMT CRP Insulin resistance	CV outcome or death	↑ CRP and cIMT and ↓ FMD in parallel with eGFR decline ($p < 0.001$ for all) CRP, iPTH, and eGFR were strong determinants of FMD (adjusted $r^2 = 0.70$) and IMT (adjusted $r^2 = 0.53$) Univariate analysis: FMD, IMT, and CRP were significant predictors of outcome Multivariate Cox model excluding IMT: FMD (HR 0.52 [95% CI: 0.37–0.73] per %) and CRP (1.07 [1.03–1.11] per mg/L) predicted CV outcomes independently of confounders. In a model excluding FMD, only CRP (and not cIMT) was a significant predictor
Kruger et al. [28]	LDF	70	70 hemo-dialysis	2 years	LDF measurements (LDPM during PORH and TH) CRP Framingham and Cardiorisk scores	CV mortality and surrogate endpoints (CAD, MI, cerebrovascular accidents, peripheral arterial disease, and CHF)	Framingham and Cardiorisk scores were near equivalent for low-risk patients, but more divergent as risk increased CRP levels and LDF parameters (amplitude of TH and area under the curve of TH) showed significant abnormality in high-risk versus low-risk patients calculated using either Framingham or Cardiorisk scores Patients with abnormal LDF parameters showed increased CV mortality but had similar risk assessments (Framingham, Cardiorisk, CRP, and homocysteine) to those with unimpaired LDF tracings CV mortality and development of CHF were associated with the decreased amplitude of the first thermal peak and the nadir (CV mortality: $p = 0.041/0.045$ and CHF: $p = 0.017/0.038$, respectively) Development of CAD was associated with the postocclusive recruitment of dermal capillaries ($p = 0.016$)
Nemcsik et al. [57]	LDF	105	105 hyper-tensive CKD patients	66.6 (39.8–80.4) months	LDF measurements (coupled with PORHHA and acetylcholine and nitroprusside administration) Biomarkers (Ang-2, ADMA, and SDMA)	Combined CV outcome (CV mortality and CV events) Combined total outcome (all-cause mortality and CV events)	Univariate models: lnPORHHA and lnAng-2 predicted the combined CV outcome besides age, diabetes, baseline CV disease, PP, and logCRP Multivariate analysis: lnPORHHA (HR 0.66, 95% CI [0.49–0.89] per ln [mU s]), age (1.03 [1.01–1.06] per year), logCRP (1.31 [1.06–1.64] per ln [mg/L]), and diabetes (3.33 [1.70–6.53]) remained significant predictors of the CV outcome, whereas lnAng-2 did not enter the model Neither of the LDF nor biomarkers were an independent predictor of the combined total outcome

ADMA, asymmetric dimethylarginine; Ang-2, angiotensin-2; BP, blood pressure; CAD, coronary artery disease; CHF, congestive heart failure; cIMT, carotid intima-media thickness; CRP, C-reactive protein; CKD, chronic kidney disease; CV, cardiovascular; FBF, forearm blood flow; FDR, flow debt repayment; eGFR, estimated glomerular filtration rate; FMD, flow-mediated dilatation; HR, hazard ratio; LDF, laser Doppler flowmetry; LVMI, left ventricle mass index; PORH, postocclusive reactive hyperemia; PORHHA, postocclusive reactive hyperemia area; PTH, parathormone; PWV, pulse wave velocity; RR, risk ratio; TH, thermal hyperemia; SDMA, symmetric dimethylarginine; VOP, venous occlusion plethysmography.

baseline and at end-diastole in order to determine the response of the brachial artery to increase in flow [28]. FMD is expressed as a percentage change from baseline [14]. The changes in brachial artery diameter are caused by shear-stress-induced generation of endothelial-derived vasoactive mediators; this phenomenon is mainly attributed to endothelial release of NO [26]. FMD in the forearm is significantly correlated with the endothelial function in the coronary arteries [30], and it is considered a reliable indicator of NO bioavailability in various populations, including CKD and essential hypertensive patients [2]. Moreover, reactive hyperemia flow and induced shear stress (the stimuli for FMD) are considered to be a valid measure of peripheral microvascular function [2]. Indeed, hyperemia-induced shear stress and velocity changes have shown stronger correlations with cardiovascular risk factors than FMD [2]. Even though this method is commonly applied in clinical research and its principle seems to be simple, its application is challenging; it has high inter- and intraobserver variability that can affect the method reproducibility, so it requires good standardization, adherence to strict protocols, and well-trained and experienced operators (Table 1) [2, 14, 27]. Moreover, appropriate subject preparation (physical and environmental factors and medication) is vital in order to control any confounders that may affect vascular reactivity [27, 29].

Many observational studies used FMD for assessment of microvascular function in individuals with CKD. FMD levels were found to be lower in CKD patients compared to healthy individuals, and they were significantly decreased from stage 1 to stage 5 CKD, while they increase after renal transplantation [31–34]; BP levels were the major determinant of FMD in patients with CKD and no other comorbidities [35], while in other works, fibroblast growth factor-23 (FGF23), 25-OH vitamin D, and serum phosphate were independently associated with FMD [36].

In a seminal study, Verbeke et al. [37] studied variations of vasomotor tone in response to hand warming by comparing shear-stress-mediated changes in brachial artery diameter obtained by FMD between 35 ESKD patients with and without known cardiovascular disease and 22 healthy controls; a negative association between changes in FMD and presence of ESKD or cardiovascular disease was noted. In another study, FMD was reversely correlated with CRP and left ventricle mass index (LVMI); the association between LVMI and FMD was independent of other traditional risk factors (i.e., age, diabetes, and smoking) [38]. Similar observations were also evi-

dent in a cross-sectional study in 149 individuals with ESKD [39]. Other studies have indicated that FMD is associated with hemoglobin, IL-10, IL-33, suppression of tumorigenicity 2 (ST2), and serum sclerostin and visfatin levels [40–43]. In a cohort of 304 nondialyzed CKD patients, decreased FMD was strongly associated with cardiovascular events and mortality (HR = 0.52 with 95% CI [0.37–0.73]) (Table 2) [33].

Furthermore, several clinical trials have applied FMD in order to test the effects of different interventions on microvascular function in CKD. In an 8-week randomized, double-blind, placebo-controlled trial in 70 CKD patients, rosiglitazone reduced significantly insulin resistance and endothelial dysfunction markers but not FMD and arterial stiffness parameters [44]. In CKD stage 3–5, atorvastatin and gemfibrozil were not associated with improvement in FMD and endothelial-independent vasodilatation compared to placebo [45]. In addition, in a double-blind randomized trial in 60 patients with advanced diabetic kidney disease, paricalcitol did not affect FMD and other inflammation biomarkers compared to placebo [46]. These findings are expanded in a meta-analysis of studies examining the effect of vitamin D supplementation in patients with CKD, where no significant differences in levels of FMD between the active and control groups were observed [47]. Finally, in a prospective study in 42 ESKD patients, forearm FMD was significantly increased in patients undergoing high-efficiency online hemodiafiltration compared to high-flux hemodialysis [48].

Laser Doppler Flowmetry

Laser Doppler flowmetry (LDF) is a reliable indicator of skin microvasculature function; it is based on diffusion and refraction of laser beam light [49]. The main principle relies on the Doppler effect; by changing the direction of the laser beam, the alterations in light wavelength reflect the number and velocity of erythrocytes inside the microvessels [11]. Many techniques can be associated with LDF involving postocclusive reactive hyperemia, thermal hyperemia, iontophoresis, and microdialysis. Local thermal hyperemia leads to a temperature-dependent rise in skin blood flow and achieves a maximal vasodilatation which corresponds to the maximal vasodilator vessel capacity [49]. Iontophoresis is based on the principle that electrically charged drugs in solution will migrate across the skin under the influence of a direct low-intensity electric current [50]. When coupled with LDF, iontophoresis enables the detection of changes in skin blood flow in response to vasoactive drug administration (i.e., acetylcholine and sodium nitroprusside) [11]. Acetylcholine and

sodium nitroprusside are used to assess endothelium-dependent and -independent vasodilation, respectively [11]. Microdialysis allows pharmacological compounds to be delivered directly to an area of the skin vessels without exerting systemic effects, even if these compounds are nonpolar and have higher molecular weight that would be difficult to deliver by iontophoresis [51]. Coupling this technique with LDF allows for real-time measurement of skin blood flow changes during localized infusion of pharmacological agents [51]. Although simple and easy to perform, LDF is considered to be poorly reproducible (Table 1) [52].

As of this writing, several studies have used LDF alone or combined with one of the 4 aforementioned methods to evaluate microvascular function in individuals with CKD. In a cross-sectional study including hypertensive CKD patients and hypertensive controls without CKD, CKD patients had lower response to postocclusive reactive hyperemia. LDF response to both postocclusive reactive hyperemia and thermal hyperemia was strongly associated with the presence of albuminuria and CKD, independent of diabetes and BP levels [53]. Another study showed that young CKD patients had impaired endothelium-dependent and endothelium-independent vasodilation (evaluated by iontophoresis) compared to similar age healthy controls but similar microvascular function to elderly CKD patients [54]. Mitochondria-derived reactive oxygen species suggested to play a major role in this microvascular dysfunction [55]. Farkas et al. [56] observed that hemodialysis patients with hypertension have impaired endothelium-dependent and endothelium-independent vasodilation and higher levels of endothelial dysfunction markers compared to essential hypertensives and healthy individuals.

In a cohort of 105 nondialysis CKD patients, postocclusive reactive hyperemia area changes evaluated by LDF independently predicted cardiovascular events and mortality (Table 2) [57]. Similar associations with cardiovascular disease were also observed in other studies in ESKD patients [58, 59]. Finally, in a recent clinical trial including 36 nondialysis CKD patients, 12 weeks of aerobic exercise improved microvascular function and maintained conduit artery function measured by LDF coupled with microdialysis [60].

Arterial Glycocalyx Functional Assessment

Glycocalyx is a complex of glycosaminoglycans and proteoglycans covering the surface of endothelial cells and is considered to protect the vasculature wall against pathogenic insults in cardiovascular disease [52]. It plays

a significant role in transferring shear stress into shear-dependent endothelial responses, leading to NO release [14]. Both invasive and noninvasive techniques can be used to assess glycocalyx function [14]. Historically, one of the first methods described to estimate systemic glycocalyx volume was the tracer dilution method [61]. In this technique, systemic glycocalyx volume is measured using the glycocalyx permeable tracer (i.e., Dextran 40) versus a glycocalyx impermeable tracer (i.e., fluorescein-labeled erythrocytes) [14, 61]. Despite the fact that this technique can directly estimate the whole-body glycocalyx volume, its application is limited due to its invasiveness and time-consuming preparations (Table 1) [14]. New techniques have also emerged in the field of optics, using orthogonal polarization spectral (OPS) and sidestream dark field (SDF) imaging at the superficial microvasculature [14]. These procedures are simple, noninvasive, painless, and rapid, and they can be executed at bedside (Table 1) [62, 63]. These methods use the erythrocyte-endothelium gap of the capillaries in the image to quantify glycocalyx thickness [64]. Perfused boundary region (PBR) (calculated as the distance between red blood cell column and red blood cell impermeable glycocalyx) is an SDF-measured parameter that is inversely related to glycocalyx thickness; large PBR values are an indirect measure of an impaired glycocalyx [65].

Studies using arterial glycocalyx to assess endothelial dysfunction in CKD are scarce. In a rat model with proteinuric CKD, loss of endothelial glycocalyx was linked to albuminuria and vascular dysfunction [66]. In a human study, Vlahu et al. [67] used SDF to detect differences in glycocalyx dimension between 40 hemodialysis patients and 21 healthy controls and showed that PBR was increased in hemodialysis patients, suggesting an impaired glycocalyx barrier.

Laser Speckle Contrast Imaging

LSCI is a novel, fast, noninvasive technique that provides continuous measurement of skin blood flow. It is based on the principle of tracking the “speckle pattern” (i.e., the backscattered light from a tissue that is illuminated with laser light forms a random interference pattern at the detector) (Fig. 1). When coupled with reactivity tests such as postocclusive reactive hyperemia and local thermal hyperemia, LSCI showed improved reproducibility compared to the aforementioned method of LDF [68]. So far, it has been used to evaluate different circulatory beds [69, 70] and for assessment of endothelium-dependent vasodilator responses in patients with type-1 DM and coronary artery disease (CAD) [71, 72].

Fig. 1. Assessment of skin endothelial function with LSCI depicting the position of the forearm under the laser light beam and the skin sites used for microvascular response recording. LSCI, laser speckle contrast imaging.

Fig. 2. Assessment of skin endothelial function with nailfold video capillaroscopy: capillaries are depicted in the middle of the nailfold, ~4.5 mm near the terminal capillary line on the skin of the distal phalanx of the dorsal fourth finger of the right upper hand.

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Color version available online

Color version available online

To date, there is only one study which used LSCI to evaluate microvascular function in patients with CKD. In this study, Alexandrou et al. [73] included 38 hemodialysis patients, 38 peritoneal dialysis patients, and 38 controls matched in a 1:1:1 ratio and found that patients under hemodialysis and peritoneal dialysis exhibit similar time to peak response, increase (%) from baseline to peak perfusion, peak cutaneous vascular conductance, cutaneous vascular conductance increase (%) at peak, and post-occlusive reactive hyperemia response, all of which were significantly impaired compared to controls, findings suggesting that skin capillary recruitment and endothelial function in response to hyperemia are severely disturbed in ESKD patients.

Nailfold Capillaroscopy

Nailfold capillaroscopy is an easily applicable, noninvasive technique used to evaluate microcirculation and endothelial function [74–76]. It provides information for multiple microcirculatory parameters, including capillary morphology and density and flow velocity [74, 76]. The reduction in the density of capillaries in any given visual field is called capillary rarefaction and is categorized into 2 types: structural (i.e., decrease in overall number of capillaries) and functional (i.e., reduction in the number of perfused capillaries) rarefaction (Fig. 2) [74, 76]. Video capillaroscopy offers the advantage of evaluation of capillary density in 3 phases: baseline, during hyperemia after arterial occlusion (postocclusive reactive

hyperemia), and after venous congestion (Table 1) [76]. Capillary density during venous congestion is considered the best method to objectively assess the anatomic capillary number, as it can expose nonperfused capillaries that are missed with simple capillaroscopy. Furthermore, the postocclusive hyperemic phase detects the functional recruitment of initially nonperfused capillaries (measure of structural and functional integrity) [77].

To date, few studies have used nailfold video capillaroscopy to explore the subject of capillary rarefaction in CKD. Thang et al. [78] evaluated 35 CKD stage 5 (20 hemodialysis and 15 peritoneal dialysis) patients, 19 predialysis patients, and 19 controls and observed that baseline capillary density was impaired in both CKD groups. Capillary recruitment during postocclusive reactive hyperemia and venous occlusion was also lower in both CKD groups compared to controls; these alterations were independently associated with increased serum phosphorus and bicarbonate [78]. Furthermore, Edwards-Richards et al. [79] studied 19 pediatric hemodialysis patients and 20 controls and showed that capillary rarefaction was strongly associated with biomarkers of altered mineral metabolism. Finally, in a very recent study of our group in 96 CKD stage 2–4 patients, we have demonstrated that both structural and functional capillary density progressively decreased with advancing CKD stages (Fig. 3); reduced eGFR, diabetes, and increased PTH levels are independently and inversely associated with functional capacity density [76].

Fig. 3. Capillary density during baseline, after postocclusive reactive hyperemia, and during venous congestion among different stages of CKD (reprinted with permission from Schoina et al. [76], *Hypertens Res.* 2020).

In a 3-month randomized, double-blind, placebo-controlled trial in 36 subjects with moderate CKD, paricalcitol treatment was associated with a trend toward improvement in toe video capillaroscopy parameters [80]. In another nonrandomized, pilot study including 15 CKD stage 3–4 patients and 15 controls, short-term administration of recombinant human growth hormone resulted in increase of capillary blood flow at baseline and during reactive hyperemia in both groups [81].

Near-Infrared Spectroscopy

NIRS is a relevant new, costless, noninvasive method that assesses local tissue oxygenation and can provide valuable information about local oxygen consumption and blood flow [51]. It is based on the “modified Beer-Lamberts Law” [82]. A simple NIRS device consists of a light source (that produces light in the near-infrared range into the examined tissue) and a detector; hemoglobin and myoglobin are oxygen carriers in blood and skeletal muscles, respectively, and their absorbance of near-infrared light differs depending on their oxygenation state [82]. NIRS allows to assess microvascular reactivity and skeletal muscle oxygenation at rest and during exercise, via continuous monitoring of functional changes in oxygenated hemoglobin dissociation [83]. Using postocclusion reactive hyperemia, NIRS technology provides information on skeletal muscle’s oxidative capacity, microvascular function, and muscle oxygenation at rest and during exercise [83]. With regard to cerebral oxygenation, NIRS noninvasively monitors alterations and assesses relative changes from baseline for oxygenated, de-

oxygenated, and total hemoglobin [84]. Due to its ability to assess microvascular function, NIRS has been applied in various populations with impaired microvascular function, including patients with essential hypertension, DM, CAD, and ESKD [83, 85–87].

As shown in Table 3, only one small study (24 cases and 6 controls) up to date has evaluated muscle oxygenation via NIRS in nondialysis CKD patients. In this study, Wilkinson et al. [88] described NIRS-derived skeletal muscle O_2 saturation changes during and following exercise and found that CKD patients have dysfunctional kinetics that may indicate reduced mitochondria capacity to perform oxidative phosphorylation. Moreover, there are few studies examining muscle oxygenation via NIRS in ESKD individuals. In one of them, the presence of dialysis access resulted in decreased muscle oxygenation and strength even in the absence of clinically overt hand ischemia [89]. In another study, regional saturation of oxygen (rSO_2) of muscles was significantly lower in hemodialysis patients compared to healthy controls, and this was associated with serum inorganic phosphate and albumin levels [90]. In an EXerCise-Introduction-To-Enhance-performance-in-dialysis (EXCITE) substudy, resting muscle oxygen consumption was significantly lower in patients with ESKD compared to healthy controls, but it was improved after a 6-month exercise program [87]. NIRS has been also utilized to examine the alterations in microvascular function and muscle oxygenation during the hemodialysis session; the findings indicate that hemodialysis brings about major changes in skeletal muscle oxygenation, blood flow, microvascular compliance, and

Table 3. Studies using NIRS for evaluation of muscle oxygenation and microvascular function in CKD [144–146]

Study	Study type	N	Participants	Study variables	Results
Vaux et al. [144]	Phase-2, double-blind RCT	26	26 HD (13 in the carnitine group and 13 in the control group)	$T_{1/2}$ of muscle reoxygenation measured via NIRS and other parameters measured via ^{31}P magnetic resonance spectroscopy and ^1H magnetic resonance imaging at baseline and at 16 weeks	L-Carnitine had no statistically significant effect on any of the parameters measured
De Blasi et al. [91]	Observational	20	20 HD (10 DM and 10 non-DM) and 22 healthy controls for baseline comparisons	Once hourly during dialysis [HbT], [HbO ₂], and [HHb] mBF, microvascular compliance, and mVO ₂ after venous occlusion StO ₂ and CtO ₂ , microvascular bed volume	<p>↑ [HbT] and [HHb] during dialysis in patients without and with diabetes</p> <p>In diabetic: ↑ [HbO₂] and CtO₂ during dialysis but left mVO₂ unchanged</p> <p>StO₂ significantly positively correlated with HbO₂ and negatively with mVO₂</p> <p>Dialysis increased mBF only in diabetic patients</p> <p>Microvascular compliance decreased rapidly and significantly during the first hour of dialysis in both groups</p>
Manfredini et al. [87]	Parallel-group RCT	59	59 HD (31 in the exercise group and 28 in control) (in addition, normative data for rmVO ₂ were obtained from a group of 19 healthy subjects)	rmVO ₂ at baseline and after 6 months	<p><i>Baseline</i></p> <p>rmVO₂ was higher ($p < 0.001$) in ESKD (0.083 ± 0.034 mL/100 g/min) than in healthy subjects (0.041 ± 0.020 mL/100 g/min)</p> <p>rmVO₂ correlated with resting HR ($r = 0.34$, $p = 0.009$)</p> <p><i>Study end</i></p> <p>Exercise group: ↓ rmVO₂ [0.064 ± 0.024 mL/100 g/min (-23%, $p < 0.001$)]</p> <p>Control group: rmVO₂ no change (0.082 ± 0.032 vs. 0.082 ± 0.031 mL/100 g/min)</p>
Pipili et al. [145]	Observational	28	11 HD, 9 hemodiafiltration, 8 healthy controls	Thenar StO ₂ at baseline and after venous occlusion test (rate of Hb desaturation [oxygen consumption rate], StO ₂ growth curve rate [endothelial function], hyperemia phase [vascular reactivity] before and after the dialysis session	<p>Predialysis endothelial function (9.1 ± 5.6 vs. 15.7 ± 6.3, $p = 0.003$) and vascular reactivity were lower in ESKD compared to healthy</p> <p><i>HD</i></p> <p>↑ oxygen consumption rate after HD (24.5 ± 7.5 vs. 40 ± 17.7, $p = 0.03$) correlated with Kt/V ($r = 0.74$, $p = 0.03$).</p> <p>Predialysis StO₂ correlated with PTH ($r = 0.90$, $p < 0.001$)</p> <p><i>Hemodiafiltration</i>: no differences</p>
Miyazawa et al. [90]	Observational	81	67 HD and 15 healthy controls	rSO ₂ of the gastrocnemius before dialysis	<p>rSO₂ values were lower in HD compared to healthy (50.0 ± 1.7 vs. $76.8 \pm 2.5\%$, $p < 0.001$)</p> <p>rSO₂ was independently associated with serum inorganic phosphate (standardized coefficient: 0.27) and serum albumin concentrations (standardized coefficient: 0.24)</p> <p>No differences in rSO₂ between diabetic and nondiabetic HD patients</p>
Malik et al. [97]	Observational	44	27 HD and 17 age-matched healthy controls	rSO ₂ was measured at the brain frontal lobe and at the hand with dialysis access at rest and during HD	<p>Dialysis patients had ↓ rest brain rSO₂ (51.5 ± 10.9 vs. $68 \pm 7\%$, $p < 0.001$) and hand rSO₂ (55 ± 16 vs. $66 \pm 8\%$, $p = 0.03$)</p> <p>Both values ↓↓ during the first 35 min of HD (brain: rSO₂ to $47 \pm 8\%$, $p < 0.001$, and hand to $45 \pm 14\%$, $p < 0.001$)</p> <p>Brain rSO₂ decrease was related to the UF rate and hand rSO₂ decrease to the finger pressure and to Hb</p>
Kmentova et al. [89]	Observational	79	52 HD and 27 healthy controls	rSO ₂ , handgrip strength, and finger systolic BP performed in both the hands before HD session	<p>Hands with dialysis access had ↓ values of handgrip strength, systolic finger pressure, and of thenar rSO₂ (45.8 ± 12.9 vs. $42.5 \pm 13.3\%$, $p = 0.002$)</p> <p><i>On access hand</i>: handgrip strength was related to the thenar oxygenation ($r = 0.36$; $p = 0.014$) and to the finger systolic pressure ($r = 0.38$; $p = 0.007$)</p> <p><i>On the nonaccess hand</i>: handgrip strength was inversely related to age ($r = -0.41$, $p = 0.003$), dialysis vintage ($r = -0.32$, $p = 0.02$), and RDW ($r = -0.37$, $p = 0.01$)</p>

Table 3 (continued)

Study	Study type	N	Participants	Study variables	Results
Ookawara et al. [146]	Observational	53	53 HD	rSO ₂ and HbT signal strength in the brain, liver, and lower-limb muscle during HD	↓ brain versus liver rSO ₂ from HD start to the end (HD start 46.5±1.3 vs. 52.4±1.7%, <i>p</i> = 0.031) ↑ liver and muscle HbT signal strength ratio compared to brain during HD
Wilkinson et al. [88]	Observational	30	24 nondialysis CKD patients and 6 healthy controls	Muscle SMO ₂ % before, during, and after (recovery) ISWT	↓ SMO ₂ % during exercise, followed by rapid ↑ upon recovery CKD patients with higher exercise capacity (ISWT distance: 727.1±38.1 m) took 45% longer to reach their minimum SMO ₂ % (<i>p</i> = 0.038) and recovered 79% faster (<i>p</i> = 0.046) compared to patients with lower exercise capacity (ISWT: 727.1±38.1 m) CKD patients took significantly 56% longer to recover (<i>p</i> = 0.014) compared to controls

NIRS, near-infrared spectroscopy; DM, diabetes mellitus; BP, blood pressure; CKD, chronic kidney disease; CtO₂, oxygen tissue content; ESKD, end-stage kidney disease; Hb, hemoglobin; HD, hemodialysis; [HHb], deoxygenated Hb concentration; [HbO₂], oxygenated Hb concentration; [HbT], total Hb concentration; HR, heart rate; ISWT, incremental shuttle walk test; mBF, muscular blood flow; mVO₂, muscle oxygen consumption; PTH, parathormone; RCT, randomized clinical trial; RDW, red cell distribution width; rmVO₂, resting muscle oxygen consumption; rSO₂, regional saturation of O₂; SMO₂, skeletal muscle O₂; StO₂, tissue oxygen saturation; UF, ultrafiltration.

tissue metabolic rate, and that all except from microvascular compliance were more impaired in patients with DM compared to patients without DM [91].

Studies applying cerebral NIRS in CKD patients are scarce. In early studies, patients undergoing hemodialysis show significantly lower cerebral rSO₂ levels compared to healthy individuals; cerebral oxygenation was more impaired in diabetic than nondiabetic hemodialysis patients [92, 93]. The presence of severe anemia deteriorated cerebral oxygenation in this population [94, 95]. In an observational study in 23 hemodialysis and 9 peritoneal dialysis patients scheduled to undergo surgery, peritoneal dialysis patients had significantly higher rSO₂ levels compared to hemodialysis [96]. In a retrospective study including 49 patients (9 hemodialysis and 40 nonhemodialysis) that underwent coronary artery bypass graft surgery, hemodialysis patients had lower rSO₂ levels, even after adjusting for age, hemoglobin, and LV ejection fraction [97]. A cross-sectional study including 104 hemodialysis patients indicated that cerebral rSO₂ significantly decreased as aortic-arch calcification progressed and was independently associated with serum phosphate and history of smoking [98]. Finally, studies evaluating cerebral oxygenation during dialysis session have shown that cerebral oxygenation deteriorates after the beginning of hemodialysis [93, 99]; cerebral ischemia cannot be predicted from BP alterations during dialysis, and this leads to high risk of stroke and cognitive dysfunction development [100, 101].

Assessment of Endothelial Dysfunction in CKD: Biochemical Markers

Over the years, several circulatory substances have been used as markers of endothelial function in human studies. The most important of them are summarized in Table 4. A detailed description of all these biomarkers is beyond the scope of this review. In the following lines, we concisely discuss the most important biomarkers used to assess endothelial dysfunction in CKD patients.

Asymmetric Dimethylarginine

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO synthase and a strong marker of atherosclerosis [102]. Several studies demonstrated that ADMA is also strongly and independently associated with noninvasive measurements of endothelial dysfunction [103]. Patients with CKD have significantly higher ADMA levels than healthy individuals [103]. Furthermore, among patients with CKD, ADMA is inversely related to GFR [104]. For example, in a previous study of our group, ADMA levels were higher in patients with autosomal-dominant polycystic kidney disease (ADPKD) and eGFR <70 mL/min/1.73 m², compared to age- and sex-matched patients with ADPKD and eGFR >70 mL/min/1.73 m², and higher in the 2 aforementioned groups compared to age- and sex-matched controls with preserved eGFR. A negative association between ADMA levels and eGFR (*r* = −0.460, *p* < 0.001) was observed [105].

Table 4. Summary of the most commonly used endothelial dysfunction circulating biomarkers in human studies

NOS inhibitors
ADMA
SDMA
Cell adhesion molecules
VCAM-1
ICAM-1
E-selectin
P-selectin
Coagulation pathway molecules
tPA
Fibrinogen
PAI-1
VWF
Thrombomodulin
ADAMTS13
Microparticles
EMPs
PMPs
Arterial glycocalyx
Heparin sulfate
Syndecan-1
Hyaluronan
Biomarkers of inflammation
CRP
ILs
TNF- α
TWEAK
PTX3
MCP-1
Visfatin
TLRs
Ang
CD40 ligand
EPC
Endocan
Oxidative stress
Oxidized LDL
MicroRNAs
MiR-126

NOS, nitric oxide synthase; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intracellular adhesion molecule 1; tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor 1; VWF, von Willebrand factor; EMPs, endothelial microparticles; PMPs, platelet microparticles; CRP, C-reactive protein; ILs, interleukins; TNF- α , tumor necrosis factor- α ; TWEAK, TNF-like weak inducer of apoptosis; PTX3, pentraxin 3; MCP-1, monocyte chemoattractant protein-1; TLRs, Toll-like receptors; Ang, angiopoietin; EPC, endothelial progenitor cell.

In patients with CKD, ADMA levels have been associated with carotid intima-media thickness (cIMT) and coronary calcification [106–108] as well as with proteinuria and LVH [109–111]. Therefore, it was no surprise that ADMA was found to be an independent predictor of

progression to dialysis, cardiovascular outcomes, and death in these individuals [104, 112–116]; a detailed description of these studies can be found in Table 5. Of note, both ADMA levels and endothelial dysfunction improve after renal transplantation [117]; in renal transplant, recipient ADMA levels are associated with graft function deterioration and mortality [118].

Cell Adhesion and Coagulation Pathway Molecules

Vascular adhesion molecule 1 (VCAM-1), intracellular adhesion molecule 1 (ICAM-1), and endothelial selectin (E-selectin) are considered endothelial-specific biomarkers [119]. Compounds involved in the coagulation cascade (i.e., fibrinogen and von Willebrand factor [VWF]) are considered related but not specific markers of endothelial dysfunction [120]. In the general population, eGFR is inversely associated with circulating adhesion molecules [121]. In nondialysis patients with CKD, creatinine clearance was inversely correlated with VCAM-1 and VWF levels [122]. Levels of VCAM-1 and ICAM-1 increase in parallel with the progression to CKD to ESKD [123]. Therefore, patients undergoing hemodialysis have elevated levels of VCAM-1 that are not affected by the dialysis procedure; patients undergoing peritoneal dialysis have also higher levels of VCAM-1 and ICAM-1 compared to controls and hemodialysis subjects [124, 125].

In nondialysis CKD patients, VCAM-1 levels were associated with higher LVMI [126]. In ESKD patients, E-selectin was reversely related to cardiovascular events, LVH, and total mortality, while a specified E-selectin gene polymorphism was associated with carotid atherosclerosis [127–129]. In a previous work, E-selectin and ICAM-1 were also correlated with salt and water retention in ESKD [130].

Endothelial Microparticles

Endothelial microparticles (EMPs) are vesicles shed from plasma membranes following endothelial cell activation or apoptosis; elevated EMP levels are considered the most specific marker of endothelial dysfunction [14]. Endothelial NOS uncoupling and low shear stress are linked with EMP levels [14]. In a seminal study in 34 ESKD patients, Boulanger et al. [131] showed that EMPs were elevated through low shear stress, and this was also linked to the presence of anemia. Activation of the alternative complement pathway is considered to be a significant mechanism of EMP elevation, as it is strongly related to endothelial dysfunction and CKD indices (eGFR and albumin/creatinine ratio) [132]. Faure et al. [133] examined the association between EMPs and uremic toxins

Table 5. Cohort studies using ADMA/EMPs to evaluate the associations of endothelial dysfunction with outcomes

Study	Bio-marker	N	Participants	Follow-up duration	Study variables	Outcome	Results
Zoccali et al. [112]	ADMA	225	225 hemodialysis	33.4±14.6 months	ADMA	All-cause mortality CV events (fatal and nonfatal)	Univariate analysis: ADMA was related to fibrinogen, L-arginine, dialysis vintage, serum cholesterol, and albumin Multivariate analysis: ADMA was correlated with fibrinogen ($p = 0.0001$) and albumin ($p = 0.04$) ADMA was the second factor predicting overall mortality (HR 1.26, 95% CI [1.11–1.41]) and CV events (HR 1.17, 95% CI [1.04–1.33])
Mallamaci et al. [116]	ADMA	224	224 hemodialysis	42.3 (0.2–70.5) months	ADMA Plasma norepinephrine	All-cause mortality CV events (fatal and nonfatal)	ADMA was strongly correlated with norepinephrine ($p < 0.001$) Cox models not including ADMA: norepinephrine was a predictor of death (HR 1.06, 95% CI [1.01–1.12]) and CV events (HR 1.08, 95% CI [1.02–1.15]) ADMA introduced into the model: norepinephrine was an insignificant predictor of outcomes, and ADMA was a significant predictor of death (HR 1.22, 95% CI [1.11–1.35]) and CV events (HR 1.19, 95% CI [1.08–1.32])
Ravani et al. [104]	ADMA	131	131 CKD stage 2–5 patients	27 (3.4–36) months	ADMA	Progression to ESRD (halving GFR or dialysis start) All-cause mortality Combined outcome	Univariate analysis: ADMA (HR 1.23, 95% CI [1.076–1.412]), proteinuria, GFR, hemoglobin, and CaxP product correlated with outcomes. Patients with ADMA levels <median had survival advantage in both event-specific strata ($p < 0.001$) Multivariate analysis: ADMA (HR 1.20, 95% CI [1.071–1.350]) predicted combined outcome independent of confounders: GFR, proteinuria, hemoglobin, and homocysteine
Young et al. [115]	ADMA	820	820 CKD stage 3–4 patients	9.5 (0.25–11.60) years	ADMA	All-cause mortality CV mortality	Higher ADMA tertiles were more likely to have CVD, lower DBP, and lower GFR Univariate analysis: a 1-SD increase in ADMA increased the risk for all-cause (HR 1.18, 95% CI [1.04–1.35]) and CV mortality (HR 1.25, 95% CI [1.06–1.47]) After adjustment for demographic, randomization, CVD, and kidney disease factors, HR = 1.09 with a trend toward statistical significance
Abedini et al. [118]	ADMA	2,102	2,102 kidney transplant recipients from the ALERT study	Mean: 6.7 years	ADMA	Renal endpoint: the time to graft failure or doubling SCr MACE (cardiac death, nonfatal MI, or coronary intervention procedure) Cerebrovascular event (fatal and nonfatal stroke) All-cause mortality	The number of renal, MACE, cerebrovascular, and all-cause mortality endpoints increased significantly by ADMA quartiles ADMA was an independent predictor of renal endpoint (HR 2.78, 95% CI [1.22–5.82]), MACE (HR 2.61, 95% CI [1.03–6.61]), cerebrovascular events (HR 6.63, 95% CI [2.52–23.13]), and all-cause mortality (HR 4.87, 95% CI [2.12–11.18])
Shi et al. [109]	ADMA	76	76 CKD patients and 15 controls	15 (6–24) months	ADMA LVMI	CV events	ADMA: independent marker of LVMI ADMA was an independent risk factor for CV events (HR = 1.18, 95% CI [1.07–1.29])

Table 5 (continued)

Study	Bio-marker	N	Participants	Follow-up duration	Study variables	Outcome	Results
Lu et al. [114]	ADMA	298	298 CKD stage 3–4 patients	2.9±1.2 years	ADMA	All-cause mortality Combined: all-cause mortality, nonfatal MI, or stroke	↑↑ ADMA in patients who showed all-cause death and the composite outcome. ROC analysis→ ADMA cutoff point = 0.47 μmol/L for all-cause death and composite outcome <i>Multivariate analysis</i> ADMA >0.47 versus ≤0.47 μmol/L was associated with increased risk of all-cause mortality (HR 2.87, 95% CI [1.14–7.22]) and the combined outcome (HR 2.45, 95% CI [1.18–5.09]) ADMA (as continuous variable) was associated with the combined outcome (HR 1.37, 95% CI [1.09–1.73])
Kanbay et al. [113]	ADMA	259	259 CKD stage 1–5 patients	Median of 38 months	ADMA Uric acid FMD	All-cause mortality CV events (CV mortality, nonfatal MI, or stroke) Combined outcome	Univariate analysis, uric acid, and ADMA >median → ↑ risk all-cause mortality (HR 5.06, 95% CI [2.01–12.76]), CV events (HR 4.75, 95% CI [2.98–7.59]), and the composite outcome (HR 4.13, 95% CI [2.66–6.43]) After adjustment for renal-specific risk factors (GFR, proteinuria, and hsCRP)→ this association only for CV and composite outcome Addition of uric acid and ADMA into a model with traditional and renal-specific risk factors→ not increase the prediction ability for none of the outcomes
Amabile et al. [135]	EMPs	81	81 hemodialysis	50.5 months (5–72)	Microparticles (platelet-derived MPs, erythrocyte-derived MPs, EMPs: leukocytes MPs) Framingham and European SCORE	All-cause mortality CV mortality (fatal MI, stroke, acute pulmonary edema, and sudden death) MACE (nonfatal ACS, stroke, acute pulmonary edema, and arrhythmias)	EMPs (separated in tertiles): significant differences in CV (log-rank $p < 0.01$) and all-cause mortality (log-rank $p < 0.001$) <i>All-cause mortality</i> Multivariate analysis: age (HR 1.10, 95% CI [1.02–1.19]) and EMPs (HR 21.7, 95% CI [4.23–111.18]) were independent predictors <i>CV mortality</i> Multivariate analysis: age (HR 1.09, 95% CI [1.02–1.16]) and EMPs (HR 20.0, 95% CI [3.86–103.5]) were independent predictors <i>MACE</i> Multivariate analysis: history of CVD (HR 7.15, 95% CI [2.33, 20.0]), age (HR 1.06, 95% CI [1.02–1.10]), and EMPs (HR 5.03, 95% CI [1.67–15.15]) were independent predictors
Carmona et al. [138]	EMPs	160	160 hemodialysis (80 with DM and 80 without DM)	5.5 years	EMPs Monocyte subpopulations Ang-1, Ang-2	All-cause mortality	EMPs level, monocyte subpopulations (CD14/CD162 and CD142/CD16), and Ang-2-to-Ang-1 ratios increased in patients with DM compared with non-DM EMP level <median → improved survival versus EMP levels >median (log-rank $p < 0.001$). Similar significant associations observed in subgroup analyses (DM and non-DM) After adjustment for DM and traditional CV risk factors, EMP level >median → ↑ all-cause mortality (HR 2.36, 95% CI [1.40–4.01])

Table 5 (continued)

Study	Bio-marker	N	Participants	Follow-up duration	Study variables	Outcome	Results
Green et al. [139]	EMPs	123	123 CKD stage 3–5 patients	3.9±2.2 years	EMPs ΔEMPs (longitudinal EMP change)	All-cause mortality MACE (including MI, stroke, CABG, limb amputation for ischemic reason, noncoronary revascularization, and hospitalization for heart failure or CV death) RRT (dialysis or transplantation)	<i>All-cause mortality</i> Univariate analysis: EMP (HR 3.31, 95% CI [1.12–9.76]) and not ΔEMP (HR 0.64, 95% CI [0.21–1.94]) associated with death. Other parameters that were associated: age, DM, Karnofsky score, eGFR, PTH, CRP, and hemoglobin Multivariate analysis: EMP (HR 8.20, 95% CI [1.67–40.2]), age, and eGFR were significantly associated. ΔEMP was not significantly associated (HR 2.69, 95% CI [0.04–165]) <i>MACE or CV death</i> Univariate analysis: age was associated (HR 1.05 [1.00–1.09]) Multivariate analysis: none of the parameters showed significance <i>RRT</i> Univariate analysis: none of the parameters showed significance Multivariate analysis: albumin was an independent predictor (HR 0.90, 95% CI [0.83–0.98])

ADMA, asymmetric dimethylarginine; Ang, angiotensin; ACS, acute coronary syndrome; CKD, chronic kidney disease; CABG, coronary artery bypass grafting; CVD, cardiovascular disease; CV, cardiovascular; CRP, C-reactive protein; DM, diabetes mellitus; EMPs, endothelial microparticles; ESRD, end-stage renal disease; FMD, flow-mediated dilatation; GFR, glomerular filtration rate; LVMI, left ventricular mass index; MPs, microparticles; MACE, major adverse cardiovascular events; MI, myocardial infarction; PTH, parathyroid hormone; RRT, renal replacement therapy; SCr, serum creatinine.

and found that EMPs were significantly higher in CKD patients than in healthy controls and that uremic toxins induced high levels of EMP release. In a study in CKD patients under different dialysis modalities, EMP levels were increased only in nondialysis and hemodialysis patients and not in the peritoneal dialysis group and controls [134]. In a previous study, Amabile et al. [135] demonstrated that EMP levels are inversely correlated with FMD and also with increased pulse wave velocity (PWV) and augmentation index; impairment of NO release was proposed as the mechanism through which EMPs impair endothelium-dependent vasodilation [136]. A pediatric study yielded similar results; EMPs were found to be strongly correlated with cIMT and PWV in children with predialysis CKD and ESKD [137].

With regard to outcome prediction (Table 5) in the aforementioned prospective cohort study of Amabile et al. [136] in 81 hemodialysis subjects, patients with higher levels of EMPs had significantly higher probability of cardiovascular and total death; EMP levels were independent predictors of cardiovascular outcomes and all-cause mortality after adjustment for confounding factors. Similar observations were made in another study in 160 hemodi-

alysis patients; EMP levels above the median were associated with increased all-cause mortality, after adjustment for diabetes and other traditional cardiovascular risk factors [138]. Despite the associations of EMPs with hard outcomes, the longitudinal EMP change was not associated with mortality [139].

Arterial Glycocalyx Breakdown Products

Apart from functional methods to assess the integrity of arterial glycocalyx discussed above, estimates of glycocalyx's breakdown products (e.g., heparan sulfate, syndecan-1, and hyaluronan) through serum assays provide a readily assessable and reliable measure of glycocalyx dysfunction [65]. Few studies have examined the levels of such markers in CKD. In the aforementioned study of Vlahu et al. [67], hemodialysis patients had increased levels of syndecan-1 and hyaluronan. In a following study, Padberg et al. [140] demonstrated a robust inverse association between renal function and serum levels of syndecan-1 and hyaluronan in a cohort of different stage CKD patients. Finally, in a cohort study including 84 hemodialysis patients, lower syndecan-1 levels were associated with cardiovascular events and total mortality [141].

Biomarkers of Inflammation

Following a well-established, bidirectional association between endothelial dysfunction and inflammation, the circulating levels of biomarkers of inflammation parallel the severity of endothelial dysfunction. This category includes several markers, including CRP, interleukins (ILs), tumor necrosis factor- α (TNF- α), TNF-like weak inducer of apoptosis (TWEAK), pentraxin 3 (PTX3), monocyte chemoattractant protein-1 (MCP-1), and others, that have been variably used in studies in CKD that have also evaluated endothelial function. In a study including 38 non-dialysis, 18 hemodialysis, and 22 kidney transplant patients and 65 healthy controls, CRP was higher in the CKD group compared to controls, and it was significantly correlated with FMD and cIMT; hemodialysis patients had the highest CRP of all CKD groups studied [34]. In a cohort study of 403 subjects with stage 1–5 CKD, IL-10 levels increased along with the reduction of kidney function and FMD, while higher IL-10 levels were associated with cardiovascular events [40]. Both PTX3 and TWEAK levels are associated with the endothelial dysfunction observed with progressive kidney failure, as well as with cardiovascular events [142]. TNF- α and MCP-1 were also shown to be significantly higher in CKD patients with established cardiovascular disease [143].

Conclusions

Cardiovascular disease is the main cause of death in CKD with many traditional and nontraditional risk factors playing important roles in its development. Through the years, noninvasive functional methods, most commonly VOP and FMD, have been used to assess endothelial dysfunction in patients with CKD and examine associations with clinical outcomes. In addition, several studies have used biomarkers of endothelial function, most commonly ADMA and cell adhesion molecules, to investigate such associations. Accumulated evidence from such works suggests that endothelial dysfunction occurs in the early stages of CKD and deteriorates with eGFR reduction. Furthermore, in several observational studies, impaired endothelial function was shown to be associated with markers of target organ damage (i.e., LVH, cIMT, or albuminuria) in these subjects. In addition, a few cohort studies indicate that endothelial dysfunction is associated with cardiovascular events and mortality and progression of CKD toward ESKD. Despite the above data on the relationship of endothelial dysfunction with cardiovascular or kidney disease progression, the use of endothelial dys-

function markers in prediction of cardiovascular outcomes is currently far away from clinical practice. Future studies examining the value of adding relevant markers on available risk scores for cardiovascular risk prediction could be particularly useful. Moreover, over the last years, novel, noninvasive methods evaluating microvascular function, such as LSCI, video capillaroscopy, and NIRS, are increasingly used, but studies in CKD patients are still scarce. As such methods can offer a detailed, real-time assessment of various factors related to vascular function, such as changes in blood flow, capillary recruitment, and tissue oxygenation, and can be easily combined with standardized procedures for vascular reactivity testing (arterial and venous occlusions, exercise, and others), future studies with proper design and adequate power can open new avenues of valuable information on the precise roles of endothelial dysfunction on cardiovascular and renal disease progression in patients with CKD.

Conflict of Interest Statement

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