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Atherosclerotic Renovascular hypertension: Results of The METRAS study (Medical and Endovascular Treatment of Atherosclerotic Renal Artery Stenosis)

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BACKGROUND

Renal artery stenosis (RAS) is a vascular lesion causing narrowing of the renal artery thereby impairing blood flow to the kidney. Atherosclerotic renal artery stenosis (ARAS) is by far the most common renovascular lesion accounting for about 90% of all renal artery lesions[1] and typically involving the proximal third of the renal artery including the aorta and ostium. Other less frequent causes of RAS are listed in Table 1.

Table 1: Major causes of vascular occlusion producing renovascular hypertension[2]
Unilateral disease **Unilateral atherosclerotic renal-artery stenosis** **Unilateral fibromuscular dysplasia (FMD)** **Medial fibroplasia** **Perimedial fibroplasia** **Intimal fibroplasia** **Medial hyperplasia** **Renal artery aneurysm** **Arterial embolus** **Arteriovenous fistula (congential/traumatic)** **Segmental arterial occlusion (post-traumatic)** **Extrinsic compression of renal artery (e.g. pheochromocytoma)** **Renal compression (e.g. metastatic tumor)**
Bilateral disease or solitary functioning kidney **Stenosis to a solitary functioning kidney** **Bilateral renal arterial stenosis** **Aortic coarctation** **Systemic vasculitis (e.g. Takayasu's, polyarteritis)** **Atheroembolic disease** **Vascular occlusion due to endovascular aortic stent graft**

The prevalence of ARAS increases with age: significant ARAS can be detected in 6.8% of community-based subjects above age 65 years[3]. It also increases with clinically manifest disease in the coronary arteries (18-20%), the aorta, or peripheral vascular beds (35-50%); patients with refractory congestive heart failure and/or end-stage renal disease may have demonstrable ARAS in 40–50% of cases, as recently reviewed[4] (Table 2).

Table 2: Prevalence of Atherosclerotic Renal Artery Stenosis in Different Subgroups[5]	
Subgroups	Prevalence of ARAS (>60% of renal artery lumen)
General population	0.5%
Age >65 years (Doppler)	7%
Healthy kidney donors	3-5%
Chronic kidney disease	5.5%
Suspicion of renovascular hypertension	14%
Coronary angiography	19%-24% (7% bilateral)
ESRD	12%-14% (2%-5% as cause of CKD)
Peripheral arterial disease	28%-59%
Abdominal aortic aneurysm	33%
Elderly with CHF	34%
Refractory CHF	40%-50%
Diffuse arterial disease	50%
Note: Please note that data are based on heterogeneous studies with different methods of assessment. Abbreviations: CHF, congestive heart failure; CKD, chronic kidney disease; ESRD, end-stage renal disease.	

Despite antihypertensive drug therapy and control of risk factors for atherosclerosis, ARAS is a progressive disease, leading to renal ischemia and loss of renal function[6].

Pathophysiology and clinical presentations

The clinical spectrum of ARAS is broad and since RAS can be 'silent', it might go undetected or become manifest as renovascular hypertension, ischemic nephropathy, and recurrent 'flash' pulmonary edema (Fig. 1).

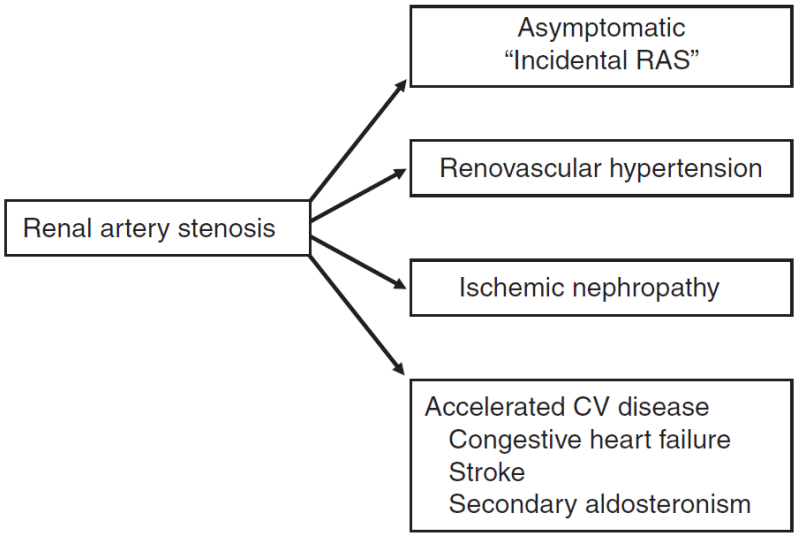


Fig. 1. Manifestations of renal arterial disease. CV, cardiovascular; MR, magnetic resonance; PRA, plasma–renin activity; RAS, renal artery stenosis[2].

ARAS is a common cause of **secondary hypertension**. Unilateral experimental renovascular disease with a functioning "contralateral kidney" that excretes sodium as function of "pressure natriuresis" (identified as 2-kidney-1-clip hypertension) serves as a major model of angiotensin-dependent hypertension. This theory dates back to the 1930s, when Goldblatt et al performed a series of studies examining the impact of unilateral and bilateral RAS on blood pressure (BP)[2] (Fig. 2).

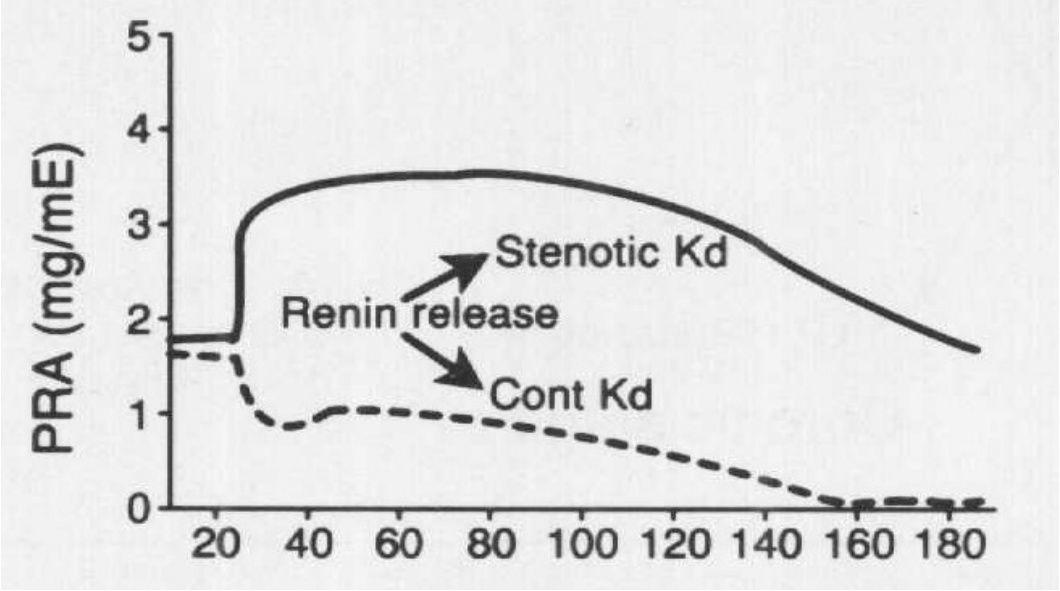


Fig. 2. Adaptive mechanism in two-kidney, one-clip Goldblatt hypertension. Cont= contralateral, Kd=kidney, PRA= plasma renin activity.

By clamping one renal artery in dogs, Goldblatt et al demonstrated a systemic pressor effect: in the early stages BP increases within hours after clipping, with maximum levels reached at later stages (approximately the 36th week). By contrast, renin levels are overtly elevated only in the early stages, with gradual return to baseline. This might explain why at the time of diagnosis, as many as one-third of patients with renovascular hypertension do not have high plasma renin, either in the peripheral or in renal vein blood from the affected side[8]. Activation of the renin-angiotensin-aldosterone system (RAAS) is transient and leads to recruitment of additional pressor pathways, including oxidative stress, sympatho-adrenergic activation, and impaired vasodilatory responses both within the renal and systemic microcirculation[9]. Instead, the mechanism behind bilateral or unilateral RAS with a solitary kidney is due to extracellular fluid overload secondary to decreased diuresis rather than a renin-mediated mechanism.

It should be emphasized that the release of circulating renin depends on a substantial reduction in kidney perfusion pressure. Unilateral or bilateral hypersecretion of renin is associated with 80% or

greater reduction of renal artery lumen diameter[10]. De Bruyne at al by means of renal artery balloon occlusion in humans and expressing stenosis severity as the ratio of distal pressure (Pd) corrected for aortic pressure (Pa), demonstrated a Pd/Pa ratio of 0.90 is the threshold level below which the stenosis is likely responsible for an up-regulation of renin production and a Pd/Pa ratio of 0.90 corresponds approximately to a systolic gradient of 25 mm Hg[11] (Fig.3).

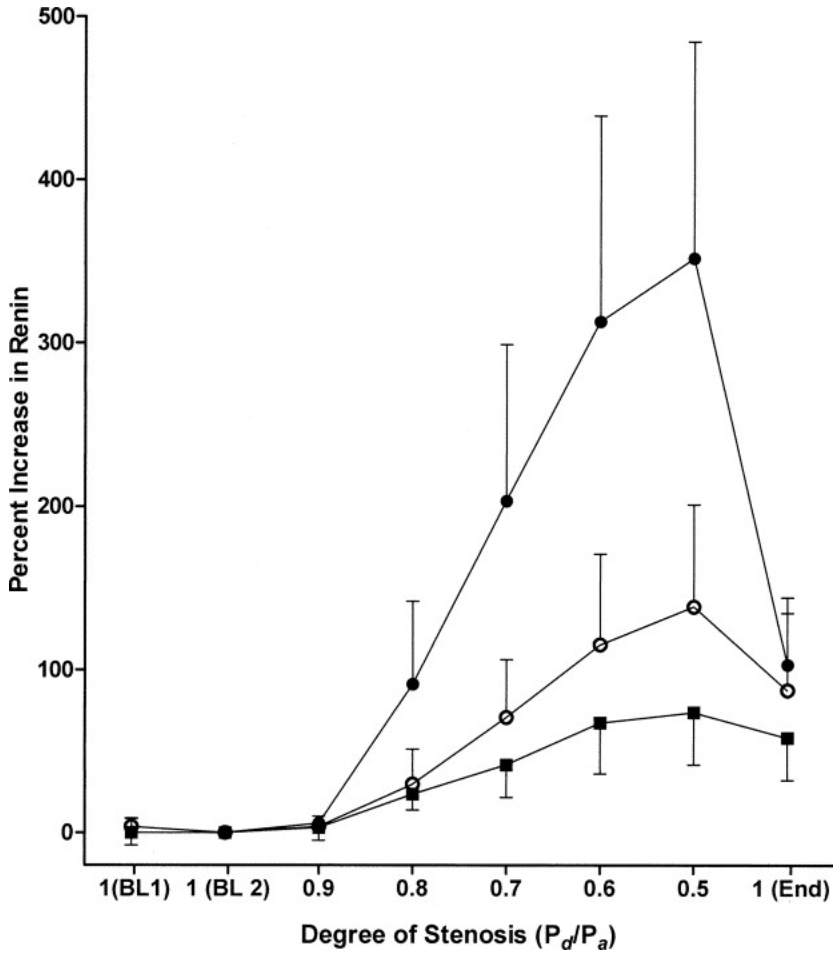


Fig. 3. Relationship between the individual values of mean aortic pressure (Pd)/mean pressure distal to the renal artery stenosis (Pa) ratios and the corresponding systolic pressure gradients (closed circles) and mean pressure gradients across the stenosis (open circles)[11].

The assessment of pressure gradient is a conflicting item and the optimal method for determining it has not been established. Currently, translesional pressure gradients are often measured by placing a 4Fr or larger catheter distal to the lesion while simultaneously measuring pressure in the aorta. Unfortunately, the catheter itself may partially obstruct flow and thereby artifactually increase the pressure gradient[12]. By reducing the size of the device used to measure the pressure using a 0.014'' guidewire, Coyler demonstrated that the translesional pressure gradient obtained

by pressure-sensing guidewire correlate more strongly with angiographic minimal lumen diameter (r^2 = 0.801) than those obtained by 4 Fr catheter (r^2 = 0.360)[12].

When reduced kidney perfusion activates pressor mechanisms, systemic blood pressure often rises, sometimes leading to acceleration of pre-existing essential hypertension. As a result, the clinical manifestations of ARAS most commonly develop in previously treated hypertensive subjects.

Ischemic nephropathy is due to decreased perfusion caused by the obstruction of the renal artery with subsequent excretory dysfunction. The cause of ischemic nephropathy has not been fully elucidated and it has to be underlined that, in most cases, ARAS develops in a setting of preexisting vascular changes affecting the kidney as a result of aging, hypertension, diabetes, dyslipidemia, and occasionally atheroembolism. The severity of stenosis has not been correlated with kidney function, decline in glomerular filtration, or results of revascularization[13,14], indicating that other mechanisms are implicated in ischemic nephropathy.

Several pathways have been proposed to explain how a hemodynamically significant lesion ultimately results in interstitial fibrosis (Fig. 4 and 5). Recurrent local ischemia and global hypoperfusion cause tubulointerstitial injury and microvascular damage and RAAS activation with subsequent vasoconstriction. In addition to kidney hypoperfusion, systemic atherosclerosis leads to activation of proinflammatory cytokines and oxidative stress, causing microvascular rarefaction, inflammatory infiltration, glomerulosclerosis, and tubulointerstitial fibrosis, which lead to proteinuria and glomerular filtration reduction. The renal lesion in ischemic nephropathy usually is accompanied by hypertensive nephrosclerosis and atheroembolic disease, which contribute to the reduction of glomerular filtration[15,16].

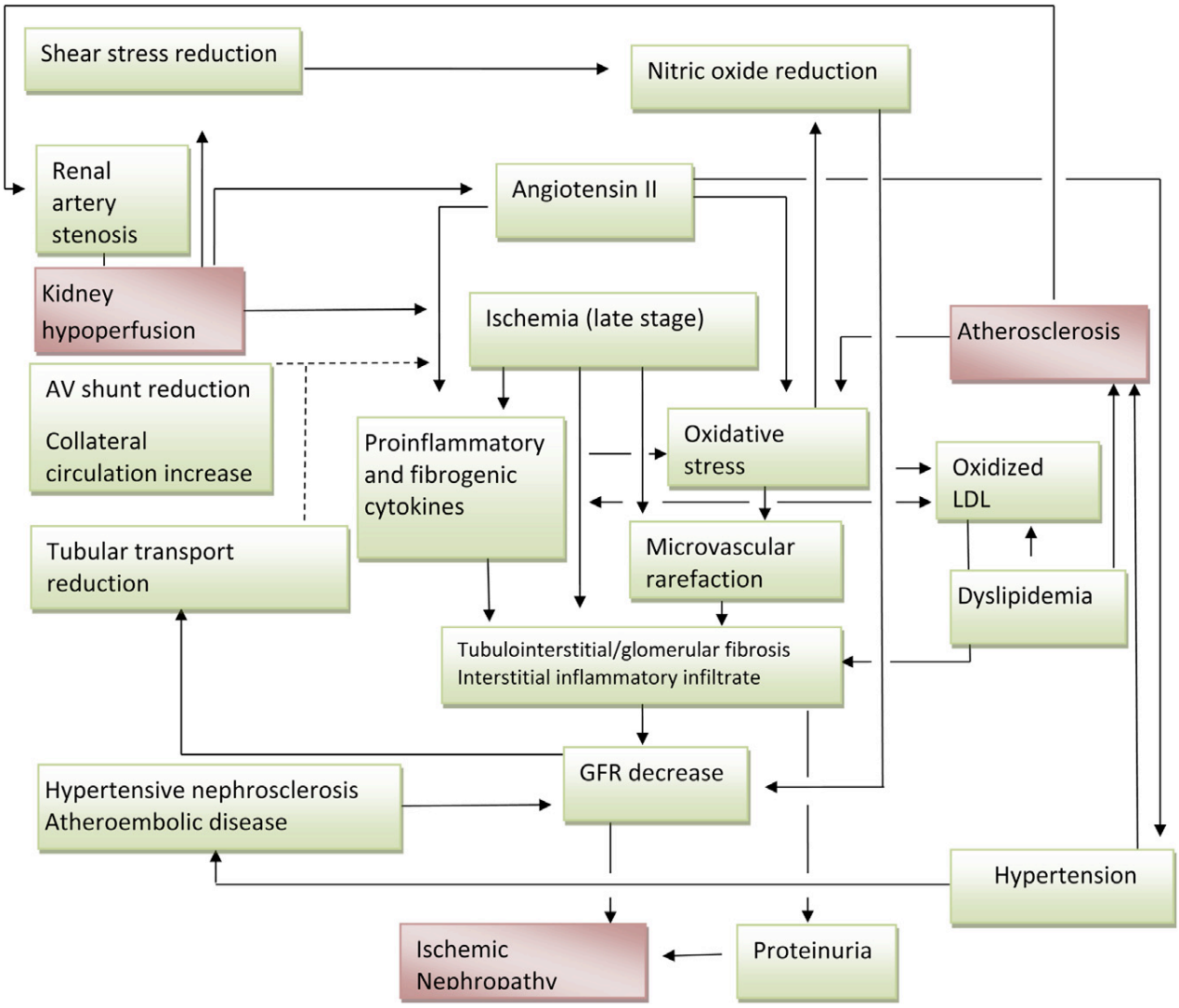


Fig. 4. Pathophysiology of ischemic nephropathy. Abbreviations: AV, arteriovenous; GFR, glomerular filtration rate; LDL, low-density lipoprotein. Dotted line: protective mechanisms[16].

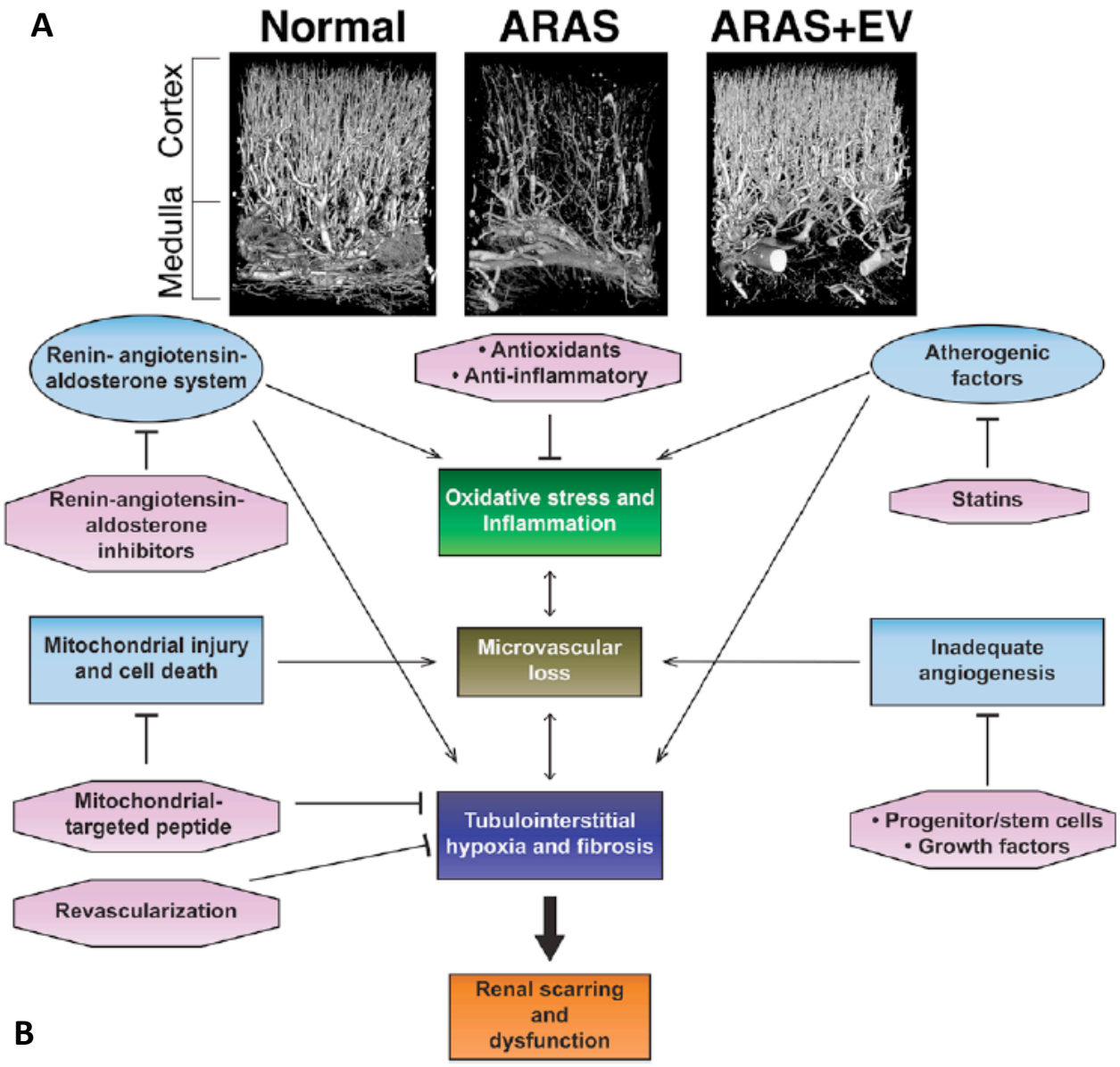


Fig. 5. **A** Micro–computed tomographic images showing microvascular loss in the poststenotic kidney, which was rescued using mesenchymal stem cell–derived extracellular vesicles. **B** Schematic of injurious mechanisms activated in the poststenotic kidney in ARAS, and experimental therapeutic interventions that can potentially blunt them[17].

Ischemic nephropathy is particularly interesting in the light of recent results of randomized clinical trials (RCTs), which failed to demonstrate a superiority of revascularization compared to medical therapy alone. This could be in part explained by ischemic nephropathy and renal interstitial fibrosis, which develop beyond a vascular lesion and could make revascularization ineffective.

Saad et al recently demonstrated that severe renovascular disease is associated with tissue hypoxia and increased renal venous markers of inflammatory cytokines (monocyte chemoattractant protein-1 and tumor necrosis factor-α) and tissue injury (neutrophil gelatinase-

associated lipocalin). Revascularization might reduce hypoxia and partially restore blood flow, but fails to alter the markers of inflammation, suggesting that additional measures may be needed to reverse the process of kidney injury[18].

Eirin and coworkers evaluated the relationship between net renovascular cytokine release and tissue inflammation in pigs' post-stenotic kidney. Net release of TNF-α, IF-γ, and MCP-1 was higher in RAS compared with normal and to the contralateral kidney[19]. More recently Eirin et al hypothesized that chronic renal damage involves mitochondrial injury and that mitochondrial protection would reduce renal fibrosis and dysfunction in ARAS pigs. They studied 28 pigs after 10 weeks of ARAS or sham, treated with vehicle or Bendavia, a tetrapeptide that preserves cardiolipin content in the mitochondrial inner membrane. They found that Bendavia restored cardiolipin content in ARAS and improved vascular density, oxygenation, renal blood flow, and GFR and that oxidative stress and fibrosis were improved, and renovascular endothelial function normalized both *in vivo* and *in vitro*[20].

Ebrahimi et al tested the hypothesis that addition of mesenchymal stem cells (MSC) to percutaneous transluminal renal angioplasty (PTRA) can restore stenotic kidney medullary tubular transport function and attenuate its remodeling in swine. MSC delivery in addition to PTRA reduces inflammation, fibrogenesis and vascular remodeling, and restores oxygen-dependent tubular function in the stenotic-kidney medulla[21,22].

Most recent researches are focused on microRNAs, small non-coding RNAs that are important regulators of gene expression and have been implicated in atherosclerosis. Park et al hypothesize that it might be implicated in modulating renal injury in ARAS[23].

These recent studies may in part explain the failure of RCTs to demonstrate the superiority of percutaneous transluminal renal angioplasty plus stenting (PTRAS) over medical therapy in ARAS treatment in whom, beyond the simple restore of blood flow, is also implicated an ischemic alteration of the renal tissue. The failure of renal artery revascularization to restore renal function in ARAS provides the impetus to explore underlying mechanisms and treat the poststenotic kidney directly[17]. Further studies are needed to demonstrate the benefit of the addition of a renal ischemia "protective" vehicle to PTRAS and identification of the basic mechanisms that lead to kidney tissue injury in ARAS can assist in the development of targeted therapies.

ARAS may either cause or exacerbate unstable angina and congestive heart failure. A rapid rise in arterial pressure caused by peripheral arterial vasoconstriction combined with reduced renal

perfusion and enhanced sodium reabsorption can lead to rapidly developing circulatory congestion (**flash pulmonary edema**). This phenomenon was first reported by Pickering in 1988[24]. Flash pulmonary edema does occur in unilateral RAS, but tends to occur more often in patients with bilateral RAS (Fig. 6). Acute angiotensin-mediated increase in afterload is the physiopathologic mechanism that explains unstable angina in RAS due to increased myocardial work and oxygen demand, resulting in myocardial ischemia.

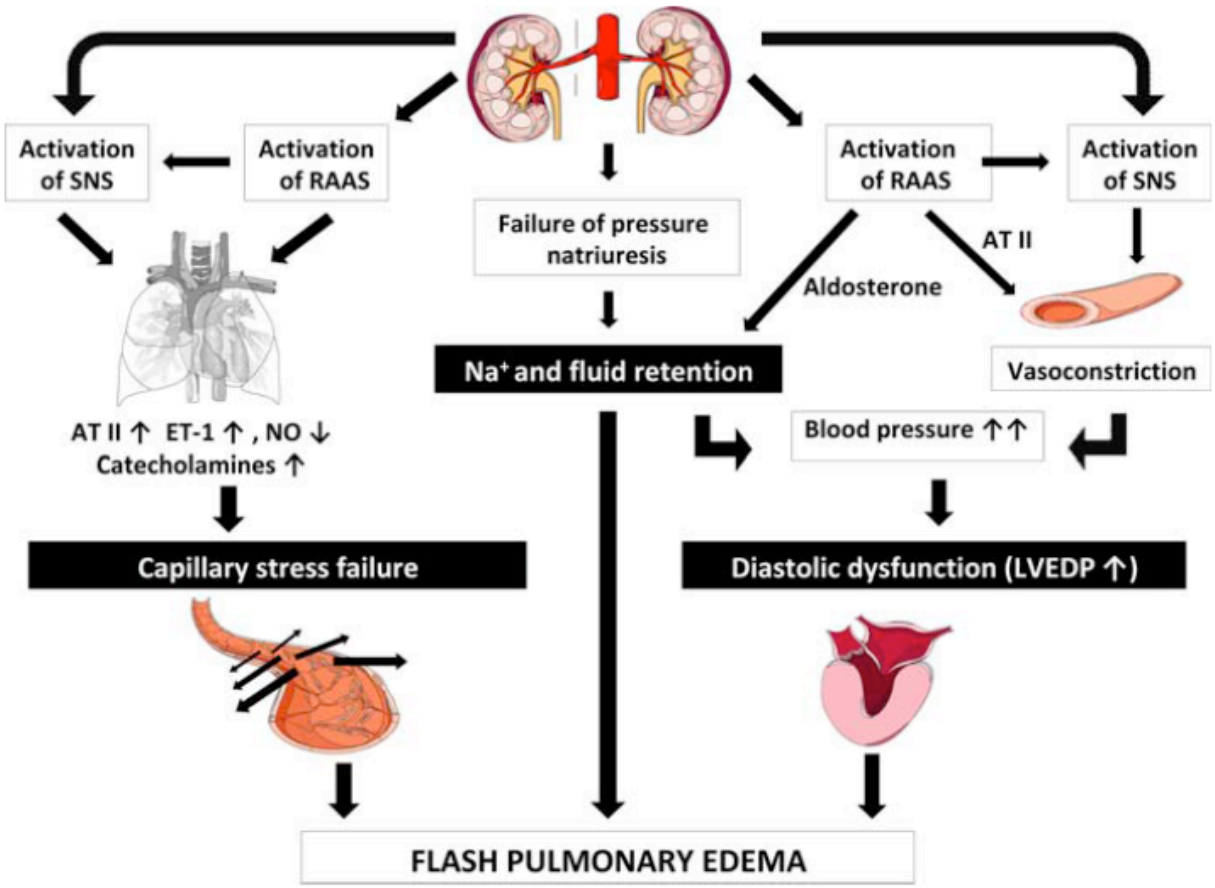


Fig. 6. The Pickering Syndrome. Three main pathophysiological mechanisms contribute to the development of flash pulmonary oedema: defective pressure natriuresis with sodium and fluid retention, increased left ventricular end-diastolic pressure associated with left ventricular hypertrophy and stiffening, and failure of the pulmonary capillary blood–gas barrier. RAAS, renin–angiotensin–aldosterone system; SNS, sympathetic nervous system; Na+, sodium; AII, angiotensin II; ET-1, endothelin-1; NO, nitric oxide[25].

Diagnosis

According to the American College of Cardiology/American Heart Association (ACC/AHA) Clinical Practice Guidelines[26] clinicians may perform diagnostic studies to identify clinically significant RAS (class I with level of evidence B) in patients with the onset of hypertension before the age of 30 years or after the age of 55 years, in patients with accelerated, resistant, or malignant hypertension (level of evidence C), in patients with new azotemia or worsening renal function after the administration of an ACE inhibitor or an angiotensin receptor blocking agent, in patients with an unexplained atrophic kidney or a discrepancy in size between the 2 kidneys greater than 1.5 cm and in patients with sudden, unexplained pulmonary edema.

The performance of diagnostic studies to identify RAS is reasonable (class IIa level of evidence B) in patients with unexplained renal failure, including individuals starting renal replacement therapy (dialysis or renal transplantation) and may be reasonable (Class IIb) in patients with multivessel coronary artery disease and none of the clinical clues or peripheral artery disease at the time of arteriography (level of evidence: B) and in patients with unexplained congestive heart failure or refractory angina (level of evidence: C).

According to the ACC/AHA guidelines, duplex ultrasonography, computed tomographic angiography (CTA), and magnetic resonance angiography (MRA) all received a class I indication (level B evidence) as a screening test to establish the diagnosis of RAS[26].

Duplex ultrasonography allows direct visualization of the renal arteries (B-mode imaging) and Doppler velocity measurements of blood flow. The advantages of this technique are that it is not invasive, is not affected by medications, is low-cost and does not require the use of intravenous contrast. Duplex is particularly useful for identifying restenosis of metallic stents after implantation. However, Duplex is not without limitations. The test is time-consuming with prolonged examination times, it can be technically challenging in obese patients or in the presence of abdominal gas, and is also significantly operator-dependent. Detection of stenosis is determined by the measurement of a high peak systolic velocity (PSV) (>180 or >200 cm/second) or by the renal to aortic ratio, which is calculated by dividing the PSV of the renal artery by the PSV of the adjacent aorta (normal ratio <3.5). Duplex may be used also to measure the renal artery resistive index (RRI). An increased RRI suggests structural abnormalities in the small blood vessels e.g. in the context of longstanding hypertension associated with nephrosclerosis or glomerulosclerosis. There are conflicting reports regarding the usefulness of RRI to predict the

response to revascularization. Radermacher et al in their retrospective study demonstrated that an elevated resistance index >0.80 predicted a lack of improvement in terms of mean arterial pressure and renal function, but this study had some limitations such as the retrospective design and the inclusion of a large majority of patients who received balloon angioplasty without stent placement[27]. Zeller et al analyzed a subgroup of patients treated with stent angioplasty for severe (≥70%) ostial ARAS who had diabetes mellitus and nephrosclerosis. They did not find any difference in terms of improvement in mean blood pressure in patients with RRI <0.7 or RRI >0.8 and serum creatinine decreased significantly in both subgroups during follow-up[28]. Therefore ACC/AHA guidelines concluded that RRI may prove useful in identifying severe parenchymal disease, but could not be considered as predictors of an adverse or beneficial clinical outcome to renal revascularization[26].

CTA has proven to be the most frequent diagnostic test used to identify RAS. Multidetector computed tomography (MDCT) increased speed of image acquisition as well as spatial resolution. The disadvantages are the difficulties in estimating the degree of stenosis with extensive vessel wall calcification and the requirement of potentially nephrotoxic iodinated contrast (100 to 150 cc), especially harmful in ARAS patients, a population with increased prevalence of renal failure. Compared to MRA, CTA has higher spatial resolution and less artifacts due to implanted metal stents.

MRA requires intravascular contrast to enhance the imaging of blood vessels; however, gadolinium contrast is less nephrotoxic than the ionized contrast used for CTA. Therefore, MRA can be performed in patients with some degree of renal failure (although not in patients with severe renal insufficiency or those undergoing dialysis treatment due to the risk of nephrogenic systemic fibrosis with gadolinium contrast in subjects with a GFR <30 mL/minute/1.73 m^2), congestive heart failure, and dye allergy. The disadvantages are the cost, the impossibility to be used in patents with pacemakers or other metallic objects and to evaluate in-stent restenosis.

Arterial angiography remains the gold standard for the diagnosis of RAS, and is typically used only after a positive noninvasive screening test. Although more invasive than other techniques, the risk of complications is low (access-related complications, embolization, contrast-related allergic reactions, and contrast-induced nephropathy).

Receiving a Class III recommendation, **captopril scintigraphy**, **selective renal vein renin measurements**, **plasma renin activity**, and **captopril test** (measurement of plasma renin activity

after captopril administration), are not recommended as a screening test to diagnose RAS. Renal vein renin measurements may have some utility in establishing the indication for nephrectomy in patients with renal artery occlusion rather than in identifying patients with RAS who may benefit from revascularization.

Treatment

Crucial in ARAS treatment is an adequate risk factors' control for all patients. This includes glycemic control optimization, cholesterol decrease, smoking cessation, blood pressure reduction, and primary prevention with antiplatelets. According to ACC/AHA guidelines (Table 3), ACE inhibitors, angiotensin receptor blockers, calcium channel blockers, and beta-blockers all receive a class I indication for the treatment of hypertension associated with RAS[26]. The role of ACE inhibitors/angiotensin receptor blockers is crucial in ARAS treatment because of blocking RAAS system but specific attention has to be paid in patients with solitary functioning kidneys, severe bilateral stenoses, or advanced chronic kidney disease for the potential to induce acute renal failure. Under these conditions a rise in creatinine develops both because blood flow is affected and because angiotensin II supports filtration. Hence, the loss of glomerular filtration rate (GFR) reflects a functional signal that blood flow is threatened and filtration requires the supportive action of angiotensin. Most patients with hemodynamically relevant ARAS tolerate RAAS blockade without adverse effects. Recommendations for the use of these agents include re-evaluation of serum creatinine and potassium level soon (within a week) after initiating therapy, particularly in patients with reduced kidney function[29].

PTRAS is the treatment of choice for symptomatic ARAS. It had been demonstrated that balloon angioplasty alone was hampered by lower procedural success rate and a higher restenosis rate[30-34]. The superiority of renal stent placement over balloon angioplasty was confirmed in a RCT in hypertensive patients by van de Ven and coworkers[35], in whom patients with ostial ARAS were assigned to receive PTRA or PTRAS. Primary success rate (<50% residual stenosis) of PTRA was 57% compared with 88% for PTRAS and at 6 months, the primary patency rate was 29% for PTRA, and 75% for PTRAS. Restenosis after a successful primary procedure occurred in 48% of patients for PTRA and 14% for PTRAS.

Endovascular treatment is considered in case of hemodynamically significant stenosis. According to ACC/AHA guidelines[26] 'significant stenosis' is defined as a narrowing in lumen diameter:

(a) ≥50% and <70% by visual estimation with a peak translesional gradient (measured with a less than or equal to 5-Fr catheter or pressure wire) ≥20 mm Hg or a mean gradient ≥10 mm Hg,

(b) any stenosis ≥70%[36].

Indications for PTRAS are listed in Table 3.

Table 3: ACC/AHA guidelines[26]
Asymptomatic stenosis **Class IIb** **1. PTRAS may be considered for treatment of an asymptomatic bilateral or solitary viable kidney with a hemodynamically significant RAS. (Level of Evidence: C)** **2. The usefulness of PTRAS of an asymptomatic unilateral hemodynamically significant RAS in a viable kidney is not well established and is presently clinically unproven. (Level of Evidence: C)**
Hypertension **Class IIa** **1. PTRAS is reasonable for patients with hemodynamically significant RAS and accelerated hypertension, resistant hypertension, malignant hypertension, hypertension with an unexplained unilateral small kidney, and hypertension with intolerance to medication. (Level of Evidence: B)**
Ischemic nephropathy **Class IIa** **1. PTRAS is reasonable for patients with RAS and progressive chronic kidney disease with bilateral RAS or a RAS to a solitary functioning kidney. (Level of Evidence: B)** **Class IIb** **2. PTRAS may be considered for patients with RAS and chronic renal insufficiency with unilateral RAS. (Level of Evidence: C)**
Cardiac destabilization syndromes **Class I** **1. PTRAS is indicated for patients with hemodynamically significant RAS and recurrent, unexplained congestive heart failure or sudden, unexplained pulmonary edema. (Level of Evidence: B)** **Class IIa** **2. PTRAS is reasonable for patients with hemodynamically significant RAS and unstable angina. (Level of Evidence: B)**

Surgery for ARAS is limited to patients with clinical indications for intervention, especially those with multiple small renal arteries or early primary branching of the main renal artery and for patients with ARAS in combination with pararenal aortic reconstructions (in treatment of aortic aneurysms or severe aortoiliac occlusive disease).

Previous randomized clinical trials

The major RCTs published to date have been unable to prove a clinical benefit of endovascular revascularization for the treatment of ARAS. However, significant design flaws and selection bias affect many of these studies, limiting the usefulness of available data (Table 4 and 5).

Detailed below are the most important RCTs' bias:

- **SELECTION CRITERIA**

  Most of RCTs included patients without resistant hypertension or with only mild hypertension. The aforementioned practice guidelines recommendations[26] suggest that PTRAS is reasonable for patients with hemodynamically significant RAS and resistant hypertension, defined as the failure to achieve goal BP in patients who are adhering to full doses of an appropriate three-drug regimen that includes a diuretic. The mean number of antihypertensive drugs in the patients recruited in these trials was <3 in ASTRAL[37], 2 in DRASTIC[38] and ≤2 in the EMMA study[33]. In terms of renal function, the EMMA study[33] included patients with normal serum creatinine (mean levels 103 mcmol/L), in DRASTIC[38] and in CORAL[39] patients had preserved levels of eGFR (63.5 and 58 ml/min respectively). Therefore, considering the preserved renal function, easily controlled BP and exclusion of high risk patients (with recent congestive heart failure, unstable angina, stroke or TIA), the RCTs population represented a 'low-risk' atherosclerotic cohort. Finally, the most striking bias is represented by the following ASTRAL[37] inclusion criterion: "if the patient's doctor was uncertain that the patient would definitely have a worthwhile clinical benefit from revascularization". In practice, if a patient was thought to need revascularization within 6 months, he was excluded.

- **ENDPOINTS**

  Most of RCTs determined as primary endpoint the renal function but they assessed indexes of global renal function (as creatinine and/or creatinine clearance), and not separate estimates of GFR in the ischemic and the contralateral kidney. However, as a hemodynamically significant RAS is expected to lower GFR in the ischemic kidney and to

induce hyperfiltration in the contralateral kidney that is exposed to high BP, it is probably naïve to expect any improvement of indexes of overall GFR after lowering BP with PTRAS. Furthermore, the primary endpoint in ASTRAL[37] was the rate of decline in renal function assessed by measuring the mean slope of the reciprocal of the serum creatinine level over time. White in his well known Editor's page "Kiss my ASTRAL" declared: "How can revascularization improve renal function when 25% of the patients enrolled in the trial had normal renal function and another 15% had nearly normal renal function? Moreover, the hypertensive patients enrolled in this trial were taking an average of only 2.8 antihypertensive medications, with BP averaging in the 150/75 mmHg range. Are these patients likely to benefit from a renal artery stent?"[40]. In STAR[41] it is also worth noting that >50% had unilateral disease. This is important because bilateral disease is usually required to observe changes in creatinine clearance, which was the primary endpoint.

- **DEFINITION OF SIGNIFICANT STENOSIS**

  The older RCTs included patients with non-significant RAS according to later published ACC/AHA guidelines[26]. In SNARSCG[42] and DRASTIC[38] RAS was defined as greater than 50%, indicating that many non-hemodynamically significant lesions were treated. In STAR[41] of the 140 patients, 33% had only mild RAS (50%-70%). In ASTRAL[37] 41% had stenosis <70%.

- **TYPE OF INTERVENTION**

  DRASTIC[38] evaluated only the effect of PTRA without stenting like SNRASCG[42] and EMMA[33] trials (Table 5). Angioplasty without stenting represents suboptimal treatment in stenting era.

- **ANALYSIS OF RESULTS**

  Most RCTs performed an intention-to-treat analysis (with the exception of EMMA[33] and SNRASCG[42]) and the percentage of patients who crossed over from medical to endovascular treatment was 44% in DRASTIC[38] (because of persistent hypertension despite treatment with three or more drugs or because of deterioration of renal function), and 6% in ASTRAL[37] but 17% of those originally randomized to endovascular treatment did not receive it. In STAR[41] 12 of 64 patients (19%) in the stenting arm had RAS <50% and did not receive a stent but were still analyzed on a intention-to-treat basis. An additional 6 patients in the stent arm did not receive a stent (one received balloon angioplasty, one died before stent placement, two declined the stent, and two had technical failures) but all were analyzed in endovascular group.

- **PERI-PROCEDURAL ADVERSE EVENTS**

  In ASTRAL[37] the major adverse event rate of 9% is fourfold higher than the 2.4% observed in the ODORI Registry[43] and the ASPIRE-2 Study[44]. During the 7 years of recruitment, 24 centers randomized between one and five patients (42% of all randomized patients) and 35 centers randomized 10 patients or less (65% of all randomized patients). This means that 65% of all participating centers randomized fewer than one patient per year[40].

Table 4: Randomized clinical trials characteristics: definition of ARAS and inclusion/exclusion criteria[45]

Study Name/ Year	N° pts	Definition Of "Substantial" ARAS	Inclusion Criteria	Exclusion Criteria
EMMA 1998	49	Unilateral ≥75% without thrombosis or ≥60% with positive lateralization test*	- DBP >95 mmHg - GFR ≥50 ml/min	- malignant hypertension - stroke or AMI in previous 6 months - pulmonary edema - DBP >109 mmHg at end of run-in
SNRASCG 1998	55	Uni- or bilateral ≥50%	- DBP >95 mmHg with at least 2 drugs	- SCr >500 µmol/L - stroke or AMI in previous 3 months
DRASTIC 2000	106	Uni- or bilateral ≥50%	- DBP >95 mmHg with at least 2 drugs	- SCr>200 µmol/L - cancer - other forms of secondary hypertension - HF or unstable angina - single kidney with SCr >150 µmol/L - stenotic kidney <8 cm - renal artery occlusion - aortic aneurism needing surgery
STAR 2009	140	Uni- or bilateral ≥50%	- GFR (Cockcroft Gault) <80 ml/min	- stenotic kidney <8 cm - renal artery <4 mm - GFR <15 ml/min - diabetes with proteinuria >3 g/die - malignant hypertension
ASTRAL 2009	806	Uni- or bilateral 'substantial anatomical stenosis'	'if the patient's doctor was uncertain that the patient would definitely have a worthwhile clinical benefit from revascularization'	- need of surgery or high revascularization probability in 6 months - non atherosclerotic cardiac disease - previous renal revascularization
CORAL 2013	947	Uni- or bilateral with: - ≥60% with a ≥20 mmHg systolic pressure gradient, or - if ≥80% (no pressure gradient required)	SBP ≥155 mmHg on ≥2 drugs	- DBP ≥120 mmHg and/or SBP ≥200 mmHg - stroke or TIA within 3 months or known carotid stenosis ≥70% - major surgery, trauma, revascularization procedure, unstable angina, or AMI in previous 30 days - hospitalization for HF within 3 months - ejection fraction <30% - diabetes with either: a. proliferative retinopathy and ≥1+ protein on urine dipstick, or b. ≥1+ protein on urine dipstick and urine protein/Cr ratio >0.5 - kidney size <8 cm - SCr >3.0 mg/dl - aneurysm of the abdominal aorta >4.0 cm - previous renal artery bypass surgery or angioplasty or stent intervention or kidney transplant - intolerance to iodinated contrast, statin or antiplatelets

*venouspielography, renal scintigraphy, renal venous renin concentration; ABPM=ambulatory BP monitoring; AMI=acute myocardial infarction; BP=blood pressure; CKF=chronic kidney failure; DBP=diastolic BP; DDD=defined daily dose; GFR=glomerular filtration rate; HF= heart failure; OBP=official BP; SCr=serum creatinine; n.a.=not available.

Table 5: Randomized clinical trials characteristics: endpoints, follow-up timing and types of analysis[45]

Study Name	Primary Endpoints	Outcome	Follow-Up (Months)	Intention-To-Treat	Crossing-Over N° (%)	Stenting (%)	Bilateral Stenosis (%)
EMMA	- BP by means of ABPM	No difference in BP and creatinine clearance. DDD: 1.78 in Med vs. 1.0 in PTRA group (P=0.009)	6	no	7 (27%)	8.7	0
SNRASCG	- OBP - SCr	No BP difference in unilateral ARAS. Lower BP in bilateral ARAS: 171/91 in Med vs. 152/83 in PTRA (P<0.01)	12	no	0	0	50.9
DRASTIC	- OBP	No difference in BP at 3 and 12 months. N° drugs: 2.4 in Med vs. 1.9 in PTRA group (P<0.01)	12	yes	22 (44%)	3.6	22.6
STAR	- GFR increase ≥20%	No difference in rates of developing a fall in GFR	24	yes	1 (1.3%)	71.8	48
ASTRAL	- mean slope of the reciprocal of serum SCr over time	No difference in BP, serum creatinine, mortality, CHF at 33 months (median)	60	yes	24 (6%)	95	53.5
CORAL	- event-free survival from cardiovascular and renal adverse events (composite of cardiovascular or renal death, stroke, AMI, hospitalization for HF, progressive renal insufficiency, or need for permanent renal replacement therapy)	No significant difference in regards to the composite endpoint, any of the individual components of the composite endpoints, or all-cause mortality. SBP favoring the PTRA group (−2.3 mmHg P=0.03)	60	yes	19 (4%)	100	20

ABPM=ambulatory BP monitoring; AMI=acute myocardial infarction; BP=blood pressure; CKF=chronic kidney failure; DBP=diastolic BP; GFR=glomerular filtration rate; HF=heart failure; OBO=official BP; SBP=systolic BP; SCr = serum creatinine; n.a. =not available. In underlined the significant differences between groups.

Meta-analysis

Meta-analysis published to date including CORAL trial[45-49], reported that the available data are insufficient to conclude that revascularization in the form of balloon angioplasty, with or without stenting, is superior to medical therapy for the treatment of ARAS. However, PTRAS does seem to have a small drug-saving effect and may result in a small improvement in diastolic BP. The clinical importance of these benefits is unclear as there is no evidence that these improvements translate into improved cardiovascular and renal outcomes. Therefore, balloon angioplasty appears safe and results in similar numbers of cardiovascular and renal adverse events compared to medical therapy.

There remains a need for further identification of subjects and appropriate indications in hopes of improving outcomes and avoiding unnecessary procedures in patients who would not benefit from treatment.

The Cochrane's authors concluded that: "Further well-conducted randomized controlled trials comparing the effect of medical therapy and balloon angioplasty in patients with atherosclerotic renal artery stenosis should be performed to overcome methodological errors evident in the published literature. These trials should ensure a sufficient number of participants are included to provide statistical power, unambiguous participant selection criteria, blinded outcome assessment, and follow-up of three or more years in order to evaluate the long-term effect of the interventions on the preservation of renal function"[46].

MATERIALS AND METHODS

METRAS study design

The decision to revascularize renal arteries in ARAS usually is based on the assumption that ischemia is partially responsible for the decrease in kidney function and that correcting the stenosis and restoring kidney perfusion will stabilize or improve glomerular filtration. The ultimate aim of the treatment is to avoid or at least delay the need for renal replacement therapy. The results of published RCTs[37,39] discouraged physicians to perform PTRAS except for patients with compelling indications to treatment, as indicated in ACC/AHA guidelines[26].

The primary objective of METRAS was to evaluate the GFR modification after PTRAS compared to medical therapy in order to increase the knowledge on blocking or delaying ischemic nephropathy. The trial protocol has been published[49] and is available online (https://clinicaltrials.gov/ct2/show/NCT01208714).

1. OBJECTIVES

The primary objective of the METRAS study is to determine if PTRAS is superior or equivalent to optimal medical treatment for preserving GFR in the ischemic kidney.

Secondary objectives are to determine if the two treatments are equivalent in:

1) lowering BP;

2) preserving overall renal function, as assessed by global estimated GFR and the reciprocal of serum creatinine, and indexes of Ca^{2+} and PO_4^{3-} metabolism;

3) decreasing the damage in target organs of hypertension, including cardiac hypertrophy, microalbuminuria, and aortic stiffness;

4) improving quality of life.

2. STUDY DESIGN

METRAS Study is a prospective multicenter randomized, unblinded two-arm study.

a. Enrolment.

Hypertensive patients of both genders (age >18 years) were eligible if they had radiological evidence of unilateral or bilateral RAS. Patients with clinical evidence suggestive of RAS underwent angio-CT to identify RAS. Clinical evidence of RAS was defined as unexplained renal

dysfunction (GFR <60 ml/min)[50], uncontrolled or refractory hypertension, and/or significant worsening of renal function (defined as 20% increase of serum creatinine) after administration of a renin-angiotensin system blockers (ACE inhibitors or ARBs), and/or an abdominal bruit, and/or evidence of atherosclerotic involvement of other sites. Eligible subjects were offered enrolment in the study and must sign a written consent.

b. Inclusion Criteria

Patients were recruited if they had RAS determining an area stenosis of the main renal artery lumen or its major branches either ≥70% or, if <70, with post-stenotic dilatation at angio-CT. Fulfillment of these criteria will be assessed at the core laboratory of the coordinating center.

c. Exclusion Criteria

Exclusion criteria were:

1) detection of non-significant acceleration (PSV <1.8 m/sec) in renal arteries and homogeneity between the Doppler resistance indexes of the upper, medium, and lower interlobar arteries (when clearly visualized) or evidence of ischemic kidney diameter <10 cm, and/or differences between kidney diameters >20%,

2) compelling indication to PTRAS, for example, sub-occlusive stenosis of the renal artery (>95%) and/or rapidly worsening renal function,

3) refusal to participate in the study,

4) previous endovascular or surgical treatment of RAS,

5) fibromuscular RAS,

6) planned or ongoing pregnancy, or childbearing potential without adequate measures to prevent pregnancy,

7) life expectancy <2 years,

8) current participation to another trial possibly influencing the safety of the patient and/or the outcomes of the study,

9) co-morbid conditions or any other circumstances likely to limit participation and availability to long-term follow-up studies.

d. Run-in period

Eligible patients underwent a 4-8 weeks run-in period during which BP treatment was optimized, LDL-cholesterol was lowered to below 2.17 mmol/L (80 mg/dL), homocysteine if elevated was lowered possibly to less than 15 μmol/L (with folate and vitamin B6/B12 supplementation), and treatment for diabetes was optimized to achieve, if feasible, a HbA1c <6.5% (Fig 7).

As these patients are to be considered at high-risk anti-hypertensive treatment was adjusted to attain a BP <130/85 mmHg. However, due to vascular remodeling this target BP value will be difficult to reach in these patients[51], therefore, a target BP level <140/90 mmHg was acceptable. All classes of antihypertensive drugs were allowed paying utmost attention to the changes in serum creatinine during treatment, particularly if ACE-inhibitors and ARB were needed. Full doses of antihypertensive medications, including a diuretic, were expected to allow most patients to reach the target value.

Statins and/or ezetimibe (in patients with history of rabdomyolysis with statins) were titrated to achieve a LDL-cholesterol <2.07 mmol/L (80 mg/dl). Treatment with oral antidiabetic agents and/or insulin was adjusted to reach post-prandial glycaemia <11 mmol/L. All patients received antiplatelet treatment with the same dose of aspirin (100 mg o.d.) or clopidogrel (75 mg o.d.), or, if intolerant to ASA and clopidogrel, ticlopidine (250 mg b.i.d), throughout the study period. Cessation of smoking was strongly recommended.

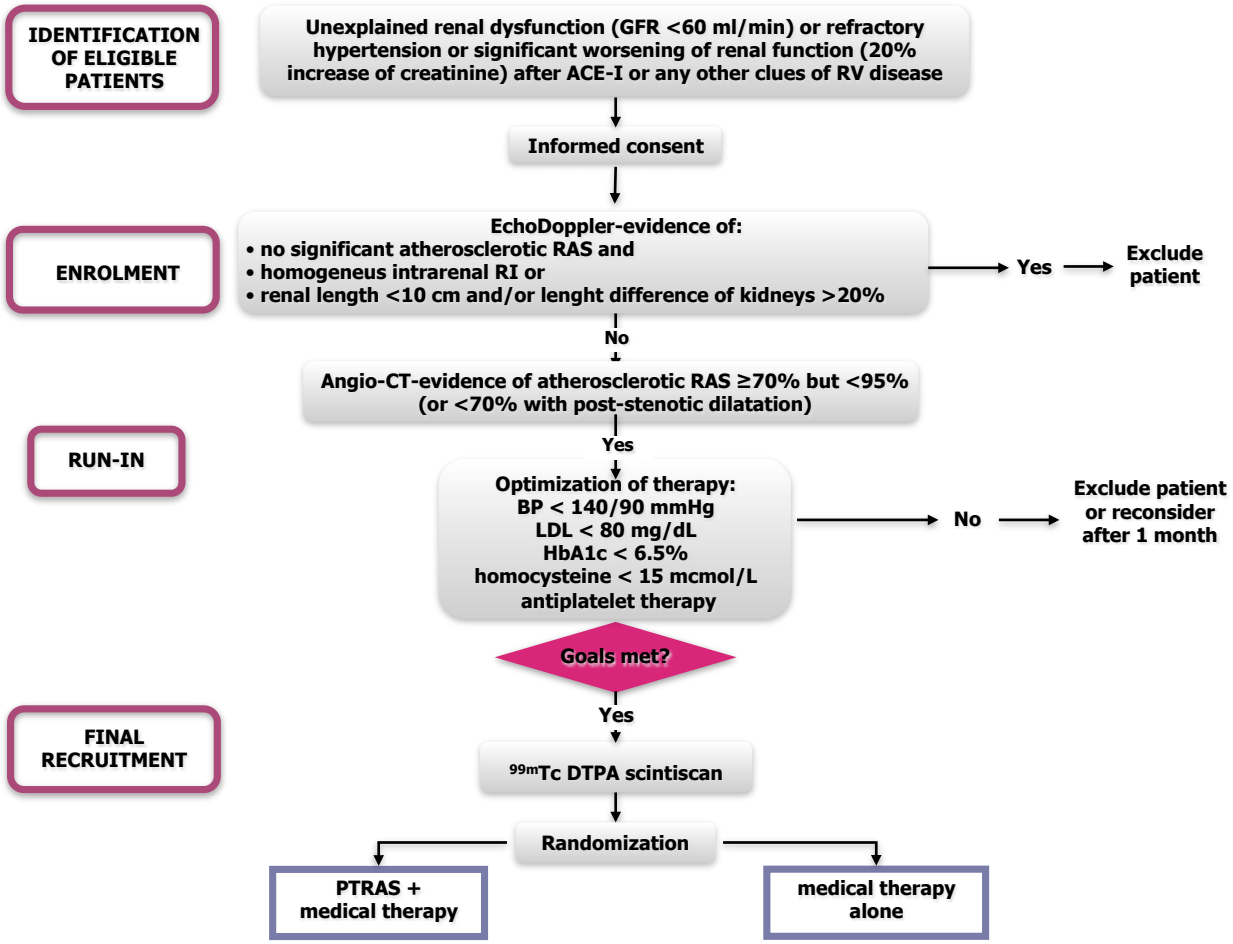


Fig. 7. Study design

e. Imaging and renal function.

Angio-CT was performed to assess the abdominal aorta and renal arteries anatomy and pathology. The evaluation of grade of stenosis was performed by means of the assessment of cross sectional area with a specific software (Vessel analysis, Syngo Siemens, Erlangen, Germany). Utmost precautions were adopted to minimize the chances of worsening renal function with angio-CT. The site of the RAS was assessed by two experienced radiologists. Post-stenotic dilatation was also determined and taken as a surrogate marker of hemodynamically relevant RAS whenever the luminal narrowing was <70% at biplane angiography.

When more renal arteries were detected, the patient was enrolled if a stenosis ≥70% or post-stenotic dilatation was found in the largest artery.

All on-site measurements from imaging were validated by the core laboratory in Padova.

f. Assessment of kidney perfusion.

^{99m}Tc-DTPA renal scintigraphy was performed on 2 consecutive days, before and after captopril administration, according to guidelines (http://interactive.snm.org/docs/pg_ch16_0403.pdf). If serum creatinine level after angio-CT did not differ significantly from that measured before, the patient discontinued ACEI and/or ARBs for 1 week and then underwent ^{99m}Tc-DTPA sequential renal scintigraphy. If an increase in serum creatinine levels >20% from baseline occurred after angioCT, scintigraphy was delayed until full restoration of renal function.

The captopril dose (50 mg per os) was given 60 min before injection of ^{99m}Tc-DTPA (3.7 MBq/kg body weight) and a similar dose was injected for the basal study, at least after 24 h. BP was checked every 15 min after captopril administration. Before the scintigraphy ACEI/ARB were stopped for 3-7 days (depending on drug half-life); if withdrawal was judged to be unsafe, the captopril scintigraphy was omitted and only baseline ^{99m}Tc-DTPA scintigraphy was performed.

Scintigraphy data analysis and split GFR were calculated with a specific software on a nuclear medicine workstation. The captopril scintigraphy was considered positive when a reduction of 10% or more in split renal function was observed. All scintiscan data were evaluated and validated at the core laboratory in Padova.

g. Assessment of renal function.

Global renal function was assessed by means of glomerular filtration rate (GFR) using the CKD-EPI formula[50], and by urinalysis, and measurement of serum (S)-erythropoietin, S-electrolytes, S-creatinine, S-urea, S-homocysteine, cystatin C, uric acid, PTH, PTH-related peptide (PTHr), 25-OH and 1-25-OH vitamin D.

h. Assessment of target organ damage (TOD).

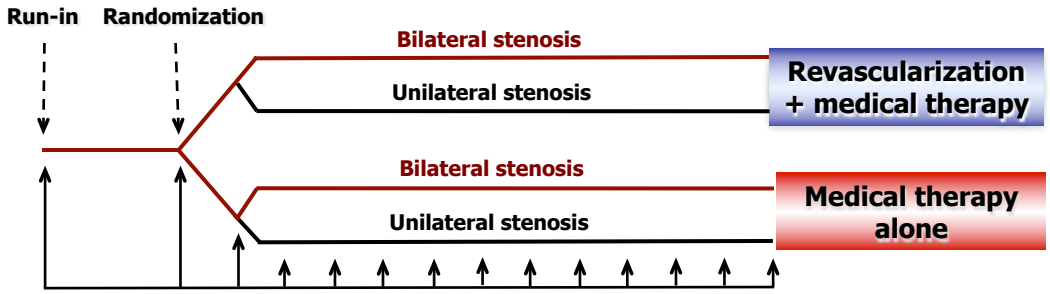
Besides the anthropometric and clinical data, total serum homocysteine, glycosylated hemoglobin, lipids (total cholesterol, HDL- and LDL-cholesterol, triglycerides) were determined as indexes of metabolic risk. Transthoracic echocardiogram with Doppler to assess left ventricular mass index (LVMI), relative wall thickness (h/r), the E/A wave peak flow velocity rate and tissue Doppler were used to detect changes in LV remodeling and diastolic filling changes[52-53]. Left ventricular mass was calculated by means of the Devereux's formula[54] normalized for BSA and height.

i. Assessment of blood pressure.

Clinic and 24 hours ambulatory blood pressure monitoring (ABPM) were performed to evaluate the changes of BP values in each treatment arm.

j. Randomization.

The patients were randomly assigned to either PTRAS on top of optimal medical therapy (revascularization arm) or to optimal medical therapy alone (medical therapy arm) by means of an algorithm at the core laboratory (see later). Because the number of the patients with bilateral stenosis was smaller than that with unilateral stenosis, the algorithm considered unilateral/bilateral stenosis to achieve an equal number of patients in each treatment arm (Fig. 8)



Time (mo)	1	0	1	3	6	12	18	24	30	36	42	48	54	60
	t-1	t0	t1	t2	t3	t4	t5	t6	t7	T8	T9	t10	T11	t12
Clinical evaluation	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Lab tests	x	x	x	x	x	x		x		x		x		x
Scintiscan		x			x	x		x		x		x		x
Renal echoDoppler	x		x	x		x		x		x		x		x
AngioTC	x					x								x
Echocardiography		x			x	x		x		x		x		x
ABPM	x	x	x		x	x		x		x		x		x

Fig. 8. Flow-chart

k. Treatment arms

Revascularization. In the patients randomized to PTRAS lesions involving the ostium and/or the main renal artery were treated; more distal stenoses were recorded for data analysis purposes but not treated. A balloon-expandable stent (Palmaz Genesis on Cordis AMIIA Delivery System) was

implanted during PTRA. After PTRAS the patients continued on their antihypertensive drug regimen. The dose and number of drugs were down-titrated, if necessary, to reach a target BP values <130/80 mmHg for systolic and diastolic.

Medical therapy. The patients randomized to medical treatment were continued on the drug regimen optimized during the run-in period. Adjustment of antihypertensive drug therapy allowed to achieve the best possible control of BP; changes of treatment (number and doses of each drug) were recorded for the purpose of data analysis.

l. Follow-up.

Visits were scheduled at 1 (t1), 3 (t2), 6 (t3), 12 (t4) and 24 (t5) months after revascularization or beginning of the medical therapy (t0). The study then continued in an observational setting with outpatient visits scheduled at 1 year intervals for a total of 5 years.

m. Duration of the Study

The METRAS Study will last for 5 years. This time interval is expected to be sufficient to register renal and major cardiovascular events.

3. EXPERIMENTAL ENDPOINTS

The GFR value assessed by ^{99m}Tc-DTPA in the ischemic kidney was used as a quantitative variable and compared between groups at each time point during follow-up. For the purpose of Cox regression analysis a categorical definition of kidney loss, defined as fall of GFR of the ischemic kidney to <5 ml/min, was used; rate of achievement of such endpoint were compared.

4. HANDLING OF POTENTIAL CONFOUNDERS

To minimize the untoward effect of differences in CV risk management, all patients were given anti-platelet, lipid-lowering, and anti-diabetic agents if needed. These treatments were up-titrated to reach the desired effect or to the daily defined dose (DDD)[55] if tolerated. They were thereafter maintained at the dose achieved at the end of the run-in period throughout the study, unless otherwise indicated.

5. DATA COLLECTION and STATISTICAL ANALYSIS

Data were collected using specifically predefined forms in an *ad hoc* designed database. The building up of the database was monitored in real time at the core laboratory.

Power calculations.

Power calculation (nQuery Vers, 6.0, Statistical Solutions) showed that assuming a 16% drop-out rate, a common GFR standard deviation (SD) of 8.0 ml/min, an equal sample size in each treatment arm, using a two group t-test with a 0.05 2-sided significance level, with 60 patients per arm our study will have a 99% power to detect a difference in means of GFR in the vascularized (or control untreated kidney) of 7.5 ml/min.

To obtain homogeneous distribution of characteristics of patients between arms, we used the Treatment Allocation Procedure for Sequential Clinical Trial[56]. The allocation was performed considering baseline total GFR at NAFS, presence of diabetes mellitus, stenosis <70 or >=70%, age <68 or >=68 years, and gender.

Continuous variables are expressed as means and SD and were compared at Student's t-tests. Comparison of GFR by NAFS in the ischemic kidney between groups (revascularization vs. medical therapy) at each time point was performed using Student's t-test, repeated measures ANOVA with adjustment of potential confounders, and a repeated measures generalized linear model analysis (GLM), Method A. GFR in ischemic kidney was also derived from eGFR (with CKD-EPI formula[50]) multiplied for percentage of filtration assessed by NAFS, obtaining new GFR in ischemic kidney at each time-point (Method B).

We used mean substitution to replace missing values at each time-points, because not all the patients completed the follow-up.

All analyses were performed with the use of SPSS software, (IBM).

5. EXPECTED RESULTS.

With its high power this study should clarify whether PTRAS on top of optimal medical therapy is superior or equivalent to the latter alone in preventing deterioration of GFR in the ischemic kidney. Assessment of secondary endpoints will elucidate some clinically relevant issues concerning BP lowering and preserving renal function.

RESULTS

Patients

From June 2010 through April 2015, thirty patients were evaluated for inclusion in the METRAS trial at Hypertension Clinic in-patient or out-patient facilities, Centre of Excellence of the European Society of Hypertension, Department of Medicine, University of Padua. Ten of these patients did not meet the inclusion criteria and therefore were excluded: six for compelling indication to PTRAS according to the AHA/ACC guidelines[26] (for sub-occlusive renal artery stenosis with rapid declining renal function), two for non-significant stenosis, one for fibromuscular dysplasia; one refused to participate to the study.

During the study, two additional patients were excluded for lack of adherence to the protocol as they failed to attend the regular outpatient visits and follow-up exams.

Up to April 2015, eighteen patients were enrolled, ten in the endovascular and eight in the medical treatment arm (Fig. 9). All the patients had unilateral stenosis with a mean percentage of stenosis of 76% in both groups. 90% of patients had a cross sectional area stenosis >70%. The two patients with stenosis <70% had also a post-stenotic dilation, which according to the protocol was held to indicate a hemodynamically significant stenosis.

No crossover of patients from one to the other treatment arm occurred.

The baseline features of the patients assigned to the PTRAS and the medical therapy arms are shown in the table 6. The mean age of the cohort was 68±9 years, there was a trend toward more females and diabetics in the PTRAS group, but the difference was not significant.

Mean 24 hours BP was 138/73 mmHg in the medical treatment group and 131/70 mmHg in the endovascular group with a mean number of drugs of 3.6 and 3.1, respectively.

Renal function did not differ between groups assessed by NAFS in terms of total GFR and GFR measured separately in two kidneys nor by bioumoral parameters (GFR estimated with CKD-EPI formula, serum creatinine and cystatin-c).

Left ventricular mass assessed at echocardiography was similar in the two arms.

None of the baseline characteristics differed between the two groups.

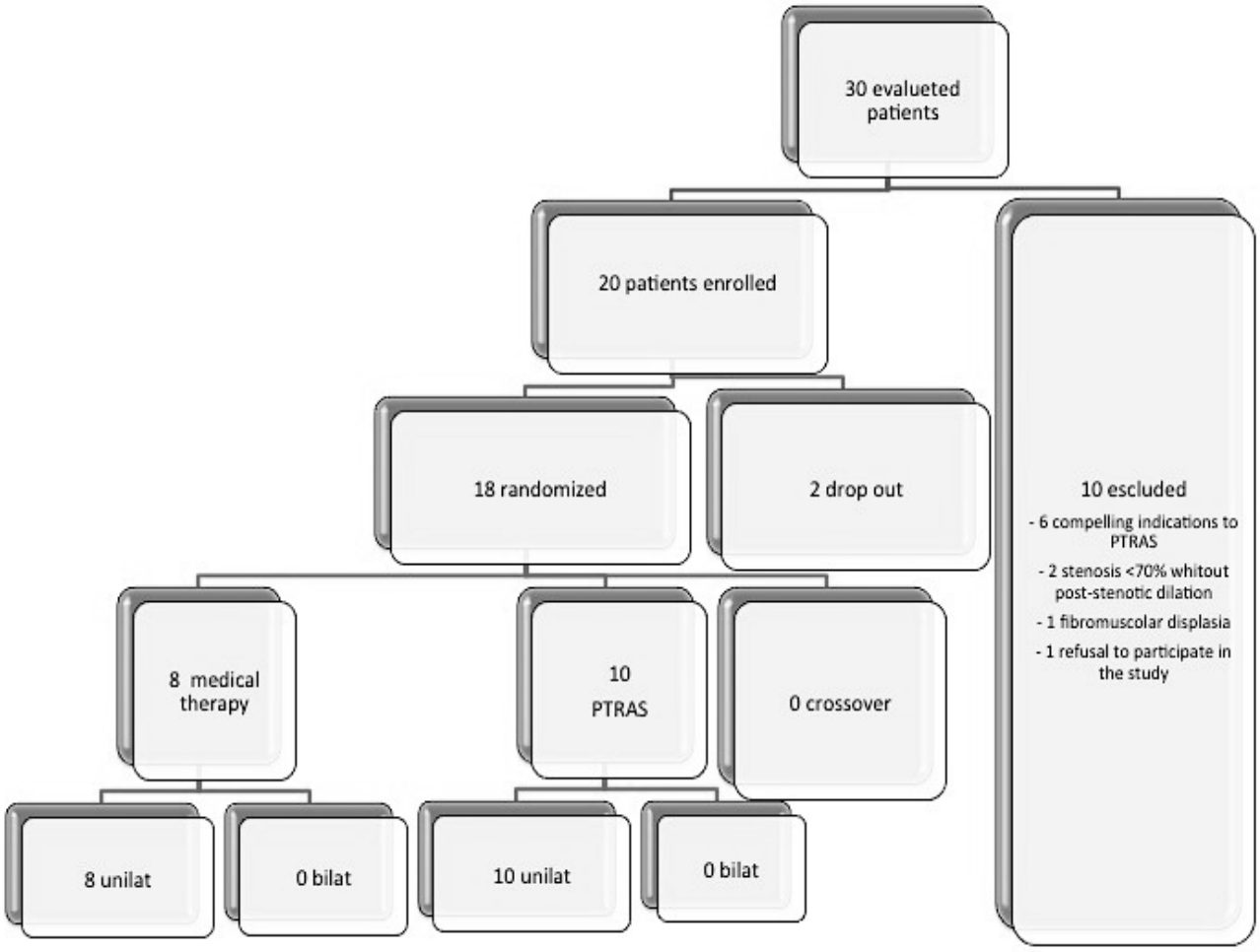


Fig. 9. Screening, Randomization, and Follow-up.

At completion of my PhD course the median follow-up, which is still ongoing, was 36 months.

In the revascularization group all the patients underwent angiography plus stenting without major complications. The success rate of the procedure was 100% without residual stenosis. There was an intra-stent restenosis <50% at 6 month after PTRAS in 1 patient who did not require re-intervention for the stability of clinical, bioumoral, and scintigraphyc parameters. This patient got closer follow-up visits.

All the patients in both groups were treated with antiplatelets therapy according to the METRAS protocol and with antihypertensive agents including an ACE-I or ARB (except 2 in PTRAS group under therapy with calcium channel blocker and/or beta-blocker).

Tab. 6 Baseline characteristics of population		
VARIABLES	**MEDICAL THERAPY (n=8)**	**PTRAS (n=10)**
Age (years)	69±10	67±8
Male/Female (%)	4 (50) / 4 (50)	2 (20) / 8 (80)
% of stenosis	76±7.3	76.1±7.6
Degree of Stenosis: (n. of patients and %)		
<70%	1 (12.5)	1 (10)
70-80%	5 (62.5)	5 (50)
>80%	2 (25)	4 (40)
Body Mass Index (Kg/m^2)	26.2±5.6	24.9±3.5
Diabetes (%)	1 (12.5)	3 (30)
24 hours SBP (mmHg)	138±14	131±14
24 hours DBP (mmHg)	73±8	70±9
Number of Drugs	3.6±1.5	3.1±1.4
total GFR by NAFS (ml/min/m^2)	71.4±22.5	80±21.2
GFR in ischemic kidney by NAFS (ml/min/m^2)	33.4±12.7	29.7±10.4
GFR in contralateral kidney by NAFS (ml/min/m^2)	38±14.3	50.3±19.2
Serum Creatinine (mcmol/L)	83.7 ±23	70 ±11
Serum Cystatin-c (mg/L)	0.90±0	0.92±0.23
eGFR with CDK-EPI (mg/ml/1,73m^2)	81.1±15.4	88.2±15.1
Microalbuminuria (mg/g creatinine)	33.7±35	15.7±11
Glycosylated hemoglobin (mmol/mol)	44.7±14.4	43.1±6.7
Total Cholesterol (mmol/L)	4.2±0.8	4.7±1.3
HDL (mmol/L)	1.2±0.3	1.3±0.3
Triglycerides (mmol/L)	1±0.3	1.3±0.1
LDL (mmol/L)	2.5±0.7	2.9±1.1
Homocysteine (mcmol/L)	17±6.1	11.8±4.9
Left ventricular mass normalized for Body Surface Area (g/m^2)	114.7±16.7	110±15.8
Left ventricular mass normalized for height (g/m$^{2.7}$)	52.2±7.7	50.1±9.5
Relative Wall Thickness	0.55±0.15	0.49±0.07
Resistive index in ischemic kidney (%)	71.4±9.4	69.7±7.7
Resistive index in contralateral kidney (%)	73.2±8.1	75±4.6
None of variables differed between groups.		

PRIMARY ENDPOINT

GFR in the ischemic kidney assessed by NAFS: method A

At repeated measures generalized linear model analysis (GLM) that included age and DBP at baseline as covariates, GFR in the ischemic kidney as assessed at NAFS increased compared to medical treatment at 3 years follow-up, albeit it was borderline significant (p=0.07).

GFR in the ischemic kidney assessed by NAFS: method B

At GLM that included age and DBP at baseline as covariates, GFR in the ischemic kidney as assessed at NAFS increased compared to medical treatment at 3 years follow-up (p=0.048).

GFR in the ischemic kidney assessed by NAFS excluding the 3 years time-point: method B

We performed the previous analysis excluding the 3 years time-point, in whom the number of patients was too small. At GLM that included age and DBP at baseline as covariates, GFR in the ischemic kidney as assessed at NAFS increased compared to medical treatment at 3 years follow-up (p=0.027) (Fig. 10 and Tab. 7).

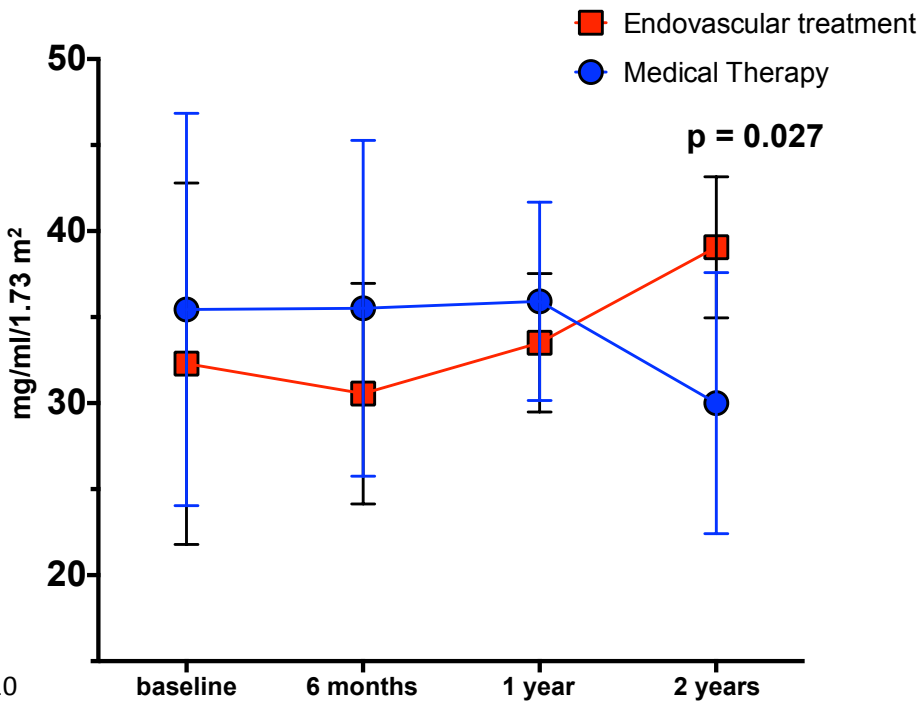


Fig. 10

Tab. 7 GFR in ischemic kidney from eGFR X % by NAFS (ml/min/1.73m^2)								
	BASELINE		6 MONTHS		1 YEAR		2 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**35.4**	11.4	**35.5**	9.8	**35.9**	5.8	**30.0**	7.6
PTRAS	**32.1**	10.0	**29.0**	7.7	**31.0**	8.5	**39.0**	3.9

GFR in the contralateral kidney assessed by NAFS: method A

At GLM including as covariates age and DBP at baseline, GFR in the contralateral kidney as assessed at NAFS did not differ in the two groups at 3 years follow-up (p=0.642).

GFR in the contralateral kidney assessed by NAFS: method B

At GLM including as covariates age and DBP at baseline, GFR in the contralateral kidney as assessed at NAFS did not differ in the two groups at 3 years follow-up (p=0.140) (Fig. 11 and Tab. 8).

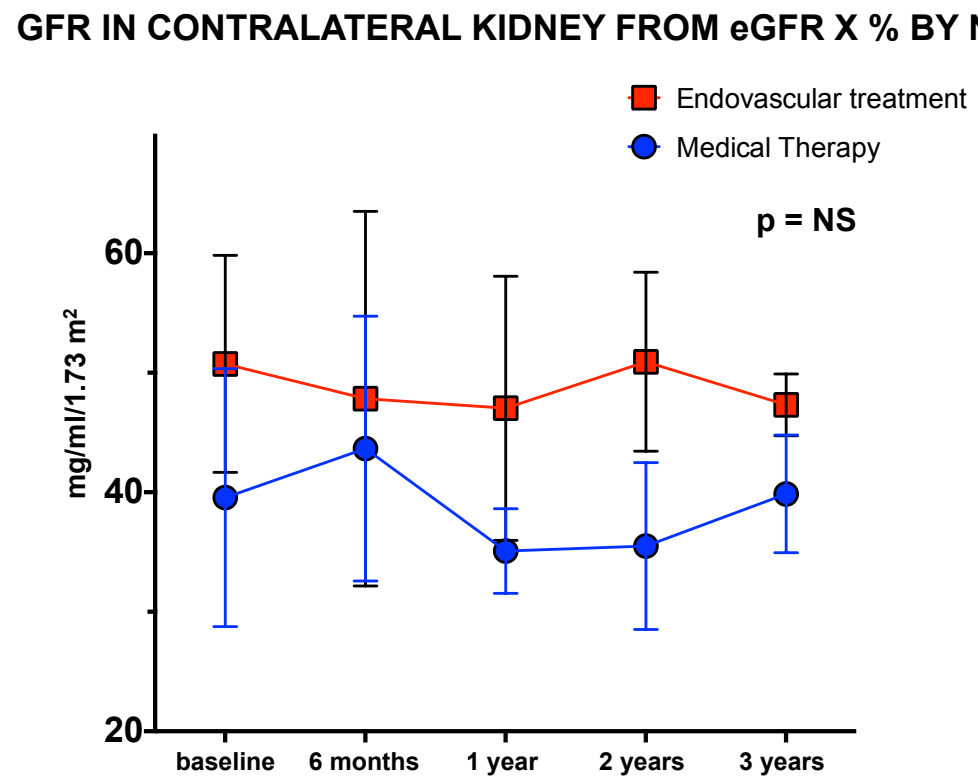


Fig. 11

Tab. 8 GFR in contralateral kidney from eGFR X % by NAFS (ml/min/1.73m^2)										
	BASELINE		6 MONTHS		1 YEAR		2 YEARS		3 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**39.5**	10.8	**43.6**	11.1	**35.1**	3.5	**35.5**	7.0	**39.9**	4.9
PTRAS	**50.7**	9.1	**47.8**	15.7	**47.0**	11.0	**50.9**	7.5	**47.3**	2.6

Total GFR assessed by NAFS

At GLM including as covariates age and DBP at baseline, total GFR as assessed at NAFS did not differ in the two groups at 3 years follow-up (p=0.713) (Fig. 12 and Tab. 9).

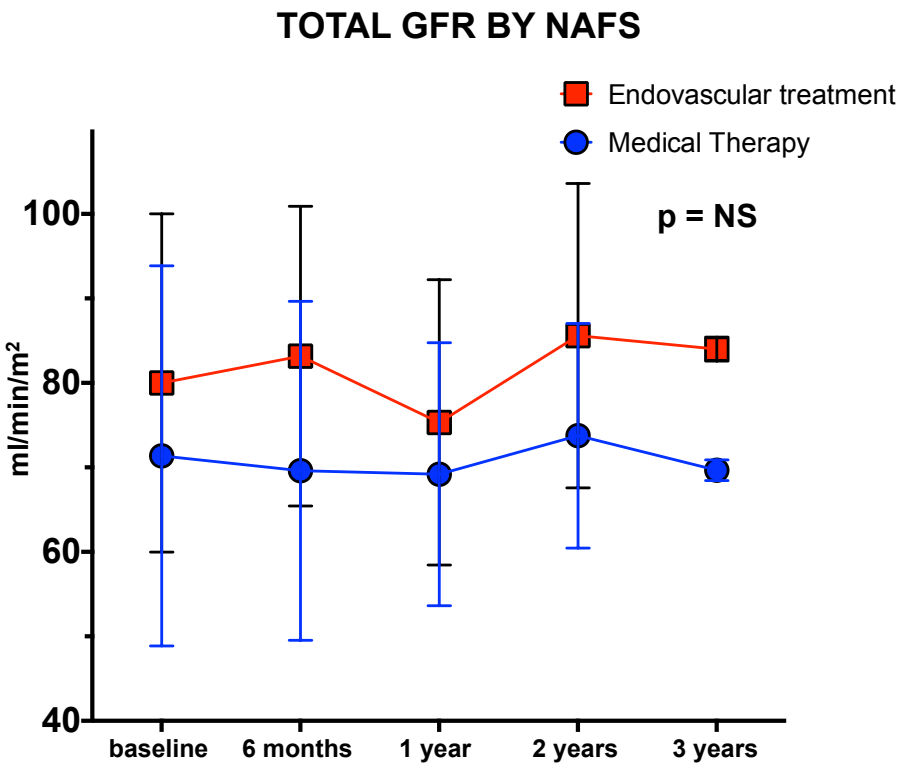


Fig. 12

Tab. 9 Total GFR assessed by NAFS (ml/min/m^2)										
	BASELINE		6 MONTHS		1 YEAR		2 YEARS		3 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**71.4**	22.5	**69.6**	20.1	**69.2**	15.6	**73.7**	13.3	**69.7**	1.2
PTRAS	**80.0**	20.0	**83.2**	17.7	**75.3**	16.9	**85.6**	18.0	**84.0**	1.4

SECONDARY ENDPOINTS

Blood pressure control: 24 hours Systolic Blood Pressure assessed by ABPM

At GLM including as covariates age, sex, and DBP at baseline, 24 hours SBP did not differ between the groups during 3 years follow-up (p=0.117) (Fig. 13 and Tab. 10).

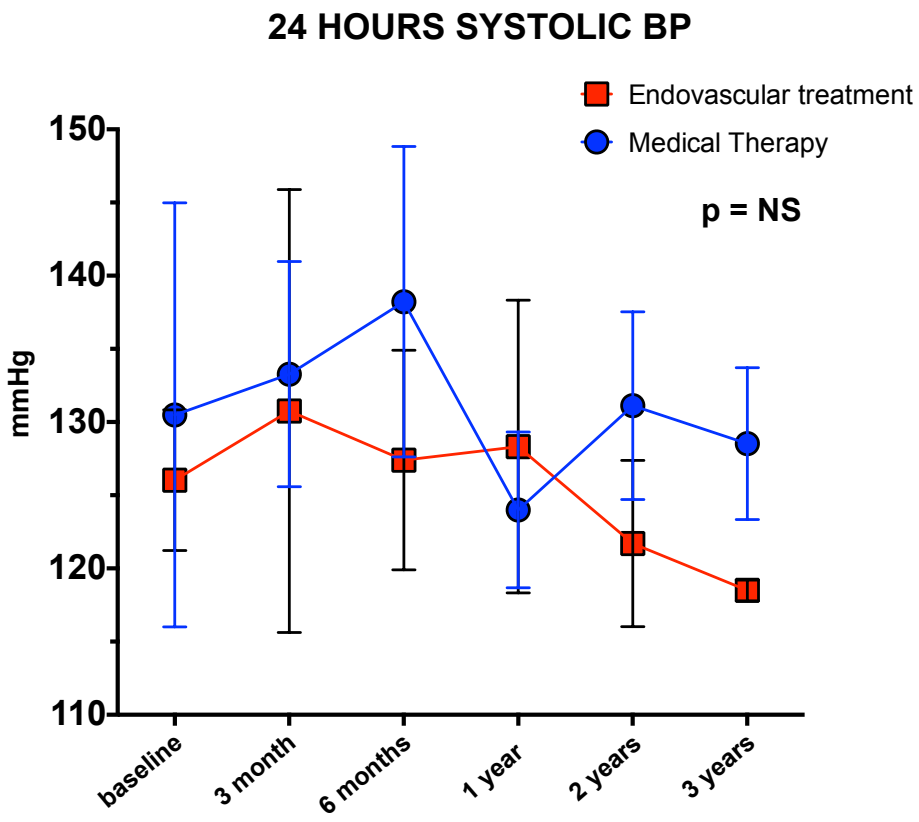


Fig. 13

Tab. 10 24 hours SBP (mmHg)												
	BASELINE		3 MONTHS		6 MONTHS		1 YEAR		2 YEARS		3 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**130.5**	14.5	**133.3**	7.7	**138.2**	10.6	**124.0**	5.3	**131.1**	6.4	**128.5**	5.2
PTRAS	**126.0**	4.8	**130.7**	15.1	**127.4**	7.5	**128.3**	10.0	**121.7**	5.7	**118.5**	0.7

Blood pressure control: 24 hours Diastolic Blood Pressure assessed by ABPM

At GLM including as covariates age and DBP at baseline, 24 hours DBP diverged between the PTRAS group and the medical therapy starting from 6 months post-revascularization. At 3 years follow-up it remained significantly lower (p=0.029) (Fig. 14 and Tab. 11).

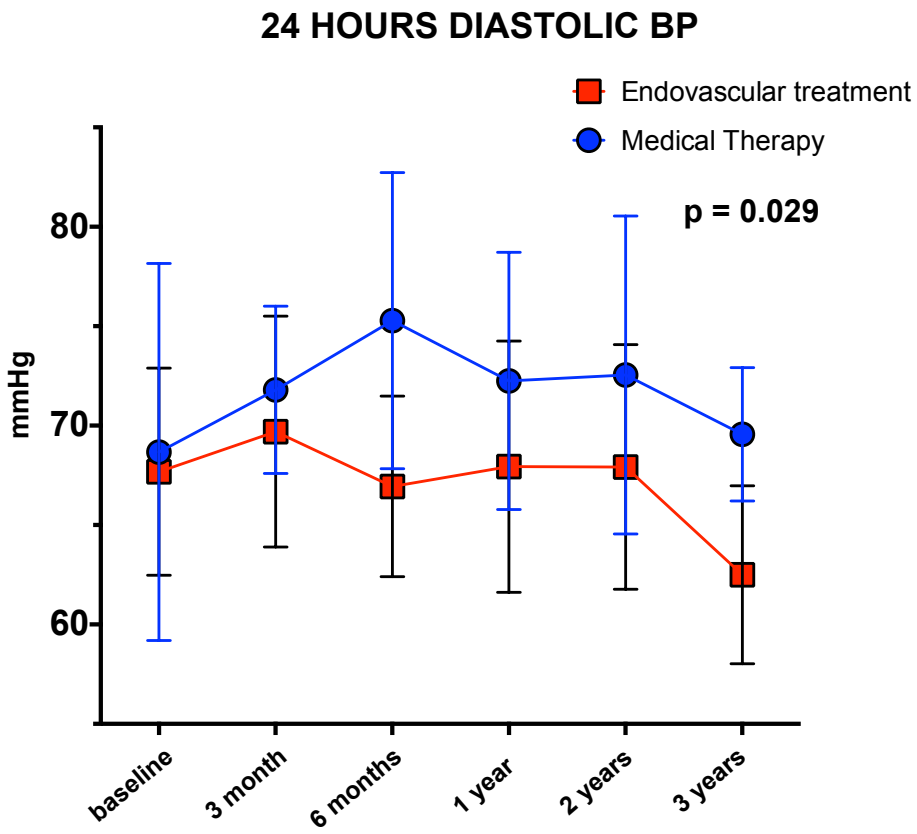


Fig. 14

Tab. 11 24 hours DBP (mmHg)												
	BASELINE		3 MONTHS		6 MONTHS		1 YEAR		2 YEARS		3 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**68.7**	9.5	**71.8**	4.2	**75.3**	7.4	**72.2**	6.5	**72.5**	8.0	**69.6**	3.3
PTRAS	**67.7**	5.2	**69.7**	5.8	**66.9**	4.5	**67.9**	6.3	**67.9**	6.1	**62.5**	4.5

Blood pressure control: number of drugs

At GLM including as covariates age and DBP at baseline, number of drugs decreased compared to medical treatment at 3 years follow-up, although it was borderline significant (p=0.055) (Fig. 15 and Tab. 12). The ANOVA test revealed a decreased number of drugs in the PTRAS group at 3 months, 1 year, and 2 years compared to the medical therapy group (p=0.047 at 6 months, p=0.016 at 1 year, and p=0.048 at 2 years).

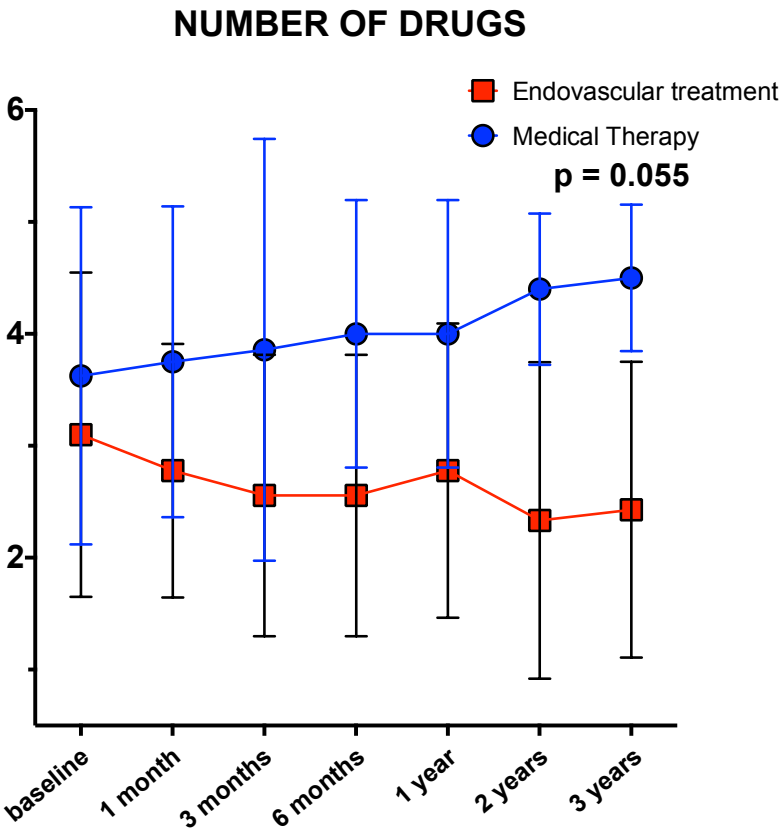


Fig. 15

Tab. 12 Number of Drugs														
	BASELINE		1 MONTH		3 MONTHS		6 MONTHS		1 YEAR		2 YEARS		3 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**3.6**	1.5	**3.7**	1.4	**3.8**	1.9	**4.0**	1.2	**4.0**	1.2	**4.4**	0.7	**4.5**	0.6
PTRAS	**3.1**	1.4	**2.8**	1.1	**2.5**	1.2	**2.5**	1.2	**2.8**	1.3	**2.3**	1.4	**2.4**	1.3
ANOVA (p)	0.142		0.144		0.047		0.094		0.016		0.048		0.158	

Renal function: estimated GFR by CKD-EPI

At GLM including as covariates age, eGFR, and DBP at baseline, eGFR as assessed with CKD-EPI did not differ in the two groups at 3 years follow-up (p=0.159) (Fig. 16 and Tab 13).

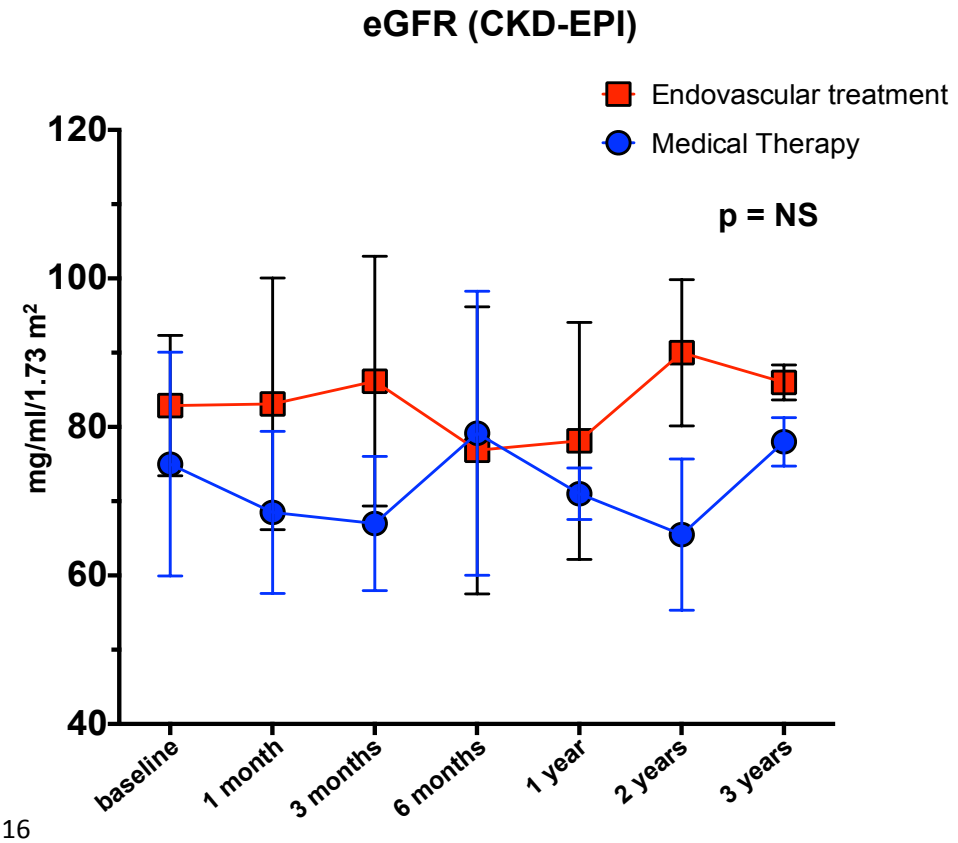


Fig. 16

Tab. 13 estimated GFR by CKD-EPI (mg/ml/1.73²)														
	BASELINE		1 MONTH		3 MONTHS		6 MONTHS		1 YEAR		2 YEARS		3 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**75**	15	**68.5**	10.9	**67**	9	**79.2**	19.1	**71**	3.5	**65.5**	10.2	**78**	3.2
PTRAS	**82.9**	9.4	**83.1**	16.9	**86.2**	16.8	**76.8**	19.3	**78.1**	16	**90**	9.8	**86**	2.3

Renal function: serum creatinine

At GLM including as covariates age, DBP, and creatinine at baseline, serum creatinine was lower in PTRAS group compared to medical therapy at 3 years follow-up (p=0.035) (Fig. 17 and Tab. 14).

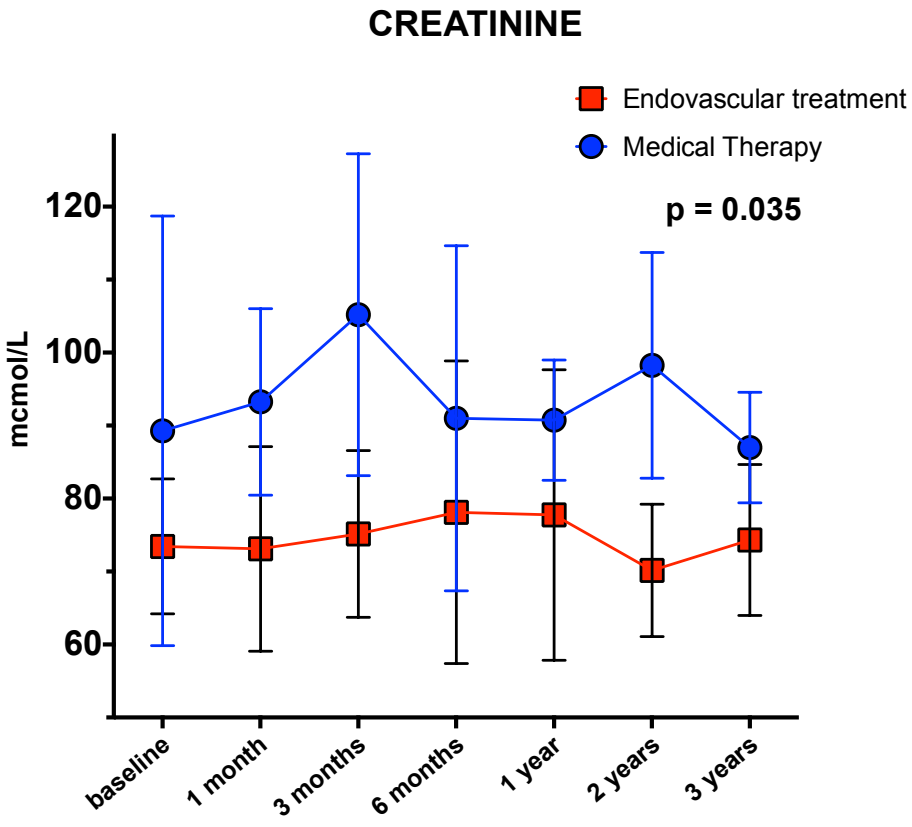


Fig. 17

Tab. 14 Serum creatinine (mcmol/L)

	BASELINE		1 MONTH		3 MONTHS		6 MONTHS		1 YEAR		2 YEARS		3 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**89.3**	29.4	**93.2**	12.8	**105.2**	22.1	**91**	23.6	**90.7**	8.2	**98.2**	15.4	**87**	7.6
PTRAS	**73.4**	9.2	**73.1**	14	**75.2**	11.4	**78.1**	20.7	**77.7**	19.9	**70.2**	9.1	**74.3**	10.3

Renal function: cystatin-c

At GLM including as covariates age DBP and cystatin-c at baseline, serum cystatin-c was lower in PTRAS group compared to medical therapy at 2 years follow-up (p=0.02) (Fig. 18 and Tab. 15).

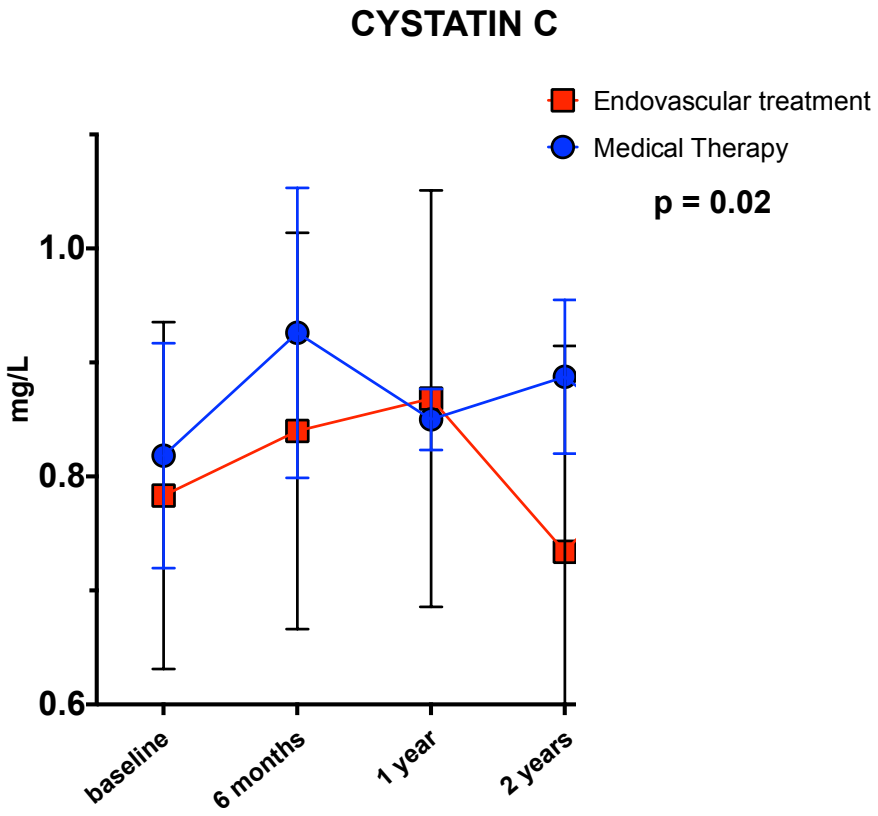


Fig. 18

Tab. 15 Cystatin-c (mg/L)								
	BASELINE		6 MONTHS		1 YEAR		2 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**0.8**	0.1	**0.9**	0.1	**0.8**	0.20	**0.9**	0.1
PTRAS	**0.8**	0.1	**0.8**	0.2	**0.9**	0.2	**0.7**	0.2

Metabolic control: LDL

At GLM including as covariates age BMI and LDL at baseline, LDL did not differ in the two groups at 3 years follow-up (p=0.701) (Fig. 19 and Tab. 16).

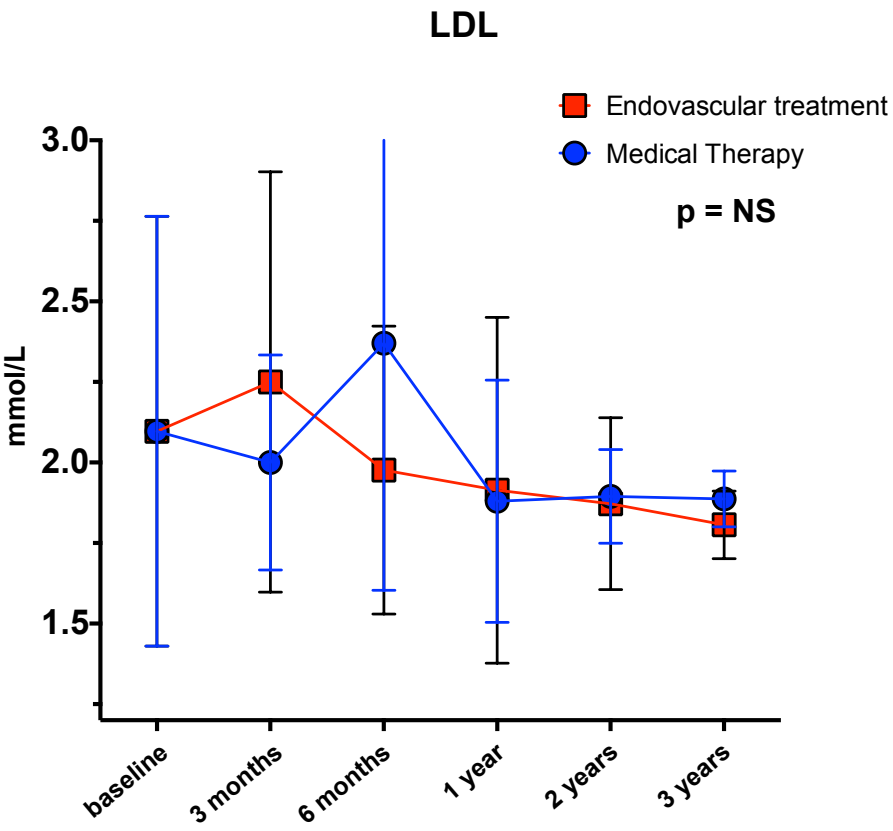


Fig. 19

Tab. 16 LDL-cholesterol (mmol/L)												
	BASELINE		3 MONTHS		6 MONTHS		1 YEAR		2 YEARS		3 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**2.1**	0.7	**2**	0.3	**2.4**	0.8	**1.9**	0.4	**1.9**	0.1	**1.9**	0.1
PTRAS	**2.4**	0.5	**2.2**	0.6	**2**	0.4	**1.9**	0.5	**1.9**	0.3	**1.8**	0.1

Metabolic control: HBA1c

At GLM including as covariates age, presence of diabetes and HbA1c at baseline, HBA1c was lower in PTRAS group compared to medical therapy at 3 years follow-up (p=0.034) (Fig. 20 and Tab. 17).

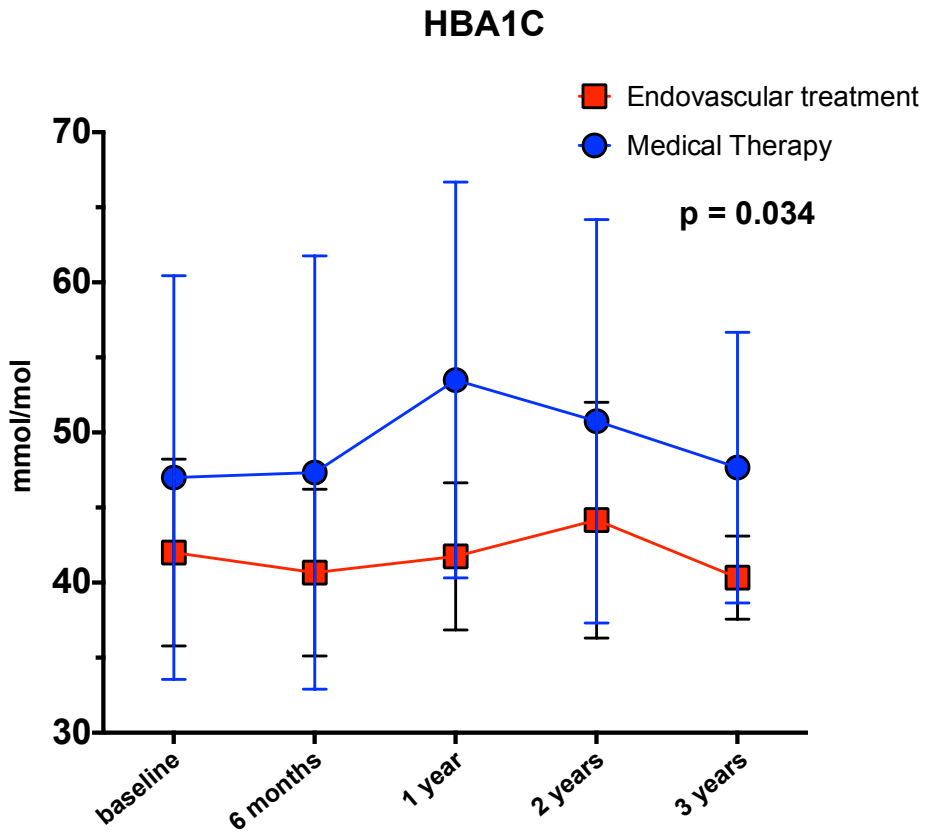


Fig. 20

Tab. 17 HBA1c (mmol/mol)										
	BASELINE		6 MONTHS		1 YEAR		2 YEARS		3 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**47**	13.4	**47.3**	14.4	**53.5**	13.9	**50.7**	13.4	**47.7**	9
PTRAS	**41.3**	6.0	**40.3**	4.3	**40.4**	2.7	**42.3**	5.6	**40.3**	3.4

Metabolic control: homocysteine

At GLM including as covariates age, BMI, and homocysteine at baseline, homocysteine did not differ in the two groups at 3 years follow-up (p=0.197) (Fig. 21 and Tab. 18).

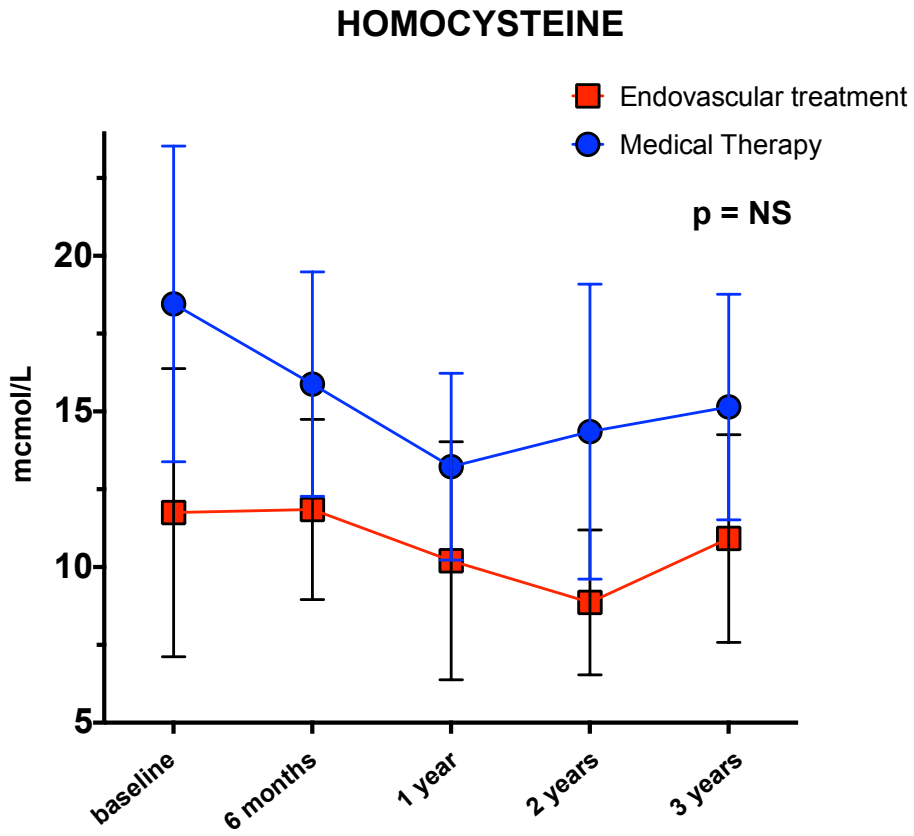


Fig. 21

Tab. 18 Homocysteine (mcmol/L)										
	BASELINE		6 MONTHS		1 YEAR		2 YEARS		3 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**18.4**	5.1	**15.9**	3.6	**13.2**	3	**14.3**	4.7	**15.1**	3.6
PTRAS	**11.7**	4.6	**11.8**	2.9	**10.2**	3.8	**8.9**	2.3	**10.9**	3.3

TARGET ORGAN DAMAGE

Kidney – Microalbuminuria

At GLM including as covariates age DBP and microalbuminuria at baseline, microalbuminuria was lower in the PTRAS group at 3 years follow-up (p=0.033) (Fig. 22 and Tab. 19).

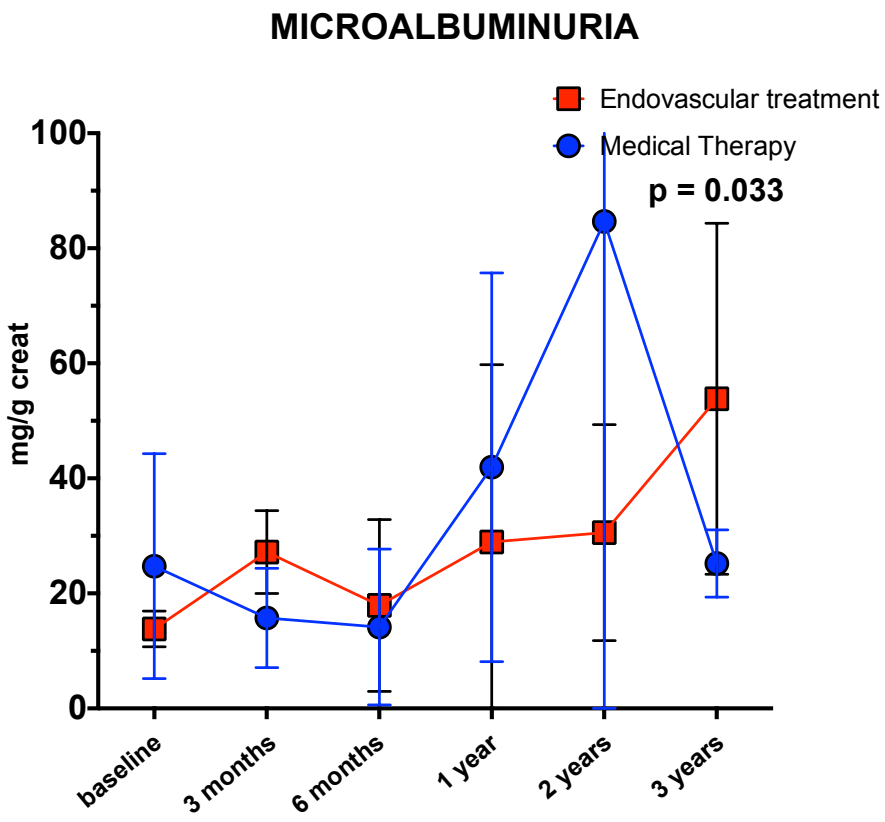


Fig. 22

Tab. 19 Microalbuminuria (mg/g creatinine)

	BASELINE		3 MONTHS		6 MONTHS		1 YEAR		2 YEARS		3 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**24.7**	19.6	**15.7**	8.6	**14.2**	13.5	**41.9**	33.8	**84.6**	94.5	**25.2**	5.8
PTRAS	**13.8**	3.1	**27.2**	7.2	**17.9**	14.9	**28.9**	30.8	**30.6**	18.8	**53.8**	30.5

Kidney – Resistive index assessed by Echo-Doppler

At GLM including as covariates age and DBP at baseline, IR in ischemic kidney was lower in the medical therapy compared to the PTRAS group, although it was borderline significant (p=0.052). IR in contralateral kidney was not different at 3 years follow-up (p=0.240) (Fig. 23-24 and Tab. 20-21).

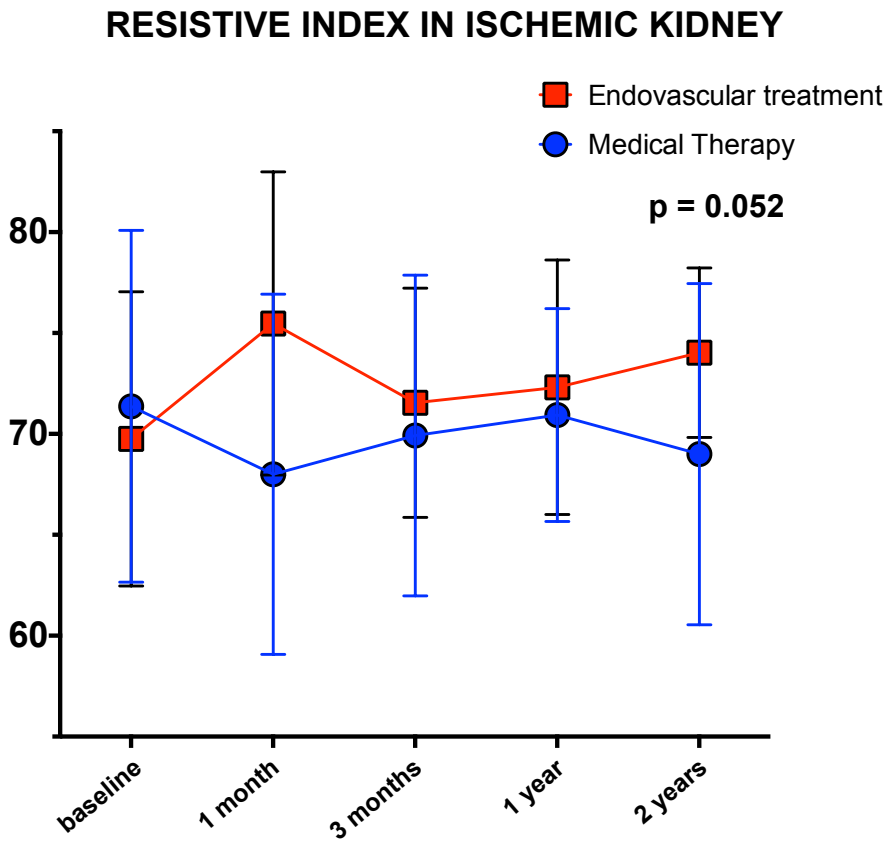


Fig. 23

Tab. 20 IR in ischemic kidney										
	BASELINE		1 MONTH		3 MONTHS		1 YEAR		2 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**71.4**	8.7	**68**	8.9	**69.9**	7.9	**70.9**	5.3	**69**	8.4
PTRAS	**69.7**	7.3	**75.5**	7.5	**71.5**	5.7	**72.3**	6.3	**74**	4.2

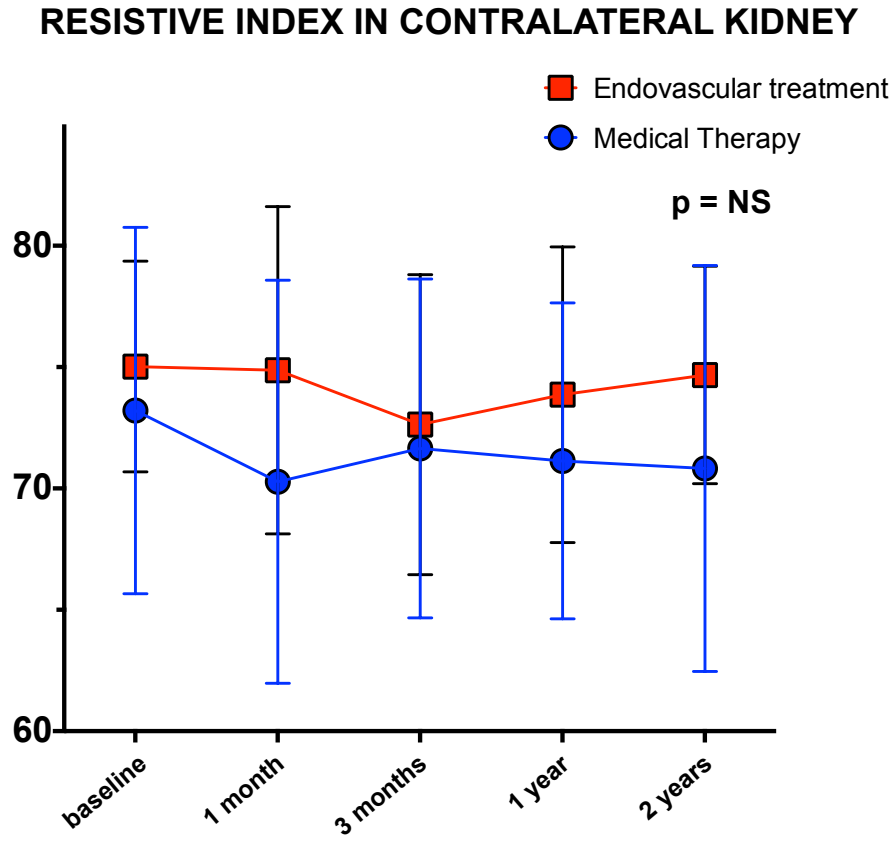


Fig. 24

Tab. 21 IR in contralateral kidney										
	BASELINE		1 MONTH		3 MONTHS		1 YEAR		2 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**73.2**	7.5	**70.3**	8.3	**71.6**	7	**71.1**	6.5	**70.8**	8.4
PTRAS	**75**	4.3	**74.9**	6.7	**72.6**	6.2	**73.9**	6	**74.7**	4.5

Heart - Left Ventricular Mass

At GLM including as covariates age, sex, and DBP at baseline, LVM normalized for BSA did not differ in the two groups (p=0.791) at 2 years follow-up (Fig. 25 and Tab. 22).

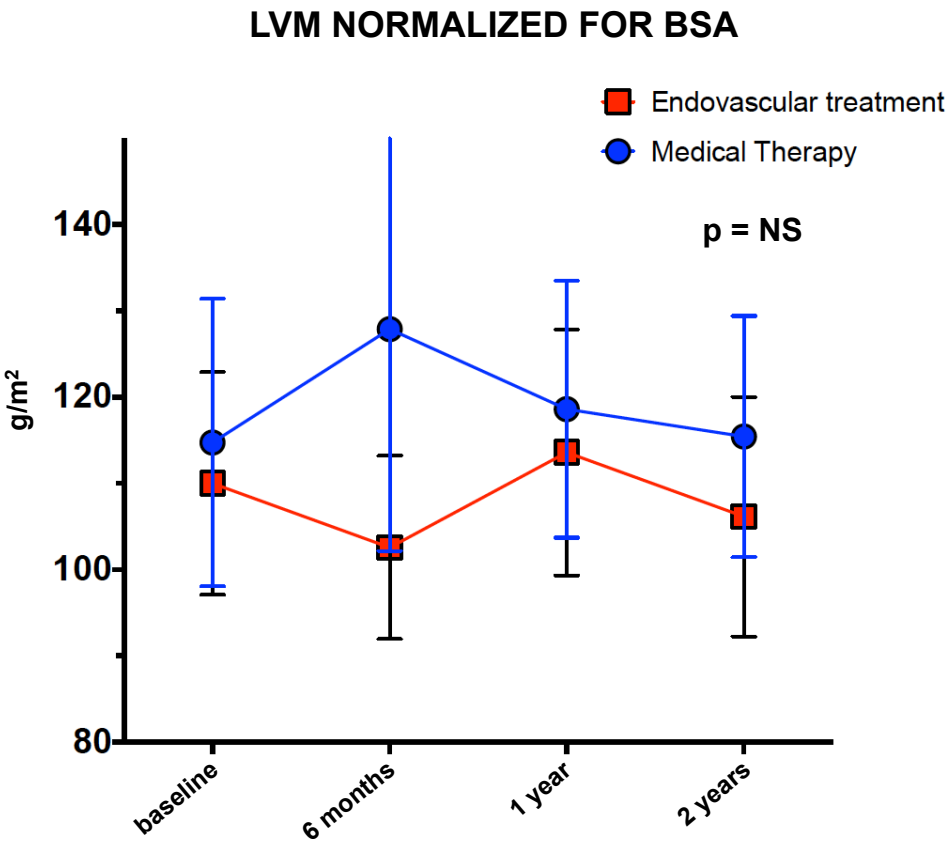


Fig. 25

Tab. 22 LVM normalized for BSA g/m^2								
	BASELINE		6 MONTHS		1 YEAR		2 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**114,7**	16,7	**127,9**	25,7	**118,6**	14,9	**115,4**	14
PTRAS	**110**	12,9	**102,6**	10,6	**113,6**	14,2	**106,1**	13,9

At GLM including as covariates age, sex, DBP, and LVM at baseline, LVM normalized for height was lower in the PTRAS group (p=0.058) at 2 years follow-up (Fig. 26 and Tab. 23).

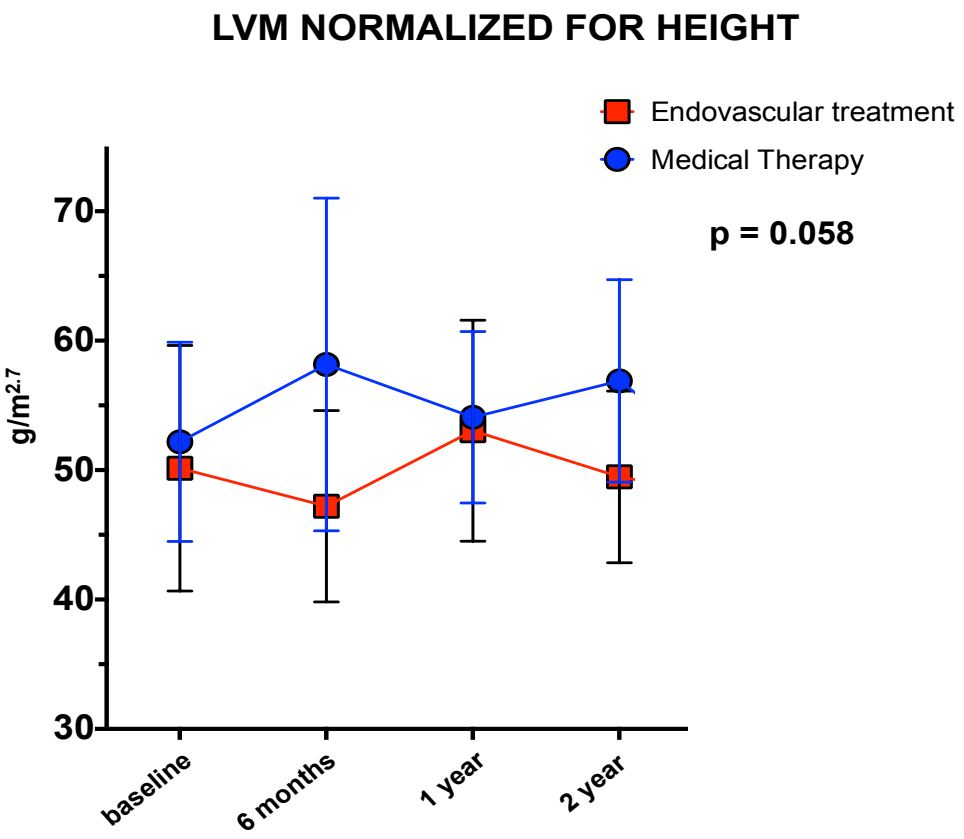


Fig. 26

Tab. 23 LVM normalized for height ($g/m^{2.7}$)

	BASELINE		6 MONTHS		1 YEAR		2 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**52.2**	7.7	**52.2**	7.7	**54.1**	6.6	**49.5**	6.6
PTRAS	**50.1**	9.5	**50.1**	9.5	**53**	8.5	**52.8**	7.9

At GLM including as covariates age and DBP at baseline, relative wall thickness did not differ in the two groups at 3 years follow-up (p=0.390) (Fig. 27 and Tab. 24).

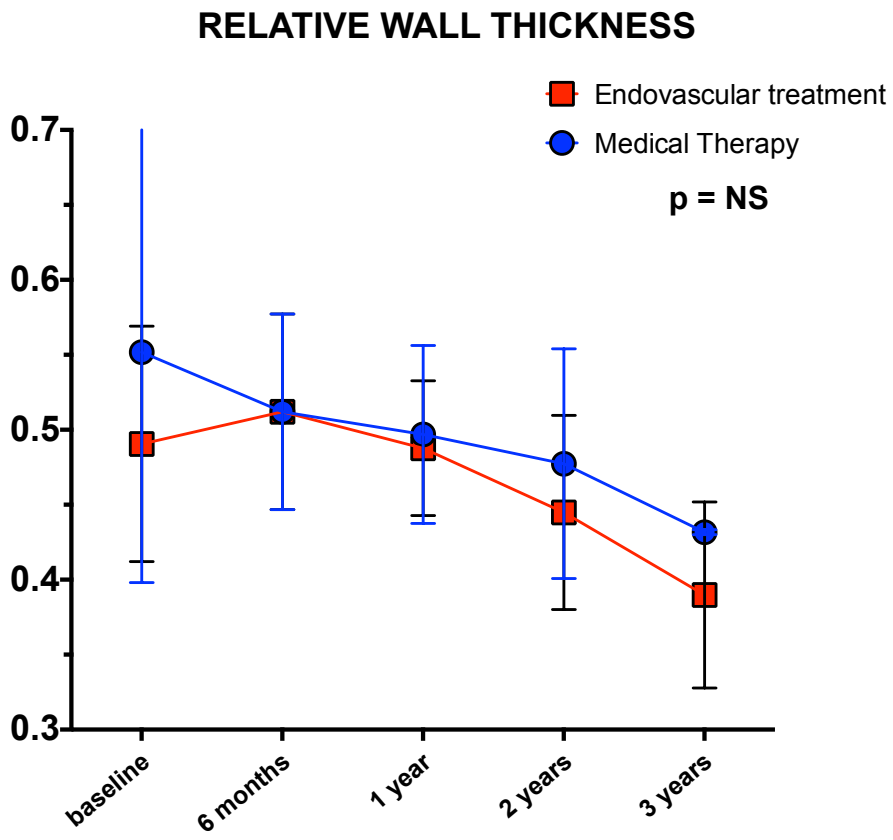


Fig. 27

Tab. 24 Relative wall thickness

	BASELINE		6 MONTHS		1 YEAR		2 YEARS		3 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**0.55**	0.15	**0.51**	0.06	**0.49**	0.06	**0.48**	0.08	**0.43**	0.001
PTRAS	**0.49**	0.08	**0.47**	0.07	**0.49**	0.04	**0.44**	0.06	**0.39**	0.06

Heart – Diastolic dysfunction

At GLM including as covariates age and DBP at baseline, E/E' was lower in the PTRAS group compared to the medical therapy group at 2 years follow-up (p=0.011) (Fig. 28 and Tab 25).

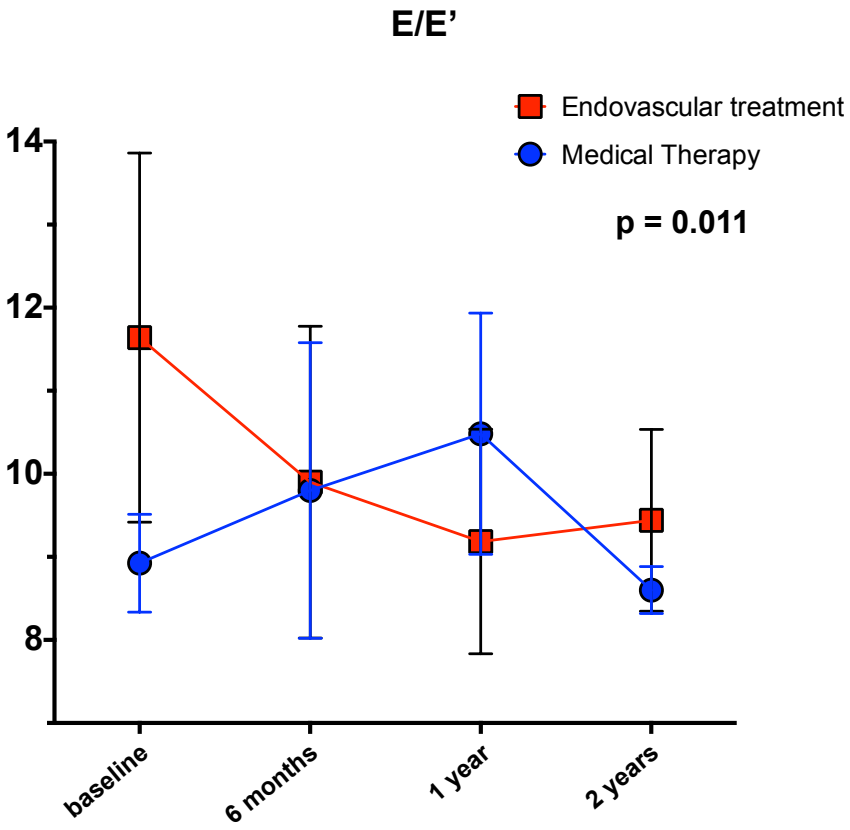


Fig. 28

Tab. 25 E/E'								
	BASELINE		6 MONTHS		1 YEAR		2 YEARS	
	mean	SD	mean	SD	mean	SD	mean	SD
MEDICAL THERAPY	**8.9**	0.6	**9.8**	1.8	**10.5**	1.4	**8.6**	0.3
PTRAS	**11.6**	2.2	**9.9**	1.9	**9.2**	1.3	**9.4**	1.1

DISCUSSION

1. Novelty of METRAS study

The novelty of the METRAS study includes the assessment of the two kidneys function separately as primary endpoint rather than an index of global renal function as carried out in previous trials[33,37-39,41,42] (reciprocal of serum creatinine or eGFR). None of the previous trials considered the GFR separately in the two kidneys as primary endpoint. This is an important issue in particular for unilateral renal artery stenosis (all patients included to date in the METRAS trial have unilateral stenosis). In fact, it is well known that in significant unilateral stenosis the GFR of the ischemic kidney is lower compared to the hyperfiltration in the contralateral kidney exposed to a higher blood pressure; after PTRAS, with BP decrease, the filtration in the unaffected kidney declines and consequently total GFR remains stable. Therefore, indexes of global renal function as eGFR and reciprocal of serum creatinine are endpoints not well suited to detect a change of renal function in unilateral RAS (Fig. 29). This was already reported in 2004 by Coen et al[57], who demonstrated in an analysis of the separate renal function in unilateral RAS that after PTRAS an increase in the percentage of total GFR in the affected kidney occurred, with ensuing decrease in the filtration of the contralateral kidney. Coen et al attributed the decrease in contralateral kidney function to the decline of ultrafiltration in the non-stenotic kidney after revascularization of the affected renal artery. Moreover, hemodynamic factors following the decreased RAAS activity might be involved, as suggested by the significant fall in systolic and diastolic blood pressure after the procedure[57]. It has to be underlined that all previous RCTs had chosen as endpoints indexes of global renal function: creatinine clearance in EMMA[33], STAR[41] and CORAL[39], serum creatinine in SNRASCG[42] and CORAL[39], mean slope of the reciprocal of serum creatinine in ASTRAL[37].

Another important novelty in METRAS trial is the modality of assessment of the renal artery stenosis. All the previous RCTs[33,37-39,41,42] evaluated the degree of stenosis by means of CT or angiography with a biplanar measurement based on diameter of the renal artery. In the METRAS study the assessment of percentage of stenosis is performed by means of the reduction of lumen in cross sectional area. This type of measurement is more accurate in evaluation of stenosis because it is not affected by error measurements in eccentric plaques.

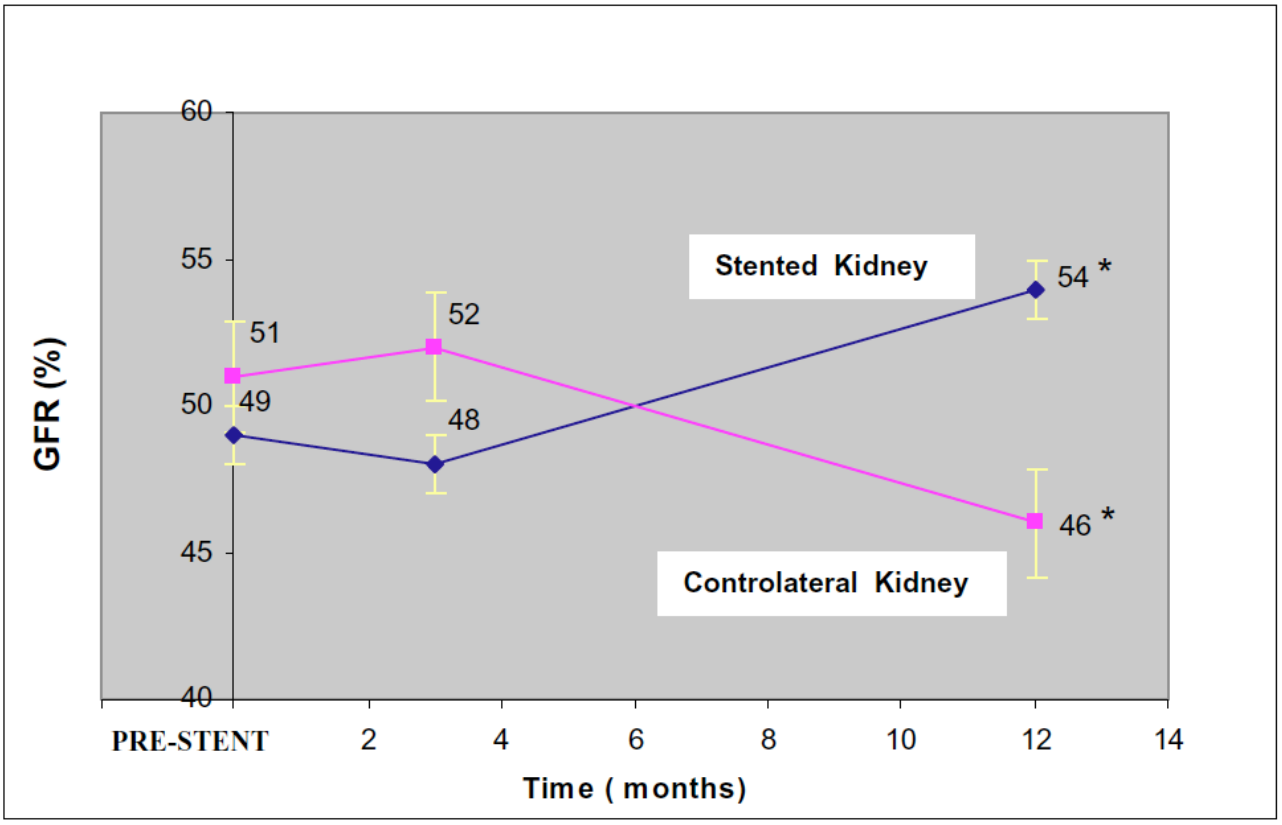


Fig. 29. Evolution of percent GFR in the stented and contralateral kidneys. *Significance of the difference compared to basal value ($p<0.05$)[57].

2. Effects on primary endpoint: improvement of ischemic kidney function with PTRAS

The primary objective of the METRAS study was to determine if PTRAS was superior or equivalent to optimal medical treatment in preserving the GFR in the ischemic kidney by means of ^{99m}Tc-DTPA scintigraphy. The crucial issue was the importance of restoring blood flow in the ischemic kidney and its evaluation by means of GFR as assessed at renal scintigraphy, not only for preservation of renal function in ischemic kidney but also for the benefits in the contralateral organ. Indeed, an important observation was that pathways of injury in a stenotic kidney contribute to significant risks for the non-stenotic kidney, the cardiovascular system, and arterial pressure. The post-stenotic kidney in both animals and humans releases inflammatory injury signals[58-60], and their levels rise not only in the stenotic but also in the contralateral kidney, suggesting reno-renal crosstalk and systemic effects of ARAS. Consequently, damage and inflammatory markers in the contralateral kidney are greater than those induced by simple nephrectomy or even angiotensin-II infusion[61]. Furthermore, cardiac hypertrophy and dysfunction are magnified in patients with RVH compared with essential hypertension[62].

The METRAS study demonstrated that GFR in the ischemic kidney increases progressively after

PTRAS compared to the medical therapy group, in which GFR remains stable. These results were significantly different (p = 0.048) when the GFR was estimated by means of the CKD-EPI formula and the filtration of the ischemic kidney determined at NAFS (method B) that is more accurate compared to the assessment of the GFR directly at NAFS (method A), which was borderline significant (p = 0.07). METRAS trial is the first RCT evaluating the function in the two kidneys separately assessed at NAFS. There is only one prospective trial, above mentioned, in whom 27 patients with unilateral RAS underwent stenting and 19 patients remained on medical treatment, who were followed for 1 year to assess the variation of GFR in the two kidneys separately[57] (Fig. 29). METRAS trial confirms the results reported by Coen et al in whom in the stented kidneys GFR increased significantly.

These results are interesting considering the small number of patients enrolled to date in METRAS trial (10 in the endovascular and 8 in the medical therapy group). The initial power calculation of the METRAS trial showed that at least 60 patients per arm were needed to reach an adequate statistic power. Moreover, it should be considered that not all the patients completed the follow-up. The analysis with method B excluding T6, in whom the number of patients was very small, proved the difference to be even more significant (p=0.027) (Fig. 10).

In the unaffected kidney the GFR remained stable during time in both arms (Fig. 11). In our study we could not confirm the reduction in hyperfiltration post-PTRAS as reported by Coen[57].

3. Effects on secondary endpoint: global renal function

Global renal function was evaluated by means of GFR assessed at NAFS and estimated by CKD-EPI formula, serum creatinine, and serum cystatin-c. Global GFR at NAFS and eGFR were similar in the two arms at follow-up (Fig. 12 and 16). After 2 years of follow-up creatinine and cystatin-c decreased in the PTRAS compared to the medical therapy group, in whom it increased (serum creatinine from 89.3 to 98.2 mcmol/L in medical group and from 73.4 to 70.2 mcmol/L in PTRAS group, p=0.035; cystatin-c from 0.8 to 0.9 mg/L in medical group and 0.8 to 0.7 mg/L in PTRAS group, p=0.02) (Fig. 17 and 18). Cystatin-c, a marker of renal function whom serum concentration depends on glomerular filtration and is not influenced by gender, age, and muscle mass, is more accurate than creatinine to assess global renal function.

These results are interesting compared to data reported from previous RCTs. In fact, none of them demonstrated an improvement in terms of global renal function in PTRAS group. EMMA[33] failed to demonstrate a difference between groups in GFR calculated with Cockcroft formula at 6

months follow-up (p=0.73). Scottish trial[42] observed no significant differences, or trend in serum creatinine between or within the groups during follow-up (192 vs. 152 mcmol/L in endovascular and medical arm respectively in bilateral stenosis and 146 vs. 168 mcmol/L in endovascular and medical arm respectively in unilateral stenosis). The Dutch trial[38] showed a difference in terms of serum creatinine and GFR estimated between the groups at 3 months follow-up (serum creatinine 1.2 vs. 1.3 mg/dl in endovascular and medical arm respectively; p=0.03 and GFR 70 vs. 59 ml/min in endovascular and medical arm, respectively; p=0.05) but it was not confirmed at 1 year follow-up (serum creatinine 1.3 vs. 1.2 mg/dl in endovascular and medical arm, respectively, and GFR 58 vs. 65 ml/min in endovascular and medical arm respectively; p=0.11 for both). The Dutch trial performed also renal scintigraphy with use of technetium-99m-labeled mercaptoacetyltriglycine, but they reported only the results in terms of the probability of renovascular disease (low, indeterminate, or high) and not the total and separate GFR in the two kidneys[38]. STAR trial[41] considered as primary endpoint the worsening of renal function, defined as a 20% or greater decrease in estimated creatinine clearance according to the Cockcroft and Gault formula, compared with baseline, based on 2 repeated measurements. Difference between groups did not reach the significance. ASTRAL trial[37] reported during the 5-year study period, an overall mean slope of the reciprocal of the serum creatinine concentration of −0.07×10−3 liters per micromole per year in the revascularization group, as compared with −0.13×10−3 liters per micromole per year in the medical-therapy group, results borderline significant favoring revascularization (p=0.06). However, the mean serum creatinine level did not reach the significance. The authors performed also a per-protocol analysis and a post hoc subgroup analysis between patients with severe anatomical disease (bilateral renal-artery stenosis of more than 70% and with renal-artery stenosis of more than 70% in a single functioning kidney) and patients without such severe anatomical disease, but they also found no significant differences in the primary outcome[37]. However, significant design flaws and selection bias affect many of these studies[63], limiting the usefulness of available data, as previously mentioned in the background paragraph.

It is interesting that only METRAS trial evaluated global renal function by means of GFR estimated at NAFS and is the first RCT that considered renal function expressed as level of cystatin-c.

4. Effects on secondary endpoint: blood pressure control

The METRAS trial demonstrated a better BP control in the PTRAS arm considering DBP and number of antihypertensive drugs (Fig. 14 and 15).

The mean 24 hours DBP assessed at ABPM decreased in PTRAS group at 3 years follow-up (63 vs. 70 mmHg in the endovascular arm and the medical arm, respectively; p=0.029) despite a reduction in the number of hypertensive drugs (2.4 vs. 4.5 in the endovascular and the medical arm, respectively; p=0.055). Although the GLM for the number of drugs was borderline significant (p=0.055), the ANOVA test revealed a decreased number of drugs in the PTRAS group at 3 months, 1 year, and 2 years compared to the medical therapy group (p=0.047 at 6 months, p=0.016 at 1 year, and p=0.048 at 2 years) (Figure 15). We would like to underline that patients available at 3 years follow-up are few and this could have affected the results.

The decrease of DBP and number of antihypertensive drugs in the PTRAS group highlights the favorable effect of the revascularization procedure in ARAS patients and, if this trend will be confirmed upon completion of the trial, it will translate in a better compliance of patients to therapy and possibly to a reduction of cost for National Healthcare System.

The benefit of revascularization in ARAS on BP control has been demonstrated in some RCTs and in a recent meta-analysis[45]. Plouin et al in EMMA trial[33] reported a reduction in number of drugs expressed as defined daily doses in the PTRAS group compared to the medical group (1.0 vs. 1.78 respectively, p=0.009) during 6 months follow-up. Dutch study[38] also showed a difference in drugs in endovascular and medical group at 1 year follow-up (1.9 vs. 2.4 respectively, p=0.02). In SNRASG trial[42], Webster et al reported lower BP values in bilateral stenosis in endovascular group compared to medical treatment (152/83 vs. 171/91 mmHg respectively, p<0.01) and in recently published CORAL trial[39], SBP resulted lower in PTRAS group compared to medical therapy (-2.3 mmHg, p=0.03).

Recently published meta-analysis[45-48], showed that PTRAS seems to have a small drug-saving effect and may result in a small improvement in diastolic BP. These results are concordant with our data.

5. Effects on secondary endpoint: metabolic control

The METRAS study demonstrated a decrease of glycosylated hemoglobin in patients treated with PTRAS. Although the two groups had different levels of glycosylated hemoglobin at baseline (47 mmol/mol in the medical therapy vs. 41 mmol/mol in the PTRAS group), we performed a GLM analysis including as covariates the percentage of diabetic patients at baseline and baseline levels of glycosylated hemoglobin. At 3 years follow-up glycosylated hemoglobin was lower in the PTRAS compared to the medical arm, in whom values tended to increase (from 47 to 47.7 mmol/mol in medical group and from 41.3 to 40.3 mmol/mol in PTRAS group, p=0.034) (Fig. 20).

LDL, HDL, total cholesterol, and triglycerides were similar across groups, probably because both arms at randomization and during the trial were aggressively treated with lipid-lowering drugs. Similarly, homocysteine was comparable between arms, which was not unexpected being a level exceeding 15 mcmol/L an exclusion criterion (Fig. 19 and 21).

6. Target organ damage

The METRAS study assessed renal and cardiac organ damage.

Patients treated with PTRAS had lower renal damage assessed by means of microalbuminuria. It is worth highlighting that at 2 years time-point microalbuminuria was higher in the medical therapy arm probably because of one patient with uncompensated diabetes mellitus and urinary infection with a transient increase of microalbuminuria. However, after exclusion of the 2 years time-point, the level of microalbuminuria increases in medical therapy group while appears stable with time in the PTRAS group (Fig. 22).

In the METRAS study design resistive index was not an inclusion criterion to avoid a potential confounder. At follow-up patients treated with PTRAS showed an increased RI from baseline compared to medical therapy. This effect might be explained by an increased RI due to removal of the stenosis in the treated kidney after the procedure. On the other hand, analyzing the time-course of RI in contralateral kidney no between group differences could be detected and RI appeared stable (Fig. 23 and 24).

Cardiac damage was evaluated at echocardiography assessing LVMI for BSA and height. We found a regression of LVH in both groups during 3 years follow-up (from 52.2 to 47.5 and from 50.1 to 47.8 $g/m^{2.7}$ in the medical and endovascular group, respectively), but there were no differences between groups in terms of regression of LVH, although the LVM indexing for height seems to be lower in PTRAS group at follow-up (borderline significant p=0.058). In both groups there was a reduction in relative wall thickness (from 0.55 to 0.43 in the medical therapy and from 0.49 to 0.39 in the PTRAS group) (Fig. 25, 26 and 27).

Diastolic dysfunction, assessed as E/E', decreased in the PTRAS compared to the medical arm (p=0.011) (Fig. 28). The improvement in diastolic function in the PTRAS group might reflect the improved DBP control in this group during time.

These results are consistent with those of RAS-CAD trial, a clinical trial aimed at testing whether renal artery revascularization, compared with medical therapy, affects left ventricular hypertrophy

progression. It included 84 patients randomly assigned to the revascularization arm (43 patients) and to the medical therapy arm (41 patients). The authors found that there was a mild but statistically significant regression of left ventricular mass in patients on medical therapy and those who underwent renal revascularization. In patients on medical therapy, LVM indexed to body surface area (LVMI) decreased from 113 to 107 g/m^2 after 1 year (ΔLVMI, -6.1; 95% CI, -11.4 to -0.8 g/m^2; p=0.03). This change was similar (p=0.6) to that registered in the revascularization arm, from 124 at baseline to 116 g/m^2 at 1 year (ΔLVMI, -8.2; 95% CI, -14.3 to -2.1 g/m^2; p=0.01)[64].

7. Limitations and strengths of the study

The METRAS study has some **limitations** that deserve to be mentioned**.**

First of all, as above stated, the number of patients enrolled is limited. In the design of the study, the minimum number of patients in each arm was planned to be sixty, instead we enrolled only 10 patients in PTRAS and 8 in medical group.

This could be explained in part by the strict enrollment criteria, which require a predefined percentage of atherosclerotic stenosis and exclude patients with compelling indication to PTRAS. In fact, among 30 patients evaluated for trial inclusion, only 20 were eligible. Moreover, the METRAS trial was originally conceived as a multicentric trial, but none of the centers originally involved in study design concurred to patient enrolment. Major issues were the ASTRAL[37] and more recently CORAL[39] trials results, which discouraged PTRAS procedure except in patients with compelling indications to PTRAS.

Among the most important problems in METRAS trial, which might also explain the lack of patients enrolment in other centers, is the complexity of the trial due to the number of tests and follow-up visits required, particularly during the first year (planned at baseline, after 4-8 weeks, at 1 month, 3 months, 6 months, 1 year). Patients often complained about the numerous tests requested and, among others, about NAFS, which was erroneously perceived as a “dangerous exam”. ARAS is an asymptomatic disease and patients often hesitate to be adherent to follow-up compared to diseases greatly affecting quality of life. Moreover, the majority of patients were referred to our center and lived far from Padua with logistic problems with visits, particularly old patients with limited mobility. In addition, the cost of the trial was covered by the Healthcare system only for a subset of patients, because the tests were considered part of a standard follow-up in patients undergoing PTRAS. Thus, patients who were required to pay out of their pocket, had poor compliance to follow-up tests and visits due to excessive costs, which explains the non-trivial drop out rate (10%). In particular, cystatin-c is a not reimbursable exam from National Healthcare

System, and the patient had to pay all the cost of the exam. This was a not negligible problem for adherence to follow-up visits and exams of patients in our trial and for this reason the cystatin-c available values at each time-point are fewer compared to other blood exams.

Finally, the METRAS study did not assess major clinical endpoints as cardiovascular and renal events and deaths because the patients required to assess these end-points far exceeded the number that was reasonable to expect to enroll in such a thorough trial.

In the METRAS study, one **strong point** is the standardization of therapy at baseline in order to obtain a strict control of BP and metabolic parameters in both groups, in addition to randomization, assured a homogeneity between groups at baseline (as showed by the characteristics of the cohort at baseline in table 6 in whom there are no difference at baseline between medical and endovascular group). This permitted a balanced comparison between groups and this uniformity could have emphasized the difference between groups during time, difference not demonstrated in previous trials.

Furthermore, in METRAS study our center had the availability of a first level radiology, which allowed an accurate evaluation of grade of stenosis by means of the assessment of cross sectional area with a specific software and not on the basis of a biplane measurement, as all the previous trials. Moreover, the percentage of complications (0%) and the success rate (100%) of procedure underlined the experience of radiologists of the Radiology Institute of Padua University.

CONCLUSIONS

Our study demonstrated an improvement in GFR in ischemic kidney in patients treated with PTRAS in association to optimal medical therapy compared to patients treated with medical therapy alone.

Patients treated with PTRAS showed an improvement of DBP control in spite of a decreased number of antihypertensive drugs during 3 years follow-up.

Index of global renal function (serum creatinine and cystatin-c) and glycemic control (glycosylated hemoglobin) appeared to improve in PTRAS compared to medical therapy group.

Diastolic dysfunction showed a regression in PTRAS patients compared to medical treated patients.

In my view these results are encouraging and suggest the need to continue the trial in order to confirm them with higher number of patients and longer follow-up.