Summary

The endothelium is the monolayer of endothelial cells lining the lumen of all blood vessels.

The endothelium is an active dynamic organ that fulfills many crucial roles.

First, it is a physical barrier, ultra-structurally defining the components of the vessel wall and the contents of the vessel lumen.

Second, this barrier affords movement of some small solutes (O2, glucose, etc.), in preference to large molecules (e.g., albumin), therefore, involved in tissue autoregulation.

Third, the endothelium affords an antithrombotic environment and mediates vasoactivity.

Finally, the endothelium mediates adherence of platelets and white blood cells to the vessel wall during injury and inflammation, respectively.

Endothelial function may be tested by two approaches: *The first*, is functional in nature and is based on the forearm haemodynamic response to acetylcholine (a pharmacological stimulus impinging upon the enzyme NO synthase) or to ischaemia [flow mediated vasodilatation (FMD) a physiological stimulus to the same enzyme]. Endothelium-dependent vasodilation can be assessed in the coronary circulation in humans and is used for assessment of endothelial function. *The second*, approach rests on the measurement of the plasma concentration of specific biomarkers, i.e. a series of compounds,

synthesized within the endothelium and that are released into the systemic circulation when endothelial integrity is hampered by noxious factors: LOX-1, CD40 ligand, CRP, and ADMA, Long pentraxin 3 (PTX3) ET-1 Microalbuminuria. The intercellular (ICAM) and vascular (VCAM) adhesion molecules, endothelial selectin (E-Selectin) and vonWillebrand Factor (vWF) are currently held as the most reliable biomarkers of endothelial dysfunction/damage.

The two approaches look at different aspects of endothelial function and therefore haemodynamic and biomarker-based tests provide complementary rather than overlapping information on endothelial integrity.

Haemodynamic studies appear of particular value in clinical research, because altered endothelium dependent vasoregulatory control predicts cardiovascular complications in a variety of clinical settings.

The definition of endothelial cell dysfunction, its pathophysiology and therapy remain poorly defined.

Based on the diversity of endothelial functions, it is logical to expect that the definition of the syndrome of endothelial cell dysfunction (ECD) should be broad enough to encompass disturbances in the barrier function of the vascular endothelium; its impaired antithrombogenic properties; perturbed angiogenic competence; inappropriate regulation of vascular smooth muscle tonicity, proliferative capacity and migratory properties; perturbed synthetic functions and deterrent of neutrophils and monocytes from diapedesis.

The pathophysiology of endothelial dysfunction is complex and involves multiple mechanisms. However, some of these seem to be common to most conditions.

- Reduced Nitric oxide (NO).
- Elevated Asymmetric Dimethylarginine .
- Oxidative excess.
- Ang II.
- OXLDL.
- Endothelin system.
- Alterations in the local balance of angiogenic and endothelial survival factors.
- Hyperhomocysteinemia.
- Diabetes mellitus.
- The adipose tissue.
- Hyperuricemia.
- Inflamation.

The most common causes of CKD are atherogenic diseases (hypertension, dyslipidemia, and type 2 diabetes mellitus), diseases in which the underlying histologic alteration is commonly represented by nephroangiosclerosis.The most typical finding in nephroangiosclerosis is represented by intimal hyperplasia of medium and small renal arteries. Such an alteration is the expression of systemic endothelial damage being extended to the whole arterial system, from small vessels to the aorta.Therefore endothelial damage seems to be the

basic anatomic alteration that eventually leads to disastrous vascular events in the kidney.

The pathophysiology of endothelial dysfunction and the kidney involves multiple mechanisms:

1-Hypertension.

Arterial hypertension is the most common cause of mild to moderate renal insufficiency in the general population and one of the major causes of ESRD. Renal dysfunction per se may be an underlying abnormality conducive to hypertension because hypertension "goes with the kidney" in cross-transplantation experiments, and a congenital reduction in the number of nephrons likely represents an important cause of glomerular endothelial dysfunction and hypertension in humans.

2-Diabetes mellitus.

Hyperglycemia leads to advanced glycation end products (AGE), which were shown to quench NO and impair endothelial function. AGE induce ROS and promote vascular inflammation, with enhanced expression of interleukin-6, VCAM-1, and MCP-1. This turns into a vicious circle in diabetic nephropathy, because in renal failure, clearance of AGE is delayed, which further promotes vascular and renal injury.

3-Reduced Nitric oxide (NO).

NO contributes to the control of renal hemodynamics and the process of urine formation, by interfering at multiple and physiologically critical steps of nephron function. NO dilates both the afferent and the efferent arteriole, may augment the glomerular filtration rate (GFR) and influence renal sodium handling along various tubule segments from the

thick ascending limb to the distal tubule and the collecting duct.Thus inhibition of NO synthesis has profound effects at systemic and renal levels.

4-ADMA.

An endogenous competitive inhibitor of eNOS. Reduced NO bioavailability may contribute to renal disease progression. Cross-sectional studies documented that high ADMA is associated both with oxidative stress and endothelial dysfunction (i.e., compromised flow mediated vasodilation). Relatively higher ADMA levels (i.e., ADMA > median vs. ADMA <median) predict not only renal survival but also all cause death.

5-Oxidative stress status.

Renal dysfunction is frequently associated with oxidative stress, as levels of different markers including plasma F2-isoprostanes, advanced oxidation protein products and malonyl dialdehyde are increased in patients with varying degrees of renal function, including patients with end-stage renal failure. In addition, high levels of oxidized LDL have been reported. This increase in oxidized LDL can favor the atherosclerotic process. It appears that ROS increase in a graded manner as renal function deteriorates, as different studies have reported inverse correlations between different markers of oxidative stress and glomerular filteration rate. Excessive ROS levels can produce cellular damage by interacting with biomolecules (proteins, lipids, and nucleic acids) and thus have negative effects on tissue function and structure. As such, they are implicated in different pathological situations including a variety of renal diseases In addition, graded increases in oxidative stress have been reported with increasingly longer durations of dialysis therapy, thus

suggesting that oxidative stress could accelerate renal injury progression by inducing cytotoxicity.

6-Chronic ischemia.

Ischemia could occur via several mechanisms, such as by intrarenal vasoconstriction (secondary to increased local angiotensin II or endothelin or a local loss of nitric oxide) or via structural lesions that impair blood flow delivery to the tubules. The latter could result from arteriolar disease (such as in diabetes or hypertension), from intraglomerular lesions (such as in rapidly progressive glomerulonephritis) or from loss of the peritubular capillaries. Interstitial fibrosis itself may lead to local ischemia by impairing the diffusion gradient from the capillary to the tubule.

7-The loss of the microvasculature.

The loss of the glomerular endothelium predisposes to activation of platelets and the coagulation system that favors capillary collapse and the development of glomerulosclerosis.

8-OXLDL.

Functional consequences of OxLDL-induced oxidative stress extend to atherosclerosis, vasomotor regulation, and endothelial dysfunction. OxLDL affects endothelial function and impairs endothelium-dependent dilations. The impact of OxLDL on apoptotic cell death may be a clue to its role in the development of atherosclerosis.

9-Endothelin system.

In patients with CKD, selective ETA receptor antagonism produces an increase in RBF and a decrease in renovascular resistance suggesting that ET-1 acting *via* ETA receptors is involved in the increased renovascular tone. These changes are accompanied by a fall in effective filtration fraction (EFF), suggesting that ET-1, acting *via* ETA receptors,

exerts a preferential efferent arteriolar vasoconstrictive effect, raising the possibility that ET-1 promotes hyperfiltration with its consequent potential for renal injury. Upregulation of the renal ET system exacerbates. Through its hemodynamic effects, ET-1 causes glomerular capillary hypertension, an increase in glomerular permeability, and excessive protein filtration. Excess protein filtration at the glomerulus leads to increased tubular reabsorption. This can activate tubular-dependent pathways of interstitial inflammation and fibrosis, with progressive renal scarring.

10- proangiogenic and antiangiogenic factors.

Several growth factors have been identified to have a critical role in promoting or inhibiting endothelial cell proliferation and survival. Growth factors, such as vascular endothelial growth factor (VEGF), have trophic, survival, and angiogenic properties, whereas factors such as thrombospondin-(TSP-1) not only inhibit endothelial proliferation but also cause endothelial cell death.

11-Proteinuria.

Significant proteinuria has emerged as a powerful predictor of renal disease progression and proteinuria reduction is an important strategy to retard or prevent renal functional loss**.**

12-microalbuminurea.

Increasing evidence has been accrued that microalbuminuria is a risk factor of paramount importance for cardiovascular complications and renal disease. This abnormality is currently seen as the renal expression of a primary or secondary systemic disturbance in endothelial function which determines an increased transcapillary escape of this protein. A series of observational and interventional studies in humans supports the concept that the link between microalbuminuria and cardiovascular and

renal outcomes is causal in nature and potentially modifiable, implying that the hypothetical systemic endothelial dysfunction responsible for the renal loss of albumin may be reversed by appropriate treatment. Experimental studies offer a solid support to the concept that albuminuria is a marker of endothelial dysfunction in the kidneys. Microalbuminuria is also a feature of human hypertension where this alteration signals a situation of high cardiovascular and renal risk. Interestingly, recent observations in the Framingham Heart Study cohort have nicely shown that a subtle increase in urinary albumin excretion antedates the clinical outset of arterial hypertension in healthy individuals in the general population.

13-Inflammation.

Numerous studies have reported an association between renal impairment and different mediators and markers of inflammation including CRP, IL-6, TNF-a, and fibrinogen, even among patients with moderate renal impairment, suggesting that CKD is a low-grade inflammatory process with peripheral polymorphonuclear leukocyte and CD14/CD16 cells being key mediators in this process. In fact, persistent inflammation may also be risk factor per se for progression of CKD, as inflammatory markers are predictors of kidney function deterioration.This could be a consequence of inflammatory mediators such as TNF-a or IL-6 being able to act as toxins participating in uremia complications. In addition, CRP formed locally in the renal inflammatory process reduces nitric oxide production, stimulates endothelin-1 formation, and induces some of the steps involved in the atherosclerosis process (monocyte recruitment and foam cell formation).

Long pentraxin 3 (PTX3) is a recently discovered multimeric inflammatory mediator that is structurally linked to short pentraxins, such

as C-reactive protein (CRP) and serum amyloid P component. PTX3 is produced by a variety of tissues and cells, including vascular endothelial cells and macrophages. Because of its extrahepatic synthesis (in contrast to CRP), the PTX3 level is believed to be a true independent indicator of disease activity because PTX3 is produced at sites of inflammation and is intimately linked to ED. PTX3 levels are elevated in patients with chronic kidney disease (CKD) and represent a novel mortality risk factor in stage 5 CKD.

14-Uric Acid.

Extensive epidemiologic and experimental evidence now suggests that serum uric acid (UA) is a relevant and independent risk factor for cardiovascular and renal disease, particularly in patients with hypertension, heart failure, or diabetes. Hyperuricemia predicts mortality in patients with heart failure or coronary heart disease, cerebrovascular events in individuals with diabetes, and cardiac ischemia in hypertension.The mechanism(s) by which UA may engender organ damage is still incompletely understood, but there is increasing evidence that endothelial dysfunction is a fundamental mechanism whereby this substance may affect cardiovascular and renal function and structure. In a series of elegant experiments in rats, it was demonstrated that hyper-uricemia, induced by an uricase inhibitor, triggers hypertension and impairs nitricoxide(NO) generation in the macula densa, whereas both hypertension and renal injury are reduced by treatment with the NO precursor l-arginine.

15-Hyperhomocysteinemia.

Cellular, animal, and human, suggest that homocysteine reduces NO bioavailability by oxidative excess. There is now also evidence that homocysteine may cause ADMA accumulation by inhibition of DDAH. These mechanisms may explain the increased cardiovascular risk of patients with hyperhomocysteinemia. This is of special importance for patients with chronic renal failure, who often have increased homocysteine levels, which were shown to predict cardiovascular outcomes in a recent study.

Compared with the general population, patients with chronic kidney disease (CKD) have an unacceptably high risk for premature death, primarily as a result of cardiovascular disease (CVD). The cardiovascular risk is increased very early on in the evolution of CKD (at a GFR of about 75 ml/min) and increases continuously with decrease in renal function. In accordance, recent reports have shown that patients with moderate to severe CKD are at a high risk of developing congestive heart failure (CHF) and that the majority of these patients have coronary heart disease or risk equivalents. Whereas traditional risk factors (age, lifestyle, left ventricular hypertrophy, dyslipidemia, hypertension, and diabetes mellitus) predict cardiovascular mortality in patients with mild to moderate CKD, so-called novel risk factors for CVD, such as inflammation, endothelial dysfunction, sympathetic overactivation, protein-energy wasting (*i.e.*, the proposed new term for loss of body protein mass and fuel reserves, oxidative stress, vascular calcification, and volume overload, are highly prevalent in these patients and seem to play a far more important role for vascular disease than in the general population. The association between traditional risk factors and

cardiovascular outcome in CKD is also complicated by the so-called reverse epidemiology phenomenon: i.e., the well-known association between established risk factors in the general population, such as hypertension, hypercholesterolemia, obesity, and hyperhomocysteinemia, does not exist, or even appears to be reversed, in patients with advanced CKD.

We also propose that future research protocols, whenever possible, should use a global approach by studying whole pathogenetic pathways rather than associative studies linking a single risk factor to morbidity and mortality. Mechanistic experimental and interventional studies designed to test whether biomarkers are not only markers but also etiological risk factors may provide further information that could lead to novel treatment options. As traditional and novel risk factors are not likely to operate in separate rigid compartments, experimental studies identifying the impact of traditional risk factors, such as hypertension, diabetes, and obesity, on the atherogenic potential of novel risk factors in the uremic milieu are needed. Finally, new areas of research that aim to use innovative tools, such as proteomics and epigenetics, may be helpful in the identification of new vascular markers or factors, unravel existing pathogenetic pathways, and facilitate the more rapid development of novel, safe, and effective therapies.

Treatment of the underlying disease may restore endothelial function.

Angiotensin receptor blockers and angiotensin-converting enzyme inhibitors have been shown to be especially beneficial.

Proliferator-activated receptor-gama activators (insulin sensitizers, *e.g.*, the glitazones pioglitazone and rosiglitazone) and peroxisome proliferator-activated receptor-alpha activators (fibrates, *e.g.*, feno-fibrate).

Decrease of homocysteine levels in hyperhomocysteinemia by supplementation with folic acid can improve endothelial dysfunction.

L-arginine and tetrahydrobiopterin, as well as tetrahydrobiopterin mimetics may improve endothelial function via increased NO bioavailability. However, some studies have not found L-arginine administration to improve endothelial dysfunction.

Acetyl salicylic acid has been suggested as an agent that can reduce oxidative stress and improve endothelial function.

Statins have proved to have beneficial effects on endothelial dysfunction which may be the result in part of lipid lowering but also of their pleiotropic anti-inflammatory effects.

ET receptor antagonists have been shown to reduce BP, improve arterial stiffness and ED, and retard the progression of atherosclerosis.

Transfer of endothelial progenitor cells may represent a new approach to therapy.