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Arterial Stiffness Is Related to Oxidative Stress, Inflammation, and Calcium-Phosphate Metabolism in Haemodialysis Patients

Dissertation
zur Erlangung des Doktorgrades der Medizin des Fachbereichs Medizin der Johann Wolfgang Goethe-Universität Frankfurt am Main

vorgelegt von

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geboren am 25.07.2007, Bukarest

Frankfurt am Main, 2007

ABSTRACT

Die progressive Einschränkung der Nierenfunktion führt zu einer vermehrten Versteifung der Gefäße (Arteriosklerose), unabhängig von und zusätzlich zur Atherosklerose, die eine erhöhte kardiovaskuläre Komplikationsrate zur Folge haben kann. Das durch die Urämie veränderte metabolische Milieu führt zu einem erhöhten oxidativen Stress, damit assoziert zu einer beträchtlichen inflammatorischen Belastung sowie zu einer gestörten Calcium-Phosphat-Homöostase, Konstellation die für vaskulären Veränderungen verantwortlich ist. Ein in klinischen Studien etablierter Surrogatparameter der arteriellen Gefäßsteifigkeit ist die Pulswellengeschwindigkeit (PWV). Ziel dieser Studie war, weitere Einblicke in den Pathomechanismus der beschleunigten Arteriosklerose von Hämodialyse-Patienten zu gewinnen, insbesondere dabei die Relation zwischen der PWV und Marker für oxidativen Stress, Prokoagulation und Inflammation sowie des Calcium-Phosphat-Produktes zu überprüfen.

Wir haben eine Querschnittsstudie mit 53 stabilen Patienten, 30 Männer und 20 Frauen, im Alter von 59 ± 16 Jahren, an Hämodialyse seit 68 ± 48 Monaten, durchgeführt. Die Carotis-Radialis PWV wurde mithilfe eines semiautomatischen Gerätes, Complior SP (Artech Medical, France) gemessen. Die Advanced glycosylation end-products (AGE) und Advanced oxidation protein products (AOPP) wurden mittels schon beschriebener Methoden bestimmt. Das hochsensitive CRP wurde mittels ELISA gemessen, während für die anderen biochemischen Parameter, Fibrinogen, Albumin, Calcium, Phosphat, Cholesterol und Trigylzeride Routine-Methoden verwendet wurden. Die statistischen Berechnungen wurden mittels SPSS (Statistical Package of Social Science, 12.0, 2003) durchgeführt. Die Korrelationen zwischen PWV (abhängige Variable) und mehreren unabhängigen Variablen wurden mittels multipler Regressionsanalyse bestimmt. Dabei wurde der Einfluß traditioneller kardiovaskulärer Risikofaktoren und der Medikation der Patienten auf die abhängige Variable, PWV, berücksichtigt.

Die Pulswellengeschwindigkeit korrelierte signifikant mit CRP ($p=0.003$), LDL-Cholesterin ($p<0.001$), Triglyzeride ($p<0.001$), AGE ($p=0.002$), Calcium ($p<0.001$),

Phosphat (p=0.001), and Fibrinogen (p=0.020). Zwischen PWV und Dauer der Dialysebehandlung (Monate) wurde eine interessante quadratische Beziehung beobachtet. Die multiple Regressionsanalyse zeigte eine negative Korrelatin zwischen AOPP und PWV (p=0.001). Wir konnten keine signifikante Korrelation zwischen der PWV und dem Alter, dem systolischen Blutdruck oder der Herzfrequenz nachweisen. Von der Gruppe der konventionellen kardiovaskulären Risikofaktoren wurde in dieser Studie eine positive Korrelation ausschließlich zwischen dem LDL-Cholesterinspiegel und der PWV belegt.

Wir konnten in dieser Querschnittserfassung zeigen, dass bei Hämodialyse-Patienten die anhand der PWV abgeschätzte arterielle Steifigkeit positiv und signifikant mit Surrogatparametern der Inflammation, Prokoagulation (Fibrinogen) und mit dem Phosphatspiegel korreliert. Die vaskuläre Architektur scheint synergistisch durch die für die Urämie charakteristische Konstellation – erhöhte inflammatorische und prokoagulatorische Aktivität und gestörter Knochenstoffwechsel –, modelliert zu werden.

ABSTRACT

End-stage renal disease has been denominated a vasculopathic state, owing to the accelerated arterial stiffening, which occurs in addition to and independent of atherosclerosis and bears an increased cardiovascular risk. The altered metabolic milieu in uraemia leads to an increased oxidative stress, heightened inflammatory burden, and an abnormal calcium-phosphate metabolism, which are thought to be responsible for the vascular changes. The pulse wave velocity (PWV) is a widely employed surrogate parameter of arteriosclerosis. The purpose of this study was to gain more insight into the pathogenesis of arterial stiffness, by investigating the influence of markers of oxidative stress, procoagulation, and inflammation, and of the calcium-phosphate product on the PWV.

We conducted a cross-sectional study in 53 stable patients aged 59 ± 16 years, who had been on haemodialysis for at least 4 months (68 ± 48). Carotid-radial PWV was measured using a semi-automated device, Complior SP (Artech Medical, France). Advanced glycosylation end-products (AGE) and advanced oxidation protein products (AOPP), were quantified according to previously described methods. High sensitive CRP was measured using ELISA, whereas the other biochemical parameters, i.e. fibrinogen, albumin, calcium, phosphate, cholesterol, and triglycerides, were determined using routine methods. For statistical calculations we employed SPSS (Statistical Package of Social Science, 12.0, 2003). The correlations between PWV, as the dependent variable, and many dependent variables were assessed by means of multiple regression analysis, in which we controlled for the influence of the traditional cardiovascular risk factors and some of the patients' medication (calcium-channel blockers and statins).

PWV was found to be significantly correlated to serum CRP ($p=0.003$), LDL-cholesterol ($p<0.001$), triglycerides ($p<0.001$), AGE ($p=0.002$), calcium ($p<0.001$), phosphate ($p=0.001$), and fibrinogen ($p=0.020$). Between PWV and dialysis duration (months) an interesting quadratic relationship ($p=0.058$) was noted. Against expectation, regression analysis showed a negative correlation between AOPP and PWV ($p=0.001$). We failed to confirm the correlation between PWV

and age, systolic blood pressure, or heart rate. Among traditional cardiovascular risk factors only LDL-cholesterol was positively correlated to PWV.

In this cross-sectional analysis we could put forward that PWV correlates positively and significantly with fibrinogen, CRP, AGEs, calcium, phosphate, and LDL-cholesterol in haemodialysis patients. It seems procoagulatory and proinflammatory pathways, oxidative stress, and the calcium-phosphate product exert a synergistic effect on disturbances of vascular architecture in ESRD patients.

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GLOSSARY OF ABBREVIATIONS

ACE – angiotensin-converting enzyme
ADMA – asymmetrical dimethylarginine
AGE – advanced glycosylation end-products
AOPP – advanced oxidation protein products
BMI – body mass index
CaPP – calcium phosphate product
CML – carboxymethyllysine
CKD – chronic kidney disease
DBP – diastolic blood pressure
DM – diabetes mellitus
E_{inc} – incremental elastic modulus
ESRD – end-stage renal disease
GFR – glomerular filtration rate
GSH – gluthatione (reduced)
GSSG – gluthatione (oxidised)
HD – haemodialysis
HDL – high-density lipoproteins
IDL – intermediate-density lipoproteins
IL – interleukin
IMT – intima-media thickness
LDL – low-density lipoproteins
LV – left ventricle
MAPKs – mitogen-activated protein kinases
MIA – malnutrition-inflammation-atherosclerosis
NADP(H) – nicotineamide adenosine??
NF-κB – nuclear factor-κB
NO – nitric oxide
PBS – phosphate buffered saline
PTH – parathormone
PWV – pulse wave velocity
RAGE – receptors of AGE
ROS – reactive oxygen species
SBP – systolic blood pressure
TG – triglycerides
TNF-α – tumour necrosis factor-α
VLDL – very low-density lipoproteins
VSMC – vascular smooth muscle cells

1. INTRODUCTION

1.1 Motivation

End-stage renal disease (ESRD) patients undergoing maintenance haemodialysis (HD) experience lower quality of life, significantly greater morbidity, higher hospitalisation rates, and higher mortality compared with the general population.[1;2] The most frequent cause of death in patients with advanced chronic kidney disease (CKD) is with 40% cardiovascular (CV) disease.[3] The risk is 10 to 20-fold higher than in the background population[4] and even much higher than in diabetic patients, a group with notoriously high cardiovascular risk. Accumulating evidence strongly suggests that chronic kidney disease is an independent risk factor for cardiovascular disease. The risk of death from myocardial infarction is five times higher than in the general population. In 1998, the National Kidney Foundation (NKF) Task Force on Cardiovascular Disease in Chronic Renal Disease recommended that patients with CKD be considered in the "highest risk group" for subsequent cardiovascular events.[5]

Studies show that a progressive decline in glomerular filration rate (GFR) is associated with occlusive atherosclerotic lesions. Even starting with stage 3 renal failure the risk of myocardial infarction and death rise independently of clinical variables, or baseline angiographic evidence of coronary disease and therapy.[5;6] Data strongly suggest that cardiovascular complications accelerate as renal function is progressively lost. Anavekar et al. found that below 81 ml per minute per 1,73 m^2, each 10-unit reduction in the baseline estimated GFR was associated with a 10 percent increase in the relative risk of death or nonfatal cardiovascular complications.[7] Coronary-artery calcification, as assessed by electron-beam computed tomography, is common and progressive in young adults (30 years old or younger) with ESRD who are undergoing dialysis.[8] Furthermore, independent of and in addition to atherosclerotic lesions, cardiovascular remodelling phenomena occur, characterized by left ventricular (LV) hypertrophy and arteriosclerosis,[9-11] i.e. stiffening, dilation, and hypertrophy of large conduit arteries.[12] Epidemiological studies have shown that left ventricular hypertrophy and arterial stiffening are tightly associated and that both are independent cardiovascular risk factors.[13-16]

Chronic kidney disease has been thus denominated a vasculopathic state.[12] Notably, a high percentage of dialysis patients already have a substantial burden of cardiovascular disease as they start haemodialysis.[4] The high incidence of cardiovascular events in patients undergoing long-term dialysis led Linder to the supposition that atherosclerosis may be accelerated.[15] This assumption was sustained by both post-mortem[17] and clinical[18] investigations which documented an increased incidence of atherosclerotic plaques in coronary vessels of uraemic patients compared to matched non-uraemic patients. Also, it is uncertain whether atherosclerosis itself is accelerated, or if these complications in HD patients are accounted for by different plaques morphology or by other modifications in the arterial properties. Another issue of debate is the role of HD itself in the evolution of the cardiovascular complications, since an abnormally high incidence of CV complications is already observed in the praedialysis state. Of note, kidney transplantation does not reverse loss of elastic and morphologic properties of arteries found in patients with ESRD.[19]

The aetiology of vascular disease in end-stage renal disease patients is very likely multifactorial. Traditional risk factors, such as dyslipidaemia, diabetes, and inactivity are highly prevalent in patients undergoing maintenance HD. The proportions of patients with hypertension and diabetes mellitus increased with worsening estimated GFR, systemic hypertension being the rule in patients in over 50 to 60% of haemodialysis patients, and hyperreninemia is also frequently present.[7;20] Many of the classic cardiovascular risk factors, predominantly diabetes mellitus and hypertension, perpetuate renal disease, and progressive renal decline increases the potency of such risk factors, building up a vicious circle. Furthermore, the proportion of patients with chronic kidney disease who receive appropriate risk-factor modification and intervention is lower than in the general population, a concept designated "therapeutic nihilism".[7;21] Nonetheless, the cardiovascular risk is disproportionately high relative to the prevalence and severity of the above factors, and several “non-traditional” factors, like hyperhomocystinaemia, hyperfibrinogenaemia, volume overload, albuminuria inflammation, anemia, oxidative stress, altered nitric oxide/endothelin balance and malnutrition, all of which develop early in the course of CKD, are very likely involved.[5;22] The long-term pressure and/ or flow overload characteristic of the uraemic state join the metabolic disturbances in their noxious influence.

Better knowledge of the pathogenesis would help in the diagnostic challenge of detecting patients at risk for abnormal structure and function in the vascular system before the development of symptoms or sequels of cardiovascular disease. The importance of prevention of arteriosclerosis cannot be emphasized enough, because it could not only improve life expectancy of dialysis patients, but also decrease total medical cost required for the patients with end-stage renal diseases on haemodialysis.

1.2 Proposed Approach

The purpose of this study was to investigate the pathogenesis of vascular alterations in haemodialysis patients, by exploring the influence of several factors on arteriosclerosis. We chose as a tool the widely employed surrogate parameter of the latter, namely the pulse wave velocity (PWV).

We conducted a cross-sectional study enrolling 52 stable HD patients. The extent of arteriosclerosis was assessed by means of the carotid-radial PWV, which can be easily and automatically determined. Its measurement is accurate and highly reproducible.[23] We weighed up first of all the traditional cardiovascular risk factors and those factors known from many studies to influence PWV, explicitly age, systolic blood pressure (SBP), pulse pressure (PP), and heart rate (HR). Subsequently, we considered the impact of oxidative stress, inflammation, malnutrition, and haemodialysis duration, next to serum calcium and phosphate concentrations on arteriosclerosis. As a surrogate parameter of inflammation we used the level of C-reactive protein (CRP). The advanced glycosylation end-products (AGE) and the advanced oxidation protein products (AOPP) were chosen as oxidative stress markers. The influence of fibrinogen, both an indicator of procoagulation and an acute phase reactor, and of malnutrition, reflected by albumin levels, on arterial stiffness were also analysed.

Measurement of aortic PWV could help, not only in risk assessment strategies, but also in risk reduction stratagems by monitoring arterial stiffness under different pharmacological regimens.[24]

1.3 Thesis Outline

Following the introductory chapter (1), the thesis is organized in the classical form of five main parts: state of the art, methods, results, discussion, and conclusions.

2. State of the Art

The second chapter presents the background theoretical and evidence-based information. It contains several subchapters addressing the most important issues, such as the pathogenesis and consequences of arteriosclerosis, oxidative stress, inflammation, malnutrition, procoagulation, calcification and dyslipidaemia. The pulse wave velocity, its significance, and major determinants are outlined. The other surrogate parameters used in this study and their relevance are also discussed in depth, i.e. the CRP, AOPP, AGE. It is also meant to be a justification of the proposed approach, i.e. the choice of the study design and goals.

3. Methods

The third chapter describes the study design and portrays the enrolled patients. It is also dedicated to the methodology of PWV measurement, the biochemical aspects of AGE, AOPP assessment, and the statistical analysis.

4. Results

In the fourth chapter the results of the clinical and biochemical measurements are presented and the statistical analysis is explained. The biochemical and clinical characteristics of the patients, including aetiologies of ESRD and medication, are enlisted in tables. The simple regression and the three models of the multiple regression, involving two sets of variables and two entry methods of the variables, are outlined.

5. Discussion

The fifth chapter discusses the results of the study and their implications. The emphasis lies on the correlations between PWV and the other biochemical parameters and risk factors. We also attempt an integration of our results in the current paradigms on the pathogenesis of arteriosclerosis in haemodialysis patients.

6. Conclusions

Finally, the sixth chapter is dedicated to the conclusions, the limitations of the study, and the proposed further research. This chapter is completed by a model representing our proposed integrated view upon arteriosclerosis in haemodialysis patients.

2 STATE OF THE ART

The causes of the increased vascular stiffness in dialysis patients are complex and intertwined (Table 1), all the pathogenic factors influencing one another and acting in a vicious circle. As previously noted, traditional atherosclerotic risk factors do not account completely for the arteriosclerosis of terminal renal disease.[5;25-27]

2.1 ARTERIOSCLEROSIS

Most of the cardiovascular events in the general population are causally connected to the atherosclerotic process; nevertheless, many CKD or ESRD patients suffer from cardiovascular complications without evidence of clinically significant atherosclerosis.[28] In CKD a second vasculo-pathological process takes place. With progressive decline in renal function the arterial walls undergo remodelling associated with stiffening, i.e. arteriosclerosis,[9-11] that is independent of and occurs in addition to atherosclerotic lesions.[5;12] This structural alteration of the arterial system is in many aspects similar to aging and is characterized by dilation, hypertrophy, and stiffening of the aorta and major arteries.[13;16] Importantly, remodelling of the arterial system is closely coupled to cardiac remodelling.[13] A prospective study showed serum creatinine levels to be a strong independent determinant of the progression of aortic stiffness.[29]

Atherosclerosis is a patchy, focal, intimal disease, which primarily affects the conduit function of the arteries.[16] In contrast, arteriosclerosis is a medial degenerative disease, which mainly impairs the cushioning function.[30] It is generalized throughout the thoracic aorta and central arteries, and more pronounced in the central elastic-type arteries than in the muscular-type limb arteries,[16] but it does not spare the latter, which are usually devoid of atherosclerosis. It results in diffuse fibroelastic intimal thickening, an increase in medial ground substance and collagen, and fragmentation of elastic lamellae with secondary fibrosis and calcification of the media.[8;31] Remodeling may be due either to pressure overload, characterized by wall hypertrophy and an increased wall-to-lumen ratio, or flow overload, which causes a proportional increase in arterial diameter and wall thickness.[5] The central, capacitative arteries undergo dilatation and, to a lesser degree, hypertrophy, while peripheral muscular conduit arteries suffer mostly wall hypertrophy.[13] It is ac-

wall hypertrophy.[13] It is accompanied by an abnormal increase in incremental elastic modulus (E_{inc}), characteristic of the intrinsic properties of the biomaterials of arterial walls.[11;13;32]

Table 1: Potential cardiovascular risk factors in ESRD patients

A	**Risk factors in the general population**
	hypertension
	anaemia
	hypercholesterolaemia
	lipoprotein(a) and apolipoprotein(a) isoforms
	insulin resistance
	hormonal disturbances (low estrogen, high parathyroid hormone)
	enhanced erythrocyte fragility
	recurrent bacterial and/or viral infections
	immunologic alterations (transcriptional disturbances of cytokine genes)
B	**CKD-associated risk factors**
	secondary hyperparathyroidism and increased calcium-phosphate product
	low levels of fetuin (potent calcification inhibitor)
	nephrotic syndrome (severe hyperlipemia and hypercoagulable state)
	chronic anaemia of renal disease
	altered nitric oxide/endothelin balance
	alterations in calcium and phosphate metabolism
	increased ADMA concentrations with subsequent low NO bioavailability
	microinflammation
	malnutrition
	alterations in blood lipids
	decreased adiponectin levels (protein with anti-inflammatory properties)
	albuminuria
C	**Haemodialysis-associated risk factors**
	haemolysis
	transmembrane passage of bacteria-derived products
	loss of antioxidant vitamins (C & E) and nutrients (amino acids)
	hypotensive episodes
	anaemia (chronic low-level blood loss)
	high shear rates
	substitutive therapies (erythropoietin, iron)
	hyperhomocysteinaemia
	increased C-reactive protein plasma levels
	extracellular volume overload

Thus, the structural changes of vascular walls described in uraemic patients are different from those observed in aging, atherosclerosis, or hypertension.[33] It is noteworthy that these abnormalities in arterial wall occur even in childhood.[34] Furthermore, the standard cardiovascular risk factors such as diabetes, dyslipidaemia, obesity, and cigarette smoking do not influence significantly arterial compliance in the face of a decreased creatinine clearance,[10;35] suggesting an important role of the renal failure-related factors.[33]

The relationship between arteriosclerosis and atherosclerosis is not completely elucidated; they may be two separate and distinct conditions, even though they are often seen together in older Western subjects.[36] However, arteriosclerosis correlates with the prevalence of atherosclerosis.[37]

The direct effect of arterial sclerosis is an elevated pulse wave velocity (PWV); some authors maintain that the intimal atherosclerosis does not induce vascular stiffening.[38;39]

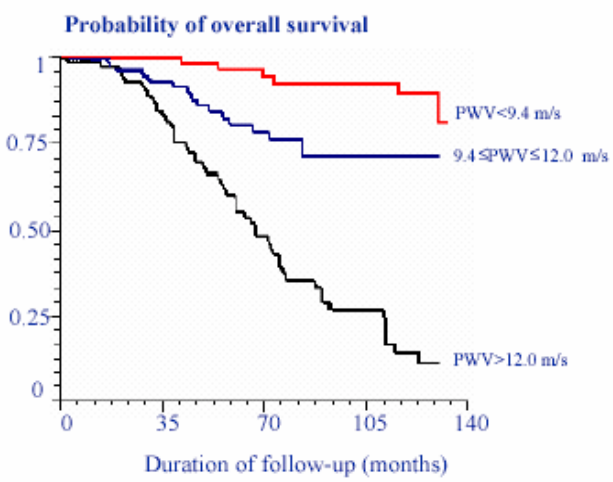
2.1.1 Marker of Arteriosclerosis: Pulse wave velocity

As already stated above, a well established clinical marker of arterial stiffness is the pulse wave velocity (PWV). The heart activity generates a wave which propagates along the arterial conduits. Its velocity depends upon the geometric and structural features of the arterial wall, an increased stiffness of the wall causing the wave to travel faster.[32]

The consequences of the arterial remodelling inherent to arteriosclerosis are different from those attributed to the presence of atherosclerotic plaques. Arteriosclerosis affects the so-called cushioning function of the arteries. This term refers to the role of arteries to dampen the pressure and flow oscillations resulting from intermittent ventricular ejection and to transform the pulsatile flow within arteries into the steady flow required in peripheral tissues and organs.[40] The wave generated by the heart, called incident wave, is reflected at any points of discontinuity along the arterial tree, thus initiating a reflected wave travelling backward towards the ascending aorta. The interaction and summing up of the incident and reflected pressure waves create the measured pressure wave. The amplitude of the measured pressure wave is determined by the timing between the component waves. When optimally timed, the reflected wave returns to the

central aorta in diastole and therefore enhances diastolic perfusion pressure in the coronary arteries. An increased PWV due to arterial stiffening leads to an early return of the reflected wave from the periphery to the aorta, leading to its impaction on the central arteries during systole rather than diastole. The deleterious consequence is the amplification of aortic and ventricular pressures during systole and the reduction of aortic pressure during diastole with the inherent enhancement of left ventricular workload and compromising of the coronary blood flow.[41-43] Furthermore, changes in blood flow pattern might impair endothelial function and interfere with peripheral microcirculation, resulting in end-organ damage.[44] Higher SBP and pulse pressure, lower DBP, and LV hypertrophy have been identified as independent factors of cardiovascular morbidity and mortality in the general population as well as in ESRD patients.[45]

Aortic PWV has been shown to be a strong predictor of cardiovascular and all-cause mortality in patients with ESRD on HD[46] independently of other factors known to affect the outcome of uraemic patients, namely age, overall duration of ESRD, number of years on HD, pre-existing cardiovascular disease, degree of LV hypertrophy, BP, and serum albumin and haemoglobin levels.[47] Among hypertensive patients, it has even been shown to be a stronger predictor for any type of cardiovascular risk than plasma creatinine, left ventricular hypertrophy, and total/ HDL (high density-lipoproteins) cholesterol. Blacher et al[47] even showed that each aortic PWV enhancement of 1 m/s increased the all-cause mortality by 39% (Figure 1).



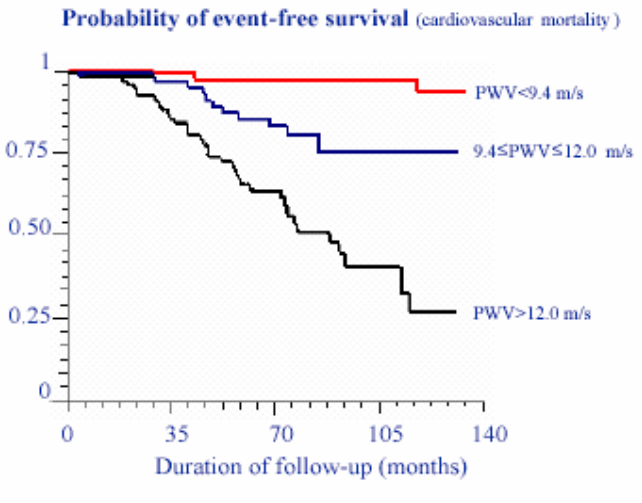


Figure 1: Probability of overall and event-free survival in relation to PWV. (adapted from Blacher et al.[47])

Furthermore, for a given age, PWV was the strongest predictor for cardiovascular mortality,[35] still more important than SBP and even displaced SBP as a prognostic factor.[48] Consequently, aortic PWV is probably acting further along the causal pathway for arterial disease and may represent a useful integrated index of vascular status and hence cardiovascular risk.[48] Moreover, Shoji et al.[44] demonstrated that the influence of arterial stiffening on mortality outweighed the role of diabetes.

2.1.2 Pathogenesis of Arteriosclerosis

Both atherosclerosis and arteriosclerosis are dynamic processes, involving continuous remodelling over decades, with various stages being present in the same individual. The complex process called vascular remodelling comprises the amendment of arterial wall structure in response to various factors, including direct injury, atherogenic factors, growth factors, vasoactive substances, or to changes in haemodynamic burden.[49] It is highly adaptive and comprises the activation, proliferation and migration of smooth muscle cells, and rearrangements of cellular elements and extracellular matrix of the vessel wall.[50] Notably, aging and hemodynamic stress lead to thinning, fraying, and eventual fragmentation of elastic lamellae, followed by secondary fibrous remodelling.[36]

The key-initiating phenomenon in both arteriosclerosis and atherosclerosis seems to be endothelial dysfunction.[25;51] Both coronary and systemic endothelial dysfunction are predictive of adverse cardiovascular events.[25] The endothelium lines vessel walls and plays a role of utmost importance for communication between the blood and the vessel wall. The endothelium is strategically located to serve as a sensory cell assessing haemodynamic and humoral signals, as well as an effector cell eliciting biologic responses that may eventually affect the structure of the vessel.[49] Healthy endothelium regulates blood flow by releasing vasodilatating (NO, nitric oxide, bradykinin), or vasoconstricting factors (e.g. endothelin) according to physiologic needs.

The endothelium influences the geometric and structural properties of large conduit arteries, and alterations of endothelial function might lead to arterial remodelling as follows.[25;49] Abnormal control by the endothelial cells of the tone of the underlying vascular smooth muscle and of its proliferation may originate from a reduced release and/or a diminished action of endothelium-derived vasodilators. The term endothelial dysfunction mainly refers to impaired vasodilatation secondary to a loss of endothelial production and/or bioavailability of NO. Evidence suggests that there is reduced bioavailability because of oxidative inactivation by excessive production of the superoxide anion (O_2^-) in the vascular wall, in diverse disease states such as heart failure, diabetes, hypercholesterolaemia, atherosclerosis, hypertension, and cigarette smoking.[52] Moreover, uraemic plasma contains higher than expected levels of asymmetrical dimethylarginine (ADMA) that inhibit NO synthesis. Early in the pathologic process, arterial wall changes are not only present as impaired endothelial function but also as functional changes in smooth muscle cell responses to endothelium-derived NO. Importantly, endothelial dysfunction occurs in arteries without visible characteristics of atherosclerotic lesions such as fatty streaks or plaque formation. Hence, even macroscopically intact arteries may show signs of chronic inflammation, characterized by enhanced endothelial permeability, expression of adhesion molecules, monocyte adhesion, and migration.

Possible causes of endothelial dysfunction include elevated and modified low-density lipoproteins (LDL); free radicals; genetic alterations; elevated plasma homocysteine concentrations; and infectious microorganisms such as herpesviruses or Chlamydia pneumoniae.[25] Some studies in children with either familial hypercholesterolaemia or severe obesity have directly related increased stiffness

with impaired endothelial function, leading to the hypothesis that cardiovascular risk factors may exert their detrimental effects on arterial stiffness through endothelial dysfunction.[41]

Studies have demonstrated an abnormal endothelial function in CKD, even in patients with mild renal insufficiency and those without atherosclerotic vascular disease.[53;54] The forearm musculocutaneous vasodilator response to various stimuli including ischaemia, hyperthermia, or acetylcholine is decreased in ESRD patients.(reviewed in reference[14]) Furthermore, the impairment in EDV found in CKD was not due to hypertension, hypercholesterolaemia or diabetes mellitus, the culprit being uremia itself.[53] Moreover, the endothelial dysfunction was strongly correlated to the number of years spent on HD.[14]

The mechanisms responsible for the endothelial dysfunction leading to arterial stiffening are not completely understood. Some may be directly related to the functions of NO.

PWV studies indicate that another factor contributing to increased arterial stiffening is hypertension.[23] The high incidence of isolated systolic HT in ESRD, conditions such as anaemia, arteriovenous shunts, and overhydration induce a state of chronic volume overload associated with increased systolic and regional blood flow and flow velocity, creating the prerequisites for arterial remodelling.[13] Remarkably, renal transplantation appears to improve arterial stiffness.[55]

Arterial elasticity has two major elements: a passive component that reflects the structural composition of the artery wall and an active component related to arterial tone.[56]

It was hypothesized that before structural vascular transformations occur functional changes play a major role in decreasing arterial compliance.[57] Functional stiffness without structural changes may result from various mechanical agents, such as high BP, hypervolaemia; or increased levels of nonmechanical factors, i.e. LDL, parathyroid hormone, homocysteine, and increased vasoconstrictor activity, in particular endothelin[58] and angiotensin II.[40;57;59] In that regard, angiotensin II may be one of the mediators of this endothelial dysfunction.[60] Volume overload increases arterial stiffness by increasing arterial distension (Laplace's law).[57] Interdialytic weight gain, a marker of sodium and water overload is associated with increased aortic PWV.[61] Endothelin levels were shown to be elevated

in ESRD patients and to correlate with left ventricular hypertrophy and carotid intima media thickness (IMT), ~~another surrogate parameter of PWV.~~[58] de schimbat!!

Functional stiffness can be reversed by BP lowering using ACE-inhibitors, β blockers, or calcium-channel inhibitors.[32;40;62] ACE inhibitors by impairing the accumulation of angiotensin II in the vascular wall would attenuate this type of endothelial dysfunction.[60] Both volume correction and ACE inhibition decrease the aortic PWV. Volume correction alone had no significant effect on PWV, presumably because of AT II stimulation.[57]

Arterial stiffness may also be determined by structural changes, evidenced by increased arterial wall thickness.[57;62] In the presence of such alterations, the stiffening is less dependent on BP. Guerin et al showed that survival of ESRD patients was significantly better for patients whose aortic PWV declined in response to BP lowering. They concluded that arterial stiffness, beside being a risk factor contributing to the development of cardiovascular disease, is also a marker of established, more advanced, less reversible arterial changes.[62]

One of the major early determinants of the establishment of structural stiffness is vascular smooth muscle cell proliferation. NO exerts potent homeostatic and antiproliferative effects on different cell types, by augmenting the production of the inhibitor of nuclear factor κB (NF-κB), a transcription factor involved in the expression of the genes encoding many proinflammatory functions of endothelial cells. Nitric oxide tonic release by endothelial cells ~~and may serve to~~ maintain the mitogenic quiescence of subintimal mesenchymal cells. In addition to its vasodilatatory functions, NO can impair platelet aggregation.(reviewed in references ~~51;60~~)

~~One recent study[63] demonstrated that high sodium intake was indeed associated with hypertrophy of the media. They pointed out that the deleterious effects of increased salt intake did not impair arterial compliance by altering the collagen content of the vascular wall, but by reducing the arterial-wall hyaluronan. This could be reversed using the diuretic compound indapamide, which normalized the interstitial hyaluronan contents~~.

Many studies, focusing on the major determinants of PWV, have been conducted. (Table 2)

Table 2. PWV Studies

	Study design	Subjects	Positive Correlations	No Correlations
London et al. 1990[32]	Cross-sectional	92 patients on HD; 90 healthy controls	Age, MAP, borderline rel: aortic calcifications	Dialysis duration, total cholesterol, TG, CaPP, BMI
Asmar et al. 1995[23]	Cross-sectional	499 healthy subjects	Age, SBP	
London et al. 1996[13]	Cross-sectional	70 ESRD patients	IMT	
Blacher et. al 1998[59]	Cross-sectional	74 patients on HD	Aortic calcifications, diabetes, age, SBP	
Taniwaki et al. 1999[64]	Cross-sectional	271 diabetes type 2 patients	Age, BP, smoking, diabetes	
Guerin et al. 2000[31]	Cross-sectional	120 patients on HD	Age, SBP, aortic calcifications	
Shoji et al. 2001[44]	Prospective	265 patients on HD	Age, diabetes, SBP, nonHDL cholesterol	CRP, HDL, smoking status
Benetos et al. 2002[29]	Prospective	187 hypertensives, 296 healthy controls	Age, PP, HR, creatinine	
Kimoto et al. 2003[65]	Cross-sectional	161 diabetics; 129 healthy controls	Age, SBP, sex, smoking, lipids, diabetes	
Stompor et al. 2003[66]	Cross-sectional	43 patients on peritoneal dialysis	Age, SBP, bFGF	Time on HD

The most important factor contributing to increased PWV in human populations is said to be age.[23;67] Avolio et al. demonstrated the dependence of aortic PWV on age in a group of 480 normal subjects in a population with low prevalence of hypercholesterolemia.[68] In another study enrolling 146 healthy volunteers aortic PWV increased 2,5-fold from 20 to 91 years of age.[67]

In a cross-sectional study involving 400 patients without cardiovascular complications, Asmar et al. identified age and systolic pressure as the two major determinants of PWV.[23] A prospective study conducted by Shoji et al. showed that PWV was positively associated with age, diabetes, systolic BP, and non-HDL cholesterol, whereas CRP did not associate with aortic PWV.[44]

London et al found no correlations between PWV or its variability and dialysis duration, total cholesterol, triglycerides, apo B, Ca-PP, body mass index (BMI). There was a borderline correlation between PWV and aortic calcifications.[32] Blacher et al.[59] showed that four parameters, i.e. the presence of aortic calcification, the presence of diabetes mellitus, and increased plasma endothelin and homocysteine levels, constituted a substantial proportion of the PWV variability.

Benetos et al found age, BMI, and MAP in normotensives; and age, PP, and HR in hypertensives to be independent determinants of baseline PWV. They also showed that in patients with uncontrolled blood pressure levels throughout the follow-up PWV progression was more than 3 times higher than in well-controlled hypertensives.[29]

Additionally, serum creatinine levels during the first visit were a strong predictor for the long-term progression of PWV in hypertensive but not in normotensive subjects, implying that hypertensive are more vulnerable than normotensive subjects to the deleterious effects of certain risk factors.[29] Wang et al. have demonstrated a stepwise increase in PWV with advancing severity of CKD from stages 1 to 5. [69]

The influence of HR on PWV is subject to intense disputes. Lantelme et al.[70] revealed that in a given subject, the HR greatly influences the measurement of PWV, while others maintain that the observed effect was solely dependent on the device used to assess the PWV (i.e. Complior®, Colson).[71] However, HR may shape arterial stiffness due to a series of mechanisms. Increased HR may be sign of increased sympathetic tone, which may increase the stiffness of large arteries

directly. Furthermore, decreased large artery stiffness leads to reduced baroreceptor sensitivity, which could amend sympathetic tone and HR. Chronically increased HR may amplify large artery stiffness by accelerating elastin breakdown in the arterial wall.(reviewed in reference [72])

Aortic PWV is greater in patients with diabetes or glucose intolerance than in controls,[48] in accordance to the fact that arterial stiffness is an early feature of diabetic vasculopathy.[73] Furthermore, microalbuminuria was shown to be a significant determinant of PWV in type 2 diabetics without cardiovascular disease.[74]

Several studies tried to investigate the stiffness in different arterial segments. One of them demonstrated that the effects of diabetes and age are greater on the heart-carotid PWV and the heart-femoral PWV than on the heart-brachial and the femoral-ankle PWV.[65] This result is not unexpected, as the brachial and radial arteries are considered to remain free from atherosclerosis,[57] and in muscular peripheral arteries, the elastin components of the wall appear to be "protected" by smooth muscle and by collagen and as such they show a lesser degree of vascular dilation as compared to central arteries.[36] It was also hypothesized that stiffness of peripheral arteries may be more strongly controlled by the endothelium-dependent mechanisms.[65]

2.1.3 Influence of Therapy on PWV Progression

A growing body of evidence from studies in essential hypertensive non-uraemic and uraemic subjects suggests that long-term antihypertensive therapy induces reverse arterial remodelling with improvement of the arterial viscoelastic properties,[75] decreased aortic PWV and wave reflections.[76]

The drugs most commonly used in such trials are calcium-channel blockers and angiotensin-converting enzyme (ACE)-inhibitors.

Tycho Vuurmans and colleagues showed that dialysis-related volume reduction and ACE inhibitors both improve the aortic PWV. Combining volume reduction and ACE inhibition had an enhanced effect and induced a consistent decrease to a completely normal PWV value.[57]

Valsartan administration for 12 months has been shown to decrease left ventricular hypertrophy and PWV in a double-blind study in 24 CAPD patients.[77]

Saito et al administered nifedipine to 47 haemodialysis patients for 2 years. While the PWV of the control group was gradually amplified by 10%, the PWV of the nifedipine group decreased by 2%.[78] They reported that the one-year progression of PWV positively correlated with age, high blood pressure, CaPP, and serum cholesterol levels and was negatively correlated with HDL levels.

The effect of medication on arteriosclerosis is not restricted to the aorta. In elderly patients with hypertension, the intima-media thickness of a peripheral muscular artery, the radial artery, and the stiffness of a proximal elastic artery, the common carotid artery, were reduced after 9 months of a perindopril or hydrochlorothiazid plus amiloride treatment.[75]

Several studies have pointed that the reduction of PWV goes beyond SBP normalization and continues to decline despite constant SBP, suggesting the occurrence of a pressure-independent pharmacological remodelling of the arterial wall. One of these studies involved perindopril, the other nitrendipine.[76;79]

Another approach to normalizing PWV involves the lipid-lowering and antioxidant effects of statins. In a placebo-controlled study in diabetic HD patients with normal lipid levels, a six months administration of fluvastatin caused a significant decrease in PWV, oxidised LDL serum levels, and CRP serum levels.[80] Of note, changes in PWV were not associated with any changes in blood pressure in this study. Six months may be too short a period for structural changes to occur. Hence, the effect may be explained at the level of endothelial function, as in another study six months of treatment with fluvastatin significantly improved brachial artery flow-mediated vasodilatation as a measure of endothelial function.[81]

2.2 OXIDATIVE STRESS

Oxidative stress occurs when the balance between free radical production and scavenger levels is altered. Mounting evidence outline its role the development of complications related to long-term HD such as atherosclerosis, amyloidosis, malnutrition, anaemia, and infection. Free radicals include chemical derivatives

of oxygen, called reactive oxygen species (ROS), reactive nitrogen species, as well as chlorinated compounds.

Compared with controls, the patients with renal insufficiency have higher levels of diene conjugates, lipid hydroperoxide, and lower GSSG/GSH ratio.[82] Stenvinkel et al.[83] and Miyata et al.[84] also showed that predialysis patients are exposed to an augmented oxidative stress. This is further exacerbated by haemodialysis and peritoneal dialysis, as evidenced by increased lipid peroxidation and low antioxidant levels.[85-87]

Himmelfarb et al hypothesized that the increased oxidative stress and its sequels is a major process linking inflammation to increased cardiovascular events found in uraemia.[88]

2.2.1 Causes of Oxidative Stress

2.2.1.1 Excess Production of Reactive Oxygen Species

The mechanism of uraemia-associated oxidative stress is not completely understood, but the haemodialysis procedure itself seems to be a source of oxidants[85;89], due to both the dialyzer membrane[90;91] and the microbiological contamination and pyrogen content of the dialysate.[92] Both lead to complement and phagocyte activation with subsequent respiratory burst and ROS production. Indeed, even a single session of haemodialysis significantly increases lipid peroxides and decreases the concentration of antioxidants.[93]

Ward and McLeish[94] have recently shown that neutrophils obtained from uraemic patients were primed for superoxide (O_2^-) production. Another important phagocyte-derived oxidant in uraemic patients is hypochlorous acid (HOCl) produced in a myeloperoxidase – catalysed reaction.(reviewed in reference [88])

Some authors underscore the importance of increased levels of oxidisable substrates, notably triglycerides with their unsaturated fatty acids.[89]

Another not to be underestimated source seems to be the administration of intravenous iron, a potent pro-oxidant.[95] Lim et al. conducted a study demonstrating that increased oxidative stress in the blood circulation of the uraemic patients on haemodialysis is exacerbated by the elevated baseline serum ferritin

levels and intravenous iron infusion.[96] Furthermore, erythropoietin enhances stimulated superoxide production in neutrophiles both in vivo and in vitro.[26]

2.2.1.2 Defective Defence Mechanisms

Antioxidative protection results from the complex interplay of both nonenzymatic factors (tocopherols, carotenoids, ascorbic acid, selenium and others) and enzymatic systems (catalase, superoxide dismutase, glutathione peroxidase) acting as oxygen radical scavengers.[92] These mechanisms are vital to prevent free radicals from irreparably damaging lipids, proteins and nucleic acids. It has been conjectured that patients with chronic kidney disease have antioxidant deficiencies, which increase their risk for cumulative injury to multiple end organs.[97]

The potential of plasma components to scavenge reactive oxygen species, especially the glutathione system, is likely to be overwhelmed, and there is a severe defect in antioxidant enzyme cofactors such as zinc or manganese (for superoxide dismutase) and selenium (for glutathione peroxidase), and antioxidant vitamins.[98] Several studies in predialysis and haemodialysis patients demonstrated profound deficiencies in the activity of the glutathione system[99] and almost complete abolishment of GSH-peroxidase activity[100], an enzyme that can detoxify hydrogen peroxide and lipid hydroperoxides in the presence of reduced glutathione. There seems to be a decreased renal synthesis of antioxidant enzymes among which, GSH peroxidase.[84;99]

HD is a non-selective process, whose benefits of clearing waste products cannot be separated from undesirable diffusive losses of essential substances, including antioxidants[92] like zinc, selenium, and vitamins E[101] and C[102]). Further plausible explanations to the impaired antioxidant defence are dietary restriction of fresh fruits and vegetables to avoid hyperkalaemia.[84;99] In addition, Himmelfarb et al have proved that plasma protein thiols, which constitute the essential extracellular defence against oxidative stress, are extensively oxidized in uraemic patients, compared to healthy subjects.[103] Moreover, Morena et al. demonstrated qualitative abnormalities of HDL-associated enzymes and consequently, the reduction of HDL protective capacity against oxidative stress in HD patients.[104]

As albumin is the major plasma protein target of excess oxidative burden in CKD and chronic haemodialysis patients, oxidative damage of albumin will decrease plasma antioxidant defences and increase the susceptibility to oxidative injury. [105] This may provide a possible link between hypoalbuminaemia and oxidative stress.[105]

2.2.2 Consequences of the Oxidative Stress

Several physiological functions, such as regulation of blood flow, inhibition of platelet aggregation, inhibition of leukocyte adhesion and control of smooth muscle cell proliferation are influenced by oxidant stress.[52;93] The mechanisms may be explained at the level of nitric oxide, as augmented release of superoxide anion (O_2^-) may annihilate nitric oxide by forming peroxynitrite and thereby neutralizing its vasodilator capacity.[106] Peroxynitrite vasodialatory potence is far less than that of NO.[52] Thus, a central pathophysiological consequence of oxidative stress is the disruption of nitric oxide signalling.

Recent reports demonstrated that NO dependent flow-mediated vasodilatation is blunted in patients undergoing haemodialysis.[93] Reduced NO would not only produce vasoconstriction, which would reduce compliance, but would also facilitate vascular smooth muscle growth that would add a structural component to the increase in arterial stiffness. Impairment of the antiproliferative role of NO is one way by which the oxidative stress could contribute to accelerated arteriosclerosis.[107]

Furthermore, a current theory implies that oxidative signals modulate the expression of vascular inflammatory genes which could promote vascular alterations.[107;108] Hydrogen peroxide (H_2O_2), for example, can activate the NFκB pathway and thus induce the synthesis of proinflammatory cytokines and amplify the inflammatory cascade.[87] Hence, ROS and/or their modified target biomolecules (ie, oxidised LDL) then serve as true second-messenger coupling molecules to transmit these extracellular signals to elevated expression of certain gene products such as adhesion molecules and other vascular inflammatory molecules.[107;108]

The utmost importance of oxidative stress in the impairment of endothelial function was substantiated many studies. One study proved that a single session of

haemodialysis raised oxidised LDL and impairs the endothelium-dependent vasodilatation levels, while treatment with vitamin E-coated-dialyser restored it.[93;109] Another study[82] showed that patients with renal insufficiency compared with controls, the had an impaired endothelium-dependent vasodilatation, which was positively correlated with total antioxidative activity, GSH and negatively correlated with GSSG and diene conjugates.

Last but not least, free radicals also impair the myocardium contractile function and may contribute to uraemic cardiomyopathy.[110]

2.2.3 Markers of Oxidative Stress

The great endeavour to understand the underlying in vivo mechanisms of oxidative injury has led to the emergence of powerful strategies based on identifying stable end-products of oxidation.

2.2.3.1 Advanced Oxidation Protein Products (AOPP)

Aminoacid residues in proteins are vulnerable to oxidation. The impaired redox balance burden bears the consequences of direct oxidative injury of proteins, which thus may be altered either directly by ROS with the eventual formation of oxidized amino acids or indirectly by reactive carbonyl compounds formed by autoxidation of carbohydrates and lipids[111] (see below).

Witko-Sarsat et al isolated dityrosine-containing protein cross-linking products in the plasma of dialysis patients and designated them advanced oxidation protein products (AOPP).[111] Plasma levels of AOPP were found to be elevated in chronic kidney disease patients, compared to healthy controls, increasing with progression of the disease and culminating in patients on haemodialysis.[112] AOPP levels were correlated with plasma concentrations of dityrosine and AGE pentosidine, which are indices of oxidant-mediated damage.[113]

AOPP are closely related to the monocyte activation state in chronic kidney disease patients.[113] In the generation of AOPP, the myeloperoxidase (MPO) produced chlorinated oxidants, previously solely considered as microbicidal agents, seem to play an important part.[114] AOPP levels are also raised by oxidative stress related to intravenous iron administration.[115]

AOPP were shown to trigger the oxidative burst in neutrophils as well as in monocytes,[113] that is, to act as mediators of inflammation and oxidative stress in chronic uraemia, providing both one of the molecular links between oxidative stress and inflammation and a means of upholding the oxidative burden. Uraemic patients suffer from a dysregulation of the immune system, characterized by the paradoxical coexistence of a state of profound immunodeficiency and chronic inflammation.[116] AOPP may be implicated in this process by activating monocyte respiratory burst and TNF production.[113]

However, AOPP themselves do not have oxidative properties and one study failed to show any correlation between AOPP and CRP.[112]

Kaneda et al. found that the severity score of coronary artery disease (CAD) had good correlation with AOPP quartiles,[117] hypothesizing a role of oxidative stress in the pathogenesis of CAD.

Witko-Sarsat et al proposed the measurement of AOPP as a reliable marker to estimate the degree of oxidant-mediated protein damage in uraemic patients and to predict the potential efficacy of therapeutic strategies aimed at reducing oxidative stress.[112;113]

2.2.3.2 Advanced Glycosylation End-Products (AGE)

Advanced glycation end products (AGE) are a heterogeneous group of molecules that accumulate in plasma and tissues with advancing age, diabetes, Alzheimer's disease and renal failure.[118]

In the nonenzymatic glycosylation, called the Maillard reaction, glucose attaches to the amino group of proteins, forming the reversible Schiff bases, that may rearrange to form more stable, Amadori-type early glycosylation products.

The early glycosylation products on collagen and other long-lived proteins in interstitial tissues and arterial walls undergo a slow series of chemical rearrangements to form irreversible AGE, which accumulate over the life-time.[119] This process occurs increasingly with time, thus representing a form of molecular senescence. Two such structures have been identified: **pentosidine** and **carboxymethyllysine** (CML) (Figure 2).[120]

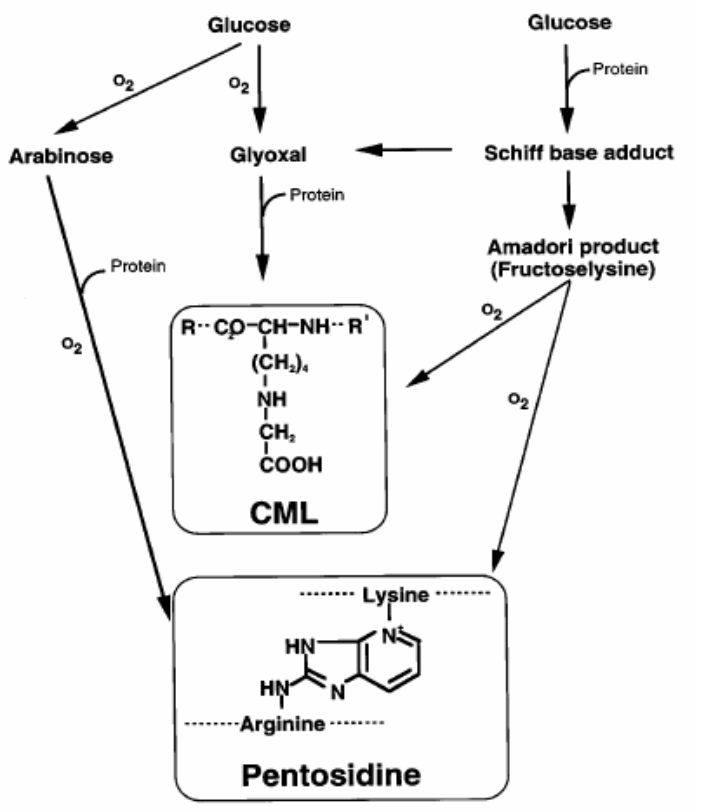


Figure 2. Reaction pathway of AGE formation. The first intermediate in the Maillard reaction occurring between glucose and protein is the Amadori product fructoselysine. Both pentosidine and carboxymethyllysine (CML) are formed by sequential glycation and oxidation reactions. CML is formed in two pathways: an oxidative cleavage of fructoselysine and a reaction of protein with glyoxal, an autoxidative product of glucose. Pentosidine is formed by a crosslink between lysine and arginine residues resulting from the glycoxidation of Amadori products or a reaction of arabinose, another autoxidative product of glucose. [120]

New evidence has challenged the former theories which stated that AGE formation involves primarily long-lived extracellular proteins. The current view is that AGE arise on short-lived molecules as well, including circulating plasma proteins and lipids.[121] They can form rapidly on nucleic acids and cytoplasmic proteins, leading to disrupted molecular conformation, impaired enzymatic activity, decreased degradation, and abnormal recognition and clearance by scavenger receptors. [122]

The plasma of CKD and dialysis patients, independently of diabetes mellitus, contains increased levels of AGE.[123] The importance of ESRD itself in the pathogenesis of AGE formation was underlined by one study enrolling HD patients,

which found no difference in AGE levels between diabetics and nondiabetics.[124] In CKD patients, AGE may comprise an important component of the uraemic middle molecule and may constitute a potential cause of the tissue damage and complications.[118] A significant relationship between AGE peptides and serum creatinine was observed and AGE concentration decreases rapidly after transplantation.[125] Hence, one could conclude that an important reason for the AGE accumulation is the decreased renal clearance, but the complete mechanisms are still enigmatic. For example, pentosidine is mainly linked to albumin, hence its accumulation cannot be fully attributed to a decreased removal by glomerular filtration.[126]

The most likely conceivable mechanism may involve the uraemia-specific metabolic alterations with subsequent oxidative stress leading to increased concentration of reactive carbonyls, derived from carbohydrates and lipids, such as glyoxal, methylglyoxal, malondialdehyde and 3-deoxyglucosone.[127] Like AOPP, AGE-pentosidine is closely related to the monocyte activation state in chronic kidney disease patients.[113]

Miyata et al have introduced the term "carbonyl stress" to denominate the exaggerated oxidative production of carbonyl groups in proteins, lipids, and sugars in CKD patients.[111] Reactive carbonyl compounds, which are unable, under normal circumstances, to augment the AGE production, can be detected in uraemia in concentrations up to tenfold higher of healthy subjects.[88]

AGE may have a role in the cardiovascular complications of uraemia,[118] enhancing both atherosclerosis and arteriosclerosis at many levels. AGE accumulation in long lived proteins such as vascular collagen reduce the elasticity of vessel walls.[128] AGE play a critical role in the development of vascular wall hyperplasia in uraemic subjects.[120]

2.3 INFLAMMATION

The altered metabolic milieu in uraemia may sustain a state of chronic inflammation.[26] Indeed, compelling studies have pointed out that chronic uraemia is associated with an inflammatory syndrome, which occurs early in the course of renal failure, is accentuated with the progression of uraemia, and reaches its zenith during dialysis therapy.[129;130]

Studies have indicated that pro-inflammatory cytokine levels are increased in predialysis. Furthermore, the levels of these cytokines and acute phase proteins rise significantly when patients are treated by HD.[131;132] Extracorporeal circulation of blood during HD may act as a repeated stimulus for an inflammatory response. The causes for the inflammatory syndrome in HD patients are complex. (Table 3) Bioincompatible membranes, such as cellulosic membranes, activate white blood cells and complement and can even exert effects on residual renal function.[101] Moreover, compatible membranes may activate inflammation as well. Some studies demonstrated very rapid and similar increases in the levels of inflammatory markers, regardless of the biocompatibility status of the used membranes, while others failed to confirm ~~these~~ results. It should be highlighted that even though HD using ultrapure dialysate and biocompatible membranes reduces CRP levels, it does not normalize it.[133]

Table 3. Mechanisms of the inflammatory syndrome in HD patients[88;101;129;132;134;135]

1. ~~exposure of circulating immunocompetent cells to the dialysis membrane~~
2. ~~generation of complement due to plasma protein-membrane contact~~
3. ~~membrane bioincompatibility~~
4. backfiltration of LPS/pyrogen-contaminated dialysate to the blood compartment
5. ~~Gore-tex~~ vascular access
6. prolonged use of catheters for vascular access
7. unrecognised infections
8. dialysis-related priming of mononuclear cells ~~so that they respond more vigorously to subsequent exposure to endotoxin~~
9. increased risk of infection as a consequence of impaired humoral and cellular immunity
10. decreased clearance of TNF and IL-1
11. increased carbonyl stress
12. angiotensin II

Several acute-phase proteins, such as C-reactive protein and fibrinogen, are directly associated with vascular disease.[136-138] Both CRP and fibrinogen are elevated in a significant proportion of ESRD patients,[139] independently the modality of renal replacement therapy. [140;141]

2.3.1 CRP as a Marker of Inflammation

CRP is an acute phase protein with still not completely elucidated functions. It is a marker of inflammation, a predictor of lesion formation, and an acute-phase reactant with proinflammatory, proatherogenic, and immune activities. It seems to be important for non-specific host defence, acting as an opsonin for bacteria, parasites, and immune complexes, and activating the classical pathway of complement.[142] It has also been recently shown to bind LDL and VLDL and to induce adhesion molecule expression in endothelial cells. In addition, CRP directly modulates endothelial dysfunction, down-regulating the NO production while augmenting endothelin-1 synthesis. Furthermore, CRP is enriched in atherosclerotic plaques, with a possible role in the pathogenesis of lesion formation, plaque rupture, and coronary thrombosis.(reviewed in [135;143])

CRP should be regarded as an indirect, albeit important, measure of endothelial function.[143] CRP, as an indicator of inflammation, is associated with an increased risk of cardiovascular death.[136] This is true for both apparently healthy subjects[138] and patients with established cardiovascular disease.[130] Ridker et al have shown that even a single point measurement of CRP predicts cardiovascular death several years in advance, independently of other risk factors.[138] A breakthrough study in the general population suggest that even CRP increases previously deemed to be insignificant, up to 3 mg/l, are associated with increased cardiovascular risk.[144] A 3-year longitudinal study in 424 HD patients showed CRP to be a significant predictor of mortality even after adjustment for diabetes, albumin levels, ferritin and, age.[87] However, it is still a matter of debate, how can a single measurement of a protein with a short half-life deliver important information concerning the long-term prognosis of patients.[145] A very recent study by Ridker et al. yielded an even more astonishing result. Patients who have low CRP levels (lower than 2 mg/l) after statin therapy have better clinical outcomes than those with higher CRP levels, regardless of the resultant level of LDL cholesterol.[146]

Various studies have demonstrated that CRP is elevated in a significant proportion of praedialysis and ESRD patients on either haemodialysis or peritoneal dialysis.[139] Furthermore, the increase in CRP levels has been shown to be related to renal function in patients with chronic kidney disease not on dialysis: the lower the creatinine clearance, the higher the CRP levels.[147] The acute phase reactants and inflammatory cytokins may lead to the increased morbidity and mortality.[1]

The link between inflammation and cardiovascular complications may be endothelial dysfunction. The question arises whether inflammation merely reflects the inflammatory nature of endothelial dysfunction leading to atherosclerosis and arteriosclerosis or it contributes directly to the development of vascular alterations. The current paradigm is that inflammation causes endothelial injury or dysfunction, leading to impaired endothelial dependent vasodilatation, smooth muscle proliferation, and possibly increased stiffness.[25] CRP has inflammatory effects on human endothelial cells and may injure them directly.[87;134] Cleland et al observed that increasing CRP concentration is accompanied by significant ~~decreasing~~ of basal NO generation, concluding that there is an association between endothelial dysfunction and low-grade chronic inflammation.[148] Fichtlscherer et al revealed that an elevated CRP level was associated with impairment of endothelium-dependent but not endothelium-independent vasodilatation in patients with coronary artery disease and that CRP normalization led to improvement.[149] They hypothesised that the temporary endothelial activation with ensuing vasodilator dysfunction in response to systemic inflammatory stimuli might play a role as a transient risk factor for acute cardiovascular events. The same may be true for reversible functional arterial stiffness.

Additionally, inflammation can enhance cardiovascular mortality through many mechanisms, by leading to and upholding oxidative stress, procoagulation, and malnutrition. Indeed, a study led by Mezzano et al indicated significant, positive correlations of acute-phase proteins with most markers of oxidative stress, endothelial dysfunction and haemostatic activation.[150] The exact mechanisms linking inflammation to vascular disease are poorly understood. The modern trend is to consider atherosclerosis an inflammatory disease.[25] The cellular interactions in atherogenesis are fundamentally no different from those in chronic inflammatory–fibroproliferative diseases such as cirrhosis, rheumatoid arthritis, glomerulosclerosis, pulmonary fibrosis, and chronic pancreatitis. In addition, inflamma-

tion has been recognized as a critical component in determination of the atherosclerotic plaque stability.[25] Moreover, CRP may play a role in the recruitment of monocytes during atherosclerosis. The inflammatory response may modify plasma protein composition and raise fibrinogen levels.[87] CRP was identified in necrotic atherosclerotic lesions, thickened intimas, and early plaques. CRP stimulates tissue factor production by monocytes.

2.4 MALNUTRITION AND MIA

Malnutrition is common in patients with chronic kidney disease; its incidence varies according to different studies between 23 and 76% in haemodialysis patients. They have reduced body weight, loss of muscle protein, depleted energy stores, and low levels of serum albumin, transferrin, pre-albumin and other visceral proteins.[151]

There are many determinants of protein malnutrition, e.g. poor food intake because of anorexia, nausea and vomiting due to uraemic toxicity, hormonal derangements, acidosis and increased resting energy expenditure, nutrient losses in the dialysate, co-morbidity, including chronic heart failure, inflammatory and infectious complications.[1;151-153] An additional cause of the exaggerated protein wasting may be the excess of parathyroid hormone (PTH).[152] Many investigators pointed out that uraemia is a catabolic state, while others maintain that there are few data to support this fact.[152] They sustain that other than protein and amino acid losses, additional hypercatabolism related to dialysis is not of a dramatic degree. Nevertheless, data regarding albumin synthesis are controversial; Caglar et al.[131] reported an increase in the albumin fractional synthetic rate during haemodialysis.

Among the most important determinants of malnutrition is cited inflammation with the inherent cytokine release. Tumour necrosis factor not only suppresses protein synthesis, but also induces anorexia.[1] Several studies show malnutrition to be associated with increased levels of CRP,[130;153] in accordance to the concept of albumin being a negative acute phase protein.

It has been conjectured that there may be two forms of malnutrition in HD patients: (1) a malignant form that is induced by inflammation and is associated

with poor prognosis and (2) a more benign form unrelated to inflammation with little or no important consequences for clinical outcome.[151]

It is well established that a low serum albumin level is a strong independent predictor of total and cardiovascular mortality in HD patients.[130;151;154] Furthermore, a recent cross-sectional study showed a strong association of albumin level with prevalence of atherosclerosis.[155] Stenvinkel et al.[2] proposed the existence of a syndrome consisting of malnutrition, inflammation and atherosclerosis (MIA syndrome) in some patients with CKD. The correlation between the high prevalence of inflammation, malnutrition and cardiovascular disease may be accounted for by the elevated levels of pro-inflammatory cytokines. Himmelfarb et al. put forth the paradigm according to which the link between inflammation, hypoalbuminemia, and subsequent cardiovascular risk may be through the process of oxidative stress.[105] However, it is actually not clear if malnutrition itself is an independent cause of poor outcome and cardiovascular disease or just a secondary marker.[156]

The extent and the nature of the correlation between malnutrition, cardiovascular complications, and inflammation is not exactly known. Albumin and CRP levels are not always correlated. It is likely that the inflammatory cytokines represent the cause of malnutrition, but inflammation itself may be induced by inadequate protein intake due to anorexia. However, which one ever is the initial cause, in the end a vicious circle may develop, in which inflammation begets malnutrition and vice-versa.

2.5 PROCOAGULATION

CKD is characterised by disturbances in the haemostatic balance, including both bleeding diathesis and thromboembolism. Among the multiple haemostatic abnormalities present in uraemia, to be mentioned are increased levels of factor VII, factor VIII, von Willebrand factor (vWF) activity, fibrinogen, and D-dimer and decreased activities or levels of clotting inhibitors (antithrombin III, protein C and S), diminished fibrinolytic activity, and platelet hyperaggregability. (reviewed in [157;158])

Hyperfibrinogenaemia is a risk factor for cardiovascular events in the general population.[137;159] Its levels correlate with vascular disease and predict the severity

and incidence of ischaemic complications, of carotid artery stenosis.[160] Plasma fibrinogen is significantly elevated in CKD patients compared to healthy controls, independently of replacement therapy.[140;141] Furthermore, among CKD patients, those who suffered cardiovascular events had significantly higher fibrinogen levels than those who did not.[141]

One factor leading to high plasma fibrinogen levels in dialysis patients can be inflammation, as IL-6 can stimulate the transcription of fibrinogen genes and suppress albumin synthesis, as part of the acute phase response.[140] Furthermore, fibrinogen levels rise in response to monocyte-derived cytokines, such as IL-1 and TNFα.[141] Haemodialysis, with extracorporeal circulation and exposure to heparin, may also contribute to the prothrombotic state due to recurrent platelet stimulation leading to their hyperaggregability, reduced levels of heparin cofactor II, and increased concentrations of coagulation factors.[161] Moreover, HD enhances fibrinogen and IL-6 production.[131]

Fibrinogen may contribute to atherogenesis by increasing plasma viscosity, red blood cell and platelet aggregation.[140] The increased plasma fibrinogen level in dialysis patients was found in association with markers of endothelial dysfunction.[141] and by direct incorporation into the injured endothelium where it localizes with LDL.[140]

2.6 CALCIFICATION

Another issue in ESRD patients is the altered calcium-phosphate metabolism, which leads to both more intensively calcified atheromatous plaques and diffuse medial calcification, i.e. premature and accelerated aging.[162;163] Calcification is described at two sites in the arterial wall: the intima and the media. The intimal calcification constitutes an advanced stage of atherosclerosis and is associated with the development of plaques and occlusive lesions.[164] The heavy calcification of the plaques in uraemic patients may also explain the notoriously high reocclusion rate following PTCA.[162] In contrast, medial calcification, or Mönckeberg's arteriosclerosis, observed with predilection in muscle-type conduit arteries, is commonly associated with aging, diabetes, hyperparathyroidism, and ESRD.[164;165] Diffuse calcification of the media is non-obstructive, but it could contribute to the increased stiffness. Another distinction between the two forms is

based on the fact that medial calcification appears to be amorphous rather than organized bone tissue. However, areas of atherosclerosis-associated calcification can appear amorphous, particularly in early stages. Hence, there seems to be a great extent of clinical and pathogenic overlap between the two conditions.[166]

Arterial medial calcification is a powerful and independent prognostic marker for all-cause and CV mortality in chronic HD patients.[167] Mediacalcosis of the aorta was found to lead to an increased aortic PWV and to be associated with an elevated CaPP.[32] The presence of medial calcification is linked to increased CV risk.[168] While intimal calcification was shown to be apparently associated with generalized atherosclerosis and not specifically attributable to HD, medial calcification seems to be much more closely associated with HD treatment and its duration. Chronic low-grade inflammation and malnutrition favours the occurrence of both types of calcifications in ESRD.[167]

Of note, a recent study using the non-calcium-containing compound sevelamer has shown that the administration of this compound, but not that of calcium binders, can prevent the progression of arterial calcifications in dialysis patients, as detected by electron beam computed tomography.[169]

2.7 DYSLIPIDAEMIA

The lipid status is altered in patients with advanced renal failure, the main abnormality being hypertriglyceridaemia, in the form of triglyceride(TG)-rich lipoproteins (very low-density lipoproteins, VLDL, and intermediate-density lipoproteins, IDL). The cause seems to be a reduced lipolysis of TG-rich VLDL that leads to the accumulation of partially metabolised ‘remnant' lipoproteins. This lipoprotein catabolism impairment is usually associated with reduced levels of HDL, affecting reverse cholesterol transport.[104] The results of the Monitored Atherosclerosis Regression Study[170] indicate the importance of triglyceride-rich lipoproteins as an independent risk factor for the progression of coronary artery disease. Their accumulation in ESRD may considerably contribute to progression of atheromatous disease in renal patients. Indeed, Shoji et al.[171] found IDL to be independent risk factors for aortic arteriosclerosis, as assessed by means of PWV in haemodialysis patients.

Although the LDL levels are largely within the normal range, LDL from HD patients is enriched with dense LDL and an electronegative LDL (LDL–) subfractions, representing potentially atherogenic subpopulations of LDL. Both dense LDL and LDL– are characterized by impaired binding to the LDL receptor and high susceptibility to oxidation. (reviewed in [172]) Furthermore, Kronenberg et al. demonstrated higher levels of serum lipoprotein(a) (Lp(a)) in dialysis patients as compared to healthy controls.[173] Lp(a) is stated to be an independent risk factor for atherosclerotic complications in haemodialysis patients.[2]

Additionally, ongoing or prior nephrotic syndrome is also associated with hyperlipidaemia and hypercoagulability, which increase the risk of occlusive vascular disease.[174]

Although until now mainly the roles of lipoproteins in atheroma development have been highlighted, their pathogenic roles are very likely also exercised at the levels of endothelial cells and as such may also induce functional arterial stiffening even before the establishment of full-blown atherosclerosis.

Hypercholesterolaemia belongs to the group of cardiovascular risk factors, including smoking and hyperhomocysteinaemia, which have been reported to be significantly related to endothelial dysfunction, as indicated by decreased flow-dependent vasodilatation. Furthermore, increased oxidative stress and impaired anti-oxidant activity may lead to enhanced formation of oxidised LDL, which is known to have proinflammatory effects and to lead to endothelial dysfunction.[25;109]

3 STUDY DESIGN AND METHODS

3.1 Subjects

52 Caucasian stable ambulant end-stage renal disease patients, aged 59,37 ± 15,81 years, who had been on maintenance haemodialysis for more than 4 months (67,92 ± 47,76 months) were included in the study. Among them there were 22 women (42,3%) and 30 men (57,7%). Patients with active systemic diseases, malignant tumours, clinical signs of acute infection, or taking antibiotics were not enrolled. The patients were dialyzed three times per week either in the Darmstadt Hospital (n=10) or in another outpatient dialysis centre in Darmstadt (n=42). The duration of the dialysis sessions was individually adjusted (3-5 hours) to maintain body fluid homeostasis and to achieve a Kt/V of > 1,2. Patients were dialysed with a bicarbonate dialysate. The dialysate calcium (1.25, 1.5 or 1.75 mmol/l) was chosen according to the serum calcium–phosphate equilibrium and the necessity to use 1,25-dihydroxy vitamin D3 to control the parathyroid hormone (PTH) levels. When necessary, the patient were administered intravenous iron and erythropoietin preparations. Antihypertensive drugs and statins were prescribed when necessary.

All the subjects gave their written informed consent and the study protocol was approved by our Ethical Committee. Anamnestic information was collected regarding lifestyle, antecedent cardiovascular events, including myocardial infarction, angina pectoris, cerebrovascular disease, and peripheral vascular disease. We are aware that designation of coronary artery disease would have been more reliable had it been performed by coronary angiography. Data regarding primary renal disease, comorbid conditions, and medication were recorded.

3.2 Pulse Wave Velocity

First, the patient's resting supine brachial blood pressure was measured with a standard sphygmomanometer, considering phases I and V of the Korotkoff sounds as the systolic BP (SBP) and diastolic BP (DBP), respectively. Arterial blood pressure may be divided into 2 components: a steady component (mean blood pressure [MBP]), which is the pressure that may be present in the vascular system during a given cycle when cardiac output is nonpulsatile, and a pulsatile

component (pulse pressure [PP]), which is the oscillation around mean arterial blood pressure.[175] MBP and PP were calculated by using the following formulas: MBP=2⁄3DBP+1⁄3SBP and PP=SBP-DBP.[176]

The biomechanics of blood flow makes the velocity of the heart-generated wave a valuable marker for the assessment of arteriosclerosis.[32] Since fluid is contained in a system of elastic conduits, energy propagation occurs predominantly along the arterial wall and not through the incompressible blood. The material properties of the arterial wall, its thickness, and the lumen diameter thus become the major determinants of PWV. This concept has been formalized in a mathematical model in which PWV is given by the Moens-Korteweg equation:

$$PWV = \sqrt{Eh/2\rho R}$$

where E is Young's modulus of the arterial wall; h is wall thickness; R is arterial radius at the end of diastole; ρ is blood density.[23]

The carotid-radial pulse wave velocity was measured non-invasively, using a semiautomatic device, Complior SP, Artech Medical, France. The device records the same pulse wave simultaneously at the carotid and radial artery, respectively, using direct-contact pulse sensors and displays the waveforms. When the operator observes a pulse waveform of sufficient quality on the computer screen, calculation of the time delay between the two pressure upstrokes is initiated. The device determines the mean transit time delay (Δt) of the pulse wave between those two points from 10 consecutive cardiac cycles and calculates the PWV automatically as the quotient PWV = D/Δt (Figure 3). The distance (D) travelled by the by pulse wave was measured over the body surface. The measurements were made during the haemodialysis session, since a previous study has shown haemodialysis not to have acute effects on PWV.[177] Since the PWV is independent of blood pressure in patients with ESRD[62] the PWV data were not standardized by blood pressure in the present study. All the measurements were performed by the same investigator. The method, extensively employed in clinical studies, was shown to be precise and reproducible, with intraobserver and interobserver repeatability coefficients of 0,935 and 0,890, respectively.[23]

The accuracy of the PWV value depends on the precise measurements of the transit time and the length of the vascular segments. The maximum error regarding the measured pulse wave transit time is less then 1%. The more signifi-

cant source of errors may be the determination of the vessel length over the body surface, an approximation that might underestimate the vascular length, especially in elderly patients with unfolded tortuous aorta. However, the overall estimated maximum error is less than 10% and despite these limitations, measurement of PWV is strongly correlated to direct measurements of arterial distensibility.[13]

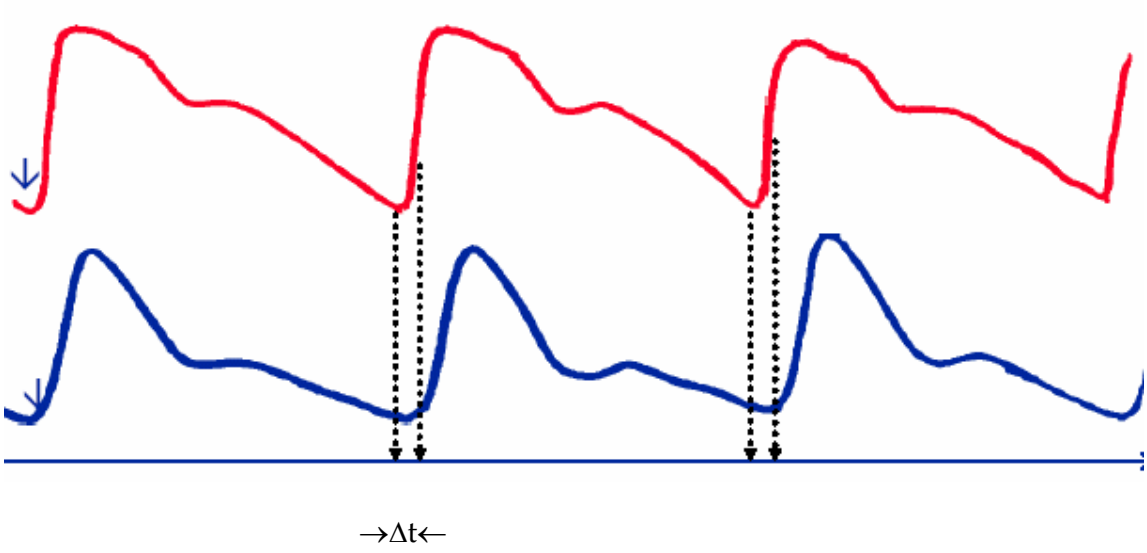


Figure 3. The upper and the lower curves represent the proximal, carotid, and the distal, brachioradial, pulse waves, respectively.

Lantelme et al.[70] raised substantial methodological concern about the accuracy of aortic PWV determination, pointing out to an intraindividual variability of PWV at different heart rates. However, we measured peripheral PWV, which was shown in a large cohort study[72] to be considerably less dependent on heart rate than aortic PWV.

3.3 Blood Parameters

Blood was drawn from the fistula needle using the appropriate vacuum tubes immediately before starting the midweek dialysis session. Most of the biochemical parameters were assessed by using routine laboratory methods.

The parameters AGE, AOPPs, and CRP were assessed in the Gambro Medical and Biological Research laboratory, Hechingen. For that purpose 5 ml blood was

collected in standard sterile EDTA polystyrene vacuum tubes. Following centrifugation at 2000 g for 10 minutes, the plasma was stored in 500 µl aliquots, frozen at –26 degrees until analysis. AGE were measured as plasma fluorescence intensity according to Henle et al[124]. The plasma samples were diluted 1:50 with phosphate buffered saline (PBS). Fluorescent AGE intensity was then measured using a spectrofluorimeter at 430 nm after excitation at 350 nm.

The methodology for AOPP quantification relies on their spectrophotometric properties, which are the absorbance at 340 nm in acidic conditions, as described by Witko-Sarsat et al. [113] Briefly, AOPP were measured by spectrophotometry on a microplate reader and were calibrated with chloramine-T solutions that in the presence of potassium iodide absorb at 340 nm. In test wells, 200 µl of plasma diluted 1/5 in PBS was placed on a well microtiter plate, and 20 µl of acetic acid was added. In standard wells, 10 µl of 1.16 M potassium iodide was added to 200 µl of chloramine-T solution (0–100 µmol/liter) followed by 20 µl of acetic acid. The absorbance of the reaction mixture is immediately read at 340 nm on the microplate reader against a blank containing 200 µl of phosphate-buffered saline (PBS), 10 µl of potassium iodide, and 20 µl of acetic acid. The chloramine-T absorbance at 340 nm being linear within the range of 0 to 100 µmol/liter, AOPP concentrations were expressed as micromoles per liter of chloramine-T equivalents.

As standard assays for CRP lack the sensitivity needed to determine levels of inflammation within normal range,[144] we measured high sensitive CRP (hs-CRP) using ELISA.

3.4 **Statistics**

Statistical analysis was performed using the computer software SPSS for Windows (Statistical Package of Social Science, 12.0, 2003, SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± standard deviation of the mean (SD). Simple regression analysis and the Spearman univariate correlation coefficient (r) were used to determine the relationships between the various parameters. Correlations between PWV as the dependent variable and various independent variables were assessed by multiple regression analysis.

Attempts to explore the relationships of these and other factors with arterial disease face the inherent difficulty of multicolinearity. To avoid this predicament established statistical techniques which control for confounding factors are used.

Two sets of variables were used with two different variable selection methods yielding three models of multiple regression. For the first set we chose the "enter" and for the second models both the "enter" and the "backward" method. The two methods allow different ways for the independent variables entry into the analysis. Using the "enter" method, the variables are entered in a single step into the model. The "backward" belongs together with "stepwise" and "forward" to the stepping methods. All the variables enter in the block in a single step and are then removed one at a time based on either the significance (probability) of the F value or the F value itself. All variables must pass the tolerance criterion to be entered in the equation, regardless of the entry method specified. The default tolerance level is 0,0001. Also, a variable is not entered if it would cause the tolerance of another variable already in the model to drop below the tolerance criterion.

The variables entered in the first model were the ones known to influence PWV from other studies (see above) age, PP, heart rate, dialysis duration. To account for their influence on PWV, the common cardiovascular risk factors, i.e. diabetes hypertension, and the serum levels of total cholesterol, HDL, LDL, triglycerides were included in the model. Finally, the variables most interesting for this study, serum levels albumin, fibrinogen, CRP, AGE, AOPP, phosphate, and calcium also entered the first model. However, the results may be affected by another confounding factor, the patients' medication. As summarized above, different drugs have a beneficial effect on the pulse wave velocity. Many patients were taking such drugs, i.e. ACE-inhibitors or angiotensin II receptor-1 blocker, calcium-channel blocker, 3-hydroxy-methyl-glutaryl-CoA-synthetase inhibitors (statins), and calcium containing phosphate-binding drugs. Only those taking such medication on a chronic basis were considered. We are aware that it is not a homogeneous standardized approach, that some had been taking one or the other drug for a longer period than the others. However, we could not analyse data and draw conclusion as to the pathogenesis of arteriosclerosis without taking the effects of medication into account. Hence, in the second model we used all the variables already studied the first model plus whether the patients had been taking calcium-based phosphate binder, ACE-inhibitors, statins, or cal-

cium-channel blocker. Smoking, though an important cardiovascular risk factor, known to enhance the PWV[31;65] did not enter any of our models because only 4 patients smoked.

The patients were divided in groups according to the presence or absence of hypertension, diabetes mellitus, and antecedent arterial disease, to investigate whether and which of the biochemical and clinical parameters differed. As most parameters compared did not have a normal distribution, nonparametric tests (Mann Whitney U and the Wilcoxon W) were used to weigh up the differences between these groups.

The significance level was set at a value of 0,05.

4 RESULTS

4.1 Patient Clinical and Biochemical Characteristics

The aetiologies of the ESRD in our study group are listed in Table 4. The mean age was 59,37±15,8 (range 24-83). The patients had spent a minimum of 4 months on HD, mean 67,92 ± 47,76 months. The body mass index (BMI) was 25,2 ± 3,74 kg/m². A total of 18 subjects (34%) had antecedent cardiovascular events, as follows: 10 patients (19,2%) had coronary artery disease, with 7 infarcts; 7 patients (13.4%) suffered from peripheral artery disease, and 7 (13.4%) cerebrovascular disease, with 3 strokes. (Table 5) With regards to smoking habits only 4 patients (7,66%) were current smokers. Blood pressure, pulse pressure, heart rate, and pulse wave velocity can be seen in Table 6.

Table 4. Aetiologies of the ESRD

Aetiology	Frequency	Percent
Acute septic-toxic renal failure	1	1,9
ADPKD	5	9,6
Alport syndrome	1	1,9
Anephric	1	1,9
Chronic pyelonephritis	3	5,8
Cystic renal disease	2	3,8
Diabetic nephropathy	8	15,4
Interstial nephritis	2	3,8
Medullary cystic disease	1	1,9
Membranoproliferative glomerulonephritis	3	5,8
Mesangial IgA glomerulonephritis	1	1,9
Nephrosclerosis	6	10,5
Pauciimmune glomerulonephritis cANCA+	1	1,9
Systemic sclerosis	1	1,9
Vascular cause	1	1,9
Unknown	12	23,1

Table 5– The clinical details concerning cardiovascular risk factors and events.

Clinical Details	**Patients**
Age (years)	59,37 ± 15,81
Gender M/ F	30/ 20
Time on HD (months)	67,92 ± 47,76
BMI	25,2 ± 3,74
Diabetes	11 (21%)
High cholesterol	18 (34,61%)
Hypertension	42 (80,8%)
Hypoalbuminaemia	2 (4%)
Angina	10 (19,2%)
MI	7 (13,4%)
TIA	7 (13,4%)
Stroke	3
PVD	7 (13,4%)

MI is myocardial infarction, TIA transitory ischemic attack, and PVD peripheral vascular disease.

Table 6. Clinical Parameters

	Minimum	Maximum	Mean	Std. Deviation
SBP	80	180	131,69	17,625
DBP	50	100	71,35	9,408
PP	30	107	60,35	16,954
Heart rate	56	117	70,38	17,484
PWV	3,6307	18,82350	8,7050546	2,0889123

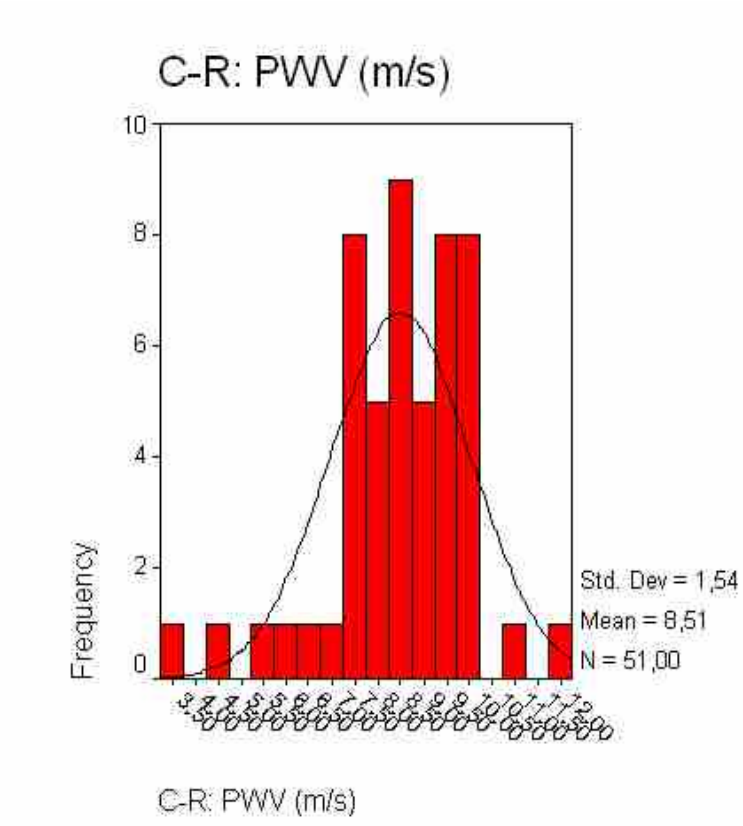
Some patients received erythropoietin preparations (75%) and intravenous iron (63,5%) for renal anaemia. As expected, the ones receiving iron had significantly higher ferritin levels. Patients regularly took vitamin supplements. Phosphate binders (calcium acetate, calcium carbonate, algeldrat) were used to try to maintain serum phosphate within normal range. The patient's drugs are listed in Table 7. Note that a large percentage of the group were receiving antihypertensive or lipid-lowering therapy. Furthermore, 25% patients took aspirin on a regular basis. This highlights the intrinsic difficulty of studying this patient group, for the reason that there are a number of confounding factors such as treatment and pre-existing vascular disease, which may independently affect vascular structure and function.

Table 7. Drugs

Drug Category	**Pat. Number (%)**
Statins	11 (21,15%)
ACE-inhibitors	16 (30,7%)
Angiotensin II receptor-1 antagonists	7 (13,46%)
Calcium-channel blockers	16 (30,7%)
β-blockers	36 (69,23%)
Diuretics	12 (23%)
Erythropoietin-preparations	39 (75%)
Intravenous iron	33 (63,5%)
Phosphate-binders (total)	40 (77%)
Calcium-based phosphate-binders	36 (69,23%)
Activated vitamin D preparations	17 (32,7%)
Aspirin	13 (25%)

The carotid-radial PWV was 8,7 ± 2,08 m/s (range 3,6 to 18,8 m/s) with no significant differences between diabetics versus non-diabetics, patients with antecedent

cardiovascular events versus those without. (Figure 4) Of note, one patient had a very high PWV, 18,8 m/s, possibly explained by her underlying disease, systemic sclerosis. As an outlier, her PWV value was excluded from the graphic.



The biochemical parameters can be examined in the Table 8. Hs-CRP levels were significantly elevated, such that 54% patients had levels over 3 mg/ l, marking increased cardiovascular risk, according to Ridker et. al.[138;144] The high fibrinogen levels fit this result: 25% of the patients had fibrinogen levels higher than the upper margin of our laboratory. Note that only 4% of our patients were malnourished, although numerous reports indicate that there is a high rate of protein-energy malnutrition, ranging from 18% to 75%, in HD patients.[1] 67,3% of the patients (N=35) had hyperphosphataemia. The total cholesterol levels were elevated in 35,3% of the study participants. However, as described in other studies involving HD patients highlighted above, the predominant lipid abnormality was not the high LDL levels (prevalent only in 17,6%), but the low HDL (in 61,76%) and high triglyceride values (in 38,46%). The AOPP levels were 469,67 ± 43,82, higher than in other described ERSD populations (137,6 +/- 11,1 μmol/l in haemodialysis

patients versus 29,4 +/- 4,9 μmol/l in controls[113] or 74±8 μmol/l in coronary artery disease patients versus 21±4 μmol/l in controls.[117])

Table 8. Biochemical Parameters

Parameter	Mean	Standard Deviation
Calcium (mmol/l)	2,26	0,2
Phosphate (mg/dl)	5,7	1,7
CaPP	65,81	78,9
Parathormone (pmol/l)	34,6	41,39
Creatinine (mg/dl)	9,7	2,07
Urea (mg/dl)	146,67	33,2
Hemoglobine (g/dl)	11	1,34
Hematocrit	33,96	4,07
Triglycerides (mg/dl)	211,84	144,28
Cholesterol (mg/dl)	186	46,9
HDL (mg/dl)	45,68	13,74
LDL (mg/dl)	114,7	40,74
Albumin (g/dl)	41,08	3,78
Fibrinogen (mg/dl)	390,5	89,3
hs CRP (mg/l)	6,94	9,99
AGE (IU)	574,17	111,94
AOPP (nmol/ml)	469,67	43,82

4.2 Correlations Between Parameters

The correlation matrix (Table 9) shows the interrelationships between various biochemical and clinical parameters.

Note the positive correlations between fibrinogen and CRP; AGE and albumin; fibrinogen and CaPP; fibrinogen and AOPP; CaPP and AGE. No correlation was found between AOPP and AGE, although they are both markers of oxidative stress and AGE-pentosidine was shown by Witko-Sarsat et al to be significantly associated to AOPP[112], suggesting that the oxidative pathways and susceptibilities of the molecules are not the same in different patients. Only AGE, not AOPPs were found to be correlated to serum albumin.

We did not find any correlation between albumin and CRP, or fibrinogen, in spite of many reports in literature, as emphasized above. However, the incidence of malnutrition in our patient collective was very low (4%).

The negative correlation between AOPP and PWV can be seen in Figure 6, which displays the simple regression.

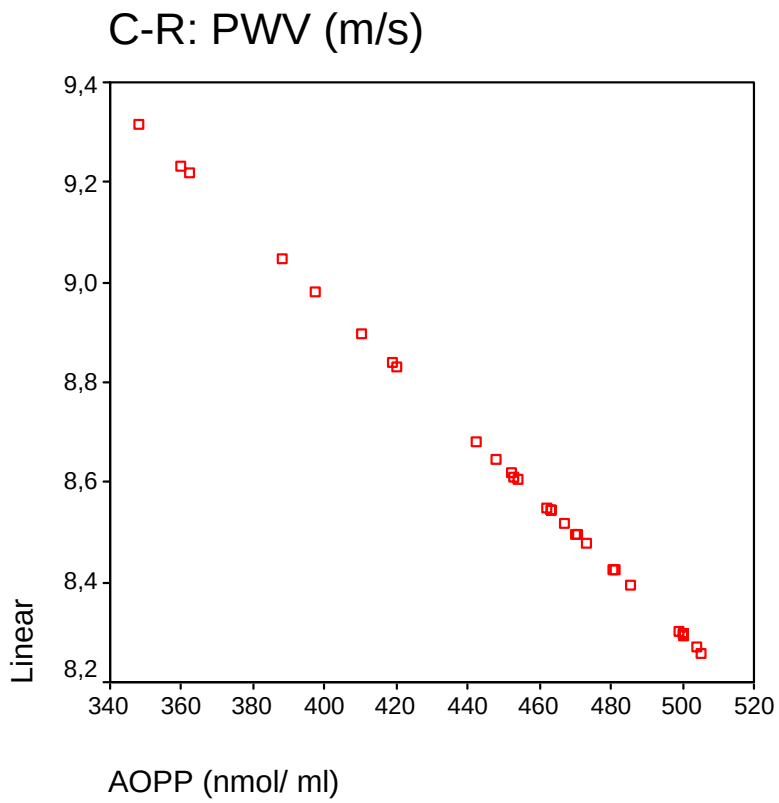


Figure 6. Simple regression. The negative correlation between AOPP and PWV

Table 9. Bivariate Correlations

		CRP	AGE	AOPP	Albumin	Fibrinogen	CaPP	Calcium
CRP	Pearson Correlation	1	-,180	,117	-,139	,515(**)	-,006	-,287(*)
	Sig. (2-tailed)	.	NS	NS	NS	,000	NS	NS
AGE	Pearson Correlation	-,180	1	,162	,304(*)	-,098	,317(*)	,323(*)
	Sig. (2-tailed)	NS	.	NS	,036	NS	,024	,021
AOPP	Pearson Correlation	,117	,162	1	,244	,311(*)	,003	,182
	Sig. (2-tailed)	NS	NS	.	NS	,031	NS	NS
Albumin	Pearson Correlation	-,139	,304(*)	,244	1	-,014	,388(**)	,257
	Sig. (2-tailed)	NS	,036	NS	.	NS	,006	NS
Fibrinogen	Pearson Correlation	,515(**)	-,098	,311(*)	-,014	1	,284(*)	,158
	Sig. (2-tailed)	,000	NS	,031	NS	.	,048	NS
CaPP	Pearson Correlation	-,006	,317(*)	,003	,388(**)	,284(*)	1	,389(**)
	Sig. (2-tailed)	NS	,024	NS	,006	,048	.	,004
Calcium	Pearson Correlation	-,287(*)	,323(*)	,182	,257	,158	,389(**)	1
	Sig. (2-tailed)	,041	,021	NS	NS	NS	,004	.

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level (2-tailed).

In multiple regression analysis CRP was additionally correlated to the dialysis duration (p=0,04) and the association between CaPP and AGE remained statistically significant (p=0,027).

4.3 Multiple Regression Analysis

4.3.1 First Model

In a multiple linear regression model (Table 10), PWV was found to be positively, significantly correlated to serum calcium (p=0,004) phosphate (p=0,013), triglycerides (p=0,002), LDL (p=0,003), AGE (p=0,045). The correlation to CRP was of borderline significance (p=0,059). Intriguingly, we found a strong negative association between PWV and AOPP levels (p = 0,005). The dialysis duration (months spent on haemodialysis) was also negatively correlated to PWV (p = 0,005); however, using curve estimation regression, a quadratic relationship (Figure 5) became apparent (significance 0,02). We were unable to show a correlation between carotid-radial PWV and age, BP, or heart rate.

The variable most strongly associated with PWV in this model (i.e. highest t value) was the triglyceride level.

The unstandardized coefficients are the coefficients of the estimated regression model. The standardized coefficients or betas are an attempt to make the regression coefficients more comparable, because often the independent variables are measures in different units. The t statistics yields the relative importance of each variable in the model. Useful predictors have t values well below -2 or above +2.

This model was shown to be highly significant (p=0,004) and to explain 87,8% of the variance of PWV. (Tables 11 and 12)

Table 10. Multiple Linear Regression Coefficients(a) (Model)

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	-33,848	15,385		-2,200	,048
age	-,001	,046	-,007	-,028	NS
PP	-,070	,048	-,405	-1,459	NS
Heart rate	-,002	,043	-,010	-,036	NS
Dialysis duration	-,048	,014	-,754	-3,450	,005
Calcium	31,359	8,729	2,753	3,592	,004
Phosphate	7,835	2,683	5,019	2,921	,013
Triglycerides	,028	,007	1,109	4,053	,002
HDL	,058	,028	,337	2,091	NS
LDL	,044	,012	,723	3,681	,003
Albumin	-,088	,131	-,124	-,670	NS
Fibrinogen	,011	,009	,303	1,213	NS
CRP	,203	,097	,453	2,091	,059
AGE	,009	,004	,445	2,238	,045
AOPP	-,054	,016	-,871	-3,395	,005
P SYS	,026	,045	,168	,568	NS
diabetes	-2,130	1,018	-,333	-2,092	NS
Cholesterol total	-,056	,016	-,973	-3,442	,005

a Dependent Variable: C-R: PWV (m/s)

Table 11. Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	,938	,881	,702	1,40281317

R is the multiple correlation coefficient. The larger the R, the stronger the correlation. R squared represents the proportion of the variance in the dependent variable explained by the regression model. Because it tends to be an optimistic marker the adjusted R square, which is more realistic is also displayed.

Table 12. ANOVA(a)

	Sum of Squares	df	Mean Square	F	Sig.
Regression	174,442	18	9,691	4,925	,004
Residual	23,615	12	1,968		
Total	198,056	30			

a Dependent Variable: C-R: PWV (m/s)

This table summarizes the results of an analysis of variance (ANOVA). The sum of squares, degrees of freedom (df), and mean square are displayed for two sources of variation, regression and residual. The output for Regression displays information about the variation accounted for by our model. The output for Residual displays information about the variation that is not accounted for by our model. A model with a large regression sum of squares in comparison to the residual sum of squares indicates that the model accounts for most of variation in the dependent variable. Our first model explains 88% of the variance of PWV. Very high residual sum of squares indicate that the model fails to explain a lot of the variation in the dependent variable. The small significance value of the F statistic (smaller than 0,05) shows that the independent variables accurately explain the variation in the dependent variable.

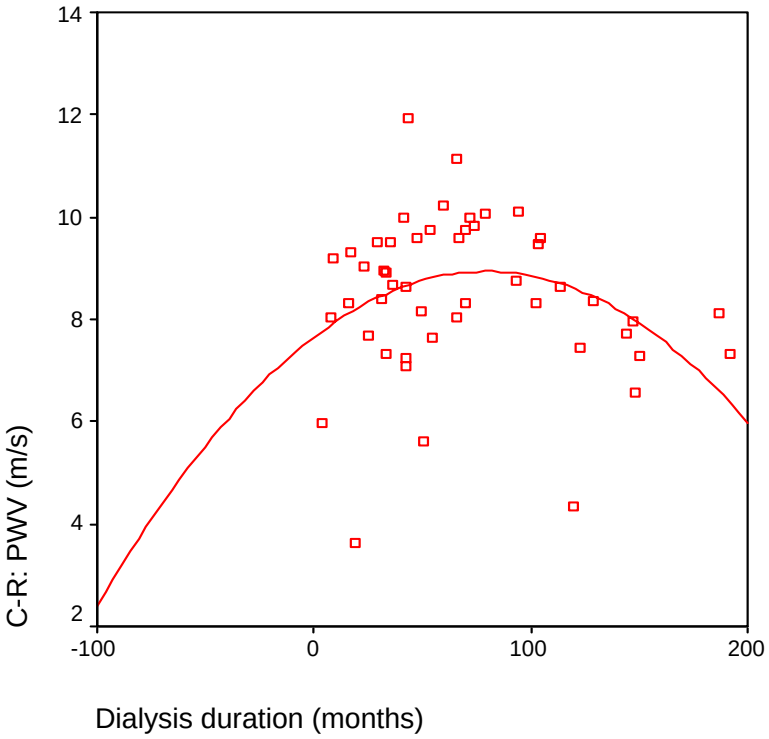


Figure 5. The quadratic relationship between PWV and the dialysis duration in months

4.3.2 Second Model: Enter Method

The results of the multiple regression analysis using the second set of variables and the enter method are shown in Table 13. The results coincide to a large extent with the first model. The variables positively correlated to PWV are calcium (p=0,000), phosphate (P=0,001), triglycerides (p=0,000), LDL (p=0,001), total cholesterol (p=0,003), AGE (p=0,002), CRP. Of note, the significance level of the CRP-PWV link is 0,003. Between PWV and AOPP levels there is a negative relationship (p=0,000). Among the drugs, the only ones correlated to PWV were the calcium-channel blocker (p=0,039). As expected, the patients taking calcium-channel blocker had lower PWV. A novel correlation unraveled in this model is the one with fibrinogen (p=0,015). The variables most strongly associated with PWV were triglyceride, calcium, and AOPP levels.

As outlined in tables 14 and 15, the second model is statistically superior to the first, i.e. its level of significance is better and it explains more of the variance of the dependent variable, PWV. The adjusted R square is 0,808 in the second model versus 0,702 in the first, the sum of squares amounts to 181,900 versus

174,442, and the significance is 0,003 versus 0,004. The second model explains thus 95% of the variance of PWV. Accordingly, this model will be discussed further.

Table 13. Multiple Regression Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	-59,773	15,203		-3,932	,004
PP	,0205	,029	,115	,716	NS
Heart Rate	-,03984	,024	-,244	-1,649	NS
Hypertension	,546	,702	,087	,777	NS
Age	-,01208	,041	-,067	-,293	NS
Dialysis duration	-,102	,019	-1,602	-5,423	,001
Calcium	40,689	6,467	3,461	6,292	,000
Phosphate	10,576	2,010	6,555	5,262	,001
Triglycerides	,03583	,005	1,463	7,095	,000
Cholesterol (total)	-,05747	,014	-,991	-4,254	,003
HDL	,03199	,027	,179	1,204	NS
LDL	,05090	,009	,834	5,386	,001
Albumin	,144	,108	,200	1,336	NS
Fibrinogen	,02681	,009	,689	3,092	,015
Diabetes	-2,047	,860	-,325	-2,380	NS
CRP	,451	,107	,872	4,229	,003
AGE	,01776	,004	,818	4,707	,002
AOPP	-,07411	,011	-1,206	-6,653	,000
Statins	-1,311	,747	-,238	-1,755	NS
ACE-inhibitors	-,07904	,668	-,014	-,118	NS
Ca-bas phos-bind	-,446	,631	-,083	-,708	NS
Ca-channel block	-3,067	1,244	-,573	-2,466	,039

a Dependent Variable: C-R: PWV (m/s)

Table 14. Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
2	,977	,954	,808	1,12426381

Table 15. ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Regression	181,900	21	8,662	7,737	,003
Residual	8,956	8	1,119		
Total	190,856	29			

4.3.3 Third Model: Backward Method

Backward regression analysis yields many models, each containing one variable less than the former. All the variables are entered first and then removed one at a time based on either the significance of the F value or the F value itself. ANOVA is given for each model (not shown). The last model is the one containing only the variables closest correlated to the dependent variable. The last model, including ANOVA is displayed in tables 16 and 17.

The significance values displayed are based on fitting a single model. Therefore, the significance values are generally invalid when the backward, like any stepwise method, is used. However, the results are important, for the reason that the remaining variables are the ones which influence PWV most. These were: dialysis duration, calcium, phosphate, triglycerides, total cholesterol, LDL, fibrinogen, CRP, AGE, AOPP, statins, calcium channel blockers. They are the same as with the enter method, except that statins are also negatively correlated to PWV.

Table 17. Multiple Linear Regression Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	-54,242	13,724		-3,952	,001
Dialysis duration	-0,09333	,013	-1,461	-6,915	,000
Calcium	41,168	6,234	3,502	6,604	,000
Phosphate	10,559	1,932	6,544	5,464	,000
Triglycerides	0,03326	,005	1,358	7,074	,000
Cholesterol total	-0,04871	,010	-,840	-4,807	,000
LDL	0,04179	,008	,685	5,330	,000
Fibrinogen	0,02639	,007	,678	4,052	,001
CRP	,437	,089	,846	4,921	,000
AGE	0,01615	,003	,744	4,848	,000
AOPP	-0,07056	,010	-1,148	-6,885	,000
Statins	-,960	,535	-,174	-1,794	,094
Ca-channel blocker	-2,628	,685	-,491	-3,837	,002

a Dependent Variable: C-R: PWV (m/s)

Table 16. ANOVA

	Sum Squares	ofdf	Mean Square	F	Sig.
Regression	175,933	15	11,729	11,003	,000
Residual	14,923	14	1,066		
Total	190,856	29			

4.4 Differences Between Groups

Using Mann-Whitney U and Wilcoxon W nonparametric tests, there were no differences in any biochemical parameter when comparing men versus women, patients with versus without diabetes mellitus, or patients with versus without hypertension. The patients with antecedent vascular events differed from those without only in respect to the age (p<0,0001). (Table 18) As expected, the ones with prior vascular events were older.

Table 18 Grouping Variable: Arterial disease?

	CRP	AGE	AOPP	PWV	Albumin	Fibrinogen	Cholesterol total	Age	Dial duration
Mann-Whitney U	253,000	240,000	269,500	273,000	219,000	261,500	256,000	86,500	270,000
Wilcoxon W	749,000	430,000	459,500	801,000	390,000	432,500	784,000	614,500	798,000
Z	-,830	-1,089	-,526	-,604	-1,086	-,181	-,935	-4,241	-,042
Asymp. Sig. (2-tailed)	,407	,276	,599	,546	,277	,856	,350	,000	,966

The Mann-Whitney U and the Wilcoxon W statistics yield identical conclusions. Small significance values (<0,05) indicate that the two groups are different for the analysed variable.

Between men and women two differences were noted (Table 19): the women in our group were older (p=0,002) and had lower cholesterol levels than the men (p=0,002). Other investigators have shown that women have 5% to 10% lower stiffness than men of the same age.[178] (334A) However, in our study that was not the case.

Table 19

	Cholesterol total	HDL	LDL	Albumin	Fibrinogen	CRP	AGE	AOPP	PWV	Age
Mann-Whitney U	165,000	171,000	139,500	210,000	292,500	298,000	268,000	296,000	278,000	160,000
Wilcoxon W	630,000	424,000	370,500	441,000	698,500	551,000	521,000	731,000	531,000	625,000
Z	-3,056	-,735	-1,395	-1,697	-,030	-,399	-,970	-,459	-,963	-3,152
Asymp. Sig. (2-tailed)	,002	,462	,163	,090	,976	,690	,332	,646	,335	,002

Grouping Variable: Gender

The diabetic patients surprisingly had significantly higher CaPP values (p=0,047).
The implications of this result remains to be clarified. (Table 20)

Table 20

	Triglycerides	Cholesterol total	HDL	LDL	Albumin	Fibrinogen	CRP	AGE	AOPP
Mann-Whitney U	223,000	218,000	113,500	121,500	194,000	200,500	217,000	201,000	172,500
Wilcoxon W	1084,000	284,000	158,500	586,500	935,000	266,500	1037,000	1021,000	238,500
Z	-,056	-,168	-,843	-,450	-,359	-,204	-,069	-,435	-1,141
Asymp. Sig. (2-tailed)	,955	,867	,399	,653	,719	,839	,945	,663	,254

Grouping Variable: Diabetes

Table 21

	Triglycerides	Choles. total	HDL	LDL	Albumin	Fibrinogen	CRP	AGE	AOPP	PWV
Mann-Whitney U	73,500	82,000	70,500	89,000	84,000	57,000	67,500	78,000	85,000	78,000
Wilcoxon W	94,500	547,000	535,500	554,000	549,000	522,000	532,500	99,000	106,000	543,000
Z	-,700	-,340	-,829	-,042	-,255	-1,402	-,955	-,509	-,220	-,509
Asymp. Sig. (2-tailed)	,484	,734	,407	,966	,799	,161	,339	,610	,826	,610

Grouping Variable: Hypertension

The hypertensive patients did not show any differences toward the normotensive ones with regard to biochemical or clinical parameters, including the pulse wave velocity (Table 21).

The same tests were applied to groups made according to medication. These tests were made only for observational and descriptive purposes only, as we are conscious that such a nonstandardized, cross-sectional study cannot make any statements as to the influence of various drugs on biochemical or clinical parameters. The analyses did not yield any differences and are not shown. Some examples: the patients taking statins did not have lower LDL levels than the one who were not, or the patients taking calcium-acetate did not have a greater CaPP than the ones who took algeldrat, or the ones taking aspirin did not have lower CRP levels.

5 DISCUSSION

Most studies report an association between chronic kidney disease and cardiovascular events. This is partly due to an abnormally increased arterial wall stiffness, which characterises CKD and ESRD patients, accounted for by either structural or functional changes in the vessel wall. The complex pathogenesis of arteriosclerosis in end-stage renal disease patients is not completely elucidated. The functions of the vascular endothelium are altered in CKD patients, as shown by means of impaired endothelial dependent vasodilatation.[14;53;54] This seems to be the first step which leads to medial thickening and ensuing arterial stiffening. Importantly, the increased arterial wall stiffness correlates with the left ventricle hypertrophy.

The associations between chronic kidney disease and traditional cardiovascular risk factors have been conclusively demonstrated. However, current investigations and opinions sustain that traditional atherosclerotic risk factors may not suffice to explain the endothelial dysfunction in ESRD patients.[179] Apart from elevated and modified LDL, cigarette smoking, hypertension, and diabetes mellitus, endothelial dysfunction is also caused by the concurrence of inflammation, oxidative stress, altered calcium phosphate metabolism, and volume overload. Hence, the inevitably arising question pertains to which of these influences arterial stiffening most. On the other hand, the independence of a risk factor is exceedingly difficult to demonstrate, particularly when its linkage to other known risk factors is very strong.

The measurement of the pulse wave velocity proved to be an effective clinical tool and has been widely used in trials. PWV might represent an integrated index of vascular structure and function, on and through which other risk factors might operate (e.g., high BP, lipid disturbances, increased CaPP, and oxidative stress), all cumulative over a lifetime.[48] Many prevalence studies report higher PWV in patients across a range of risk factors, including age, sex, smoking, lipid profiles, and peripheral arterial disease.[180] The importance of this increase in wall thickness of large and medium-sized arteries grows, as it parallels the development of left ventricular hypertrophy in patients with hypertension and could potentate the development of atherosclerosis at some arterial sites, like the carotid artery [75]

In the present study we set out to gain more insight into the pathogenesis of arteriosclerosis in ESRD patients and to investigate its relationship to markers of oxidative stress, inflammation, malnutrition, and dyslipidaemia. The palpable benefit would be the individualization of risk assessment and risk reduction strategies, which would allow detection of early vascular dysfunction before the advent of clinically apparent cardiovascular disease. Such research is crucial to the development of effective preventive strategies.

The presence of atherosclerotic plaques is accompanied by stiffening of the arterial wall. Therefore, it would be interesting to evaluate an arterial segment devoid of atherosclerosis in order to detect factors influencing only stiffness, which is the specific pathological change in CKD. Studying the aortic PWV is surely more interesting from another prognostic point of view, as it is an independent predictor of mortality[46]; however, as a means of elucidating the pathogenesis of arterial stiffness, the carotid-radial PWV may be more appealing, as it allows the assessment of arteriosclerosis in the absence of atherosclerosis. Mitchell et al.[72] showed in a Framingham Heart Study offspring cohort of healthy adults a marked age-related increase in aortic stiffness and little change in peripheral arterial stiffness.

The brachial and radial arteries are considered to remain free from atherosclerosis.[57] However, the presence of ESRD is accompanied by significantly elevated stiffness of the radial artery wall, compared to healthy controls and hypertensive patients.[11] London et al.[13] and Blacher et al.[59] showed that the presence of ESRD markedly influenced the level of PWV regardless of the site of measurement, the aorta, the lower, or the upper limb, as a result of altered intrinsic elastic properties,[11] associated with medial thickening and calcifications.[17]

The relationship between arteriosclerosis and atherosclerosis is not completely elucidated; they may be two separate and distinct conditions, even though they are often seen together in older Western subjects.[36] There has been a qualitative association between the process of atherosclerosis and arterial "rigidity".[23;37] Several investigations have brought to light strong associations between aortic stiffness and the degree of coronary artery disease assessed at coronary angiography.[16;181] Lehmann et al. suggest that a significant inverse relation exists between presumed atherosclerotic load (as assessed by the number of cardiovascular risk factors and events) and arterial compliance determined noninvasively on the

basis of ultrasonographic aortic pulse wave velocity measurements.[42] A few scenarios are plausible. It may be that atherosclerosis has two key components: fatty degeneration (atherosis) and stiffening (sclerosis) of arterial wall.[36;64] In many cases arteriosclerosis may occur completely independently of atherosclerosis. Also, arteriosclerosis may be an initial stage of the process leading to atherosclerosis; an increase in PWV could be an early indicator of atherosclerosis development (as in diabetes, see below). Arteriosclerosis is an early manifestation of essential hypertension and arterial wall hypertrophy can be demonstrated in the absence of organ damage.[175] This would render the investigation of upper limb arterial stiffness even from the prognostic point of view attractive. Several lines of evidence sustain the hypothesis of arteriosclerosis being an early stage of atherosclerosis. Kimoto et al. have recently shown that diabetes and age exert a more pronounced effect on the compliance of the central arteries than of the limb arteries and suggested that stiffness of peripheral arteries may be more strongly controlled by the endothelium-dependent mechanism.[65] An unexpected result yielded a study made by Shige et al.[182] Aortic PWV was clearly unaffected by 4 weeks therapy with simvastatin despite substantial, approximately 40% falls in LDL cholesterol, while lower limb PWV was influenced by the reduction in LDL cholesterol, consistent with the hypothesis that in the aorta the increased stiffness is more advanced, accounted for by atherosclerosis, while in the peripheral arteries it may be in an earlier stage and more readily reversible.

Furthermore, arteriosclerosis of the brachial artery may mark the presence of generalized endothelial stiffness, tightly correlated to atherosclerosis of vital arterial segments. The carotid arterial intima media thickness is used as a surrogate parameter for atherosclerosis of the coronary arteries.[183;184] Hashimoto and colleagues[185] demonstrated a significant relationship between carotid artery intima-media thickness and flow-mediated vasodilatation (FMD) in the brachial artery, used to detect endothelial dysfunction. Yokohama et al.[74] showed that microalbuminuria was positively associated to brachial-ankle PWV and carotid artery IMT, suggesting that they may all indicate an early generalized vascular dysfunction caused by structural alterations, such as a reduction in the density of heparan sulphate-proteoglycan and/or the sulphating of heparan sulphate within the extracellular matrix.

The major determinants of aortic PWV in most studies were age and systolic BP [23]; age, PP, HR [29]; age, diabetes, systolic BP, and non-HDL cholesterol[44]; age, BP,

and calcification score.[31] No correlations have been found between aortic PWV and CRP[44], dialysis duration, total cholesterol, triglycerides, apo B, Ca-PP, BMI.[32]

Prior studies that used pulse wave velocity to estimate the stiffness of arterial segments have indicated that the aorta stiffens progressively at an accelerated rate compared with other arterial segments.[68] However, age-related changes in the vasculature are not confined to large arteries but involve small arteries and arterioles as well. McVeigh et al.[186] demonstrated for the first time consistent and predictable changes in arterial pulse contour of the brachial and the radial arteries with age, independent of blood pressure.

However, we were unable to provide evidence of any influence of age, BP, or heart rate, which are usually correlated to aortic PWV, on carotid-radial PWV. Nevertheless, our result is compatible with those of Takenaka et al., who did not observe a significant relationship between blood pressure and changes in PWV.[187] There are two possible explanations for this. The BP was measured only once at the beginning of the dialysis session; a more appropriate approach would have been the 24 hours measurement. Furthermore, most of the hypertensive patients had been chronically on antihypertensive medication, including ACE-inhibitors and calcium-channel blocker, both known to normalize the pulse wave velocity after 24 weeks of treatment.[76] As to the influence of age on medium-sized arteries, the relatively small number of patients in our study may be one of the reasons for the lack of connection between PWV and age. Another rationale may be the fact that our patient group did not have a homogeneous age distribution; e.g. women were significantly older than men.

5.1 Lipids

The lipid abnormalities are not severe in HD patients, being primarily characterized by a slight increase in serum triglyceride-rich lipoproteins levels, a reduction in HDL levels, and a small increase in LDL levels.[101;104]

Hypercholesterolaemia was the first pathological condition shown to be associated with an impaired endothelium-dependent vasorelaxation both in animals and humans.[52;60] The infiltration and retention of LDL in the arterial intima initiate an inflammatory response in the artery wall.

Data regarding the relationship between arterial stiffness and blood lipids are controversial. Some studies did not show any correlations[23;31;68], while London et al.[32] reported an inverse relationship between aortic PWV and HDL cholesterol, and Shoji et al.[171] a positive relationship between aortic PWV and IDL cholesterol. Relf et al.[188] found an inverse weak relationship between HDL-cholesterol and PWV. Studies of large groups of Chinese and German populations have failed to demonstrate any association between PWV and total plasma cholesterol.[23] A very recent Japanese study found PWV increments after one year to be positively associated with total cholesterol and triglyceride levels and negatively with HDL.[187] Another study reported a positive correlation between one-year progression of PWV and total cholesterol levels and a negative correlation with HDL.[78] The heterogeneity in the effects of raised LDL cholesterol levels on arterial compliance has, therefore, been attributed partly to the absence or presence of significant atherosclerosis, only those subjects with demonstrable arterial disease showing reduced compliance that correlate with raised cholesterol concentration. (reviewed in reference [182])

Regarding the effect of triglycerides, Takenaka et al very recently showed changes in PWV to be determined by triglyceride level.[189]

In the present study we were able to demonstrate a significant positive correlation between PWV and triglycerides and LDL, while the relationship to total cholesterol levels was negative. This negative association and its physiological significance are difficult to interpret, especially since the analysed segment is the upper limb arteries. At first glance, it may seem counterintuitive; however, total cholesterol levels may be a marker of better nutrition. It would be interesting to investigate it in a larger patient cohort. Moreover, although total cholesterol (and LDL cholesterol) levels are powerful predictors of vascular disease in the general population, Lowrie et al. have proved the inverse to be true for dialysis patients.[154]

It is remarkable, that LDL and triglyceride levels influence stiffness of an arterial segment devoid of atherosclerosis. This effect may be explained at the level of endothelial dysfunction, as LDL impairs endothelium-dependent vasodilatation not only in large conductance vessels.[52] Furthermore, treatment of endothelial cells in culture with native LDL may increase their production of superoxide.[52] Additionally, triglyceride and LDL may comprise an oxidisable substrate pool. It

is worth mentioning that a single session of haemodialysis increased plasma level of oxidized LDL.[109] Oxidized LDL downregulate the expression of endothelial NO synthase.[60] And indeed, oxidized LDL have been reported to predict arterial stiffness in healthy men.[190] Besides oxidation, several other qualitative LDL changes have been shown to occur in dialysis patients, including increased carbamylation and AGE transformation, which all might favour the development of arteriosclerosis.[16]

Oxidatively modified lipids induce osteoblastic differentiation and mineralization of calcifying vascular cells.[166] Remarkably, multiple linear regression (LDL as the dependent variable) unravelled a positive correlation between LDL cholesterol and CaPP (p=0,013).

5.2 Calcium and Phosphate

In ESRD, aortic stiffening is associated with the presence of calcifications, increased calcium deposits in the arterial media, and an elevated CaPP[8;31;32], all of them leading to increased arterial stiffness. The CaPP was also positively correlated with the one-year progression of PWV in one study[78]. Medial calcification is almost exclusively associated with vascular smooth muscle cells in contrast to intimal calcification, which occurs in macrophage- and lipid-rich atherosclerotic lesions.[164] Factors associated with medial calcification are diabetes, high serum phosphate, high doses of calcium-containing phosphate binders, low serum albumin, and higher CRP.[163;167] Vascular calcification does not constitute a passive deposition of calcium-phosphate crystals in the vessel wall; it relies on active processes in which vascular cells acquire osteoblastic function.[164;191]

In a double-blind, placebo-randomized study involving 40 hypertensive HD patients, the antihypertensive effect and the stiffness reversal were conditioned by the presence or absence of aortic calcification. In that study, a 16 weeks course of nitrendipine reduced systolic blood pressure significantly more in patients with aortic calcium deposits. Aortic PWV decreased significantly in patients with aortic calcifications, but remained unaffected in patients with noncalcified vessels[192], suggesting calcification played the determinant role of arterial stiffness in those patients and that maybe the calcium-channel blocker antagonised this.

Interestingly, not only the CaPP, but also elevated phosphate per se was implied to induce vascular calcification, by regulating the expression of bone-associated proteins, among which osteocalcin.[191] Hence it is not surprising that phosphate was identified as an independent mortality predictor.[193] Older theories viewed phosphate levels only as a physico-chemical modulator of mineralization. However, new insight indicates that phosphate regulates and coordinates cell signalling and gene expression by dynamic transport processes. Inorganic phosphate induces mineralization-related genes, increasing expression of bone regulatory proteins in vascular smooth muscle cells and, at the same time, downregulates smooth muscle lineage markers.[166] Furthermore, elevated phosphate is thought to increase vascular smooth muscle cells proliferation[162], thus leading to increased stiffness. Kawagishi et al. showed carotid and femoral artery IMT to be associated to serum phosphate level, next to age and cigarette smoking.[194] In agreement with data reported in the literature and to pathophysiological expectations, we found strong, significant correlation between PWV and serum calcium and phosphate levels.

The transformation of vascular smooth muscle cells to the phenotype of osteoblast-like cells is enhanced by numerous factors, including inflammatory cytokines (such as transforming growth factor-ß, TGF-ß), oxidized lipids and lipoproteins, cyclic AMP, cholesterol, AOPP, leptin, AGE-transformed proteins, calcitriol, and glucocorticoids.[163;166] We found a significant correlation between AGE and CaPP, which, in this context, appears very interesting, especially since the it remained statistically significant (p=0,027) even after adjusting for possible confounding factors in a multiple regression model (not shown).

Another notable correlation was between fibrinogen and CaPP, in concordance with the investigations of Guerin et al[31], which yielded a statistically significant correlation between serum fibrinogen and the arterial calcification score, calculated on the basis of ultrasonographically detected calcified plaques.

Barenbrock et al. reported a significant negative correlation between the distensibility of the common carotid artery and plasma PTH concentrations.[195] Serum PTH levels were not included in our multiple regression models, because big part of the patients had undergone parathyroidectomy, which may introduce a serious bias in the analyses concerning PTH.

5.3 AGE

AGE levels are significantly elevated in diabetic patients and in patients with impaired renal clearance.[121] Schwedler et al.[196] found similar levels of AGE in diabetic and non-diabetic haemodialysis patients, implying that uraemia-associated oxidative or carbonyl stress are probably of greater importance than glycaemic control.

AGE may have a role in the cardiovascular complications of uraemia,[118] enhancing both atherosclerosis and arteriosclerosis at many levels. AGE accumulation in collagen and other long lived vascular wall proteins can reduce the elasticity of vessel walls.[120;128;128] Studies in diabetic patients have demonstrated that collagen linked fluorescence in the aorta and myocardium correlated with aortic pulse wave velocity.[128] Hence, AGE play a critical role in the development of arteriosclerosis in uraemic subjects. Indeed, immunohistochemical examination reveals the presence of AGE in thickened arterial walls.[197] Aortic collagen AGE content correlates with stiffness of the aorta.[128] AGE are potent generators of ROS via NADPH/NADP-dependent processes.[145] Furthermore, recent studies have shown that protein-bound AGE can react with and inactivate NO via a direct chemical reaction, hence the hypothesis that the accumulation of subendothelial AGE interferes with the antiproliferative activity of NO, resulting in myointimal and mesangial proliferation.[51] The cross-linking of matrix components renders them resistant to proteolysis, increasing protein deposition. AGE binding to the receptors (RAGE) on many cell types induce platelet aggregation, chemotaxis of inflammatory cells, release of cytokines and growth factors from macrophages, increased procoagulant activity of the endothelium, increased proliferation of vascular smooth muscle cells and fibroblasts,[198] and increased oxidant stress.[118] AGE, upon interaction with the receptor for AGE, trigger the signaling involving P21ras, mitogen-activated protein kinases (MAPKs), and nuclear factor-κB (NF-κB).[197] It has been speculated that intracellular accumulation of AGE may promote phenotypic conversion of smooth muscle cells and foam cell formation within the atherosclerotic plaque.[122]

The AGE modifications take also place on the low-density lipoproteins (LDL) and render LDL not recognizable by LDL receptors; however, LDL is taken up by monocytes and macrophages and initiates oxidative damage to vessel walls by generating free radicals.[16;118]

Nevertheless, Zoccali et al. could not find any correlation between the AGE pentosidine and IMT or the number of plaques in the carotid artery.[199]

The fact, that an AGE cross-link breaker reduced the stiffness of aorta but not systemic arterial resistance, led Kimoto et al. to the hypothesis that AGE are involved in the preferential stiffening of the central (elastic) over peripheral (muscular) arteries.[65] However, our results demonstrate a strong significant correlation between upper limb PWV and AGE total fluorescence, after adjustment for traditional cardiovascular risk factors.

Surprisingly, Schwedler et al.[196] found increased levels of AGE to be associated to a better survival, concluding AGE could represent better nutrition, provided the results are not an epiphenomenon. In our case, simple regression analysis yielded a positive link between AGE and albumin levels (p=0,036). This finding is not surprising, since AGE, such as pentosidine and carboxymethyllysine are more than 90% albumin-bound and albumin is the major target for carbonyl formation in uraemia.[105] Hence, AGE could reflect better nutrition; nonetheless, we showed that increased AGE levels correlate with greater arterial stiffness.

In addition to the direct roles in atherosclerosis and arteriosclerosis, AGE may maintain the inflammation present in CKD and HD patients by stimulating CRP and fibrinogen synthesis in the liver via macrophage IL-6 production.[200;201] However, in our population there was not nexus between AGE and CRP or fibrinogen.

5.4 AOPP

Witko-Sarsat et al proposed the measurement of AOPP as a reliable marker to estimate the degree of oxidant-mediated protein damage in uraemic patients.[112;113] Complementary to the results of Witko-Sarsat et al.[112], we did not find any correlation between AOPP and CRP. Conversely, we were able to show a significant correlation between AOPP and fibrinogen, underlining that, although both CRP and fibrinogen are part of the acute phase response, they do not share the same physiological significance. In another study involving CKD patients AOPP were significantly correlated with markers of endothelial dysfunction, hemostatic activation (PAP, plasmin antiplasmin complexes), and acute phase proteins, CRP and fibrinoge[152]

AOPP were revealed as an independent risk factor for coronary artery disease. Their levels seem to be correlated to the degree of coronary atherosclerosis obtained by the Gensini scoring system[117] speculating for a role of oxidative stress in the pathogenesis of arterial diseases. This hypothesis is sustained by evidence that the oxidized protein moiety is more potent than its lipid counterpart in the induction of macrophage activation and apoptosis, thus providing indirect evidence that AOPP-LDL could be of importance in the inflammatory process associated with atherosclerosis.(reviewed in [98])

Given the data in literature and the role of oxidative stress in endothelial dysfunction and inflammation, it would have been intuitive to find a positive correlation between AOPP and PWV. However, the correlation was negative. Whether this result bears any physiological significance or is just an epiphenomenon is beyond the elucidation capability of a cross-sectional study. It would be interesting to observe this correlation longitudinally.

Moreover, there is still the possibility of internal confounding factors. All the patients with AOPP levels > 500 nmol/ml were assigned the value 500, because of getting out of range of measurement.

Witko-Sarsat et al.[113] reported a link between AOPP levels and AGE pentosidine. However, we could not confirm any link between AOPP and AGE total fluorescence.

Oxidative stress related to intravenous iron administration also raised AOPP levels.[115] Furthermore, Drüecke et al showed a strong correlation between ferritin, AOPP, and the cumulative annual dose of intravenous iron.[202] In this study we did not find any correlation between ferritin and AOPP levels and the patients receiving intravenous iron therapy did not show greater AOPP levels than the one who did not.

5.5 CRP

Epidemiological studies in the general population suggest that inflammation, documented by elevated CRP as a measure of the acute phase response, is coupled with increased risk of cardiovascular mortality. Even CRP increases previously deemed to be insignificant, up to 2 mg/l, are associated with increased car-

diovascular risk.[146] The CREED investigators showed a strong correlation between the number of atherosclerotic plaques detected sonographically and CRP levels.[139] By means of intravascular ultrasonography, Nissen et al.[203] showed that under statin therapy atherosclerosis regressed in patients with the greatest reduction in CRP levels, but not in those with the greatest reduction in LDL cholesterol levels. Stenvinkel et al. detected a significantly increased intima media thickness in carotid arteries of predialysis patients with elevated CRP levels (CRP greater than 10 mg/L) compared with patients with CRP levels less than 10 mg/L.[2] Zoccali et al.[204] demonstrated that CRP levels predicted the evolution of the carotid artery intima-media thickness.[204] Nevertheless, others[130] have failed to identify CRP as an independent predictor of survival for ESRD patients. Whether CRP is simply a marker of cardiovascular disease and mortality or whether it plays an important part in the causal pathway of the disease remains an open question. Recent data are in favour of a direct involvement in the pathogenesis of disease, since binding of CRP to degraded LDL enhances complement activation and induces the expression of tissue factor.[145] Moreover, CRP has inflammatory effects on human endothelial cells and may injure them directly.[87;134] CRP renders oxidized LDL more susceptible to be scavenged by macrophages, induces the expression of vascular-cell adhesion molecules, stimulates the synthesis of tissue factor, and impairs the production of nitric oxide.[203]

Shoji et al. did not find a significant relationship between CRP and aortic PWV in the ESRD patients. However, recent reports[2] showed that CRP was an independent predictor for increased intimal thickening and the number of atherosclerotic plaques of carotid arteries in haemodialysis patients. Taken together, these results led them to the conclusion that CRP has greater effects on morphologic and functional changes of arterial intima, i.e. atherosclerosis than those of arterial media, i.e. arteriosclerosis.[44] Nevertheless, a recent longitudinal study[205] in HD patients reached another conclusion. They found that both baseline PWV, and the changes of PWV and LV mass during the follow-up were correlated with serum CRP levels, concluding that the existence of low-grade inflammation diminishes the efficiency of therapeutic attempts to improve cardiovascular outcome and contributes in the maintenance of the haemodynamic burden.

In this study CRP emerged as an independent correlate to PWV (p=0,003), even after adjustment for conventional cardiovascular risk factors. Furthermore, mul-

tiple regression analysis showed that patients who had been longer on HD had greater CRP levels (p=0,04).

5.6 Fibrinogen

Increased levels of fibrinogen, as an acute phase protein and procoagulation marker, is a risk factor for cardiovascular events in the general population,[137;159] predicting the severity and incidence of atherosclerotic complications.[160] Fibrinogen was an independent risk factor for high mortality in 412 diabetic HD patients, next to apolipoprotein A-I, age, and stroke history.[206]

Fibrinogen, like CRP, may have properties that accelerate arteriosclerosis,[207] e.g. via endothelial dysfunction. The increased plasma fibrinogen level in dialysis patients was found in association with markers of endothelial dysfunction, i.e. increased levels of proconvertin and type 1 plasminogen activator inhibitor (PAI-1), promoters of atherogenesis.[141] In our second multiple regression model fibrinogen emerged as an important predictor of arteriosclerosis (p=0,015).

The strong correlation between fibrinogen and CRP in our study is pathophysiologically intuitive. One factor leading to high plasma fibrinogen levels in dialysis patients can be inflammation, as IL-6 can stimulate the transcription of fibrinogen genes as part of the acute phase response.[140]

Fibrinogen levels are inversely correlated to albumin levels in CKD patients.[140] The intensity of hypercoagulability is thought to be related to the extent of hypoalbuminaemia, being more apparent at albumin levels of less than 2 g/dl. In this context, the associated hypertriglyceridaemia and changes in arachidonic acid metabolism, in response to hypoalbuminaemia, play participatory role.[208] In this study, however, no correlation between albumin and fibrinogen levels was evident.

In the general population fibrinogen is correlated with cholesterol, age, BMI, smoking, menopausal status and blood pressure, and inversely related HDL cholesterol and albumin.(reviewed in [140]) In our study fibrinogen was associated with CRP, CaPP, and AOPP levels. The correlation to the CaPP is in line with the results of Guerin et al. showing that arterial calcifications density increases with the fibrinogen level.[31]

5.7 Malnutrition

Protein-caloric malnutrition often occurs during the course of many chronic diseases, such as chronic kidney disease, protracted infections, and cancer. Its extent in such conditions typically exceeds that observed in starvation alone. The current view sustains that the malnutrition of chronic diseases is mediated in part by the release of one or more proinflammatory cytokines that are usually part of the acute phase reaction.(reviewed in [130]) In our patient group, the extent of hypoalbuminaemia was, however, less pronounced. Only 2 patients (4%) were considered malnourished.

It is well established that the adequacy of the body's content of protein is strongly associated with the patient's likelihood of long-term survival,[130] a low serum albumin level being a strong independent predictor of total and cardiovascular mortality in HD patients.[130;151;154;155] Due to the association of malnutrition with cardiovascular disease,[155] Stenvinkel et al.[2] proposed the existence of a syndrome consisting of malnutrition, inflammation and atherosclerosis (MIA syndrome) in some patients with CKD.

Hypoalbuminaemia may increase cardiovascular disease via increased known risk factors such as lipoproteins or fibrinogen,[151] or may simply be a marker of inflammation. Pannier et al. reported that decreased albumin level was an independent factor associated with endothelial dysfunction in ESRD patients.[14] Hypoalbuminaemia may affect blood viscosity or endothelial function as a consequence of increased concentrations of free lysophosphatidylcholine that alters erythrocyte structure. [14]

The thiol groups on albumin constitute an important line of defence against oxidative stress by scavenging hypochlorous acid and other ROS.[88] Albumin has been shown to be selectively oxidized by a variety of oxidants, functioning as a "suicide scavenger", which prevents oxidative injury to both lipoproteins and the vascular wall.[209] Indeed, Cha et al showed that albumin has preventive effects against the peroxidation of lipid.[210] Oxidation of free thiol groups on albumin seems to be one of the features of uraemic oxidative stress, in both HD and predialysis patients.[103;105] Additionally, albumin is the major target for carbonyl

formation in uraemia.[105] Himmelfarb et al. [88] proposed that albumin may be regarded as a surrogate for the level of other antioxidants.

As a factor associated both with endothelial dysfunction and with clinically manifest cardiovascular disease, it would have been expected that hypoalbuminaemia be associated with increased arterial stiffness. However, we did not find any correlation between albumin level and upper limb PWV. It would be interesting to investigate the influence of albumin concentration on arterial stiffness in truly malnourished patients and, perhaps, also at the level of the aorta.

5.8 Dialysis Duration

The role of the haemodialysis duration, meaning the time in months spent on HD, on arterial changes is still under debate. Data in literature show that CKD patients have an increased incidence of cardiovascular complications before starting HD.[10] The number of years on haemodialysis influence patients' survival to a large degree.[47]

Nevertheless, most of previous cross-sectional studies showed no significant relationship of duration of haemodialysis with thickness,[194] or stiffness[32;171] of the vascular wall. Neither did haemodialysis duration correlate to the cardiovascular mortality.[44;47] By measuring carotid artery intima media thickness, Shoji et al pointed out that the presence of renal failure and the consequent metabolic alterations, not haemodialysis itself have an adverse effect on carotid atherosclerosis.[211] Furthermore, Shinohara et al. showed aortic stiffening to be present in uraemic patients before starting haemodialysis treatment. The haemodialysis group showed a lower PWV than the predialysis one, suggesting no adverse effect of haemodialysis and emphasizing the important roles of renal failure in arterial stiffness.[212] Nonetheless, other studies indicate that arterial function worsens with time on dialysis.[17;31;78] Takenada et al very recently showed dialysis duration to be one of the most important determinants of PWV changes.[189]

The haemodialysis procedure seems to accentuate the oxidative stress and inflammatory syndrome of uraemia.

We found an interesting quadratic relationship between PWV and haemodialysis duration (Figure 5). The first, ascending, part of the curve shows a positive

correlation and may point out to the fact that HD does promote arterial stiffening. On the other hand, the second descending part, which pictures a negative relationship may need to be interpreted otherwise. It might indicate that those patients who were on HD for a very long time may comprise a low-risk group, who have survived thus far. They may show lesser degrees of inflammation and oxidative stress.

5.9 Diabetes

The close overlap of the risk factors for atherosclerosis and adult-onset diabetes and the proclivity of diabetic patients to have premature atherosclerosis has initiated a hypothesis, which suggests, in part, that both of these disorders share a mutual inflammatory and possibly genetic basis. The large-scale Women's Health Study involving apparently healthy individuals substantiated that baseline levels of both CRP and interleukin-6, which were previously shown to predict the onset of cardiovascular disease, also predicted the onset of type II diabetes.[213]

Many studies reported that diabetic patients had a significantly greater aortic PWV than the nondiabetic patients,[44;59;64] as well as significantly higher mortality, including a higher rate of fatal cardiovascular events.[44] Another study, led by Cruickshank et al.[48] demonstrated that aortic PWV was higher at any level of SBP in diabetic patients than in controls. Furthermore, their results illustrated the prognostic value of PWV in type 2 diabetes.

A very recent study showed type 2 diabetic patients with microalbuminuria have significantly higher values of IMT and PWV than those with normoalbuminuria. A slight elevation of albuminuria emerged as an independent predictor of IMT and PWV after adjustment for conventional cardiovascular risk factors.[74]

In our group, the presence of diabetes was not a predictor of arterial stiffness in the upper limb. Our result is in line with another study[65] which showed that diabetes was significantly associated only with PWV of the central arteries.

On the other hand, one source of inaccuracy may be the fact that diabetes is included in statistical models as a categorical variable, i.e. presence of diabetes, yes

or no. Perhaps a more appropriate tactic would have been using the Hb A_{1c} as a variable. However, most studies also use the variable "diabetes" as we did.

5.10 Medication

As delineated in Chapter 1.1.3, several lines of evidence in essential hypertensive and uraemic subjects suggests that long-term therapy with ACE-inhibitors, calcium channel blocker, or statins induce reverse arterial remodelling with improvement of the pulse wave velocity.[57;60;76;78-81;182;192] Indeed, treatment of patients with ACE inhibitors, angiotensin receptor type 1 inhibitors, statins and fibrates improves endothelial function.[214] However, although improvement of arterial stiffness in response to antihypertensive drugs is associated with the regression of LVH and the improvement of the survival, it is achieved in less than half of uraemic patients.[62;215]

ACE inhibitors by impairing the accumulation of angiotensin II in the vascular wall would attenuate the endothelial dysfunction, which leads to arterial stiffening.[60] In addition, the potentiation of the effects of bradykinin, an endogenous regulator of the release of endothelium-derived vasodilators, appears to play a major role in the restoration of endothelial function by ACE inhibitors.[60]

Guerin et al. showed that the extent of arterial calcifications increased with the use of calcium-based phosphate-binders,[31] in contrast to the non-calcium-containing compound sevelamer, which can prevent the progression of arterial calcifications in haemodialysis patients.[169] Furthermore, Takenada et al. have shown that the administration of sevelamer halted the acceleration of PWV observed during calcium carbonate treatment.[216]

The patients in our group, taking calcium based phosphate binder did not have a higher serum calcium level, CaPP, or PWV, suggesting that the choice of phosphate binder type was appropriate.

We included medication in our regression model, although it is not a standardized, controlled, longitudinal study; in order to control for the effect of various drugs on PWV. Our results pertaining to the influence of calcium channel blocker on PWV are in concordance with the current concepts. In the third model, using backward regression, there was also a strong negative correlation

between statins and PWV. Due to the design of the study, the lack of connection between ACE-inhibitors and PWV is not surprising. Only longitudinal, placebo-controlled, randomised studies can yield persuasive results.

6 CONCLUSIONS

6.1 Integration

The key phenomenon in the pathogenesis of arteriosclerosis is endothelial dysfunction, whose main implication is a reduced NO bioavailability. Initially, arterial stiffness is only functional and reversible with BP normalization, achievement of dry body weight, or reversal of other parameters. If the offending phenomenon persists, or if possibly other factors come into play, then the endothelial dysfunction advances to the structural stage. The structural arterial stiffening can consist of increased collagen and extracellular matrix deposition, vascular smooth muscle cell (VSMC) proliferation, and/ or calcium deposition. Calcium crystal deposition entails the osteoblastic differentiation of VSMC, under the influence of various factors, among which AOPP, AGE, oxidised LDL. The process occurs in the presence of an increased calcium phosphate product. Chronic low-grade inflammation and malnutrition favours the occurrence of media calcification in ESRD.[167]

Both inflammation and oxidative stress lead to endothelial dysfunction. CRP, fibrinogen, AGE, AOPP, and oxidised LDL have inflammatory effects on human endothelial cells.[87;134;207] Angiotensin II may be one of the mediators of endothelial dysfunction because it induces resistance to the vasodilator action of NO.[60]

Systemic inflammation, augmented oxidative stress, endothelial cell dysfunction and haemostatic activation are closely associated.[150] The inflammation in ESRD leads to endothelial dysfunction either through the direct effect of the cytokines and acute phase reactants (CRP, fibrinogen), or indirectly. Zimmermann et al have suggested that elevated Lp(a) and decreased HDL-cholesterol and apolipoprotein A-I are partly consequence of an activated acute phase response.[129]

Inflammation is linked to oxidative stress by at least two mechanisms. Firstly, the oxidative burst occurring in the phagocytes during inflammation increases the free radical production. H_2O_2 can induce the synthesis of proinflammatory cytokines[87] feeding a vicious circle. Stenvinkel et al. showed a positive correlation between CRP and oxidised LDL.[2] Secondly, inflammation is accompanied by decreased plasma levels of certain antioxidants. Consistent with this hypothesis, Van Lenten et al.[217] described alterations in HDL composition during

an acute-phase response, which change it from an antioxidant to a prooxidant lipoprotein. Nguyen-Koa et al. recently demonstrated a negative correlation between plasma CRP levels and alpha tocopherol levels.[218] Furthermore, the acute phase response leads to decreased albumin levels, which will decrease plasma antioxidant defences and increase the susceptibility to oxidative injury, since albumin is the major plasma protein target of excess oxidative burden in chronic haemodialysis patients.[105]

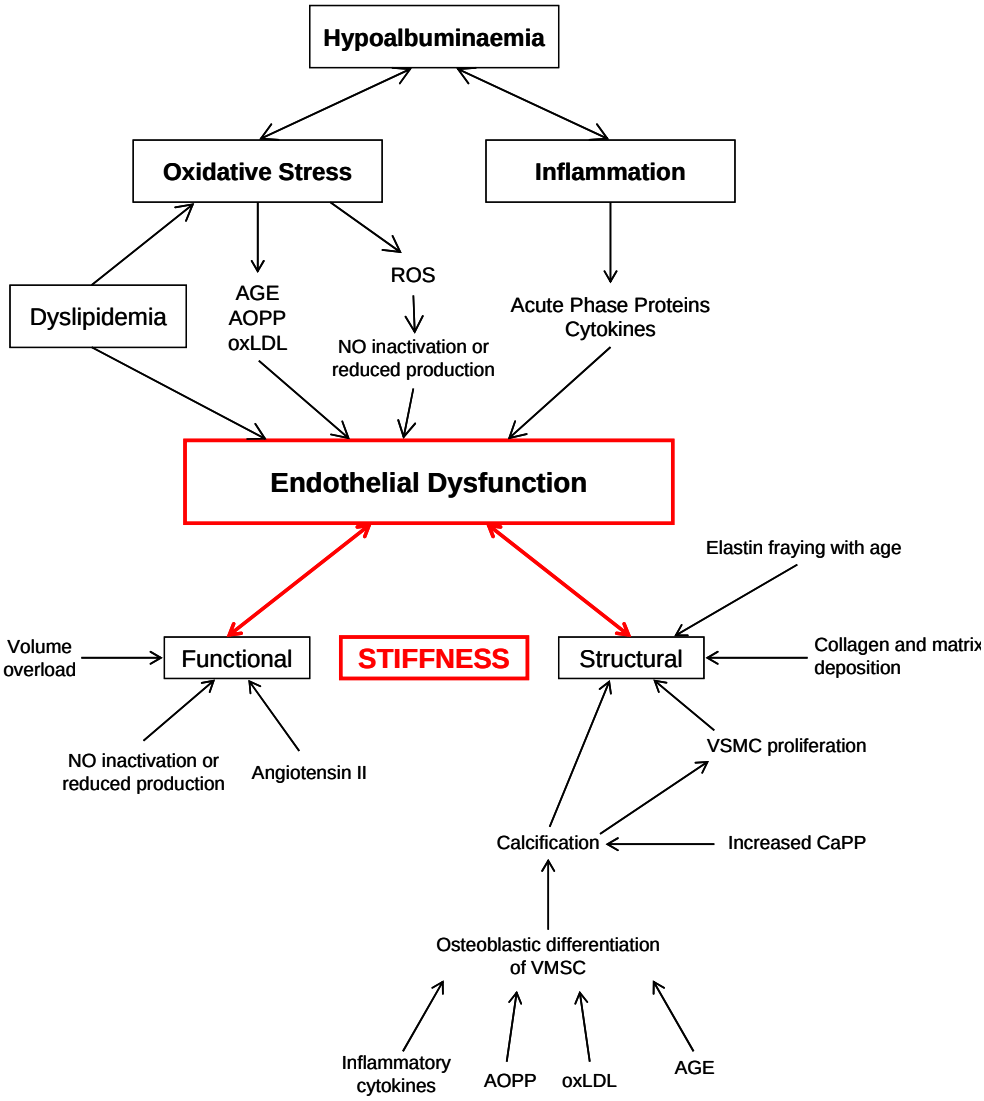


Figure 7. Integrative, simplified view on the pathogenesis of arterial stiffness based on endothelial dysfunction.

In addition to the direct roles on the vascular wall, AGE may maintain the inflammation present in CKD and HD patients by stimulating CRP and fibrinogen synthesis in the liver via macrophage IL-6 production.[200;201] AOPP were shown to trigger the oxidative burst in neutrophils and monocytes,[113] i.e. to act as mediators of inflammation and oxidative stress in chronic uraemia, providing both one of the molecular links between oxidative stress and inflammation and a means of upholding the oxidative burden.

Furthermore, AGE, AOPP, inflammatory cytokines, and oxLDL stimulate the osteoblastic differentiation of VSMC, which leads to calcium-phosphate deposition in the arterial media.

6.2 Concluding Remarks

Most patients with CKD have a higher prevalence of traditional and non-traditional CVD risk factors when compared to the general population. The relative contribution of these risk factors for the development of arteriosclerosis remains uncertain.

PWV might represent an integrated index of vascular structure and function, on and through which other standard risk factors might operate, all cumulative over a lifetime.[48] In ESRD additional specific factors come into play. Vascular architecture, an important outcome predictor in ESRD patients, is related to the interaction between procoagulatory and proinflammatory pathways, oxidative stress, dyslipidaemia, and the calcium-phosphate product.

It seems that chronic kidney disease is both an independent risk factor for cardiovascular disease and a setting which promotes the aggravation of already present cardiovascular disease. It is also clear that inflammation, oxidative stress, dyslipidaemia, and hypoalbuminaemia exert a synergistic effect with regard to the cardiovascular remodelling.

This study was meant to shed some light into the complicated pathogenesis of arteriosclerosis in ESRD patients. The elements of this pathophysiological process are intertwined in a complex network, which makes the detection of their association and interplay and then the translation into meaningful paradigms a difficult task.

The results of this cross-sectional study suggest that arterial stiffness of the brachial-radial segment is less influenced by age, BP, or heart rate, factors, which are usually correlated to aortic PWV. Conversely, in haemodialysis patients the major determinants of arteriosclerosis in peripheral arteries devoid of atherosclerosis seem to be inflammation and oxidative stress. Our results are in accordance to the hypothesis of Kimoto et al [65], proposing stiffness of peripheral arteries to be more strongly controlled by the endothelium-dependent mechanism than that of central arteries. In addition to that, the lipids, calcium and phosphate levels seem to be equally important factors in peripheral arteries.

6.3 Limitations of this Study and Further Research

The principal limitation of this study is its cross-sectional design, such that cause and effect are not discriminated. Furthermore, correlation does not necessarily imply causation and the complexity of factors interplaying may mask some correlations, while making others of lesser importance become apparent. It is exceedingly difficult for studies using this (or retrospective) design to exclude the possibility that the observed associations are due to confounding rather that to any particular causal pathway.

Other drawbacks may be the relatively low number of patients, the use of surrogate parameters, and the absence of healthy controls. Whether measurement of upper limb stiffness has a prognostic value regarding cardiovascular events still remains to be elucidated. We believe it does, as arteriosclerosis of the peripheral arteries reflects a generalized vascular dysfunction. Arteriosclerosis may be an initial stage on the way to atherosclerosis and its analysis would be of enormous help. Furthermore, its measurement at the radial artery is more readily accessible in the outpatient setting in a dialysis room with both women and men.

One possible error source is the distance travelled by the pulse wave that it is difficult to measure and can only be an approximation, and may affect the calculation of PWV.

We intend to carry out a longitudinal study, enrolling a larger number of patients, to assess the questions arisen after this cross-sectional study and to confirm the observed correlations.

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Sonstige Erfahrungen

EDV: Word, Excel, Programmiersprache C++
Statistische Auswertung: SPSS

Fremdsprachen

Englisch (Schrift und Sprache): sehr gut
Französisch (Schrift und Sprache): gut
Spanisch (Schrift und Sprache): mittel

Wissenschaftliche Aktivitäten

- 10/2001-02/2004 – Studententutorin für das Physikum, das Erste und das zweite Staatsexamen, Dekanat der Universität Frankfurt/ Main
- 06/2002: Teilnahme am Benjamin Franklin Contest Berlin im Team der Universität Frankfurt
- Seit Oktober 2002: Promotionsarbeit: „Die arterielle Steifigkeit wird bei Hämodialyse-Patienten von oxidativem Stress, Inflammation und Calcium-Phosphat Metabolismus beeinflusst“ unter der Leitung von Prof. Dr. med. W. Riegel, Klinikum Darmstadt
- 09/2005: Poster „Die Pulswellengeschwindigkeit (PWV) korreliert mit Fibrinogen, Phosphat und CRP bei Hämodialyse-Patienten“ beim Kongresses für Nephrologie 2005 der Gesellschaft für Nephrologie Deutsche und der Arbeitsgemeinschaft für Klinische Nephrologie, Saarbrücken