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Renal nerve denervation reduces blood pressure in resistant hypertension: the role of full four quadrant ablation technique and number of ablations

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To what remains

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1. SUMMARY

Background: Approximately 10% of hypertensives have resistant hypertension, even if adequate pharmacological therapy is established. In this regard renal nerve denervation (RDN) could represent a valid alternative treatment. **Methods:** In a retrospective analysis with a follow-up of 6, 12 and 24 months we investigated the efficacy and safety of catheter-based renal artery ablation in 57 patients undergoing RDN with multiple renal nerve ablation in both renal arteries. In addition to medical antihypertensive therapy (4.2±1.4 drugs per patient), RDN using three different ablation systems was performed in patients with confirmed resistant hypertension (systolic blood pressure >140 mmHg in spite of three drugs including a diuretic). The primary endpoint was the change in office ambulatory systolic blood pressure from baseline to 6, 12 and 24 months follow-up after RDN. The primary safety endpoint was the change in plasma creatinine levels after 12 and 24 months compared to baseline. **Results:** The mean office systolic blood pressure at baseline was 167.6±22.4and after 6, 12 and 24 months averaged 143.5±21.1 ($p<0.05$), 141.1±21.1 ($p<0.05$) and 139.4±19.6 mmHg ($p<0.05$) respectively, with an average of 15.1±5, three nerve ablations performed. No significant changes in plasma creatinine levels were observed at 12 ($p=0.421$) and at 24 months ($p=0.217$). There were no complications after RDN nor any relevant adverse vascular, renal or cardiovascular events were observed except in one patient in whom a covered stent had to be placed at the femoral puncture site. **Conclusions:** In this study in all comers with resistant hypertension RDN, if performed adequately in number of ablations and energy delivery, is an efficient and safe treatment option to lower office and 24h blood pressure. Whether these blood pressure lowering effects will lead to a reduction of the cardiovascular morbidity and mortality will require further studies.

2. LIST OF ABBREVIATIONS USED

RDN= renal nerve denervation

BP= blood pressure

HBPM= home blood pressure measurement

ABPM= ambulatory blood pressure measurement

SBP= systolic blood pressure

DBP= diastolic blood pressure

ACE= angiotensin converting enzymee

AT= angiotensin

ACTH= adrenocorticotropic hormone

MRI= magnetic resonance imaging

RAAS= renin angiotensin aldosterone system

ESC= European society of cardiology

eGFR= estimated glomerular filtration rate

BMI= body mass index

CAD= coronary artery disease

CKD= chronic kidney disease

COPD= chronic obstructive pulmonary disease

AF= atrial fibrillation

OAC= oral anticoagulation

CA= calcium antagonists

3. INTRODUCTION

3.1 Relevance of the problem

Arterial hypertension is highly prevalent in the overall population, in particular in adults and in the elderly, and represents one of the major cardiovascular risk factors for myocardial infarction and stroke [1, 2]. Both these events exhibit a linear relationship with blood pressure and particularly stroke is tightly linked to elevated blood pressure and age [3,4]. Moreover, high blood pressure carries an increased risk of developing vascular dementia [5]. In over 95% of patients, no apparent cause for the elevated blood pressure values can be found, a condition that has been defined as essential hypertension [6]. Despite lifestyle modification and the availability of effective antihypertensive drugs, blood pressure control remains suboptimal worldwide. Several factors account for that problem, among them non-compliance of physicians and patients, untreated secondary forms of hypertension and true treatment-resistant hypertension (i.e. blood pressure (BP) > 140/90 mmHg in spite of 3 antihypertensive drugs including a diuretic).

3.2 Hypertension

3.2.1 Definition

According to the latest American College of Cardiology Guidelines [7] BP should be categorized as normal, elevated, or stages 1 or 2 hypertension to prevent and treat high BP. Normal BP is defined as <120/<80 mm Hg; elevated BP 120-129/<80 mm Hg; hypertension stage 1 is 130-139 or 80-89 mm Hg, and hypertension stage 2 is ≥140 or ≥90 mm Hg. Prior to labeling a person with hypertension, it is important to use an average based on ≥2 readings obtained on ≥2 occasions to estimate the individual's level of BP. Out-of-office and self-monitoring of BP measurements are recommended to confirm the

diagnosis of hypertension and for titration of BP-lowering medication, in conjunction with clinical interventions and telehealth counseling. Corresponding BPs based on site/methods are: office/clinic 140/90, home blood pressure measurement (HBPM) 135/85, daytime ambulatory blood pressure measurement (ABPM) 135/85, nighttime ABPM 120/70, and 24-hour ABPM 130/80 mm Hg. In adults with an untreated systolic BP (SBP) >130 but <160 mm Hg or diastolic BP (DBP) >80 but <100 mm Hg, it is reasonable to screen for the presence of white coat hypertension using either daytime ABPM or HBPM prior to diagnosis of hypertension. In adults with elevated office BP (120-129/<80) but not meeting the criteria for hypertension, screening for masked hypertension with daytime ABPM or HBPM is reasonable. According to the European Guidelines, Hypertension [8] is defined as a systolic blood pressure value at rest of> 140 mmHg and / or a diastolic blood pressure value at rest of> 90 mmHg (Table 1).

Table 1. Definition and classification of blood pressure according to Mancia et al, 2013.

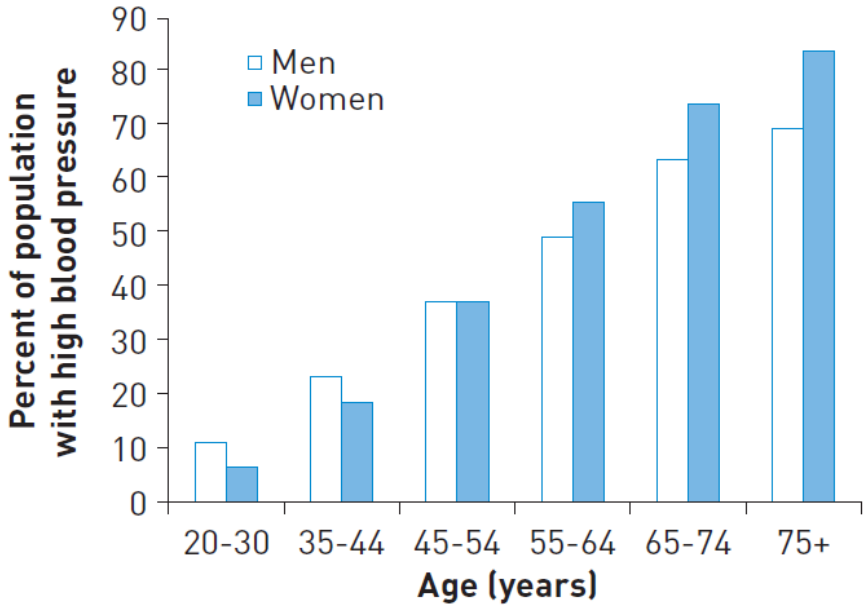
Classification	**SBP (mmHg)**		**DBP (mmHg)**
Optimal	<120	And	<80
Normal	<140	and/or	<90
Hypertension Grade 1 (mild)	140-159	and/or	90-99
Hypertonie Grade 2 (moderate)	160-179	and/or	100-109
Hypertonie Grade 3 (severe)	≥180	and/or	≥110
Isolated systolic Hypertension	≥140	And	<90

3.2.2. Epidemiology

Arterial hypertension is highly prevalent in the overall population, but particularly in adults and the elderly (**Figure 1**) [1]. Systolic blood pressure especially increases with age due to a loss in elasticity of the vasculature and hence a loss of the "White Coat Effect". In

Western countries, around 20% of the adult population have elevated blood pressure values (above 140/90 mmHg) making it one of the most important cardiovascular risk factors.

Figure 1. Percent of population with high blood pressure according to age (from Rosamond et al., Circulation. 2008;117: e25-146.



3.2.3 Etiology

In patients considered for renal sympathetic denervation, secondary forms of hypertension must be excluded prior to the procedure [9]. In over 95% of the patients, no apparent cause for the elevated blood pressure values can be found, a condition, which is referred to as essential hypertension [6]. It is likely that this condition has a hereditary basis, as it is seen to run in families. In fact, the risk of developing high blood pressure with advancing age increases three to five–fold if one or two parents respectively are hypertensive [10]. Recently, it has been identified that genes themselves account for small changes in blood pressure [11]. Furthermore, the condition is more prevalent in certain populations over others (the so-called "low blood pressure populations") [12, 13]. Besides a genetic disposition, dietary factors such as sodium and potassium intake as well as obesity have

been linked to essential hypertension. Of note, an over-activation of the sympathetic nervous system is often associated with essential hypertension. Renovascular hypertension is the most common curable form of secondary hypertension. Its prevalence ranges from between 1-3% in the hypertensive population but is more common in referral centers [14]. Two forms of renovascular hypertension can be distinguished: 1) Fibromuscular dysplasia and 2) atherosclerotic renal artery stenosis [15]. Both are amenable to percutaneous renal angioplasty [16, 17], and in the case of atherosclerotic lesions, to stenting as well [18]. Most forms of chronic kidney disease involve both kidneys, and lead to a steady decline in renal function over years and decades. Hypertension is associated with all forms of chronic kidney disease, in particular diabetic glomerulosclerosis (characterized by Kimmelstiel-Wilson lesions on renal biopsy) and chronic glomerulonephritis [19]. Specific treatment modalities are rarely available, although inhibitors of the renin-angiotensin system (i.e., ACE-inhibitors and AT-receptor antagonists) reduce proteinuria and delay the decay in kidney function over time [9, 20]. In 1955 Jerome Conn described patients with hypertension, hypokalemia and adenomas of the adrenal cortex (the so-called "Conn's syndrome") [21]. Mineralocorticoid hypertension is characterized by elevated plasma aldosterone levels and suppressed plasma renin activity reflecting an autonomous aldosterone secretion by the adenoma [22]. Localization of the adenoma is best performed with computer tomography [12]. Patients with phaeochromocytoma typically experience palpitations, sweating and sometimes headaches due to sudden releases of catecholamines from the tumor. The diagnosis involves either computer tomography or magnetic resonance imaging [9, 23, 24]. Catecholamine levels in plasma or in urine (metanephrine, vanillinic acid) are typically elevated [23, 25]. Hypertension is one of the most distinguishing features of endogenous Cushing's syndrome. The diagnosis is based on clinical observations and laboratory parameters (i.e., morning plasma cortisol, 24-hour

cortisol metabolites in urine) [6]. The latter often requires a cortisol-suppression test to distinguish Cushing's syndrome from elevated cortisol values in simple obesity. True Cushing's disease due to a pituitary adenoma producing ACTH should be distinguished from a cortisol-producing adrenal adenoma or bilateral adrenal hyperplasia, primarily by using imaging techniques such as MRI and/or adrenal scintigraphy. In the former clinical condition, removal of the tumor by trans-sphenoidal hypophysectomy is the treatment of choice, whereas adrenal tumors are managed by unilateral adrenalectomy. Determining possible secondary causes of hypertension is an important part in diagnosing patients with elevated blood pressure. All secondary causes of hypertension, including the less common (renal parenchymal disease, renovascular disease, phaeochromocytoma, primary aldosteronism (Conn's syndrome), Cushing's syndrome, coarctation of the aorta, thyroid dysfunction (hypo or hyperthyroidism), primary hyperparathyroidism, acromegaly, obstructive sleep apnea, drug/toxin-induced, monogenic renal tubular syndromes, pre-eclampsia), should always be excluded in severe hypertension, resistant hypertension or those aged <40 before starting or continuing long-term conventional pharmacological treatment.

3.2.4 Diagnostic

Although blood pressure itself was discovered by William Harvey as early as the 17th century, it could not be quantified until about 100 years later, when Stephen Hales used a cannula inserted into the carotid artery to determine blood pressure in a horse. After all, it was Scipione Riva-Rocci who, over a hundred years later, developed a first practical cuff for blood pressure measurement. Nicolai Korotkoff improved the method of Riva-Rocci by the additional use of a stethoscope, which allowed an auscultatory rather than palpatory determination of blood pressure. For blood pressure determination, auscultatory or

oscillometric semi-automatic sphygmomanometers should be used. The measurement on the upper arm is preferred and attention should be paid to the use of the appropriate sleeve size. For example, a cuff that is too small primarily leads to an overestimation of blood pressure in adipose patients. The clinical investigation includes a comprehensive medical examination with pulse status and auscultation of the vessels after flow sounds. Furthermore, the abdominal and/or hip circumference is measured while standing. In addition, the eye fundus is also examined in diabetics and hypertensives of the third degree. Blood pressure should be measured in a quiet environment after a three to five minute break. At least 2 measurements are taken every 1-2 minutes. At the first examination, the blood pressure on both arms is measured to exclude a side difference. In the auscultatory blood pressure determination, care must be taken that the decompression proceeds at a rate of 2 mmHg/s. The diastolic blood pressure value is reached as soon as the sounds disappear. In elderly patients, orthostatic hypotension must be precluded by taking one measurement each after standing for 1 and 3 minutes. When measuring blood pressure, a distinction is made between inpatient and outpatient blood pressure measurements, with the measured blood pressure in most cases being higher than the one measured on an outpatient basis, which is interpreted as a stress response to the tense situation during inpatient blood pressure measurement. This "white-coat hypertension" occurs in about 13% of all patients and is associated with increased age, female gender and non-smokers. White coat hypertension prevalence is lower if blood pressure is measured multiple times or by a nurse. Furthermore, "white-coat hypertension" is dependent on the degree of hypertension, which is more common in first-degree hypertension than in higher-grade hypertension. Inpatient and outpatient blood pressure should always be evaluated together. In addition, there is also a masked hypertension, in which controversial outpatient blood pressure is higher than the measured in-patient. These are more likely to be young

male patients with characteristics such as nicotine abuse, alcohol intake, physical activity, stress, and obesity. Masked hypertension often remains undetected, so the incidence of cardiovascular events in this patient group is about twice that of normotonic patients. The ambulatory measured blood pressure is more like the true blood pressure value [9].

During the 24-hour blood pressure measurement, the patient wears a portable blood pressure monitor, which generates information about blood pressure during daily activities and the nightly sleep phase. Blood pressure is usually measured at 15-minute intervals during the day and at 30-minute intervals during the night. Overly long intervals should be avoided as they limit the accuracy of the 24h blood pressure measurement. Blood pressure usually drops during the night, and a drop in blood pressure in excess of 10% of daily blood pressure during the night is considered dipping. Patients who fail to dipping suffer from secondary hypertension more frequently [26].

3.2.5 Therapy

If lifestyle modification alone can not adequately reduce hypertension in the blood pressure, drug therapy is initiated. Basically, it does not matter which drug is given, as long as adequate blood pressure reduction is achieved, because the largest meta-analysis in this regard shows no clinically relevant differences between the different drug groups. Thus, it can be confirmed that the use of diuretics, beta-blockers, calcium antagonists, ACE inhibitors and angiotensin receptor blockers is suitable for the initiation and maintenance of anti-hypertensive therapy [9, 27]. In 1958, the first beta-blocker, dichloroisoproterenol, was developed by Eli Lilly Laboratories. But it was Sir James Black who made a breakthrough in the treatment of angina pectoris with propranolol in the 1960s [28]. Beta-blockers act by their competitive inhibition of beta-receptors, which has the effect of catecholamine mediating activity. Today, about 60 years after the first beta-blocker was

introduced, it can be said that no other synthetic drug group has ever been so widely used over time in the treatment of cardiovascular and non-cardiovascular disease as the drug group of beta-blockers. Diuretics form the basis of any antihypertensive drug therapy. Diuretics were introduced in the 1950s and are still considered to be the primary choice when starting pharmacological hypertension therapy [9]. In addition to an increased excretion of water, a decrease in the peripheral vascular resistance leads to a reduction in blood pressure. Today, diuretics represent the second most prescribed drug group in antihypertensive therapy [29]. ACE inhibitors, which are more effective and more tolerated antihypertensive, were first used in the 1970s. They block the conversion of angiotensin I to angiotensin II and thus inhibit the activity of RAAS [30]. A decade later, the angiotensin receptor antagonists were added, which block the activation of angiotensin II receptors. Among other things, this leads to vasodilation and decreased secretion of vasopressin and aldosterone. These two drug groups are also among the most commonly used in hypertension treatment. Renin inhibitors have only been on the market for almost 10 years. Aliskiren, the only renin inhibitor to make it into the third phase of a clinical trial, was approved as a therapeutic against hypertension by the US Food and Drug Administration and the European Medicine Agency in 2007 [31, 32]. Renin inhibitors inhibit RAAS by binding to the active site of renin, thus preventing the binding of renin to angiotensinogen [33]. Central active antihypertensive and alpha-receptor blockers are also effective drugs in blood pressure therapy [34].

3.3 Therapy-resistant hypertension

Despite widespread antihypertensive drugs, approximately 10% of all hypertensives are resistant to antihypertensive and fail to achieve adequate blood pressure reduction [35]. Hypertension in patients who can not reach systolic and diastolic blood pressures of

<140/90mmHg, despite therapy containing lifestyle modification, a diuretic, and two other antihypertensive drugs of different classes, is termed therapy-resistant hypertension [1, 9]. Therapy-resistant hypertension is associated with a higher probability of cardiovascular and renal events, which is why new methods for lowering blood pressure in this disease must be sought. However, this is extremely difficult because of the complex pathophysiology.

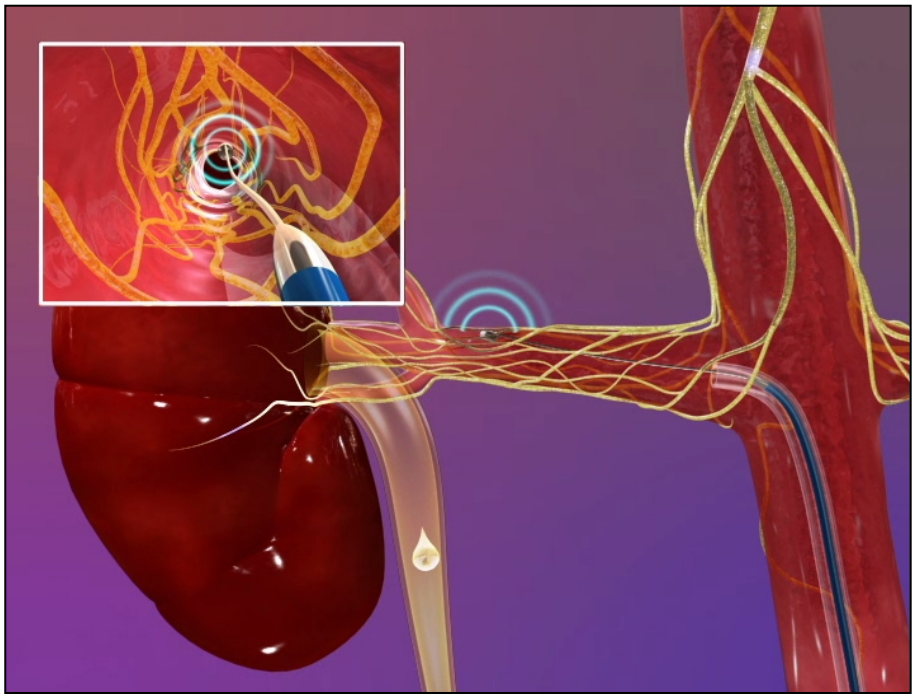
3.4 Renal denervation

In the search for new treatment options for refractory hypertension, the focus continued to be on the kidney. It has long been known that no one can control blood pressure via afferent and efferent nerves, [36] and that the sympathetic nervous system plays a major role in the regulation of circulating blood [37]. Thus, activation of the sympathetic nervous system leads to an increase in heart rate and blood pressure [38]. Efferent sympathetic fibers activate renin release in the kidney, thus regulating kidney and water excretion. Afferent nerves from the kidneys can affect the sympathetic activity in the brain stem [36]. Already in 1953, it could be shown experimentally that a sympathectomy leads to a lowering of the blood pressure, which led to a reduced mortality [39, 40]. Based on this, RDN via ablation catheter was invented in 2009 by the research group led by Murray Esler, with whose help the sympathetic-efferent and sensory-afferent signal transmission to and from the kidney can be interrupted [41-43]. A radiofrequency ablation catheter (**Table 2**) is introduced through the femoral artery, which then bilaterally ablates the sympathetic nerves along the renal arteries into the adventitia (**Figure 2**).

Table 2. Devices, Producer, Technology and methods of energy delivery of each of the three systems used in our study. The table reports also the mean values of systolic and diastolic blood pressure (SBP and DBP respectively) achieved after RDN at 6, 12 and 24 months for each of these three devices.

	Device	Producer	Technology	Methods	n	SBP/DBP	SBP/DBP (mmHg) 6m	SBP/DBP (mmHg) 12m	SBP/DBP (mmHg) 24m
	Symplicity	Medtronic	Single electrode monopolar	2 min per ablation	24	173.4±20.9/ 89.2±15.8	148.1±19.4/ 84.7±14.0	151.2±21.7/ 85.5±14.7	151.6±23/ 87.6±13.9
	EnligHTN	St. Jude Medical	Multielectrode basket, monopolar	90s up to 4 ablation points	14	174.5±17.5/ 94.3±16.4	145.5±19.7/ 85.4±6.2	138.6±19.7/ 83.1±12.2	133.0±5.7/ 80.8±9.9
	Vessix	Boston Scientific	Over-the-wire based catheter, bipolar	30 s up to 6 ablation points	19	155.3±22.4/ 87.3±21.3	136.6±21.3/ 78.9±12.4	126.4±16.5/ 80.4±12.0	126.2±12.8/ 74.5±3.2

Figure 2. Renal denervation. The ablation catheter is located in the renal artery, where it locally destroys sympathetic nerve fibers (according to Krum H et al., Circulation. 2011; 123:209-215).



RDN has shown antihypertensive effects in four mammalian species (rat, dog, rabbit, and pig) [44], so why should not the method work in humans [45]? Several studies worldwide have shown better blood pressure reduction after RDN compared to drug therapy alone in

refractory hypertensive [46-50], which leads to high expectations this new therapy method has. However, RDN was questioned after the large Symplicity-HTN-3 study did not show significantly better blood pressure reduction after RDN [51]. However, this study has some limitations, which could explain it as non-inclusive [52]. In particular, a sub-analysis of the Symplicity-HTN-3 study has shown that patients who received 12-16 ablations showed a comparable blood pressure reduction as in the registries [53]. The randomized French study DENER-HTN confirmed the effect of the RDN [50]. Furthermore, long-term results of this new antihypertensive method are not yet known and further studies are needed to make a final judgment on the efficiency and safety of RDN.

4. MATERIAL AND METHODS

4.1 Patients

All consecutive 57 patients with pharmacologically resistant hypertension (BP>140/90 mmHg) according to the last ESC guidelines [54] with therapy of at least 3 antihypertensive drugs, one of which one had to be a diuretic, all at the maximal tolerated dose, referred to the Department of Cardiology of the University Hospital of Zurich from August 2010 and April 2017 were enrolled in the current study with a rate of 8 patient/year (**Table 1**). A secondary form of hypertension was formally excluded in all the patients considered in this cohort, during at least two outpatient clinical evaluations. Patients with moderate to severe renal impairment (eGFR < 45 ml/min), anatomical contraindications to percutaneous renal denervation, anatomical variant of the renal arteries, short and small renal arteries with a length inferior to 20 mm or a diameter inferior to 4 mm were excluded. In all patient's antihypertensive therapy had to remain stable for at least 4 weeks before the procedure. All the patients were under diuretic therapy and 20 out of 57 already assumed an aldosterone antagonist. Systolic blood pressure upon admission was >140 mmHg in all the patients. Routine blood analyses and blood pressure measurement were performed before the intervention and at 6, 12 and 24 months in all patients, this latter with an automatic oscillometric device (Microlife® or Omron®) according to current guidelines. Patients' characteristics are resumed in **Table 3**.

Table 3. Patients characteristics.

Characteristics (Pts N=57)	
Age yrs mean ± SD	61.26±12.25
Male	35/57 (61.4%)
BMI kg/m^2 mean ± SD	30.93±5.19
Smokers	20 (35.1%)
Diabetes	18 (31.6%)
Dyslipidemia	24 (42.1%)
CAD	10 (17.5%)
CKD	10 (17.5%)
COPD	3 (5.3%)
AF	4 (7%)
Previous Stroke	7 (12.3%)
Aspirin	27 (47.4%)
OAC	5 (8.8%)
Diuretics	57 (100%)
Renin Inhibitors	7 (12.3%)
RAAS Inhibitors	49 (86%)
CA-Antagonists	44 (77.2%)
Beta Blockers	45 (78.9%)
Alpha Blockers	22 (38.6%)
Nitrates	5 (8.8%)
Aldosterone-Antagonists	20 (35.1%)
Avarage Medications mean ± SD	4.24±1.39

CAD= coronary artery disease. CKD= chronic kidney disease. COPD= chronic obstructive pulmonary disease. AF= atrial fibrillation. OAC= oral anticoagulation. RAAS= Renin-Angiotensin-Aldosterone-system. SD= standard deviation.

4.2 Technique

Three different types of renal denervation systems have been used for the procedures by experienced operators (**Table 2**): 1) the Symplicity™-RDN-System from Medtronic (single electrode, monopolar), 2) the EnligHTN™-Multi-Electrode-Renal-Denervation-System (multielectrode basket, monopolar) from St. Jude Medical and 3) the Vessix™-Renal-Denervation-System from Boston Scientific (over-the-wire based catheter, bipolar). Radiofrequency was erogated through a generator. With the first generation Symplicity^R system, the ablation catheter was advanced over a guidewire through a 6F catheter deep close to the bifurcation of the main renal artery, with final application of six ablations in a spiral fashion starting from the distal part of the renal artery up to its origin from the aorta

[41]. Commonly, 5-8 watts are applied for 2 minutes at each of the at least six ablations sites. Impedance may be used to assure good wall contact (optimal range: 300 – 350 Ω). The St. Jude basket contains four electrodes and once placed distally in the renal artery before the bifurcation, 4 ablations are automatically applied for 60 sec. In contrast to the Symplicity system, the St. Jude system is temperature driven. After the first ablation series, multiple ablations are possible by turning the node at the steering end of the ablation catheter and slightly pulling towards the ostium of the renal artery [55]. The Vessix balloon catheter (Boston Scientific Corporation Natick, MA) is an over the wire system using bipolar energy, consisting in a low-pressure balloon (3 atm) available in 4, 5, 6, 7 mm diameter sizes with offset electrode pairs placed in helical pattern. With simple anatomy, the balloon can be easily advanced into the renal artery over a 0.014 F guidewire [56]. Blood pressure was measured with an automatic oscillometric device (Microlife® or Omron®) while sitting for 5 minutes and using a 24h blood pressure recorder (SpaceLab®) before and at 6, 12 and 24 months after the RDN procedure. RDN was performed using a full four-quadrant ablation technique on both renal arteries, from the distal to the proximal segment, with energy delivery performed for all the time required from each system.

4.3 Statistics

The primary endpoint was the change in systolic and diastolic office blood pressure values at 6, 12 and 24 months compared to baseline immediately before the index procedure. The primary safety endpoint was the change in plasma creatinine levels at 12 and 24 months compared to baseline immediately before the index procedure. The 6 months follow-up was left optional for the patients; 24 patients completed follow-up with a 24hours ABPM and28 only with an office measurement. At 24 months, only 11 patients were investigated with a 24hours ABPM, while 21 accepted a clinical follow-up. All the results were

reported as mean ± standard deviation. A t-test was performed to analyze and for all the statistical analyses a $\alpha=0,05$ was considered, with statistical significance with a $\alpha\leq0,05$. A non-parametric test with paired samples was used to compare the BP values series. All these statistical analyses were performed with the SPSS Version 22.0.

4.4 Informed consent and privacy

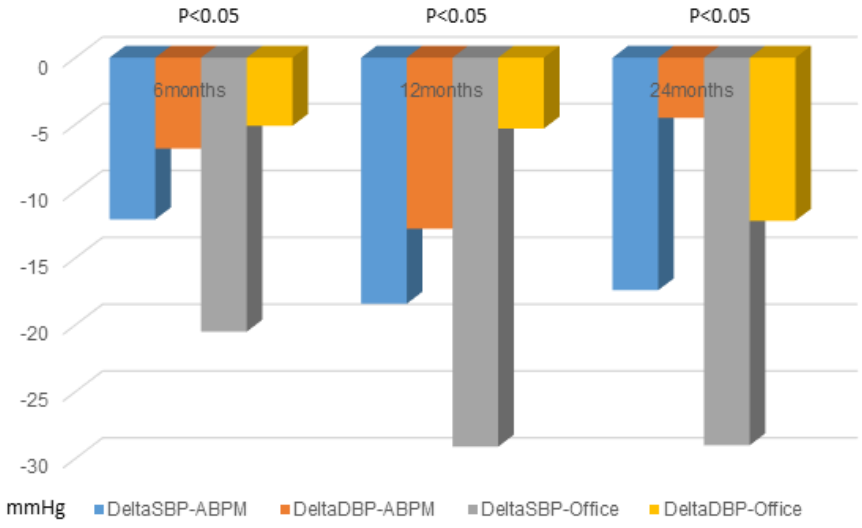
The data were collected during a medical consultation and used in a pseudo-anonymous form. A written consent for the RDN was obtained in writing for all patients prior to the procedure and the results obtained were analyzed retrospectively.

5. RESULTS

5.1. Outcome

After RDN, a significant reduction of systolic and diastolic blood pressure was observed at 6, 12 and 24 months (**Figure 3**).

Figure 3. Mean systolic and diastolic blood pressure reduction according to 24hours ABPM and office measurements. DeltaSBP-ABPM= systolic blood pressure difference according to 24h ambulatory blood pressure measurement (-12.1, -18.4 and -17.4 mmHg); DeltaDBP-ABPM= diastolic blood pressure difference according to 24h ambulatory blood pressure measurement (-6.8, -12.8 and -4.5 mmHg); DeltaSBP-Office= systolic blood pressure difference according to office blood pressure measurement (-20.5, -29.1 and -29 mmHg); DeltaDBP-Office= diastolic blood pressure difference according to office ambulatory blood pressure measurement (-5.1, -5.3 and -12.2 mmHg). Compared to baseline all these differences were statistical significant ($p<0.05$).



The mean office systolic blood pressure value was 167.6±22.4mmHg at baseline and 143.5±21.1, 141.1±21.1 and 139.4±19.6 mmHg after 6, 12 and 24 months, respectively (all $p<0.05$, compared to baseline). The mean 24hours systolic ABPM values at baseline averaged 154.8±18.4mmHg and 142.4±21.8, 137.7±17.4 and 139.9±11.8 mmHg at 6, 12

and 24 months, respectively (all p<0.05, compared to baseline). Similar results were observed also when considering the mean diastolic values both the for office and 24h measurements. Compared to baseline value of 89.8±18.36mmHg diastolic blood pressure fell to 83.3±12.2 (p<0.05), 83.0±12.2 (p<0.05) and82.5±12.3 mmHg (p<=0.05) at office measurements and from 89.8±18.3mmHg to 83.3±12.2 (p=0.02) 83.0±12.2 (p<=0.05) and 82.5±12.3 (p<=0.05) for the 24hours ABPM (**Table 4** and **Table 5**).

Table 4. 24hours ABPM at baseline, 6,12 and 24 months after RDN. P-values refer to baseline.

ABPM (Pts N=57)	
SBP Baseline (Pts N=46)	154.8±18.4
DBP Baseline	88.7±13.6
SBP 6 months (Pts N=32)	142.4±21.8 (p<0.05)
DBP 6 months	81.0±12.4 (p<0.05)
SBP 12 months (Pts N=19)	137.7±17.4 (p=0.05)
DBP 12 months	78.6±9.8 (p=0.02)
SBP 24months (Pts N=9)	139.9±11.8 (p=0.19)
DBP 24 months	82.0±8 (p=0.32)

ABPM= ambulatory blood pressure measurement. SBP= systolic blood pressure. DBP= diastolic blood pressure. SBP and DBP values are expressed in mmHg ± standard deviation

Table 5. Office blood pressure measurement at baseline, 6, 12 and 24 months after RDN. P-values refer to baseline.

Office Blood Pressure Measurement (Pts n=57)	
SBP Baseline (Pts N=57)	167.6±22.4
DBP Baseline	89.8±18.3
SBP 6 months (Pts N=44)	143.5±21.1 (p=0.02)
DBP 6 months	83.3±12.2 (p=0.05)
SBP 12 months (Pts N=29)	141.1±21.1 (p=0.45)
DBP 12 months	83.0±12.2 (p>0.05)
SBP 24months (Pts N=23)	139.4±19.6 (p=0.45)
DBP 24 months	82.5±12.3 (p>0.05)

SBP= systolic blood pressure. DBP= diastolic blood pressure. SBP and DBP values are expressed in mmHg ± standard deviation.

The small difference between ABPM and office values is due to the limited number of AMBP measurements available (9 patient out of 57 at 24 months). A non-parametric test

with related samples was applied to both ABPM and office measurements. Regarding the ABPM we found a significant reduction from the baseline for SBP at 6 ($p<0.05$) and at 12 months ($p=0.05$), but not at 24 months ($p=0.19$) as well as for DBP at 6 ($p<0.05$), 12 ($p=0.02$) and 24 ($p=0.32$) months. Analyzing the office blood pressure measurements, we found a statistical significant reduction for SBP at 6 ($p=0.02$), but not at 12 ($p=0.45$) and 24 ($p=0.45$) months as well as for DBP at 6, 12 and 24 months (all $p>0.05$). The average number of ablations was 15.1±5.3 (11.9±2.8 until 2013, 19.4±4.6 from 2014) performed with the Symplicity™-RDN-System from Medtronic (n=24 patients, 2 minutes per ablation), with the EnligHTN™-Multi-Electrode-Renal-Denervation-System from St. Jude Medical (n=14 patients, 90 seconds up to 4 ablation points) and with the Vessix™-Renal-Denervation-System from Boston Scientific (n=19 patients, 30 seconds up to 6 ablation points). There was no statistical significant difference in lowering efficacy between the three different systems used (all $p>0.05$). There was no significant correlation between baseline characteristics and BP reduction, except for the assumption of an aldosterone antagonist prior to RDN and the reduction of both office SBP and DBP at 6 ($p= 0.046$ and 0.003 respectively) months, but not at 12 and 24 months. The lack of significance at the follow-up is probably due to the limited number of patients. Follow-up was the major limitations of the study: ABPM is available for 39, 26 and 12 patients at 6, 12 and 24 months respectively, while an office measurement for 44, 31 and 23 patients. Considering the office measurement, according to the international guidelines, we found that at 6, 12 and 24 months only 16 (SBP 119.9±12.7 mmHg, DBP 76.9±10.5 mmHg), 11 (SBP 118.2±10.4 mmHg, DBP 73.6±9.3 mmHg) and 13 (SBP 126.3±8.8 mmHg, DBP 77±6.6 mmHg) out of 57 patients presented controlled blood pressure after the RDN. No significant changes were observed regarding the number of medications at 6 12 and 24

months (all > 0.05, Table 5). Of notice only 3 out of 37 patients without a previous anti-aldosterone therapy were treated with this drug.

5.2 Safety aspects

There were no short- or long-term complications after the intervention and no relevant adverse vascular, renal or cardiovascular, were observed except in one patient in whom the procedure had to be postponed after introducing the sheet due to marked bleeding from the puncture site which was treated with implantation of a covered stent. The RDN procedure was then performed a few weeks later. All 57 patients were discharged at home the day after the procedure. Plasma creatinine levels remained stable throughout the observation period, with no significant changes at 12 (p=0.421) and 24 months (p=0.217).

6. DISCUSSION

6.1 Main results

In this registry of consecutive patients treated in a single center by one operator performing an average of 15 ablations in both renal arteries, renal nerve ablation led to a marked reduction of systolic and diastolic blood pressure without changes in antihypertensive medication or significant side effects. RDN using 3 different ablation systems was performed in patients with confirmed resistant hypertension: 1) Symplicity™-RDN-System from Medtronic (n=24); 2) the EnligHTN™-Multi-Electrode-Renal-Denervation-System from St. Jude Medical (n=14) and 3) the Vessix™-Renal-Denervation-System from Boston Scientific (n=19). Our results confirm the results of some international studies on renal denervation [47-50].

6.2 Comparison with other publications

Indeed, the Prague-15 Study and the DENER-HTN study, which had a comparable number of patients, achieved similar blood pressure reductions of 12.4±4.6 mmHg and 15.1±5.5 mmHg, respectively with a less pronounced reduction at 6 months compared to our results. Furthermore, our study led to similar systolic and diastolic blood pressure reductions 24 months after RDN (28.4±23.95 mmHg vs. 28.9±4.6 mmHg) as the Symplicity HTN-1 registry [47]. Also, in the randomized Symplicity HTN-2 trial, comparable results were obtained [48]. In contrast, the Symplicity-HTN-3 study failed to achieve the primary endpoint and revealed an overall non-significant reduction in blood pressure after RDN compared to the drug only therapy group.

6.2.1 The Symplicity HTN-3 Study

The negative result of the large Symplicity HTN-3 Study, lead to a wide interpretation of the study results. Several aspects of the study have been criticized: first, Symplicity HTN-3 included patients in whom blood pressure and antihypertensive drugs had not been stabilized before the intervention. Second, the percentage of Afro-American was with 25% much higher than in other studies. This is particularly in contrast to European and Australian studies as well as the current Swiss registry [57]. Indeed, Afro-Americans often have low-renin and volume-dependent hypertension, which may not respond to RDN. Third, the majority of Symplicity HTN-3 patients had already been treated with an average of 5.2±1.4 antihypertensive drugs [58], which makes it difficult to provide a further blood pressure lowering with any intervention. Finally, one of the major issues, on which the researchers of the field focused their attention in the last years, regards the technical performance of RDN in the Symplicity HTN-3 study. Of note, most of the cardiologists involved in the study had no previous experience with the procedure and in most of the cases they accounted only for 1-2 interventions of this type in the trial. Furthermore, the number of ablations which correlates directly with the degree of blood pressure reduction have been quite variable in the Symplicity HTN-3 trial ranging from 1 to 18 ablations. Importantly, only 84% of the procedures produced complete ablations of 120 seconds and a bilateral 4-quadrant ablation was only achieved in 6% of all patients. In a small subpopulation of 19 patients, which received bilateral 4-quadrant ablation, the blood pressure reduction also averaged 24.3 ±10.3 mmHg), similar to other studies and the current Swiss registry. Indeed, in the present series of patients, the experienced single operator performed an average of 15 ablations, a number that also showed in the sub analysis of Symplicity HTN-3 a marked and sustained blood pressure lowering effect of similar size. Thus, it appears that it is essential to perform a large number of ablation in order to destroy the renal nerves within the adventitia and to achieve a relevant blood

pressure lowering effect. A post-hoc analysis identified predictors of systolic blood pressure change in the subjects of the SIMPLICITY HTN-3, particularly severe baseline systolic hypertension (SBP > 180 mmHg), aldosterone antagonist use, non-use of vasodilators and, in the denervation group, the number of ablation, which if delivered in a four quadrant-pattern led to greater reduction of office and ambulatory SBP and heart rate in this population [53].

6.2.2 The SPYRAL HTN-OFF MED Study

The results of the SPYRAL HTN-OFF MED trial confirmed blood pressure lowering efficacy of RDN and encouraged to design larger pivotal trial [59]. In the 80 patients analyzed there was a significant reduction in office and 24-h-ABPM at 3 months in patients with mild to moderate hypertension after RDN without antihypertensive therapy that was not observed in the sham control group (24-h SBP -5.0 mm Hg (95% CI -9.9 to -0.2; p=0.041), 24-h DBP -4.4 mm Hg (-7.2 to -1.6; p=0·002), office SBP -7.7 mm Hg (-14.0 to -1.5; p=0·015), and office DBP -4.9 mmHg (-8.5 to -1.4; p=0·007). The retrospective and observational approach, together with the absence of a control arm, pharmacological or sham-procedure, represent the main limitation of the study, considering the profound placebo effect of RDN shown in controlled trials.

6.3 Limitations

Our study confirms findings from previous studies demonstrating safety and efficacy of RDN in a cohort of patients with resistant hypertension. The retrospective analysis, the lack of a control group and the limited number of patients available at follow-up represents the major limitations of this study. Repeated AMBP represented the major complaint for the patients.

6.4 Importance of the study

The study results of the University Hospital of Zurich confirm the results of a number of other studies [46-50] that RDN can be used as an effective and safe therapy option for treatment-resistant hypertension, overall if performed adequately in number of ablations and energy delivery.

6.5 Unanswered questions

Based on the current controversy on the effectiveness of RDN a European group of experts discussed the study design for future clinical studies during a consensus conference on the RDN [60]. According to this expert opinion paper, RDN should be performed in relatively young patients with mild hypertension as young patients present a higher sympathetic activity. In the current registry patients were relatively young with a median age of around 60 years. Indeed, in elderly hypertensives, which usually present with isolated systolic hypertension, RDN has been shown to be much less effective [61]. The type of system, which should be used for RDN, the type of ablation and its duration represent other major issues. Balloon-based catheters, as used partially also in this study, are probably better and provide a more consistent bilateral 4-quadrant ablation in the distal part of the artery. Also, the 24hours ABPM should be the only measurement tool to assess changes in blood pressure. As recently report, indeed, the 24hours ABPM is important to validate resistant hypertension [62]. Recent study results on RDN have led to more questions than answers. Future studies will provide a definitive assessment of the efficiency and safety of RDN. It remains unclear whether all patients should receive the same antihypertensive drugs before intervention. Furthermore, in the case of medication, the question arises as to whether a washout phase should be carried out so that the pre-therapeutic blood pressure values are

reached again in advance. However, the European expert group denies this because otherwise the risk of stroke and other cardiovascular events would increase during this time. Such a washout phase, if any, should only be realized by experienced research centers. Noncompliance of patients taking medication and the lack of strategies to eliminate this confounder in large study populations pose further problems. The focus of future study planning should be on standardization. This should include study design and population, therapy, technique, compliance and success markers. This is the only way to compare study results free from confounding factors.

In conclusion, research on the efficiency and safety of RDN is still ongoing. Nevertheless, it should be noted that although subsequent studies should prove that RDN is not an effective and safe antihypertensive therapy, it does not necessarily mean the end of RDN. In addition to the hypotensive effect, a decrease in the left ventricular mass, an improvement in diastolic function and antiarrhythmic effects after RDN were also observed [63, 64]. If not in hypertension therapy then this may be the future of RDN.

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8. STATEMENT

PhD Thesis

I expressly declare that the present work entitled "Renal nerve denervation reduces blood pressure in resistant hypertension: the role of full four quadrant ablation technique and number of ablations" is a Phd's thesis written by myself and without unauthorized assistance and in my own words.

Use of sources

I expressly declare that I have identified all references to foreign sources contained in the above-mentioned work. In particular, I confirm that, without exception and to the best of my knowledge, I have stated authorship both in the case of statements taken verbatim (citations) and in the statements made in other words by other authors (paraphrases).

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I note that works that violate the principles of self-declaration - particularly those containing citations or paraphrases without indications of origin - are considered as plagiarized and can lead to the corresponding legal and disciplinary consequences

I confirm with my signature the correctness of this information.

Name: Andrea Denegri, MD, PhD Candidate

Signature: 

Date: February 20th, 2018

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