



YRJÖ LESKINEN

# Manifestations and Risk Factors of Atherosclerosis in Chronic Renal Failure



ACADEMIC DISSERTATION

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## **ACADEMIC DISSERTATION**

University of Tampere, Medical School  
Tampere University Hospital, Department of Internal Medicine  
Finland

### **Supervised by**

Professor Amos Pasternack  
University of Tampere  
Docent Heikki Saha  
University of Tampere

### **Reviewed by**

Docent Raimo Kettunen  
University of Kuopio  
Docent Kaj Metsärinne  
University of Helsinki

Distribution



University of Tampere  
Bookshop TAJU  
P.O. Box 617  
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Finland

Tel. +358 3 215 6055  
Fax +358 3 215 7685  
taju@uta.fi  
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*to my family*



## ABSTRACT

Cardiovascular mortality is 10 to 20 times higher in patients with uremia than in the general population, even after stratification by age, sex, and presence of diabetes. The high prevalence of cardiovascular disease (CVD) in patients with chronic renal failure (CRF) may be caused by the accumulation of recognised risk factors for CVD or by factors characteristic of CVD in these patients. The aims of the present study were to study the characteristics as well as the prevalence and diagnostics of clinical manifestations of atherosclerotic vascular disease in patients with CRF. Particularly, the significance of risk factors for atherosclerosis was examined.

The study population was designed to include all CRF patient groups: 58 predialysis patients, 36 dialysis patients, and 41 renal transplant recipients. In addition, 58 control subjects were examined. In this cross-sectional study, methods included a thorough characterisation of the atherosclerotic manifestations by using clinical criteria, lower-extremity blood pressure measurements and carotid artery ultrasound examination. In addition, the analysis of thoracic aortic atherosclerosis was performed for 118 study patients with CRF by using transesophageal echocardiography (TEE).

The results of **study I** show that peripheral arterial disease (PAD) and medial arterial calcification are common in patients with CRF. Furthermore, a reliable diagnosis of PAD requires the use of both ankle and toe pressure measurements in patients with CRF. The novel finding in the examination of aortic atherosclerosis in **study II** is the association between hypercholesterolemia and atherosclerosis in CRF. In addition, age, duration of hypertension and renal disease contributed to atherosclerosis. In **study III**, it was shown that the characteristic alterations of the carotid arteries include increased plaque burden and calcification as well as increased arterial stiffness. In contrast, no difference in intima-media thickness was found between the controls and the patient groups. In **study IV**, no significant association between elevated total homocysteine level and carotid atherosclerosis or vascular disease in CRF was found. However, the effect of renal function may confound the results of this analysis in cross-sectional studies on patients with CRF or the general population. Randomised, placebo-controlled trials on the effect of homocysteine lowering for CVD events are required to resolve the significance of moderately elevated homocysteine level. In **study V**, the analysis of aortic atherosclerosis in TEE proved useful in predicting coronary artery disease in patients with CRF.

In conclusion, the extent of atherosclerosis is increased in patients with CRF. The treatment of hypercholesterolemia as well as other classic risk factors are important to improve the outcome of patients with CRF.

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## ABBREVIATIONS

ABI	ankle brachial index
AMI	acute myocardial infarction
CABG	coronary artery bypass grafting
CAD	coronary artery disease
CEVD	cerebrovascular disease
CHF	congestive heart failure
CRF	chronic renal failure
CRP	C-reactive protein
CT	computed tomography
CVD	cardiovascular disease
ECG	electrocardiogram
HDL	high-density lipoprotein
IMT	intima-media thickness
IMT <sub>fixed</sub>	IMT at a fixed point (1 cm from bifurcation)
IMT <sub>max</sub>	maximum IMT of 3 cm section in the common carotid artery
LAPs	large aortic plaques ( $\geq 3.0$ mm in diameter)
LDL	low-density lipoprotein
LVH	left ventricular hypertrophy
MAC	medial arterial calcification
PAD	peripheral arterial disease
PTCA	percutaneous transluminal coronary angioplasty
TBI	toe brachial index
TEE	transesophageal echocardiography
tHcy	plasma total homocysteine



## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following five original publications, which are referred to in the text by their Roman numerals **I-V**. In addition, some unpublished data are presented.

- I** Leskinen Y, Salenius JP, Lehtimäki T, Huhtala H, Saha H (2002): The prevalence of peripheral arterial disease and medial arterial calcification in patients with chronic renal failure: requirements for diagnostics. *Am J Kidney Dis* 40: 472-479.
- II** Leskinen Y, Groundstroem K, Virtanen V, Lehtimäki T, Huhtala H, Saha H (2003): Risk factors for aortic atherosclerosis determined by transesophageal echocardiography in patients with chronic renal failure. *Am J Kidney Dis* 42: 277-285.
- III** Leskinen Y, Lehtimäki T, Loimaala A, Lautamatti V, Kallio T, Huhtala H, Salenius JP, Saha H (2003): Carotid atherosclerosis in chronic renal failure — the central role of increased plaque burden. *Atherosclerosis* 171: 295-302.
- IV** Leskinen Y, Lehtimäki T, Loimaala A, Huhtala H, Salenius JP, Oja SS, Saha H (2004): Homocysteine and carotid atherosclerosis in chronic renal failure — the confounding effect of renal function. *Atherosclerosis* 175: 315-323.
- V** Leskinen Y, Groundstroem K, Virtanen V, Lehtimäki T, Huhtala H, Saha H:  
The prediction of coronary artery disease by transesophageal echocardiographic detection of thoracic aortic plaque in patients with chronic renal failure (submitted).

## INTRODUCTION

Patients with chronic renal failure (CRF) have a markedly reduced life expectancy in comparison to subjects with no renal disease. A large part of the reduced survival of these patients results from excessive cardiovascular mortality (de Lemos and Hillis 1996). The high risk for cardiovascular disease (CVD) in patients with CRF may be caused by the high prevalence of recognised risk factors for CVD or by factors characteristic of CRF in these patients (Longenecker et al. 2002a). However, knowledge of the development of CVD in patients with CRF has been insufficient. Thus, there has been a need for further studies on the prevalence of cardiovascular disorders or on risk factors for CVD in patients with CRF (Parfrey 1993, Foley et al. 1998).

Despite the fact that CRF has been described as a “vasculopathic state” (Luke 1998), there are few reports on the progression of angiographically defined coronary artery disease (CAD) in patients with CRF (Goldsmith and Covic 2001). Due to the invasiveness of angiography B-mode ultrasound of arterial vessels has been used as an alternative non-invasive method to examine atherosclerosis in epidemiological studies. Additionally, B-mode ultrasound provides reliable and valid estimates of early disease of the arterial wall (Crouse and Thompson 1993).

The high prevalence of CVD among incident dialysis patients suggests that CVD begins in earlier stages of CRF and that the implementation of risk factor reduction strategies earlier in the course of CRF may provide an opportunity to better prevent CVD in CRF (Parfrey 1993, Sarnak and Levey 2000). The present study was designed to include all CRF populations: predialysis patients, dialysis patients and renal transplant recipients. Methods included a thorough characterisation of the atherosclerotic manifestations of the study patients by using clinical criteria, lower-extremity blood pressure measurements or high-resolution B-mode ultrasound examination. In addition to classic risk factors for atherosclerosis (advanced

age, hypertension, diabetes, hyperlipidemia, smoking history, family history of CAD), the significance of novel or CRF-related risk factors (inflammation, duration of renal disease, calcium-phosphate disorders, homocysteine) for the development of atherosclerosis were evaluated.

# REVIEW OF THE LITERATURE

## 1. Cardiovascular disease in chronic renal failure

Between 1985 and 1998, the five-year survival rate of Finnish dialysis patients was 15 % and of renal transplant recipients 88 % (Finnish Registry for Kidney Diseases 1999). The notable difference in survival between these CRF patient groups results from the fact that renal transplant recipients are younger (US Renal Data System 2000, Finnish Registry for Kidney Diseases 2001) and they are selected from a patient group with no unstable conditions (de Lemos and Hillis 1996). In addition, renal transplantation has a beneficial effect on the prognosis of these patients (Schnuelle et al. 1998).

In 2000, the proportion of mortality due to cardiovascular causes in Finnish dialysis patients was 46% and in renal transplant recipients 55% ( Finnish Registry for Kidney Diseases 2001). Excessive cardiovascular morbidity and mortality in comparison to the general population have been shown in patients with chronic renal insufficiency, in dialysis patients and in renal transplant recipients (Foley et al. 1998, Kasiske 2000, Henry et al. 2002, Muntner et al. 2002, Fried et al. 2003). It has been presented that cardiovascular mortality is 10 to 20 times higher in patients with uremia in the USA, even after stratification by age, gender, race, and presence of diabetes (Foley et al. 1998).

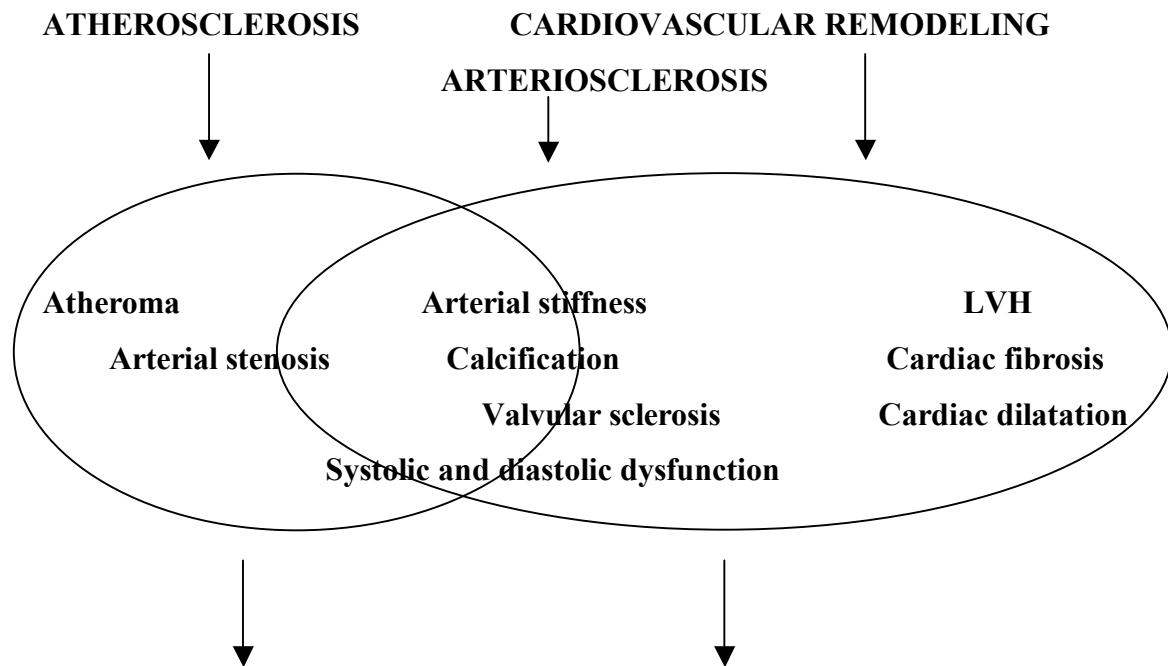
Several cardiovascular conditions reduce the survival in CRF. Nearly all patients with CRF have hypertension prior to the initiation of dialysis treatment (Raine et al. 1998). Hypertension predisposes for the development of cerebrovascular disease (CEVD) as well for coronary artery disease (CAD) and peripheral arterial disease (PAD). Increased arterial stiffness, a common finding in chronic renal failure (London et al. 1996, Mourad et al. 1997, Blacher et al. 1999), elevates mean systolic, peak systolic and end-systolic blood pressure in the ascending aorta. This will increase myocardial oxygen consumption. Furthermore, diastolic

coronary blood supply is impaired. In the long run, increased systolic blood pressure induces myocardial hypertrophy, and impairs diastolic left ventricular filling and ventricular ejection (London 1994). Consequently, both ischemic and non-ischemic systolic dysfunction are common in CRF (Rostand et al. 1991), promoting the development of congestive heart failure (CHF). The prevalence of LVH is around 75 % in patients starting dialysis therapy (Foley et al. 1995). CHF worsens prognosis, and the two-year survival rate of these patients is only 33% (Parfrey et al. 1990a).

In the study by Parfrey et al. (1990b), the prevalence of dilated cardiomyopathy in dialysis patients was 6 %. The cardiac alterations in CRF also include myocardial fibrosis leading to disturbed ultrastructure in the myocardium (Mall et al. 1988, Mall et al. 1990). This, together with ischemia or the typical disturbances in electrolyte homeostasis in CRF, makes these patients prone for cardiac arrhythmias or cardiac arrest, which account for the majority of the immediate causes of cardiac death in these patients (Herzog 2002, Näppi et al. 2000). Other common cardiovascular conditions in this patient population include cardiac valve or arterial calcifications and an increased risk for the development of pericardial effusions (Maher et al. 1987, Goodman et al. 2000, Schwarz et al. 2000, Wood and Mahnensmith 2001).

The cardiovascular changes leading to excessive cardiovascular mortality in patients with CRF are summarised in Figure 1. These various alterations are not mutually exclusive — on the contrary, they are often present simultaneously (London et al. 1996). *Atherosclerosis* (page 37) is primarily an intimal disease in the arteries and is characterised by the presence of plaques causing disturbed conduit function (London and Drüeke 1997). *Arteriosclerosis*, on the other hand, is primarily a medial degenerative condition causing dilatation, diffuse hypertrophy and stiffening of the arteries. Arteriosclerosis and the structural and functional alterations in the heart together represent the *cardiovascular remodeling* typical of patients with CRF.

## CARDIOVASCULAR CHANGES IN CHRONIC RENAL FAILURE



## CARDIOVASCULAR MORTALITY IN CHRONIC RENAL FAILURE

- Myocardial ischaemia / infarction
- Congestive heart failure
- Arrhythmias and cardiac arrest
- Cerebrovascular events
- Other cardiac

**Figure 1.** The cardiovascular changes leading to excessive cardiovascular mortality in patients with chronic renal failure.

## **2. Clinical manifestations of atherosclerotic vascular disease in chronic renal failure**

### **2.1. Coronary artery disease**

Coronary artery disease (CAD) is more prevalent in uremia, more difficult to diagnose and less rewarding to treat, as compared to non-uremic subjects (Goldsmith and Covic 2001). Among incident dialysis patients, the prevalence of angina pectoris symptoms was 21% and the prevalence of a history of an acute myocardial infarction (AMI) 15% in 2001 (Finnish Registry for Kidney Diseases 2002). A previous revascularisation intervention was performed for 9 % of these patients. AMI accounts for approximately 20 % of cardiac deaths in dialysis patients (Herzog 2002). According to Herzog et al. (1998) the one-year mortality after myocardial infarction was 59% and the two-year mortality 73% for 34,189 dialysis patients in the USA between 1977 to 1995. The survival after an AMI was more favourable in 3,079 renal transplant recipients, since their two-year mortality was 30%.

It is unclear to what degree the dismal long-term survival of dialysis patients after an AMI reflects the vulnerability of these patients to cardiac death or the consequences of therapeutic nihilism and deficiencies in the delivery of modern cardiac care to dialysis patients (Herzog 2002). The exclusion of patients with CRF from the clinical trials on the treatment of acute coronary syndromes may have caused this therapeutic nihilism, as data on the safety and efficacy of modern pharmacological agents for the treatment is non-existent in dialysis patients. There appears to be an underutilization of thrombolytic and other pharmacological therapies among dialysis patients (Beattie et al. 2001, Fernandez et al. 2003). However, the medical management of coronary artery disease or acute coronary syndrome is generally the same as it is for the general population (Jardine and McLaughlin 2001).

The operative mortality of coronary artery bypass grafting (CABG) is about 10 %, and the survival and event-free survival rates are lower than those for non-dialysis patients (Jardine

and McLaughlin 2001). However, in these high-risk patients, CABG reduces the risk for subsequent AMI and improves survival (Solomon and Gersh 1998). The results of early studies on coronary revascularization implied that CABG was better than conventional percutaneous transluminal coronary angioplasty (PTCA) in dialysis patients (Vaitkus 2000). The high occurrence of restenosis after PTCA may be partly explained by the high proportion of diabetic patients or the excessive calcium deposition in coronary arteries of the patients with CRF (Goldsmith and Covic 2001). Coronary angioplasty with stenting has given more promising results and is now considered the method of choice for coronary revascularization in dialysis patients, if lesions are accessible to stenting (Le Feuvre 2000, Vaitkus 2000, Goldsmith and Covic 2001).

Because atherosclerotic CAD — whether symptomatic or asymptomatic — is associated with an increased incidence of perioperative or postoperative mortality a general practise today is to evaluate the presence of CAD in those patients considered for renal transplantation (de Lemos and Hillis 1996, Goldsmith and Covic 2001). The American Society of Transplant Physicians recommends screening of all potential transplantation patients except those at the very lowest risk (Murphy et al. 1998). For this purpose, the non-invasive diagnostic methods for CAD include the use of stress-testing, preferably using nuclear scintigraphic screening or stress-echocardiography. For high-risk patients with symptomatic CAD or diabetic patients older than 50 years, a coronary angiogram is recommended as the first diagnostic method (de Lemos and Hillis 1996, Jardine and McLaughlin 2001).

#### *Diagnosis of coronary artery disease*

The diagnosis of CAD is based on symptoms typical of myocardial ischemia, clinical manifestations of CAD and a history of an acute myocardial infarction (WHO MONICA Project Principal Investigators 1990). Questionnaires for the detection of the classic anginal chest pain may be used for epidemiological purposes (Rose et al. 1982). In the WHO-Monica



study, ECG criteria for a definite or possible AMI were presented (WHO MONICA Project Principal Investigators 1990). Recently, due to improvement in the diagnostic markers of myocardial damage, the role of significant changes in the levels of these markers is emphasised in the diagnosis of an AMI (The Joint European Society of Cardiology/American College of Cardiology Committee 2000). The diagnosis of CAD may also be achieved by detecting reversible ischemia in stress-ECG testing. The specificity and sensitivity of stress-testing improves with the use of nuclear scintigraphic screening or by stress-echocardiography. The golden standard for the detection of the morphologic findings of CAD is coronary angiography. Additionally, post-mortem analysis of coronary artery disease is possible in autopsy (The Joint European Society of Cardiology/American College of Cardiology Committee 2000).

One of the problems concerning the diagnostics of CAD in patients with CRF is that anginal symptoms may be present in this patient group even without the existence of CAD (Rostand et al. 1991) — or, on the other hand, these symptoms may be absent in spite of significant CAD (Weitz et al. 1996). Changes in the rest ECG are commonly present in patients with CRF. Part of the changes are caused by LVH, a common phenomenon in CRF. Consequently, the diagnosis of CAD using stress-ECG testing is unreliable in CRF (de Lemos and Hillis 1996). Moreover, a considerable proportion of these patients cannot reach the required exercise level or pulse frequency in stress testing. Therefore, pharmacological stress testing may have advantages. In one study, dipyridamole-exercise thallium imaging examination had a sensitivity of 92% and a specificity 89% for CAD in patients with CRF (Dahan et al. 1998). The findings also showed prognostic value for future coronary events. The sensitivity of 96% and specificity of 86% has been reported in dobutamine-stress echocardiography in patients with CRF (Reis et al. 1995). Finally, the use of coronary angiography is often needed for the diagnosis of CAD in these patients.

## 2.2. Cerebrovascular disease

Cerebrovascular events, both haemorrhagic strokes and ischemic infarctions, are common in patients with CRF. According to the Registry of the European Dialysis and Transplantation Association (1995), stroke accounted for 11% of deaths in end-stage renal disease. In Finland, 10% of the patients have a history of a cerebral infarction or a haemorrhagic stroke at the onset of renal replacement therapy (Finnish Registry for Kidney Diseases 2002). According to Kawamura et al. (1998) the incidence of cerebrovascular events and, especially, cerebral haemorrhage is higher in dialysis patients than in the general population. Similarly, in the study by Oliveras et al. (2003), cerebral haemorrhage was more common among renal transplant recipients than in the general population.

### *Diagnosis of cerebrovascular disease*

Cerebrovascular events are characterised by the neurological defects found in the clinical examination. A stroke may be the consequence of cerebral haemorrhage or ischemic infarction. The computed tomography scan of a subarachnoid haemorrhage and a haemorrhagic cerebral infarction have distinctly different findings. The differential diagnosis of cerebral haemorrhage is important, since the etiology of a subarachnoid haemorrhage is different from that of a cerebral infarction — the former being a consequence of an anatomic vascular defect, while the latter is a consequence of atherosclerotic vascular disease (Osborn 1994). Symptoms typical of amaurosis fugax are also highly representative for atherosclerotic vascular disease (Amaurosis Fugax Study Group 1990).

### 2.3. Peripheral arterial disease

Knowledge on the prevalence of peripheral arterial disease (PAD) in patients with CRF is limited because of a lack of uniformity in disease definition and recognition. According to clinical criteria, the prevalence of PAD was 15% among incident dialysis patients and 2.4% among incident renal transplant recipients in the USA (US Renal Data System 2000). However, on the basis of history and physical findings, estimates of the prevalence of PAD are probably underestimates of the true prevalence of PAD among patients with CRF. Therefore, a more accurate screening strategy for PAD in patients with CRF is required (O'Hare and Johansen 2001).

The incidence of non-traumatic limb amputations is approximately 10 times higher in end-stage renal disease than among the general population (Eggers et al. 1999). In Finland, limb amputations were performed for 5% of male and 3% of female incident dialysis patients in 2001 (Finnish Registry for Kidney Diseases 2002). The outcomes of limb revascularization are lower in patients with end-stage renal disease compared to the general population (Reddan et al. 2001). The factors associated with limb loss include advanced generalised atherosclerosis, extensive tissue necrosis, failed limb revascularisation and poor cardiac functional status (Simsir et al. 1995). Therefore, a carefully planned surgical approach is needed, and a more liberal use of primary amputation may be important in both limb salvage rates and overall operative mortality (Johnson et al. 1995, Simsir et al. 1995, Donayre 1996, Leers et al. 1998). However, screening programs for high-risk patients earlier to provide regular foot care are essential (Eggers et al. 1999).

#### *Diagnosis of peripheral arterial disease*

In epidemiologic studies, the prevalence of intermittent claudication may be achieved using questionnaires, such as the WHO/Rose questionnaire or the Edinburgh claudication

questionnaire (Rose et al. 1982, Fowkes et al. 1991). However, the claudication symptom is very insensitive in the diagnosis of PAD (Criqui et al. 1985, Hirsch et al. 2001), and a considerable amount of patients may have claudication without the existence of PAD (Fowkes et al. 1991, Meijer et al. 1998). The reproducibility of the palpation of pulses in the clinical examination is poor (Brearley et al. 1992). A suitable method for the non-invasive diagnosis of PAD is the ankle brachial systolic pressure index measurement (ABI), according to which the prevalence of PAD appears to be threefold higher when compared to the use of a questionnaire (Fowkes et al. 1991, Weitz et al. 1996). ABI has been shown to predict all-cause and cardiovascular mortality in the general population (Newman et al. 1993, Newman et al. 1999) and in dialysis patients (Ono et al. 2003). However, ABI values can be falsely elevated due to medial arterial calcification (MAC), which renders the diagnosis of PAD by an ABI measurement unreliable (Carter 1993, Orchard and Strandness 1993, Zierler and Sumner 2000). In this circumstance, a toe brachial index measurement (TBI) is recommended for the diagnosis of PAD, because MAC does not interfere with toe pressure measurement (Carter 1993, Zierler and Sumner 2000). In the diagnosis of PAD, the established criteria are  $ABI \leq 0.90$  or  $TBI \leq 0.60$  (Weitz et al. 1996). In renal transplant recipients, low TBI has been shown to indicate shortened patient survival (Mäkisalo et al. 1998). Finally, when invasive treatment such as percutaneous angioplasty or surgery is considered, an angiography of the lower extremities is indicated.

#### *Medial arterial calcification*

Medial arterial calcification (MAC) may cause falsely elevated blood pressures at ankle level — a phenomenon that is common among diabetic patients. The concept of MAC, also described as Mönckeberg's arteriosclerosis, has been presented both in studies concerning elevated blood pressure at ankle level (Carter 1993, Orchard and Strandness 1993, Zierler and Sumner 2000) and studies presenting the radiological findings of lower-extremity arteries

(Everhart et al. 1988, Gray et al. 1996). Typical radiological findings of MAC are uniform linear railroad track-type calcifications, which can be distinguished from the discrete plaque-like intimal-type calcifications (Lindbom 1950). Histological examination of these lesions shows calcification in the medial arterial wall, instead of the intimal calcification found in atheromatous disease (Lachman et al. 1977). An ABI value greater than 1.3 or incompressible arteries at ankle level suggest the presence of MAC (Carter 1993, Zierler and Sumner 2000).

MAC is common among patients with diabetes, and it has been shown to associate with male sex, nephropathy, and neuropathy (Edmonds et al. 1982, Maser et al. 1991). Patients with diabetes and MAC are at high risk for developing both macrovascular and microvascular complications, and their total or cardiovascular mortality is increased (Everhart et al. 1988, Lehto et al. 1996).

### **3. Risk factors for atherosclerosis**

#### 3.1. General population

Atherosclerosis is characterized by the accumulation of lipids and fibrous elements in the large and medium-sized arteries, leading to pathological thickening of vessel wall and to arterial luminal obstruction. In atherosclerosis, the intima of the muscular and elastic arteries is affected, most commonly of the coronary arteries, aorta, iliac, femoral and cerebral arteries (Ross and Glomset 1973).

The cause of atherosclerosis is multifactorial. To date, convincing evidence exists on a number of risk factors for atherosclerosis in the general population. For a risk factor to be considered causal, the marker of interest must precede the onset of disease and must have biological plausibility (Ridker et al. 2001). Several risk factors such as hyperlipidemia and hypertension are modifiable, and trials have demonstrated that lowering these factors reduces vascular risk. The conventional risk factors for atherosclerosis include hyperlipidemia, smoking, hypertension, insulin resistance and diabetes, physical activity and obesity; whereas the novel atherosclerotic risk factors include homocysteine, fibrinogen, and lipoprotein(a) as well as indices of fibronolytic function and markers of inflammation (Ridker et al. 2001). The classic risk factors for CAD include the presence of diabetes, dyslipidemia, hypertension, advanced age, family history for CAD and smoking (Finnish Cardiac Society Task Force 2000).

As an example, the association between elevated cholesterol level and atherosclerosis was first shown in a number of large-scale epidemiologic studies (Kannel et al. 1964, Steinberg 1989, Pekkanen et al. 1990). In subsequent studies, starting in the 1980ies, the benefit of lipid-lowering medication was first shown in the angiographic studies, in the which slowing down of progression and the regression of atherosclerosis were introduced (Brensike et al. 1984,

Nikkilä et al. 1984, Blankenhorn et al. 1987, Brown et al. 1990, Kane et al. 1990, Watts et al. 1992). Starting from the 1990s, the results of the large-scale statin treatment studies have led to widespread use of lipid-lowering medication in the prevention of atherosclerotic complications in the general population (Scandinavian Simvastatin Survival Study Group 1994, Shepherd et al. 1995, Heart Protection Study Collaborative Group 2002, Sever et al. 2003).

### *Homocysteine*

Elevated total homocysteine (tHcy) level has recently been presented as a significant risk factor for atherothrombotic vascular disease in the general population. Originally, the influence of elevated tHcy was demonstrated in the genetic deficiency of homocystinuria in 1962 (Carson and Neill 1962, Gerritsen et al. 1962). This autosomal recessive genetic disorder causes a deficit in cystathionine  $\beta$ -synthase and subsequently, the blood tHcy level is very high ( $\geq 100 \mu\text{mol/l}$ ) compared to the normal range (5-15  $\mu\text{mol/l}$ ) (Nygård et al. 1997, Robinson 2000). If untreated, appr. 50 % of patients have thromboembolic events, and mortality is about 20 % before the age of 30 (Nygård et al. 1997). The interest for moderately elevated tHcy level ( $>15 \mu\text{mol/l}$ ) for the pathogenesis of atherothrombotic vascular disease in the general population started when case-control studies demonstrated that patients with premature peripheral and cerebrovascular disease have higher levels of tHcy (Boers et al. 1985, Clarke et al. 1991). Most but not all studies have demonstrated an association between elevated levels of total homocysteine and CVD or markers of atherosclerosis (Alfthan et al. 1994, Verhoef et al. 1994, Perry et al. 1995, Nygård et al. 1997, Dierkes et al. 1998, Bostom et al. 1999, Taylor et al. 1999, Whincup et al. 1999, Tsai et al. 2000, Schnyder et al. 2001).

The suspected pathogenetic mechanisms of homocysteine for cardiovascular disease include effects on platelet function, clotting factors and the endothelium with vascular smooth muscle cell migration and proliferation (Mayer et al. 1996, Robinson 2000). The tHcy level

may be influenced by vitamin B<sub>12</sub>, B<sub>6</sub> and folate supplementation through their role in the metabolism of homocysteine. The genetic polymorphism of the thermolabile methylenetetrahydrofolate reductase enzyme has also been shown to have an effect on the tHcy level. The high dose of vitamin supplementation in the VISP study (The Vitamin Intervention for Stroke Prevention) consisted of 2.5 mg folic acid, 25 mg vitamin B<sub>6</sub> and 0.4 mg vitamin B<sub>12</sub> (Toole et al. 2004).

Epidemiologic studies suggest that the influence of elevated tHcy is independent of other cardiovascular risk factors (Clarke et al. 1991). However, elevated tHcy has been shown to relate positively to major components known to be associated with atherogenesis and cardiovascular risk — age, male sex, smoking, blood pressure, elevated total cholesterol, and lack of exercise (Nygård et al. 1995, Brattström and Wilcken 2000). Furthermore, renal function has an effect on tHcy, since in renal insufficiency, tHcy level is increased. Thus, it has been debated whether elevated tHcy really acts as a causal factor in the pathogenesis of atherosclerosis or rather as a marker of atherosclerosis (Kuller and Evans 1998, Brattström and Wilcken 2000).

Though it has been shown that folate, vitamin B<sub>6</sub> and B<sub>12</sub> supplementation lower tHcy, the effect of this medication for atherothrombotic vascular disease has not been demonstrated in large-scale placebo controlled trials on CVD events. The results of high-dose vitamin supplementation in the first such trial did not show advantages for recurrent cerebral infarction, coronary artery disease events or death during two-years' follow up (Toole et al. 2004). Therefore, there remains controversy on the influence of moderately elevated tHcy for CVD.



### 3.2. Risk factors for atherosclerosis in patients with chronic renal failure

Early studies on the hemodialysis population suggested that atherosclerosis is accelerated in patients with uremia (Lindner et al. 1974, Ibels et al. 1979). A magnitude of risk factors for atherosclerosis is present among patients with CRF, and the development of renal damage includes similar pathogenetic mechanisms with the development of CVD (Kasiske 1987, Diamond 1991, Longenecker et al. 2002a). The impact of these risk factors is clearly additive and the proof that CRF, per se, is the cause for accelerated atherosclerosis in CRF has not been clarified (London and Drüeke 1997). Due to the hemodynamic and metabolic changes in CRF, the significance of the risk factors for atherosclerosis may be altered in this population. Factors specific for uremia, “uremic toxins”, may promote the development of atherosclerosis in these patients (Vanholder et al. 2003).

The evidence of the link between CVD risk factors, such as elevated cholesterol level, and atherosclerosis in patients with CRF is limited, even though recent years have increased the knowledge on this topic. There are few studies using angiography for this patient population, and, in particular, the studies concerning risk factor interventions in patients with CRF are sparse. However, a growing number of studies has reported results concerning the association between various risk factors for CVD and all-cause or cardiac mortality, CVD morbidity, or markers of atherosclerosis, such as findings of atherosclerotic changes in the carotid arteries.

#### *Hypertension*

The significance of hypertension for atherosclerosis is well-established in CRF. Nearly all patients with CRF have hypertension prior to the initiation of dialysis treatment (Raine et al. 1998). Hypertension may be the cause and the consequence of CRF. In patients with CRF, hypertension has been associated with iliac artery atherosclerosis, all cause and cardiovascular

mortality as well as cardiovascular morbidity (Vincenti et al. 1980, Degoulet et al. 1982, Kasiske 1988, Zager et al. 1998). However, low blood pressure has also been shown to predict CVD mortality in hemodialysis patients, and a J-shaped or a U-shaped relationship between blood pressure and CVD mortality has been suggested (Degoulet et al. 1982, Zager et al. 1998).

Hypertension has been shown to be related to cerebrovascular accidents or haemorrhage in dialysis patients and renal transplant recipients (Degoulet et al. 1982, Kawamura et al. 1998, Oliveras et al. 2003). According to Degoulet et al. the relation between elevated diastolic blood pressure and cerebrovascular accidents is particularly obvious. Kawamura et al., on the other hand, reported that both elevated systolic and diastolic blood pressure are risk factors for cerebral haemorrhage in dialysis patients. In an earlier study, elevated diastolic blood pressure has also shown to be associated ischemic heart disease in dialysis patients (Rostand et al. 1982). Moreover, it has been demonstrated, that elevated mean arterial blood pressure is independently associated with the development of LVH (Foley et al. 1996), a strong factor associated with poor outcome in CRF. Few studies have reported on the association between blood pressure and morphologic atheromatous changes in CRF (Malatino et al. 1999, Zoccali et al. 2000, Maeda et al. 2003).

Recent findings suggest that low rather than high diastolic blood pressure is associated with advanced atherosclerosis (Levin et al. 2001, Foley et al. 2002, Tozawa et al. 2002), which emphasises the role of high pulse pressure for atherosclerosis (Zoccali et al. 2000, Maeda et al. 2003). Studies on the effect of interventions of blood pressure level on CVD are limited. However, it has been reported that good control of hypertension prolongs survival for maintenance dialysis patients (Charra et al. 1983). Furthermore, London et al. (2001) reported that active treatment of hypertension leading to LVH regression has a favourable and independent effect on-all cause and CVD survival in hemodialysis patients.

### *Diabetes*

The role of diabetes in patients with CRF is important since a major proportion of patients with CRF have diabetes — for example, 23% of Finnish dialysis patients or renal transplant recipients had diabetes in 1999 (Finnish Registry for Kidney Diseases 2000). In the USA, the proportion of dialysis patients with diabetes is even higher, 42% (US Renal Data System 2000). The presence of diabetes is associated with poor outcome among patients with CRF (Degoulet et al. 1982, Foley et al. 1997, Foley et al. 1998). As in the general population, in CRF, the risk for PAD is increased among patients with diabetes (Kasiske et al. 1996, Cheung et al. 2000, O'Hare et al. 2002).

There are few reports on the association between diabetes and morphological atherosclerotic findings in CRF. Among 110 diabetic candidates for renal transplantation the duration of diabetes predicted CAD in angiography (Manske et al. 1992). Few reports have described increased carotid intima-media thickness (IMT) among diabetic patients with CRF (Shoji et al. 2000, Lim et al. 2001).

### *Smoking*

The detrimental effect of smoking, one of the classic risk factors for atherosclerosis, has also been shown in patients with CRF. Smoking has adverse effects for renal function, worsens graft survival among renal transplant recipients (Orth 2002), and increases the risk for CVD events (Kasiske et al. 1996, Jungers et al. 1997). Furthermore, smoking is associated with PAD in dialysis patients (Wakeen and Zimmerman 1998, Cheung et al. 2000). In morphological studies on atherosclerosis in CRF, smoking has been shown to be associated with carotid atherosclerosis in some studies (Kawagishi et al. 1995, Malatino et al. 1999, Lim et al. 2001).

## *Dyslipidemia*

The typical lipid level alterations in uremia include a decrease in the high-density lipoprotein (HDL) cholesterol level and an increase in the triglyceride level — furthermore, the level of atherogenic small dense low-density lipoprotein (LDL) particles is elevated (Attman et al. 1993, Wanner and Quaschnig 2001). Total cholesterol levels are often found to fall within the normal range. The pathogenetic mechanisms behind these changes include a decrease in the activity of the lipolytic enzymes; lipoprotein lipase, hepatic triglyceride lipase and lecithin-cholesterol acyltransferase. Characteristic alterations in the apolipoprotein profile (apoA-I, apoA-II, apoB, apoC-I, apoC-II, apoC-III) are also present (Attman et al. 1993). Moreover, the level of lipoprotein(a) is increased. The characteristic dyslipidemia is present in early stages of renal disease (Grützmacher et al. 1988, Attman and Alaupovic 1991). Some factors may modify this dyslipidemia — for example, nephrotic patients usually have elevated total cholesterol, LDL-cholesterol and triglyceride levels, but the HDL-cholesterol levels are normal (Appel 1991). Similarly, the treatment modality may have effects on lipids, the dyslipidemia in peritoneal dialysis patients may differ from hemodialysis patients (Avram et al. 1992).

In spite of the quantity of abnormal lipoprotein particles in uremia, Wanner and Quaschnig (2001) suggest that a specific strategy based on LDL-cholesterol, HDL-cholesterol and triglyceride levels has to be developed for renal patients in order to identify those patients with a high cardiovascular risk in whom lipid-lowering treatment is mandatory.

## *Dyslipidemia, outcome and cardiovascular disease in chronic renal failure*

The significance of hypercholesterolemia in patients with CRF has been controversial. For example, the studies on the significance of total cholesterol level for outcome have demonstrated an inverse (Degoulet et al. 1982) or U-shaped (Iseki et al. 2002) relationship between total cholesterol level and overall or CVD mortality in hemodialysis patients. In these studies, malnutrition, a condition often accompanying uremia, was considered to explain these

findings. However, two recent studies have reported that apolipoprotein(a) size (Longenecker et al. 2002b) and non-HDL-cholesterol level (Nishizawa et al. 2003) predict mortality in patients with end-stage renal disease.

There are a few reports supporting the association between CVD and dyslipidemia in CRF (Table 1). Cressman et al. reported that lipoprotein(a) is a risk factor for CVD (1992) and CVD mortality (1994) in hemodialysis patients. In addition, Koch et al. (1997) reported that apolipoprotein B level and low HDL-cholesterol level as well as apolipoprotein(a) phenotypes predict CAD in hemodialysis patients. In the follow-up study by Kronenberg et al. (1999), apo(a) phenotype predicted CAD. In predialysis patients, low HDL-cholesterol level was identified as an independent risk factor for developing CVD events (Jungers et al. 1997). In renal transplant recipients, elevated total cholesterol level predicted post-transplant vascular disease (Kasiske 1988), and low HDL-cholesterol level predicted ischemic heart disease (Kasiske et al. 1996). In summary, the studies presented in Table 1 show that an association between elevated total cholesterol level and CVD may generally be found in renal transplant recipients, but not among predialysis or dialysis patients.

The data on the relationship between dyslipidemia and morphological findings of atherosclerosis is still not convincing, even though some reports on an association between dyslipidemia and atherosclerosis in CRF have been presented. Among the few coronary angiography studies, Rostand et al. (1984, 1986) found no independent association between hypercholesterolemia and CAD. However, a model which included race, sex, total cholesterol level and anginal symptoms predicted the presence of CAD. Of the several studies on carotid atherosclerosis, positive results on the relation between dyslipidemia and carotid artery findings in CRF are presented in Table 2.

The benefit of lipid-lowering medication for CVD was not shown in any CRF subpopulation before the presentation of the ALERT (Assessment of LEscol in Renal Transplantation) study, in which the use of fluvastatin reduced the incidence of cardiac deaths

and non-fatal AMI in renal transplant recipients (Holdaas et al. 2003). In recent years, the use of lipid-lowering medication has become general in patients with CRF in Finland, since 35% of patients are currently receiving lipid-lowering medication at the start of renal replacement therapy (Finnish Registry for Kidney Diseases 2002). This may also explain the decrease in the cholesterol level in Finnish patients on renal replacement therapy between 1999 and 2002 (Finnish Registry for Kidney Diseases 2003).

**Table 1.** Summary of studies reporting positive findings on the association between dyslipidemia and CVD in CRF

Reference	Risk factor	Endpoint	Patients	Setting
Kasiske et al. 1988	Cholesterol	CVD	464 RT	follow-up 46.1 ± 36.2 months
Cressman et al. 1992	Lp(a)	CVD event	129 HD	follow-up 4 years
Cressman et al. 1994	Lp(a)	CVD mortality	129 HD	follow-up 4 years
Kasiske 1996	Low HDL	ischemic heart disease	706 RT	follow-up 7.0 ± 4.2 years
Jungers et al. 1997	Low HDL	CVD events	147 PRED	follow-up 10 years
Koch et al. 1997	ApoB, low HDL	CAD	607 HD	cross-sectional
Massy et al. 1998	Apo(a) phenotypes Cholesterol	CVD events	79 RT	follow-up 18 ± 5 months
Kronenberg et al. 1999	Apo(a) phenotype	CAD	440 HD	follow-up 5 years
Aakhus et al. 1999	Cholesterol	ischemic heart disease	406 RT	cross-sectional
Nizhizawa et al. 2003	Non-HDL	CVD mortality	525 HD	follow-up 64 months

Abbreviations: CVD, cardiovascular disease; CRF, chronic renal failure; Lp(a), lipoprotein(a); HDL, high-density lipoprotein cholesterol; ApoB, apolipoprotein B; Apo(a), apolipoprotein(a); CAD, coronary artery disease, HD, hemodialysis; RT, renal transplant recipient; PRED predialysis.

**Table 2.** Summary of studies reporting positive findings on the association between dyslipidemia and carotid atherosclerosis in CRF

Reference	Risk factor	Endpoint	Patients	Setting
Kronenberg et al. 1994	Apo(a) phenotype, Lp(a)	Plaque	167 HD	cross-sectional
Jogestrand et al. 1996	Cholesterol	Plaque	16 RT	cross-sectional
Rossi et al. 1996	Triglycerides	Carotid lesion (IMT + plaque)	55 PRED, 33 HD, 15 RT	cross-sectional
Fujisawa et al. 2000	Lp(a)	IMT, plaque score	12 PRED, 51 HD	cross-sectional
Cofan et al. 2002	Cholesterol, triglycerides ApoB	Plaque	50 RT	cross-sectional
Ohkuma et al. 2003	Lp(a)	IMT, plaque score	59 PD	cross-sectional

Abbreviations: CRF, chronic renal failure; Apo(a), apolipoprotein(a); Lp(a), lipoprotein(a); ApoB, apolipoprotein B; IMT, intima-media thickness; HD, hemodialysis; RT, renal transplant recipient; PD, peritoneal dialysis; PRED, predialysis.



### *Oxidation*

Lipoprotein oxidation is involved in the genesis of atherosclerosis (Ross 1999). In CRF, oxidative stress is enhanced because of an imbalance between pro-oxidant and antioxidant systems (Drüeke et al. 2001, Galle 2001). In particular, oxidative damage to LDL and endothelial cells are important in the development of fatty streaks, the early lesion in atherosclerosis, and may represent an alternative pathway in the pathogenesis of atherosclerosis in CRF. In studies on hemodialysis patients, oxidative stress has shown to be associated with the severity of CVD (Usberti et al. 2002). Oxidative stress has also been shown to be related to endothelial function in CRF (Annuk et al. 2001). Stenvinkel et al. (1999) showed that the presence of carotid plaques are associated with oxidized LDL among predialysis patients. Moreover, a high titer of antibodies against oxidized LDL is protective for CVD in end-stage renal disease (Shoji et al. 2002), and these antibodies are inversely associated with carotid artery IMT (Shoji et al. 2003). In a secondary prevention study, the use of high-dose vitamin E supplementation had a favourable effect for CVD during 1.4 years' follow-up among 196 hemodialysis patients (Boaz et al. 2000). To date, such results have not been confirmed in larger-scale studies in CRF.

### *Inflammation*

Atherosclerosis has been described as an inflammatory disease (Ridker et al. 1997, Ross 1999). Inflammation may enhance the development of atherosclerosis also in CRF, since it has been presented that uremia, in fact, is an inflammatory state, and markers of inflammation may be elevated in uremia even without a known cause (Cavaillon et al. 1992, Pereira et al. 1994, Arici and Walls 2001, Ducloux et al. 2002, Fine 2002, Panichi et al. 2002).

Reports on the association between inflammation and mortality or CVD mortality in patients with CRF have been presented (Zimmermann et al. 1999, Yeun et al. 2000, Qureshi et al. 2002), though in the largest study of 988 hemodialysis patients, Owen and Lowrie (1998)

could not confirm this result. Furthermore, in this study CRP level was associated with nutritional parameters (Owen and Lowrie 1998). Some studies have also reported on an association between CRP or markers of inflammation and CVD morbidity (Kim et al. 2002, Stefoni et al. 2002). The fact that the number of acute transplant rejection episodes has an effect on CVD events in renal transplant recipients may also be considered as one sign of the detrimental effect of inflammation for CVD (Kasiske et al. 1996).

In studies on morphological findings of atherosclerosis, associations between markers of inflammation (fibrinogen, CRP, interleukin-6) and carotid atherosclerosis have been found (Rossi et al. 1996, Stenvinkel et al. 1999, Zoccali et al. 2000, Kato et al. 2002, Zoccali et al. 2002, Ohkuma et al. 2003). The association between chlamydiae pneumoniae seropositivity and carotid atherosclerosis in dialysis has been described (Stenvinkel et al. 2002). Stenvinkel et al. (1999) have also demonstrated a strong association between malnutrition, inflammation and carotid atherosclerosis in predialysis patients.

### *Homocysteine*

In the end-stage renal disease population on hemodialysis, median levels of homocysteine are markedly elevated, often within the range of 25 – 30  $\mu\text{mol/l}$  (Robinson 2004). The underlying cause of hyperhomocysteinemia in renal disease is not entirely understood but seems to involve reduced clearance of plasma homocysteine and defects in the metabolism of homocysteine (Friedman et al. 2001). Since CVD is very common in CRF and the prevalence of elevated tHcy in CRF is high, elevated tHcy has been presented as a potential “uremic toxin” influencing to the high prevalence of CVD in CRF (Perna et al. 2003). The beneficial effect of folate, vitamin B<sub>6</sub> and vitamin B<sub>12</sub> substitution for tHcy level in the CRF population has been presented (Bostom et al. 1997, Perna et al. 1997, Jungers et al. 1999, Massy 2003). However, the tHcy level of dialysis patients has been found refractory for high dose folate or vitamin B

substitution, whereas a better response to this treatment is found in renal transplant recipients (Bostom et al. 2001).

A summary of studies on the association between elevated tHcy level and CVD or carotid atherosclerosis in CRF is presented in Table 3. The studies have produced conflicting results. Elevated tHcy level has been proposed to be a predictor of increased cardiovascular morbidity or mortality in some studies (Table 3), whereas others have failed to observe such a relationship. In the study by Suliman et al. (2000), low tHcy level constituted a risk for the presence of CVD and overall mortality. The authors considered that this opposite result of the significance of tHcy for CVD may be explained by poor nutritional status, which was more common in patients with CVD. To date, there are no consistent results supporting the association between elevated tHcy level and carotid atherosclerosis in CRF (Table 3). Furthermore, the results of the benefit of folate and vitamin B supplementation for CVD events in CRF are not available. Thus, the significance of elevated tHcy level for CVD in patients with CRF still remains unclear.

**Table 3.** Summary of studies on the association between elevated tHcy level and CVD or carotid atherosclerosis in CRF

Reference	Endpoint	Patients	Setting	Result
Robinson et al. 1996	CVD	176 PD	cross-sectional	positive
Bostom et al. 1997	CVD events	73 HD, PD	follow-up 17 months	positive
Moustapha et al. 1998	CVD events or CVD mort.	130 HD, 46 PD	follow-up 17.4 ± 6.4 months	positive
Sulman et al. 2000	mort.	117 HD	follow-up 4 years	opposite
Ducloux et al. 2000	CVD events	207 RT	follow-up 21.2 ± 1.9 months	positive
Hagen et al. 2001	mort.	189 RT	follow-up 2.3 ± 0.7 years	negative
Wrone et al. 2001	CVD	430 HD, 29 PD	cross-sectional	opposite
Mallamaci et al. 2002	CVD mort.	175 HD	follow-up 29 ± 12 months	positive
Massy et al. 1998	Carotid plaque CVD events	79 RT	plaque, cross-sectional CVD, follow-up 18 ± 5 months	negative negative
Zoccali et al. 2000	Carotid IMT, plaque	92 HD, 46 PD	cross-sectional	positive
Suwelack et al. 2000	Carotid IMT	53 RT	follow-up 12 months	negative
Haraki et al. 2001	Carotid IMT, plaque	43 HD, 12 PD	IMT, plaque; cross-sectional	negative
Kennedy et al. 2001	CVD events	69 HD, 60 PD	CVD follow-up 31 ± 3 months	positive
Zoccali et al. 2002	Carotid IMT	90 HD	cross-sectional	negative
Kato et al. 2003	Carotid IMT mort., CVD mort.	219 HD	follow-up 15 months IMT, cross-sectional	negative negative
Maeda et al. 2003	Carotid plaque	226 HD	mort., follow-up 5 years	negative
Ohkuma et al. 2003	Carotid IMT	59 PD	cross-sectional	negative
	Plaque score			positive

Abbreviations: tHcy, total homocysteine; CVD, cardiovascular disease; CRF, chronic renal failure; mort., mortality; IMT, intima-media thickness; PD, peritoneal dialysis; HD, hemodialysis; RT, renal transplant recipients. Definitions: positive result, elevated tHcy associated with endpoint; negative, no association; opposite, low tHcy associated with endpoint.

## 4. Arteriosclerosis in chronic renal failure

### 4.1. Definition

London and Drüeke (1997) describe *atherosclerosis* as primarily an intimal disease, focal and patchy in its distribution, occurring preferentially in the carotid bifurcation, coronaries, renal arteries, infrarenal aorta, and femoral arteries. They describe *arteriosclerosis* as primarily a medial degenerative condition that is generalised throughout the thoracic aorta and central arteries, causing dilatation, diffuse hypertrophy and stiffening of arteries but not occlusion. Another description divides atherosclerosis into two components: *atherosis* (morphologic wall thickening) and *sclerosis* (functional stiffening) (Blankenhorn and Kramsch 1989, Nishino et al. 1994, Shoji et al. 1998). Thus, this latter description of atherosclerosis includes the concept of arteriosclerosis. In any case, these conditions frequently coexist (London and Drüeke 1997).

### 4.2. Arterial stiffness

The stiffness of an arterial segment can be determined by changes in circumference or diameter, induced by a change in pressure. Increased arterial stiffness can be the “passive” consequence of increased blood pressure or a result from structural or functional alterations of the arterial wall (London 1994). Increased arterial stiffness predisposes to the development of LVH and to a decrease in the subendocardial flow (London and Drüeke 1997). The methods for evaluating increased arterial stiffness include determination of parameters such as pulse-wave velocity (PWV), distensibility, compliance, incremental elastic modulus and the stiffness index  $\beta$  (Hirai et al. 1989, London et al. 1990, London 1994, Mourad et al. 1997, Guerin et al. 2000). All these parameters may be evaluated using either Doppler or M-mode ultrasound measurements.

The results of studies on arterial stiffness indicate that arterial stiffness is increased in CRF (London et al. 1990, Mourad et al. 1997, Shoji et al. 1998, Groothoff et al. 2002) and related to vascular calcifications (Guerin et al. 2000) and to the presence of LVH (London et al. 1990). Moreover, increased arterial stiffness predicts cardiovascular and all-cause mortality (Blacher et al. 1998, Blacher et al. 1999). In risk factor analyses, increased arterial stiffness has been shown to be associated with triglyceride rich intermediate density lipoproteins (Shoji 1998) and hyperphosphatemia (Marchais et al. 1999). Parameters describing arterial stiffness may also be used as vascular endpoints in follow-up studies (London et al. 2003).

#### 4.3. Vascular calcification

Calcification of the intimal atheroma generally occur in advanced lesions (Stary et al. 1992). This process is focal, and adjacent regions of the vessel wall may be remarkably normal. Another type of calcification, medial arterial calcification (MAC), results in diffuse mineral depositions throughout the vascular tree, occurring predominantly in the media of the vessel, with no relationship to atheromatous plaque formation. This form of calcification is characteristic of ageing, but is also found in diabetes and CRF (Davies and Hruska 2001). The intimal-type calcifications and MAC have distinctly different radiological findings (Lindbom 1950).

In 1979, Ibels et al. (1979) showed that calcification was typical in arterial samples of uremic patients. Recently, electron beam computed tomography findings of the coronary arteries have revealed calcification in uremic patients (Braun et al. 1996, Goodman et al. 2000). A similar finding has also been found in autopsy (Schwarz 2000). Calcification of carotid artery plaques have shown to be common in dialysis patients (Savage et al. 1998). Recently, in hemodialysis patients, the presence of intimal-type calcifications or MAC reduced

all-cause and CVD mortality, but the survival of patients with MAC was longer than that of patients with intimal-type calcifications (London et al. 2003).

#### *Effect of calcium-phosphate disorders*

The following factors specific for CRF may contribute to the development of vascular calcification in patients with CRF: secondary hyperparathyroidism, disordered calcium and phosphate homeostasis, and use of vitamin D and high-dose calcium preparations (Davies and Hruska 2001). Block et al. have showed that the changes in the calcium-phosphate metabolism are associated with poor outcome in CRF. In their large-scale two-year follow-up study of 2,134 hemodialysis patients, elevated phosphate level and calcium-phosphate product predicted mortality, but not calcium level. Ganesh et al. (2001) have also showed that elevated phosphate, calcium-phosphate product and parathyroid hormone predict cardiac mortality in hemodialysis patients.

Further studies have shown that phosphorous level, calcium phosphorous product and the daily intake of calcium are higher among hemodialysis patients with coronary calcification (Goodman et al. 2000). London et al. (2003) demonstrated that the risk factors of MAC differ from the risk factors for intimal-type calcifications. MAC was associated with the duration of dialysis treatment and disorders in calcium-phosphate metabolism. The risk factors for intimal-type calcifications resembled typical risk factors associated with atherosclerotic disease. In other studies, vascular calcification in CRF has been associated with age and hypertension (Braun et al. 1996); high triglyceride and low HDL-cholesterol level (Tamashiro et al. 2001); as well as age, diabetes, lipoprotein(a) and homocysteine levels (Kronenberg et al. 2003).

The evidence from arterial calcification has suggested an active process similar to osteogenesis (Watson et al. 1997, Moe et al. 2002). The recent findings on the importance of the disorders in the calcium-phosphate metabolism for vascular calcification in CRF have also

altered the traditional management of these disorders in patients with CRF (Kidney Diseases Outcomes Quality Initiative Guidelines 2003).

## **5. Ultrasound examination of arterial vessels**

### **5.1. Carotid artery ultrasound examination**

The primary goal of the carotid artery ultrasound examination is the estimation of carotid artery stenoses for possible surgical repair in patients with CEVD symptoms. In addition, a high resolution B-mode ultrasound measurement of the carotid intima-media thickness (IMT) gives a reliable and valid estimate of the arterial wall (Crouse and Thompson 1993) and is considered more appropriate than angiography when risk factors for atherosclerosis are studied. In B-mode carotid ultrasound, it is possible to examine the prevalence and nature of carotid artery plaques (Salonen and Salonen 1993, Savage et al. 1998). Furthermore, the estimation of carotid artery stiffness may be achieved by the determination of parameters such as incremental elastic modulus and the stiffness index  $\beta$  (Hirai et al. 1989, Guerin et al. 2000).

In recent years a growing number of carotid ultrasound studies have been performed on the general population and in patients with CRF. Most commonly, IMT has been used in the description of early atherosclerosis. However, there is variation in the methods and the site of the IMT measurement (Ebrahim et al. 1999). IMT measurement of the common carotid artery alone has been considered to be more reliable and reproducible than measurements from external and internal carotid arteries (Crouse and Thompson 1993). In addition to cross-sectional studies, the use of carotid artery atherosclerotic findings have also been established in follow-up studies and interventional studies (Salonen and Salonen 1990, Smilde et al. 2001). Recently, it has been presented that carotid plaque status explains prevalent cardiovascular



disease more accurately than IMT, and carotid plaque score predicts atherothrombotic cerebral infarction more effectively than IMT (Ebrahim et al. 1999, Nagai et al. 2002).

## 5.2. Thoracic aortic examination with transesophageal echocardiography

Transesophageal echocardiography (TEE) has become a widely used imaging technique for evaluating cardiac structure, function, and valvular anatomy. Furthermore, TEE provides excellent images of the thoracic aorta, which cannot be visualised by transthoracic echocardiography. TEE has been used in identifying thoracic aortic aneurysms and dissections, potential aortic sources of embolism and abnormal vascular connections as well as in the diagnosis of endocarditis (Fazio et al. 1993, Blanchard et al. 1994). It has also been utilised to avoid vascular complications during bypass surgery and intra-aortic balloon pump replacement. The close proximity of the transesophageal probe to the thoracic aorta allows reliable analysis of the degree of thoracic aortic atherosclerosis.

The studies on thoracic aortic atherosclerosis have shown that the presence of thoracic aortic plaques predict CAD in the general population (Fazio et al. 1993, Tribouilloy et al. 1994, Khoury et al. 1997, Belhassen et al. 2002). Transesophageally determined thoracic aortic atherosclerosis have also been used as markers of atherosclerosis when risk factors for atherosclerosis have been studied. In the general population, common risk factors for atherosclerosis are related to the atheromatous thoracic aortic disease (Nishino et al. 1994, Tribouilloy et al. 1998, Peltier et al. 2002). Due to the insertion of the transesophageal probe, TEE is not entirely non-invasive, but has been shown to be safe (Daniel et al. 1991). Furthermore, in their study of 10,419 patients, Daniel et al report that in 3.4% of the examinations, the insertion of the TEE probe was not successful, and in 0.9 % TEE had to be interrupted because of discomfort.

## **AIMS OF THE STUDY**

The significance of the disease states related to atherosclerosis seems enhanced in patients with CRF, yet the knowledge of the prevalence of these disease states as well as the risk factors for atherosclerosis in this patient group is limited. Therefore, among patients with CRF, the specific goals of the present study were:

1. To examine the diagnostics and the prevalence of peripheral arterial disease and medial arterial calcification.
2. To study the severity and risk factors for thoracic aortic atherosclerosis.
3. To examine the extent and nature of carotid artery atherosclerosis.
4. To investigate the significance of elevated total homocysteine level and other cardiovascular risk factors for carotid atherosclerosis.
5. To study whether the atherosclerotic findings of the thoracic aorta detected by transesophageal echocardiography are useful in predicting coronary artery disease.

## SUBJECTS

### *Patients*

The study population was selected from among patients living in Tampere or neighbouring municipalities, an area with a total population of 304,190 inhabitants. In the Tampere University Hospital District, information on all adult (>16 years) predialysis patients with chronic renal failure (creatinine  $\geq 200$   $\mu\text{mol/l}$ ) and renal transplant recipients is collected in the Pirkanmaa District Kidney Register. The study population in **study I** consisted of the predialysis patients and renal transplant recipients in this register. In addition, patients on hemodialysis or peritoneal dialysis therapy were invited to participate in the study. Age was limited to younger than 70 years. The study subjects were in stable condition and had no acute infections at the time of the examination.

In the recruitment, all eligible patients in the register in 1998 were invited twice by mail to participate in the study. In addition, eligible dialysis patients were invited in person during the study period. As a result, all patients willing to attend took part in the study (Table 4). The proportion of patients studied in relation to the parent population accounted for 63% for the 59 predialysis patients and 48% for the 41 renal transplant recipients. The age and sex distribution of the patients studied was comparable to that of the patients not included in these two subgroups (**study I**). The 36 dialysis patients represent a sample of the dialysis population, for which corresponding participation cannot be accurately defined.

The primary causes of CRF were chronic glomerulonephritis in 37 (27%) patients, diabetic nephropathy in 32 (24%), polycystic kidney disease in 18 (13%), hypertensive nephropathy in 12 (9%), chronic interstitial nephritis in 11 (8%), chronic pyelonephritis in 7 (5%), congenital nephropathy in 3 (2%), amyloidosis in 2 (1.5%) and other, defined cause in 8 (6%) patients. In 6 patients (4.5%), the primary cause of CRF remained unknown.

**Studies III and IV** (Table 4) include the same patient population with the exception of one predialysis patient. This patient was excluded from risk factor analysis and following studies, because the previously elevated creatinine level had been normalised at the time of examination. **Studies II and V** (Table 4) include those 118 study patients (87%) who participated in the TEE examination and for whom TEE was successfully performed.

#### *Control subjects*

The 59 control patients in **study I** were invited from among the orthopaedic patients in the Tampere University Hospital or Tampere Municipal Hospital. The control group was matched by age, sex, and body mass index with the predialysis group, the largest subgroup among the study patients. Except for their orthopaedic disability, the control subjects had no previously known chronic illness, which was confirmed by a brief interview before they attended the study. Similarly to the predialysis patient group, one control subject was excluded from **studies III and IV** (Table 4).

**Table 4.** Demographic characteristics of the study subjects in studies I – V

Study	Group	Number of subjects	Male	Age (years)	Presence of diabetes	Presence of hypertension
III, IV (I*)	Predialysis	58	66%	55 ± 11	26%	97%
	Dialysis	36	78%	51 ± 11	42%	94%
	Transplantation	41	56%	49 ± 11	22%	95%
	Control	58	66%	55 ± 11	2%	16%
II, V	Predialysis	52	69%	55 ± 12	27%	96%
	Dialysis	32	81%	51 ± 12	41%	94%
	Transplantation	34	53%	49 ± 12	24%	94%
	Control	-				

Data are number, percentage or mean ± SD

\* An additional predialysis patient and control subject were included in study I.

*Ethical aspects of the study*

The study was approved by the Tampere University Hospital ethical committee and Tampere City ethical committee (control subjects), and all subjects gave an informed consent before their examination.

## **METHODS**

### **1. Study protocol**

The study subjects were examined during March 1998 and December 2000. In general, two study patients and one control subject was examined on a weekly basis. The study protocol of the study patients included three separate visits to the researcher (Y.L.). The first visit included a careful interview and clinical examination with an evaluation of patient history based on hospital and outpatient records. In addition, transthoracic echocardiography was performed (results not presented in this study). The following two examinations included a visit for a TEE examination and a visit for the carotid ultrasound examination as well as for the blood pressure measurements from upper and lower extremities using photo-plethysmography. Blood samples were drawn before the TEE examination. The study protocol of the control subjects was similar with the exception that TEE was not performed.

### **2. Evaluation of patient history and interview**

Prior to the first visit, patient history was evaluated according to hospital records. If necessary, outpatient records were analysed later for sufficient information of patient history. The data on patients' chronic illnesses and medication was obtained. The duration of CRF was measured from the time the initial creatinine value exceeded 200  $\mu\text{mol/l}$ . The duration of predialysis CRF, dialysis treatment and the duration of a functioning renal transplant were recorded. The duration of a functioning renal transplant was not included in the duration of CRF, which included both the duration of predialysis CRF and dialysis treatment in risk factor analysis.

The interview included information on the patients' marital status, educational level and present occupation. The presence of classic exercise angina and the presence of claudication

were determined by an interview according to the WHO/Rose questionnaire (Rose et al. 1982). Furthermore, family history for CAD and smoking history were recorded. Smokers or ex-smokers were defined as heavy smokers if they had a history of smoking 20 cigarettes daily for 10 years.

#### *Definitions of hypertension and diabetes*

Hypertension was diagnosed when a study subject had received medical treatment for hypertension, or had systolic blood pressure  $\geq 160$  mmHg or diastolic blood pressure  $\geq 95$  mmHg at the time of the examination. Diabetes was diagnosed when a previous or current 12-hour fasting blood glucose level was 6.7 mmol/l or greater (WHO Study Group 1985). The duration of hypertension was defined from the time the patient started receiving medical treatment for hypertension and the duration of diabetes from the time of initial diagnosis.

#### *Definitions of coronary artery disease and cerebrovascular disease*

The criteria for coronary artery disease in **studies I-IV** included a history of a documented myocardial infarction (possible or definite AMI; WHO MONICA Project Principal Investigators 1990), a coronary angiogram showing significant occlusive disease ( $\geq 50\%$  stenosis), a history of classic exercise angina, or Minnesota codes 1.1 – 1.2 present in the ECG (Rose et al. 1982). In the follow-up **study V**, in which the relation between transesophageally determined thoracic aortic atherosclerosis and CAD was analysed, stricter criteria for CAD were used. In **study V**, CAD was defined by a history of a documented myocardial infarction, a coronary argiogram or a post-mortem autopsy finding showing significant occlusive CAD by the end of the follow-up period. The analysis of the coronary angiograms that were performed for the study patients prior to their examination or during follow-up (**study V**) was performed by a cardiologist unaware of the patient's clinical history.

The definition for cerebrovascular disease (CEVD) included a history of a documented stroke or transient ischemic attack, as well as symptoms typical of amaurosis fugax. Patients who had experienced a subarachnoid haemorrhage were excluded from the CEVD group.

### **3. Clinical examination**

A clinical examination was performed with attention to the cardiovascular status of the study subject. Blood pressure was measured as the mean of three successive blood pressure measurements with the patient lying in supine position. Measurements were performed using an aneroid sphygmomanometer which was calibrated within three-month intervals. An electrocardiogram was taken shortly prior to the clinical examination, and the findings were classified according to the Minnesota codes (Rose et al. 1982).

### **4. Laboratory measurements**

Venous blood samples were drawn after a 12-hour overnight fast. Blood hemoglobin, glucose, serum creatinine, ionized calcium, phosphate, albumin, and fibrinogen were measured using the standard method at Tampere University Hospital. Laboratory tests for hemodialysis patients were performed during a non-dialysis day, and tests for insulin-dependent patients, after a light breakfast. A 24-hour urine collection was performed, and creatinine clearance was calculated (Van Lente and Suit 1989).

Plasma samples for lipid, sensitive C-reactive protein (CRP) and total homocysteine (tHcy) determinations were obtained at the time of the examination and stored at -70°C until analysis. The plasma tHcy (free, bound, reduced and oxidized) level was measured using a previously described method (Wirta et al. 1998). Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and CRP were analyzed by Cobas Integra 700 automatic



analyzer (Hoffmann-La Roche Ltd., Basel, Switzerland). Low-density lipoprotein (LDL) cholesterol was calculated using Friedewald's formula when the triglyceride level was less than 4.0 mmol/l. Forty-four (33%) of the study patients in **study IV** and 38 (32%) in **study II** had been administered lipid-lowering medication (statins) for a mean of  $2.0 \pm 1.5$  years. In these cases, lipid values before the initiation of medication were used in the analysis.

## **5. Plethysmographic examination for ABI and TBI**

Ankle brachial index (ABI) and toe brachial index (TBI) were measured using Nicolet VasoGuard<sup>®</sup>, a device which allows simultaneous systolic blood pressure measurements from the upper and lower extremities using photoplethysmography. The ABI and TBI measurements were performed in optimal conditions: the measurements were performed while the study subject was in supine position at room temperature after a rest of at least 30 minutes before the pressure measurements. The photoplethysmographic probes were attached to the tips of the thumbs in the upper extremities and to tips of the great toes in the lower extremities. The cuffs were placed in the arms and the legs above ankle level or to the base of the great toes. The higher value of the simultaneously measured brachial systolic blood pressure values was used, and in case of an arteriovenous shunt, the brachial blood pressure was measured from the arm free of the shunt. The values of a single ABI or TBI measurement of a good quality were used, but no pathologic or near-pathologic values were accepted unless they could be repeated.

### *Definitions of peripheral arterial disease and medial arterial calcification*

The definition of peripheral arterial disease (PAD) included the existence of any of the three following criteria: a positive ankle-brachial index (ABI) in either leg, a positive toe-brachial index (TBI) for PAD in either foot, or a history of positive lower extremity angiogram. ABI values  $\leq 0.90$  and TBI values  $\leq 0.60$  were considered positive findings in diagnosing PAD

(Weitz et al. 1996). ABI values  $\geq 1.3$  or incompressible arteries at ankle level were considered to indicate medial arterial calcification (MAC) (Carter 1993). If the ABI indicated MAC, a TBI measurement was used for the diagnosis of PAD. Study subjects with claudication that met the criteria for PAD were defined to have true claudication.

## **6. Carotid ultrasound examination**

Examination of the carotid arteries was performed with a 7 MHz linear probe using a commercially available ultrasound system (Acuson 128 XP, Acuson Inc., Mountain View, CA). The B-mode scanning of the carotid arteries included the scanning of the right and left common carotid arteries (3 cm section before the carotid bifurcation), carotid bifurcation, as well as of the internal and external carotid arteries — both 2 cm distally from the carotid bifurcation. The maximum intima-media thickness (IMT<sub>max</sub>) was measured from the far wall of the common carotid artery (3 cm section before bifurcation) and from a fixed (IMT<sub>fixed</sub>) position 1 cm proximal to the bifurcation. Three IMT measurements of the right and left common carotid arteries were performed from anterolateral, lateral and posterolateral angles of the far wall (Salonen and Salonen 1993). IMT was not measured at the carotid bulb or at the site of a carotid plaque.

In addition, carotid artery plaques were examined from a suitable longitudinal and transverse view. A plaque was defined as a focal thickening relative to the adjacent segment with protrusion into the lumen and with a minimum diameter of 1.2 mm. The plaque score was calculated by summing up the thickness of all plaques from both carotid systems (Fujisawa et al. 2000). Carotid artery plaques were classified qualitatively as soft, mixed or calcified plaques according to their calcification (Savage et al. 1998).

The maximum pulsed Doppler velocities at the site of carotid plaques were used to classify the stenoses of the internal carotid artery (Moneta et al. 1993). The stiffness index  $\beta$

was calculated according to Hirai et al. (1989);  $\beta = \ln(Ps/Pd) \times Dd/(Ds-Dd)$ . In this formula, the diastolic diameter (Dd) and systolic diameter (Ds) represent the mean of five consecutive internal diameters in the M-mode image of the common carotid artery at 1 cm proximal to the bifurcation. Ps and Pd represent systolic and diastolic blood pressure measurements at the time of the M-mode imaging.

The intraobserver reproducibility of the carotid artery examination was assessed by a repeated examination of 15 study subjects (**study III**). In addition, the interobserver repeatability of the off-line measurements was performed.

## **7. Transesophageal echocardiography**

Transesophageal echocardiography (TEE) was performed after an overnight fast at between 07.00 and 08.00 in the morning and for insulin dependent diabetic patients, after at least four hours' fast between 01.00 and 02.00 o'clock in the afternoon. The study was performed with Acuson 7 MHz multiplane transesophageal probe, which was connected to Acuson 128 XP 10 ultrasound system (Acuson Inc, Mountain View, CA). Oropharyngeal xylocaine anesthesia without intravenous sedation was used before the insertion of the probe. The thoracic aorta was examined with a short axis view of the ascending aorta, aortic arch, and the descending aorta. The atherosclerotic changes of the thoracic aorta were graded (grade I-IV, **study II**) using a modification of previously described classifications (Fazio et al. 1993, Khoury et al. 1997). Atherosclerotic plaques  $\geq 3.0$  mm in diameter (grades III-IV) were defined as large aortic plaques (LAPs). The tracings of 15 consecutive patients were selected for the intraobserver and interobserver reproducibility measurements (**study II**).

## 8. Statistical methods

Results are expressed as mean  $\pm$  SD or number and percentage. Comparison of continuous variables between study groups was performed using unpaired t-test or one-way analysis of variance (ANOVA), followed by least significant difference method for multiple comparisons. When a variable was not normally distributed, Mann-Whitney *U* test or Kruskal-Wallis test was used. In such a case, Bonferroni correction was used for multiple comparisons. Comparison of the frequencies of classified variables was performed using chi-square test and, in the 2 x 2 tables, Fisher's exact test. The analysis of correlation was performed either by using Pearson's or Spearman's (non-parametric) correlation test. Multivariate analyses were performed using binary logistic regression analysis. A p-value  $< 0.05$  was considered statistically significant. Statistical analyses were performed using the SPSS version 9.0 (SPSS Inc, Chicago, IL) or Statistica version 5.0 (StatSoft Inc, Tulsa, OK) computer software.

## RESULTS

### 1. Peripheral arterial disease and medial arterial calcification

#### *Peripheral arterial disease*

The prevalence of PAD was 22.0% in patients with predialysis CRF, 30.6% in patients on dialysis, 14.6% in renal transplant recipients and 1.7% in control subjects (Table 5). The differences in prevalence between the study groups were significant showing a difference between the control group and all the CRF patient groups. There was no significant difference in the prevalence of PAD between male or female patients with CRF (24% vs. 17%,  $p=0.39$ ). Similarly, no significant difference was found between patients  $\geq 55$  years and younger patients (29% vs. 17%,  $p=0.14$ ). The presence of diabetes constituted a risk for PAD (44% vs. 13%,  $p<0.001$ ). The prevalence of claudication, according to the questionnaire, was 6.6% ( $n=9$ ) among the 136 patients. The prevalence of true claudication was 4.4% ( $n=6$ ), since three patients with claudication did not meet the criteria for PAD.

**Table 5.** The prevalence of PAD and MAC in the study groups

	Predialysis $n=59$	Dialysis $n=36$	Transplantation $n=41$	Control $n=59$	p
PAD	22.0% (13)	30.6% (11)	14.6% (6)	1.7% (1)	0.001
MAC	23.7% (14)	41.7% (15)	23.1% (9)	3.4% (2)	$<0.001$

Data are percentage and (number). PAD, peripheral arterial disease; MAC, medial arterial calcification.

### *Medial arterial calcification*

Medial arterial calcification was observed in 23.7% of the patients with predialysis CRF, in 41.7% of the patients on dialysis, in 23.1% of the renal transplant recipients and in 3.4% of the control subjects (Table 5). The difference in the prevalence of MAC between the control group and all the CRF patient groups was significant. Most of the patients with MAC were male (87%,  $p=0.002$ ). MAC was strongly associated with diabetes (58% vs. 17%,  $p<0.001$ ). However, the prevalence of MAC did not differ between patients  $\geq 55$  years and younger patients (35% vs. 24%,  $p=0.18$ ). No significant difference in the presence of PAD was found between the patients with and without MAC (26% vs. 19%,  $p=0.35$ ).

## **2. Carotid atherosclerosis in the study groups**

The maximum intima-media thickness (IMT<sub>max</sub>) of the common carotid artery did not differ between any of the study groups (predialysis  $0.83 \pm 0.23$ ; dialysis  $0.78 \pm 0.20$ ; transplantation  $0.79 \pm 0.21$ ; control  $0.80 \pm 0.20$  mm;  $p=0.764$ ). In contrast, the plaque prevalence (predialysis 64%; dialysis 61%; transplantation 51%; control 28%;  $p<0.001$ ) and plaque score (predialysis  $3.3 \pm 4.3$ ; dialysis  $3.0 \pm 3.4$ ; transplantation  $2.5 \pm 3.2$ , control  $0.8 \pm 1.7$  mm;  $p<0.001$ ) of all CRF patient groups were significantly higher than those of the control group. However, no difference was found in these parameters between the CRF patient groups. The patient groups had more calcified plaques and early stage internal carotid artery stenoses than the control group (**study III**, Table 2). The comparison of stiffness index  $\beta$  revealed that carotid artery stiffness was increased in all the patient groups in comparison to the control group, whereas there was no significant difference in  $\beta$  between the patient groups.

### 3. Risk factors for atherosclerosis

The aim in **studies II and IV** was to examine the association of the various risk factors for atherosclerosis with the development thoracic aortic atherosclerosis (**study II**) and carotid atherosclerosis (**study IV**). These risk factors included both clinical parameters and laboratory findings of the study subjects.

#### *Clinical parameters*

Of the clinical parameters, advanced age (**study IV**) was significantly associated with carotid IMTmax in all the study groups (predialysis,  $r=0.69$ ,  $p<0.001$ ; dialysis  $r=0.53$ ,  $p<0.001$ ; transplantation  $r=0.67$ ,  $p<0.001$ ; control  $r=0.67$ ,  $p<0.001$ ). In contrast, the presence of diabetes, family history for CAD and the parameters describing tobacco consumption were not associated with IMTmax, though among renal transplant recipients a heavy smoking history showed a borderline correlation ( $p=0.084$ ) with IMTmax. Moreover, the smokers were younger than ex-smokers or non-smokers ( $46.3 \pm 10.1$  vs.  $53.5 \pm 11.4$  years,  $p=0.003$ ). In dialysis patients, the duration of dialysis treatment was related to IMTmax ( $r=0.36$ ,  $p=0.033$ ). The duration of hypertension was related to IMTmax in predialysis ( $r=0.27$ ,  $p<0.001$ ) and dialysis patients ( $r=0.36$ ,  $p=0.037$ ), and in renal transplant recipients this relation was of borderline significance ( $r=0.27$ ,  $p=0.094$ ).

The relation between age and the highest quartile of carotid plaque score was significant in all patient groups (**study IV**, Table 3). The duration of hypertension correlated significantly with a high plaque score among the dialysis patients and renal transplant recipients, and a similar trend was found in the predialysis group. Diabetes, family history for CAD or smoking history were not associated with a high plaque score. The analysis of the relation of risk factors and high plaque score was not possible for the control group, because

only two control subjects had a high plaque score. However, instead of plaque score, age and lower creatinine clearance were related to plaque prevalence in the control group (**study IV**).

The TEE examination (**study II**) of the patients with CRF revealed that advanced age, duration of hypertension, duration of dialysis treatment and the duration of a functioning renal transplant were associated with the presence of large thoracic aortic plaques (LAPs,  $\geq 3.0$  mm), as shown in Table 6. Male gender, body mass index, the presence of hypertension or diabetes and the duration of diabetes or CRF were not associated with the presence of LAP. The analysis of blood pressure showed that high pulse pressure and low diastolic blood pressure were related to LAPs. In the multivariate analysis, the duration of hypertension was significantly associated with the presence of LAPs (odds ratio 1.07 per year,  $p=0.016$ ).



**Table 6.** Univariate analysis of clinical parameters related to large aortic plaques among the study patients

	LAPs (n=39)	No LAPs (n=79)	p <sup>a</sup>
Age (year)	60.0 ± 7.7	48.1 ± 11.7	<0.001
Men (%)	74	65	0.305
Body mass index	26.9 ± 5.1	25.8 ± 4.4	0.242
Current smoker (%)	13	22	0.320
Heavy smoker (%)	28	27	1.000
Family history for CAD (%)	59	42	0.116
Diabetic patients (%)	33	28	0.669
Duration of diabetes (year)	20.8 ± 13.7	19.3 ± 10.3	0.798
History of hypertension (%)	97	94	0.662
Duration of hypertension (year)	18.0 ± 9.2	12.0 ± 8.8	0.002
Duration of CRF (year)	5.5 ± 7.9	4.4 ± 3.4	0.193
Duration of dialysis (year)	2.0 ± 2.1	1.0 ± 1.0	0.048
Duration of transplant (year)	12.4 ± 6.8	7.8 ± 6.5	0.048
Systolic blood pressure (mmHg)	163 ± 26	155 ± 25	0.113
Diastolic blood pressure (mmHg)	84 ± 10	90 ± 9	0.007
Pulse pressure (mmHg)	78 ± 22	65 ± 20	0.002

Data are mean ± SD or %. LAPs, large aortic plaques (≥ 3.0 mm in diameter) found in transesophageal echocardiography; No LAPs, no large aortic plaques found; CAD, coronary artery disease; CRF, chronic renal failure. Definitions: Heavy smoker, history of smoking 20 cigarettes daily for 10 years.

<sup>a</sup>t-test, Mann-Whitney U-test or Fisher's exact test.

### *Lipids*

When carotid IMT was used as a continuous variable, no association between total cholesterol, HDL-cholesterol, LDL-cholesterol or the triglyceride level was found in the CRF patient groups, though carotid IMT correlated with LDL-cholesterol in the control group ( $r=0.32$ ,  $p=0.02$ ). However, when the CRF patient groups are combined, LDL-cholesterol level associated with the highest quartile of IMT ( $4.0 \pm 1.0$  mmol/l vs.  $3.6 \pm 1.1$  mmol/l,  $p=0.041$ ).

Elevated LDL-cholesterol level correlated significantly with a high plaque score in the predialysis ( $4.1 \pm 1.1$  vs.  $3.5 \pm 1.1$  mmol/l,  $p=0.041$ ) and dialysis group ( $4.3 \pm 1.2$  vs.  $3.6$  mmol/l,  $p=0.023$ ), whereas the association was not significant among renal transplant recipients ( $4.0 \pm 1.6$  vs.  $3.5 \pm 1.0$  mmol/l,  $p=0.485$ ).

In **study II**, significant association between total cholesterol level as well as LDL-cholesterol level and the presence of LAPs was found (Table 7). In the multivariate analysis of the risk factors, total cholesterol level remained significantly associated with LAPs (odds ratio 1.47 per 1 mmol/l,  $p=0.040$ ), while the LDL-cholesterol level (odds ratio 1.55,  $p=0.07$ ) showed borderline association.

**Table 7.** Univariate analysis of the relation between laboratory findings and large aortic plaques among the study patients

	LAPs (n=39)	No LAPs (n=79)	p <sup>a</sup>
Hemoglobin (g/l)	128 ± 17	127 ± 16	0.726
Albumin (g/l)	37.3 ± 5.0	38.1 ± 4.4	0.353
Ionized calcium (mmol/l)	1.28 ± 0.07	1.26 ± 0.07	0.222
Phosphate (mmol/l)	1.41 ± 0.43	1.27 ± 0.36	0.087
Calcium phosphate product	1.81 ± 0.60	1.60 ± 0.45	0.054
Fibrinogen (g/l)	4.9 ± 1.4	4.2 ± 1.1	0.036
C-reactive protein (mg/l)	6.4 ± 7.8	3.4 ± 5.0	0.004
Total cholesterol (mmol/l)	6.4 ± 1.8	5.9 ± 1.3	0.049
HDL-cholesterol (mmol/l)	1.3 ± 0.4	1.4 ± 0.5	0.283
LDL-cholesterol (mmol/l)	4.0 ± 1.2	3.5 ± 1.1	0.025
Triglycerides (mmol/l)	2.2 ± 1.2	2.3 ± 2.5	0.090

Data are mean ± SD. LAPs, large aortic plaques ( $\geq 3.0$  mm in diameter) found in transesophageal echocardiography; No LAPs, no large aortic plaques found; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

<sup>a</sup>t-test or Mann-Whitney U-test

### *Markers of inflammation and variables of calcium-phosphate metabolism*

In **study II**, CRP-level (Table 7) and fibrinogen level were significantly associated with the presence of LAPs among patients with CRF. In **study IV**, no such association of CRP or fibrinogen level with a high carotid plaque score was not found. However, in all patients with CRF, CRP-level showed borderline association with IMT ( $r=0.16$ ,  $p=0.07$ ). The correlation between IMT and CRP ( $r=0.38$ ,  $p<0.01$ ) or fibrinogen ( $r=0.37$ ,  $p<0.01$ ) in the control group was significant.

Calcium-phosphate product showed borderline association (Table 7) with LAPs among patients with CRF in **study II**. Furthermore, a trend between phosphate level and the presence of LAPs was present.

### *Homocysteine*

In **study IV**, the tHcy level had no association with IMT in the CRF patient groups: predialysis ( $r = -0.04$ ,  $p = 0.756$ ); dialysis ( $r = -0.01$ ,  $p = 0.940$ ); and transplantation ( $r = -0.11$ ,  $p = 0.485$ ). In contrast, in the control group, there was a significant correlation between tHcy level and IMT ( $r = 0.31$ ,  $p = 0.019$ ) and plaque prevalence ( $10.2 \pm 3.2$  vs.  $8.2 \pm 2.3$   $\mu\text{mol/l}$ ,  $p = 0.010$ ). However, the analysis of tHcy level and a high carotid plaque score revealed no apparent relation between these parameters in the CRF patient groups. Similarly, the analysis of the relation between tHcy level and the presence of CAD, CEVD or PAD in the CRF patient groups revealed no consistent association between tHcy level and CAD, CEVD or PAD.

The analysis of the determinants of tHcy in each study group showed that creatinine clearance had the strongest correlation with tHcy (predialysis,  $r=-0.459$ ,  $p<0.001$ ; dialysis  $r=-0.260$ ,  $p=0.131$ ; transplantation  $-0.551$ ,  $p<0.001$ ; control  $r=-0.409$ ,  $p=0.001$ ) in all study groups except for the dialysis group. However, tHcy level was significantly higher in anuric dialysis patients compared to those with some residual renal function ( $39.5 \pm 22.2$  versus  $25.3$

$\pm 14.0 \mu\text{mol/l}$ ,  $p = 0.027$ ). In addition, folate level and vitamin B<sub>12</sub> showed associations with tHcy level in the study groups (**study IV**). Advanced age was not related to tHcy level in the patient groups, whereas it correlated strongly with tHcy level in the control group ( $r=0.402$ ,  $p=0.002$ ).

#### **4. Relation of thoracic aortic atherosclerosis and coronary artery disease (study V)**

Of the 118 study patients, 31 (26%) met the criteria for CAD (**study V**). The presence of aortic plaques had a sensitivity of 100% and a specificity of 37% for CAD. The positive predictive value was 36% and the negative predictive value 100%.

Significant coronary artery stenosis was found in 26 (73%) of those 36 patients for whom the morphological analysis of the coronary arteries by angiogram or autopsy was available. Thoracic aortic plaques  $\geq 3.0$  mm in diameter had a 73% sensitivity and 90% specificity for significant coronary artery stenosis, and the positive predictive value was 95%, while the negative predictive value was 56%.

## DISCUSSION

### 1. Demographic characteristics of the study subjects

The data from the Pirkanmaa District Kidney Register made it possible to reach not only renal transplant recipients, but also predialysis patients for this study. A considerable proportion of the patients invited took part in the study. The age and sex distribution in these two study groups did not differ substantially from their parent population. Therefore, it is possible to generalise the results to all predialysis patients and renal transplant recipients in the Pirkanmaa district, whereas the dialysis patients represent a non-selected sample of the dialysis patients in Pirkanmaa. According to the current criteria for chronic kidney disease, the predialysis patients represent stage 3 to 4 and the dialysis patients stage 5 chronic kidney disease (Kidney Diseases Outcomes Quality Initiative Guidelines 2002).

Control subjects were selected from the orthopaedic patients living in the same area. Except for previously known chronic diseases, the control group was not selected based on other cardiovascular risk factors. The confounding influence of risk factors associated with obesity was diminished when the controls were matched with body mass index. Their tobacco consumption and educational level (**study III**) did not differ from those of the patient groups. Consequently, the orthopaedic patients constitute a suitable control group representing the general population with no previously known chronic illness in the same area.

The clinical characteristics of the study patients show that almost all patients with CRF had hypertension, as is commonly reported (Raine et al. 1998). Similarly, the proportion of study patients with diabetes (29%) is close to the proportion of diabetic patients in end-stage renal disease (25%; Finnish Registry for Kidney Diseases 2003) in Finland. No control patients were excluded when a new diagnosis of a chronic illness was made. The proportion of hypertension among the control subjects who had no previously known chronic disease was 16%

(**study III**). This demonstrates that hypertension is an underdiagnosed condition in the general population. The criteria for diabetes represents the criteria for the definition of diabetes (WHO Study Group 1985) used at the time of the initiation of the study. In the course of the study, new, stricter criteria for diabetes were introduced (Alberti and Zimmet 1998).

The criteria for hypertension included a history of medical treatment for hypertension or the casual blood pressure measurement of 160/95 or greater. The casual blood pressure cut-point level is conservative ( $\geq 160/95$ ), since stricter criteria for hypertension (140/90; Finnish Hypertension Society Working Group 2002) have recently been presented. However, at least at the time of the initiation of the study, a single blood pressure measurement of this level did not consistently lead to initiation of medication for hypertension for these patients in our unit. The cut-point level of 160/95 has been used in Tampere University Hospital previously as well (Wirta 1992).

## **2. Peripheral arterial disease and medial arterial calcification**

The results of the present study show a high prevalence of PAD among predialysis patients, dialysis patients and renal transplant recipients. These findings are consistent with the earlier results (O'Hare 2001), though only one study has previously reported the prevalence of PAD by using both ABI and TBI measurements in this population (Mäkisalo et al. 1998). The prevalence of PAD in CRF seemed to be higher than that in the general population (Fowkes et al. 1991, Stoffers et al. 1996, Meijer et al. 1998). This difference can also be found when the presence of diabetes is taken into consideration (Walters et al. 1992). Therefore, it may be justified to propose that CRF predisposes to the development of PAD both in patients with or without diabetes.

In addition, the present study indicates that asymptomatic PAD is common among patients with CRF, since only nine patients (6.6%) had claudication. Claudication was particularly

insensitive among dialysis patients despite the highest prevalence of PAD in this group (**study I**, Table 2). Furthermore, one third of the patients with claudication did not meet the criteria for PAD, which is in accordance to the previous findings (Fowkes et al. 1991, Meijer et al. 1998). Therefore, the use of ankle-brachial index (ABI) measurement is important in the non-invasive diagnosis of PAD and the detection of high-risk patients for the implementation of active risk factor strategies.

This is the first study to present the prevalence of MAC by using ABI measurements in patients with CRF. The results show that the prevalence of MAC is high among the patient groups, suggesting that the presence of CRF predisposes to the development of MAC. Our data suggest that MAC is an entity of its own, because three out of four patients with MAC had no PAD. The concept of MAC has been presented both in studies concerning elevated blood pressure at ankle level (Carter 1993, Orchard and Strandness 1993, Zierler and Sumner 2000) and studies presenting the radiological findings of medial arterial calcification (Everhart et al. 1988, Gray et al. 1996). Therefore, it seems that these findings describe the same phenomenon, or they cannot be separated from each other. The known disturbances in the calcium phosphate metabolism are likely to contribute to the development of MAC in patients with CRF (Davies and Hruska 2001, London et al. 2003).

Medial arterial calcification may cause falsely elevated blood pressures at ankle level; a phenomenon that is common among diabetic patients. Since up to one third of the patients with CRF had diabetes, it was important to take into account the effect of MAC in the diagnosis of PAD among the study patients. In case of MAC, toe-brachial index measurement has been recommended, because toe pressure measurement is not similarly affected by arterial calcification as ankle pressures (Carter 1993, Zierler and Sumner 2000). The recommended threshold values for ABI and TBI were used for the diagnosis of PAD and MAC (Carter 1993, Weitz et al. 1996). The criteria for a previously performed angiogram was particularly necessary for the patients with lower extremity amputations. Even though there is no gold

standard available for the diagnosis of PAD, the chosen criteria may serve as the best estimate of PAD available to date for patients with CRF.

### **3. Ultrasound findings of the carotid arteries and the thoracic aorta**

#### *Carotid ultrasound examination*

In previous studies, both carotid intima-media thickness and the prevalence of carotid plaques have been used in the description of atherosclerosis in CRF. Though the majority of studies on carotid atherosclerosis have reported an increase in IMT among patients with CRF compared to a control group (Kawagishi et al. 1995, London et al. 1996, Stenvinkel et al. 1999, Fujisawa et al. 2000, Haraki et al. 2001, Kennedy et al. 2001), negative (Rossi et al. 1996, Savage et al. 1998, Rossi et al. 2002), and opposite results (Pascazio et al. 1996) have also been reported.

In the present study, no significant difference in IMT between any of the CRF patient groups and the control group was found, which indicates that an increase in IMT is not the characteristic alteration in patients with CRF. In previous studies, the varying results of the comparison of IMT between CRF patient groups and a control group may be due to differences in the methodology of the carotid ultrasound examination or in the selection of the control group. There is variation in the site of the IMT measurement, in the exclusion of measurement at the site of a plaque, or in the description of a plaque. In the present study, a recognised method of the IMT measurement of the far wall of the common carotid artery was used, with good reproducibility (**study III**). The control group of the present study was matched with age, sex and BMI — furthermore, no difference in tobacco consumption and educational level was found in comparison to the patient groups (**study III**).

In contrast to IMT, increased plaque score and plaque prevalence proved to be characteristic of CRF patient groups when compared to controls. Similarly, internal carotid artery stenoses were more frequent in the CRF patient groups. The finding of increased plaque



burden is consistently supported by previous reports on carotid atherosclerosis (Pascazio et al. 1996, Rossi et al. 1996, Savage et al. 1998, Stenvinkel et al. 1999, Haraki et al. 2001), though plaque score has not commonly been used in the description of atherosclerosis in CRF. The significance of a high plaque score is also supported by the fact that a high plaque score was more closely associated with the disease states related to atherosclerosis (CAD, CEVD, PAD) than IMT in patients with CRF (**study III**).

According to the stiffness index  $\beta$ , arterial stiffness is increased in CRF — a finding which represents arteriosclerosis, which is one clinical form of an accelerated ageing process among CRF patients (London et al. 2002). Increased arterial stiffness has been shown to be a strong predictor of all-cause and cardiovascular mortality in patients with CRF (Blacher et al. 1998, Blacher et al. 1999). Another characteristic feature was calcification of plaques in patients with CRF in the present study. This is in agreement with previous results (Savage et al. 1998, Haraki et al. 2001). Recently, intimal-type calcifications have been shown to increase all-cause and CVD mortality in dialysis patients (London et al. 2003).

The results of **study III** show that the characteristic alterations of the carotid arteries in CRF include increased plaque burden and calcification as well as increased arterial stiffness. The use of parameters describing these alterations are suitable for risk factor analysis of accelerated atherosclerosis in patients with CRF. No significant differences in these parameters was found between the CRF patient groups, which implies that these alterations are present at early stages of renal disease. This supports the statement that the implementation of risk factor reduction strategies should start early in the course of CRF to prevent the development of CVD (Parfrey 1993, Sarnak and Levey 2000).

### *Transesophageal echocardiography*

No previous reports on the significance of aortic atherosclerosis determined by TEE in patients with CRF have been published. In **studies II and V**, TEE proved feasible in identifying atheromatous aortic disease. No complications occurred. Only four patients (3.2%) were excluded because of unsuccessful insertion of the probe, and for additional two patients (1.6%) the examination had to be interrupted due to discomfort — both figures being comparable to the reported success rate of the TEE examination (Daniel et al. 1991). The reproducibility of the measurements and the classification of the atherosclerotic changes of the aortic wall was acceptable. The comparison of thoracic aortic findings with a control group was not possible, since TEE was not performed for the controls for ethical reasons.

#### **4. Risk factors for atherosclerosis**

The goal in **study II and IV** was to examine the association between the various risk factors for atherosclerosis and thoracic aortic atherosclerosis (**study II**) or carotid atherosclerosis (**study IV**) in patients with CRF. The relation of a risk factor and aortic atherosclerosis was examined in the study population consisting of all subgroups of patients with CRF (**study II**). The subgroups were combined in order to increase the power of the statistical analysis. This was also considered rational, because the treatment modality subgroups are far from pure — all study patients have a history of predialysis CRF, some dialysis patients had a history of a functioning renal transplant, and all renal transplant recipients had a history of dialysis treatment. Furthermore, the analysis was focused mainly on risk factors that had no marked differences between the subgroups. In **study IV**, the variable of key interest, the tHcy level, differed markedly according to renal function and, thus, between the study groups. Therefore, in **study IV**, the risk factor analysis was performed separately for each study group.

### *Clinical parameters*

The relation of age and atherosclerosis was obvious for all the parameters describing atherosclerosis in this study. Diabetes constituted a major risk for PAD, but it was surprising that the presence of diabetes and the duration of diabetes were not associated with carotid or thoracic aortic atherosclerosis — the reason for this remains obscure. However, few studies have reported the association between diabetes and carotid atherosclerosis in CRF (Shoji et al. 2000, Lim et al. 2001). Moreover, the arterial disease in diabetes seems different and is characterised by the development of MAC. Distal or small vessel disease may be more common in diabetes than atheromatous changes in large-diameter vessels, such as the aorta or carotid arteries.

The variables describing tobacco consumption did not confound the comparison between the various groups in the study, since there were no significant differences in these parameters between the groups. No consistent association between variables of tobacco consumption and carotid or thoracic aortic atherosclerosis was found. The fact that smokers were younger than ex-smokers or non-smokers in the patient groups may have an effect on these results. Of the parameters, only a history of heavy smoking showed borderline association with IMT among renal transplant recipients. Therefore, the verification of the known risk of smoking for atherosclerosis may require the use of continuous parameters that combine the history of current or previous smoking and the amount of cigarettes smoked more accurately than the parameter of heavy smoking history, or this may require reasonably larger-scale studies.

In a previous study (Zoccali et al. 2000), duration of dialysis treatment predicted carotid IMT in dialysis patients. In **study II**, the duration of dialysis treatment and a functioning renal transplant were associated with advanced aortic atherosclerosis, suggesting that the duration of renal disease is associated with the development of atherosclerosis. In the multivariate analysis, the odds ratio for advanced atherosclerosis was considerably greater in

dialysis patients than renal transplant recipients. This is most likely explained by the fact that renal transplant recipients are selected from a patient group with no unstable conditions.

### *Hypercholesterolemia*

The results of **study II** show that elevated total cholesterol level is a risk factor of quantitatively measured atheromatous disease in the CRF population. LDL-cholesterol level showed borderline association with aortic atherosclerosis in the multivariate analysis. Furthermore, in **study IV**, elevated LDL-cholesterol level associated with a high carotid plaque score among predialysis and dialysis patients. Because this is the first report on the association between elevated total cholesterol level or elevated LDL-cholesterol and atheromatous disease in CRF, it is reasonable to ask why similar findings have not been shown in previous studies on patients with CRF.

Firstly, one explanation may be the fact that according to BMI and albumin level, malnutrition did not have a major confounding effect for the results presented in **studies II and IV**. Secondly, some of the earlier studies have been performed on Japanese patients (Kawagishi et al. 1995, Fujisawa et al. 2000, Haraki et al. 2001), whose lipid levels are considerably lower than those of Finnish patients. Thirdly, in **studies II and IV** atherosclerosis was defined by using markers which represent advanced atherosclerosis (LAPs, high plaque score). As was shown in **study III**, atherosclerosis in CRF is characterised by increased plaque burden. Yet, carotid IMT has commonly been used as a marker of atherosclerosis in patients with CRF. In previous studies, the positive associations between dyslipidemia and atherosclerosis have been achieved by using the prevalence of plaques as markers of carotid atherosclerosis (Table 2, page 32). However, since the majority of patients with CRF have plaques (**study II and III**), the prevalence of plaques does not represent advanced atherosclerosis as accurately as the parameters of LAPs or the highest quartile of carotid plaque score.

Recently, further support for the importance of hypercholesterolemia for patients with CRF have been shown in the ALERT study. In this study, the benefit of lipid lowering medication was demonstrated among renal transplant recipients, as the use of fluvastatin reduced the incidence of cardiac deaths and non-fatal AMI (Holdaas et al. 2003).

A limitation of the present study is that part of the patients had been on statin treatment, although for a relatively short time. The lipid values prior to the initiation of the lipid-lowering medication were used in the analysis, because the pretreatment level was considered to reflect atherogeneity in these cases. The exclusion of these patients would have led to the exclusion of a considerable amount of hypercholesterolemic patients from the present study. Currently, the availability of study patients receiving no treatment for their lipid disturbances is limited for ethical reasons.

### *Hypertension*

Hypertension, one of the classic risk factors for atherosclerosis, is very common in the CRF population (Raine et al. 1998). Nevertheless, few studies report an association between hypertension and morphological atheromatous changes in patients with CRF (Malatino et al. 1999, Zoccali et al. 2000). In **studies II and IV**, the duration of hypertension predicted the atheromatous changes in the thoracic aorta and carotid arteries. Furthermore, **studies II and III** reported the association of low rather than high diastolic blood pressure and advanced atheromatous disease, as recently suggested (Levin et al. 2001, Tozawa et al. 2002). This finding emphasises the role of high pulse pressure, which was closely related to the atheromatous disease.

In **study III**, the association of blood pressure and carotid atherosclerosis in the control group was similar, with the exception that high diastolic blood pressure constituted a risk for high IMT. The different behaviour of diastolic blood pressure is explained by the characteristic change in arterial stiffness, because increased arterial stiffness causes a decline in

diastolic blood pressure in addition to an increase in systolic blood pressure (London 1994). On the basis of the present cross-sectional study, the duration of hypertension is likely to act as a causal factor for atherosclerosis. Conversely, the finding of low diastolic blood pressure and increased pulse pressure reflect an association with atherosclerosis, rather than causality.

#### *Inflammation, variables of calcium-phosphorous metabolism*

Previous reports have shown that markers of inflammation, such as CRP (Stenvinkel et al. 1999, Zoccali et al. 2000, Stenvinkel et al. 2002) and fibrinogen (Rossi et al. 1996) are associated with carotid atheromatous disease in CRF. In **study II**, both CRP and fibrinogen were associated with advanced atherosclerosis. Current research also indicates that cardiovascular morbidity and mortality in CRF is linked to the disorders in the calcium and phosphorous metabolism, such as hyperphosphatemia, high calcium phosphorous product and the use of calcium containing phosphate binders (Block et al. 1998, Goodman et al. 2000, Guerin et al. 2000). In **study II**, a tendency towards a relation of high phosphorous or high calcium phosphorous product was found. However, there were marked differences in the levels of CRP, fibrinogen and the variables of the calcium and phosphate metabolism between the study subgroups. Therefore, a more definite conclusion on the importance of these parameters cannot be drawn on the basis of the present study.

#### *Homocysteine*

The results of **study IV** show no support for an association between elevated tHcy level and carotid atherosclerosis in patients with CRF. In addition, no consistent association between elevated tHcy and the disease states related to atherosclerosis (CAD, CEVD, PAD) was found. Though folate and vitamin B<sub>12</sub> levels were associated with tHcy level, renal function showed the closest association with tHcy level. Even among dialysis patients, tHcy level was associated with residual renal function.

Studies on the relationship between elevated tHcy level and vascular disease in patients with CRF have given conflicting results (Table 3, page 36). Renal function has been considered an important confounding factor for the results and conclusions from studies on the importance of tHcy level in the development of atherothrombotic vascular disease (Clarke et al. 1991, Kuller and Evans 1998, Bostom 2000, Brattström and Wilcken 2000). However, the effect of renal function has not commonly been taken into account in studies on the importance of tHcy level for CVD in patients with CRF or the general population. In the present study, no optimal method for adjusting the effect of renal function was found. Furthermore, no adjustments could have taken into account the time-dependent changes in renal function and tHcy level during in the course of renal disease.

In view of the confounding effect of renal function on tHcy level, the significance of tHcy for vascular disease is difficult to demonstrate in patients with CRF. Since the same determinants are involved in the development of CRF, CVD and an elevation in tHcy level, tHcy may simply act as a marker of CVD rather than as a significant risk factor in the pathogenesis of CVD (Kuller and Evans 1998, Brattström and Wilcken 2000). Due to differences in renal function and a number of other confounding factors it seems obvious that no cross-sectional, observational study will ever adequately resolve the role of tHcy for CVD (Bostom 2000). Randomised, placebo-controlled trials on the effect of tHcy lowering for the development of CVD are required to resolve this issue. To date, such results do not exist for patients with CRF.

## **5. Transesophageal echocardiography and the prediction of CAD in CRF**

For a variety of reasons, the non-invasive screening for CAD is difficult in the CRF population. Yet patients with both CRF and CAD constitute a patient group with a very poor prognosis. The detection of these high-risk patients would be useful for improving their prognosis by using aggressive therapeutic and preventive interventions.

In **study V**, TEE proved useful in the prediction of CAD in patients with CRF. Normal thoracic aorta in TEE excluded CAD, and large thoracic aortic plaques predicted significant coronary artery stenosis. These results are in agreement with the corresponding findings in the general population (Fazio et al. 1993, Tribouilloy et al. 1994, Parthenakis et al. 1996, Khoury et al. 1997, Belhassen et al. 2002). Since echocardiography is indicated for most patients with CRF, TEE provides a suitable and relatively non-invasive method for the analysis of thoracic aortic atherosclerosis in the risk evaluation for CAD. Consequently, TEE may also be useful in the selection of suitable patients for renal transplantation. However, the development of other non-invasive screening methods, such as magnetic resonance imaging or CT-scan, may provide optional screening methods for defining CAD in this population in the near future (Nikolaou et al. 2003).



## SUMMARY AND CONCLUSIONS

The major findings and conclusions are:

1. Asymptomatic PAD and MAC are common in patients with CRF, suggesting that the presence of CRF predisposes to the development of these manifestations. A reliable diagnosis of PAD requires the use of both ABI and TBI measurements in patients with CRF.
2. The risk factors of aortic atherosclerosis evaluated by TEE include age, duration of hypertension, hypercholesterolemia as well as the duration of dialysis treatment or a functioning renal transplant.
3. The characteristic alterations of the carotid arteries in CRF include increased plaque burden and calcification as well as increased arterial stiffness. No differences were found between the patient groups, which implies that the alterations are present at an early stage of renal disease.
4. Classic risk factors for atherosclerosis (age, duration of hypertension, elevated LDL-cholesterol level) contribute to the development of carotid atherosclerosis in patients with CRF. The tHcy level showed no apparent association with carotid atherosclerosis or vascular disease in CRF. However, the strong confounding effect of renal function may not be adequately controlled for the analysis of the significance of elevated tHcy for CVD in patients with CRF.
5. TEE proved useful in the prediction of CAD in patients with CRF. Among patients with CRF, normal thoracic aorta in TEE very likely excludes CAD, and large thoracic aortic plaques are often accompanied with significant coronary artery stenosis.

In summary, the extent of the disease states related to atherosclerosis is clearly increased in patients with CRF. Similar cardiovascular risk factors to the general population are acting in the pathogenesis of atherosclerosis in patients with CRF. This is, however, more difficult to demonstrate due to a number of confounding factors: heterogeneity of the primary cause of CRF, a variety of treatment modality phases in the course of renal disease, malnutrition and anaemia accompanied with renal disease, and the multiplicity of cardiovascular risk factors present. The haemodynamic burden associated with hypertension due to renal disease causes marked excessive load to the cardiovascular system in this patient group. Furthermore, disorders in the calcium-phosphorous metabolism or inflammatory processes may have additive effects on this population.

However, to date, the evidence of strong “uremic toxins” causing the excessive development of atherosclerosis in this patient group is insufficient. For example, the role of elevated tHcy level for the pathogenesis of atherosclerosis is still an open issue in patients with CRF. Therefore, it seems that the existence of such factors cannot explain the development of atherosclerosis in patients with CRF. As in the general population, the pathogenesis of atherosclerosis is multifactorial in CRF. The pronounced burden of cardiovascular disease is, most likely, the cause of the additive impact of multiple cardiovascular risk factors acting simultaneously in this patient group. Consequently, the preventive measures to improve the outcome of these patients include active treatment of all the cardiovascular risk factors present in a patient with CRF.

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