

Cardiovascular risk factors in renal artery stenosis

**Effects of renal angioplasty and
angiotensin II receptor antagonism**

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Cover illustration: Renal artery stenosis in magnetic resonance angiography of a patient from our study. The examination has been performed in the Department of Radiology at Sahlgrenska University Hospital, Gothenburg, Sweden.

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*Dedicated to my friend **Ewa Nebeski***

*Although you are no longer with us, you will always remain in my heart.
Thank you for your friendship and support.*

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ABSTRACT

Renovascular hypertension (RVH) caused by atherosclerotic renal artery stenosis (ARAS) is one of the most common forms of secondary hypertension. The prognosis for patients with RVH is much worse compared to patients with primary hypertension, and caused by a high cardiovascular morbidity. The aim of this thesis was to increase our knowledge about the pathophysiology of RVH and to identify novel treatment targets that could reduce cardiovascular risk in these patients. We investigated: 1) whether systemic inflammation and endothelin-1 (ET-1) are increased in patients with RVH and evaluated how treatment with percutaneous transluminal renal angioplasty (PTRA) affected these variables; 2) lipoprotein abnormalities in patients with atherosclerotic renovascular disease (ARVD) and analyzed whether angiotensin II (Ang II) receptor antagonism with candesartan influenced lipoprotein levels; 3) whether plasma levels of brain natriuretic peptides (BNP) are increased in patients with ARAS and may predict favorable outcome of PTRA; and 4) the long-term effects of candesartan on kidney function, inflammatory biomarkers and ET-1 in patients with ARVD and residual hypertension after PTRA.

In patients with significant renal artery stenosis (RAS) we found increased plasma levels of inflammatory biomarkers and ET-1 compared to healthy subjects. Intervention with PTRA triggered a rapid, transient increase in hs-CRP and IL-6. However, one month after PTRA, both IL-6 and ET-1 had decreased compared to before intervention. Patients with ARVD had elevated levels of atherogenic, triglyceride-rich, ApoC-III-containing lipoproteins in spite of ongoing treatment with statins. Treatment with candesartan did not correct these abnormalities. Patients with ARAS had increased plasma levels of BNP compared to healthy controls, but BNP concentrations were not affected by PTRA. Plasma levels of BNP could not be used to predict the outcome of PTRA on blood pressure. Candesartan did not have any significant effects on kidney function, inflammatory biomarkers or ET-1 in patients with ARVD during 35 months of follow up.

In conclusion, patients with ARAS had increased levels of inflammatory biomarkers, ET-1, and ApoC-III-containing lipoproteins that may contribute to progressive atherosclerosis and accelerated cardiovascular disease. Intervention with PTRA reduced plasma levels of IL-6 and ET-1 indicating beneficial effects on inflammation and the endothelin system. Plasma concentrations of BNP could not be used to identify patients with a favorable outcome to PTRA.

Keywords: inflammation, endothelin-1, apolipoprotein C-III, brain natriuretic peptides, angiotensin II receptor antagonism, renal angioplasty, renovascular hypertension, atherosclerotic renal artery stenosis

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POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

Förträngning av njurartären (njurartärstenos) är orsak till högt blodtryck (hypertoni) upp till 5% av alla patienter med hypertoni. Den vanligaste orsaken till att en förträngning uppstår är åderförkalkningssjukdom (ateroskleros). Prognosen för patienter med njurartärstenos (NAS) är betydligt sämre än för patienter med primär hypertoni och är associerad med hög hjärt-kärl sjuklighet och dödlighet. Minskning av blodtillförseln till njuren leder till aktivering av renin-angiotensin-aldosteron systemet (RAAS) som bidrar till att höja blodtrycket. Orsaken till den höga hjärt-kärl sjukligheten hos dessa patienter är inte helt klarlagd. Vi tror att riskfaktorer som inflammation, och specifika blodfettsrubbingar, sekundära till aktivering av RAAS eller nedsatt njurfunktion, ligger bakom en accelererad åderförkalkning. Trots ballongvidgning (PTRA) av förträngningen och förbättrat blodflöde till njuren är det tveksamt om PTRA förbättrar njurfunktionen och minskar hjärt-kärlhändelser. Angiotensin II (Ang II), som är den aktiva substansen i RAAS, stimulerar också produktion av brain natriuretic peptide (BNP), ett hormon som frisätts från hjärtmuskelceller och som motverkar Ang II:s blodtryckshöjande effekter.

Den övergripande målsättningen med detta arbete var att öka kunskapen om patofysiologin vid NAS och att förbättra behandlingen och minska risken för hjärt-kärlsjuknande. Vi studerade om inflammation är ökad vid NAS och hur intervention med PTRA påverkade inflammationsmarkörer. Vidare undersökte vi blodfetsprofilen hos patienter med NAS och undersökte om behandling med candesartan, som blockerar RAAS systemet, påverkade denna. Sedan undersökte vi om BNP är förhöjt hos patienter med NAS och om BNP kunde användas för att identifiera patienter som har klinisk nytta av PTRA. I sista arbetet analyserade vi långsiktiga effekter av candesartan på njurfunktion och inflammation hos patienter med NAS.

Arbetet baseras på en prospektiv, randomiserad, öppen studie, där vi studerade effekterna av candesartan på patienter med aterosklerotisk NAS som genomgått behandling med PTRA under år 2003-2008. Vi inkluderade 178 patienter med misstänkt NAS, varav 108 genomgick PTRA. Fyra veckor efter PTRA randomiserades patienter med kvarstående hypertoni (>130/80 mmHg) antingen till candesartan eller till antihypertensiv behandling utan RAAS-blockerare. Patienterna följdes upp i 3 år efter PTRA.

Vi fann att patienter med signifikant NAS hade förhöjda inflammationsmarkörer jämfört med friska kontroller. PTRÄ åstadkom en snabb övergående ökning av inflammationsmarkörer. En månad efter PTRÄ hade vissa av de markörerna sjunkit till en lägre nivå än innan ballongvidgning tydande på en gynnsam effekt av PTRÄ. Vi fann också att patienter med NAS har en särskilt farlig rubbning i blodfetterna trots behandling med kolesterolsänkande läkemedel. Candesartan påverkade inte blodfetsbildningen under 11 månaders behandling efter PTRÄ. Vår studie visade att BNP var signifikant förhöjt hos patienter med NAS jämfört med friska kontroller, men inte påverkades av PTRÄ. Studien ger inget stöd för användning av BNP-analyser för att identifiera patienter som kommer att ha nytta av PTRÄ. Vår hypotes var att candesartan kunde ha njurskyddande och antiinflammatoriska effekter. Resultaten visade att candesartan inte påverkade njurfunktion, eller inflammatoriska markörer under 35 månaders behandlingstid hos patienter med NAS, som genomgått PTRÄ. Candesartan tolererades dock väl utan njurbiverkningar.

Sammanfattningsvis visade vår studie att patienter med NAS har ökad inflammation jämfört med friska individer samt en särskilt farlig blodfetsbild trots statinbehandling. Dessa avvikelser skulle kunna bidra till snabb utveckling av åderförkalkning och en accelererad hjärt-kärlsjukdom. Det krävs fler studier med ett större antal patienter samt längre uppföljningstid för att undersöka vilka kliniska konsekvenser dessa rubbningar kan ha. Vår studie visar att PTRÄ kan ha gynnsam effekt på inflammation även om ingreppet ger upphov till en övergående inflammatorisk svar. BNP kan inte användas för att förutsäga vilka patienter som har klinisk nytta av PTRÄ. Hos patienter med NAS som genomgått PTRÄ kunde vi inte påvisa någon antiinflammatorisk effekt av candesartan, men den var väl tolererad utan njurbiverkningar och kan användas med säkerhet hos denna patientgrupp.

LIST OF PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals.

- I. Renal angioplasty causes a rapid transient increase in inflammatory biomarkers, but reduced levels of interleukin-6 and endothelin-1 1 month after intervention.**
Alaa Alhadad, Gregor Guron, Elzbieta Nowakowska-Fortuna, Aso Saeed, Ingrid Mattiasson, Gert Jensen, Bengt Lindblad, Anders Gottsäter and Hans Herlitz.
Journal of Hypertension 2007 Sep;25(9):1907-14.
- II. Lipoprotein abnormalities in patients with atherosclerotic renovascular disease.**
Elzbieta Nowakowska-Fortuna, Hans Herlitz, Aso Saeed, Per-Ola Attman, Gert Jensen, Petar Alaupovic and Gregor Guron.
Kidney Blood Pressure Research 2011; 34(5):311-9;
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- III. Brain natriuretic peptides in atherosclerotic renal artery stenosis and effects of renal angioplasty.**
Elzbieta Nowakowska-Fortuna, Aso Saeed, Gregor Guron, Michael Fu, Ola Hammarsten, Gert Jensen and Hans Herlitz.
Kidney Blood Pressure Research 2013;37(6):657-66;
doi:10.1159/000355746.
- IV. Effects of candesartan on kidney function and inflammatory biomarkers in hypertensive patients subjected to renal angioplasty of atherosclerotic renal artery stenosis.**
Elzbieta Nowakowska-Fortuna, Aso Saeed, Gregor Guron, Gert Jensen, Anders Gottsäter and Hans Herlitz.
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ABBREVIATIONS

ACE	angiotensin converting enzyme
ACEI	angiotensin converting enzyme inhibitor
ABP	ambulatory blood pressure
ADBP	ambulatory diastolic blood pressure
Ang II	angiotensin II
AP	arterial pressure
APO	apolipoprotein
ARAS	atherosclerotic renal artery stenosis
ARB	angiotensin receptor blocker
ARVD	atherosclerotic renovascular disease
ASA	acetylsalicylic acid
ASBP	ambulatory systolic blood pressure
BMI	body mass index
BNP	brain natriuretic peptide
BP	blood pressure
CD40L	CD40 ligand
CDS	color duplex sonography
CKD	chronic kidney disease
CTA	computed tomography angiography
CV	cardiovascular
CVD	cardiovascular disease
DBP	diastolic blood pressure
eGFR	estimated glomerular filtration rate
ESRD	end-stage renal disease
ET	endothelin
FMD	fibromuscular dysplasia
GFR	glomerular filtration rate
HDL-C	high-density lipoprotein-cholesterol
hs-CRP	high-sensitivity C-reactive protein
IL	interleukin
LDL-C	low-density lipoprotein-cholesterol
MAPG	mean arterial pressure gradient
MRA	magnetic resonance angiography
NT-proBNP	N-terminal proBNP
PP	pulse pressure
PPAR	peroxisome proliferator-activated receptor
PRA	plasma renin activity
PTRA	percutaneous transluminal renal angioplasty
RAS	renal artery stenosis
RAAS	renin-angiotensin-aldosterone system

RVH	renovascular hypertension
RI	resistive index
SBP	systolic blood pressure
TC	total cholesterol
TG	triglycerides
TNF- α	tumor necrosis factor-alfa
UAE	urinary albumin excretion
VLDL-C	very-low-density lipoprotein-cholesterol

1 INTRODUCTION

1.1 The kidneys

The kidneys exert a variety of homeostatic functions through their capacity to regulate the content of water and electrolytes in the body fluids. The kidneys also remove waste products, maintain acid base balance, regulate blood pressure and synthesize hormones such as erythropoietin, active vitamin D and renin. The present thesis focuses on the role of the kidneys in blood pressure regulation.

1.1.1 Renin-angiotensin-aldosterone system in blood pressure regulation

The kidneys control blood pressure (BP) through multiple mechanisms. Already in 1898 Tigerstedt and Bergman [1] described the occurrence of renin, a BP-raising substance from rabbits renal cortex. The role of renal artery stenosis in the development of renovascular hypertension was clarified by Goldblatt in 1934 [2] who demonstrated that reduced perfusion of the kidney produced a sustained elevation of BP. Later work identified activation of the renin-angiotensin-aldosterone system (RAAS) as a central component of this process [3, 4]. Reduced pressure in afferent renal arterioles is sensed by juxtaglomerular cells resulting in the release of the enzyme renin [5]. Renin then acts upon circulating angiotensinogen to produce angiotensin I which is subsequently converted to angiotensin II (Ang II) by the action of angiotensin converting enzyme (ACE). Renin release is considered to be the rate-limiting step in the RAAS cascade. Angiotensin II, the main effector peptide of the RAAS, raises BP by multiple mechanisms. For instance, Ang II causes arterial vasoconstriction and increases total peripheral resistance, stimulates secretion of aldosterone from the adrenal glands, potentiates the sympathetic nervous system, enhances thirst, and triggers release of antidiuretic hormone [6]. Angiotensin II also blunts the pressure-natriuretic response to elevated BP.

1.2 Renovascular hypertension

Hypertension is defined as BP $\geq 140/90$ mmHg. Hypertension is an important risk factor for cardiovascular disease, stroke and renal failure. Renovascular hypertension (RVH) is defined as hypertension induced by renal artery

stenosis (RAS) which causes decreased renal perfusion and an activated RAAS.

1.2.1 Epidemiology of renal artery stenosis

Renovascular hypertension is one of the most common forms of secondary hypertension and occurs in 1-5% of all patients with hypertension [7]. However, the true prevalence is hard to estimate given the asymptomatic nature of majority of cases. The prevalence may be considerably higher in specific patient groups. In resistant hypertension the prevalence may be 15-20% [8]. The prevalence increases with age [9] and is higher in patients with established cardiovascular diseases (CVD), e.g. coronary artery stenosis, congestive heart failure or peripheral vascular disease [10-16]. For example, the prevalence of renal artery stenosis in patients with coronary artery stenosis undergoing coronary angiography is 15-19% [10, 16]. No racial differences have been reported in the prevalence of RAS [17].

Etiology of RAS

There are two main causes of RAS: atherosclerosis and fibromuscular dysplasia (FMD). In Western populations, atherosclerosis is the leading cause of RAS, constituting up to 90% of the cases [18, 19]. The stenosis is typically located either in the ostium or in the proximal segment of the renal artery and may be unilateral or bilateral. The frequency increases after 50 years of age and males are more commonly affected than females. Atherosclerotic RAS (ARAS) is usually a manifestation of generalized atherosclerosis. In approximately 10% of cases, RAS is caused by FMD, which is more frequent in women and with a highest incidence in the age 25- to 50- years. The right renal artery is more commonly affected and the changes are located distally. Fibromuscular dysplasia may occur simultaneously in other vascular beds (e.g. carotid, vertebral, iliac, subclavian, visceral and coronary arteries) [7, 18, 19]. The etiology of FMD is unknown, but a number of factors have been suggested, such as genetic predisposition, hormonal influence, mechanical factors (stretching and trauma to the blood vessel wall), and ischemia of the vascular wall due to fibrotic occlusion of the vasa vasorum [18, 19]. Less frequent causes of RAS include vasculitis (e.g. Takayasu's arteritis), aortic dissection, renal artery aneurysm, arterio-venous fistula and antiphospholipid syndrome.

1.2.2 Pathophysiology of renovascular hypertension

Renin-angiotensin-aldosterone system

Renal artery stenosis is an anatomical narrowing of the artery lumen, but this does not in itself embrace the existence of its pathophysiological consequences i.e. hypertension and reduced renal function. For a stenosis to affect renal blood flow or perfusion pressure resulting in the release of the renin a reduction of at least 75% of the cross-sectional area of the vessel is required. This corresponds to a reduction of the blood vessels diameter by 50% [20, 21]. Some studies show that also a pressure gradient across the stenosis is required to cause renin release. This occurs when a pressure distal to the stenosis is reduced at least 10-20% below the pressure (AP) in aorta. This corresponds to a trans-stenotic mean arterial pressure gradient (MAPG) of at least 10-20 mmHg [20, 22].

In unilateral RAS, the contralateral non-stenotic kidney responds to the elevated BP by increasing sodium excretion (i.e. pressure-natriuresis). Hypertension in this condition is Ang II dependent. In bilateral RAS, or when a RAS is present to a solitary kidney, there is no non-stenotic kidney to excrete sodium and water in response to increased BP. Hypertension in this situation is due mainly to volume expansion, which finally leads to feedback inhibition of the RAAS. In this setting hypertension is considered Ang II independent [5].

1.2.3 The clinical manifestations of RAS

The RAS activates the RAAS resulting in hypertension and also often decreased glomerular filtration rate (GFR). RAS is a progressive disease and causes a spectrum of clinical syndromes ranging from asymptomatic lesion (“incidental RAS”), by symptomatic RAS (renovascular hypertension) to more advanced disease as ischemic nephropathy with decreased GFR and accelerated cardiovascular disease [5]. Many patients with ARAS have years of preexisting primary hypertension, active smoking histories and coexisting diabetes mellitus [16].

The deterioration of renal function in RAS

The pathophysiological mechanisms leading to decreased GFR in patients with RAS are multiple, complex and not clearly understood. The term “ischemic nephropathy” has been commonly used to describe the impairment in renal function beyond an occlusive disease of the main renal artery [5, 23]. It is defined as an obstruction of renal blood flow that leads to ischemia and excretory dysfunction. Histologically, it is characterized by arteriolar

nephrosclerosis, collapsed glomeruli and interstitial fibrosis, therefore the picture is unspecific [23]. The injury of the stenotic kidney may be induced by the activation of vasopressor systems such as the RAAS and ET-1, in addition to a direct damaging effect of hypoxia [5, 23]. The pre-existing long-term primary hypertension in this patient group may also contribute to renal injury. Interestingly, FMD rarely results in impaired renal function despite similar degrees of stenosis compared with ARAS. This observation suggests that atherosclerosis and comorbidity play an important role in kidney damage in RVH [23].

When to suspect RAS in the hypertensive patient?

The typical clues to suggest the diagnosis RVH include: 1) treatment resistant hypertension (>140/90 mmHg) despite 3 or more antihypertensive drugs in the maximum dose, 2) treatment failure, accelerated (previously well-controlled hypertension, which suddenly becomes difficult to treat), or malignant hypertension, 3) severe hypertension in a young individual (<30 years in males and <50 years in women), 4) severe hypertension and progressive deterioration of renal function of unknown origin, 5) hypertension associated with reduced renal function or worsening of renal function during treatment with RAAS-blockers, 4) unexplained asymmetry in renal size, or 5) recurrent pulmonary edema associated with hypertension [18].

1.2.4 Screening methods

The first-hand screening tests for RAS are color duplex sonography (CDS), magnetic resonance angiography (MRA) and computed tomography angiography (CTA). These methods have similar sensitivity and specificity. The choice of method can be determined in part from local competence and tradition.

Color duplex sonography

Doppler ultrasound is a safe, inexpensive non-invasive screening test for RAS and provides high sensitivity and specificity (80-95%) in experienced laboratories [24]. The other advantage of this method is that patients avoid exposure to radiation or contrast and can be used in patients with renal failure. This method can provide reliable hemodynamic assessment of arterial stenosis [25], which is important to know before making a decision about revascularization. The limitation of this approach is that its diagnostic accuracy depends on the investigator's experience and the patient's body characteristics. Using this method blood flow is tested at the renal hilum and in the intraparenchymal renal arteries. Furthermore, measurement of

intrarenal resistive index (RI) can indicate small vessels disease. Rademacher et al suggested a RI of ≥ 0.80 as a negative predictor for clinical outcome after revascularization since a RI of ≥ 0.80 associated with reduced likelihood of improved BP or renal function by PTRAs [26]. However, these results were not confirmed in other studies [27].

Magnetic resonance angiography

Magnetic resonance angiography with gadolinium contrast provides good morphological information about the aorta and other abdominal blood vessels including renal arteries. It has a high sensitivity (around 95%) and specificity (around 90%) for detecting proximal RAS, but lower sensitivity in detecting stenotic lesion in the middle and distal part of renal artery. Therefore, MRA is a very useful method for screening atherosclerotic RAS but has limited value in diagnosing FMD [5, 24]. The limitation of this method is the risk of nephrogenic systemic fibrosis caused by gadolinium-based contrast agents in patients with severe renal failure. Furthermore, MRA may overestimate the degree of stenosis.

Computed tomography angiography

Computed tomography angiography provides similar diagnostic accuracy as MRA with the same sensitivity and specificity [5, 24]. It is a better method to follow up patients with stent. Limitations include exposure to the radiation and the risk of contrast nephropathy with reduced renal function.

ACE inhibitor renography

It is a functional screening test, which is performed by intravenously injected isotope, which is excreted by glomerular filtration and/or tubular secretion. Renal uptake of the isotope is recorded by a gamma camera. The procedure is performed before and after an oral dose of the angiotensin converting enzyme inhibitor (ACEI) captopril. If the side difference between renal uptake is more pronounced after ACEI, a significant RAS is considered to be present. The sensitivity and specificity of ACEI renography is in the range of 85% [24]. However, this procedure is much less sensitive and specific in patients with bilateral RAS, impaired renal function, urinary obstruction, and chronic intake of ACEI [24] and therefore is less suitable for this patient group. This method is not recommended as the first-hand test.

The goal of the further investigation should be an aim at receiving the appropriate decision concerning whether the patient in question should be subject to revascularization or not. If the initial screening was performed with a CDS it is wise to complement this investigation with a CTA or a MRA-examination to delineate the vascular anatomy before the renal angiography

is done. Conversely, it is valuable to complement a CTA or a MRA-examination with a CDS investigation to prove that the stenosis is hemodynamically significant.

1.2.5 Digital subtraction angiography

Renal angiography (digital subtraction angiography) is the gold standard in diagnosis of RAS. This method gives a good morphological assessment and by measuring the trans-stenotic arterial pressure gradient it is also possible to assess the hemodynamic impact of the stenosis [28]. A trans-stenotic pressure gradient of at least 20 mmHg in systolic AP, or at least 10 mmHg in mean arterial pressure (MAPG), has been used to determine whether a stenosis is hemodynamically significant [20, 22]. The advantage of this method is the ability to perform a therapeutic intervention by percutaneous transluminal renal angioplasty (PTRA) during the same séance. Some data indicate that a MAPG \geq 10 mmHg is able to predict those patients with RAS responding to PTRA with reduced BP or a reduced need for antihypertensive drugs [29]. This method is the gold standard, but is expensive and its use is limited by the invasive nature of the procedure. It is also associated with some risks and the potential adverse effect of intravenous contrast.

1.2.6 Management of renovascular hypertension

Management of RVH involves both an invasive treatment as PTRA and a non-invasive medical therapy depending on etiology and degree of comorbidity.

Treatment of fibromuscular dysplasia of the renal arteries

The primary treatment of patients with RVH caused by FMD is PTRA, since a meta-analysis has shown that almost half of the patients are cured by the procedure and the majority of the remaining patients obtain an improved AP control [30].

Treatment of atherosclerotic renal artery stenosis

The management of atherosclerotic RAS in patients with hypertension or impaired renal function remains a clinical dilemma. It is still unclear if restoring vessel patency by PTRA in ARAS improves outcomes in these patients [18]. Our study was initiated in 2003, i.e. before the publication of two large randomized clinical trials the ASTRAL [31] and the CORAL [32]. These studies have been performed in patients with ARAS, where PTRA plus medical treatment has been compared to medical treatment only. Variables in

the studies have been changes in BP and renal function and a combination of renal and cardiovascular outcomes. The results of these studies show that PTRAs are not advantageous over medical treatment. In addition, PTRAs procedure was associated with substantial serious complications. The conclusion of these studies was that the majority of patients with ARAS should not be further investigated and revascularized but be subjected to aggressive medical treatment. Maybe revascularization could be indicated in special groups of ARAS patients.

Medical management

Aggressive antihypertensive treatment plays an elemental role in medical management of all patients with RVH independent of the patient will be a candidate for revascularization or not. Previously, RAAS blockade in patients with RVH was considered contraindicated due to fear of inducing renal ischemia [33, 34]. At present, pharmacological blockade of the RAAS with either ACEI or ARB (angiotensin II receptor antagonist) is considered a first-line therapy in RVH to counteract activation of RAAS [35-38]. Patients with RVH often have other indications for ACEI and/or ARB treatment in addition to hypertension, as diabetes, congestive heart failure, or high cardiovascular risk. Clinical data show that RAAS blockers may reduce the risk of cardiovascular events and reduce mortality in ARAS patients and can be used in this patient group without risks [35-37]. However, it needs to be remembered, that these agents may accelerate the damage to the stenotic kidney. Therefore RAAS blockade should not be used in patients with bilateral RAS or stenosis of a solitary kidney. However, these patients may be considered for revascularization [5, 18].

Atherosclerotic RAS represents a clinical manifestation of atherosclerotic disease, and is often associated with hyperlipidemia and smoking. Therefore, the use of cholesterol lowering therapy by statins, antiplatelet agents and life style modifications such as smoking cessation, reduced dietary intake of salt and increased exercise are paramount to reduce these risks [39].

Renal revascularization

Interventional treatment of renal artery stenosis includes PTRAs with or without stent placement and in some rare cases surgery. Endovascular therapy became implemented in 1978 [40] and has developed a lot since then and has replaced surgery [41]. It has been shown that PTRAs with stenting are more efficacious than PTRAs without stent regarding restenosis and improved technical success [42].

1.3 Cardiovascular morbidity in patient with atherosclerotic renal artery stenosis

The prognosis of RVH is much worse than for patients with primary hypertension, and is associated with high cardiovascular morbidity and mortality [43, 44]. The mortality in this patient group is increased six-fold compared to an age-matched population [43]. Renal artery stenosis is a common manifestation of atherosclerosis and is frequently associated with other atherosclerotic diseases such as coronary artery disease, cerebrovascular disease, and peripheral vascular disease [35, 45-53]. In addition, the presence of RAS is a strong independent predictor of mortality, and increasing severity of RAS has an incremental effect on mortality in patients undergoing coronary angiography [54].

Renal artery stenosis not only gives rise to activation of RAAS resulting in hypertension, but also to a decrease in renal function [55, 56]. Both hypertension, activation of the RAAS, and renal insufficiency are known cardiovascular risk factors [52, 57, 58] (Figure 1).

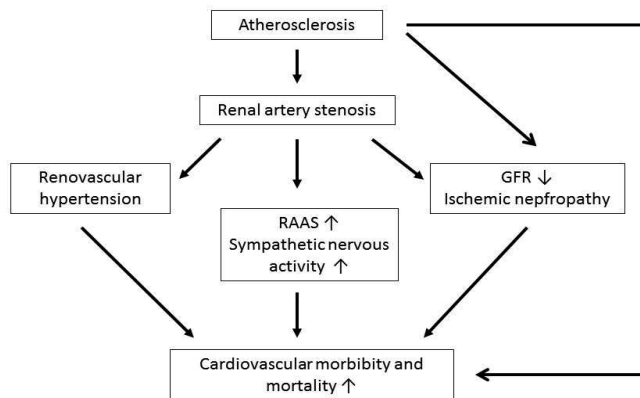


Figure 1. The clinical manifestations of renal artery stenosis. Abbreviations are: GFR, glomerular filtration rate; RAAS, renin-angiotensin-aldosterone system.

The reason for the increased cardiovascular (CV) morbidity and mortality in patients with renovascular hypertension is not clearly understood. It is reasonable to speculate that the high CV risk in this patient group is most

likely multifactorial and the other additional, non-traditional risk factors may contribute to the high CV morbidity and mortality in this patient group.

Angiotensin II may contribute to hypertension and end-organ damage by triggering a number of downstream effector pathways [5]. These proposed mechanisms include increased inflammation and oxidative stress, enhanced endothelin 1 (ET-1) production, and arterial wall re-modeling [5] (Figure 2). In addition, Ang II has been shown to increase the oxidation of low-density lipoprotein cholesterol, metallo-proteinase production, and lipid peroxidation [59-61].

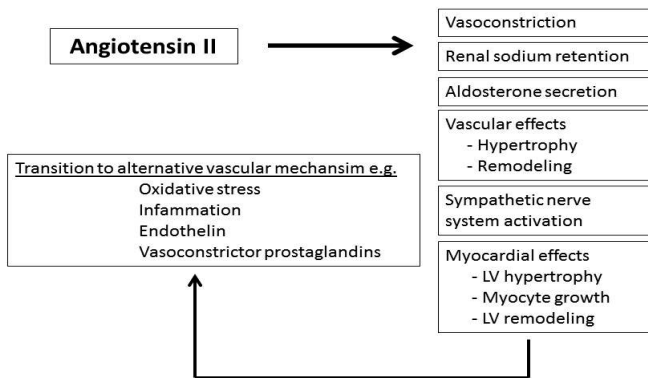


Figure 2. Schematic illustration of pressor mechanisms identified in renovascular hypertension. Abbreviations are : LV, left ventricle; (Modified from: Garovic VD et al. Renovascular hypertension and ischemic nephropathy. *Circulation* 2005; 112:1362-74).

1.3.1 Additional mechanisms in renovascular hypertension

Endothelin-1

Angiotensin II stimulates the synthesis of ET-1, a potent vasoconstrictor and pressor peptide produced by vascular endothelial cells [62] which is involved in the initiation and progression of atherosclerosis [63]. The biological actions of ET-1 are mediated via two receptors: type A (ETA) and type B

(ET_B) [64]. In the kidney, ET-1 exerts direct effects on tubular epithelial cells by ET_A and/or ET_B receptors and regulates sodium and water reabsorption [65]. In addition, ET-1 influences salt and water homeostasis also through its effects on the RAAS, vasopressin and atrial natriuretic peptide and stimulates the sympathetic nervous system [66]. The physiological actions of ET-1 relevant to cardiovascular disease take place in various organs, such as: the systemic vascular bed, the pulmonary vascular bed, the heart, the kidney, and the endocrine system [66].

Inflammation

At the molecular and cellular levels, Ang II stimulates key components of atherosclerosis [67]. Atherosclerosis is a chronic inflammatory disease, which involves vascular cells, immune system, and several organs [68]. The RAAS serves an important role in promoting inflammation [67, 69]. Impairment of the endothelium is the first physiological alteration in the pathophysiology of atherosclerosis which is manifested by enhanced vascular constriction triggered by Ang II and endothelin. Furthermore, Ang II induces the production of reactive oxygen species, inflammatory cytokines, and adhesion molecules [70]. Therefore, inflammatory processes are manifested by increased biosynthesis of mediators of inflammation and thrombosis [71]. Several inflammatory mediators such as tumor necrosis factor- α (TNF- α) [72], neopterin [73], interleukin-6 (IL-6) [74], and CD40 ligand (CD40L) [75, 76] are involved in atherogenesis. In particular, increased levels of IL-6 and high-sensitivity C-reactive protein (hs-CRP) have been shown to predict cardiovascular disease [77].

Dyslipidemia

In patients with ARAS, dyslipidemia could be a primary event leading to the development of peripheral stenotic lesions involving renal arteries. However, dyslipidemia can also develop as a consequence of reduced GFR (i.e. renal dyslipidemia) [78] and may thus be superimposed on atherosclerotic renovascular disease (ARVD). Notably, renal dyslipidemia is not always reflected in hyperlipidemia but in altered concentrations of individual lipoprotein subclasses classified according to their apolipoprotein (Apo) composition [79-81]. Renal dyslipidemia is characterized by the accumulation of atherogenic ApoB- and ApoC-containing lipoproteins [80, 81] and could hence add to pre-existing perturbations of lipoprotein metabolism. The classification system of lipoproteins recognizes two classes, one of which is characterized by ApoA and the other by ApoB as the major lipoprotein constituents (Figure 3). The former lipoprotein class consists of two major lipoprotein subclasses, lipoprotein A-I (LpA-I) and lipoprotein A-I:A-II (LpA-I:A-II), whereas the latter encompasses five major subclasses

called lipoprotein B (LpB), lipoprotein B:E (LpB:E), lipoprotein B:C (LpB:C), lipoprotein B:C:E (LpB:C:E), and lipoprotein A-II:B:C:D:E (LpAII:B:C:D:E) [79, 82]. In addition to their unique apolipoprotein composition, each of these lipoprotein subclasses has specific metabolic and functional properties [79, 82]. Furthermore, previous studies have indicated that ApoB-containing lipoprotein subclasses may differ in their atherogenic capacities [79, 83, 84] and ApoA-containing lipoproteins in their antiatherogenic potentials [79, 85-87]. While lipoprotein subclasses have been analysed in detail in patients with chronic kidney disease (CKD) [80, 81, 88, 89], such analyses have to our knowledge not been carried out in patients with ARAS.

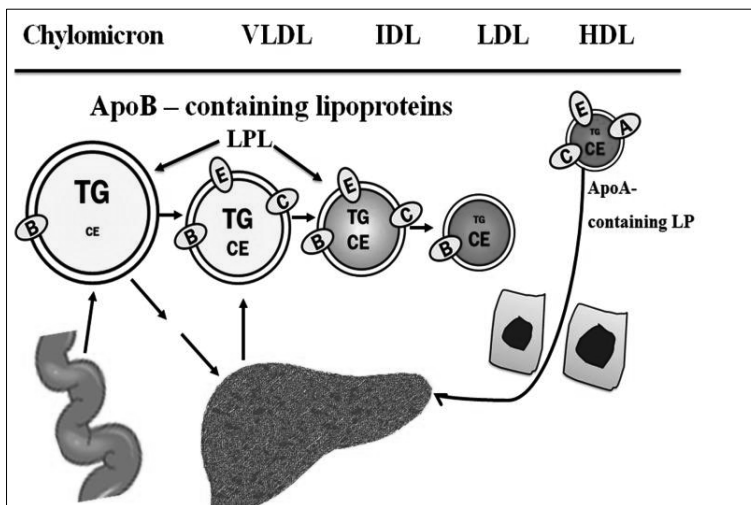


Figure 3. The normal lipoprotein metabolism and the concept of lipoprotein particles. A lipoprotein particle consists of a core of lipids, predominantly triglycerides and cholesterol esters. On the surface, the particles have proteins of specific types, apolipoproteins with different functions. These apolipoproteins determine the metabolic function of the individual particle. TG, triglycerides; CE, cholesterol ester; A, apolipoprotein A; B, apolipoprotein B; C, apolipoprotein C; E, apolipoprotein E. (Modified from: Per-Ola Attman and Ola Samuelsson. *Dyslipidemia of kidney disease. Current Opinion in Lipidology* 2009,20:293-9).

Brain natriuretic peptide in ARAS

Brain natriuretic peptide (BNP) belongs to the vasoactive peptides that are synthesized, stored, and secreted from the ventricular myocytes in response to atrial or ventricular stretch [90] in the setting of volume expansion or pressure overload and neurohormonal activation [91]. Brain natriuretic

peptide is produced as prohormone (pro-BNP) which is cleaved to two parts during secretion: (1) the active hormone BNP and (2) the inactive hormone with the N-terminal part NT-proBNP. Both BNP and NT-proBNP are secreted in equimolar amounts during hemodynamic and neurohormonal stress of the heart [92]. Because of their close correlation with the severity of symptoms they have been developed as markers of heart failure [93, 94]. The elimination of these two parts differs. Serum half-life of BNP is 20 minutes [95], while the corresponding value for NT-proBNP is 120 minutes. Both BNP and NT-proBNP plasma concentrations increase in renal failure. Clearance of NT-proBNP is likely more dependent on glomerular filtration and increases more than BNP in response to renal failure [96, 97]. The most important physiological actions of BNP take place in the kidney where it exerts natriuretic and diuretic effects, causes renal vasodilation, increases glomerular filtration rate and inhibits renin release [98]. Hence, BNP counteracts the RAAS. In addition, in vitro data have demonstrated that Ang II may directly induce the synthesis and release of BNP [99]. Hence, plasma BNP may be increased in patients with RVH at least partly due to an enhanced activity of the RAAS [99-101].

Adiponectin in ARAS

Overweight and obesity lead to adverse effects on AP, lipids and insulin resistance and a great part of cardiovascular disease could be accounted for this [102]. The adipose tissue is not a passive energy depot, but in fact an active endocrine organ secreting a variety of hormones, i.e. adipokines. Dysregulation of these adipokines is believed to be important for the development in cardiovascular disease in obesity [103]. One of these adipokines, adiponectin was identified as a 244 amino acid protein in 1996 [104]. It is highly expressed and actively secreted by adipocytes and is present in human plasma [104]. Adiponectin has a variety of protective functions and is believed to exert anti-inflammatory, anti-atherogenic, and anti-diabetogenic effects [105]. It is reasonable to speculate that patients with ARAS may have reduced plasma levels of adiponectin that could contribute to the increased cardiovascular risk.

2 AIMS

The overall aim of this study was to increase our knowledge about the pathophysiology of RVH and to identify novel treatment targets that could reduce cardiovascular risk in this patient group.

The specific aims of the papers included in this thesis were:

Paper I

To examine whether systemic inflammation and ET-1 are increased in hypertensive patients with RAS and to evaluate how treatment with PTRAs affected these variables

Paper II

To investigate lipoprotein abnormalities in patients with ARVD who had undergone PTRAs and to analyze whether Ang II receptor antagonism influenced lipoprotein levels

Paper III

To evaluate whether plasma levels of BNP are increased in patients with ARAS and may predict a favorable outcome of PTRAs

Paper IV

To analyze the long-term effects of Ang II receptor antagonism on kidney function and inflammatory biomarkers in patients with ARVD and residual hypertension after PTRAs

3 PATIENTS AND METHODS

3.1 Patients

All patients were recruited from the (Candesartan in Renal Artery Stenosis-CARLAS) study program, a randomized, open, investigator-initiated trial in which the effects of the ARB, candesartan, was examined in patients with ARVD who had undergone treatment with PTRA with or without stenting. This study was carried out at two Swedish centers (Department of Nephrology at Sahlgrenska University Hospital in Gothenburg, and Department of Vascular Diseases at Malmö University Hospital). Between 2003 and 2008, 178 patients at these centers, undergoing renal angiography for suspected RAS were considered for this study.

Indications for renal angiography were hypertension (accelerated, refractory, malignant or with intolerance to medication), or a progressive increase in plasma creatinine concentrations (unexplained or during treatment with an ACEI or ARB, or recurrent hypertensive pulmonary oedema, together with a positive screening test for RAS as (a) duplex ultrasonography showing delta-resistive index (RI) >0.05 with lower RI at the stenotic side, or systolic pulse acceleration $<2.3\text{m/s}^2$, or (b) CT-angiography, or MR-angiography indicating $\geq 50\%$ diameter stenosis. The exclusion criteria are shown in Table 1.

Table 1. Exclusion criteria

Renal size < 7.5 cm at the stenotic side
Age > 80 years
Pregnancy or nursing
CKD stage 5 (eGFR $< 15\text{ml/min/1.73m}^2$)
RAS of other etiology than atherosclerosis
Urinary albumin excretion $> 1\text{g/day}$
Diabetes mellitus with urinary albumin excretion $> 0.3\text{g/day}$
Congestive heart failure
Strong indicated treatment with ACEI and/or ARB and/or aldosterone receptor antagonist
Contraindication for renal angiography/angioplasty (e.g. serious contrast allergy)
Other form of secondary hypertension
Malignant disease
Treatment with immune modulating drugs, e.g. cyclosporine or oral steroids

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate according to the 4-variable equation from the Modification of Diet in Renal Disease (MDRD) study; RAS, renal artery stenosis; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

A significant RAS was defined as a lesion with a trans-stenotic MAPG of at least 10 mmHg, or >50 % diameter stenosis on angiography in those cases in which the MAPG was not measured because of technical difficulties due to high-grade stenosis and luminal occlusion during the procedure. One hundred and eight patients with significant RAS underwent angioplasty, whereas 70 patients had no significant RAS and were therefore only subjected to the diagnostic procedure.

Sixty-three patients with residual hypertension (office BP >130/80 mmHg) four weeks after PTRAs underwent randomization to antihypertensive treatments based on either the ARB candesartan (ARVD-CAN, n=33) or a regimen without direct inhibitors of the RAAS (ARVD-C, n=30), i.e. ACE inhibitors, ARBs, renin inhibitors or aldosterone receptor antagonists. The randomization procedure was carried out by the use of sequentially numbered, opaque sealed envelopes. The targeted candesartan dose was 16 mg daily and the trough BP goal was <140/90 mmHg. All patients had discontinued treatment with direct inhibitors of the RAAS and had been on HMG-CoA reductase inhibitor (i.e. statin) therapy for at least two weeks prior to PTRAs. One-two days before angiography all patients who had no antiplatelet therapy were started on either acetylsalicylic acid (ASA) or clopidogrel. The patients were followed up for 3 years after PTRAs (see study flow chart in figure 4).

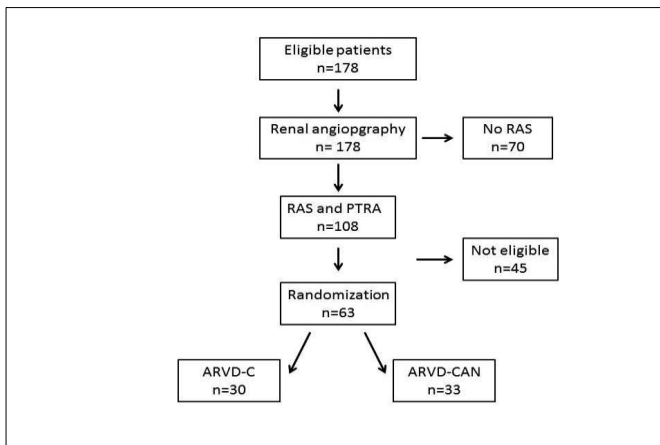


Figure 4. Study flow chart. Eligible individuals were patients with a clinical suspicion of atherosclerotic renal artery stenosis (RAS) together with a positive diagnostic screening test (see above). Abbreviations are: PTRAs, percutaneous transluminal renal angioplasty; ARVD, atherosclerotic renovascular disease; ARVD-C, control group; ARVD-CAN, candesartan group.

In this thesis patient data from both centers were included in paper I. In papers II-IV only patients from Sahlgrenska University Hospital were included as the number of patients randomized in Malmö was small.

3.1.1 Patients and protocol paper I

In this study 100 consecutive patients that fulfilled the inclusion criteria underwent renal angiography. 61 patients had significant RAS and underwent PTRAs, whereas 39 patients had no significant RAS and were only subjected to the diagnostic procedure. A population-based control group of 219 healthy individuals (median age 68 years, 117 women and 102 men) from a follow-up program for the Preventive medicine project in Malmö [106], without symptomatic cardiovascular disease or hypertension, was analyzed for comparison of inflammatory biomarkers. Office BP measurements, routine laboratory markers and plasma levels of inflammatory biomarkers and ET-1 were measured immediately before, and 1 day and 4 weeks after PTRAs.

3.1.2 Patients and protocol paper II

In this study we investigated abnormalities in a subgroup of 42 ARVD patients at the time of randomization 4 weeks after PTRAs. These patients were selected as they were the only participants with a sufficient amount of appropriately collected and stored plasma for lipid analyses. All patients had been on treatment with statins at least 6 weeks before randomization. This treatment was maintained unaltered throughout the study period. Following randomization to group ARVD-CAN (n=21) or group ARVD-C (n=21), patients were followed for 11 months at which time point new lipid analyses were carried out. Lipid analyses were performed in 32 of 42 patients at 11 months (16 patients from each group) due to 3 deaths, 2 individuals ended their participation, and in 5 patients there was an insufficient amount of plasma. In addition, 20 age-matched healthy subjects from the general population without any medications were examined at one time point and served as controls.

3.1.3 Patients and protocol paper III

Ninety-one patients with hypertension and suspected RAS were included in this study and underwent renal angiography. Angioplasty was carried out on 47 patients with significant atherosclerotic stenosis (ARAS-group). Forty-four individuals had no significant RAS and were subjected only to diagnostic angiography (non-RAS group). All patients were subjected to baseline measurements one day before angiography. Routine laboratory analyses, BNP, NT-proBNP and adiponectin were measured immediately

before renal angiography in all patients. In patients that were subjected to PTRA, analyses were repeated 4 weeks after intervention (except for adiponectin). Office BP was measured immediately before, 1 day after and 4 weeks after renal angiography. Ambulatory (24h) BP (ABP) was measured one day before angiography and 4 weeks after PTRA. The same healthy control group as in study II (n=20) was studied at one time-point and data were compared with baseline values from hypertensive patients.

3.1.4 Patients and protocol paper IV

All randomized patients from the Sahlgrenska University Hospital (n=48) were included in this study (24 patients in each group). Measurements were carried out at randomization and after 11 and 35 months. Analyses included office BP, ABP, inflammatory biomarkers, estimated glomerular filtration rate (eGFR) and renography for assessment of split kidney function. Thirty-eight patients completed the study (19 patients from each group). The study flow chart is shown in figure 5.

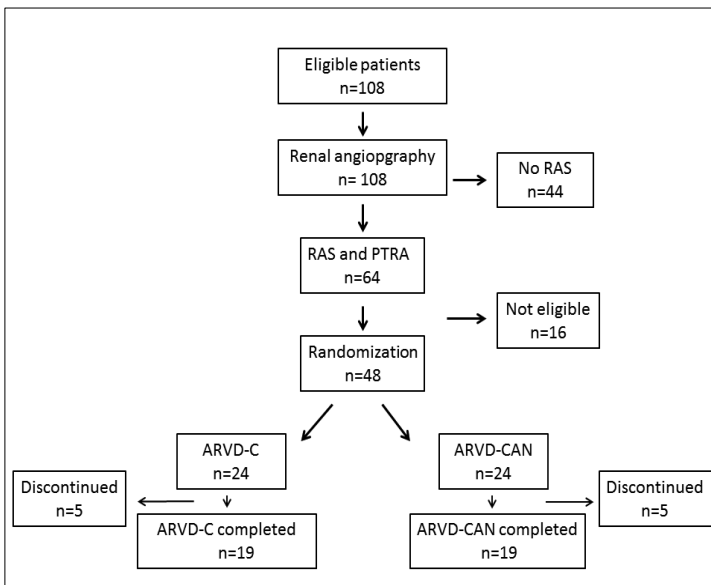


Figure 5. Study flow chart. Patients from Sahlgrenska University Hospital. Eligible individuals were patients with a clinical suspicion of atherosclerotic renal artery stenosis (RAS) together with a positive diagnostic screening test. Abbreviations are: PTRA, percutaneous transluminal renal angioplasty; ARVD, atherosclerotic renovascular disease; ARVD-C, control group; ARVD-CAN, candesartan group.

3.1.5 Ethical statement

The Ethics Committee of the University of Gothenburg and Lund approved the study and all participants gave their written consent to participate.

3.2 Measurements

Systolic (SBP) and diastolic (DBP) office BP were measured in the non-dominant arm, in a sitting position after 5 minutes rest in the morning before drug intake. Ambulatory SBP (ASBP) and DBP (ADBP) were measured for 24 h by an ambulatory BP device (Model 90217, Spacelabs Healthcare). ABP was measured every twenty minutes between 6 am and 10 pm and every sixty minutes between 10 pm and 6 am. Estimated GFR was calculated based on serum creatinine concentrations according to the 4-variable equation from the Modification of Diet in Renal Disease Study (MDRD) [107].

3.3 Biochemical analyses

3.3.1 Routine laboratory methods

Standard laboratory methods at the Departments of Clinical Chemistry at the Sahlgrenska University Hospital and the Malmö University Hospital (SWEDAC approved according to European norm 45001) were used for routine analyses. Plasma renin activity (PRA) was measured by a radioimmunoassay (RIA) kit (Dia Sorin, Stillwater, MN, USA), with inter- and intra-assay coefficients of variation (CV)s less than 10%. Plasma concentrations of Ang II (Euro-Diagnostica, Malmö, Sweden) were also measured by RIA.

3.3.2 Inflammatory biomarkers and endothelin-1

Serum high-sensitivity C-reactive protein (hs-CRP) was analysed by rate turbidimetry at the Department of Clinical Chemistry at Malmö University Hospital. The detection limit was 0.2 mg/l, and the inter assay coefficients of variation were 6% at 15 mg/l and 5% at 85 mg/l. All other inflammatory biomarkers and ET-1 were analysed at the Wallenberg Laboratory, Malmö University Hospital. ET-1 (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) was measured by RIA kits. The detection limit for ET-1 was 0.25 pg/ml, the intra-assay CV based on pooled samples was 11.3%, and the inter-assay CV was 22%. Plasma TNF α and IL-6 were measured by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Pharmingen, San Diego, California, USA).

Detection limits were 0.12 and 0.70 pg/ml respectively and the intra- and inter assay CV were 8.8 and 16.7% for TNF α and 4.2 and 6.4% for IL-6. P-neopterin was determined by ELISA (Henning, Berlin, Germany). The detection limit was 2 nmol/l, and the intra- and inter-assay CV were 1.7 and 8.2%, respectively. P-CD40L was analysed by an immunoassay using a commercially available kit (R&D Systems Inc, Minneapolis, USA). The detection limit was 2.1 pg/ml.

3.3.3 Lipoproteins

Venous blood for analyses of lipids, lipoproteins and apolipoproteins was collected into ethylenediaminetetraacetate-containing vacutainer tubes after an overnight fast and with individuals in the recumbent position. Plasma samples were recovered by low-speed centrifugation for 10 min at 4°C. A preservative solution (0.13 % *e*-aminocaproic acid and 0.1 % thiomersal) was added (10 μ l/ml) to all plasma samples and samples were frozen at -70°C until shipped on dry ice by air express mail to the Lipid and Lipoprotein Laboratory, Oklahoma Medical Research Foundation for analyses. Total cholesterol (TC), triglycerides (TG), and HDL-cholesterol (HDL-C) were determined by standardized enzymatic procedures as described previously [108]. Very-low-density lipoprotein-cholesterol (VLDL-C) was assumed to equal one-fifth of the plasma TG concentration, and LDL-cholesterol (LDL-C) levels were calculated by the procedure of Friedewald et al [109]. Measurements of ApoA-I, ApoB, ApoC-III, and ApoE and the quantification of ApoC-III bound to ApoA-containing (HDL) and ApoB-containing (VLDL+LDL) lipoproteins, performed on heparin Mn²⁺ supernates (ApoC-III heparin manganese supernate) and heparin Mn²⁺ precipitates (ApoC-III heparin manganese precipitate), were carried out as described previously [82, 108].

Plasma concentrations of LpA-I and LpA-I:A-II subclasses of high-density properties were determined by a differential electroimmunoassay [110]. Determination of plasma levels of individual ApoB-containing lipoprotein subclasses, LpB, LpB:C, LpB:C:E, and LpAII:B:C:D:E, was performed by sequential immunoaffinity chromatography of ApoB-containing lipoproteins as previously described [111]. Methods for analyses of plasma lipids, apolipoproteins and lipoprotein subclasses have been described elsewhere in detail [82, 111, 112].

3.3.4 BNP, NT-proBNP and adiponectin

Brain natriuretic peptide was analysed using the manual Shionoria BNP method (CIS Bio international, Gif-sur-Yvette, France) on freshly thawed

EDTA-plasma samples. The inter-sample CV was between 5.4 and 7.0 %. The EDTA-plasma for BNP testing was frozen at -70 C within 2 h after collection.

NT-proBNP was analysed in serum with the Roche Elecsys system on Modular E 2551 with a CV between 3.7 and 5.0 %. An ELISA kit from LINCO Research Inc. (St. Charles, MO, USA) was used for the quantification of plasma adiponectin (CV 8.55%).

3.4 Renal angiography and angioplasty

Digital subtraction angiography was used for evaluating renal arteries. A 6 French (F) vascular sheath was placed percutaneously in the femoral artery after 3000-5000 IU of heparin was administered, and the target renal artery was selectively engaged with a multipurpose introducer catheter. A 4 French catheter was used for measurements of intra-arterial pressure gradients. The diameter of stenosis was estimated manually in all cases. Indications for stent placement were angioplasty failure (elastic recoil or flow-limiting dissection resulting in >30% residual luminal narrowing, absence of antegrade flow, or significant residual MAPG), or restenosis.

3.5 Renography

Renographic examinations were performed on hydrated patients in the supine position using a large field gamma camera (APEX 415, Elscint, Israel). Ninety-six frames (64x64 pixels) of 10 seconds each were recorded after an intravenous bolus injection of 100 MBq⁹⁹Tc^m-DTPA. Time-activity curves for the regions of interest over the kidneys were created. Renograms were corrected for the extrarenal background signal and normalized for kidney area. Relative function was estimated by means of the uptake index as previously described [113].

3.6 Statistics

Analyses were performed using one-way analysis of variance (ANOVA). If data were not normally distributed, Kruskal-Wallis one-way ANOVA on ranks was used. Differences between groups were analyzed with unpaired t-test or the Mann-Whitney U test and the chi-square test for categorical data. Bonferroni corrections were made for multiple comparisons. Paired t-test or Wilcoxon signed rank test were used for within group analyses. The Pearson correlation (or Spearman correlation when data did not meet assumption

about normality) coefficient was used to evaluate correlations. In study II multiple linear regression analysis was used to examine the association at baseline between apolipoproteins or lipoproteins and demographic, clinical and laboratory variables. Variables that were significantly correlated were included in multiple regression models. All tests were two-tailed and P-values <0.05 were considered statistically significant. Data are presented as means \pm SD. Software SPSS 17.0 (SPSS Inc., Chicago, Illinois, USA) was used.

4 REVIEW OF RESULTS

The following is a brief overview of the main results found in each study.

4.1 Inflammatory biomarkers and ET-1 in patients with RAS and effect of renal angioplasty during the first month after intervention (paper I)

4.1.1 Baseline characteristics and biomarkers prior to angiography

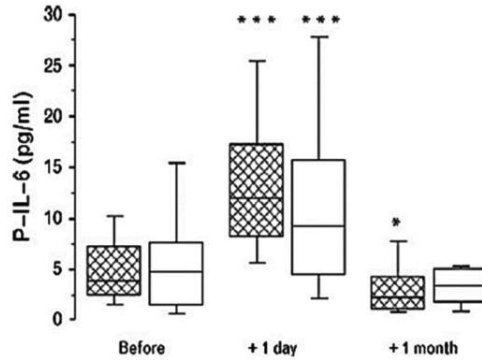
Patients with significant RAS had higher SBP at baseline ($p=0.030$) and were more often men ($p=0.024$) compared to patients without significant RAS.

There were no significant differences in inflammatory biomarkers or ET-1 between the RAS and non-RAS group. However, inflammatory biomarkers and ET-1 were significantly elevated in patients with significant RAS compared to healthy controls ($p<0.001$).

4.1.2 Effect of angioplasty on IL-6 and hs-CRP

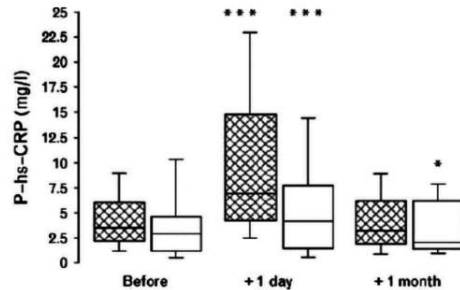
Interleukin-6 (Figure 6) and hs-CRP (Figure 7) had increased both in patients with significant RAS and in patients without significant RAS, who undergoing angiography only one day after angiography compared to baseline. At this time point IL-6 and hs-CRP were significantly elevated in the RAS group compared to patients subjected to angiography only.

One month after PTR, IL-6 levels had decreased significantly compared to before intervention (Figure 6).



P-interleukin (IL)-6 before and 1 day and 1 month after renal angiography in 61 patients undergoing percutaneous transluminal renal angioplasty (PTRA, shaded boxes) because of renal artery stenosis (RAS) and 39 patients undergoing diagnostic angiography only (white boxes). * $P < 0.05$, * $P < 0.001$ compared to before angiography.**

Figure 6. Effect of angioplasty on Interleukin-6 (IL-6) in patients with significant renal artery stenosis (RAS) vs. patients without significant RAS.

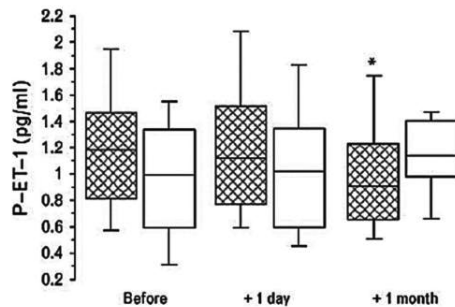


Plasma (P)-high-sensitivity C-reactive protein (hs-CRP) before and 1 day and 1 month after renal angiography in 61 patients undergoing percutaneous transluminal renal angioplasty (PTRA, shaded boxes) because of renal artery stenosis (RAS) and 39 patients undergoing diagnostic angiography only (white boxes). * $P < 0.05$, * $P < 0.001$ compared to before angiography.**

Figure 7. Effect of angioplasty in high-sensitivity C-reactive protein (hs-CRP) in patients with significant renal artery stenosis (RAS) vs. patients without significant RAS.

4.1.3 Effect of angioplasty on endothelin-1

One day after PTRAs plasma levels of ET-1 were not significantly different compared to before intervention. However, 1 month after PTRAs ET-1 levels had decreased significantly compared to before intervention (Figure 8).



P-endothelin (ET)-1 before and 1 day and 1 month after renal angioplasty in 61 patients undergoing percutaneous transluminal renal angioplasty (PTRAs, shaded boxes) because of renal artery stenosis (RAS) and 39 patients undergoing diagnostic angiography only (white boxes). * $P < 0.05$, compared to before angioplasty.

Figure 8. Effect of angioplasty in endothelin-1 (ET-1) in patients with significant renal artery stenosis (RAS) vs. patients without significant RAS.

4.2 Lipoproteins abnormalities in patients with atherosclerotic renovascular disease (paper II)

4.2.1 Patients characteristics at baseline

ASBP and ADBP, serum creatinine concentration, leukocyte count, fasting plasma glucose concentration, and urinary albumin excretion (UAE) were significantly elevated in patients with ARVD compared to controls ($p < 0.05$). In addition, eGFR was significantly reduced in ARVD patients compared to controls (59 ± 18 vs. 79 ± 14 ml/min/1.73m², $p < 0.05$).

4.2.2 Plasma lipids, apolipoproteins and lipoproteins at baseline

Lipids and lipoproteins (Table2)

Table 2. Lipids and lipoproteins at baseline in patients with ARVD treated with statins, and in age-matched healthy controls.

	ARVD (n=42)	Controls (n=20)
TC (mg/dl)	177±36*	217±36
HDL-C (mg/dl)	49±13*	65±14
LDL-C (mg/dl)	96±28*	130±38
VLDL-C (mg/dl)	32±14*	23±10
TG (mg/dl)	166±88*	116±49

TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; VLDL-C, very low-density lipoprotein-cholesterol; TG, triglycerides. Values are means±SD. * denotes $p<0.05$.

Apolipoproteins (Table 3)

Table 3. Plasma concentrations of apolipoproteins at baseline in patients with ARVD treated with statins, and in age-matched healthy controls

	ARAS (n=42)	Controls (n=20)
ApoA-I (mg/dl)	141±13	141±11
ApoB (mg/dl)	103±19	95±11
ApoA-I/ApoB	1.4±0.3	1.5±0.2
ApoC-III (mg/dl)	12.7±4.6*	8.8±2.6
ApoC-III-HS (mg/dl)	8.9±3.2*	5.3±1.8
ApoC-III-HP (mg/dl)	3.9±1.8*	3.0±1.2
ApoC-III-ratio	2.5±0.9	2.4±1.6
ApoA-I/ApoC-III	12.1±3.5*	17.2±4.6
ApoE (mg/dl)	8.2±2.3*	6.7±1.1

Apo, apolipoprotein; HS, heparin-manganese supernate; HP, heparin-manganese precipitate. The ApoC-III ration was calculated as ApoC-III-HS/ApoC-III-HP. Values are means±SD. * denotes $p<0.05$.

Lipoprotein subclasses (Table 4)

Table 4. Lipoprotein subclasses at baseline in patients with ARVD treated with statins, and in age-matched healthy controls.

	ARVD (n=42)	Controls (n=20)
LpA-I, mg/dl	35±4	33±2
LpA-I:A-II, mg/dl	107±10	108±9
LpB, mg/dl	57.0±7.8	57.7±4.5
LpB:C, mg/dl	11.1±4.1	9.5±4.1
LpB:C:E, mg/dl	13.3±5.4*	8.4±4.3
LpA-II:B:C:D:E, mg/dl	21.4±8.9	19.2±7.0
ApoC-III-containing Lp:s, mg/dl	46±15*	37±8

ApoC-III-containing Lp:s were calculated as LpB:C + LpB:C:E + LpA-II:B:C:D:E. Values are means±SD. * denotes $p < 0.05$.

4.2.3 Multiple regression analysis

As the most striking findings in this study were increase in ApoC-III, and ApoC-III-containing lipoproteins in patients with ARVD, we analysed which variables that were significantly correlated with ApoC-III. Only ASBP, ADBP, and PRA were significantly correlated to ApoC-III and these variables were included in a multiple regression analysis as predictors. In this model only PRA was significantly associated with ApoC-III ($p < 0.001$, $r = 0.74$, figure 9). Notably, ApoC-III was not correlated to age, BMI, fasting plasma glucose, serum creatinine, eGFR, UAE, leukocyte count or serum albumin levels.

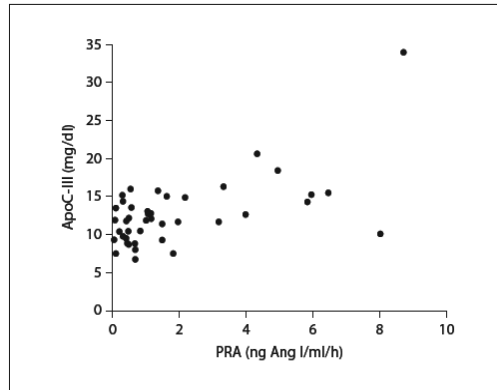


Figure 9. Correlation between PRA and ApoC-III in patients with ARVD ($n=42$) at baseline 4 weeks after PTR. In a multiple regression model, only PRA was significantly associated with ApoC-III ($p<0.001$, $r=0.74$). Abbreviations are: ApoC-III, apolipoprotein C-III; PRA, plasma renin activity; ARVD, atherosclerotic renovascular disease; PTR, percutaneous transluminal renal angioplasty.

4.2.4 Effects of candesartan on lipoproteins in ARVD patients

Analyses 11 months after randomization showed that candesartan treatment had no statistically significant effects on lipoproteins levels.

4.3 Brain natriuretic peptides in atherosclerotic renal artery stenosis and effects of renal angioplasty (paper III)

4.3.1 BNP, NT-proBNP and adiponectin at baseline

Plasma BNP and NT-proBNP concentrations were elevated in patients with significant ARAS compared to healthy controls (82 ± 99 vs. 28 ± 20 , $p<0.05$ for BNP and 521 ± 737 vs. 87 ± 55 , $p<0.05$ for NT-proBNP), but not in comparison to hypertensive individuals without RAS (82 ± 99 vs. 48 ± 68 for BNP and 521 ± 737 vs. 350 ± 808 for NT-proBNP). Plasma adiponectin was significantly

reduced in the ARAS group compared to controls (9.3 ± 7.5 vs. 12.0 ± 4.9 , $p < 0.05$), however, this difference did not reach statistical significance when patients with diabetes had been excluded from the analysis.

Correlation analyses at baseline

In patients with ARAS, baseline BNP and NT-proBNP levels were correlated significantly to age, ASBP, ambulatory PP, serum creatinine, eGFR and UAE (Table 5). However, in multiple regression analysis only serum creatinine levels showed a statistically significant relationship to both BNP and NT-proBNP ($r = 0.42$, $p < 0.05$ for BNP; and $r = 0.67$, $p < 0.001$ for NT-proBNP).

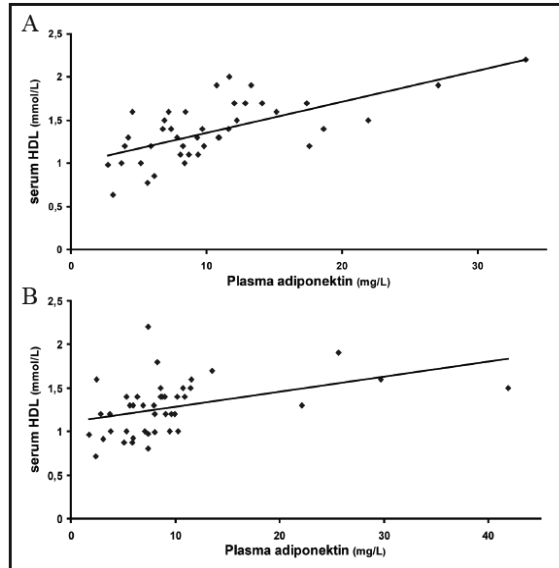
Table 5. Correlation between BNP, NT-proBNP, adiponectin and other variables at baseline in patients with significant atherosclerotic renal artery stenosis.

	S-BNP	S-NT-proBNP	P-Adiponectin
Age, years	$r = 0.506^{**}$	$r = 0.522^{**}$	ns
BMI, kg/m ²	ns	ns	$r = -0.340^*$
ASBP, mmHg	$r = 0.374^{**}$	$r = 0.520^*$	ns
ADBP, mmHg	ns	ns	$r = 0.308^*$
Ambulatory PP, mmHg	$r = 0.515^{**}$	$r = 0.640^{**}$	ns
S-creatinine, $\mu\text{mol/L}$	$r = 0.428^{**}$	$r = 0.476^{**}$	ns
eGFR, ml/min/1.73m ²	$r = -0.547^{**}$	$r = -0.644^{**}$	ns
UAE, mg/day	$r = 0.327^*$	$r = 0.365^*$	ns
S-HDL-C, mmol/L	ns	ns	$r = 0.538^*$

Statistically significant correlation coefficients are presented. BNP, brain natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; ASBP, ambulatory systolic blood pressure; ADBP, ambulatory diastolic blood pressure; PP, pulse pressure; eGFR, estimated glomerular filtration rate according to the 4-variable equation from the Modification of Diet in Renal Disease Study (MDRD); UAE, total 24-h urinary albumin excretion; S-HDL-C, serum high-density lipoprotein-cholesterol. * $p < 0.05$; ** $p < 0.01$.

Plasma adiponectin levels showed a significant correlation to BMI, ADBP and S-HDL-C (Table 5, figure 10B), but in multiple regression analysis only S-HDL-C showed a significant correlation. Notably, there was a statistically significant, and similar, correlation between plasma adiponectin and S-HDL-C also in the non-RAS group (Figure 10A).

Figure 10. Relationship between plasma adiponectin concentration and serum levels of high-density lipoprotein (HDL) cholesterol in patients undergoing renal angiography because of suspected atherosclerotic renal artery stenosis (RAS). Out of these, 41 patients had no significant RAS (A) and 43 patients had atherosclerotic renal artery stenosis (ARAS)(B). There was a statistically significant correlation between plasma adiponectin levels and S-HDL cholesterol in both groups ($p<0.01$).



4.3.2 Effects of PTRA on blood pressure, kidney function and biomarkers

ASBP, ADBP and ambulatory PP had decreased significantly 4 weeks after PTRA ($p<0.01$) (Figure 11). Serum creatinine levels and eGFR were not significantly affected by PTRA (Figure 11).

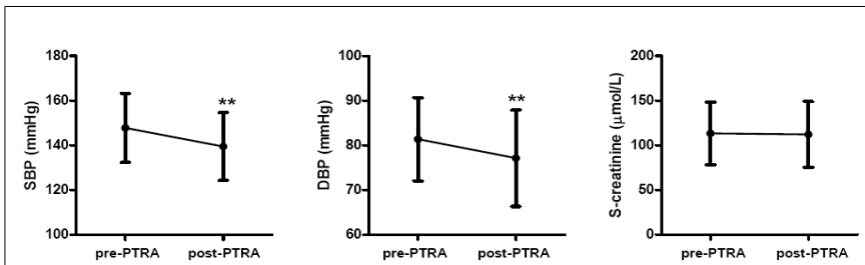


Figure 11. Ambulatory systolic (SBP) and diastolic (DBP) blood pressure and serum creatinine levels before (pre-PTRA) and 4 weeks after (post-PTRA) percutaneous transluminal renal angioplasty (PTRA) in patients with atherosclerotic renal artery stenosis. Values are means \pm SD. **denotes $p<0.01$ versus baseline.

Effects of PTRA on BNP and NT-proBNP

PTRA had no statistically significant effects on serum BNP or NT-proBNP when analyzed 4 weeks after intervention (Figure 12).

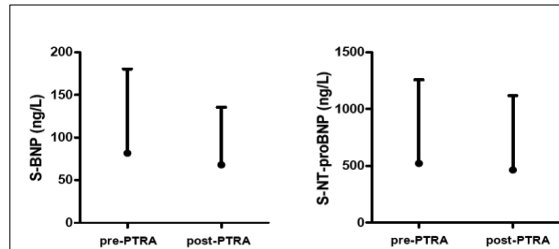


Figure 12. Serum concentration of brain natriuretic peptide (BNP) and N-terminal pro-BNP (NT-pro BNP) before (pre-PTRA) and 4 weeks after (post-PTRA) percutaneous transluminal renal angioplasty (PTRA) in patients with atherosclerotic renal artery stenosis. Values are means \pm SD. There were no statistically significant effects of PTRA.

4.3.3 Correlation of baseline data to changes in ASBP and BNP in response to PTRA

Changes in ASBP in response to PTRA were significantly correlated to baseline ASBP, ambulatory PP, eGFR, plasma glucose, and plasma Ang II. However, in a stepwise multiple regression analysis, only baseline ASBP, plasma glucose and Ang II significantly predicted changes in ASBP.

There was no threshold plasma BNP concentration above which PTRA was shown to cause a more pronounced BP reduction. There was no significant difference in BP response to PTRA between patients from the highest and the lowest quartiles of baseline BNP values. However, there were statistically significant correlations between changes in ASBP and delta BNP (Figure 13) and delta NT-proBNP in response to PTRA.

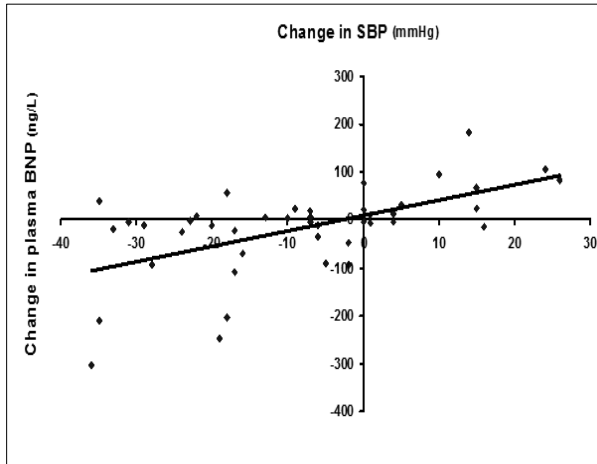


Figure 13. Correlation between change in ambulatory systolic blood pressure (SBP) and change in serum brain natriuretic peptide (BNP) concentration in response to percutaneous transluminal renal angioplasty ($r=0.57$, $p<0.001$) in patients with atherosclerotic renal artery stenosis.

4.4 Effects of candesartan on kidney function and inflammatory biomarkers in hypertensive patients subjected to renal angioplasty of atherosclerotic renal artery stenosis (paper IV)

4.4.1 Patient follow-up

Of the 48 patients randomized 38 patients (19 patients from each group) completed the study. In group ARVD-CAN 4 patients died (2 from malignant disease, 1 from head injury and 1 from a PTRA-related complication) and 1 patient did not want to participate further. In group ARVD-C 1 patient died (from pulmonary embolism) and 4 discontinued for other reasons (2 violations of study protocol, 1 had a major stroke and could not cooperate, 1 needed dialysis).

4.4.2 Effects of candesartan on blood pressure and kidney function

Ambulatory SBP and ADBP decreased significantly in both groups following randomization. However, the relative reduction in ASBP was significantly greater in group ARVD-CAN vs. ARVD-C ($p<0.05$). There was no difference between groups in ASBP or ADBP at 35 months after randomization (Table 6).

Table 6. Blood pressure and kidney function in patients with atherosclerotic renovascular disease (ARVD) at baseline and 35 months after randomization.

	ARVD-C (n=19)			ARVD-CAN (n=19)		
	baseline	35 months	% change	baseline	35 months	% change
Office SBP, mmHg	150±22	148±14	0.1±14.0	159±21	146±17*	-7.1±12.0
Office DBP, mmHg	84±11	83±9	-0.6±14.6	88±11	85±15	-3.4±14.1
ASBP, mmHg	132±12	126±10*	-4.4±8.4	144±14	128±13*	-12.2±9.7†
ADBP, mmHg	75±8	70±8*	-5.7±8.3	79±12	74±11*	-6.9±8.5
S-creatinine, µmol/L	101±24	100±31	-1.3±15.3	115±46	113±35	2.6±21.9
eGFR, ml/min/1.73m ²	62±15	63±17	0.8±18.6	58±21	57±21	1.8±27.3
UAE mg/day	103±224	287±649	240±950	117±157	49±72	3±113

Abbreviations are: ARVD-C, control group; ARVD-CAN, candesartan group; SBP, systolic blood pressure; DBP, diastolic blood pressure ; ASBP, ambulatory systolic blood pressure; ADBP, ambulatory diastolic blood pressure; eGFR, estimated glomerular filtration rate according to the 4-variable equation from the Modification of Diet in Renal Disease Study (MDRD); UAE, urinary albumin excretion. Values are means±SD. *denotes $p<0.05$ within group effect 35 months after randomization vs. baseline; and † denotes $p<0.05$ ARVD-CAN vs. ARVD-C.

Total eGFR, eGFR of the revascularized kidney, and urinary albumin excretion, did not change significantly during 35 months follow-up in any of the groups and there were no significant between group differences (Figure 14).

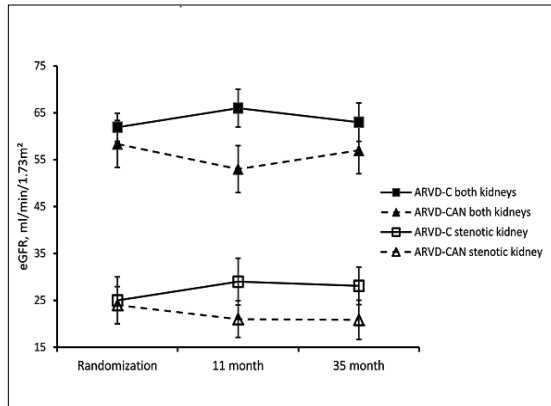


Figure 14. Estimated glomerular filtration rate in patients with atherosclerotic renovascular disease. Estimated glomerular filtration rate (eGFR) was determined at the time of randomization, and 11 and 35 months thereafter. Presented values are for both kidneys and for the previously stenotic kidney that had been subjected to revascularization four weeks prior to randomization. Estimated glomerular filtration rate according to the 4-variable equation from the Modification of Diet in Renal Disease Study (MDRD); Single kidney eGFR was calculated from split-kidney function data derived from DPTA renography. Values are means \pm SD. Abbreviations are: ARVD, atherosclerotic renovascular disease; ARVD-C, control group; ARVD-CAN, candesartan group.

4.4.3 Effects of candesartan on inflammatory biomarkers, PRA, Ang II and ET-1

There was no statistically significant difference between candesartan, and control treatment, on plasma levels of inflammatory biomarkers or endothelin-1. Plasma renin activity increased significantly in the ARVD-CAN group vs. ARVD-C (Table 7).

Table 7. Plasma biomarkers in patients with atherosclerotic renovascular disease (ARVD) at baseline and 35 months after randomization.

	ARVD-C (n=19)			ARVD-CAN (n=19)		
	baseline	35 months	% change	baseline	35 months	% change
Leukocytes, 10 ⁹ /L	7.2±2.1	7.2±1.5	2.6±18.3	7.9±2.1	7.7±1.4	0.3±14.9
hs-CRP, mg/dl	3.5±2.7	2.3±1.0	1.5±61.4	5.5±6.6	7.0±10.7	39.4±81.6
IL-6, pg/ml	4.0±8.5	2.7±1.9	161±308	3.6±3.0	3.5±2.9	95±254
TNF α , pg/ml	1.4±0.9	1.3±0.8	16.7±79.9	1.5±0.5	1.7±0.8	23.4±72.6
Uric acid, μ mol/L	342±64	357±81	6.2±24.9	377±105	390±99	8.6±19.7
ET-1, pg/ml	1.0±0.4	1.1±0.4	24.0±74.4	1.1±0.4	1.2±0.6	15.0±62.2
PRA, ngAngI/ml/h	1.3±1.5	1.2±1.0	36±133	2.1±2.6	10.6±18.8*	1463±3113†
Ang II, pg/ml	9.7±4.2	17.2±10.5*	121±200	13.2±7.6	34.0±39.1*	176±248

Abbreviations are: ARVD-C, control group; ARVD-CAN, candesartan group; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; TNF α , tumor necrosis factor- α ; PRA, plasma renin activity; Ang II, plasma angiotensin II; ET-1, endothelin 1. Values are means \pm SD.

*denotes $p < 0.05$ within group effect and † denotes $p < 0.05$ ARVD-CAN vs. ARVD-C.

5 DISCUSSION

Inflammation and endothelin-1 in patients with renal artery stenosis and effects of renal angioplasty and candesartan

In our first study we investigated inflammatory biomarkers and ET-1 in patients with RAS and evaluated how treatment with PTRAs affected these variables during the first month after intervention. We found that all patients with suspected RAS had increased levels of inflammatory biomarkers compared to healthy controls, with the exception of CD40L. As previously reported for IL-6 and CRP [114], we found no differences in inflammatory activity between patients with hemodynamically significant RAS and those without significant RAS. These data suggest that the increase in inflammatory activity is related to generalized atherosclerosis and not to the renal artery stenosis per se. Moreover, we observed that leukocyte count, hs-CRP, and IL-6 increased transiently after renal angiography, irrespective of whether PTRAs with or without stenting was performed. This inflammatory reaction was, however, significantly more pronounced in patients subjected to PTRAs compared to those undergoing only angiography. Previously, studies have shown that renal artery stent placement triggers an inflammatory response with increased CRP and IL-6 levels during the first 24 hours after PTRAs [114], and we extend those findings in a larger patient group. It must be kept in mind that all our patients were on antiplatelet therapy (ASA or clopidogrel) and statin treatment, which may have attenuated the inflammatory response. Statins are known to inhibit increases in IL-6 and hs-CRP after percutaneous coronary intervention [115]. Interestingly, in our study IL-6 had decreased compared to baseline one month after PTRAs, suggesting beneficial effects of PTRAs upon inflammatory activity in patients with RAS [69].

The impact of PTRAs on other inflammatory biomarkers was varied. TNF α levels decreased significantly on the day after PTRAs. TNF α responses have been investigated previously after femoral percutaneous transluminal angioplasty [116, 117]. A prompt increase followed by an exhaustion of TNF α levels 4 h after the intervention was shown. Such a transient increase cannot be excluded in our patients, as we did not collect blood samples until the day after intervention.

The levels of another inflammatory biomarker, such as CD40L, were lower at baseline in patients with RAS compared to the healthy controls. This surprising finding might be explained by the fact that all patients were on statins, which in some [118], but not all [115] studies has been shown to

weaken CD40L responses. CD40L levels are related to platelet activation [75, 115, 118, 119], and have been reported to reach a peak after one month in patients undergoing percutaneous coronary intervention [120]. Our results are compatible with this finding since we demonstrated elevated CD40L levels one month after intervention. This might have been due to late platelet and inflammatory responses caused by mechanical vessel dilatation and, in some patients, stent placement.

There is still controversy regarding ET-1 in renovascular hypertension. In several earlier studies [121-123] it has been shown that circulating ET-1 does not differ significantly between patients with renovascular and primary hypertension, even though the activity of the renin-angiotensin system is clearly increased in RVH [115]. Other studies [124] have reported increased ET-1 levels in patients with RAS. In our study only patients with significant RAS, who then underwent PTRA had increased plasma ET-1 levels at baseline (i.e. before angioplasty) compared to healthy controls. ET-1 levels did not change one day after angiography/angioplasty. However, one month after PTRA levels of ET-1 had decreased significantly in the group with significant RAS compared to before intervention, suggesting a beneficial effect of PTRA on the endothelin system. The fact that ET-1 levels were no longer elevated compared to healthy controls one month after intervention suggests that a hemodynamically significant RAS is associated with increased ET-1 levels [124], which may be lowered by endovascular correction of the stenosis.

In study 4 we investigated inflammatory biomarkers and ET-1 in patients with ARVD and residual hypertension after revascularization of renal artery stenosis. The aim of this study was to examine long-term effects of angiotensin II receptor antagonism, with candesartan, on inflammation and ET-1.

Hypertension and atherosclerosis are disorders associated with inflammation and studies have demonstrated that ARBs exert anti-inflammatory effects [125]. Several mechanisms have been proposed for these beneficial actions of ARBs, such as reduced production of mitochondrial reactive oxygen species [126] and cytokines [127], and via activation of the peroxisome proliferation-activated receptor-gamma [128]. In the present study candesartan did not affect inflammatory biomarkers and ET-1 during 35 months follow up and hence did not support a specific anti-inflammatory effect of ARBs in this cohort of ARVD patients. All patients in the present study were taking statins, a class of drugs known to have anti-inflammatory actions. It is

possible that the use of statins made it more difficult to detect anti-inflammatory effects of candesartan.

In conclusion, we found that patients with significant RAS had increased levels of inflammatory biomarkers and ET-1 compared to healthy subjects. PTRAs trigger a rapid, transient, increase in hs-CRP and IL-6. However, one month after PTRAs, both IL-6 and ET-1 had decreased compared to before intervention, indicating beneficial effects of PTRAs on inflammation and the endothelin system. However, candesartan did not show beneficial effects on inflammatory biomarkers or the endothelin system during 35 months of follow up.

Lipoprotein abnormalities in patients with atherosclerotic renovascular disease and effects on candesartan

In study 2 we examined lipoprotein abnormalities in patients with ARVD who had undergone PTRAs and analyzed whether angiotensin II receptor antagonism influenced these abnormalities. We found that patients with ARVD who underwent PTRAs four weeks earlier had increased plasma concentrations of TG, VLDL-C, ApoC-III and ApoC-containing lipoproteins compared to healthy controls in spite of ongoing treatment with statins. These lipoproteins were elevated even though concentration of TC and LDL-C were significantly reduced in this group most likely as a consequence of ongoing treatment with statins.

The ApoC-III-containing lipoproteins that in addition to ApoB also contain ApoC-III have triglycerides as the major lipid constituent and are generally referred to as triglyceride-rich. The lipoprotein abnormalities in ARVD patients show clear similarities to the renal dyslipidemia described in patients with CKD and reduced GFR. It is characterized by increased levels of triglyceride-rich ApoB- and ApoC-containing lipoproteins that typically express ApoC-III [80, 81]. However, ARVD patients showed an increase mainly in LpB:C:E in contrast to CKD patients in which specifically LpB:C particles are highly increased [111]. Abnormalities in the spectrum and composition of individual lipoprotein particles in renal dyslipidemia are present in both normolipidemic and hyperlipidemic CKD patients and can be detected already when kidney function is moderately reduced [89, 129]. In study 2, patients had an average eGFR of 59 ml/min/1.73 m², and it is possible that the reduction in GFR, per se, could have contributed to dyslipidemia. However, using multiple regression analysis we could not detect a significant association between eGFR and ApoC-III levels in ARVD patients, suggesting that other mechanisms were involved.

It should be noted that all ARVD patients were on statin treatment in the present study and that this could have masked underlying lipoprotein abnormalities. In previous studies it has been shown that statin treatment effectively reduces cholesterol-rich ApoB-containing lipoproteins including their characteristic lipid and apolipoprotein constituents (TC, LDL-C, ApoB, and ApoE) in CKD patients [108, 111] as well as in other patient groups [130, 131]. However, statins are much less effective in reducing triglyceride-rich ApoB- and ApoC-containing lipoproteins such as LpB:C and LpB:C:E and their constituents VLDL-C, TG, and ApoC-III [108, 111]. Although the present study was not designed to examine the effects of statins on lipoprotein abnormalities, our results are in agreement with earlier findings in other patient groups and suggest that statin treatment does not normalize triglyceride-rich ApoB- and ApoC-containing lipoproteins in ARVD patients.

Taking into consideration that cardiovascular disease is the major cause of death in patients with ARVD [44] it is intriguing that lipoprotein abnormalities in ARVD patients on statin treatment in the present study have previously been shown to be atherogenic [132-135]. In the Monitored Atherosclerosis Regression Study (MARS), elevated levels of ApoB- and ApoC-containing triglyceride-rich lipoproteins, and ApoC-III, contributed significantly to the progression of coronary artery disease as evaluated by sequential coronary angiography [132]. In addition, in the Cholesterol and Recurrent Events (CARE) trial increased ApoC-III concentration in VLDL and IDL (intermediate-density lipoprotein-cholesterol) was a strong predictor of coronary events [134]. Furthermore, recent analyses from the Hoorn study showed that an increased plasma ApoC-III concentration independently was associated with cardiovascular mortality [135]. In the current study, both total ApoC-III and ApoC-III-HP (i.e. ApoC-III in triglyceride-rich VLDL+LDL) were elevated in ARVD patients and these alterations may play a pathophysiological role both in the development of dyslipidemia and in the vascular disease process in these individuals. ApoC-III is a major regulator of lipolysis primarily by inhibiting endothelial-bound lipoprotein lipase (LPL), the principal enzyme necessary for the hydrolysis of triglyceride-rich lipoproteins [136]. Thus, the finding of increased ApoC-III levels in the present study may reflect the accumulation of intact and partially metabolized ApoB- and ApoC-containing lipoproteins due to a retarded catabolism of these lipoproteins. In addition, increased ApoC-III per se may promote the development of atherosclerosis by stimulating the activation and adhesion of peripheral monocytes to endothelial cells [137, 138].

A potentially effective therapy in patients with ARVD would be the combination of statins with fibrates, although other therapies could also be

considered. Fibrates and other peroxisome proliferator-activated receptor (PPAR) agonists have the potential to beneficially affect the catabolism of triglyceride-rich ApoB- and ApoC-containing lipoproteins [139]. Although PPAR agonists have the potential to correct dyslipidemia in ARVD, this hypothesis, and the impact on clinical endpoints need to be tested in clinical trials.

In the present study there was a significant association between PRA and ApoC-III levels in ARVD patients suggesting that an activation of the renin-angiotensin system could increase ApoC-III. Still, treatment with candesartan for 11 months did not significantly influence plasma levels of ApoC-III or ApoC-containing lipoproteins, indicating that Ang II, via the Ang II type-1 receptor, was not causatively involved. However, the number of patients in the present study was small and larger studies are warranted.

In conclusion, we found that patients with ARVD had elevated levels of triglyceride-rich ApoC-III-containing lipoproteins and increased ApoC-III in spite of ongoing treatment with statins. These lipoprotein abnormalities, which were not corrected by candesartan treatment, have previously been shown to be atherogenic and may contribute to progressive atherosclerosis in ARVD patients.

Brain natriuretic peptides in atherosclerotic renal artery stenosis and effects of renal angioplasty

In study 3 we found that hypertensive patients with ARAS had significantly elevated plasma concentrations of BNP and NT-proBNP compared to healthy controls, but not in comparison to hypertensive patients without RAS. This observation clearly suggests that other causes than RAS *per se* were responsible for the increased BNP levels in ARAS patients. In fact, multiple regression analysis showed that serum creatinine was the only variable that was significantly correlated to both plasma BNP and NT-proBNP levels in ARAS patients. Hence, differences in kidney function likely explained the increase in BNP and NT-proBNP in ARAS subjects vs. healthy controls since patients with ARAS had reduced GFR. On the contrary, serum creatinine levels were similar in hypertensive patients with or without RAS.

The other main finding in this study was that plasma BNP and NT-proBNP could not be used to predict the outcome of PTRAs on blood pressure and we could not identify a pre-interventional plasma BNP or NT-proBNP level above which a successful revascularization could be expected. We have observed that although PTRAs did not significantly affect BNP or NT-proBNP concentrations in patients with ARAS, there were a significant

correlation between changes in plasma BNP and changes in blood pressure in response to intervention. The mechanisms underlying this correlation remain speculative but could theoretically be explained by reduced left ventricular afterload secondary to the fall in blood pressure, decreased activity of RAAS, or restoration of pressure-natriuresis in the previously stenotic kidney.

Our data do not confirm the results of two previous studies in which baseline BNP levels were shown to predict the blood pressure response after revascularization in patients with RAS [140, 141]. There are a number of possible explanations for the discrepant results in our study compared to those previously published. First, in both the earlier studies [140, 141] patients with bilateral RAS were included whereas we only investigated patients with unilateral RAS. In the study by Silva et al [140] the proportion of patients with bilateral RAS was as high as 33%. It is possible to speculate that patients with bilateral RAS, who in general have a more volume-dependent hypertension, would have increased plasma levels of BNP at baseline and respond to revascularization with a more pronounced BNP reduction. Secondly, Staub et al [141] included patients with FMD, who made up 22% of all patients with a successful blood pressure response, while only patients with ARAS participated in study 3. Thirdly, the majority of patients included in the two previous studies were on treatment with ACEI or ARBs whereas none of the patients in our study were on medications that inhibited the RAAS. It is well known that cardiac BNP synthesis and release is stimulated by both hemodynamic mechanisms and by direct, non-hemodynamic, pathways via neuroendocrine factors such as Ang II [98, 99]. In our study, revascularization had no significant effects on either plasma Ang II levels or PRA and it is therefore possible that Ang II driven BNP production prevented reductions in plasma BNP levels following intervention.

Chrysochou et al [142] examined the prognostic role of NT-proBNP in patients with ARAS and found that elevated NT-proBNP levels were associated with a greater likelihood of death when subdivided by CKD stage. Thus, although these authors showed that kidney function was a more important risk factor for CV events and death, there was an additive risk effect of NT-proBNP. Therefore, according to these authors, measurements of BNP might be helpful in identifying ARAS patients with increased CV risk and in need of intensified management.

Another interesting finding in our study was reduced adiponectin in patients with ARAS compared to healthy controls. We observed a significant

correlation between low plasma adiponectin levels and low S-HDL-C concentrations in both hypertensive groups (ARAS and non-RAS), which is compatible with previous findings in patients with the metabolic syndrome and type 2 diabetes [143]. It has previously been shown that low adiponectin levels predict elevated plasma VLDL-ApoB concentrations through impaired catabolism of triglyceride rich lipoproteins [144]. Hence, it is possible that suppressed adiponectin levels in ARAS patients may contribute to the dyslipidemia described in study 2.

In conclusion, we found that hypertensive patients with ARAS have elevated plasma concentrations of BNP and NT-proBNP, but reduced adiponectin levels, compared to healthy controls. However, concentrations of these biomarkers were not significantly different in ARAS patients compared to hypertensive subjects without RAS. The increases in plasma BNP and NT-proBNP vs. healthy controls were most likely explained by reduced kidney function in ARAS patients. In addition, baseline levels of BNPs could not be used to predict the outcome of PTRAs on blood pressure.

Effects of candesartan on kidney function in hypertensive patients subjected to renal angioplasty of atherosclerotic renal artery stenosis

In study 4 we examined long-term effects of candesartan on kidney function in patients with ARVD and residual hypertension after revascularization of renal artery. We found that candesartan treatment during three years had no statistically significant effects on kidney function compared to antihypertensive treatment without RAAS inhibitors.

Several studies have provided evidence that renin-angiotensin system blockade with ACEIs or ARBs has specific renoprotective effects and are the drugs of choice for treatment of hypertension in patients with CKD and albuminuria [145]. In our study, the level of albuminuria increased more than 2-fold in the control group during follow-up whereas albuminuria levels decreased in candesartan-treated patients. Although this difference between groups did not reach statistical significance, it suggested a renoprotective effect of ARBs also in this category of patients. Interestingly, the rate of urinary albumin excretion 35 months after randomization was numerically lower in group ARVD-CAN, compared to controls, in spite of ambulatory BPs being somewhat higher in the ARVD-CAN group. This suggests that the effect of candesartan on albuminuria was caused by an intrarenal mechanism independent of systemic hemodynamics. The pathophysiological explanation of this observation could be that the intrarenal RAAS remains activated

following revascularization due to the existence of significant stenosis in smaller arteries located more distally.

Pharmacological blockade of the RAAS in hypertensive patients with ARAS was initially considered contraindicated due to fear of inducing renal ischemia, hence accelerating renal fibrosis and progression towards end stage renal disease (ESRD) [33, 34]. However, in several studies BP control was demonstrated to be remarkably improved by the use of ACEI [37]. Losito et al [37] evaluated cardiovascular and renal survival in a long term follow-up study of 195 patients with renal artery stenosis, of which 135 patients underwent revascularization. Multivariate analysis disclosed that revascularization was not associated with reduced mortality or improved renal survival when compared to medical treatment. However, the use of ACEI was associated with longer survival ($p=0.002$) in both revascularized and medically treated patients [37], indicating that RAAS-blockade is important in the management of ARVD patients.

In our study, total eGFR, and eGFR of the previously revascularized kidney, did not change significantly during 35 months follow-up in any of the groups and there was no significance between group differences. Hence, candesartan did not impair renal function during 35 months of follow up after revascularization and no patient developed acute kidney injury. It is feasible to speculate that RAAS-blockade may attenuate disease progression in ARVD patients although this was not observed in the present study. Obviously, a much larger study with a longer follow up would be needed to address this issue appropriately.

The obvious limitation of this study was the small number of patients. A main reason for this was the stringent inclusion criteria. In addition, the number of patients with ARAS undergoing PTRAs has decreased dramatically during the last 10-years due to the negative results from randomized clinical trials that were published during the period when patients were recruited to the present study [31, 32].

In conclusion, in patients with ARVD who had undergone revascularization of renal artery stenosis, candesartan-based antihypertensive therapy did not show any significant effects on kidney function compared to control treatment. However, candesartan-based therapy was well tolerated and without renal side-effects.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

What did we know before our study and what do we know now?

Before we started our study we knew that patients with RVH caused by atherosclerotic RAS had a much worse prognosis than patients with primary hypertension. In addition, we knew that this difference in prognosis was explained by an elevated cardiovascular risk [43, 44]. However, the reason for the increase in cardiovascular events was not clearly understood. We also did not know how to treat these patients ideally to reduce cardiovascular risk and to improve survival.

We speculated that non-traditional cardiovascular risk factors may contribute to the high cardiovascular morbidity and mortality in this patient group. We investigated these factors and examined whether treatment with PTRAs or candesartan affected them.

Hypertension and atherosclerosis are disorders associated with inflammation [68]. We found that patients with ARAS have increased levels of inflammatory biomarkers and ET-1 compared to healthy controls. We also observed that these patients have an atherogenic lipoprotein profile in spite of ongoing treatment with statins. This abnormality was characterized by elevated levels of ApoC-III-containing lipoproteins. We speculate that these alterations may contribute to progressive atherosclerosis and an accelerated cardiovascular disease.

We showed that IL-6 and ET-1 decreased after PTRAs during the first month of follow-up suggesting beneficial effects of this intervention. However, the potential consequences of reduced plasma levels of inflammatory biomarkers and ET-1 on clinically relevant outcomes clearly need to be examined further. Previous studies [125] have demonstrated that ARBs exert anti-inflammatory effects, but in our study candesartan did not affect inflammatory biomarkers and ET-1 during 35 months follow up and hence did not support specific anti-inflammatory effects of ARBs in patients with ARVD. In addition, we observed that candesartan did not impair renal function and was well tolerated. It is feasible to speculate that RAAS-blockade may attenuate disease progression in these patients although this was not observed in the present study. Naturally, a much larger study with a longer follow up would be needed to address this issue appropriately.

The lipoprotein abnormalities in ARVD patients observed in our study show similarities to the renal dyslipidemia described in patients with CKD and reduced GFR. How to effectively treat this dyslipidemia is still uncertain. A potentially effective therapy in patients with ARVD would be the combination of statins with fibrates. Fibrates and other peroxisome proliferator-activated receptor (PPAR) agonists have the potential to beneficially affect the catabolism of triglyceride-rich ApoB- and ApoC-III-containing lipoproteins. Although PPAR agonists have the potential to correct dyslipidemia in ARVD, this hypothesis, and the impact on clinical outcomes need to be tested in clinical trials.

During our studies, two large clinical trials: ASTRAL [31] and CORAL [32] have been published. These studies have failed to show statistically significant benefits of revascularization, over optimal medical treatment, on blood pressure, kidney function, and cardiovascular outcomes. These two trials, despite their limitations, have influenced medical decision making away from invasive interventions [146, 147]. Patients with stable kidney function and adequate blood pressure control should not be considered for revascularization. Pharmacological management with antihypertensive agents, including ACEI/ARBs, antiplatelet drugs, cholesterol lowering therapy, together with lifestyle modifications, remain the basic care for all patients with ARAS. But the conclusion of these trials should not be generalized to all patients with ARAS. For example, patients with recurrent episodes of flash pulmonary edema without an obvious cardiac cause, or with bilateral RAS or stenosis to a single functioning kidney combined with progressive renal impairment may be subject to intervention. Patients with a strong indication for treatment with RAAS-blockade, e.g. heart failure, who develop progressive renal failure during this treatment, may be also considered for intervention [146, 147].

There is a need to accurately identify individuals who may benefit from renal revascularization. We found that plasma BNP levels are increased in patients with ARAS compared to healthy control, but that BNP could not be used to predict the outcome of PTRR on blood pressure.

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