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Renal Sympathetic Denervation: Renal Nerve Stimulation

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TABLE OF CONTENTS

CHAPTER I	Introduction and outline of the thesis	9
RENAL NERVE STIMULATION		
CHAPTER II	Blood pressure response to renal nerve stimulation in patients undergoing renal denervation: a feasibility study <i>J Hum Hypertens. 2015 May;29(5):292-5</i>	27
CHAPTER III	Renal Nerve Stimulation-Induced Blood Pressure Changes Predict Ambulatory Blood Pressure Response After Renal Denervation <i>Hypertension. 2016 Sep;68(3):707-14</i>	39
CHAPTER IV	Persistent Increase in Blood Pressure After Renal Nerve Stimulation in Accessory Renal Arteries After Sympathetic Renal Denervation <i>Hypertension. 2016 Jun;67(6):1211-7</i>	57
CHAPTER V	Renal Nerve Stimulation Identifies Aorticorenal Innervation and Prevents Inadvertent Ablation of Vagal Nerves During Renal Denervation <i>Blood Press. 2018 Oct;27(5):271-279</i>	71
CHAPTER VI	Renal Nerve Stimulation as a procedural end point for Renal Nerve Denervation <i>Curr Hypertens Rep. 2018 Mar;19;20(3):24</i>	87
FUTURE RESEARCH		
CHAPTER VII	Treatment of atrial fibrillation in patients with enhanced sympathetic tone by pulmonary vein isolation or pulmonary vein isolation and renal artery denervation: clinical background and study design <i>Clin Res Cardiol. 2018 Jul;107(7):539-547</i>	97
CHAPTER VIII	Summary, conclusions, and perspective	115
CHAPTER IX	Nederlandse samenvatting	125
APPENDIX	List of publications	129
	Dankwoord	135
	Curriculum vitae	139

CHAPTER I

**General introduction
and outline of the thesis**

HYPERTENSION

Hypertension is a common medical condition and its incidence is increasing. The available data on the prevalence of hypertension and the temporal trends of blood pressure (BP) values in different European countries suggest that the overall prevalence of hypertension appears to affect approximately 30–45% of the general population, with a clear increase with ageing. There also appears to be noticeable differences in the average BP levels across countries, with no systematic trends towards BP changes in the past decade^{1–21}. Globally, the overall prevalence of raised BP in adults aged 25 and over was around 40% in 2008. The proportion of the world's population with high BP (>140/90 mmHg), or uncontrolled hypertension, fell modestly between 1980 and 2008. However, because of population growth and ageing, the number of patients with uncontrolled hypertension rose from 600 million in 1980 to nearly 1 billion in 2008²². Across the World Health Organization (WHO) regions, the prevalence of raised BP was highest in Africa, where it was 46% for both sexes combined. Both men and women have high rates of elevated BP in the Africa region, with prevalence rates over 40%. The lowest prevalence of raised BP was in the WHO region of the Americas at 35% for both sexes. Men in this region had higher prevalence than women (39% for men and 32% for women). In all WHO regions, men have slightly higher prevalence of raised BP than women. This difference was only statistically significant in the Americas and Europe.

The BP taken during a visit to the treating physician (Office BP) is a known independent risk factor for major cardiovascular events such as atrial fibrillation (AF), stroke, myocardial infarction, sudden death, left ventricular hypertrophy, heart failure and peripheral artery disease as well as of end-stage renal disease^{23–25}. This relationship has been established at all ages and in all ethnic groups^{26,27}. A continuous relationship with events is also exhibited by out-of-office BP values, such as those obtained by ambulatory blood pressure measurements (ABPM).

CONTROL OF HYPERTENSION

Despite the high prevalence of hypertension and its associated complications, control of the condition is by far not satisfying. Although the awareness rate of hypertension increased and both treatment and control of hypertension have improved over the past few decades, data from NHANES 2005–2008 show that only 50.1% of persons with hypertension have their BP under control. Control of hypertension was defined as a level below 140/90 mmHg. This demonstrates that treatment still has a long way to go²⁸. In a large Dutch registry HELIUS similar differences in hypertension control among the native Dutch population and ethnic minorities were observed. The native Dutch population demonstrated hypertension control in 53% of men and 61% of women. Minorities within the Dutch population demonstrated adequate control in 43%

of the South-Asian Surinamese population, but only 23% in Ghanaian men. These data show that hypertension control can be improved in the native Dutch population, but many ethnic minorities could benefit from even closer attention and subsequent treatment²⁹.

MEDICAL TREATMENT OF HYPERTENSION

Large randomized trials have shown that antihypertensive therapy is associated with a nearly 50 percent relative risk reduction in the incidence of heart failure. The relative risk reduction in stroke is estimated between 30 to 40 percent. Although less pronounced, antihypertensive treatment produces a 20 to 25 percent relative risk reduction for myocardial infarction.³⁰ These relative risk reductions correspond to the following absolute benefits: antihypertensive therapy for four to five years prevents a coronary event in 0.7 percent of patients and a cerebrovascular event in 1.3 percent of patients for a total absolute benefit of approximately 2 percent³¹. Thus, 100 patients must be treated for four to five years to prevent a complication in two patients.

However, data for this estimation are derived from trials with a follow up of five to seven years. Therefore, we assume that the true benefit of antihypertensive treatment is underestimated.

Medical therapy aims to lower BP to avoid the mentioned cardiovascular events. Multiple guidelines have been developed to help set up goals for BP reduction. Meta-analyses conclude that primarily the degree of BP reduction, and not a specific drug regimen, is the major determinant of cardiovascular risk reduction in patients with hypertension^{30,32-34}. Recommendations for the use of specific classes of antihypertensive medications are based upon clinical trial evidence of decreased cardiovascular risk, BP-lowering efficacy, safety, and tolerability. Most patients with hypertension will require more than one BP medication to reach the determined goal BP. Medical therapy should be based upon individual patient characteristics and physician's preferences.

RESISTANT HYPERTENSION VS. SECONDARY HYPERTENSION

Resistant hypertension is defined as BP that is not controlled, despite adherence to an appropriate $\geq$ three-drug regimen (including a diuretic) in which all drugs are dosed at 50 percent or more of the maximum recommended antihypertensive dose; or BP that requires at least four medications to achieve control³⁵.

Approximately 15 percent of patients diagnosed with hypertension appear to have resistant hypertension. However, many patients who appear to have resistant hypertension may actually display pseudo resistance rather than true resistance.

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Pseudo resistance may result from one or more of the following issues:

- Inaccurate BP measurement (e.g., use of an inappropriately small BP cuff).
- Inadequate screening methods for secondary hypertension
- Poor compliance to BP medications
- Poor compliance to lifestyle and dietary approaches to lower BP
- Suboptimal antihypertensive therapy, due either to inadequate doses or exclusion of a diuretic from the antihypertensive regimen
- White coat resistance; as an example, a Spanish study found that 35 percent of patients with apparent treatment-resistant hypertension actually had well-controlled BP by ambulatory monitoring, suggesting they had white coat hypertension as a cause of their resistance³⁶

Patients with resistant hypertension referred for renal denervation were systematically screened for secondary causes. In Isala Hospital, resistant hypertensive patients are screened according to a vigorous protocol. One of the most common causes to be found through screening is hyperaldosteronism. The hormonal screening performed in our hospital is an effective approach to exclude this frequently encountered and otherwise asymptomatic cause of hypertension. A different approach is to treat all patients referred for resistant hypertension with a drug regimen including an MRA (mineralocorticoid receptor antagonist) and monitor the effect. We diagnosed hyperaldosteronism in approximately 10% of the so called resistant hypertension patients with. Medical therapy is the mainstay of treatment in these patients. A trial period including an MRA is limited by drug compliance. We believe that hormonal screening is preferred over adding another drug to the current regimen, lowering compliance. Resistant hypertension and the research regarding this field are plagued by poor drug compliance. Researchers from the University Medical Center Utrecht (UMCU) have published the results of the SYMPATHY study. A unique feature of this study was measurements of plasma levels of the prescribed antihypertensive drugs. A shocking 20% of patients had adequate levels of all their prescribed drugs, leaving 80% of the patients without adequate levels (or in a much smaller portion with plasma levels of different antihypertensive drugs than currently prescribed)³⁷. The treating physicians should do what is in their power to improve drug adherence, however achievement of an adherence of 100% is not realistic. Therefore, interventional therapy might be a good alternative to a life time challenge of drug adherence, especially in a patient population dealing with such poor drug adherence.

When true resistance has been established, another issue rises. Many underlying diseases can manifest as drug resistant hypertension. These types of disorders are classified as secondary hypertension. There is a number of general clinical clues that are suggestive of secondary hypertension and require further investigation:

- An acute rise in BP developing in a patient with previously stable values.
- Age less than 30 years in non-obese, non-black patients with a negative family history of hypertension and no other risk factors (e.g., obesity) for hypertension.
- Malignant or accelerated hypertension (e.g., patients with severe hypertension and signs of end-organ damage such as retinal haemorrhages or papilledema, heart failure, neurologic disturbance, or acute kidney injury).
- Proven age of onset before puberty.

Renovascular hypertension is the most common potentially correctable cause of secondary hypertension. The incidence varies with the clinical setting. It probably occurs in less than 1 percent of patients with mild hypertension³⁸. By comparison, between 10 and 45% of white patients with severe or malignant hypertension has renal artery stenosis.

Renal artery stenosis can be detected in many individuals with other manifestations of atherosclerosis, such as coronary artery disease (10 to 14 percent) and peripheral arterial and aortic disease (24 to 35 percent)³⁹.

Several clinical features should bring the physician to exclude renovascular disease. For example, new onset of severe hypertension (BP ≥ 180 mmHg systolic and/or 120 mmHg diastolic) after the age of 55 years. Also, an unexplained deterioration of kidney function during antihypertensive therapy, especially an acute and sustained elevation in the serum creatinine concentration by more than 50 percent that occurs within one week of starting a drug regimen that includes an angiotensin-converting enzyme (ACE) inhibitor or angiotensin II receptor blocker (ARB) should alert physicians. Due to the vasoconstrictive effect of these agents, severe kidney function deterioration can be observed. Hypertension in patients with other atherosclerotic diseases, particularly those over age 50 is another clue. Severe hypertension in patients with recurrent episodes of acute (flash) pulmonary edema or refractory heart failure with impaired renal function should prompt physicians as well⁴⁰. In a series of 55 patients with renovascular hypertension, for example, 23 percent had recurrent episodes of pulmonary edema requiring hospitalization. Flash pulmonary edema was more common in patients with bilateral compared with unilateral renal artery stenosis. Factors that contribute to acute cardiac decompensation include a hypertension-induced increase in afterload, inability of a hypertrophied left ventricle to relax in diastole (i.e., diastolic with or without systolic LV dysfunction), sodium retention due to activation of the renin-angiotensin-aldosterone system, and associated renal dysfunction^{41,42}. Registry data from the United Kingdom indicate that patients with episodes of pulmonary edema and renal artery stenosis have a benefit of reduced mortality after successful renal revascularization⁴³. Since antihypertensive drug therapy can lead to kidney function deterioration, imaging is often indicated.

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Hormonal disturbances can cause drug resistant hypertension in different ways. As discussed earlier, hyperaldosteronism is the most common hormonal cause. The main clinical clue suggestive of primary hyperaldosteronism is otherwise unexplained or easily provoked hypokalaemia. High levels of aldosterone induce potassium wasting in the kidneys in exchange for sodium. However, more than one-half of patients have a normal serum potassium levels. Therefore, nearly all patients with suspected secondary hypertension should be evaluated for primary hyperaldosteronism. Primary hyperaldosteronism should also be suspected in the presence of slight hypernatremia, drug-resistant hypertension, and/or hypertension with an adrenal incidentaloma. Measurements of the ratio of the plasma aldosterone concentration to plasma renin activity can help identify such patients⁴⁴, although inappropriate elevation of aldosterone is also a common feature in obese patients⁴⁵.

Obstructive sleep apnoea syndrome (OSAS) is another cause of secondary hypertension that can be treated, for example by continuous positive airway pressure. Patients have multiple apnoeic episodes at night due to passive collapse of the pharyngeal muscles during inspiration, such that the airway becomes occluded from the apposition of the tongue and soft palate against the posterior oropharynx. Other symptoms of OSAS include headache, daytime somnolence and fatigue, morning confusion with difficulty in concentration, personality changes, depression, persistent systemic hypertension, and potentially life-threatening cardiac arrhythmias⁴⁶. Patients with obstructive sleep apnoea often retain sodium and fail to respond optimally to antihypertensive drug therapy⁴⁷. Correction of the sleep apnoea may improve BP control⁴⁸. Other causes of secondary hypertension, including primary kidney disease, use of oral contraceptives, and the more exotic underlying diseases such as pheochromocytoma, Cushing's syndrome, and coarctation of the aorta must also be excluded in the appropriate settings.

RENAL SYMPATHETIC DENERVATION

When all causes of apparent resistant hypertension have been excluded, a subset of patients is diagnosed with true resistant hypertension. This subset of patients is at high risk of developing cardiovascular complications. For this group medical therapy falls short and other solutions are warranted. Renal denervation (RDN) is a non-pharmacological treatment option for patients with resistant hypertension.

RDN is a procedure using a percutaneous transarterial approach. Catheter ablation of the renal sympathetic nerve tissue is performed by delivering RF energy through electrodes positioned in the renal arterial wall. Ablation lesions are rotationally and longitudinally applied in a spiral pattern to the renal artery to ablate the renal nerves under fluoroscopic guidance (Figure 1 and 2).

Figure 1. The RDN catheter set up consists of three parts. The generator supplies the energy for radio frequency ablation. The guiding wire helps position the ablation catheter. The actual ablation catheter delivers the radio frequency energy to the renal arterial wall for subsequent destruction of the surrounding nerve tissue. Two different types are shown: EnligHTN by St. Jude Medical in the left and in the right panel Spyral by Medtronic.

RDN is supported by a solid rationale, including reports from the years 1940-60⁴⁹⁻⁵². During those times, radical surgery was performed for sympathetic denervation, successfully lowering BP. However, also in these patients a percentage appeared to be non-responders. Nammass et al. described the cardiorenal sympathetic interaction⁵³. Deleterious effects of chronically increased sympathetic tone on the cardiorenal axis are well known⁵⁴. However, neural connections between the central and peripheral autonomic system remain very complex. Tsai et al. provided further insights into the cardiorenal neural connections in a canine model⁵⁵. The investigators provided histopathological proof of damage to nerves in the stellate ganglion and medulla in healthy dogs in the weeks following RDN. Besides the histological proof, functional measurements with radio transmitters on the stellate ganglia and vagal nerve demonstrate decreased stellate ganglion activity and a significant reduction of both duration and frequency of atrial tachyarrhythmia episodes after RDN. This study clearly demonstrated that RDN leads to central as well as to peripheral sympathetic nervous system denervation. Among other cardiovascular diseases, AF is a condition that is influenced by the autonomic nervous system. Therefore this study provides insight in the crosstalk between the autonomic nervous system, the heart and the possibility to influence this system by targeting the autonomic nervous system. The autonomous nerve connections to heart are visualized in Figure 2.

Figure 2. The cardiac sympathetic ganglia consist of cervical ganglia, stellate (cervicothoracic) ganglia, and thoracic ganglia. Parasympathetic innervation originate from the vagal nerves. Reprinted from Shen et al⁶² with permission of the publisher. Copyright © 2011, Nature Publishing Group. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

The initial successes of studies regarding renal sympathetic denervation created a promise of one form of solution for resistant hypertension. Several unblinded studies suggested that renal denervation could substantially lower BP in patients with resistant hypertension⁵⁵⁻⁶⁴. These studies have raised quite an interest in RDN.

As an example, an open-label randomized trial (DENERHTN) compared stepped antihypertensive therapy alone with stepped antihypertensive therapy plus renal denervation in 106 patients with confirmed resistant hypertension despite therapy with indapamide, amlodipine, and ramipril (or irbesartan if allergic to angiotensin-converting enzyme inhibitors)⁶⁵. Stepped antihypertensive therapy consisted of spironolactone, bisoprolol, prazosin, and rilmenidine added (in that order) at monthly intervals if home BP remained above 135/85 mmHg. At six months, the decrease in 24-hour ambulatory systolic pressure was significantly more in the renal denervation group (-15.4 versus -9.5 mmHg). However, this study had several limitations. In addition to the lack of a sham intervention, all patients lost to follow-up were in the denervation group and were not analysed, also the number of antihypertensive medications used at six months was similar in both groups. Baseline

BP was higher in the denervation group, implying that the results could be due in part to regression to the mean. Regression to the mean is a prominent pitfall in hypertension studies. A considerably smaller trial found that spironolactone lowered 24-hour ambulatory systolic pressure more than renal denervation⁶⁶.

A blinded randomized trial (SYMPPLICITY-HTN-3) failed to demonstrate benefit of RDN^{67,68}. In SYMPPLICITY-HTN-3 trial, 535 patients with treatment-resistant hypertension (systolic pressure >160 mmHg despite three or more antihypertensive medications, including a diuretic) were assigned to renal denervation or a sham procedure; BP decreased to a similar degree in both groups at six months, and there was no difference in the incidence of serious adverse events. Due to the lack of benefit, the inclusion in a larger ongoing international trial (SYMPPLICITY-HTN-4) was stopped prematurely. Two additional, smaller sized sham-controlled trials confirmed the findings of the SYMPPLICITY-HTN-3 trial^{69,70}.

The current state of RDN in the setting of uncontrolled hypertension is established in the Joint Position Paper on Renal Denervation. Further research is encouraged to better select patients that might benefit from renal denervation. Patient selection can be divided into different factors that differ between patients. For example, the anatomy of the renal vasculature might be a reason to refrain from RDN (Okada classification). Another factor that should be considered is vascular resistance as a contributor to the development of hypertension. And major concern regarding RDN is the lack of a procedural endpoint⁷¹.

The autonomic nervous system, particularly the imbalance between sympathetic and vagal hyperactivity, plays an important role in the initiation, maintenance and perpetuation of atrial tachycardia/atrial fibrillation^{72,73}. Animal models, for example the rabbit model for atrial fibrosis reported by Wei and colleagues, demonstrated less inducibility of AF after RDN⁷⁴. An interesting study by Yu et al. demonstrated the effect of renal sympathetic stimulation (RSS) on the effective refractory period and the window of vulnerability. This paper concludes that RSS leads to an increase in sympathetic tone and an increase in inducibility of AF in a dog model⁷⁵. Coumel et al. first reported on the importance of vagal and sympathetic hyperactivity in the genesis of atrial arrhythmias in humans⁷⁶⁻⁷⁸. Arora et al demonstrated an abundance of parasympathetic fibres in the posterior wall of the left atrium which have distinctive anatomic, molecular, and electrophysiological properties that favour the development and maintenance of AF⁷⁹. The mechanism of arrhythmia triggering through the cardiorenal axis may be initiated by activation of the mechanoreceptors in the renal pelvic wall and chemoreceptors in the renal interstitium with stimuli, such as ischemia, hypoxia or intrinsic renal disease. Stimulation of these receptors may lead to renal afferent sympathetic signalling through the hypothalamus, followed by increased central sympathetic outflow and efferent sympathetic nerve signalling to the heart, which leads to enhanced automaticity and triggered activity. Figure 3 summarizes the discussed interactions elegantly. The currently

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available data on the role of RDN in cardiac arrhythmogenesis have been summarized well in a review by Linz et al., suggesting an ongoing role for RDN in the setting of atrial (and ventricular) arrhythmia⁸⁰.

Figure 3. Mechanisms by which autonomic tone can promote AF. Top, Action potential changes showing cellular mechanisms by which adrenergic activation can lead to focal ectopic firing. Black dotted tracings represent normal reference action potentials in each panel. A, Enhanced automaticity. B, Early afterdepolarizations (EADs). C, Delayed afterdepolarization (DADs). Contributions from adrenergic activation alone are shown by red tracings, whereas that from cholinergic activation (combined with adrenergic activation) by green tracings. Adrenergic stimulation in the setting of impaired repolarization reserve can cause phase-2 EADs (red dashed tracings in B). Most phase-3 EADs are also associated with prolonged action potential duration (APD; blue dashed tracings in B). Combined adrenergic/vagal discharge can produce late phase-3 EADs (green dashed tracings in B) because of a prolonged and enhanced Ca^{2+} transient that outlasts I_{KACH} -induced accelerated repolarization. D, Tissue-level arrhythmia mechanisms, with focal ectopic activity maintaining AF as a driver or acting on vulnerable re-entrant substrates. Parasympathetic firing discharges acetylcholine, producing spatially heterogeneous action potential and refractory period abbreviation that promotes the occurrence and maintenance of re-entrant activity. CaT indicates calcium transient; LA, left atrium; NCX, sodium calcium exchanger; RA, right atrium; RyR, ryanodine receptor; and SR, sarcoplasmic reticulum.

AIM AND OUTLINE

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Excluding the general introduction (**chapter 1**) and the discussion (**chapter 8**), the thesis consists of six chapters. Each chapter discusses a different aspect of RNS and RDN. **Chapter 2** discusses the feasibility of RNS during RDN procedures in humans while studying different output settings and pacing settings. Renal nerve stimulation was only performed in animal models prior to this publication. We used RNS to establish it as a predictor of clinical response to RDN based on ABPM data in **chapter 3**. The correlation between the RNS-induced changes in BP and the differences in 24 hour ambulatory BP proved to be significant. **Chapter 4** demonstrates that accessory arteries elicit an impressive increase in BP in response to RNS, possibly explaining the mechanism behind the sympathetic innervation of accessory arteries. Therefore, renal denervation is less likely to be effective in patients with a more complex renal vasculature involving smaller accessory arteries that cannot be denervated with the current technology.

Chapter 5 discusses the different patterns of BP response to RNS. The different patterns possibly explain the different physiological structures that are being stimulated during RNS. An invited review discussing RNS as a procedural endpoint to RDN is described in **chapter 6**. A final step in this thesis is the start of a currently ongoing trial investigating the role of RDN in the setting of AF. The ASAF study design is described **chapter 7**, discussing the clinical background and rationale of investigating RDN as a treatment option on top of pulmonary vein isolation in a multicenter randomized clinical trial.

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CHAPTER II

Blood pressure response to renal nerve stimulation in patients undergoing renal denervation: a feasibility study

Cardiovascular effects of renal nerve stimulation

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ABSTRACT

During renal sympathetic denervation (RDN), no mapping of renal nerves is performed and there is no clear endpoint of RDN. We hypothesized high-frequency renal nerve stimulation (RNS) may increase blood pressure (BP), and this increase is significantly blunted after RDN. The aim of this study was to determine the feasibility of RNS in patients undergoing RDN. Eight patients with hypertension undergoing RDN were included. A quadripolar catheter was positioned at four different sites in either renal artery. RNS was performed during 1 minute with a pacing frequency of 20 Hz. Subsequently, all patients successfully underwent RDN. After RDN was performed, RNS was repeated at the site of maximum BP response before RDN in either renal artery. Mean age was 66 years. During RNS, BP increased significantly from 108 / 55 to 132 / 68 mmHg ($P < 0.001$). After RDN, systolic BP response at site of maximum response to RNS was significantly blunted (+43.1 mmHg vs. +9.3 mmHg, $P = 0.002$). In 25% of patients, a systolic BP increase > 15 mmHg after RDN. In conclusion, RNS resulted in an acute temporary increase in BP. This response was significantly blunted after RDN. RNS may potentially serve as an endpoint for RDN.

Keywords: Renal denervation, renal nerve stimulation, therapy-resistant hypertension

INTRODUCTION

Endovascular renal sympathetic denervation (RDN) is a promising treatment modality in the treatment of resistant, essential hypertension.^{1,2} Ablation lesions are rotationally and longitudinally applied in a spiral pattern to the renal artery to ablate the renal nerves under fluoroscopic guidance. However, no anatomical or functional mapping of renal nerves is performed during ablation. There is no functional test to guide RDN and no functional endpoint is defined to establish a successful RDN procedure. The potential therapeutic role of RDN in hypertensive patients is being questioned after announcement of failure of the Symplicity HTN-3 study to meet its primary efficacy endpoint.³ This suggests a modest overall effect of RDN and highlights the crucial need for a functional test to define a clear cut RDN procedural endpoint. Studies are needed to delineate the mechanisms of RDN and to optimize the technique before further deployment in patients. High-frequency renal nerve stimulation (RNS) in an animal model resulted in an acute temporary rise in blood pressure (BP), which was significantly blunted after RDN.⁴ However, no data is available on RNS in humans. The aim of this study was to design an RNS protocol and determine the effects of high-frequency electrical stimulation in the renal arteries on BP before and after RDN.

METHODS

Eight consecutive patients were included. Inclusion criteria were: office systolic BP exceeding 140 mmHg and systolic BP on 24-hour ambulatory BP recording exceeding 130 mmHg, despite the use of at least three anti-hypertensive drugs, including at least one diuretic. Patients were excluded in case of secondary hypertension (e.g. hyperaldosteronism), or if a preprocedural CT scan revealed renal arteries that were not suitable for RDN. In case of dual renal arteries, RNS was performed in the renal artery with the largest diameter.

The study was approved by the institutional review board and written informed consent was obtained from all patients. All procedures were performed under general anaesthesia, supervised by a cardiac anaesthesiologist.

RNS protocol

A quadripolar catheter (EPXT, Bard, U.S.A.) was introduced in the renal artery via femoral access. The first renal artery to undergo RNS was alternated between left and right among patients. RNS was performed at four sites (distal – cranial, distal – caudal, proximal – cranial, proximal – caudal) in both arteries for 1 minute. Pacing frequency was set at 20 Hz⁴⁷, pacing output at 20 mA and pulse duration at 2 ms. The pacing output setting was based on data in four patients (data not shown), in whom pacing output was gradually increased (5, 10, 15, 20 mA respectively). BP response was only observed with a pacing output setting exceeding 10 mA,

and pacing output was set at twice the threshold. In the first four patients, RNS duration was set at 3 minutes. However, BP response was observed after 30 seconds in these patients (data not shown). Therefore, RNS duration was set at twice the threshold, thus at 1 minute. During RNS, vasoactive medication was not altered. Figure 1 displays a fluoroscopic image of the setup.

Figure 1. Fluoroscopic image of renal nerve stimulation catheter setup. Panel A displays the renal angiogram. Panel B displays the quadripolar catheter, which has been inserted in the femoral artery, was maneuvered through the aorta and is positioned at the distal – caudal renal nerve stimulation site of the right renal artery.

Renal denervation protocol

The renal ablation catheter^{1,2} (Symplicity, Medtronic, Minneapolis, MN, USA) was introduced into each renal artery via femoral access. Discrete, radiofrequency (RF) ablations lasting up to 2 minutes each and ≤ 8 Watts were applied, with up to six ablations within each renal artery, separated both longitudinally and rotationally under fluoroscopic guidance. During RF energy application, tip temperature and impedance were monitored. RF energy delivery was regulated by a predetermined algorithm. The results of RNS were not used during the ablation procedure.

Post ablation RNS protocol

After RDN was performed, the sites of maximum BP response in either renal artery were relocated, guided by fluoroscopy. RNS was performed at the same settings as before RDN, i.e. pacing frequency of 20 Hz, pacing output was set at 20 mA and pacing duration was set at 2 ms. RNS at the subsequent site was performed after the BP returned to its baseline value.

Statistical analysis

Continuous variables were expressed as mean $\pm$ standard deviation or median with range where appropriate. Significances in differences were analyzed by a repeated measures analysis using mixed models. BP and heart rate response to RNS after RDN were compared to the BP and heart rate response at the corresponding site before RDN. Statistical analysis was performed using IBM SPSS statistics version 20 (IBM inc., Armonk, NY, USA). A P-value of ≤ 0.05

was considered statistically significant.

RESULTS

Eight consecutive patients were included, baseline characteristics are shown in Table 1. There was one patient with an accessory left renal artery and one patient with an accessory right renal artery.

Table 1. Baseline characteristics

Age (years)	66.3 (± 5.6)
Male gender (n)	5
Body mass index (kg m^{-2})	29.3 (± 1.3)
Systolic office (mm Hg)	172.3 (± 20.0)
Diastolic office BP (mm Hg)	96.1 (± 20.1)
24-h Ambulatory systolic BP (mm Hg)	153.3 (± 12.9)
24-h Ambulatory diastolic BP (mm Hg)	89.0 (± 3.5)
Antihypertensive drug use (range)	3.9 (3-6)
ACE-inhibitors (n)	3
Angiotensin II receptor blockers (n)	6
Renin inhibitors (n)	1
Beta blockers (n)	6
Calcium channel blockers (n)	5
Diuretics (n)	8
Other (n)	2
Diabetes mellitus (n)	1
Serum creatinine ($\mu\text{mol l}^{-1}$)	86.4 (± 15.7)

Abbreviations: ACE, angiotensin-converting enzyme; BP, blood pressure.

Data are presented as number of patients or mean $\pm$ s.d. or range where appropriate.

Blood pressure response to renal nerve stimulation

Mean BP increased during RNS from 107.6 (± 13.0) / 54.5 (± 7.4) mmHg before RNS to 132.3 (± 25.0) / 67.6 (± 11.8) mmHg after RNS ($P < 0.001$), as displayed in Figure 2. At 19.2% of sites where RNS was performed, BP increase was 5 mmHg or less, which were distributed evenly among RNS sites. There were four patients who showed a BP increase exceeding 5 mmHg at all RNS sites. The RNS sites that displayed the maximum BP increase were distributed evenly.

Renal denervation

RDN was successfully performed in all patients, with a median of 5 (range 4-6) RF applications per renal artery using the SymplicityTM catheter system. Mean catheter tip temperature was 54.3 (± 3.8) °C.

Figure 2. Systolic blood pressure before and after renal nerve stimulation

This figure displays the systolic BP before and after RNS. Mean systolic BP increased from 107.6 (± 13.0) mmHg to 132.3 (± 25.0) mmHg ($P < 0.001$) during RNS. The horizontal line indicates the mean systolic BP, the whiskers represent the range of systolic BP among patients. RNS: renal nerve stimulation; BP: blood pressure. See text for details.

Blood pressure response after renal denervation

BP response to RNS at the site of maximum response was +43.1 (± 14.7) mmHg before RDN, compared to +9.3 (± 10.5) mmHg after RDN, $P = 0.002$, as displayed in figure 3. In two patients at three sites, RNS after RDN increased systolic BP by more than 15 mmHg (15, 16 and 33 mmHg). Before RDN, RNS resulted in an increase of 42, 54 and 57 mmHg in systolic BP in these patients.

Figure 3. Systolic blood pressure response to renal nerve stimulation before and after renal denervation. RNS induced systolic BP increase at site of maximum response before renal nerve denervation (RDN) and after RDN. After RDN, systolic BP increase after RNS was significantly blunted: RNS induced systolic BP increase was +43.1 (± 14.7) mmHg before RDN compared to +9.3 (± 10.5) after RDN, $P = 0.002$. The horizontal line indicates the mean systolic BP response during RNS, the whiskers represent the range of observed systolic BP responses among patients. RDN: renal denervation; RNS: renal nerve stimulation; BP: blood pressure. See text for details.

Heart rate response to renal nerve stimulation

2 patients were in atrial fibrillation during the procedure. The sinus cycle length shortened significantly during RNS from 1286.9 (± 172.8) ms to 1069.2 (± 274.0) ($P < 0.001$). After RDN, sinus cycle length shortening during RNS was significantly reduced (+210.7 ms vs.

44.7 ms, $P = 0.015$), as displayed in figure 4. Of note, maximum sinus length shortening after RDN was 152 ms. This patient also displayed a BP increase after RDN of 33 mmHg.

Figure 4. Heart rate increase during renal nerve stimulation before and after renal denervation

This figure displays the sinus cycle length shortening during RNS before and after RDN. After RDN, the sinus cycle length shortening was significantly reduced compared to the sinus cycle length shortening before RDN (-210.7ms vs. -44.7 ms, $P = 0.015$). The horizontal line indicates the mean sinus cycle length reduction during RNS, the whiskers represent the range of observed sinus cycle length reductions among patients. RDN: renal denervation; RNS: renal nerve stimulation.

DISCUSSION

This is, to our knowledge, the first clinical study in patients with resistant hypertension, showing that high-frequency RNS is feasible and results in an acute, temporary increase in BP. BP response was significantly blunted after RDN. RNS may be an important tool to assess successful RDN.

Blood pressure response to renal nerve stimulation

This study aimed to show the feasibility of RNS. We hypothesized stimulation of renal nerves produces an increased sympathetic nervous tone, resulting in an increase in blood pressure and heart rate. Earlier reports have shown stimulation of afferent sympathetic nerve increases vasoconstriction⁸⁻¹¹ and induces metanephrine release¹¹⁻¹⁴. Moreover, in a recent report⁴, RNS in an animal model was reported to produce an increase in BP and to induce epinephrine and norepinephrine release.

Renal nerve anatomy

Atherton et al.¹⁵ showed, in a histologic study, 90% of renal nerves were located in the renal artery wall within the first 2 mm from the renal artery lumen, making them suitable for ablation. In the present study, we used high-frequency pacing to stimulate these renal nerves, resulting in an acute, temporary BP increase. Atherton et al. reported the number of nerves

tended to increase along the length of the renal artery. In the current study, the sites that displayed a BP increase less than 5 mmHg were evenly distributed among proximal and distal sites. However, our study was not designed to determine the association between the anatomical location of renal nerves data and the BP response to RNS.

2

Non-responders to renal denervation

Recently, failure of the Symplicity HTN-3 study to meet its primary efficacy endpoint was announced and the results of this study will be presented at the ACC convention in March 2014³. This preliminary announcement suggests a modest overall effect of RDN and highlights the crucial need for a functional test to define a clear cut RDN procedural endpoint. Furthermore, the procedure needs optimization to allow reliable denervation of the renal arteries with reproducible clinical results. Studies are needed to delineate the mechanisms of RDN before further deployment of the technique. Previous studies demonstrated that about 23% of patients did not show a BP reduction after RDN (non-responders)⁶. Hypothetically, a possible explanation is that not all renal nerves were successfully ablated during RDN. Since RDN is performed empirically, there is no clear cut functional endpoint to ascertain if all renal nerves have been successfully ablated. The present study showed RNS increased systolic BP after RDN in two patients. Potentially, ablation was incomplete in these patients, resulting in a BP increase up to 33 mmHg during RNS after RDN. Hypothetically, these patients are among the non-responders, although this feasibility study was not designed to prove this hypothesis. Potentially, RNS may be a technique to identify patients with incomplete ablation lesions and provide rationale for RNS guided application of additional ablation lesions to denervate the renal arteries.

Future directions

In this study, RNS did not result in an acute BP rise in 19.2% of RNS sites. Hypothetically, the absence of BP response to RNS is indicative of local absence of sympathetic nerve fibers. Whether RNS can be used to map renal nerves and identify possible target sites for ablation, is yet to be delineated.

The currently used catheter systems to perform RDN do not allow performing RNS. Potentially, a catheter system can be designed capable to both stimulate and ablate renal nerves, similarly to the currently used RF ablation systems that are used to perform cardiac ablations in patients with arrhythmias.

Limitations

With regard to interpreting our data, the following limitations should be considered. This is a single center study with a limited number of patients. We did not use a 3D anatomical mapping system, since it was not available at the time of this study, RNS sites after RDN were identified using fluoroscopy. Patients were not followed up after RDN.

Conclusion

High-frequency electrical stimulation of nerves in the renal arteries results in a temporary increase in BP. RDN significantly blunted the BP response to RNS. Potentially, RNS can be used to identify target ablation sites and can be used as an endpoint of successful RDN.

Acknowledgments

None.

Conflict of interest

The authors declare no conflict of interest.

2

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CHAPTER III

Renal Nerve Stimulation-Induced Blood Pressure Changes Predict Ambulatory Blood Pressure Response after Renal Denervation

Creating an endpoint for renal nerve denervation

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ABSTRACT

Blood pressure (BP) response to renal denervation (RDN) is highly variable and its effectiveness debated. A procedural endpoint for RDN may improve consistency of response.

The objective of the current analysis was to look for the association between renal nerve stimulation (RNS)-induced BP increase before and after RDN, and changes in ambulatory blood pressure monitoring (ABPM) after RDN.

Fourteen patients with drug-resistant hypertension referred for RDN were included. RNS was performed under general anaesthesia at four sites in the right and left renal arteries, both before and immediately after RDN. RNS-induced BP changes were monitored and correlated to changes in ambulatory BP at a follow-up of 3 to 6 months after RDN. RNS resulted in a systolic BP increase of 50 ± 27 mmHg before RDN and systolic BP increase of 13 ± 16 mmHg after RDN ($p<0.001$). Average systolic ABPM was 153 ± 11 mmHg prior to RDN and decreased to 137 ± 10 mmHg at 3 to 6 months follow-up ($p=0.003$). Changes in RNS-induced BP increase before vs. immediately after RDN, and changes in ABPM before vs. 3 to 6 months after RDN were correlated, both for systolic BP ($R=0.77$, $p=0.001$) and diastolic BP ($R=0.79$, $p=0.001$). RNS-induced maximum BP increase before RDN had a correlation of $R=0.61$ ($p=0.020$) for systolic and $R=0.71$ ($p=0.004$) for diastolic ABPM changes.

RNS-induced BP changes before vs. after RDN were correlated with changes in 24-h ABPM 3-6 months after RDN. RNS should be tested as an acute endpoint to assess the efficacy of RDN and predict BP response to RDN.

Keywords: sympathetic renal denervation, 24h ABPM, renal nerve stimulation, drug resistant hypertension

List of abbreviations

BP	Blood pressure
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
RDN	Renal sympathetic denervation
ABPM	Ambulatory blood pressure monitoring
RNS	Renal nerve stimulation
RF	Radio-frequency

INTRODUCTION

Renal sympathetic denervation (RDN) has been reported as a successful treatment of resistant hypertension in multiple trials studying the efficacy of RDN.¹⁻⁶ The sham-controlled Symplicity HTN-3 trial, however, failed to show significant RDN induced changes in BP versus control,⁷ raising serious doubts about the efficacy of RDN.⁸⁻¹¹ Variable effects of RDN on BP have been reported, with a wide spectrum ranging between non-response to marked reduction in BP.¹² The absence of an ablation procedural endpoint for RDN is a potential explanation for variable responses and the successful implementation of a reliably predictable endpoint could improve procedural success and clinical response.

Recently we reported on the feasibility of high frequency electrical renal nerve stimulation (RNS) in patients with resistant hypertension and demonstrated that RNS-induced BP increase was significantly blunted after RDN.¹³ We hypothesized that the difference between RNS-induced BP rise before and after RDN could be used as a procedural endpoint for RDN, and may prove a reliable functional test to predict BP response to RDN. The main goal of the current analysis was to investigate the 24-h ambulatory BP changes after RDN and the relation to RNS before and after RDN.

METHODS

Patients

All patients with treatment-resistant hypertension referred for renal denervation between May 2014 and February 2015 were screened for inclusion in the RNS study. Patients were eligible if they were on a stable antihypertensive drug regimen of at least 3 antihypertensive drugs (preferably with a diuretic) for at least 1 month, guided by hypertension specialists. Office systolic BP was >140 or diastolic BP >90 mmHg and 24-h systolic BP was >130 or diastolic BP >80 mmHg despite stable treatment, excluding patients with white coat hypertension. Patients were screened for eligibility by a multi-disciplinary team, including cardiologists, hypertension specialists and a radiologist. All patients were willing and able to comply with the protocol and had provided written informed consent. Age range for possible inclusion was 18-80 years and glomerular filtration rate had to be >45 mL/min/1.73m² according to the MDRD (Modification of Diet in Renal Disease) formula.¹⁴

Exclusion criteria were an unsuitable anatomy for RDN assessed by CT-angiography (main renal artery lumen diameter ≤ 3 millimetre, or a total length <20 millimetre of the main arteries), type 1 diabetes mellitus, chronic oxygen use, primary pulmonary hypertension, pregnancy and a mental or physical inability to participate. Patients enrolled in another investigational drug or device study were also excluded. Secondary hypertension was

excluded by a rigorous protocol excluding hyperaldosteronism, pheochromocytoma, renal artery stenosis (>50% stenosis in one or both arteries on CT-angiography), drug or substance abuse induced hypertension when appropriate, according to the European guidelines for the management of hypertension.¹⁵ The study was approved by the local medical ethical committee (ABR number 47172) and was conducted according to the declaration of Helsinki. The trial is registered under ClinicalTrials.gov ID: NCT02496117.

Outcomes

The primary endpoints of the current study were invasively measured RNS-induced BP changes before and after RDN, the change in 24-h ABPM before RDN versus 3 to 6 months follow-up after RDN, and the correlation between these variables.¹⁴ Secondary endpoints were office BP measurements during follow up and number of antihypertensive drugs. To guard safety during RNS we set the maximum systolic BP during RNS at 180 mmHg, at which point RNS was immediately discontinued.

Procedures

RDN was performed under general anaesthesia. Throughout the RDN procedure, no changes were made in use of vasoactive medication and no inotropic medication was necessary. The procedure was performed by experienced cardiac electrophysiologists. Two sheaths were placed in the right femoral artery, one for continuous BP measurement and one for catheter access. 5000 IU of heparin were administered during the procedure. In addition, in patients not previously on acetylsalicylic acid, we administered 500 mg of acetylsalicylic acid. We performed angiography with a pigtail catheter to visualize the aorta and renal arteries. Initially, a conventional quadripolar catheter with a tip electrode of 2 mm, other electrodes of 1 mm and interelectrode spacing of 5 mm (EP-XT, C. R. Bard, Inc., Murray Hill, NJ, USA) was introduced in the renal artery, under fluoroscopic guidance. Unipolar stimulation was performed from the tip of the catheter. This catheter was used only when performing RDN with the single-electrode ablation catheter (Symplicity Flex Renal Denervation Catheter, Medtronic, Minneapolis, MN, USA). In patients who were ablated with the multi-electrode basket ablation catheter (EnligHTN, St Jude Medical, Saint Paul, MN, USA), bipolar stimulation was performed by the ablation catheter itself, with bipolar stimulation from poles 1-2 and 3-4 proximally and distally in the renal artery. The first renal artery to undergo RNS was alternated between left and right among consecutive patients. RNS was performed at multiple sites with a minimum of 4 sites in both arteries, ensuring that different quadrants of the arterial circumference were stimulated in proximal and distal areas of the renal artery, in which ablations were usually performed. Pacing frequency was set at 20 Hz, pacing output at 20 mA with a pulse duration of 2 ms, based on earlier research.^{13,16-19} Stimulation duration was 60 sec, or shorter when systolic BP increased beyond 180 mmHg. We waited for the BP to return to baseline values before proceeding to the next stimulation site.

After RNS in both arteries (total of at least 8 stimulation sites), we performed a standard RDN procedure. The single-electrode Medtronic renal ablation catheter was used in 3 patients and the St. Jude multi-electrode EnligHTN catheter in 11 patients. In one patient, a combination of both single electrode and multi-electrode RDN catheters was used. In this latter patient, the left renal artery was denervated with the multi-electrode basket catheter. We switched to the flexible single electrode catheter due to anatomical variation prohibiting the introduction of the multi-electrode basket catheter in the right renal artery. The use of the single electrode catheter (Symplicity, Medtronic) has been described previously.¹³ The multi-electrode RDN catheter contains 4 electrodes separated from each other in a basket configuration. Up to 4 discrete radiofrequency (RF) ablations were applied simultaneously, each application lasting up to 120 seconds with a power ≤ 8 Watts (W) and a target temperature of 70 degrees Celsius (158 degrees Fahrenheit). Electrodes that did not reach the target temperature or impedance drop were switched off. In each renal artery, up to 3 sets corresponding to 12 ablation points were performed, separated both longitudinally and rotationally under fluoroscopic guidance. During RF energy application, tip temperature and impedance were monitored. RDN was considered successful when the maximum number of anatomically possible ablation points was reached, in accordance with standard care.

Importantly, the results of RNS were only monitored and did not influence the number of RF applications or the RDN procedure. After RDN we repeated the RNS procedure, fluoroscopically placing the pacing electrodes at the site at which the maximum BP response to RNS was observed prior to RDN. We chose to check the site of maximum BP response to ensure that the difference between patients that still showed an increase in BP after RDN and patients that no longer showed an increase in BP in response to RNS would become clear. The Bard EP system (Labsystem Pro, Bard, USA) was used to record and monitor BP changes throughout the procedure. Additionally we documented all RNS-related BP changes on a study work list.

Patients were followed and monitored as an outpatient by a vascular internist, who was informed that RDN was successfully performed, but was unaware of the results of the RNS. In all patients a validated 24-h ABPM with 30 min BP measuring intervals was obtained (Spacelabs, Snoqualmie, WA, USA) at 3 and/or 6 months after the procedure to assess the effect of RDN.²⁰ Office BP measurements were executed either by repeated automated oscillometry measurements (intrinsically blinded) or by sphygmomanometry during outpatient contacts. BP measurements by sphygmomanometry were performed by technicians not involved in analysis of the study and were regarded blinded. Antihypertensive drug therapy was left unchanged, unless symptomatic hypotension or out of range hypertension (>180 mmHg systolic BP) warranting immediate control were present.

Statistical analysis

Categorical variables were summarized by frequencies and percentages. Continuous variables (i.e. ABPM, office systolic BP, office DBP) are reported as mean and standard deviation or median and interquartile range where appropriate. Variables were tested for normality of distribution. A paired t-test was used to compare continuous variables, Wilcoxon signed rank test was applied to compare the difference in number of antihypertensive drugs. Pearson's correlation was used to assess the relation of RNS-induced BP changes assessed by office BP and 24-h BP measurements. To compare the different correlations, a Fisher's R to Z transformation was used. Statistical analysis was performed using IBM SPSS statistics version 20 (IBM Inc. Armonk, NY, USA). A P-value of ≤ 0.05 was considered statistically significant.

RESULTS

Demographics

Fourteen patients were included in the RNS study. Patients underwent their RDN procedure between May 2014 and February 2015. The demographic and clinical characteristics at baseline are shown in Table 1. The anatomy of the renal vasculature is represented by the Okada classification.

Table 1. Baseline characteristics (n=14 patients)

Variable	Baseline	Follow up (3 to 6 months)	P-value
Age (years)	66 [39-76]	-	-
Male gender	7/14	-	-
Office systolic BP (mmHg)	166 $\pm$ 23	149 $\pm$ 19	0.003
Office diastolic BP (mmHg)	98 $\pm$ 14	90 $\pm$ 15	0.017
24-h systolic ABPM (mmHg)	153 $\pm$ 11	137 $\pm$ 10	0.003
24-h diastolic ABPM (mmHg)	88 $\pm$ 8	80 $\pm$ 9	0.018
Hypercholesterolemia	10/14		
BMI (kg/m ²)	29.2 $\pm$ 2.9	-	-
Creatinine (μ mol/L)	84 $\pm$ 16	86 $\pm$ 17	0.570
GFR (MDRD, mL/min/1.73m ²)	76 $\pm$ 16	75 $\pm$ 18	0.682
Smoking	1/14		
CAD	2/14		
Atrial fibrillation	2/14		
Diabetes mellitus type II	1/14		
Heart rate (bpm)	66 $\pm$ 8	66 $\pm$ 7	0.716
# anti-hypertensive drugs	4 [3-6]	4 [2-6]	0.206
• diuretic	9/14	8/14	
• ARB	13/14	13/14	
• ACEi	1/14	1/14	

Table 1 continued.

Variable	Baseline	Follow up (3 to 6 months)	P-value
# anti-hypertensive drugs			
• β blocker	9/14	10/14	
• CCB	12/14	10/14	
• α_1 blocker	6/14	4/14	
• renin inhibitor	1/14	1/14	
• aldosterone antagonist	6/14	5/14	
• CAS	2/14	1/14	
Okada classification right			
• A1	9/14		
• A2	1/14		
• A3	3/14		
• B1	1/14		
• B2	0/14		
Okada classification left			
• A1	11/14		
• A2	1/14		
• A3	2/14		
• B1	0/14		
• B2	0/14		
Accessory artery	6/14		

Values between brackets indicate range, other values represent standard deviation, BP = blood pressure, ABPM = ambulatory blood pressure monitoring, BMI = body mass index, GFR = glomerular filtration rate, MDRD = modification of diet in renal diseases, CAD = coronary artery disease, ARB = angiotensin receptor blocker, ACEi = angiotensin converting enzyme inhibitor, β blocker = beta receptor blocker, CCB = calcium channel blocker, α_1 blocker = α_1 receptor blocker, CAS = centrally acting sympatholytic

Procedural data

In all 14 patients denervation of the renal arteries was performed. The median number of ablations was 8 in both arteries. The number of RF ablations ranged from 4 to 16 in the right renal artery and from 4 to 12 in the left renal artery. Six out of the 14 patients had accessory renal arteries. Details of renal vasculature based on the Okada classification are summarized in Table 1. The accessory arteries were deemed too small for additional denervation. Depending on the RDN system used, RF energy was delivered based on tip temperature or impedance drop. EnligHTN multi-electrode RDN system (St Jude Medical) simultaneously delivers RF energy at 4 electrodes of the basket catheter in a temperature controlled mode with a target 70 degrees Celsius (158 degrees Fahrenheit). In patients denervated with the Symplicity RDN system, RF energy delivery is fully automated and the system displays changes in impedance during RF energy application. An impedance drop of >10% was considered successful. If target ablation point did not reach adequate impedance drop or target temperature, the RF application was automatically turned off. Delivered RF energy ranged from 2.0 W to 6.6 W and was not allowed to exceed 8.0 W.

Of the cumulative 105 sites that were successfully stimulated, an increase in systolic BP of more than 10 mmHg in response to RNS was elicited at 78 sites (74.3%). Procedural data on the RNS sites is summarized in Table 2. Of note, in one patient no RNS has been performed in the right renal artery due to technical failure of the stimulator (Micropace). Response to RNS varied between patients with a median number of responding sites of 4.5, ranging from 3 to 12 RNS sites per patient. In the responding patients increases ranged from no response to a systolic BP increase of 118 mmHg. In one patient we observed no increase in BP exceeding 10 mmHg. In this patient, SBP increases ranged from no response to 9 mmHg increase in BP. We observed a preference for the site of maximum response to be in the upper quadrants, both distally and proximally (22/27 vs. 5/27 locations, $p=0.001$). No clear differences were observed between the right and left renal artery. There were no complications.

Table 2. Procedural data (n=14 patients)

Variable	Left renal artery (n=54 sites)	Right renal artery (n=51 sites)
# sites with SBP increase >10 mmHg	41 (76%)	37 (73%)
# sites with no BP response to RNS	13 (24%)	14 (27%)
Site of maximum SBP response to RNS	N = 14 arteries	N = 13 arteries
Distal lower quadrant	7/14 (50.0%)	6/13 (46.2%)
Distal upper quadrant	0/14 (0.0%)	0/13 (0.0%)
Proximal lower quadrant	4/14 (28.6%)	4/13 (30.8%)
Proximal upper quadrant	2/14 (14.3%)	2/13 (15.4%)
Mid renal roof	0/14 (0.0%)	1/13 (7.7%)
Mid renal bottom	1/14 (7.1%)	0/13 (0.0%)
# RF ablation points	8 [4-12]	8 [4-16]

Table 2 summarizes the procedural data of the patients. In one patient no RNS was performed in the right renal artery due to technical failure of the stimulator (Micropace). No significant differences between the left and right renal arteries were observed.

In agreement with our previous work,¹³ the mean increase in systolic BP in response to RNS at the site of maximum response significantly decreased from 50 ± 27 mmHg before RDN to 13 ± 16 mmHg after RDN ($p<0.001$). See Figure 1 for a visual depiction of the data.

At a median follow-up of 4.5 months (3-6 months), office SBP decreased from 166 ± 23 mmHg at baseline to 149 ± 19 mmHg ($p=0.003$). DBP decreased from 98 ± 14 mmHg to 90 ± 15 mmHg during follow-up. At the same time point, 24-h systolic BP decreased from 153 ± 11 mmHg at baseline to 137 ± 10 mmHg ($p=0.003$). Antihypertensive drugs were increased in 2 patients and decreased due to symptomatic hypotension in 5 patients. However, the median number of anti-hypertensive drugs remained unchanged.

Figure 1. The maximum increase in systolic BP before the RDN procedure was 50 ± 27 mmHg and decreased to 13 ± 16 mmHg after the RDN procedure ($p < 0.001$).

Changes in ABPM systolic BP documented at median follow-up of 4.5 months after RDN were correlated with RNS-induced systolic BP rise before RDN at the site of maximum response ($R=0.610$, $p=0.020$). This correlation further increased after subtracting the RNS-induced BP rise measured immediately after RDN (ΔBP at site of maximal response = RNS induced BP increase prior to RDN - RNS induced BP increase after RDN) at the same site ($R=0.769$, $p=0.001$). These findings hold true for DBP as well (see Figure 2). Changes in 24-h ambulatory DBP were strongly correlated with RNS-induced DBP rise before RDN at the site of maximum response ($R=0.734$, $p=0.003$). In contrast, no significant correlation was observed between ABPM BP changes after RDN and residual RNS-induced BP rise after RDN considered alone.

Figure 2. The Pearson's correlation between the difference in systolic blood pressure increase induced by renal nerve stimulation before versus immediately after renal denervation, and the difference in mean 24-h systolic pressure before versus 3 to 6 months after renal denervation was $R=0.769$ ($p=0.001$, left upper quadrant). The

Pearson's correlation between the initial RNS-induced increase in systolic blood pressure prior to RDN procedure and the difference in mean 24-h systolic blood pressure was $R=0.610$ ($p=0.020$, right upper quadrant). The Pearson's correlation between the difference in diastolic blood pressure increase induced by renal nerve stimulation before versus after renal denervation, and the difference in mean 24-h diastolic pressure at a median of 4.5 months of follow-up was $R=0.664$ ($p=0.010$, left lower quadrant). The Pearson's correlation between the initial RNS-induced increase in diastolic blood pressure prior to RDN procedure and the difference in mean 24-h diastolic blood pressure was $R=0.734$ ($p=0.003$).

3

Changes in office BP were correlated with the acute BP data during the procedure. The difference in systolic BP increase before versus after RDN was significantly correlated with the clinical outcome based on office systolic BP ($R=0.724$, $p=0.003$). The difference in DBP increase before versus after RDN was significantly correlated with office DBP outcome during follow-up ($R=0.539$, $p=0.047$). See Figure 3 for depiction of the data. However initial RNS induced BP increase before RDN was not correlated with the DBP outcome. The increase in BP after RDN was not correlated with office BP data during follow-up.

Figure 3. The Pearson's correlation between the difference in systolic blood pressure increase induced by renal nerve stimulation before versus immediately after renal denervation, and the difference in office systolic pressure before versus 3 to 6 months after renal denervation was $R=0.724$ ($p=0.003$, left upper quadrant). The Pearson's correlation between the initial RNS-induced increase in systolic blood pressure prior to RDN procedure and the difference in office blood pressure was $R=0.446$ ($p=0.110$, right upper quadrant). The Pearson's correlation between the difference in diastolic blood pressure increase induced by renal nerve stimulation before versus after renal denervation, and the difference in office diastolic pressure at a median of 4.5 months of follow-up was $R=0.539$ ($p=0.047$, left lower quadrant). The Pearson's correlation between the initial RNS-induced increase in diastolic blood pressure prior to RDN procedure and the difference in office diastolic blood pressure was $R=0.238$ ($p=0.412$).

DISCUSSION

In this study, we have demonstrated that high frequency RNS before and after RDN is feasible and safe in patients. In addition, it is suggested that the use of RNS may facilitate prediction of the completeness of RDN and subsequent BP changes after the procedure.¹³ The current work describes for the first time the significant association between RNS-induced BP responses during RDN, and the clinical ABPM response to RDN at 3 to 6 months after RDN. This reflects the potential of using RNS as a functional test to evaluate the efficacy of renal nerve ablation and predict the clinical outcome of the RDN procedure.

Since the publication of the Symplicity HTN-3 trial,²¹ a study that failed to demonstrate BP reduction above and beyond a sham procedure, RDN has been the topic of renewed and ongoing debate. Some have questioned the efficacy of RDN, and others sought reasons for the negative findings. It has been argued that the poor efficacy of RDN in Symplicity HTN-3 may be partly explained by suboptimal renal nerve ablation.¹⁰ In contrast, in the recent DENERHTN trial,⁶ RDN was performed by a limited number of experienced operators, and drug adjustment was fully standardized, with a moderate but significant overall benefit of RDN on daytime ABPM. A recent meta-analysis by Fadl Elmula and colleagues including all published randomised controlled trials testing RDN against stable or intensified medical treatment showed little if any benefit of RDN.²² The troubling implication is that the BP benefit directly assignable to RDN may have been substantially overestimated in Symplicity HTN-1 and 2 and other non-randomised unblinded studies using office BP as primary endpoint.³⁻⁵

RDN is supported by a solid rationale, including reports from the years 1940-60²³⁻²⁶. During those times radical surgery was performed for sympathetic denervation, successfully lowering blood pressure, however, also in these patients a percentage appeared to be non-responders. High frequency electrical stimulation of the sympathoexcitatory renal afferent pathways is the most likely cause of the rise in BP induced by RNS. This is because the gradual rise in BP 10-20 sec after starting RNS is consistent with an increase in adrenergic response through stellate ganglion stimulation in canine studies as reported previously.²⁷ Experimental studies indicate that the afferent nerves play an important role in the sudden increase in BP in response to RNS.^{28,29} The afferent nerves are involved in the increase of central sympathetic tone. Peripheral renal denervation has a peripheral sympatholytic effect and alters the level of activation excitation of central noradrenergic pathways but does not alter sodium or water intake or excretion, plasma renin activity or creatinine clearance, suggesting that efferent renal nerve function does not play an important role in the maintenance of this form of hypertension. Selective lesioning of the renal afferent nerves attenuates the development of hypertension, thus providing direct evidence that the renal afferent nerves, at least in part, participate in the pathogenesis of renovascular hypertension.³⁰ Therefore, RNS influences this BP regulating mechanism by increasing the central sympathetic tone through an afferent

mechanism. The efferent nerve fibres would take more time to increase BP and renal artery vasoconstriction alone cannot explain a transient increase in BP in the femoral artery where BP was measured. We believe that the increase in central sympathetic tone accounts for an increase in systemic vascular resistance. One might argue that pain sensation invokes the increase in BP, but we did not observe an increase in the level of consciousness which would be expected when pain sensation increased greatly. All of the procedures were performed supervised by a cardiac anaesthesiologist, ensuring that pain was prevented. This study demonstrates a relationship between the increase in BP before vs. after a RDN procedure and the decrease in 24-h ambulatory BP. It is very unlikely that this can be an effect of the ablation of pain fibres surrounding the renal arteries. RNS enables the operator to determine the location of the renal nervous fibers, in which both afferent and efferent fibers are located. Both are to be targeted during a RDN procedure to diminish hypertension. Differences in BP increase are attributed to the differences in distribution of sympathetic nerve fibers in the tunica adventitia of the renal artery. The between-patient differences herein account for differences in response to high frequency electrical stimulation.

Failure to improve BP after RDN may reflect a poor contribution of the renal nerve sympathetic system to the pathophysiology of hypertension in a particular patient, or alternatively failure to ablate a sufficient proportion of sympathetic fibers.³¹ Unfortunately, so far, RDN has been performed unguided, mostly using unipolar electrodes, in the absence of a straightforward method allowing quantification of the renal sympathetic traffic before RDN and assessment of the completeness of renal nerve ablation after RDN. We hypothesized that peri-procedural high frequency RNS could be an attractive candidate to address both issues. One heavily criticized RDN study in the treatment of AF has used this modality to in the setting of catheter ablation in patients with atrial fibrillation and hypertension.³² While acute RNS-induced BP rise before RDN may reflect the contribution of renal sympathetic nervous system, the difference between RNS-induced BP rise before and after RDN may be a reliable indicator of the efficacy of the RDN procedure. The patient that did not show an increase in BP exceeding 10 mmHg in response to RNS at any point was a non-responder with office BP unchanged. This could theoretically be predicted by RNS since no sympathetic overdrive was observed prior to RDN. More importantly, patients that do show an impressive increase in BP before RDN and still do after RDN might require more extensive ablation. However, this has not been addressed in this explorative study.

Notably, RNS-induced BP changes were better correlated with ambulatory than with office BP, enforcing our belief that “true” responders to RDN are those defined according to ambulatory BP, which should be used as primary criterion both for patient selection and evaluation of efficacy.³³ The fact that office BP measurements have not been carried out as standardized as the 24-h ABPM may have played a part as well, leading to overestimation of the effect of white coat hypertension during office measurements.

Our results are in line with the pioneering experimental study of unilateral RDN in dogs by Chinushi and his colleagues.¹⁶ However, to the best of our knowledge, we are the first to describe this electrical stimulation-guided renal nerve mapping technique along with clinical outcome in patients with resistant hypertension.

The presence of an accessory artery might be another explanation why patients fail to respond to regular RDN. Previous reports showed significantly lower BP reduction in patients with accessory arteries that could not be denervated because of small size (<3-4 mm in diameter).³⁴ Because the sympathetic plexus is complex, when the accessory renal artery originates far from the main renal artery, an extra plexus may accompany the accessory artery. The remaining nerve tissue may lead to a possible sympathetic response via this pathway.³⁵

Admittedly, our study has limitations. First, the sample size is small. Secondly, our study is unblinded and purely observational, and the results may have been obscured by various patient- and physician-related biases. However, the impact of white coat effect was limited by the use of 24-h ambulatory blood pressure, and other potential confounders such as changes in drug adherence have been closely monitored. Finally, although we tried to perform RNS in several quadrants proximally and distally in the renal artery, we cannot exclude that some renal nerves were not stimulated, possibly decreasing the association between RNS and clinical response to RDN. In experimental studies norepinephrine spill over has been measured to assess efficacy of RDN. A suggestion for future research would be to correlate our findings with norepinephrine spill over or other ways to estimate sympathetic overdrive. In conclusion, this study demonstrates that the difference between acute RNS-induced BP rise before and after RDN is a significant predictor of BP outcome after RDN, probably because it conveys information on two major determinants of BP response to RDN: the importance of renal sympathetic activity at baseline and the completeness of renal nerve ablation after RDN. Future research should aim to demonstrate whether RNS-guided ablation - performed with the goal to decrease as much as possible RNS-induced BP response - allows the procedure to achieve more important and consistent BP decreases than conventional RDN.

Perspectives

Competency in medical knowledge: this study demonstrates that the difference between acute RNS-induced BP rise before and after RDN is a significant predictor of BP outcome after RDN, probably because it conveys information on two major determinants of BP response to RDN: the importance of renal sympathetic activity at baseline and the completeness of renal nerve ablation after RDN.

Competency in procedural skills: renal nerve stimulation (RNS) may be used as a functional test to identify sympathetic nerve fibers which can be subsequently ablated during a RDN procedure.

Translational outlook

Future research should aim to demonstrate whether RNS-guided ablation - performed with the goal to decrease as much as possible RNS-induced BP response - allows the procedure to achieve more important and consistent BP decreases than conventional RDN. This would change the procedural approach radically.

Role of the funding source

St. Jude Medical (Saint Paul, MN, USA) has provided funds for the EnligHTN system used to deliver electrical stimulation and RF energy for ablation. St. Jude Medical was not involved in the design of the study, patient recruitment, or data collection/analysis. St. Jude Medical provided critical comments.

Declaration of interest:

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CHAPTER IV

Persistent Increase in Blood Pressure Following Renal Nerve Stimulation in Accessory Renal Arteries After Sympathetic Renal Denervation

Role of residual sympathetic overdrive after RDN

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ABSTRACT

Blood pressure response to renal denervation is highly variable and the proportion of responders is disappointing. This may be partly due to accessory renal arteries too small for denervation, causing incomplete ablation. Renal nerve stimulation before and after renal denervation is a promising approach to assess completeness of renal denervation and may predict blood pressure response to renal denervation.

The objective of the current study was to assess renal nerve stimulation-induced blood pressure increase before and after RDN in main and accessory renal arteries of anaesthetized patients with drug-resistant hypertension. The study included 21 patients. Nine patients had at least one accessory renal artery in which renal denervation was not feasible. Renal nerve stimulation was performed in the main arteries of all patients and in accessory renal arteries of 6/9 patients with accessory arteries, both before and after RDN.

Renal nerve stimulation before renal denervation elicited a substantial increase in systolic blood pressure, both in main ($25.6 \text{ mmHg} \pm 2.9 \text{ mmHg}$, $p < 0.001$) and accessory ($24.3 \pm 7.4 \text{ mmHg}$, $p = 0.047$) renal arteries. After renal denervation, renal nerve stimulation-induced systolic blood pressure increase was blunted in the main renal arteries ($\Delta \text{SBP } 8.6 \text{ mmHg} \pm 3.7 \text{ mmHg}$, $p = 0.020$) but not in the non-denervated renal accessory renal arteries ($\Delta \text{SBP } 27.1 \pm 7.6 \text{ mmHg}$, $p = 0.917$).

Renal nerve stimulation-induced blood pressure increase after renal denervation of main renal arteries was significantly blunted in main renal arteries but not in accessory renal arteries. This residual source of renal sympathetic tone may result in persistent hypertension after ablation and partly account for the large response variability.

Keywords: renal sympathetic denervation, renal nerve stimulation, accessory artery, therapy-resistant hypertension

List of abbreviations

BP	Blood pressure
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
RDN	Renal sympathetic denervation
ABPM	Ambulatory blood pressure monitoring
RNS	Renal nerve stimulation
RF	Radio-frequency
ABR	Algemene beoordeling en registratie (Dutch study registration)

INTRODUCTION

Renal sympathetic denervation (RDN) has been accepted as a treatment for resistant hypertension^{1,2} because of the promising first results.^{3,5} However, failure of the RDN to lower blood pressure over and above the sham control group in the large randomised double-blinded Symplicity HTN-3 trial⁶ has raised doubt about the efficacy of the technique.⁷ Besides crucial design differences between Symplicity HTN-3 and previous, unblinded and mostly observational studies,⁸ the disappointing results of Symplicity HTN-3 may have been partly due to insufficient renal ablation.^{9,10} The possible role of insufficient renal ablation is further highlighted by the demonstration of a lesser response to RDN in patients with multiple¹¹ and accessory¹² renal arteries, which may contribute to the large variability of response observed after RDN.¹³ However, the mechanisms underlying these clinical observations have not yet been elucidated.¹⁴

Recently we showed that blood pressure increase elicited by high-frequency renal nerve stimulation (RNS) is significantly blunted after RDN.¹⁵ This approach may allow to gain further insights into the differences in blood pressure response to RDN in patient with or without renal accessory arteries. We hypothesized that after successful RDN there will be no significant increase in blood pressure after RNS in the main arteries while there will still be an increase in blood pressure in response to RNS in the accessory arteries. In order to test our hypothesis we investigated the blood pressure response to RNS in patients with only two renal arteries, as well as in patients with accessory renal arteries too small for renal nerve ablation, both before and after RDN of the main renal arteries.

METHODS

Primary objective

The main goal of this clinical study was to investigate the blood pressure response to high frequency electrical stimulation in accessory renal arteries before and after denervation of the main renal arteries in patients with resistant hypertension.

Study design

We performed a prospective single-center intervention study.

Patient population, inclusion and exclusion criteria

All patients between May 2014 and February 2015 with treatment-resistant hypertension referred for renal denervation were screened for inclusion in the renal nerve stimulation (RNS) study. Patients were eligible if they were on a stable antihypertensive drug regimen of at least 3 antihypertensive drugs (preferably with a diuretic) for at least 1 month, guided

by experienced physicians in hypertension treatment. Office SBP should be >140 or DBP >90 mmHg and 24-h ABPM SBP should be >130 or DBP >80 mmHg despite stable treatment, excluding patients with white coat hypertension. Patients were screened for eligibility by a multi-disciplinary team, including cardiologists, internists with hypertension subspecialty and a radiologist. All patients were willing and able to comply with the protocol and had provided written informed consent. Age range for possible inclusion was 18-80 years and glomerular filtration rate had to be >45 mL/min/1.73m² according to the MDRD formula.

Exclusion criteria were an unsuitable anatomy for RDN assessed by CT-angiography (main renal artery lumen diameter ≤ 3 millimeter, or a total length of less than 20 millimeter of the main arteries), type 1 diabetes mellitus, chronic oxygen use, primary pulmonary hypertension, pregnancy and a mental or physical inability to participate. Patients enrolled in another investigational drug or device study were also excluded. Secondary hypertension was excluded by a rigorous protocol excluding hyperaldosteronism, pheochromocytoma, renal artery stenosis ($>50\%$ stenosis in one or both arteries on CT-angiography), drug or substance use induced hypertension when appropriate, according to the European guidelines for the treatment of hypertension.¹⁶ The study was approved by the local medical ethical committee (ABR number 47172) and was conducted according to the declaration of Helsinki.

Endpoints

The primary end points of our study were twofold. The first endpoint was the difference between blood pressure response to RNS applied in accessory renal arteries before and after RDN, based on the intra-arterially measured blood pressure one minute after RNS-application. In daily routine this concerns accessory arteries with a lumen diameter of 3 millimeter or less. The second endpoint was the response in arterial blood pressure to RNS before and after RDN in the main renal arteries.

Procedure

In all patients, RDN and RNS were performed under general anesthesia with propofol administered intravenously, accompanied by fentanyl to reduce possible pain sensation. The administration of epinephrine was indicated only in case of severe hypotension, which proved to be unnecessary. No other medication acting on blood pressure or on vascular tone were administered during the procedure. Two sheaths were placed in the right femoral artery, one for continuous BP measurement and one for catheter access. 5000 IU of heparin were administered during the procedure. In addition, in patients not previously on acetylsalicylic acid, we administered 500 mg of acetylsalicylic acid intravenously.

The RNS protocol has been described in detail previously.¹⁵ Briefly, a quadripolar catheter (EPXT, Bard, Murray Hill, NJ, USA) was introduced in the renal artery via femoral access. The first renal artery to undergo RNS was alternated between left and right among patients. RNS

was performed at four sites (distal – cranial, distal – caudal, proximal – cranial, proximal – caudal) in both arteries for 1 minute. Pacing frequency was set at 20 Hz, pacing output at 20 mA and pulse duration at 3 ms. This protocol was established in our earlier research.¹⁵ To assess blood pressure response to RNS in the accessory artery, the same quadripolar catheter (EPXT, Bard, USA) was introduced in the accessory renal artery under fluoroscopic guidance. RNS was performed ostially in the accessory renal artery for 1 minute. The intra-arterially measured BP was determined immediately after cessation of RNS which was administered for one minute, or less in the case of severe BP increase with systolic BP reaching values that would exceed the maximum limit of 180 mmHg.

The renal ablation catheter by Medtronic (Symplicity, Medtronic, Minneapolis, MN, USA) was used in 12 patients and later the catheter by St. Jude Medical (EnligHTN, St. Jude Medical, Saint Paul, MN, USA) in 9 patients. The RDN catheter was introduced into each renal artery via femoral access. The use of the single electrode catheter (Symplicity, Medtronic) has been described previously.¹⁵ The multi-electrode RDN (EnligHTN, St Jude Medical) catheter contains 4 electrodes separated from each other in a basket configuration. Up to 4 discrete radiofrequency (RF) ablations were applied simultaneously, each application lasting up to 120 seconds and ≤ 8 Watts with a target temperature of 70 degrees Celsius. Electrodes that did not reach the target temperature or impedance drop were switched off. In each renal artery, up to 12 ablations were produced, separated both longitudinally and rotationally under fluoroscopic guidance. During RF energy application, tip temperature and impedance were monitored. The results of RNS were only monitored and did not influence the number of RF applications. Figure 1 displays a typical example of a fluoroscopic image of the aorta, main renal arteries and an accessory renal artery that was not suitable for RDN, but eligible for RNS. RDN of these relatively small accessory renal arteries (diameter < 3 mm) was not possible with the currently available catheters. Therefore, RDN in the accessory renal artery was not performed. After the RDN procedure of the main renal arteries, RNS in the main renal arteries was repeated according to the same stimulation protocol. Then a standard steerable quadripolar catheter (EPXT, Bard Electrophysiology) was placed in the accessory renal artery under fluoroscopic guidance. Once the quadripolar catheter was inserted in the accessory renal artery, RNS was performed with the same settings as for the other RNS locations. Blood pressure was recorded continuously during RNS.

Statistical analysis

All results are shown for the whole group and the subsets with and without accessory renal arteries. Categorical variables are summarized by frequencies and percentages. Continuous variables (i.e. ABPM, office SBP, office DBP) are reported as mean and standard deviation or median and interquartile range where appropriate. Variables were tested for normality of distribution. Main study endpoint was the arterial blood pressure response to RNS prior to RDN and decrease or absence of blood pressure increase in response to pacing in the renal

artery after RDN. The decrease or absence of blood pressure increase in response to pacing was defined as no or minimal blood response increase (<10 mmHg systolic blood pressure) in response to RNS. The percentage of patients without blood pressure increase are presented. Responses to RNS at the site of maximal response were taken into account in the analysis. Statistical analysis was performed using IBM SPSS statistics version 20 (IBM inc., Armonk, NY, USA). A P-value of ≤ 0.05 was considered statistically significant.

RESULTS

Our study included 21 patients with resistant hypertension who underwent RDN, either using the Symplicity RDN catheter (Medtronic, $n=12$), or the EnlighTN catheter (St. Jude Medical, $n=9$). 9/21 patients had an accessory artery deemed too small for denervation (diameter <3 mm, A2 or A3 subtype according to the Okada classification).¹⁷ Of these 9 accessory arteries 6 could be reached with the smaller size standard quadripolar stimulation catheter and RNS was performed in those 6 patients. RNS was performed both before and after RDN in all main renal arteries, as well as in non-denervated accessory arteries in which this proved feasible using the smaller size standard quadripolar stimulation catheter.

The demographic and clinical characteristics, renal anatomy, blood pressure level and antihypertensive treatment at baseline are shown in table 1, both for the whole group and in patients with or without renal accessory arteries. No significant difference was observed between both groups, with the exception of daytime systolic blood pressure, which was higher in the accessory renal artery group. A fluoroscopic image showing a set of main renal arteries and one accessory renal artery is displayed in figure 1.

Table 1. Baseline characteristics

Characteristic	All patients (n = 21)	Patients without accessory renal artery (n = 12)	Patients with accessory renal artery (n = 9)
Age (years)	64 (± 6)	63 (± 6)	65 (± 7)
Sex			
Males	15 (71.4%)	8 (66.7%)	7 (77.8%)
Body-mass index (kg/m ²)	29.6 (± 3.1)	29.5 (± 3.1)	29.6 (± 3.3)
Current smokers	1 (4.8%)	1 (8.3%)	0 (0%)
Medical history			
Hypercholesterolemia	13 (61.9%)	7 (58.3%)	6 (66.7%)
Type 2 diabetes mellitus	3 (14.3%)	1 (8.3%)	2 (22.2%)
Coronary artery disease	5 (23.8%)	3 (25%)	2 (22.2%)
Atrial fibrillation	11 (52.4%)	7 (58.3%)	4 (44.4%)
Number of antihypertensive medications	4 [2–6]	4 [3–6]	4 [2–5]

Table 1 continued.

Characteristic	All patients (n = 21)	Patients without accessory renal artery (n = 12)	Patients with accessory renal artery (n = 9)
Class of antihypertensive medication			
Diuretic	16 (76.2%)	9 (75%)	7 (77.8%)
Aldosterone receptor blocker	2 (9.5%)	1 (8.3%)	1 (11.1%)
Beta-blocker	16 (76.2%)	11 (91.7%)	5 (55.6%)
Calcium channel blocker	14 (66.7%)	8 (66.7%)	6 (66.7%)
ACE-inhibitor	6 (28.6%)	3 (25%)	3 (33.3%)
Angiotensin receptor blocker	17 (81%)	10 (83.3%)	7 (77.8%)
Aliskiren	1 (4.8%)	0 (0%)	1 (11.1%)
Centrally acting α_2 -sympatholytics	1 (4.8%)	0 (0%)	1 (11.1%)
A1-receptor blockers	5 (23.8%)	4 (33.3%)	1 (11.1%)
Other antihypertensive drugs	0 (0%)	0 (0%)	0 (0%)
Serum creatinin ($\mu\text{mol/L}$)	88 [69–124]	88 [69–113]	87 [74–124]
GFR (mL/min/m^2)	97.1 [46.5–141.5]	95.8 [74.3–114.7]	108.2 [46.5–141.5]
Ambulatory blood pressure (mm Hg)			
24-hour systolic	144 (± 16)	142 (± 17)	147 (± 16)
24-hour diastolic	82 (± 12)	81 (± 10)	83 (± 17)
Daytime systolic	144 (± 14)	137 (± 11)	151 (± 14)*
Daytime diastolic	85 (± 14)	83 (± 9)	88 (± 16)
Nighttime systolic	133 (± 16)	132 (± 17)	136 (± 16)
Nighttime diastolic	75 (± 13)	75 (± 11)	76 (± 16)
Office blood pressure (mm Hg)			
Systolic	158 (± 15)	157 (± 13)	160 (± 17)
Diastolic	95 (± 14)	96 (± 14)	93 (± 15)
Heart rate (bpm)	65 (± 16)	65 (± 19)	66 (± 15)
Okada classification			
Right			
A1	13 (61.9%)	12 (100%)	1 (11.1%)
A2	2 (9.5%)	0 (0%)	2 (22.2%)
A3	6 (28.6%)	0 (0%)	6 (66.7%)
B1	0 (0%)	0 (0%)	0 (0%)
B2	0 (0%)	0 (0%)	0 (0%)
Left			
A1	18 (85.7%)	12 (100%)	6 (66.7%)
A2	1 (4.8%)	0 (0%)	1 (11.1%)
A3	1 (4.8%)	0 (0%)	1 (11.1%)
B1	0 (0%)	0 (0%)	0 (0%)
B2	0 (0%)	0 (0%)	0 (0%)

Data are expressed as mean ($\pm$ SD), median [range], or number (%). ACE = angiotensin converting enzyme. eGFR: estimated glomerular filtration rate calculated using the MDRD formula. bpm = beats per minute.

*: $p < 0.05$ compared to patients without renal accessory arteries

The following data have been calculated with a mixed model analysis, for we obtained repeated measurements in multiple patients before and after an intervention. This provided us with a mean and a standard error of the mean. Before RDN, RNS of the main arteries elicited a significant systolic BP increase of 31.5 ± 14.1 mmHg. After RDN, RNS elicited a systolic BP increase of 9.2 ± 6.3 mmHg ($p = 0.020$, Figure 2). When the subgroup of 9 patients with one

or more accessory was studied, comparable results were observed. RNS in the main renal arteries elicited a systolic BP increase of 21.0 ± 3.6 mmHg prior to RDN. After RDN, RNS of the main renal arteries in these patients elicited a systolic BP increase of 8.2 ± 4.7 mmHg ($p=0.002$, Figure 3A). Notably, RNS in the accessory arteries resulted in a systolic BP increase of 24.5 ± 7.4 mmHg prior to RDN. After RDN, RNS still elicited a significant BP response of 27.1 ± 7.6 mmHg ($p=0.917$, Figure 3B). When the individual data were studied only in 1 out of 6 patients with an accessory artery demonstrated a blunted effect of RNS in the accessory artery.

Figure 1. Fluoroscopic image of the renal vasculature. There is a single left renal artery (*). The ostium of the main right renal artery is slightly lower than that of the left main artery (†). At the same height as the left main artery is the ostium of a smaller accessory renal artery (‡).

Figure 2. The mean SBP increase after RNS of the main renal arteries in the whole cohort before RDN is 31.5 mmHg with a standard error of ± 14.1 based on the mixed models analysis. The mean SBP increase after RNS of the main renal arteries in the whole cohort after RDN is 9.2 mmHg with a standard error of ± 6.3 mmHg ($p=0.020$).

Figure 3A: In the subset of 6 patients with accessory arteries RNS in the main renal arteries elicited a systolic BP increase of 21.0 with a standard error of ± 3.6 mmHg based on mixed models prior to RDN. After RDN, RNS of the main renal arteries in these patients elicited a systolic BP increase of 8.2 with a standard error of ± 4.7 mmHg ($p=0.002$).

Figure 3B: In the subset of 6 patients with accessory arteries RNS in the accessory renal arteries elicited a systolic BP increase of 24.5 with a standard error of ± 7.4 mmHg prior to RDN. After RDN, RNS of the accessory renal arteries in these patients elicited a systolic BP increase of 27.1 with a standard error of ± 7.6 mmHg ($p=0.917$).

DISCUSSION

Our study confirms that high frequency electrical stimulation of nerves in renal arteries (RNS), both main and accessory, elicits a substantial blood pressure increase. The amplitude of blood pressure increase after RNS in accessory arteries was similar to that observed in main renal artery trunks, suggesting that sympathetic fibers innervating accessory arteries may play an important role in patients with resistant hypertension. In agreement with our previous work,¹⁵ successful RDN of main renal arteries led to a substantial blunting of RNS-related BP increase after electrical stimulation of denervated arteries. However, RNS of (non-denervated) accessory arteries was associated with an unchanged blood pressure increase. This residual source of sympathetic activity may explain the lesser benefits of incomplete RDN limited to the main renal artery trunks in patients with accessory arteries.^{11,12}

Our findings may prove to be of major practical relevance. Indeed, the prevalence of renal accessory and multiple arteries varies between 15 and 30% in most populations.^{11,12,18} In a recent series of 941 hypertensive patients who underwent coronary angiography, 21% of patients had either aberrant or accessory renal arteries, and the mean diameter of non-principal renal arteries was 2.9 mm,¹⁹ which means that in a substantial proportion, RDN would not have been feasible. The proportion of patients with multiple renal arteries reached 34% in a Dutch monocentric cohort of 126 hypertensive patients referred for RDN.¹¹ It has been hypothesized

that the smaller diameter of accessory renal arteries may lead to localized hypoperfusion and subsequent activation of the renin angiotensin system and increased blood pressure,²⁰ thus explaining the higher prevalence of accessory renal arteries in severe hypertensive patients referred for RDN.¹¹

Furthermore, our results confirm that blunting of SBP increase after RNS is specific of denervated renal arteries, as already suggested by Chinushi et al. in their pioneering study of unilateral RDN in dogs.²¹ As such, they reinforce the hypothesis that RNS can serve as an intermediate end point to assess the completeness of RDN, which in its turn may be a predictor of blood pressure response.^{15, 22} In other words, a persistent increase in blood pressure in response to RNS after RDN might already indicate that a patient is likely to be a non-responder. As shown by Vink and colleagues,²³ RDN does not always result in circular lesions completely destroying the renal nerve tissue. By applying RNS to these areas the remaining functional nerve tissue might be detected and ablated afterwards. From a clinical perspective, RNS-guided RDN may allow to optimize renal nerve ablation and to achieve further BP decrease after the procedure. From a more research-oriented perspective, this strategy may improve the completeness and reproducibility of RDN, and thus facilitate the identification of clinical predictors of response on this more homogeneous background. Notably, after the disappointing performance of RDN in Symplicity HTN-3⁶ and the modest overall results of the technique in other recent randomised controlled trials,²⁴⁻²⁶ identification of true responders to RDN is a major priority.

The fact that smaller catheters are being developed to allow access to the smaller accessory arteries might improve the clinical outcome for these patients. Current studies are investigating these catheters and results are pending. However, this will not provide the operators with a procedural endpoint.

Admittedly, in this study, the number of denervated patients with accessory renal arteries and the number of accessory renal arteries in which RNS was performed was small. We used two different types of stimulation catheter and a sensitivity analysis is not implemented due to the small number of patients. Still, the results were extremely consistent. While RNS-related SBP increase was maintained in accessory arteries after RDN of main renal artery trunks in 5 out of 6 patients, only one patient showed a blunted effect in the accessory artery. Notably, the latter accessory renal artery was located very close to the main renal artery and both arteries might have shared common sympathetic nerve tissue, thus explaining this apparent discrepancy. In the other 5 patients we hypothesize that afferent signaling influences the central sympathetic tone, in line with the dog model by Chinushi and colleague,²¹ since efferent effects will not take place this quickly and transiently.

Other limitations include the following: (I) RNS was performed at only 4 sites per main renal artery; (II) detailed renal nerve mapping in the renal arteries was not performed; (III) all procedures were performed under general anesthesia, which may have influenced blood pressure response to RNS; and (IV) our findings have not yet been correlated with long-term follow up to clarify clinical relevance.

Perspectives

Our results support the concept that in patients undergoing RDN of main renal arteries, enhanced sympathetic tone can still be elicited by electrical stimulation of nerve tissue in the accessory renal arteries, with an increase in arterial blood pressure in these patients. These findings may at least in part explain why patients with aberrant or accessory arteries too small for RDN are more likely to be non-responders and our findings contribute to the ongoing debate about the position of renal denervation in patients with a more complex renal vasculature. We believe that the consensus regarding renal denervation procedures in patients with a more complex renal vasculature should be re-evaluated. Future studies with larger number of patients are warranted to assess the clinical and prognostic value of mapping of renal nerves with RNS in patients undergoing RDN. Finally, in order to develop a novel approach in which RNS-guided RDN can be performed, one has to make sure that the ablation catheter is positioned at the exact spot where the increase in blood pressure was observed. Developing a catheter that could do both mapping and ablation in one setting would allow to tackle this issue.

Conflict of interest

None declared

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CHAPTER V

Renal Nerve Stimulation Identifies Aorticorenal Innervation and Prevents Inadvertent Ablation of Vagal Nerves During Renal Denervation

Avoiding ablation of renal vagal nerves

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ABSTRACT

Background: Recently we reported the use of renal nerve stimulation (RNS) during renal denervation (RDN) procedures. RNS induced changes in blood pressure (BP) and heart rate are not fully delineated yet. We hypothesized that electrical stimulation of the sympathetic nerve tissue in the renal artery would lead to an increase in BP and vagal stimulation would cause a decrease in BP. We report the different patterns of BP and heart rate responses elicited by RNS prior to RDN.

Methods and results: 35 patients with drug-resistant hypertension were included. RNS was performed under general anesthesia at four sites in the right and left renal arteries, both before and immediately after RDN. RNS-induced BP and heart rate changes were monitored.

A total of 289 RNS sites in 35 patients were analyzed. An increase in systolic BP of >10 mmHg was regarded as a positive BP response to RNS. This pattern of response was observed in 180 sites (62%). 86 RNS sites (30%) showed an indifferent response with BP changes $\leq$ 10 mmHg. At 13 sites (4.5%) RNS elicited a decrease in BP up to -8 mmHg. However, 10 RNS sites (3.5%) showed a pronounced vagal response with hypotension and sinus cycle lengths ranging between 422-1027 milliseconds. These sites were distributed among two patients.

Conclusion: RNS identified sympathetic and parasympathetic nerve tissue in the renal arteries. RNS can be potentially used to map nerve bundles and guide selective ablation of sympathetic nerve fibers and prevent inadvertent ablation of parasympathetic nerve tissue during RDN.

Keywords: sympathetic renal denervation, vagal response, renal nerve stimulation, drug resistant hypertension

List of abbreviations

BP	Blood pressure
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
RDN	Renal sympathetic denervation
RNS	Renal nerve stimulation
ABPM	Ambulatory blood pressure measurements
CT	Computed tomography

INTRODUCTION

Renal sympathetic denervation (RDN) has been accepted as an experimental treatment for resistant hypertension because of the promising first results.¹⁻⁶ Recently, we reported the use of renal nerve stimulation (RNS) in patients undergoing a RDN procedure.⁷ During RNS high frequency electrical stimulation is applied to identify nerve bundles in the renal artery. The initial increase in blood pressure (BP) in response to RNS can be used to assess the amount of sympathetic overdrive in these patients. This report aims at describing the patterns of BP response to RNS and the potential clinical implications of our findings. We believe that RNS can possibly be used to identify both sympathetic and parasympathetic nerve fibers. This identification can prevent subsequent ablation of parasympathetic fibers. It implies a major change in the way RDN procedures are performed. Targeting the right sites for ablation is an important aspect of successful RDN procedures. We hypothesized that stimulation of the sympathetic nerve tissue would lead to an increase in BP and stimulation of parasympathetic nerve fibers would elicit a decrease in BP prior to RDN. Our findings highlight once more the importance of RNS.

METHODS

Patient population, inclusion and exclusion criteria

Between May 2014 and March 2016, RDN procedures were performed with the use of RNS in 35 patients. These patients were referred for a RDN procedure to treat drug-resistant hypertension. Patients were eligible if they were on a stable antihypertensive drug regimen of at least 3 antihypertensive drugs (preferably with a diuretic, or less when not tolerated) for at least 1 month, guided by experienced physicians specialized in hypertension treatment. Office SBP had to be >140 or DBP >90 mmHg and 24-h ABPM SBP had to be >130 or DBP >80 mmHg despite stable treatment, excluding patients with white coat hypertension. Patients were screened for eligibility by a multi-disciplinary team, including cardiologists, internists with hypertension subspecialty and a radiologist. All patients were willing and able to comply with the protocol and had provided written informed consent. Age range for possible inclusion was 18-80 years and glomerular filtration rate had to be >45 mL/min/1.73m² according to the MDRD formula.

Exclusion criteria were an unsuitable anatomy for RDN as assessed by CT-angiography (main renal artery lumen diameter ≤ 3 millimeter, or a total length of less than 20 millimeter of the main arteries), type 1 diabetes mellitus, chronic oxygen use, primary pulmonary hypertension, pregnancy and a mental or physical inability to participate. Patients enrolled in another investigational drug or device study were also excluded. Secondary hypertension was excluded by a rigorous protocol excluding hyperaldosteronism, pheochromocytoma, renal

artery stenosis (>50% stenosis in one or both arteries on CT-angiography), drug or substance use induced hypertension when appropriate, according to the European guidelines for the treatment of hypertension.⁸ The study was approved by the local medical ethical committee (ABR number 47172) and was conducted according to the declaration of Helsinki.⁹

Endpoints

The endpoints were acute per-procedural heart rate and BP measurements in response to RNS.

Procedure

All patients were under general anaesthesia induced by Propofol, supervised by a cardiac anaesthesiologist. Throughout the RDN procedure, no changes were made in use of vasoactive medication and no inotropic medication was necessary. The procedure was performed by experienced interventional cardiac electrophysiologists. Two sheaths were placed in the right femoral artery; one for continuous BP recording and one for RNS/RDN catheter access. 5000 IU of heparin was administered during the procedure.

In addition, in patients not using acetylsalicylic acid, 500 mg of acetylsalicylic acid IV was administered. Aorto-renal angiography was performed using a pigtail catheter.

Initially, a conventional quadripolar catheter with a tip electrode of 2 mm, 3 proximal electrodes of 1 mm and interelectrode spacing of 5 mm (EP-XT, C.R. Bard, Inc., Murray Hill, NJ, USA) was introduced in the renal artery, under fluoroscopic guidance. Bipolar stimulation was performed between the distal electrode pair of the catheter. This catheter was used only when performing RDN with the single-electrode ablation catheter (Symplicity Flex Renal Denervation Catheter, Medtronic, Minneapolis, MN, USA). In patients who were ablated with the multi-electrode basket ablation catheter (EnligHTN, St Jude Medical, Saint Paul, MN, USA), bipolar stimulation was performed by delivering electrical pulses through the electrodes of this multi-electrode basket catheter, with bipolar stimulation from pole 1-2 and 3-4 proximally and distally in the renal artery. The first renal artery to undergo RNS was alternated between left and right among consecutive patients. RNS was performed at multiple sites with a minimum of 4 sites in each artery, ensuring that different quadrants of the arterial circumference were stimulated in proximal and distal areas of the renal artery, in which ablations are usually performed. Pacing frequency was set at 20 Hz, pacing output at 20 mA with a pulse duration of 2 ms, based on earlier research.^{7,10-13} Stimulation duration was 60 sec, or shorter when systolic BP increased beyond 180 mmHg. We chose this as a cut-off point as we experienced additional BP rises up to >200 mmHg after stopping stimulation in previous research. We waited for the BP to return to baseline values before proceeding to a next stimulation site. After RNS in both arteries (total of at least 8 stimulation sites) a standard RDN procedure was performed. The single-electrode Medtronic renal ablation catheter was used in 14 patients and the St. Jude multi-electrode EnligHTN catheter in 20 patients. In 1 patient, a combination of both single

electrode and multi-electrode RDN catheters was used. In this latter patient, the left renal artery was denervated with the multi-electrode basket catheter. We switched to the flexible single electrode catheter due to anatomical variation prohibiting the introduction of the multi-electrode basket catheter in the right renal artery.

The use of the single electrode catheter (Symplicity, Medtronic) has been described previously.⁷ The multi-electrode RDN catheter contains 4 electrodes separated from each other in a basket configuration. The protocol has been described previously as well.^{14,15} In each renal artery, between 8 and 24 ablation points were delivered by subsequent sets of RF energy applications. During RF energy application, tip temperature and impedance were monitored to ensure proper ablation.

Patients were followed and monitored as an outpatient by a vascular internist. In all patients a validated 24h ABPM with 30 min. BP measuring intervals was obtained (Spacelabs, Snoqualmie, WA) at 3, 6 and 12 months after the procedure to assess the effect of RDN. Office BP measurements were executed by repeated automated oscillometry measurements (intrinsically blinded) or by sphygmomanometry by technicians not involved in the analysis of the study (regarded blinded).

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RESULTS

The baseline characteristics of the 35 patients are summarized in Table 1. RNS was applied prior to RDN at 289 sites in 35 patients (left and right renal arteries combined). We observed three patterns of response to RNS:

1. Sympathetic response with a SBP increase of >10 mmHg during RNS.
 - Sympathetic response is defined as an increase in systolic BP of >10 mmHg. The increase in BP started half way RNS and continued slightly after RNS was discontinued. We observed a RNS-induced decrease in cycle length from 1210 ± 201 ms to 1170 ± 203 ms before RDN ($P = 0.028$) without a change of the depth of general anaesthesia determined by bispectral index (BIS).
2. Indifferent response to RNS with no or negligible change in BP, i.e. BP increase ≤ 10 mmHg
 - Indifferent response is defined as an increase in systolic BP of ≤ 10 mmHg. We observed no increase in heart rate, or in change of the depth of general anaesthesia determined by BIS.
3. Vagal response to RNS with bronchospasm and BP drop due to bradycardia (cycle length increasing up to 10272 ms).

Table 1. Characteristics

Characteristic	All patients (n = 35)	Follow up at 3 or 6 months
Age (years)	63 (± 8)	
Sex		
Males	20 (57%)	
Body-mass index (kg/m ²)	29.9 (± 4.0)	
Current smokers	2 (5.7%)	
Medical history		
Hypercholesterolemia	21 (60%)	
Type 2 diabetes mellitus	10 (28.6%)	
Coronary artery disease	7 (20.0%)	
Atrial fibrillation	13 (37.1%)	
Number of antihypertensive medications	4 [2–6]	4 [1–6]
Class of antihypertensive medication		
Diuretic	26 (74.3%)	22/33 (66.6%)
Aldosterone receptor blocker	8 (22.9%)	8/33 (24.2%)
Beta-blocker	27 (77.1%)	25/33 (75.8%)
Calcium channel blocker	23 (65.7%)	19/33 (57.6%)
ACE-inhibitor	9 (25.7%)	6/33 (18.2%)
Angiotensin receptor blocker	27 (77.1%)	24 (72.7%)
Aliskiren	1 (2.9%)	1/33 (3.0%)
Centrally acting α2-sympatholytics	3 (8.6%)	3/33 (9.1%)
A1-receptor blockers	11 (31.4%)	7/33 (21.2%)
Other antihypertensive drugs	0 (0%)	0/33 (0%)
Serum creatinin (μmol/L)	76 [51–124]	
GFR (mL/min/m ²)	76.6 [39.8–113.0]	
Ambulatory blood pressure (mm Hg)		
24-hour systolic	144 (± 14)	135 (± 13), P = 0.004
24-hour diastolic	81 (± 12)	77 (± 11), P = 0.070
Daytime systolic	147 (± 13)	138 (± 12), P = 0.008
Daytime diastolic	83 (± 12)	79 (± 11), P = 0.080
Nighttime systolic	133 (± 13)	128 (± 13), P = 0.074
Nighttime diastolic	73 (± 12)	69 (± 10), P = 0.070
Heart rate (bpm)	69 (± 14)	
Office blood pressure (mm Hg)		
Systolic	161 (± 19)	144 (± 21), P = 0.002
Diastolic	92 (± 14)	82 (± 13), P = 0.023
Okada classification		
Right		
A1	24 (68.8%)	
A2	3 (8.6%)	
A3	7 (20%)	
B1	1 (2.9%)	
B2	0 (0%)	
Left		
A1	26 (74.3%)	
A2	6 (17.1%)	
A3	2 (5.7%)	
B1	1 (2.9%)	
B2	0 (0%)	

Data are expressed as mean (± SD), median [range], or number (%). ACE = angiotensin converting enzyme. bpm = beats per minute. eGFR: estimated glomerular filtration rate calculated using the MDRD formula.

Either a site would demonstrate an increase in BP following RNS, or it would demonstrate an indifferent reaction without a change in BP. Notably sinus cycle length decreased from 1210 ± 201 ms to 1170 ± 203 ms ($P = 0.028$) before RDN. After RDN sinus cycle length did not decrease significantly (991 ± 590 ms to 986 ± 588 ms, $P = 0.583$). When an increase in BP was observed there appeared to be two distinct patterns. Either there was an immediate increase in BP or the increase in BP started slightly later, but continued to increase even though RNS had been terminated. It was this latter observation that made us discontinue RNS when a systolic BP of 180 mmHg was reached.

An increase in systolic BP of >10 mmHg was regarded a positive response to RNS. This pattern of response was seen in 180 sites (62%). 86 RNS sites (30%) showed an indifferent response with BP changes ≤ 10 mmHg. At 23 sites (8.0%), RNS elicited a decrease in BP. At 13 of these sites BP drops ranged from -1 to -8 mmHg. 10 of these 23 RNS sites showed a pronounced vagal response with sinus arrests ranging between 4224-10272 ms. These sites were distributed among two patients. Both patients demonstrated a mixed response to RNS, ranging from sites that elicited a large BP increase to the mentioned vagal response. We observed the vagal response both distally and proximally in the renal arteries. These results have been summarized in a flowchart (Figure 1).

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Figure 1. This figure displays the flowchart that summarizes the results that we have obtained in 35 patients. A total number of 289 have been analyzed. 180 sites demonstrated a BP increase. 86 sites demonstrated an indifferent response. 23 sites demonstrated BP decrease. 10 of these sites demonstrated a vagal response consisting of bradycardia/asystole with hypotension.

Details of a representative example of a pronounced increase in BP are illustrated in Figure 2. This figure displays a pronounced effect of BP increase in response to RNS. Panel A demonstrates normal sinus rhythm with a cycle length of 1152 ms and a BP of 100/46 mmHg. RNS is applied (RNS artefacts on the ECG). Panel B shows termination of RNS and a new situation in which the BP is increased to 149/59 mmHg and no effect on sinus cycle length (1184 ms). An example of an indifferent response is shown in Figure 3. This figure displays an indifferent effect of BP increase in response to RNS. Panel A demonstrates normal sinus rhythm with a cycle length of 1216 ms and a BP of 111/49 mmHg. RNS is applied (RNS artefacts on the ECG). Panel B shows termination of RNS and an unchanged situation in which the BP is 109/49 mmHg and no effect on sinus cycle length (1312 ms).

Figure 2. This figure displays a pronounced effect of BP increase in response to RNS. Panel A demonstrates normal sinus rhythm with a cycle length of 1152 ms and a BP of 100/46 mmHg. RNS is applied (RNS artefacts on the ECG). Panel B shows termination of RNS and a new situation in which the BP is increased to 149/59 mmHg and no effect on sinus cycle length (1184 ms).

Figure 3. This figure displays an indifferent effect of BP increase in response to RNS. Panel A demonstrates normal sinus rhythm with a cycle length of 1216 ms and a BP of 111/49 mmHg. RNS is applied (RNS artefacts on the ECG). Panel B shows termination of RNS and an unchanged situation in which the BP is 109/49 mmHg and no effect on sinus cycle length (1312 ms).

An illustrative example of the vagal response we have observed is depicted in Figure 4. This figure displays a vagal effect on BP in response to RNS. Panel A demonstrates a RNS-induced sinus cycle length prolongation of 10272 ms. RNS was terminated immediately and sinus rhythm was restored. The pronounced drop in BP from 110/50 mmHg to 70/35 mmHg was noted. Panel B demonstrates response to RNS at a different site in the same patient. It resulted in extreme bradycardia (maximum cycle length 9376 ms) and subsequent drop in BP to 80/40 mmHg. After termination of RNS, BP increased and regular sinus rhythm was restored. All RNS effects on sinus rate and BP were reversible.

Figure 4. This figure displays a vagal effect on BP in response to RNS. Panel A demonstrates a marked RNS-induced RR interval increase to 10272 ms. RNS was terminated immediately and sinus rhythm was restored. The pronounced drop in BP from 110/50 mmHg to 70/35 mmHg can be noted. Panel B demonstrates response to RNS at a different site in the same patient. It resulted in extreme bradycardia (maximum RR interval 9376 ms) and subsequent drop in BP to 80/40 mmHg. After termination of RNS regular sinus rhythm was restored.

As a group, BP of our patients responded significantly at 3 to 6 months of follow up. Mean systolic BP declined from 144 (± 14) to 135 (± 13), $P = 0.004$. Daytime systolic BP declined from 147 (± 13) to 138 (± 12), $P = 0.008$. However night-time systolic BP declined from 133 (± 13) to 128 (± 13), which did not reach statistical significance, $P = 0.074$. Diastolic BP did not reach statistical significance, but declined numerically. See Table 1.

Detailed description of the 2 patients that demonstrated a vagal response pattern are presented here. The first patient was a 66 year old woman referred by her general practitioner to an internist with hypertension subspecialty due to difficulty with establishing an effective antihypertensive drug regimen to control hypertension. Average office BP measurements was 165/60 mmHg when she was initially screened. The subsequent 24h ABPM daytime BP was 132/55 mmHg whilst being put on four different types of antihypertensive medication (diuretic, β -blocker and the combination of an ACE-inhibitor and AT₁-antagonist, which was switched to a calcium channel antagonist during the screening). Because she did not tolerate

her medication and she was found to have micro albuminuria she was regarded a suitable patient for RDN. Screening for secondary hypertension did not show any abnormalities. This patient did not experience (symptoms of) orthostatic hypotension before RDN. She did not have other traditional risk factors for cardiovascular disease (i.e. smoking, diabetes, hypercholesterolemia, vascular disease or obesity). This patient did not demonstrate a beneficial effect of RDN during follow up; 24h ABPM daytime BP was 141/58 mmHg.

The second patient with a pronounced vagal response to RNS was a 70 year old woman referred to the internist with hypertension subspecialty by the cardiology department to screen her for secondary hypertension and eligibility for RDN. Besides hypertension she suffered from type 2 diabetes and hypercholesterolemia. Average office BP measurements were 155/80 mmHg when she was initially screened. The 24h ABPM daytime BP was 146/79 mmHg whilst being put on five different types of antihypertensive medication (combination of two diuretics, β -blocker, an ACE-inhibitor and an α_1 -blocker). Because she was still hypertensive despite extensive medical therapy she was referred for RDN as well. During follow up this patient demonstrated a slight beneficial effect of RDN; 24h ABPM daytime BP was 136/72 mmHg whilst being on the same drug regimen.

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DISCUSSION

Electrical stimulation in renal arteries may identify different patterns of response to RNS. Even though we cannot proof histologically that parasympathetic fibres are responsible for the observed vagal effect, it is very likely that activation of the aortico-renal parasympathetic ganglia elicited the observed pronounced vagal response. Potentially, RNS technique can be used to map nerve bundles in renal arteries and guide selective ablation of sympathetic nerve tissue. In addition, RNS may probably prevent inadvertent ablation of parasympathetic nerve tissue during RDN. Taking into account the number of non-responders to RDN, this is an interesting possibility that warrants further analysis⁶.

We previously demonstrated that RNS is feasible in patients with drug-resistant hypertension undergoing RDN.⁷ Furthermore, we showed that an increase in BP can still be elicited in accessory arteries when denervation of the main renal arteries was performed.¹⁴ Finally, we demonstrated in a small cohort that RNS predicts 24h ABPM data during follow up after a RDN procedure.¹⁵ During our data collection we encountered an unexpected, but very interesting finding; the occurrence of bradycardia/sinus arrest and hypotension during RNS. The rationale of RNS comprehends the activation of the sympathetic nerve tissue surrounding the renal arteries and its afferent coupling with the central sympathetic nervous system.¹⁰ Also renal nerves play a key role in the communications between the kidneys and the central nervous system to maintain homeostasis. An increased sympathetic nerve activity to the kidney and

the renal afferent nerve activity to the CNS may contribute to the outcome of diseases, such as hypertension¹⁷. Through this mechanism RNS elicits the observed BP increase and sinus cycle length decrease. In two patients we observed the opposite response. They developed sinus bradycardia/sinus arrest with subsequent hypotension and bronchospasm at the same time. Since data on RNS in human hypertensive patients are scarce and this observation has only been described in one case,¹⁸ we believe this information is essential to report. This finding underlines the rationale and importance of RNS in RDN procedures both before and after RDN. An extensive ablation at the sites that show a vagal response will presumably not lower BP during follow up. The ablation might even cause an increase in BP after RDN, due to ablation of parasympathetic nerve fibres, potentially enhancing sympathetic overdrive. We did not perform ablation at these specific sites to avoid these consequences, preventing us to compare the effect of RNS before versus after RDN at these specific sites. We observed vagal responses in 2 out of 35 patients (5.7% of the cases). This may even be one of the contributing factors that help to explain the variable drop in arterial BP after RDN.¹⁹

We analysed the two patients that demonstrated a vagal response pattern in more detail to determine if we could or should have foreseen their profound vagal response to RNS. After analysing their reported patient characteristics we concluded that both patients are no exception to the population referred for RDN.

Ablation of the parasympathetic fibres from the aorticorenal ganglia is an undesirable but possible result of a conventional 'blind' RDN procedure.²⁰ Since the innervation of the renal artery is variable in patients²¹ there is no empiric possibility of preventing the ablation of vagal fibres from these ganglia, unless ablation points are placed in distal parts in the renal arteries.²² We believe that a vagal response was found only in those patients in whom the parasympathetic nerve fibres were located in close proximity to the vessel wall and were identified by RNS. The point of RDN is not to target these fibres, even though not much is known about their ablation. We are relieved to not demonstrate the effect more often, and assume that the majority of the patients do not have parasympathetic fibres in very close proximity to the renal artery ostia. A limitation is that our findings are based on a clinical response to RNS, but we lack the anatomical proof of the location of the parasympathetic fibres in these two patients. The fact that this phenomenon has not been described before is related to the very limited use of RNS during RDN procedures in other centres. The use of RNS enables detection of vagal responses, preventing inadvertent ablation of parasympathetic nerve tissue. RF ablation of sites with indifferent response to RNS, i.e. sites that do not demonstrate an enhanced BP response to RNS seems ineffective and undesirable. We advocate the use of RNS to identify sympathetic nerve tissue for selective ablation instead of conventional 'blind' placement of ablation points in the renal arteries.

Some may argue that the vagal response is due to activation of pain fibres. We do not believe

this assumption because of the stable bispectral index (BIS), measured to monitor the depth of anaesthesia. It remained stable in all patients, and in particular during the episodes of vagal response to RNS, ensuring a stable depth of anaesthesia. In addition, we administered intravenous opioids to prevent pain reaction to heating of the tissue by ablation. Of note, ablation of pain fibres innervating the kidneys is of no use in the treatment of drug resistant hypertension. RNS could possibly be used to avoid that, if this would be the case.

We believe that use of RNS during RDN has a strong rationale and that patient selection and conventional 'blind' ablation technique are the main reasons why previous RDN studies reported such divergent results. In our previous work we demonstrated residual physiologically active sympathetic nerve tissue in accessory arteries.¹⁴ These accessory arteries may contribute to enhanced sympathetic drive and increased BP after ablation of the main renal arteries. We demonstrated that the initial increase in BP in response to RNS might serve as an indicator of sympathetic overdrive. The difference between the response before RDN and after RDN was significantly correlated with the decrease in 24h BP. The extent of this difference might serve as an endpoint to assess completeness of RDN. During these procedures we discovered the important possibility of eliciting a response to RNS which consists of bronchospasm, asystole and subsequent hypotension which appeared to be completely reversible. We believe that this response is not caused by stimulating the sympathetic nerve tissue through the renal arteries, but the stimulation of the parasympathetic fibres in the aorticorenal ganglia which are located ostially adjacent to the renal arteries¹⁸.

Conclusion

RNS enables functional identification and localization of both sympathetic and parasympathetic nerve tissue and use of the RNS technique may guide selective ablation of the sympathetic nerve fibres adjacent to the renal artery and improve the efficacy of RDN. In addition, RNS may help in preventing inadvertent ablation of parasympathetic nerve tissue in close proximity to the renal arteries.

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Declaration of interest

The authors received research support from St. Jude Medical Inc. Otherwise we declare no conflict of interest.

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CHAPTER VI

Renal Nerve Stimulation as procedural end point for Renal Nerve Denervation

Review

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Purpose of review: Renal nerve denervation (RDN) as treatment option for hypertension has a strong rationale, however variable effect on blood pressure (BP) have been reported ranging between non response to marked reductions in BP. The absence of a procedural end point for RDN is a possible explanation for the variable response, it is suggested that renal nerve stimulation (RNS) can be used to solve this problem. This review aims to summarize on the clinical and non-clinical data available of the effects of renal nerve stimulation (RNS).

Recent findings: Animal studies have shown that high frequency electrical stimulation of the renal arteries leads to increase in BP and increased norepinephrine spillover as a marker of increased sympathetic activity and that these effects of stimulation are blunted after RDN. In a human feasibility study using RNS both before and after RDN same responses on blood pressure have been showed. On top of that in a small study RNS induced changes in blood pressures appeared to be correlated with 24-hour BP response after RDN.

Key words: renal nerve stimulation, renal nerve denervation, drug-resistant hypertension

Abbreviations

ABPM	Ambulatory blood pressure measurements
BP	Blood pressure
DBP	Diastolic blood pressure
ENS	Electrical nerve stimulation
HR	Heart rate
RDN	Renal nerve denervation
RNS	Renal nerve stimulation
SBP	Systolic blood pressure

INTRODUCTION

Dysregulation of the autonomic nervous system, particularly imbalance between sympathetic and vagal tone, has been implicated in the development and maintenance of hypertension.^{1,2} In this context renal nerve denervation (RDN) has been developed as a potential treatment for resistant hypertension;³⁻⁵ its rationale originates in denervating the renal sympathetic efferent and afferent coupling with the central autonomic nervous system. In 2009, the first patient with treatment resistant hypertension who underwent RDN was reported.⁶ After the RDN procedure the patient had substantially reduced blood pressure (BP) and whole-body norepinephrine concentrations as a parameter of decreased sympathetic outflow. Thereafter, Krum and colleagues showed in a proof of concept study in a cohort of 45 patients with resistant hypertension the safety and effectivity of RDN with a significant decline in office BP of 32/12 mmHg at 12 months follow-up.⁷ This study was followed by the Symplicity HTN-2 trial in which the investigators established in 106 patients who were randomly allocated to RDN or control that office BP significantly decreased by $32\pm 23/12\pm 11$ mmHg in the RDN group ($p<0.0001$), whereas the BP in the control group remained unchanged compared to baseline at 6 months follow-up.⁸ After the HTN-2 trial the ESC Guidelines stated that RDN may be considered as a possible treatment option for patients with resistant hypertension.⁹ After these promising results of RDN on lowering BP the last step before definite proof of the efficacy of RDN was a blinded, randomised, sham-controlled controlled trial. The Symplicity HTN-3 trial was subsequently started and included 535 patients assigned in a 2:1 ratio to RDN or a sham procedure. The trial confirmed again the safety of the RDN procedure; however it failed to demonstrate a benefit on RDN on reduction in 24-h ambulatory blood pressure measurements (ABPM) during follow-up.⁴ This discrepancy between the results of the first blinded, sham-controlled HTN-3 trial and previous studies raised concern and a number of possible explanations for the results of the HTN-3 trial were suggested. Part of the explanations were procedural related since there is no procedural end point for the RDN procedure; probably effective denervation had not occurred in all patients randomised to the RDN group due to inadequate radio frequent lesions,¹⁰ wrong locations were denervated due to anatomical variations of perivascular nerves and ganglia¹¹ or due to the presence of aberrant renal arteries there was still residual autonomic nerve activity post RDN.¹² The other part of the explanations for the results of the Symplicity HTN-3 trial were patient or physician related.¹³ Subsequently many papers have been published addressing these issues. However, very recently the results from another single-blind, randomised, sham-controlled trial has been published and showing again proof of principle for the BP lowering efficacy of RDN. The RDN group ($n=38$) showed a decrease of $5.5[-9.1, -2.0]/4.8 [-7.0, -2.6]$ mmHg on 24-h ABPM at 3 months follow-up, whereas the BP in the control group ($n=42$) remained unchanged.⁵

Summarizing, overall the rationale of RDN as possible new treatment remains very attractive and there is evidence supporting this rationale. However, results on lowering blood pressure

have been variable with a wide spectrum ranging between non response to a marked reduction for different reasons. These reasons have highlighted the crucial need for a functional test to define the optimal denervation target site and a clear procedural end point for RDN. In order to create a procedural end point for the RDN procedure, renal nerve stimulation (RNS) has been developed and studied and this review will focus on the data available to use RNS during the RDN procedure.

Renal Nerve Stimulation in animal models

Chinushi et al. reported in dogs about the use of electrical nerve stimulation (ENS) of the renal arteries aiming to study the role of the autonomic nerves and radio frequent (RF) ablation of the renal artery in the regulation of BP.¹⁴ They electrically stimulated the proximal portion of the renal arterial nerves before and after RF catheter ablation. Dogs were anesthetized with tiopental and to minimize pain sensitization during electrical stimulation pentazocine intravenous (i.v.) was administered. Before RDN, they showed a significant increase in BP (from $146\pm17/89\pm17$ mmHg to $170\pm23/103\pm19$ mmHg, $p<0.001$) and HR (from 119 ± 9 bpm to 131 ± 7 bpm, $p<0.001$). After RDN, no significant changes in either BP ($150\pm20/90\pm16$ mmHg to $152\pm20/92\pm17$ mmHg) or heart rate (HR) (from 124 ± 14 bpm to 124 ± 14 bpm) were observed. Besides these ENS-induced BP effects, the authors showed that the serum epinephrine and norepinephrine concentrations significantly increased due to ENS before RDN, whereas after RDN the concentrations only minimally increased. This finding suggests that the ENS-induced increase in BP and HR were attributed to an increase in systemic sympathetic nervous activity. Importantly, no angiographic damages to the renal arteries were observed after ENS. In a following study Chinushi et al. aimed to demonstrate that these BP responses to ENS could be used as an indicator of successful modulation of the renal autonomic nerve function. Again in a canine model, they showed with histological proof that the suppressive effects of ENS-induced increase in BP by RDN may be related to the severity of tissue injury.¹⁵ So, functional RDN seems performable with the guidance of ENS. In the corresponding letter to this article authors highlight the standardization of stimulation protocols and systematic evaluation of anaesthetic use considering disappointing results of ENS in their porcine model.¹⁶ Sun et al.¹⁷ showed also in dogs comparable results as Chinushi et al.¹⁴, i.e. a significant increase in systolic BP after applying ENS to the renal arteries. However, HR did not significantly change in their model and they hypothesize that this is due to a result of baroreceptor independent sympathetic activation.¹⁸ These results on BP are also confirmed by Lu et al. who assessed again in a canine model the efficacy of ENS-guided RDN and showed that ENS-guided targeted ablation can achieve apparent BP reduction and central sympathetic inhibition measured by plasma norepinephrine changes.¹⁹

Summarizing, animal models, mainly using dogs, have given supportive evidence that ENS can be used to localize the sympathetic nerves before RDN and ENS-induced BP effects are blunted after RDN. Of note, the autonomic anatomy of the renal nerves shows important

differences between dog and man.²⁰

Renal Stimulation Protocol

In 2015 for the first time a clinical study using high-frequency RNS before and after a RDN procedure in patients with resisted hypertension was reported.²¹ The study aimed to design a RNS protocol and determine the effects of RNS in the renal arteries on BP and HR before and after RDN. The latter will be discussed in the next section, in this paragraph we will focus on the pacing and output settings during RNS. Pacing frequency was set at 20 Hz, conform previous studies from Chinushi et al.¹⁴. In 4 patients pacing output settings were gradually increased (5, 10, 15, 20 mA) to determine the pacing output threshold. Only with a pacing output setting exceeding 10 mA a BP response was observed and pacing output was set at twice the threshold. In the first four patients RNS duration was set at three minutes; however BP response was observed already after 30 seconds. Therefore RNS duration was set at twice the threshold, at 1 minute. In three patients the repeatability of RNS was tested with mentioned settings and no significant differences were found between the two measurements.

Renal Stimulation in human

In the mentioned feasibility study, RNS has been used both before and after RDN in eight patients.²¹ Patients in this study had a mean age of 66 ± 5.6 years, baseline ABPM of $153 \pm 13/89 \pm 4$ mmHg and were using an average of 4 [3-6] antihypertensive drugs. In accordance with the mentioned animal models,^{14,15,17,19} RNS-induced a systolic BP response of $+43 \pm 15$ mmHg before RDN compared with $+9 \pm 10.5$ mmHg after RDN, $p=0.0002$. Also sinus cycle length significantly shortened before RDN due to RNS (1286.9 ± 173 ms to 1069 ± 274 ms, $p < 0.001$). And after RDN sinus cycle length shortening was significantly reduced ($+211$ ms versus $+45$ ms, $p=0.015$). This study showed for the first time in man that RNS resulted in a temporarily increase in BP, and this effect is blunted after RDN. In order to implement RNS as a reliably predictable procedural end point for the RDN procedure it should also be correlated to BP responses at follow-up. So, De Jong et al.²² investigated whether 24-h ABPM changes after RDN correlated with acute per-procedural RNS-induced BP responses. In the analysis were fourteen patients included with a mean age of 66 [39-76] years, half of their population was male and the average 24-h ABPM was $153 \pm 11/88 \pm 8$ mmHg at baseline. In agreement with the results from the feasibility study²¹ RNS induced a systolic BP increase (50 ± 27 mmHg) before RDN, and this effect was again blunted after RDN (13 ± 16 mmHg) ($p < 0.001$). At 3 to 6 months follow-up ABPM was significantly decreased to $137 \pm 10/80 \pm 9$ mmHg, $p = 0.003/0.018$. With a Pearson correlation authors show subsequently that the RNS-induced SBP rise before RDN at the site of maximum response is significantly correlated with changes in systolic ABPM at a median of 4.5 months follow-up ($R=0.610$, $p=0.020$). The correlation further increased after subtracting the RNS-induced BP rise measured immediately after RDN, (delta BP at site of maximum response defined as RNS-induced BP before RDN minus the RNS-induced BP after RDN) showing a significant correlation with 24-h BP response at follow-up ($R=0.769$, $p=0.001$). These results

show the potential of using RNS as functional test to evaluate the efficacy and predict the clinical outcome of the RDN procedure. The same research group showed that RNS of non-denervated accessory arteries was associated with an unchanged BP increase both before and after RDN.²³ This residual source of sympathetic activity may partly explain why some patients are non-responders to RDN due to anatomical variations. But these results give also supportive evidence using RNS as a procedural end point; because of a persistent increase in BP in response to RNS after RDN might indicate that a patient is likely to be a non-responder due to an incomplete RDN.

Pathophysiological mechanism of RNS

The exact pathophysiological mechanism of as well RDN as RNS is yet not completely understood. As established in the animal studies RNS leads to increase in serum epinephrine and norepinephrine concentrations as markers of an increased sympathetic activity.^{1,14} Nammias et al.²⁴ described the cardio/renal sympathetic interaction, showing afferent renal sympathetic nerve signaling regulating central sympathetic outflow. We believe described RNS-induced changes in BP are due to increasing the central sympathetic tone through an afferent mechanism. The sympatho-excitatory renal afferent reflex is a likely cause of the rise in blood pressure induced by electrical stimulation, because the rise in blood pressure 15-30 seconds after starting electrical stimulation which was consistent with an increase in sympathetic nervous activity through stellate ganglion stimulation in canine studies as reported previously.²⁵ Other experimental data indicate also that the afferent nerves play an important role in the RNS-induced BP response.^{26,27} Plasma renin activity and creatinin clearance are not affected by RDN, suggesting that efferent renal efferent nerve activity does not play an important role. On top of that efferent nerve fibers would take more time to increase BP, so therefore likely not to explain the transient BP increase due to RNS.²⁸ Since BP was measured with a femoral artery line during the RNS procedure^{21,22} renal vasoconstriction cannot explain either the temporarily RNS-induced BP increase. However, further research regarding the pathophysiological mechanisms is needed.

Clinical applications

Up to now, there is evidence from small studies that RNS can be used to identify sympathetic nerve fibers in patients with treatment resistant hypertension undergoing RDN and that it predicts BP response at follow-up. In the future RNS-guided RDN may possibly allow optimizing RDN and achieving further BP decrease after the RDN procedure.

Conclusions

RNS has proven in to induce increases in BP and HR before RDN and these effects are blunted after RDN. Moreover there is also evidence that the RNS-induced BP increases predict outcome on 24-h ABPM at follow-up. However, studies are small and limited. The exact pathophysiological mechanism of RNS increasing BP is not well studied.

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CHAPTER VII

Treatment of atrial fibrillation in patients with enhanced sympathetic tone by pulmonary vein isolation or pulmonary vein isolation and renal artery denervation: clinical background and study design

The ASAF trial: ablation of sympathetic atrial fibrillation

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ABSTRACT

Background: Hypertension is an important, modifiable risk factor for the development of atrial fibrillation (AF). Even after pulmonary vein isolation (PVI), 20-40% experience recurrent AF. Animal studies have shown that renal denervation (RDN) reduces AF inducibility. One clinical study with important limitations suggested that RDN additional to PVI could reduce recurrent AF.

Objective: The goal of this multicenter randomized controlled study is to investigate whether RDN added to PVI reduces AF recurrence.

Methods: The main end point is the time until first AF recurrence according to EHRA guidelines after a blanking period of 3 months. Assuming a 12 month accrual period and 12 months of follow-up, a power of 0.80, a 2-sided alpha of 0.05 and an expected drop-out of 10% per group, 69 patients per group are required. We plan to randomize a total of 138 hypertensive patients with AF and signs of sympathetic overdrive in a 1:1 fashion. Patients should use at least 2 antihypertensive drugs. Sympathetic overdrive includes: obesity, exercise-induced excessive blood pressure (BP) increase, significant white coat hypertension, hospital admission or fever induced AF, tachycardia induced AF and diabetes mellitus. The interventional group will undergo PVI + RDN and the control group will undergo PVI.

Results: Patients will have follow-up for 1 year, and continuous loop monitoring is advocated.

Conclusion: This randomized, controlled study will elucidate if RDN on top of PVI reduces AF recurrence.

Keywords: RDN, PVI, AF, hypertension, sympathetic overdrive

INTRODUCTION

Atrial fibrillation (AF) is the most common arrhythmia and its prevalence rises exponentially after the age of 60 years. It is expected that the prevalence of AF will increase in the ageing population of Europe in the upcoming years. Besides its disabling symptoms, AF is associated with serious morbidity (e.g. thromboembolism, CVA, heart failure) and increased mortality.¹ Results of medical therapy to prevent AF are very modest, and reported as 30% success in achieving long term sinus rhythm. Catheter ablation of AF (i.e. pulmonary vein isolation, PVI) is significantly more successful in obtaining and maintaining sinus rhythm with a success rate of 60-80% after one or more interventions. The rationale of PVI for AF lies in the fact that arrhythmogenic foci arising from the pulmonary veins induce fibrillatory electrical activity in the left atrium, hereby initiating and perpetuating AF. It is hypothesized that this mechanism is most important in patients with paroxysmal AF. If AF recurs more than once but terminates spontaneously within seven days, the term paroxysmal AF is used. This is also used when the episode is less than 48 hours in duration and is terminated with electrical or pharmacological cardioversion. Persistent AF is defined as recurrent AF that is sustained for more than seven days. A patient who is electrically or pharmacologically cardioverted after more than two days is also diagnosed with persistent AF. The AF recurrence rates are even higher in patients with persistent AF (40-50%). Experimental and clinical research have shown that the autonomic nervous system has an important role in the induction and persistence of AF, and on the histopathological and electrical substrate in the atria (fibrosis, functional conduction blocks).^{2,3} This has drawn the attention of cardiac electrophysiologists to the treatment of modifiable factors in order to prevent AF episodes.⁴ It has been reported that patients with sympathetically induced (supra)ventricular arrhythmias in the setting of resistant hypertension remained free of arrhythmias after renal artery denervation (RDN).^{2,5-7}

It is hypothesized that by applying ablational therapy in the renal arteries, the connection of the sympathetic nervous system within the renal arterioles is at least partially severed.⁸ See Figure 1 for a summary of the mechanisms between the central nervous system, the kidneys and the heart that initiate arrhythmia. By applying this new interventional therapy in patients with therapy resistant hypertension, it was demonstrated in several reports that the sympathetic overdrive inducing hypertension was prevented, and significant reductions in blood pressure were achieved.^{6,8-13} Furthermore a beneficial effect on cardiac (diastolic) function was shown.¹⁴ Pre-clinical trials carried out by Liang et al demonstrate that RDN prevents AF inducibility in a dog model.¹⁵ Similar results have been found in a rabbit heart failure model.¹⁶ Furthermore, in a small clinical study, 27 patients with AF were randomised to PVI or PVI + RDN. Patients who underwent PVI + RDN had significantly less episodes of AF than those undergoing PVI alone.¹⁷ This report however involved a very limited number of patients, had important study limitations rendering its results less reliable, and consisted of a very selected group. Loop recorder data of these patients demonstrated a lower AF burden

in patients that underwent RDN.¹⁸ We hypothesize that patients who have paroxysmal or persistent AF, with hypertension (systolic >140 mmHg or >130/80 mmHg in diabetics and patients with chronic renal disease according to the ESC guideline on arterial hypertension¹⁹) and signs of sympathetic overdrive, will benefit from RDN on top of the standard ablative therapy (PVI). Therefore, we have initiated a randomised controlled trial that will assess the effects of PVI and RDN+PVI on the success of preventing AF recurrence. Furthermore we will assess the effect of these therapies on several aspects of the cardiovascular system and the autonomic nervous system. The study is listed at clinicaltrials.gov (NCT02115100).

Figure 1. Mechanism of arrhythmia triggering through the reno-cardiac axis (re-use courtesy of Heart Rhythm).

METHODS

Primary aim

To investigate if renal artery denervation in combination with pulmonary vein isolation, prevents AF recurrence significantly better in patients with out of range hypertension and signs of sympathetic overdrive.

Secondary aim(s):

Investigate the effects of renal artery denervation on the sympathetic innervation of the heart and the general sympathetic drive and the effects of RDN on arterial stiffness and

hemodynamic parameters.

Study design

The ASAF trial is a prospective, randomised, controlled, multicenter, international clinical trial. Patients are not blinded for their procedure.

Patient population, inclusion and exclusion criteria

The target group of patients are subjects aged <75 years, with paroxysmal or persistent AF, who have out of range hypertension (systolic >140 or >130/80 mmHg in diabetics and patients with chronic renal disease) and signs of sympathetic overdrive. Sympathetic overdrive is defined when patients show one or more of the following features: obesity (BMI >30), excessive blood pressure rise (peak systolic blood pressure >250 mmHg) on exercise stress testing, white coat hypertension (normal ambulatory blood pressure measurements despite hypertension in a clinical setting which is associated with an increase in sympathetic drive²⁰), raised serum cortisol during illness or admission, hospital admission or fever induced AF, tachycardia induced AF during Holter monitoring or telemetry and/or diabetes mellitus. Patients should use at least 2 antihypertensive drugs or should be intolerant for medication. The following medication is seen as antihypertensive medication: angiotensin II blocker, ACE inhibitor, alpha blocker, beta blocker, calcium antagonist, diuretics, central acting agents and renin inhibitors. Apparent secondary causes for hypertension and/or AF should be excluded by a vigorous protocol. We hypothesize that the proportion of AF free survival after a single procedure of PVI will be 60% after one year compared to 80% in the PVI + RDN group. We will perform a log-rank test to test a hazard ratio of 0.437 (RDN + PVI versus PVI). 69 patients per group are required for a log-rank test to test a hazard ratio of 0.437, with a power of 0.80, a 2-sided alpha of 0.5 and an expected drop-out of 10% per group assuming a 12 month accrual period and 12 months follow-up. For the sample size calculation the approach of Schoenfeld and Richter was used.

In order to be eligible to participate in this study, a subject must meet all of the following criteria: 1. The patient is willing and able to comply with the protocol and has provided written informed consent. 2. The patient falls within the target group as stated earlier. 3. Patient is an acceptable candidate for renal denervation treatment. 4. Patient is <75 year of age. A potential subject who meets any of the following criteria will be excluded from participation in this study: 1. Documented left atrial diameter on trans thoracic echocardiography (PLAX > 4.5 cm). 2. Contraindication to chronic anticoagulation therapy or heparin. 3. Previous left heart ablation procedure for AF. 4. Acute coronary syndrome, cardiac surgery, PCI or stroke within 3 months prior to enrolment. 5. Untreated hypothyroidism or hyperthyroidism. 6. More than grade 1/3 valvular regurgitation and/or significant valve stenosis (modest or severe). 7. LVEF <45% and/or grade 3/4 diastolic dysfunction. 8. Enrolment in another investigational drug or device study. 9. Woman currently pregnant or breastfeeding or not using reliable

contraceptive measures during fertile age. 10. Mental or physical inability to participate in the study. 11. Planned cardiovascular intervention. 12. Life expectancy ≤ 12 months. 13. Renal artery stenosis $>50\%$ of the arterial lumen, or renal artery lumen ≤ 3 mm. 14. Dual or triple ipsilateral renal artery ostia not suitable for RDN. 15. Obvious secondary cause of hypertension.

Patients who satisfy the entry criteria will be randomised by an automated system in order to minimize bias based on patient selection and baseline characteristics. The randomization will be generated with random permuted blocks with a 1:1 allocation of treatments.

The control group will be the group that underwent pulmonary vein isolation. See figure 2 for the randomization into the two arms of this study.

Figure 2. Each arm of the study contains 69 patients. The investigational groups will receive PVI + RDN. The control group will receive PVI only.

Interventional group (1)

These patients will undergo renal artery denervation and pulmonary vein isolation. Renal artery denervation will be performed by femoral arterial catheterization, and cannulation of the renal arteries. A number of ablations will be performed in the left and right renal artery according to our previously described protocol. In a subset of patients, renal nerve stimulation (RNS) will be used to assess the completeness of ablation. Pulmonary vein

isolation will be performed by femoral vein cannulation, transseptal puncture, and circular antral radiofrequency or cryo-ablation of the pulmonary veins, with confirmation of isolation.

Control group (2)

These patients will undergo pulmonary vein isolation. Pulmonary vein isolation will be performed as stated above. No other sham procedure will be performed in the control group.

Pre procedural measurements

Pre-treatment: 24 hour Holter monitoring or cardiac rhythm monitoring with a loop recorder (AF burden), lab tests, number of antihypertensives, 24-h ambulatory blood pressure monitoring, office blood pressure measurement. An internist with a special interest in hypertension treatment assesses the patients prior to randomization.

Procedure

Shortly the procedure is as follows. A complete workup including CT or MRI scan of the renal arteries is performed before the intervention and eligible candidates with symptomatic AF are included. The right femoral artery is punctured, and via a Seldinger technique a guiding wire is introduced. Afterwards a sheath is introduced. A pigtail catheter is introduced through the sheath and a contrast angiogram of the abdominal aorta is made depicting the renal arteries. Subsequently, the renal artery is cannulated with the multi-electrode Symplicity Spyral catheter through a guiding sheath. This catheter has the advantage of activating up to 4 electrodes to simultaneously deliver radio frequency energy, reducing the ablation time to 2 minutes. The ablation catheter is introduced up to the first bifurcation and extensive ablations according to our previously described protocol in both renal arteries, with 0.5 cm distance between ablation points. In a subset of patients the renal nerve stimulation (RNS) technique is used to assess completeness of renal denervation, providing us with more data and stimulation properties in a non-resistant hypertensive population²¹⁻²³.

7**Periprocedural measurements**

Blood pressure measurement before and after intervention. Periprocedural blood pressure before intervention and after intervention.

Follow up

Post treatment and follow up (3, 6 and 12 months): 24 hour Holter monitoring or cardiac rhythm monitoring with a loop recorder, lab and urine tests, number of antihypertensive drugs, 24 hour ambulatory blood pressure measurement, office blood pressure measurements. Specific drug adherence testing was not included in the ASAF study protocol. Although very important, this drug adherence issue is beyond the scope of our current study. A drug up-titration scheme is not part of the ASAF study, most patients with atrial fibrillation who suffer from hypertension referred for treatment do not have uncontrolled or resistant hypertension.

Main study end point

Time to first detection of AF >30 seconds, with the monitoring period starting 3 months after the intervention.

Secondary end points

1. AF burden after 12 months of follow-up, expressed in % of the monitoring period, in patients with continuous rhythm monitoring. The monitoring period starts 3 months after the intervention. 2. Blood pressure at 3, 6, 12 months after the intervention, and change in blood pressure compared to measurement before the intervention.

Other endpoints

The other end points include laboratory measurements, ambulatory blood pressure monitoring, Holter monitoring, rhythm assessments (AF burden) from implantable continuous loop recorder and exercise stress tests.

Use of co-intervention

In all groups, Vaughan-Williams class 1c, class II, class III (only sotalol) and/or class IV antiarrhythmics can be used. The goal is to stop class 1c or class III antiarrhythmic medication after 3 months after the intervention. The physician can re-initiate these antiarrhythmics when needed, with proper documentation of these changes in medication. If used, amiodarone should be stopped at least 3 months before the intervention. Antihypertensive medication should not be changed, unless grade 3 hypertension (office BP >180/110 mmHg) persists, or symptomatic orthostatic hypotension is present. Physicians are expected to use only antihypertensive drugs of the following classes: angiotensin II blocker, ACE inhibitor, beta blocker, calcium antagonist and diuretics.

7**Early termination of the study**

The study will be terminated if safety data indicate that treatment is associated with important AEs or in case of unacceptable patient risk exposure. In that case an unscheduled interim analysis will be performed and the decision to stop the trial will be at the discretion of the DSMB.

Statistical analysis**Intention-to-treat (ITT) population**

The ITT population consists of patients who have met the study entry criteria and have granted informed consent to participate in this study. These criteria are a prerequisite to randomization. This analysis population is also referred to as the all Randomised Subjects Set (RSS), or Enrolled Set. All analyses will be based on this population if there is post-randomization data for every patient. All baseline characteristics (e.g. summary of demographics) will be summarized on this analysis set. All results listings will be based on

the ITT population.

Full Analysis Set (FAS)

The FAS consists of patients from the ITT population who have treatment-related data post randomization. This analysis set is as complete as possible and as close as possible to the ITT population. If the FAS population is not, in practice identical to the ITT population, the FAS will be used for all non-baseline analyses.

Per Protocol Set (PPS)

The PPS population consists of all subjects from the FAS set with evaluable data and who have demonstrated full compliance with the study protocol (i.e. there is absence of any major protocol violations including the violation of entry criteria). Minor protocol deviations would be judged at the Data Review Meeting and deemed as such, before subjects can be retained in this population. The problems that lead to the exclusion of subjects to create the PPS, and other protocol violations, will be fully identified and summarized per treatment group, indicating frequencies, date and time of occurrence, where applicable.

Description of Statistical Analysis

We will perform an intention-to-treat analysis, if data permits. Otherwise the next most exhaustive study population (FAS) will be used. The eventual study population used will be explicitly stated for all results tables and figures generated. All line listings will be based on the ITT population and the study population would not need to be specified in the listings.

Descriptive statistics will be provided for all variables considered in the analysis. Continuous variables will be summarized descriptively providing, where applicable, the number of patients (N), mean, standard deviation (SD), median, minimum (min) and maximum (max), and interquartile range. Categorical variables will be expressed as counts and percentages (%). Percentages will be calculated according to the number of patients for whom data are available. P-values and accompanying Effect Size (Eta-squared values) statistics will be presented where applicable. The former will be used as a flag to filter for subsets of statistically significant results during comparison of treatment groups, if required. Unless otherwise stated, calculations will be performed per treatment group.

Primary endpoints

For key results changes (and percentage change, if deemed appropriate) from baseline will be summarized descriptively providing, the N, Nmiss (number missing), mean, SD, median, min and max; where applicable. The primary study endpoint is time to first detection of AF >30 seconds, with the monitoring period starting 3 months after the intervention. The primary objectives are to investigate (I) if RDN in combination with PVI prevents AF; (II) if there is superiority of the RDN procedure in combination with the PVI procedure in contrast to the

PVI procedure alone. The primary outcome measure will be tested by Kaplan Meier curves and log-rank tests. The Kaplan Meier curves will indicate freedom of AF >30 seconds after 1 year of follow-up with the monitoring period starting 3 months after the intervention.

For the first part of the primary objective, we will test the absolute outcome values (incidence of success vs incidence of non-success for the PVI+RDN group) statistically by using Pearson's chi-squared test. In case of rare events (the expected number per cell lower than 5 in more than 20% of the cells) the Fisher Exact test will be used. Two-sided 95% confidence intervals of the difference in percentages between the incidence groups will be calculated using exact methods. For the second part of the primary objective, we will test for significance of differences between PVI+RDN versus PVI. We will compare the survival distributions of PVI+RDN versus PVI using a log-rank test.

Differences in secondary endpoint absolute outcome values (incidences) will be statistically tested between groups by using Fisher's exact test or Pearson's chi-squared test. Differences in means of continuous data will be statistically tested by performing Student's t-test or, in case the data are not normally distributed, the Mann-Whitney-Wilcoxon test. Changes in continuous secondary endpoints compared to measurements before the intervention (blood pressure and heart rate response, heart rate variability, biomarkers, arterial stiffness measures) will be tested by paired t-tests. If the normality assumption is not met we will perform Wilcoxon Signed Rank Tests.

For blood pressure (systolic and diastolic) effects over time a two way repeated measures ANOVA will be performed. If the normality assumption is not met we will transform the data. We will test the interaction between treatment (PVI+RDN versus PVI) and time.

ANOVA assumes sphericity (compound symmetry), i.e. all the correlations of the outcome variable between repeated measurements are equal and all the variances of the outcome variable at each of the repeated measurements are equal. If the sphericity assumption is violated we will perform ANOVA with a Greenhouse and Geisser correction.

DISCUSSION

This study is a multicenter randomised controlled trial in which RDN on top of PVI is one of the arms as a therapeutic procedure for AF. This study will elucidate the added benefit of renal denervation to standard AF ablation in the prevention of AF recurrence in hypertensive patients with signs of sympathetic overdrive.

In animal studies, renal denervation resulted in less inducibility of AF with aggressive programmed electrical stimulation. Furthermore, it resulted in less persistent AF when AF

was still inducible in dogs. Besides, in post myocardial infarction rats, renal denervation resulted in a higher left ventricular ejection fraction, significantly improved hemodynamic indicators, and lower NT-pro-BNP, as compared with controls.²⁴⁻²⁶

In a small sample of patients with paroxysmal and/or persistent AF and resistant hypertension, renal denervation in addition to pulmonary vein isolation, resulted in significantly less patients with AF episodes, compared to patients undergoing pulmonary vein isolation only.¹⁷ This study however, had very important limitations, i.e. the small number of patients included, the changes that have been made to the design of the study and the change in end points. Most of these issues have been addressed by Staessen et al.²⁷ Apart from one change in the study design, dropping the RDN only arm, none of the mentioned limitations are present in the ASAF study. In several studies, including randomised controlled trials, it was shown that renal denervation resulted in a significant and durable reduction of blood pressure and less need for antihypertensive medication use and a higher proportion of patients reaching blood pressure goals. Renal denervation resulted in a reduced renal resistive index and incidence of albuminuria without adversely affecting glomerular filtration rate or renal artery structure. In all studies, renal denervation appeared to be a safe, with a <1% complication rate (renal artery dissection, groin bleeding, etc.), without any development of renal artery stenosis.²⁸⁻³⁰ Importantly, there was zero mortality associated with the intervention.

The Symplicity HTN-3 trial however, a sham controlled randomised blinded study, failed to meet both its primary (office systolic blood pressure reduction) and its secondary (mean 24-h ambulatory blood pressure) endpoints.³¹ One of the most important concerns raised after this trial, was the lack of an acute endpoint. Our group assessed the feasibility of renal nerve stimulation (RNS) before and after the procedure to assess this endpoint.²¹ Recently we demonstrated that RNS can be used to predict the outcome to RDN in a small cohort.²² RNS also elicited an increase in BP in accessory renal arteries after denervation of the main arteries, underlining a proof of concept.²³ Question remains whether a RDN without a procedural endpoint will be effective, but that is beyond the scope of this trial.

We have chosen different inclusion criteria than the classic one for resistant hypertensive patients, since our primary goal is to prevent AF, rather than treating resistant hypertension. If higher sympathetic tone is present through the renal nervous system, a rise in peripheral resistance and thus hypertension should be present. We therefore include patients who use two or more antihypertensives and have out of range hypertension.

Additionally, we chose to target patient groups in which previously a higher sympathetic tone was proven, such as diabetics, hypertensive patients with significant white coat effect, patients with tachycardia or illness mediated AF and patients with excessive blood pressure rise on exercise testing.

Our primary endpoint will be recurrence of AF/AFL/AT post-ablation, after a blanking period of 3 months after the interventional procedure, which is a frequently used and well known endpoint. Recurrence of AF/AFL/AT after ablation will be defined according to EHRA guidelines. In patients with continuous loop monitors, we will assess AF burden as a secondary endpoint. Treating AF by RDN can be mediated through different mechanisms. First, one of the most important risk factors for incident AF, but also for recurrent AF after treatment by ablation, is hypertension. We expect that after an added RDN procedure, both resting and stress blood pressure will be lower. Furthermore, RDN resulted in improved diastolic function and thus less atrial pressure and stretch, which is associated with less inducible AF in different studies.³² Finally, a reduction of sympathetic tone by RDN resulted in less atrial and ventricular ectopic beats, and even less ventricular tachycardia. By the reduction of ectopic beats, fewer trigger moments for the initiation of AF could be present, aiding in the prevention of recurrent AF.³⁰ A single blind study is currently investigating the effect of RDN on AF burden compared to anti arrhythmic drug regimens.³³ This study will contribute to assessing the effect of RDN on AF, but does not cover the additional effect of RDN on PVI. Another interesting trial currently recruiting patients is the Simplicity AF.³⁴ The main goal of the Simplicity AF trial is to test the feasibility and safety of performing both renal denervation and pulmonary vein isolation. ASAF is an investigator initiated trial. The primary endpoint of ASAF trial is AF free survival after a combination of pulmonary vein isolation and renal denervation compared to pulmonary vein isolation only. The most important difference between the Simplicity AF trial and the ASAF is the emphasis on hypertension in the Simplicity AF trial. Hypertension is a modifiable risk factor contributing in many patients who suffer from atrial fibrillation. By including patients with office systolic BP >150 mmHg whilst on a drug regimen with 3 antihypertensives, this study automatically targets a truly hypertensive population. ASAF trial focuses on the enhanced sympathetic drive in patients with atrial fibrillation. Independent of hypertension, other conditions are accompanied by sympathetic overdrive. We test the hypothesis that sympathetic overdrive can be targeted by RDN. In addition to blood pressure lowering effect, RDN may have anti-arrhythmic effects. Since the publication of the Simplicity HTN 3 trial, several other important trials have been published. These studies partly restore the efficacy of RDN in lowering BP. For example the DENERHTN trial by Azizi et al.³⁵ demonstrate the additive effect of RDN on top of stepped care standardized antihypertensive treatment. Another more fundamental randomised trial (SPYRAL HTN OFF-MED) took resistant hypertensive patients off their drug regimen and divided them between RDN and sham procedures. RDN significantly lowered BP in the RDN arm.³⁶ In the light of these recent landmark studies we are confident that RDN is still a viable option to interfere with possibly multiple factors influencing and maintaining AF.

Concluding, our present trial is the first multicenter randomised, controlled trial, assessing the effect of RDN as adjunct therapy on top of standard AF ablation therapy, in the prevention of recurrent AF.

Conflict of interest

The study receives sponsoring from the Medtronic External Research Program.

Dr. J. Toquero Ramos reports his membership of the Medtronic European Advisory Board. The other authors declare no conflict of interest.

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CHAPTER VIII

**Summary, conclusions,
and perspective**

HYPERTENSION

Patients affected with hypertension can be graded into different categories of severity ranging from 'high normal' to grade III hypertension. A subset of patients remains hypertensive despite being treated with a drug regimen containing at least three different classes of drugs or when there is documented intolerance for these classes. These patients suffer from resistant hypertension. Resistant hypertension is diagnosed in approximately 15% of the hypertensive population. These patients are at high risk of developing cardiovascular complications and require extensive medical attention, since it is hard to diagnose resistant hypertension without careful monitoring the effects of the intended treatment. Patients are often well aware of their risk and are very motivated to undergo invasive treatment for their medication resistant hypertension.

The hypertension community has awaited other solutions than lifestyle interventions and medical therapy to treat resistant hypertension. With the emergence of renal denervation, a solution seemed to have been found. The initial reports based on unblinded studies on blood pressure lowering effects of renal denervation were very promising. Renal denervation (RDN) could be a new treatment modality in patients with resistant hypertension. The SYMPPLICITY-HTN-2 trial suggested a major reduction with RDN in systolic BP based on office-based measurement of BP. However, ambulatory BP is a state-of-the-art technique for measuring BP in patients with treatment-resistant hypertension. Poor drug adherence is common in treatment-resistant hypertension, but this was not monitored in SYMPPLICITY-HTN-2 which made the study vulnerable to the Hawthorne effect (i.e. a change in behaviour when people know they are being observed). The magnitude of response varied between nonresponse to the so called super-response to renal denervation in a subgroup of patients.

However, data from 3 blinded randomised trials failed to demonstrate a benefit of RDN, and therefore an ongoing large international trial was stopped prematurely. SYMPPLICITY-HTN-3 (5) included sham and ambulatory BP measurement that balanced the Hawthorne and other patient and investigator unspecific effects but BP lowering caused by RDN was not significant in this trial. It was then suggested that SYMPPLICITY-HTN-3 was not rigorously executed and that RDN was suboptimal due to various technical issues related to the renal artery anatomy and technique for delivery of energy. But analyses were post-hoc, and SYMPPLICITY-HTN-3 findings were in line with results of other trials, and the meta-analyses of the first wave of properly done randomized studies of RDN did not show BP lowering effects of RDN whether SYMPPLICITY-HTN-3 was included or not, or whether sham was a part of the design or not. This had led to uncertainty of the place of RDN in the treatment of resistant hypertension. In the most recent ESC/ESH Arterial Hypertension (Management of) guidelines, device-based therapies, such as RDN, are not recommended (class III, level of evidence B) for the routine treatment of (resistant) hypertension, unless in the context of clinical studies and RCTs, until

further evidence regarding their safety and efficacy becomes available. As one of the many RDN investigators stated: “We have seen the rise and fall of renal artery denervation, and we hope that we shall witness the resurrection.” (Bhatt, Deepak L. MD, in *Ruminations about Renal Denervation*).

This thesis describes the development of renal nerve stimulation (RNS) in the setting of RDN. After publication of the disappointing results of the HTN-3 trial, many explanations were given to clarify the results of this study. Some people believed it was due to the learning curve of newer centres, inspired by the initial results. More experienced centres seemed to have a more pronounced drop in blood pressure. Another explanation is inefficient ablation by not applying enough radiofrequency energy to the wall of the renal arteries. This is in analogy with ablation for AF in which measuring and adjusting to contact force might contribute to the effectiveness.

However, we chose a different path. From the perspective of a cardiac electrophysiologist, performing a renal denervation without a procedural endpoint does not make sense. Repeating the analogy of pulmonary vein isolation for AF ablation, isolation of the veins is checked with a circular mapping catheter after ablation to make sure the ablation has been performed successfully. In Isala Heart Centre the renal denervation procedures are carried out by cardiac interventional electrophysiologists, in contrast to majority of centres where these procedures were performed by interventional cardiologists or radiologists.

Looking for a procedural endpoint for renal denervation we came up with the development of renal nerve stimulation. The technique enables the operator to perform renal denervation using a more functional approach.

This thesis describes our clinical studies regarding potential mechanisms underlying the variable response to renal denervation and the results of a novel approach using renal nerve stimulation to guide renal denervation. Both of them are aspects that have to be addressed to determine the role of renal denervation in the treatment of resistant hypertension. RNS is an important improvement of the RDN technique and provides operators with a procedural endpoint. This sets our studies apart from the large negative studies studying the effect of RDN. Our studies provide more insight into the effect of RDN, after the large negative studies on this topic. In addition, we try to elucidate the potential mechanisms why RDN might work in hypertension, which may also shed light on the results of earlier studies in this field.

THE DEVELOPMENT OF RNS

This thesis describes the development of RNS as a tool to localize renal perivascular nerves.

The first step in the development of RNS was the feasibility study in patients with resistant hypertension. Chapter 2 elaborates on the process of different setting for pacing output, pacing frequency and duration of the electrical stimulation. Studying the effects of different settings was a very interesting experience since human data on RNS is scarce and most of the ideas have only been tested in animal models, such as a canine model. We carefully monitored the effect of RNS on the cardiovascular system and concluded that RNS in the setting of a RDN procedure was safe in our patient population. The most important ‘serious adverse event’ was a temporary decline in renal function in one patient, which normalised after discontinuation of nephrotoxic medication.

One may argue that renal nerve stimulation is painful and that it will cause a pronounced vagal reaction to pain induced by pacing. However, our acute blood pressure findings were related to the post RDN outcome based on 24 hour ambulatory blood pressure monitoring. These findings have been explored in chapter 3. Because of the significant relationship between the RNS data and the blood pressure lowering effect, it is unlikely that RNS induced blood pressure decrease is related to pain.

Chapter 4 builds a bridge between RNS and predictors of response to RDN. We demonstrated an important aspect of RDN in patients with accessory renal arteries. Since these accessory arteries are common and their role not fully delineated, we investigated them with RNS.

After a ‘successful’ denervation of the main renal arteries, an increase in BP can still be invoked by RNS in the accessory arteries. A successful denervation was defined as an increase in BP due to RNS before RDN and a blunted increase in BP after RDN of the main arteries. Even after denervation of the main arteries, a BP increase could be invoked with RNS in the accessory arteries. These findings demonstrate an important relationship between renal vasculature and responder rate.

Chapter 5 describes the acute effects of RNS on the blood pressure response in patients. Including patients in the RNS study yielded interesting information with regards to blood pressure response patterns. In accordance with our hypothesis, blood pressure increased at most sites (almost two-third of the sites), due to activation of sympathetic nerve tissue. There was no blood pressure change after RNS at other sites. This procedure enables the operator to map sympathetic nerve bundles along the perivascular space of the renal arteries. In response to stimulation at sites without sympathetic nerves, no alteration in blood pressure was observed. High frequency electrical stimulation is a functional approach and exact relationship between anatomic localisation of nerves and RNS response remains to be elucidated.

Interestingly, we identified sites at which the blood pressure dropped in response to RNS.

We speculate that blood pressure decrease was elicited by stimulation of vagal nerves since, anatomically, the parasympathetic ganglia are located in close proximity to these RNS sites. Ablation of these ganglia would serve no purpose in the treatment of hypertension.

RENAL DENERVATION AND HYPERTENSION

Since the clinical evidence of RDN as an effective BP-lowering technique is conflicting, and based on large RCTs currently not recommended for the routine treatment of (resistant) hypertension, the place of RND in the treatment of drug resistant hypertension is uncertain. The multifactorial underlying pathophysiology in these patients makes it hard to establish one factor that will solve hypertension in the entire 15% of drug resistant patients.

In our database, approximately one third of the patients responded well to RDN (substantially lower blood pressure, reduction in number of antihypertensive drugs). One third responded moderately (slightly lower blood pressure or reduced dosages of the same drug regimen). One third of the patients responded poorly with no change in blood pressure, or even an increase in blood pressure in some patients. There are several factors that need to be taken into account before we can interpret these data. None of these patients had a strict check of drug compliance. Some centres admitted their patients to the general ward and checked the BP lowering effect of clinically provided medication intake before diagnosing drug resistant hypertension. Other hypertension centres put their patients on a trial period with spironolactone to exclude hyperaldosteronism. Our patients were screened vigorously to rule out secondary causes of hypertension before diagnosing drug resistant hypertension. Hereby, we aimed to exclude hormonal disturbances such as hyperaldosteronism. Patients eligible for RDN after careful screening were very motivated to undergo the invasive procedure. We assumed these patients were compliant to the prescribed drugs. Of note, a drug compliance of 100% is not realistic in any population, so this issue remains arguable. Those patients who responded well to the renal sympathetic denervation therapy might have a lower risk of developing cardiovascular endpoints, optimal management of blood pressure with a reduced number of antihypertensive drugs and as a consequence may experience less side effects. We believe that in the future there may possibly be a place for renal denervation in the treatment of drug resistant hypertension, if (preferably sham-) controlled studies are positive. The prospective, open-label randomised controlled trial with blinded endpoint evaluation DENERHTN study and the single-blind, randomised, sham-controlled, proof-of-concept SPYRAL HTN OFF MED study demonstrated a positive result for RDN. And we have to take into account that RDN only requires a one-time procedure instead of drug adherence with possible side effects. RDN is possibly the treatment of choice for younger patients, that would otherwise have to use a certain drug regimen for lifetime. Our reflections on the role of RNS in the setting of RDN have been summarised in the review (chapter 6).

RENAL DENERVATION (RDN) IN ATRIAL FIBRILLATION

This thesis includes one of the future aspects of renal denervation. AF is subject to a different discussion regarding the role of the autonomic nervous system in triggering and maintaining AF. Medical therapy falls short in treating AF since long-term sinus rhythm is achieved in only 30-40% of patients. Catheter ablation is the next step to establish and maintain sinus rhythm and has a success rate ranging from 60-70% of AF-free survival after a single procedure. This leaves a significant number of patients who remain symptomatic post-ablation and could probably benefit from additional treatment. One of adjunct therapy options is renal sympathetic denervation. The concept behind renal denervation for AF is to suppress triggers caused by fluctuations in the sympatho-vagal balance. Majority of patients who wake up with a paroxysm of AF do not have overt structural heart disease and a vagal component is quite likely in these patients. In patients with enhanced sympathetic tone and AF, risk factors such as hypertension, obesity, diabetes mellitus can be identified. Furthermore, some patients develop sympathetic AF during episodes of fever for example. Enhanced sympathetic tone may induce AF. A similar effect is seen when patients develop AF during exercise. For this reason, the potential role of RDN on top of pulmonary vein isolation is discussed in chapter 7. Renal denervation is a possible adjunct treatment in patients with AF and enhanced sympathetic tone undergoing catheter ablation. We hypothesised that if patients are identified with sympathetic AF, they could possibly benefit from renal denervation. The ASAF study is an ongoing international multicentre randomised controlled trial which tests the role of renal denervation in achieving and maintaining sinus rhythm on top of pulmonary vein isolation compared to pulmonary vein isolation alone.

If renal denervation combined with pulmonary vein isolation proves to be superior to pulmonary vein isolation alone, a potential anti-arrhythmic role for renal denervation will be established. As of May 2019 only one meta-analysis by Atti and colleagues in the Journal of Cardiovascular Electrophysiology has analysed 6 studies, 4 of which were randomised studies. The total number of patients was 432 patients. RDN on top of PVI did contribute to a significantly lower recurrence of AF in patients with a history of hypertension (pooled RR 0.58 [0.47-0.72], $P < 0.001$).

The ASAF trial is important because of the currently recruited number of patients and the scarcity of studies investigating the role of RDN in AF that are actively including patients. As of October 2018 only a few studies are recruiting patients. The Simplicity AF trial is currently recruiting patients with paroxysmal and persistent AF in the United States. The Chinese SWAN-cpAF study has been recruiting patients since 2012 without reaching the intended 100 patients. A South-African study (RDPAF1) is investigating the preventive effect of RDN on the development of AF in hypertensive heart disease.

RENAL DENERVATION IN THE LIGHT OF NEW RESEARCH

This thesis referred to the following statement: “we have seen the rise and fall of renal denervation and we hope to see its resurrection”. This is a statement from 2016 when the HTN-3 trial devastated the initial hope that RDN would solve resistant hypertension, and led, among others, to the class III recommendation (i.e. “not recommended”) in the latest ESC/ESH guidelines for the routine treatment of (resistant) hypertension. However, the SPYRAL OFF and ON MED studies have shined new light on the efficacy of RDN. The results of the SPYRAL OFF MED demonstrated a significant effect of RDN when compared to sham control. The SPYRAL ON MED had similar, but even more pronounced results. It is reassuring that the procedure can bring about a blood pressure lowering effect. However, we have come a long way from the initial procedure and the current procedure.

When RDN was first performed, 4-8 ablation points per renal artery were placed, in hopes of permanently destroying the surrounding sympathetic nerve tissue. The SPYRAL ON MED has a staggering average of 45.9 ablation points.

The evolution from carefully selected point to ‘as many as you can fit in’ has definitely changed the extent of damage done to the sympathetic nerves. The procedure has become more effective with more pronounced blood pressure drops in larger proportions of the included patients. However, the more damage you do, the more worried you should be about long term effects like kidney function and renal artery stenosis. With progressive insight in the number of ablation points, the currently available follow-up is insufficient to determine these effects. The role of RDN in accessory arteries has been studied as well. With the advancement of technique and catheters, smaller and smaller arteries could be denervated without compromising the artery. Initially, patients with a more complex vasculature were not included in the RDN studies, or these patients demonstrated a less impressive blood pressure lowering effect. Currently accessory arteries are being treated in the same setting as the main branches, whenever that seems feasible.

Other groups have diverted their focus to contact force, to ensure proper contact between the catheter and the renal arteries for subsequent ablation. An even more different approach has been chosen in the RADIANCE-HTN-SOLO, to replace radiofrequency with ultrasound energy to destroy the sympathetic nerves. The initial results favour RDN over sham procedures. Investigators relied on patient-reported antihypertensive drug use. Reported medication use was more common in the sham group, but hidden use was not evaluated by drug measurements in blood. Assuming that hidden drug use was not more common in the treatment group, RADIANCE-HTN-SOLO together with the SPYRAL-OFF and ON-MED trials may provide the first true trial evidence that RDN lowers BP. Another important study is the ULTRASOUND-HTN, comparing three techniques to denervate the renal arteries. Patients

with daytime ambulatory BP more than 135/85 mmHg and less than 170/105 mmHg after a four-week discontinuation of 1-2 antihypertensive drugs were randomised to RDN (n=74) or sham (n=72). The primary endpoint was change in daytime ambulatory systolic BP at 2 months in the intention-to-treat population. Endovascular ultrasound RDN reduced daytime ambulatory systolic BP with 6.3 mm Hg more than sham ($p=0.0001$). Perhaps a switch in energy source is an important step in perfecting RDN procedures, especially after the REDUCE HTN: REINFORCE study failed to meet its primary efficacy endpoint. The latter study investigated ablation with radiofrequency energy, but was terminated because a preliminary analysis demonstrated no difference between the interventional and the sham procedure groups.

However, there is one major difference between the empiric RDN results and the development of RNS. No matter how many ablation points have been placed, no matter which arteries were targeted for denervation (main, accessory or both) and no matter what technology is used to denervate the arteries with, none of these improvements identify and localise the perivascular nerve bundles. A successful RDN procedure hinges on successfully destroying the targeted nerve fibres. RNS combines localising the sympathetic nerves with preventing inadvertent ablation of other tissue. Therefore RNS should be explored in a wider variety of patients.

CONCLUSION

The aim of our studies was to increase our insights into the mechanisms of RDN, improve patient selection and technique to increase response rate to RDN. We were the first assessing the feasibility of RNS in humans with resistant hypertension, undergoing RDN. We showed a significant temporary rise in BP due to RNS, and a large reduction of RNS-induced BP rise after RDN. Furthermore, we reported that the magnitude of reduction of RNS-induced BP changes after RDN were strongly associated with reductions of BP measured with 24-h ABPM during follow-up after RDN. The underlying pathophysiological mechanisms of RNS and the effects on arterial blood pressure dynamics remain to be fully elucidated. Future studies exploring the role of RNS guided RDN in larger cohorts are warranted.

CHAPTER IX

Nederlandse samenvatting

Hoge bloeddruk is een van de meest voorkomende risicofactoren voor het ontwikkelen van hart- en vaatziekten. Hoge bloeddruk is goed behandelbaar met medicatie. Er is echter een groep patiënten welke niet de streefwaarde van de bloeddruk bereikt met drie middelen waaronder een plastablet. Deze groep patiënten dient gescreend te worden voor een onderliggende oorzaak van de hoge bloeddruk (bijv. hormonaal bepaald, op basis van nierslagadervernauwing, slaap-apneu syndroom etc.). Als deze oorzaken niet worden gevonden resteert er een groep patiënten met medicatie-resistente hoge bloeddruk. Deze groep mensen heeft allicht baat bij een ingreep aan de zenuwen die de nierslagaders omgeven. Deze ingreep wordt renale denervatie (RDN, renal sympathetic denervation) genoemd. Het idee dat de nieren een te hoge bloeddruk maken op basis van een verhoogde zenuwactiviteit is niet nieuw. Vroeger werd deze ingreep operatief verricht, maar met de opkomst van de medicatie tegen een hoge bloeddruk (antihypertensiva) raakte de RDN procedure uit zwang. Met de opkomst van catheters en daarmee de mogelijkheid om via de liesslagader bij de nierslagaders te komen, kreeg renale denervatie een nieuw leven. Vanuit de binnenzijde van het bloedvat kan de ingreep verricht worden. Dit biedt hoop aan patiënten die gebonden zijn aan een uitgebreid schema met antihypertensiva. De eerste resultaten van de studies die werden verricht om de effectiviteit van RDN te onderzoeken waren erg goed. Dit gaf de behandeling een enorme boost. Echter een grote studie waarin patiënten werden verdeeld tussen een echte RDN procedure en een 'nep-procedure', resulteerde in een teleurstelling. De bloeddrukdaling was vergelijkbaar in beide groepen. Er is binnen de wereld van onderzoekers naar RDN veel gediscussieerd over wat de oorzaak zou kunnen zijn en verscheidende facetten van deze discussie zijn onderzocht. Er is o.a. gekeken naar patiëntenselectie, een vast schema titreren van de medicatie, stoppen met de medicatie vooraf aan de behandeling en de deelnemende centra. Om de ingreep zelf te verbeteren is gekeken naar het aantal ablatie punten, ablatie voorbij de eerste splitsing van de nierslagader en zelfs naar de kracht waarmee de catheter contact maakt met de wand van de nierslagader. Wat nog ontbrak was een manier om de zenuwvezels welke doorgebrand moeten worden (de sympathische zenuwvezels, van het actieve zenuwstelsel) op te sporen tijdens de ingreep. Het onderzoek dat wordt beschreven in dit proefschrift gaat in op een uitbreiding van de RDN procedure met een stimulatietechniek die de zenuwvezels activeert. Tijdens de procedure wordt met elektrische energie de sympathische zenuwvezels geactiveerd, welke op hun beurt weer een stijging van de bloeddruk veroorzaken. De uitbreiding hebben wij renale zenuwstimulatie genoemd en afgekort tot RNS. In hoofdstuk 1 van dit proefschrift wordt uitgelegd hoe het proefschrift is opgezet. In hoofdstuk 2 wordt beschreven dat de nieuwe RNS-techniek mogelijk is bij mensen. In hoofdstuk 3 wordt aangetoond dat als de procedure een succes is volgens de RNS-techniek, de daling in de gemiddelde bloeddruk over 24 uur ook groter is dan bij de groep bij wie de RDN volgens RNS minder goed is gelukt. In hoofdstuk vier laten we zien dat als de RNS techniek een goede RDN procedure voorspelt in de hoofdslagaders naar de nieren, er nog steeds sympathicusweefsel kan bestaan in de zijtakken. Dit past goed bij de bestaande literatuur waarin bekend is dat patiënten met een ingewikkeldere bloedvat voorziening

naar de nieren minder snel een goede reactie op RDN laten zien. De ervaring die is opgedaan met RNS gaf meer inzicht in de verschillende manieren waarop gereageerd wordt op RNS. Er zijn drie patronen te onderscheiden. In de eerste plaats is er de stijging in de bloeddruk welke ook wordt verwacht en aldaar zal ablatie (het wegbranden van de zenuwvezels) dan ook nodig zijn. Naast de stijging is er ook géén bloeddrukreactie mogelijk. Dit komt overeen met een stuk nierslagader waar geen sympathicusweefsel omheen zit. Ablatie is hier dan ook niet zinvol. We ontdekten ook plaatsen waar er een tegenovergestelde reactie plaatsvond met een vertraging in het hartritme en een bloeddrukdaling. Dit kan berusten op activatie van het ‘parasympathische’ zenuwstelsel, welke de tegenhanger is van de sympathicus en gebruikt wordt in rust en bij het verteren van voedsel. Ablatie aldaar is onwenselijk en kan met RNS voorkomen worden. In hoofdstuk 6 bespreken we de techniek als eindpunt voor een goede RDN procedure waarin wordt betoogd dat de techniek routinematiger toegepast zou moeten worden. Tot slot wordt in hoofdstuk 7 besproken dat gezien de heropleving van RDN procedures ook naar toepasbaarheid bij andere ziektes gekeken moet worden. Atriumfibrilleren (boezemfladderen) is een ritmestoornis van de voorkamers van het hart. Dit geeft een verhoogde kans op een herseninfarct, maar is ook een voorspeller van slechte uitkomst bij allerlei andere ziektebeelden. Met medicijnen alleen wordt maar in beperkte mate het hartritme hersteld naar het normale hartritme (sinusritme). Om de ritmestoornis te behandelen kan er in het hart een ablatie uitgevoerd worden. Echter uit dierexperimenteel onderzoek een kleine onderzoeken bij mensen blijkt dat er mogelijk ook een grotere kans is op het behoud van sinusritme als RDN wordt toegevoegd aan de ablatie in het hart. Om dit uit te zoeken is de ASAF studie opgezet. De studie loopt momenteel nog. Concluderend beschrijft dit proefschrift de mogelijkheid om RNS toe te voegen aan de huidige RDN procedure om zo het resultaat van de behandeling te voorspellen en tijdens de procedure te beoordelen of de ingreep beëindigd kan worden. De waarde van RNS maar ook de keerzijde worden besproken in de verschillende hoofdstukken. Het proefschrift eindigt met een voorzet voor de verbetering van de behandeling van de ritmestoornis atriumfibrilleren.

APPENDIX

List of publications

Dankwoord

Curriculum Vitae

LIST OF PUBLICATIONS

1. Gal P, **de Jong MR**, Smit JJ, Adiyaman A, Staessen JA, Elvan A. Blood pressure response to renal nerve stimulation in patients undergoing renal denervation: a feasibility study. *J Hum Hypertens*. 2015 May;29(5):292-5.
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DANKWOORD

Het tot stand komen van een proefschrift is niet het werk van een individu. Vele mensen hebben in meer of mindere mate bijgedragen aan mijn 'boekje'. Ik wil stilstaan bij een aantal mensen die hier in het bijzonder aan hebben bijgedragen

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CURRICULUM VITAE

Mark Roland de Jong (1989) is geboren te Groningen (Martini Ziekenhuis) en opgegroeid in Haren. Hij heeft zijn gymnasiumdiploma cum laude behaald aan het MaartensCollege te Haren in 2007. Vervolgens is hij geneeskunde gaan studeren (2007-2013) aan de Rijksuniversiteit Groningen. Hij volgde coschappen in het Martini Ziekenhuis en Isala te Zwolle). In 2013 is hij aan de slag gegaan als ANIOS cardiologie in Isala en vervolgens als arts-onderzoeker bij de afdeling cardiologie vanaf 2014. In 2015 is hij begonnen aan de specialisatie tot cardioloog en is het onderzoek onder de vleugels van de opleiding verder afgemaakt. De specialisatie loopt nog tot 2021. Het verrichte onderzoek is op meerdere internationale congressen gepresenteerd en o.a. bekroond met de Gert van Montfrans-prijs 2017 voor meest innovatieve onderzoek binnen het vakgebied hypertensie.

Mark Roland de Jong (1989) was born in Groningen (Martini Hospital) and he grew up in Haren. He graduated with honors from the MaartensCollege Gymnasium in 2007. Subsequently he went to Medical School at the University of Groningen. He did his internships at the Martini Hospital and at Isala in Zwolle. He accepted a position as ANIOS at Isala in 2013 and as researcher with the cardiology department from 2014. He started his specialization to a cardiologist in 2015 and continued to work on his research. The specialization will be finished in 2021. The data in this thesis have been presented at several international conferences and amongst others has been awarded with the Gert van Montfrans-award 2017 for most innovative research in the field of hypertension.