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Endothelial Dysfunction and Cardiovascular Risk in Chronic Kidney Disease

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Title

Endothelial Dysfunction and Cardiovascular Risk in Chronic Kidney Disease

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Abstract

Cardiovascular disease is the main cause of death in patients with chronic kidney disease and its incidence and severity increases in direct proportion with kidney function decline. This relationship seems to be bidirectional, leading to a vicious circle. Cardiovascular risk prevention should be implemented since the earliest stages of kidney dysfunction because death due to cardiovascular disease is much higher than that from kidney failure. Non-traditional risk factors for cardiovascular diseases, including endothelial dysfunction, are highly prevalent in this population and play an important role in cardiovascular events. Endothelial dysfunction is the first step, yet potentially reversible, in the development of atherosclerosis and its severity has prognostic value for cardiovascular events. Several risk markers have been associated with endothelial dysfunction and some of these have been implicated in its ethiopathogeny. Nitric oxide reduced bioavailability plays a central role linking kidney disease to endothelial dysfunction, atherosclerosis and cardiovascular events. Inflammation is closely related to endothelial dysfunction in chronic kidney disease. Insulin resistance strongly promotes both endothelial and renal dysfunction progression. Residual renal function is inversely correlated to the levels of serum pro-inflammatory molecules, endothelial damage and function markers, particularly on dialysis patients. Endothelial dysfunction may be followed by structural damage and remodeling that can precipitate both bleeding and thrombotic events. Evaluation of endothelial function is nowadays possible, by several different methods, and may have major clinical diagnostic and therapeutic implications. The emerging non-invasive techniques, namely peripheral arterial tonometry, are particularly attractive, allowing clinical routine evaluation and may change current's clinical practice.

Key-Words: endothelial dysfunction; cardiovascular risk factors; chronic kidney disease; cardiovascular disease; peripheral arterial tonometry.

INTRODUCTION

Cardiovascular disease (CVD) is the main cause of death in patients with chronic kidney disease (CKD) (1). Maintenance hemodialysis (MHD) patients in the United States have a mortality rate of >20% year, almost half of which are due to CVD (2). Even in children with end-stage renal disease (ESRD), CVD is the primary (45 percent) cause of death (3). Cardiovascular risk (CVR) increases in direct proportion with kidney function decline (4), since the early beginning of CKD [glomerular filtration rate (GFR) of about 75ml/min] (5). There is an independent, graded association between lower levels of the estimated GFR (eGFR) and the risk of death, cardiovascular events, and hospitalization (6). Stenvinkel *et al.* suggest that the relationship between CKD and CVD may be reciprocal or bidirectional and that this association leads to a vicious circle (7). Even subtle kidney dysfunction should result in intensive prevention of CVR since death due to CVD is much higher than that from ESRD (8).

In patients with mild to moderate CKD, traditional (i.e., Framingham) risk factors (age, lifestyle, left ventricular hypertrophy, dyslipidemia, hypertension, and diabetes mellitus) may predict cardiovascular mortality (9). However, in MHD, markers of kidney disease wasting (KDW) such as hypoalbuminemia, anorexia, body weight and fat loss, rather than traditional cardiovascular risk factors (CVRF), appear to be the strongest predictors of early death (2). The KDW is closely related to oxidative stress, which is associated with pro-inflammatory cytokines and poor survival in MHD patients (2). Dysfunctional lipoproteins such as a higher ratio of the high-density lipoprotein inflammatory index (HII) may engender or aggravate the KDW, whereas functionally intact or larger lipoprotein pools, as in hypercholesterolemia and obesity, may mitigate the KDW in MHD patients (2). Hence, a reverse epidemiology or "bad-gone-good" phenomenon may be observed (10-11). Genetic polymorphism, nutrition and their complex interaction may lead to higher proportions of pro-inflammatory or oxidative

lipoproteins resulting in the aggravation of the oxidative stress and inflammatory processes, ED and subsequent atherosclerotic CVD and death in MHD patients (2).

Non-traditional or novel risk factors for CVD are highly prevalent in these patients and seem to play a far more important role for vascular disease than in the general population (12). These include: endothelial dysfunction (ED), inflammation, sympathetic overactivation, protein-energy wasting, oxidative stress, vascular calcification, and volume overload (13). However, for assessing individual risk, the use of 10 contemporary biomarkers showed to add only moderately to standard risk factors (14).

Despite the progress in the understanding about the pathogenesis of CVD in CKD patients, no survival benefit has been achieved from new treatment strategies, such as: intensive HD (15), high dose peritoneal dialysis (PD) (16), parenteral nutrition (17), homocysteine-lowering (18), normalization of hemoglobin (19), lipid lowering with statins (20) and treatment with angiotensin-converting enzyme inhibitors (21). Thus, it seems likely that the risk profile in these patients is different from the general population (7).

ETHIOPHATHOGENY OF ENDOTHELIAL DYSFUNCTION

ED is an early phenomenon that precedes structural changes and clinical manifestations of atherosclerosis (22-23), contributing to both plaque initiation and progression (24). It is regarded as the first step, yet potentially reversible, in the development of atherosclerosis and it is present as early as in children at all stages of renal failure (25).

The severity of ED has been shown to have prognostic value for cardiovascular events (24). Additionally, several risk markers have been associated with ED in CKD (Table 1) (26). Some of these markers have also been shown to be implicated in the pathogenesis of ED (27). ED associated with atherosclerosis is a recognized complication of uremic patients (28).

Table 1 - Risk markers have associated with endothelial dysfunction in CKD.

ADMA (29)
Hyperhomocysteinemia (30)
Uremic retention solutes (p-cresol and indoxyl sulphate) (31)
Albuminuria (32)
EMP (33)
EPC decrease in number and function (50).
Anoikis of endothelial cells (24)
Pentraxin-related protein PTX3 (34)
Inflammatory biomarkers: hs-CRP (35); IL-6, MMP-9, TNF-α (28)
Uric acid (36)
VCAM (37)

Abbreviations are: CKD, chronic kidney disease; ADMA, asymmetric dimethylarginine; EMP, endothelial microparticles; EPC, endothelial progenitor cells; hs-CRP, high-sensitivity C-reactive protein; VCAM, vascular cell adhesion molecule.

The strong correlation between markers of ED and soluble adhesion molecules, in patients with renal insufficiency and on dialysis, strengthen the view that an ongoing stress on endothelial cells is present in these groups of patients and may play a pathophysiological role in the development of CVD (38). In fact, upregulation of adhesion molecules, generation of chemokines such as macrophage chemoattractant peptide-1 (MCP-1), and production of plasminogen activator inhibitor-1 (PAI-1) participate in the inflammatory response and contribute to a prothrombic state (24). Vasoactive peptides such as angiotensin II and endothelin-1; the accumulation of ADMA; hypercholesterolemia; hyperhomocysteinemia; altered insulin signaling; and hyperglycemia can contribute to these different mechanisms (24).

Nitric Oxide (NO)

ED is associated with an impairment of endothelium-dependent relaxation (EDR), due to the reduced bioavailability of NO, a molecule with anti-atherogenic properties produced by endothelial NO synthase (eNOS) (26). Evidence indicates that NO deficiency contributes to cardiovascular events and progression of kidney damage (39). Nitric oxide production is reduced in renal disease, partially due to decreased endothelial NO production (39), which may be caused by substrate (L-arginine) limitation and increased levels of circulating endogenous inhibitors of eNOS, particularly ADMA (39). Decreased L-arginine availability in CKD is, at least partially, due to perturbed renal biosynthesis of this amino acid (39). In addition, inhibition of transport of L-arginine into endothelial cells and shunting of L-arginine into other metabolic pathways (e.g. those involving arginase) might also decrease availability (39). In contrast, eNOS overexpression with hypercholesterolemia may promote atherogenesis via increased superoxide generation from dysfunctional eNOS (26).

Various mechanisms have been implicated in the impaired EDR (Table 2).

Table 2 - Mechanisms implicated in the impaired endothelium-dependent relaxation.

ADMA-induced competitive inhibition of eNOS (27)
Hcy-induced inactivation of eNOS (40).
AGE-induced inhibition of eNOS (41).
Superoxide generated from dysfunctional eNOS (26).
Vascular O-2(-) production by an NADH-dependent oxidase that inactivates NO (42).
Reduced production of Endothelium-Derived Hyperpolarizing Factor (24).

Abbreviations are: ADMA, asymmetric dimethylarginine; eNOS, endothelial nitric oxide synthase; Hcy,homocysteine; AGE, advanced glycation end product.

Asymmetric Dimethylarginine (ADMA)

ADMA is an endogenous NOS competitive inhibitor, which is elevated in: CKD (29, 43); hypercholesterolemia (44); older age; higher mean arterial blood pressure and; reduced glucose tolerance (27). Despite some studies suggesting that elevated ADMA is primarily a consequence rather than a cause of endotheliopathy, there is recent evidence that dimethylarginine dimethylaminohydrolase (DDAH) is inhibited by endothelial oxidative stress. Consequently, the rise in ADMA can exacerbate this dysfunction by inhibiting eNOS (45).

In CKD, plasma and tissue levels of ADMA are elevated not only because of the reduced catabolism by DDAH but also because of reduced renal excretion (39). The former might be associated with loss-of-function polymorphisms of a DDAH gene, functional inhibition of the enzyme by oxidative stress in CKD and ESRD, or both (39). These findings provide the rationale for novel therapies, including supplementation of dietary L-arginine or its precursor L-citrulline, inhibition of non-NO-producing pathways of L-arginine utilization, or both. Because an increase in ADMA has emerged as a major independent risk factor in ESRD (and probably also in CKD), lowering ADMA concentration became a major therapeutic goal and interventions that enhance the activity of the ADMA-hydrolyzing enzyme DDAH are under investigation (39). PD-treated patients exhibit significantly lower ADMA levels than HD-treated patients. This difference may be caused by differences in the dialytic clearance of ADMA between the two treatment methods or by interference with the metabolism of ADMA (29).

Homocysteine (Hcy)

Several studies have shown that Hcy induces ED by an increased inactivation of NO and identified hyperhomocysteinemia as an independent risk factor for the development of atherosclerosis (40). Levels of reduced Hcy (rHcy) and other aminothiols are markedly increased in patients with CKD (46). In fact, in dialysis patients, rHcy/(total Hcy) ratio is markedly elevated when compared to patients with

CKD and healthy subjects (46). rHcy is believed to cause ED and may be part of the accelerated atherosclerosis observed in CKD patients (46). Thus rHcy level could be a more relevant marker of cardiovascular disease risk in CKD patients than total Hcy level (46).

In patients with chronic renal failure (CRF), the administration of folic acid or its metabolites reduces but does not normalize plasma homocysteine concentrations (40). On the other hand, 5-methyltetrahydrofolate (5-MTHF) administration improves ED in patients undergoing PD, but this effect appears to be independent of the reduction in homocysteine plasma levels (40).

Uremic Retention Solutes

A representative of protein-bound uremic retention solutes, p-Cresol, has been shown to be implicated in uremic immunodeficiency and ED, associated with mortality in HD-treated patients (47).

Albuminuria

Albuminuria is an independent risk factor for CVD (32). A significant risk is observed well below the microalbuminuria cutoff of 2 mg/mmol and increases progressively with the albumin/creatinine ratio (ACR) (32). In clinical practice, albuminuria has been used to identify subjects at high risk for CV events and the regular measurement of urinary albumin excretion is recommended in hypertensive patients (32). Actually, microalbuminuria (ACR> upper decile - 1.07 mg/mmol), was validated as the strongest predictor of ischemic heart disease, with a relative risk of 3.5 when adjusted for other usual atherosclerotic risk factors (48). On the other hand, macroalbuminuria has been shown to be a better risk marker than low eGFR or erythrocyturia in the identification of individuals at risk for accelerated GFR loss in the general population (49). Albuminuria may, therefore, be the glomerular reflection of the increased permeability that is associated with ED. This dysfunction also induces alterations in haemostasis, fibrinolysis and blood pressure (50).

Endothelial Microparticles (EMP)

EMPs may be a reliable marker of subclinical atherosclerosis and arterial stiffness (24). In children on continuous ambulatory PD (CAPD), lower levels of EMPs and more favorable cardiovascular indices were observed when compared to patients on HD (24). In these studies, mean blood pressure (MBP) was the most important risk factor for atherosclerosis, and EMPs and MBP the most important predictors for arterial stiffness (24).

Endothelial Progenitor Cells (EPC)

EPC contribute to the repair and structural maintenance of the vascular system. From stage 1 CKD, a decrease in EPC number and function is observed (hampered adherence, reduced endothelial outgrowth potential and reduced antithrombogenic function). This alteration, that may hinder vascular repair and add to the CVD risk, becomes more significant with CKD progression (51). In patients with terminal CKD, dialysis only partially improved EPC impairment (51). Furthermore, EPC number and function could not be correlated to the degree of coronary calcifications in HD patients (52).

Inflammation

The causes for the high prevalence of inflammation in ESRD are multifactorial and include: decreased renal function, volume overload, comorbidities, intercurrent clinical events and dialysis associated factors (35). An acute-phase reaction may be a direct cause of vascular injury (53). Proinflammatory cytokines play a central role in the genesis of both CVD and malnutrition in ESRD (53). As the prevalence of inflammation varies considerably between continents and races, dietary and/or genetic factors may have impact on inflammation in ESRD (54). Inflammation (which is interrelated to oxidative stress, ED, wasting and insulin resistance) has been suggested to be a significant contributor to CVD in ESRD.

It is difficult to define systemic inflammation (SI) in CKD patients because there is not a gold standard inflammatory marker (28). Several different inflammatory biomarkers, such as **high-sensitivity C-reactive protein** (hs-CRP), have been shown to independently predict mortality in these patients (35). Although C-reactive protein (CRP) is the most used in clinical trials, it is at the end of the inflammatory cascade and many early inflammatory processes are underdiagnosed (28). Besides, at least in part, inflammatory markers are retained by the lack of renal function making it difficult to set a threshold for inflammation and any definition of SI in dialysis patients may be discussed (28). Therefore several authors adopted the coincidental elevation of CRP and a pro-inflammatory cytokine plasma level as definition of SI (IL-6, MMP-9, TNF-α) (28). Elevated CRP relates to long-term prognosis in both patients with coronary artery disease (CAD) and in apparently healthy men, due to a blunted systemic endothelial vasodilator function (55). CRP has also been suggested to be a mediator of atherogenesis (35). Therefore, the identification of elevated CRP levels, as a transient independent risk factor for ED, might provide an important clue to link a systemic marker of inflammation to atherosclerotic disease progression (55).

Inflammation seems to be an important part of both ED and albuminuria. An association of CRP and fibrinogen with urinary albumin excretion, in the microalbuminuric range, in type 2 diabetic and nondiabetic individuals has been demonstrated (56). Chronic inflammation therefore emerged as a potential mediator between microalbuminuria and macrovascular disease.

Theoretical therapies for reducing inflammation in ESRD include: drugs interfering with the angiotensin system or with adrenergic activity; anti-inflammatory and antilipidic agents; vitamins and antioxidants; L-arginine, the NO aminoacid precursor and; antibiotics. However, very few controlled trials have been performed or are in progress to support this hypothesis (57).

Pentraxin 3 is an inflammatory mediator produced by endothelial cells that may have a role in atherogenesis (34). In two cohorts: stage 5 CKD and type 2 diabetes with

normal renal function, pentraxin 3 was found to be independently associated with proteinuria. Moreover, both pentraxin 3 and proteinuria were associated with ED in patients with type 2 diabetes (34).

Metabolic and Endocrinologic Alterations

Metabolic syndrome and **insulin resistance** strongly promote macrovascular complications, ED and renal dysfunction progression in CKD. In chronic PD patients, these metabolic alterations may correlate with changes in peritoneal solute clearance and solute transport rate but do not seem to be related to the maintenance of the RRF (58).

Secondary complex dyslipidemia occurs in ESRD consisting of: increased levels of serum triglycerides; low high-density lipoprotein (HDL) cholesterol (59); often, normal low-density lipoprotein (LDL) cholesterol, but the atherogenic small and dense LDL subclass (sdLDL) may be elevated (59). The apolipoprotein B (apoB)-containing part of the lipoprotein may undergo modifications (enzymatic and AGE-peptide modification, oxidation or glycosilation) that contribute to impaired LDL receptor-mediated clearance from plasma and promote prolonged circulation time (59). Although most studies failed to demonstrate an influence of serum lipids in the development of CVD (which in part may be due to the interference with deteriorating aspects of the activated acute-phase response), the very high CV risk observed in CKD probably justify the metabolic control of dyslipidemia in these patients (59). **Uric acid** augmentation is related to ED and is independently correlated with impaired flow-mediated dilation (FMD) (36).

Adiponectin is a hormone that modulates several metabolic processes; it has antiatherogenic properties and attenuates endothelial inflammatory responses. It may be used to assess the risk of CAD since it is decreased in the presence of some CVDRF such as male sex, obesity and diabetes mellitus (60). Adiponectin levels are elevated in patients with CRF, in CAPD (61) and HD (62). In CRF, adiponectin is elevated and related to CD146 expression, a novel cell adhesion molecule localized at

the endothelial junction (61). This correlation may be the expression of a counterregulatory response aimed at mitigating the consequences of endothelial damage and increased CVR in renal failure (61).

In kidney allograft recipients, ED and atherosclerosis are almost universal and, accordingly, markers of endothelial cell injury are significantly increased (60). Moreover, ED and alterations suggesting a procoagulant state are more pronounced in kidney transplant recipients with CAD, particularly in those with lower GFR (60). In patients with stage 3-5 CKD, renal function had a significant non-linear inverse association with adiponectin levels and was in fact the strongest predictor of this hormone expression (63). However, plasma adiponectin and leptin levels did not explain the correlation between eGFR and some markers of ED, making less probable the etiological involvement of the two hormones in this process (63).

Ghrelin is an orexigenic gastric hormone that is increased in disease states associated with wasting, such as ESRD. Markedly elevated plasma ghrelin (PGhr) levels are found in advanced renal failure and correlate with fat mass, plasma insulin and serum leptin levels (64). PGhr levels were also shown to be moderately increased in patients undergoing both HD and PD. In both uremic patients and healthy controls, age acted as a strong determinant of PGhr levels, whereas dialysis adequacy, RRF and inflammation appeared to have no influence in ghrelin levels. The negative correlation between PGhr levels and nutritional markers suggests that a low dietary intake may be involved in increased ghrelin secretion in dialysis patients (65).

RENAL REPLACEMENT THERAPY

Residual renal function

The role of residual renal function (RRF) in the elimination of the excess of pro-inflammatory molecules has not motivated much attention (28). Moreover, dialysis does not eliminate adequately the cytokine overproduction (28). On the contrary, peritoneal

membrane or HD filters are important sources of cytokine production (28). Accordingly, it seems that RRF protects dialysis patients from the excess of pro-inflammatory molecules (28). Effectively, the changes in endothelial damage and function markers caused by SI are more severe in those with less RRF (28). Volume overhydration is independently associated with worse EF in CAPD patients (66). In addition, normalized extracellular water, together with the product of phosphate times calcium (Ca×P), and dialysis vintage, were independent determinants of FMD in CAPD patients, suggesting that ED might link volume overhydration and cardiovascular events in dialysis patients (66).

Peritoneal Dialysis *vs.* Hemodialysis

A number of studies have reported that PD compared to HD is associated with lower levels of oxidative stress and inflammation. However, association with vascular or myocardial structure and function could not be established (67). Some studies have shown that PD is associated with a lower mortality than HD in the first one-two years but, thereafter, it may actually be higher on PD than HD. However most registry data does not support this (67). In PD patients SI induces ED, as estimated by elevation of endothelial damage markers. In addition, pro-inflammatory cytokines are associated with elevations in procoagulable and proatherosclerotic mediators in plasma (28). PD patients without known atherosclerosis show ED and their advanced oxidation protein products (AOPP) levels independently predict EF level (68). Acute SI, assessed by hs-CRP, is associated with a temporary increase in peritoneal solute transport rate (PSTR), determined by peritoneal equilibration test (PET), in chronic peritoneal dialysis (CPD) patients (69). This may be caused by intraperitoneal inflammation through the IL-6 system or AOPP (69).

Kidney Transplant

EF was shown to be better in transplanted patients than in dialysis patients (70). However, despite correction of uremia after renal transplantation, substantial ED,

structural alterations of the arterial wall and disturbed mechanical vessel wall properties persist (71). ED is more prominent among patients with failed transplants, which are usually complicated by inflammation, than naïve PD cases, suggesting that the failed allograft may be responsible for this abnormality (72). Patients with active CVD when starting PD show higher protein and albumin levels in peritoneal effluent (73). Daily peritoneal protein clearance on initiating PD is significantly and independently related to the presence of PAD appearing to be a possible new marker of systemic ED (73).

ENDOTHELIAL DYSFUNCTION PATHOPHYSIOLOGIC EFFECTS

Endothelial Damage

Endothelial injury is followed by release of NO, von Willebrand factor (vWF) and thrombomodulin (TM). **vWF** is an acute phase reactant, whose synthesis is increased by cytokines, that stimulates platelet aggregation and thrombus formation (28). It is not removed by the kidneys, thus the lack of renal excretion should not affect plasma levels. However, in dialysis patients, elevated vWF plasma levels have been found spontaneously and not associated with SI, suggesting that other uremia-related factors regulate vWF plasma concentration (28).

Due to its renal excretion, **TM** plasma levels are high in uremia and its levels are also affected by inflammatory molecules, proteolysis and oxidative stress. It activates protein-C inhibiting fibrin formation (28). The elevated levels of **adhesion molecules** (P-selectin, E-selectin, ICAM and VCAM) in CAPD patients may reflect inadequate clearance, enhanced synthesis/release and/or endothelial cell injury (74). In renal failure patients, particularly on CAPD, there is evidence of endothelial cell injury and a high degree of hypercoagulation that may lead to fibrin deposition in the vascular wall, thrombus formation and atherosclerosis (74). Platelet Gp IIb-IIIa is increased in CAPD and HD patients, what may promote dysfunctional aggregation (75).

Endothelial Remodeling

SI increases plasma levels of TGF-β which, in normal conditions, is an anti-inflammatory agent. However, in pathologic situations, it acts as pro-fibrotic and, among other mechanisms, induces endothelial damage by increasing PAI. In advanced atherosclerosis it is characteristically diminished what is consistent with a low-remodeling status (28).

Inflammation causes an increase in the hepatic synthesis of acute phase reactants such as Lp(a) and Hcy, CVRFs that are frequently elevated in dialysis patients. Persistently elevated Lp(a) levels induce a procoagulable status and inhibit TGF-β generation in smooth muscle cells. Hcy induces ED and atherosclerosis by different mechanisms: stimulating the vessel smooth cell proliferation and mitogenesis and; platelet adhesion (28). Inflammation is pointed as the specific factor responsible for cachexia and pro-atherogenic mechanisms in uremia (28).

Kennedy et al. have shown that the atherosclerotic burden in patients with renal failure, as indicated by an increased intima-medial thickness, may reflect effects of uremia that are independent of CVRF (76).

Hematologic Alterations

ESRD is paradoxically associated with both bleeding tendency and thrombotic events (HD vascular access thrombosis, ischemic heart disease, and renal allograft thrombosis). Several different mechanisms that influence thrombosis play a role in a complex interaction between platelets, endothelium and coagulation factors (Table 3) (77). In addition, non-traditional risk factors for thrombosis, such as hyperhomocysteinemia, ED, inflammation, and malnutrition must be taken under consideration (77). Several ESRD treatment factors such as recombinant erythropoietin (EPO) administration, dialyzer bioincompatibilty, and calcineurin inhibitor administration may have prothrombotic effects (77).

Table 3 – Hematologic factors influencing thrombosis in ESRD. Adapted from Casserly *et al.* (77).

ANTITHROMBOTIC	
Platelet factors	Reduced dense granule content and release
	Reduced serotonin uptake and release
	Reduced thromboxane synthesis
	Increased intracellular cAMP
	Abnormal Ca2+ mobilization
	Abnormal platelet arachidonic acid metabolism
	Impaired glycoprotein IIb–IIIa receptor activation
Endothelial factors	Anemia
	Increased endothelial nitric oxide production
	Increased endothelial prostacyclin production
	Increased homocysteine
PROTHROMBOTIC	
Platelet factors	Hemodialyzer-induced platelet aggregation
Endothelial factors	Elevated PAI-1
	Increased VWF release
	Oxidative stress
Coagulation factors	Increased fibrinogen
	Increased activity of factors VII, VIII, IX–XII
	Increased thromboplastin (tissue factor)
	Increased fibrinopeptide A
	Reduced protein C

Chronic inflammation and protein-energy wasting may contribute to ED in PD patients (78) and, consequently, to prothrombotic and proatherogenic processes (79). In PD

patients there is a baseline ED, characterized by a low fibrinolytic capacity, disorders in endothelial vasoactive function and elevated plasma markers of endothelial injury (28). Moreover, the contact of leucocytes with the HD membrane is able to stimulate the cytokine production (28).

The low fibrinolytic capacity is characterized by tissue plasminogen activator (t-PA) deficiency and PAI elevation (28). A clear association between elevated pro-inflammatory molecules (such as TNF-α) and a decrease in tPA has been shown and uremia *per se* is associated with poor tPA response (28). Both IL-1 and TNF-α induce elevation of PAI (28). The elevation of PAI associated with inflammation should be considered as a CVRF due to the predisposition to thrombotic events (28). Furthermore, malnourishment and patients with inflammation, show hepatic acute hyperproduction of half-life proteins such as CRP, prealbumin, fibronectin and PAI, among others (28).

CLINICAL METHODS FOR EVALUATION OF ENDOTHELIAL FUNCTION

Several methods are available nowadays to evaluate EF. Coronary arteries ED focuses on the circulation bed with greatest clinical relevance and it is a predictor for future cardiovascular events (80). Intracoronary studies evaluating endothelium-dependent vasodilatation after infusion of acetylcholine are in fact considered the gold standard for this purpose. However these tests are invasive and require cardiac catheterization what holds back to their routine use (81).

Evaluation of ED in peripheral vessels, including the brachial, femoral, and carotid arteries, correlates with measurements in the coronary arteries and is abnormal in subjects at risk for atherosclerosis and in patients with CAD (82). This fact is not surprising since atherosclerosis is a well-established systemic disease.

Flow-dependent vasodilation was first reported in 1992 by Celermajer et al. (22) and has been widely used since, because it is noninvasive, repeatable and reproducible. It represents a good marker of vascular NO availability. The increase in laminar shear stress caused by increased blood flow during reactive hyperemia, results in a rapid activation of eNOS leading to vasodilation and over a longer period it results in increased eNOS expression. FMD is measured through high resolution ultrasonography. However, this assessment has significant test-to-test variability, requires skilled technicians, and lacks technique standardization and well-established cut-off values. FMD in the brachial artery has emerged as a widely utilized clinical tool. Reduced FMD is independently associated with cigarette smoking, older age, male gender and larger vessel size but not with total cholesterol level, blood pressure or family history (83). It was shown that impairment of FMD in the brachial artery, a marker of systemic EF, is closely related to the angiographic extent of CAD (84).

ESRD patients have significantly higher carotid intima media thickness (CIMT) and lower endothelium-dependent FMD. CIMT was higher in diabetic ESRD patients, but FMD was similar. Serum inorganic phosphate is an independent risk factor for atherosclerosis and was significantly correlated with CIMT. The noninvasive CIMT and FMD tests can be used to monitor atherosclerosis and ED (85).

Recent non-invasive techniques include pulse wave of arterial stiffness and Peripheral Arterial Tonometry (PAT). As mentioned before, atherosclerosis may be preceded by ED and increased arterial stiffness and measures of arterial stiffness such as aortic pulse wave velocity predict morbidity and mortality (67).

Increased arterial stiffness in renal patients may be a consequence of vascular calcification, chronic volume overload, inflammation, ED, oxidative stress and several other factors (86). Increased arterial stiffness has significant clinical consequences: isolated systolic hypertension, left ventricular hypertrophy (and failure), and reduced myocardial perfusion (86).

Peripheral Arterial Tonometry

PAT is an emerging, noninvasive method for assessment of vascular function. It is based on the plethysmographic recording of digital arterial pulse wave amplitude (PWA) (87). Fingers are highly vascular, with dense arteriovenous shunts and have great blood flow variability making it very suitable to this measurement. Endothelium provocation may be achieved by several different methods and the vasodilator response results in a hyperemic flow and pulse amplitude increase. This response has been shown to depend primarily on nitric oxide (NO) release (88). PAT has been shown to have a good correlation with coronary EF (sensitivity=80%; specificity=85%) (89).

The Framingham Heart Study has incorporated PAT since 2003. Hamburg *et al.* demonstrated a significant inverse relationship with PAT ratio and multiple CVRF, including: male sex, body mass index (BMI), ratio of total to HDL cholesterol, diabetes mellitus, smoking, and lipid-lowering treatment (87). It has also been associated with family history of CAD, evidence of previous CAD, and hypertension (82).

Assessment of PWA with PAT demonstrates patterns of abnormality similar to that of brachial arterial ultrasound (BAUS) assessment of flow mediated dilation (FMD) (82). The main advantages of PAT are that it is: non-invasive, validated, user-independent and reproducible (82, 89). Nonetheless, there are also some limitations to this test: blood flow may be altered by systemic changes, namely the sympathetic and autonomic nervous systems. Thus it is possible that room temperature, mental stress or even conversation alters vascular tone. These confounders should be reduced as far as possible, even though the contralateral control arm is used as a control for these influences.

CONCLUSIONS

Even initial CKD should result in intensive prevention of CVR since death due to CVD is much higher than that from ESRD. Non-traditional risk factors for CVD are highly prevalent in CKD patients and seem to play an important role in cardiovascular events. ED is the first, yet potentially reversible, step in the development of atherosclerosis and its severity has prognostic value for cardiovascular events. Therefore, evaluation of ED may have major clinical diagnostic and therapeutic implications. Several methods are available nowadays to evaluate endothelial function. For this purpose, the emerging non-invasive methods are noticeably attractive, mainly for opening the possibility for routine evaluation of EF. In this perspective, PAT is particularly promising, and may change current's clinical practice.

Disclosure

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