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To my family

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ROOSA LANKINEN: Predictors of Cardiovascular Disease and Mortality in Patients with Advanced Chronic Kidney Disease
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ABSTRACT

Cardiovascular disease is the leading cause of high mortality in advanced chronic kidney disease (CKD) patients. Treatment and prevention of cardiovascular disease should be in focus to improve prognosis in this high-risk population.

The aim of the study was to assess cardiovascular determinants of mortality, to study the effect of diabetes on arterial endothelial function and structure, to evaluate exercise capacity and abdominal aortic calcification (AAC) in patients with advanced CKD, not on dialysis.

210 participants of the Chronic Arterial Disease, quality of life and mortality in chronic KIDney injury -study underwent maximal bicycle ergometry stress test, echocardiography, lateral lumbar radiograph to study AAC score, ultrasound measures of arterial structure and function, and laboratory measures.

Determinants of mortality were stress ergometry performance, AAC score, E/e' ratio of echocardiography, and cardiac biomarkers and albumin. Diabetes was a significant determinant of AAC but did not associate with endothelial dysfunction or increased carotid intima-media thickness. Maximal stress ergometry performance was associated with troponin T (TnT) and AAC. The progression of AAC was rapid and the increment rate was similar in patients transitioning to different modalities of renal replacement therapy.

To conclude, stress ergometry performance, AAC, E/e' of echocardiography, and cardiac biomarkers and albumin predict mortality, and diabetes is associated with AAC in advanced CKD. TnT and AAC are associated with maximal ergometry stress test and AAC progresses at a comparable rate across the CKD treatment groups.

KEYWORDS: Chronic kidney disease, cardiovascular disease, aortic calcification, ergometry stress test, endothelial dysfunction, carotid intima-media thickness, echocardiography, troponin T

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Sisätautioppi
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TIIVISTELMÄ

Loppuvaiheen munuaisten vajaatoimintapotilailla sydän- ja verisuonitaudit ovat johtava kuolleisuuden syy. Sydän- ja verisuonitautien hoito ja ennaltaehkäisy tulisi olla keskiössä tämän korkean riskin ryhmän ennusteen parantumiseksi.

Tutkimuksessa arvioitiin kuolleisuuteen vaikuttavia sydän- ja verisuonitautitekijöitä, diabeteksen vaikutusta verisuonen sisäkalvon toimintaan ja suonirakenteeseen, suorituskykyä, sekä vatsa-aortan kalkkisuutta ei-dialyysihoitoisessa loppuvaiheen munuaisten vajaatoiminnassa.

210 potilasta osallistui Krooninen valtimotauti, elämänlaatu ja mortaliteetti vaikeaa munuaisten vajaatoimintaa sairastavilla -tutkimukseen. Tutkittaville tehtiin polkupyörärasituskoe, sydämen ultraäänitutkimus, lannerangan röntgenkuvaus aortan kalkkisuuden määrittämiseksi, verisuoniultraäänitutkimus verisuonen rakenteen ja toiminnan tutkimiseksi sekä otettiin laboratoriokokeet.

Kuolleisuutta määrittivät suorituskyky, vatsa-aortan kalkkisuus, sydämen ultraäänitutkimuksen E/e' suhde, sydänmerkkiaineet ja albumiini. Diabetes määritti vatsa-aortan kalkkisuutta merkittävästi, mutta ei ollut yhteydessä verisuonen laajenemiskykyyn eikä kaulavaltimon seinämäpaksuuteen. Maksimaalinen suorituskyky oli yhteydessä troponiini T:hen (TnT) ja vatsa-aortan kalkkisuuteen. Vatsa-aortan kalkkisuus kehittyi nopeasti ja kehityksen suuruus oli sama eri keinomunuaishoitomuodoissa.

Yhteenvetona todetaan, että suorituskyky, vatsa-aortan kalkkisuus, sydämen ultraäänitutkimuksen E/e' suhde, sydänmerkkiaineet ja albumiini ennustavat kuolleisuutta, ja diabetes on yhteydessä vatsa-aortan kalkkisuuteen, mutta ei verisuonen laajenemiskykyyn tai sisäkalvopaksuuteen, loppuvaiheen munuaisten vajaatoiminnassa. TnT ja vatsa-aortan kalkkisuus ovat yhteydessä maksimaaliseen suorituskykyyn, ja vatsa-aortan kalkkisuus etenee samalla nopeudella eri munuaiskorvaushoitomuodoissa.

AVAINSANAT: krooninen munuaisten vajaatoiminta, sydän- ja verisuonitauti, vatsa-aortan kalkkiutuminen, polkupyörärasituskoe, verisuonen sisäkalvon toimintahäiriö, sisemmän kaulavaltimon paksuus, sydämen ultraääni, Troponiini T

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Abbreviations

AAC	Abdominal aortic calcification
ACE	Angiotensin converting enzyme
AGE	Advanced glycation end products
AIC	Arterial intimal calcification
AMC	Arterial medial calcification
ARB	Angiotensin receptor blocker
AUC	Area under the curve
BMI	Body mass index
BMP	Bone morphogenic protein
CAC	Coronary artery calcification
CAD	Coronary artery disease
Cbfa	Core binding factor A1
CKD	Chronic kidney disease
CKD-MBD	Chronic kidney disease-mineral and bone disorder
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CKMB	Creatine kinase MB
CPP	Calciprotein particle
CRP	C-reactive protein
CT	Computed tomography
CV	Cardiovascular
DKD	Diabetic kidney disease
EBCT	Electron beam computed tomography
EDF	Endothelial dysfunction
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
ESKD	End stage kidney disease
FGF23	Fibroblast growth factor 23
FMD	Flow-mediated dilatation
GLP-1	Glucagon-like peptide-1
GLS	Global longitudinal strain
HD	Haemodialysis

IL	Interleukin
IMT	Intima-media thickness
LDL	Low density lipoprotein
LV	Left ventricle
LVEDD	Left ventricular end diastolic dimension
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
LVMI	Left ventricular mass index
MACE	Major cardiovascular event
MDRD	Modification of Diet in Renal Disease
MGP	Matrix Gla protein
MRI	Magnetic resonance imaging
MSCT	Multislice computed tomography
NO	Nitric oxide
NT-proBNP	NT-pro-brain natriuretic peptide
OPG	Osteoprotegerin
PD	Peritoneal dialysis
PET	Positron emission tomography
PTH	Parathyroid hormone
PWV	Pulse wave velocity
RAAS	Renin-angiotensin-aldosterone system
RANK	Receptor activator of NK-κB
RRT	Renal replacement therapy
SD	Standard deviation
SGLT2	Sodium-glucose co-transporter 2
TNF	Tumour necrosis factor
TnT	Troponin T
VC	Vascular calcification
VO_{2peak}	Peak oxygen uptake
VSMC	Vascular smooth muscle cell

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals I-IV:

I Lankinen R, Hakamäki M, Metsärinne K, Koivuviita NS, Pärkkä JP, Hellman T, Kartiosuo N, Raitakari OT, Järvisalo MJ. Cardiovascular Determinants of Mortality in Advanced Chronic Kidney Disease. *Am J Nephrol*. 2020;51(9):726–735. doi: 10.1159/000509582.

II Hellman T, Lankinen R, Järvisalo MJ, Hakamäki M, Koivuviita NS, Raitakari OT, Metsärinne K. Arterial endothelial function, carotid artery intima-media thickness and abdominal aortic calcification in diabetic and nondiabetic CKD stage 4–5 patients not on dialysis. *Diabetes Res Clin Pract.* 2021 Jan;171:108559. doi: 10.1016/j.diabres.2020.108559.

III Lankinen R, Hakamäki M, Metsärinne K, Koivuviita N, Pärkkä JP, Saarenhovi M, Hellman T, Järvisalo MJ. Association of maximal stress ergometry performance with troponin T and abdominal aortic calcification score in advanced chronic kidney disease. *BMC Nephrol.* 2021 Feb 4;22(1):50. doi: 10.1186/s12882-021-02251-y.

IV Lankinen R, Hakamäki M, Hellman T, Koivuviita NS, Metsärinne K, Järvisalo MJ. Progression of Aortic Calcification in Stage 4–5 Chronic Kidney Disease Patients Transitioning to Dialysis and Transplantation. *Kidney Blood Press Res.* 2022;47(1)23–30. doi: 10.1159/000518670.

1 Introduction

Chronic kidney disease (CKD) is a global health burden with substantially high estimated prevalence of 11–13%.[1] Patients with advanced chronic kidney disease are at high risk for cardiovascular and all-cause mortality compared to general population. Cardiovascular mortality is 10- to 30-fold higher than in age-matched populations, and the incidence of maintenance dialysis dependency is exceeded by cardiovascular mortality.[2,3] The enhanced mortality risk is best treated with renal transplant, not a preference for all CKD patients.

Progression of vascular calcification in CKD is expeditious, and is only incompletely explained by traditional risk factors.[4] Atherosclerosis of the abdominal aorta is a significant cardiovascular risk factor in the setting of CKD.[5,6] Noninvasive radiographic detection and quantification of abdominal aortic calcification is a useful method for high-risk patient identification.[7] The best estimate of abdominal aortic calcification, used in a number of contemporary studies[8,9], is proposed by Kauppila et al.[10]

In addition to the aortic artery, the carotid artery is susceptible to calcification. Ultrasonographically assessed intima-media thickness of carotid artery has been used as a surrogate marker of cardiovascular risk in the general population.[11,12,13,14] Increased carotid intima-media thickness has been associated with poor cardiovascular outcomes in advanced CKD.[15] As an early marker of subclinical atherosclerosis, the function of the endothelium deteriorates as the function of the kidneys declines.[16] Inflammation and reduced nitric oxide (NO) bioavailability have been suggested as mediators of endothelial dysfunction in CKD[16,17] but the exact cause remains unknown.

CKD population suffer from declined exercise tolerance which is predictive of survival.[18] After renal transplantation, exercise capacity improves, still remaining at level comparable to normal sedentary people.[19] Cardiorespiratory fitness is the most tested outcome reported in exercise studies on CKD using exercise tolerance testing.[20] Roshanravan et al. compared different methods to study physical performance in patients with mild to severe chronic kidney disease, and they concluded that impaired physical performance of the lower extremities is common, and strongly associates with all-cause mortality in CKD.[21]

In summary, a comprehensive cardiovascular risk assessment of individual patients with CKD could improve prognosis and provide more personalised treatment of this high-risk patient group. Thus, we studied the cardiovascular sequalae, determinants of mortality and their associations in a cohort of advanced kidney disease patients, and concluded, that detecting individual risk with a comprehensive selection of imaging and other methods could aid in clinical decision-making and targeting treatment strategies.

2 Review of the Literature

2.1 Chronic kidney disease

2.1.1 Definition

In 2002, the National Kidney Foundation (NKF) Kidney Disease Outcomes Quality Initiative (KDOQI) introduced the definition and classification of chronic kidney disease (CKD).[22] In 2012, The Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline served an updated guideline for evaluation and management of chronic kidney disease.[23]

CKD is defined as the presence of kidney damage or glomerular filtration rate (GFR) ≤60 ml/min/1.73 m^2 for ≥3 months. Kidney damage refers to abnormalities detected by histology or by imaging of kidneys, abnormalities in urinary sediment, or history of kidney transplant.

The best index for the measurement of kidney function is GFR. Declining GFR is characteristic of progressive kidney disease. Precise GFR may be measured by assessment of urinary clearance of an ideal filtration marker, such as inulin or iothalamate. However, in clinical practise, glomerular filtration rate is estimated (eGFR) by prediction equation models that incorporate sex, race, age, and body size to serum concentrations of endogenous filtration markers such as creatinine or cystatin C. As a more precise equation, Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)[24] has mostly replaced the Modification of Diet in Renal Disease (MDRD) -study equation. The serum cystatin C level is recommended for confirmatory measurement of eGFR and for screening of adults with eGFRs 45–59 ml/min/1.73 m^2 who do not have markers of kidney damage.[23] In 2021, new eGFR equations without race were developed.[25] These new equations that incorporate creatinine and cystatin C are more precise in estimating measured GFR.

Albuminuria is the most commonly assessed kidney damage marker and can be assessed using different measurement methods.[26] Normal urine contains small amounts of albumin and proteins. An excretion rate of urine albumin ≥ 30 mg/day corresponds to moderately increased and an excretion rate ≥300 mg/day to severely increased albuminuria. Albumin-to-creatinine -ratio in an untimed spot urine has many advantages and is considered sufficient for risk stratification and clinical

decision making.[22] End-stage kidney disease (ESKD) means chronic kidney failure treated with dialysis or transplantation.

2.1.2 Staging

CKD staging help guide management of and risk stratification for CKD progression and complications. CKD is categorised according to cause of the kidney disease, six GFR categories and three albuminuria categories; see, **Table 1**.

Table 1. CKD categories and prognosis by GFR and range of albuminuria.

Stage	**Description**	**GFR**	**Albuminuria,** normal to mildly increased	**Albuminuria,** moderately increased	**Albuminuria,** severely increased
			<30 mg/g <3 mg/mmol	30–300 mg/g 3–30 mg/mmol	>300 mg/g >30 mg/mmol
G1	Normal	≥90	Low	Moderate	High
G2	Mildly decreased	60–89	Low	Moderate	High
G3A	Mildly–moderately decreased	45–59	Moderate	High	Very high
G3B	Moderately–severely decreased	30–44	High	Very high	Very high
G4	Severely decreased	15–29	Very high	Very high	Very high
G5	Kidney failure	≤15	Very high	Very high	Very high

GFR=glomerular filtration rate (ml/min/1.73 m^2)

Identifying the cause of the kidney disease help in directing specific therapy for and halt the progression of the kidney disease. Albuminuria increases the risk of mortality[27] and ESKD,[28] as well as progression of CKD[29] independent of eGFR, and consequently has been added to CKD staging.[22]

2.1.3 Aspects of diabetic kidney disease

Diabetes is the leading cause of chronic kidney disease and end-stage kidney disease worldwide.[30] Aetiology of diabetic kidney disease (DKD) comprises glomerular hemodynamic changes, oxidative stress and inflammation, and interstitial fibrosis and atrophy.[31] In diabetes, the renin-angiotensin-aldosterone system (RAAS) is activated and results in an elevated glomerular filtration rate further leading to sclerotic changes in the kidney.[32]

Any observable albuminuria predicts an increased risk for kidney and cardiovascular disease in diabetic patients. The risk rises substantially with severely increased albuminuria levels,[33] and is even higher at levels above 1000 mg/day.[34] In most DKD patients, the risk of death is higher than that of developing kidney failure.

2.1.4 Progression of chronic kidney disease

The kidney can adapt to damage by increasing the filtration rate, a phenomenon called adaptive hyperfiltration, which in the long term appears to result in damage to the glomeruli. Adaptive hyperfiltration manifests by proteinuria and progressive kidney failure.

Several factors including GFR, albuminuria and cause of the kidney disease influence the likelihood and rate of CKD progression. Modifiable factors should be distinguished and provide treatment considering the association with CKD progression, mortality, and quality of life.[16] Proteinuria is characteristic of overt nephropathy and associates with faster decline in kidney function and increased cardiovascular risk.[34] Decreased GFR and enhanced albuminuria synergistically associate with higher rates of CKD progression, and thus assessment of GFR and albuminuria should both be undertaken to evaluate the progression of CKD.

Two significant factors to diminish CKD progression can be considered: treatment of the primary kidney disease and treatment of secondary factors, such as blood pressure and proteinuria. Progression of CKD is considered in part to be attributable to intraglomerular hypertension and glomerular hypertrophy, which are responsible for adaptive hyperfiltration and glomerular scarring. Interstitial fibrosis and focal segmental glomerulosclerosis are responsible for the histologic manifestations of the secondary causes of kidney damage. Genetic risk factors, such as risk alleles in the apolipoprotein L1 encoding gene, may play a part in CKD progression.[35]

Stabilisation of kidney function is probable with available protective therapies including RAAS-system blockers and sodium-glucose co-transporter 2 (SGLT2) inhibitors. Blood pressure control is important in CKD progression, and a relationship prevails between blood pressure and the risk for adverse kidney outcomes.[36]

Two large renal outcome trials demonstrated the renoprotective effect of angiotensin receptor blockers beyond their effect on blood pressure.[36,37] Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) mitigate the hyperfiltration state as they decrease arteriolar resistance in the efferent arteriole, thereby lowering glomerular pressure.[38] This declining of arteriolar resistance is considered as the fundamental mechanism by which these pharmaceuticals slow the rate of CKD progression. ACE inhibitors

reduce the incidence and extent of glomerulosclerosis which may halt the progression of renal failure.[39]

Modifiable risk factors to halt CKD progression include obesity, smoking and glycaemic control. Obesity culminate in a form of secondary focal segmental glomerulosclerosis, and is a significant and modifiable risk factor in CKD progression.[40] Smoking has been associated with endothelial dysfunction, oxidative stress, and inflammation, the same pathogenic pathways that manifest in diabetic kidney disease.[41] Glycaemic control affects the risk for incident and progressive diabetic kidney disease[42] and restoration of glycaemic control with pancreatic transplantation in patients with type 1 diabetes can improve the prognosis of kidney disease.[43]

In addition to hypertension, type 2 diabetes is a major cause of renal failure. Aside from ACE inhibitors and ARBs, no other renoprotective treatment have been previously available. In albuminuric diabetic patients, SGLT2 inhibitor should be included in medication. SGLT2 inhibitors reduce blood glucose by increasing glucose excretion in urine. Reabsorption of glucose and sodium at the proximal convoluted tubule is inhibited resulting in increased sodium delivery to the macula densa. The juxtaglomerular apparatus senses this as a stimulus of low circulating volume. Tubuloglomerular feedback is restored by vasoconstriction of the afferent arteriole, in an effort to reduce kidney plasma flow and hyperfiltration.[44]

SGLT2 inhibitors reduce the risk of kidney disease progression and cardiovascular disease among patients with DKD who are already taking ACE inhibitors (or ARBs).[45] The best data come from two large trials, CREDENCE[46] and DAPA-DKD[47].

Considerable evidence has shown that treatment with glucagon-like peptide 1 (GLP-1) reduces albuminuria, and thus can also be suggested as renoprotective. Evidence on hard renal outcomes is still lacking.[48]

2.1.5 Physical activity in chronic kidney disease

Chronic kidney disease results in alterations in physical function and structure assessed subjectively in quality-of-life studies[49] as well as objectively[50]. Prior studies have shown that physical inactivity is associated with increased mortality in CKD as well as non-CKD populations; see, **Figure 1.**[51] Preservation of functional independence is of significance in CKD patient-reported questionnaires.[52]

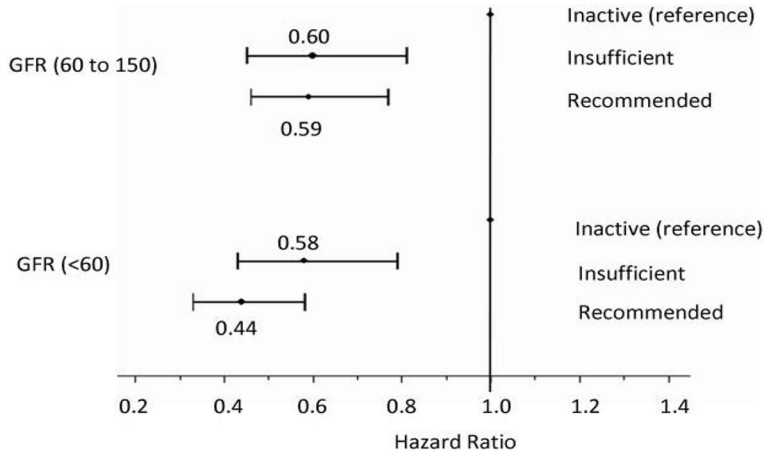

Figure 1. Association of physical activity with mortality in patients with and without CKD represented in NHANES study (Beddhu et al. 2009). Reproduced with the permission of American Journal of Nephrology.

Chronic kidney disease patients experience impaired physical function that is associated with reduction of peak exercise oxygen consumption.[53] Physical function deteriorates in accordance with kidney function impairment[54] but may improve after kidney transplantation.[50] Decreased exercise capacity is an independent predictor of mortality in various populations, including dialysis patients[18], and has been shown to predict mortality better than kidney function or commonly measured serum biomarkers in the setting of CKD.[21] Exercise programs for CKD and haemodialysis patients have shown to improve physical capacity and components of quality of life.[55,56]

Dialysis initiation can possibly lead to improvement of physical function through resolution of uraemia, inflammation and volume overload. However, few studies have assessed alterations in subjective and objective changes in physical function. An ongoing study of advanced non-dialysis CKD patients showed that physical function as measured by the chair stand test declines promptly after the transition to dialysis.[57] This emphasises a demand for interventions to preserve the physical capability of the dialysis population. An intriguing conclusion was that no change in self-reported physical activity level was found in this study of Rampersad et al.[57]

The K/DOQI Clinical Practice Guideline of Cardiovascular Disease in Dialysis Patients recommends evaluation of physical function and encourages physical activity.[58] Physical function assessment is of great value for identification of high-risk persons and patients who might benefit from preventive interventions.

A wide spectrum of measurement possibilities exists for physical functioning, cardiorespiratory fitness being the most-tested outcome in the setting of CKD.[20] Maximal oxygen uptake is a typical measure of cardiorespiratory fitness and serves

as an index of exercise capacity. It is indicative of the cardiovascular system's ability to use oxygen at peak performance. CKD patients infrequently achieve maximal levels; thus, the tests are called symptom-limited, and the obtained measure is peak oxygen uptake (VO_{2peak}). VO_{2peak} is a powerful predictor of survival in ESKD population.[18] One of the mechanisms behind reduced cardiovascular reserve is considered cardiac dysfunctional changes, including elevated left ventricular (LV) filling pressure and LV mass.[53]

2.1.6 Cardiac biomarkers in chronic kidney disease

Circulating cardiac biomarkers play a significant role in the early detection of cardiovascular disease. Previous investigations have proved an association between cardiac biomarkers and cardiovascular events and death[59,60], including populations with advanced CKD.[61] The use of troponin T (TnT) for identification of mortality risk of CKD patients is approved by the Food and Drug Administration.[62]

However, conflicting results in a post hoc analysis of data concerning 543 dialysis patients concluded that the predictive power of troponin T, high-sensitivity C-reactive protein, albumin and a number of other biomarkers, was relatively weak for the presence of cardiovascular (CV) disease and risk of death.[63]

Cardiac biomarkers TnT and N-terminal-proB-type natriuretic peptide (NT-proBNP) assays are standardised and readily available, compared to invasive and noninvasive methods, to detect subclinical cardiovascular changes.

NT-proBNP originates from cardiac myocytes responding to the stretch and tension of the ventricular wall and corresponds to left ventricular hypertrophy and volume overload. In CKD patients, elevated NT-proBNP may reflect underlying heart disease, such as coronary artery disease or LV hypertrophy, independent of heart failure.[64,65]

NT-proBNP is a strong predictor of incident heart failure and an independent predictor of adverse outcomes in patients with CKD.[66,67] In a study of Astor et al, the association of elevated NT-proBNP levels and higher CV risk was strong among black, hypertensive patients with eGFR of 20 to 65 mL/min/1.73 m^2, and the association was more powerful in individuals with significant albuminuria.[67]

NT-proBNP concentrations are typically higher in CKD, including in patients treated with dialysis, than in the general population.[64,68] The diagnostic use of natriuretic peptides in CKD has been confounded by concurrent volume overburden as well as by reduced renal clearance. However, the elevated levels do not necessarily reflect a reduced clearance of the peptide or chronic fluid overload but rather constitute a true finding, corresponding to the presence and severity of heart disease. When evaluating CKD patients with acute heart symptoms compared to

those with preserved renal function, higher decision limits of NT-proBNP are necessary.[64,68]

TnT and NT-proBNP are substantially predictive of heart failure in the general population, but also in individuals with CKD.[67,69] Even after adjustment for traditional cardiovascular risk factors, elevated TnT and NT-proBNP have been associated with heart failure assumably indicative of early subclinical changes in volume and myocardial stress. In dialysis-dependent patients, NT-proBNP may be used as a biomarker to identify left ventricular dysfunction.[68]

Cardiac TnT appears into the circulation with cardiomyocyte injury, can be detected by highly sensitive assays, and is used as a biochemical marker for the detection of myocardial necrosis. TnT is a simple, cost-effective test that might be considered as a tool for cardiovascular risk stratification of CKD patients, who need further cardiac evaluation.

Comparable to NT-proBNP, prevalence of detectable TnT is frequently high, associates strongly with alterations of left ventricular structure, and has a modest association with diastolic dysfunction in the setting of CKD.[70,71] It remains unclear whether the detection, release, or clearance of troponin is altered by uraemia. Nonetheless, the utility of TnT for detecting patients with CKD for cardiac structural and functional abnormalities is limited.

2.2 Cardiovascular disease and vascular calcification in chronic kidney disease

2.2.1 Natural history, prevalence, and clinical significance

Vascular calcification (VC) is a considerable risk factor for cardiovascular events and mortality in the general population as well as in patients with chronic kidney disease. Causal relationship between VC and enhanced cardiovascular risk seems obvious. Previous data indicate that the pathophysiology of vascular calcification is an active rather than degenerative process.[72] New evidence has addressed a broad spectrum of responsible mechanisms and regulatory courses for vascular calcification. Accelerated vascular ageing may be predisposed by several risk factors, commonly divided into classic and non-classic factors. Classic risk factors comprise smoking, hypertension, dyslipidaemia, age, gender, CKD and dialysis vintage, state of inflammation, calcium-phosphate disorders, and diabetes. Non-classic factors include abnormal levels of bone-related proteins[73] and other humoral factors.

In CKD and dialysis patients, the incidence and prevalence of VC has been consistent over the last decades.[74–77] The reported prevalence of VC detected by computed tomography (CT) scans is >80 percent among dialysis patients[76,77] and

somewhat less among CKD patients with eGFR <60 ml/min/1.73 m^2.[78] Even in ESKD patients of young age the incidence of coronary artery and valve calcifications is remarkably high compared to subjects of the same age and sex, older adults with normal renal function, or patients with suspected and documented coronary artery disease.[76] Kidney transplantation alleviates cardiovascular risk. Nevertheless, cardiovascular sequalae remain the primary death cause even after successful renal transplantation.[79,80]

It is considered that VC is a consequence of a wide variety of different biological processes, but also of pharmacological interference[73] and it contributes to increased risk of cardiovascular disease and mortality in the CKD population.[2,74] Currently no effective treatment is attainable for vascular calcification, and thus the target of treatment is to mitigate symptoms and delay progression.

Chronic kidney disease is an independent risk factor for coronary artery disease (CAD). Previous data have shown that vascular calcification is common in already moderate stages of CKD, maintenance haemodialysis, and kidney transplant recipients, and progresses even after successful transplantation.[81,82]

Congestive heart failure caused mainly by CAD also explains a substantial proportion of the cardiovascular disease associated events in CKD. Vascular calcification precipitate significant adverse effects such as systolic hypertension, hypertrophy of the left ventricle and coronary ischemia. Arterial medial inflammation, fibrosis, hypertrophy, and calcification result in increased arterial stiffness, which precipitates vascular ageing. Calcification of large conduit arteries such as the aorta, increases arterial stiffness and pulse pressure, a phenomenon which may predict left ventricular dysfunction, heart failure, stroke and cardiovascular events in CKD patients.[83,84]

Cardiovascular disease mortality is 20- to 30-fold higher in CKD population compared to age-, gender- and race-matched controls. In end-stage kidney disease patients with diabetes, the burden of cardiovascular disease and mortality is even emphasized.

Limited evidence supports the use of therapies to ameliorate cardiovascular outcomes in milder stages of CKD. Overactivation of the mineralocorticoid receptor is closely linked with cardiovascular and kidney diseases. A recent trial of patients with CKD and type 2 diabetes receiving finerenone, a selective non-steroidal mineralocorticoid receptor antagonist, showed an improvement in cardiovascular outcomes compared with placebo.[85]

2.2.2 Intimal versus medial calcification

Vascular calcification can emerge in different histological and anatomical sites. It can arise either in the intimal or the medial wall of the vessel, and different subtypes

may prevail in same location. However, it is not achievable to distinguish vascular calcification subtypes with current clinical tools.

The clinical significance of medial and intimal vascular calcification may be very different. Intimal calcification is part of atherosclerosis and seems to appear as a secondary phenomenon of inflammation. Intimal calcification can culminate in plaque rupture and acute vessel occlusion, which are considered the primary consequences of this subtype of vascular calcification.

Arterial medial calcification (AMC) is considered an active process responding to various risk factors, such as diabetes, aging and inflammation.[73] Vascular smooth muscle cells are discovered to transform to osteoblast-like cells as a consequence of calcification-regulating humoral factors. Increased arterial stiffness is the outcome for medial calcification, the hallmark of arteriosclerosis.[73]

Pathogenesis of arterial medial calcification and arterial intimal calcification (AIC) are somewhat diverse. AMC is a non-occlusive condition affecting young and middle-aged patients without traditional atherosclerotic risk factors. It affects hemodynamic and is highly predictive of all-cause and CV mortality in HD patients. In end-stage renal disease, AMC seems to closely associate with HD treatment and its duration, diabetes, high serum phosphate, high doses of calcium-containing phosphate binders and low serum albumin.[74] Contrary to AMC, AIC is predominantly observed in older individuals, can be observed in atherosclerotic plaques and associates with clinical history of diabetes and atherosclerosis.[74]

ESKD patients represent arterial calcification in the form of medial calcinosis; see, **Figure 2**. The mineral deposition of large- and medium sized arteries represents the fundamental component of the premature vascular ageing seen in CKD patients.[86] Arterial medial calcification is associated with expression of "bone" matrix proteins, namely osteopontin, type I collagen, bone sialoprotein, and alkaline phosphatase.[87]

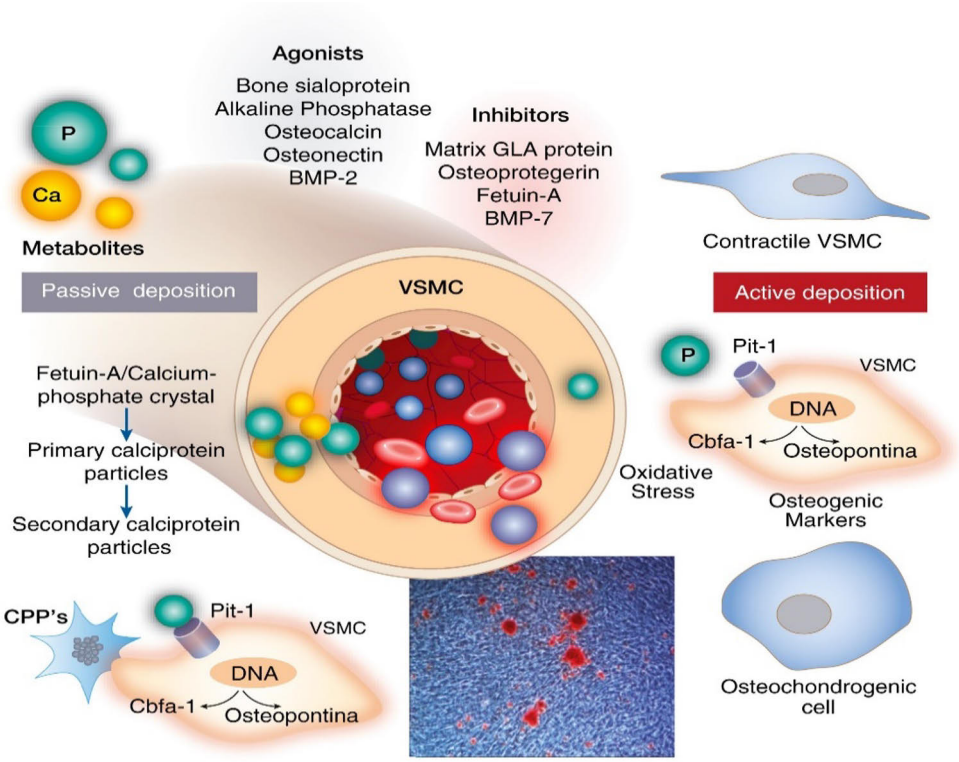

Figure 2. Illustration of medial calcification of a medium-sized artery. Leukocytes (blue cells) are involved in intimal and medial calcification. Calcium (yellow) and phosphate ions (green) play a key role in initiation and progression of calcification. Agonist and inhibitors of vascular calcification on the top of the vessel. Passive deposition and including factors on the left. BMP, bone morphogenic protein; Cbfa, core binding factor A1; CPP, calciprotein particle; Pit-1, phosphate cotransporter; VSMC, vascular smooth muscle cell. (Vervloet et al. 2017). Reproduced with the permission of Elsevier.

2.2.3 Traditional risk factors

Traditional risk factors, such as hypertension, diabetes, dyslipidaemia and smoking have higher prevalence among patients with chronic kidney disease compared to people with preserved kidney function. For example, in patients with CKD, the prevalence of hypertension was about 70 % in the third National Health and Nutrition Evaluation Study.[88]

In advanced CKD, the benefits of risk factor management are considered to be weakened.[89] Two large trials concerning haemodialysis patients showed no benefits of statin use to CV outcome.[90,91] On the contrary, a study by Baigent et al. showed a reduction in major atherosclerotic events in CKD patients of wide range as a result of reduction of low density lipoprotein by statin use.[92]

Diabetes has a strong propensity for vascular calcification with various mechanisms.[93] Mechanistic considerations of vascular calcification in diabetes might be the formation of advanced glycation end products (AGE), release of osteoprogenitor cells, as well as reduction of vitamin K-dependent activation of the calcification inhibitor, matrix Gla protein. Presumably a combination of multiple diabetic factors coincides in the progress of vascular calcification.[94] Many studies have shown that diabetes increases the risk for vascular calcification up to 55.7 percent in the setting of CKD.[78]

Increasing age and longer dialysis period are associated with increased prevalence of vascular calcification,[75] and progression of VC is rapid in older patients. Cardiovascular mortality is increased by dialysis vintage as a consequence of accelerated progression in cardiovascular disease.[95] Vascular calcification is already present up to nearly 40% of patients at dialysis onset and becomes steadily more prevalent over time.[84]

2.2.4 Uraemia-related risk factors

2.2.4.1 Role of bone and mineral metabolism

CKD-mineral and bone disorder (CKD-MBD) is a complication of CKD comprising abnormalities of serum phosphorus, calcium, parathyroid hormone (PTH) and vitamin D. Hyperphosphatemia has systematically been shown to associate with poor outcome in a variety of populations, particularly in CKD patients,[96] although few evidence-based recommendations exist to guide hyperphosphatemia management.

CKD-stimulated onset of vascular calcification is shown to emerge even before hyperphosphatemia,[97] and sustained hyperphosphatemia hastens the progression of vascular calcification.[75] Intestinal uptake of dietary phosphate, renal reabsorption, and switching between extracellular and bone storage pools determine the phosphate homeostasis. Alteration of phosphate metabolism is one of the key amendments in chronic kidney disease. Serum phosphorus concentration increases with declining kidney function. Commonly phosphorus remains close to normal until late in stage 4 or 5 CKD. Development of hyperphosphatemia is preceded by adaptive mechanisms, such as high concentrations of PTH and fibroblast growth factor (FGF23).

In CKD, even normal-range phosphorus has been associated with cardiovascular and all-cause mortality.[98] In maintenance haemodialysis patients, a benefit for non-calcium-based phosphate-binders with a parallel reduction in vascular calcification and mortality has been demonstrated in a recent meta-analysis.[99,100] In predialytic CKD patients, the reduction in phosphorus observed with calcium- and non-calcium-

based phosphate binders as well as with sevelamer, has been insignificant and no improvement in VC has been observed.[101-103] Although evidence-based data are lacking, growing epidemiological evidence supports treatment of hyperphosphatemia considering that reduction in phosphate concentration has been associated with reduced mortality.[96]

The use of calcium-based as well as non-calcium-based phosphate binders has been shown to associate in reduction of urinary phosphorus excretion, a surrogate of intestinal phosphate absorption, and attenuation of progressive secondary hyperparathyreoidism.[104] CKD stage 3–5D patients using calcium-based phosphate-binders are at an increased risk for hypercalcemia, indicating that homeostatic adaptations are exceeded.[77,99] Non-calcium-based phosphate binders have been associated with lower all-cause mortality compared to calcium-based binders within patients with CKD stage 3-5D.[99] However, the mechanism have remained unclear. In a meta-analysis of Liu et al, non-calcium-based binders had an equal effect on serum phosphate, but the incidence of coronary artery calcification was lower in a cohort of 3676 HD-patients.[100]

2.2.4.2 Role of secondary hyperparathyroidism

Mineral metabolism imbalance is associated with CKD-MBD and higher PTH levels with increased morbidity, mortality and vascular calcification.[84,105] Thus prevention and treatment of secondary hyperparathyroidism is of great importance. Hyperparathyroidism does not seem to play a role in the pathogenetic mechanisms of VC. Rather, an inverse relationship has been observed between the serum PTH level and bone histomorphometry indices. It has been suggested that reducing excessive parathyroid activity with pharmaceuticals could terminate in lower bone turnover and adynamic bone disease. This could further influence the development and progression of vascular calcification. The association between moderate vascular calcification and parathyroid activity could result from the direct action of hyperphosphatemia on vascular calcification and parathyroid activity.[105]

Cinacalcet is a calcimimetic agent that acts by activating the calcium-sensing receptor on parathyroid tissue. This pharmaceutical may mitigate vascular and cardiac valve calcification in haemodialysis patients with moderate to severe secondary hyperparathyreodism.[106] However, in a previous analysis concerning cinacalcet, no significant reduction in the risk of death or major cardiovascular events was seen in a study cohort of haemodialysis patients who established moderate to severe secondary hyperparathyroidism.[96]

2.2.4.3 Inflammation and oxidative stress

CKD and cardiovascular disease (CVD) are both characterised by enhanced inflammation.[94] A low-grade systemic inflammation characteristic of a uremic state may further translate into increased risk for cardiovascular disease. An association between serum albumin, a marker of poor nutrition and possibly inflammation in CKD, and all-cause mortality has been shown in previous studies.[107] Oxidative stress emerges when there is a disparity in free-radical production and antioxidants. Patients with CKD represent greater oxidative stress, associated with endothelial function and poor cardiovascular outcomes.[17]

Inflammation has obtained growing interest as a contributor to the increased CVD risk in the CKD population. Serum inflammation biomarkers high-sensitivity C-reactive protein (CRP) and interleukin (IL)-6 are associated with increased CVD risk in the general population, but also in patients with CKD.[108] Macrophages, derived primarily from blood monocytes,[109] play an important role in all stages of atherosclerosis, from lesion establishment and expansion, to necrosis and rupture. In addition to systemic inflammation, local inflammatory activity of the arterial wall can be detected with positron emission tomography (PET) computed tomography (CT) in patients with CKD without known atherosclerotic or inflammatory disease, and with few traditional risk factors and comorbidity.[110] The inflammatory state of the arterial wall correlates to measures of kidney function, namely eGFR and plasma urea.

In uraemia, the inflammatory state may be defined by the combination of an impaired immune response in addition to persistent immune stimulation leading to low-grade inflammatory state. Substantial data suggest cytokine alterations in dialysis and non-dialysis ESKD patients reporting elevated, but considerably different concentrations of proinflammatory cytokines IL-6 and TNF-α, and anti-inflammatory IL-10 in cardiovascular disease and in uremia.[111]

2.2.4.4 Bone-related proteins and humoral factors

In chronic kidney disease, vascular calcification is considered an active and biologically regulated process comparable to bone formation. Imbalance between promoters and inhibitors of calcification process may contribute to vascular calcification. Inhibitors of calcification are essential in preventing calcium and phosphorus deposition in vivo. Lack of endogenous anti-calcification factors, such as matrix Gla protein, osteoprotegerin, fetuin-A, fibroblast growth factor (FGF) 23, and Klotho possibly play an important role in calcium-phosphate deposition in soft tissues in CKD.[112,113]

In experimental Klotho deficiency and in renal failure, soft-tissue calcification and premature ageing are a marked feature. Klotho was originally identified within

the process of ageing. Klotho functions in an endocrine fashion and is widely expressed, and its level is highest in the kidney.[114] Klotho is an early biomarker of CKD, and also plays a pathogenic role in CKD progression. Klotho is regarded to ameliorate the calcification process of the vasculature by increasing phosphaturia, preserving glomerular filtration, and restricting phosphate uptake by vascular smooth muscle. Patients with early stage 1–2 CKD already have significantly lower urinary Klotho, which is progressive lowered with declining GFR.[115]

Osteoprotegerin (OPG) was first discovered as a protein that played a role in bone density regulation. On osteoclast precursor cell membranes, OPG competes with the receptor activator of NK-κB ligand (RANKL) and its receptor, RANK. The RANKL/RANK/OPG system may mediate important and complex links between the vascular, skeletal, and immune systems, and thus promote and protect against vascular calcification.[116] OPG also shows a relationship to inflammation, and observations have been made that osteoporosis and vasculopathy are often linked via inflammatory pathomechanisms.[111]

Extracellular matrix Gla protein (MGP) is a member of the vitamin K-dependent protein family with unique structural and physical properties. MGP supresses vascular calcification and arises in vascular and other soft tissues. In experimental models, MGP leads to accelerated arterial calcification, resulting in expeditious death. MGP does not inhibit calcification without sufficient vitamin K.[73]

Fetuin-A is a circulating acute-phase, calcium-regulatory glycoprotein.[109,117] It inhibits vascular calcification[118] and is associated with malnutrition and inflammation.[111] Fetuin-A is related to poor outcome in CKD patients receiving renal replacement therapy.[119] Patients who present with carotid plaques may have markedly low fetuin-A levels supporting the hypothesis of the association of low fetuin-A, accelerated atherosclerosis and vascular calcification.[119]

In observational studies concerning CKD populations, vitamin K deficiency has been shown to promote vascular calcification and to have a potential role in cardiovascular-related outcomes.[94] In most studies patients are restricted in foods rich in potassium that are good sources of vitamin K, and these studies have been rather small. Warfarin, an anticoagulant that depletes functional vitamin K reserves, is known to promote the development of atherosclerosis and vascular calcification.[120]

Vitamin K status can be estimated by various biomarkers, such as MGP, which is the most studied vitamin K-dependent protein in the regulation of vascular calcification. Compiled evidence suggests that dietary vitamin K deficiency or its antagonism may induce VC, and that vitamin K supplementation is a sound approach. Determining whether Vitamin K supplementation can reduce CKD complications, will require more interventional trials.[121,122]

2.2.5 Cardiac structure and function in chronic kidney disease

Chronic kidney disease and ESKD treated with dialysis are highly associated with altered echocardiographic indices, namely left ventricular hypertrophy (LVH). Attenuated cardiac systolic[123] and diastolic[124] functions are strongly associated with poor outcomes in dialysis as well as predialysis CKD. LVH is already present in moderate CKD, and prevalence increases as renal dysfunction progresses; by the initiation of dialysis, up to 70–80–% of patients manifest with LVH.[70,125,126] Carotid plaques, coronary calcification, and their severity associate with left ventricular mass index (LVMI), implicating that vascular calcification is a risk factor for LVH in the CKD population.[126]

In haemodialysis patients, the most accurate method for detection and quantification of LVH, as measured by LVMI, is cardiac magnetic resonance imaging (MRI).[127] By cardiac MRI, cardiac structure may be assessed independent of interdialytic variations of volume status. However, echocardiography is easily available and inexpensive compared to MRI, for the LVH assessment.

The pathogenetic mechanisms of LVH in CKD include afterload- and preload-related factors, and wide variety of others. Afterload-related factors involve blood pressure and large-vessel compliance,[126] while preload-related factors include anaemia and intravascular volume overload, in part associated to arteriovenous fistula of haemodialysis patients.

Anaemia is responsible for decreased peripheral resistance. It is hypothesised that anaemia results in higher availability of the endothelium-derived relaxing factor nitric oxide, which further leads to increased cardiac output.[128] Treatment of anaemia with epoetin has shown to be of advantage on partial regression of LVH.

Treatment of other factors, such as secondary hyperparathyroidism, hyperphosphatemia, oxidative stress and microinflammation, seems beneficial as these are important components in the pathogenetic mechanisms of LVH.[125]

Only half of the patients commencing conventional dialysis therapies show a regression in LVH. Eventually, LVH results in cardiac fibrosis, diastolic dysfunction, chamber dilatation and disturbances in intraventricular conduction predisposing patients to ventricular arrhythmias and further to sudden cardiac death.[125]

2.2.6 Endothelial function in chronic kidney disease

Endothelium is comprehended as single layer of squamous endothelial cells which line the inside surface of blood and lymphatic vessels. Normal endothelium responds to physical and chemical signals by production of a wide variety of factors adequate for vascular tone regulation, cellular adhesion, smooth muscle cell proliferation,

vessel wall inflammation and thrombosis resistance.[129] Endothelial dysfunction is a hallmark of vascular diseases and the development of atherosclerosis.

The significance of endothelium was recognised by its impact on vascular tone in an *in vitro* –study concerning acetylcholine-induced relaxation of preparations of rabbit thoracic aorta. The study of Furchgott and Zawadzki was the first to demonstrate nitric oxide (NO) as an endothelium-derived relaxing factor and attenuation of vasodilatation was associated to specific stimuli, such as acetylcholine or bradykinin.[130]

Endothelial cell injury has been established as an early marker of atherosclerosis in consequence of reduced release of vasodilators from the endothelium in subjects with diabetes, hypertension and dyslipidaemia. Endothelial dysfunction has been associated with microalbuminuria regardless of the degree of renal function impairment,[131] and the dysfunction of the endothelium is usually extensive in diabetic as well as in non-diabetic subjects. This supports the finding that impairment in endothelial nitric oxide synthesis takes part in the association between microalbuminuria and cardiovascular disease risk.[132]

In a study of Yilmaz et al, altered circulating vasoactive peptides such as adiponectin, was associated with endothelial function and low-grade proteinuria in type 2 diabetes contributing to increased cardiovascular disease risk.[131] It was hypothesized that these vasoprotective peptides were lost in the urine. It has been postulated that endothelial dysfunction anticipates microalbuminuria, but the causality of this association has not been fully determined.

In CKD endothelial function is highly abnormal in predialysis, dialysis and even after kidney transplantation.[16,133,134] Flow-mediated dilation, a phenomenon reflecting the vasodilatation following reactive hyperaemia, reduces in progressive kidney disease. Flow-mediated dilation has been considered a useful detection method of atherosclerosis concerning type 2 diabetic individuals without overt cardiovascular disease[135] as well as healthy subjects with and without traditional cardiovascular risk factors.[152] Inflammation has been suggested as a mediator of endothelial dysfunction.[16,17] Endothelial dysfunction has also been associated with abnormal response to shear stress, but the exact cause in CKD is unknown.

Traditional risk factors, such as tobacco use, high pulse pressure, and advanced age have consistently associated with increased cIMT.[134,136] In patients with diabetes, carotid IMT is increased and endothelial function altered already in childhood.[137] Endothelial dysfunction and increased cIMT are associated with poor cardiovascular outcome in diabetes as well as in the CKD population in previous studies.[138-140] After kidney transplantation, endothelial function in peripheral arteries seems to improve but not to the level of healthy, non-CKD patients.[141,142]

2.3 Methods to study cardiovascular disease and vascular calcification in chronic kidney disease

2.3.1 Screening for cardiovascular disease and vascular calcification

In general as well as CKD population, increased levels of coronary artery calcium, reduced ankle-brachial index, and increased carotid intima-media thickness are prognostic of increased cardiovascular events and mortality. No agreement prevails on the most suitable detection method to diagnose vascular calcification in CKD. Vascular calcification is frequently detected coincidentally on imaging acquired for other purposes.

Various noninvasive methods have been established for the detection and quantification of vascular calcification including cardiac computed tomography (CT), projection (plane) radiography, vascular ultrasound, and echocardiography. Additionally, tonometry for pulse wave velocity assessment, magnetic resonance imaging (MRI) and Doppler ultrasound may be used to estimate functional parameters of cardiovascular health, arterial stiffness, and endothelial function. A significant obstacle is the absence of evidence-based consensus on the appropriate screening and monitoring technique and what would be the most representative vascular bed to image.

An ideal detection method of vascular calcification in CKD should be easily accessible, accurate, reproducible, safe, and cost-effective. An optimal technique would detect the progression and the effect of medical interventions on VC, but most of all, would predict hard outcomes. Imaging methods should simplify in differentiating patients at low or high risk for adverse events. Currently no specific therapy to prevent or facilitate the regression of vascular calcification is available; thus no attempts to quantify vascular calcification in the CKD population have been done.

A significant part of the data on vascular calcification in CKD come from studies that have applied noninvasive imaging techniques to detect arterial wall and cardiac valve calcification.[143] As a limitation, current imaging methods are insufficient to clearly differentiate intimal from medial calcification in the vessel wall.

2.3.2 Radiography

2.3.2.1 Projection radiography

Evaluating calcification of the abdominal aorta may identify CKD patients at high risk for cardiovascular events. Plain radiography is the simplest, yet insensitive and

mainly qualitative, technique in vascular calcification detection of large conduit arteries. Compared to computed tomography, plain radiography is cheap, readily available, and relatively specific. Plain radiographic images are positively associated with the quantity of vascular calcification and the risk of clinical outcomes including all-cause and cardiovascular mortality in general, CKD and ESKD population .[7,73] It has been suggested that planar radiography is able to detect and distinguish patchy, intimal and railroad-like medial forms of calcifications, but is unable to quantify the intensity of calcification.[74]

Atherosclerosis develops first in the aorta before progressing to femoral, carotid or coronary arteries. Abdominal aortic calcification (AAC) is an important risk factor for congestive heart failure, independent of coronary heart disease. Patients with diabetes typically present with aortic calcification.[144] Noninvasive radiographic detection and quantification of atherosclerosis of abdominal aorta is a useful method for high-risk patient identification.[7] In a study of Wilson et al, middle-aged men and women with abdominal aorta calcific deposits are likely to develop coronary heart disease, cardiovascular disease, and cardiovascular mortality.[145]

Assessing AAC score on lateral spine radiographs of general[8] as well as CKD[9] patients identifies people at increased risk of long-term atherosclerotic vascular disease, hospitalisations and deaths. Also in the haemodialysis population, abdominal aortic calcification evaluated by radiography of the lateral abdomen is significantly associated with all-cause and cardiovascular mortality.[146]

The best semiquantitative method to assess AAC score from plain lumbar radiography is proposed by Kauppila et al.[10] A lateral X-ray of the lumbar spine is given a score of 0-24 based on the number and extent of calcified deposits adjacent to the aortic segment of the first through fourth lumbar vertebrae; see, **Figure 3**. This method provides a fast and low-cost assessment of location, severity, and progression of aortic calcification.

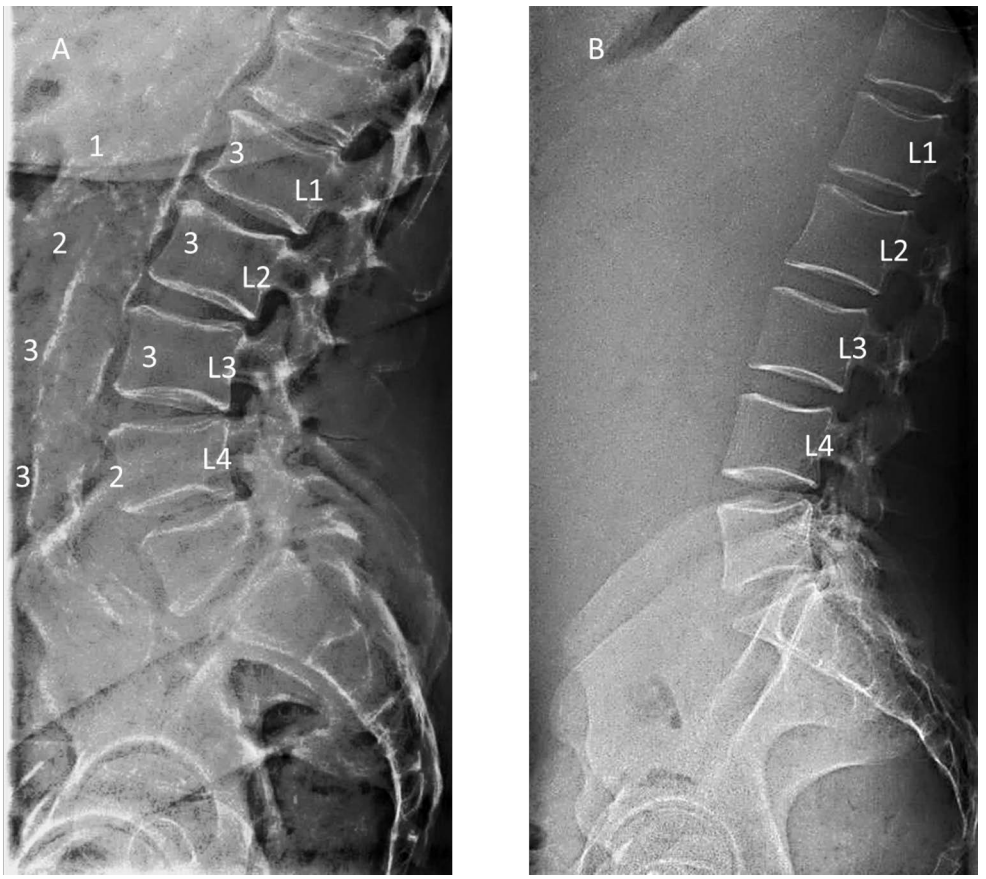

Figure 3. Example of the abdominal aortic calcification assessment from a plain lateral lumbar radiograph. A) Severe calcification, AAC score 20/24 in a 60-year-old male with CKD 5. B) No calcific deposits, AAC score 0/24 in a 40-year-old woman with CKD 5. (Lankinen et al. 2021). Reproduced with the permission of Creative Commons licence.

An additional method to study vascular calcification by projection radiography was proposed by Adragao et al at year 2004.[147] The Adragao score represents a simple method to assess cardiovascular risk related to vascular calcifications in chronic HD patients. This uncomplicated approach applies plain radiographic films of pelvis and hands, to detect any calcification. In this method, vascular calcifications are evaluated in muscular arteries of the iliac, femoral, radial, and digital regions, because muscular arteries are more susceptible to linear calcification. Any linear calcification that was present, is counted as 1. The final score is the sum of all the calcified sections of pelvis and hands, ranging from 0 to 8. The Adragao score has been independently associated with coronary artery calcification, peripheral artery disease and vascular disease.[147]

2.3.2.2 Computed tomography

Electron beam computed tomography (EBCT) and multislice computed tomography (MSCT) are considered the reference methods for coronary artery calcification (CAC) and valvular calcification detection. These methods are well-validated, noninvasive, and with no contrast administration requirement. Ultrafast computed tomography scans that can be without contrats, detect and quantify vascular calcification[148] but do not differentiate between intimal and medial calcium deposition, although both have a negative prognosis for cardiovascular events.[77,87]

Powerful evidence indicates that CAC and calcification of the aortic arch have predictive value when evaluated with CT concerning a spectrum of populations, including patients with CKD.[143] CAC has been also suggested as a valid surrogate marker for research based on studies comparing the impact of phosphate-binders, calcimimetics and vitamin D on CAC.[143] In the haemodialysis population, CT based techniques appear to be more sensitive at detecting peripheral and aortic vascular calcification compared with plain X-rays.[149]

CAC has been widely studied using CT,[75,76] although the reproducibility of CAC score based on CT is somewhat suboptimal.[73] The Agatston score is the most frequently used and reported method for quantification of CAC on CT. Nevertheless the total score being associated with the burden of atherosclerosis, only a modest correlation exists between CAC score and the severity of coronary artery obstructions in CKD patients.[143] CT angiography is increasingly utilized test for CAD assessment. This method is accompanied by radiation exposure and is somewhat expensive, and given the risk of contrast-induced nephropathy in CKD patients, is mostly performed only after dialysis initiation.

2.3.3 Ultrasound

2.3.3.1 Vascular structure

Vascular ultrasound is a noninvasive imaging technique. This method allows anatomical as well as functional assessment of subclinical atherosclerosis in all vascular beds. Ultrasound-based imaging methods can be used to assess superficial vessels, such as femoral and carotid arteries, in evaluating vascular disease in patients with CKD.

Quantitative plaque evaluation in other than coronary arterial beds has attracted curiosity as potential CV risk modifier. Equivalent to X-ray, ultrasound assessment is qualitative or semiquantitative. However, the differentiation of intimal from medial calcification remains difficult. In chronic kidney disease, ultrasound

assessment of vascular calcification has been associated with abnormal arterial function and adverse cardiovascular outcome.[143]

Besides the aortic artery, the carotid artery is also susceptible to calcification. The carotid intima-media thickness (cIMT) is possible to measure, and echogenic plaques can be detected with high-resolution ultrasound transducers.[143] Lumen diameter and intima-media thickness can easily be assessed and evaluated, while the intima and media layers of the vessel wall cannot be discriminated by ultrasound.

The ultrasonographically assessed cIMT, the distance from the lumen-intima interface to the media-adventitia interface of the artery wall, has been used as a surrogate marker of cardiovascular health in the general population, as well as in diabetics.[11-14,137] An increased cIMT is associated with subsequent cerebrovascular and cardiovascular events.[12] Evaluation of cIMT by ultrasonography has achieved recognition as a nonivasive method to assess the extent of atherosclerosis even by adolescence.[11]

Traditional cardiovascular risk factors, such as systolic blood pressure, low density lipoprotein (LDL)-cholesterol level, cigarette smoking, and body mass index (BMI) measured in adolescence have been independently associated with adult IMT.[11] In type 2 diabetes, IMT predicts complications of diabetes and mortality.[93]

Although cIMT is a risk marker for cardiovascular events in the general population, data on CKD is limited, and the utility of cIMT assessment in CKD remains conflicting. In patients with advanced pre-dialysis CKD, higher carotid IMT is associated with traditional cardiovascular risk factors and cardiovascular death.[138] Additional research is needed to examine the clinical utility of cIMT in the risk stratification and clinical management of CKD patients.

Limited data have accumulated regarding the prognostic role of the association of femoral artery plaque presence and clinical CV outcomes. Arterial wall plaque formation and increased intima-media thickness of the femoral artery are among surrogate markers of subclinical atherosclerosis and may provide prognostic information for predicting cardiovascular disease in primary prevention.[150] However, femoral artery investigation by ultrasound for CV risk modification has not become a customary clinical routine.

2.3.3.2 Endothelial function

The endothelium has appeared as a significant regulator of vascular homeostasis. Change in function of the endothelium precedes morphological atherosclerotic alterations. Early pathophysiological arterial changes can be studied by assessment of arterial elasticity by ultrasound.

Reduced arterial elasticity is associated with traditional CV risk factors prevalent already in childhood and adolescence, and may reflect increased atherosclerotic

burden.[151] Clinical assessment of the endothelial function of the brachial artery supplies independent prognostic information on atherosclerotic risk factors. Endothelial function measurement also predicts adverse cardiovascular events in healthy subjects.[152]

Endothelium-dependent vasomotion, induced by pharmacological and/or physiological stimulation of nitric oxide (NO) or other vasoactive release, is the most common assessment method to study endothelial function.[13,129] In this method, brachial artery diameter is measured before and after an increase in shear stress that is induced by reactive hyperaemia, resulting in a measurement called flow-mediated dilation (FMD). Original clinical studies of endothelial function were conducted in coronary vasculature.[129]

The presence of proteinuria with or without diabetes has been associated with attenuated FMD in previous studies. [131,135,153,154]

Deterioration of kidney function is associated with oxidative stress, thickening of carotid intima-media, and endothelial dysfunction.[17,134] In the CKD population FMD has been shown to be severely impaired, even after the exclusion of overt cardiovascular disease.[155] Previous studies have been supportive of the use of FMD over IMT assessment to monitor high risk CKD patients.[134]

2.3.3.3 Echocardiography

The most common cardiac amendment in CKD is left ventricular hypertrophy (LVH). This cardiomyopathy of advanced CKD is an independent risk factor for cardiac death and is facilitated by anemia[156] and vascular noncompliance. The prevalence of LVH increases with declining renal function,[156] and the incidence is up to 70% in patients initiating dialysis indicating that cardiovascular disease must be present long before commencing to dialysis in a significant proportion of patients.[156]

Echocardiography has been widely used for coronary artery disease risk stratification in CKD, and is accepted as a primary instrument for left ventricle (LV) mass assessment in clinical practice and in research. An increased LV mass has been associated with the CKD population even when compared to hypertensive controls, confirming that LV mass is not solely hypertension-related but is partly connected to uremia.[53]

Echocardiography is considered the gold standard technique for noninvasive evaluation of ventricular structure and function as well as cardiac valves. Valvular calcification assessed by echocardiography has been proved to predict mortality and morbidity in CKD patients on dialysis.[143] Echocardiography is movable and generally obtainable. However, valvular calcification can be evaluated only

qualitatively or subjectively. The accuracy also depends on the timing relative to dialysis session and volume changes.

CKD patients may present with lower LV ejection fraction, but the most common finding is an increased LV filling pressure, compared to normal. This change is associated with impaired LV relaxation, a hallmark of diastolic dysfunction, which is a prevalent finding in patients with CKD and is associated with heart failure and mortality. The ratio of early diastolic mitral inflow velocity to early diastolic mitral annulus velocity (E/e' ratio) is used for the evaluation of LV filling and thus for the diagnosis of diastolic dysfunction. The E/e' ratio can predict mortality and cardiovascular events in patients with CKD and diastolic dysfunction.[157]

2.3.4 Functional imaging

Aortic pulse wave velocity (PWV) is recognised as the gold standard measure of arterial stiffness, and is the most-used stiffness parameter for predicting CV diseases in a substantial number of studies.[56] Age, diabetes, hypertension, and CKD promotes arterial wall thickening, fracture of elastic fibres, calcification and endothelial dysfunction, leading to an increase in arterial stiffness, a phenomenon which increases the velocity of pressure waves along the arterial walls.

Structural changes within the medial arterial wall caused by abnormal calcification are believed to relate to arterial stiffening. Arterial tonometry measures pressure wave forms typically at sites of the carotid and the femoral artery simultaneously with an electrocardiographic lead. PWV is the velocity measured from a pressure wave generated by the ejection fraction of the left ventricle and the back reflected waves at arterial branching points. The velocity of the forward and reflected waves increases along with the increment of arterial stiffening. The faster-returning reflected wave imposes an increase on the left ventricle afterload, resulting in left ventricular hypertrophy.

PWV has been associated with cardiovascular risk outcome prediction in the CKD population[17] and is widely used as a research tool, but currently no recommendation for utilisation of PWV measurement in clinical practise prevails.[143] Additionally, PWV is not a direct measure of calcification but rather a surrogate marker of vascular calcification, and may be influenced by several other factors.

2.3.5 Assessment of physical performance

CKD patients often present with muscle-wasting along with low physical activity and diminished physical performance,[19] which strongly associate with all-cause mortality.[21] In nephrology practice, the measurement of physical function and

activity has not been the focus of care but could provide information on quality of life and help assess well-being.

In the CKD population, physical performance limitations predict impairment and mortality regardless of the test used.[20] Particularly, lower extremity physical performance measured by usual gait speed, timed up and go, and six-minute walking distance, has been associated with poor clinical outcome in the CKD population not treated with dialysis.[20,21]

The gold standard measure of cardiorespiratory fitness, and the most commonly tested outcome reported in exercise studies in CKD, is exercise testing by either incremental treadmill or a cycle ergometer protocol.[20] The typical measurement in these protocols is maximal oxygen uptake,[56] which has been indicated as a strong predictor of survival of ESKD patients.[18] Typically, peak oxygen uptake (VO_{2peak}) is reduced in CKD,[158] including dialysis patients, as a result of exercise intolerance that probably is mediated in part by increased diastolic LV stiffness, low peak heart rate and anemia.[53,158] Aerobic capacity, namely VO_{2peak}, can be improved by moderate intensity exercise training in the setting of CKD.[56]

CKD patients rarely achieve maximal exercise levels, and thus tests reported for maximal exercise are called symptom-limited. Heart rate and blood pressure are regularly reported outcomes while being highly dependent on medication, volume status and timing in relation to dialysis treatment.

After successful renal transplantation exercise capacity ameliorates to levels of normal sedentary individuals.[19] The improvement in functional capacity by removal of uraemia may be a result of several factors, such as reduction of excessive left ventricular work and removal of uremic toxins.

Because a cardiac stress test has been necessity before kidney transplantation, much of the analysis is on asymptomatic CKD patients. Despite high cardiovascular disease burden in the CKD population, the diagnostic implementation of cardiac stress testing is poor.[159] Exercise stress testing has a high rate of false-positive and false-negative outcomes when evaluating possible ischemia in CKD and ESRD partly due to suboptimal performance and poor value of the electrocardiogram compromised by LV hypertrophy.[159] Functional testing appears to perform better as a prognostic rather than diagnostic test in CKD populations.

3 Aims

This thesis was designed to study cardiovascular disease, mortality, and their determinants in a cohort of CKD stage 4–5 patients.

The aims of the study were:

1. To study the cardiovascular determinants of mortality in stage 4–5 CKD patients not on dialysis (I).
2. To examine the effect of diabetes on endothelial function, cIMT and AAC, and their determinants in stage 4–5 CKD patients not on dialysis (II).
3. To study maximal physical performance and its determinants in patients with stage 4–5 CKD not on dialysis (III).
4. To study progression of abdominal aortic calcification, associated risk factors, and outcomes in stage 4–5 CKD patients transitioning to dialysis and kidney transplantation, and compare abdominal aortic calcification progression between different modalities of renal replacement therapies (IV).

4 Materials and Methods

4.1 Study sample

The primary study cohort included 210 consecutive voluntary subjects referred to the Kidney Center predialysis outpatient clinic of Turku University Hospital, and recruited between August 2013 and September 2017 into the Chronic Arterial Disease, quality of life and mortality in chronic KIDney injury (CADKID) – study. The target of study population was set to a minimum of 200 patients in the initiation of recruitment. The CADKID -study is an ongoing, prospective, follow-up study assessing arterial disease, quality of life, mortality, and their predictors in patients with chronic kidney disease (CKD-KDIGO 4-5) (https://www.ClinicalTrials.gov NCT04223726).

Inclusion criteria for the study participants were ≥ 18 years of age and CKD stage 4–5 with an eGFR < 30 ml/min per 1.73 m^2 calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. At the time of study enrollment, none of the patients were on renal replacement therapy. There were no exclusion criteria.

The study subjects were followed-up regularly at Kidney Center predialysis outpatient clinic at 1–2 month intervals. Laboratory variables were collected and recorded every three months from the beginning of the study spanning to the end of follow-up. Biochemical data were provided by Turku University Hospital laboratory service (TYKSLAB). Major adverse cardiovascular events were specified as cardiovascular death, myocardial infarction, stroke, and coronary artery revascularisation were documented.

The individual data were collected from the hospital's patient documents during study and clinical control visits. The data from the hospital software were combined, and the patient identity numbers were removed before statistical analyses.

The study was approved by the Medical Ethics Committee of the Hospital District of Southwest Finland. All procedures were in accordance with the Helsinki Declaration. All patients gave written informed consent before entering the study.

4.2 General study design

Study I

A total of 210 patients were recruited for the CADKID study. 174 study participants underwent maximal bicycle stress testing, echocardiography, and planar lateral lumbar radiography to assess AAC score and were included in study I analyses. Data on carotid and femoral artery IMT, elasticity, and brachial artery FMD were collected on 156 patients (of the 174). The remaining subjects were unable to attend the lateral lumbar radiography or the vascular ultrasound study due to severe illness or other contraindication.

Study II

Of all study patients, 199 were assessed for plain lumbar radiography and included in study II. Data on cIMT were available for 172 patients and on FMD for 161. The remaining subjects were incompetent to attend vascular ultrasound assessment due to severe disability or another contraindication.

Study III

Of all the CADKID-study patients, 194 were invited to stress ergometry in the beginning of the study. Of these 194 patients, 16 were incompetent to undergo a stress test, considering severe disability, illness or another contraindication. 180 study subjects agreed to attend but in three patients the stress ergometry was cancelled and was considered contraindicated in the morning of the study. Thus, a total of 174 underwent a standard maximal bicycle stress test. Abdominal aortic calcification, echocardiography and laboratory studies were assessed for all 174 study subjects.

Study IV

In study IV 199 patients of the study population (n=210) underwent lateral lumbar radiography for AAC score assessment at the beginning of the study before commencing to dialysis therapy (AAC1). 150 study patients underwent a second lateral lumbar radiography at follow-up of 3 years from the recruitment (AAC2). Of all study subjects, 150 who had two consecutive measurements of AAC were included to the study. The remaining patients who were unable to undergo AAC assessment had deceased, had severe disability or another contraindication. Echocardiography was assessed in the beginning of the study and was repeated at 3

years of follow-up (available in 143 patients). Major adverse cardiovascular events (MACE) was defined as a composite of cardiovascular death, myocardial infarction, stroke and coronary artery revascularisation and were documented.

4.3 Assessment of abdominal aortic calcification

Abdominal aortic calcification was calculated after recruitment in the beginning of the study for each subject from lateral lumbar radiography performed in the standing position and using standard radiographic equipment. A second lateral lumbar radiography was taken at three years of follow-up.

Calcification of the abdominal aorta was evaluated in the region of the abdominal aorta corresponding to the first through fourth lumbar vertebrae using a previously validated 24-point scale by Kauppila et al.[10] The location and severity of anterior and posterior aortic calcification were graded on a 0–3 scale for each lumbar segment, and the results were summarised. Lesions were graded as follows: 0, no aortic calcific deposits; 1, small scattered calcific deposits filling <1/3 of the longitudinal aortic wall; 2, calcific lesions filling 1/3 or more, but <2/3 of the longitudinal aortic wall; 3, two-thirds or more of the longitudinal wall of the aorta calcified. The scores of individual aortic segments for both anterior and posterior walls were summarised so that the ultimate score ranged from 0 to 24. All radiographs were read independently by two researchers, and the mean was utilised in the analyses.

AAC increment per year (ΔAAC) was defined as [follow-up AAC (AAC2) – baseline AAC (AAC1)] / [time between AAC1 and AAC2 (years)].

4.4 Maximal stress ergometry

Maximal stress ergometry was performed as an incremental, symptom-limited bicycle exercise test according to clinical standards.[160] The initial workload and the workload increase/minute were 10, 15, or 20 W. Workload and increment rate were determined according to estimated maximum workload with the goal of achieving symptom limitation within 6–10 minutes from the start. A continuous monitoring of ECG was assessed, and frequent blood pressure determinations were made through exercise.

Each patient started with a 30 s warm-up during which the target speed of 60 rpm was achieved. The increase of the workload per minute was accomplished automatically by the ergometer software. Participants were encouraged to cycle at a stable speed of 60 rpm and were advised to cycle until exhaustion. The perceived exertion was the highest reported rating from 1 to 20 on the Borg scale, a psycho-physical tool to assess subjective perception of effort.[161]

The mean proportion workload of the last four minutes of the age, sex, and body size predicted value (WMAX%) and the corresponding workload in watts (WMAX) were used in the analyses. The normal considered values for the expected maximal performance measured as watts were derived and extrapolated from large data sets of the Mini Suomi -study, a survey of a population representative of apparently healthy adult Finns.[162] The algorithm for expected exercise performance was incorporated in the cycle ergometer software and is used in daily clinical practise.

4.5 Echocardiography

Comprehensive echocardiography was performed at rest before the exercise test at the Department of Physiology of Turku University Hospital. Stress echocardiographic images were not obtained. The collected data comprised systolic and diastolic dimensions and function of left ventricle (LV), left ventricular wall thickness, aortic and left atrial dimensions, LV mass index (LVMI), LV ejection fraction (LVEF), global longitudinal strain (GLS), and early maximal ventricular filling velocity, and the late filling velocity (E/A-ratio).

For E/e', the transmitral early diastolic inflow velocity (E wave) was measured using pulsed-wave Doppler in the apical four-chamber view, and the peak early (e') diastolic mitral annular velocity was acquired using tissue Doppler imaging at the septal mitral annulus.

Ultrasound examinations were performed using a commercially available ultrasound system (Vivid E9; GE Vingmed Ultrasound, Horten, Norway) with a transducer of 3.5-Mhz phased-array (M5S).

4.6 Ultrasound assessment of vascular structure and function

Vascular ultrasound examinations were performed using Sequoia 512 ultrasound mainframe (Acuson) with a 13.0-MHz linear-array transducer. For intima media dimensions, the left common carotid artery ca. 1 cm proximal to the carotid bulb and the right femoral artery ca. 2 cm proximal to the bifurcation of the deep femoral artery were scanned using B-mode. The image was focused on the posterior (far) wall of the artery, and gain settings were adjusted for optimum image quality. A magnified image was recorded with five seconds duration, and a minimum of four measurements were taken.

Compliance of the artery was surveyed from the left common carotid and right femoral arteries at locations comparable to the IMT measurement sites using M-mode. The means of the two measurements were employed as the end-diastolic and

end-systolic diameters. Multiple images for each variable and artery were digitally saved.

Brachial flow-mediated dilation (FMD) was assessed and evaluated from the right brachial artery diameter at rest and during reactive hyperaemia. Hyperaemia, namely increased flow of blood, was provoked by inflation of a pneumatic torniquet located around the forearm. Pressure was amplified to 250 mmHg for 4.5 minutes, followed by release. Three measurements of arterial diameter were attained at the end-diastole at a determined site from an anatomic marker at rest and at 40, 60 and 80 s after cuff release. After reactive hyperaemia, the vessel diameter was represented as the percentage relative to the vessel diameter at rest. The maximum FMD was adapted from the mean of the three measurements at each time-point. The reproducibility of the methods described have been previously published.[151,13,11]

The scans were digitally stored and manually analysed by one researcher blinded to the details of study subjects. The cardiac cycle of best quality was selected for subsequent offline analysis.

4.7 Biochemical analyses

Laboratory analyses including blood haemoglobin, leukocytes, thrombocytes, glycated haemoglobin, pH, bicarbonate, base excess, plasma C-reactive protein, glucose, alanine aminotransferase, alkaline phosphatase, creatinine, urea, albumin, sodium, potassium, phosphorus, total and ionised calcium and parathyroid hormone were collected and recorded every three months from the beginning of the study spanning to the second AAC imaging, until the patient deceased or until the end of study in every patient. Troponin T, CKMB-mass and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were collected at the recruitment of the study and at follow-up of. Serum low-density lipoprotein values were not included in the original study protocol and were collected from the patient's medical records after the study enrolment. Quantification of renal function was based on the eGFR equation from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) study.[24]

4.8 Statistical analyses

All statistical analyses were performed using Statistical Analysis System, version 9.3 (SAS Institute Inc., Cary, NC, USA). $p<0.05$ was considered statistically significant.

The results are presented as mean ± standard deviation (SD) for the normally distributed variables and as median [(interquartile range (IQR)] for skewed variables if not otherwise stated. For skewed variables, different transformations (log_e-

transformation, square root transformation, and square transformation) were examined to normalise distributions, and the best transformation for each variable was chosen according to tests for normality (Shapiro-Wilk and Kolmogorov-Smirnov) and visual examination. If none of the transformations did not reduce the skewness based on the test and visual examination, it was determined, that there was no suitable transformation.

Study I

In study I, square root transformation was used for FMD and square transformation for ejection fraction. eGFR, TnT, CK-MB-mass, proBNP, E/A ratio, E/e' ratio were $\log_e$-transformed. For variables that had negative values, $\log(X + a + 1)$ transformation was used, where X is the value of the variable and a is the minimum value of the variable in the dataset in order to eliminate missing values. For the variables with values between (0, 1), $\log(X + 1)$ transformation was used.

Analysis of variance (ANOVA) was used for continuous variables for the group-wise comparisons between subjects alive through follow-up, patients deceased in >2 years and patients deceased within 2 years follow-up. The χ^2 test for used for categorical variables. A non-parametric Kruskal-Wallis test was used for skewed variables that were without a suitable transformation (CRP, albumin, AAC, carotid IMT, femoral IMT). When the two deceased groups were compared with survivors, multiple comparisons were accounted for by adjusting the p values in ANOVA using Bonferroni correction.

The association between mortality and exposure variables of interest was investigated by univariable and multivariable Cox proportional hazards models. All variables were standardized to have a mean 0 and SD 1 for the Cox models to compare the magnitudes of the hazard ratios between the exposure variables. The multivariable Cox models included age, sex, previous coronary artery disease as covariates together with a single variable of interest (e.g., TnTm proBNP, WMAX, AAC and E/e' ratio) in each model respectively. None of the study subjects were lost to follow-up.

Study II

Univariable associations between the study variables were analysed by estimating Spearman's correlation coefficients. Linear regression calculation was done in multivariable analysis. Variables with significant univariable correlations with FMD, cIMT and AAC were included as covariates in separate stepwise multivariable linear regression models. Parameters were included without transformation in the multivariate models to help interpretation of the results.

With diabetes as a covariate, univariable and multivariable Cox proportional hazards models were used to examine the association between FMD, cIMT and AAC and cardiovascular mortality. Receiver operating characteristics (ROC) curve analyses were used to estimate the area under the curve (AUC) as a measure of differentiate capability of AAC and cIMT for incident cardiovascular death.

Study III

In study III, student's t-test was used to compare continuous normally distributed covariates and Chi-square test for categorical covariates. Univariable associations were examined by calculating Spearman's correlation coefficients. For some skewed variables a suitable transformation was not found and comparisons between groups a non-parametric Kruskal-Wallis test was used.

Multivariable analysis was done with the linear regression technique. The potential existence of multicollinearity was accomplished by examining variance inflation factors. Variables that had significant univariable correlations with WMAX% (diastolic blood pressure, troponin T, proBNP, haemoglobin, leukocytes, AAC, E/e' ratio and GLS) as well as diabetes and previous coronary artery disease were included as covariates in stepwise multivariable linear regression models. The multivariable associations were analysed between exposure variables and WMAX. Age, height and gender were included as covariates in the stepwise multivariable model for WMAX.

ROC curve analyses were conducted to evaluate the AUC as a measure to discriminative capability of TnT and AAC for WMAX% <50%. An AUC >0.90 was considered remarkable, an AUC 0.80–0.90 excellent, an AUC 0.70–0.80 acceptable, and an AUC <0.70 poor for discrimination.[163]

Study IV

In study IV, comparisons between AAC1 and AAC2 were calculated using the Wilcoxon signed-rank test. Differences between the treatment groups (conservative treatment, peritoneal dialysis, haemodialysis and kidney transplant) in ΔAAC were examined using a non-parametric Kruskal-Wallis test followed by the Dwass-Steel-Critchlow-Fligner method for pair-wise comparisons.[164]

The mean of two consecutive echocardiographic measurements and the mean of blood pressure measurements during the study was used in the analyses of echocardiographic measures and blood pressure, respectively. For laboratory variables, mean values measured during the interval between AA1 and AAC2 assessments were included in the univariate models.

Univariable and multivariable associations between exposure variables and ΔAAC were assessed using linear regression models. Respective univariate models were used to study the associations between ΔAAC and exposure variables.

The significant univariate exposure variables (phosphorus, albumin, LVMI, pulse pressure, smoking status) for ΔAAC were included in the multivariable model. Potential existence of multicollinearity was evaluated by examining variance inflation factors. Associations between ΔAAC and outcomes were studied using univariate Cox proportional hazards models. The all-cause mortality number was low and did not allow for multivariable models.

5 Results

5.1 Cardiovascular determinants of mortality in chronic kidney disease (Study I)

5.1.1 Patient characteristics

A total of 210 patients with a mean age of 61 ±14 years and a mean eGFR of 12.8 ±3.4 mL/min/1.73 m^2 were recruited in the CADKID -study. Of those 210, 174 study subjects underwent a standard maximal bicycle ergometry stress test, echocardiography, and plain lateral radiograph for AAC score assessment, and were included in the analyses. In all, 165 patients (of the 174) had data on carotid and femoral artery IMT and elasticity and on brachial artery FMD. Baseline clinical and laboratory data; see, **Table 2.**

Study patients were followed-up for 42 ± 17 months (range 134–2,217 days). Thirty-one (21%) patients deceased during follow-up, but only three within the first year; 139 (80%) patients commenced dialysis, and 59 (34%) received a kidney transplant during follow-up. Twenty-eight (78%) of those, who perished, had started dialysis, and two (6%) had received transplants before death. Causes of death were cardiovascular, 17 (47%); infection, 5 (14%); malignancy, 8 (22%); trauma, 2 (6%); pulmonary, 1 (3%); gastrointestinal, 2 (6%); and urinary, 1 (3%).

The mean workload of the last four minutes of the maximal stress ergometry was on average 55.7 ± 21.5% of the age, sex and body-size predicted value. At the time of the recruitment, five patients had a diagnosed malignancy including three patients who died during follow-up and two who survived.

Table 2. Baseline characteristics in study I.

Variable	Value
Subjects (female), N	174 (54)
Age, years	60.9±13.7
CAD, n (%)	21 (12)
Antihypertensive medication	141
Beta blockers, n (%)	128 (73.6)
ACE inhibitors/angiotensin II receptor blockers	43/82
Diuretics, n (%)	131 (75.3)
Aspirin, n (%)	47 (27)
Clopidogrel/ticagrelor	9/1
Warfarin/novel oral anticoagulant	30/1
Statin use, n (%)	111 (63.8)
Height, m	1.72±0.09
Weight, kg	83.5±18.4
BMI, kg/m^2	28.0±5.6
Systolic blood pressure, mmHg	152±25
Diastolic blood pressure, mmHg	82±14
Creatinine, µmol/L	413±100
eGFR, mL/min	12 (11–15)
Urea, mmol/L	22.7±6.1
HAemoglobin, g/L	115±12
CRP, mg/L	2.0 (1.0–4.0)
Albumin, g/L	34.9 (32.4–37.6)
Sodium, mmol/L	141 (140–143)
Potassium, mmol/L	4.3±0.5
Ionised calcium, mmol/L	1.20±0.07
Phosphorus, mmol/L	1.47±0.29
Parathyroid hormone, ng/L	182.5 (126.0–299.0)
TnT, ng/L (n=168)	31.0 (21.0–55.5)
CKMBmass, µg/L (n=169)	2.5 (1.6–3.7)
proBNP, ng/L (n=167)	1030 (450–2560)
pH	7.38±0.04
Bicarbonate, mmol/L	22.4±2.6
Total cholesterol, mmol/L	4.48±1.26
HbA1c, %	5.4 (5.1–6.5)

*Values are mean±SD for normally distributed and median (IQR) for skewed variables. CAD, coronary artery disease; CRP, C-reactive protein; BMI, body mass index; eGFR, estimated glomerular filtration rate; proBNP, N-terminal pro-B-type natriuretic peptide; TnT, troponin T. Modified from Lankinen et al, 2020 (I).

5.1.2 Determinants of mortality

In study I we compared patients who survived the follow-up period, those who perished after two years, and those who died within two years. In the comparison between these subgroups, the subjects who deceased during the two year follow-up were older and had significantly higher TnT, proBNP, CRP, E/e' ratio, LVMI, and AAC score, and lower WMAX. No substantial discrepancy existed in eGFR, blood pressure, total cholesterol, or arterial ultrasound measures, namely carotid and femoral IMT and compliance and FMD of brachial artery, between those who perished and those who survived.

Mortality was not associated with possible dialysis initiation, continuing in conservative care, or with HD- and PD modalities (haemodialysis [HD] or peritoneal dialysis [PD]) (23.5 vs. 32.1%; p= 0.36). Mortality was similar in patients on HD and on PD treatment (35.6 vs. 27.8%; p= 0.46). Among those who received kidney transplants, mortality was significantly lower (3.4%) compared with others (p <0.005 for all group-wise comparisons). When comparing all the patients that had started dialysis at some point during follow-up (n= 139, of which 58 received kidney transplant later) to those who never started dialysis (n= 35, of which 1 received pre-emptive kidney transplant), no differences in mortality was found (20.1 vs. 22.9%; p= 0.72).

The incidence of study subject who had HD catheter was comparable in patients who survived compared with those who deceased (survivors: 60/43.5% vs. deceased: 20/55.6%; p =0.020), and the HD catheter was not associated with mortality (HR: 1.58 [95% CI: 0.82–3.05], p= 0.17).

Univariable and multivariable Cox proportional hazards models were used to assess the predictors of mortality. The significant predictors of all-cause mortality in two-year follow-up in univariable models were male sex, age, diabetes, CAD, TnT, proBNP, left ventricular end-diastolic diameter, GLS (global longitudinal strain), E/A ratio, E/e' ratio, AAC, carotid IMT, low diastolic blood pressure, low albumin, and low WMAX. In multivariable proportional hazards models the significant determinants of mortality were TnT, proBNP, WMAX, AAC, E/e' ratio, and albumin.

In the multivariable proportional hazards models adjusted for age, sex and CAD as covariates including a single variable of interest in each respective model the significant determinants of mortality were TnT, proBNP, WMAX, AAC, E/e' ratio and albumin; see, **Table 3**. Carotid IMT seemed to be significant (HR 1.45 [95%CI 1.06–1.98], p= 0.02), but after eliminating an outlier with a value of 4.31 mm (plaque), no significant association was found (HR 1.23 [95%CI 0.85–1.78], p= 0.28).

The multivariable results remained equivalent when stage 4 CKD patients were excluded, with a total of 125 stage 5 CKD patients left in the models. Significant

multivariable determinants of mortality in stage 5 CKD patients were TnT (HR 1.97 [95% CI 1.20–3.21], p= 0.007), proBNP (HR 1.90 [95%CI 1.21–2.96], p= 0.005), WMAX (HR 0.44 [95%CI 0.27–0.73], p= 0.001), AAC (HR 1.70 [95%CI 1.19–2.43], p= 0.004), and albumin (HR 0.46[95%CI 0.29–0.75], p= 0.002). The association between mortality and E/e' ratio became nonsignificant (HR 1.56 [95%CI 0.87–2.78], p= 0.14). When subjects with prior malignancy were excluded from the analyses, the independent predictors of mortality remained similar.

Mortality was not associated dialysis initiation during follow-up (HR 0.62 [95%CI 0.28–1.38], p= 0.24) or with the time to dialysis initiation (HR 0.75 [95%CI 0.52–1.09], p= 0.13). At the beginning of the study, none of the subject had HD catheters. 80 (46.0%) of the study subjects received a HD catheter at some point during the study. The incidence of HD catheters was similar in subjects who survived and those who died [survivors: 60 (43.5%) vs. deceased: 20 (55.6%), p= 0.20]. Presence of HD catheter was not associated with mortality (HR 1.58 [95%CI 0.82–3.05], p= 0.17.

Table 3. Multivariable predictors of all-cause mortality.

Variable	HR (95%CI) adjusted for age, sex, and CAD	*p* value
TnT	1.81 (1.16–2.82)	0.009
proBNP	2.07 (1.42–3.01)	<0.001
WMAX	0.40 (0.25–0.65)	<0.001
AAC	1.75 (1.25–2.43)	<0.001
E/e' ratio	1.73 (1.10–2.72)	0.02
Albumin	0.61 (0.41–0.91)	0.01

* Hazard ratios are for standardised variables with mean 0 and SD 1. CAD, coronary artery disease; WMAX, mean workload of the last 4 min of maximal stress ergometry; AAC, abdominal aortic calcification score; TnT, troponin T; proBNP, N-terminal pro-B-type natriuretic peptide. Modified from Lankinen et al, 2020 (I).

5.2 Arterial endothelial function, carotid artery intima-media thickness and abdominal aortic calcification in diabetic and non-diabetic chronic kidney disease (Study II)

5.2.1 Patient characteristics

Altogether 199 patients, with a median age of 65 (IQR 54–76) years and eGFR of 12 (IQR 10–14) mL/min/1.73 m^2 were recruited. 68 (34.2%) study subjects were female, and 88 (44.2%) had diabetes. 23 patients died during a mean follow-up of 50.6 ±19.9 months (range 4.2-84.5 months).

Patients with diabetes had higher pulse pressure, a higher degree of proteinuria and lower plasma LDL cholesterol, and had a higher proportion of betablocker and statin therapy compared to non-diabetic patients. There was no difference in prevalence and use of warfarin, FMD or cIMT in individuals with and without diabetes.

Diabetes types 1 and 2 were not distinguished as the majority of study participants with diabetes were on insulin treatment. Cardiovascular mortality was defined as death due to coronary artery disease, heart failure, peripheral artery disease or cerebrovascular disease. Plasma lipids were not included in the original protocol of the CADKID-study and thus, the low-density lipoprotein (LDL) values included in the analyses of study II were values that were available from the patients' medical records 12 months prior to study recruitment. AAC score was significantly higher in the diabetic group; see, **Figure 4.**

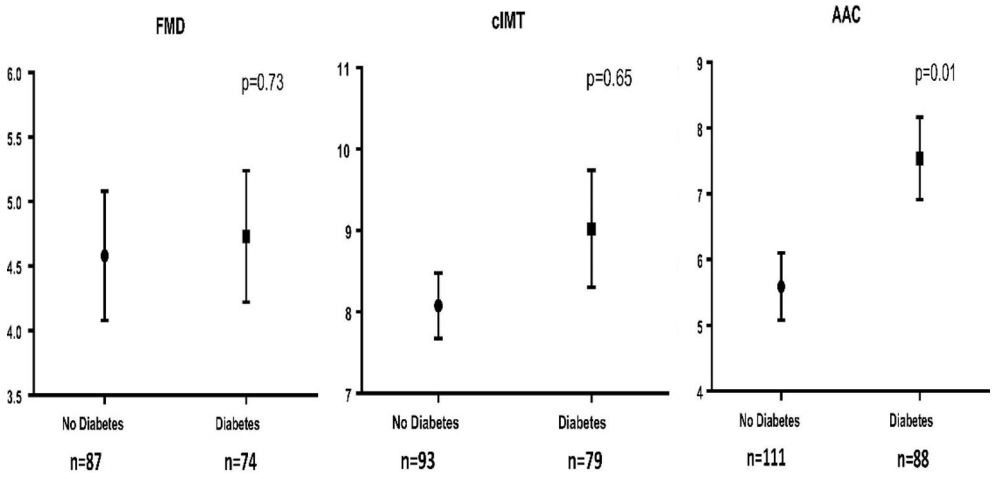

Figure 4. Flow mediated dilatation (FMD), carotid intima-media thickness (cIMT) and abdominal aortic calcification (AAC) compared between patients with and without diabetes. Values are mean (standard error of mean). Modified from Hellman et al. 2021 (II).

5.2.2 Determinants of vascular variables and abdominal aortic calcification

Associations of FMD, cIMT and AAC with risk factors were markedly different in diabetic and non-diabetic CKD patient groups.

In patients with diabetes FMD was inversely associated with urinary protein excretion (r= -0.38, p= 0.008), whereas cIMT had no significant univariate associations with assessed risk factors. FMD was not associated with proBNP or HbA1c. AAC was associated with proBNP (r= 0.38, p= 0.001) in diabetic as well as non-diabetic patients. In diabetics, LDL was not associated with cIMT, FMD or AAC.

In non-diabetic patients elevated proBNP was significantly and inversely associated with FMD (r= -0.36, p= 0.0007) and directly with cIMT (r= 0.22, p= 0.04) and AAC (r= 0.31, p= 0.005). HbA1c was directly associated with cIMT (r= 0.31, p= 0.004) and AAC (r= 0.27, p= 0.01) and tended to be inversely associated with FMD (r= -0.21, p= 0.06). Pulse pressure was associated with cIMT (r= 0.25, p= 0.02), AAC (r= 0.24, p= 0.03) and LDL cholesterol with cIMT (r= 0.29, p= 0.03).

In diabetic study subjects, FMD was lower in those with urinary protein excretion ≥ 0.5 g/l compared to others [2.9 (1.3-6.5) vs. 6.1 (4.0-8.9) %, p= 0.03]. In non-diabetics, association between FMD and urinary protein excretion was not found.

In the multivariate analysis, urinary protein excretion was the only significant determinant of FMD (β= -0.19, p= 0.03). Older age (β= 0.008, p= 0.01), higher pulse pressure (β= 0.004, p=0.01), use of tobacco (β=0.40, p= 0.02) and higher LDL

cholesterol (β=0.10, p= 0.01) associated independently with cIMT in the multivariate analysis. AAC was significantly associated with older age (per year β= 0.22, p< 0.0001), pulse pressure (per mmHg β= 0.05, p= <0.0001), diabetes (β= 1.33, p= 0.04) and proBNP (per µg/l β= 0.18, p= 0.0008). Association between AAC and diabetes remained significant after controlling for betablockers, calcium channel blockers and statins (β= 1.65, p= 0.05). A modest correlation between cIMT and AAC (r= 0.34, p< 0.0001) was found.

Patients who were on warfarin therapy had increased AAC compared to those without [8.5 (5.0–14.5) vs. 5 (0.5–9.75), p= 0.001]. Warfarin use did not significantly associate with AAC in the multivariate model (p= 0.42).

During follow-up 23 patients died, and the death reasons were cardiovascular. AAC (HR 1.19 95%CI 1.11–1.27, p< 0.0001] and cIMT (per 0.1 mm HR: 1.12 95% CI 1.05–1.19, p= 0.0005) were associated with cardiovascular mortality. FMD did not associate with cardiovascular mortality. In the Cox models, the association between AAC (HR 1.18 95%CI 1.10–1.26, p< 0.0001) and cIMT (per 0.1 mm: HR 1.10 95%CI 1.03–1.17, p= 0.003) and cardiovascular mortality remained significant after controlling for diabetes.

In ROC curve analyses, AAC showed good predictive power for incident cardiovascular death with an AUC of 0.81 (95%CI 0.71–0.92). AUC for cIMT was modest, 0.67 (95%CI 0.53–0.81).

The intraobserver variation of AAC measurements was 4.8% and the interobserver variation 11.5%. The mean differences between intra- and interobserver AAC measurements were 0.5 and 1.0 points, respectively.

5.3 Association of maximal stress ergometry performance with troponin T and abdominal aortic calcification (Study III)

5.3.1 Patient characteristics

A total of 174 patients with a mean age of 60.9 ±13.7 years and median eGFR of 12.9 ±3.4 mL/min/1.73 m^2 underwent a maximal bicycle stress test, echocardiography, and lateral lumbar radiography for assessment of AAC score. Nearly half (43%) had diabetes and 21 (12%) had coronary artery disease. All, excluding one were on antihypertensive medication. The mean workload of the last four minutes of maximal stress (WMAX) was 83 ± 36.5 W, and the proportional maximal workload (WMAX%) 55.7 ± 21.5% of the age, sex and body size predicted normal value.

The patients not attending (n= 36) stress ergometry were mostly women, had a higher proportion of CAD, were older and had higher TnT, compared to those that did attend (n= 174) stress ergometry, but no differences were observed in AAC or proportion of diabetes.

5.3.2 Determinants of maximal stress ergometry performance

The study population was divided into two groups according to WMAX% <50% versus ≥50% of the expected age, sex and body size predicted normal value.
The patients with WMAX% < 50% of expected values were older, mostly men, and they presented with high prevalence of diabetes and coronary artery disease. They had higher TnT, proBNP, AAC, left ventricular end-diastolic diameter, LVMI, E/e', and pulse pressure, and lower GLS, compared to the group of better performance. No significant differences were established in body mass index, eGFR, haemoglobin, albumin, total cholesterol, LVEF or use of beta blockers or calcium channel blockers between groups. Only five patients out of the whole study cohort had below normal (<50%) LVEF.

Univariable correlates of WMAX% are shown in **Table 4**. TnT, proBNP, E/e', LVMI, AAC, pulse pressure, leukocytes, erythrocyte sedimentation rate (ESR) and CRP had negative correlation and GLS positive correlation to relative exercise performance (WMAX%). WMAX% was significantly lower in patients with diabetes and CAD compared to those without (No diabetes or CAD: 65.2 ± 21.0%, Diabetes 45.6 ± 15.7%, CAD 42.5 ± 18.3%, p <0.0001 for both comparisons).

In the stepwise multivariable linear regression model, TnT (β= -0.09, p= 0.02), diabetes (β= -0.09, p= 0.02) and AAC (β= -1.67, p <0.0001) remained as significant predictors for WMAX%. When WMAX instead of WMAX% was included as the dependent variable in the multivariable model, the significant explanatory variables were TnT (β= -0.13, p= 0.046), AAC (β= -1.44, p= 0.001), age (β= -0.97, p <0.0001), male gender (β= 31.0, p< 0.0001), haemoglobin (β= 0.42, p= 0.01), leukocytes (β= -2.09, p= 0.046), diastolic blood pressure (β= 0.30, p= 0.05) and diabetes (β= 16.1, p= 0.0005); see, **Table 5.**

TnT associated with LVMI (r= 0.34, p< 0.0001), E/e' (r= 0.28, p= 0.002), GLS (r= -0.27, p= 0.003) and creatinine (r= 0.20, p= 0.01). No association was found with TnT and LVEF or eGFR.

In ROC curve analyses, TnT and AAC showed fair predictive power for WMAX% less than 50%. TnT showed an AUC of 0.75 (95%CI 0.68–0.83) and AAC an AUC of 0.70 (95%CI 0.62–0.79).

Table 4. Univariable correlations of WMAX% of exercise performance test.

Variable	Correlation coefficient	*p* value
TnT	-0.52	<0.0001
proBNP	-0.39	<0.0001
AAC	-0.46	<0.0001
E/e'	-0.41	<0.0001
LVMI	-0.25	0.001
GLS	0.27	0.002
Diastolic blood pressure	0.35	<0.0001
Pulse pressure	-0.34	<0.0001
Haemoglobin	0.17	0.02
Leukocytes	-0.35	<0.0001
ESR	-0.25	0.0009
C-reactive protein	-0.22	0.003

TnT, troponin T; proBNP, N-terminal pro-B-type natriuretic peptide; AAC, abdominal aortic calcification; E/e', ratio of transmitral Doppler early filling velocity to tissue Doppler early diastolic mitral annular velocity; LVMI, left ventricular mass index; GLS, left ventricular global longitudinal strain; ESR, erythrocyte sedimentation rate. Modified from Lankinen et al. 2021 (III).

Table 5. Stepwise multivariable analyses of WMAX and WMAX%.

Variables in the final model (significance <0.15)	β	*p* value
Stepwise multivariable model for WMAX		
AAC score	-1.44	0.001
Diabetes	-16.06	0.0005
Troponin T	-0.13	0.046
Leukocytes	-2.09	0.046
Haemoglobin	0.42	0.01
Diastolic blood pressure	0.30	0.05
Male gender	31.00	<0.0001
Age	-0.97	<0.0001
Stepwise multivariable model for WMAX%		
AAC score	-1.67	<0.0001
Diabetes	-11.7	<0.0001
Troponin T	-0.09	0.02
Leukocytes	-1.47	0.08

Exposure variables included in the initial stepwise model for WMAX%: diabetes; coronary artery disease; diastolic blood pressure; troponin T; N-terminal pro-B-type natriuretic peptide; haemoglobin; leukocytes; abdominal aortic calcification score; E/e' ratio of transmitral early filling velocity; left ventricular global longitudinal strain. Exposure variables included in the initial stepwise model for WMAX: The exposure variables for WMAX% concluding age, gender and height. Modified from Lankinen et al. 2021 (III).

5.4 Progression of abdominal aortic calcification in chronic kidney disease (Study IV)

5.4.1 Patient characteristics

A total of 150 study subjects of the CADKID-study had two consecutive AAC measurements, one at the beginning of the study (AAC1) and one at follow-up (AAC2) at a median interval of 37 (29–44) months. During follow-up of 5.0 ±1.4 years 15 (10%) died, and MACEs were observed in 30 patients (22%) (myocardial infarction n=9; stroke n=8; coronary artery revascularisation n=13).

At the time of the follow-up AAC, 33 patients had remained on conservative treatment for chronic kidney disease, 39 were on haemodialysis, 39 on peritoneal dialysis, and 39 had received kidney transplant. For those who commenced dialysis treatment, the time from dialysis initiation to AAC2 was 19 (15–27) months and in the transplanted patients the time from transplantation was 14 (7–22) months.

In the conservative treatment group, the eGFR was 13 ± 6 ml/min/1.73 m^2 and in those who had received a kidney transplant, eGFR was 60 ±23 ml/min/1.73 m^2 at the time of the AAC2 assessment.

5.4.2 Progression of abdominal aortic calcification

Median baseline AAC score (AAC1) was 4.8 (0.5–9.0) and median follow-up AAC score (AAC2) 8.0 (1.5–12.0), (p<0.001). Altogether median AAC increment per year (ΔAAC) was 0.48 (0.00–1.43). The median ΔAAC was 0.41 (0.00–0.97) in conservatively treated patients, 0.34 (0.00–0.95) in transplanted patients, 0.53 (0.00–1.57) in HD patients and 1.0 (0.00–1.83) in PD patients; see, **Figure 5.** The differences in ΔAAC between the groups were nonsignificant (p=0.19).

Patients without calcification at baseline had lower ΔAAC in comparison to patients with any calcification at baseline (0 [0-0] vs. 0.81 [0.26–1.61] per year, p< 0.0001). A significant but modest correlation was found between AAC1 and ΔAAC (r= 0.25, p= 0.002).

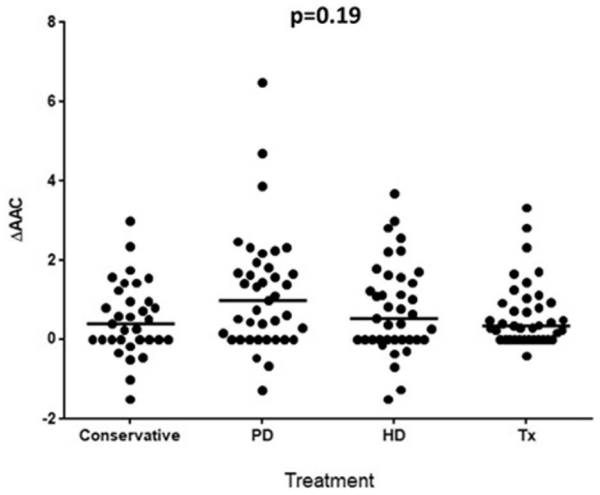

Figure 5. Abdominal aortic calcification progression (ΔAAC) per one year in between conservative, PD, HD and transplantation groups. Modified from Lankinen et al. 2021(IV).

AAC1 (8.5 [5.9–15.5] vs. 4.0 [0.0–8.5], p= 0.004), AAC2 (11.5 [8.5–18.0] vs. 7.0 [1.0–11.5], p= 0.003) and ΔAAC (1.67 [0.0–2.25] vs. 0.43 [0.00–1.27], p= 0.04) were higher among patients who deceased during follow-up compared to others.

ΔAAC (HR 1.427, 95%CI 1.044–1.950, p= 0.03), AAC1 (HR 1.128, 95%CI 1.043–1.220, p= 0.003) and AAC2 (HR 1.140, 95%CI 1.050–1.237, p= 0.002) were associated with all-cause mortality in univariate Cox proportional hazards models. AAC1 (HR 1.065 95%CI 1.003–1.130, p= 0.04) and AAC2 (HR 1.067 95%CI 1.010–1.128, p= 0.02) associated with incident MACEs whereas ΔAAC did not (HR 1.194 95%CI 0.921–1.549, p= 0.18).

In univariate analysis ΔAAC was significantly associated with mean values of phosphorus (log phosphorus: β= 1.79, p= 0.001), albumin (β= -0.09, p= 0.0005), LVMI (log LVMI: β= 1.43, p= 0.0005), pulse pressure (β= 0.02, p= 0.0009) and smoking (non-smoker, ex-smoker, current smoker: β= 0.31, p= 0.02); see, **Table 6.** Serum lipids, glycated haemoglobin, ionised or total calcium, PTH or other repeated laboratory variables were not significantly associated with ΔAAC.

In the multivariable model the only significant explanatory variables for ΔAAC were mean LVMI (log LVMI: β= 0.97, p= 0.02) and phosphorus (log phosphorus: β= 1.19, p= 0.02). The progression of LVMI was not associated with ΔAAC.

Use of calcium-based phosphate binders or their dose was not associated with ΔAAC nor was the use of non-calcium-based phosphate binders. Moreover, warfarin use was not associated with ΔAAC.

Table 6. Univariate and multivariable associations of ΔAAC and risk factors.

Variable	Univariate analysis		Multivariate analysis	
	β	*p* value	β	*p* value
Phosphorus, log	1.79	0.001	1.19	0.02
Albumin, g/L	-0.09	0.0005	-0.04	0.17
LVMI, log	1.43	0.0005	0.97	0.02
Smoking status	0.31	0.02	0.19	0.16
Pulse pressure, mmHg	0.02	0.009	0.005	0.41

log, logarithmic transformation; smoking status (non-smoker, ex-smoker, current smoker); LVMI, left ventricular mass index; AAC, abdominal aortic calcification. Values are expressed as mean. Modified from Lankinen et al. 2021 (IV).

In subjects with a kidney transplant, the time from the beginning of the study to transplantation had an association with ΔAAC (per month on the waiting list: β= 0.04, p= 0.001).

The time from transplantation to the assessment of AAC2 was not associated with ΔAAC (β= -0.000, p= 0.53) and the duration of dialysis therapy was not associated with ΔAAC in dialysis patients (β= 0.009, p= 0.48).

6 Discussion

6.1 Cardiovascular determinants of mortality in advanced chronic kidney disease (I)

Regardless of improved medical care and diagnostics, the risk of death of advanced chronic kidney disease patients remains high. Cardiovascular diseases are the leading cause of death being 10- to 30-fold higher than in age-matched populations.[2] End stage renal disease frequently culminate in death rather than initiation of renal replacement therapy.[3] It would be invaluable to be able to assess individual risk and prognosis, and to personalise treatment of CKD stage 4–5 patients.

We studied demographic, laboratory, echocardiographic, and nonivasive arterial imaging measures, and maximal aerobic stress ergometry performance to determine all-cause mortality, and assessed individual risk factors and prognoses in patients with advanced chronic kidney disease.

The results of our study indicate that stress ergometry performance, AAC, E/e' of echocardiography, TnT and proBNP, as well as albumin, a marker of poor nutritional status and inflammation, predict mortality in advanced CKD. Conversely, specific vascular ultrasound markers, namely IMT and FMD, did not have an association.

Traditional risk factors explain only part of the increased cardiovascular risk of CKD patients. In study I blood pressure was not predictive of mortality. However, all study participants but one were on antihypertensive medication, though only 16% of the patients reached the target blood pressure of ≤130 mmHg, guided by KDIGO guidelines.

The mortality risk associated with CKD is best treated with kidney transplantation, which is unfortunately not available for every patient. Kidney transplant recipients in study I had considerably lower mortality (3.4%) compared with those who were treated with dialysis or continued on conservative care emphasizing the significance of kidney transplantation in decreasing mortality risk in the advanced CKD population.

Apart from kidney transplantation, mortality was comparable between patients preceding in conservative care or commencing in dialysis treatment. No differences

in mortality was found when the patients who started dialysis at some point of follow-up were compared with those who remained without dialysis.

6.1.1 Abdominal aortic calcification

Arteriosclerosis and vascular calcification have a propensity for accelerated progression, and traditional risk factors incompletely explain the arterial disease sequalae in the high-risk CKD population.[4]

Abdominal aortic calcification precedes the occurrence of coronary artery calcification, and develops usually at younger ages in dialysis patients, presumably in consequence of uremic milieu.[4] Atherosclerosis of the abdominal aorta is an important cardiovascular risk factor and has been associated with all-cause and cardiovascular mortality in CKD including patients on renal replacement therapy.[5,6,146] Peeters et al. showed an association between AAC and cardiovascular events in a study cohort of 280 non-dialysis CKD patients.[9]

Several methods, including computed tomography, ultrasound, and echocardiography, are available for detection of cardiovascular calcification. Planar X-ray is readily available, easy to perform, and a cost-effective method of studying vascular calcification of abdominal aorta and other vascular beds.

AAC can be noninvasively detected and quantified on lateral lumbar radiographs to identify subjects with high cardiovascular risk, as presented in the Framingham Heart Study.[7] The best known method to quantify radiopaque lumbar calcifications of abdominal aorta, used in a number of contemporary studies,[8,9] is proposed by Kauppila et al.[10]

In study I, AAC assessed at the beginning of the study was independently associated with all-cause mortality in the multivariable Cox regression model in advanced chronic kidney disease patients.

Findings of the present study are consistent with Okuno et al. who reported that examination of calcification at a single site, the abdominal aorta, of end-stage kidney disease patients, can predict all-cause and cardiovascular mortality.[5] Our findings for the prevalence of abdominal aortic calcification by lateral lumbar X-ray and its association with cardiovascular events in non-dialysis CKD patients are in line with previous studies.[9]

6.1.2 Physical performance

Altered structures of myocardium and arteries in the setting of CKD may result in exercise intolerance.[165,18] Declined exercise tolerance predicts survival in end-stage renal disease.[18] After renal transplant, exercise capacity improves, still remaining only on a level equal to normal sedentary people.[19] Cardiorespiratory fitness is the

most-tested outcome reported in exercise studies in CKD using exercise tolerance testing.[20]

We concluded that maximal ergometric capacity may be used to assess the likelihood of death in non-dialysis, advanced CKD patients. Our results are consistent with the results of Roshanravan et al.[21] They compared measures of handgrip strength, usual gait speed, timed up and go, and six-minute walking distance to normative values to study physical performance in CKD 2–4 patients. The study concluded that declined physical performance of the lower extremities is ordinary in CKD and has strong association with all-cause mortality. However, association between lower extremity function and mortality may have been confounded by uncaptured comorbid conditions, such as peripheral artery disease and the study population had milder degree of chronic kidney disease. A similar relationship between declined exercise capacity and survival status in the CKD population has been identified in several other studies,[18,53] but no previous studies, to our knowledge, have shown an association between maximal aerobic exercise capacity and mortality in a non-dialysis CKD population.

6.1.3 Vascular function and structure

Vascular ultrasound assessment of intima-media thickness (IMT), brachial artery flow-mediated dilation and vascular elasticity are widely used measures of subclinical arteriosclerosis and associated with cardiac events and death in general population.[12,14] In the current study, we demonstrated an association between carotid IMT and mortality in advanced CKD, which is in line with the previous study of Zoungas et al.[15] Our study did not find associations with arterial elasticity, brachial FMD, or femoral IMT and mortality in our study cohort of advanced CKD.

There are few previous studies concerning femoral artery IMT as a surrogate of subclinical atherosclerosis, compared to carotid IMT, in the non-CKD population.[150] To our knowledge, no previous studies concerning association of femoral IMT with cardiovascular outcome in advanced CKD have been published.

Our present work adds to the mixed results from previous studies concerning endothelial function as a predictor of cardiovascular adverse events in high-risk patients.[152] Endothelial function, namely FMD, deteriorates as kidney function declines.[17] FMD appears to be severely impaired in CKD,[155] but data are scarce on advanced CKD patients not on dialysis.

6.1.4 Cardiac biomarkers

Brain natriuretic peptide parallels the presence and severity of cardiovascular disease[64,68] and associates to cardiovascular events[69] in CKD, but associations with

all-cause mortality have been infrequent. Troponin T has been associated with subclinical cardiac changes in CKD,[71,70] and has been suggested as a helpful marker to identify patients at increased risk for cardiovascular events[59] and cardiovascular deaths.[61]

We demonstrated an independent association between TnT and NT-proBNP, and all-cause mortality, although cardiovascular mortality accounted only for 47% of all fatalities. Our finding parallels that of the African American Study of Kidney Disease and Hypertension concerning 994 study subjects with a glomerular filtration rate of 20–65 ml/min/1.73 m^2 and showing an association between elevated NT-proBNP and higher CV risk, but not with all-cause mortality.[67]

Elevated NT-proBNP levels have been linked with peripheral artery disease suggesting that arterial luminal narrowing may result in increased arterial resistance and cardiac afterload.[166] The study of Untersteller et al. confirming natriuretic peptide as an independent predictor of adverse outcomes in patients with mild to moderate CKD,[66] is in line with our conclusions. Our results of the association of cardiac biomarkers with all-cause mortality underlies the contribution of the cardiovascular burden to increased non-cardiac mortality risk in advanced CKD.

6.1.5 Echocardiography

Cardiac structure and function, detected by echocardiography, become altered in advanced CKD. Cardiac systolic and diastolic functions attenuate and have been associated with mortality in CKD patients dependent and independent of dialysis.

The only significant echocardiographic measure independently associated with mortality in our study was increased E/e' ratio. The increased E/e' ratio was considered to be a consequence of fluid and sodium retention, along with ventricular stiffness, typical findings in CKD.[128,126] Results of our study are comparable to previous findings of higher E/e' ratio being predictive of mortality and cardiovascular events in candidates waiting for renal transplant[124] as well as in non-dialysis CKD patients.[157]

6.2 Effect of diabetes on vascular structure and function, and abdominal aortic calcification in advanced chronic kidney disease (II)

Flow-mediated dilation and cIMT

Endothelial dysfunction and increased carotid IMT are considered early markers of subclinical atherosclerosis and can be detected in diabetic patients compared to healthy controls. Our study showed no associations between diabetes and FMD or

carotid IMT (cIMT). We speculated that the CKD-associated cardiovascular risk factors may negate those related to diabetes. The presence of proteinuria independently predicted attenuated FMD in our study cohort, results being in line with previous studies demonstrating an association between proteinuria and impaired endothelium-dependent, flow-mediated dilatation on diabetic as well as non-diabetic patients.[132,140,153]

In our study diabetes per se was not associated with FMD, a strong predictor for subclinical atherosclerosis. Current study suggests that the presence of diabetes may not be a significant factor for endothelial dysfunction among patients with advanced CKD. Also, no association between diabetes and cIMT was detected. However, our study cohort had relatively good glycaemic control, which likely explains the absence of association between diabetes and FMD. The current study did not assess the duration of diabetes. In patients without diabetes, higher blood glucose has been associated with cIMT in previous studies.[167,168]

Our study cohort consisted of CKD stage 4–5 patients. While we detected no association between endothelial dysfunction or cIMT and diabetes, the studies demonstrating a positive correlation between diabetes and cIMT predominantly included patients with less severe CKD or lower prevalence of diabetes.[169,138]

Abdominal aortic calcification

Abdominal aortic calcification, a noninvasive cardiovascular risk marker, is associated with diabetes, declining kidney function as well as cardiovascular morbidity and mortality. Not surprisingly, prevalence of AAC was high in our cohort of comorbid CKD patients, nearly half of which had diabetes.

Previous studies have shown association between AAC and advanced atherosclerosis in multiple vascular sites, and AAC has better predicted cardiovascular disease and CV events compared to cIMT or FMD.[170-172] In our study, older age, higher pulse pressure, and diabetes were predictors of high AAC. Our findings are in concordance with the study of Petchey et al. demonstrating an association between diabetes and AAC, but not between diabetes and cIMT or FMD.

6.3 Associations of cardiac biomarkers and abdominal aortic calcification on maximal stress ergometry performance in advanced chronic kidney disease (III)

CKD is characterised by inferior exercise capacity which predicts poor outcomes. The decline in physical functioning is already developing in the predialysis period.[54]

Previous data have shown that elevated troponin T associates with cardiovascular mortality in advanced CKD.[61,62,173]

The present study shows that maximal stress ergometry performance is associated with TnT and AAC in non-dialysis CKD patients. Our results suggest that increased TnT represents cardiovascular burden by limiting maximal physical performance. In line with our results is a study of Porter et al. showing an association between TnT and perfusion defects in single photon emission cardiac tomography after pharmacologic and/or exercise stress in end-stage renal disease patients.[174] Elevations in TnT may indicate subclinical changes in volume and myocardial stress that subsequently contribute to markers of cardiac dysfunction.[69,70]

In a cohort of CKD 4-5 patients not on dialysis, TnT was inversely associated with the physical composite score of the Short Form 36 Items Health Survey indicating an association of TnT and cardiovascular burden.[175] Our results are in line with other studies indicating that increased TnT may be a marker of silent myocardial ischemia, which could explain the reduced physical performance.[69] However, concerning advanced CKD and maximal physical performance stress testing and associations with cardiac biomarkers, data are still scarce.

Only 5 patients in our study cohort had below normal LVEF (<50%) which may explain why proBNP was not independently associated with WMAX%, marker of proportional maximal ergometry workload.

We found, that AAC has an impact on ability to tolerate exercise. Association of AAC and inferior exercise tolerance may be attributed to silent myocardial ischemia, universal atherosclerosis, peripheral artery disease and cardiac dysfunction. Former studies have shown associations between AAC and coronary artery disease, as well as AAC and incident claudication, both of which may culminate in inferior exercise tolerance.[176,177] To our knowledge, no previous data are available on the predictive association of AAC and physical stress tests in patients with advanced CKD.

6.4 Progression of abdominal aortic calcification and associated risk factors in advanced chronic kidney disease (IV)

Progression of aortic calcification is significantly related to an increased all-cause and cardiovascular mortality in advanced CKD.[178] Our study showed for the first time that AAC progression is similar in CKD stage 4–5 patients transitioning to different modalities of renal replacement therapies (RRT) or continuing conservative treatment. The increased rate of AAC was independently associated with plasma phosphorus but not with ionised calcium, PTH, alkaline phosphatase, serum lipids, glycated haemoglobin, or other laboratory variables.

Progression of AAC

The progression of vascular calcification in the setting of CKD has been assessed in multiple studies, showing that progression is already rapid in earlier stages of CKD,[179] accelerated in advanced CKD[179] and haemodialysis patients,[178] and proceeds even after successful kidney transplantation.[180] Until now, comparative data have been lacking on patients assessed for AAC during advanced CKD prior to RRT and prospectively after transitioning to different treatment modalities. In a study by Noordzij et al., aortic calcification progressed in almost a third of patients in haemodialysis or peritoneal dialysis, and progression was significantly associated with mortality.[178]

In the present study, patients who presented with abdominal aortic calcification at baseline had higher rate of AAC progression (ΔAAC) compared to those who were free of calcification at the beginning. This is in line with previous studies assessing aortic calcification on patients initiating dialysis during follow-up,[178] and before and after renal transplantation.[82] Patients who deceased during follow-up of our study had higher AAC scores at baseline as well as at follow-up.

Our current findings are consistent with previous research demonstrating that a set of transplant patients show progression of AAC after kidney transplantation in a mean follow-up of 1–3 years.[82,180]

In study IV we concluded that ΔAAC was positively associated with the time on the transplant waiting list, but the time from transplantation to the control AAC was not associated with ΔAAC. These findings suggest that at least in the early years following kidney transplantation the process of vascular calcification continues similarly to the way it does in those on maintenance dialysis or conservative care. Progression of vascular calcification after kidney transplantation has been associated with older age, calcium, and post-transplant eGFR in previous studies.[81,82,180]

Association of phosphorus and AAC progression

CKD-mineral and bone disorder precipitate to vascular calcification. Previous data concerning maintenance haemodialysis patients have shown an association between accelerated progression of VC and hypercalcemia and hyperparathyreosis.[178,179] Most studies assessing AAC progression have concerned patients already in maintenance haemodialysis or with kidney transplant, whereas studies on predialysis patients have been scarce.

Hyperphosphatemia and hyperparathyroidism have been associated with all-cause, and cardiovascular morbidity and mortality.[98] However, the few studies of calcium-based and non-calcium-based phosphate binders have been disappointing in regards to vascular calcification in coronary arteries and in the abdominal aorta.[104]

In the present study plasma phosphorus was the only repeated laboratory variable independently associated with ΔAAC. Higher phosphate levels have independently determined a high calcification score in the non-dialysis CKD population.[9] However, established medical therapies to attenuate vascular calcification are limited, and there is currently no specific therapy to reverse vascular calcification in humans.

Treatment with the phosphate binder lanthanum carbonate did not result in any difference in arterial stiffness or aortic vascular calcification compared with placebo in a recent randomised, placebo-controlled trial by Toussaint et al.[101] Potential benefits of phosphate-lowering therapies in nondialysis CKD patients are still lacking.

Both vascular calcification and high plasma phosphorus might be dependent on the high levels of PTH, although no relationship between PTH and the calcification process was observed in this study. In study IV the increase rate of aortic calcification was not associated with PTH. This is in line with previous data linking elevated phosphate and aortic calcification but without a direct action of PTH on aortic calcification.[105]

6.5 Limitations of the study

This study has limitations. The study sample was rather small. However, the findings concerning the predictors of mortality in advanced CKD were distinct, and limited sample size is not likely to detract from the validity of our main conclusions. A full-scale selection of imaging and other methods to detect vascular calcification and assess all-cause mortality risk was used in the present study. All study subjects were followed-up for at least two years, or until death, though, follow-up time differed between patients.

Peak oxygen uptake is a typical measurement used as a surrogate of exercise tolerance in exercise studies of the CKD population. As we did not perform spiroergometry to define peak oxygen uptake, the maximal ergometry performance was limited by subjective exhaustion and was estimated by the relation of achieved power to subjects body weight, age and sex. We consider our results of maximal exercise performance reliable as the detected mean workload value of 55.7 ± 21.5% of the age predicted value parallels the reduction in maximal oxygen uptake reported in a study concerning maintenance dialysis patients.[19]

A high proportion of study patients were on betablockers, and the ergometry was performed without medication pauses, which has probably influenced the workloads achieved. However, betablocker use was similar between patients with WMAX% ≤50% compared to others.

Previous cross-sectional data from two large cohorts have shown that the urinary albumin/creatinine -ratio is inversely associated with physical activity.[19]

Unfortunately, urinary albumin/creatinine -ratio was not included in the CADKID study protocol. Only spot urine samples were available for urinary protein excretion analysis. However, a low qualitative cut-off value was used to avoid the inaccuracy issues in quantitative interpretation of spot urine samples.

Assessing aortic calcification based on plain radiographs may overlook some elusive calcifications and changes compared with CT techniques. However, intra- and interobserver reproducibility of AAC measurements was acceptable. As several patients deceased prior to the follow-up measure of AAC, the association or lack between ΔAAC and adverse events is likely to be affected by the study design and should be interpreted with caution.

FMD and cIMT data were partially unavailable in the cohort and the decreased total sample size may slightly weaken definitive conclusions. However, the patients were conservatively recruited and extensively studied, and the quality of the data was good.

The data included in this study were cross-sectional and from a single centre which may limit the generalisability of our results. There was a degree of expected selection bias in attending stress ergometry as the patients not attending were more often women and had CAD, were older and had higher TnT, but no differences were observed in AAC or proportion of diabetics. Nevertheless, in our opinion the studied cohort with a high degree of comorbidities represents the overall CKD stage 4–5 population at our centre well.

6.6 Future aspects

Detecting the increased individual risk with methods presented in our study could aid in clinical decision-making and targeting treatment strategies to the individuals at the highest mortality risk possibly translating to a beneficial outcome. A more precise clinical risk assessment could also be helpful when considering if the patient will benefit from a renal transplant and be applicable for the transplant waiting list.

The finding that TnT and AAC are independently associated with maximal physical performance in advanced CKD may have clinical implications in recognizing patients at risk and targeting treatment to increase the functionality and quality of life of affected patients.

Concerning advanced CKD patients with diabetes, measurements of vascular function, namely IMT and FMD, may not give any further use to cardiovascular risk prediction. In advanced CKD, AAC may better depict the combined result of atherosclerosis due to traditional cardiovascular risk factors and uremic vascular calcification than cIMT and FMD. This finding supports the contemporary Kidney Disease Global Outcomes (KDIGO) clinical guidelines in recommending the use of

AAC in screening for vascular calcification as a part of cardiovascular risk assessment in CKD.[181]

7 Summary

I Stress ergometry performance, abdominal aortic calcification score, E/e' of echocardiography, plasma cardiac biomarkers TnT and proBNP, and albumin predict mortality in advanced CKD. Vascular ultrasound assessment of IMT and FMD do not have an association with mortality.

II Diabetes in a cohort of advanced CKD patients is associated with increased AAC, but not with increased cIMT or attenuated FMD

III Cardiac biomarker TnT and AAC are independently associated with maximal ergometry stress test workload in patients with advanced CKD not on dialysis.

IV AAC progresses at a comparable rate in patients on different renal replacement modalities or continuing conservative treatment and the increment rate is independently associated with phosphorus.

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