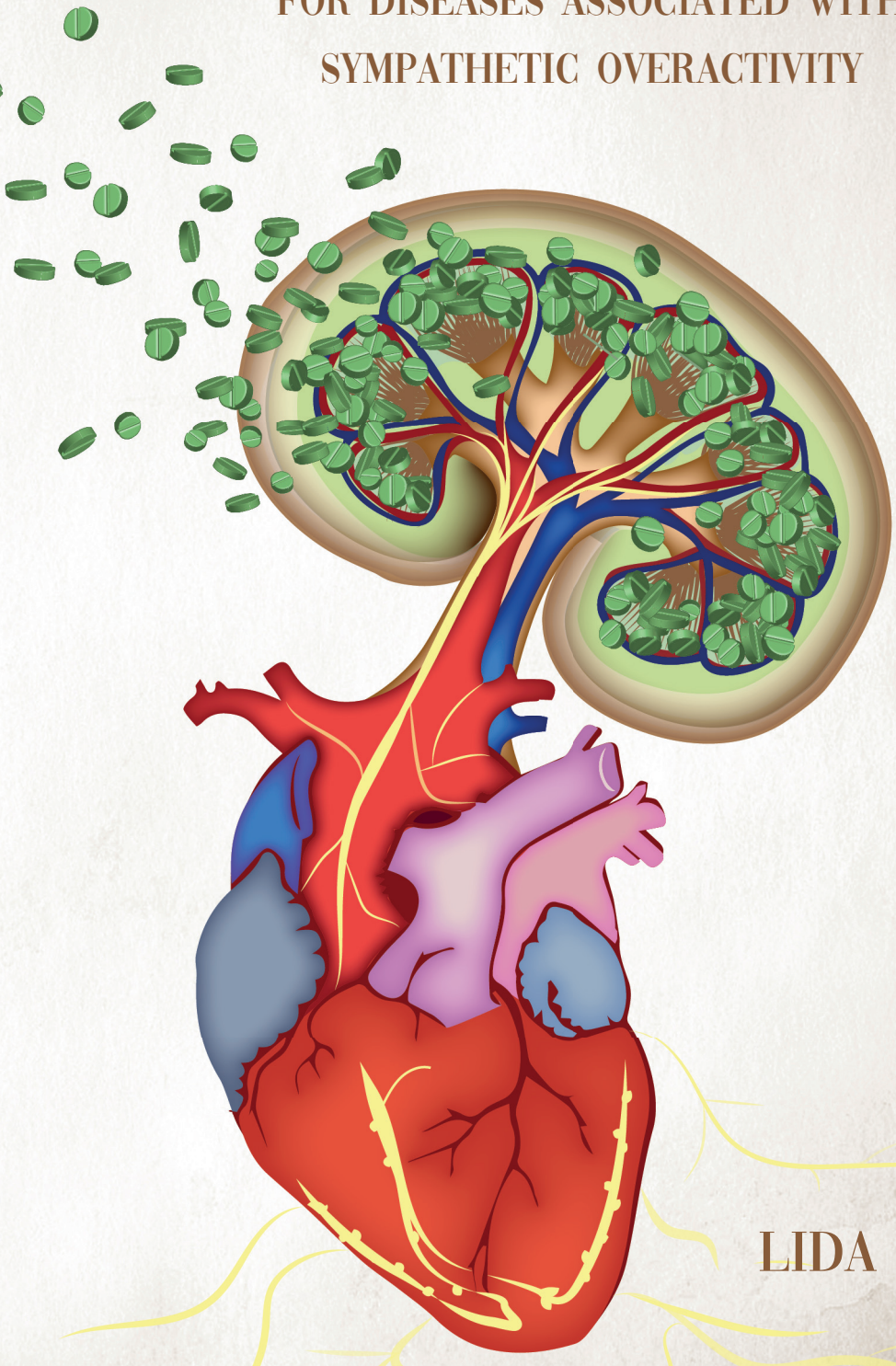
ADVANCES IN THE WORKUP AND TREATMENT FOR DISEASES ASSOCIATED WITH SYMPATHETIC OVERACTIVITY



LIDA FEYZ

Advances in the workup and treatment for diseases associated with sympathetic overactivity

Nieuwe inzichten in de diagnostische work-up en behandeling voor aandoeningen geassocieerd met sympathetische overactiviteit

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Promotor:	Prof. dr. F. Zijlstra
Overige leden:	Prof. dr. J. W. Deckers
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	Prof. dr. P. J. Blankestijn
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	Dr. J. Versmissen

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“To achieve great things, two things are needed: a plan and not quite enough time”

- Leonard Bernstein -

To my parents

Contents

CHAPTER 1

General Introduction

The autonomic nervous system consists of different pathways of neurons to control organ systems. It is divided into the sympathetic and parasympathetic nervous system. The sympathetic component is known as the quick 'fight or flight' stress-response system. To activate this stress response, the hypothalamus stimulates the sympathetic nerves to send out impulses to vascular smooth muscles and the adrenal medulla. Subsequently, catecholamines (norepinephrine, epinephrine) are released into the bloodstream. These stress hormones cause several changes in the body, such as an increase in heart rate, blood pressure and in blood glucose levels (Figure 1). The parasympathetic nervous system conversely is known as the rest system. Parasympathetic nerve activity results in lower heart rate, vasodilatation, increase intestinal activity and increase in urine production.

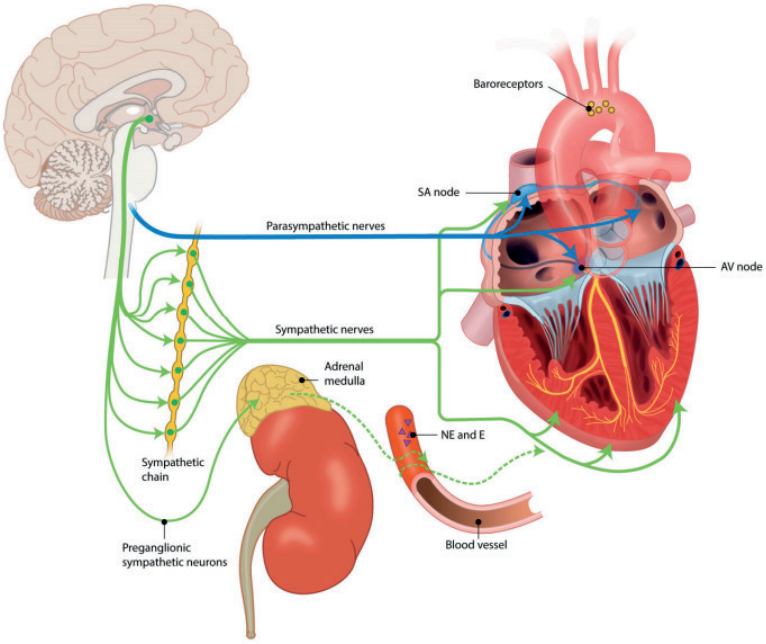

Figure 1. Sympathetic and parasympathetic nervous system (1). AV: Atrioventricular, E: epinephrenine, NE: norephinephrine, SA: sinoatrial

Autonomic cardiovascular control is impaired in hypertension, leading to an increase in sympathetic control to the heart and peripheral vessels with a reduction in parasympathetic tone (2). The latter, the so-called sympathetic overactivity depends on a variety of reflex and non-reflex mechanisms and plays an important role at development and progression of the essential hypertensive state (2, 3). Hypertension is the most

important risk factor for cardiovascular morbidity and mortality (4). Additionally, sympathetic overactivity is related to poor clinical outcome in patients with heart failure and progression of kidney failure (5-7). Despite significant advances in the understanding and management of hypertension, a large number of patients have blood pressure levels above the recommended treatment targets. A phenomenon that has been explained by a variety of reasons that appeared to be difficult to control. The latter triggered the field to innovate more advanced tools and techniques to treat hypertension.

At first, non-adherence to antihypertensive drugs proved to be an important reason for not reaching treatment targets, which also has an important impact on cardiovascular prognosis (8). Specifically, several publications reported a high proportion of non-adherence in patients with apparently resistant hypertension (also called pseudo-resistant hypertension) (9). The term pseudo-resistance refers to lack of blood pressure control with appropriate treatment in a patient who does not have resistant hypertension. It is thought that 'resistance to therapy' is present in about 50% of the patients with pseudo-resistance (10-12). Therefore, evaluation of adherence should become an integral part of assessment in patients with resistant hypertension. Many factors contribute to non-adherence to medication and a single approach will not be effective for all patients (13). Major predictors of non-adherence to medication were previously described to be side effects of medication (14), presence of psychological problems, patient's lack of belief in benefit of treatment (15) and/or inadequate follow-up or discharge planning (16). Several tools were developed to identify non-adherence, with therapeutic drug monitoring (TDM) as the most accurate method to monitor drug concentrations (12). However, TDM must be performed at the exact moment of blood pressure measurement to correlate drugs levels directly to the blood pressure. The latter is not feasible in clinical practice. To overcome this logistic and time-consuming burden, the dried blood spot (DBS) method should be implemented. The advantage of DBS above plasma measurement is the fact that sampling can take place at the same moment when increased blood pressure is measured. The latter prevents white coat adherence, which is seen when an additional visit to the phlebotomy unit is needed. Second, complementary therapeutic pathways are needed when blood pressure still remains uncontrolled after optimizing pharmacotherapy and excluding secondary causes of hypertension. The exact mechanisms responsible for the sympathetic overactivity are still unknown when focusing on the pathophysiological role of the sympathetic nervous system (SNS) in the genesis of hypertension and hypertension-related cardiovascular disease. Roughly, the SNS can be divided into an afferent- and

efferent pathway. The afferent nerves of the SNS transmit sensory information from the internal organs to the central nervous system. Oppositely, the efferent nerves transmit information from the central nervous system to the various organs (17) (Figure 2).

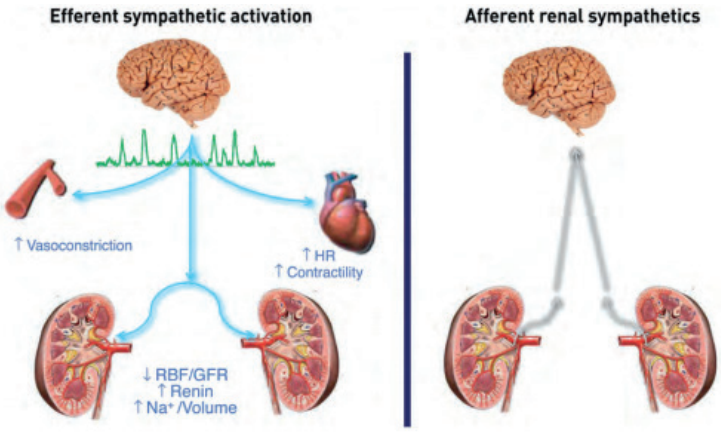

Figure 2. Renal afferent and efferent sympathetic activation. GFR: glomerular filtration rate, HR: heart rate, RBF: renal blood flow (18).

The renal sympathetic nerves have an important role in the initiation and maintenance of the sympathetic overactivity (19). The sympathetic nerve fibers are located within and adjacent to the adventitia of the renal arteries. These norepinephrine-containing (efferent) nerves are in contact with juxtaglomerular cells which in turn stimulate renin secretion to regulate arterial pressure trough sodium resorption and volume expansion (20, 21). The renal afferent nerves consists of chemo- and mechanosensitive fibers, which are the physiological conduit of central sympathetic drive (6). This regulatory mechanism helps to maintain the circulatory homeostasis under normal conditions. However alternations in efferent and afferent renal nerve activity result in changes in renal function and are known to contribute to the development and maintenance of hypertension (22). Several hypothesis have been studied previously which can change the activity of the nerves, such as changes in renal blood flow, changes through humoral factors (norepinephrine, angiotensin II) and/or changes in baroreflexes (23-25). When considering the pathophysiologic mechanisms described above, one might argue that renal sympathetic denervation (RDN) might work to decrease SNS activity and improve for instance blood pressure control (19, 21, 26) (Figure 3).

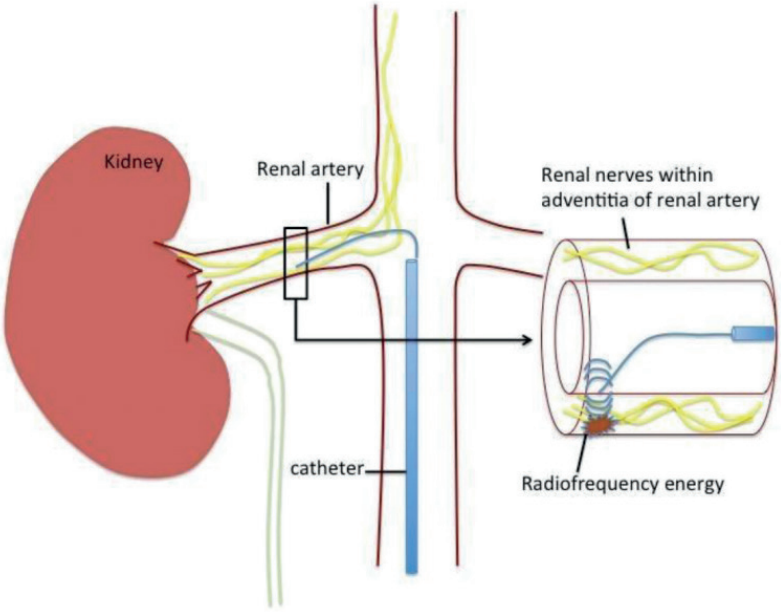

Figure 3. Schematic overview of the catheter-based RDN procedure (27).

In 1953, Reginald Smithwick introduced surgical splanchnicectomy for essential hypertension (28). Significant blood pressure reduction was observed, with a benefit in the mortality rate at five year follow-up as compared to patients who only received available pharmacological therapy. Despite being effective in a vast number of patients, the therapy had significant side-effects (eg. bladder dysfunction and orthostatic hypotension) and the therapy was quickly abandoned after the introduction of effective antihypertensive pharmacotherapy in the early ’60 (29). Nevertheless, almost half a decade later, blood pressure control rates remain poor despite optimal medical treatment using multiple combinations of the available agents and the risk for cardiovascular disease remains high. In a search towards novel means of blood pressure reduction the concept of the surgical denervation from the early 50’s trigger several pathophysiologist to perform experimental studies assessing the effect of less invasive denervation such as RDN.

The first proof-of-principle catheter-based RDN study was published in 2009 (26). The treatment modality was introduced to optimize blood pressure control in patients with resistant hypertension (26, 30). Resistant hypertension in these studies was defined as a baseline office systolic blood pressure of ≥160 mm Hg despite a regimen of ≥3

antihypertensive drugs (with at least one diuretic) in a maximally-tolerated dose. Although the procedure showed to be efficacious and safe, many questions remain about the exact pathophysiological mechanisms by which RDN lowers blood pressure. Several clinical and experimental evidence described that RDN only interrupts the function of efferent nerves, which results in a decrease in renal vascular resistance and a decrease in renin activity (31, 32). However, more robust data is needed and a bidirectional relationship between the RAAS and the SNS is more assumable (33-35).

Previous clinical data on RDN

Several outcome studies have focused on the safety and efficacy of catheter-based RDN. The Symplicity HTN-I was the first-in-man pilot study, in which a total of 45 patients with resistant hypertension underwent RDN (26). The authors reported a mean reduction in office blood pressure of -22/-11mmHg (95%CI 10/5) at 6 months follow-up. At this moment, there was no focus on the 24h ambulatory blood pressure measurement (24h-ABPM), which seems to be very important nowadays. In the latter study, only 9 24h-ABPM measurements were performed which turned out to show a reduction in mean systolic ABPM of -11mmHg, 95%CI 7. The 24-months results of the trial showed a persistent blood pressure lowering effect after RDN (by -32/-14mmHg versus baseline) (36). Following these promising findings the Symplicity HTN-II trial was conducted, a multi-center prospective randomised trial, allocating 106 patients to RDN or control group (30). At 6 months follow-up, 84% of the patients who underwent RDN had a reduction of ≥ 10mmHg in office systolic blood pressure as compared with 35% in the control group ($p<0.001$). The procedure proved to be safe with minor adverse events. There was a need to conduct larger, randomised sham-controlled trials after these initial promising results. Therefore, the Symplicity HTN-III was executed. In the latter study, RDN failed to achieve its efficacy endpoint (37). This prospective, single-blind, randomised, sham-controlled trial showed no difference in efficacy and safety between RDN therapy versus control group. These results were surprising and disappointing for researchers who had to reevaluate their ongoing study programs. In-depth analyses on the latter trial found several potential explanations for the lack in efficacy (38-40). Including, *patient-related aspects*: heterogeneous patient baseline characteristics, changes in antihypertensive drugs and adherence to antihypertensive drugs was not assessed; and *procedural aspects*: low number of ablation attempts and a non-circumferential ablation was observed. Addressing these

issues, the DENERHTN trial in 2015 was the first randomised controlled trial showing a significant blood pressure lowering effect of RDN (41). The authors excluded the confounding effect of changes in antihypertensive drugs during the course of the study by a standardized drug regimen. Adherence to antihypertensive drugs was assessed by using a questionnaire (42), no difference was observed in drug-adherence assessed in both groups. A sub analysis from the DENERHTN trial showed that the prevalence of non-adherence to antihypertensive drugs at 6 months was around 50%, which was similar in the treatment-arm (RDN + standardized stepped-care therapy) and control-arm (standardized stepped-care therapy alone) (43). However, the variability in adherence to antihypertensive therapy was not known, due to the fact that no drug levels in plasma or urine were measured (44).

Pre-clinical assessment of the SNS

Given the lack of direct post procedural feedback, RDN remains a so-called blind procedure and measuring the effect of RDN on the SNS proved to be challenging. The two most used methods to quantify sympathetic activity include the measurement of noradrenaline (NA) spillover in plasma and/or urine, and the assessment of muscle sympathetic nerve activity (MSNA) (26, 31, 45). An advantage of measuring NA levels is that it is easy to apply, however this method is hampered by limited reproducibility (45). The NA levels measured will represent a fraction (5-10%) of the total amount adrenergic activity (46). Therefore it is only a static indicator of the sympathetic function (47). Measurement of regional rates of NA release allows assessment of organ-specific sympathetic tone. However the major problem in interpreting regional NA spillover measurements is the fact that extraneous factors might affect the spillover, such as the possible influence of blood flow on NA washout (48). MSNA assesses nerve activity by microelectrodes which are inserted in a peroneal or brachial nerve. These microelectrodes will record spontaneous bursts over time. Assessing MSNA, quantifies directly the efferent sympathetic nerve activity (49, 50). Important advantages are the possibility to evaluate the adrenergic activity continuously and it has a better reproducibility rate than NA levels measurement (51). However, the method is invasive (although safe), complex and laboratory-dependent. From a technical point of view, the RDN procedure is designed to disrupt the renal sympathetic nerves in order to reduce blood pressure. However, there is no virtual test to assess procedural success. An efficacy (procedural) marker could be electrical renal nerve stimulation (52). Whereas

a previous study showed that the electrical stimulation of the renal nerves increased blood pressure (53). The latter response might be helpful to determine the target ablation site and it could also be applicable as an endpoint for the RDN procedure.

Clinical outcome and unsolved questions

Despite current data, no conclusive evidence is available to prove the effectiveness of RDN. Several unsolved issues remain to be addressed. Though, the search towards answers will be complicated by the fact that the exact pathophysiological mechanism of RDN-induced blood pressure reduction is still unknown.
The first issue; the identification of appropriate patients who may benefit the most from the procedure. Subjects in previous studies were included after extensive screening, which resulted in a selective study population. The office blood pressure was used at inclusion. It should be acknowledged that enrolling subjects for complex hypertension trials is a challenge. By screening extensively in a search towards the appropriate patient for undergoing RDN, only a fraction of the population was suitable (41).
Secondly; the evaluation of an (objective) outcome measure to assess efficacy, since blood pressure is a very variable parameter which is influenced by several factors such as stress and lifestyle. To add, blood pressure response varies after RDN, distinguishing 'responders' and 'non-responders' to the therapy. The first clinical trials on RDN already showed that the non-response to therapy varies between 8-37% (30, 36). Non-response was defined as the failure of a specific treatment, roughly it was defined as a reduction of office systolic blood pressure < 10mmHg at 6 months follow-up (54). However, this definition remains arbitrary. The cause for non-response is thought to be multifactorial such as inappropriate patient selection, ineffective RDN procedure and non-adherence (54). Whereas non-adherence to antihypertensive drugs could be an important contributor to highly variable response to RDN therapy (55). Assessment of adherence to antihypertensive drugs should be a requirement at inclusion in a clinical trial, however the question remains which method of assessment should be used. In previous studies, changes in antihypertensive drugs were not adjusted, and finally, assessment of adherence to antihypertensive drugs was not performed consequently (32, 54).
Thirdly, the research field should also focus on developing pre- and post-procedural tests to determine and optimize therapy response. Several methods were described previously to assess the efficacy of RDN (monitoring by measuring NA spillover, MSNA), however more data is needed on direct neural stimulation of renal nerves.
Finally, several studies described some potential cardiovascular outcome after RDN,

however the place of RDN in the treatment of chronic heart failure and atrial fibrillation is debatable (56-58).

Aim of this thesis

In this thesis, we aimed to study the efficacy and safety of RDN in (resistant) hypertension and other sympathomimetic driven cardiovascular disease. **Chapter 2** includes a review on the work-up of patients presenting with uncontrolled hypertension focused on identifying the aetiology of the problem and providing guidance on when secondary invasive treatment options might come in to play.
Part II of this thesis focuses on patient screening and eligibility for RDN. **Chapter 3** acknowledges the complexity of enrolling subjects for complex hypertension trials and reports on the use of social media to recruit study participants for a clinical trial in which device based therapy for hypertension is studied. **Chapter 4** describes the consequences of extensive diagnostic work-up prior to interventional cardiovascular procedures, with a specific focus on incidental findings and their clinical relevance. In **Chapter 5** we assess a novel add on technology for RDN allowing mapping of the renal nerves to identify potential responders to therapy in order to provide immediate feedback on the potential of adequate renal nerve denervation. **Chapter 6** reports on the additive value of several biomarkers to assess the treatment effect of RDN.
Part III of this thesis focuses on drug adherence to antihypertensive medication and the role of measuring drug levels. **Chapter 7** compares two different methods to evaluate adherence to antihypertensive drugs in patients with assumed resistant hypertension. **Chapter 8** and **Chapter 9** describe the clinical and analytical validation of a dried blood spot method to measure antihypertensive drug levels and the clinical implications of measuring antihypertensive drug levels.
Part IV of this thesis focuses on clinical outcome studies on RDN. **Chapter 10** describes the effect of RDN in patients with mild hypertension (multi-center randomised trial). **Chapter 11** includes a case series studying the effect of a redo RDN procedure in patients who were considered non-responders to a first RDN procedure for resistant hypertension. **Chapter 12** describes the effect of RDN on myocardial mechanics. **Chapter 13 and chapter 14** describe the effect of RDN in patients with vasospastic angina. **Chapter 15** describes the effect of RDN on atrial fibrillation burden. **Chapter 16** describes the effect of RDN in patients with chronic heart failure. The epilogue (**Chapter 17**) contains a general discussion of the results described in the present thesis and provides suggestions for further research.

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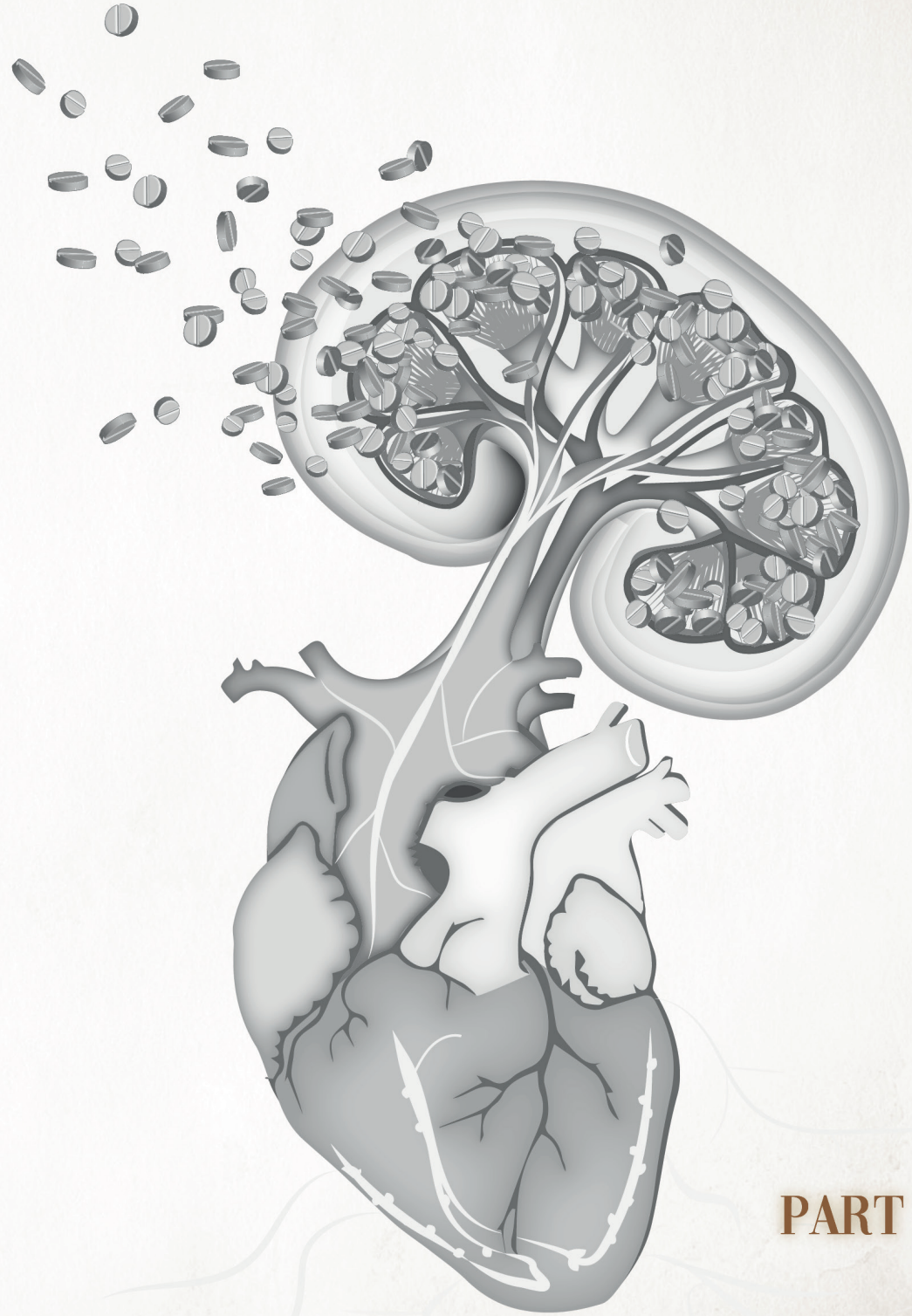
RESISTANT HYPERTENSION

Chapter 2

Diagnostic and (new) therapeutic options in resistant hypertension



PART I

CHAPTER 2

Diagnostic and (new) therapeutic options for resistant hypertension: a short review

Lida Feyz, MD; Laura Peeters, MSc; Joost Daemen, MD, PhD;
Jorie Versmissen, MD, PhD

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Abstract

Hypertension is a major risk factor for ischaemic heart disease and stroke. Despite the availability of numerous pharmacological treatment options, blood pressure (BP) targets are often not achieved. The inability to reach a BP levels below 140/90 mmHg despite the use of three or more antihypertensive drugs is defined as resistant hypertension (RH). The etiology for RH is multifactorial. First, BP should be appropriately measured. In order to improve BP control, lifestyle modification should be recommended, adherence should be carefully assessed to exclude pseudo-resistance, and efforts should be made to exclude secondary causes of hypertension before initiating new drugs or considering device-based treatment strategies. This short review will highlight several aspects of RH management along with a focus on several new treatment options.

Case presentation to introduce the topic

A 55-year-old female with a history of Graves' disease and thyroidectomy was referred due to severe hypertension from which she suffered for over 20 years. The patient reported that several antihypertensive drugs were replaced due to side effects, including perindopril, which was discontinued due to a dry cough and doxazosin, which was stopped due to palpitations. Despite a regimen of valsartan 320 mg once a day (qd), hydrochlorothiazide 25 mg qd, amlodipine 10 mg qd and metoprolol 100 mg qd, her blood pressure (BP) remained uncontrolled. She reported to be fully adherent to all medications. In addition to antihypertensive drugs, she was taking levothyroxine 100 microgram qd. Most of the time when she was late from work, she ordered ready-meals (3-4 times a week). Physical and laboratory examination including 24 hr urine sodium measurement and thyroid function tests revealed, in addition to a severely elevated BP (200/120 mmHg, heart rate 75 beats/min) and a high-salt intake of 170 mmol/24 hrs, no further abnormal findings. Additional workup showed no relevant secondary causes of hypertension.

Twenty-four-hour ambulatory blood pressure monitoring (24h ABPM) confirmed true resistant hypertension with mean daytime BP 145/98 mmHg. Her physician proposed to intensify her antihypertensive drug regimen and offered her dietary support to reduce salt intake. However, the patient was curious about a new technology that she had read about on Facebook, something with heat and nerves that could result in a better blood pressure control: would she be a candidate for this technique?

Definitions

With the recent changes in the European and American hypertension guidelines, blood pressure (BP) targets are lower than ever (1, 2). However, the 2018 European Society of Cardiology/European Society of Hypertension (ESC/ESH) and the 2017 American College of Cardiology/American Heart Association (ACC/AHA) guidelines vary in the recommendations when treatment should be initiated. The ESC/ESH guideline recommends to initiate treatment at an office systolic BP of ≥140 mmHg and/or a diastolic BP ≥90mmHg, with 24h ABPM values, in general, being lower than the office BP values (diagnostic threshold for hypertension is an mean daytime ABPM ≥ 135/85mmHg) (1). The ACC/AHA guidelines advocate an even lower thresholds for commencing treatment (office BP ≥ 130/80mmHg, daytime 24h ABPM >130/80mmHg) (2). These thresholds were based on multiple clinical trials and meta-

analyses, showing the beneficial effects of BP lowering therapy on cardiovascular events (3-7). Regardless of the final target value, Ettehad et al. showed that every 10 mmHg reduction in systolic BP significantly reduced the risk for all major cardiovascular events by 20% and led to a significant 13% reduction in all-cause mortality (3).

BP that remains uncontrolled (>140/90 mmHg) despite the prescription of three antihypertensive drugs (with at least one diuretic) in a maximally tolerated dose is considered "resistant hypertension" (RH) (8). The definition RH was initially established to identify a high-risk patient population that would benefit from more specialized care and specific diagnostic testing (9). However, the definition remains rather nonspecific and could include patients with either true or pseudo- RH. Several factors play a role in persistent uncontrolled hypertension, therefore a stepwise approach is recommended.

Stepwise approach

General clinical characteristics

The American Heart Association emphasizes that RH is a multifactorial problem; individual patient characteristics including lifestyle factors, potential secondary causes of hypertension including drug-related causes, and potential pseudo-resistance should be assessed (9, 10).

Several patients characteristics such as older age, obesity, excessive sodium intake, diabetes, black race, female gender, high baseline systolic BP and target organ damage (chronic kidney disease and left ventricular hypertrophy) proved to be strong predictors for uncontrolled BP (9). Lifestyle factors such as excessive sodium intake and heavy alcohol intake should be discussed and discouraged. Pimenta et al. studied the effect of dietary salt restriction on office and 24h ABPM in patients with RH (11), whereas low versus high-salt diet decreased both office BP (systolic and diastolic decrease of 22.7/9.1mmHg, respectively) and 24h ABPM (20.7/9.6mmHg). Additionally, the degree of BP reduction induced by salt restriction in the cohort with RH was larger than reductions observed in the normotensive cohort. These results demonstrate the importance of salt restriction in patients with RH. A recent published randomized trial showed the effect of lifestyle change and weight management in football fans, in which the lifestyle program helped to improve weight, waist circumference, vitality and significantly reduced diastolic BP at 12 months (mean

difference between intervention and control-group -1.2mmHg (95% CI: -2.1 to -0.4, p=0.004) (12).

Furthermore, several pharmacological agents that could increase BP such as the use of nonsteroidal anti-inflammatory agents, oral contraceptives, sympathomimetic agents (cocaine) should be dissuaded (13). Furthermore, care should be taken in performing accurate BP measurement and general clues that could reveal secondary causes of hypertension should be identified during outpatient clinic follow-up (14). Additionally, non-adherence to prescribed antihypertensive drugs should be assessed (10, 15).

Appropriate BP measurement

Inaccurate BP measurements proved to be a frequent cause of pseudo-resistance (8). Automatic office BP measurement is recommended and BP should be measured in both arms at the first visit. As a rule of thumb, the health care provider should measure BP three times with intervals of at least 1 minute. The average of the last two measurements in the arm with the highest BP value should be used as a reference at follow-up (2, 16). In the most optimal setting BP should be measured unattended (17). Frequent mistakes comprise measuring BP before the patient could sit quietly for a couple of minutes and second, using to small BP cuffs, which will result in falsely high BP measurements (18).

The gold standard, to rule out white coat and masked hypertension, is a 24h ABPM, which should be performed in all patients with suspected RH (16, 19, 20).

Furthermore, home BP assessment proved to be a better predictor for cardiovascular morbidity than office BP (21, 22). When home BP is combined with physician counseling, the adherence to antihypertensive drugs could improve, resulting in better BP control (23-25). Home-BP measurement is recommended when the patient is able to measure his/her BP by an automatic BP monitor.

Exclude secondary causes for hypertension

Secondary causes of hypertension can be found in up to 10% of the cases (26). True RH should be the trigger towards more extensive workup (14). Attention should be paid to symptoms of snoring, daytime sleepiness and morning headache, especially in overweight and obese patients in order to detect obstructive sleep apnoea (OSAS)

which proved to be twice as common in RH as in non-RH patients, with incidences reported to be more than 30% (8, 14, 27). Furthermore, other relatively common causes should be excluded based on clinical and laboratory findings such as renal parenchymal disease (e.g., especially in patients with diabetes or smokers; generalized atherosclerosis; and previously renal failure, for which, screening should be done with kidney ultrasound) and primary aldosteronism (6-23% of RH cases; clinical findings such as muscle weakness could be observed, the patient could complain about fatigue; constipation; polyuria and polydipsia) (Figure 1). Finally, renal artery stenosis is a known secondary cause of RH present in 2-20% of the RH cases and either caused by atherosclerosis or fibromuscular dysplasia. Occurrence of flush pulmonary edema might unravel bilateral renal artery stenosis. The importance of treatment, and in particular renal artery stenting or balloon dilatation, remains debated, but should be considered, especially in patients with rapidly decreasing kidney function or patient with only one functional kidney (28).

More uncommon causes to be ruled out include Cushing's syndrome (<1%), as well as hypo- as hyperthyroidism, coarctation of the aorta (<1%) and phaeochromocytoma (<1%) (14). Of note, while helpful in lowering BP and improving patient prognosis, treatment of secondary causes does not always lead to normalisation of BP and pharmacotherapy remains necessary (29).

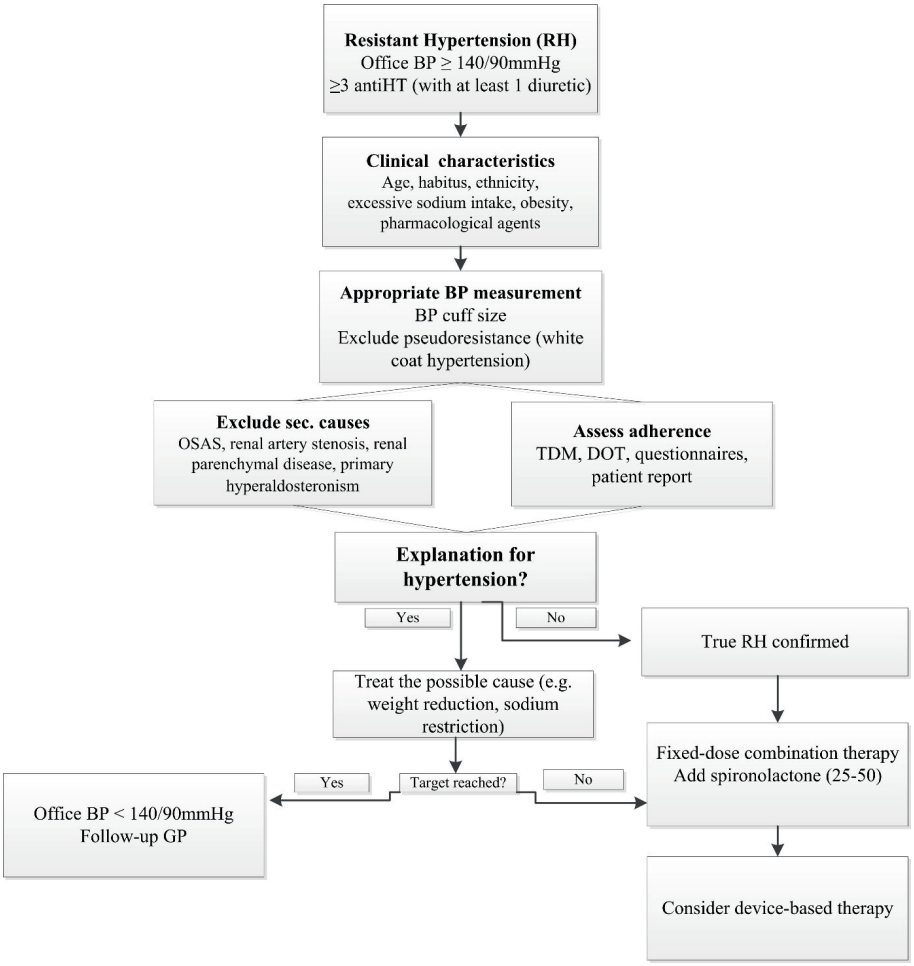

Figure 1. Stepwise approach in patients with RH

*Based on the recent results of the RADIANCE-HTN SOLO and SPYRAL OFF-MED trial, RDN may be considered earlier than mentioned in the flowchart (e.g. patients with a high-risk cardiovascular profile). BP = blood pressure ; DOT = directly observed therapy ; GP = general practitioner ; OSAS = obstructive sleep apnoea syndrome ; RH = resistant hypertension ; TDM = therapeutic drug monitoring

Exclude non-adherence

One of the main reasons of pseudo-RH is non-adherence, with a reported prevalence ranging from 23-66% (30). Non-adherence can mean that a patient is non-adherent to all drugs (full non-adherence) or to a limited number of drugs (partial non-adherence).

As a matter of fact, non-adherence to antihypertensive drugs, as underlying cause is the most difficult to treat cause for RH (31). A crucial step in the management of hypertension and especially in patients with RH, is the assessment of adherence to antihypertensive drugs. Assessing adherence has been recognized to be the first step in improving non-adherence and clinical outcome (32). Scotti et al. showed that an enhancement of adherence from 52% to 60-80% led to a reduction in cardiovascular events from 85 to 83, and 77 events every 10,000 person-year, respectively (32).

Clinicians in general tend to overestimate adherence rate (33). Several methods are available to assess adherence such as pill counts, patient self-reports, directly observed therapy and measurement of drug- or metabolite levels. (34, 35). Direct methods (such as directly observed therapy, electronic-monitoring and therapeutic drug monitoring (TDM) are more accurate and reliable than indirect methods (such as questionnaires or pill-count) (30). Direct methods, however, are more expensive and more labor intensive (30, 34). In more complex cases, a combination of therapeutic drug monitoring (TDM) and directly observed therapy might provide a better understanding and facilitate further counseling on drug adherence (36). Once non-adherence has been objectified, efforts should be made to find the underlying cause to help the patient in finding a solution (37). In a small pilot study, it appeared that using TDM to identify non-adherence, followed by counselling, led to improvement of adherence and BP regulation (38). Currently, the effect of TDM combined with counselling based on finding the underlying cause for non-adherence to improve BP regulation in pseudo- RH is being assessed in the Resistant Hypertension: Measure to Reach Targets (RHYME-RCT) *(trialregister.nl; NTR6914, RHYME-RCT)*.

Treatment options

Pharmacotherapy

The 2018 ESC/ESH guideline recommends the initiation of pharmacologic treatment within the five major antihypertensive classes: ACE-inhibitors, angiotensin II receptor inhibitors (ARBs), calcium- channel blockers (CCBs), diuretics or beta-blockers in which an overview per drug class and possible side effects can be found (16).

When focusing on patients with RH, without finding a secondary cause and after confirming adherence to the prescribed antihypertensive drugs, BP targets can often

still be achieved by optimizing antihypertensive drug regimens (including a fixed-dose combination therapy) (1, 39). As a next step to an ACE-inhibitor or an ARB, a CCB and a diuretic, the addition of spironolactone has shown to be effective in lowering BP (40). In the PATHWAY-2 study, the BP lowering effect of spironolactone (25-50mg) was greater than doxazosin and bisoprolol. New antihypertensive drugs, which are being studied, mostly targeting the renin-angiotensin-aldosterone system (RAAS), such as aldosterone synthase inhibitors or non-steroidal mineralocorticoid receptor blockers, with the aim to reduce the anti-androgenic side effects (41). Although vaccines targeting RAAS components were considered as promising treatment options to avoid adherence issues, pharmacokinetic and pharmacodynamics issues hampered further development (42). A promising treatment option to date, is the combination of angiotensin II receptor blockade and neprilysin inhibitor (ARNI), currently registered for heart failure (41, 43). ARNIs showed significantly greater reductions in BP as compared with an ARB alone and have been proven safe and well-tolerated.

Device-based therapy

Despite currently available interventions targeting lifestyle and pharmacotherapy including drug adherence, it is often not possible to reach the target BP. A recent study by Patel et al. reported that when causes for pseudo-resistance including non-adherence were excluded, 15% of patients with RH may be eligible for device-based therapies for hypertension. Moreover, recent studies showed that patients would prefer device-based therapy that may diminish the need for more antihypertensive drugs. Hutchins et al. showed that approximately one out of three respondents would be willing to trade two years of their life to avoid taking drugs. Additionally, respondents were willing to pay an average of $1445 to avoid medication daily (44). At present, several device-based therapies have been studied to control BP as an alternative or add-on therapy in patients with RH, by primarily targeting the autonomic nervous system (45).

Renal denervation

The most studied device-based therapy to date is renal sympathetic denervation (RDN). The percutaneous treatment targets renal sympathetic nerves at the renal artery level and demonstrated reduction of sympathetic overactivity (46). Although several promising trials have been published, the treatment faced a significant decline in enthusiasm following the neutral results of the first sham-controlled RDN trial, the

Symplicity HTN-3 trial in 2014 (47-49). Dissecting the trial design identified several factors that could potentially have led to the failure of the trial of meeting its primary endpoint (50). As such, inadequate screening, frequent changes in antihypertensive regimens and the use of a first-generation RDN device along with a lack of operator experience were suggested to be responsible (51-53). Subsequently, three proof-of-principle studies with more advanced trial designs, proved the overall efficacy and safety of the technique (54-57). Both RDN techniques used in these studies seem efficacious in achieving a significant drop in blood pressure of approximately 6mmHg as compared to a sham comparator arm at 2 to 3 months. The body of evidence supporting the efficacy of the treatment in patients with RH is steadily increasing. Given the positive data available thus far, referring patients with true RH to specialized centers participating in dedicated RDN trials should be strongly encouraged.

Several studies on BP efficacy and safety of renal sympathetic denervation, with strict entry criteria, are ongoing *(RADIANCE II Pivotal; TRIO; NCT03614260; NCT02649426; REQUIRE study NCT02918305).*

Barostim Neo system

Carotid baroreflex activation therapy a relative new surgical implantable device which stimulates the carotid baroreceptors and therefore down-regulates the sympathetic outflow with an increase in parasympathetic tone (58, 59). The first sham-controlled studies in patients receiving bilateral implants showed significant BP reduction with a clear on/off phenomenon (58, 60). A recently published study on the safety profile and efficacy of a second generation of the device, the Barostim neo, showed that side effects such as syncope, hypertensive crisis and arrhythmias occurred in 28% of the patients. A significant BP drop was seen at 6 months in patients treated with Barostim neo (from a mean of 169 ± 27 to 148 ± 29mmHg, $p<0.001$) and 1 year (a further decrease to 145 ± 24mmHg as compared with baseline, $p<0.001$) follow-up with a significant decrease in prescribed antihypertensive drugs (59). However, due to current lack of randomized controlled trials, the side-effects, the high-costs and the need for frequent battery replacement, there was a quest for alternative methods to stimulate the baroreflex and lower the BP (61, 62).

Mobius HD system

Carotid baroreceptor amplification therapy, the Mobius HD system, is an endovascular carotid implant of which the mechanism of action is based on passive activation of the baroreceptor by irreversibly changing the carotid sinus shape, resulting in pulsatile wall stretch and a linear increase in firing rate when the BP increases (62). The latter phenomenon suppresses the sympathetic outflow and consequently decreases the BP. The CALM-FIM study showed that the Mobius HD device significantly reduced BP (by 21/12 mm Hg at 6 months, $p<0.001$)., however this was a non-randomized, open-label study (63). Two patients developed neurological symptoms after implantation. CALM-2 study is now enrolling to further study the efficacy and safety *(ClinicalTrials.gov NTC03179800)*.

Other non-pharmacological interventions

A number of non-pharmacological therapies are in the pipeline at different stages of development, such as the alcohol-mediated perivascular renal denervation with the Peregrine System™ (Ablative Solutions) (64) and the ROX AV coupler (ROX Medical) which creates an arteriovenous (AV) anastomosis between the external iliac artery and vein to reduce vascular resistance and the effective arterial volume, that could immediately result in significant reductions of BP (65). Several studies are ongoing to evaluate their safety and efficacy further (Peregrine system, *TARGET BP OFF-MED trial, NCT03503773*) ; ROX coupler (*CONTROL HTN-2 NCT02895386)*.
In general, based on the recent 2018 ESC/ESH guideline, device-based therapy for hypertension is not recommended for the routine treatment of hypertension, unless performed within the context of a clinical (randomized) trial. The results of currently ongoing larger clinical trials will provide more details on the safety and efficacy of the technology (1).

Back to the case

Despite a substantial decrease in urine sodium excretion from 170 to 130mmol/24h, blood pressure remained uncontrolled. Further work-up of our patient included drug adherence testing using TDM in which we measured the drug levels by venous sampling. After confirming full- adherence, spironolactone was added to her drug regimen resulting in daytime average 24h ABPM of 141/90mmHg (mean office BP 165/100mmHg). Due to a lack of response to the therapy above, the patient was enrolled in a (double-blind) randomized controlled trial to assess the efficacy and safety of RDN in patients with RH.

Conclusion

The management of RH should contain advise on lifestyle modifications including the reduction of sodium intake, and accurate BP measurement, preferably by 24h ABPM, to rule out pseudo-RH. Additionally, assessment for secondary causes of hypertension should be considered and non-adherence to prescribed antihypertensive drugs should be ruled out. To note, adding a mineralocorticoid receptor blocker to the existing antihypertensive regimen could lead to an additional BP drop, also in essential hypertension. New drug- and device-based treatment options have been studied extensively over the past years, with promising results in the general hypertensive population. More evidence is warranted in order to determine the clinical relevance and cost-effectiveness of device-based therapies as compared with (existing) pharmacological treatment options.

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CHAPTER 3

Using social media to recruit study participants for a randomised trial for hypertension

Lida Feyz, MD; Yale Wang, MD; Atul Pathak MD, PhD;
Manish Saxena, MBBS, MSc; Felix Mahfoud, MD; Kintur Sanghvi, MD;
Rose Peterson; Ajay J. Kirtane, MD, SM; Michel Azizi, MD, PhD;
Mano Iyer; Joost Daemen, MD, PhD

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Abstract

Aims The present study aimed to evaluate the potential of social media as an approach to recruit hypertensive subjects.

Methods and results In addition to conventional trial recruitment, Facebook ads were run. Over a 115-day recruitment period, Facebook reached 5.3 million people in 168 separate campaigns run in the proximity of 19 sites in the US and 14 sites in Europe. A total of 182 839 participants (3.4%) clicked on the ad; of those 10 483 subjects (5.7%) completed a dedicated questionnaire. This resulted in 3632 potential candidates. A total of 285 potential candidates were recruited by various recruitment strategies in the specified time period, of which 184/285 (64.6%) came from Facebook. When comparing Facebook with a 7-day radio spot in the same time period, 48 radio spots were launched; resulting in nine inquiries with eventually five potential candidates and two consents.

Conclusion Targeted social media was a successful and efficient strategy to recruit hypertensive subjects.

Introduction

Great and costly efforts are required to recruit potential participants into clinical trials. Using social media may make the recruitment process more efficient. Merely 20% of clinical trials are completed on time, a finding mostly linked to challenges in patient recruitment (1). Recruitment through social media is increasingly being recognized as a tool to efficiently identify eligible subjects at lower costs (2, 3). One of the key reasons for its success is the strong adherence of users to specific social media platforms. Facebook for instance has over 2.38 billion active monthly users of which about 75% access the network on a daily basis (4). As such, the platform and other like it offer great potential to quickly and affordably enroll patients into clinical trials and surveys (3, 5-7).
At present, little evidence is available on the efficacy of using social media to recruit patients into cardiovascular and hypertension trials (8). The aim of the present study was to evaluate the efficacy of social media as an approach to recruit hypertensive patients into the RADIANCE-HTN SOLO trial.

Methods

The RADIANCE-HTN SOLO (*NCT02649426*) is a multicenter, randomised study that was designed to demonstrate the efficacy and safety of endovascular ultrasound renal denervation (RDN) to reduce ambulatory blood pressure at 2 months in patients with combined systolic–diastolic hypertension in the absence of medications. Between 28 March 28 2016 and 28 Dec 2017, 803 patients were screened for eligibility and 146 were randomised to undergo RDN (n=74) or a sham procedure (n=72) (9). Key entry criteria included: age 18-75 years with essential hypertension using 0-2 antihypertensive drugs. Patients were recruited from 21 hospitals in the USA and 18 hospitals in Europe. The study was approved by local ethics committees or institutional review boards and was performed in accordance with the declaration of Helsinki. All participants provided written informed consent. All recruitment materials including social media campaigns was approved by local ethics committees of the involved sites.

Recruitment strategies included social media (Facebook), conventional advertisements (ads) (magazine, brochure/poster, radio, newspaper), web search (the clinical website, craigslist and web-browsing), and physician referral. Both newspaper ads and posters contained brief information about study entry criteria. Newspapers were distributed at public transport places and posters were displayed in outpatient cardiology and

hypertension clinics. Radio ads were run for 30 or 60 seconds providing a short summary of the study, entry criteria and contact information. Ads were run in major metropolitan areas on radio stations with large adult listener bases during popular days and times.

Facebook ads were targeted towards subjects >45 years old within a certain distance from a recruitment site (range 20-50 miles). Criteria were modified over time in order to increase response rates [i.e. distance was increased or decreased, age was increased to >55 year]. Facebook ads referred to a dedicated study website translated into country specific languages. If interested, subjects could complete an anonymous online screening questionnaire which provided direct automatic feedback on study eligibility. The questionnaire included questions such as age, blood pressure, number of antihypertensive drugs, diabetes (yes/no), stroke (yes/no), willing to consider a minimally invasive procedure (yes/no), pregnancy, involvement in other trials (Supplementary material online). Eligible subjects were asked to provide contact details (name and telephone number) to receive additional information, a process coordinated via a secure online portal (*Galen Gateway Patient Recruitment Portal, Galen Patient Recruitment, Inc., Cumberland, RI*). Study site were only able to contact potential candidates within their area. The study sponsor was not able to access any personal data. Trained local site personnel or contracted secondary screeners contacted candidates by phone to verify eligibility and answer potential questions. A subsequent outpatient clinic visit was scheduled during which the study was explained in greater detail and the informed consent form could be signed.

Statistical analysis

Categorical variables were expressed as percentages and counts. Continuous variables were described as mean ± standard deviation (SD) when normally distributed, data was compared using an Independent-Samples or Paired-Samples T test to analyze the difference between recruitment methods. In case of non-normal distribution, median data was presented with the interquartile range [IQR]. All statistical tests are 2-tailed. A *P*-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS statistical analysis (version 24.0).

Results

Facebook ads were active during a 115-day recruitment period between August and November 2017. A total of 285 potential candidates were recruited by different recruitment strategies in this specific time period, of which 184 (65%) were consented through Facebook (Table 1). The average age of the subjects consented through Facebook was 59 ± 8 years and 51% were male (Table 2).

Table 1 Screened individuals by recruitment strategy

Recruitment strategy		**N (%)**
	Total	**285**
Social media	Facebook	184 (64.5)
Traditional media	Newspaper	26 (9.1)
	Poster	2 (0.7)
	Radio	2 (0.7)
	Web search	8 (2.8)
	Internal referral	3 (1.1)
	Word of mouth	6 (2.1)
	Physician referral	44 (15.4)
	Other*	10 (3.5)

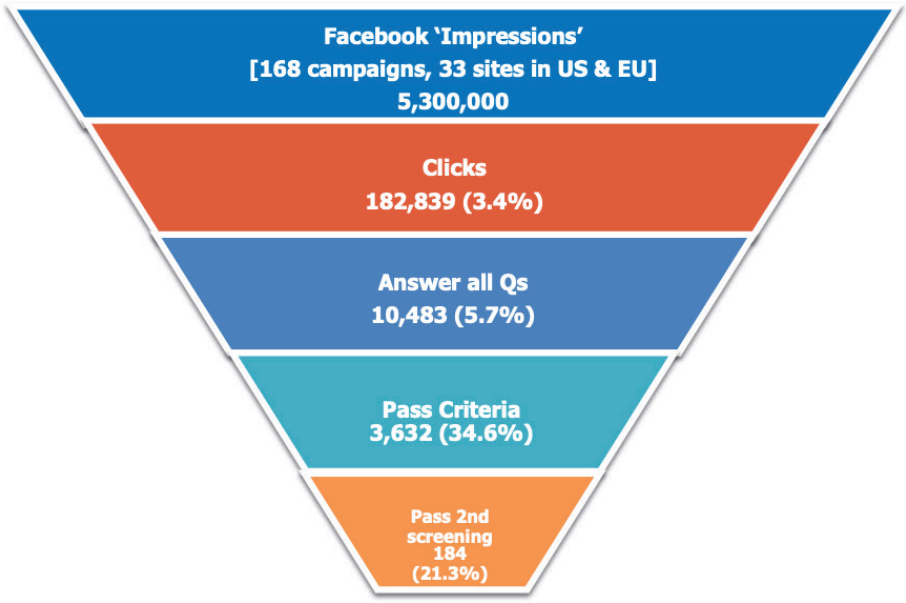
*=clinical website, clinicaltrials.org, craigslist

Table 2 Baseline characteristics

	Total population recruited N=285	**Population recruited via Facebook N=184**	**Population recruited via other methods N=101**	**p-value**
Age, years	57 ± 10	59 ± 8	54 ± 12	<0.001
Male gender, (%)	151 (53)	93 (50.5)	58 (57.4)	0.267
BMI, kg/m^2	30.6 ± 5.9	30.8 ± 6.2	30 ± 5.4	0.273
Caucasian, n (%)	226 (79.3)	151 (82.1)	75 (74.3)	0.004
Systolic office BP, mmHg	141 ± 17	141 ± 16	141 ± 20	0.739
Diastolic office BP, mmHg	91 ± 11	91 ± 11	91 ± 12	0.572
Mean number of antihypertensive drugs	1.3 ± 0.7	1.3 ± 0.7	1.2 ± 0.8	0.160

Facebook reached 5.3 million people in 168 separate campaigns run in proximity to 19 sites in the US and 14 sites in Europe. The number of candidates per site was variable with a median of 23 [17 – 26] candidates per site that passed the questionnaire (Figure 1). A total of 27/184 subjects were eventually randomised.

Figure 1 Recruitment funnel



Total cost for the Facebook ads was $152,412; costing $907/campaign and $0.83/click. This resulted in a total cost of $828/consent. During the same recruitment period, 7-day radio spots were launched with a total cost of $2,870; resulting in nine inquiries with eventually five potential candidates and two consents ($1,435/consent).

Discussion

The use of targeted social media through Facebook was an efficient strategy to find candidates for a prospective randomised hypertension trial. Our findings add to previous studies assessing the efficacy of Facebook campaigns to recruit participants into an observational blood pressure trial by Nash et al. in Australia and Tasmania,

in which the authors concluded that Facebook ads were associated with a significant increase in the recruited number of patients/month (8). In the latter study, an increase in the recruited number was found to be strongly location dependent with a higher yield in densely populated regions. The average age of the potential study candidates recruited by using Facebook in the present study was significantly higher as compared to candidates recruited by conventional methods. A finding that can be explained by the higher overall age of Facebook users. The latter was also in line with the study of Nash et al., in which Facebook was successful in recruiting a cohort of patients at higher age (8). According to the data of the Pew Research Center in 2018, more older people use Facebook and Youtube whereas younger people use more Instagram (4).

In the present study, Facebook ads generated an overwhelming amount of interest in a trial on a novel device-based treatment option for hypertension. Using an online screening portal in RADIANCE-HTN SOLO allowed to automatically exclude over 65% of subjects prior to consent. Perhaps one of the features contributing to successful recruitment efforts in this study was including the ability to filter patients using both the online questionnaire and secondary screeners. The need for an additional online screening portal as a filter between Facebook clicks and study referral should be put into perspective to strict rules and regulations limiting the amount of information to be provided in the add itself and the complexity of the entry criteria of the associated study. The low percentage of patients that were finally enrolled in the trial was determined by another set of stringent (clinical) entry criteria for the RADIANCE SOLO trial that could not be ruled out by online questionnaires.

Finally, Facebook appeared to be less expensive than radio ads, however with the present available data and the small sample size of responders to radio ads, a reliable cost-effectiveness comparison could not be performed.

Limitation

Our study has several limitations. First, social media could only be compared with the radio spots in the same time period relative to cost because robust data on costs of other conventional ads were not available. Second, a large number of potential candidates passed the online questionnaire (via Galen), however only a small percentage of these candidates were finally randomised, likely due to an inability to be reached for follow-up screening or changing their minds on their availability and interest in participating in the study. Finally, by using Facebook, we were selecting a certain type of patients (e.g. who have access to internet, middle aged participants).

Conclusion

Targeted social media was a successful and efficient strategy to find potential candidates for a multicenter blood pressure clinical trial. Whether this approach can be replicated across other disease states or demographics remains to be studied.

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CHAPTER 4

Prevalence and consequences of noncardiac incidental findings on preprocedural imaging in the workup for transcatheter aortic valve implantation, renal sympathetic denervation, or MitraClip implantation

Lida Feyz, MD ; Nahid El Faquir, MD;
Miguel E. Lemmert, MD, PhD; Karan Ramdat Misier, BSc;
Laurens J.C. van Zandvoort, BSc; Ricardo P.J. Budde, MD, PhD;
Eric Boersma, PhD; Felix Zijlstra, MD, PhD; Peter de Jaegere, MD,
PhD; Nicolas M. Van Mieghem, MD, PhD; Joost Daemen, MD, PhD

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Abstract

Introduction Dedicated data on the prevalence of incidental findings (IF) stratified according to overall clinical relevance and their subsequent correlation to outcome is lacking. The aim of the present study was to describe the prevalence and consequences of non-cardiac IF on computed tomography (CT) or magnetic resonance imaging (MRI) in the workup for interventional cardiovascular procedures.

Methods A total of 916 patients underwent pre-procedural CT or MRI in the workup for transcatheter aortic valve implantation (TAVI), renal sympathetic denervation (RDN) or MitraClip implantation.

Results IF were found in 395/916 patients (43.1%) with an average of 1.8 IF per/patient. Classifying the IF resulted in 155 patients with minor-, 171 patients with moderate- and 69 patients with major IF. The intended procedure was delayed or cancelled in only 15/916 (1.6%) of the patients, due to the presence of potential malignant IF. In patients that did undergo the intended procedure (n=774), the presence of a moderate or major IF (23.8%) did not impact 1-year mortality compared to no or minor IF (adjusted HR 0.90; 95% CI 0.56 – 1.44; p-value 0.65). These findings were consistent among patients referred for either TAVI, RDN or MitraClip.

Conclusions IF are frequent in patients referred for cardiovascular procedures. IF did not result in a delay or cancellation of the intended procedure in the vast majority of cases, irrespective of their clinical relevance. The presence of a major or moderate IF did not significantly impact 1-year mortality.

Introduction

The number of incidental findings (IF) detected by diagnostic imaging modalities used for screening patients with a variety of cardiovascular diseases rapidly increased during the past two decades. This has been explained by an expanding number of percutaneous treatment options, in which detailed pre-procedural radiological assessment using high resolution computed tomography (CT) or magnetic resonance imaging (MRI) is of paramount importance to guarantee optimal treatment results (1, 2). While IF have been reported in 25 to 85% of patients referred for cardiovascular interventions their clinical implications most often remain elusive (3-6). Thereby, the definition of (clinically relevant) IF remains a topic of debate, explaining the large heterogeneity in reported prevalences (7, 8). At present, dedicated data on the prevalence of IF stratified according to overall clinical relevance and their subsequent correlation to outcome is lacking. The aim of the present study is to describe the prevalence and consequences of non-cardiac IF on CT or MRI in the work-up for transcatheter aortic valve implantation (TAVI), renal sympathetic denervation (RDN) or MitraClip implantation.

Methods

Study population

Between May 2009 and December 2016, a total of 1194 patients were referred for TAVI (n=1060), RDN (n=110) or MitraClip (n=24) implantation. A total of 278 patients were excluded due to either mortality in the screening process before radiological evaluation, or a clinical decision not to proceed with screening for the respective procedure. Eventually, 916 patients with pre-procedural workup including CT (n=869) or MRI (n=47) were included in the final analyses, of which 85.4% were screened for TAVI, 12.0% were screened for RDN and 2.6% were screened for MitraClip implantation. Following the completion of the diagnostic work-up and in accordance with European Society of Cardiology guidelines (9), all patients referred for TAVI and MitraClip implantation were discussed in a multi-disciplinary team including interventional cardiologists, cardiothoracic surgeons, anesthesiologists and geriatricians. Patients undergoing RDN in the context of treatment resistant hypertension, atrial fibrillation and heart failure with reduced ejection fraction were screened according to recent recommendations (10). A total of 142 patients did not undergo the procedure for which they were initially referred (Figure 1).

For the purpose of this study patients were not subject to study interventions, neither was any mode of behavior imposed, otherwise than as part of their regular treatment. Therefore according to Dutch law, written informed consent for a patient to be enrolled in this study was not required. This study was conducted according to the privacy policy of the Erasmus MC and to the Erasmus MC regulations for the appropriate use of data in patient orientated research, which are based on international regulations, including the declaration of Helsinki. All patients consented to the use of their data for scientific research. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper and its final contents.

Definition and methodology

IF were defined as any radiological abnormality not related to the illness or causes that prompted the diagnostic imaging test. IF were stratified according to clinical importance as being either minor (eg. cystes or osteoarthritis), moderate (eg. pulmonary nodules or adrenal adenomas) or major (eg. aneurysms, lymphadenopathy according to a recently proposed classification described by Lumbreras et al (8)). In case multiple IF were found, the patient was categorized in highest ranked cohort (major > moderate > minor) for further analysis. The impact of the presence of moderate or major IF on outcome 1 year mortality was based on the cohort of 789/916 patients that either underwent the procedure or were deferred due to the presence of IF; excluding the 127 patients that did not undergo the intended procedure due to non IF related issues (frailty, vascular access site issues etc.).

Data extraction and follow-up

Data on IF were acquired by evaluating radiologic findings from pre-procedural CT or MRI by specialized (cardiovascular) radiologists.

Patients were evaluated with CT prior to TAVI in order to achieve appropriate valve sizing and to evaluate the best access pathway before the procedure. The scan protocol was made up of three scans on a second or third generation Dual Source CT scanner (Siemens Healthineers, Forchheim, Germany) for the analysis of the peripheral access (from the extracranial carotids up to the femoral bifurcation) and aortic valve assessment. The valve sizing scan was reconstructed at 0.75 mm slice thickness and 0.4mm increment at every 5% of the RR-interval of the available data. Peripheral

artery access was analyzed on 1mm slices with 0.5mm increment data.

Patients were evaluated with CT prior to MitraClip when echocardiographic findings were in question, a diagnostic CT-angiography scan was performed of the aorta, heart and proximal peripheral access vessels on a dual source CT scanner (Siemens Healthineers, Forchheim, Germany). The scan was performed in dual source flash mode in order to obtain maximum temporal resolution and thereby minimizing motion artefacts of the heart. Scan timing was set to 8 seconds after contrast arrival in the ascending aorta and the systolic phase of the RR interval. Evaluation was performed on data which was reconstructed at ≤0.75 mm slices with 30-50% slice overlap.

Pre-procedural imaging for RDN includes an abdominal (including arterial phase) CT or MR to confirm anatomical suitability and as a part of an evaluation for secondary causes of hypertension. The MR scan protocol prior RDN was performed on a 1.5T scanner (Discovery MR450, GE Medical systems, Milwaukee, Wisconsin USA). A 3D Vasc Fast TOF Spoiled Gradient Echo sequence was used to acquire images of the renal vasculature. To determine the arterial scan-delay for the CEMRA scan, a test-bolus sequence was acquired. Images were acquired during a 20 second- breath hold, depending on the number of slices per slab. A MR compatible contrast injector (Medrad Spectris, Warrendale, Pennsylvania, USA) was used to inject gadobutrol (Gadovist 1.0mmol/ml, Bayer, Mijdrecht, the Netherlands).

In case of an abdominal CT for RDN, a standard diagnostic abdominal CT-angiography was performed on a multi-slice CT scanner of minimal 128 slices (Somatom AS+, Edge, Drive or Force, Siemens Healthineers, Forchheim, Germany). The scan covered the diaphragm trough lesser trochanters. Evaluation was performed on data which was reconstructed at ≤0.6 mm slices with 30-50% slice overlap.

Baseline characteristics of all patients were obtained from local procedural databases and additional chart review in case of missing data. Survival information was obtained through chart review and contact with the municipal civil registries. Median follow-up period post-procedure was 424 days [131 – 830].

Statistical analysis

Continuous variables are presented as mean ± standard deviation. Categorical variables are expressed as percentages. Continuous variables were compared using

Student's t test or one- way ANOVA in patients with versus without IF. Comparisons among the three groups (major, moderate, minor) were performed by the F-test from an analysis of variance for continuous variables and Pearson's Chi-Square test for categorical variables. All statistical tests are 2-tailed. The incidence of mortality over time was studied with the use of the Kaplan-Meier method, whereas log-rank tests were applied to evaluate differences between the groups. Patients lost to follow-up were considered at risk until the date of last contact, at which point they were censored. Cox proportional-hazards regression analyses were applied to adjust for potential confounders. Baseline variables with $p < 0.10$ in univariate analyses were entered in a multivariable Cox proportional hazards models. Final results are presented as adjusted hazard ratios with 95% confidence interval. Variables with $p < 0.05$ were considered as statistical significant. Statistical analyses were performed using SPSS (version 21.0).

Results

Prevalence and types of IF

Mean age of the total population was 78 ± 10 years, 53.8% of the patients were male and 18.4% had a history of neoplasms. IF were found in 395 of the 916 patients (43.1%). No relevant differences were observed in the baseline characteristics of patients with versus without IF on pre-procedural imaging (Table 1).

Table 1. Baseline characteristics of the total study population.

	All patients N=916	**Patients with IF** N=395	**Patients without IF** N=521	**p-value** (with vs.)
Age, years	78 ± 10	78 ± 10	77 ± 11	0.13
Male n, (%)	493 (53.8)	216 (54.7)	277 (53.2)	0.65
BMI, kg/m^2	27.4 ± 8.1	27.6 ± 10.8	27.2 ± 5.2	0.53
CV risk factors n, (%)				
Diabetes	268 (29.3)	118 (29.9)	150 (28.8)	0.92
Hypertension	701 (76.5)	290 (73.4)	411 (78.9)	0.13
Dyslipidemia	553 (60.4)	232 (58.7)	321 (61.6)	0.64
Smoker, current	73 (8.0)	27 (6.8)	46 (8.8)	0.04
Prior MI, n (%)	209 (22.8)	82 (20.8)	127 (24.4)	0.17
Prior PCI, n (%)	304 (33.2)	115 (29.1)	189 (36.3)	0.04
Prior CVA, n (%)	93 (10.2)	42 (10.6)	51 (9.8)	0.24
eGFR, mL/min	52 ± 26	52 ± 28	52 ± 25	0.99
COPD	192 (21.0)	80 (20.3)	112 (21.5)	0.23
Neoplasm in history,n (%)	168 (18.4)	76 (19.3)	92 (17.7)	0.61
Breast cancer	29 (3.2)	12 (3.0)	17 (3.3)	
Colorectal cancer	25 (2.7)	12 (3.0)	13 (2.5)	
Prostate cancer	23 (2.5)	11 (2.8)	12 (2.3)	
Skin cancer	20 (2.2)	10 (2.5)	10 (1.9)	
Non-Hodgkin lymph.	12 (1.3)	6 (1.5)	6 (1.2)	
Hodgkin's lymph.	8 (0.9)	6 (1.5)	11 (2.1)	
Bladder cancer	11 (1.2)	-	6 (1.2)	
Lung cancer	10 (1.1)	4 (1.0)	4 (0.8)	
Gyn. cancer	8 (0.9)	4 (1.0)	2 (0.4)	
Laryngeal cancer	5 (0.5)	2 (0.5)	3 (0.6)	
Leukemia	4 (0.4)	3 (0.8)	1 (0.2)	
Neuroendo. cancer	4 (0.4)	1 (0.3)	3 (0.6)	
Multiple Myeloma	3 (0.3)	2 (0.5)	1 (0.2)	
Esophageal cancer	2 (0.2)	2 (0.5)	-	
RCC	2 (0.2)	2 (0.5)	-	
Vestibular Schwannoma	2 (0.2)	-	2 (0.4)	

Values are mean ± SD or n (%). BMI=body mass index, CV=cardiovascular, CVA= cerebrovascular accident, eGFR= estimated glomerular filtration rate, Gyn.cancer= Gynaecological cancer, IF=incidental findings, MI=myocardial infarction, (non)Hodgkin-lymph.=(non)Hodgkin lymphoma, Neuroendo. cancer=neuroendocrine cancer, PCI=percutaneous coronary intervention, RCC= renal cell carcinoma.

The total number of IF was 698, resulting in an average of 1.8 IF/patient with IF in the overall cohort. The number of IF/patient varied from 1.8 (range 1-5) in the TAVI cohort to 1.4 IF/patient in the RDN (range 1-3) and to 1.3 in the MitraClip (range 1-2) cohort per IF patient respectively.

The 3 most frequent IF were renal cysts, pulmonary nodules and pulmonary consolidations in 16.3%, 13.3% and 6.6% of the patients with IF, respectively (Table 2). Classifying the IF resulted in 155 patients (16.9%) with minor findings, 171 (18.7%) with moderate findings and 69 patients (7.5%) with major IF. No significant differences in baseline characteristics were found between patients identified with either class of IF (Supplement, Table S1). The total number of moderate or major IF was 476, resulting in an average of 2.0 IF/patient with moderate or major IF (range 1-5). The number of moderate or major IF/patient varied from 2.0 (range 1-5) in the TAVI cohort to 1.7 IF/patient in the RDN (range 1-3) and to 1.3 in the MitraClip (range 1-2) cohort per IF patient respectively.

Table 2. Specification of IF per category (n=698)

Organ system	Major (90)	Moderate (287)	Minor (321)
Head-chest	Laryngeal mass (2)	Thyroid IF (39)	
Vascular	Aortic aneurysm (22)	Abdominal aortic ectasia (2) Hepatic haemangioma (16)	R. artery stenosis (31) FMD (3) MVD (24) Carotid disease (29)
Reticuloendothelial	Lymphadenopathy (43)	Splenomegaly (1)	Splenic cyst (2)
Thoracic cavity	Pulmonary mass (7) Pulmonary embolism (2)	Pulmonary nodules (93) Pulmonary consolidation (46)	Pleural plaques (32)
Hepatobiliary		Pancreatitis (2) Pancreatic calcification (1) Liver cirrhosis (2)	Cholelithiasis (26) Pancreatic cyst (6) Hepatic steatosis (7) Liver cyst (40)
Peritoneal cavity	Renal mass (9) Pelvic mass (3)	Adrenal adenoma (15) Adrenal mass (benign) (23)	Renal cyst (114) Adrenal cyst (1)
Gastrointestinal tract		Diverticulosis (44)	
Gynaecological	Breast mass (2)	Uterine enlargement (3)	Ovarian cyst (4) Breast cyst (2)

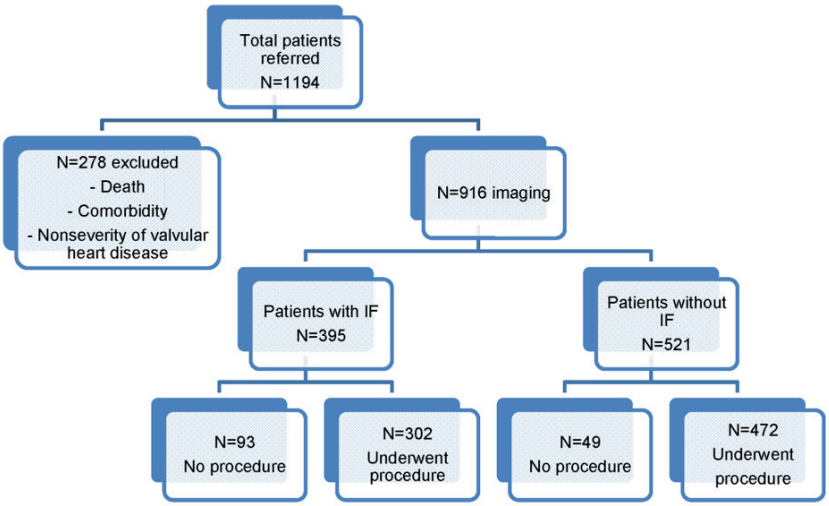
FMD= Fibromuscular disease, MVD=mesenteric vascular disease, R.artery disease= renal artery disease.

In the 395 patients with IF, active malignancies were found in 15 cases (3.8%). Malignancies included lung carcinoma (n=8), breast cancer (n=3), bladder cancer (n=1), renal carcinoma (n=1), non-Hodgkin lymphoma (n=1) and Multiple Myeloma (n=1). In patients with a history of neoplasms (n=168), IF were present in 76 cases (45%) and proved to be malignant in 7 cases (4.2%).

Consequences

A total of 142 patients out of the initially screened cohort of 916 patients eventually did not undergo the procedure for which they were initially referred (Figure 1).
Procedures were delayed (n=7) or cancelled (n=8) in 15/916 cases (1.6%) due to the presence of IF (breast cancer; lymphoma; renal cell carcinoma; lung cancer; bladder cancer). Mean delay was 130 ± 120 days. Procedures did not take place in the remaining cases due to either a variety of non-IF related issues (comorbidity, vascular access issues, renal anatomy not compatible for current intravascular treatment options for RDN, non-severe valvular heart disease) or death while waiting for the intended procedure.

Figure 1 Total patients referred for TAVI, RDN and MitraClip implantation



Impact on mortality

Unadjusted cumulative 1-year mortality was comparable in patients (N=789) with versus those without IF (14.9% vs. 16.1%; log-rank p=0.82). Unadjusted cumulative 1-year mortality rates were 14.8% in patients with no or minor IF and 18.5% in patients with moderate or major IF (p=0.25).

In patients that eventually underwent the procedure (n=774), 1-year mortality rates were identical in patients with versus without moderate or major IF (14.3% vs. 14.8% respectively; log rank p=0.87); adjusted HR 0.90; 95% CI 0.56 – 1.44; p=0.65) (Figure 2). Renal insufficiency and COPD at baseline appeared to be the sole independent predictors of 1-year mortality (Table 3). Finally, also the total number of moderate or major IF was not a predictor for 1-year mortality (adjusted HR 0.91; 95% CI 0.73 – 1.14; p=0.41).

Table 3. Multivariate predictors of 1-year mortality in patients who underwent the intended procedure (N=774**)**

Variable	Unadjusted HR HR (95% CI; p-value)	Adjusted HR HR (95% CI; p-value)
Age	1.00 (0.98 – 1.02; 0.93)	
Female sex	0.89 (0.60 – 1.33; 0.57)	
Hypertension	0.72 (0.46 – 1.13; 0.15)	
Diabetes Mellitus	1.28 (0.85 – 1.93; 0.24)	
Dyslipidemia	0.68 (0.46 – 1.00; 0.05)	0.69 (0.46 – 1.02; 0.06)
Smoking	1.00 (1.00 – 1.01; 0.30)	
Prior PCI	0.85 (0.56 – 1.30; 0.45)	
Prior MI	1.36 (0.89 – 2.06; 0.16)	
Prior CVA	1.46 (0.85 – 2.49; 0.17)	
COPD	1.67 (1.12 – 2.48; 0.01)	1.58 (1.06 – 2.35; 0.023)
Malignancy in history	0.95 (0.67 – 1.38; 0.79)	
Renal insufficiency	1.73 (1.06 – 2.83; 0.03)	1.65 (1.01 – 2.70; 0.046)
Moderate- Major IF	0.96 (0.60 – 1.54; 0.87)	0.90 (0.56 – 1.44; 0.65)

PCI=percutaneous coronary intervention, MI=myocardial infarction, CVA= cerebrovascular accident, IF=incidental finding.

In the cohort of patients that did not undergo the intended procedure (n=142), 1-year mortality rates were numerically higher in the cohort with moderate or major IF (57.9%) versus the cohort with no or minor IF (31.1%) (adjusted HR 1.51; 95% CI 0.81 – 2.82; p=0.20) (Figure 3). Renal insufficiency appeared to be the sole independent predictor of 1-year mortality (Table 4). Also in this cohort, the total number of moderate or major IF was not a predictor for 1-year mortality (adjusted HR 1.14; 95% CI 0.89 – 1.46; p=0.29).

Figure 2 Cumulative one-year mortality in patients that underwent the intended procedure (n=774) stratified according to the presence of a moderate or major IF

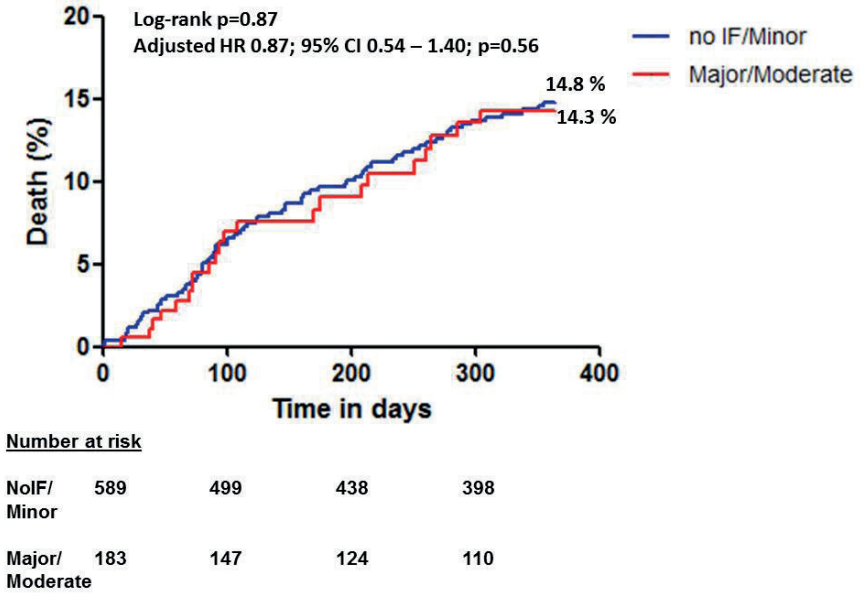
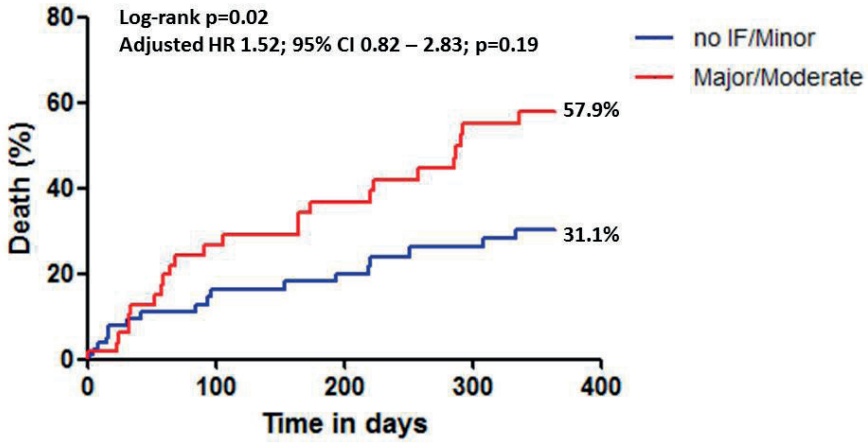


Table 4. Multivariate predictors of 1-year mortality in patients who did not underwent the intended procedure (N=142)

Variable	Unadjusted HR HR (95% CI; p-value)	Adjusted HR HR (95% CI; p-value)
Age	1.05 (1.02 – 1.08; <0.01)	1.02 (0.98 – 1.06; 0.29)
Female sex	1.35 (0.74 – 2.46; 0.33)	
Hypertension	0.86 (0.46 – 1.61; 0.64)	
Diabetes Mellitus	1.65 (0.91 – 3.02; 0.10)	
Dyslipidemia	1.63 (0.88 – 3.02; 0.10)	
Smoking	1.01 (1.00 – 1.01; 0.08)	1.00 (1.00 – 1.01; 0.23)
Prior PCI	0.98 (0.52 – 1.84; 0.94)	
Prior MI	0.81 (0.44 – 1.51; 0.51)	
Prior CVA	1.40 (0.52 – 3.76; 0.51)	
COPD	0.85 (0.38 – 1.89; 0.68)	
Malignancy in history	1.00 (0.94 – 1.07; 0.98)	
Renal insufficiency	5.40 (1.93 – 15.1; <0.01)	3.62 (1.16 – 11.3; 0.03)
Moderate- Major IF	2.02 (1.11 – 3.70;0.02)	1.51 (0.81 – 2.82; 0.20)

PCI=percutaneous coronary intervention, MI=myocardial infarction, CVA= cerebrovascular accident, IF=incidental finding.

Figure 3: Cumulative one-year mortality in patients that did not underwent the intended procedure (n=142) stratified according to the presence of a moderate or major IF



Number at risk				
No IF	85	48	41	36
Major/ Moderate	55	31	25	17

In patients in whom the intended procedure was delayed or cancelled due to the presence of IF (n=15), 14 died within 1 year. Ten patients (67%) died due to a malignancy underlying their IF while 4 patients died due to their initial cardiac condition while waiting for additional analyses of the IF.

Sensitivity analyses in the cohort of patients that underwent TAVI revealed similar findings with an adjusted 1-year mortality rate for the presence of moderate or major IF of 1.37 95% CI; 0.96 – 1.96; p=0.09. Renal insufficiency was the only independent predictor of 1-year mortality, adjusted HR 1.76, 95% CI 1.12 – 2.81; p=0.02 (Supplement, Table S2). A sub-analyses in the cohort of patients that underwent RDN or MitraClip showed also that the presence of a moderate or major IF was not a predictor for 1- year mortality (adjusted HR 0.98 95% CI 0.11 – 8.13; p=0.98).

Additionally, the total number of moderate or major IF did not impact 1-year mortality in patients that underwent TAVI (adjusted HR 1.04; 95% CI; 0.81 – 1.34; p=0.75).

Discussion

In this large-scale retrospective single center registry we demonstrate that IF are frequently detected in patients screened for TAVI, RDN or MitraClip implantation. In 43.1% of the patients referred, non-cardiac IF were found. While the majority of the total number of IF were minor, the presence of moderate or major IF resulted in a delay or cancellation of the intended procedure in only a fraction of the patients and did not significantly impact 1-year mortality.

Pre-procedural screening can identify an asymptomatic disease, a risk factor or a harmful disease such as a malignancy (11-13). The present study demonstrates that a broad spectrum of IF can be found in patients referred for percutaneous cardiovascular interventions. Out of the 698 IF found in a total of 395 patients with IF, 39.2% of the patients were diagnosed only with a finding of minor clinical relevance, mostly determined by the presence of renal cysts (114/698). Although this is in line with previous studies showing that most of the IF are believed to be benign, it could explain the lack of a correlation between the presence of an IF per se and outcome in previous studies (2, 7, 12, 14, 15).

To the best of our knowledge the present study is the first to assess whether IF stratified according to clinical relevance could predict 1-year mortality. To stratify the IF, we used a classification into 3 categories; minor, moderate or major, as previously described by Lumbreras et al (8). In their systematic review comprising 44 studies, the authors reported a mean IF frequency of 23.6%. Follow-up was initiated in only 64.5% and with a lack of specific outcome data in the vast majority of the cases, the authors concluded that the optimal management strategy for these findings remains elusive.

The present study demonstrates that the presence of a moderate or major IF did not significantly impact 1-year mortality rates in patients undergoing TAVI, RDN or MitraClip implantation. This finding was consistent among the individual subgroups of TAVI, RDN or MitraClip. Instead, renal insufficiency and COPD at baseline appeared to be the sole independent predictors for 1-year mortality.

Previous work by Orme et al. concluded that a higher number of potentially pathological IF/patient might impact 2 year mortality in 424 patients screened for TAVI (16). In the subgroup of patients from our study that were screened for TAVI (n=782), we were not able to confirm that the number of moderate or major IF per patient significantly

predicts 1-year mortality. This discrepancy might be explained by the exceptionally high number of IF detected in the work by Orme et al. The authors reported an average of at least 5.3 IF/patient as compared to 1.8 in the present study, 0.3 in a large registry by Koonce et al. and 0.4 in previous work specifically focusing on TAVI patients (2, 6).

Untreated symptomatic severe aortic stenosis has been associated with 1-year mortality rates of up to 50% (17). In patients that actually underwent the intended procedure in the present study, survival curves in patients with moderate or major IF as compared to those with minor- or no IF were superimposed.

Additional analyses in patients that did not undergo the intended procedure revealed an increased crude mortality rate when moderate of major IF were present. The mortality difference in this subset appeared to be explained by the fact that 14/15 patients with moderate or major IF died; 10 due to the consequences of the IF, however 4 patients died due to their underlying cardiac condition for which the treatment was delayed for IF screening purposes. While the latter is in agreement with recent guidelines for valvular heart disease in which TAVI is only indicated in patients with a life expectancy of at least 1 year, it also illustrates that delaying a potentially lifesaving cardiovascular intervention could have important clinical implications (9).

Nevertheless, despite the high number of total IF, the intended procedures were delayed or cancelled in only a fraction of the patients (15/916) due to the presence of active malignancies deserving further attention in a study cohort in which the average age was 78 years. Although these numbers might appear trivial, a total of 698 IF were found in the present study comprising 916 patients. Unfortunately we do not have specific details on the screening process of patients referred for additional diagnosis. Nevertheless, it is likely that the presence of IF might have caused a significant clinical and economic burden to both patient and health care parties.

Currently, there is a broad variety in the quality of international guidelines for follow-up of specific IF. Some are clear from a radiological perspective whilst other need extensive clinical data which is usual not readily available to radiologist or even local operator. The latter leads to extensive heterogeneity in how these recommendations are being followed-up in clinical practice. Lee et al. described that only in one-third of the patients in which IF were found, radiologists recommended further follow-up (18). In the vast majority of radiology reports in patients with IF, explicit follow-up recommendations are lacking. On the other hand, the same study reports that frequently, in case follow-up recommendations were explicitly reported, clinical and imaging follow-up were not

performed. While precise reporting by radiologists is essential, we should realize that previous imaging data from patients referred for either TAVI, RDN of MitraClip in tertiary referral centers is usually not readily available complicating validated follow-up recommendations. Finally, radiologists are frequently not part of multi-disciplinary teams involved in decision making regarding high-risk cardiovascular procedures (19).

Finally, the total number of IF will only increase along with the improving image quality of current generation CT or MRI scanners. With the exception of clear active malignancies, there seems currently no reason to delay or cancel potential lifesaving procedures as TAVI based on the presence of IF. Furthermore, with a prevalence of 43.1% of non-clinically evident radiological findings, there is need for larger prospective studies focusing on the sense and nonsense of follow-up of a broad spectrum of these findings allowing the development of practical guidelines helping physicians in deciding whether or not to refer a patient for additional screening. Until then, it remains important that for every IF a follow-up plan is made along with a decision as to whether or not the intended procedure should be delayed. Based on the data presented above, the presence of a moderate or major IF did not impact 1-year mortality.

Limitations

The present study has several limitations. First, detailed data on the percentage of patients with IF that did undergo additional tests is unknown precluding any statements on their potential clinical and economic consequences. This limitation was inherent to the nature of our institution, being a tertiary referral site for the previously mentioned procedures. Dedicated follow-up for IF was left to the discretion of the referring physician. Second, we might have underestimated the actual incidence of IF due to a lack of structural reporting and the use of different scan protocols associated with the different imaging modalities used. Third, the classification of IF in three categories remains disputable and has not been considered a strict rule to stratify these abnormalities. Moderate IF as adrenal adenomas or breast nodules might become major clinical problems in case associated with an active malignancy. In the present study however, the prevalence of active malignancies was only 1.6% suggesting that in the vast majority of cases IF were benign. Besides these known limitations we were the first to assess whether classification of IF into these 3 categories resulted in different 1-year mortality rates potentially justifying a differential screening approach to those with moderate of major IF.

Conclusion

IF are frequent in patients referred for percutaneous cardiovascular procedures. IF did not result in a delay or cancellation of the intended procedure in the vast majority of the cases, irrespective of their severity. The presence of a major or moderate IF did not significantly impact 1-year mortality in patients referred for TAVI, RDN or MitraClip implantation.

Impact on daily practice

The total number of IF detected on current generation CT or MRI scanners will increase due to improving image quality. The strongest predictor for prognosis in patients as described in this study remains whether or not they undergo the procedure for which they are initially referred. There is need for larger prospective studies focusing on the sense and nonsense of follow-up of a broad spectrum of these findings allowing the development of practical guidelines helping physicians in deciding whether or not to refer a patient for additional screening.

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CHAPTER 5

Safety and performance of diagnostic electrical mapping of renal nerves in hypertensive patients

Konstantinos P. Tsioufis, MD, PhD, Lida Feyz, MD;
Kyriakos Dimitriadis, MD, PhD, Dimitris Konstantinidis, MD,
Dimitrios Tousoulis, MD, Michiel Voskuil, MD, PhD;
Felix Mahfoud, MD, PhD; Joost Daemen, MD, PhD

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Abstract

Aim To evaluate the safety and performance of renal nerve stimulation (RNS) for diagnostic mapping of the renal nerves.

Methods and results In this first-in-man study, twenty hypertensive patients underwent RNS using the ConfidenHT™ system. Bilateral stimulations were performed at 3 to 4 sites per artery at 2 and 4 mA. The primary endpoint was change in systolic blood pressure (SBP). Mean office blood pressure was 156/89 mmHg. No peri-procedural adverse events occurred. Stimulation with 2 mA resulted in a maximum change of 8.3 ± 6.3 mmHg in SBP (based on 119 stimulations; $p < 0.001$), while stimulating with 4 mA resulted in a maximum change of 10.1 ± 7.8 mmHg (based on 61 stimulations; $p < 0.001$). The mean change in SBP did not vary between mid, distal or branch sites when stimulating at 2mA but was significantly higher at ostial (23 ± 14 mmHg) than in non-ostial locations (9 ± 7 mmHg) when stimulating at 4 mA ($p = 0.003$).

Conclusion RNS can be performed safe and effective along the renal artery and results in a large variation in temporary BP changes per patient and per anatomic location. RNS might help in optimizing treatment effect and selecting potential responders to renal sympathetic denervation.

Introduction

Hypertension forms a major health problem worldwide associated with a significantly increased risk for cardiac- and cerebrovascular events (1, 2). While the effect of pharmacological treatment is accepted, a large proportion of patients remains uncontrolled due to non-adherence, side effects and/or failure to reach blood pressure (BP) targets despite maximum tolerated regimens (3, 4). Several novel device-based treatment strategies were recently introduced to help controlling BP by modulating the sympathetic nervous system (SNS) (5). Among those, renal denervation (RDN) has been the most widely studied. When performing RDN, one of the major challenges is the lack of landmarks along the renal arteries identifying the exact location of the renal nerves. The treatment itself remains "blinded" as no intra-procedural feedback or guidance is provided on which patient will benefit nor on if technical success is achieved. The importance of the latter was demonstrated by more recent studies showing non-response rates of up to 37%, mostly attributed to inadequate patient selection and incomplete denervation (6, 7).

The ConfidenHT™ system is an add-on technology for RDN allowing mapping of the renal nerves with the objective to identify potential responders and to provide peri-procedural guidance. The aim of the present study is to demonstrate the safety and performance of RNS using the ConfidenHT™ system in stimulating the renal sympathetic nerves in patients with hypertension.

Methods

Study design

The present study is a prospective, feasibility, open-label, single-arm, multicenter study (three European sites: Hippocration Hospital, Athens, Greece, Erasmus Medical Centre, Rotterdam and Utrecht Medical Center in the Netherlands). The ConfidenHT™ system (Pythagoras Medical Ltd, Israel) was used for diagnostic mapping of the renal nerves through electrical renal nerve stimulation (RNS). Clinical follow-up visits were scheduled at 30 days and 3 months post-procedure. The study protocol was approved by local ethical committees (*ClinicalTrials.gov: NCT02777216; approval IRB MEC-2016-542 for the Netherlands and 167CLP for Greece*), all patients signed written informed consent.

Study population

Patients were included when the following criteria were met (N=20): age >18-75 years with hypertension (office SBP ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg), potential candidates for RDN or a planned elective cardiac catheterization, glomerular filtration rate (eGFR) > 45 ml/min and a main renal artery with a diameter ≥ 4.0 mm. Patients were excluded when there was relevant renal artery disease (renal artery stenosis of >30%, aneurysm or fibromuscular dysplasia), a history of RDN or renal artery stenting, triple ipsilateral renal artery ostia, known secondary causes of hypertension, diabetes mellitus type I or an active implantable medical device (e.g. ICD or CRT-D; baroreflex stimulator).

Study measurements

Prior to the procedure and during follow-up visits, office BP was measured and laboratory measurements (creatinine) were performed. Serious adverse events (SAE's) and adverse events (AE's) were reported spontaneously by the subject or observed at regular follow-up, or any time in between, were to be recorded by the investigator. A SAE was defined as an AE that has led to death, led to serious deterioration in the health of the subject that either resulted in (1) a life-threatening illness or injury, or (2) a permanent impairment of a body structure or a body function, or (3) inpatient or prolonged hospitalization, or (4) medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function. Device related deficiencies were also reported. The primary safety outcome was defined as the occurrence of serious adverse events (system and/or procedure related events). The primary performance outcome was the change in arterial BP in response to RNS.

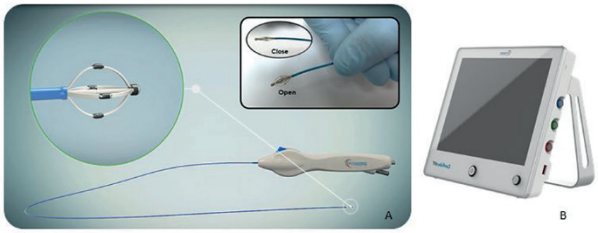
Study procedure

All patients were preloaded with 300 mg aspirin, if naïve, and advised to continue with aspirin for at least 1 month. Pre-procedurally, 100 IU heparin/kg were administered to achieve an active clotting time >250 s. In 17 out of 20 patients the procedure was performed under local anesthesia. Midazolam (up to 15mg) and fentanyl (125µg) were used only in case of discomfort (Table S1, Supplemental). The remaining 3 procedure were performed under general anesthesia using propofol and remifentanil or alfentanil. After administration of local or general

anesthesia common femoral artery access was achieved according to local clinical practice and an 8-Fr sheath was then introduced. In case of scheduled RDN (N=7), either the Symplicity Spyral multi-electrode catheter (N=6) (Medtronic, Galway, Ireland) or the EnligHTN™ ablation catheter (N=1)(St. Jude Medical, St. Paul, MN, USA) were used according to standard instructions for use.

The ConfidenHT™ system consists of two main parts; the console and the catheter (Figure 1). The console delivers electrical energy to the catheter using a multi-channel stimulator, which produces controlled electrical stimulation during the mapping procedure. The console independently controls each electrode on the stimulation catheter. The following stimulation parameters were pre-programmed; stimulation amplitude, frequency, pulse duration and stimulation duration. During stimulation, arterial BP and MAP were continuously measured invasively through a side port of the guiding catheter manifold and analyzed and displayed on the console. The ConfidenHT™ catheter consists of a flexible catheter shaft, catheter tip and an ergonomic handle with a wheel to open and close the basket. The monorail catheter is compatible with an 8-Fr guiding catheter and 0.014" guide wire. The catheter distal-end is composed of an expandable non-occlusive nitinol basket with four peripheral radiopaque stimulation electrodes, mounted on each basket's strand. The basket size when expanded is 8 mm (Figure 1).

Figure 1. The ConfidenHT™ system



The ConfidenHTTM catheter consists of a flexible catheter shaft, catheter tip and an ergonomic handle with a wheel to open and close the basket (A). The ConfidenHTTM console delivers electrical energy to the ConfidenHT catheter using a multi-channel stimulator (B).

The ConfidenHT™ system's principal concept is based on monitoring of hemodynamic response elicited by site-specific electric stimulation in order to map the location of the renal nerves. Electrical stimulation of sympathetic renal nerves induces a sympathetic response by activating afferent and efferent nerve pathways which in turn drives a transient change in several physiological measurable parameters such as heart rate

(HR) and BP (8). Stimulations were performed for 120 s each in the left and right renal arteries, including branches at 3 to 4 locations per artery (branches, distal, mid and proximal) at a frequency of 20 Hz. In all anatomical sites, stimulations were performed at 2 mA (n=119), while in some anatomical sites additional stimulation with 4 mA was performed (n=61). Each stimulation generated an electrical signal in all 4 electrodes (configuration as in Figure 1) for the given duration. Changes in arterial BP response and HR were continuously monitored and recorded during each stimulation. For analytical purposes, arbitrary response thresholds were defined for the change in SBP and MAP. The thresholds used to categorize the rate of response were (ΔSBP ≥8.1 mmHg or a ΔMAP ≥5.6 mmHg) for high response, (ΔSBP 4.1-8 mmHg or ΔMAP 2.6-5.5 mmHg) for medium response, or (ΔSBP 0-4mmHg or ΔMAP 0-2.5 mmHg) for low/no response.

The duration of the procedure was defined as the time interval between inserting of the RNS catheter and the time when the catheter was retrieved outside the body of the patient.

Statistical analysis

All measured variables and derived parameters were listed individually, if appropriate summarized using descriptive statistics. Categorical variables were expressed in absolute, relative frequencies and percentages. Continuous variables were expressed as mean ± standard deviation (SD). The incidence of SAE's were presented with percentages and 95% CI. Continuous variables were compared using Student's t test. All statistical tests are 2-tailed. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using SAS (SAS Institute, Cary North Carolina) (version 9.3).

Results

A total of 20 patients were enrolled. Baseline characteristics are presented in Table 1. In brief, 9 patients were male and mean age was 60±11 years. Mean duration of antihypertensive treatment was 6.7 years.

Table 1. Baseline characteristics of the study population

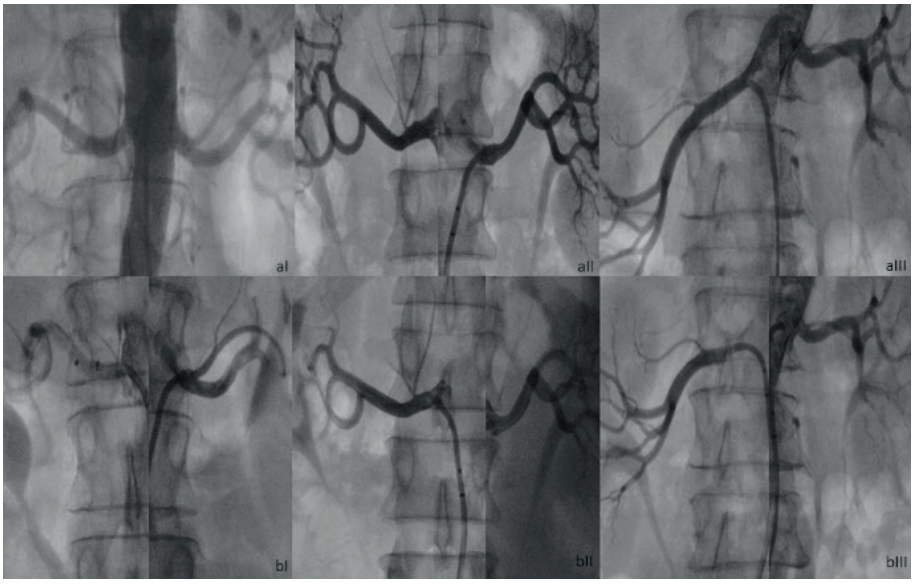
	Total study population (N=20)
Age, years	60 ± 11
Male n, (%)	9 (45)
Race n, (%)	
Caucasian	19 (95)
Other	1 (5)
Diabetes Mellitus type II	1 (5)
BMI, kg/m^2	28.8 ± 4.0
Heart rate, bpm	72 ± 11
Office blood pressure, mmHg	
Mean SBP	156 ± 23
Mean DBP	89 ± 15
MAP	115 ± 18
eGFR, ml/min/1.73m^2	81 ± 19
Antihypertensive drugs n, (%)	
Mean number of antihypertensive drugs	2
Diuretics	9 (45)
ACE-inhibitor	3 (15)
ARB	15 (75)
CCB	12 (60)
Beta-blocker	8 (40)
Aldosteron-antagonist	1 (5)

Variables were expressed in mean ± SD or %. ACE=angiotensin- converting enzyme inhibitor, ARB=angiotensin receptor blocker, BMI= Body Mass Index, CCB=calcium channel blocker. DBP=diastolic blood pressure. eGFR=estimated glomerular filtration rate. MAP= mean arterial pressure. SBP=systolic blood pressure.

Safety outcome

No peri-procedural adverse events occurred. No signs of angiographically visible spasms/thrombus or dissection were observed post procedure (Figure 2). No severe adverse events were reported at 30 days follow-up (N=20). Device-related events occurred in 1/20 patients (reported as myalgia in the back), and procedure related events occurred in 3/20 patients (Table 2). All device and/or procedure related events were resolved at 3 months follow-up. Renal function remained unchanged during follow-up (baseline eGFR 81 ±19 ml/min vs. 78± 16 ml/min at 30 days (p=0.17), and 78 ± 17 at 3 months (p=0.43).

Figure 2. Renal angiograms pre- and post RNS using the ConfidenHT system



No signs of angiographically visible spasms/thrombus or dissection were observed post renal nerve stimulation. aI-aIII pre-procedure, bI-bIII postprocedure.

Table 2. Adverse events (AE) by relationship to the device or procedure

AE class	AE described	Relationship			N patients (%)	Events (%)
		Device	Procedure	Time to event (days)		
Gastrointestinal	Diarrhea	-	-	96	1 (5)	10
Musculoskeletal	Chest pain	-	-	97	1 (5)	30
	Myalgia	+	+	1	1 (5)	
	Joint swelling	-	-	39	1 (5)	
Immune system	Contrast allergy	-	+	1	1 (5)	10
Nervous system	TIA	*	*	28	1 (5)	10
Renal and urogenital	Renal impairment	*	+	26	1 (5)	10
Skin	Pruritus	-	-	1	1 (5)	30
	Rash	-	-		2 (10)	

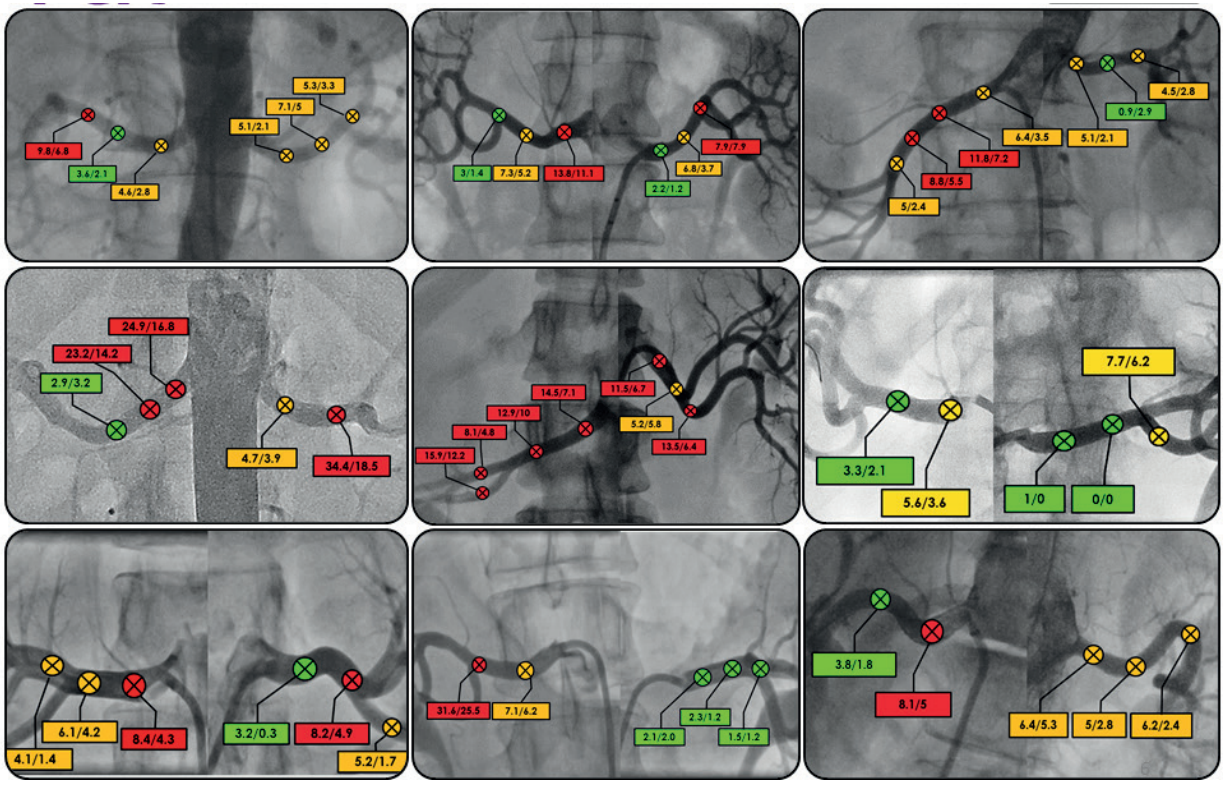
Variables are presented in frequencies and %. The relationship to device and/or procedure are presented with (-)=unrelated, (+)=possible, (*)=unlikely. Renal impairment included a creatinine rise from 1.4mg/dl at baseline to 1.6mg/dl at 30 days, which resolved at follow-up. TIA= Transient Ischemic Attack.

Performance outcome

A total of 194 stimulations were performed; 119/194 stimulations were performed with the 2 mA amplitude and 61/194 with the 4 mA. The remaining 14/194 stimulations had different amplitudes in the range of 2 mA to 10 mA, which were performed in the first 3 patients in order to set the stimulation amplitude to either 2 mA and 4 mA. An average of 9.7 stimulations/patient were performed.

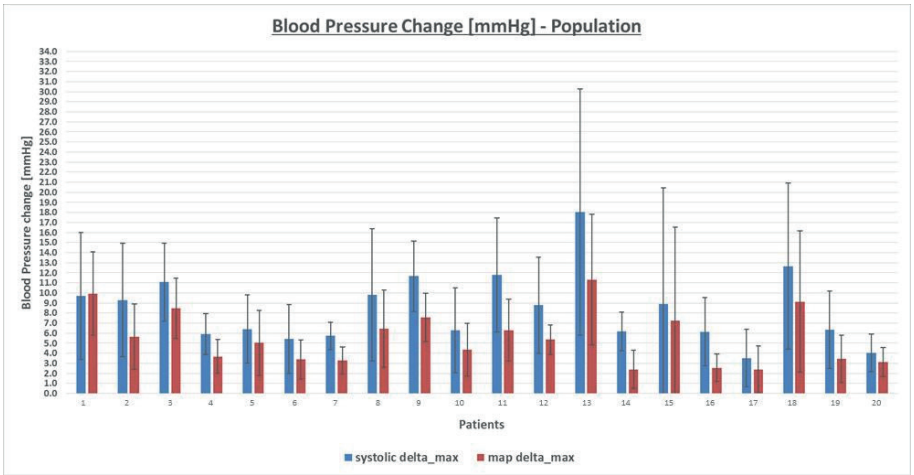
Response to electrical stimulation varied among different anatomic locations within the arteries as well as in-between patients (Figure 3). The mean change in SBP in the overall study population was 9 ± 7 mmHg, with a maximum change in SBP of 34 mmHg. The mean change in MAP in the overall study population was 6 ± 5 mmHg, with a maximum change in MAP of 26 mmHg. The mean change in SBP per patient varied between 4 and 21 mmHg while the mean change in MAP varied between 3 and 14 mmHg (Figure 4).

Figure 3. Response variation between different anatomical locations per patient in delta SBP and delta MAP



Definitions for a response site (Δ SBP/ΔMAP): red=high response (ΔSBP ≥8.1 or a ΔMAP ≥5.6), orange=medium response (ΔSBP 4.1-8 or ΔMAP 2.6-5.5) or green=low/no response (ΔSBP 0-4mmHg or ΔMAP 0-2.5). MAP=mean arterial pressure. SBP=systolic blood pressure.

Figure 4. BP change within patients induced by renal nerve stimulation (RNS)



Change in SBP and MAP due to RNS per patient, demonstrating different response to stimulation. Patient 1-17: local anesthesia, patient 18-20: general anesthesia

A high response (ΔSBP ≥8.1 mmHg or a Δ MAP ≥5.6 mmHg) to stimulation with 2 mA was observed in 53 sites with a mean change in SBP of 13 ± 6 mmHg; medium response (ΔSBP 4.1-8 mmHg or ΔMAP 2.6-5.5 mmHg) was observed in 47 sites with a mean change in SBP of 5 ± 2 mmHg and low/no-response (ΔSBP 0-4 mmHg or ΔMAP 0-2.5 mmHg) was seen in 19 sites with a mean change in SBP of 2 ± 2mmHg. Procedural data on the RNS sites and numbers with both 2 mA and 4 mA are summarized in Table 3. Per patient analyses showed a lack of high response with 2 mA to any of the stimulations in 1/20 patients. All other patients showed at least ones a high response to one of the stimulations. Overall, the maximum change in SBP to 2 mA stimulation was 8 ± 6 mmHg, while stimulating with 4 mA resulted in a maximum change in SBP of 10 ± 8 mmHg (p=0.09).Distribution of the stimulations along the renal arteries was as follows: 47/194 (24%) at the ostium/proximal part of the artery, 54/194 (28%) at the middle part, 51/194 (26%) at the distal part and 42/194 (22%) at the branches. No significant change was observed in SBP response between proximal, mid, distal or branch locations at 2 mA (p=0.77). While stimulation with 4 mA showed a significant difference in SBP response between ostial (23 ± 14 mmHg) and non-ostial locations (9 ± 7 mmHg), p=0.003.

Table 3. Mapping renal nerve stimulation (RNS) data by the ConfidenHT™ system based on response rate

Response/site	**RNS**		
	2 mA (N=119)	**4 mA (N=61)**	**p-value**
No. of sites with 'high response'	53	35	-
No. of sites with 'medium response'	47	13	-
No. of sites with 'no/low response'	19	13	-
Site of max. SBP response, mmHg			
Bifurcation	9	-	-
Ostium	14 ± 8 (n=5)	23 ± 14 (n=3)	0.34
Proximal	7 ± 5 (n=32)	11 ± 7 (n=12)	0.03
Middle	9 ± 7 (n=39)	10 ± 8 (n=20)	0.60
Distal	8 ± 6 (n=34)	8 ± 5 (n=20)	0.89

Definitions for a response site: high response (ΔSBP $\geq$ 8.1 or a ΔMAP $\geq$ 5.6), medium response (ΔSBP 4.1-8 or ΔMAP 2.6-5.5) or low/no response (ΔSBP 0-4mmHg or ΔMAP 0-2.5). SBP=systolic blood pressure. RNS= renal nerve stimulation.

No difference was found in the response to stimulation in patients that did or did not undergo subsequent RDN. The change in SBP due to mapping at 2mA in patients who underwent RDN was 8.4 ± 8.0mmHg versus 8.2 ± 5.0mmHg in the remaining patients (p=0.91).

Because 40% of the patients used beta-blockers, which might have influenced BP response during stimulation, a subanalysis was performed in patients with versus without beta-blockers. SBP change in patients without beta-blockers was 8.6 ± 6.2mmHg versus 7.7 ± 6.4mmHg in patients with beta-blockers (p=0.48) versus 9.2 ± 9.1mmHg versus 11.1 ± 6.0mmHg (p=0.34), respectively, when stimulations were performed at 4mA.

The average time to maximal response was 45 seconds. Out of the 20 patients, one subject withdrew informed consent 2 months post procedure due to personal reasons and was considered as lost to follow-up at 3 months visit.

Procedure

The mean amount of contrast used during the procedure was 174.0 ± 69.7 ml. The mean fluoroscopy time was 12 ± 6 minutes. Mean time of the mapping procedure was 44 ± 11 minutes. Mean total procedure time was 63 ± 20 minutes.

Discussion

This feasibility study demonstrated that RNS using the ConfidenHT system is safe and effective along the renal artery. A large variation in temporary BP changes was observed per patient and per anatomic location within the artery in response to RNS, suggesting that it may be used to identify anatomic areas for effective RDN.

Previous work showed that RDN could contribute to better BP control, however there is still a number of patients who do not seem to benefit from the treatment (9, 10). The latter leaves an unmet need in the possibility to identify potential non-responders (11). Next to patient selection the blind nature of the RDN procedure has been hypothesized to be one of main reasons for non-response (12, 13). A way to convert the blind nature of the procedure in targeted therapy is to identify the locations along a renal artery at which a patients' BP reacts to RNS. The concept was first introduced in pathophysiological work from Chinushi et al. who demonstrated that electrical stimulation of the renal nerves in the proximal renal arteries can evoke action potentials and a change in BP (14). These findings were taken further by Lu et al. who published the feasibility of targeted RDN guided by RNS in the proximal renal artery, resulting in both BP reduction and sympathetic inhibition as measured by plasma norepinephrine levels (8). In addition, both animal studies support basic evidence that renal sympathetic nerve locations are distributed unequally between different animals and anatomic locations along a particular renal artery (8, 14, 15). More recently, two small pivotal in-vivo studies demonstrated that the BP response to RNS could significantly be blunted following RDN, hypothesizing that RNS could even serve as an endpoint for RDN (16, 17). The first study by Gal et al. investigated the feasibility of RNS in eight patients with resistant hypertension undergoing RDN and showed that BP increased significantly during RNS (from 108/55 mmHg to 132/68 mmHg; $p<0.001$) with stimulation outputs of 10 mA and 20 mA. After RDN the maximum BP response to RNS was blunted significantly (from +43 mmHg to + 9 mmHg after RDN, $p<0.01$) (16). The second study, performed by de Jong and coworkers described the association between RNS pre- and post RDN and change in ambulatory BP measurement at 3 to 6 months post RDN. The authors described a correlation between the change in RNS-induced BP change pre- versus immediately post-RDN and the change in ABPM at follow-up, both for SBP ($R=0.77$, $p<0.001$) and DBP ($R=0.79$, $p<0.001$). However, in both studies RNS was performed using either a conventional quadripolar EP-XT catheter (C. R. Bard, Inc, Murray Hill, NJ) which only allowed stimulation with a single electrode at the tip of the catheter or a reprogrammed version of the multi-electrode basket ablation catheter (EnligHTN; St. Jude Medical, Saint Paul, MN) which is no longer on the market.

To the best of our knowledge, the ConfidenHT™ system is the first dedicated RNS system and proved, in the present study, to successfully identify locations with positive SBP change during RNS. We found a large variability in the change in BP in-between different stimulated locations and in-between patients in line with previous work (8, 14). More specifically, low or no change of BP to any of the stimulations at either 2 or 4 mA (<4 mmHg in SBP was found in up to 30% of the patient in the present study) was also reported in previous studies (16, 17). The estimation of the non-responders based on arbitrary RNS thresholds in this study is in line with previously published results of several RDN studies, reporting on a non-response rates between 8 and 37% of the patients (7).

The highest BP change was observed in the ostial part when comparing stimulation with 2 mA and 4 mA, however probably due to low stimulation numbers the change did not reach statistical significance. Stimulation with 4 mA showed a significant difference in SBP response between ostial and non-ostial locations. The latter supports other pathophysiological studies, demonstrating that renal nerves at the ostial sites are located more distally from the arterial lumen as compared to more distal locations (15), thus higher energy is needed for effective RNS in these sites.

With the potential of RNS reported above, the change in SBP to electrical stimulation could be used as a diagnostic tool to identify the patient with a high likelihood of response. In addition, responsiveness to RNS at different locations may indicate treatment targets along the renal artery with the potential to identify and increase technical success of RDN.
Little is known of the relationship between stimulation or ablation of the nerves travelling along the renal artery lumen and pain. DeWolf and Fraley described in 1975 that stretching of the renal capsule, pelvis, artery, or vein or distention of the pelvis or upper ureter produces the sensation of pain (18). Of note, in a previous RNS study (17), all procedures were performed under general anesthesia that could confound the effects on sympathetic tone and BP response. However, the authors successfully stimulated 105 sites, whereas 78/105 sites (74.3%) showed >10 mmHg SBP increase. Herein, we studied three patients under general anesthesia who demonstrated the same variability in response between patients and within different renal loci in the same patient, as seen in those studied under local anesthesia, which further mitigates the potential lack of response due to general anesthesia.

Limitations

Our study has several limitations. First, the present first-in-man report included 20 patients and therefore warrants larger studies with adequate statistical power confirming–the usability of RNS. Second, the duration of renal nerve stimulation (120sec per stimulation at both 2 and 4mA) resulted in an average mapping time of 44 minutes. Given the findings of our study, this could be reduced substantially in the future. At present, a second generation ConfidenHT™ catheter is under development incorporating also the ability to perform RDN. Subsequent studies are needed to identify the feasibility of the device to determine treatment response. Third, temporal changes to stimulation at 2 and 4mA were assessed. The catheter was not moved in between stimulations at 2 and 4 mA at the same spot providing a reasonable level of assurance that mapping was performed at the exact same location. Nevertheless, we cannot ascertain that minimal changes in catheter position might have occurred.

Conclusion

In the present study we demonstrated that the ConfidenHT system constitutes a safe and effective method to achieve RNS. Diverse temporary BP changes were observed per patient and per anatomic location within the artery in response to RNS. These results suggest that the ConfidenHT system may be used to identify renal nerve loci with better RNS-induced BP response in order to achieve effective RDN and help in identifying responders to RDN.

Impact on daily practice

The change in SBP to electrical stimulation could be used as a diagnostic tool for an appropriate patient selection for RDN. In addition, responsiveness to RNS at different locations may indicate treatment targets along the renal artery with the potential to identify and increase technical success of RDN.

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CHAPTER 6

The effect of renal denervation on catecholamines and the renin-angiotensin-aldosterone system

Lida Feyz, MD; Sjoerd van den Berg, PhD; Robert Zietse, MD, PhD; Isabella Kardys, MD, PhD; Jorie Versmissen, MD, PhD; Joost Daemen, MD, PhD

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Abstract

Introduction The effect of renal sympathetic denervation (RDN) on neurohormonal responses is largely unknown. We aimed to assess the effect of RDN on the renin-angiotensin-aldosterone system (RAAS) and endogenous catecholamines.

Methods A total of 60 patients with hypertension underwent RDN and remained on a stable antihypertensive drug regimen. Samples for plasma aldosterone, plasma renin and urine (nor)metanephrine were collected at baseline and at 6 months post-procedure. Ambulatory blood pressure (BP) recordings were obtained pre- and 6 months post-procedure.

Results Mean age was 64±9yrs and 30/60 patients was male. At 6 months, average daytime systolic- and diastolic ABP decreased by 10 and 6mmHg ($p<0.001$), respectively. No significant change was observed in plasma aldosterone (median[IQR]: 248.0[113.3-369.5] pmol/l vs. 233.0[110.3 – 360.8] pmol/l; $p=0.66$); renin (19.5[6.8 – 119.5] μU/mL vs. 14.3[7.2 – 58.0] μU/mL; $p=0.32$), urine metanephrine (0.46[0.24 – 0.77] μmol/L vs. 0.46[0.22-0.88] μmol/L; $p=0.75$) and normetanephrine (median 1.41[0.93 – 2.00] μmol/L vs. 1.56[0.74-2.50] μmol/L; $p=0.58$) between baseline and 6 months, respectively. No correlation was found between the decrease in mean systolic daytime BP and changes in RAAS hormones or endogenous catecholamines.

Conclusion Despite significant reductions in ambulatory BP, RDN did not result in a significant change in endogenous catecholamines and in RAAS hormones at 6 months.

Introduction

Recent randomised, sham-controlled studies have proven the blood pressure (BP) lowering effect of renal sympathetic denervation (RDN) in patients with mild- and uncontrolled hypertension (1-3). Although the procedure showed to be efficacious and safe, many questions remain about the exact pathophysiological mechanisms by which RDN lowers BP (4). The antihypertensive effect of reducing sympathetic tone by RDN is hypothesized to be driven by the interaction between renal sympathetic nerves and the renin-angiotensin-aldosterone-system (RAAS) (5). The renal afferent nerves are the physiological conduit of central sympathetic drive and are involved in the control of peripheral vascular resistance (6). The efferent nerves increase renin secretion by juxtaglomerular cells using norepinephrine (NE) as a neurotransmitter, stimulate tubular reabsorption of sodium and directly increase renal vascular resistance (6, 7).

Previous studies have shown that there is a causal relationship between renal norepinephrine spillover, renin release and arterial plasma renin activity (8). Moreover, the first proof-of-concept trial for RDN showed a mean reduction of 47% in renal noradrenaline spillover accompanied with a significant BP drop in patients with hypertension (9). Conversely, the effects of RDN on the RAAS has only been studied in a limited number of preclinical studies failing to demonstrate a correlation between BP reduction and change in sodium excretion or in RAAS activity (10, 11). To date, dedicated studies focusing on the link between RDN and neurohormones in vivo are lacking. Therefore, in the present study, we aimed to assess the effect of RDN on RAAS hormones and endogenous catecholamines as an attempt to understand the BP lowering effect.

Methods

Study population

This is a single-center (Erasmus Medical Center) prospective study including 63 hypertensive patients undergoing RDN between November 2012 and February 2016. Patient had to be on a stable antihypertensive drug regimen for at least four weeks prior to inclusion. Patients were excluded when their antihypertensive drug regimen was changed during the course of the study (n=3). The study was approved by the Erasmus Medical Centre Ethics Committee, and written informed consent was obtained from

all participants.

RAAS hormones and endogenous catecholamines were prospectively measured at baseline, after a 4-week stabilization protocol, and at 6 months follow-up. Enrolment occurred when the following criteria were met: a mean office blood pressure of ≥ 140 and/or 90mmHg (based on three consecutive measures), use of ≥2 antihypertensive drugs, age ≥ 18-75 years and an estimated glomerular filtration rate (eGFR) > 45 ml/min/1.73m^2. Secondary causes for hypertension were ruled out in all patients after extensive laboratory analysis and Computed Tomography Angiography (CTA) or Magnetic Resonance Imaging (MRI) assessment.

Study measurements and endpoints

Laboratory data were drawn pre-procedurally (baseline) and at 6 months post-procedure (RDN), during morning hours; laboratory analyses based on blood samples were obtained in a sitting position. Blood sampling was done at four to eight weeks prior to procedure. Urine portions were collected to measure the levels of (nor) metanephrine. The blood- and urine sampling protocol was similar for all time points. Creatinine corrected urinary sodium and potassium levels were obtained at baseline and at 6 months in order to correct for possible confounding by changes in diet and sodium intake.

Antihypertensive drugs that could interfere with the plasma renin and/or aldosterone remained unchanged during sampling at both baseline and at 6 months follow-up.

The primary endpoint was the change in plasma renin, plasma aldosterone and endogenous catecholamine (urine (nor)metanephrine) concentrations at 6 months post-procedure as compared to baseline.

Secondary analyses included the change in renal function as measured using both eGFR (ml/min) and cystatin C (mg/L).

eGFR was estimated based on the following the following: GFR in mL/min per 1.73 m2 = 175 x serum creatinine-1.154 x age-0.203 x 1.212 (if patient is black) x 0.742 (if female).

Plasma aldosterone, urinary metanephrine and normetanephrine concentrations were measured by liquid chromatography-mass spectrometry (LC-MS/MS) (Waters Xevo

TQS, Milford, Massachusetts, USA). Plasma renin concentration was measured using IDS-iSYS assay system (Immunodiagnostic System, The Boldons, UK).

Automated seated office blood pressure measurement was performed with the Omron M10-IT (Omron Healthcare Co., Ltd. Kyoto, Japan). An average value of three consecutive BP measurements was used (measured on the upper arm with the highest BP value). The 24h ambulatory blood pressure measurements (24h ABPM) were performed with an oscillometric device (Spacelabs Healthcare, model 90217A), the mean (systolic and diastolic) 24h ABPM and mean daytime (systolic/diastolic) ABPM were included in the analysis. A patient with a decrease of ≥ 5mmHg in mean daytime systolic ABPM at 6 months was considered as a responder to RDN (12). Patients in which samples were only available either at baseline or at follow-up were excluded.

RDN

All patients not using aspirin were preloaded with 300mg aspirin and were advised to continue with aspirin for at least 1 month. Pre-procedurally, 100IU heparin/kg were administered to achieve an active clotting time>250s. All procedures were performed under conscious sedation. After administration of local anesthesia, RDN was performed according to standard instructions for use using either the Paradise™ (N=13; Recor Medical, Palo Alto), Vessix V2™ (N=5; Boston Scientific, Natick, MA), Symplicity™ (N=19; Medtronic, Minneapolis, MN), OneShot™ (N=3; Covidien, Campbell, CA, USA) or EnligHTN system (N=20; St. Jude Medical, St Paul, MN, USA) (13, 14).

Statistical analysis

Continuous data was expressed as mean ± standard deviation (SD) when normally distributed, or as median [interquartile range, IQR] when non-normally distributed. Categorical data was presented as percentages. Changes in blood pressure between baseline and 6 months were assessed by using the paired t-test. Changes in RAAS hormones and endogenous catecholamines between baseline and 6 months were assessed by using the Wilcoxon signed-rank test. The Spearman- or Pearson correlation coefficient was used when appropriate to evaluate the relationship between the change in RAAS hormone concentration and the change in blood pressure. The change in RAAS hormones and endogenous catecholamines in responders versus non-responders was tested by using the Mann-Whitney U test. All statistical test were two-tailed. A p-value

of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS statistical analysis (version 24.0).

Results

Mean age was 64 ± 9yrs and 30/60 patients was male. Mean number of antihypertensive drugs at baseline was 3±1. The antihypertensive drug regimen did not change between baseline and 6 months follow-up in any of the patients. Mean office BP was 172±23/94±14mmHg and mean 24h ABPM was 144±17/81±12mmHg. Baseline characteristics are presented in Table 1. No differences were found in baseline characteristics between responders and non-responders.

Table 1. Baseline characteristics

	Total study population N=60
Age, years	64 ± 9
Male gender n, (%)	30 (50.0)
BMI, kg/m^2	30.2 ± 5.3
Caucasian n (%)	51 (85.0)
eGFR, ml/min/1.73m^2	72 ± 16
Diabetes mellitus n, (%)	13 (21.7)
Office BP, mmHg	172 ± 23/94 ± 14
24h ABPM, mmHg	144 ± 17/81 ± 12
Mean number of antihypertensive drugs	3 ± 1
Type of antihypertensive drugs, n (%)	
ARB	37 (61.7)
ACEi	12 (20.0)
CCB	39 (65.0)
β-receptor blockers	41 (68.3)
Thiazide diuretics	45 (75.0)
Loop diuretics	2 (3.3)
Aldosterone receptor blockers	7 (11.7)
α-receptor blockers	12 (20.0)
Direct renin inhibitor	2 (3.3)

Values are presented in mean ± SD or n (%). ARB= angiotensin receptor blocker, ACEi=angiotensin converting enzyme inhibitor, 24h ABPM= 24h ambulatory blood pressure measurement, BP= blood pressure, CCB=calcium channel blocker, eGFR=estimated glomeral filtration rate

Change in RAAS hormones and endogenous catecholamines

Venous blood sampling and urine collection was performed at a median of 78 days [34 -150] prior to procedure (considered as 'baseline') and at 6 months follow-up at a median of 192 days [183 – 199] after RDN. Plasma aldosterone was 248.0[113.3 -369.5] pmol/l at baseline vs. 233.0[110.3 – 360.8] pmol/l; p=0.66 at 6 months, and plasma renin was 19.5[6.8 – 119.5] μU/mL at baseline vs. 14.3[7.2 – 58.0] μU/mL at 6 months; p=0.32. Urine metanephrine was 0.46[0.24 – 0.77] μmol/L at baseline vs. 0.46[0.22-0.88] μmol/L at 6 months; p=0.75, and urine normetanephrine was 1.41[0.93 – 2.00] μmol/L at baseline vs. 1.56[0.74-2.50] μmol/L at 6 months follow-up; p=0.58 (Table 2).

Table 2. Renin and aldosterone, (nor)metanephrine at baseline and 6 months post-procedure

	Pre-procedure	**6 months**	**p**
Aldosterone (pmol/l) (N=42)	248.0 [113.3 – 369.5]	233.0 [110.3 – 360.8]	0.66
Renin (μU/mL) (N=40)	19.5 [6.8 – 119.5]	14.3 [7.2 – 58.0]	0.32
Metanephrine (μmol/L) (N=18)	0.46 [0.24 – 0.77]	0.46 [0.22 – 0.88]	0.75
Normetanephrine (μmol/L) (N=18)	1.41 [0.93 – 2.00]	1.56 [0.74 – 2.50]	0.58
Metanephrine/creatinine (μmol/mol) (N=18)	0.06 [0.04 – 0.09]	0.05 [0.04 – 0.07]	0.09
Normetanephrine/ creatinine (μmol/mol) (n=18)	0.17 [0.14 – 0.24]	0.16 [0.13 – 0.19]	0.42
Urinary sodium excretion/creatinine (mmol/l) (N=48)	10.3 [5.30 – 15.5]	10.2 [6.13 – 16.7]	0.72
Urinary potassium excretion/creatinine (mmol/l) (N=48)	7.0 [4.3 – 9.3]	6.9 [4.9 – 9.6]	0.60

Values are presented in median [IQR].

Safety endpoint

Renal function remained unchanged between baseline (70.8 ± 15.9ml/min) and 6 months follow-up (69.4 ± 17.6ml/min), (p=0.33). Also, Cystatin C remained unchanged between baseline (1.10 ± 0.24mg/L) and 6 months follow-up (1.11 ± 0.26mg/L) (p=0.54).

Change in BP and correlation with neurohormones/catecholamines

Mean office BP decreased significantly between baseline and 6 months (-12±23/-8±12mmHg, p<0.01). Similar results were observed in mean (-8±14/-5±9mmHg) and mean daytime ABP, (-10±16/-6±9mmHg) (both p<0.01) (Table 3).

No correlation was found between the change in mean daytime systolic ABP and change in plasma renin, r^2=0.26; p=0.28 (Figure 1). A similar lack of correlation was found for plasma aldosterone and change in mean daytime systolic ABP, r^2=0.34; p=0.14 and similar findings were observed in urine normetanephrine r^2=-0.28; p=0.72 and urine metanephrine r^2=0.20; p=0.80. When comparing responders (n=36/57) and non-responders (n=24/57), no significant difference in changes between 6 months and baseline were observed in plasma renin and aldosterone levels and urine catecholamines (Table 4).

Table 3. Change in blood pressure at follow-up measured in office and ambulant

	Pre-procedure	6 months	p*
Systolic office BP, mmHg (N=60)	172 ± 23	160 ± 26	<0.01
Diastolic office BP, mmHg	94 ± 14	85 ± 13	<0.01
Systolic 24h ABPM, mmHg (N=57)	144 ± 17	135 ± 19	<0.01
Diastolic 24h ABPM, mmHg	81 ± 12	76 ± 10	<0.01
Daytime systolic ABPM, mmHg	146 ± 19	137 ± 20	<0.01
Daytime diastolic ABPM, mmHg	83 ± 13	77 ± 10	<0.01

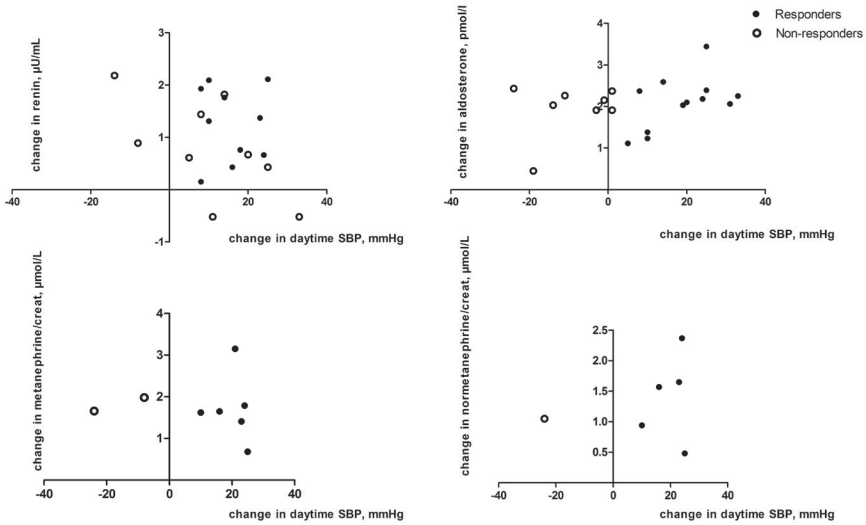
Values are presented in mean ± SD or n (%).

Table 4. Change in RAAS hormones and endogenous catecholamines in responders vs. non-responders to RDN

	Responders (N=36)	Non-responders (N=24)	p-value
Δ Plasma aldosterone (pmol/l)	13 [-118 – 150]	-0.10 [-84.8 – 116.7]	0.95
Δ Plasma renin (µU/mL)	-0.60 [-14.4 – 21.0]	2.85 [-0.57 – 22.7]	0.33
Δ Urine metanephrine (µmol/L)	-0.12 [-0.22 – 0.27]	-0.30 [-0.36 – 0]	0.60
Δ Urine normetanephrine (µmol/L)	-0.87 [-1.19 – 0.82]	-0.41 [-0.86 – 0]	0.37

Values are presented in median [IQR].

Figure 1. Change in plasma renin and aldosterone and endogenous catecholamines after RDN in responders versus non-responders (on a logarithmic scale)



Discussion

In the present study the significant reduction in ambulatory and office blood pressure induced by RDN, in patients with moderate to severe hypertension, could not be explained by the change in endogenous catecholamines and in RAAS hormones at 6 months. Additionally, no correlation was found between the response to RDN as measured using the mean daytime systolic ABP and change in both RAAS hormones and endogenous catecholamines.

In a search towards a better understanding of the pathophysiological mechanisms behind the blood pressure lowering effect by RDN, we attempted to search for potential differences in RAAS hormones and endogenous catecholamines as induced by RDN. While our findings are in line with several preclinical studies (10, 11), previous animal data by Zhao et al. suggested that RDN might suppress an increase in plasma aldosterone in twenty-eight dogs with progressive heart failure at three weeks (15). Additionally, Hong et al. demonstrated a clear suppression in aldosterone release following RDN in rats with Ang-II-induced hypertension after 14-17 days. Conversely, no change was found in renin levels, which could probably be explained by the negative feedback effect of Ang-II on renin(16, 17). With the latter evidence, a relation between the renal sympathetic nerves and aldosterone regulation was suggested, a process that could act independently of renin. As such, a small clinical study (N=17) found a significant change in plasma noradrenaline 6 months post RDN, however, no change was found in BP, nor in renin levels (18).

To the best of our knowledge, the present work represents the largest body of evidence to date on the topic, which failed to show a significant impact of RDN on RAAS hormones and endogenous catecholamines release in vivo. Based on the available evidence, it could be that endogenous catecholamines are not sensitive enough to detect a difference which would correlate with blood pressure decrease (19). Furthermore, it should be noted that catecholamines are the shortest lived signaling molecules in plasma with an initial biologic half-life of approximately 1-2 minutes (20).

It is well known that activation of the sympathetic nervous system and stimulation of the RAAS leads to an increase in blood pressure (21, 22). Blocking the RAAS with angiotensin (Ang)-converting enzyme (ACE) inhibitors and angiotensin II type 1 receptor (AT1R) antagonists subsequently became one of the most frequent ways to treat hypertension and proved to significantly improve cardiovascular morbidity and lower mortality (23-25).

ACE-inhibitors, AT1R-antagonists, beta-blockers and aldosterone receptor blockers, are known to affect plasma renin and aldosterone levels (26, 27). Therefore, in the present study we paid specific attention to keep the confounding effect of drugs as low as possible. For this reason we only included patients on a stable drug-regimen for at least 4 weeks before blood samples were taken and RDN was performed. Second, patients in which the antihypertensive drugs were changed during the course of the study were excluded. Furthermore, we attempted to correct for potential differences in the change of dietary sodium and/or potassium intake, which are known to affect plasma renin and aldosterone (28, 29).

Finally, the present work confirms the difficulty in linking RDN treatment success. Along these lines, even studies correlating RDN response to muscle sympathetic nerve traffic (MSNA), which has been considered the gold standard for measuring systemic sympathetic nerve activity due to its good sensitivity and reproducibility (30), however other studies showed conflicting results(31, 32) . MSNA measurement however, proved significantly more invasive and complex than measuring neurohormones.

Limitations

The present study has several limitations. First, most recent RDN trials identified primary endpoints at two- to three months post procedure in order not to expose patients to unnecessary long periods off-drugs (for trials performed in an off-med population) and in order to reduce the likelihood of the impact of changes in antihypertensive drug regimens in on-med trials in patients on pre-defined drug regimens (1-3). At the time this study was designed (2012) there was an overall believe that 6 months of follow-up were needed to observe the actual effect of RDN (9). The latter explains the decision to perform RAAS and neurohormone levels at the same timepoint, 6 months post procedure. Future trials with assessment of RAAS and neurohormone levels at shorter term timepoints are to be awaited, though it almost technically not feasible to determine the real-time rate of neurohormone production. Second, two thirds of the patients in our study received an ACE inhibitor, an AT1R antagonist or a diuretic which could have influenced our findings. The latter bias however was considered to be minimal due to a similar degree of interference at baseline and follow-up in a population in which no changes in drug regimen occurred. Third, we did not test for drug adherence at baseline and at 6 months follow-up. Fourth, since no sham control group was included in the present study, the potential of a placebo effect on BP could not be ruled out.

Finally, procedures were performed with different devices and different procedural strategies. A total of 25/60 RDN-procedures were performed with RDN-systems that are no longer available. A sensitivity subgroup analyses into radiofrequency vs. ultrasound catheter-based RDN did not reveal any heterogeneity in the findings on RAAS hormones and endogenous catecholamines. However, we could not perform a logistic regression, due to a small sample size.

Conclusion

Despite significant reductions in ambulatory blood pressure, RDN did not result in a significant change in endogenous catecholamines and in RAAS hormones at 6 months post procedure.

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ASSESSING ADHERENCE TO ANTIHYPERTENSIVE DRUGS

Chapter 7

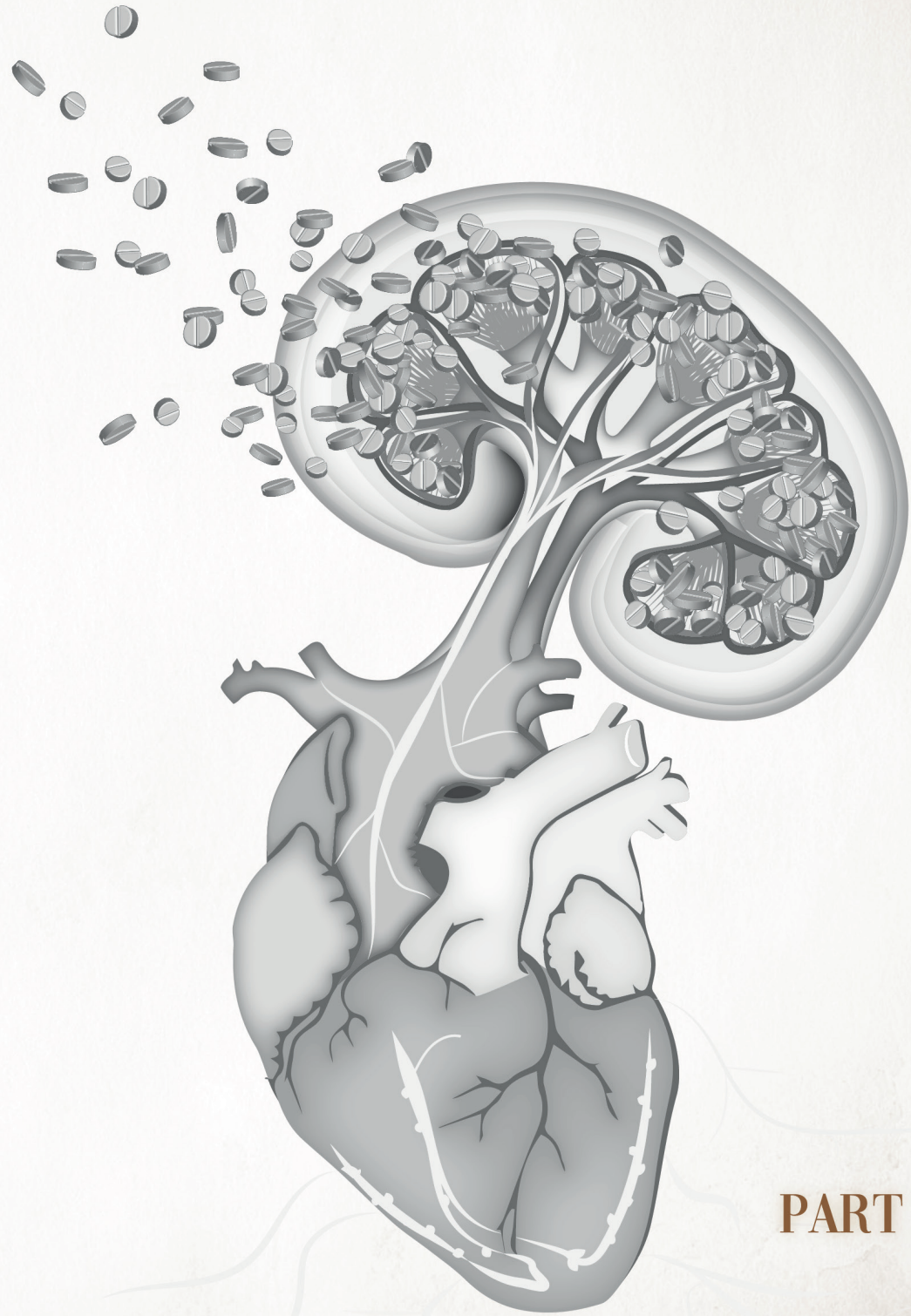
Therapeutic Drug Monitoring to assess drug adherence in assumed resistant hypertension: a comparison with directly observed therapy in three nonadherent patients

Chapter 8

Clinical validation study of a dried blood spot assay for eight antihypertensive drugs and four active metabolites

Chapter 9

The clinical applicability of monitoring antihypertensive drug levels in blood



PART III

CHAPTER 7

Therapeutic Drug Monitoring to assess drug adherence in assumed resistant hypertension: a comparison with directly observed therapy in three nonadherent patients

Lida Feyz, MD; Soma Bahmany, MSc, Joost Daemen, MD, PhD;
Anton H. van den Meiracker, MD, PhD; Birgit C.P. Koch, PhD;
Teun van Gelder, MD, PhD ; Jorie Versmissen, MD, PhD

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Abstract

Resistant hypertension (RH) is a common health problem leading to suboptimal cardiovascular prevention. A large number of patients with RH have poor medication adherence explaining their assumed resistance to therapy. We combined directly observed therapy (DOT) with therapeutic drug monitoring (TDM) in three patients at several time points to enable an extensive feedback on blood pressure (BP) and drug levels. BP was measured with an automatic oscillatory device at regular intervals of 5 minutes (directly before and after drug intake) and at 30 minute intervals (at night) during admission. Blood samples were obtained at different time points (t=in hours; t=0, 2, 4, 6, 12, 24 after drug intake). DOT was performed under supervision of the physician. In 2 out of the 3 patients automated BP decreased directly after DOT, -10/0mmHg and -5/-5mmHg, respectively. Plasma drug levels for several drugs or active metabolites were 0 at t=0 while plasma levels were positive at t=24 hours after observed intake. We recommend a more frequent use of TDM combined with repeated BP measurements in clinical practice, since this is a convenient, objective method of measurement and to ensure that actual drug levels reflect the BP at the time of measurement.

Introduction

Resistant hypertension (RH), defined as uncontrolled blood pressure (BP) despite a medication regimen of antihypertensive drugs from at least three classes including a diuretic, is a challenging clinical problem associated with high costs and suboptimal cardiovascular prevention (1, 2). About 40 to 60% of patients with RH have a problem with adherence to their medication explaining their assumed resistance to therapy (3, 4). Non-adherence is a multifactorial problem and has been associated to adverse cardiovascular events, even independently of BP control (5). Barriers to adherence differ between patients and can be divided into either intentional or unintentional, or into perceptual (e.g. beliefs about the condition and the drugs) and practical (e.g. inconvenient drug dosing scheme) (6). While assessing adherence has been recognized to be the first step in improving non-adherence and clinical outcome, this remains extremely complex (4).

In general, indirect and direct methods can evaluate adherence. Indirect methods like questionnaires, patient diaries and pill counts are simple to perform but unreliable (7). Examples of direct methods are directly observed therapy (DOT) under supervision of a healthcare professional and measurements of levels of drugs and if needed metabolites in blood or urine, so-called therapeutic drug monitoring (TDM) (4, 8). DOT is time-consuming and as a consequence expensive, it is susceptible to manipulation and can be potentially hazardous if a patient has been non-adherent for the antihypertensive drugs prior to DOT. TDM is more objective and reliable, but requires a sensitive assay and knowledge of the pharmacokinetic parameters of drugs to ascertain that a negative value rules out adherence for that drug (4, 9). Previous retrospective analyses suggest that clinical use of TDM might improve BP control in initially non-adherent patients (10). In the present work, we demonstrate three patients with RH who denied non-adherence and had doubts about the efficacy of the prescribed drugs. We agreed with the patients to combine DOT with TDM at several time points to enable an extensive feedback on BP and drug levels. The results enabled us to compare both methods.

Description and verification of method

Three non-adherent patients (patient A, B, C) were admitted to the department of Internal Medicine (Erasmus Medical Centre, Rotterdam, The Netherlands). Patients were requested not to take antihypertensive drugs prior to admission, when DOT and

TDM were performed. Patient instructions explaining the whole procedure were re-established in a written letter. During admission, BP was measured for at least 20 minutes (every 5 minutes) before and after intake of their antihypertensive medication (method performed: DOT) with an automatic oscillatory device (Datascope Accutorr V™, Pacific Medical, CA) followed by 24h ABPM using an embedded oscillometric device by SpaceLabs Healthcare (90207-1Q) measuring BP at 20 minute intervals during daytime (between 7:00 AM and 23:00 PM) and at 30 minute intervals during nighttime.
Blood samples were obtained at t=0, followed by DOT , and blood sampling at 2, 4, 6, 12 and 24 hours after drug intake. Drug levels (method performed: TDM) were measured using a previously published FDA validated liquid chromatography tandem mass spectrometry multi-drug assay (LC-MS/MS) (11). Using this assay, we measured eight hypertensive drugs (amongst which amlodipine, valsartan, spironolactone, hydrochlorothiazide) and four active metabolites in case the half-life was short, for instance canrenon (active metabolite of spironolactone). We summarized timing of measurements and drug administration in Table 1. Written informed consent was obtained from all patients.

Table 1. Overview of measurements and drug administration.

1.	Admission to the department in the early morning No antihypertensive drug should have been taken by the patient as described in a letter sent in advance
2	20 minutes repeated BP measurements (Datascope) First blood sample t=0
3.	Supervised drug intake
4.	20 minutes repeated BP measurements Start of 24h ABPM
5.	Blood sampling at 2, 4, 6, 12 and 24 hours (or less dependent on patients'agreement)

The demographic characteristics of the patients are presented in Table 2. In brief, two patients were female and mean automated BP was 168/113 mmHg at previous outpatient clinic visits.

Table 2 Baseline characteristics

Characteristics	**Patient A**	**Patient B**	**Patient C**
Age, year	47	23	55
Ethnicity	Female	Female	Male
Length, cm	174	165	171
Weight, kg	80	80	79.8
BMI, kg/m²	26.4	29.4	27.3
Mean automated BP, mmHg*	159/114	200/120	144/105
Mean automated BP, mmHg	170/111	150/97	144/98
eGFR, ml/min	>90	75	70
Antihypertensive drugs			
ACE inhibitor	No	No	No
Angiotensin receptor blocker	Valsartan 320mg qd	Valsartan 320mg qd	Valsartan 320mg qd
Diuretic	HCT 25mg qd	HCT 25mg qd Furosemide 40 tid	HCT 25mg qd
Calcium channel blocker	Amlodipine 10mg qd	Amlodipine 10mg qd	Amlodipine 10mg qd
Alpha blocker	No	No	No
Beta blocker	Metoprolol 100mg qd	Labetalol 800mg tid	Metoprolol 100mg qd
Aldosteron receptor antagonist	Spironolactone 25mg qd	Spironolactone 25mg qd	Spironolactone 25mg qd
Vasodilator	No	No	No
BP change			
Automated BP after DOT, mmHg	160/111	145/92	154/101
24h ABPM after DOT, mmHg	133/86	146/98	128/84
Change in automated BP, mmHg	-10/0	-5/-5	+10/+3
Adherence	Fully NA	Partially NA	Partially NA

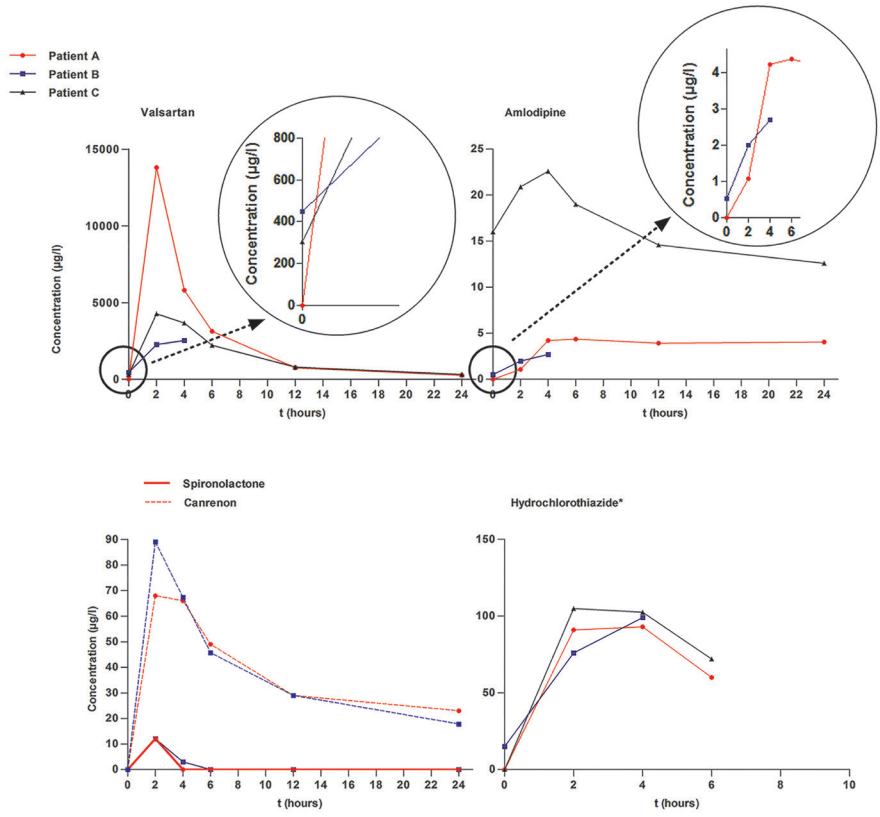
* measured during previous visits, prior to admission. BMI=body mass index, BP=blood pressure, eGFR=estimated glomerular filtration rate, ACE=angiotensin converting enzyme, HCT=hydrochlorothiazide, DOT=directly observed therapy, NA=non-adherent

The first patient, patient A, had a history of Morbus Graves and bilateral renal artery stenosis, which were previously successfully treated. After DOT, automated BP decreased with -10/0mmHg (average of 6 measurements) as compared to the automated BP prior to DOT. The 24h ABPM average improved to 133/86mmHg. Drug levels were undetectable at t=0 hours for amlodipine, valsartan and canrenon, while at t=24 hours all were detectable (Figure 1, red line). As expected, because of the short half-life, spironolactone and hydrochlorothiazide were below the lower limit of quantification after the 12 hours time-point. The second patient, patient B, underwent previously bilateral renal sympathetic denervation due to severe RH. She had been admitted earlier with the suspicion of non-adherence and TDM was previously applied in another hospital with feedback on potential non-adherence. She had persistent hypertension despite high doses of medication (Table 2). Average BP during automated measurements at admission was 150/97, much lower than at the last visit to the outpatient clinic (Table 1). After DOT, automated BP decreased with -5/-5mmHg (average of 5 measurements) as compared to the automated BP prior to DOT. The 24h ABPM improved to 146/98mmHg. Amlodipine, valsartan and hydrochlorothiazide were detectable at t=0 hours. The patient reported not to use the prescribed spironolactone because of dizziness at home, although she had not mentioned this at earlier outpatient clinic visits. Blood sampling in this patient was challenging, therefore samples only were obtained at t=0, t=2 and t=4 hours (Figure 1, blue line).

The last patient, patient C, had severe RH with secondary retinopathy despite antihypertensive drugs (Table 2).

After DOT, automated BP increased with +10/+3mmHg (average of 9 measurements) as compared to the automated BP prior to DOT. The 24h ABPM improved to 128/84mmHg. During observed intake it appeared that patient was unaware of the prescription of spironolactone, as confirmed by the undetectable canrenon concentration at t=0 (Figure 1, black line).

Fig.1 Drug levels of valsartan, amlodipine, spironolactone (and metabolite canrenon) and hydrochlorothiazide in three patients after observed intake



*Hydrochlorothiazide drug levels were below the lower limit of detection after six hours

Discussion of its applications

This anecdotal overview supports the reliability and accuracy of TDM on individual patient level. To the best of our knowledge DOT and TDM were not earlier combined at an individual level giving a lot of information on inter-individual differences in drug levels and response to treatment, even in this limited number of patients.

TDM allowed us to differentiate between complete and partial non-adherence. DOT alone would have led to the conclusion of non-adherence. The first patient was fully non-adherent to her antihypertensive medication. Drug levels were undetectable at t=0 hours for all measured drugs while the drug levels should have been similar to

those obtained 24 hours after DOT. The second patient was aware of TDM and DOT, leading to temporary (partial) adherence, which resulted in lower BP than usually measured at outpatient clinic visits on admission ("white coat adherence"). Whether or not TDM can be applied unannounced is an ethical discussion. In this particular case, applying TDM urged the patient to disclose not to use spironolactone, enabling adjustment of the medication regime. The last patient was partially non-adherent, which was confirmed by TDM. If we would have obtained only DOT followed by 24h ABPM, the conclusion of partial versus full adherence would completely rely on self-reporting.

Although in the presented patients drug levels were measured at several time points, TDM enables sampling at one random time point when combined with actual BP values. For reliable TDM a sensitive well-validated method is necessary to prevent false negative results. The difference in drug levels between patients A, B, and C, most strikingly for amlodipine and valsartan, shows that knowledge of normal reference levels is necessary and that the lower limit of detection needs to be low to allow measurement at a random time point within 24 hours after drug intake. DOT is a useful tool to improve BP, however time-consuming, potentially hazardous when the BP drops abruptly and the presence of a physician is mandatory. To directly visualize the decrease in BP after DOT an observation period of a couple of hours dependent on the T_{max} of the drugs taken is recommended (8).

Previous reports suggest that direct feedback of undetectable drug levels to the patient might even improve BP control (10, 12). Although the results in the present study were assessed on individual level, we showed the potential value of TDM in non-adherent patients. We recommend a more frequent use of TDM combined with repeated BP measurements in clinical practice. We propose to ask for consent at first visit to the hypertension clinic to measure drug levels combined with repeated BP measurements at unannounced time points during follow-up. TDM is a convenient and objective method of measurement and it ensures that actual drug levels reflect the BP at the time of measurement. Due to relatively low costs per sample, its implementation was earlier concluded to be cost-effective, assuming improved adherence leads to less complications. (13, 14). In complex cases when uncertainty in both patient and physician hamper drug adherence, the combination of TDM and DOT can give useful information, facilitating further counseling on drug adherence.

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CHAPTER 8

Clinical Validation of a Dried Blood Spot Assay for 8 Antihypertensive Drugs and 4 Active Metabolites

Laura E.J. Peeters, MSc†; Lida Feyz, MD,†; Edon Hameli, BSc; Tom Zwart, BSc; Soma Bahmany, BSc; Joost Daemen, MD, PhD; Teun van Gelder, MD, PhD; Jorie Versmissen, MD, PhD; Birgit C.P. Koch, PharmD, PhD

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Abstract

Background Drug non-adherence is one of the major challenges faced by resistant hypertension patients, and identification of this problem is needed for optimizing pharmacotherapy. Dried blood spot (DBS) sampling is a minimally invasive method designed to detect and determine the degree of non-adherence. In this study, a DBS method for qualifying 8 antihypertensive drugs (AHDs) and 4 active metabolites was developed and validated using ultra high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS).

Method The DBS assay was validated analytically and clinically, in accordance with FDA requirements. Analytical validation was accomplished using UHPLC-MS/MS. For clinical validation, paired peak and trough levels of DBS and plasma samples were simultaneously collected and comparatively analyzed using Deming regression and Bland-Altman analyses. All concentrations below the set lower limit were excluded. Deming regression analysis was used to predict comparison bias between the collected plasma and DBS samples, with DBS concentrations corrected accordingly.

Results The UHPLC-MS/MS method for simultaneously measuring 8 AHDs and their metabolites in DBS, was successfully validated. With Deming regression no bias was observed in N = 1; constant bias was seen in N = 6 and proportional bias in N = 11 of the AHDs and metabolites. After correction for bias, only one metabolite (canrenone) met the 20% acceptance limit for quantification, after Bland-Altman analyses. In addition, amlodipine, valsartan, and [enalaprilate] met the 25% acceptance limit.

Conclusions A novel DBS assay for simultaneously qualifying and quantifying 8 AHDs and their metabolites, has been successfully developed and validated. The DBS assay is therefore a suitable method to detect drug non-adherence. However, with the exception of canrenone, the interchangeable use of plasma and DBS sampling to interpret drug quantities should be avoided.

Background

Hypertension is associated with increased risks of cardiovascular events and end-organ damage (1, 2). The use of antihypertensive therapy to decrease blood pressure (BP) reduces stroke rates in as high as 30% of the respective patient population (3). Antihypertensive drugs (AHDs) are an effective hypertension therapy. However, non-adherence to antihypertensive medication is one of the most common causes of uncontrolled BP, hence the incorrect label 'resistant hypertension' in as high as 50% of the respective patient population (4, 5). Ultra high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) is a reliable method for measuring plasma AHD concentrations (6-8). However, its use is hampered by logistic challenges and its vulnerability to bias, attributable to white-coat adherence. The dried blood spot (DBS) method is a more convenient and patient-friendly technique for drug concentration measurements. DBS can be performed by a simple finger prick, enabling immediate sampling (in the general practitioner's office) when non-adherence is suspected, minimizing the risk of white-coat adherence (9-11). However, minute differences exist between DBS and plasma samples, and should be taken into account when using this method. Firstly, whole blood as opposed to plasma, is used for the DBS assay, and is beneficial to drugs that adhere to red blood cells, which normally have lower plasma concentrations. Furthermore, because only a drop of blood is sampled, the blood viscosity, and thereby hematocrit, influences the quantity used for measurements. More so, the shape of the drop is important for obtaining reliable results during drug concentration measurements. It was therefore necessary to validate this method extensively, to observe for the similarities/equality in drug concentrations, compared to plasma-only measurements. Although the DBS method has been used to accurately measure immunosuppressive and antipsychotic drug concentrations (12-14), it has not been validated for AHD measurements (15-17). In this study, we developed (following analytical validation) and clinically validated a DBS method for 8 commonly used AHDs from the 4 most frequently prescribed drug classes, by qualifying and quantifying the AHD and active metabolite (displayed within [brackets]) concentrations, using UHPLC-MS/MS. Validation of the DBS-assay will give clinicians a reliable and valuable new tool to address non-adherence in patients with hypertension, which will benefit the patients by preventing cardiovascular events.

Materials and Methods

2.1 Analytical validation of the DBS method to determine reliability

2.1.1 Method Development

A sensitive UHPLC-MS/MS (Thermo Scientific, Waltham, Massachusetts, USA) assay was developed for the quantification of 8 AHDs and [their metabolites], in DBS. These include angiotensin converting enzyme (ACE) inhibitors; enalapril [enalaprilate] and perindopril [perindoprilate], angiotensin II receptor blockers (ARB); losartan [losartan carboxylic acid (ca)] and valsartan, diuretics; hydrochlorothiazide and spironolactone [canrenone], and calcium channel blockers; amlodipine and nifedipine. The UHPLC setup consisted of a Dionex Ultimate system connected to a TSQ Vantage MS with a triple quadrupole and heated electrospray ionization (HESI) probe. Plasma and DBS samples were prepared (plasma only) and analyzed (plasma and DBS) as previously described (7). For DBS sampling, filter papers (Whatman protein saver 903 card, Cardiff, UK) were used, and one blood spot contained one drop of whole blood. Using a manual disk puncher, a 6 mm diameter sample was punched out of the blood spot. Enalaprile-d5-maleate dissolved in a mixture of acetonitrile and methanol (1:1), was used as internal standard, and added to 7 AHD samples. For hydrochlorothiazide, an internal standard of hydrochlorothiazide-13CD2 was used. Thereafter, samples were sonicated, centrifuged, and diluted with eluent (7). DBS assay validation was performed following the FDA/EMA guidelines on bioanalytical method validation (18, 19), and the concept guidelines of the alternative sampling committee of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDCMT), and as previously described (12, 20, 21).

2.1.2 Hematocrit

To investigate the influence of blood viscosity on different AHD concentrations, blood with different hematocrit levels (0.3, 0.35, 0.4, and 0.45 L/L) was administered to increasing AHD concentrations on DBS (22, 23). Samples were measured using the UHPLC-MS/MS method, as previously described (7).

2.1.3 Stability

The DBS samples were analyzed in batches. Although studies indicate the relative stability of these drugs in DBS (20, 24-26), we studied AHD stability in our samples. Hence, quality control (QC) samples (QC low, medium, and high) were used, and then stored in a desiccator for 11 and 26 days after sampling, at room temperature. The 11 day storage period was considered clinically acceptable. Sample concentrations were comparable to those mentioned on supplementary Table 1. Measurements were done in 2-fold. Based on the guidelines of the alternative sampling committee of IATDCMT, a ±15% degradation in the nominal value was considered acceptable (27).

2.2 The clinical validation methods: DBS concentrations versus plasma concentrations

2.2.1 Patients

Samples were collected between October 2016 and July 2018. A total of 135 patients were included, aiming for 40 paired samples/drug to validate the DBS method and ensure statistical test validity, when comparing DBS and plasma AHD/[metabolite] concentrations (24). Patients were selected from the outpatient clinic (internal medicine, cardiology, and nephrology) at the Erasmus Medical Center, Rotterdam, The Netherlands.

As inclusion criteria, the automated BP measurement (ABPM) (Datascope Accutor Plus, Paramus, NJ, USA) or office BP had to be <135/85 mmHg, to optimize the chances of sampling an adherent patient, and the use of ≥2 AHDs for which the DBS method had been developed. All available drug dosages were exploited, in a bid to test and increase their usability in clinical practice. Exclusion criteria included the inability to provide written informed consent and proven non-adherence, after sampling and inclusion; based on non-measurable drug concentrations derived from plasma and DBS samples when a peak drug level was expected. This study was approved by the medical ethical committee of the Erasmus Medical Centre, Rotterdam, The Netherlands (MEC-2016-162).

2.2.2 DBS and Plasma Sampling

DBS samples were obtained using the finger prick method, following predetermined instructions; washing hands, finger massage before and after puncture, and sampling without card contact. The spots were allowed to dry for at least 20 minutes, and the cards folded to keep the blood spots away from light, in order to prevent the degradation of the photo labile compounds amlodipine and nifedipine (28). The DBS cards were then stored at room temperature in plastic zipper bags containing silica desiccant, transported to the laboratory, and placed in a desiccator until further analyses. Venous whole blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes during a regular venipuncture, preferably obtained within the hour before or after DBS sampling. For study purposes, an extra tube of blood was drawn (asides that requested by the physician), sent to the pharmacy laboratory, centrifuged, and the resulting plasma stored at - 80°C until analyses.
For each AHD, 2 hospital visits were required, since the trough and peak levels of the same patient were measured to validate the entire potential drug concentration range (24, 29). The first visit, where BP was measured using ABPM, was planned in the morning to ensure drug levels were measured approximately 1-7 hours after AHD intake (peak levels). During the second visit (approximately 2 weeks later), venipuncture and DBS were performed to measure trough levels. Here, patients were asked to delay their AHD intake until after the appointment, to maximize trough levels at an interval of ≥20 hours post intake. After attaining 20 samples (peak and trough levels from 10 patients) per drug, the peak levels for the remaining samples were then measured, as required for validation. This adaptation improved the convenience of study participation, as only 1 visit to the hospital was required. Furthermore, it limited the number of negative values (trough levels >24 hours after the last intake were not observed). Additional trough levels were obtained solely from patients who accidentally failed to self-medicate on the day of measurement. Patients were allowed to participate more than once.

2.3 Data analysis

2.3.1 Analytical Validation

To determine DBS method reliability, the following quantification limits were calculated, following the FDA/EMA guidelines on bioanalytical method validation

(18, 19): linearity (by means of a calibration curve), limits of quantification, intra- and inter-day accuracy, precision, and stability. Matrix effects were measured as a whole, as opposed to individually, since different matrices were used for subsequent days. Reproducibility data were then used to determine any matrix effects.

2.3.2 Clinical Validation

To compare quantitative data from DBS and plasma samples, all samples below the LLOQ (lower limit of quantification) were discarded. Outliers were determined using boxplot analyses, as samples with extreme values were excluded in further analyses. Deming regression analyses were then performed to assess any constant and/or proportional bias between plasma and DBS AHD/[metabolite] measurements (12). A proportional bias was obtained if the 95% confidence interval (95%-CI) of the regression line slope did not contain 1. When the 95%-CI of the regression line intercept did not contain 0, the data was considered to have a constant bias. These results were used to calculate the 'estimated plasma concentration' (30). The following formula was used, and adjusted with respect to constant and/or proportional bias:

Estimated plasma concentration = (DBS concentration - intercept of Deming regression line)/slope of Deming regression line.

Using a Bland-Altman plot, the plasma AHD/[metabolite] concentration measured from venipuncture samples was compared to the 'estimated plasma concentration' derived from DBS samples (24, 31). According to the European Guidelines on Bioanalytical Method Validation, ≥67% of the measurements per drug should be within 20% of the mean of the differences between the both methods (18). This difference was adopted as the acceptance limit, although alterations could be made, based on clinical relevance (12, 32). Previous work showed that a within 25% difference is also acceptable in AHDs, owing to their wide therapeutic range (16). More so, to determine the suitability of the DBS method in clinical practice, the number of DBS false negatives, compared to those of plasma, were calculated. Here, all samples were included independent of their LLOQ.

Analyses were performed using the IBM SPSS statistics v24.0 software for Windows (IBM, Armonk, NY, USA) and GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA).

Results

3.1 Analytical validation of the method

A summary of the quantification limits is presented on supplementary Tables 1, 2, and 3. The lower limit of detection (LLOD) and LLOQ for all drugs measured with DBS were determined during analytical validation. For most analytes, the lowest QC was higher, compared with the lowest measured DBS concentration. Hence, almost all low QCs failed to meet the requirements. Therefore, the LLOQ was raised (in comparison with the plasma method) to an acceptable value, in accordance with the QCs.

3.1.2 Hematocrit

No differences were observed in AHD concentrations, despite increasing hematocrit quantities. Therefore, no correction was required for measured DBS AHD/[metabolite] concentrations, with regard to hematocrit.

3.1.3 Stability

DBS cards with low, middle, and high concentrations of the 8 AHDs and their metabolites were prepared on different dates, and assessed for stability (supplementary Table 4). Amlodipine, losartan, [losartan-ca], [perindoprilate], and enalapril, at all concentrations, showed no relevant differences in almost all QCs, after 11 and 26 days (<15% from the nominal value). [Canrenone] displayed a difference in QC low and medium after 11 and 26 days, which exceeded the acceptance limit. Although valsartan and spironolactone showed no relevant differences at low and median concentrations, their QC high values were above the acceptance limit after 11 and 26 days. Nifedipine stability data showed >20% degradation at medium and high concentrations. Hydrochlorothiazide and [enalaprilate] proved unstable at different concentrations at both time points.

3.2 Clinical validation of the DBS method

3.2.1 Sampling

A total of 195 samples were obtained from 135 unique patients. Patients (N = 3) were excluded when negative plasma and DBS analyses were found in peak drug levels, an indication of total or partial non-adherence.

3.2.2 Deming Regression Analyses

Prior to these analyses, samples below the LLOQ for DBS and/or plasma were initially excluded (Table 1), followed by the exclusion of 2 [enalaprilate] sample outliers. These outliers were seen in the venipuncture, and corresponding DBS samples. The results of the Deming regression analyses are presented on Table 1. Constant and proportional biases were observed for the following 6 drugs of which [1 metabolite]: hydrochlorothiazide, losartan, nifedipine, perindopril, [perindoprilate], and spironolactone. Proportional bias was observed for the following 5 drugs of which [3 metabolites]: amlodipine, [canrenone], [enalaprilate], valsartan, and [losartan-ca] (Figure 1 A1-3).

Table 1 Results of Deming Regression analyses after correction with the conversion formula for the comparison of plasma and estimated plasma concentrations (derived from DBS)

Analyte	N*	N ≤ LLOQ DBS	N ≤ LLOQ Veni	Conversion formula	Slope	95% Confidence interval	Y-intercept	95% Confidence interval
Amlodipine	57	7	4	EPC = DBS* (1/slope)	1.443	1.258 – 1.628	2.537	-0.990 – 6.606
Enalapril	25	12	7	No bias	0.960	0.737 – 1.182	6.272	-3.901 – 16.450
Enalaprilate	27**	9	0	EPC = DBS* (1/slope)	0.416	0.349 – 0.484	4.348	-2.822 – 11.520
Hydrochloro-thiazide	42	26	42	EPC = ((DBS-intercept)* (1/slope))	3.540	2.225 – 4.856	-105.900	-276.600 – 64.680
Losartan	24	18	10	EPC = ((DBS-intercept)* (1/slope))	0.359	0.889 – 0.428	29.660	3.226 – 56.100
Losartan-ca	24	18	0	EPC = DBS* (1/slope)	0.506	0.330 - 0.682	5.337	-86.460 – 97.14 0
Nifedipine	15	25	4	EPC = ((DBS- intercept)* (1/slope))	0.622	0.330 – 0.915	29.250	10.580 – 47.910
Perindopril	14	26	23	EPC = ((DBS- intercept)* (1/slope))	0.745	0.623 – 0.866	5.782	2.360 – 9.204
Perindoprilate	19	22	1	EPC = ((DBS- intercept)* (1/slope))	0.485	0.328 – 0.642	3.688	0.480 – 6.896
Spironolactone	9	30	18	EPC = ((DBS- intercept)* (1/slope))	0.527	0.242 - 0.812	18.650	10.530 – 26.780
Canrenone	22	20	2	EPC = DBS* (1/slope)	0.876	0.764 – 0.988	6.748	-7.330 – 20.840
Valsartan	42	0	0	EPC = DBS* (1/slope)	0.553	0.479 – 0.627	-6.017	-211.200 – 199.100

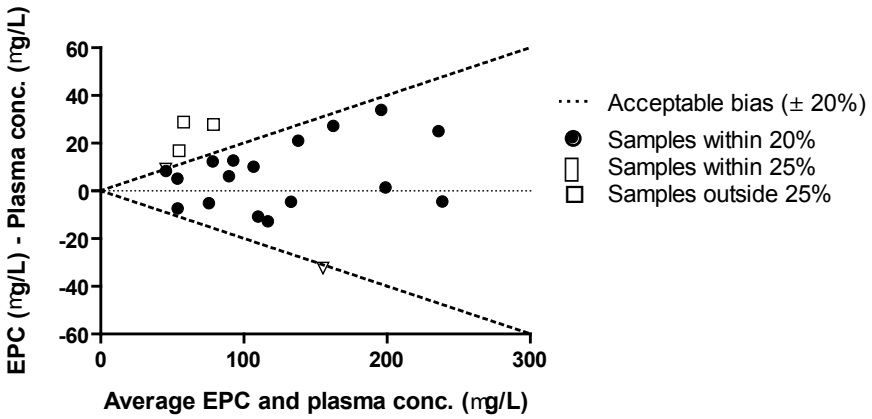
After exclusion of samples below the LLOQ of DBS and/or plasma. Enalaprilate numbers were decreased as a result of two outliers*
N = number of samples, EPC = estimated plasma concentrations, DBS = Dried Blood Spot, ca = carboxylic acid, LLOQ = Lower Limit of Quantification, Veni = sample collected by venipuncture.

3.2.3 Bland-Altman Analyses

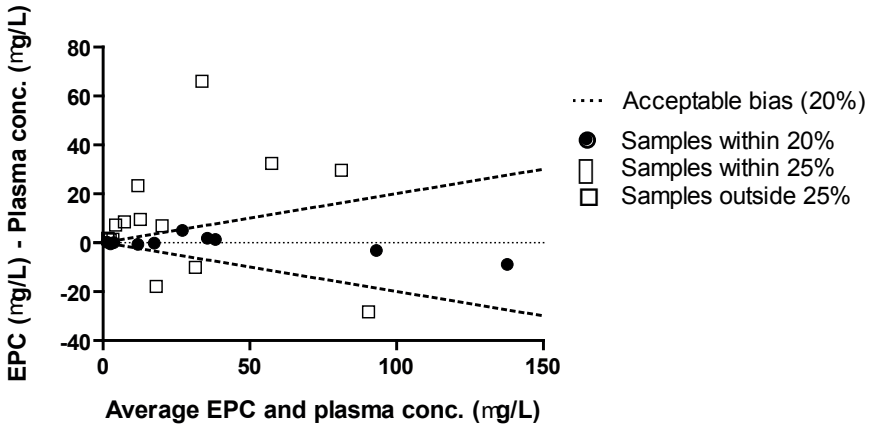
Results of the proportion within the 20 and 25% range calculated using Bland-Altman analyses are presented in Table 2. Only [canrenone] met the 20% acceptance limit (Figure A1). For the 25% acceptance limit, additional AHDs; amlodipine, valsartan, and one metabolite; [enalaprilate], met the criteria. Moreover, the metabolites showed a better agreement, compared with their respective parent drugs (appendix; enalapril versus [enalaprilate], Figure A2 versus A3).

Figure 1: Bland-Altman analyses of [canrenone] (A1), enalapril (A2), and [enalaprilate] (A3). The dotted line in each graph depicts 20% acceptable bias when comparing plasma and dried blood spot measurements. Enalaprilate and canrenone are the active metabolites of respectively enalapril and spironolactone and are shown within brackets. EPC= estimated plasma concentrations.

A1. Bland-Altman [Canrenone] (N = 22)



A2. Bland-Altman Enalapril (N = 25)



A3. Bland-Altman [Enalaprilate] (N = 27)

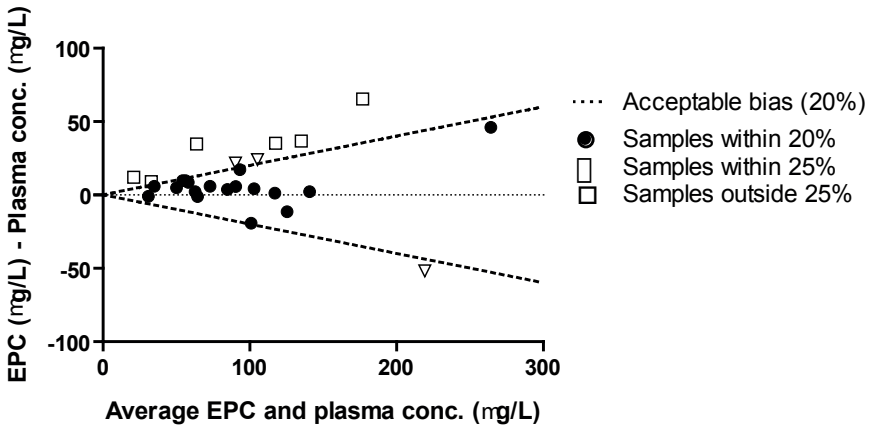


Table 2 Results of Bland-Altman analysis of plasma versus estimated plasma concentrations (derived from DBS)

Analyte	N*	Mean bias	95% Limits of agreement	Δ Within 20% of Average (%)	Δ Within 25% of Average (%)
Amlodipine	57	1.76	-6.36 – 9.87	57.9	**71.9*****
Enalapril	25	5.17	-30.13 – 40.48	36.0	44.0
Enalaprilate	27**	10.45	-32.41 – 53.32	66.7	**77.8*****
Hydrochlorothiazide	42	-0.03	-113.58 – 113.51	45.2	52.4
Losartan	24	-0.01	-291.88 – 291.86	12.5	16.7
Losartan-ca	24	10.61	-435.21 – 456.43	41.7	45.8
Nifedipine	15	0.00	-48.53 – 48.54	26.7	33.3
Perindopril	14	0.00	-11.99 – 11.99	28.6	28.6
Perindoprilate	19	0.01	-12.77 – 12.78	31.6	31.6
Spironolactone	9	35.39	14.82- 55.96	33.3	33.3
Canrenone	22	7.69	-24.44 – 39.82	**77.3*****	**86.4*****
Valsartan	42	-0.09	-1457.49 – 1457.31	61.9	**73.8*****

** After exclusion of samples below the LLOQ of DBS and/or plasma. ** Enalaprilate numbers were decreased as a result of two outliers. *** Values that are in the agreement of the acceptance limit of >67% of the samples within 20% or 25% difference of the mean. DBS = Dried Blood Spot, ca = carboxylic acid*

3.2.4 DBS False Negatives

False negatives for DBS compared to plasma are displayed on supplementary Table 5. Overall, most negative values were observed for the parent drugs: enalapril (5.2%), losartan (4.8%), perindopril (4.9%), and spironolactone (20.9%). Amlodipine, nifedipine, and [enalaprilate] only had a single false negative, while valsartan, [canrenone], and [losartan-ca] had no false negatives. Nevertheless, hydrochlorothiazide and [perindoprilate] had more false negatives for DBS (9.4% and 9.8%, respectively), compared with other measured drugs. These false negatives were found in random samples, independent of drug dosage.

Discussion

In this study, a clinically convenient DBS method for qualifying and quantifying 8 AHDs and 4 metabolites from the most frequently prescribed drug classes, was validated using UHPLC-MS/MS. To the best of our knowledge, this is the first study that compared AHDs in DBS and plasma in an extensive number of samples from actual patients, instead of spiked plasma samples. A good agreement was observed with Deming regression analyses, after correction for outliers and bias. Based on Bland-Altman analyses, only [canrenone] met the 20% acceptance limits, implying that DBS and plasma sampling could be used interchangeably when quantifying [canrenone]. Also, the 25% acceptance limit was met by valsartan, amlodipine, and [enalaprilate].

4.1 Analytical validation

DBS is a reliable method for qualifying and quantifying 8 AHDs and 4 of their metabolites. However, the LLOD and LLOQ were higher in DBS, compared to plasma (Supplementary Table 3). This was mainly due to the deviation in the lowest QC of most drugs, during analytical validation. If the DBS method is used for AHD quantification, accurate measurement of trough levels could be a challenge.

Our stability data showed that spironolactone and [canrenone] were close to the acceptance limit of a <15% degradation. This reduced stability was expected for spironolactone, as shown by van der Nagel et al. for plasma (7). [Canrenone] exceeded the limits for stability in QC low and medium after 11 and 26 days, contrasting an earlier study by Suyagh et al. showing its DBS stability for as long as 1 month post-sampling (33). Also, [enalaprilate] and hydrochlorothiazide stability data were hard to interpret

as they were stable at different concentrations and time points. Technical issues in sample collection, capable of influencing the quality of the spot, might have influenced stability outcome. Nifedipine was the only unstable AHD after 11 days. Generally, these inconsistencies can be attributed to the fact that DBS sampling is more susceptible to inter-individual variability. Therefore, it is thought that most drugs are more stable than observed, with the exception of nifedipine.

4.2 Clinical Validation

Clinical validation was necessary to determine if DBS and plasma can be interchangeably used to quantify drugs. Here, the LLOQ is one of the most important parameters. Quantitative measurement is only accurate above the LLOQ. Therefore, only measurements above the LLOQ should be used in clinical validation studies. Given the raise in DBS LLOQ, more samples than expected had to be excluded. Prior to sample measurements, approximately 40 samples per drug were collected to meet the guideline-based clinical validation criteria. However, only 3 of the 8 drugs measured in this study had the appropriate amount of samples after excluding samples below the LLOQ, and outliers. For nifedipine, perindopril, [perindoprilate], and spironolactone in particular, the number of excluded samples was so high, denting the results from the Bland-Altman plot. Spironolactone is rapidly converted to [canrenone] after intake, a probable reason why only few samples of spironolactone were above the LLOQ. Therefore, for better quantitative value interpretation, it is necessary to include [canrenone] when measuring spironolactone. Fortunately, [canrenone] had more samples above LLOQ and could be used to determine plasma concentration post spironolactone intake.

However, for enalapril, losartan, and [losartan-ca], which failed to meet the Bland-Altman criteria and had <40 working measurements, it is unlikely that adding samples will enable the attainment of the required acceptance limits.

The number of measurable concentrations for hydrochlorothiazide was low in our study population, for DBS and plasma, compared to the quantity of samples collected. Approximately half of all samples were excluded, owing to a relatively high LLOQ, established via analytical validation. This is probably due to a rapid decrease in plasma concentrations, whereby hydrochlorothiazide levels are already below the LLOQ at 12 hours post intake (34, 35). UHPLC-MS/MS optimization is therefore

necessary for improving hydrochlorothiazide LLOQ. However, in clinical practice, hydrochlorothiazide is often administered as a combination tablet; hence, the presence of other drugs can, in this case, be used as indication of hydrochlorothiazide intake.

When comparing blood drug concentrations without adjustment for bias, higher DBS concentrations, compared to plasma, were found for amlodipine, hydrochlorothiazide, nifedipine, and perindopril. Conversely, for [perindoprilate], [enalaprilate], losartan, [losartan-ca], and valsartan, higher plasma concentrations were observed. This was independent of the drugs' protein binding or lipophilicity properties. However, the higher drug concentrations in whole blood have been attributed to strong adhesion to red blood cells (36, 37). It is unclear if these findings are related to the outcomes of the Bland-Altman analyses.

The Deming regression analyses of losartan and perindopril showed a great variation between plasma and DBS at peak levels. The latter could be attributed to the fact that not all plasma and DBS samples were taken simultaneously, but often 30-60 minutes apart. Losartan and perindopril peak concentrations have reportedly been attained an hour post intake (38, 39). [Losartan-ca] and [perindoprilate], the active metabolites of their parent drugs, attain peak concentrations at 3-4 hours post drug intake (40, 41). In this study, sampling was often done between 1-5 hours post drug intake, corresponding to the metabolite and parent drug peak concentration times. At peak concentrations, steep slopes exist, emphasizing the importance of time between sampling. This is one possible explanation for not meeting the Bland-Altman acceptance criteria.

Also, perindopril and [perindoprilate] DBS and plasma values were much lower, compared to valsartan for instance (highest concentration for [perindoprilate] vs. valsartan measured with DBS: 23.51 μg/L vs. 3883.06 μg/L). This difference is mainly attributable to the settings of the UHPLC-MS/MS method. As a result, deviations in [perindoprilate] plasma and DBS concentrations have a much greater impact on the Bland-Altman outcomes.
False negatives can result in wrong assumptions towards patient non-adherence. [Perindoprilate] had the most false negatives, compared to other metabolites. However, these results can be attributed to the sampling time post drug intake. For instance, one sample was taken <1 hour post drug intake, and another, 36 hours after intake (trough sample). This explains why both samples showed no [perindoprilate] for the DBS

method. Wrong patient information on the time of drug intake may have also skewed results. Here, [perindopril] was totally absent in the plasma and DBS sample, and low [perindoprilate] concentrations were observed in the plasma samples. These findings were in accordance with samples used for trough level measurements, indicating that the information given by the patients on time of drug intake was inaccurate. Other false negatives were mainly observed for parent drugs, and were nullified by results from their corresponding metabolites. It is therefore very important for any non-adherence detection method to include the parent drug, as well as their metabolites.

4.3 Considerations when using DBS

The main advantage DBS has above plasma measurements is the ability of sampling to occur at the same time as BP measurements, and at any given location, without the need for an additional visit to a blood sampling facility. DBS sampling is less invasive and less time-consuming for the patient, compared to venipuncture. For the 8 AHDs measured, the metabolites were more often detected, compared with the parent drugs. Furthermore, for the AHDs with the best correlation, like [canrenone] and [enalaprilate], DBS could also be useful for PK/pharmacodynamic (PD) modeling, with more intense sampling. This could be convenient for patients who have great variation in drug concentrations when using the same dose, including the elderly and female patients (42).

Differences between DBS and plasma can be explained from several angles; stability, DBS sampling method, and blood spot quality (43), with the sampling method and blood spot quality being of great importance. According to previous studies, measuring a different spot on the same card, which theoretically contains the same drug concentration, showed a difference in mean, from -25% to almost 40% (43). This could be attributed to different spot sizes, irregularities, spot overlap, or multiple spots in one area. Some suboptimal spots were also seen in our samples, particularly in the drug concentrations that deviated from the Bland-Altman acceptance limits. It is therefore recommended to evaluate spot quality before analyses, as not all blood spots are similar and/or of good quality. This is also the most likely explanation for the stability results observed in this study, during analytical validation. Stability challenges were observed for nifedipine when the drug was sampled with DBS, which may explain why it failed to meet the acceptance limits for Bland-Altman analyses. Despite the possible stability and technical sampling problems, all AHDs in DBS were

measured, with some agreement in the Bland-Altman analyses, when DBS and plasma measurements were compared. This implies the efficiency of DBS in determining non-adherence for all measured AHDs over all available dosages, as it could measure the presence of a drug with a high accuracy for as long as 24 hours post intake; the most important factor. This can preserve a good patient-physician relationship, which is key to properly discussing the outcome of the absence of drugs in the blood. The use of DBS, as well as discussions on the outcome, to improve non-adherence, is currently studied in a large multicenter trial called RHYME-RCT (NL6736). However, to quantify drug concentrations, it is important to choose one of the methods (DBS or plasma) and avoid interchanges. Also, a more detailed analyses with respect to the quantitative values, might be considered in the future, to determine long-term adherence.

Conclusion

DBS is a reliable and accurate method for measuring AHDs, and is therefore applicable for non-adherence assessment/detection. DBS sampling enables AHD quantification. However, when measuring trough levels, venipuncture is more accurate. Additionally, DBS and plasma should not be used interchangeably when quantifying AHDs, with the exception for valsartan.

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CHAPTER 9

Clinical applicability of monitoring antihypertensive drug levels in blood

Laura E.J. Peeters, PharmD, Lida Feyz, MD; Eric Boersma, PhD,
Joost Daemen, MD, PhD; Teun van Gelder MD, PhD;
Birgit C.P. Koch PharmD, PhD; Jorie Versmissen, MD, PhD

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Abstract

Dried blood spot (DBS) analysis is a novel analytical method for therapeutic drug monitoring to identify non-adherence to antihypertensive drugs. This study was conducted to evaluate the clinical applicability of measuring drug concentrations of eight antihypertensive drugs, using DBS and venipuncture. Furthermore, this study aimed to provide more insight into the between-patient variability in drug concentrations.

False negative values from DBS compared to a venipuncture were determined to assess drug adherence. A Generalized Estimating Equation (GEE) was used to estimate the model parameters including sex, dose, age, weight and the time interval between drug intake and sampling, on the C_{plasma} (drug concentration in plasma).

No false negative values were found when measuring non-adherence using DBS compared to venipuncture. A high variability in C_{plasma} between patients was observed, especially at peak concentrations with a fold change reaching from 2.3 to 35.2. The time of intake was significantly related to the height of the C_{plasma} in 7 of the 8 measured drugs with a p-value < 0.05, but the influence of dose, weight, age and sex on drug levels differed largely between the measured drugs.

DBS is a reliable and convenient method to assess non-adherence to antihypertensive drugs in clinical practice. The C_{plasma} of the eight antihypertensive drugs in this study show a large inter-individual difference and therefore low plasma concentrations do not necessarily mean non-adherence. Non-adherence can only be confirmed if drug levels are undetectable, that is, values below the lower limit of detection.

Introduction

Optimal (pharmacological) treatment of hypertension is of major importance to reduce the incidence of cardiovascular and kidney disease (1, 2). The inability to reach blood pressure (BP) targets despite the use of three or more antihypertensive drugs is defined as resistant hypertension (RH), of which the etiology is multifactorial (1, 3). While exact figures are lacking, non-adherence has been reported in up to 50% of patients with resistant hypertension (4). These non-adherent patients are not truly resistant to their medication but have so-called pseudo-resistant hypertension (pseudo-RH). It is assumed that about half of the patients with pseudo-RH can be explained by non-adherence (2, 5, 6). Assessment of non-adherence and improving adherence is of major importance to reduce disease burden and costs (7). Over the years several tools have been developed to identify non-adherence, including (self-reported) questionnaires, the use of prescription record views and electronic monitoring with devices such as the medication electronic monitoring system caps (MEMS), all with inherent strengths and weaknesses (6, 8, 9). With Therapeutic Drug Monitoring (TDM) drug concentrations can be measured in blood or other body fluids, to identify non-adherence to prescribed drugs (6). Ideally, TDM must be performed at the time of BP measurement to link drug concentrations directly to the BP. However, in clinical practice, this is logistically often not possible, as blood or urine samples are mostly retrieved at another time point at a phlebotomy department. To address the aforementioned limitations we developed a dried blood spot (DBS) method to measure plasma concentrations of eight antihypertensive drugs and four of their metabolites using ultra-performance liquid chromatography- tandem mass spectrometry (UPLC-MS/MS). DBS can easily be obtained directly in the office by a finger prick, and is therefore a convenient and patient friendly method to assess non-adherence (10). It does allow blood sample collection in close proximity to the blood pressure measurement. In our previous study the DBS method was analytically and clinically validated, in patients with controlled hypertension who were therefore assumed to be adherent (11). While using DBS in routine clinical practice requires adequate diagnostic performance in order to prevent false acquisitions of non-adherence. Therefore, it is important to check whether or not the DBS method is subject to false negative results compared to the golden standard. Although qualitative data (present yes or no) are most important to determine non-adherence, quantitative data might give more information (12). However, the effects of the time interval between drug intake and blood sampling on drug concentrations,

and on between-patient variability in drug concentrations need to be known to draw conclusions from quantitative data. For some antihypertensive drugs the drug metabolism is such that over a 24-hour dosing interval in the elimination phase the parent drug is no longer detectable, while (active) metabolites can be detected. In that case, the interpretation of the data detection of active metabolites was/is sufficient to conclude the patient was adherent. Therefore, in this study the clinical implication of the use of DBS sampling and the variance in quantitative values between patients derived by TDM, for eight antihypertensive drugs of which four of active metabolites (displayed within brackets) was studied.

Methods

The data that support the findings of this study are available from the principal investigator upon reasonable request. Requests to access the dataset may be sent to J. Versmissen at j.versmissen@erasmusmc.nl.

Study design

A single center, cross-sectional observational study was conducted to assess drug concentrations of antihypertensive drugs using UPLC-MS/MS in simultaneously collected DBS and plasma samples (11, 13). Eight drugs from the four most frequently prescribed drug classes and their active [metabolites] were measured: Angiotensin Converting Enzyme inhibitors (ACEi): 1) (enalapril [enalaprilate]; 2) perindopril [perindoprilate]), angiotensin II receptor blockers (ARB): 3) (losartan [losartan carboxylic acid], 4) valsartan), diuretics: (5) hydrochlorothiazide and 6) spironolactone [canrenone]) and calcium channel blockers: (7) amlodipine and 8) nifedipine) (13). To measure drug concentrations, only patients that were likely to be adherent were selected. Therefore, patients were eligible if they used at least 2 of the drugs that were measured with DBS and had an office blood pressure <135/85 mmHg at time of the screening (11). Analysis of the DBS and plasma samples have been described in our clinical validation paper (11). For each drug, at least 10 trough levels were sampled. For the trough concentrations patients were asked to postpone their once daily dosage until after the blood sampling. Peak levels were sampled up until a total of 40 samples per drug were collected including samples from trough levels. As fixed-dose combination tablets were often used, sample numbers varied between 38 – 85 for each drug. The study was approved by the medical ethical committee of the Erasmus Medical Centre

(MEC-2016-162) and written informed consent of each patient was obtained before samples were collected (11).

Blood pressure measurement

An unattended BP measurement was performed previously to the physician's appointment with an automated office blood pressure (AOBP) measurement (Accutor Plus, Datacope , Paramus, USA). An appropriate cuff size was used with the adapted cuff placed at the level of the brachial artery. The AOBP measurements were done according the recommended guidelines (14) and during at least 30 minutes BP measurements, spaced with 5 minutes apart, were performed. Target blood pressure was defined as the average of all measurements <135/85 mmHg.

Data analyses

DBS method

The most important parameter to assess adherence using measurement of drug concentrations, i.e. whether a drug is present in blood, is the lower limit of detection (LLOD). This is the lowest concentration of a specific drug that can be detected reliably, i.e. statistically distinguished from a blank sample with a 99%-confidence level. Approximately 10 trough levels for each drug were obtained in order to assess whether or not after an interval of 24 hours a positive drugs concentration could still be found. DBS samples were compared to the plasma samples obtained by venipuncture to determine the number of false negative values in DBS. False negative values of plasma samples were also determined as either the parent drug or metabolite was available to confirm intake of a drug or combination tablets were used. False positive values of DBS could therefore be excluded when false negative plasma samples were present.

Clinical implication of measuring plasma concentrations

Quantitative values derived using TDM can provide more insight on whether a patient had taken a dose in the previous 24 hours. Because less samples could be quantified with the DBS method, plasma drug concentrations (venipuncture) were used to gain more insight in the relation between drug concentration and time after intake (11). For this purpose C_{plasma} vs time graphs were made and assessed visually.

To investigate the variability in drug concentrations between patients, several samples around the supposed t_{max} from literature were compared. Since values from both methods cannot be used interchangeably, as shown in the clinical validation study, for this purpose C_{plasma} were used. The drug concentrations were plotted over time to determine variability in C_{plasma} and thereby taking into account drug dose and time after intake.

Linear regression analyses were applied to determine the influence of age, sex, dose, weight and time since intake of the drug on C_{plasma}. Generalized estimating equations (GEE) were used to estimate the model parameters, thus accounting for clustering of data within individuals.

Analyses were performed with IBM SPSS statistics 24.0 for Windows (IBM Corp, Armonk, NY) and GraphPad Prism 5 software (GraphPad Software, La Jolla, CA).

Results

Study population

Patient characteristics

In total 135 patients were included from the vascular, nephrology and cardiology outpatient clinic from the Erasmus Medical Centre, Rotterdam. All patients had well controlled blood pressures with a mean BP of 132 ± 15 mmHg systole and 78 ± 10 mmHg diastole based on an average of 8 ± 2 measurements (Table 1). On average the population was overweight (BMI 29.0± 4.5 kg/m^2) and kidney function was not impaired (N = 7 (5.2%) with eGFR < 30 mL/min/1.73m^2). Female participants used perindopril in 27.5% of the cases and amlodipine in 30.5% of the cases which is less often compared to the female participants in the study (40.8%) (Table 3). For nifedipine the opposite was found, as more than 50% of the nifedipine users were female. For the other measured drugs distribution between males and females were equal (Table 3).

Table 1 General characteristics of the study population

	Total study population N=135
Age, years	59 ± 12
Male n, (%)	87 (59.2)
BMI, kg/m²	29.0 ± 4.5
CKD-EPI eGFR, mL/min/1.73m² (median (IQR))	70 (33.1)
Mean SBP, mmHg	132 ± 15
Mean DBP, mmHg	78 ± 10
Nr. of AHDs	3 ± 1
Nr. of AHDs measurable by DBS	2 ± 1
Other drugs, n (%)	
Antiplatelet	58 (39.7)
Oral anticoagulant	21 (14.4)
Cholesterol lowering drugs	96 (65.8)
Antidiabetics	38 (26.0)

Values are mean ± SD or n (%) except for the eGFR which is depicted with the median and IQR. AHD = antihypertensive drug, BMI=body mass index, CKD-EPI=Chronic Kidney Disease Epidemiology Collaboration, eGFR=Estimated Glomerular filtration Rate, IQR=Interquartile Range, DBS=Dried Blood Spot. SBP= systolic blood pressure/DBP= diastolic blood pressure (both measured through datascope)

DBS method

The trough drug levels with DBS could be detected within a median time of 25 hours [IQR 17 – 27] after the last reported drug intake.

Negative values (<LLOD) of the antihypertensive drugs with inclusion of the active metabolites were present in both the DBS and the plasma samples (17-36 hours after intake). The number of samples per drug that showed negative values for DBS were comparable to the number of negative values in plasma (Table S1). After evaluation the few negative values found in DBS compared to plasma were not of clinical relevance as they would not change the verdict of being adherent or non-adherent.

Clinical applicability of measuring quantitative plasma concentrations

In Figure 1, 2 and 3 and Figure S3-S9 (Supplementary data) drug levels for different dosages obtained at different time points after reported intake are shown. Based on these figures, drug levels seem highly variable. On the t_{max} of the measured drugs or metabolites there was a median fold-change between the lowest and highest measured plasma concentration (C_{plasma}) of the same dosage of 3.52 [IQR 1.54-7.43] (Table 2).

Table 2 Fold-change in plasma concentrations at T_{max} of antihypertensive drugs with a same dosage

Drug [metabolite]	Dose of drug to measure fold-change (mg)	Fold-change plasma concentration at supposed T_{max}* (mcg/L)			Supposed T_{max} (hours)
		Lowest concentration	highest concentration	Fold - change	
Amlodipine	10	11.00	46.1	4.19	6-12
[Enalaprilate]	20	51.36	139.6	2.71	4
Hydrochlorothiazide	12.5	39.59	105.3	2.66	4
[Losartan-ca]	100	18.10	637.0	35.2	4
Nifedipine	60	20.86	89.8	4.3	1.6-4.2
[Perindoprilate]	8	4.72	45.3	9.61	3-4
[Canrenone]	25	42.0	98.0	2.3	4.3
Valsartan	320	1310.87	6876.4	5.25	2-4

* Fold-change plasma was calculated with data from approximately 0.5 hours around the supposed Tmax if no range was supposed

Figure 1. Plasma concentration-time curve amlodipine, N=55

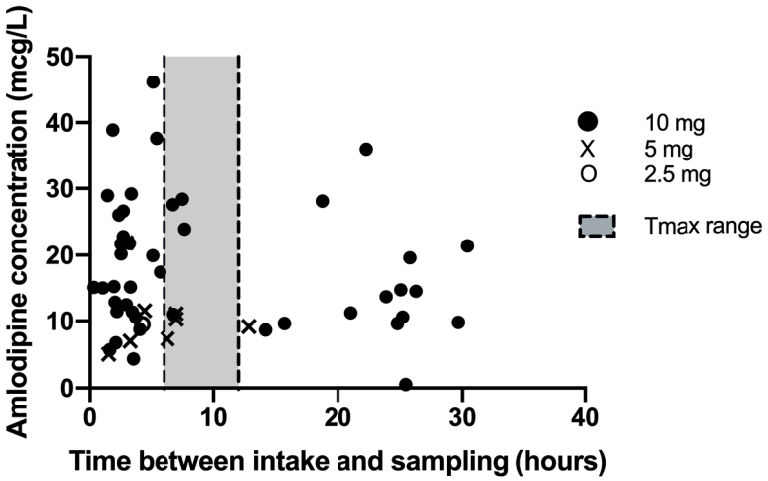


Figure 2. Plasma concentration-time curve canrenone, N=40

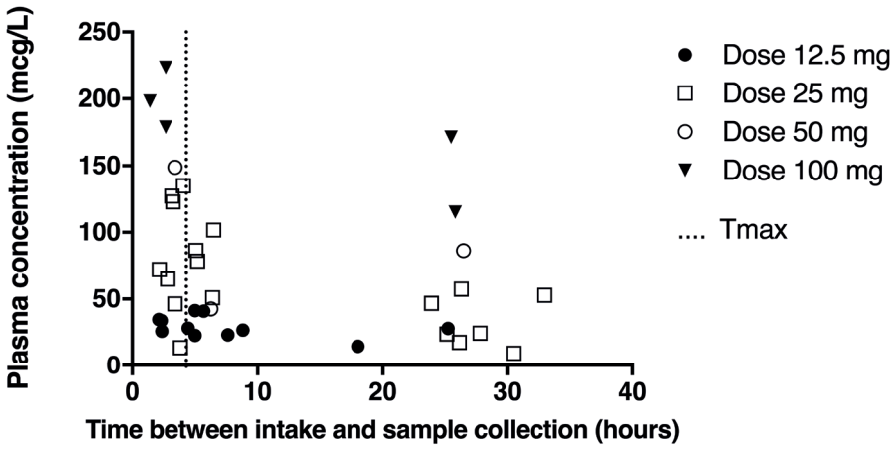
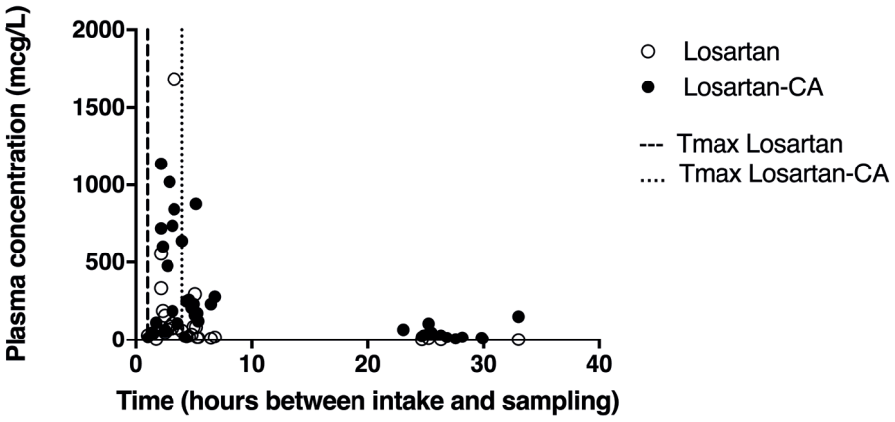


Figure 3. Plasma concentration-time curve losartan, N=32 and losartan-CA, N=41



The low C_{plasma} at the t_{max} from certain patients were in the same range as the C_{plasma} measured at t = 24 h (trough level) for other patients that used the same drug. Also, C_{plasma} did not seem to predict the blood pressure measured on the time of sampling (Figure S2, Supplementary data).

The influence of several parameters on the C_{plasma} was analyzed using linear regression models (Table 3). The dosages and time between intake and sampling were in 7 out of 8

drugs significantly related to the C_{plasma} ($P<0.05$). On average a higher dosage was related to an increase in drug concentration. An increase of 1 mg drug led to the lowest increase average of 0.12 mcg/L for nifedipine and highest increase of 8.3 mcg/L of valsartan. Also, weight had a significant influence on the differences in C_{plasma} of losartan-ca. In this specific case increased weight of the patients seemed to be negatively correlated with the C_{plasma}, resulting in lower concentrations of losartan-ca (-6.28 mcg/L per 7 kg increase in weight). Furthermore, there was a significant relation between age and the C_{plasma} of canrenone and perindoprilate. However, higher age was negatively correlated to the C_{plasma} of canrenone, so an increase in age led to a lower C_{plasma} (-3.33 mcg/L/ year older) and positively correlated to the C_{plasma} of perindoprilate (+0.26 mcg/L/ year older). Only in case of canrenone a significant difference was found between the plasma concentrations in males vs females. At the same time point, dosage, weight and age, females had a higher plasma concentration compared to males with an average difference of 30 mcg/L.

Table 3 Average difference in plasma concentrations in relation to sex, time after intake, dosage, body weight and age

Drug [metabolite] (mcg/L)	Female (%)	Male vs female	Time (h)	Dose (mg)	Weight (kg)	Age (year)
Amlodipine (mcg/L)	30.5	-4.8	-0.21*	1.5‡	-0.16	0.12
[Enalaprilate] (mcg/L)	39.4	-12	-3.1†	-1.9	-0.93	-0.03
Hydrochlorothiazide (mcg/L)	43.5	-31	-3.0	0.74	-0.75	1.1
[Losartan-ca] (mcg/L)	47.6	17	-9.4‡	5.8†	-6.28†	12
Nifedipine (mcg/L)	51.4	-4.5	-1.0	0.12	0.20	0.73
[Perindoprilate] (mcg/L)	27.5	1.9	-0.50‡	0.55	-0.03	0.26*
[Canrenone] (mcg/L)	42.3	30*	-2.1‡	0.17*	-0.32	-3.33†
Valsartan (mcg/L)	48.8	408	-109‡	8.3†	24	12

P-values: * 0.01-0.05; † 0.001-0.01; ‡ <0.001Results are based on multivariable linear regression, including the listed covariates

Discussion

A new method to assess non-adherence needs to be reliable to prevent false accusations of non-adherence that can potentially damage the patient doctor relationship. DBS is a newly developed method for measuring plasma concentrations of antihypertensive drugs and was found to be an appropriate and accurate method to monitor adherence. The advantage of DBS above plasma measurements is that sampling can take place at the exact moment the increased blood pressure is measured which prevents white coat adherence. Additionally, using multi-drug analysis with TDM, several antihypertensive drugs can be measured simultaneously, which allows the physician to distinguish partial and complete adherence in clinical practice. To use drug concentrations for assessment of drug adherence, knowledge of pharmacokinetic parameters of different drugs and influence of different parameters leading to intra-patient and inter-patient variability is paramount (9).

Applicability of DBS in clinical practice

The developed DBS method is suitable for measuring and proving non-adherence, at least 24 hours after intake of the measured antihypertensive drugs. This was due to the sensitivity of the method with a LLOD lower than the trough levels 24 hours after intake.
Both the LLOD and LLOQ were higher in DBS compared to plasma which is common when using this sampling technique (15). This implicates that DBS is less sensitive for measuring trough levels of antihypertensive drugs, however, this was not generally applicable, as our data showed higher concentrations in DBS for amlodipine, hydrochlorothiazide, nifedipine and perindopril when compared to the plasma samples collected at the same time point. It was thought that the discrepancy between the concentrations measured in plasma and DBS was due to the physical chemical properties of the different drugs. However, no relation could be found between the physical chemical properties and the measured concentrations. A previously published validation study was designed to correct for the different values found in plasma compared to DBS, which is measured in whole blood. In whole blood total drug levels (bound and unbound) are measured whereas plasma measurements do not include intracellular drug levels. Unfortunately, no fixed correction could be found to correct for the different results measured in whole blood and plasma. Because of this, these methods cannot be used interchangeably and it indicates that the medium is not the only cause of the high variability in drug concentration.

Clinical applicability of DBS results

A few false negative results were found for DBS in this study, which could lead to false acquisitions of non-adherence in clinical practice. However, almost all false negative results could be explained by the time between intake and sampling due to the pragmatic design of the study, for instance. Sampling was done before reaching t_{max} or trough levels >24 hours after last intake (amlodipine >25 hours, enalaprilate >17 hours and perindoprilate >36 hours after intake). Only few samples could not be explained by the time between intake and sampling including hydrochlorothiazide samples, 1 sample of nifedipine and 2 samples of perindoprilate. The false negative results for hydrochlorothiazide were seen in both DBS and plasma samples and can be explained be the high LLOQ in combination with a rapid decline of hydrochlorothiazide concentrations in blood (11). To improve this the UPLC-MS/MS needs to be optimized. The false negative samples of nifedipine and perindoprilate are most likely caused by a discrepancy between self-reported time of intake and true time of intake. Only low concentrations of these drugs were found in plasma which were not in accordance with the values of other samples with the same reported time after intake. Unfortunately, no combination tablets were used in this case to confirm real non-adherence. Only three patients were deemed partially non-adherent, but this did not lead to alarmingly high blood pressure values (12). This could be due to white coat adherence as a results of this research.

When zooming in to the plasma and DBS concentrations, more negative results in plasma as compared to DBS were measured for amlodipine (N=3), hydrochlorothiazide (N=5), perindoprilate (N=1) and canrenone (N=1). These values are not assigned as false positive, because of extensive analytical validation of the method. The negative values found in plasma suggest that DBS is a better method to measure adherence for these specific drugs as compared with plasma. This also indicates that with DBS, it is possible to detect the drugs over a longer time period. An explanation for these higher concentrations found with DBS is the uptake of drug into red blood cells. However, this was only described in literature for amlodipine (16).

As explained earlier, hydrochlorothiazide outcomes are less reliable due to suboptimal measurement at low concentrations in both plasma and DBS. The positive result for perindoprilate is due to sampling at trough level, more than 26 hours after intake and canrenone is possible a mistake in the measurement of the plasma sample.

All methods to assess adherence have their own advantages and disadvantages (17). One of the main limitations of TDM to assess non-adherence is the short period of time that is covered. Methods like microelectromechanical systems (MEMS®) are able to monitor adherence over a long period of time. However, white coat adherence and the costs are still a problem with this method. The most important advantage of DBS is that it can be carried out immediately when the physician suspects non-adherence. Thereby, avoiding white coat adherence. Another limitation is when patients refer one dose of a three times daily regime of drugs, like nifedipine. For this more information is needed on the relation between drug dose and the quantitative values measured in blood.

Clinical applicability of quantitative plasma concentrations

To interpret quantitative values correctly, knowledge of pharmacokinetic parameters of the different drugs is important. Also, an exact value of the drug concentration is needed, which should be above LLOQ. The LLOQ is most often the lowest concentrations of a calibration curve, which can be measured with a certain accuracy, precision and reproducibility. Measurements below the LLOQ are inaccurate when quantitative values are needed. In the clinical validation study for DBS, the LLOQ for all measured antihypertensive drugs in DBS was determined. The lower limit of quantification (LLOQ) for the antihypertensive drugs in DBS was higher compared to the LLOQ determined for plasma (13). Also, our previous work, as mentioned before, showed that results from DBS and plasma cannot be used interchangeably. This was the reason to exclude the results obtained with DBS.

When looking at the C_{plasma} of the ADHs obtained with a venipuncture a large variation was seen for all eight AHDs and their metabolites and when plotted in graphs C_{plasma} seemed visually independent of blood pressure, dose, age and weight. An important confounder in the blood pressure measurements is the fact that all patients in this study used more than one antihypertensive drug, and that selection was based on controlled blood pressure selection in good responders. Furthermore, blood pressure was not measured at the exact time of sampling and office blood pressure does not reflect the real blood pressure well due problems like white coat hypertension. It remains unclear to which extent certain C_{plasma} are correlated with a drop in blood pressure and therefore the use of quantification of antihypertensive drugs in clinical practice remain limited.

The influence of parameters on the plasma concentration

GEE was used to determine the true influence of parameters like age and weight on the plasma concentrations of each individual antihypertensive drug. It was found that time between intake and sampling and the dose of the drug were directly related to the height of the C_{plasma} for almost all measured antihypertensive drugs. Also, age, weight and sex seemed of influence on the C_{plasma}, but only significantly related to the plasma concentration for certain drugs. The relatively large differences in mcg/L between the height of the plasma concentrations are due to the settings from the UPLC-MS/MS method. This is the reason why relatively small changes in plasma concentration were significant, for example the dosage of canrenone was of significant influence on the plasma concentration with a value of 0.17 mcg/L/mg dosage. Weight was an important parameter of influence when looking at the C_{plasma} of losartan-ca. Solubility of Losartan-ca in water is low (determined experimentally: <150 mg/L), but it is assumed that due to high protein binding properties in blood, the distribution volume is low (12 L) (18). Although counterintuitive when taking the low distribution into account we postulate that losartan-ca has relatively lipophilic properties based on the solubility and will distribute more to fat tissue in patients with higher weights. This could explain why the plasma concentrations of losartan-ca decrease with increasing weight.
Increasing age was of influence on the C_{plasma} of perindoprilate and canrenone. This was in accordance with earlier findings which showed that the elderly patients had increased plasma concentrations from perindoprilate and canrenone compared to younger patients (19, 20).

In general males and females were equally distributed over the different drug classes. However, in the group using perindopril and amlodipine the females were underrepresented compared to the other measured antihypertensive drugs. This could be due to the fact that woman experience more adverse reactions when using amlodipine and perindopril (21-23). Also, women are overrepresented in the group of nifedipine users. It is assumed that the clearance of nifedipine in women is higher, but this would not explain this finding (24). A possible explanation could be that woman are switched to nifedipine when adverse events are seen with amlodipine. Side effects are more common in woman as stated before (21, 22). Another factor that may favor the use of certain drugs in females is that nifedipine is one of the preferred drugs used during pregnancy, and after delivery this drug is then continued (25).

When taking into account the time between intake and sampling, the dose of the drug, weight and age, there was a significant difference between the plasma canrenone concentrations of males compared to females. Females seemed to have higher C_{plasma} at the same time point with a difference of 30 mcg/L. Higher C_{plasma} can lead to an increased risk of adverse events which could explain why, on average, females are treated less with spironolactone compared to males (26). This was also seen in our population were only 35% of the canrenone users were female compared to a 40% to 60% distribution of female en male in our total population. All but one female participant used a dosage of 12.5 or 25 mg spironolactone, while one third of the males used 50 mg or even higher dosages. Higher C_{plasma} in females seemed to have led to decreased tolerability to the drug and thereby lower prescribed dosages of canrenone.
Furthermore, the sample size to determine the parameters with GEE was relatively small and could have possibly led to overfitting. However, all parameters were directly related to the C_{plasma} and the outcomes do not show this. Therefore, all parameters were used in further analyses. As only limited effects were seen of the parameters on the C_{plasma} of antihypertensive drugs, the exact time of intake of the dose could not be deduced from the drug concentrations. It is therefore recommended to ask the patient to record the time of intake to interpret drug concentrations. It should be noted that the time of drug intake is self-reported and some of the results may suffer from response bias. This also applies to the time after which the LLOD is reached. Due to self-reported time of intake, it is not clear when the LLOD is reached for each drug. To confirm the influence of the measured parameters on the plasma concentration and to determine the time of reaching the LLOD, it is advisable to include observed intake of antihypertensive drugs and to measure drug concentrations at set time points. Population pharmacokinetic modelling could be used as a patient-friendly solution to address this problem. To assess adherence in clinical practice, the time of intake is of less importance as this is only used to interpret the measured drug level.

Perspectives

It is important to be aware of the high inter-individual variability in plasma levels, making a clear cut-off of 0 most reliable to prevent false accusations of non-adherence. Combining drug levels with the time of intake for simultaneously used drugs makes the interpretation even more reliable. Usage of drug levels rather than absence or presence is vulnerable to false accusations due to the shown high inter-individual

variability which also might explain low levels rather than irregular intake (12). Also, false negative results, although not very common with this validated DBS method, can be minimized when taking combination tablets. When finding one drug, often the drug with the longest half-life, of a combination tablet, it can be assumed that the patient also ingested the other drugs as the compounds are inseparable.

We expect that a frequent use of DBS in patients with RH combined with personal feedback could improve adherence for antihypertensive medication and therefore result in lower disease burden and health care costs (27). To investigate this, a large government-funded clinical trial called RHYME-RCT is now being conducted, which includes patients with RH. With this trial the DBS method is tested in patients with uncontrolled blood pressure. Also, DBS can be used to monitor non-adherence in research with devices to lower blood pressure to elucidate the true blood lowering effect of the device only (28, 29). Furthermore, due to its non-invasive, quick, accurate and fast performance the test could also be performed in a primary health care setting. The general physician or assistant could perform the test in the office and for analyses the sample can be sent to a hospital (pharmacy) laboratory. Furthermore, more drugs can be added to the DBS method, such as the beta blockers, to increase the applicability in clinical practice and to use this method in a larger group of patients. However, it should be noted that results are not directly available like the DBS for glucose, but are available within a few days after sampling.
The fact that this method is extensively validated, results in accurate measurements and prevents false acquisitions towards to patients. This is important to maintain the physician /patient-relationship, while this has a direct influence on adherence (30, 31).
When quantification of a certain antihypertensive drug is required, plasma sampling is preferred over DBS sampling due to a lower LLOQ and thereby higher sensitivity at trough levels.

Conclusion

DBS is a convenient and accurate method to assess non-adherence in the clinical (outpatient) practice. When measuring non-adherence the total absence of a drug, so a value smaller than the LLOD, should be used for indisputable outcomes. Also, because a high inter-individual difference in C_{plasma} around the same time point were observed, quantitative values of antihypertensive drugs cannot be used to interpret time after intake or drug dosages.

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MEASURING THE EFFICACY OF RENAL SYMPATHETIC DENERVATION

Chapter 10

Endovascular ultrasound renal denervation to treat hypertension (RADIANCE-HTN SOLO): a multicentre, international, single-blind, randomised, sham-controlled trial

Chapter 11

Redo renal denervation using a multi-electrode radiofrequency system in patients with persistent therapy-resistant hypertension

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Chapter 13

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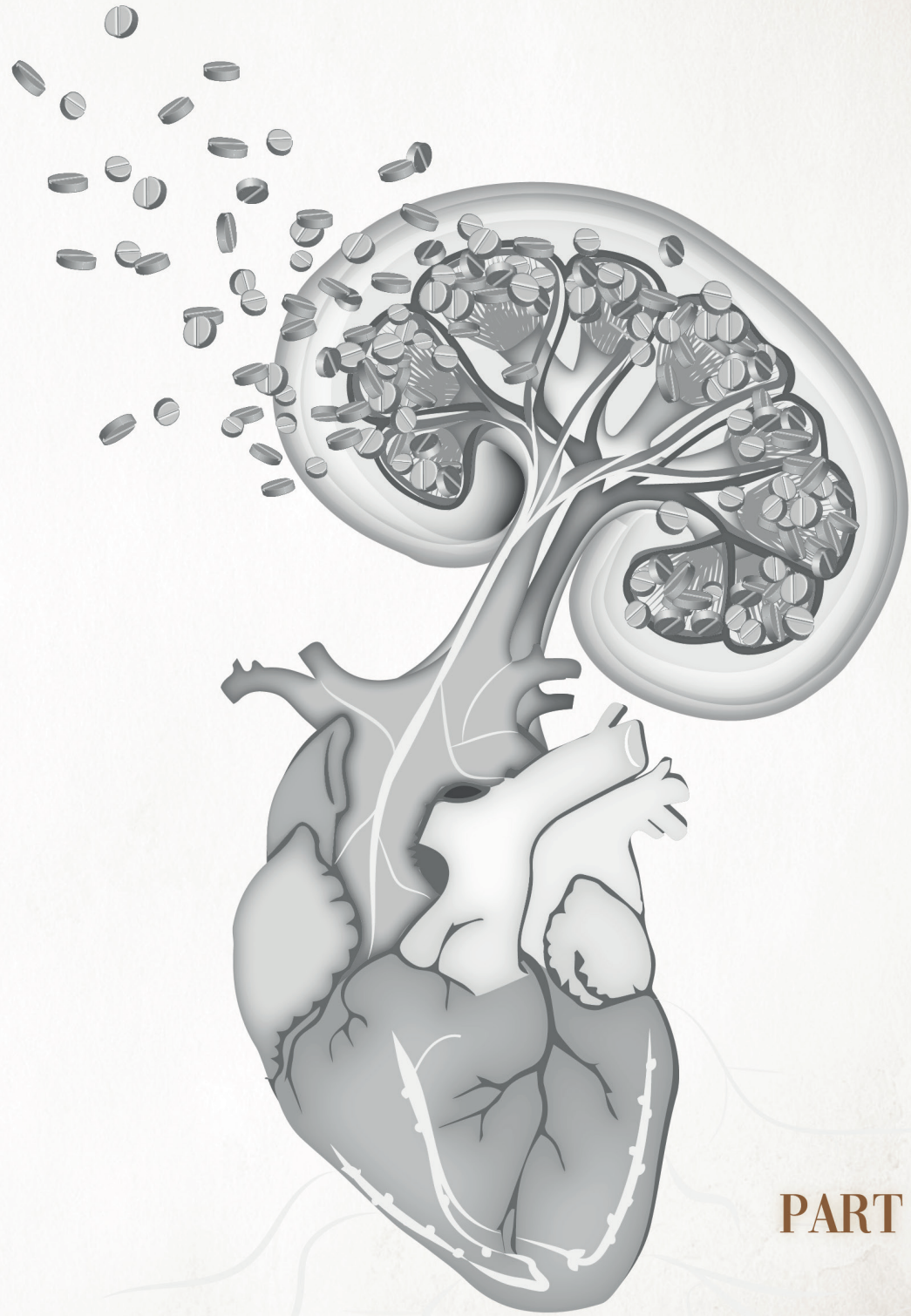
Chapter 15

Atrial fibrillation reduction by renal sympathetic denervation: 12 months results of the AFFORD study

Chapter 16

Endovascular renal sympathetic denervation to improve heart failure with reduced ejection fraction: the IMPROVE-HF-I study



PART IV

CHAPTER 10

Endovascular ultrasound renal denervation to treat hypertension (RADIANCE-HTN SOLO): a multicentre, international, single-blind, randomised, sham-controlled trial

Michel Azizi*, Roland Schmieder, Felix Mahfoud, Michael Weber, Joost Daemen, Justin Davies, Jan Basile, Ajay Kirtane, Yale Wang, Melvin Lobo, Manish Saxena, Lida Feyz, Florian Rader, Philipp Lurz, Jeremy Sayer, Marc Sapoval, Terry Levy, Kintur Sanghvi, Josephine Abraham, Andrew Sharp, Naomi Fisher, Michael Bloch, Helen Reeve-Stoffer, Leslie Coleman, Chris Mullin, and Laura Mauri*, on behalf of the RADIANCE-HTN Investigators**

***Contributed equally to the manuscript**

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Abstract

Background Limited adherence to antihypertensive therapy may increase morbidity and mortality. We investigated whether catheter-based renal denervation reduces blood pressure in subjects with hypertension.

Methods Hypertensive subjects aged 18–75 years with ambulatory blood pressure ≥135/85 and <170/105 mm Hg after a four week discontinuation of antihypertensive medications and with suitable renal artery anatomy were randomized (1:1) to renal denervation (via an endovascular ultrasound catheter) or a sham procedure. The primary effectiveness endpoint was the change in daytime ambulatory systolic blood pressure at 2 months in the intention-to-treat population. Subjects were to remain off antihypertensive medications through 2 months unless office or home blood pressures exceeded 180/110 or 170/105 mm Hg, respectively.

Results A total of 146 subjects were randomized to renal denervation (N=74) or sham procedure (N=72). Renal denervation reduced daytime ambulatory systolic blood pressure more than the sham (-8.5 mm Hg vs. -2.2 mm Hg; difference: -6.3 mm Hg, 95%CI -9.4 to -3.1, p<0.001). Consistent reductions were observed for daytime diastolic, 24-hour, and nighttime ambulatory systolic and diastolic blood pressures. Fewer subjects in the renal denervation group received antihypertensive medication prior to 2 months [5/74 (6.8%) vs. 13/72 (18.1%), P=0.04]. In the denervation group, 15/74 (20.3%) subjects attained controlled daytime ambulatory blood pressure (<135/85 mm Hg) in the absence of antihypertensive medications, vs. 2/72 (2.8%) in the sham group (p=0.001).

Conclusions Endovascular ultrasound-based renal denervation reduced blood pressure in subjects with hypertension and without antihypertensive medication at 2 months.

Introduction

Hypertension is the leading cause of premature death and disability:adjusted life years (1) and is associated with increased risks of myocardial infarction, heart failure, ischemic or hemorrhagic stroke, and end-stage renal disease (1-3). Lifestyle interventions and lifelong antihypertensive drug treatment are two well established strategies to lower blood pressure, and are associated with prevention of these adverse events (4). However, hypertension remains poorly controlled worldwide, and its prevalence is increasing due to the aging of the population and the obesity epidemic (5-8). Indeed, despite the availability and prescription of various antihypertensive drugs, many patients with hypertension are not adequately treated, for various reasons including limited adherence to available therapy (9, 10).

Targeted endovascular catheter-based denervation of the renal efferent and afferent nerves with minimally invasive approaches has been investigated as a new blood pressure lowering treatment for resistant hypertension (11). A initial randomized trial using catheter-directed radiofrequency ablation to achieve denervation appeared to overestimate efficacy due to the lack of a sham control (12), as a subsequent sham controlled study did not show improvement in blood pressure control (13).

Additional features of these trials may have limited their ability to demonstrate blood pressure reduction following renal denervation (14). These include uncertainty regarding the completeness of denervation, variable adherence to antihypertensive medications among subjects in follow-up, and a study population with advanced resistant hypertension that may be difficult to reverse (15-17). Subsequently, trials have been designed with more systematic attention to procedural technique and have included subjects with early stage hypertension in order to examine the blood pressure lowering efficacy of catheter-based renal denervation in the absence of medications. Interim results of one such study suggest the ability of renal denervation with a multi electrode radiofrequency ablation device to reduce blood pressure (18). Another catheter has been developed to percutaneously deliver ultrasound energy to thermally ablate the renal sympathetic nerves (Paradise® Renal Denervation System, ReCor Medical, Palo Alto, CA, USA). Placed within the proximal segment of the renal arteries and centered by a low pressure, water-filled balloon (Supplementary Appendix, Figure S1), this endovascular catheter achieves a circumferential ring of ablation at a depth of 1-6 mm from the vessel lumen in animal models (data on file ReCor Medical, Inc.)

(19), designed to match the expected location of the renal nerves in the adventitia (20, 21). In subjects with resistant hypertension, feasibility studies have shown reduction in blood pressure with a low incidence of adverse events, yet without comparison to a sham procedure (22, 23).

We designed the international, multicenter RADIANCE-HTN Trial to compare the blood pressure lowering efficacy of the endovascular ultrasound renal denervation system with a sham procedure in two separate cohorts 1) subjects with early hypertension, randomized while off antihypertensive medications (SOLO cohort), and 2) subjects with resistant hypertension, randomized while on 3 antihypertensive medications (TRIO cohort) (24). Each cohort was independently powered to detect a significant difference between denervation and sham procedure on the primary endpoint of change in daytime ambulatory systolic blood pressure at 2 months. We herein report the primary results of the RADIANCE-HTN randomized trial in the SOLO cohort.

Methods

Study design

The RADIANCE-HTN trial design has been described previously (24). The study was approved by local ethics committees or institutional review boards and conducted in accordance with the declaration of Helsinki. All participants provided written informed consent. Between March 28, 2016, and December 28, 2017, participants were recruited into the SOLO cohort of the RADIANCE-HTN trial from 21 centers in the United States and 18 in Europe (France, Germany, the Netherlands, Belgium, and the United Kingdom).

Study population

Briefly, eligible subjects were men or women aged 18–75 years with grade I to II combined systolic-diastolic primary hypertension either uncontrolled on 0 to 2 hypertensive medications (average seated office systolic and diastolic blood pressure of 140/90 mm Hg or higher but less than 180/110 mm Hg) or controlled on 1 to 2 antihypertensive medications (average seated office blood pressure <140/90 mm Hg) with no history of cardiovascular or cerebrovascular events and with an estimated glomerular filtration rate (eGFR) of greater than or equal to 40 mL/min/1.73m^2 (Modification of Diet in Renal Disease formula). All antihypertensive medications were discontinued for 4 weeks prior to the qualifying ambulatory blood pressure assessment. Subjects with ambulatory systolic blood pressure of at least 135 mm Hg and diastolic blood pressure at least 85 mm Hg and less than 170/105 mm Hg and suitable renal artery anatomy (accessory arteries with diameter ≥2mm <4mm and >8mm were excluded) on a pre-randomization renal CT- or MR-angiography underwent renal angiography to confirm anatomic eligibility. Eligible subjects were randomized in a 1:1 ratio to receive renal denervation or a sham procedure immediately following randomization.

Randomization and blinding

The randomization sequence was generated by computer and stratified by centers using randomized blocks of small size and permutation of treatments within each block. Treatment assignment was masked for 6 months after randomization for subjects and for clinical and research staff responsible for follow-up.

Procedures

Renal denervation was performed with the endovascular ultrasound renal denervation system. A minimum of two sonications, separated by 5 mm, were delivered in the main branch of the right and left renal artery according to individual pre-specified treatment plans developed by the sponsor based on the pre-randomization CT- or MR-angiography. The sham procedure consisted of renal angiography. To prevent unblinding, subjects were sedated and had headphones and eye covers. Subjects completed a blinding questionnaire at discharge and 2-month follow-up. Pain was assessed pre- and post-procedure using the visual analog scale.

Subjects were evaluated at clinical visits at one and two months after randomization. Seated office blood pressure and heart rate, adverse events, concomitant medications were recorded and laboratory assessments were performed at each visit.

Research staff measured office blood pressure after a 5-min rest in the seated position during the outpatient visits. Subjects were requested to measure their blood pressure at home after a 5-min rest in the sitting position in the morning and the evening during seven consecutive days before every outpatient visit. The same validated electronic device (Omron M10-IT, Kyoto, Japan) was used for both office and home blood pressure measurements. All subjects remained off antihypertensive medications until two months after randomization unless office blood pressure exceeded 180/110 mm Hg or home blood pressure exceeded 170/105 mm Hg prior to the two-month evaluation, in which case subjects received "escape" antihypertensive treatment. At two months, a specific drug titration protocol was initiated if the average blood pressure at home was greater than or equal to 135 mm Hg, systolic, or 85 mm Hg, diastolic and subsequently was confirmed by office blood pressure ≥140, systolic, or ≥ 90mmHg, diastolic.

Ambulatory blood pressure measurements were performed to assess eligibility and at 2 months post-randomization (Microlife WatchBP, Taipei, Taiwan). Blood pressure was recorded every 20 minutes during daytime (07:00–22:00 hours) and every 30 minutes during nighttime (22:00–07:00 hours). The ambulatory blood pressure measurement was repeated if the number of daytime blood pressure measurements was less than 21. All ambulatory blood pressure recordings were sent to a core laboratory (dabl Ltd., Dublin, Ireland), with treatment assignment masked. Renal duplex ultrasound was performed at 2 months in all randomized subjects (except for 4 subjects in one center who underwent MR angiography), with treatment assignment masked. Subjects with

elevated peak systolic velocities or increased aortic to renal artery velocity ratios on duplex ultrasound underwent renal CT- or MR-angiogram to assess for renal artery stenosis. All randomized subjects were consented to complete 3 years follow-up.

Outcomes

The primary efficacy endpoint was the mean change in daytime ambulatory systolic blood pressure from baseline to 2 months. Secondary efficacy endpoints included change in daytime diastolic, and 24-hour and night time ambulatory systolic and diastolic blood pressures. Additional specified efficacy endpoints included change in ambulatory heart rate, office and home systolic and diastolic blood pressures, and the proportion of subjects with controlled blood pressure: less than 135/85 mm Hg for daytime ambulatory, 130/80 mm Hg for 24-hour ambulatory, or 140/90 mm Hg for office blood pressure. We also assessed change in eGFR at 2 months.

Specified major adverse events were all-cause mortality, renal failure (eGFR less than 15mL/min/1.73m^2 or need for renal replacement therapy or doubling of serum creatinine), an embolic event with end-organ damage, renal artery, or other major vascular complications requiring intervention, or hospitalization for hypertensive crises within 30 days; and new renal artery stenosis (greater than 70%) within 6 months. Additional specified safety endpoints included: hypotensive emergency; hospitalization for heart failure; stroke, transient ischemic attack, or cerebrovascular accident; acute myocardial infarction (STEMI/non-STEMI); any coronary revascularization; procedure-related pain lasting greater than 2 days; new renal artery stenosis greater than 50% by duplex ultrasound and confirmed by renal CT- or MR-angiography; and need for renal artery angioplasty and/or stenting.

Sites were required to report all adverse events. All potential device or procedural and/or serious adverse events were sent for independent adjudication. An independent data safety and monitoring board reviewed study data quarterly for all enrolled subjects.

Statistical analysis

The study was designed to compare the blood pressure lowering effect of renal denervation versus a sham procedure. Assuming a 6 mm Hg difference in change in daytime ambulatory systolic blood pressure at 2 months between the renal denervation

and the sham groups (25), a common SD of 12 mm Hg, 1:1 randomization, and a type I error rate of 5%, a sample size of 128 evaluable subjects yielded 80% power. To account for up to 10% missing data on the primary endpoint, we planned to randomize a total 146 subjects in the study.

The primary analysis was performed according to the intention-to-treat (ITT) principle. The per-protocol population excluded the following subjects: 1) subjects not meeting baseline daytime ambulatory blood pressure or renal artery anatomic inclusion criteria, 2) subjects in the denervation group who did not receive bilateral denervation, 3) subjects who were treated with antihypertensive medications before the 2-month ambulatory blood pressure measurement (according to protocol criteria, or according to patient or physician decision) or 4) subjects who did not complete the 2-month ambulatory blood pressure assessment. We also performed a modified intention-to-treat analysis, which only excluded subjects from the ITT population who met protocol criteria for receiving antihypertensive drug treatment before 2 months. The as-treated population excluded subjects who received no denervation treatment in either renal artery from the denervation group of the ITT population.

For the primary ITT analysis, subjects who met protocol criteria for antihypertensive drug treatment before 2 months and subjects with missing 2-month ambulatory blood pressure data were assigned their baseline value of ambulatory systolic blood pressure at 2 months. Evaluable data was used for all other analyses. We assessed treatment effect (change in blood pressure parameters, heart rate or eGFR from baseline) using analysis of covariance, including the baseline value as a covariate. When the change in blood pressure parameters, heart rate or eGFR from baseline was not normally distributed, a baseline-adjusted analysis of covariance on the ranks was also performed. Treatment interaction was assessed using linear regression adjusting for baseline daytime ambulatory systolic blood pressure for prespecified subgroups (age, ethnicity, sex, baseline daytime ambulatory systolic blood pressure, baseline office blood pressure, abdominal obesity). Bang and James blinding indices were calculated (26, 27).

Continuous variables are expressed as mean ± standard deviation (SD), unless otherwise specified, and between-group differences are expressed with their two-sided 95% confidence intervals (CI). We used the statistical analysis system (SAS) software version 9.4 (SAS Institute, Cary, NC, US). We deemed a P value lower than 0.05 to be significant.

Role of the funding source

The study was funded by ReCor Medical, Inc. (Palo Alto, CA, USA). The trial executive committee designed the protocol in conjunction with the sponsor. The sponsor was responsible for selection of clinical sites in collaboration with the executive committee, as well as collection, monitoring and analysis of the data. The authors had unrestricted access to the data, and statistical analyses were independently validated (Baim Institute for Clinical Research, Boston, MA, USA). The manuscript was written by the two lead authors (LM, MA) with significant contributions from the coauthors. The sponsor assisted in figure and table generation, copy editing and formatting. The authors had full responsibility for the decision to submit for publication.

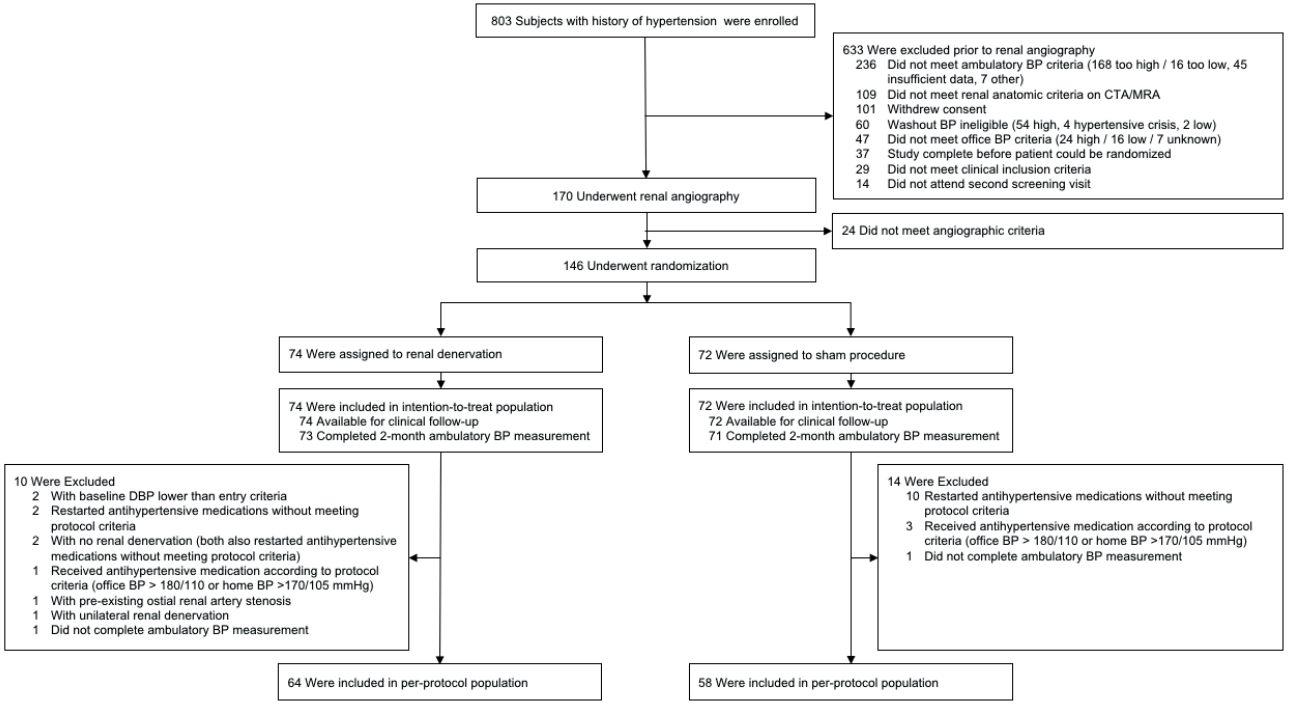
Results

Study population

A total of 803 subjects with a history of hypertension were enrolled in the study. After a 4-week period where all antihypertensive therapy was discontinued, a total of 170 subjects met daytime ambulatory blood pressure and non-invasive imaging criteria and underwent renal angiography. A total of 146 subjects met angiographic criteria for randomization (renal denervation, N=74; sham procedure, N=72, Figure 1). There were no subjects lost to follow-up at 2 months.

Baseline characteristics are shown in Table 1 and were similar across both study groups. The mean age was 54.1±10.1 years. Female subjects represented 41.8%, and black subjects represented 17.1% of randomized subjects. Abdominal obesity was present in 58.2% of randomized subjects. At the time of enrollment, 28 subjects (19.2%) were not receiving any antihypertensive medications (9 were drug naïve, 19 were drug intolerant or had chosen not to take antihypertensive medications), and 61 (41.8%) and 55 (37.7%) were receiving 1 or 2 medications, respectively (Table 1).

Figure 1. Trial Subject Flow. Between March 2016 and December 2017, subjects with hypertension on the basis of their office blood pressure were assessed for eligibility. A total of 803 subjects were consented for participation in the trial. After 4-week medication wash-out period, 170 subjects had confirmed hypertension with daytime ambulatory blood pressure > 135/85 mmHg and suitable renal artery anatomy on non-invasive renal imaging and underwent renal angiography prior to randomization. After renal angiography, 24 subjects had unsuitable renal anatomy and 146 subjects were randomized to the renal denervation (N=74) or the sham group (N=72).



BP: blood pressure; ABP: ambulatory blood pressure; CTA: computed tomography angiogram; MRA magnetic resonance angiogram; DBP: diastolic blood pressure

Table 1. Baseline Demographic and Clinical Characteristics of the Intention-to-Treat Population.

Characteristic	Renal Denervation (N=74)	Sham Procedure (N=72)
Age – yr	54.4±10.2	53.8±10.0
Female sex – no. (%)	28/74 (37.8)	33/72 (45.8)
Race – no. (%)		
White	60/74 (81.1)	52/72 (72.2)
Black	12/74 (16.2)	13/72 (18.1)
Other	2/74 (2.7)	7/72 (9.7)
Body mass index – kg/m²	29.9±5.9	29.0±5.0
Abdominal obesity†	41/73 (56.2)	44/72 (61.1)
eGFR – ml/min/1.73m²	84.7±16.2	83.2±16.1
eGFR <60 ml/min/1.73m² – no. (%)	1/73 (1.4)	3/69 (4.3)
Diabetes – no. (%)		
Type 1	0/74 (0)	0/72 (0)
Type 2	2/74 (2.7)	5/72 (6.9)
Obstructive sleep apnea – no. (%)	6/74 (8.1)	8/71 (11.3)
Office SBP prior to antihypertensive medication washout – mm Hg	142.6±14.7	144.6±15.9
Office DBP prior to antihypertensive medication washout – mm Hg	92.3±10.1	93.6±8.3
Office heart rate prior to antihypertensive medication washout – bpm	73.2±12.4	73.2±12.4
Number of antihypertensive medications at screening – no. (%)		
0††	12/74 (16.2)	16/72 (22.2)
1	33/74 (44.6)	28/72 (38.9)
2	28/74 (37.8)	27/72 (37.5)
3†††	1/74 (1.4)	1/72 (1.4)
Types of medication at screening – no. (%)		
Renin Angiotensin System Blockers	41/62 (66.1)	40/56 (71.4)
Angiotensin converting enzyme inhibitor	27/62 (43.5)	27/56 (48.2)
Angiotensin receptor blocker	13/62 (21.0)	13/56 (23.2)
Direct renin inhibitor	2/62 (3.2)	0/56 (0)
Calcium channel blocker	19/62 (30.6)	19/56 (33.9)
Diuretic	9/62 (14.5)	5/56 (8.9)
Beta blocker	5/62 (8.1)	7/56 (12.5)
Alpha-1 receptor blocker	3/62 (4.8)	1/56 (1.8)
Spironolactone	0/62 (0)	1/56 (1.8)

Data displayed as mean ±SD. eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; DBP: diastolic blood pressure, bpm: beats per minute.† Abdominal obesity is defined as a waist circumference >102 cm for men and > 88 cm for women †† 9 subjects were drug naïve in the denervation and sham groups (3 and 6, respectively), and 19 were drug intolerant or had chosen not to take antihypertensive medications (9 and 10, respectively). ††† Two subjects were discovered to have been on 3 antihypertensive medications at screening.

Subjects in the denervation group who received treatment received an average of 5.4±1.0 total ultrasound emissions with a total average ablation time of 37.9±6.7 seconds. Including angiography, the average total procedure time was 72 min for the denervation group vs 38 min for the sham procedure (P<0.001; Supplementary Appendix, Table S1). Successful bilateral renal ablation (minimum of 2 emissions bilaterally) treatment was performed in 71 (95.9%) subjects. One subject in the denervation group received only unilateral denervation due to ostial renal artery tortuosity. Two subjects received no denervation; one was due to ostial renal artery tortuosity; the other was due to a nonfunctioning generator ascertained after randomization but prior to denervation catheter insertion. There was no difference between groups in post-procedure pain (Supplementary Appendix, Table S1). The Bang and James blinding indices are shown in the Supplementary Appendix, Table S2.

Efficacy Endpoints

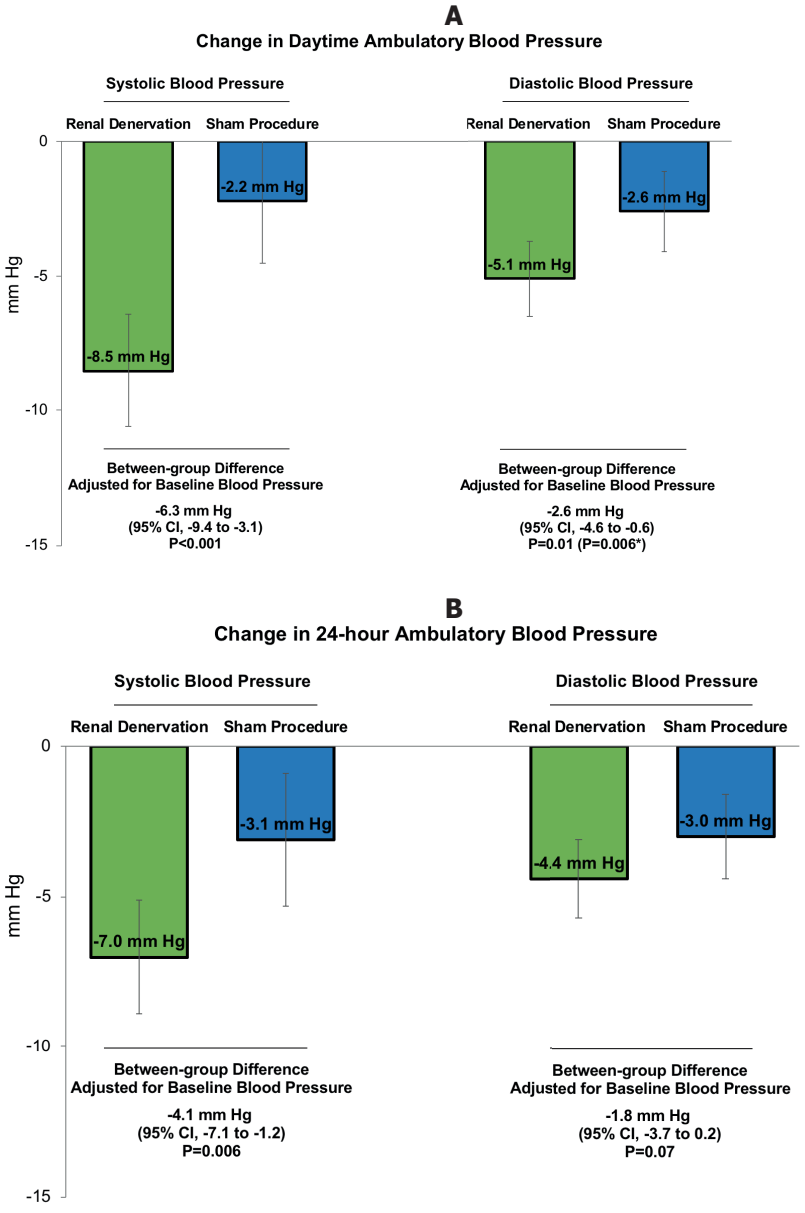
In the intention-to-treat population, there was a greater reduction in the primary endpoint of daytime ambulatory systolic blood pressure at 2 months in the denervation group (-8.5 ± 9.3 mm Hg) compared with the sham group (-2.2 ± 10.0 mm Hg; between-group difference of -6.3 mm Hg, 95%CI, -9.4 to -3.1 mm Hg, P<0.001; Table 2 and Figure 2). Changes in daytime ambulatory diastolic blood pressure, 24-hour ambulatory, nighttime ambulatory, home and office systolic and diastolic blood pressures were consistent (Table 2).

Table 2. Primary and Secondary Efficacy Endpoints: Change in Ambulatory, Office, and Home Blood Pressure at 2 Months Following Renal Denervation or Sham Procedure (Intention-to-Treat Population). The primary efficacy endpoint of reduction in daytime ambulatory systolic blood pressure at 2 months was greater in the renal denervation group compared with the sham group (between group difference: -6.3 mm Hg, 95CI -9.4 to -3.1, P<0.001).

	Renal Denervation			Sham Procedure			Mean between-group difference adjusted for baseline blood pressure (95% CI)	P Value[†]
	Random-ization	2 months	Difference	Randomiza-tion	2 months	Difference		
Daytime ABP – mm Hg	(N=74)	(N=74)	(N=74)	(N=72)	(N=72)	(N=72)		
SBP	150.3±7.8	141.9±11.9	-8.5±9.3	150.0±9.8	147.9±13.3	-2.2±10.0	-6.3 (-9.4, -3.1)	<0.001
DBP	93.1±4.8	87.9±7.1	-5.1±5.9	93.5±5.5	90.9±7.9	-2.6±6.5	-2.6 (-4.6, -0.6)	0.012 (0.006*)
24-hour ABP – mm Hg	(N=74)	(N=74)	(N=74)	(N=72)	(N=72)	(N=72)		
SBP	142.6±8.1	135.6±11.4	-7.0±8.6	143.8±10.4	140.7±11.8	-3.1±9.7	-4.1 (-7.1, -1.2)	0.006
DBP	87.3±5.0	83.0±6.8	-4.4±5.8	88.6±5.7	85.7±7.1	-3.0±6.1	-1.8 (-3.7, 0.2)	0.07
Nighttime ABP – mm Hg	(N=74)	(N=74)	(N=74)	(N=71)	(N=71)	(N=71)		
SBP	130.3±11.9	125.6±12.8	-4.8±11.7	132.5±13.7	129.4±13.1	-3.1±11.5	-2.5 (-6.0, 0.9)	0.15
DBP	78.2±8.0	74.8±8.5	-3.3±8.5	80.0±8.1	77.3±8.5	-2.7±7.3	-1.4 (-3.8, 1.0)	0.25
Office BP – mm Hg	(N=74)	(N=74)	(N=74)	(N=72)	(N=72)	(N=72)		
SBP	154.5±12.4	143.7±16.1	-10.8±13.6	153.6±15.7	149.7±17.4	-3.9±17.4	-6.5 (-11.3, -1.8)	0.007 (<0.001*)
DBP	99.7±7.7	94.2±10.1	-5.5±8.4	99.1±9.4	98.0±10.0	-1.2±10.0	-4.1 (-7.0, -1.3)	0.005
Home BP – mm Hg	(N=71)	(N=71)	(N=71)	(N=72)	(N=72)	(N=72)		
SBP	147.5±8.8	139.4±11.7	-8.1±9.7	147.7±12.3	146.6±15.4	-1.1±10.6	-7.1 (-10.4, -3.8)	<0.001 (<0.001*)
DBP	94.8±6.9	89.9±7.8	-4.9±6.7	94.6±7.0	93.3±8.5	-1.3±6.2	-3.6 (-5.6, -1.5)	<0.001

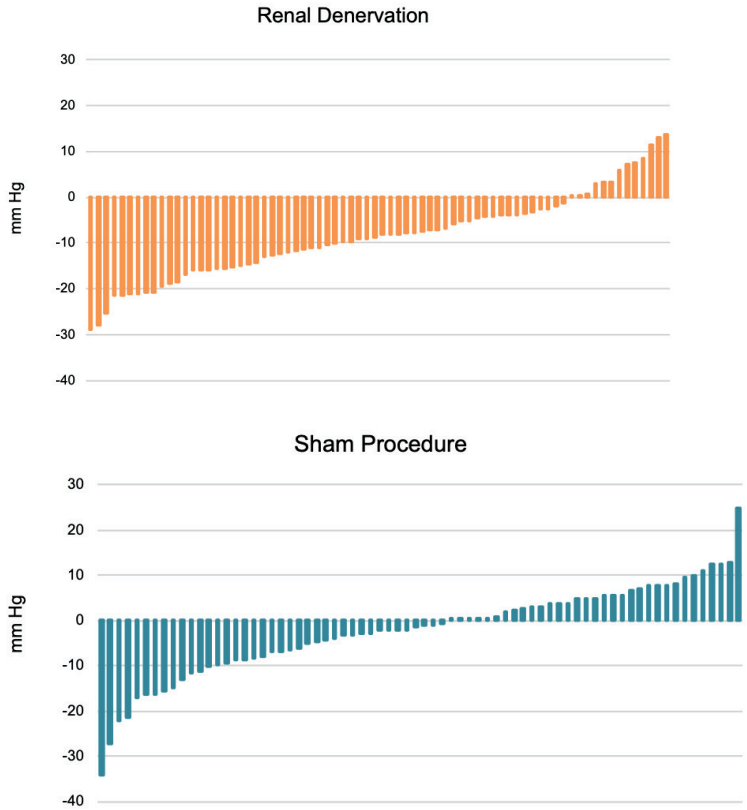
Data displayed as mean ±SD unless otherwise noted.[†] P value by analysis of covariance, adjusting for baseline value. *P value by baseline adjusted analysis of covariance on the ranks. ABP: ambulatory blood pressure; CI: Confidence interval; SBP: systolic blood pressure; DBP: diastolic blood pressure; SD: standard deviation.

Figure 2. Change Ambulatory Blood Pressure from baseline to 2 months in the Intention-to-Treat population. A greater change from baseline to 2 months was observed in the primary endpoint of daytime ambulatory systolic blood pressure in the renal denervation group (N=74) compared with the sham procedure group (N=72) (Panel A). A similar greater reduction with renal denervation compared with sham was observed in the secondary endpoint of 24-hour ambulatory blood pressure (Panel B). Data are presented as mean and 95% confidence intervals.



Five subjects in the denervation group and 13 in the sham group had been treated with antihypertensive medications prior to the 2-month ambulatory blood pressure measurement (1 and 3 respectively were treated after meeting protocol-defined "escape" criteria, and 4 and 10 respectively were treated according to patient or physician preference despite not meeting criteria; Supplementary Appendix, Table S3). After completion of the 2-month ambulatory blood pressure collection, a total of 41/74 (55.4%) subjects in the denervation group compared with 57/72 (79.2%) subjects in the sham group received antihypertensive treatment (P=0.002; Supplementary Appendix, Table S3). Individual subject changes in daytime ambulatory systolic blood pressure are shown according to treatment group in Figure 3.

Figure 3. Individual Subject Changes in Daytime Ambulatory Systolic Blood Pressure from Baseline to 2 Months (Intention-to-Treat Population). A total of 49 of 74 (66.2%) renal denervation subjects had a decrease of at least -5 mm Hg compared with 24 of 72 (33.3%) in the sham procedure group (P<0.001).



A total of 49 of 74 subjects (66.2%) had a decrease of at least -5 mm Hg in daytime ambulatory systolic blood pressure in the renal denervation group compared with 24 of 72 (33.3%) in the sham procedure group (P<0.001). In the denervation group, 15/74 (20.3%) subjects attained controlled daytime ambulatory blood pressure (<135/85 mm Hg) in the absence of antihypertensive medications, vs. 2/72 (2.8%) in the sham group (P=0.001; Supplementary Appendix, Table S4). There was no significant difference in heart rate in any group at 2 months following randomization (Supplementary Appendix, Table S5).

Baseline characteristics were similar across both study groups in the per-protocol population (Supplementary Appendix, Table S6). In the per-protocol analysis, the change in daytime ambulatory systolic blood pressure was -8.5 ± 9.6 mm Hg in the denervation group (N=64) and -0.1 ± 8.5 mm Hg in the sham group (N=58; between-group difference of -8.2 mm Hg, 95%CI, -11.5 to -5.0 mm Hg, P<0.001) and analysis of other blood pressure measures were consistent (Supplementary Appendix, Table S7). A total of 14/64 (21.9%) subjects attained controlled daytime ambulatory blood pressure without added antihypertensive medication at 2-month ambulatory blood pressure measurement, vs 2/58 (3.4%) in the sham group (P=0.003, Supplementary Appendix, Table S4). The between-group differences in changes in daytime and 24-hour ambulatory systolic blood pressure were also consistent in the modified intention-to-treat and as-treated analysis populations (Supplementary Appendix, Table S8).

The treatment effect on the primary efficacy endpoint was consistent across several pre-specified subgroups, with the exception of the abdominal obesity subgroup where a greater treatment effect was observed (Supplementary Appendix, Figure S2). While subjects with and without abdominal obesity in the renal denervation group both experienced reduction in daytime ambulatory blood pressure, this interaction appeared to be related to a larger than expected daytime ambulatory blood pressure reduction in subjects without abdominal obesity who were in the sham group (Supplementary Appendix, Figure S3). Number of emissions was not a predictor of reduction in daytime ambulatory systolic blood pressure in the renal denervation group (P for interaction = 0.33; Supplementary Appendix, Figure S4).

Safety Outcomes

There were no major adverse events in either group at 2 months. Procedure-related pain lasting greater than 2 days occurred at a similar frequency (11%) in both groups (Supplementary Appendix, Table S9). Two-month non-invasive renal artery imaging was available in 71/74 renal denervation subjects and 68/72 sham subjects. No new renal artery stenosis greater than 50% was detected in either group at 2 months. Six-month imaging was available in 43 denervation subjects and 41 sham subjects. At 6 months, one subject in the renal denervation group underwent renal artery stent placement. Independent review of the pre-procedure MR angiography and renal angiography showed a preexisting ostial renal artery stenosis (40-50% on MR, 44%% on angiography) which would have met criteria for exclusion but was not correctly evaluated at the time of randomization and 57% stenosis on angiography prior to stent placement. Finally, at 2 months there was no significant difference in changes in eGFR between the treatment groups (Supplementary Appendix, Table S10).

Discussion

In subjects with combined systolic–diastolic primary hypertension grade I to II who were not receiving antihypertensive medications, renal denervation achieved a greater reduction in daytime ambulatory systolic blood pressure at 2 months compared with a sham procedure. This blood pressure lowering effect was consistent for daytime ambulatory diastolic, 24-hour and nighttime ambulatory and diastolic, and office and home blood pressures. Consequently, at 2 months, subjects receiving renal denervation were more likely to achieve daytime ambulatory blood pressure control without the addition of antihypertensive medications than subjects in the sham group (20.3% vs. 2.8%, respectively). The higher blood pressure control rate in the renal denervation group was achieved even though 83.8% of the subjects had been receiving with 1 to 2 antihypertensive drugs prior to study enrollment. The treatment effect of renal denervation was consistent across sex, ethnicity, and varying baseline blood pressures.

The renal denervation group experienced an 8.5 mm Hg reduction in daytime ambulatory systolic blood pressure, and a 6.3 mm Hg greater reduction compared with the sham procedure group in the intention-to-treat analysis. However, the intention-to-treat analysis included ambulatory systolic blood pressure measurements in subjects who had received antihypertensive medications, more commonly in the sham

group (N=13) than in the denervation group (N=5) as per-protocol safety criteria or as subjects/physician preference. The per-protocol analysis excluding such subjects from both groups is, therefore, a more accurate reflection of the true "off medication" blood pressure lowering treatment effect. Indeed, the change in daytime ambulatory systolic blood pressure was similar in the renal denervation group to the intention-to-treat analysis (-8.5 mm Hg) but much smaller in the sham group (-0.1 mm Hg) amplifying thus the between-group difference from 6.3 to 8.2 mm Hg in favor of the renal denervation group. In the per-protocol analysis, subjects receiving renal denervation were more likely to achieve daytime ambulatory blood pressure control than subjects in the sham group (21.9% vs 3.4%, respectively, P=0.003; number needed to treat: 5 to 6 subjects). The between-group differences in favor of the renal denervation group were also amplified for all other blood pressure parameters (Supplementary Appendix, Table S7).

Interestingly, despite the large decrease in ambulatory blood pressure achieved with renal denervation while subjects were off-antihypertensive medications, there was not any increase in ambulatory heart rate or reduction in eGFR at 2-months. While direct measures of sympathetic nerve activity and cardiac and renal physiology were not directly tested, these results suggest that the denervation-induced reduction in blood pressure did not result in baroreflex activated tachycardia or in decreased renal perfusion because it may directly impact sympathetic regulation.

This blinded randomized trial to evaluate renal denervation has several strengths. First, the study was prospectively powered to demonstrate superiority of renal denervation to lower daytime ambulatory systolic blood pressure over a sham procedure, and is the largest randomized trial of renal denervation in subjects not on antihypertensive medications. Second, we carefully selected subjects for randomization, by ensuring that they remained hypertensive by both systolic and diastolic ambulatory blood pressure criteria after a 4 week off medication period, and were aged less than 75 years, to avoid isolated systolic hypertension and increased arterial stiffness (28, 29), and to avoid the confounding effect of antihypertensive medications and variable subject adherence to treatment.[30,31] Third, we took great care to reduce between-center variability 1) in procedural performance by establishing pre-specified treatment plans on the CT- or MR-angiography, 2) in office, home and ambulatory blood pressure measurement technique as shown by the concordance between blood pressure levels at baseline and changes in blood pressure at 2 months using the different blood pressure measurement

methods, and 3) patient care, by establishing protocol-defined escape criteria for treatment of hypertension during the follow-up period.

The reduction in 24-hour ambulatory systolic blood pressure with renal denervation and the difference compared with sham observed at 2 months in our trial was similar (-7.1 mm Hg and -4.1 mm Hg, respectively) to that observed at 3 months in the SPYRAL HTN-OFF MED study (-5.5 mm Hg and -4.6 mm Hg, respectively) using the same modified intention-to-treat population (18). While both studies enrolled hypertensive subjects off antihypertensive medications and utilized a sham procedure for the control group, there were some differences in study populations and conduct. Our study enrolled a greater proportion of women and black subjects, and the average ambulatory systolic blood pressure prior to randomization was lower.

The method used for renal nerve ablation differed in our study compared to prior studies (endovascular ultrasound vs. radiofrequency catheter ablation). The ultrasound catheter is designed to be placed in the main renal arteries, prior to the first bifurcation, and ablative energy can be targeted below the endothelial and medial surface at a 1-6 mm depth (data on file ReCor Medical, Inc.) (19). In contrast, the recently investigated multielectrode radiofrequency Spyral catheter is designed to target the main distal and branch renal arteries and delivers energy through the endothelium at a depth of 2.15 mm (32). The endovascular ultrasound catheter delivers energy circumferentially, rather than from individual radiofrequency electrodes. It is possible, therefore, that there is less intra- and interoperator variability in the completeness of reaching renal nerves with endovascular ultrasound denervation than for the multielectrode radiofrequency-based denervation. Indeed, a similar blood pressure lowering effect was achieved despite a smaller number of renal ablations with the endovascular ultrasound catheter (5.4±1.0 ablations in the main and large accessory renal arteries only) than in the SPYRAL HTN-OFF MED study (43.8 ± 13.1 total ablations in the main renal arteries and branch vessels) (18). Overall, however, these two study designs enrolled largely similar patient populations, and yielded concordant results, demonstrating that catheter-based renal denervation, independently of the method of renal ablation, lowers blood pressure in early stage hypertension.

This study was designed to evaluate the effect of renal denervation on blood pressure rather than clinical endpoints. Nonetheless, the 6 mm Hg greater reduction in daytime ambulatory systolic blood pressure with renal denervation compared with sham

procedure was chosen to calculate our study sample size as it may be expected to contribute to a reduction in cardiovascular morbidity based on results from previous randomized trials of antihypertensive agents, if maintained in the long term (4, 33). Indeed, we observed an average of ~6 mm Hg greater reduction in daytime ambulatory and office systolic blood pressures in ITT analysis and ~8 mmHg greater reductions in the per-protocol analysis, respectively, in favor of renal denervation compared with sham.

The magnitude of reduction in blood pressure was consistent across multiple prespecified subgroups, yet not all individuals received uniform benefit (see Figure 3), which may be due to a variable involvement of prevailing renal sympathetic nerve activity and neural mechanisms to the pathophysiology of hypertension (34).

Our study has limitations. First, additional follow-up beyond 2 months will be required to determine whether the magnitude of the blood pressure lowering effect remains over time. Scheduled follow-up is planned through 3 years. Also, while in this study no major adverse event occurred and reported adverse events were infrequent, longer follow-up of this trial and additional numbers of treated subjects with renal denervation will provide greater assurance of safety to exclude rare adverse events. While this study was conducted in a well-defined population, whether the results will extend to the ongoing study of subjects with resistant hypertension (TRIO cohort) is not yet known (24).

In conclusion, in subjects with combined systolic–diastolic primary hypertension who were not receiving antihypertensive medications, renal denervation using endovascular ultrasound, safely reduced ambulatory, home and office blood pressure more than a sham procedure at 2 months. Ongoing follow-up as well as additional studies will be important to evaluate the durability and long term clinical impact of renal denervation in subjects with hypertension.

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CHAPTER 11

Redo renal denervation using a multi-electrode radiofrequency system in patients with persistent therapy-resistant hypertension

J. Daemen, MD, PhD, L. Feyz, MD, L. van Zandvoort, PhD,
N.M. Van Mieghem, MD, PhD

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Abstract

Objectives Renal sympathetic denervation has been studied as a potential therapeutic option for patients with therapy resistant hypertension, however a significant proportion of patients do not show a significant reduction in blood pressure and are classified as non-responders. The objective of the present study was to assess whether a redo renal denervation procedure increases response rates.
Methods We present a case series of 3 consecutive renal denervation non-responders treated with the multi-electrode radiofrequency St. Jude EnligHTN™ catheter after an average of 22 months. Patients were followed for 6 months.
Results Mean age was 66 years and 2 patients were male. Patients were previously treated using either Recor's Paradise™ system, the Vessix V2™ system or the Covidien Oneshot™ system. Mean office blood pressure 1 year after the initial procedure was 187/102mmHg with a mean 24h ambulatory blood pressure of 166/102mmHg. All patients underwent a successful redo procedure using the EnligHTN™ system because of persistent therapy resistant hypertension. At 6 months a significant drop in both office and ambulatory blood pressure of -27/-6mmHg and -15/-13mmHg respectively was observed. No significant renal artery stenosis was observed at 6 months.
Conclusions In patients with therapy resistant hypertension not responding to a first renal denervation procedure, a redo procedure using the St. Jude EnligHTN™ system might help to significantly improve blood pressure control.

Introduction

Controlling blood pressure in hypertensive patients remains a challenge and treatment targets are frequently not achieved despite multiple antihypertensive drugs (1). Renal sympathetic denervation (RDN) has been studied as a potential therapy to decrease blood pressure in patients with therapy resistant hypertension (2-4). Several catheter based RDN techniques exist and proof of adequate denervation and efficacy is mounting (5, 6). However, the individual magnitude of the treatment effect proved to vary extensively with a significant proportion of patients not responding to the therapy in terms of blood pressure reduction – irrespective of the technology used. A substantial number of both patient and procedural characteristics have been hypothesized to account for this so-called non-responsiveness (7, 8). While characteristics like increased age along with a non-compliant arterial system as well as inadequate renal nerve destruction with first generation devices are clear theoretical causes of this "non-responsiveness", scientific evidence is still scarce. Previous work already demonstrated that a higher degree of renal nerve disruption leads to a higher inhibition of the sympathetic nerve system (9). Building further on this hypothesis, performing a redo procedure could theoretically make sense in a proportion of the patients not responding to a first RDN procedure. Besides one case report on a successful redo procedure using the Symplicity Flex system and one study on the effect of cryoablation as a second line option in RDN non-responders no data is available on the additional effect of a redo RDN procedure on blood pressure reduction (10, 11). We present a series of 3 consecutive RDN non-responders treated with a second procedure using the next generation multi-electrode St. Jude Medical EnligHTN system.

Methods

Patient selection and definitions

We present a series of 3 consecutive patients who did not show a relevant reduction in both office and ambulatory blood pressure after renal sympathetic denervation because of therapy resistant hypertension. All procedure were performed at the Erasmus Medical Center, Rotterdam, the Netherlands, between December 2012 and March 2013. Initial denervation was performed with the Paradise™ system (Recor Medical, Palo Alto, CA), the Oneshot™ system (Covidien, Campbell, CA) and the Vessix V2™

system (Boston Scientific, Natick, MA) respectively (12). In order to qualify as non-responders, patients had to fulfil the following criteria: mean systolic 24-h ambulatory blood pressure>150mmHg despite treatment with >3 antihypertensive drugs or failure to show a reduction of >10 mmHg in mean systolic 24h ambulatory blood pressure at 12 months after the initial RDN procedure.

Office blood pressure was collected according to the Standard Joint National Committee VII Guidelines and ESC/ESH Guidelines (13). 24-h ambulatory blood pressure was obtained by using an Ambulatory Blood Pressure System (Spacelabs Healthcare, Inc., Issaquah,WA, USA).

Redo procedures were performed at 22.3±4.6months post the initial procedure. At the time of the redo procedure all patients were free of adverse events related to the index procedure, showed no signs of renal artery stenosis as diagnosed using either CT or MR angiography and had a preserved renal function.

Redo denervation procedure

All patients were preloaded with 300mg aspirin, if naïve, and advised to continue with aspirin for at least 1 month. All procedures were performed under conscious sedation by using 1 to 3mg of midazolam and 50 to 100mcg of fentanyl. After local anaesthesia common femoral artery access was achieved successfully after an echo guided puncture and the introduction of a 6Fr sheath. Under fluoroscopic guidance the short 6Fr sheath was exchanged for a 8Fr RDN or IMA tipped guiding sheath which is recommended for a more easy use of the St. Jude EngligHTN system. Pre-procedurally, 100IU heparin/kg were administered to achieve an active clotting time>250s. After smoothly engaging the renal arteries by using a no-though technique with the help of a standard high-torque BHW coronary guidewire, selective renal artery angiograms were made and an appropriate basket size was chosen (small basket 4.0–5.5 mm diameter/large basket 5.5–8.0 mm diameter). The BHW guidewire was exchanged for the EnligHTN™ ablation catheter with its tip proximal to the bifurcation of the main renal artery. The basket catheter, containing four bipolar Platinum-Iridium electrodes, was then opened with the impedance of each electrode on the basket monitored. After a total of 4 ablations were performed successfully the basket was collapsed and retracted proximally while another 4 ablations were performed in the same artery. A total of 8 ablations were performed in each vessel, except for 1 artery in 1 patient in which the basket could not be engaged due to a very steep take-off of the right renal artery.

Follow-up

All patients were discharged home the following day. All patients were followed according to a dedicated follow-up protocol including office visits at 1, 3 and 6 months along with office blood pressure measurement at each time point and ambulatory blood measurements at 3 and 6 months. Additionally a 12-lead ECG, blood- and urine collection and a 6-month assessment of renal artery patency using either CTA or MRA were performed in all patients.

Results

Patient demographics and baseline characteristics are depicted in Table 1. Procedural characteristics in Table 2 and clinical follow-up parameters in Table 3.

Table 1. Baseline characteristics

	Patient 1	**Patient 2**	**Patient 3**
Age	76	59	60
Gender	Female	Male	Male
Race	Hindu	Caucasian	Caucasian
Length, cm	168	196	170
Weigth, kg	99	98	76.9
BMI, kg/m2	35.2	25.6	26.6
Mean office blood pressure (mmHg)	193/93	173/115	195/98
Mean ambulatory blood pressure (mmHg)	173/101	161/107	164/98
Diabetes	No	No	Yes
Hypercholesterolemia,	No	No	Yes
Current smoking	No	No	Yes, 20PY
eGFR, ml/min	56	72	90
Coronary artery disease	No	No	No
Peripheral artery disease	Yes	No	No
History of stroke	Yes	No	No
Antihypertensive medication			
Ace inhibitor	Yes	No	No
Angiotensin receptor blocker	No	Yes	Yes
Diuretic	Yes	Yes	Yes
Calcium channel blocker	Intollerant	Yes	Yes
Alpha blocker	No	Yes	Yes
Beta blocker	Yes	Yes	Yes
Aldosteron receptor antagonist	No	No	No
Vasodilator	No	No	Yes

	Patient 1	Patient 2	Patient 3
Previously used RDN device	Recor Paradise 3 ablations/ artery	Vessix 8 ablations/ artery	Covidien 1 ablation/ artery
Office blood pressure before initial treatment	207/93	165/100	184/103
Ambulatory blood pressure before initial treatment	155/75	161/109	162/98
Change in office blood pressure 1 year after 1st procedure	-14/0	+8/+15	+11/-5
Change in ambulatory blood pressure after 1st procedure	+18/26	0/-2	+2/0

Table 2. Redo procedure characteristics

	Patient 1	Patient 2	Patient 3
Number of ablations	8 (left side only)	8+8	8+8
Duration of the procedure, min	64	81	45
Contrast usage, ml	70	120	90
Renal artery dissection	No	No	No
Renal artery spasm	No	No	Yes
Renal artery thrombus	No	No	No

Table 3. Clinical follow-up parameters

	Patient 1		Patient 2		Patient 3	
	3 months	6 months	3 months	6 months	3 months	6 months
Drop in mean office blood pressure (mmHg)	-31/+2	-62/-18	+8/-5	+28/+10	-50/-23	-30/-10
Drop in ambulatory blood pressure (mmHg)	-44/-28	-16/-22	-2/+3	-9/-4	-2/-4	-20/-12
Change in heart rate (bpm)	0	0	-8	-2	-19	-9
Change in eGFR (ml/min)	0	+5	+6	-5	0	0
Antihypertensive medication						
Ace inhibitor	Yes	Yes	No	No	No	No
Angiotensin receptor blocker	No	No	Yes	Yes	Yes	Yes

	Patient 1		Patient 2		Patient 3	
	3 months	6 months	3 months	6 months	3 months	6 months
Diuretic	Yes, dose decreased	Yes	Yes	Yes, dose increased	No	No
Calcium channel blocker	Intollerant	Intollerant	Yes	Yes	No	No
Alpha blocker	No	No	Yes	Yes	No	No
Beta blocker	Yes	Yes	Yes	Yes	yes	yes
Aldosteron receptor antagonist	No	Yes	No	No	No	No
Vasodilator	No	No	No	No	No	No
Adverse events						
Death	No	No	No	No	No	No
Myocardial infarction	No	No	No	No	No	No
Stroke/TIA	No	No	No	No	No	No

Patient 1 was a 76 year-old obese female with persistent headaches, known atrial fibrillation and hypertension since the age of 36. Due to repetitive episodes of life-threatening gastrointestinal bleeding Coumadin's were stopped and replaced by Aspirin. Secondary causes of hypertension were excluded. With a mean office blood pressure of 207/93mmHg (24h ABPM 155/75mmHg) despite the use of 3 antihypertensive drugs, she underwent a first RDN using the ReCor Paradise™ system. After an uneventful recovery her blood pressure remained uncontrolled and she suffered from an ischemic stroke. One-year after the initial treatment office blood pressures remained 193/93mmHg and given the clear hypertension related comorbidities a second procedure combining a redo RDN procedure and a percutaneous left atrial appendage closure were performed. Due to a very steep take off of the right renal artery only a left sided ablation was performed successfully. After a successful recovery office blood pressures significantly dropped to 131/75mmHg (ambulatory 129/73 at 3 months, 157/79mmHg at 6 months and 110/64mmHg at 12 months). The headaches resolved and renal function increased from 56ml/min at baseline to 61ml/min at 6 months.

Patient 2 was a 59 year-old Caucasian male with persistent headaches and a history of hypertension since the age of 19 for which he received 6 antihypertensive drugs. Renal function was preserved. With a mean office blood pressure of 165/100mmHg and a 24h ambulatory blood pressure of 161/109mmHg he underwent a successful bilateral RDN procedure using the Vessix V2™ RDN system after secondary causes

of hypertension were excluded. One year after his initial procedure his clinical condition remained stable with no improvement in blood pressure control. Both office and ambulatory blood pressure remained unchanged (office mean 173/115mmHg, ambulatory 161/107mmHg) despite a stable medication regimen. Given his persistent headaches and persistent refractory hypertension a redo denervation was scheduled. After intensive follow-up at 1, 3 and 6 months, ambulatory blood pressures started to slightly decrease to 159/110 at 3 months and 152/103 at 6 months while office blood pressure remained stable.

Patient 3 was a 60-year-old Caucasian male suffering from insulin-dependent diabetes, hypercholesterolemia, and hypertension since more than 10 years. The patient was a heavy smoker (over 20 pack years). Despite two percutaneous coronary interventions the patient suffered from stable angina CCS Class II and severe fatigue. Both renal and cardiac functions were preserved. The patient was on 6 antihypertensive drugs and was referred for RDN with an office and ambulatory blood pressure of 184/103mmHg and 162/98mmHg respectively. One year after a successful bilateral RDN using the Covidien OneShot™ system blood pressures remained unchanged and angina worsened to class III. Given the extensive number of comorbidities, persistent grade III hypertension with a mean office blood pressure of 195/98mmHg (ambulatory 164/98mmHg) a redo procedure was scheduled in combination with a new coronary angiogram. After a successful bilateral RDN using the EnligHTN™ system the coronary angiogram showed several 3-vessel disease for which the patient was discussed in the heart team and accepted for coronary artery bypass surgery which was performed soon there-after. The patient recovered quickly and uneventfully. Along with a significant reduction in both office and ambulatory blood pressure to 165/99mmHg and 146/86mmHg complaints of fatigue resolved and angina reduced to class I. The number of antihypertensive drugs was reduced to 2 at 6 months.

Discussion

In the present case series we report the outcome of 3 consecutive patients with persistent grade III hypertension despite an initial technically successful RDN procedure 1 year before while still being on optimal medical therapy. A second RDN procedure using the St. Jude EnligHTN™ system proved to be safe in all patients with no procedure related adverse events up to 6 months. Drops in ambulatory systolic blood-pressure ranged from 9mmHg in patient 2 to 20mmHg in patient 3.

Uncontrolled hypertension is independently associated with the incidence of stroke, coronary- and peripheral artery disease, heart failure, sudden death and renal insufficiency (14, 15). Conversely, only a small portion of the hypertensive patients has an elevated blood pressure alone, with the majority suffering from additional cardiovascular risk factors. Furthermore the presence of hypertension and other cardiovascular risk factors may potentiate each other resulting in an exponentially elevated risk for future adverse events (13). The latter is reflected by the patients presented in this case series, in which a persistently uncontrolled blood pressure might have contributed to their comorbidities and adverse events. Despite extensive follow-up in a tertiary referral center in which secondary causes of hypertension were excluded in all patients and drug-regimens were improved blood pressure levels remained unacceptably high. The latter illustrates the need for more effective antihypertensive therapies in patients with drug-resistant hypertension. In the past years a wide variety of (non)invasive treatment options have been evaluated reducing the sympathetic nervous system in order to optimize blood pressure control (16, 17). Of these, percutaneous RDN is currently the most widely studied option with studies reporting 24h ambulatory systolic blood pressure reduction in the range of 2 to 16mmHg (4, 18, 19). Individual treatment response however proved to vary extensively from unchanged hypertension and thus non-responsiveness to frank hypotension and need to taper pharmacological antihypertensive treatment. The frequency of "non-responder" rates reportedly varies between 15 and 30% when a definition of a lack in decrease in office systolic blood pressure >10mmHg was used (9, 20, 21).

Despite the growing body of evidence supporting the potential additional value of renal sympathetic denervation in patients with therapy resistant hypertension, responders have not been clearly identified yet. The hypothesis of the present study was to test if a second denervation using the multi-electrode EnligHTN system could lead to a higher degree of sympathetic nerve damage and subsequently lead to a higher level of blood pressure reduction as a surrogate endpoint. Two previous studies evaluated the effectiveness of a second denervation procedure. A case report by Lambert et al. reported on a 79-year-old hypertensive patient who experienced a drop of 40mmHg in systolic office based blood systolic blood pressure 6 months after an initial procedure using the Medtronic Symplicity™ system (11). With blood pressure levels back to baseline levels at 1-year post procedure the decision was made to perform a redo procedure using the same device. Three months later office systolic blood pressure levels were again

40mmHg lower. The case suggests a positive effect of a redo denervation procedure but also illustrates the high variability of office blood pressure precluding any firm conclusions. A second study evaluated the safety and effectiveness of cryoablation in 10 RDN non-responders (10). The study reported an impressive reduction in both mean systolic office blood pressures as well as ambulatory blood pressure of 61mmHg and 52mmHg respectively at 12 months.

In the Symplicity HTN-I study, a mean reduction in renal norepinephrine spillover was reported of 47%. However, the 95% confidence interval ranged from 28 to 65% (20). The hypothesis that this first generation device was potentially not as technically efficacious as initially hypothesized was soon confirmed by interesting post-mortem work by Vink et al. (22) In a patient who died 12 days after a RDN procedure, the authors demonstrated a dome-shaped distribution field with limited penetration leaving a large part of the nerves in (peri-)adventitial areas unaffected. The authors concluded that it was unlikely that RDN, using the first generation Symplicity device, would result in complete interruption of the continuity of all adventitial nerve bundles around the renal arteries. As a consequence several new devices quickly appeared to the market with data showing substantially higher degrees of (peri)adventitial nerve damage along with higher drops in norepinephrine levels.[5, 6] Remarkably, non-responder rates did not clearly improve, suggesting that also specific patient related factors might account for part of the non-response. The fact that the redo procedure was effective in all 3 patients in the present study creates a new dilemma since all of them were treated with second-generation devices. On the other hand, it suggests that a redo procedure could be an option for all non-responders – at least for now.

To the best of our knowledge this is the first report of the potential performance of a redo RDN procedure using the EnligHTN™ RDN system in 3 patients with persistent refractory hypertension. Despite their rather distinct baseline characteristics and risk profile the redo procedure significantly helped in improving blood pressure control and physical condition in 2 patients and modestly reduced blood pressure in 1 patient. Given the lack of validated alternative treatment options in these high-risk patients we believe that a redo RDN using the EnligHTN™ system can be a safe and effective option to improve blood-pressure and potentially reduce future adverse cardiovascular events.

Limitations

The present study was limited by a small number of patients with extensive comorbidities. Two out of 3 patients received an additional treatment in combination with the redo denervation procedure. It is however unlikely that the left atrial appendage closure and/or coronary artery bypass surgery led to the significant blood pressure reductions observed in both patients with known hypertension since over 20 years.Finally, we cannot confirm that a similar effect of the redo procedure might have occurred with other devices than the EnligHTN™ system.

Conclusion

Redo RDN using the EnligHTN™ system can be a safe and effective option to improve blood-pressure control in patients with persistent therapy resistant hypertension after a first RDN procedure.

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CHAPTER 12

Effect of catheter-based renal denervation on left ventricular function, mass and (un)twist with two-dimensional speckle tracking echocardiography

Lida Feyz, MD; Bas van Dalen, MD, PhD; Marcel L. Geleijnse, MD, PhD; Nicolas M. Van Mieghem, MD, PhD; Ron T. van Domburg, PhD; Joost Daemen, MD, PhD

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Abstract

Background Speckle tracking echocardiography (STE) is an echocardiography modality that is able to measure LV characteristics including rotation, strain and strain rate. Strain measures myocardial fibre contraction and relaxation . This study aims to assess the effect of renal sympathetic denervation (RDN) on functional myocardial parameters including STE and to identify potential differences between responders and non-responders.

Methods The study population consisted of 31 consecutive patients undergoing RDN in the context of treatment for resistant hypertension. Patients were included between December 2012 and June 2014. Transthoracic echocardiography and speckle tracking analysis was performed at baseline and at six months follow-up.

Results The study population consisted of 31 patients with treatment resistant hypertension treated with RDN (mean age 64 ± 10 y, 15 men). The total study population could be divided into responders (n=19) and non-responders (n=12) following RDN. RDN reduced office blood pressure by 18.9 ± 26.8/8.5 ±13.5 mmHg ($p<0.001$). A significant decrease was seen in left ventricular posterior wall thickness (LVPWd) 0.47 ± 1.0 mm ($p=0.020$), without a significant change in LV mass index (LVMI). In the total cohort, only peak late diastolic filling velocity (A-wave velocity) decreased significantly by 5.3 ± 13.2 cm/s ($p=0.044$) and peak untwisting velocity decreased significantly by 14.5 ± 28.9 degrees/s ($p=0.025$).

Conclusion RDN reduced blood pressure and significantly improved functional myocardial parameters such as A-wave velocity and peak untwisting velocity in patients with treatment resistant hypertension, suggesting a potential beneficial effect of RDN on myocardial mechanics.

Background

Hypertension is associated with a significantly increased risk for adverse cardiac and cerebrovascular events as well as chronic kidney disease (1). Despite optimal medical treatment, blood pressure control often remains poor and the risk for cardiovascular disease remains high. With of prevalence ranging between 15 and 30%, treatment resistant hypertension remains an important medical challenge and leads to intrinsic changes in the heart muscle and is associated with LV hypertrophy and diastolic dysfunction (2, 3).

Catheter-based renal denervation (RDN) has been introduced as a treatment modality to optimize blood pressure control in patients with treatment resistant hypertension by reducing sympathetic nerve activity. Unfortunately, the exact blood pressure lowering effect of renal sympathetic denervation remains disputed demonstrated by non-responder rates varying between 8 and 37% depending on study specific cohorts and definitions used (4). However, sympathetic hyperactivity has been directly associated with LV remodeling and heart rate, which makes it imperative to look at the effects of renal sympathetic denervation beyond blood pressure (5). Speckle tracking echocardiography (STE) is a new echocardiography modality that is able to measure LV characteristics including rotation, strain and strain rate. Strain measures myocardial fibre contraction and relaxation (6). This study aims to assess the effect of RDN on functional myocardial parameters including STE and to identify potential differences between responders and non-responders.

Methods

Study population

The study population consisted of 31 consecutive patients undergoing RDN in the context of treatment for resistant hypertension according to recent recommendations (7). Patients were included between December 2012 and June 2014. All patients underwent non-invasive pre-procedural renal artery imaging and were discussed in a multi-disciplinary team including interventional cardiologists, radiologists and hypertension specialists. As part of routine practice all patients referred for RDN underwent extensive blood- and urine analyses, 24-hour ambulatory blood pressure measurement (24h ABPM), echocardiography, ECG, and magnetic resonance imaging

(MRI) to assess renal artery eligibility and exclude renal artery stenosis in order to be able to exclude secondary causes of hypertension. Informed consent was obtained from all patients.

Definitions and endpoints

Office blood pressure measurements were recorded three times in a resting situation with intervals of 5 minutes using an Omron automated blood pressure monitor. Patients were classified as responders in case the drop in 6-months office systolic blood pressure was 10mmHg or higher. In order to identify subtle changes in LV function, STE was used to obtain apical rotation, basal rotation, LV twist, twist velocity, peak untwisting velocity, time to peak untwisting velocity, global longitudinal strain (GLS), global circumferential strain (GCS), peak early and late longitudinal diastolic strain rate, peak early and late circumferential diastolic strain rate. LV twist is defined as the maximal value of the apical systolic rotation - basal systolic rotation (8).

Transthoracic echocardiography

Echocardiography measurements were performed before the RDN-procedure (baseline) and six months after the RDN-procedure. Two-dimensional grayscale images were obtained in the left lateral decubitus position using a commercially available ultrasound system (iE33, Philips, Best, The Netherlands), equipped with a broadband (1-5MHz) S5-1 transducer (frequency transmitted 1.7MHz, received 3.4 MHz). Data were analyzed by two experienced echocardiographers according to the recent recommendations (9). The following echocardiographic parameters were acquired: LV end-diastolic septal (IVSd) and posterior wall thickness (LVPWd), LV end-diastolic (LVEDD) and end-systolic dimension (LVESD). LV mass was calculated with the Devereux formula (10). Body surface area (BSA) was calculated according to the Mosteller formula (11). LV mass was indexed by BSA as recommended in the guidelines (12).

From the mitral-inflow pattern, peak early (E-wave velocity) and late (A-wave velocity) filling velocities, E/A ratio and E-wave velocity deceleration time were measured. Tissue Doppler was applied end-expiratory in the pulsed-wave Doppler mode at the level of the inferoseptal side of the mitral annulus from an apical 4-chamber view, to obtain Em septal (peak early diastolic wave velocity of the mitral annulus) and E/Em ratio.

To acquire the highest wall tissue velocities, the angle between the Doppler beam and the longitudinal motion of the investigated structure was adjusted to a minimal level. The spectral pulsed-wave Doppler velocity range was adjusted to obtain appropriate scale. To optimize speckle tracking echocardiography settings were adjusted to obtain a frame rate of 50-70 frames/s. The echo images were transformed to a QLAB Advanced Quantification Software workstation (version 10.0, Philips, Best, The Netherlands) for offline analysis.

Speckle tracking analysis

STE is an approved echocardiographic modality that provides information on regional and global ventricular function (13). In order to obtain this information apical long-axis views (4-, 3- and 2-chamber view) and parasternal short-axis views (at apical, mid-ventricular and basal LV level) were assessed. The aortic valve closure was assessed in a parasternal long-axis view and added manually. After selecting the appropriate view, the endocardial border was automatically recognized and the tracking points were positioned. When this auto-trace function was not optimal the tracking points were re-positioned manually on an end-diastolic frame. Next, the software automatically tracked these points using speckle tracking. LV ejection fraction was assessed using this automated endocardial border detection. Most components of LV systolic function (rotation [clockwise rotation as viewed from the apical level has a positive value and counterclockwise rotation from the basal level has a negative value], twist, GCS and global circumferential strain rate (GCSR)) and diastolic function (peak untwisting velocity, time to peak untwisting velocity, peak early and late circumferential diastolic strain rate) were abstracted from parasternal short-axis views, whereas others were derived from the apical views (systolic function: GLS and global longitudinal strain rate (GLSR); diastolic function: peak early and late longitudinal diastolic strain rate). Data were exported to a spreadsheet program (Excel, Microsoft Corporation, Redmond, WA) to determine these parameters. In a previous study we have demonstrated the reproducibility and variability of the parameters investigated in the current study in our center (14).

RDN procedure

Procedures were performed using four different systems Paradise™ (Recor Medical, Palo Alto, CA) (n=13), Oneshot™ (Covidien, Campbell, CA) (n=3), Vessix V2™

(Boston Scientific, Natick, MA) (n=5) and Symplicity™ (Medtronic, Minneapolis, MN) (n=10). Procedures were performed according to device-specific instructions for use (7, 15). All procedures were performed under conscious sedation with midazolam and fentanyl.

Statistical analysis

All continuous variables are expressed as mean ± SD. The continuous variables were compared using Student's t test. Simple linear regression of peak untwisting velocity against heart rate was performed. Categorical variables were compared with the Chi-square test or Fisher's Exact test when appropriate. A P value < 0.05 was considered statistically significant. The statistical analysis was performed with SPSS statistical analyses (version 21.0).

Results

Study population Thirty-one patients with resistant hypertension following RDN were enrolled in this study, all of whom completed the six months follow-up period. Mean age was 64 ± 10 y, 15 patients (48%) were male. A total of 19 patients were classified as responders vs. 12 non-responders. Besides a significantly lower age of the responders 61 ± 10 y vs. 69 ± 9 y in the non-responders (p= 0.028), no significant differences in patient characteristics were seen between both groups (Table 1).

Table 1. Clinical characteristics of the study population, responder vs. non responder at baseline

	All patients baseline	**Responder** baseline	**Non-responder** baseline	**p-value** responder vs. non-responder
	(n=31)	**(n=19)**	**(n=12)**	
Age, years	64 ± 10	61 ± 10	69 ± 9	0.028
Male gender n, (%) 15 (48)	10 (53)	5 (42)	0.552	
BMI, kg/m^2	29 ± 4	29 ± 5	28 ± 3	0.639
Mean office SBP, mmHg	182 ± 18	186 ± 20	176 ± 15	0.133
Mean office DBP, mmHg	94 ± 16	97 ± 14	89 ± 18	0.149
Mean systolic ABPM, mmHg	150 ± 12	148 ± 12	152 ± 13	0.358
Mean diastolic ABPM, mmHg	83 ± 13	83 ± 11	82 ± 17	0.946
Heart rate, beats/min	68 ± 12	69 ± 12	65 ± 12	0.282
CAD, (%)	16 (52)	12 (63)	4 (33)	0.106
Atrial fibrillation, (%)	2 (7)	2 (11)	-	0.368
Cardiovascular risk factors n, (%)				
Hypercholesterolaemia	22 (71)	15 (79)	7 (58)	0.204
Smoking				
Current	11 (36)	6 (32)	5 (42)	0.619
Diabetes Mellitus	8 (26)	7 (37)	1 (8)	0.086
Number of hypertensive drugs	4 ± 1	4 ± 1	4 ± 1	0.634
Patients receiving (drug class), (%)				
ACE inhibitors/ARBs	27 (87)	17 (90)	10 (83)	0.507
Direct renin inhibitors	1 (3)	-	1 (8)	0.387
Beta-blockers	25 (81)	16 (84)	9 (75)	0.435
Alfa-blockers	9 (29)	4 (21)	5 (42)	0.168
Calcium-channel blockers	25 (81)	16 (84)	9 (75)	0.435
Aldosterone antagonist	4 (13)	3 (16)	1 (8)	0.493
Diuretics	22 (71)	13 (68)	9 (75)	0.417
Central acting agent	3 (10)	1 (5)	2 (17)	0.328

Values are mean ± SD or n (%). BMI= body mass index (kg/m2), SBP= systolic blood pressure, DBP= diastolic blood pressure. ABPM= ambulatory blood pressure monitoring. CAD= coronary artery disease, ACE= angiotensin converting enzyme, ARBs=angiotensin receptor blocker.

Blood pressure A significant decrease was seen in office based systolic blood pressure (SBP) and diastolic blood pressure (DBP) after RDN at six months follow-up 182 ± 18 mmHg vs. 163 ± 27 mmHg ($p < 0.001$) and 94 ± 16 mmHg vs. 85 ± 14 mmHg ($p=0.001$). The same applies for the systolic 24h ABPM after RDN 150 ± 12 mmHg vs. 142 ± 18 mmHg ($p= 0.017$) and diastolic 24h ABPM after RDN 83 ± 13 mmHg vs. 78 ± 11 mmHg ($p=0.006$) (Table 2).

Table 2. Clinical, echocardiographic and speckle tracking parameters in patients following renal denervation

	Baseline	Follow-Up	p-Value (6 Months)
Clinical parameters			
Mean office SBP, mmHg	182 ± 18	163 ± 27	<0.001
Mean office DBP, mmHg	94 ± 16	85 ± 14	0.001
Mean systolic ABPM, mmHg	150 ± 12	142 ± 18	0.017
Mean diastolic ABPM, mmHg	83 ± 13	78 ± 11	0.006
Heart rate, beats/min	68 ± 12	63 ± 11	0.016
Echocardiographic parameters			
LA-size, mm	45.1 ± 7.9	44.5 ± 6.8	0.523
LAVI, ml/m²	36.8 ± 10.9	35.6 ± 11.0	0.335
IVSd, mm	10.8 ± 2.1	10.3 ± 1.8	0.105
LVPWd, mm	8.8 ± 1.4	8.3 ± 1.5	0.020
LV-EDD, mm	53.8 ± 8.1	55.5 ± 7.9	0.028
LV-ESD, mm	37.7 ± 8.7	39.5 ± 8.3	0.048
LV-EF, %	59.5 ± 11.0	58.1 ± 10.1	0.123
LVMI, g/m²	105.8 ± 33.8	102.6 ± 30.2	0.133
Doppler indices			
E, cm/s	67.2 ± 20.5	65.0 ± 23.1	0.519
A, cm/s	69.7 ± 14.2	64.4 ± 14.0	0.044
E/A ratio	1.0 ± 0.3	1.0 ± 0.4	0.557
DET, ms	219.7 ± 51.6	219.7 ± 84.3	0.996
Em septal, cm/s	5.5 ± 1.8	5.1 ± 1.7	0.134
E/Em ratio	12.3 ± 4.7	12.5 ± 3.0	0.840
Speckle tracking echocardiography			
GLS, %	-19.6 ± 4.2	-19.9 ± 3.5	0.653
GCS, %	-27.3 ± 6.5	-27.2 ± 5.3	0.877
early GLSR	0.89 ± 0.25	0.89 ± 0.21	0.932
late GLSR	0.80 ± 0.23	0.82 ± 0.24	0.495
early GCSR	1.88 ± 0.62	1.80 ± 0.51	0.470
late GCSR	1.39 ± 0.50	1.32 ± 0.44	0.432
apical GR, degrees	4.8 ± 3.4	4.3 ± 2.7	0.542
basal GR, degrees	-4.2 ± 2.0	-3.5 ± 2.2	0.212
Twist, degrees	8.7 ± 4.4	7.4 ± 3.7	0.178
Twist velocity, degrees/s	15.5 ± 12.9	13.7 ± 12.1	0.665
Peak untwisting velocity, degrees/s	-70.6 ± 28.5	-56.1 ± 24.9	0.025
Time to peak untwisting velocity, s	0.08 ± 0.07	0.09 ± 0.08	0.707
LV-EDV, ml	76.1 ± 28.4	82.6 ± 34.2	0.152
LV-ESV, ml	30.5 ± 17.9	33.9 ± 22.9	0.146
LV-EF, %	61.8 ± 10.1	61.5 ± 8.5	0.803

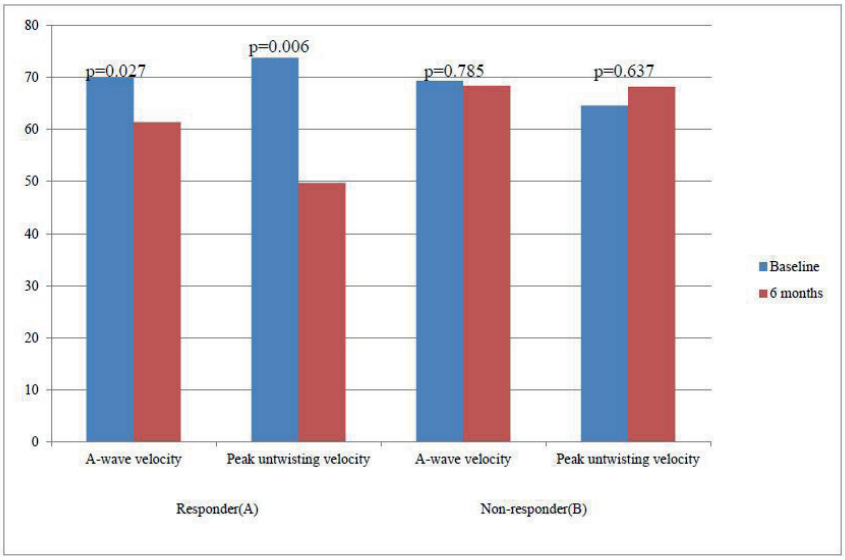
Values are mean ± SD. SBP= systolic blood pressure, DBP= diastolic blood pressure, ABPM = ambulatory blood pressure monitoring. LA = left atrial, LAVI=left atrial volume indexed, IVSd= interventricular septum thickness (diastole), LVPWd= left ventricular posterior wall thickness (diastole), LV-EDD= left ventricular end-diastolic dimension, LV-ESD= left ventricular end-systolic dimension, LV-EF= left ventricular ejection fraction, LVMI= left ventricular mass index, E= peak early phase filling velocity, A= peak atrial phase filling velocity, DET = deceleration time, Em= peak early wave velocity. GLS= Global longitudinal strain, GCS= Global circumferential strain, GLSR= Global longitudinal strain rate (early and late diastole), GCSR= Global circumferential strain rate (early and late diastole), GR= Global rotation (apical and basal level), Twist= instantaneous left ventricular peak systolic twist. Peak untwisting velocity= peak diastolic de-rotation velocity. EDV= end-diastolic volume, ESV= end-systolic volume.

Conventional echocardiography and STE

In the total cohort, significant differences at baseline vs. 6 months follow-up period were noted in LVPWd, A-wave velocity and peak untwisting velocity. LVPWd decreased significantly from 8.8 ± 1.4 mm at baseline to 8.3 ± 1.5 mm at follow-up (p=0.020). LVMI reduced by 3.2 ± 11.6 g/m² at 6 months. A-wave velocity decreased significantly from 69.7 ± 14.2 cm/s at baseline to 64.4 ± 14.0 cm/s at follow-up (p=0.044). No significant changes were seen in other conventional echocardiographic parameters. Furthermore, peak untwisting velocity decreased significantly from -70 ± 28.5 degrees/cm at baseline to -56 ± 24.9 degrees/cm at follow-up (p=0.025) (Table 2).

Stratifying the cohort in responders and non-responders did reveal significant changes from baseline to follow-up in A-wave velocity and peak untwisting velocity in the responders (Figure 1). In the non-responders LVESD increased significantly from 37.4 ± 9.3mm to 39.7 ± 10.1 mm (p=0.013), although it should be noted that LV ejection fraction (EF) showed no difference. Also, no differences were found in the more sensitive systolic parameters GLS, GCS and LV twist.

Figure 1. Change in A-wave velocity and peak untwisting velocity at baseline and 6 months follow-up, categorized to Responders (A) and Non-Responders (B)



Comparing the baseline characteristics of both cohorts, no difference was observed in the LVMI at baseline in the responders as compared to the non-responders 98 ± 25.4g/m^2 vs. 116 ± 42.9 g/m^2 (Table 3).

Predictors for response

No association was found between the clinical characteristics, conventional echocardiographic and speckle tracking parameters which could predict response.

Table 3. Baseline and six-months follow-up parameters in patients following renal denervation, responder vs. non-responder

	Responder (n=19) baseline	6 months	p-value	**Non-responder (n=12)** baseline	6 months	p-value	**p-value** responder vs. non-responder at baseline
Echocardiographic parameters							
LA-size, mm	44.6 ± 8.0	44.2 ± 7.5	0.712	45.8 ± 7.9	44.9 ± 5.7	0.585	0.706
LAVI, ml/m²	37.3 ± 11.1	36.0 ± 12.2	0.394	36.1 ± 11.3	34.9 ± 9.7	0.637	0.855
IVSd, mm	10.5 ± 2.0	10.3 ±2.2	0.408	11.3 ± 2.2	10.3 ± 1.2	0.176	0.352
LVPWd, mm	8.7 ± 1.6	8.3 ± 1.8	0.130	9.0 ± 1.2	8.4 ± 1.2	0.089	0.546
LV-EDD, mm	52.9 ± 7.8	55.1 ± 6.8	0.060	55.1 ± 8.7	56.2 ± 9.7	0.289	0.484
LV-ESD, mm	37.9 ± 8.5	39.4 ± 7.3	0.284	37.4 ± 9.3	39.7 ± 10.1	0.013	0.872
LV-EF, %	57.6 ± 10.1	57.0 ± 9.5	0.587	62.5 ± 12.2	59.8 ± 11.1	0.088	0.235
LVMI, g/m²	98.8 ± 25.4	98.8 ± 25.4	*	116 ± 42.9	108.5 ± 36.9	0.136	0.151
Doppler indices							
E, cm/s	72.2 ± 19.5	70.9 ± 24.7	0.759	59.7 ± 20.5	56.2 ± 17.7	0.555	0.103
A, cm/s	69.9 ± 17.0	61.4 ± 14.4	0.027	69.3 ± 10.0	68.4 ± 13.0	0.785	0.914
E/A ratio	1.0 ± 0.3	1.1 ± 0.3	0.374	0.9 ± 0.3	0.9 ± 0.4	0.889	0.249
DET, ms	207.5 ± 40.6	189.7 ± 41.7	0.155	238.1 ± 62.2	264.6 ± 111.3	0.400	0.113
Em septal, cm/s	5.6 ± 1.6	5.3 ± 1.7	0.515	5.5 ± 2.1	4.8 ± 1.8	0.151	0.882
E/Em ratio	12.5 ± 3.6	12.5 ± 3.3	0.872	12.2 ± 5.8	12.3 ± 2.9	0.898	0.875
Speckle tracking echocardiography							
Twist, degrees	8.0 ± 3.8	6.8 ± 3.8	0.242	10.1 ± 5.2	8.6 ± 3.5	0.492	0.278
Twist velocity, degrees/s	14.7 ± 13.2	13.3 ± 11.2	0.792	17.1 ± 13.2	14.5 ± 14.5	0.740	0.673
Peak untwisting velocity, degrees/s	-73.8 ± 30.4	-49.7 ± 23.8	0.006	-64.6 ± 25.5	-68.2 ± 23.5	0.637	0.475
Time to peak untwisting velocity, s	0.07 ± 0.08	0.09 ± 0.08	0.431	0.10 ± 0.06	0.08 ± 0.07	0.509	0.389
GLS, %	- 19.2 ± 4.7	-19.5 ± 3.9	0.661	-20.6 ± 3.0	-20.6 ± 2.4	0.938	0.480
GCS, %	- 27.2 ± 6.7	-26.5 ± 5.9	0.476	-27.5 ± 6.5	-28.5 ± 4.2	0.657	0.916
early GLSR	0.90 ± 0.28	0.89 ± 0.19	0.809	0.87 ± 0.21	0.91 ± 0.26	0.460	0.625
late GLSR	0.76 ± 0.25	0.77 ± 0.24	0.840	0.86 ± 0.19	0.92 ± 0.22	0.429	0.188
early GCSR	1.91 ± 0.56	1.81 ± 0.50	0.339	1.81 ± 0.75	1.77 ± 0.57	0.883	0.736
late GCSR	1.26 ± 0.53	1.15 ± 0.33	0.320	1.62 ± 0.34	1.62 ± 0.48	0.990	0.094

Values are mean ±SD. LA=left atrial, LAVI= left atrial volume index, IVSd= interventricular septum thickness (diastole), LVPWd=left ventricular posterior wall thickness (diastole), LV-EDD= left ventricular end-diastolic dimension, LV-ESD= left ventricular end-systolic dimension, LV-EF= left ventricular ejection fraction, LVMI= left ventricular mass index, E= peak early phase filling velocity, A= peak atrial phase filling velocity, DET= deceleration time, Em= peak early wave velocity. Twist= instantaneous left ventricular peak systolic twist. Peak untwisting velocity= peak diastolic de-rotation velocity. GLS= Global longitudinal strain, GCS= Global circumferential strain, GLSR= Global longitudinal strain rate (early and late diastole), GCSR= Global circumferential strain rate (early and late diastole). *standard error of the difference.

Discussion

The aim of this study was to assess if RDN resulted in functional and structural cardiac changes as assessed using both conventional 2D echocardiography and 2D speckle tracking echocardiography. A secondary objective was to assess any differences in these parameters between responders and non-responders. We observed a significant difference in blood pressure and heart rate at 6 months post procedure. Echocardiographically, at 6 months, a significant difference was noted in LVPWd and A-wave velocity. Additionally, STE demonstrated a significant difference in peak untwisting velocity.

Persistent sympathetic nervous system hyperactivity plays a critical role in hypertension and is associated with significant structural and functional cardiac changes (16). In this study, we found that RDN significantly reduced office blood pressure by 18.9 ± 26.8/8.5±13.5 mmHg ($p < 0.001$) and heart rate by 4.5 ± 9.9 beats/min ($p=0.016$). In line with these findings we observed a significant decrease in LVPWd 0.47 ± 1.0 mm along with a reduction in LVMI of 3.2 ± 11.6 g/m^2 in the total cohort. The fact this reduction did not reach statistical significance could be due to a lack of power.

Looking further into diastolic function, which is strongly related to hypertension and blood pressure control, revealed a pseudonormal diastolic dysfunction, based on normal E/A ratio, but increased LA-dimension and E/Em ratio in the overall study population. We observed a significant decrease in the A-wave velocity by 5.3 ± 13.2 cm/s ($p=0.044$) in the total population. In the responders, the difference in the A-wave velocity even decreased to 8.5 ± 13.8 cm/s ($p=0.027$). The decrease in A-wave velocity after renal denervation could implicate an improvement in the LV relaxation and a subsequent better diastolic function (17). Additionally, a pseudonormal diastolic function may reflect a decrease in LV compliance and a moderate increase in left atrial (LA) pressure in our population, with impaired relaxation and prolonged A-wave velocity before treatment. The link between peak untwisting velocity and A-wave velocity may be explained by the rate of uncoiling. In other words, it is likely that less force is needed for the active atrial contraction during late diastolic filling after blood pressure lowering. The relation between LV untwisting and the conventional parameters was also described in previous work, in which a positive correlation between untwisting rate and A-wave velocity was found while there was no correlation between E-wave velocity and untwisting rate (8). However, these findings should be interpreted

with caution as no differences were noted in other echocardiographic parameters determining diastolic function such as the E/Em ratio and left atrial dimensions.

Hypertensive patients have a greater risk of developing cardiac fibrosis, myocyte hypertrophy and diastolic dysfunction (18). These changes may influence the LV twist and rotation (19). LV twist is a wringing motion of the heart as the apex rotates with respect to the base around the LV long axis, which is a key element for regulating LV systolic and diastolic mechanics (20). LV twist is derived from the dynamic interaction between subendocardial and subepicardial myocardial fibres, the latter defining the direction of the LV twist. It is known that in myocardial fibrosis and LV hypertrophy, which is related to pressure overload, impairment appears frequently in the subendocardial layer leading to a dominance of the subepicardial fibres (21). This might explain the increased LV twist in patients with LV hypertrophy. LV untwisting starts after the peak LV twist. In a healthy population, the peak systolic twist is supposed to store potential energy and is thought to contribute towards diastolic suction and facilitate the early LV diastolic filling. Previous work from our group demonstrated that peak untwisting velocity is increased in hypertrophic cardiomyopathy patients with mild diastolic dysfunction as well as in aortic stenosis patients (8, 22) . Our study is the first to demonstrate a significant decrease in peak untwisting velocity of 14.5 ± 28.9 degrees/s (p=0.025) in patients undergoing RDN. This change was mainly driven by a change in peak untwisting velocity by 24.1 ± 28.7degrees/s (p=0.006) in the responders. In non-responders, no difference following RDN was observed. The decrease in untwisting velocity after renal denervation could implicate an improvement in the LV relaxation and a subsequent better diastolic function, similar to A-wave velocity. One may hypothesize that in hypertension patients, like in hypertrophic cardiomyopathy and aortic stenosis patients, increased peak untwisting velocity serves as a compensatory mechanism for abnormal relaxation and prevents the need to increase LA pressure (22, 23). RDN probably leads to an improvement of these specific changes in LV rotational and de-rotational mechanics, especially in responders. However, no significant change was seen in twist and twist velocity. Finally, we observed a significant decrease in heart rate following RDN in our population. This is in line with several randomized controlled studies which also demonstrated a decrease in heart rate following RDN (24, 25). Interestingly a similar decrease in heart rate was observed in the (sham) control arm of both studies suggesting that RDN by itself has no significant effect on heart rate. Additional exploratory analyses in our study ruled out a correlation between heart rate an untwisting velocity (R^2= 0,1%).

Future studies, comparing hypertensive patients with a healthy control group may be warranted in order to investigate myocardial geometry changes in hypertensive subjects in context of diastolic LV twisting and untwisting, blood pressure or heart rate independently.

Limitations

Data are derived from a small patient population and a lack of power might have impacted our findings. Furthermore, accurate assessment of changes in cardiac systolic and diastolic function and volumes with conventional echocardiography produces limited image quality in patients with a body mass index (BMI) above 25 kg/m^2. In some patients (n=6) automatically detected LV contours had be corrected manually which might have impacted the reliability of the measurements. Four different renal denervation systems were used. Based on individual previous studies, the blood pressure lowering effect of these individual devices remains in the same range, however, a differential effect on the echocardiographic parameters measured in our study could not be excluded.

Conclusion

RDN reduced blood pressure and heart rate, and significantly improved functional myocardial parameters such as A-wave velocity and peak untwisting velocity in patients with treatment resistant hypertension, suggesting potential pleiotropic beneficial effects of renal sympathetic denervation on myocardial mechanics. Further dedicated studies are needed to elucidate the potential role of RDN on echocardiographic parameters.

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CHAPTER 13

Renal sympathetic denervation in patients with vasospastic angina

Lida Feyz, MD; Maureen Henneman, MD, PhD; Fred Verzijlbergen, MD, PhD; Isabella Kardys, MD, PhD; Nicolas M. Van Mieghem, MD, PhD; Joost Daemen, MD, PhD

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Abstract

Background Sympathetic overactivity has been linked to vasospastic angina (VSA), although the exact pathophysiology of VSA is poorly understood. The purpose of this study is to assess if renal sympathetic denervation (RDN) reduces cardiac sympathetic nerve activity with a subsequent beneficial effect on angina relief in patients with refractory VSA.

Methods and results Cardiac sympathetic nerve activity was assessed prior to procedure and at 6 months post procedure using iodine-123 labeled meta-iodobenzylguanidine (^{123}I-MIBG) imaging. The Seattle Angina questionnaire (SAQ) was used to assess the degree to which the disease impacts quality of life. No significant change was observed in early HMR (pre-RDN: 2.74 [2.10 – 3.21] vs. 6 months post-RDN: 2.57 [2.20 – 3.00]; p=0.76), and late HMR (pre-RDN: 2.56 [2.18 – 3.20] vs. 6 months post-RDN: 2.36 [2.13 – 3.22]; p=0.22) . Additionally, no change was seen in WR (p=0.22). SAQ results revealed significant improvements in perceived physical limitation, angina frequency, and quality of life at 6 months ($p<0.05$ for all).

Conclusion RDN resulted in improvements in angina class and quality of life at 6 months in patients with refractory VSA. RDN, however, did not result in significant changes in cardiac sympathetic nerve activity as measured using ^{123}I-MIBG. The latter observation should be considered with caution given the small sample size of this study. Larger studies are needed to assess this further.

Background

Vasospastic angina (VSA) is a clinical syndrome that was first described by Prinzmetal et al. in 1959 (1). Although the syndrome has been characterized by episodes of coronary artery vasospasm, the exact pathophysiology of spasms is poorly understood. While studies from the early '80s and '90s already demonstrated that both the sympathetic and parasympathetic nervous system are responsible for coronary vasomotion, more recent work demonstrated significant hyperactivity of the sympathetic nervous system (SNS) in patients with VSA as compared to healthy controls (2-4). The majority of VSA patients present with refractory angina, however, the disease appears not benign. Syncope and ventricular arrhythmias are the first clinical presentation in up to 40% of the cases and the risk for sudden death may be increased by up to 50% (5-8). Current treatment options are mainly pharmacological and include the use of long-acting nitrates and calcium-channel blockers, which showed to provide symptom improvement in 30 to 80% of the cases respectively (9). Looking for more effective treatments, several small studies showed that surgical (cardiac) sympathetic denervation resulted in a significantly lower number of angina episodes and ST-segment deviations on 24h-holter monitoring (10, 11). In the present study, we hypothesized that renal sympathetic denervation (RDN), a percutaneous treatment that proved to significantly lower blood pressure and systemic sympathetic nerve activity in hypertensive patients, might provide additional angina relief in patients with VSA. In order to quantify the potential effect of RDN on cardiac sympathetic nerve activity iodine-123-labeled meta-iodobenzylguanidine (^{123}I-MIBG) scintigraphy was used (12, 13).

Methods

Study population and endpoints

Between April 2013 and August 2016 a total of 10 consecutive patients with refractory VSA underwent RDN. Patients were eligible in case of refractory angina. Significant residual coronary artery stenosis was ruled out and patency of previously implanted stents was confirmed in all cases by recent coronary angiography. Coronary physiologic assessment using fractional flow reserve was used in case of intermediate lesions.

VSA was confirmed in case of spontaneous or methylergonovine induced spasms with ST-segment changes and symptoms of chest pain in 6 out of 10 patients. In 4 patients the diagnosis was based on the presence of recurrent episodes of non-exercise induced angina, which resolved after sublingual nitrates and in the absence of significant atherosclerotic coronary artery disease.

Patients were screened for RDN and followed according to routine clinical practice. Work-up included 24h ambulatory blood pressure measurement (24h ABPM), laboratory analysis, echocardiography and CT (N=2), MRI (N=7) or renal duplex (N=1) to confirm renal artery eligibility.

Follow-up in the outpatient clinic at 1, 3 and 6 months post-RDN included 24h ABPM (at 3 and 6 months) and echocardiography (at 6 months). Renal function was assessed at baseline and at follow-up. Renal artery imaging was performed at 6 months to confirm renal artery patency.

For the purpose of this, study patients were not subjects to acts, neither was any mode of behavior imposed, otherwise than as part of their regular treatment. Therefore, according to Dutch law, written informed consent for study enrolment was obtained. This study was conducted according to the privacy policy of the Erasmus Medical Center and to the Erasmus Medical Center regulations for the appropriate use of data in patient-orientated research, which are based on international regulations, including the declaration of Helsinki. All patients consented to the use of their data for scientific research.

The primary efficacy endpoint was the change in cardiac sympathetic nerve activity as measured using ^{123}I-MIBG imaging at 6 months post-RDN, as compared to pre-RDN (baseline). The primary safety endpoint was defined as the occurrence of cardiovascular death, stroke, major access site bleeding and acute kidney injury or renal artery stenosis at 6 months follow-up.

Secondary endpoints included the changes in Canadian Cardiovascular Society grading (CCS class), blood pressure (24h ABPM and office blood pressure) and heart rate. The Seattle Angina Questionnaire (SAQ) was used to assess the quality of life and signs and symptoms of angina.

^{123}I-MIBG scintigraphy data acquisition and analysis

^{123}I-MIBG is a physiologic analogue of norepinephrine and acts selectively on sympathetic nerve endings. By using cardiac neurotransmission imaging global information about neuronal function can be expressed in early, but more specifically in late HMR (reflecting the storage regional distribution and release of ^{123}I-MIBG), with washout rate (WR) reflecting the neuronal integrity or sympathetic tone (14). In order to block thyroid uptake of free radioactive iodide, 200 mg potassium iodide (10%

solution) was administered. After 30 minutes, 185 MBq ^{123}I-MIBG was administered intravenously. Early and late (i.e. 15 minutes and 4 hours after tracer injection, respectively) anterior planar scintigraphic images were acquired for 10 minutes with a zoom factor of 1.0 and stored in a 256 x 256 matrix. Patients were imaged in a supine position with a dual head gamma camera (Symbia T, Siemens, Erlangen, Germany), using a medium energy collimator. An energy window of ± 10% was symmetrically centred around the 159-KeV 123I photo peak. Offline processing software (Hermes Medical Solutions Workstation) was used to draw a round region of interest (ROI) with fixed diameter over the upper mediastinum, below the thyroid gland (Figure 1). Additionally, a manual ROI over the heart was drawn, carefully excluding adjacent activity in the liver and lung. The left ventricular cavity was included in the myocardial ROI. The MIBG images were scored by a dedicated nuclear medicine specialist blinded to the timing of the scan. The HMR was computed by dividing the average number of counts within the cardiac ROI by the average number of counts within the mediastinal ROI. Calculation of WR was performed using the following formula (no correction for background): WR= (HMR_{early} – HMR_{late})/(HMR_{early}) x 100% (15).

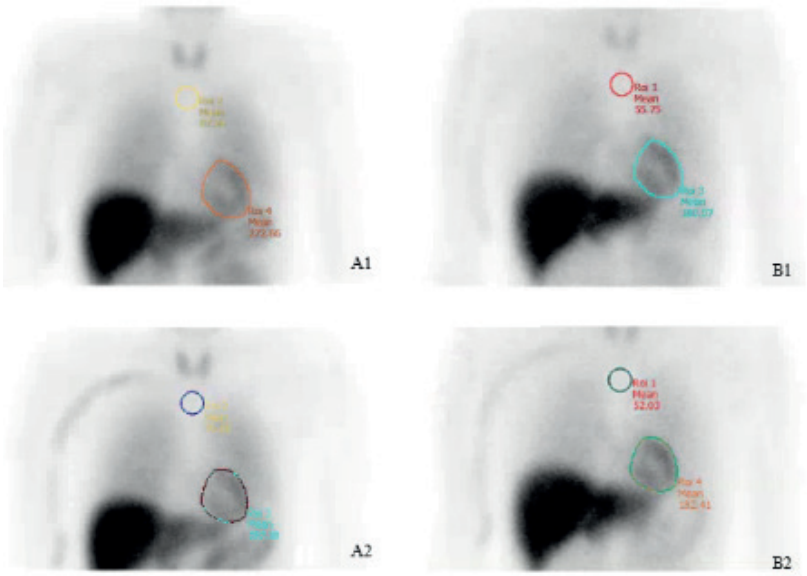

Figure 1. Example of MIBG image scoring with early and late HMR. Early HMR at baseline (A1) was 3.11 and changed to 3.35 at 6 months (A2), while late HMR at baseline was 3.23 (B1) and changed to 3.51 at 6-month (B2) follow-up. (A) 15 min after tracer injection, (B) 4 h after tracer injection

RDN procedure

Pre-procedurally, 100IU heparin/kg was administered to achieve an active clotting time >250s. All procedures were performed under conscious sedation. After administration of local anesthesia, common femoral artery access was achieved by an ultrasound-guided puncture and a 7-Fr sheath was then introduced. A 7-Fr guiding catheter was used to accommodate the Paradise™ RDN catheter (ReCor Medical, Palo Alto, CA) (16). After smoothly engaging the renal arteries by using a no-touch technique with the help of a standard high-torque BHW coronary guidewire selective renal artery angiograms were made. The Paradise™ system ablation catheter was then advanced over the BHW wire. The Paradise™ catheter has a distal balloon which is pressurized by the Paradise system to a range of 1.5-2.0 ATM using sterile circulating water. The ultrasound transducer is located within the balloon (balloon diameters 5mm – 8mm). A total of 2 to 3 ultrasound emissions of 6 to 10 seconds each were delivered per artery.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD). Continuous variables were compared using Student's t test. Categorical variables were expressed as percentages and were compared using the Chi-square test or Fisher's Exact test when appropriate. Early- and late HMR and WR were compared using the Wilcoxon signed-rank test. All statistical tests are 2-tailed. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS statistical analysis (version 22.0).

Results

Study population

Mean age of the patients was 57 ± 11 years, 90% were male and 90% were in CCS class III or IV. None of the patients had diabetes, and hypertension was present in 80% of the cases.

All patients were using anti-anginal therapy using long-acting nitrates (10/10) and calcium channel-blockers (8/10). Most patients had a history of prior coronary revascularization (Table 1).

Table 1 Baseline characteristics

Total study population N=10	
Age, years	57 ± 11
Male n, (%)	9 (90)
BMI, kg/m^2	26.6 ± 5.0
eGFR, ml/min	76 ± 17
Cardiovascular risk factors (%)	
Diabetes	0 (0)
Hypertension	8 (80)
Dyslipidemia	9 (90)
Smoker, current	3 (30)
Family history of premature CVD	7 (70)
Cardiovascular history (%)	
Prior MI	7 (70)
Prior PCI	8 (80)
24h ABPM, mmHg	121 ± 16/72 ± 8
Office BP, mmHg	143 ± 19/80 ± 10
Heart rate, bpm	62 ± 8
Angina grading scale	
CCSI	-
CCSII	1 (10)
CCSIII	8 (80)
CCSIV	1 (10)
Echocardiographic parameters	
LVEF, %	59 ± 9.6
LVEDD, mm	50 ± 6.1
LVESD, mm	34 ± 4.9
Pharmacological therapy, n (%)	
Nitrates,	10 (100)
Calcium channel blockers*	8 (80)*
Selective beta-blockers	5 (50)
ACE/ATII	8 (80)
Aspirin	10 (100)
Diuretics	4 (40)
Statins	10 (100)

Variables are presented in mean ± SD or %. ABPM=ambulatory blood pressure measurement, BP=blood pressure, BMI=body mass index, CCS=Canadian cardiovascular society grading of angina pectoris, CVD=cardiovascular disease, eGFR=estimated glomerular filtration rate, MI=myocardial infarction, LVEF=left ventricular ejection fraction, LVEDD=left ventricular end-diastolic diameter, LVESD=left ventricular end-systolic diameter, PCI=percutaneous coronary intervention. *N=2 were intolerant for calcium channel blockers.

Primary efficacy endpoint

^{123}I-MIBG change

No significant change was observed in early HMR (pre-RDN: 2.74 [2.10 – 3.21] vs. 6 months post-RDN: 2.57 [2.20 – 3.00]; p=0.76) and in late HMR (pre-RDN: 2.56 [2.18 – 3.20] vs. 6 months post-RDN: 2.36 [2.13 – 3.22]; p=0.22). No significant change was observed in WR (pre-RDN: 15.0 [10.5 – 18.5] vs. 6 months post-RDN: 13.0 [6.0 – 22.0]; p=0.22) (Table 2).

Table 2 Cardiac sympathetic nerve activity measured by ^{123}I-MIBG pre- and 6 months post-procedure, expressed in median [IQR]

	Pre-RDN	6 months	p
Early HMR	2.74 [2.10 – 3.21]	2.57 [2.20 – 3.00]	0.76
Late HMR	2.56 [2.18 – 3.20]	2.36 [2.13 – 3.22]	0.22
WR	15.0 [10.5 – 18.5]	13.0 [6.0 – 22.0]	0.22

Variables are presented in mean ± SD. HMR=heart/mediastinum ratio, WR=washout rate

Primary safety endpoints

There were no peri-procedural complications. No adverse events including death, stroke or renal artery stenosis occurred out to 6 months follow-up. Renal function remained unchanged, estimated glomerular filtration rate (eGFR) was 76 ± 17 ml/min pre-RDN vs. 75 ± 18 ml/min at 6 months follow-up (p=0.68).

Secondary endpoints

Outcome on angina and quality of life

CCS class improved significantly from 3.00 ± 0.47 (pre-RDN) to 1.80 ± 0.92 at 6 months follow-up; p=0.005.

The SAQ results showed significant improvements in 3/5 subscales at 3 and 6 months follow-up; patients were less limited in daily activities due to angina, angina frequency decreased significantly and quality of life improved as compared to pre-RDN. Angina stability and treatment satisfaction remained unchanged at 3 and 6 months follow-up (Figure 2).

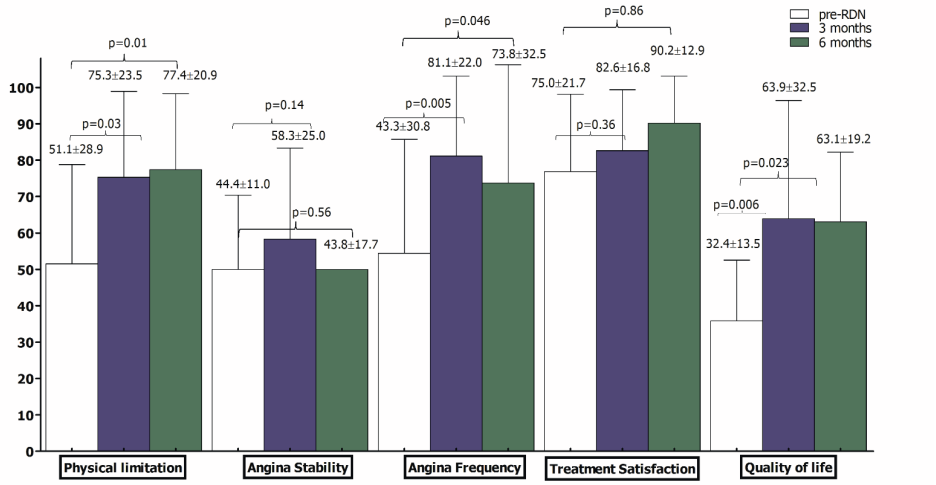

Figure 2. Seattle Angina Questionnaire (SAQ) SAQ scale (each scale is a score of 0 to 100, wherein higher scores indicate better function or less angina/limitation and better quality of life)

Blood pressure change

A numerical decrease in both office- and ambulatory BP at 6 months was found as compared to pre-RDN. Office BP changed from 143 ± 19/80 ± 10mmHg pre-RDN to 132 ± 12/77 ± 7mmHg at 6 months follow-up and 24h ABPM decreased from 121 ± 16/72 ± 8mmHg to 112 ± 8/70 ± 7mmHg (p=ns for all).

Change in medication

During the course of the study, long-acting nitrates and calcium-channel blockers were decreased or stopped in 4 patients, while dosages were increased in 3 patients.

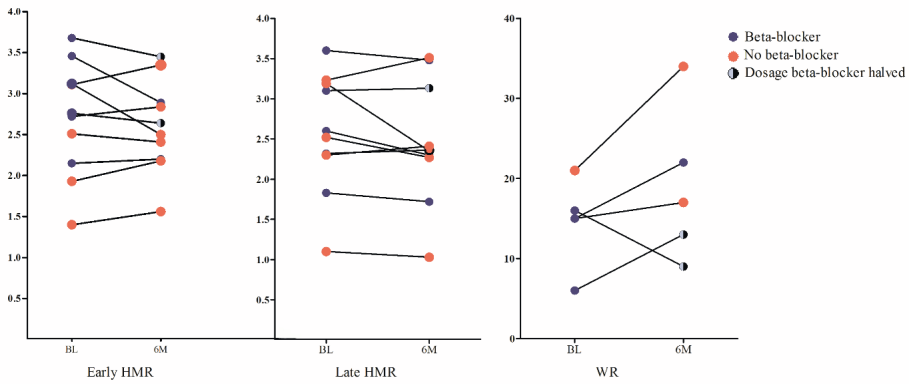

Figure 3. Change in early and late heart-to-mediastinum ratio (HMR), and washout rates (WR) at 6-month follow-up. Legend: 5 patients were off beta-blocker (red bullet) during the course of the study; 2 patients were on beta-blockers (same dose) at baseline and follow-up; 3 patients had betablockers at baseline and their dosage was halved (N = 2) and N = 1 was stopped at 6 months. In 5 patients, no washout rate (WR) could be calculated at baseline or 6 months.

Discussion

This present single centre pilot study demonstrated that RDN might significantly improve quality of life at 6 months post procedure. However, no significant change was observed in cardiac sympathetic nerve activity as assessed by ^{123}I-MIBG at 6 months post procedure.

The autonomic nervous system is a key regulator of coronary vasomotion and an imbalance between the sympathetic and parasympathetic nervous system has been reported in patients with VSA (4, 17, 18). Provocation testing with parasympathetic agents such as acetylcholine or ergonovine can be performed in an attempt to confirm the diagnosis (19). In vitro, sympathetic agents like norepinephrine were used to test the severity of vasoconstriction or spasm (20).

In the present study, we directly assessed cardiac sympathetic nerve activity by ^{123}I-MIBG imaging, in line with previous work demonstrating the potential use of the technique in diagnosing VSA (21). ^{123}I-MIBG is a physiologic analogue of norepinephrine and acts selectively on sympathetic nerve endings. By using cardiac neurotransmission imaging global information about neuronal function can be expressed in early, but more specifically in late HMR (reflecting the storage regional distribution and release of ^{123}I-MIBG), additionally, the WR reflects the neuronal integrity or sympathetic tone (14). ^{123}I-MIBG uptake is quantified by calculating an HMR after drawing regions of interest over the heart and mediastinum. Normal values for HMR and WR are 2.5 ± 0.3 or greater and 20% ± 10% or less, respectively (22). Little data is available on reference values in patients with VSA. Taki et al. reported higher WR in patients with VSA, probably due to increased sympathetic tone resulting in increased turnover of MIBG or impaired MIBG reuptake at the nerve endings (23, 24). In addition, Arbab et al. showed a late HMR of 1.80 ± 0.60 in nine patients with VSA which was numerically lower as compared to healthy controls (2.00 ± 0.36) (25). In the present study, pre-RDN HMR and WR were in line with previously published reference values for healthy control groups. Even in the cohort of patients with positive methylergonovine testing, mean late HMR was within the normal range. Our observations question whether HMR and WR in patients with VSA are truly significantly different than in healthy controls. Of note, several minor differences in imaging acquisition between this and other studies should be acknowledged such as the use of different collimators and radioactive compounds.

RDN has been studied since 2008 for its potential to control blood pressure in patients with therapy resistant hypertension, in which increased SNS activity has been hypothesized to play an important role (26). Only very recently, the results of several sham-controlled randomized trials demonstrated that the therapy, conducted using a radiofrequency ablation catheter, might significantly decrease blood pressure (27). At the same time, a series of studies were conducted to assess potential pleiotropic effects of RDN (28, 29). In the present study, we attempted to further extend these findings in a cohort of VSA patients without residual regular therapeutic options. Given the limited options in objectively assessing the severity of signs and symptoms of these patients, we decided to further study the effect of RDN by using ^{123}I-MIBG imaging. We hypothesized that post-RDN, late HMR would increase and WR would decrease as compared to pre-RDN(23, 25). However, 6 months post RDN we found no meaningful differences in both early and late HMR and WR. The latter thus again questions the role of the SNS in patients with VSA and the potential of RDN to decrease intra-cardiac sympathetic nerve activity.

Nevertheless, we found significant improvements in angina class and quality of life at 6 months along with a numerical decrease in BP. Following treatment with the Paradise ultrasound balloon catheter, mean systolic ambulatory blood pressure decreased with 9mmHg at 6 months. Although these figures did not reach statistical significance in the present small single-center study, the primary results of the RADIANCE SOLO trial assessing the safety and efficacy of the same device in a multicentre randomized sham-controlled setting showed a significant decrease in daytime 24h ABPM as compared to sham (30).

Limitations

There are several limitations that should be taken into account. First, results were based on a small pilot study (N=10) and should be considered hypothesis generating. Using the results of the present study along with the findings of previous work by Arbab et al, a total of 70 patients would be needed to support that RDN results in a 20% increase in late HMR. Second, WR was calculated using one of the methods as described by Flotats et al. (31), in which we used the actual heart counts for calculating WR, instead of ratios. Third, while the positive finding of a significant reduction in angina class could be interpreted as promising, a potential placebo effect of the treatment cannot be ruled out. In the recently published sham-controlled ORBITA trial, a sham procedure was able to significantly improve at least 3 subscales of the SAQ in patients with

stable angina and significant coronary artery disease (32). Fourth, anti-anginal drug-regimen was changed in 7/10 of the patients (including an increase in nitrates (n=1) and diltiazem (n=1)). The impact of medication on MIBG uptake should be taken into account. Jacobson et al. described that the MIBG uptake could be inhibited mostly by the use of beta blockers such as labetalol, less evidence is available on the MIBG-inhibitory effect by calcium channel blockers (33). Furthermore, we acknowledge the fact that half of the patients were still using selective beta-blockers that were deliberately continued mostly due to the presence of a myocardial infarction in the past, in patients (N=3) beta-blockers were stopped or dosages changed during follow-up. However, also in patients in whom beta-blockers were either stopped or dosages were changed, no change in late HMR was found which was in line with results in patients without changes in drug regimen.

Finally, we cannot exclude the fact that apart from autonomic nervous system tone, multiple other alternative factors might play a role in triggering coronary spasms such as endothelial dysfunction or microvascular dysfunction (34, 35).

Conclusion

RDN resulted in significant improvements in angina class and quality of life at 6 months in patients with refractory vasospastic angina. RDN did not result in significant changes in cardiac sympathetic nerve activity as measured using ^{123}I-MIBG.

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CHAPTER 14

Renal denervation as a treatment strategy for vasospastic angina induced ventricular tachycardia

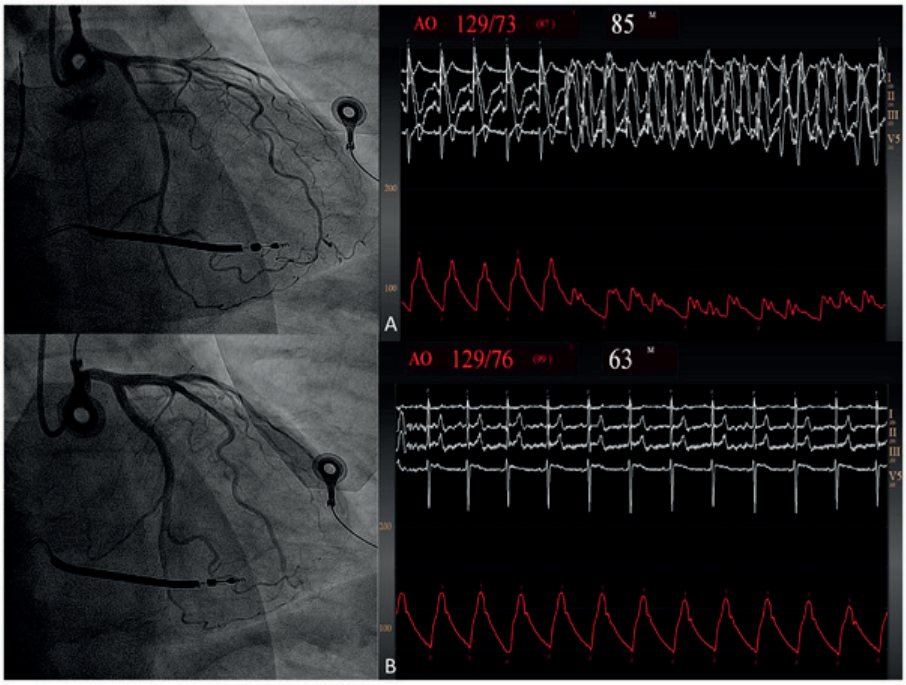
Lida Feyz, MD; Sip Wijchers, MD; Joost Daemen, MD, PhD

Neth Heart J. 2017 Oct;25(10):596-597

Heart beat

Polymorphic ventricular tachycardia (VT) can be a detrimental consequence of coronary vasospasm (1). Although the pathophysiology of vasospastic angina (VA) is poorly understood, sympathetic hyperactivity has been linked to the disease (2-4). We present a 52-year-old male smoker with ventricular fibrillation (VF) 2 years ago. Work-up revealed no signs of structural heart disease or obstructive coronary disease. An implantable cardioverter-defibrillator (ICD) was implanted and several episodes of non-sustained polymorphic VT and ICD shocks due to VF ensued, despite maintenance therapy with isosorbide 100mg, metoprolol 50mg, verapamil 300mg, amiodarone 200mg. Methylergometrine testing confirmed VA as the cause of VF. *(Fig.1 A-B).* To reduce sympathetic hyperactivity bilateral renal denervation (RDN) was performed using the ReCor Paradise™ system. The patient remained free from episodes of angina and ventricular arrhythmias at 6 and 12 months. Blood pressure and mean heart rate remained stable between baseline and 6 months (108/60mmHg vs. 113/71mmHg and 60 bpm and 60 bpm respectively). RDN might be a safe and effective treatment for normotensive patients with severe VA despite optimal medical therapy.

Figure 1 Methylergometrine testing revealed severe coronary spasms in multiple coronary segments resulting in VT and hemodynamiccollapse *(Panel A),* which quickly revolved after intracoronary nitrates *(Panel B)*



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CHAPTER 15

Atrial fibrillation reduction by renal sympathetic denervation: 12 months' results of the AFFORD study

Lida Feyz, MD; Dominic A. Theuns, PhD; Rohit Bhagwandien, MD; Mihai Strachinaru, MD, PhD; Isabella Kardys, MD, PhD; Nicolas M. Van Mieghem, MD, PhD; Joost Daemen, MD, PhD

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Abstract

Aim The purpose of this pilot study was to assess whether renal sympathetic denervation (RDN) decreases atrial fibrillation (AF) burden in hypertensive patients with symptomatic AF at 6 and 12 month follow-up, as measured using an implantable cardiac monitor (ICM).

Methods and results A total of 20 patients with symptomatic paroxysmal or persistent AF (EHRA ≥ II) and primary hypertension with a mean office systolic blood pressure (BP) of >140mmHg were enrolled. After enrolment, an ICM was implanted 3 months pre-RDN to monitor AF burden. Quality of life (QOL) was assessed using the Atrial Fibrillation Effect on QualiTy-of-life (AFEQT) questionnaire. Mean age was 64 ± 7yr and 55% was female. AF burden in min/day decreased from a median [IQR] of 1.39 [0-11] pre-RDN to 0.67 [0- 31.6] at 6 months (p=0.64) and to 0.94 [0 – 6.0] at 12 months (pre-RDN vs. 12 months; p=0.03). QOL improved significantly at both 6 months (+11 ± 15 points, p=0.006) and 12 months (+10 ± 19, p=0.04) as compared to pre-RDN. Office BP decreased significantly at 12 months follow-up (-20 ± 19/-7±10mmHg), p<0.01) as compared to pre-RDN.

Ambulatory BP decreased -7 ± 16/-3 ± 9mmHg (p>0.05) at 12 month follow-up as compared to pre-RDN .

Conclusion This pilot study suggests that RDN might be able to decrease AF burden in min/day as measured using an ICM, with a positive effect on QOL. Large-scale randomized trials are needed to prove the definite value of RDN in hypertensive patients with atrial fibrillation.

Introduction

AF is the most common arrhythmia worldwide, occurring in 2-3% of the adult population (1). The incidence of AF, along with an inherent risk for thromboembolic events, increases with age and the presence of hypertension (2). Vice versa, hypertension is the most common cardiovascular condition responsible for the development and recurrence of AF (3). AF is associated with an impaired quality of life (QOL) and is known to increase the risk of hospitalization by 2- to 3-fold resulting in increased health care costs (4). In general, the current treatment options for AF can be divided into either pharmacological and/or ablation therapy (i.e. pulmonary vein isolation, PVI), performed either by percutaneous or surgical techniques. Despite improving tools and techniques, many patients remain symptomatic and side effects of pharmacological treatment are common with high recurrence rates following catheter ablation, especially in patients with hypertension (5-7). Furthermore, major complications as tamponade and stroke have been reported in up to 4.5% of the patients treated with percutaneous techniques, while the impact on survival associated to any of the therapies mentioned above is still disputed (8). Previous pathophysiological studies described a correlation of AF burden to hyperactivity of the sympathetic nervous system (SNS) and demonstrated that, by modulating the SNS directly, AF control might improve significantly (9-11). While renal sympathetic denervation (RDN) has been studied to help control hypertension (12), its potential value in improving signs and symptoms of AF is currently unknown. The aim of the present pilot study is to assess if RDN decreases occurrence and symptoms of AF in patients with symptomatic paroxysmal or persistent AF at 6 and 12-month follow-up.

Methods

Study design and patient population

This study is a single-arm pilot study including 20 patients. Patients were eligible for enrolment if all of the following inclusion criteria were met: paroxysmal or persistent AF, hypertension (mean office systolic BP ≥ 140mmHg), use of ≥2 antihypertensive drugs, age ≥ 18 years, estimated glomerular filtration rate (eGFR) > 45 ml/min/1.73m^2. Paroxysmal AF was defined as an episode of AF that terminated spontaneously in less than seven days. Persistent AF was

defined as AF that fails to terminate in seven days and an intervention was needed to restore sinus rhythm (pharmacologic or electrical cardioversion). Patients with permanent AF, renal artery abnormalities, first episode of AF, comorbidities with a life expectancy of less than one year or unwillingness to undergo RDN or ICM implantation or follow-up visits were excluded. Secondary causes for hypertension were excluded prior to enrolment. The study was approved by our local ethics committee and all patients provided written informed consent *(trialregister.nl, NTR number: NTR5329).*

Study measurements and endpoints

Clinical and laboratory data were obtained at 1, 3, 6 and 12 months post-RDN; annual follow-up will be continued up to 3 years. The primary efficacy objective, AF burden, was measured with the SJM Confirm DM2102 ICM (St. Jude Medical, St Paul, MN, USA) featuring both an automatic and a manual activation trigger, AF triggers, heart rate histograms, mean heart rate and ventricular rate response (VRR) monitoring during AF. The primary safety objective was defined as a composite of death from cardiovascular causes, stroke, major access site bleeding, acute kidney injury or renal artery stenosis.

Serious adverse events (SAE's) and adverse events reported spontaneously by the subject or observed at each follow-up or any time in between, were recorded by the investigator. A SAE was defined as: any untoward medical occurrence, or effect, which in any dose results in events that were fatal or life-threatening, or that required a prolonged hospitalization; as well as any other important medical event that required intervention.

Secondary outcomes included change in office BP, ambulatory blood pressure measurements (ABPM), change in 24h-holter monitoring, change in echocardiographic parameters (change in left ventricular (LV) and left atrial (LA) volumes and dimensions and change in LV diastolic function) and QOL measurements (using the AFEQT questionnaire). The AFEQT questionnaire is an AF-specific health related QOL questionnaire designed to be used in different clinical settings and for research purposes to assess the impact of AF on patients QOL. Overall or subscale scores range from 0 (complete disability or limitations) to 100 (no disability or limitations) (13).

Office BP was measured at each follow-up visit with an automatic blood pressure monitor (Omron M10-IT). ABPM was performed using the Ultralite Ambulatory Blood Pressure monitor (Spacelabs Healthcare, model 90217A) and 24h-holter monitoring was performed with the evo digital recorder (Del Mar Reynolds, Spacelabs Healthcare) to assess changes in (supra)ventricular ectopic beats (S)VE beats. We aimed to maintain the antihypertensive and antiarrhythmic drug regimen during the course of the study in all patients, however, changes were allowed in case of hypotension, hypertension or frequent AF episodes.

ICM implantation and interrogation

ICM (SJM Confirm DM2102 (St. Jude Medical, St Paul, MN, USA)) implantation was performed by an electrophysiologist under local anesthesia 3 months pre RDN. A small incision (about 2-3cm) was made lateral to the sternum at the level of the fourth and the fifth intercostal spaces. After the procedure, patients were instructed to use the activator in case of symptoms. To measure AF burden, the ICM was interrogated at each study visit per protocol by an expert (DT) to obtain the following parameters: functional status of the device, device battery level, analysis of any abnormal heart rhythms (AF, tachycardia, bradycardia and asystole) along with the highest VRR during AF episodes.

Final AF burden was assessed by confirming the AF episodes on the ECG readings in order to prevent false positive and false negative results. The minimum arrhythmia duration for an appropriate AF episode to be recorded was 30 seconds and a tachycardia cut-off rate of 120bpm was applied. Final outcomes were based on 3-month intervals; pre-RDN (0-3 months pre- procedure), 6 months (3-6 months post-RDN) and 12 months (9-12 months post-RDN). AF burden was defined as the average minutes/day spent in AF. Prior to procedure a total of 2/20 patients with persistent AF progressed to permanent AF when using data derived from the long-term ECG recordings through ICM, both patients were excluded from the assessment of the primary outcome due to inequivalent burdens of AF as compared to patients with paroxysmal or persistent AF.

RDN procedure

All patients were preloaded with 300mg aspirin, if naïve, and advised to continue with aspirin for at least 1 month. Pre-procedurally, 100IU heparin/kg were administered to

achieve an active clotting time>250s. All procedures were performed under conscious sedation. After administration of local anesthesia, common femoral artery access was achieved by an ultrasound-guided puncture and a 6-Fr sheath was then introduced. Under fluoroscopic guidance, the short 6-Fr sheath was exchanged for an 8-Fr RDN or an IMA tipped guiding sheath, to accommodate the St. Jude EnligHTN™ system. After smoothly engaging the renal arteries by using a no-touch technique with the help of a standard high-torque BHW coronary guidewire, selective renal artery angiograms were made and an appropriate basket size was chosen (small basket 4.0–5.5 mm diameter/large basket 5.5–8.0 mm diameter). The BHW guidewire was exchanged for the EnligHTN™ ablation catheter with its tip proximal to the bifurcation of the main renal artery. The basket catheter, containing four bipolar Platinum-Iridium electrodes, was then opened with the impedance of each electrode on the basket monitored. After a total of 4 ablations were performed successfully the basket was collapsed and retracted proximally while another 4 ablations were performed in the same artery, with the intention to achieve at least 8 successful ablations per artery.

Statistical analysis

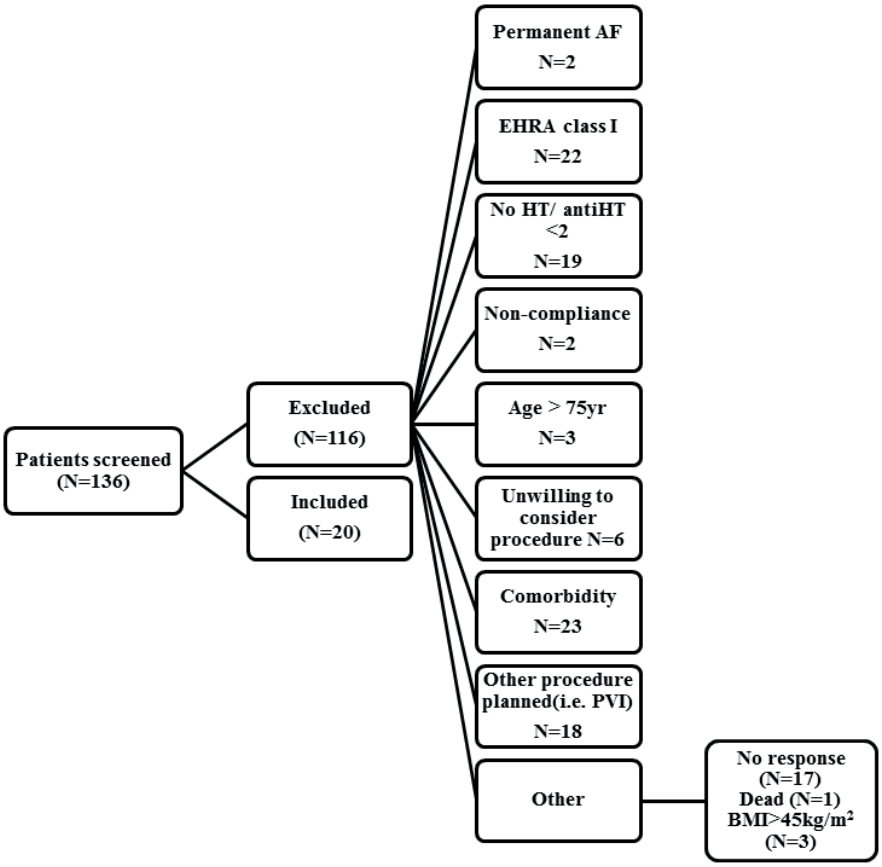
Continuous variables were expressed as mean ± standard deviation (SD) when normally distributed; non-normally distributed variables were presented as median [interquartile range, IQR]. Categorical variables were expressed as percentages. Continuous variables were compared using Student's t test. Categorical variables were compared with the Chi-square test or Fisher's Exact test when appropriate. The Wilcoxon signed-rank test or McNemar's test were performed to analyse the AF burden. Spearman correlations coefficient was used to evaluate the relationship between blood pressure drop and the change in AF burden. The Friedman test was performed to analyse EHRA class. All statistical tests are 2-tailed. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS statistical analysis (version 21.0).

Results

Between July 2014 and February 2016 a total of 136 patients were screened for eligibility. Twenty patients (14.7%) met the inclusion criteria (Figure 1). Baseline characteristics are presented in Table 1. In brief, nine patients were male and mean age was 64 ± 7

years. Mean office blood pressure was 153/88mmHg. Most patients were on rhythm control (19/20) and 20% of the patients had a history of pulmonary vein isolation. Timing between the RDN- and the previous PVI-procedure in 4/20 patients was 916 ± 116 days. Based on ambulatory BP measurements a total of 11 patients had essential hypertension according the definitions of the European Society of Hypertension (14) and 9 patients had white coat uncontrolled hypertension. None of the patients had a history of obstructive sleep apnea syndrome.

Figure 1. Screening process based on inclusion- and exclusion criteria



Legend: AF=atrial fibrillation, antiHT=antihypertensive medication, BMI=body mass index, EHRA=European Heart Rhythm Association, HT=hypertension, PVI=pulmonary vein isolation

Table 1. Baseline characteristics of the study population

	Total study population N=20
Age, years	64 ± 7
Male n, (%)	9 (45)
BMI, kg/m²	30.7 ± 5.6
Paroxysmal AF n, (%)	18 (90)
EHRA class II	15 (75)
EHRA class III	5 (25)
Mean heart rate, bpm	71 ± 15
Office BP, mmHg	153 ± 17/88 ± 11
Ambulatory BP, mmHg	130 ± 15/77 ± 9
Cardiovascular risk factors n, (%)	
Diabetes	2 (10)
Hypertension	20 (100)
Dyslipidemia	8 (40)
Smoking	1 (5)
Family history of IHD	4 (20)
Cardiovascular history n, (%)	
Prior PVI	4 (20)
Prior CVA	2 (10)
Antiarrhythmic drugs n, (%)	
Class I	4 (20)
Class II	1(5)
Class III	12 (60)
Class V	2 (10)
Antihypertensive drugs n, (%)	
ACE-i	7 (35)
ARB	8 (40)
Beta-blockers*	18 (90)
CCB	10 (50)
Alfa-blocker	3 (15)
Diuretics	13 (65)

Values are mean ± SD or n (%). AF=atrial fibrillation. BMI=body mass index. BP=blood pressure, CVA=cerebrovascular accident, EHRA=European Heart Rhythm Association, IHD=ischemic heart disease, PVI= pulmonary vein isolation. * 2/20 patients were intolerant for beta-blockers.

AF burden decreased at 6 and 12 months. AF burden (min/day) was 1.39 [0-10.9] pre-RDN versus 0.67 [0-31.6] at 6 months (p=0.64) and 0.94 [0-6.0] at 12 months (p=0.03). Changes in AF episodes and change in the total minutes in AF are presented in Table 2.

Table 2. AF burden on ICM monitors pre-RDN versus 6 and 12 months follow-up

	Pre-RDN	6 months	12 months	p*	p**
AF episodes, n	1 [0 - 11]	1 [0 – 11]	3 [0 – 16]	0.84	0.31
Total episodes AF, min	125 [2 – 978]	44 [0 – 2833]	84 [0 – 544]	0.64	0.03
AF min/day	1.39 [0 – 10.9]	0.67 [0 – 31.6]	0.94 [0 – 6.0]	0.64	0.03
Highest VRR, bpm	127 [105 – 145]	117 [104 – 141]	106 [75 – 126]	0.09	0.01

Values are in median [IQR]. AF= atrial fibrillation, ICM=implantable cardiac monitor, VRR=ventricular rate response during AF. *between pre-RDN vs. 6 months, **pre-RDN vs, 12 months. Results are based on 18/20 patients, patients with permanent AF were excluded.

Two patients progressed from persistent AF to permanent AF prior to RDN and were excluded from AF burden analysis. Both patients underwent failed attempts to restore sinus rhythm by ECV. Despite persistent EHRA class II on rate control both patients declined PVI during the course of the study. A sub analysis in patients with a history of PVI and an additional RDN showed a numerical decrease in AF burden min/day at 6 months and 1 year follow-up, from a median of 973 min [11.1-1440] to 12.9 min [3.7 – 22.8] at 6 months to 1.57 [0.5 – 27.3] at 1 year ($p>0.05$ for both).

Renal function remained unchanged at both 6 and 12 months follow-up, eGFR (ml/min) pre-RDN was 83 ± 20 vs. 86 ± 21 at 6 months ($p=0.23$) and 86 ± 23at 12 months ($p=0.14$). No cases of cardiovascular death, stroke, major access site bleeding, acute kidney injury or renal artery stenosis were reported. One peri-procedural complication was reported involving a renal artery dissection that resolved after balloon dilatation.

Office systolic BP decreased from 153±17mmHg pre-procedure to 148±17mmHg at 6 months ($p=0.13$) and to 133±16 at 12 months follow-up ($p<0.01$) (Table 3). No correlation was found between Δ BP (ABPM) and Δ AF burden (in terms of min/day or episodes); at 12 months (Δ number of AF episodes and Δ mean 24h systolic ABPM $r= -0.09$; $p=0.74$ and ΔAF min/day and Δ mean 24h systolic ABPM, $r=0.30$; $p=0.28$).

Table 3. Office and ambulatory blood pressure change pre-RDN versus 6 and 12 months follow-up

	pre-RDN	6 months	12 months	p*	p**
Office systolic BP, mmHg	153 ± 17	148 ± 17	133 ± 16	0.13	<0.01
Office diastolic BP, mmHg	89 ± 10	81 ± 11	81 ± 10	0.006	0.007
24h ABPM systolic, mmHg	131 ± 16	121 ± 9	124 ± 11	0.007	0.07
24h ABPM diastolic, mmHg	78 ± 9	72 ± 6	74 ± 9	0.006	0.16

Values are mean ± SD. ABPM=ambulatory blood pressure measurement, BP=blood pressure, *pre-RDN vs. 6 months . **pre-RDN vs. 12 months

Mean heart rate on 24h-holter monitoring remained unchanged at 6 months (-5 ±14bpm; p=0.15) and 12 months follow-up (-1±14bpm;p=0.63) as compared to baseline. A numerical decrease was seen in SVE beats at 6- and 12-month follow-up as compared to pre-procedure. VE beats remained unchanged during follow-up (Table 4). EHRA class improved significantly at both 6 and 12 months as compared to pre-RDN (p<0.01) (Figure 2).

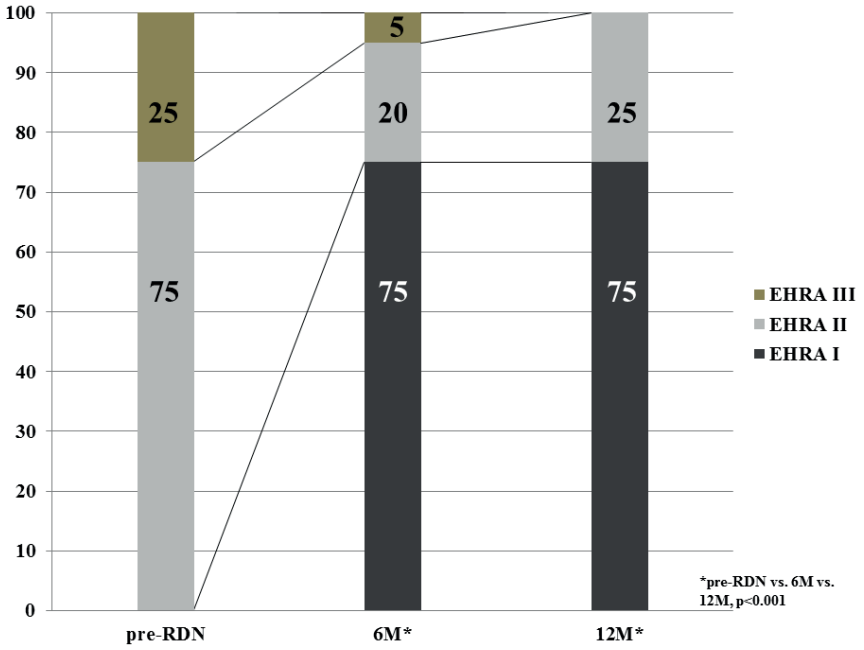
None of the patients underwent PVI within 1 year follow-up post-RDN.

Table 4. 24h-holter monitoring for (S)VE beats

	pre-RDN	6 months	12 months	p*	p**
Heart rate, bpm	71 ± 15	66 ± 8	70 ± 12	0.15	0.63
SVE, beats	187 [82 – 948]	137 [43 – 1096]	79 [13 – 763]	0.36	0.05
VE, beats	35 [3 – 153]	22 [3 – 86]	42 [5 – 134]	0.57	0.73

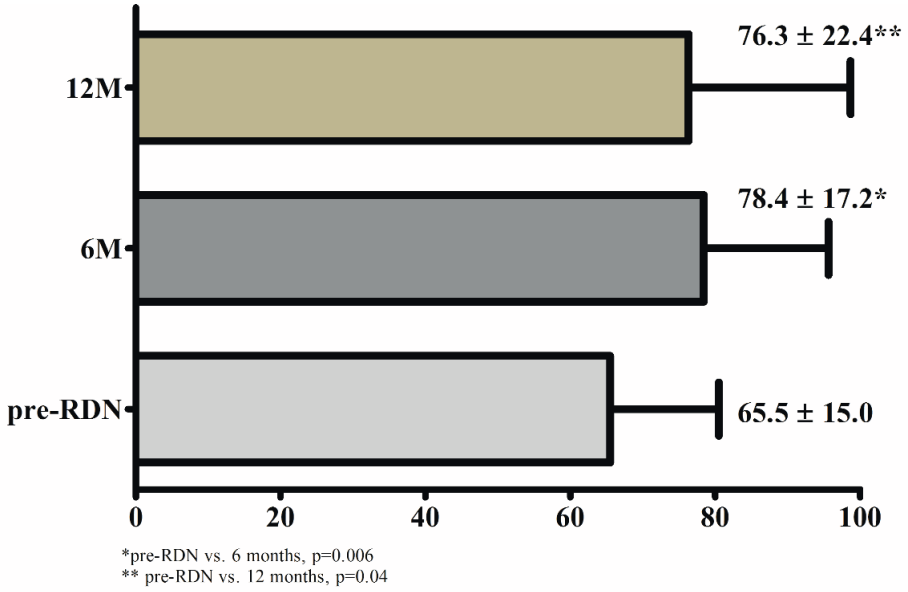
Values are mean ± SD or are median [IQR]. (S)VE=(supraventricular)ectopic beats.*pre-RDN vs. 6 months . **pre-RDN vs. 12 months

Figure 2. Change in EHRA class at follow-up visits (6- and 12 months results)



No change was found in LV and LA volumes and dimensions at 6- and 12 months post RDN (Supplement, Table S1). QOL improved significantly at both follow-up visits (as compared to pre-procedure; +11±15 points at 6 months, $p<0.01$ and +10 ± 19 at 12 months, $p=0.04$) (Figure 3).

Figure 3. AFEQT questionnaire



Legend: pre-RDN=prior to renal sympathetic denervation, 6M=6 months, 12M=12 months

A total of 3/20 (15%) patients underwent an ECV post RDN due to recurrence of AF at 6 months, the average period to perform the first ECV post-RDN in these patients was 134 ± 83 days. In 2 out of these 3 patients a second ECV was done and 1 patient received a total of 3 ECV's during follow-up. Despite efforts in maintaining initial drug regimens during the course of the study, antiarrhythmic drugs or drug dosages were increased in 3/20 patients (these were different patients from who underwent an ECV) and decreased in 6/20 patients. Antihypertensive medication was increased in 2/20 patients, and decreased or stopped in 4/20 patients.

Discussion

This single center pilot study suggests that RDN was able to significantly decrease AF burden in min/day as measured using an ICM, together with an improvement in QOL. Finally, RDN appeared safe with a positive effect on blood pressure.

Sympathetic hyperactivity has been considered an important source for the induction, and maintenance of arrhythmias (15). Previous studies provided evidence for the presence of increased atrial sympathetic activity in both persistent and paroxysmal AF, suggesting autonomic remodeling may be part of the atrial substrate for AF (16, 17).

Pathophysiological studies demonstrated that by modulating the SNS through RDN, AF control might improve (18). Linz and colleagues demonstrated the effect of RDN on heart rate and VRR in pigs with permanent AF, the authors described a reduction of 24% in VRR in the treated pigs versus sham (19). Furthermore, the potential effects of RDN on ventricular electrophysiological properties should be mentioned. Huang and colleagues demonstrated that RDN could alter the ventricular effective refractory period (ERP) and action potential duration (APD) in 8 dogs as compared to a sham operation group (N=8) (20). The authors described a prolongation of ventricular APD after RDN, which hypothetically could prevent the occurrence of fatal ventricular arrhythmia's (VA). A multicenter registry reported that RDN appeared to be safe and efficacious in reducing VA burden in patients with chronic heart failure, the procedure was performed in patients with a few or no further therapeutic options (21). Finally, successful anecdotal experience was achieved with RDN in patients with refractory vasospastic induced ventricular tachycardia (22). The findings however do suggest that RDN might be a potentially more appealing option to modify cardiac electrophysiological properties as compared to pivotal work on cardiac catheter ablation which proved to shorten the ERP and increase the incidence of premature ventricular complexes (23). Of note, in the present study, no changes were observed over time in the incidence of ventricular ectopic beats.

Several recent randomized sham controlled trials proved the blood pressure lowering effect of RDN in both hypertensive patients taken off medication as well as in patients with uncontrolled hypertension respectively (12, 24-26). The multi-electrode EnligHTN™ catheter showed to decrease BP based on ABPM and to reduce renal norepinephrine in previous animal studies (27, 28). Specifically related to the present

human study, similar BP lowering effect was observed with the same catheter in patients with treatment resistant hypertension (29).

Besides the antihypertensive effects of RDN, an experimental study by Tsai and colleagues showed the antiarrhythmic effects of RDN in six ambulatory dogs by measuring lower nerve activity at the level of the stellate ganglion with also a decrease in paroxysmal atrial tachycardia episodes and duration after RDN as compared to controls. (10). Moreover, two clinical studies assessed the antiarrhythmic effect of RDN in addition to PVI in hypertensive patients with symptomatic AF. One of these studies demonstrated a positive correlation between the decrease in mean BP and the decline in AF burden. A reduction of 5-10mmHg in mean BP led to a 7% decrease in mean AF burden as measured with an ICM (30). Unfortunately, in the present study, we were not able to show a clear correlation between the change in blood pressure and change in AF burden following RDN. Pokushalov and colleagues showed that RDN on top of PVI in patients with symptomatic AF and resistant hypertension reduced the incidence of AF recurrence rates significantly. At 1 year, based on 24h-holter monitoring, 69% of the patients in the PVI+RDN were free of AF episodes, while in the PVI only group, only 29% of the patients remained free of AF episodes (6). A dedicated prospective randomized controlled trial is currently ongoing to determine the efficacy of RDN on top of PVI in patients with hypertension (31) *(Clinicaltrials.gov, NCT02115100)*.

It can be hypothesized that the antiarrhythmic effects of RDN could be due to a synergistic effect of (a) better BP control (partly) withdrawing an important risk factor for AF recurrence and (b) improving AF control by modifying electrophysiological settings like prolongation of the atrial effective refractory period (3, 32). AF burden (in min/day and episodes) decreased significantly at 1-year follow-up, however it did not reach statistical significance at 6 months follow-up. Although the latter could be due to the fact that the variability in AF burden at 6 months was high, an increasing effect of RDN over time could not be excluded. In the recently published SPYRAL-ON MED trial, BP reduction was greater at 6 months as compared with 3 months (33).

To the best of our knowledge, the present work is the first clinical study to demonstrate that RDN, without concomitant PVI, may reduce AF burden as measured using an ICM. Measuring AF burden with an ICM is superior to intermittent AF event monitoring using 24h-holters or event recorders (34). Monitoring treatment effect based on symptoms alone is unreliable since approximately 50% of AF recurrences proved to

occur in asymptomatic patients (35). Vice versa, Padeletti and colleagues showed a high intra-patient burden variability and demonstrated that only 52% of patient symptoms appear to be correlated to documented AF (36). The latter was confirmed in our study in which we found a clear discrepancy between pre-procedural EHRA class and the actual AF episodes as measured using either 24h-holter or ICM. Nevertheless, despite a large variability in AF burden between patients we were able to demonstrate a significant reduction in AF burden post RDN.

Of note, despite their known limitations, the vast majority of previous studies assessing the efficacy of either PVI or surgical ablation used intermittent and symptom-based monitoring (i.e. 24h holter monitoring or event monitors) to demonstrate their effect (37).

Finally, we were not able to demonstrate any changes in echocardiographic parameters and diastolic function in our study population, which could be due to either the small sample size or advanced stages of LA dilatation prior to study participation.

Limitations

Despite the positive results in this present study, there are several limitations that should be taken into account. First, it concerns a small study cohort (N=20) in whom 2 patients progressed to permanent AF and were excluded from the analysis of AF burden. Second, in approximately 50% of the treated patients, we stopped or decreased drug dosages of antihypertensive and/or anti-arrhythmic drugs. Third, adherence to antihypertensive drugs was not measured and only confirmed by the physician at the outpatient clinic visit. Fourth, the ICM used in this study was not able to transmit wirelessly the ECG to the office, which could have led to an underestimation of the AF events in case of data overload and a lack of memory (which occurred in 1/20 patient). Fifth, RDN was performed with the EnligHTN™ ablation catheter which enabled the operator to ablate only in the main renal arteries and is currently no longer available for clinical use. Whether the use of current generation devices using different technologies as ultrasound, ethanol or RF sidebranch ablation would have resulted in more potent results remains to be determined. Finally, since no sham control group was included in the present pilot study, the potential of a placebo effect cannot be ruled out. Nevertheless, we showed the potential value of RDN in patients with paroxysmal AF and warrants the conduction of larger and sham controlled studies assessing the anti-arrhythmic effect of RDN.

Conclusion

This pilot study suggests that RDN was safe and able to decrease AF burden in min/day as measured using an ICM at 12 months follow-up, together with an improvement in QOL in patients with symptomatic paroxysmal or persistent AF. Large-scale randomized trials are needed to demonstrate the value of RDN in hypertensive patients with AF.

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CHAPTER 16

Endovascular renal sympathetic denervation to improve heart failure with reduced ejection fraction: the IMPROVE-HF-I study

Lida Feyz, MD; Rajiv Nannan Panday, MD; Maureen Henneman, MD, PhD; Fred Verzijlbergen, MD, PhD; Alina A. Constantinescu, MD, PhD; Bas M. van Dalen, MD, PhD; Jasper J. Brugts, MD, PhD, MSc; Kadir Caliskan, MD, PhD; Marcel L. Geleijnse, MD; Isabella Kardys, MD, PhD1; Nicolas M.Van Mieghem, MD, PhD1; Olivier Manintveld, MD, PhD; Joost Daemen, MD, PhD

Under Revision

Abstract

Objectives The aim of the present study was to assess the safety and efficacy of RDN in patients with HFrEF.

Background Heart failure with reduced ejection fraction (HFrEF) is characterized by sympathetic overactivity, which could be influenced by renal sympathetic denervation (RDN).

Methods We randomly assigned 50 patients with a left ventricular ejection fraction (LVEF) ≤ 35% and NYHA class ≥ II, in a 1:1 ratio, to either RDN and optimal medical therapy (OMT) or OMT alone. The primary safety endpoint was the occurrence of a combined endpoint of cardiovascular death, rehospitalization for heart failure, and acute kidney injury at 6 months. The primary efficacy endpoint was the change in iodine-123 meta-iodobenzylguanidine (^{123}I-MIBG) heart to mediastinum ratio (HMR) at 6 months.

Results Mean age was 60 ± 9 years, 86% was male and mean LVEF was 33 ± 8%. At 6 months, the primary safety endpoint occurred in 8.3% vs. 8.0% in the RDN and OMT groups, respectively (p=0.97). At 6 months, the mean change in late HMR was -0.02 (95% CI: -0.08 to 0.12) in the RDN group, versus -0.02 (95% CI: -0.09 to 0.12) in the OMT group (p=0.95) whereas the mean change in washout rate was 2.34 (95% CI: -6.35 to 1.67) in the RDN group versus -2.59 (95% CI: -1.61 to 6.79) in the OMT group (p-value 0.09).

Conclusion RDN in patients with HFrEF was safe, however, did not result in significant changes in cardiac sympathetic nerve activity at 6 months as measured using ^{123}I-MIBG.

Introduction

Chronic heart failure (CHF) is a major public health problem, and with a prevalence of 1-2% in the adult population, the most common cause of hospitalization in developed countries (1). While pharmacological treatment for heart failure (HF) with reduced ejection fraction (HFrEF) has shown to prevent hospitalization and improve quality of life and survival, its long-term prognosis remains poor justifying a persistent need for novel therapeutic strategies that improve both morbidity and mortality (2-6).

Increased sympathetic tone has been directly linked to severity of heart failure and adverse outcome (7, 8). In response to a chronic low-output state in HF, neurohormonal adaptations occur such as the activation of the renin-angiotensin-aldosterone-system (RAAS) and the sympathetic nervous system (SNS) in order to maintain vital organ perfusion (9, 10).

In the past decade renal sympathetic denervation (RDN) emerged as a novel minimally invasive treatment option to reduce sympathetic tone and proved to significantly reduce blood pressure in hypertensive patients (11-14). Promising findings were subsequently reported on the effects of RDN in experimental HF animal models (15, 16). As of to date, the clinical evidence for RDN in the treatment of HF is limited and restricted to several small non randomized studies indicating an improvement in 6-minute walk test, left ventricular ejection fraction and cardiac dimensions (17, 18). Furthermore, in contrast to several studies with pharmaceutical agents, data correlating the effect of RDN on cardiac sympathetic tone as measured using iodine-123 labeled meta-iodobenzylguanidine (^{123}I-MIBG) is lacking. The present study aimed to assess the safety and efficacy of RDN with a bipolar balloon based RDN system in patients with HFrEF as measured using ^{123}I-MIBG at 6 months.

Methods

Study design and patient population

This present study is a single center open label prospective randomized controlled trial designed to allocate 70 patients to treatment with RDN and optimal medical therapy (OMT) or OMT alone (1:1).

Due to the impact of several studies disputing the effect of RDN in patients with arterial hypertension, subsequent slow inclusion and the decision of the manufacturer of the device to halt further production of the Vessix™ V2 Renal Denervation System (Boston Scientific, Natick, MA, USA), inclusion was halted after the first 50 patients.

Patients were eligible for enrolment when the following inclusion criteria were met: left ventricular ejection fraction (LVEF) ≤ 35% (as assessed by echocardiography), New York Heart Association (NYHA) functional class ≥ II, age between 18 and 75 years, renal arteries suitable for RDN (i.e. baseline diameter stenosis <30%, main renal artery diameter of ≥3.5mm and ≤7.0mm for each kidney), a glomerular filtration rate (eGFR) of > 30 ml/min/1.73m^2. Exclusion criteria included: a systolic office blood pressure <110mmHg, recent (<3 months) stroke or myocardial infarction, acute HF, presence of other medical illnesses associated with a life expectancy less than one year.

Work-up at baseline included laboratory analyses, 24h ambulatory blood pressure measurement (24h ABPM), echocardiography, ^{123}I-MIBG and a Computed Tomography (CT) was performed to confirm renal artery eligibility. Clinical follow-up occurred at 1, 3 and 6 months and will be continued yearly up to 5 years. Follow-up renal artery imaging using CT was performed at 6 months in patients who underwent RDN. This study was approved by our local ethics committee and all patients provided written informed consent (trialregister.nl, NTR number: NTR5328).

Study endpoints and measurements

The primary safety endpoint included the occurrence of a combined endpoint of cardiovascular death, rehospitalization for heart failure, and acute kidney injury at 6 months. The primary efficacy endpoint was the change in ^{123}I-MIBG late heart-to-mediastinum ratio (HMR) at 6 months. Other safety parameters that were assessed at 6 months follow-up: major access site bleeding, change in renal function (measured in plasma: cystatine C and estimated by eGFR) and newly acquired renal artery stenosis and/or repeat renal artery intervention.

Secondary efficacy endpoints include (baseline vs. 6 months follow-up): change in NYHA class, 6-minute walk test (6MWT), change in quality of life, echocardiographic endpoints including LVEF, left ventricular end-diastolic (LVEDD), left ventricular end-systolic dimensions (LVESD) and indices for diastolic function (left atrial size/

volume, E- and A- wave velocity (peak early – and late filling), E/A ratio, Em (peak early diastolic wave velocity of the mitral annulus), E/Em ratio and deceleration time (DT) were measured, laboratory endpoints (change in NT-pro-BNP) and change in diuretic dosage (based on a change in the defined daily dose (DDD)) (19). The Diuretic Defined Daily Dose is expressed as the sum of the individual DDD of loop diuretics, thiazide diuretics and potassium-sparing diuretics. The Defined Daily Dose of heart failure drugs is expressed as the sum of the individual DDD of ACE-inhibitors, Angiotensin II-antagonists, β-blockers and diuretics including aldosterone receptor blockers. All echocardiograms were assessed by dedicated imaging cardiologists unaware of the treatment allocation.

Quality of life and an overall physical and mental function survey (RAND-36 and the Kansas City Cardiomyopathy questionnaire (KCCQ)) were used at baseline and at 6-months follow-up (20, 21). The RAND-36 is an indicator of overall health status, scores range from 0 – 100, whereas lower scores represent more disability (20). The KCCQ is a questionnaire that quantifies physical and social function and quality of life (21).

^{123}I-MIBG scintigraphy data acquisition and analysis

^{123}I-MIBG is a physiologic analogue of norepinephrine and acts selectively on sympathetic nerve endings. By using cardiac neurotransmission imaging global information about neuronal function can be expressed in early, but more specifically in late HMR (reflecting the storage, regional distribution and release of ^{123}I-MIBG), with washout rate (WR) reflecting the neuronal integrity or sympathetic tone (22). For detailed data acquisition and analysis, our previous work should be used as a reference (23). Calculation of WR was performed using the following formula (no correction for background): $WR = (HMR_{early} - HMR_{late})/(HMR_{early}) \times 100\%$ (24).

RDN procedure

Pre-procedurally, 100IU heparin/kg were administered to achieve an active clotting time>250 seconds. After administration of local anesthesia, common femoral artery access was achieved by an ultrasound-guided puncture and a 6-Fr sheath was then introduced. Under fluoroscopic guidance, the short 6-Fr sheath was exchanged for an 8-Fr RDN or an IMA tipped guiding sheath, to accommodate the Vessix™ V2 Renal Denervation System. The Vessix™ V2 system consists of an over-the-wire balloon

catheter and a radiofrequency generator. After engaging the renal arteries, selective renal artery angiograms were made and an appropriate balloon size was chosen (4 (4 electrodes) to 7 (6 electrodes) mm). Once balloon inflation was completed and the device was activated, the generator raised the electrode temperature to 68ºC, the temperature at which treatment is conducted, nerve denervation was done within 30 seconds.

Statistical analysis

The study was designed to compare the primary efficacy endpoint, late HMR and WR derived from ^{123}I-MIBG, in the treatment- versus control group. The sample size was originally calculated at 33 patients per arm. For this calculation we hypothesized an average increase in late HMR from 1.64 from 1.80 with a SD of 0.23 in the treatment-arm, with no change in the late HMR and WR expected in the control group; and used a power of 80%, two-sided significance (alpha) level of 0.05, and 1:1 randomisation. To account for drop-outs, patients withdrawing informed consent and patients lost to follow-up, the proposed sample size for the present study was in total 70 patients. Assumptions were based on the OLOMOUC-I study, in which an increase in LVEF from 25 to 31% (SD 14%) was found at 1-year follow-up and the work of Kasama et al. showing an increase in H/M ratio from 1.64 ± 0.20 to 1.86 ± 0.27 and LVEF of 6% in patients with HFrEF following treatment with spironolactone(17, 25). Eventually, 25 patients per arm were included; based on the assumptions above, this results in a power of 69%.

Baseline categorical variables were expressed as counts and percentages. Differences in baseline categorical variables between randomly allocated treatment groups were compared using the Chi-square test, Fisher's Exact test or Chi square test for trend (NYHA class) when appropriate. Baseline continuous variables were described as mean ± standard deviation (SD) when normally distributed. In case of non-normal data distributions, data were presented as median [interquartile range, IQR]. Continuous variables (such as HMR and WR, normally distributed) were compared between groups using Independent-Samples T test or Paired-Sample T test. To examine within-group changes, Paired-Sample T tests were used. Non-parametric tests (Wilcoxon signed rank or Mann Whitney U test, when appropriate) were used to analyse differences in case of non-normal distributions. All statistical tests are 2-tailed. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS statistical analysis (version 24.0).

Results

Clinical characteristics

A total of 343 patients were assessed for eligibility, 50/343 (14.6%) were enrolled between August 2014 and June 2018 (Figure 1). There were no significant differences in patient characteristics, hemodynamic parameters and baseline medications between both groups at the time of inclusion (Table 1). Mean age was 60 ± 9 years, 86% was male, 78% was in NYHA class II at baseline, ischemic cardiomyopathy was present in 60% of the patients. Furthermore, mean baseline LVEF was 33 ± 8%, while mean LVEDD was 70 ± 11 mm. An implantable cardioverter-defibrillator (ICD)/ cardiac resynchronization therapy (CRT) was present in 66% and 22% of the patients, respectively. Mean ambulatory blood pressure at baseline was 111/69 ± 13/7mmHg and mean heart rate was 70 ± 10 bpm.

Figure 1. Patients screened for eligibility

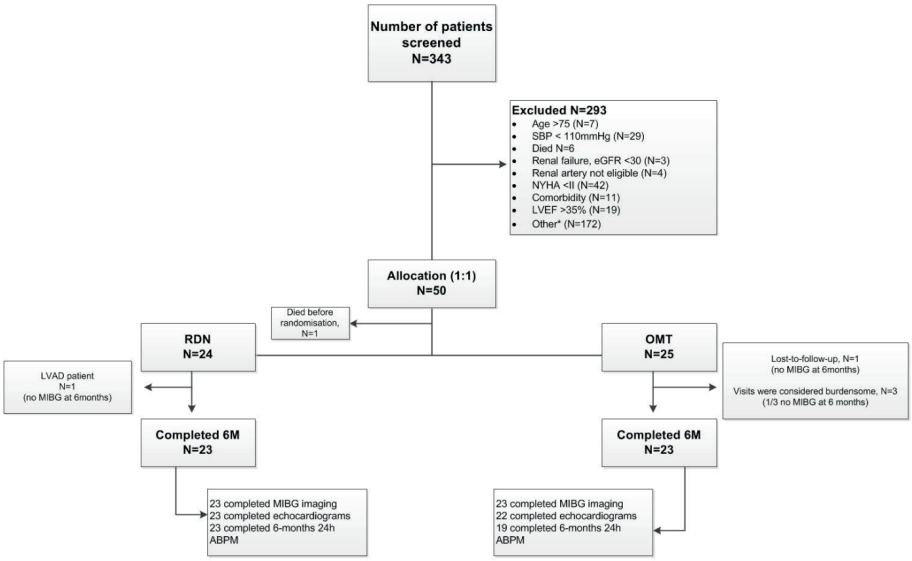


Table 1. Baseline characteristics

	RDN N=24	**OMT N=25**
Age, years	60 ± 8	59 ± 10
Male n, (%)	20 (83.3)	22 (88.0)
BMI, kg/m²	28.0 ± 4.4	27.9 ± 5.2
eGFR, ml/min	68.3 ± 17.6	69.8 ± 20.8
ICD/CRT, (%)	68/24	64/20
Cardiomyopathy		
iCMP n, (%)	15 (62.5)	14 (56.0)
DCM n, (%)	8 (33.3)	11 (44.0)
Other n, (%)	1 (4.2)	-
Cardiovascular history (%)		
Prior MI	12 (50.0)	13 (48.0)
Prior PCI	9 (37.5)	12 (48.0)
Prior CABG	5 (20.8)	-
CVA	3 (12.5)	1 (4.0)
Cardiovascular risk factors (%)		
Diabetes	6 (25.0)	10 (40.0)
Hypertension	14 (58.3)	10 (40.0)
Dyslipidemia	18 (75.0)	15 (60.0)
Smoker, current	4 (16.7)	6 (24.0)
Family history of premature CVD	7 (29.2)	9 (36.0)
Clinical parameters		
24h ABPM, mmHg	111 ± 9/69 ± 6	108 ± 9/66 ± 5
Office BP, mmHg	121 ± 11/75 ± 8	124 ± 19/75 ± 14
Heart rate, bpm	70 ± 9	67 ± 9
NYHA II, (%)	17 (70.8)	21 (84.0)
NYHA III, (%)	7 (29.2)	4 (16.0)
Echocardiographic parameters		
LVEF, %	32 ± 7	33 ± 9
LVEDD, mm	72 ± 7	69 ± 13
LVESD, mm	63 ± 8	61 ± 15
Mean number diuretics, n (%)	2 ± 1	2 ± 1
Pharmacological therapy, n (%)		
ACE-i/ATII-antagonist	15(62.5)/7(29.2)	21 (84.0)/ 4(16.0)
Calcium channel blockers	2 (8.3)	2 (8.0)
Selective beta-blockers	21 (87.5)	23 (92.0)
Diuretics/MRA	20(83.3)/21(87.5)	25 (100)/19(76.0)
Aspirin	12 (50.0)	14 (56.0)
Statins	17 (70.8)	18 (72.0)
Procedural characteristics		
Number ablations L/R, median [IQR]	11 [6 – 12]/10 [7 – 12]	-
Mean number of accessories L/R	2/1	-

Data was presented in mean ± SD or median [interquartile range, IQR] when appropriate. ABPM=ambulatory blood pressure measurement, ACE-i=angiotensin-converting-enzyme inhibitor, ATII=angiotensin-II antagonist, BMI=body mass index, BP=blood pressure, CABG= coronary artery bypass graft, CVA= cerebrovascular accident, CVD= cardiovascular disease, DCM=dilated cardiomyopathy, eGFR=estimated glomerular filtration, iCMP= ischemic cardiomyopathy, MI= myocardial infarction, MRA= mineralocorticoid receptor antagonist, NYHA=New York Heart Association, PCI=percutaneous coronary intervention

Change in ^{123}I-MIBG (primary efficacy point)

No significant change was seen in late HMR and WR at 6 months between the RDN- and OMT- groups, respectively (Table 2). At 6 months, the mean change in late HMR was -0.02 (95% CI: -0.08 to 0.12) in the RDN group, versus -0.02 (95% CI: -0.09 to 0.12) in the OMT group (p-value for mean between group difference =0.95), whereas the mean change in WR was 2.34 (95% CI: -6.35 to 1.67) in the RDN group versus -2.59 (95% CI: -1.61 to 6.79) in the OMT group (p-value for mean between group difference =0.09).

Table 2. Change in ^{123}I-MIBG (primary efficacy endpoint)

	RDN		Difference (95% CI)	OMT		Difference (95% CI)	Mean between-group difference (95% CI)	p value
	Baseline	6 months		Baseline	6 months			
Early HMR	2.14 ± 0.41	2.13 ± 0.43	-0.02 (-0.09 to 0.13)	2.44 ± 0.49	2.42 ± 0.48	-0.02 (-0.13 to 0.16)	0 (-0.18 to 0.18)	1.00
Late HMR	1.92 ± 0.43	1.90 ± 0.47	-0.02 (-0.08 to 0.12)	2.15 ± 0.47	2.13 ± 0.48	-0.02 (-0.09 to 0.12)	-0.004 (-0.14 to 0.13)	0.95
WR	11.3 ± 7.8	13.7 ± 8.2	2.34 (-6.35 to 1.67)	14.8 ± 11.5	12.2 ± 9.0	-2.59 (-1.61 to 6.79)	4.93 (-0.73 to 10.6)	0.09

Data was presented in mean ± SD, with differences presented in 95% CI. HMR= heart-to-mediastinum ratio, OMT= optimal medical therapy, RDN= renal sympathetic denervation, WR=washout rate.

Safety (primary safety endpoint)

The primary safety endpoint occurred in 2/24 patients in the RDN group (8.3%) vs. 2/25 patients in the OMT group (8.0%), respectively (p=0.97). In 3/24 (12.5%) patients a minor access site bleeding was observed (all small hematomas with no further clinical consequences), no further peri-procedural complications occurred. In the RDN group, one patient received a left ventricular assist device (LVAD) due to refractory heart failure. Safety events are described in Table 3.

eGFR remained unchanged in both cohorts; in the RDN group: 68 ± 17ml/min at baseline vs. 68 ± 20ml/min at 6 months, p=0.98, similar findings were seen in the OMT group: 70 ± 19ml/min vs. 71 ± 21ml/min, p=0.94. (Table S1, Supplemental material).

Table 3. Safety endpoint

Endpoint	RDN	OMT
Total events (%)	*7/24 (29.2)*	*10/25 (40.0)*
*Primary safety endpoint	2/24 (8.3)	2/25 (8.0)
Specific events within 6M		
Death	0/24	0/25
Myocardial infarction	0/24	2/25 (8.0)
New-onset of end-stage RD	0/24	0/25
Renal-artery intervention	0/24	0/25
Stroke	0/24	0/25
Hospitalization for HF	2/24 (8.3)**	2/25 (8.0)
Hospitalization for AF	1/24 (4.2)	1/25 (4.0)
Hospitalization non-cardiac	0/24	2/25 (8.0) (colon carcinoma, non-Hodgkin lymphoma)
New renal-artery stenosis	1/24 (4.2)	0/25
Side effects medication	2/24 (8.3) (statin-induced myalgia, amiodarone induced hyperthyroidism)	-

*the primary safety endpoint includes the occurrence of a combined endpoint of cardiovascular death, rehospitalisation for heart failure and acute kidney injury at 6 months. AF=atrial fibrillation, HF=heart failure, RD=renal disease, VT=ventricular tachycardia
** N=1 received a LVAD=left ventricular assist device

Secondary endpoints

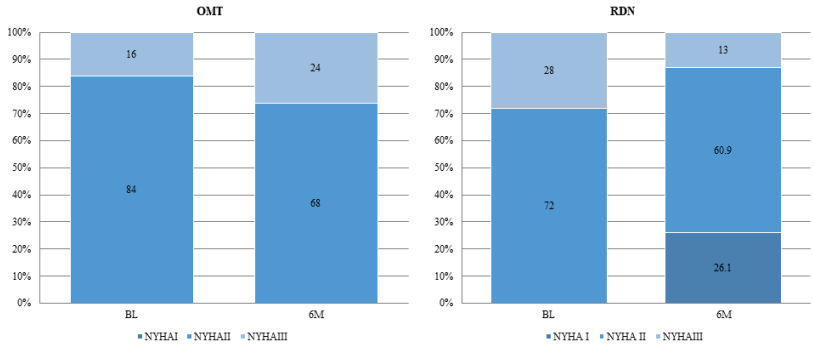
NYHA class

As compared to baseline, NYHA class at 6 months remained unchanged in the RDN group, p=0.18, however in the OMT group functional class worsened significantly (p=0.002) (Figure 2). At 6 months, 26.1% of the patients in the RDN group was in NYHA class I vs. none in the OMT group, p=0.03.

6MWT

The 6MWT remained unchanged at 6 months in both groups (RDN/OMT): +2.1m (95% CI: -29 to 24) vs. +0.1m (95% CI: -28 to 28), p-value for mean between group difference 0.91.

Figure 2. Change in NYHA class



Echocardiographic parameters

The mean change in LVEF at 6 months was +0.7% (95% CI: -2.7 to 1.3) in the RDN group as compared with +0.6% (95% CI: -2.5 to 1.3) in the OMT group (p-value for mean between group difference=0.96), whereas LVEDD significantly decreased in the RDN group -2.6mm (95% CI: -0.9 to -4.3) and remained unchanged in the OMT group -0.2mm (95% CI: -1.6 to 1.9), (p-value for mean between group difference=0.045). Furthermore, a significant change was seen in the A-wave velocity in the RDN-group, with no change in the OMT-group, from 80 cm/s [56 -100] at baseline to 65 cm/s [55 -87] at 6 months, p=0.009 vs. 61 cm/s [46 -79] to 60 cm/s [50 -72], p=0.67 in the OMT group, p-value for difference between group=0.048. No significant change was seen in other indices for diastolic function (Table S2, Supplemental Material).

Change in blood pressure

Office blood pressure as well as the 24h ABPM remained unchanged in both groups at 6 months. Mean office systolic blood pressure difference in the RDN group was 4.2mmHg (95% CI: -9.4 to 0.9) at 6 months vs. -2.8mmHg (95% CI: -5.4 to 11.0) in the OMT group, p-value for mean between group difference p=0.14. Additionally, mean systolic 24h ABPM in the RDN group changed at 6 months with -1.8mmHg (95% CI: -1.4 to 5.1) vs. 1.7mmHg (95% CI: -5.0 to 1.6) in OMT group, p-value for mean between group difference p=0.12 (Table S3 , Supplemental material).

Laboratory parameters

No change was observed in NT-proBNP (pmol/L) at baseline vs. 6 months in the RDN group (69 [30 – 128] vs. 75 [33 – 171], p=0.83) vs. the OMT group (64 [38 – 87] vs. 56 [40 – 105], p=0.61), p-value for difference between group difference= 0.81 (Table S3, Supplemental material).

Change in DDD

No change was observed in the within-group difference in DDD between baseline and 6 months in both the RDN and OMT group (Table 4a). No difference was observed between diuretic DDD and DDD of heart failure drugs between both groups at 6 months (Table 4b).

Table 4a. Defined Daily Dose (DDD) at 6 months within the groups

	RDN		**p value baseline vs. 6M RDN**	**OMT**		**p value baseline vs. 6M OMT**
	Base-line	**6 months**		**Base-line**	**6 months**	
Total antihyper-tensive drugs DDD	4.5 [3.6-6.1]	4.5 [3.4-6.8]	0.55	4.5 [3.1-6.0]	4.4 [3.0-5.4]	0.86
Diuretic DDD	2.2 [1.3-3.7]	2.3 [1.0-4.3]	0.31	2.1 [1.0-2.5]	2.0 [1.0-2.5]	0.50
Loop Diuretic DDD	2.0 [1.0-3.5]	2.0 [1.0-8.0]	0.59	2.0 [1.0-3.5]	2.0 [1.0-2.0]	0.19
Heart Failure drugs DDD	4.5 [3.5-6.1]	4.1 [3.4-6.8]	0.71	4.2 [3.1-5.4]	3.9 [3.0-5.4]	0.86

Data was presented as median [interquartile range, IQR]. OMT=optimal medical therapy, RDN= renal sympathetic denervation

Table 4b. Defined Daily Dose (DDD) at 6 months between the groups

	RDN (n=24)	**OMT (n=25)**	**p value**
Total antihypertensive drugs DDD	4.5 [3.4-6.8]	4.4 [3.0-5.4]	0.25
Diuretic DDD	2.3 [1.0-4.3]	2.0 [1.0-2.5]	0.78
Loop Diuretic DDD	2.0 [1.0-8.0]	2.0 [1.0-2.0]	0.72
Heart Failure drugs DDD	4.1 [3.4-6.8]	3.9 [3.0-5.4]	0.25

Data was presented as median [interquartile range, IQR].

Change in quality of life

Based on the RAND-36 questionnaire, two health summary scores were calculated based on eight health concepts with multi-item scales (Table S4, Supplementary material). Besides a significantly greater change in the physical functioning score in the RDN group as compared to the OMT group (p for mean group difference = 0.04) no differences were found between both cohorts.

Also, no change was found in the KCCQ (in which the overall and clinical summary score could be calculated) between the groups. The overall summary score between groups showed a mean difference of 28 points (95% CI: -0.9 to 56, p=0.06), whereas the clinical summary score showed a mean difference between the groups of 3 points (95% CI: -11 to 5, p=0.50).

Discussion

The IMPROVE-HF-I trial showed that RDN using a bipolar radiofrequency balloon catheter in patients with HFrEF did not result in a significant change in cardiac sympathetic nerve activity as measured using ^{123}I-MIBG late HMR and WR at 6 months. The therapy appeared safe with similar rates of the combined safety endpoint of cardiovascular death, rehospitalization for heart failure, and acute kidney injury at 6 months in both groups. Furthermore, a significant difference was observed in LVEDD in the RDN-group, and 26% of the patients in the treatment-group was in NYHA class I versus none in the control-group, suggesting a potential effect of RDN.

After pivotal surgical studies from the early 1940s, percutaneous RDN was introduced about 10 years ago as a potential minimal invasive treatment option for patients with therapy resistant hypertension, a condition linked to sympathetic overactivity (14, 26). Sympathetic overactivity also proved to contribute to the progression of myocardial cell injury, cardiac fibrosis, and LV dysfunction in patients with HF and a significant correlation was found between the severity of overactivity and NYHA class (8, 27). As a result to the chronic low-output state in HF, elevated sympathetic tone stimulates renin release by the kidneys, leading to sodium retention, volume expansion and renal vasoconstriction in order to maintain vital organ perfusion (28). However, due to a subsequent increase in peripheral resistance, myocardial contractility and increase in heart rate prognosis worsens. As such, an inverse association was found between norepinephrine release and survival (29).

Preliminary data on the potential efficacy of RDN in HF was derived from animal data showing that modulation of the autonomic nervous system by radiofrequency (RF)-RDN in a post-infarct rat model resulted in significant reductions in LV fibrosis and improved vascular function (15). Human data from the REACH pilot study showed that RDN in seven patients with congestive HF was safe and associated with a significant increase 6MWT (18). Finally, a randomized study presented by Taborsky et al., showed that RDN in patients with more advanced heart failure (mean LVEF was 25%, N=51) resulted in significant improvements in LVEF, LVESD and LVEDD as well as in NT-pro-BNP while no change was seen in patients with OMT alone (17). For unknown reasons the study was never published.

To the best of our knowledge the present study is the first to assess the potential therapeutic effect of RDN in patients with HFrEF using specific ^{123}I-MIBG nuclear imaging to assess cardiac sympathetic nerve activity. H/M ratios were between 1.9

and 2.4 at baseline and remained unchanged at 6 months in both arms. While we aimed to enroll patients with symptomatic HFrEF the vast majority of the patients in our study were in NYHA class II with relatively low NT-proBNP values, suggesting a less severe HF phenotype. The relatively low risk profile of our patients could explain the higher than expected baseline H/M ratio's in our study as compared to previous studies on the topic with baseline late H/M rates in the range of 1.2 to 1.6 in patients with more pronounced heart failure and late HMRs of 2.5 ± 0.3 in healthy controls (25, 30). The same applies for the WR found in our study which, being around 12%, were significantly below the threshold of 27% associated with poor prognosis (31, 32). This might suggest that the stable HF population studied in the present study was on relatively well controlled heart failure therapy in which the additional treatment with RDN did not add substantially on top of pre-existent OMT to improve cardiac sympathetic nerve activity.

Although our study was not powered to detect a difference in clinical endpoints, the overall rate of HF related events at 6 months in the present study was low which might illustrate the relatively low risk profile of the patients included. Considering the underlying disease in each of the patients included, it is likely that the severity of HF will progress over time. As all patients will be followed for 5 years, longer-term follow-up will help to understand the potential long-term clinical impact of RDN in patients with HFrEF.

Based on the data available at the time of our study design, a significant BP lowering effect of RDN was anticipated in patients with hypertension. The latter raised concerns about a potential BP lowering effect of RDN in HF patients which might have forced downtitration of HF drugs. The latter made that we refrained from including patients with a baseline systolic BP <110mmHg and might have resulted in the HFrEF population at relatively low risk. Nevertheless, we were able to show that RDN appeared safe with no effect on BP in patients with HFrEF. Moreover, extensive analyses on DDD at baseline and follow-up demonstrated no significant change in both HF drugs and diuretics at baseline and follow-up in both arms and renal function remained unchanged.

Furthermore, while NYHA class remained unchanged between baseline and 6 months in the RDN-group NYHA class worsened significantly in the OMT group at follow-up indicating the progressive nature of CHF. Of note, about a third of the patients in the RDN group improved to NYHA I.

Finally, in contrast to previous studies suggesting a significant change in LVEF following RDN, no change in LVEF was found in the present study. Conversely, we did observe a small albeit significant decrease in LVEDD following RDN. The latter results however should be interpreted with caution given the known variability in measurements derived from transthoracic echocardiography (33). However, we did observe a significant decrease in the peak late diastolic filling velocity in the treatment-arm, which could implicate an improvement in left ventricular relaxation (34).

RDN in the present study was performed with the Vessix™ system that proved to reduce both office and 24h-ABPM with 25 and 8 mmHg, respectively in the REDUCE-HTN study (35). The efficacy of the system was further studied in an off-med population in the REINFORCE study, which suffered from slow enrollment and was stopped for futility after only 51 patients were enrolled. Despite hints of a benefit at 6-month follow-up, the Vessix program was halted soon afterwards. The latter forced us to stop enrollment after 50 patients. Whether the use of a different technology in the present study would have altered our findings remains unknown. However, new RDN studies with competing technologies that proved successful in reducing BP in dedicated sham controlled randomized controlled trials in patients with hypertension, are eagerly awaited.

Study limitations

The current study has a number of limitations. First, we enrolled a smaller number of patients than first intended due to slow inclusion rates. Therefore, the study was underpowered to reach its primary efficacy endpoint. Second, we cannot exclude the fact that we might have used a less efficacious RDN system. Third, we included a lower risk HF phenotype (80% with NYHA II). Finally, the present trial was an open-label trial and not sham-controlled.

Conclusion

RDN with a dedicated bipolar radiofrequency balloon catheter in patients with HFrEF was safe, however, did not result in significant changes in cardiac sympathetic nerve activity at 6 months as measured using ^{123}I-MIBG.

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SUMMARY AND PERSPECTIVES

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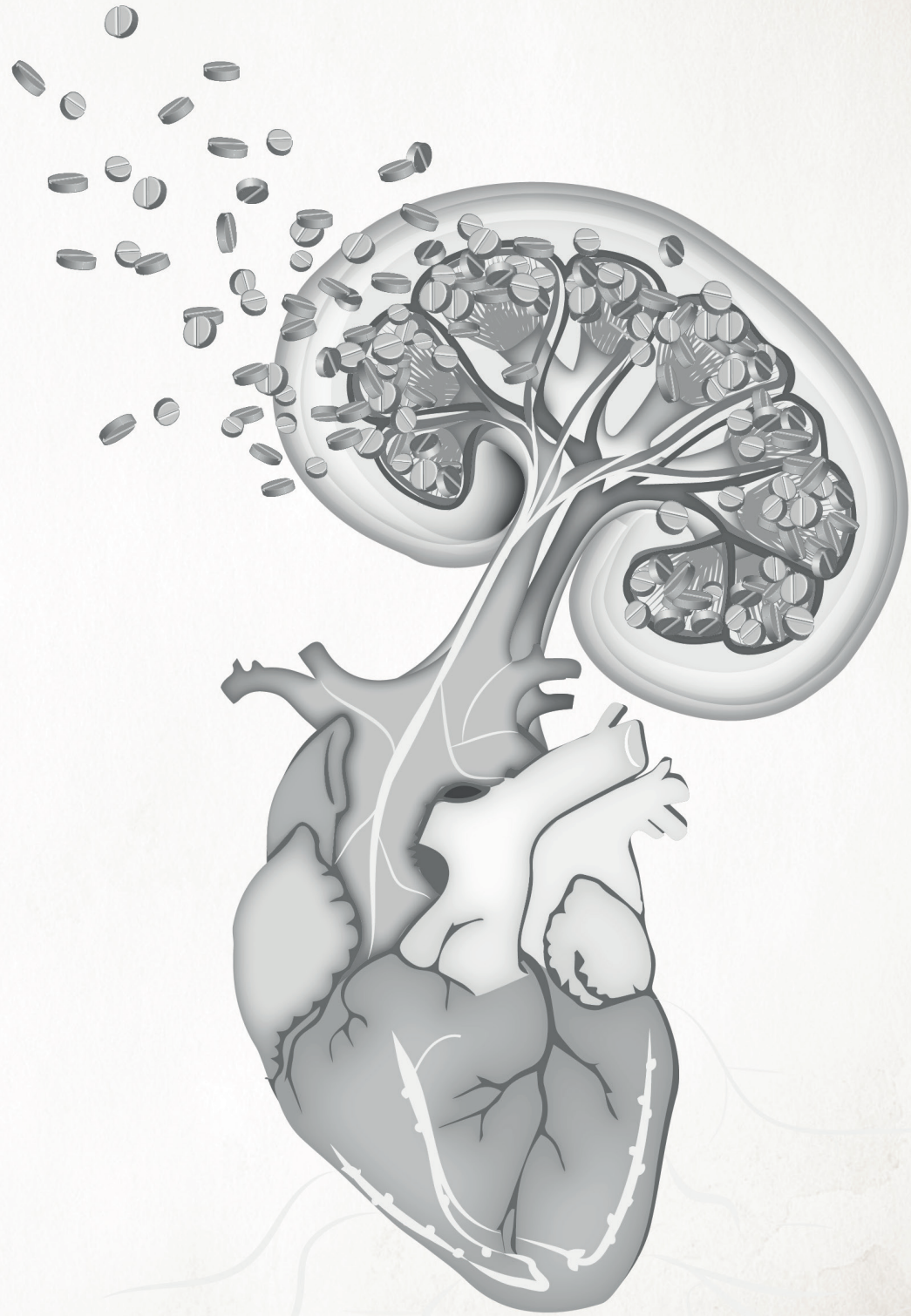

CHAPTER 17

General discussion

General discussion

Sympathetic overactivity has been documented in hypertension, chronic heart failure and arrhythmias (1-4), and it is associated with poor clinical outcome and cardiovascular mortality (5, 6). In order to modulate the latter overactivity, several pharmacological and non-pharmacological approaches have been studied; of which renal sympathetic denervation (RDN) is the most widely studied non pharmacologic approach, particularly in patients with hypertension. Initial findings demonstrated that RDN was safe and effective in lowering blood pressure (7, 8). However, the sham-controlled Symplicity HTN-3 failed to show superiority of RDN in lowering blood pressure at 6 months (9). Several concerns were subsequently raised that could have resulted in the negative findings of the later study (10). In the years that followed, a worldwide debate was triggered and our particular interest in the workup and treatment for diseases associated with sympathetic overactivity formed the basis for the present thesis. The present thesis will discuss several of these topics such as, 1) subject eligibility and screening; 2) evaluation of objective outcome measures pre- and post- RDN procedure; 3) adherence to antihypertensive drugs and; 4) clinical studies on the efficacy of RDN in diseases related to sympathetic overactivity.

Eligibility and screening

In **Chapter 2,** we present a stepwise approach in the work-up of hypertension. First, dietary and lifestyle factors must be evaluated (11). Second, white-coat hypertension needs to be ruled out by liberal use of 24h ambulatory blood pressure measurement (24h-ABPM) and secondary causes of hypertension should be assessed and excluded (12-15). Third, pharmacotherapy needs to be up titrated according the current guidelines (11). The latter actions proved to be important confounding factors in studies assessing the efficacy of RDN (16). Future studies demonstrated that the proportion of subjects eligible for RDN turned out to be around 40% after screening (8). Scrutinizing the rationale for ineligibility demonstrated that 47% of patients had controlled or substantially improved blood pressures after the adjustment of antihypertensive drugs. Anatomic reasons (renal artery not suitable for RDN) accounted for 17%, unrecognized secondary cause of hypertension for 11% (mostly primary aldosteronism), and a renal function beyond the set thresholds (i.e. lower limit of GFR<30ml/min) (9%), older age and frailty (7.9%), white coat hypertension (7.7%) and poor adherence to

antihypertensive drugs in 7.7% (17). The above findings illustrate the complexity of enrolling patients in dedicated RDN trials. Recruiting in these trials, as well as screening study participants for clinical cardiovascular and hypertension trials in general, proved time-consuming, especially with the knowledge that half of the hypertensive subjects screened for RDN finally proved to be ineligible. The latter repetitively resulted in slow inclusion rates. In order to boost inclusion rates, we studied the effectiveness of an approach using social media. In **Chapter 3,** we report the results of a targeted social media campaign using Facebook to recruit subjects participating in a clinical hypertension trial (RADIANCE HTN trial program) (18). Facebook ads generated an overwhelming amount of interest for a novel device-based treatment option for hypertension (19). We ran campaigns in a large variety of geographic areas creating better awareness on the rationale for blood pressure lowering treatment, hypertension and RDN, which resulted in improved subject enrolment and enhanced recruitment rates (20).

Having screened an extensive amount of patients, we noticed another remarkable finding, namely the occurrence of incidental findings (IF). Given the need to rule out secondary causes of hypertension and confirm renal artery eligibility a large series of high-resolution computed tomography (CT) or magnetic resonance imaging (MRI) was performed. In **Chapter 4**, we assessed the consequences of larger scale screening in patients using CT or MRI in the pre-procedural work-up for RDN, Transcatheter Aortic Valve Implantation (TAVI) and MitraClip implantation. In this large-scale retrospective single-center registry we demonstrated that non-cardiac IF could be detected in 43.1% of patients screened for TAVI, RDN, or MitraClip implantation. We found that a total of 39.2% patients were diagnosed with a minor (clinical irrelevant) IF and that the presence of a moderate or major IF resulted in a delay or cancellation of the intended procedure in only 1.6% of the patients. Moreover, the presence of a moderate or major IF did not significantly impact 1-year mortality rates. Therefore, there seems to be no reason to delay or cancel a potential lifesaving procedure as TAVI, with the exception for active malignancies. In the last two chapters (**Chapter 5 and 6**) of part II we describe methods to identify potential responders to RDN, which has been considered an important question given the fact that up to 30% of patients do not seem to respond to the treatment (16, 18). The latter triggered a search towards clinical markers that could predict procedural success. To date, RDN still remains 'blinded', as no feedback or guidance is available per-procedurally (no immediate blood pressure drop is observed after denervation, such as is fore instance seen when

stimulating carotid baroreceptors (21)). We hypothesized that if the renal nerve location could be more precisely located prior to denervation, a major shortcoming on technical success could be tackled. In **Chapter 5,** we assess a novel add-on technology for RDN, the ConfidenHT™ system, which has the potential to identify (non)-responders. The system is based on monitoring of hemodynamic response elicited by site-specific electric stimulation, in order to map the location of the renal sympathetic nerves. The electrical stimulation drives a transient change in several physiological measurable parameters such as heart rate and blood pressure. We found a large variation in temporary blood pressure changes per patient and per anatomic location within the artery in response to stimulation. The latter could convert the blind nature of the RDN procedure in targeted therapy by identifying the locations along a renal artery at which a patients' blood pressure reacts to renal nerve stimulation (22). Finally, in a search towards potential markers for success we studied the effect of RDN on neurohormonal response (**Chapter 6).** It is well known that activation of the sympathetic nervous system and stimulation of the renin-angiotensin-aldosterone system (RAAS) leads to an increase in blood pressure. In an attempt to understand the blood pressure lowering effect of RDN, we collected data on renin and aldosterone and endogenous catecholamines ((nor)metanephrines) from blood- and urine sampling pre- and post-procedurally. Monitoring these biomarkers proved to be challenging due the fact that the endogenous parameters are continuously influenced by changes in renal sympathetic outflow (including patient's anxiety), autonomic reflexes and interaction with pharmacological agents that frequently changed during the course of the study (22). In a dedicated study, we were not able to demonstrate a direct correlation between reduction in blood pressure by RDN in patients with moderate to severe hypertension and change in RAAS hormones and endogenous catecholamines. Given the above mentioned limitations in combination with the limited sample size of our study, it remains to be debated whether the complexity of the assessment of the neurohormonal response or a true lack of effect of RDN on this markers caused the negative findings of our study. The latter warrants dedicated prospective study to assess the impact of RDN on neurohormonal response.

Adherence to antihypertensive drugs

Despite clear guidelines on its management, control of hypertension is far from adequate (23, 24). A single drug therapy is only capable of reducing blood pressure to

adequate levels in 20-30% of the patients. Up-titration of antihypertensive medication is often the next step in the therapy, while it is known that the number of drugs inversely correlates with drug adherence (25, 26). Non-adherence is a multifactorial problem that is associated with poor clinical outcome (27). Several methods to assess adherence to therapy have been studied, in which therapeutic drug monitoring (TDM) proved to be the most reliable one given its ability to directly measure drug metabolites in blood or urine (28, 29). However, the availability of a sensitive assay and detailed knowledge on the pharmacokinetics of the individual drug proved to be of paramount importance to interpret the results and assess adherence (**Chapter 7**). In **Chapter 8 and 9,** we discuss the clinical validation and applicability of a minimal invasive method (dried blood spot, DBS) to measure levels of antihypertensive drugs and their active metabolites in order to assess adherence. The DBS method was validated analytically (**Chapter 8**) and clinically (**Chapter 9**). The advantage of DBS above plasma measurements is the fact that sampling can take place at the same moment when increased blood pressure is measured. The latter prevents white coat adherence, which is seen when an additional visit to the phlebotomy unit is needed. Additionally, DBS sampling can be done at random moments, even in the general practitioner office. We expect that a frequent use of DBS in patients with resistant hypertension combined with personal feedback could improve adherence for antihypertensive drugs, resulting in lower disease burden and health care costs. In both studies, DBS proved to be a reliable method to quantify drug levels and assess adherence in routine clinical practice.

Clinical outcome of RDN in hypertension

Despite an initial overwhelming success of RDN in a large variety of studies, the first dedicated prospective, randomised sham-controlled study on the topic (Symplicity HTN-3) failed to meet its primary efficacy endpoint (9). The study shocked the hypertension community and triggered new and higher standards in trial design (30). New international multicenter randomised, sham-controlled trials were subsequently designed to assess the blood pressure lowering efficacy of RDN in more controlled settings (18, 31) addressing all the potential confounders that were believed to have stimulated the negative results of HTN3. As such, the RADIANCE HTN (SOLO and TRIO) trial program was designed to evaluate patients in two cohorts in the United States and Europe (30). The SOLO cohort included patients with essential hypertension, at low cardiovascular risk, and either controlled in 1 to 2 antihypertensive drugs or

uncontrolled on 0 to 2 antihypertensive drugs. Patients underwent a 4-week drug washout period before randomisation to RDN or renal angiography (sham). The TRIO cohort included patients with resistant hypertension stabilized on fixed triple pill combination for 4 weeks before randomisation. In both cohorts the primary efficacy endpoint (based on daytime systolic ABP) was set at 2 months after the procedure to lower the chance of bias by reintroduction or change in antihypertensive drug regimens. Blood pressure recordings per center were sent to a central, independent and blinded core laboratory for validation and further analysis. In perhaps one of the most rigorously designed trials on the topic thus far, we were able to demonstrate in the RADIANCE-HTN SOLO trial (**Chapter 10**) a clinical benefit of -6mmHg (95% CI −9.4 to −3.1, p=0.0001) in daytime systolic ABP at 2 months after RDN as compared with sham (18). Furthermore, a quarter of the patients who underwent RDN had sufficient control of blood pressure (without antihypertensive drugs), whereas half of the patients treated with RDN had insufficient blood pressure control to remain off antihypertensive drugs after 2 months. The latter findings were strengthened by similar results published in the SPYRAL-HTN OFF MED trial at 3 months (31). Both proof-of-principle studies confirmed that RDN significantly reduces blood pressure at 2-3 months in a highly selected low risk patient population in which confounders were reduced to an absolute minimum. Whether these findings can be extended into real world patient scenarios with higher risk patients on multiple antihypertensive drug-regimens needs to be determined in dedicated future larger scale trials. A second item of concern is the relevance of the magnitude of the blood pressure lowering effect. While the linear relation between a decrease in blood pressure and subsequent reduction in major adverse cardiac events has been widely accepted, future cost-effectiveness analyses will be needed to assess willingness to pay threshold for RDN. Finally, more work is needed to understand the procedural and patient characteristics associated with success. In **Chapter 11,** we elaborate on the concept of incomplete denervation by testing the potential effect of a redo-RDN procedure, in order to increase response rate. In a small case series of three consecutive non-responders to the first RDN, a second RDN using a different RDN catheter system, proved to result in a significant reduction in both office- and 24h-ABPM. Either ultrasound and radiofrequency endovascular RDN were used in this case series, both with proven clinical efficacy for the treatment of hypertension (18, 31). These findings suggest an important procedural reason for non-response and warrant future work in order to optimize procedural efficacy. Along with our work on peri-procedural mapping (chapter 5), several dedicated studies are

on the way assessing the impact of different procedural treatment strategies targeting main or distal parts of the renal artery along with the necessity to also target accessories of different sizes.

Measuring the efficacy of RDN

While blood pressure has been accepted as a surrogate endpoint linked to long-term cardiovascular morbidity and mortality, the feasibility of large scale prospective clinical outcome studies with RDN seems unlikely within the near future. The latter triggered studies with mechanistic endpoints to assess potential pleiotropic effects of RDN. In **Chapter 12,** we studied the effect of RDN on functional myocardial parameters as assessed with speckle tracking echocardiography. We found an improvement in diastolic function characterized by a significant decrease in A-wave velocity and peak untwisting velocity was found after RDN at 6 months follow-up, which might suggest that less force was needed for active atrial contraction during late diastolic filling (32). However, these findings should be interpreted with caution since no differences were found in other echocardiographic parameters. Another cardiovascular entity in which an imbalance of the SNS has been described is vasospastic angina (VSA) (33). In **Chapter 13** and **14** we studied the effect of RDN in patients with refractory VSA using cardiac sympathetic nerve activity was measured using iodine-123 labeled meta-iodobenzylguanidine (^{123}I-MIBG) imaging as a recognized objective endpoint (**Chapter 13**). At 6 months follow-up, RDN resulted in angina relief with an significant improvement in quality of life. However, no change was observed in the ^{123}I-MIBG parameters questioning the impact of RDN on cardiac sympathetic activity. Given the positive effect of RDN on the symptoms of these patients, for which there are virtually no proved alternative therapeutic options, larger prospective randomized trials should be considered to evaluate the role of RDN in patients with VSA. Next to its relation with VSA, the sympathetic nervous system has been considered an important source of cardiac arrhythmias (**Chapter 14**). Atrial fibrillation (AF) is a common cardiac arrhythmia in which both hypertension as autonomic imbalance contribute to AF burden (34, 35). In **Chapter 15** we studied the effect of RDN on AF burden as measured by using an implantable cardiac monitor and a quality of life questionnaire. A decrease in AF burden was observed and an improvement in quality of life was reported. However no correlation between change in blood pressure and change in AF burden was found. Previous studies assessed the additional effect of RDN on

pulmonary vein isolation (PVI), in order to enhance long-term anti-arrhythmic efficacy (36, 37). In both studies, RDN added to PVI, compared with PVI alone, significantly increased the likelihood of freedom from AF at 12 months follow-up.

Finally, an increased sympathetic tone was described in chronic heart failure with reduced ejection fraction (HFrEF) as a response to a chronic low-output state. Some animal models showed promising findings of RDN on left ventricular ejection fraction (LVEF) (38, 39). In **Chapter 16,** we present the first dedicated prospective randomised study assessing the safety and efficacy of RDN in patients with HFrEF. RDN with a bipolar radiofrequency balloon catheter in patients with HFrEF was safe, however, did not result in significant changes in cardiac sympathetic nerve activity at 6 months as measured using ^{123}I-MIBG. The therapy appeared safe with numerically lower rates of the combined safety endpoint of cardiovascular death, rehospitalization for heart failure, and acute kidney injury at 6 months in the RDN group as compared with OMT alone. Furthermore, a significant difference was observed in LVEDD in the RDN-group, and 26% of the patients in the treatment-group was in NYHA class I versus none in the control-group, suggesting a potential effect of RDN. Despite several positive signals in the results of the present study should be put into perspective to a relatively small sample size (n=50), patients included were mostly in NYHA class II with relatively low NT-proBNP, suggesting a less severe heart failure phenotype and finally to the fact the RDN was performed using the Vessix device of which the production was halted leading to a premature termination of the present study at 50/70 patients (40). Dedicated larger studies with alternative devices are currently being designed to better understand the potential of RDN to improve signs and symptoms of heart failure.

Present and future perspectives

Interventions targeting lifestyle and therapy adherence are of paramount importance in the management of hypertension. Nevertheless, the enthusiasm for minimal invasive treatment options to improve blood pressure control within both medical professionals as well as patients, is increasing rapidly (41, 42). This new momentum was created by several positive rigorously designed proof-of principle sham-controlled randomized trials (18, 43-45). In order to understand the place of RDN in the contemporary management of hypertension or cardiovascular diseases related to sympathetic overactivity, the therapy needs to demonstrate its long-term safety, cardiovascular benefits and several important technical issues should be addressed.

As such, the exact clinically relevant blood pressure lowering effect of RDN remains to be established in light of commonly accepted willingness to pay thresholds. Two recent meta-analysis supported a reduction of 8-10mmHg in office blood pressure as clinical meaningful, which corresponds to 6-7mmHg in 24h-ABPM (46, 47). Second, a better understanding is needed on which patient will benefit the most from RDN. Third, recent clinical trials have been performed in relatively low risk patients with mild to moderate hypertension. The challenge will be to design future studies with smart designs that will be able to extent the findings of the first proof of principle studies to more real world and higher risk patient populations taking into account a broad variety of antihypertensive therapies. It is likely that the adoption of TDM, potentially with DBS testing will be a game changer in future trial designs. Fourth, when focusing on the technical issues of the therapy, two important issues remain unsolved namely, the 'blinded' nature of the therapy (no clinical marker for pre-procedural feedback or success of therapy is available) and whether all (currently available) catheter systems should have the potential to treat branches and renal accessories. The latter is likely to be resolved only if more reliable and reproducible markers of success become available. Fifth, and finally, a high number of trials fails to reach recruitment targets. Embedding social media in recruitment campaigns of cardiovascular trials has the potential to significantly boost patient recruitment.

Concluding remarks

Interventions targeting lifestyle and therapy adherence are important in the management of hypertension and cardiovascular diseases. Easier and faster compliance tests have the potential to enhance blood pressure control by increased awareness of adequate drug therapy for both patients and treating physicians. Also social media proved to be a powerful tool to increase awareness of novel blood pressure lowering therapies and could act as an important add-on for study recruitment campaigns of hypertension trials.

Renal sympathetic denervation is thus far the most widely studied invasive treatment option for hypertension. However, given the multifactorial etiology of hypertension along with a high number of factors impacting blood pressure, we demonstrated in a rigorous sham controlled randomized trial, a significant blood pressure lowering effect of RDN. While this proof of principle trial helped to build a strong foundation for the potential of the therapy, a significant number of questions related to technical

optimization of the technology, efficacy in other diseases related to sympathetic overactivity, durability and cost-effectiveness remain.

With these findings we aimed to contribute to the body of evidence supporting the importance of compliance to pharmacological therapy and the role of minimal invasive non-pharmacological therapy in hypertension and other diseases related to sympathetic overactivity in order to be finally able to improve patient outcome. Future large-scale studies are eagerly awaited.

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EPILOGUE

Summary

The autonomic nervous system consists of different pathways of neurons to control organ systems. It is divided into the sympathetic and parasympathetic nervous system. The sympathetic nervous system (SNS) regulates several hemodynamic variables, such as blood pressure, heart rate, cardiac output and vascular resistance. A strong relationship between sympathetic overactivity and essential hypertension has been described. The renal sympathetic nerves seem to have an important role in the initiation and maintenance of the sympathetic overactivity. Effective pharmacological treatment for hypertension was introduced in the early '60. However despite optimal medical treatment using combinations of all available agents, blood pressure control often remains poor and the risk for cardiovascular disease remains high. Percutaneous renal sympathetic denervation (RDN) has been reported to lower blood pressure by reducing renal sympathetic nerve activity. The initial efficacy results in non-randomised trials were promising and the therapy seemed safe. However, the first sham-controlled study showed no superiority of RDN in lowering blood pressure as compared to sham. This study explored some key factors that may have contributed to the puzzling results, namely a more pronounced response to a sham procedure. Data on changes in antihypertensive drugs, outcomes in selected subgroup populations and detailed procedural data were studied in order to detect potential confounders that may have impacted the efficacy endpoint. The recently conducted trials showed the importance of standardised care in blood pressure management and proved the blood pressure lowering of RDN in a strict selective study population. However, in order to understand the place of RDN in the management of hypertension and in other sympathomimetic driven cardiovascular disease, this thesis tried to outline several challenges at the patient level to clinical outcomes.

Part I Resistant hypertension

Resistant hypertension is a challenging problem. In order to improve blood pressure control, lifestyle modification is recommended, adherence must be carefully assessed to exclude pseudo-resistance, and secondary causes of hypertension need to be ruled out before initiating new drugs or considering device-based treatment strategies (chapter 2). Due to the fact that resistant hypertension is a multifactorial problem, a stepwise approach to reach therapy goals is recommended.

Part II Eligibility and screening

Great and costly efforts are required to recruit potential participants into clinical trials, especially with the knowledge that half of the hypertensive subjects screened for RDN are ineligible. Recruitment through social media, Facebook, is increasingly being recognized and was observed to be an efficient strategy to find candidates for a prospective randomised hypertension trial (chapter 3). Facebook appeared to be less expensive than radio ads, however with the available data no comparison with other strategies could be presented, also a reliable cost-effectiveness comparison could not be performed. Then, when included in a RDN study, detailed pre-procedural radiological assessment using a high resolution computed tomography (CT) or magnetic resonance imaging (MRI) can be performed to evaluate secondary causes for hypertension (chapter 4). Apart from the pre-procedural screening, the work-up for RDN could also identify asymptomatic disease or a harmful finding such as a malignancy (incidental finding, IF). Despite the high number of IF, only a fraction of the intended procedures were delayed or canceled. However structural reporting on these radiologic findings is important. In a search towards responders and non-responders to the RDN therapy, several clinical markers (biochemical as procedural) were studied (chapter 5 and chapter 6).

Part III Assessing adherence to antihypertensive drugs

Despite the current medication regimen of antihypertensive drugs, control of hypertension is not optimal. Up-titration of antihypertensive drugs results in a linear drop in adherence to antihypertensive drugs. Several methods, indirect as direct methods can evaluate adherence. The most reliable methods are described to be direct methods (such as directly observed therapy (DOT) under supervision of a health care professional) and measurements of levels of drugs and if needed metabolites in blood or urine, the so-called therapeutic drug monitoring (TDM). However, inter-individual differences in drug levels and response to treatment was observed (chapter 7). TDM is an objective method of measurement and it ensures that actual drug levels reflect the blood pressure at the time of measurement. TDM can also differentiate between complete and partial non-adherence, however the patient needs to visit the laboratory for venipuncture. Therefore, a minimal invasive method (dried blood spot, DBS) to measure levels of antihypertensive drugs and their active metabolites is introduced (chapter 8 and 9). DBS showed to be a reliable and convenient method to assess non-

adherence to the eight most prescribed antihypertensive drugs in clinical practice.

Part IV Clinical outcome studies on renal sympathetic denervation

The RADIANCE-HTN SOLO trial, a multicenter, international, single blind, randomised sham-controlled trial proved the efficacy and safety of RDN in subjects with hypertension in the absence of antihypertensive medications at two months follow-up (chapter 10). However, variable blood pressure response to therapy was observed. This finding could suggest that some patients have more benefit of the therapy than others (responders vs. non-responders in different subgroups). The effect of a second (redo)-RDN was studied to increase response-rate, however this was studied in patients with resistant hypertension (chapter 11).

Several studies support the potential use of RDN in the management of sympathomimetic driven cardiovascular disease, often independent of antihypertensive effects. Clinical studies were performed in order to understand the effect of RDN on myocardial parameters and left ventricular ejection fraction/left ventricular mass by (speckle tracking) echocardiography (chapter 12). Also the effect of RDN in patients with refractory vasospastic angina was described (chapter 13 and chapter 14). In order to understand the effect of RDN, clinical parameters from the iodine-123 labeled meta-iodobenzylguanidine (^{123}I-MIBG) imaging were used to observe the effect of the therapy on cardiac sympathetic activity. Furthermore, studies in patients with paroxysmal or persistent atrial fibrillation (chapter 15) and in patients with chronic heart failure with reduced ejection fraction were performed (chapter 16). Despite positive effects on quality of life measures (placebo effect could not be excluded in chapter 11 to chapter 15, no control group was available). Additionally, these studies were of small size and the data retrieved was heterogeneous. Larger study populations need to be conducted, whereas the present findings should be interpreted with caution. Though, these findings could help in creating new study designs.

Samenvatting

Het autonome zenuwstelsel bestaat uit verschillende neuronbanen om orgaansystemen te besturen. Het is onderverdeeld in het sympathisch en het parasympatisch zenuwstelsel. Het sympathische zenuwstelsel (SNS) reguleert verschillende hemodynamische variabelen, zoals bloeddruk, hartslag en vaatweerstand. De constante hyperreactiviteit van het sympathische zenuwstelsel (het “stress systeem” van het lichaam) lijkt een belangrijke rol te spelen in hoge bloeddruk. De sympathische zenuwen rondom de nierslagaders hebben een prominente rol bij het initiëren en onderhouden van de sympathische hyperactiviteit. Begin jaren ’60 werden effectieve medicijnen (bloeddrukverlagers) voor hoge bloeddruk geïntroduceerd. Ondanks deze optimale medische behandeling met combinaties van alle beschikbare middelen, blijft de bloeddruk controle vaak matig en blijft het risico op hart- en vaatziekten hoog. Een nieuwe catheter-gebonden behandeling, kan een aantal van de zenuwverbindingen uitschakelen, de zogenoemde renale sympathicus denervatie (RDN). RDN werd geïntroduceerd om de bloeddruk te verlagen door de sympathische zenuwactiviteit ter hoogte van de nierenvaten te verminderen. De initiële resultaten over de effectiviteit van de therapie, in niet-gerandomiseerde onderzoeken, waren veelbelovend en de therapie bleek veilig. De eerstvolgende studie met een controlegroep toonde echter geen superioriteit van de RDN therapie in het verlagen van de bloeddruk in vergelijking met de controlegroep. Studies onderzochten factoren die mogelijk hebben kunnen bijgedragen tot deze verrassende resultaten, waarin de controlegroep een groter bloeddruk verlagend effect liet zien. Verschillende zaken werden benoemd: veranderingen in bloeddrukmedicatie gedurende de studie, therapietrouw voor medicatie was niet gecontroleerd, de karakteristieken van de onderzochte studiepopulatie was ongelijk en gedetailleerde procedurele gegevens werden bestudeerd om potentiële verstorende factoren te detecteren die mogelijk de eindresultaten hebben beïnvloed. De recent uitgevoerde onderzoeken toonden het belang aan van gestandaardiseerde zorg bij bloeddruk behandeling. Tevens werd het bloeddruk verlagende effect van RDN in een strikt selectieve onderzoekspopulatie bewezen. Het doel van dit proefschrift was het bestuderen van screening van patiënten die geschikt zouden zijn voor de RDN studies, het belang van therapietrouw aan bloeddrukverlagers te onderzoeken en tenslotte werd het effect van de RDN therapie bestudeerd in hoge bloeddruk en in sommige hart- en vaatziekten (zoals hartritmestoornissen en chronische hartfalen).

Deel I Therapieresistente hoge bloeddruk

Therapieresistente hoge bloeddruk is een uitdagend probleem. Om de bloeddruk controle te verbeteren, moet aanpassing van de levensstijl worden aanbevolen, dient therapietrouw zorgvuldig te worden beoordeeld om pseudo-resistentie uit te sluiten, en moet worden getracht secundaire oorzaken van hypertensie uit te sluiten voordat nieuwe geneesmiddelen worden gestart of invasieve behandelstrategieën worden overwogen zoals RDN (hoofdstuk 2). Omdat therapieresistente hoge bloeddruk een multifactorieel probleem is, wordt een stapsgewijze aanpak aanbevolen om therapiedoelen te bereiken.

Deel II Screening en beoordeling van potentiele deelnemers

Enorme inspanningen zijn nodig om potentiële deelnemers voor klinische studie te werven, vooral met de wetenschap dat de helft van de hoge bloeddruk patiënten die voor RDN worden gescreend, niet in aanmerking komen. Werving via social media, zoals Facebook, wordt steeds meer erkend. Deze methode van werving lijkt een efficiënte strategie te zijn om deelnemers te vinden voor een prospectieve gerandomiseerde hoge bloeddrukstudie (hoofdstuk 3). Facebook bleek goedkoper te zijn dan radioadvertenties, maar met de beschikbare gegevens kon geen vergelijking met andere strategieën worden gepresenteerd en kon ook geen betrouwbare kosteneffectiviteitsvergelijking worden uitgevoerd. Indien eenmaal geïncludeerd in een RDN onderzoek, is een gedetailleerde pre-procedurele radiologische beoordeling met behulp van computertomografie (CT) met hoge resolutie of magnetische resonantie beeldvorming (MRI) om secundaire oorzaken van hoge bloeddruk te evalueren van belang. Tevens om de geschiktheid van de nierslagader te beoordelen (hoofdstuk 4). Afgezien van de pre-procedurele screening, kunnen er nevenbevindingen worden gedetecteerd (een asymptomatische ziekte of een schadelijke bevinding zoals een maligniteit). Ondanks het hoge aantal nevenbevindingen werd slechts een fractie van de beoogde procedures vertraagd of geannuleerd. Maar structurele rapportage van deze radiologische bevindingen is belangrijk. In een zoektocht naar responders en non-responders (patiënten die baat hebben bij de therapie versus geen baat), zijn er verschillende klinische markers (biochemisch als procedureel) bestudeerd (hoofdstuk 5 en hoofdstuk 6).

Deel III Beoordelen van therapietrouw

Ondanks het huidige medicatie regime van bloeddrukverlagende medicatie is de controle van hoge bloeddruk niet optimaal. Optitratie van bloeddruk verlagende medicatie leidt tot een lineaire afname van de therapietrouw. Verschillende methoden, zowel indirect als direct, kunnen de therapietrouw beoordelen. De meest betrouwbare methoden worden beschreven als directe methoden (zoals direct geobserveerde therapie (DOT) onder toezicht van een zorgverlener innemen van medicatie) en metingen van medicijnconcentraties en indien nodig metabolieten in bloed of urine, de zogenaamde 'therapeutic drug monitoring' (TDM). Er werden interindividuele verschillen in medicijn concentraties waargenomen bij gelijke dosering van het medicijn. Tevens was de respons op bloeddruk behandeling verschillend per patiënt (hoofdstuk 7). TDM is een objectieve meetmethode en weerspiegelt de concentraties van het medicijn met de bloeddruk op het moment van meting. TDM kan ook onderscheid maken tussen volledige en gedeeltelijke therapietrouw, maar de patiënt moet het laboratorium bezoeken voor venapunctie. Daarom zou een minimaal invasieve methode ('dried blood spot', DBS) kunnen worden geïntroduceerd om de concentraties van de medicijnen en hun actieve metabolieten te meten (hoofdstuk 8 en 9). DBS bleek een betrouwbare en gemakkelijke methode te zijn om de therapieontrouw van de acht meest voorgeschreven antihypertensiva in de klinische praktijk te beoordelen.

Deel IV Klinische uitkomsten in RDN studies

De RADIANCE-HTN SOLO-studie, een multicenter, internationale, gerandomiseerde studie met een controlegroep, bewees recentelijk de werkzaamheid en veiligheid van RDN bij proefpersonen met hoge bloeddruk bij afwezigheid van bloeddrukverlagende medicatie bij twee maanden (hoofdstuk 10). Er werd echter een variabele bloeddruk respons op de therapie waargenomen. Deze bevinding zou kunnen suggereren dat sommige patiënten meer baat hebben bij de therapie dan andere (responders versus non-responders in verschillende subgroepen). Het effect van een tweede (redo)-RDN werd bestudeerd om de respons te verhogen, maar dit werd bestudeerd bij patiënten met therapieresistente hoge bloeddruk (hoofdstuk 11).

Verschillende wetenschappelijke onderzoeken ondersteunen de behandeling van sympathicus gemedieerde hart- en vaatziekten middels RDN, vaak onafhankelijk van de bloeddrukverlagende effecten. Er zijn klinische studies uitgevoerd om het effect van

RDN op hartspier parameters en de linkerkamerfunctie te begrijpen door middel van (speckle tracking) echocardiografie (hoofdstuk 12). Ook het effect van RDN bij patiënten met refractaire vasospastische angina werd beschreven (hoofdstuk 13 en hoofdstuk 14). Om het effect van RDN te begrijpen, werden klinische parameters met jodium-123 gelabelde meta-joodbenzylguanidine (123I-MIBG) -beeldvorming toegepast. Dit werd gedaan om het effect van de therapie op cardiale sympathische activiteit te observeren. Verder werden studies uitgevoerd bij patiënten met boezemfibrillatie (hoofdstuk 15) en bij patiënten met chronisch hartfalen (hoofdstuk 16). Ondanks positieve effecten op metingen van kwaliteit van leven en kan een placebo-effect niet worden uitgesloten in hoofdstuk 11 tot 15, er was geen controlegroep beschikbaar. Bovendien waren deze onderzoeken van kleine omvang en waren de onderzochte gegevens heterogeen. Deze onderzoeken zouden moeten worden uitgevoerd in grotere onderzoekspopulaties. De huidige bevindingen zullen daarom met de nodige voorzichtigheid moeten worden geïnterpreteerd. Deze bevindingen kunnen echter helpen bij het maken van nieuwe studieontwerpen.

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PhD Portfolio

Name PhD student:	Lida Feyz
Erasmus MC department	Cardiology
Research school	Cardiovascular Research School (COEUR)
PhD period	*November 2015 – December 2018*
Title thesis	*Advances in the workup and treatment for diseases associated with sympathetic overactivity*
Promotor	Prof. Dr. F. Zijlstra
Co-promotor	Dr. J. Daemen, Dr. J. Versmissen

General academic courses	**Year**	**Workload (ECTS)**
Biostatistical Methods-I	2015	0.1
Cardiovascular Medicine	2015	1.5
Cardiovascular Pharmacology	2015	1.5
Arrhythmia Research: solving the mysteries of atrial fibrillation	2016	1.5
Research Integrity	2016	0.3
Seminar renal, cardiac and vascular aging	2016	0.5
COEUR course RV failure	2016	0.2
NIHES Summer course (biostatistics and survival analysis)	2016	2.8
Cardiovascular imaging and diagnostics (Part I - III)	2017	1.5
English biomedical writing and communication	2018	3.0
COEUR course ischemic heart disease (2 days)	2018	1.0
COEUR course aneurysmal disease (1 day + symposium)	2018	0.5
Systematic Literature Retrieval, PubMed 1	2018	0.1
Elective research courses		
BROK course	2017	1.5
Oral presentation		
EuroPCR Paris	2017	1.2
TCT Denver	2017	0.9
EuroPCR Paris	2018	1.2
TCT San Diego	2018	1.5
Journal Club: The DENERHTN trial	2017	1.0
Journal Club: The SPRINT trial	2018	1.0
Poster presentation		
TCT Washington	2016	1.5
EuroEcho Imaging Leipzig	2016	0.9
Supervising and teaching		
Supervising 2nd year medical students (writing a review)	2016	1.6

General academic courses	Year	Workload (ECTS)
Renal sympathetic denervation course for nurses	2017-2018	1.0
Supervising master students	2019	1.6
Extracurricular activities		
Speckle tracking echocardiography course (Eindhoven)	2017	0.3
Information evenings for patients (patient recruitment activities)	2017-2018	0.2
Investigator meetings with sponsors (Amsterdam/Utrecht)	2017	0.2
National congresses		
NVVC Juniorkamerdag	2018	0.3
Total		30.4

About the author

Lida Feyz was born on June 4th 1990 in Tashkent, Oezbekistan. After graduating high school in 2009, she started her medical training in Maastricht UMC. During her bachelor, she was a fulltime member of the international federation of medical students' association (IFMSA). She was chairman of the working group 'Standing Committee on Human Rights and Peace' (SCORP). In October 2012, she started her internship at the Erasmus MC University Medical Center in Rotterdam. During her internships she also participated in some small scientific research projects at the cardiology department. Her graduation research project studied the effect of renal sympathetic denervation on left ventricular function with speckle tracking echocardiography. After acquiring her Medical Doctor's degree in 2015 she started as a PhD candidate, supervised by Dr. Joost Daemen (cardiology department) and Dr. Jorie Versmissen (internal medicine department) at Erasmus MC University Medical Center in Rotterdam. During this period, she had the opportunity to present her work on several international conferences. As of December 2019, she started her cardiology training at Erasmus Medical Center in Rotterdam.

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