SYMPATHETIC RENAL DENERVATION FOR RESISTANT HYPERTENSION

IMPACT ON BLOOD PRESSURE AND ON SURROGATE MARKERS OF TARGET ORGAN DAMAGE.

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Tese para obtenção do grau de Doutor em Medicina
na Especialidade em Medicina Clínica (Cardiologia)
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SYMPATHETIC RENAL DENERVATION FOR RESISTANT HYPERTENSION. IMPACT ON BLOOD PRESSURE AND ON SURROGATE MARKERS OF TARGET ORGAN DAMAGE.

Catheter-based sympathetic renal denervation (RDN) is a new treatment option for resistant hypertension (rHTN) and its clinical impact is yet to be fully understood.

The aim of this study was to evaluate the impact of RDN in blood pressure (BP) and in 2 recognized surrogate markers of target organ damage (TOD): left ventricle (LV) hypertrophy (assessed by echocardiogram) and proteinuria (evaluated by the urinary albumin to creatinine ratio (ACR), at 1 year follow up.

All patients with rHTN under maximal tolerated antihypertensive drug therapy submitted to RDN since July-2011 were included in a prospective single centre registry. All clinical variables, medication, laboratory values, 24-hour ambulatory BP measurements (ABPM) and echocardiogram results were recorded in an electronic database at baseline and at 1-year follow-up. The following objectives were addressed: changes on office and ABPM BP, on LV mass and structure, on ACR and renal function, as well as procedure safety.

Since 2011, 318 patients with rHTN were referred for RDN, of which 65 were considered to have true rHTN refractory to drug therapy and accepted for RDN. From those, 31 had a complete 1-year follow-up data at the time of the present analysis and are reported here. At 12 months there was a significant decrease in either office and ABPM systolic and diastolic BP, with 84% of patients considered responders to RDN regarding SBP and 71% for DBP. There was also a significant decrease in LV mass, from 152.3g/m2 to 135.7g/m2 ($p<0.001$) and in ACR, from 25.9mg/g to 14.8mg/g ($p=0.007$), independent of diabetes status, with no significant changes in renal function. No clear linear correlations were found, between changes in BP and either LV mass or ACR, both surrogates of HTN related TOD. There were no major complications related with RDN. These results suggest benefits of RDN in recognized markers of HTN organ damage, which if confirmed are expected to translate to an improvement in clinical endpoints beyond BP control.

For all my masters

To a wonderful family

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Abbreviations and acronyms

ABPM – 24 hours ambulatory blood pressure measurements

ACE – Angiotensin converting enzyme

ACR – Urinary albumin to creatinine ratio

ARB – Angiotensin receptors blockers

BMI – Body mass index

BP – Blood pressure

DBP – Diastolic blood pressure

EDV – Left ventricle end-diastolic volume

eGFR – Estimated glomerular filtration rate

ESV – Left ventricle end-systolic volume

EP – Epinephrine

GFR – Glomerular filtration rate

HTN – Arterial Hypertension

IVS – Interventricular septum diameter in diastole

LV – Left ventricle

LVEDD – Left ventricle end-diastolic diameter

LVEDV - Left ventricle end-diastolic volume

LVEF – Left ventricle ejection fraction

LVESD – Left ventricle end-systolic diameter

LVESV - Left ventricle end-systolic volume

LVM – Left ventricle mass index

NE – Norepinephrine

PWT – Left ventricle posterior wall thickness in diastole

RAAS – Renin – Angiotensin -Aldosterone system

RBF – Renal blood flow

RDN – Sympathetic nerve system denervation

RWT – Left ventricle relative wall thickness

SBP – Systolic blood pressure

SNS – Sympathetic Nerve System

TOD – Hypertension target organ damages

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INTRODUCTION

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Sections:

Summary

Hypertension is a leading cause of death in developed countries, and although there have been large investments in drugs aiming its control, there is still a staggering contrast between its high prevalence and the low rates of adequate control. A subset of patients with suboptimal blood pressure control have drug-resistant hypertension, in the pathophysiology of which chronic sympathetic hyperactivation is significantly involved. Sympathetic renal denervation has recently emerged as a device-based treatment for resistant hypertension. In this review, the pathophysiological mechanisms linking the sympathetic nervous system and cardiovascular disease are reviewed, focusing on resistant hypertension and the role of sympathetic renal denervation. An update on experimental and clinical results is provided, along with potential future indications for this device-based technique in other cardiovascular diseases.

Hypertension

Hypertension (HTN) is the leading global risk factor for cardiovascular mortality, with a prevalence worldwide projected to be approximately 3.5 billion in 2015, with 7.8 million related deaths each year[1]. Its strong association with myocardial infarction, heart failure, stroke, end-stage renal disease and cardiovascular death, is well established, with 54% of stroke and 47% of ischemic heart disease attributable to high blood pressure (BP) worldwide,[2] and it seems to have a continuous relationship between BP and cardiovascular risk from values as low as 115/75 mmHg, doubling the cardiovascular risk for every 20/10mmHg in pressure.[1, 3]

Meanwhile, effective blood pressure lowering has consistently been shown to reduce overall cardiovascular risk [4], but the rates of adequate BP control remains suboptimal, despite the large amount of available antihypertensive drugs, from different classes and strong scientific evidence supporting their use, with only 37% of treated hypertensive patients, achieving recommended BP values, in European countries. [5]

The blame for such very low rates of BP control cannot be attributable only to poor treatment management. The complex pathophysiology underlying human BP control, with multiple interconnected systems, has certainly a relevant role for the failure in achieving HTN treatment control.

There are at least two main systems responsible for BP control: renal-based and vascular-based. The renin-angiotensin-aldosterone system (RAAS), plays a major role in renal control of salt and water homeostasis, but also in peripheral vascular resistance, acting directly through angiotensin but also by activating the sympathetic nerve system (SNS). Other mediators are also important, although their role is not so well understood.

For the last decades, RAAS system has been the central focus of HTN treatment and management. Availability of secure, efficacious and evidence proved drugs blocking this system, as led to neglect the contribution of other systems, namely the autonomous nervous system, for raising and maintaining high BP values.

The SNS and its possible role in the pathogenesis of HTN, has been receiving increasing attention. A more complete understanding of how the SNS could help to control the long-term level of BP has developed recently and supports a complete new approach to treat resistant HTN.

Sympathetic nerve system

More than one hundred years have passed since Gaskell and Langley first elucidated us on the structure and function of the autonomous system, the sympathetic and parasympathetic system, and could show, that they are both distributed to the same body structures, their effects are antagonistic, and they subservice the functions of organic life, and are not under the control of the will.[6] Later, Cannon has extended this, by pointing out the critical role of the SNS in preparing the body to struggle and to increase its powers of defence.[7] Under SNS stimulation, the pupil dilates to increase perception of light, the heart beats faster and forcibly to supply the muscles with blood, the visceral blood vessels area constrict, raising the blood pressure and driving the flow from the digestive area, whose functions are inhibited, to the muscles, the heart, the lungs and the brain.

Sympathetic nerve system control. Under physiological conditions, the autonomic nervous system, adjusts circulation in keeping with behaviour, environment and emotions, via rapid changes in the cardiac output and regional arteriolar resistance. The neural control of the circulation operates via parasympathetic neurons that innervate the heart, and via three main classes of sympathetic efferents, the barosensitive, the thermosensitive and the glucosensitive, that do innervate the blood vessels, the heart, the kidneys and the adrenal medullae. Their control centers are all located in the central nerve system: the rostral ventrolateral medulla, the spinal cord, the hypothalamus and the nucleus of solitary tract. The rostral ventrolateral medullae, the nodal point for most, if not all, sympathetic reflexes that involve cardiovascular regulation, is the primary centre for BP control.[8, 9] It is linked to hypo-

thalamic centres, influencing sodium and water balance, but also, to the cortex, specially the limbic cortex, responsible for rapid behaviour-related adjustments of sympathetic tone.[8]

Sympathetic nerve system effectors. The thermosensitive group of cardiovascular efferents is primarily cutaneous vasoconstrictors activated by hypothermia, emotions and hyperventilation. The glucosensitive are activated by hypoglycemia and physical exercise and controls the release of epinephrine (EP) by the adrenal medulla. These two types of efferents presumably have a secondary role in short and long-term regulation of BP.[10, 11]

The large group of barosensitive sympathetic efferents, under the input influence of arterial baroreceptors, have a dominant role on short and long-term regulation of BP. Regardless of the organ or tissue that they innervate, these neurons have an ongoing activity at rest. They control the heart, the release of norepinephrine (NE) from the adrenal medullae, vasoconstriction of resistance arterioles and play a major influence in kidney function, where they control renin secretion, sodium reabsorption and renal blood flow.[12]

The SNS has also a special relation with adrenal medullae. Preganglionic sympathetic nerve fibres pass directly from medio-lateral horn cells of the spinal cord into the two adrenal medullae, ending directly on modified neuronal cells that secrete EP and NE directly into blood.[13] Anatomically, those sympathetic adrenal nerve fibres can go along with the sympathetic postganglionic renal fibres and in this way may be affected by renal denervation procedures.

Sympathetic nerve system neurotransmitters. The sympathetic fibres secrete one of two synaptic transmitter substances, acetylcholine or NE. All preganglionic neurons are cholinergic, it means, they secrete acetylcholine as neurotransmitter that excites the postganglionic neurons. Most of those postganglionic neurons are adrenergic, meaning that they secrete NE as a neurotransmitter, with the exception of postganglionic sympathetic fibres to the sweat glands, which are cholinergic.[13]

The NE secreted by terminal nerve endings is removed from site by reuptake into the adrenergic nerve endings through an active transport mechanism, accounting for 50 to 80% of secreted neurotransmitter. The remaining neurotransmitter may diffuse away to the surrounding tissue and blood being destroyed later, by catechol-O-methyl transferase in the liver. Small amounts are also destroyed by local tissue monoamine oxidase enzyme. Although NE secret directly to tissue as a lifespan of a few seconds, once in blood, remains active from one to several minutes, until destroyed in the liver.[13]

Epinephrine and NE stimulates an effector organ through a membrane receptor, that either changes cell membrane permeability to ions (sodium, potassium or calcium) or altering an intracellular second messenger enzyme, the adenylcyclase, causing the intracellular formation of cAMP, which will initiate many different intracellular actions.

There are at least two known types of adrenergic receptors, alfa and beta receptors and many subtypes of them. For a more comprehensive review, the receptors and their function are listed in Table.1.

Table 1: Types and functions of adrenergic receptors.

<table>
<tr><th colspan="2">Alpha Receptors</th><th colspan="2">Beta Receptors</th></tr>
<tr><td>α1
postsynaptic</td><td>α2
presynaptic</td><td>β1
postsynaptic</td><td>β2
postsynaptic</td></tr>
<tr><td>Gq protein coupled
Activation phospholipase C</td><td>Gi protein coupled
Inhibits Adenyl Cyclase</td><td colspan="2">Gs protein coupled
Activates Adenyl Cyclase</td></tr>
<tr><td>Vasoconstriction:
• Skin
• Gut
• Kidney
• Brain
Smooth muscle cells contraction:
• Ureter
• Vas deferens
• Urethral spinchter
• Uterus
• Cilliary body
Glucose metabolism:
• Gluconeogenesis
• Glucolysis</td><td>Glucose metabolism:
• Inhibits insulin release
• Stimulates glucagon release
• Contraction of anal sphincter
• Inhibits release of EP</td><td>Heart:
• Chronotropic +
• Dromotropic +
• Inotropic +
• ↑ LVEF
Renine release ↑ by juxtaglomerular cells
Hunger ↑
• ↑ ghrelin release by stomach</td><td>SMC relaxation:
• Bronchus
Bronchioles
• Detrusor muscle
• Uterine muscle
Urethral sphincter Contraction:
Renin release by justaglomerular cells
Glucose metabolism:
• Inhibits insulin release
• Stimulate glucolysis and gluconeogenesis
Lipolysis</td></tr>
<tr><td colspan="2">Vasoconstriction of:
• Coronary arteries
• Veins
↓ Motility of gastrointestinal system</td><td></td><td></td></tr>
</table>

The beautifulness of this system, and its complexity, drives from the fact that using just two different kinds of neurotransmitters, NE and EP, it can initiate many different actions, depending on the target tissue, type of receptor and the relative affinity of the neurotransmitter for the receptor.

This mechanism must have been developed during a vast period, through innumerable intermediate stages. Therefore, we are now in possession of a complex mechanisms which discharge energy under strictly controlled fashion and adequate stimulation.

Sympathetic control of blood pressure

Blood pressure is primarily a function of peripheral arterial resistance, but also of cardiac output, which in turn, is dependent of heart rate, myocardial contractility and venous blood return, the later, also dependent on smooth muscle venous tonus. All under SNS control.

The neural control of BP and overall circulation uses the vast complex SNS and the more localized parasympathetic neurons that do innervate the heart and lungs.

As explained, the background activity of the barosensitive group of SNS efferents, is presumably the most crucial for long-term physiological regulation of BP and it seems, that in hypertensive humans, the rise in the activity of barosensitive sympathetic efferents is not restricted to renal nerves but is generalized,[8, 12, 14-16] partly explaining the cluster relationship held by HTN and other metabolic disturbances. Regardless of the organs or tissues they innervate, they show continues ongoing activity at rest (the sympathetic tone) and they discharge in burst synchronized with arterial pulse and respiration, being responsible for short term fluctuation of BP.[10, 17, 18] They control the heart, the release of NE from adrenal gland, constricting resistance arterioles, the kidneys, increasing renin secretion, tubular sodium reabsorption and renal blood flow, most likely, exerting a long-term control on BP.[12, 19]

Atrial stretch or volume expansion has a strong inhibition effect on the renal SNS efferents. It appears that the selective control of renal SNS efferent by volume receptors, might be the most important of these differential regulations mechanisms.[12, 19]

The afferent limb of this loop reflex mechanism involves mechanoreceptors activated by distension of the arterial wall. An increase in BP activates those receptors, inhibiting cardiac, renal and vasomotor sympathetic efferents, restoring BP to previous values, and so helping to damp short-term BP fluctuations.[8, 9] This system may also be reset, through neural and humoral mechanisms, still largely unexplored, allowing higher BP values without reduction in reflex sensitivity, for example, circulating and brain derived angiotensin II can reset these reflex mechanism.[8, 9] External, chronic electrical activation of this baroreceptor was associated to a reduction of neurohor-

monal indicators of sympathetic activity, namely NE and angiotensin II and with a presumed increase in survival,[7, 20] but apparently had little influence on long-term control of BP.[8, 21]

Besides its nearness in the carotid bifurcation, the "road" into the brain, there is a close interaction between baroreceptors and chemoreceptors in the control of sympathetic activity, whereby the baroreflex activation is inhibitory and the chemoreceptor reflex is excitatory. Besides this contra regulatory action, there is a facilitator effect between them, whereby a reduced baroreceptor activity will enhance the chemoreceptor response.[7, 18] These reciprocal sensory modulation, exerted by baroreceptors and chemoreceptors, are beneficial in states of circulatory collapse and shock. When severe hypotension and hypoxia coexists, they mutually enhanced sympathetic drive and ventilation helping to overcome the crisis.

Role of sympathetic nerve system on renal function

the role of kidneys in long-term regulation of BP, is mandatory in any discussion addressing this subject. The pressure-natriuresis relationship described several years ago by Guyton,[22] established that any increase in sodium retention produced an initial blood volume expansion, increasing cardiac output and therefore BP. The resulting peripheral tissues overperfusion leads to an increase in peripheral resistance, returning cardiac output towards normal. Accordingly, a reset of the pressure natriuresis relationship, establishing a new BP homeostatic set-point, inevitably lead do HTN, regardless of the cause of the resetting. But we need to address the fact, not known at the time, that volume expansion also promotes atrial stretch and activation of baroreceptors located there, and subsequently to a selective inhibition of renal SNS activity.[12, 19]

Renal sympathetic nerve terminals innervate the three major renal neuroeffectors, directly influencing renal tubular function, glomerular flow rate (GFR) and renal blood flow, with a clear impact in all major components of renal function. Renal sympathetic efferents are in direct contact with the peritubular basement membrane, of all renal tubular segments (α1A-drenoreceptors), as well as the juxtaglomerular granular cells affecting renin secretion (β1-adrenoreceptors) and renal arteries (α1A-drenoreceptors). This control is frequency dependent, with increases in renin secretion rate without changes in urinary sodium excretion, renal blood flow and GFR at low frequencies. At slightly higher frequencies, the increase in renin secretion is associated with an increase in tubular sodium reabsorption, still without changes in renal blood flow and GFR.[12] Much higher frequencies will decrease GFR and renal blood flow, maximizing renal ability to reabsorb sodium and water. Besides, additional neurophysiological renal studies support the existence of functionally specific renal sympathetic nerve fibers for each

neuroeffector, making the overall system more powerful and precise, in controlling the body sodium contends and thus BP.[12]

We can assume that the coupling of regulation of total body fluid volume to arterial blood pressure, depends on kidney's ability to excrete sodium in such a way as to achieve sodium internal balance in face of varying sodium intake, through the Guyton's pressure natriuresis mechanism.[22] Any defect on the kidney's ability to maintain this balance results in an increase in arterial pressure. The observation in various animal experiments,[12] that renal denervation prevents or delays the onset of HTN, implies that an increase in renal sympathetic activity, may be a final common pathway required for a defective renal sodium excretion, leading to development and maintenance of HTN.

Human sympathetic nerve system assessment

Until the early 70s, the most commonly used method to assess SNS activity, was blood measurements and urine excretion rates of NE and its derivate, a gross estimation of whole-body sympathetic activity at best.[23] Meanwhile, new methodologies emerged for measuring sympathetic nerve firing rates in subcutaneous nerves and for assaying the concentration of sympathetic transmitters in plasma.

Microneurography, a technique reported first by Hagbarth[24], provided a tool to study sympathetic nerve firing in subcutaneous tissue and skeletal muscle vessels, through tungsten electrodes inserted in the skin. It records burst of nerve activity, synchronous to heart-beat, generated by sympathetic efferent nerves. Highly reproducible and closely related to sympathetic traffic directed to other structures, it can be repeated over time, although painful, it allows the assess of interventions effects and direct quantification of sympathetic nerve traffic and vasomotor tone.

The **spillover technique**, a measurements of sympathetic NE transmitter release, first applied by Esler et al[25], this isotope dilution method calculates the clearance and spillover of NE, using an infusion of tritium labelled NE administered intravenously. The close relationship between the sympathetic nerve fibres firing rate of an organ and the rate of NE spillover into the venous effluent of that organ provides the rational for using measures of regional NE release as a surrogate of sympathetic tone in individual organs,[26] allowing the assessment of regional SNS function in humans.

This technique was on the forefront in proving the concept that heart failure patients, had sympathetic overactivity rather than sympathetic denervation, as initially thought, and opened the way to the routine use of betablockers in heart failure.[27]

Sympathetic nerve system over activity

Besides its central role in cardiovascular homeostasis, controlling the vascular tone through vasoconstriction of small resistance arteries, the SNS also interferes and regulates numerous other physiological processes (Table 2).

Table 2: Effects related to increased sympathetic nerve activity

Vascular
Smooth muscle cell hypertrophy and proliferation
Endothelial dysfunction and damage
Arterial stiffness
Posture blood pressure control impairment and syncope
Hypertension
Atherosclerosis
Cardiac
Myocyte hypertrophy
Left ventricle hypertrophy
Arrhythmia
Psychogenic heart disease
Renal Effects
Renal artery vasoconstriction
Sodium and fluid retention
Microalbuminuria
RAAS activation
Metabolic Effects
Insulin resistance
Dyslipidemia

There is growing evidence that sustained chronic changes in SNS activity are involved in the pathogenesis of many diseases states, ranging from metabolism to psychological disorders, including ischemic heart disease[28], chronic heart failure,[29, 30] HTN[31-33], kidney disease[34], type II diabetes[35], obesity[35], metabolic syndrome[35], obstruc-

tive sleep apnoea[36], depression to inflammatory bowel disease.[37, 38] A chronically overactive SNS is well known to worsen prognosis in patients with heart failure and end-stage renal disease[39, 40].

Supported by accumulating scientific evidence[41] is the fact that deleterious effects on vessels and myocardium, by an overactive SNS is independent of BP. Chronic SNS activation can cause hypertrophy and proliferation of vascular smooth muscles cells, as well as a direct trophic effect on cardiac myocytes with an increase on left ventricular mass (LVM) and wall thickness, without an increase in BP.[41] The combined structural changes on the myocardium and the direct effect of an overactive SNS, is a major contribution to the high incidence of arrhythmias commonly seen in this patients.[42]

Virtually all cardiovascular conditions and diseases, in which an increased adrenergic drive is involved are also characterized by endothelial dysfunction.[43] Nitric Oxide, one of the most important endothelial function mediators, is also an important neurotransmitter cooperating in the autonomic regulation of cardiovascular function, acting as a sympathetic-inhibitory substance within the central nerve system[44]. Acute and chronic increases in SNS activity, through endothelial dysfunction and endothelial cell damage, have been proven to contribute to subsequent development of atherosclerosis. [37, 41, 43]

SNS overactivity has also been linked to the development of metabolic disturbances, such as insulin resistance and dyslipidemia.[45] Not only, an increased SNS activity can itself led to insulin resistance, particularly in hypertensive patients,[45] but also elevations in circulating insulin levels, from insulin resistance in obese patients, can precipitate an increase in SNS activity leading to HTN.[41, 45, 46].

Sympathetic nerve system and essential hypertension

From the 1930s, surgical sympathectomy appeared to be very efficacious in lowering high BP and improving the clinical outcome in patients with severe HTN,[47, 48] but its poorly tolerated side effects and high surgical risk led to his abandon, especially after the appearance of ganglion blockers, the first efficacious antihypertensive drug class.[49]

The critical role of SNS in the pathogenesis of HTN, other cardiovascular diseases and disturbances, is unquestionable, but the existing complex and clinically impractical methodology, to assess the activity of SNS in humans, makes it difficult to adequately establish a relationship between SNS activity and HTN in a particular patient, a major reason why the SNS was so neglected until now.

A well-known consequence of an overactive SNS is an increase in BP. Renal sympathetic nerve activity is pivotal in the pathogenesis of essential HTN, through influences on renin release, sodium and water excretion, peripheral vasoconstriction, car-

diac contraction and venous capacitance.[37] In previous studies, increased sympathetic outflow to kidneys were related to the magnitude of essential HTN.[31, 33, 37, 50] Renal spill-over measurement, of sympathetic outflow to kidneys, in patients with HTN, revealed that more than 50% of them, had significant sympathetic hyperactivity.[51]

Resistant hypertension

Treatment-resistant HTN is defined as a BP persistently above 140/90 mm Hg and 130/80 mm Hg for patients with diabetes and renal disease, despite the prescription of three different antihypertensive drug classes, of complementary mechanism of action, at appropriate doses, preferably including a diuretic.[52] Patients in this category might have pseudo-resistance, that should be identified and differentiated from true resistance.

Pseudo-resistance, comprising around 50% of patients claimed as having resistant HTN, includes those with inadequate treatment regimens, suboptimal adherence to medication and inadequate clinical BP measurements.[53]

Several factors can lead to true resistant HTN, and must be addressed appropriately (Table 3).[54] Inappropriate levels of aldosterone are seen in up to 20% of patients with resistant HTN, whether or not, an adenoma is present. On such patients, aldosterone antagonists may provide relevant additional BP reduction.[54]

Table. 3: Factors leading to resistant hypertension.

- Obesity
- Male gender
- Older age
- African-American
- Insulin resistance
- Volume overload
- Renal dysfunction
- Exogenous drugs

Modified from: Masserli FH et al. Lancet 2007 Aug 18;370(9587):591-603

The prevalence of resistant HTN has been reported to be between 5 and 30%. This prevalence differs accordingly to the hypertensive population being studied and the methods used in BP assessment, with higher percentage of patients with resistant HTN in cohorts from centres specialized in the treatment of HTN as compared to general community based cohorts.[55-57] Recent evaluations on the prevalence of resistant HTN, especially if 24-hours ambulatory blood pressure measurement (ABPM) is used, points to rates less than 10% of all hypertensive patients.[58]

In Portugal, data from the PHYSA study involving 3720 adults patients, the prevalence of HTN was 42%, of which 76.6% were aware of the fact, 74,9% were treated and only 42,5 had their BP values below the recommended thresholds.[59]

Patients with resistant HTN, namely true resistant HTN exhibit a worst prognosis with a higher risk for cardiovascular events, when compared to hypertensive patients without resistant HTN.[60, 61]

In face of the available clinical studies, patients considered to be good candidates for RDN should have severe true resistant HTN, defined as office systolic BP of at least 160mmHg BP of at least 160 systolic (150mmHg in type 2 diabetes). [62-64]

Renal denervation

The finding that sympathetic renal activity was increased in spontaneous hypertensive rats, the animal model most used in essential HTN research, was of paramount for the role of renal SNS on HTN pathogenesis.[65] In an experimental model of HTN and obesity in dogs submitted to a high fat contend diet, RDN, not only prevented the appearance of HTN but also increased in 50% the urinary sodium excretion.[66] In another animal model with chronic renal failure, sympathectomy prevented HTN and was associated to a decrease in the activity of the central adrenergic nucleus.[67]

Renal lesions induced by phenol injection, without changing the GFR, was linked to a sustained rise in HTN and NE release by the hypothalamus. RDN of those animals prevented the rise on BP.[68] In different animal models, the effect of RDN has consistently showed the important role of renal SNS in the pathophysiology of HTN.

In humans, surgical sympathectomy exhibited a high efficacy in lowering high BP and improving the cardiovascular prognosis of patients with severe HTN.[47-49, 69-71] Its poorly tolerated side effects, such as: severe orthostatic hypotension, anhidrosis, bowel disturbances and sexual dysfunction, led to its abandon. Nevertheless, it has reinforced the important link between the SNS and the mechanisms controlling BP.

Clinical studies on catheter based sympathetic renal denervation

Since 2009, several clinical studies were published describing the effect of catheter based RDN for the treatment of resistant HTN (Table 4). The Symplicity HTN-1[72], a proof-of-principle study, was the first to evaluate the effects of RDN on patients with severe resistant HTN. One year after the procedure, the office BP decreased on average 27 mmHg for the systolic and 17 mmHg for the diastolic, and this was sustained until 24 months of follow-up, with 13% of non-responders to RDN therapy[73]. A subgroup analysis targeting the renal and systemic sympathetic activity, revealed an average reduction of 42% in renal NE spillover on these patients.[73]

The Symplicity HTN-2 study,[74] a multicenter clinical trial, randomized 106 patients with severe resistant HTN under medication to optimal antihypertensive medical therapy alone or in association with RDN, with the primary endpoint of changes on office BP at 6-month follow-up. The results revealed a significant decreased on office BP, in patients submitted to RDN, 32 mmHg on systolic BP and 12 mmHg on the diastolic ($p<0.01$), compared to an increase in 1 mmHg on systolic BP and a no change on diastolic BP (p=NS), observed in patients under optimal medical therapy alone. A subgroup analysis regarding ABPM measurements, revealed a similar behavior, a drop of 11mmHg on systolic BP and 7mmHg on diastolic BP, in the RDN group ($p<0.001$) compared to a drop of 3mmHg and 1mmHg respectively, in the medical therapy alone group (p=NS). The magnitude of the decrease in BP, between the RDN treated group and the optimal medical therapy alone group was maintained at 12 months follow-up.[73]

Alongside its efficacy, RDN was a very safe procedure. On both studies, only minor vascular complications occurred, mainly at the puncture site, haematomas, pseudoaneurysms and one renal artery dissection during the diagnostic procedure, successfully treated with a stent, all without sequalae. Regarding renal function, no significant changes on GFR occurred during follow-up. No major complications were identified during the follow-up.[62, 75]

Using a different RDN device, the EnlightHTN I study[64] also revealed a significant decrease in BP measured by office and by ABPM, at 6 months, with a remarkable safety profile.

The available published scientific data supports an excellent safety profile on short term, although the risk of renal artery stenosis on long term is still lacking.

The results from the simplicity HTN 1-2 trials and EnlighHTN I trial were very promising, nevertheless, their open control design made impossible to properly address the important bias made by the placebo effect on BP measurement, in either study groups. Some of those limitations were addressed in the Symplicity HTN3 Trial.[76]

In the Symplicity HTN 3 clinical trial,[77] a larger randomized study, to evaluate RDN for treatment of resistant HTN, the design included for the first time a sham con-

Table 4: Main studies and trials about catheter based renal denervation.

Study	**Symplicity-HTN 1** [72]	**Symplicity-HTN 2** [74]	
Device	RF Single electrode (Symplicity®)	RF Single electrode (Symplicity®)	No renal denervation
Total number of patients in study/patients in RDN arm	47 / 47	52	54
Randomized	No	Yes	
Sham control	No	-	No
Blacks (%)	4*	2*	4*
Mean baseline office Systolic BP (mmHg)	177 ±20	178 ±18	178±16
Mean number BP drugs	4.7±1.5	5.2±1.5	5,3±1,8
Aldosterone blockers (%)	NA	17	17
Office systolic BP change at 6 months (mmHg)	-22	-32±23	1±21
ABMP systolic BP reduction at 6 months (mmHg)	-11**	-11±15	-3±19
Rate of responders, ≥10mmHg change in office systolic BP from baseline	87%	84%	35%

RF: radiofrequency; NA- not available; **** Defined as non-whites; Only 9 RDN responder patients had adequate ABPM at

trol-group. On the primary endpoint, the change on office systolic BP from baseline to 6-month follow-up, between the RDN (n=353) and the sham control arm (n=171), the obtained average difference of -2.39mmHg between the 2 groups on office systolic BP, didn't achieved statistical significance (95% CI: -6.89 to 2.12, p=0.26), with an average reduction of 14.1 mmHg on office systolic BP reduction in the RDN arm vs 11.7 mmHg reduction in the sham control arm.

In the secondary endpoint, the change on the mean systolic BP from baseline to 6-month follow-up, measured by ABPM, between RDN and the sham control arm, a statistically non-significant difference of 1.96 mmHg (95% CI: -1.06 to 4.97, p=0.98) was achieved, with a systolic BP reduction of 6.8 mmHg in the RDN arm vs 4.8 mmHg reduction in the control arm, at 6-month follow-up.

The rate of major adverse events at 6 months, was very low, 1.4% in the RDN arm vs 0.6% in the sham control arm, much less than the 9.8% prespecified as the target for major safety events incidence. So, the primary safety endpoint was met, for a difference of 0.8% (95% CI, -0.9 to 2.5; p=0.67).

Symplicity-HTN 3 [77]		EnligHTN-1 [64]	RAPID [101]	REDUCE HTN FIM[102]
RF Single electrode (Symplicity®)	No renal denervation (sham)	RF multielectrode (EnligHTN®)	RF balloon (OneShot®)	RF balloon (Vessix®)
564	171	46	50	41
Yes		No	No	No
-	Yes	No	No	No
		2,2*	NA	7.3
179±16	180±17	176	181.6 ± 20.8	183±18.1
5.1±1.4	5.2±1.4	4.1±0.6	4.9	5.1±1.7
22.5	28.7	13	22	26.8
-14.1±23.9	-11.7±25.9	-26	-20	-27.6
-6.8±15.1	-4.8±17.2	-10	-11	-8.5
58.3%	48.5%	80%	62	85%

baseline and longer than 30 days, follow-up.

These conflicting results between the Symplicity HTN 3[77] and the previous Symplictity studies, HTN-1[72] and Symplicity HTN-2[74], were intensely analysed and discussed. Many factors were identified has being potentially related to the unexpected results. Among others, differences in the selected population with the inclusion for the first time of a large group of African American patients (24.8% of blacks on RDN arm and 29.2% on control arm), a population well known to be resistant to RAAS system blockers that could have a negative impact on RDN efficacy, indeed a subgroup analysis revealed a statistical significant difference favouring RDN arm, in non-African-American patients.

A likely technical procedural variability, due to the high number of recruiting centres in HTN-3,[77] with a low case load per operator, three procedures on average (most of them did their first and only case of RDN) were also implied. It may help to explain. the puzzling fact in HTN 3[77], of a smaller decrease in office systolic BP from baseline to 6 months in the RDN arm, about half of that observed in the RDN group in Symplicity HTN 2[74], despite similar baseline BP in the two studies. Such

discrepancy fired doubts, if the radiofrequency shots were properly delivered, in the renal arteries.

A more aggressive antihypertensive therapy and the requirement that no changes could be made in the first 6 months after the procedure. Overall, the antihypertensive medication was more intensive than in previous studies, probably reflecting the more severe hypertensive patients included.

The presence for the first time of a sham procedure, in the control arm (a renal angiography was performed in all patients, before randomization) could eventually had increased the placebo effect.

The regression to the mean effect[78], a more aggressive antihypertensive therapy allied to a bigger placebo effect, by the sham procedure may well explain the larger decrease in BP observed in the HTN 3[77] sham control-group, compared with the much smaller decrease in the HTN 2[74] control group, and subsequently the lower than expected difference between the RDN-treated and sham control groups, in HTN 3[77].

Altogether, these facts may have played a major role on HTN 3[77] final results.

The inability to assess if renal SNS was in fact denervated by the procedure, because there is no test available able to do it, is a major limitation to this and to almost all the clinical studies performed until now.

Even though Symplicity HTN 3[77] follow-up will continue as planed out to 5 years, the fact that patients were allowed to cross-over from the sham-control to the RDN arm at 6 month follow-up, will make more difficult to drive significant conclusion on the long-term clinical results of RDN therapy, if not impossible, even to evaluate the long term impact of the sham placebo effect.

The Symplicity HTN 3[77] trial was a landmark study in the development of RDN treatment, driving the development of a new set of industry sponsored proof of concept trials, like the Spyral HTN ON-MED and OFF-MED trials.[79] Such trials were designed to demonstrate the ability of RDN to influence BP in uncontrolled HTN, addressing some of the confounding factors identified from HTN-3[77], such as drug changes, the patient adherence to drug therapy; the heterogeneity in studied population and the procedural variability. The recently published Spyral HTN OFF-MED Trial[80] addressed some of those non-resolved issues from previous trials and proved the biological effect of RDN without the confounding factor of medication.

The Spyral HTN OFF-MED Trial[80] was an international, multicentre, randomized, blinded, sham controlled trial on RDN for the treatment of patients with HTN, in the absence of any antihypertensive medications. It was aimed to confirm the basic hypothesis that RDN therapy lowers BP in patients with HTN, without drug treatment, excluding in such way the most important confounding variable in previous trials - drug therapy.

Prior to randomization, patients had to undergo an antihypertensive medication washout period of three to four weeks. The trial was intended to isolate the effect of RDN on BP reduction as requested by both the US Federal Drug Administration and

the medical community. Key eligibility criteria included patient either on no antihypertensive medications or allowing discontinuation of drug therapy, or office systolic BP ≥ 150 and < 180 mm Hg, or office diastolic BP ≥ 90 mm Hg or ABPM mean systolic BP ≥ 140 and < 170 mm Hg. As on previous trials, patients with an ineligible renal artery anatomy, eGFR < 45 mL/min/1.73m2, type 1 diabetes mellitus or type 2 diabetes mellitus with HbA1C > 8.0%, and secondary causes of HTN were excluded.

The device used was the Spyral Catheter© (Medtronic™, Santa Monica), a flexible multi-electrode catheter with a quadrantic vessel contact for simultaneous ablation in up to 4 electrodes, 90° apart, with a 60-second simultaneous energy delivery, allowing renal branch treatment. The trial randomized 80 patients, 42 in the RDN group and 38 in the sham control group. The primary efficacy endpoint was the BP reduction based on ABPM measurements, from baseline to 3-month follow-up, between RDN arm (n=38) and the sham control arm (n=42). At follow-up, a reduction of 5.5mmHg on systolic BP (95% CI: -9.1 to -2.0, p=0.003) and of 4.8 in diastolic BP (95% CI: -7.0 to -2.6, p=0,<0.0001) occured in the RDN group against a reduction of 0.5mmHg on systolic BP (95% CI: -3.9 to -2.9, p=0.76) and of 0.4 in diastolic BP (95% CI: -2.2 to -1.4, p=0,65), in the sham control group. The blinding index was 0,65 at discharge and 0,59 at 3-months, indicating a proper blinding.

Once again, the RDN safety profile was outstanding, with no major procedural or clinical safety events observed throughout the 3-months follow-up: no deaths, no myocardial infarctions, no stroke, no major bleeding, no serum creatinine elevation greater than 40%, no embolic events, no vascular complications, no renal artery re-interventions or hypertensive crisis.

The Spyral HTN OFF-MED study[80] allowed the biologic proof of principle for the efficacy of RDN, in mild to moderate hypertensive patients, in the absence of anti-hypertensive medications, with a Clinically meaningful reduction of BP at 3 months, compared to the sham control group. As in previous trials, no major safety events occurred, despite a more complete denervation procedure that extended into the branches of renal arteries. Nevertheless, two major limitation need to be pointed out, the Spyral HTN OFF-MED[80] was a proof of concept trial, not powered for statistical significance; in addition, as in previous trials, there were no direct assessment of SNS activity before or after the procedure to verify the extend of nerve trafficking damages.

New devices for renal denervation

A high expectations and enthusiasm was created in the medical community, driving many device companies to develop new and improved technical solutions for RDN (Table 5).

Table 5: Catheter based sympathetic renal denervation devices.

Company	**Medtronic**	**Medtronic**	**St. Jude**
Product	Symplicity Flex™	Symplicity Spyral™	EnligHTN™
Catheter Size	**6 F**	**6F**	**8F**
Energy Type	Radiofrequency	Radiofrequency	Radiofrequency
Catheter design	Single electrode Monopolar	Multielectrode (4) Monopolar	Multielectrode Monopolar
Over Wire	No	yes	no
Energy delivery time	2 min	1 min	1 min
Total treatment time	16-24min	2 min	4 min
Vessel Obstructtion	No	no	no
Trials	Symplicity HTN1[72] Symplicity HTN 2[73] Symplicity TN3[77]	FIM[103]	EnligHTN-1[64]
ABMP systolic BP reduction at 6 months (mmHg)	-11**	-11±15	-3±19
Rate of responders, ≥10mmHg change in office systolic BP from baseline	87%	84%	35%

From predicable improvements of the original procedure to out of the box ideas, many innovations are being integrated in the new designs: a) alternative mechanisms of action, like ultrasound catheters and balloons with microinjection systems to deliver neurotoxins; b) simultaneous activation of multi-electrodes, able to shorten significantly the procedural time and to increases reproducibility, guaranteeing that all quadrants are adequately denervated; c) radial artery access to reduce vascular complications, making the procedure less demanding, as manipulating renal catheters from a cra-neo-caudal approach is generally easier and safer; d) radiofrequency catheters with bipolar electrodes and cooling systems, with their smaller precise electric field during

Maya/Covidien	ReCor Medical	Boston Scientific	Terumo	Cordis
OneShot™	Paradise™	Vessix V2™	Iberis™	Renlane™
9F	**6F**	**8F**	**6F**	**6F**
Radiofrequency	Ultrasound	Radiofrequency	Radiofrequency	Radiofrequency
Multielectrode Monopolar Irrigated	Ultrasound Balloon with cooling	Multielecrode Bipolar non-compliant balloon	Single Electrode Monopolar	Multielectrode Monopolar
yes	yes	yes	No	no
2 min	5 min	30 sec	2 min	30 sec
4 min	Unknown	2 min	16-24 min	Unknown
yes	yes	yes	No	no
RHAS[101]	PARADISE[104] REALISE[105]	Reduce HTN[102]	-	RENABLATE I[106]
-6.8±15.1	-4.8±17.2	-10	-11	-8.5
58.3%	48.5%	80%	62	85%

activation, they produces less heat and tissue burning, creating adequate nerve damage and much less pain and discomfort.

The absence of a method allowing intraprocedural assessment, of the degree of renal SNS damage and subsequent decrease in its activity, is a major limitation, of all devices under development. In such scenario, procedural success is difficult to determine and correlate with BP changes or HTN related target organ damages (TOD) response. Many efforts are being made in the search for a biomarker or physiologic test allowing a precise control over RDN procedure and renal sympathetic trafficking modulation.

Future indications for sympathetic renal denervation

The probable decrease on the overall SNS activity after RDN, may therefore make RDN, a valid alternative in clinical scenarios characterized by an increased SNS activity, other than resistant HTN. Some of these alternative applications, have already been explored with promising results.

The association between heart failure and an increased sympathetic drive is well known. Interestingly, cardiac and renal spillover of NE are more closely related with cardiovascular mortality than circulating catecholamine concentrations, although both are related to worst outcomes.[27, 81] This provides evidence that reducing NE spillover from the kidney could have a beneficial symptomatic and prognostic effects.[82, 83]

In animal models, RDN after myocardial infarction showed an improvement on sodium excretion[84], increased cardiac output, improved renal blood flow[85] and a down-regulation of angiotensin AT1 receptors mediating maladaptive responses.[86] In a multicentre study involving patients with resistant HTN treated by RDN, a subgroup with left ventricle (LV) dysfunction, with their anatomic and functional myocardial parameters assessed by magnetic resonance, had their ejection fraction and circumferential strain significantly increased, after RDN.[87]

In heart failure, the REACH pilot study provided evidence that RDN was able to improve the 6 min walk test results without affecting BP (average 120 mmHg at baseline).[88] Ongoing clinical trials will provide further evidence on the potential of RDN, to influence the course and outcome of heart failure.

Type two diabetes and insulin resistance are other diseases with a strong association with resistant HTN. About 50% of resistant HTN patients are considered to be insulin resistant, an increased risk for type II diabetes and since insulin resistance is dependent on sympathetic activity, it appears likely that it could also be a target for RDN.[89, 90] In a pilot study, along with BP reductions, RDN improved fasting glucose, insulin, and C-peptide concentrations as well as insulin sensitivity indices, in patients with resistant HTN and metabolic disease, suggesting that RDN might improve diabetic status on those patients.[91] Witkowski et al. showed a decline in glycated hemoglobin concentrations after RDN.[92]

Association between obstructive sleep apnea and resistant HTN is well known.[92] In 2011 Witkowski et al, published a pilot study on the effect of RDN in 10 patients with resistant HTN and obstructive sleep apnea. At 6 month after RDN, there was an improvement on apnea-hypopnea indexes.[92] In an experimental model, it has been shown that RDN reduces the post-apneic BP rise, the renal hypoperfusion during apnea and activation of the RAAS system in the kidney.[93, 94] The value of these findings is still controversial and confirmatory studies are needed.

In an animal model[95] for obstructive sleep apnea and induced atrial fibrillation, RDN, decreased the atrium refractory period and the recurrence of atrial fibrillation,[93]

providing a better rate control.[96] In another pilot trial, patients with resistant HTN and symptomatic paroxysmal or persistent atrial fibrillation, refractory to ≥2 antiarrhythmic drugs, were randomized to pulmonary vein isolation only or associated with RDN. At 12-month follow-up, 69% of patients treated with RDN were free of atrial fibrillation in comparison with 29% of patients treated with pulmonary vein isolation only.[97] These experimental findings support the potential usefulness of RDN on atrial fibrillation treatment.

The scientific evidence supporting the pivotal role of SNS activity on the pathophysiology of ventricular arrhythmias is overwhelming. In an animal model of ischemia-reperfusion induced arrhythmias, RDN decreased the occurrence of ventricular arrhythmias and attenuated the rise in LV end diastolic pressure during LV myocardial ischemia, with no influence on infarct size, on ventricular contractility, on BP or on reperfusion arrhythmias.[98] In small case series, of patients with dilated cardiomyopathy and electrical ventricular storm, RDN was able to reduce discharges from the implantable cardioverter defibrillators and ventricular ectopies.[99] Hoffmann et al.[100] reported that RDN can be performed safely and effectively, as an adjunct to cardiac catheter ablation, in a hemodynamically unstable patient with ventricular storm after ST elevation myocardial infarction.

Even though preliminary findings, the biological plausibility underneath them and the promising results, will certainly rise the interest for RDN on those new clinical scenarios.

Conclusion

It seems now evident and well accepted, that an overactive sympathetic nerve system has a pivotal role in the pathophysiology of several diseases, besides essential hypertension. All those clustered conditions like depression, mental stress, hypertension, diabetes, obesity, sleep apnea, metabolic syndrome, ischemic heart disease, heart failure and chronic kidney failure, have all a common “missing link”, the forgotten hyperactive sympathetic system. In this new era, with newer tools to control and treat effectively the sympathetic hyperactivity, it seems that this system will have finally, the long-deserved attention.

The inability to effectively treat hypertension is due in part to a lack of understanding over the fundamental mechanism involved in blood pressure control. A complex mixture of hormonal, neural and intrinsic factors, all acting together, in different time scales and different feedbacks control pathways, and it seems unlikely, that any of the current treatment approaches is targeting altogether, the main factors that lead to hypertension. In patients with hypertension, catheter based renal denervation is a truly innovative approach to treat hypertension. This technique was able to significantly reduce blood

pressure, as well as sympathetic nerve activity and norepinephrine spill over, with high safety standards. Those achievements are well documented on several international multicentre trials.

Alongside with its proven efficacy in blood pressure reduction, its plausible ability to improve insulin resistance, diabetes, left ventricular mass and proteinuria, a cluster of known risk factors acting altogether in the pathophysiology of atherosclerosis, may act as an added value to be consider at any time a strategy is chosen to treat hypertension.

Nevertheless, there are still, important limitations that need to be properly addressed in the future, like the impossibility to determine if the denervation procedure was effective, or which patients have a suitable phenotype to renal denervation, or what are the proper endpoints to define as successful a treatment strategy for hypertension, just blood pressure reduction or it should include other endpoints such as improvement in target organ lesions and other known risk factors.

Certainly, much has to be done and will be done, in the next years, but for sure a new window has been open not only to address hypertension but most of all, to address sympathetic system dysfunction.

Bibliography

1. Forouzanfar MH, Liu P, Roth GA, et al. Global burden of hypertension and systolic blood pressure of at least 110 to 115 mm hg, 1990-2015. *JAMA : the journal of the American Medical Association* 2017; **317**(2): 165-82.
2. Lawes CM, Vander Hoorn S, Rodgers A. Global burden of blood-pressure-related disease, 2001. *Lancet* 2008; **371**(9623): 1513-8.
3. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet* 2002; **360**(9349): 1903-13.
4. Turnbull F. Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. *Lancet* 2003; **362**(9395): 1527-35.
5. Tocci G, Rosei EA, Ambrosioni E, et al. Blood pressure control in Italy: analysis of clinical data from 2005-2011 surveys on hypertension. *Journal of hypertension* 2012; **30**(6): 1065-74.
6. Sharpey-Schäfer EAS. Text-book of physiology; ed. by E. A. Schäfer. Edinburgh & London: Y. J. Pentland; 1898.
7. Abboud FM. The Walter B. Cannon Memorial Award Lecture, 2009. Physiology in perspective: The wisdom of the body. In search of autonomic balance: the good, the bad, and the ugly. *American journal of physiology Regulatory, integrative and comparative physiology* 2010; **298**(6): R1449-67.
8. Guyenet PG. The sympathetic control of blood pressure. *Nat Rev Neurosci* 2006; **7**(5): 335-46.
9. W BW. in The Lower Brainstem and Bodily Homeostasis. *Oxford Univ Press New York* 1997: 165-268.
10. Janig W, Habler HJ. Neurophysiological analysis of target-related sympathetic pathways--from animal to human: similarities and differences. *Acta physiologica Scandinavica* 2003; **177**(3): 255-74.
11. Cao WH, Morrison SF. Differential chemoreceptor reflex responses of adrenal preganglionic neurons. *American journal of physiology Regulatory, integrative and comparative physiology* 2001; **281**(6): R1825-32.
12. DiBona GF. Physiology in perspective: The Wisdom of the Body. Neural control of the kidney. *American journal of physiology Regulatory, integrative and comparative physiology* 2005; **289**(3): R633-41.
13. Hall JE. The Autonomic Nervous System and the Adrenal Medulla in Guyton and Hall Textbook of Medical Physiology: Elsevier Health Sciences; 2015.

14. Schlaich MP, Lambert E, Kaye DM, et al. Sympathetic augmentation in hypertension: role of nerve firing, norepinephrine reuptake, and Angiotensin neuromodulation. *Hypertension* 2004; **43**(2): 169-75.
15. Lohmeier TE. The sympathetic nervous system and long-term blood pressure regulation. *Am J Hypertens* 2001; **14**(6 Pt 2): 147S-54S.
16. Esler M, Kaye D. Sympathetic nervous system activation in essential hypertension, cardiac failure and psychosomatic heart disease. *Journal of cardiovascular pharmacology* 2000; **35**(7 Suppl 4): S1-7.
17. Vallbo AB, Hagbarth KE, Wallin BG. Microneurography: how the technique developed and its role in the investigation of the sympathetic nervous system. *Journal of applied physiology (Bethesda, Md : 1985)* 2004; **96**(4): 1262-9.
18. Dempsey JA, Sheel AW, St Croix CM, Morgan BJ. Respiratory influences on sympathetic vasomotor outflow in humans. *Respiratory physiology & neurobiology* 2002; **130**(1): 3-20.
19. Coote JH. A role for the paraventricular nucleus of the hypothalamus in the autonomic control of heart and kidney. *Experimental physiology* 2005; **90**(2): 169-73.
20. Filippone JD, Bisognano JD. Baroreflex stimulation in the treatment of hypertension. *Current opinion in nephrology and hypertension* 2007; **16**(5): 403-8.
21. Osborn JW, Jacob F, Guzman P. A neural set point for the long-term control of arterial pressure: beyond the arterial baroreceptor reflex. *American journal of physiology Regulatory, integrative and comparative physiology* 2005; **288**(4): R846-55.
22. Guyton AC. Blood pressure control--special role of the kidneys and body fluids. *Science (New York, NY)* 1991; **252**(5014): 1813-6.
23. Von Euler US, Hellner S, Purkhold A. Excretion of noradrenaline in urine in hypertension. *Scandinavian journal of clinical and laboratory investigation* 1954; **6**(1): 54-9.
24. Hagbarth KE, Vallbo AB. Pulse and respiratory grouping of sympathetic impulses in human muscle-nerves. *Acta physiologica Scandinavica* 1968; **74**(1): 96-108.
25. Esler M, Jackman G, Bobik A, et al. Determination of norepinephrine apparent release rate and clearance in humans. *Life sciences* 1979; **25**(17): 1461-70.
26. Blombery PA, Heinzow BG. Cardiac and pulmonary norepinephrine release and removal in the dog. *Circulation research* 1983; **53**(5): 688-94.
27. Hasking GJ, Esler MD, Jennings GL, Burton D, Johns JA, Korner PI. Norepinephrine spillover to plasma in patients with congestive heart failure: evidence of increased overall and cardiorenal sympathetic nervous activity. *Circulation* 1986; **73**(4): 615-21.
28. Graham LN, Smith PA, Huggett RJ, Stoker JB, Mackintosh AF, Mary DA. Sympathetic drive in anterior and inferior uncomplicated acute myocardial infarction. *Circulation* 2004; **109**(19): 2285-9.
29. Kishi T, Hirooka Y. Central Mechanisms of Abnormal Sympathoexcitation in Chronic Heart Failure. *Cardiology Research and Practice* 2012; **2012**: 1-7.

30. Triposkiadis F, Karayannis G, Giamouzis G, Skoularigis J, Louridas G, Butler J. The Sympathetic Nervous System in Heart Failure. Physiology, Pathophysiology, and Clinical Implications. *Journal of the American College of Cardiology* 2009; **54**(19): 1747-82.
31. Esler M. The sympathetic system and hypertension. *Am J Hypertens* 2000; **13**(6 Pt 2): 99S-105S.
32. Esler M, Rumantir M, Kaye D, et al. Sympathetic nerve biology in essential hypertension. *Clinical and experimental pharmacology & physiology* 2001; **28**(12): 986-9.
33. Esler M. The sympathetic system in essential hypertension. *Rev Port Cardiol* 2000; **19 Suppl 2**: II9-14.
34. Neumann J, Ligtenberg G, Klein IH, et al. Sympathetic hyperactivity in hypertensive chronic kidney disease patients is reduced during standard treatment. *Hypertension* 2007; **49**(3): 506-10.
35. Esler M, Rumantir M, Wiesner G, Kaye D, Hastings J, Lambert G. Sympathetic nervous system and insulin resistance: from obesity to diabetes. *Am J Hypertens* 2001; **14**(11 Pt 2): 304S-9S.
36. Narkiewicz K, Somers VK. The sympathetic nervous system and obstructive sleep apnea: implications for hypertension. *Journal of hypertension* 1997; **15**(12 Pt 2): 1613-9.
37. Esler M. The 2009 Carl Ludwig Lecture: Pathophysiology of the human sympathetic nervous system in cardiovascular diseases: the transition from mechanisms to medical management. *Journal of applied physiology* 2010; **108**(2): 227-37.
38. Furlan R, Ardizzone S, Palazzolo L, et al. Sympathetic overactivity in active ulcerative colitis: effects of clonidine. *American journal of physiology Regulatory, integrative and comparative physiology* 2006; **290**(1): R224-32.
39. Kaye DM, Lefkovits J, Jennings GL, Bergin P, Broughton A, Esler MD. Adverse consequences of high sympathetic nervous activity in the failing human heart. *J Am Coll Cardiol* 1995; **26**(5): 1257-63.
40. Zoccali C, Mallamaci F, Parlongo S, et al. Plasma norepinephrine predicts survival and incident cardiovascular events in patients with end-stage renal disease. *Circulation* 2002; **105**(11): 1354-9.
41. Fisher JP, Young CN, Fadel PJ. Central Sympathetic Overactivity: Maladies and Mechanisms. *Autonomic Neuroscience* 2009; **15**(148 (1-2)): 5-15.
42. Lown B, Verrier RL. Neural activity and ventricular fibrillation. *N Engl J Med* 1976; **294**(21): 1165-70.
43. Bruno RM, Ghiadoni L, Seravalle G, Dell'Oro R, Taddei S, Grassi G. Sympathetic Regulation of Vascular Function in Health and Disease. *Frontiers in Physiology* 2012; **3**: 1-15.
44. Hirooka Y, Kishi T, Sakai K, Takeshita A, Sunagawa K. Imbalance of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neu-

ral mechanisms of hypertension. *American journal of physiology Regulatory, integrative and comparative physiology* 2011; **300**(4): R818-26.

45. Landsberg L. Insulin-mediated sympathetic stimulation: role in the pathogenesis of obesity-related hypertension (or, how insulin affects blood pressure, and why). *Journal of hypertension* 2001; **19**(3 Pt 2): 523-8.
46. Grassi G, Seravalle G, Quarti-Trevano F, et al. Excessive sympathetic activation in heart failure with obesity and metabolic syndrome: characteristics and mechanisms. *Hypertension* 2007; **49**(3): 535-41.
47. Hoobler SW, Manning JT, Paine WG, et al. The effects of splanchnicectomy on the blood pressure in hypertension; a controlled study. *Circulation* 1951; **4**(2): 173-83.
48. Smithwick RH, Whitelaw GP, Kinsey D. Surgical approach to the treatment of essential hypertension. Results of therapy (medical and surgical). *The American journal of cardiology* 1962; **9**: 893-9.
49. Harington M, Kincaid-Smith P, Mc MJ. Results of treatment in malignant hypertension: a seven-year experience in 94 cases. *Br Med J* 1959; **2**(5158): 969-80.
50. Esler M. The sympathetic nervous system through the ages: from Thomas Willis to resistant hypertension. *Experimental physiology* 2011; **96**(7): 611-22.
51. Esler M, Lambert E, Schlaich M. Point: Chronic activation of the sympathetic nervous system is the dominant contributor to systemic hypertension. *Journal of applied physiology* 2010; **109**(6): 1996-8; discussion 2016.
52. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Journal of hypertension* 2013; **31**(7): 1281-357.
53. Grigoryan L, Pavlik VN, Hyman DJ. Characteristics, drug combinations and dosages of primary care patients with uncontrolled ambulatory blood pressure and high medication adherence. *Journal of the American Society of Hypertension : JASH* 2013; **7**(6): 471-6.
54. Messerli FH, Williams B, Ritz E. Essential hypertension. *Lancet* 2007; **370**(9587): 591-603.
55. de la Sierra A, Segura J, Banegas JR, et al. Clinical features of 8295 patients with resistant hypertension classified on the basis of ambulatory blood pressure monitoring. *Hypertension* 2011; **57**(5): 898-902.
56. Egan BM, Zhao Y, Axon RN, Brzezinski WA, Ferdinand KC. Uncontrolled and apparent treatment resistant hypertension in the United States, 1988 to 2008. *Circulation* 2011; **124**(9): 1046-58.
57. Prugger C, Keil U, Wellmann J, et al. Blood pressure control and knowledge of target blood pressure in coronary patients across Europe: results from the EUROASPIRE III survey. *Journal of hypertension* 2011; **29**(8): 1641-8.

58. Brambilla G, Bombelli M, Seravalle G, et al. Prevalence and clinical characteristics of patients with true resistant hypertension in central and Eastern Europe: data from the BP-CARE study. *Journal of hypertension* 2013; **31**(10): 2018-24.

59. Polonia J, Martins L, Pinto F, Nazare J. Prevalence, awareness, treatment and control of hypertension and salt intake in Portugal: changes over a decade. The PHYSA study. *Journal of hypertension* 2014; **32**(6): 1211-21.

60. Daugherty SL, Powers JD, Magid DJ, et al. Incidence and prognosis of resistant hypertension in hypertensive patients. *Circulation* 2012; **125**(13): 1635-42.

61. Kleman M, Dhanyamraju S, DiFilippo W. Prevalence and characteristics of pseudohypertension in patients with "resistant hypertension". *Journal of the American Society of Hypertension : JASH* 2013; **7**(6): 467-70.

62. Symplicity HTNI. Catheter-based renal sympathetic denervation for resistant hypertension: durability of blood pressure reduction out to 24 months. *Hypertension* 2011; **57**(5): 911-7.

63. Murray ED, Krum H, Schlaich M, Schmieder RE, Böhm M, Sobotka PA. Renal Sympathetic Denervation for Treatment of Drug-Resistant Hypertension : One-Year Results From the Symplicity HTN-2 Randomized, Controlled Trial. *Circulation* 2012; **126**: 2976-82.

64. Worthley SG, Tsioufis CP, Worthley MI, et al. Safety and efficacy of a multi-electrode renal sympathetic denervation system in resistant hypertension: the EnligHTN I trial. *Eur Heart J* 2013; **34**(28): 2132-40.

65. Thoren P. Efferent renal nerve traffic in the spontaneously hypertensive rat. *Clinical and experimental hypertension Part A, Theory and practice* 1987; **9 Suppl 1**: 259-79.

66. Kassab S, Kato T, Wilkins FC, Chen R, Hall JE, Granger JP. Renal denervation attenuates the sodium retention and hypertension associated with obesity. *Hypertension* 1995; **25**(4 Pt 2): 893-7.

67. Campese VM, Kogosov E. Renal afferent denervation prevents hypertension in rats with chronic renal failure. *Hypertension* 1995; **25**(4 Pt 2): 878-82.

68. Ye S, Zhong H, Yanamadala V, Campese VM. Renal injury caused by intrarenal injection of phenol increases afferent and efferent renal sympathetic nerve activity. *Am J Hypertens* 2002; **15**(8): 717-24.

69. Grimson KS. The surgical treatment of hypertension. *Advances in internal medicine* 1947; **2**: 173-94.

70. Hafkenschiel JH, Fitts WT, Jr. The surgical treatment of hypertension with particular reference to adrenalectomy and sympathectomy. *Transactions American College of Cardiology* 1955; **5**: 107-12.

71. Hutchison GB, Evans JA. Should sympathectomy for arterial hypertension be abandoned? Review of surgical therapy at the Lahey Clinic: 1950-1954. *The Surgical clinics of North America* 1957; **37**(3): 871-89.

72. Krum H, Schlaich M, Whitbourn R, et al. Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet* 2009; **373**(9671): 1275-81.

73. Esler MD, Krum H, Schlaich M, Schmieder RE, Bohm M, Sobotka PA. Renal sympathetic denervation for treatment of drug-resistant hypertension: one-year results from the Symplicity HTN-2 randomized, controlled trial. *Circulation* 2012; **126**(25): 2976-82.

74. Esler MD, Krum H, Sobotka PA, Schlaich MP, Schmieder RE, Bohm M. Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet* 2010; **376**(9756): 1903-9.

75. Mahfoud F, Cremers B, Janker J, et al. Renal hemodynamics and renal function after catheter-based renal sympathetic denervation in patients with resistant hypertension. *Hypertension* 2012; **60**(2): 419-24.

76. Kandzari DE, Bhatt DL, Sobotka PA, et al. Catheter-based renal denervation for resistant hypertension: rationale and design of the SYMPLICITY HTN-3 Trial. *Clinical cardiology* 2012; **35**(9): 528-35.

77. Bhatt DL, Kandzari DE, O'Neill WW, et al. A controlled trial of renal denervation for resistant hypertension. *N Engl J Med* 2014; **370**(15): 1393-401.

78. Barnett AG, van der Pols JC, Dobson AJ. Regression to the mean: what it is and how to deal with it. *International journal of epidemiology* 2005; **34**(1): 215-20.

79. Kandzari DE, Kario K, Mahfoud F, et al. The SPYRAL HTN Global Clinical Trial Program: Rationale and design for studies of renal denervation in the absence (SPYRAL HTN OFF-MED) and presence (SPYRAL HTN ON-MED) of antihypertensive medications. *American heart journal* 2016; **171**(1): 82-91.

80. Townsend RR, Mahfoud F, Kandzari DE, et al. Catheter-based renal denervation in patients with uncontrolled hypertension in the absence of antihypertensive medications (SPYRAL HTN-OFF MED): a randomised, sham-controlled, proof-of-concept trial. *Lancet* 2017.

81. Petersson M, Friberg P, Eisenhofer G, Lambert G, Rundqvist B. Long-term outcome in relation to renal sympathetic activity in patients with chronic heart failure. *Eur Heart J* 2005; **26**(9): 906-13.

82. Bohm M, Linz D, Urban D, Mahfoud F, Ukena C. Renal sympathetic denervation: applications in hypertension and beyond. *Nature reviews Cardiology* 2013; **10**(8): 465-76.

83. Bohm M, Ewen S, Kindermann I, Linz D, Ukena C, Mahfoud F. Renal denervation and heart failure. *European journal of heart failure* 2014.

84. Villarreal D, Freeman RH, Johnson RA, Simmons JC. Effects of renal denervation on postprandial sodium excretion in experimental heart failure. *The American journal of physiology* 1994; **266**(5 Pt 2): R1599-604.

85. Masaki H, Imaizumi T, Harasawa Y, Takeshita A. Dynamic arterial baroreflex in rabbits with heart failure induced by rapid pacing. *The American journal of physiology* 1994; **267**(1 Pt 2): H92-9.

86. Clayton SC, Haack KK, Zucker IH. Renal denervation modulates angiotensin receptor expression in the renal cortex of rabbits with chronic heart failure. *American journal of physiology Renal physiology* 2011; **300**(1): F31-9.

87. Mahfoud F, Urban D, Teller D, et al. Effect of renal denervation on left ventricular mass and function in patients with resistant hypertension: data from a multi-centre cardiovascular magnetic resonance imaging trial. *Eur Heart J* 2014; **35**(33): 2224-31b.

88. Davies JE, Manisty CH, Petraco R, et al. First-in-man safety evaluation of renal denervation for chronic systolic heart failure: primary outcome from REACH-Pilot study. *International journal of cardiology* 2013; **162**(3): 189-92.

89. Mancia G, Bousquet P, Elghozi JL, et al. The sympathetic nervous system and the metabolic syndrome. *Journal of hypertension* 2007; **25**(5): 909-20.

90. Huggett RJ, Scott EM, Gilbey SG, Stoker JB, Mackintosh AF, Mary DA. Impact of type 2 diabetes mellitus on sympathetic neural mechanisms in hypertension. *Circulation* 2003; **108**(25): 3097-101.

91. Mahfoud F, Schlaich M, Kindermann I, et al. Effect of renal sympathetic denervation on glucose metabolism in patients with resistant hypertension: a pilot study. *Circulation* 2011; **123**(18): 1940-6.

92. Witkowski A, Prejbisz A, Florczak E, et al. Effects of renal sympathetic denervation on blood pressure, sleep apnea course, and glycemic control in patients with resistant hypertension and sleep apnea. *Hypertension* 2011; **58**(4): 559-65.

93. Linz D, Mahfoud F, Schotten U, et al. Renal sympathetic denervation suppresses postapneic blood pressure rises and atrial fibrillation in a model for sleep apnea. *Hypertension* 2012; **60**(1): 172-8.

94. Linz D, Hohl M, Nickel A, et al. Effect of renal denervation on neurohumoral activation triggering atrial fibrillation in obstructive sleep apnea. *Hypertension* 2013; **62**(4): 767-74.

95. Linz D, Schotten U, Neuberger HR, Bohm M, Wirth K. Negative tracheal pressure during obstructive respiratory events promotes atrial fibrillation by vagal activation. *Heart rhythm : the official journal of the Heart Rhythm Society* 2011; **8**(9): 1436-43.

96. Linz D, Mahfoud F, Schotten U, et al. Renal sympathetic denervation provides ventricular rate control but does not prevent atrial electrical remodeling during atrial fibrillation. *Hypertension* 2013; **61**(1): 225-31.

97. Pokushalov E, Romanov A, Corbucci G, et al. A randomized comparison of pulmonary vein isolation with versus without concomitant renal artery denervation in patients with refractory symptomatic atrial fibrillation and resistant hypertension. *J Am Coll Cardiol* 2012; **60**(13): 1163-70.

98. Linz D, Wirth K, Ukena C, et al. Renal denervation suppresses ventricular arrhythmias during acute ventricular ischemia in pigs. *Heart rhythm : the official journal of the Heart Rhythm Society* 2013; **10**(10): 1525-30.

99. Ukena C, Bauer A, Mahfoud F, et al. Renal sympathetic denervation for treatment of electrical storm: first-in-man experience. *Clinical research in cardiology : official journal of the German Cardiac Society* 2012; **101**(1): 63-7.

100. Hoffmann BA, Steven D, Willems S, Sydow K. Renal Sympathetic Denervation as an Adjunct to Catheter Ablation for the Treatment of Ventricular Electrical Storm in the Setting of Acute Myocardial Infarction. *Journal of cardiovascular electrophysiology* 2013.

101. Stefan Verheye. Rapid Renal Sympathetic Denervation for Resistant Hypertension Using the OneShot™ Ablation System (RAPID) Study: Primary Endpoint 6-Month Results. *TCT* 2013; (http://www.tctmd.com/txshow.aspx?tid=1374091&id=121514&trid=1373709).

102. Schofer J. Clinical evidence in catheter-based renal sympathetic denervation - building the data. *EuroPCR* 2013; (http://www.pcronline.com/file/download/(path)/var/www/media/pcronline/diapos/EuroPCR2013/(id)/2276-20130522_1708_Room242AB_Schofer_Joachim/(ext)/pdf/(ref)/131358).

103. R W. Early Results of First-in-Man Simultaneous Multi-Electrode Renal Denervation Treatment for Resistant Hypertension. *http://wwwpcronlinecom/Lectures/2013/Early-results-of-first-in-man-simultaneous-multisite-renal-denervation-treatment-for-resistant-hypertension* 2013.

104. Bonan R. ReCor Medical Paradise: FIM Results of a Novel Circumferencial Catheter Based Ultrasound Technology for Renal Denervation. *http://wwwpcronlinecom/Lectures/2012/ReCor-Medical-Paradise-first-in-man-results-of-a-novel-circumferential-catheter-based-ultrasound-technology-for-renal-denervation* 2012.

105. A P. Realise Clinical Trial: Preliminary results demonstrate catheter based ultrasound renal denervation is associated with a decrease in sympathetic activity *http://wwwpcronlinecom/Lectures/2014/Preliminary-results-from-the-REALISE-trial-show-endovascular-ultrasound-renal-denervation-ultrasound-associated-with-decrease-in-sympathetic-overactivity* 2014.

106. Wijns W. RENABLATE I Feasability Study: 3 months results. *http://wwwpcronlinecom/Lectures/2014/Renablate-study-presentation* 2014.

CHAPTER 1 BACKGROUND

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Sections:

Summary

In the last fifty years, albeit all efforts, anti-hypertensive drugs and the large scientific evidence supporting their use, the number of hypertensive patients with controlled blood pressure remains very low. True drug resistance and low treatment adherence from patients are major causes for such failure. In addition, some of the underlying mechanisms that play a major role in the pathophysiology of hypertension were not properly addressed until recently.

The recent development of newer catheter-based devices to tackle sympathetic hyperactivity, through the denervation of renal sympathetic nerves, opened newer perspectives to be explored in current clinical practice.

Current drug treatment-based strategies, with their different drugs, targets and mechanism of action, interacting with each other, makes the final biological results, more difficult to track and to account as a direct effect of a specific mechanisms of action. Contrary, device-based strategies, such as renal denervation, acts solely by interfering with the renal input and output of sympathetic nerve traffic, making possible to better identify human biological responses as a solely consequence of such interference.

Until now, the prevailing theory is that a chronic rise in blood pressure has a persistent direct negative impact on specific organs, namely the heart, the arterial walls and the kidneys, driving the mechanisms for damaging the left ventricle (hypertrophy, diastolic dysfunction) and the kidneys (proteinuria and kidney failure). In such setting, drug trials commonly used as surrogates off drug-treatment efficacy, changes in office blood pressure or in 24-hour ambulatory measurement. Very rarely, they add other endpoints such as the impact on target organs damages of hypertension. Contrary, the aim of this project is to evaluate in patients with severe drug resistant hypertension, refractory to drug treatment, the impact of renal denervation beyond changes on blood pressures, looking for changes on hypertension related target organ damages, such as on left ventricle function and morphology and on renal function, both as surrogate markers of treatment efficacy, a midpoint for the reduction of major clinical events.

The purpose of this chapter is to list and discuss possible unmet medical needs, in todays, hypertension treatment strategies, that may justify the need to newer treatment approaches, such as renal denervation, since this treatment strategy might become a better alternative either alone or combined to drug treatment, for patients with hypertension.

Limitations of previous therapeutic strategies on blood pressure control

HTN has a well-established and strong association with myocardial infarction, heart failure, stroke and end-stage renal disease, making it the leading global risk factor for cardiovascular mortality.[1, 2] Effective BP lowering consistently reduces overall cardiovascular risk[3]. Still the rates of adequate BP control remains suboptimal, despite the large amount of available antihypertensive drugs and the strong scientific evidence supporting their use[4].

As previously described, resistant HTN is defined as a BP persistently above 140/90 mmHg or 130/80mmHg for patients with diabetes or chronic renal disease, under treatment with at least three antihypertensive drugs, of different classes of complementary mechanism of action (one of which must be a diuretic), on adequate doses.[5] With a prevalence ranging from 5% to 30% of treated hypertensive patients it is an important cause for lack of success in BP control [5]. These patients exhibit a worst prognosis, with higher risk for cardiovascular events, when compared to hypertensive patients without resistant HTN.[6]

The blame for such low rates on BP control is multifactorial. Poor adherence to treatment, due to drug intolerance, social and cultural reasons are important, but inadequate treatment management or powerless therapy strategies are also to be blamed.[7]

From the two known main systems that control human BP, almost all efforts are targeted to the water and salt homeostasis control mechanisms, through agents blocking the RAAS axis or through diuresis, or occasionally, as second line, using agents that promote vascular dilatation. The role of the sympathetic system, though well known for a long time, was forgotten. The reason for such attitude resides in one hand, in the inability to measure and grade SNS activity and on the other hand, in the lack of tools to treat or modulate it, until now.

Limitations of previous studies on renal denervation

Compelling evidence from mid nineteen century[8] and more recent clinical and animal models[8-12] consistently proved that RDN is efficacious as a treatment approach for resistant HTN, sustaining the important role of SNS system in the pathophysiology of HTN. [13,14,15]

The recent development of a safe catheter-based technique to decrease the renal sympathetic nerve traffic by radiofrequency ablation, opened a new range of possibilities including a second line of treatment for HTN, especially in patients with severe treatment-resistant HTN.[9, 11, 16] Alongside with its efficacy, RDN is a safe procedure with only minor vascular complications, mainly at the puncture site. There were no significant

changes on GFR during follow-up[9, 11, 16], although the risk of renal artery stenosis on long term is still lacking.

Still, there are many limitations to establish RDN as a treatment of choice for HTN:

a) ***Identification of Resistant Hypertension.*** There is no reliable way to identify and select true resistant HTN patients for RDN treatment, mostly because office systolic HTN is a too simplistic criterion to properly identify true resistant HTN patients. In-addition, the evaluation of drug-treatment efficacy is frequently misleading, either due to patient non-adherence to treatment or to inadequate or suboptimal treatment strategies. [5, 17]

b) ***Blood pressure measurement.*** The recent routine use of ABPM for BP evaluation and longer run-in treatment phases before patients' selection, may partially overcome some of such limitations. ABPM is more robust than office BP measurement. It allows its recording in the entire day, with subjects engaged in their daily lives, including sleep and morning rise, avoiding the bias made by transient rise in BP measurements in response to medical environment and masked HTN, making ABPM values more reproducible, closer to the real BP load prevailing in patient's daily life.[17, 18] It is especially important in the evaluation of resistant HTN, allowing the precise definition of its diagnosis and excluding a significant number of patients initially wrongly labelled as resistant hypertensive patients.[19-21]

   ABPM has been proved to be better related to prognosis and to HTN related TOD.[22, 23] In addition, there is significant scientific evidence, that changes in ABPM values after treatment are superior to those obtained in office, to predict the effect of treatment on clinically relevant HTN related TOD and on its progression, such as LV hypertrophy or proteinuria.[23-25] There is a consensus on the superiority of ABPM to other measurements modalities, to determine eligibility criteria and assessment of treatment effects.[17]

   An important aspect of ABPM in the evaluation of a HTN treatment-effects, is that BP lowering either with drugs or devices are much less pronounced when evaluated by ABPM than by office measurements.[26] In the HTN 1 and 2 trials, the magnitude of the BP decrease after RDN, was 30 to 40% less in ABPM than in office measurements.[9-11] A possible explanation for such discrepancy may be that ABPM includes night-time values, usually lower than day-time.

c) ***Patient selection.*** Another limitation of previous studies on RDN, regards the fact that they were aimed to patients with, the so called, treatment resistant HTN, defined as a BP persistently above 140/90 mmHg, under treatment with at least three antihypertensive drugs, of different classes, on adequate doses (one of which is a diuretic). The investigators were not obliged

to frame the drug treatment strategies during the studies, in order to reduce its interference in the final BP results, making more difficult to isolate the real RDN treatment-effect from the broad variation induced by drug-treatment changes.[9-11] A possible solution, besides framing by protocol the drug-treatment choices and changes, is to use RDN to treat patients with severe resistant HTN, under maximal tolerated drug treatment. It has never been evaluated before, to the best of our knowledge.

Such task imposes the challenge of selecting patients with true resistant drug-treatment HTN to maximal tolerated drug-treatment regimens, meaning the failure of current best practice drug-treatment strategies. The advantage of such strategy is that all patients are on maximal tolerated drug therapy. Assuming there are no remaining therapeutic options, there will be naturally less variation regarding changes on drugs ant treatments strategies, between patients. Such strategy will better help to isolate the RDN effect on BP control, from the broad treatment variation effects that occurred in previous studies, overcoming some of the limitations pointed-out in previous studies.[9-11]

d) ***Operator experience.*** On previous RDN studies, there was a large difference in experience and proficiency between RDN operators, introducing variability in the procedure and potentially affecting the final results.[9-11] The use of a restricted number of a high proficiency operators could overcome such limitation.

e) ***Endpoint selection.*** Previous RDN studies used as clinical endpoints, essentially changes in BP measurements, before and after treatment, usually office BP measurements and more recently, for the reasons previously discussed, ABPM measurements. The effects on HTN related TOD, like changes on LV structure and function or changes in renal function like proteinuria, were not included previously as surrogates of RDN treatment efficacy. We may-say so, that they had never been tested before as surrogate markers of RDN treatment impact *per se*.

Target organ damage – Beyond blood pressure control

HTN changes in left ventricular function and structure. Long-standing HTN is known to be linked to myocardial hypertrophy, diastolic dysfunction, left atrial enlargement, atrial or LV arrhythmias, heart failure and ultimately myocardial infarction and stroke, but sympathetic drive is also implicated in cardiac and vascular remodeling.[27-30]

Of utmost importance, is the fact that LV hypertrophy is one of the most significant markers of HTN related TOD and it has been associated with an increase

rate of cardiovascular events, including death, independently of BP values.[31-33] So, any treatment able to positively change cardiac remodeling, with or without changes in BP values, may potentially have a favorable impact on the cardiovascular prognosis of patients with long-standing HTN.

Classically, on most studies involving HTN patients, LV hypertrophy was identified by EKG. However, its sensitivity and accuracy to detect LV hypertrophy is at most moderate. Echocardiography is superior to EKG for many reasons: it can quantify LV hypertrophy indexed to body surface area and it can give specific and accurate information on LV function systolic and diastolic.[34]

HTN related proteinuria. Renal dysfunction, namely microalbuminuria is an early marker of renal damages in hypertensive patients.[35] In several studies, microalbuminuria was observed in 17% to 46% of patients with HTN, being higher in resistant HTN.[35-38] In addition, microalbuminuria seems to be a potent "nephrotoxin" and a powerful predictor of cardiovascular morbidity and mortality in patients with resistant HTN, independent of traditional cardiovascular risk factors.[37, 39, 40] Its reduction with HTN treatment is associated with favourable impact in cardiovascular events, reinforcing the need for an early and aggressive control of BP in patients with resistant HTN.[37, 39]

Sympathetic system effects on HTN related TOD. Another powerful argument to include HTN related TOD as markers of RDN treatment success, relies in the recently published results from small proof-of-concept studies, on RDN, showing other positive clinical results besides HTN, such as an increase on sodium renal excretion, independent of renal function and antihypertensive medications[41]; an improvement on LV mass and diastolic function[42, 43]; an enhancement on glucose metabolism in patients with resistant HTN and metabolic disease, alongside with BP reduction[44], all implying that an overactive SNS may have a more important role in the pathophysiology of many disease states, than previously expected.

Indeed, a chronic SNS activation can cause hypertrophy and proliferation of vascular smooth muscles cells as well as a direct trophic effect on cardiac myocytes, increasing LV mass and wall thickness, even without an increase in BP.[45] This structural changes on the myocardium and the direct effect of an overactive SNS have a direct contribution to the high incidence of arrhythmias, commonly seen in these patients.[46] A chronically overactive SNS is linked to a worst prognosis in patients with heart failure and end-stage renal disease. [47, 48]

Redefinition of therapeutic success of hypertension

In HTN patients, the primary goal of treatment is to prevent major clinical complications, cerebral, cardiovascular and renal, usually associated to long term uncontrol-

led HTN. To evaluate the long-term impact of RDN on each one of these major clinical adverse events, trials with large number of patients and very long follow-ups are required. The shear cost of such studies, make them unreasonable to use in a catheter-based treatment for HTN.

An alternative could be the use of surrogate endpoints, moving for a 2nd step towards a more robust clinical evaluation of HTN treatment efficacy (Fig. 1).

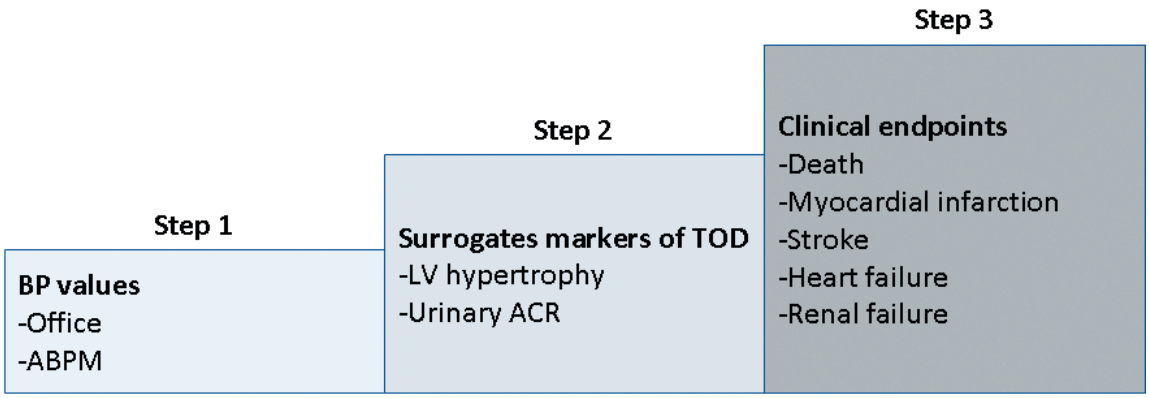


Figure 1: Different approaches to assess HTN treatment efficacy.

Chronic high BP causes morbidity and mortality. A treatment that decreases BP as bean related to a reduction in morbidity and mortality. In such a context, BP changes after treatment, was established as the most frequently used surrogate endpoint for HTN treatment success, either measured on medical office, at home or using ABPM.

Other surrogates for subclinical disease, like HTN related TOD are also known to be powerful risk factors for major clinical events, and can act as valid proxies to major disease, but have been scarcely used.[49]

Because the beneficial changes in HTN related TOD are thought to have a significant positive effect on morbidity and mortality, in the absence of such hard clinical events, the use of intermediate endpoints, as changes in LV function and structure and in renal function, like the sensitive microalbuminuria, can provide valuable insights beyond BP changes, on the potential impact of RDN in the long-term.[50, 51]

The combined use of a physiological measure, like BP, and surrogates of subclinical disease, like heart and kidney changes, to evaluate and grade the success of RDN treatment for severe resistant HTN, has never been tested before, to the best of our knowledge.

A possible advantage of such approach relies in the fact that BP as a solely endpoint to evaluate the HTN treatment success, doesn't fully capture the global effect of treatment on other important clinical adverse events. For example, antihyper-

tensive drugs have multiple effects besides lowering BP, some of which may not be reflected on BP changes. In fact, there seems to be no direct relationship between changes in BP and in concomitant changes in HTN related TOD. We can raise the hypothesis, that after RDN treatment, there will be patients with positive changes in BP values and in subclinical TOD, but also, there will be patients with changes in BP and HTN related subclinical TOD, in opposite directions. How frequent they are and what implications they have for the overall prognostic scenario of HTN, are presently still unknown.

Another difference to previous studies is the use of more robust surrogates of RDN efficacy. As previously mentioned, ABPM measurement is more robust than office evaluation to assess BP control and also proved to be better related to prognosis and to HTN related TOD.[22]

The inclusion of HTN related TOD evaluation after RDN, as a surrogate marker of RDN efficacy, is an additional difference. Although mentioned in previous studies, they were not assumed as efficacy endpoints of RDN treatment rather as "secondary effects". On contrary, in the present study and due to their important role on HTN prognostic impact and different response to different treatment strategies, changes on LV function, structure and proteinuria, should be by their own rights, surrogates of RDN efficacy in the treatment of patients with resistant HTN.[34, 50-52]

We foresee in the future, antihypertensive treatments strategies focused in the prevention of HTN related TOD, beyond BP control, introducing cardioprotective therapies, like LV hypertrophy or proteinuria improvement, independently of BP reduction.

In summary, the following arguments will support the search for subclinical organ damage in patients with chronic severe HTN, resistant to maximal tolerated antihypertensive therapy, submitted to RDN. They will be assumed as surrogate markers of RDN impact in such patients.

- HTN related TOD have been shown to have an independent prognostic impact, irrespectively of whether it involves the structure or heart function, the brain, the kidney or the vessels.[50, 51, 53]
- Resistant or refractory HTN is strongly associated with specific TOD. Similarly, TOD, particularly renal and cardiovascular may worsen the resistance to treatment of HTN.[35, 38]
- Growing scientific evidences supports the concept that a chronic increased in SNS activity, is involved in the pathogenesis of HTN,[54, 55] kidney disease,[56] type II diabetes,[57] obesity including metabolic syndrome.[57] Such effects are mediated by the chronic increase in BP, but also by direct negative effects

of SNS, in the cardiovascular system and metabolic disarrays, which are independent of BP changes.[58]

- Recent studies and registries revealed that RDN, beside lowering BP, had positive effects in HTN related TOD. In patients with severe HTN, it was associated to improvements in LV hypertrophy, diastolic function[34, 42, 59] and microalbuminuria.[52, 58] These changes were independent from BP lowering effects.[34, 42, 52, 59]
- Some studies had analysed the impact of RDN on HTN related TOD, not as endpoints of RDN efficacy, rather as "secondary effects", but few if none, had assessed simultaneously the effect of RDN on BP and multiple HTN related TOD, in the same set of patients.

Conclusion

Based on the arguments previously discussed and to clarify the therapeutic role of sympathetic nerve system modulation by renal denervation, through the application of radiofrequency energy inside the renal arteries, we assumed to be of scientific relevance, to evaluate in patients with severe resistant hypertension, under maximal tolerated antihypertensive medication, the impact of renal denervation on a composite of endpoints including: changes on blood pressure assessed by office and 24 hours ambulatory blood pressure measurement and also on changes in target organ damaged, assessed through the surrogate markers: changes in left ventricular structure and function, renal function and proteinuria.

Bibliography

1. Lim SS, Vos T, Flaxman AD, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; **380**(9859): 2224-60.
2. Lawes CM, Vander Hoorn S, Rodgers A. Global burden of blood-pressure-related disease, 2001. *Lancet* 2008; **371**(9623): 1513-8.
3. Turnbull F. Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. *Lancet* 2003; **362**(9395): 1527-35.
4. Laffin LJ, Bakris GL. Hypertension and new treatment approaches targeting the sympathetic nervous system. *Current opinion in pharmacology* 2014; **21C**: 20-4.
5. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Journal of hypertension* 2013; **31**(7): 1281-357.
6. Daugherty SL, Powers JD, Magid DJ, et al. Incidence and prognosis of resistant hypertension in hypertensive patients. *Circulation* 2012; **125**(13): 1635-42.
7. Brown MT, Bussell JK. Medication Adherence: WHO Cares? *Mayo Clinic Proceedings* 2011; **86**(4): 304-14.
8. de Sousa Almeida M, de Araújo Gonçalves P, Infante de Oliveira E, Cyrne de Carvalho H. Renal denervation for resistant hypertension. *Revista Portuguesa de Cardiologia* 2015; **34**(02): 125-35.
9. Bhatt DL, Kandzari DE, O'Neill WW, et al. A controlled trial of renal denervation for resistant hypertension. *N Engl J Med* 2014; **370**(15): 1393-401.
10. Krum H, Schlaich M, Whitbourn R, et al. Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet* 2009; **373**(9671): 1275-81.
11. Esler MD, Krum H, Schlaich M, Schmieder RE, Bohm M, Sobotka PA. Renal sympathetic denervation for treatment of drug-resistant hypertension: one-year results from the Symplicity HTN-2 randomized, controlled trial. *Circulation* 2012; **126**(25): 2976-82.
12. Townsend RR, Mahfoud F, Kandzari DE, et al. Catheter-based renal denervation in patients with uncontrolled hypertension in the absence of antihypertensive medications (SPYRAL HTN-OFF MED): a randomised, sham-controlled, proof-of-concept trial. *Lancet* 2017.

13. Kassab S, Kato T, Wilkins FC, Chen R, Hall JE, Granger JP. Renal denervation attenuates the sodium retention and hypertension associated with obesity. *Hypertension* 1995; **25**(4 Pt 2): 893-7.
14. Campese VM, Kogosov E. Renal afferent denervation prevents hypertension in rats with chronic renal failure. *Hypertension* 1995; **25**(4 Pt 2): 878-82.
15. Ye S, Zhong H, Yanamadala V, Campese VM. Renal injury caused by intrarenal injection of phenol increases afferent and efferent renal sympathetic nerve activity. *Am J Hypertens* 2002; **15**(8): 717-24.
16. Symplicity HTNI. Catheter-based renal sympathetic denervation for resistant hypertension: durability of blood pressure reduction out to 24 months. *Hypertension* 2011; **57**(5): 911-7.
17. White WB, Turner JR, Sica DA, et al. Detection, evaluation, and treatment of severe and resistant hypertension: proceedings from an American Society of Hypertension Interactive forum held in Bethesda, MD, U.S.A., October 10th 2013. *Journal of the American Society of Hypertension : JASH* 2014; **8**(10): 743-57.
18. Kikuya M, Hansen TW, Thijs L, et al. Diagnostic Thresholds for Ambulatory Blood Pressure Monitoring Based on 10-Year Cardiovascular Risk. *Circulation* 2007; **115**(16): 2145-52.
19. de la Sierra A, Segura J, Banegas JR, et al. Clinical features of 8295 patients with resistant hypertension classified on the basis of ambulatory blood pressure monitoring. *Hypertension* 2011; **57**(5): 898-902.
20. Brambilla G, Bombelli M, Seravalle G, et al. Prevalence and clinical characteristics of patients with true resistant hypertension in central and Eastern Europe: data from the BP-CARE study. *Journal of hypertension* 2013; **31**(10): 2018-24.
21. Oikawa T, Obara T, Ohkubo T, et al. Characteristics of resistant hypertension determined by self-measured blood pressure at home and office blood pressure measurements: the J-HOME study. *Journal of hypertension* 2006; **24**(9): 1737-43.
22. O'Brien E, Parati G, Stergiou G, et al. European Society of Hypertension position paper on ambulatory blood pressure monitoring. *Journal of hypertension* 2013; **31**(9): 1731-68.
23. Sega R, Facchetti R, Bombelli M, et al. Prognostic value of ambulatory and home blood pressures compared with office blood pressure in the general population: follow-up results from the Pressioni Arteriose Monitorate e Loro Associazioni (PAMELA) study. *Circulation* 2005; **111**(14): 1777-83.
24. Mancia G, Facchetti R, Bombelli M, Grassi G, Sega R. Long-term risk of mortality associated with selective and combined elevation in office, home, and ambulatory blood pressure. *Hypertension* 2006; **47**(5): 846-53.
25. Mancia G, Carugo S, Grassi G, et al. Prevalence of left ventricular hypertrophy in hypertensive patients without and with blood pressure control: data from the PAMELA population. Pressioni Arteriose Monitorate E Loro Associazioni. *Hypertension* 2002; **39**(3): 744-9.

26. Grassi G, Facchetti R, Seravalle G, Cuspidi C, Mancia G. Home and ambulatory blood pressure in resistant hypertension. *EuroIntervention* 2013; **9 Suppl R**: R35-41.

27. Cuspidi C, Vaccarella A, Negri F, Sala C. Resistant hypertension and left ventricular hypertrophy: an overview. *Journal of the American Society of Hypertension: JASH* 2010; **4**(6): 319-24.

28. James PA, Oparil S, Carter BL, et al. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). *JAMA : the journal of the American Medical Association* 2014; **311**(5): 507-20.

29. Corea L, Bentivoglio M, Verdecchia P. Echocardiographic left ventricular hypertrophy as related to arterial pressure and plasma norepinephrine concentration in arterial hypertension. Reversal by atenolol treatment. *Hypertension* 1983; **5**(6): 837-43.

30. Perlini S, Palladini G, Ferrero I, et al. Sympathectomy or doxazosin, but not propranolol, blunt myocardial interstitial fibrosis in pressure-overload hypertrophy. *Hypertension* 2005; **46**(5): 1213-8.

31. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990; **322**(22): 1561-6.

32. Ruilope LM, Schmieder RE. Left ventricular hypertrophy and clinical outcomes in hypertensive patients. *Am J Hypertens* 2008; **21**(5): 500-8.

33. Bombelli M, Facchetti R, Carugo S, et al. Left ventricular hypertrophy increases cardiovascular risk independently of in-office and out-of-office blood pressure values. *Journal of hypertension* 2009; **27**(12): 2458-64.

34. de Sousa Almeida M, de Araujo Goncalves P, Branco P, et al. Impact of Renal Sympathetic Denervation on Left Ventricular Structure and Function at 1-Year Follow-Up. *PloS one* 2016; **11**(3): e0149855.

35. Muiesan ML, Salvetti M, Rizzoni D, et al. Resistant hypertension and target organ damage. *Hypertension research: official journal of the Japanese Society of Hypertension* 2013; **36**(6): 485-91.

36. Oliveras A, Armario P, Martell-Claros N, Ruilope LM, de la Sierra A, Spanish Society of Hypertension-Resistant Hypertension R. Urinary albumin excretion is associated with nocturnal systolic blood pressure in resistant hypertensives. *Hypertension* 2011; **57**(3): 556-60.

37. Salles GF, Cardoso CR, Fiszman R, Muxfeldt ES. Prognostic importance of baseline and serial changes in microalbuminuria in patients with resistant hypertension. *Atherosclerosis* 2011; **216**(1): 199-204.

38. Cuspidi C, Macca G, Sampieri L, et al. High prevalence of cardiac and extracardiac target organ damage in refractory hypertension. *Journal of hypertension* 2001; **19**(11): 2063-70.

39. Gerstein HC, Mann JF, Yi Q, et al. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA : the journal of the American Medical Association* 2001; **286**(4): 421-6.
40. Abbate M, Benigni A, Bertani T, Remuzzi G. Nephrotoxicity of increased glomerular protein traffic. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 1999; **14**(2): 304-12.
41. Poss J, Ewen S, Schmieder RE, et al. Effects of renal sympathetic denervation on urinary sodium excretion in patients with resistant hypertension. *Clinical research in cardiology : official journal of the German Cardiac Society* 2015.
42. Schirmer SH, Sayed MM, Reil JC, et al. Improvements in left ventricular hypertrophy and diastolic function following renal denervation: effects beyond blood pressure and heart rate reduction. *J Am Coll Cardiol* 2014; **63**(18): 1916-23.
43. Tsioufis C, Papademetriou V, Dimitriadis K, et al. Effects of multielectrode renal denervation on cardiac and neurohumoral adaptations in resistant hypertension with cardiac hypertrophy: an EnligHTN I substudy. *Journal of hypertension* 2015; **33**(2): 346-53.
44. Mahfoud F, Schlaich M, Kindermann I, et al. Effect of renal sympathetic denervation on glucose metabolism in patients with resistant hypertension: a pilot study. *Circulation* 2011; **123**(18): 1940-6.
45. Fisher JP, Young CN, Fadel PJ. Central Sympathetic Overactivity: Maladies and Mechanisms. *Autonomic Neuroscience* 2009; **15**(148 (1-2)): 5-15.
46. Lown B, Verrier RL. Neural activity and ventricular fibrillation. *N Engl J Med* 1976; **294**(21): 1165-70.
47. Kaye DM, Lefkovits J, Jennings GL, Bergin P, Broughton A, Esler MD. Adverse consequences of high sympathetic nervous activity in the failing human heart. *J Am Coll Cardiol* 1995; **26**(5): 1257-63.
48. Zoccali C, Mallamaci F, Parlongo S, et al. Plasma norepinephrine predicts survival and incident cardiovascular events in patients with end-stage renal disease. *Circulation* 2002; **105**(11): 1354-9.
49. Psaty BM, Siscovick DS, Weiss NS, et al. Hypertension and outcomes research. From clinical trials to clinical epidemiology. *Am J Hypertens* 1996; **9**(2): 178-83.
50. Vernooij JW, van der Graaf Y, Nathoe HM, Bemelmans RH, Visseren FL, Spiering W. Hypertensive target organ damage and the risk for vascular events and all-cause mortality in patients with vascular disease. *Journal of hypertension* 2013; **31**(3): 492-99; discussion 9-500.
51. Currie G, Delles C. Use of Biomarkers in the Evaluation and Treatment of Hypertensive Patients. *Current hypertension reports* 2016; **18**(7): 54.

52. Sousa H, Branco P, de Sousa Almeida M, et al. Changes in albumin-to-creatinine ratio at 12-month follow-up in patients undergoing renal denervation. *Rev Port Cardiol* 2017; **36**(5): 343-51.
53. Shlomai G, Grassi G, Grossman E, Mancia G. Assessment of target organ damage in the evaluation and follow-up of hypertensive patients: where do we stand? *Journal of clinical hypertension* 2013; **15**(10): 742-7.
54. Esler M, Rumantir M, Kaye D, et al. Sympathetic nerve biology in essential hypertension. *Clinical and experimental pharmacology & physiology* 2001; **28**(12): 986-9.
55. Esler M. The sympathetic system in essential hypertension. *Rev Port Cardiol* 2000; **19 Suppl 2**: II9-14.
56. Neumann J, Ligtenberg G, Klein IH, et al. Sympathetic hyperactivity in hypertensive chronic kidney disease patients is reduced during standard treatment. *Hypertension* 2007; **49**(3): 506-10.
57. Esler M, Rumantir M, Wiesner G, Kaye D, Hastings J, Lambert G. Sympathetic nervous system and insulin resistance: from obesity to diabetes. *Am J Hypertens* 2001; **14**(11 Pt 2): 304S-9S.
58. Verloop WL, Vink EE, Spiering W, et al. Effects of renal denervation on end organ damage in hypertensive patients. *European journal of preventive cardiology* 2015; **22**(5): 558-67.
59. Mahfoud F, Urban D, Teller D, et al. Effect of renal denervation on left ventricular mass and function in patients with resistant hypertension: data from a multi-centre cardiovascular magnetic resonance imaging trial. *Eur Heart J* 2014; **35**(33): 2224-31b.

CHAPTER 2 METHODS

This chapter is partially adapted from the following articles:

Renal Denervation in patients with resistant hypertension: six months results.

H Dores, M de Sousa Almeida, P de Araújo Gonçalves, et al.

Rev Port Cardiol. 2014 33(4): 197-204.

Impact of Renal Sympathetic Denervation on Left Ventricular Structure and Function at 1-Year Follow-Up.

M de Sousa Almeida; P Araujo Gonçalves, P Branco et al.

PLoS ONE 2016 11(3): e0149855. doi:10.1371/journal.pone.0149855

Changes in albumin-to-creatinine ratio at 12-month follow-up in patients undergoing renal denervation.

H Sousa, P Branco, M de Sousa Almeida et al.

Rev Port Cardiol. 2017 36(5): 343-351.

Sections:

Summary

The aim of this study is to evaluate the impact of renal denervation, by radiofrequency application inside the renal arteries, on blood pressure and on markers of hypertension related target organ damage, such as changes on left ventricle function and structure and on renal function, in patients with severe resistant hypertension under maximal tolerated antihypertensive medication.

To achieve such purpose, a longitudinal, prospective observational study was adopted. All selected patients were submitted to renal denervation and followed by a dedicated team, under a prespecified protocol. Office blood pressure, 24 hours ambulatory blood pressure measurement, left ventricle structure and function assessed through transthoracic echocardiography, renal function and determination of urinary albumin creatinine ratio were evaluated before and at one year after renal denervation.

Aims of present study

The aim of this study was to evaluate in patients with resistant HTN under maximal tolerated antihypertensive medication, the impact of RDN on BP and on HTN related TOD, assessed through the surrogate markers, LV function and mass, renal function and proteinuria.

Contrary to previous studies, that included patients with resistant HTN defined has having uncontrolled HTN under at least three antihypertensive medications, including a diuretic on "adequate doses", in this study, the aim was to include patients under maximal tolerated antihypertensive medication, and only after the HTN medical team assumed drug treatment failure in controlling BP, the patients were candidates to RDN. This RDN treatment strategy has never been tested before, to the best of our knowledge.

Another difference to previous studies was the use of more robust surrogates of RDN efficacy. As previously mentioned, ABPM measurement is a more robust evaluation of BP control, than office BP, and proved to be better related to prognosis and to HTN related TOD.[1]

The inclusion of HTN related TOD evaluation after RDN, as a surrogate marker of efficacy, was another difference. Although mentioned in previous studies, they were not assumed as efficacy endpoints of RDN treatment, rather as "secondary effects". On the

contrary in the present study and due to their important role on HTN prognosis, they were assumed as surrogate markers of RDN efficacy (Fig. 2).

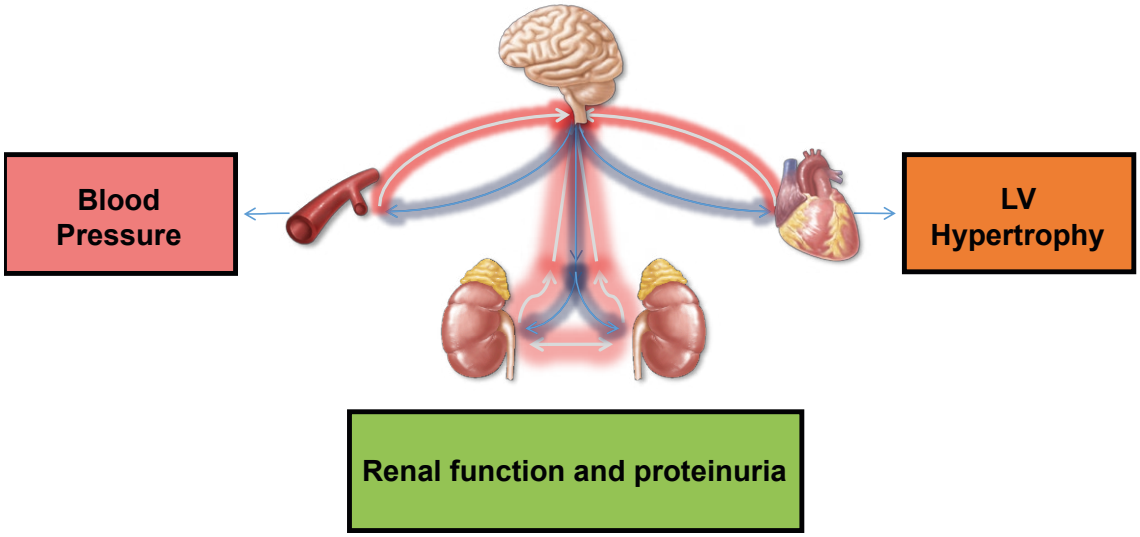


Figure 2: Impact of RDN on blood pressure and on target organ damage – left ventricle mass and renal function/proteinuria.

Specific objectives of present study

Primary objective:

- To evaluate the impact of RDN on systolic BP reduction, assessed by ABPM at 12 months follow-up after RDN.

Secondary objectives:

- To evaluate the impact of RDN on office systolic BP reduction, measured at 12-month follow-up after RDN.
- To evaluate the impact of RDN on LV mass, assessed by echocardiography at 12 months follow-up.
- To evaluate the impact of RDN on urinary albumin to creatinine ratio (ACR), at 12 months follow-up.

Secondary safety objectives:

- Rate of RDN procedure vascular access complications.
- Deterioration of renal function assessed by changes in blood creatinine, blood urea and eGFR, at 12-months of follow-up.

Study team

For the purpose of this study, an **RDN-Team** was established including interventional cardiologists (who perform the procedure), imaging cardiologists (responsible for the echocardiogram) and nephrologists (running the hypertension outpatient clinic). From a historical perspective, the HTN Outpatient Clinic at Hospital de Santa Cruz was already managed by the Nephrology Department. In such context, it was decided to implement a specific outpatient clinic for resistant HTN, for the evaluation and follow up of patients proposed and submitted to renal denervation (Fig. 3).

The mission of this RDN-team was to analyse and discuss all patients with resistant HTN, evaluated in the dedicated HTN Outpatient clinic, to properly select the patients to RDN and to follow them after the procedure. The team has regular monthly meetings to discuss patient eligibility for RDN and the follow-up results.

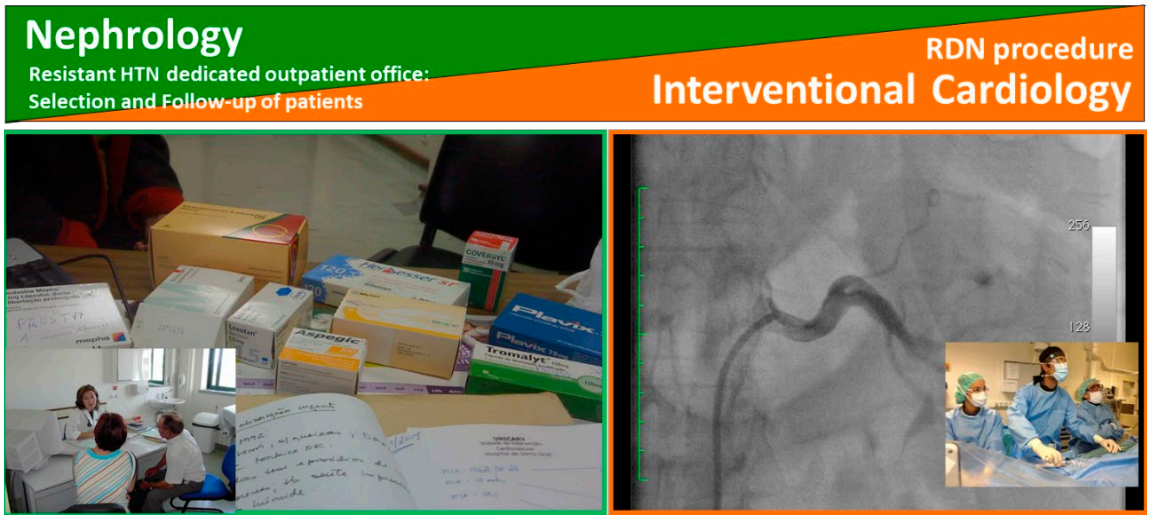


Figure 3: RDN – Team involving the Cardiology, Nephrology and Anesthesiology Departments.

Study design and patients selection

The RDN Registry. By protocol, all patients submitted to RDN in Hospital de Santa Cruz, are included in an ongoing single centre dedicated prospective registry. This registry (the RDN Registry) records all data obtained in the pre-RDN evaluation phase, in the procedure and at follow-up, including demographic, anthropometric and clinical variables, as well as medication, laboratory values and echocardiogram results. All obtained data, at baseline and at follow-up are stored in an electronic database. Procedure data and safety endpoints are also recorded, as they happen or are known by the Renal Team. Pre-specified outpatient visits are scheduled at 1-6-12 month, and

yearly after on. In these visits, a pre-specified set of tests were performed, and all efficacy endpoints were recorded.

The resistant HTN protocol evaluation, the RDN procedure and the data collection were all approved by the CHLO Ethical Commission. Dedicated informed consent is requested to all patients.

Patient selection. Since its beginning, the Renal Team decided that only patients with resistant HTN should be considered for RDN. Resistant HTN was defined according to international standards as BP > 140/90 mmHg in a patient with at least three antihypertensive drugs, of different classes (one of them must be a diuretic) on adequate doses.[16] In all patients, secondary HTN had to be ruled out. Remaining inclusion criteria (Table 6) were drawn from simplicity HTN2 trial[3] with the following major changes: the inclusion of patients with resistant HTN despite maximum tolerated antihypertensive drug therapy, with no limit to number, type or dose, as much as they were safe and tolerated by the patients. That strategy diverged from the one followed by Simplicity HTN2 trial, as he included patients with resistant HTN by classical definition, meaning that drug treatment was not used up-to its reasonable and clinical limits as we did. Our strategy was to perform RDN on top of maximal tolerated drug treatment. Another major change was to use ABPM instead of standard office BP measurements as the surrogate for drug treatment efficacy and BP control, because it is more robust than office BP measurements and its better linked with HTN long term prognosis.[1, 4]

Dimension of study population. The population dimension needed to appropriately assess the impact of RDN on BP and on HTN related TOD, was calculated assuming the following criteria: data analysed from a single centre, single arm prospective registry, single sided superiority test, assuming an expected reduction of 2 mmHg on the ABPM systolic BP measurement, as a surrogate for HTN success (value used as a secondary endpoint in Symplicity HTN 3 trial[5]), with a pre-procedure average ABPM systolic BP of 149mmHg and a standard deviation of 18mmHg (data derived from a preliminary analysis of 31 patients submitted to RDN already included in the RDN registry) with an type one error of 2,5% (single sided) and a potency of 80%, the minimal number of patients needed were 28.

Table 6: Inclusion criteria for catheter based renal denervation.

Clinical criteria

- age ≥ 18 years
- Severe resistant HTN in patients with at least ≥3 anti HTN drugs (including a diuretic) on maximal tolerated doses and
  - ABPM Systolic BP ≥ 135 mmHg
- Patients with eGFR >30ml/min/1.73m2

Anatomic criteria

- Both renal arteries:
  - ≥ 3mm diameter of a main renal artery
  - Less <50% stenosis and no previous intervention
- Anatomy documented by any of the following methods:
  - Computed Tomography angiography
  - MR angiography
  - Invasive renal angiography

Exclusion criteria

- Type 1 Diabetes
- Unstable or terminal diseases
- Pregnancy
- Secondary HTN
- Recent major surgery
- Recent active bleeding
- Unable to give informed consent

Renal denervation procedure

RDN is an invasive procedure performed primarily through the femoral arteries, exceptionally, it may be performed by radial arteries. After selective cannulation of renal arteries, using dedicated catheters, an angiography is performed on each artery. A dedicated radiofrequency catheter is then inserted inside the renal artery and adequately positioned (Fig. 4).

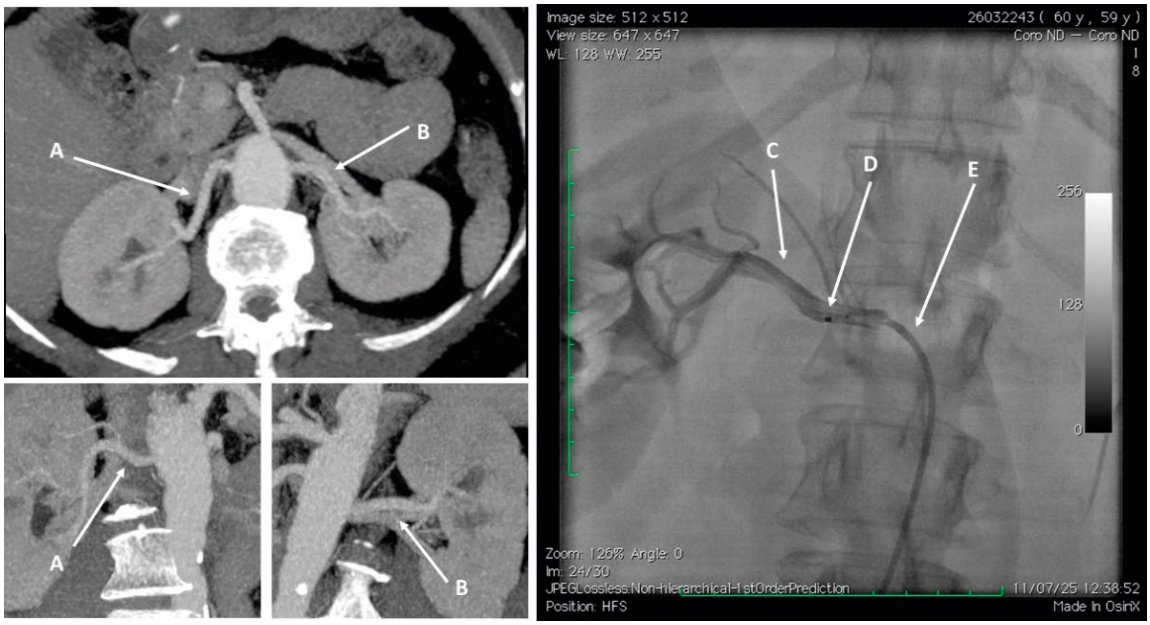


Figure 4: Evaluation by CT angiography scan of right (A) and left (B) renal artery, and angiography, displaying the ablation catheter (E) in the right renal artery (C) with its ablation tip (D) positioned in the lower artery wall near the ostium.

A radiofrequency generator was connected to the catheter and radiofrequency was delivered to the artery wall accordingly to a proprietary protocol that considers the time, the temperature and impedance. If any of pre-specified criteria values were surpassed, the radiofrequency application switched-off automatically. The aim, was to apply multiple radiofrequency shots in a 360-degree spiral circle inside the artery, avoiding unnecessary damages in the artery endothelium by multiple shots in the same spot. The *EnligHTN*® (*St. Jude Medical*, USA) and the OneShot® (Medtronic™, Santa Monica; California) devices, can apply simultaneously, multiple shots in different angles.

The systems used in the present study were Symplicity® Flex device (Medtronic™, Santa Monica; California) – Fig. 5, a single point by point radiofrequency applying system; the EnligHTN® device (Abbott Vascular™, Santa Clara, California) – Fig. 5, a multipoint radiofrequency applying system, and the OneShot® system (Medtronic™, Santa Monica; California), also a multipoint radiofrequency system. They all use a similar radiofrequency applying protocol, aimed to improve efficacy by delivering the appropriate amount of energy to vessel walls, and consequently to decrease nerve traffic in the renal SNS.

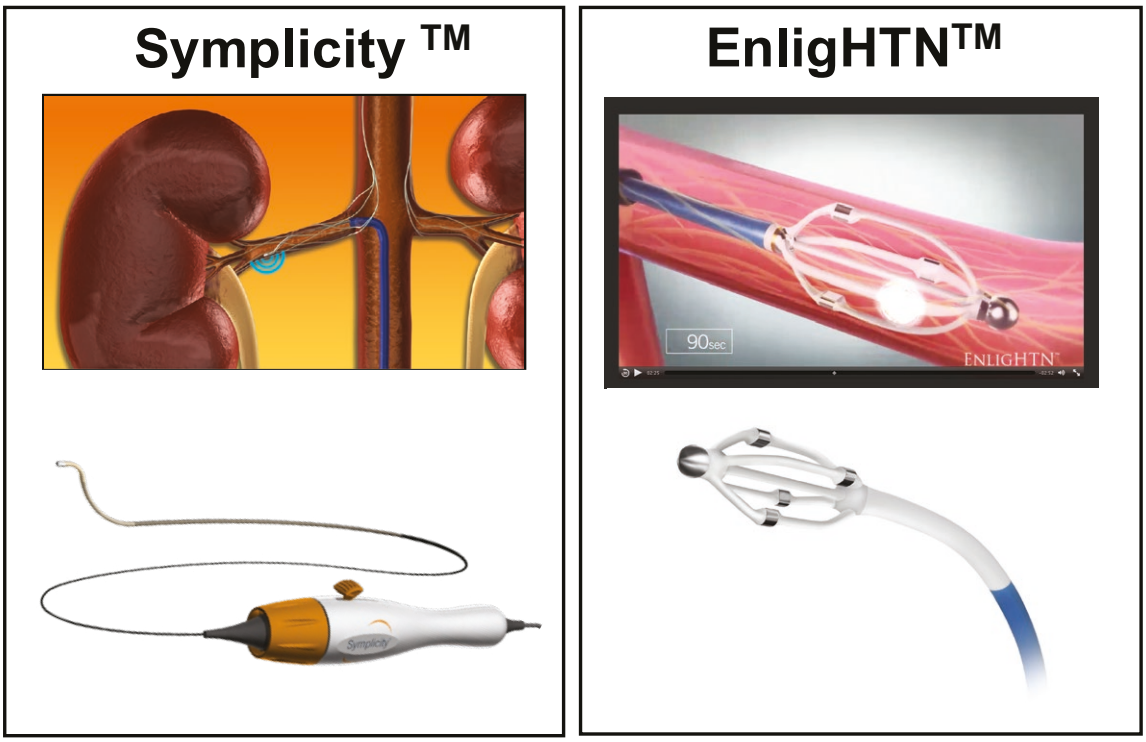


Figure 5: The most frequently used devices used in RDN along the study.

Being a painful procedure, anaesthesiology backup was needed. Weight adjusted propofol and remifentanyl were used to control the pain. Unfractionated heparin was used during the procedure to achieve an activated clotting time > 250 seconds. Saline iv was used as needed. At the end of the procedure and whenever possible, the femoral artery access was closed using a percutaneous closing device: *Angio-Seal® (St. Jude Medical,* USA), to minimize the risk of vascular access complications and to patient comfort (Fig. 6).

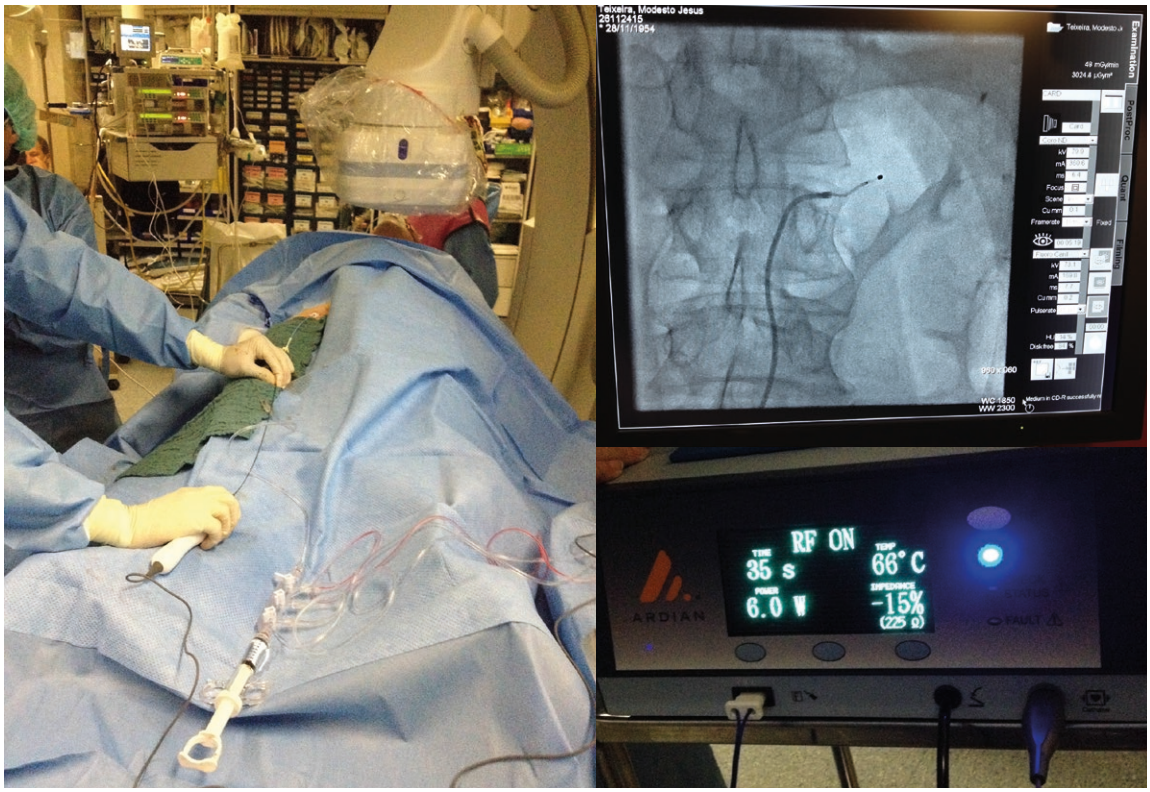


Figure 6: An overview of the cathlab setup during an RDN procedure.

All listed, likely procedure related complications, were recorded (Table 7), with a special attention to complications related to the vascular access site. The definitions were depicted from those used in HTN 3 trial.[5]

Table 7: Possible complications of catheter based renal denervation.

MAJOR complications	Kidney related complications	Insertion site complications	Minor complications
Death	Chronic loss kidney function	Long standing local pain	Hypotension
Severe bleeding	Transient loss of kidney function	Pseudoaneurysm	Orthostatic hypotension
Emboli	Kidney perforation	Arterio-venous fistula	Hypertension
Life threatening arrhythmias	Renal artery perforation	Infection	Vomiting
disturbances	Renal artery occlusion	Bleeding	Nausea
	Renal artery stenosis	Retroperitoneal bleeding	Contrast related
	Renal artery aneurism	Vessel perforation	Procedure medications related
		Vessel dissection	Electrolyte disturbances

Methodology assessment of specific objectives

Patients follow-up. After the procedure, patients were followed in the HTN outpatient's clinic. A prespecified set of visits to the HTN Outpatient clinic, were scheduled as previously mentioned, without precluding the need for further visits if clinically driven, to achieve BP control or manage drug treatment side effects.

At one year, all patients were evaluated to assess prespecified efficacy endpoints. On those visits, BP was measured, medication was reviewed and adjusted as needed. All data and changes in medication were recorded. Standard physical evaluation, anthropometrics, blood and urine analysis, ABPM measurements, echocardiogram results, were also recorded in each patient case report file.

A major change compared to previous studies, was the routine use of ABPM to assess BP values, instead of the commonly used office BP measurements. As a surrogate for RDN efficacy, ABPM is more robust than office BP measurements and its better linked to HTN long-term prognosis as previously mentioned.[1, 4]

Blood pressure measurements and definition of responders. Office BP readings were taken in a seated position with an oscillometric semiautomatic sphygmomanometer Omron HEM-907 monitor (Omron Healthcare, USA), after 5 min of rest, according to the European Guidelines for the Management of Arterial Hypertension.[6] At baseline, BP

was measured in both arms and the arm with the higher BP was used for all subsequent readings. Averages of the triplicate measures were calculated and used for analysis.

Twenty-four hours ambulatory blood pressure measurements were taken with an ABM monitor (Spacelabs Healthcare, USA), according to the current European Society of Hypertension Guidelines[6].

Blood pressure responders to RDN treatment were defined as those which had a reduction on office systolic BP of ≥5 mmHg at one year follow-up or a reduction ≥ 2 mmHg in ABPM systolic BP, according to the Symplicity HTN3 trial criteria[7].

Urinary albumin to creatinine ratio determination. As recommended[8], ACR was used as a marker of kidney damage. Urinary concentration of albumin and creatinine were measured separately by nephelometry and the Jaffé method respectively, using first morning void spot samples.[9] This value is expressed in mg/g and it is equivalent to the 24 hours value expressed in mg/day. All laboratory testing was performed by a central laboratory.

The Modification of Diet in Renal Disease (MDRD) Formula: eGFR (mL/min/1.73 m2) = 186 x (sCr/88.4)-1.154 x (Age)-0.203 x (0.742 if female) x (1.212 if of African descent) (SI units), was used estimate eGFR.

Urine spot samples to obtain ACR values were acquired before RDN (at baseline) and at 12 months follow-up.

Contrary to BP changes with RDN, regarding ACR changes and to the best of our knowledge, there is no specified standard definition to consider a patient as being a responder to RDN. In such setting, it was assumed that any decrease in ACR at one year after if RDN was consider a responder to RDN.

Transthoracic echocardiography. Comprehensive two-dimensional and Doppler transthoracic echocardiographic studies were performed at baseline and at one-year follow-up, in all patients, using a VIVID 7 ultrasound system (General Electric Healthcare). All echocardiographic recordings were stored in a digital format on a dedicated workstation for off-line subsequent analysis. The studies were performed by one of two experienced operators, while analyzed and interpreted by another operator, not involved in the images acquisition. They were all blinded to patients' clinical, BP status and sequence of images.

Left ventricular size was evaluated by both linear (using M-mode 2D guided diameters obtained perpendicular to the LV long axis) and volumetric (using the biplane method of disks summation from tracings of the blood-tissue interface in the apical four- and two-chamber views), according to accepted recommendations from the American Society of Echocardiography and the European Association of Cardiovascular Imaging [10]. LV ejection fraction was calculated using the following formula: EF = (EDV − ESV)/EDV, with LV volume estimates obtained by the biplane method of disks.

Assessment of LV mass (LVM) was performed by the linear method using the Deveraux cube formula[11] (LV mass = $0.8 \times 1.04[(IVS + LVID + PWT)^3 - LVID^3] + 0.6g$), with 2D guided M-mode measurements obtained at end-diastole from the parasternal

approach perpendicular to the LV long axis measured at the level of the mitral valve leaflet tips. LV hypertrophy was considered present when LV mass exceeded 115 g/m^2 for men and 95 g/m^2 for women.

We also calculated the relative wall thickness (RWT) measured as twice the posterior wall thickness divided by LV end-diastolic diameter and determined the LV anatomical pattern in each participant. Normal LV mass and RWT were defined as normal LV anatomy, normal LV mass and RWT >0.42 as concentric LV remodeling, increased LV mass and RWT >0.42 as concentric LV hypertrophy and increased LV mass in the presence of RWT <0.42 as eccentric LV hypertrophy [12]. Left atrial size was evaluated using M-mode 2D guided diameters and area, when the left atrium chamber was at its greatest dimension (end of LV systole).

LV diastolic function was assessed by pulsed-wave Doppler examination of mitral inflow and Doppler tissue imaging of the mitral annulus. Peak velocities of early (E) and late (A) trans-mitral flow and deceleration time were determined, and the ratio E/A was calculated. Doppler tissue imaging with pulsed-wave Doppler at the level of septal and lateral mitral annulus was used to measure e' velocities. The average of septal and lateral mitral annulus e' peak velocities, were used to calculate the E/e' ratio. The Valsalva maneuver was performed to distinguish normal from pseudo-normal patterns. Spectral recordings were obtained at a sweep speed of 100 mm/s at end-expiration, and each measurement was averaged over multiple cardiac cycles to account for inter-beat variability.

Grade 1 diastolic dysfunction (impaired relaxation) was defined by the presence of an E/A ratio <0.8, a deceleration time >200ms and E/e′ ratio <8 in the presence of an enlarged left atrium. Moderate (pseudo-normal, grade 2) diastolic dysfunction was defined as a mitral E/A ratio >0.8 and <1.5 that decreases by 50% during the Valsalva maneuver, E/e' ratio 9 to 12 and e'<8 cm/s. Finally, severe (grade 3) diastolic dysfunction corresponds to restrictive LV filling defined by E/A ratio >2, deceleration time <160ms, and average E/e'>13. All subjects with impaired LV relaxation, pseudo-normal or restrictive filling patterns were defined as having LV diastolic disfunction [13].

Based on previous studies, a patient was assumed as a responder to RDN, if a 5% reduction in the LV mass was achieved one year after the RDN procedure.[14]

Statistics

Continuous variables are reported as mean ± standard deviation. Variables' normal distribution was tested with the Kolmogorov-Smirnov test and/or Q-Q Plot visual assessment. Discrete variables are expressed as frequencies and percentages. Variables with a normal distribution were compared between baseline and at one-year follow-up, using a paired Student t test or a Wilcoxon matched-pairs test if without a normal distribution. Discrete variables are expressed as frequencies and percentages (in brackets). A p value <0.05 was considered as statistically significant. Linear regression

analysis was used to calculate the correlation between the change on blood pressure, the change on echocardiographic parameters and on ACR values.

Statistical Package for the Social Sciences®, V.21.0 (IBM SPSS Modeler, Chicago, IL) and Medcalc® V.6.0 is used for data processing and statistical analysis.

Definitions of other used variables

Height and weight were measured with the participant in light clothing without shoes.

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters

Diabetes was diagnosed if at least one of the following criteria was present: a fasting plasma glucose level ≥126 mg/dL (7.0 mmol/L), non-fasting glucose of ≥200 mg/dL (11.1 mmol/L) or an abnormal glucose tolerance test.

Obesity is a BMI greater than or equal to 30 and equal for both sexes.

Dyslipidemia defined according to the ESC guidelines.[15] Measured in a blood sample obtained under 12 hours of fasting condition and calculated, as in most studies, using Friedewald's formula (unless Triglycerides are elevated >4.5 mmol/L or more than ~400 mg/dL). In patients with low risk for cardiovascular disease (<1% CV risk score) dyslipidemia was assumed present if Low Density Lipoproteins > 190mg/dL (4.9mmol/L). In patients with high risk for cardiovascular disease (>1% CV risk score) or if known cardiovascular disease, dyslipidemia was assumed if Low Density Lipoproteins > 100mg/dL (>2.5mmol/L).

Smoking defined as smoking daily or smoking on some days (National Cancer Institute).[16]

Sleep apnea defined as a documented spontaneous breathing cessation lasting more than 10 seconds, during sleep (European Respiratory Society).[17]

Coronary artery disease defined as any of the following: con**firmed myocardial infarction**, coronary angiography showing more than 50% narrowing of at least 1 major coronary artery, diagnosis of classic angina pectoris, or concordant abnormalities on electrocardiography, echocardiography, or radionuclide scans from stress test findings concordant for ischemia.

Any vascular disease defined as any documented sign of clinical meaningful arterial obstructive disease outside coronaries. Documented either by brachial ankle index significant changes or by any imageology test showing significant artery narrowing.

Ethical approvals

The present study was submitted and approved by the Ethical Commission from Nova Medical school on September 1st, 2015 and by the Ethical Commission from the Centro Hospitalar de Lisboa Ocidental (CHLO) on July 11, 2011.

Bibliography

1. O'Brien E, Parati G, Stergiou G, et al. European Society of Hypertension position paper on ambulatory blood pressure monitoring. *Journal of hypertension* 2013; **31**(9): 1731-68.
2. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Annals of internal medicine* 1999; **130**(6): 461-70.
3. Esler MD, Krum H, Sobotka PA, Schlaich MP, Schmieder RE, Bohm M. Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet* 2010; **376**(9756): 1903-9.
4. Persu A, Azizi M, Jin Y, et al. Hyperresponders vs. nonresponder patients after renal denervation: do they differ? *Journal of hypertension* 2014; **32**(12): 2422-7; discussion 7.
5. Bhatt DL, Kandzari DE, O'Neill WW, et al. A controlled trial of renal denervation for resistant hypertension. *N Engl J Med* 2014; **370**(15): 1393-401.
6. O'Brien E, Parati G, Stergiou G, et al. European Society of Hypertension position paper on ambulatory blood pressure monitoring. *J Hypertens* 2013; **31**(9): 1731-68.
7. Kandzari DE, Bhatt DL, Sobotka PA, et al. Catheter-based renal denervation for resistant hypertension: rationale and design of the SYMPLICITY HTN-3 Trial. *Clinical cardiology* 2012; **35**(9): 528-35.
8. Astor BC, Shafi T, Hoogeveen RC, et al. Novel markers of kidney function as predictors of ESRD, cardiovascular disease, and mortality in the general population. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2012; **59**(5): 653-62.
9. Mogensen CE, Vestbo E, Poulsen PL, et al. Microalbuminuria and potential confounders. A review and some observations on variability of urinary albumin excretion. *Diabetes care* 1995; **18**(4): 572-81.
10. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging* 2015; **16**(3): 233-70.
11. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. *Circulation* 1977; **55**(4): 613-8.

12. Gaasch WH, Zile MR. Left ventricular structural remodeling in health and disease: with special emphasis on volume, mass, and geometry. *J Am Coll Cardiol* 2011; **58**(17): 1733-40.
13. Nagueh SF, Appleton CP, Gillebert TC, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography* 2009; **22**(2): 107-33.
14. de Simone G, Devereux RB, Izzo R, et al. Lack of Reduction of Left Ventricular Mass in Treated Hypertension: The Strong Heart Study. *Journal of the American Heart Association* 2013; **2**(3).
15. Reiner Ž, Catapano AL, De Backer G, et al. ESC/EAS Guidelines for the management of dyslipidaemiasThe Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *European Heart Journal* 2011; **32**(14): 1769-818.
16. Bergen AW, Caporaso N. Cigarette Smoking. *JNCI: Journal of the National Cancer Institute* 1999; **91**(16): 1365-75.
17. Jun JC, Chopra S, Schwartz AR. Sleep apnoea. *European Respiratory Review* 2016; **25**(139): 12-8.

CHAPTER 3 RESULTS

This chapter is partially adapted from the following articles:

Impact of Renal Sympathetic Denervation on Left Ventricular Structure and Function at 1-Year Follow-Up.

M de Sousa Almeida; P Araujo Gonçalves, P Branco et al.

PLoS ONE 2016 11(3): e0149855. doi:10.1371/journal.pone.0149855

Changes in albumin-to-creatinine ratio at 12-month follow-up in patients undergoing renal denervation.

H Sousa, P Branco, M de Sousa Almeida et al.

Rev Port Cardiol. 2017 36(5): 343-351.

Sections:

Summary

From 318 patients with suspected resistant hypertension, evaluated between 2011 and 2015 in a dedicated outpatient clinic, 65 were confirmed as having resistant hypertension and later submitted to renal denervation, of which 31 patients had a complete 12 months follow-up data at the time of the present analysis and are reported here.

At 12-months follow-up there was a statistically significant decrease in office and 24-hour ambulatory blood pressure values, with 84% of patients considered responders to renal denervation regarding systolic blood pressure and 71% for diastolic blood pressure.

There was also a statistically significant decrease in left ventricle mass, measured by transthoracic echocardiography, with an improvement in left ventricle mass, from 152.3g/m2 to 135.7g/m2 ($p<0.001$). Five patients (16%) were not responders in left ventricle mass reduction to renal denervation.

Regarding renal function, assessed through urinary albumin to creatinine ratio, there was also a significant decrease at one year, from 25.9mg/g to 14.8mg/g ($p=0.007$), with no significant changes in estimated glomerular filtration rate. Such significant decrease was maintained, regardless of being diabetics or not.

No clear linear correlations were found, between changes in blood pressure, after renal denervation, and changes in either left ventricular mass or urinary albumin to creatine ratio, both surrogates of hypertension related target organ damages.

There were no major complications related to the renal denervation procedure.

Study population

Between July 2011 and April 2015, a total of 318 patients with presumed resistant HTN were evaluated in a dedicated outpatient HTN clinic as probable candidates for RDN. From those, 253 patients were excluded after an extensive clinical evaluation, the majority of which due to pseudo-resistance, after controlling their BP with further treatment adjustment (n=139). Secondary HTN (n=31), unfavorable renal anatomy (n=22), renal dysfunction with an eGFR below 30ml/min/1.73m2, considered at the time, the lowest safety limit for RDN (n=85), and patients refusal for RDN(n=41), were also reasons for excluding patients from the RDN program.

The remaining 65 patients were considered good candidates and were submitted to RDN. Those patients entered a prospective registry aimed do evaluate in a standardized fashion, safety and the outcomes of RDN on a set of prespecified endpoints, including changes on BP and on HTN related TOD. (Fig. 7)

From these 65 patients treated with RDN, it was possible to obtain the complete clinical and technical data at one-year follow-up, including ABPM measurements, transthoracic echocardiogram and ACR values, in 31 consecutive patients that were the final population included at the time of this analysis.

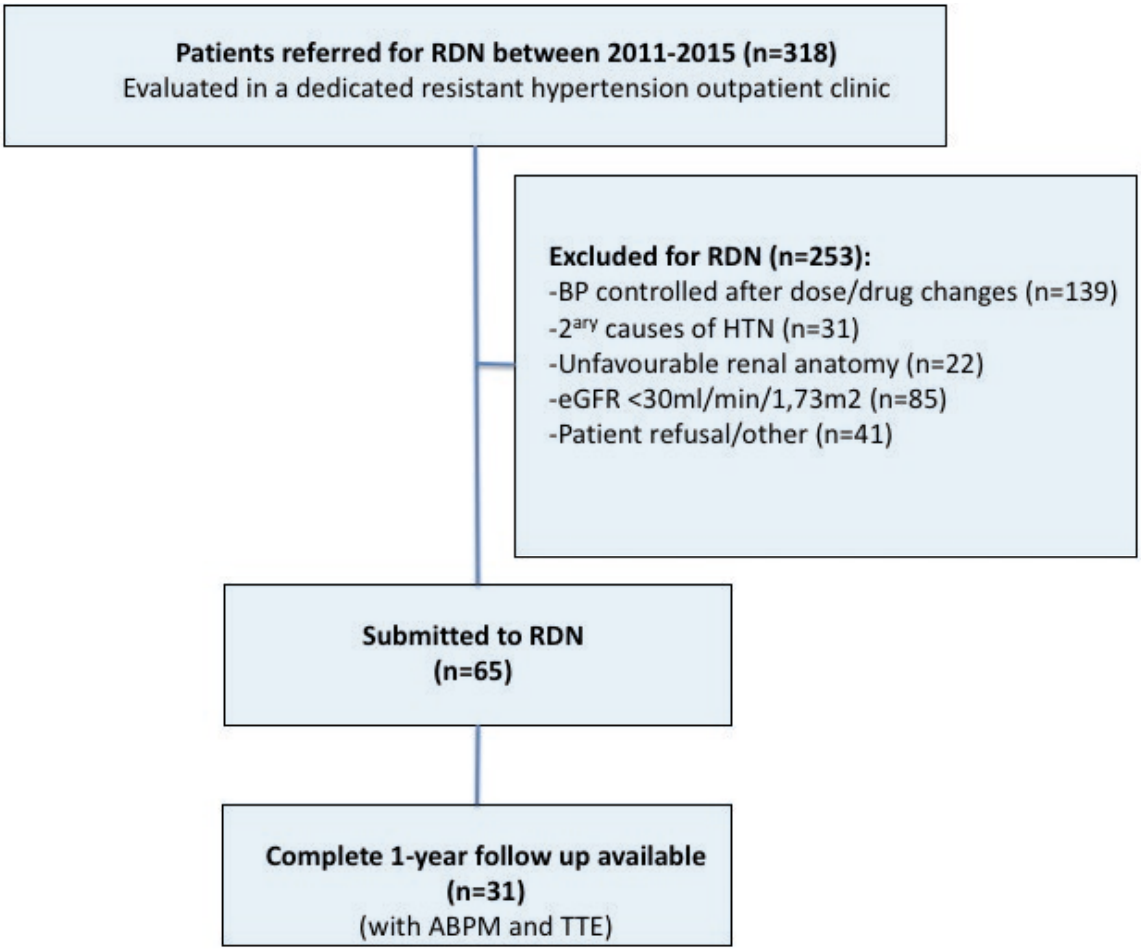


Figure 7. Flowchart of patient selection.
From the total number of patients evaluated in the outpatient HTN clinic (n=318), 31 patients with ABPM monitoring, transthoracic echocardiography (TTE) and complete data at 12-months follow-up, were select for the analysis.

The mean age of selected study population, was 65±7 years, 48% were males (n=15), all Caucasian. Concerning traditional cardiovascular risk factors, obesity was present in 68% of the patients (mean body mass index 32±6 Kg/m^2), type 2 diabetes in 71%, dyslipidemia in 68% and active smoking in one patient (3.2%). Coronary artery disease was present in 10 patients (32%) and any vascular disease in 11 (36%).

The mean eGFR at baseline, was 76.4±24.7 mL/min/1.73m2. Five patients had chronic kidney disease defined as having an eGFR < 60 mL/min/1.73m2.

Baseline, median ACR was 25.8 (IQR 9.0-574.0) and 15 patients (48,4%), had an ACR > 30 mg/g.

Patient's demographic and clinical characteristics at baseline, are shown in Table 8.

Table 8: Patient's demographic and clinical characteristics at baseline.

Demographic and clinical variables	
Age (years)	65±7
Male (%)	15 (48.4)
Caucasians (%)	31 (100)
Weight (kg)	86±16
Height (m)	1.65±0.1
BMI (kg/m2)	31.8±5.5
Obesity (%)	21 (67.7)
Atrial fibrillation (%)	1 (3.2)
Previous stroke (%)	2 (6.5)
Type 2 Diabetes (%)	22 (71)
Dyslipidaemia (%)	21 (67.7)
Smoking (%)	1 (3.2)
Sleep apnea (%)	5 (19.1)
eGFR (ml/min/1,73m2)	76.4±24.7
Chronic kidney disease* (%)	5 (16.1)
Hypertension > 10 years (%)	28 (90.3)
Coronary artery disease (%)	10 (32.3)
Any vascular disease (%)	11 (35.5)

*Chronic kidney disease (eGFR <60 ml/min/1,73m^2)

The majority of patients (90%) had HTN lasting for more than 10 years, treated with a median of 5.8 anti-hypertensive agents from a median of 5.5 different pharmacological classes. Almost all patients were treated with calcium antagonists, 96.8% (n=30), 87% with diuretics including 74% with spironolactone, 61% with ACE inhibitors, 61% with ARB inhibitors, 84% with beta-blockers and 71% with a sympatholytic drug (Table 9).

Table 9: Antihypertensive medication at baseline.

	Baseline
Mean number of antihypertensive drugs	5.8±1.1
Mean number of classes	5.5±0.9
ACE inhibitors	19 (61.3)
ARBs (%)	19 (61.3)
Beta-blockers (%)	26 (83.9)
Calcium channel blockers (%)	30 (96.8)
Diuretics (%)	27 (87.1)
Spironolactone (%)	23 (74.2)
Sympatholytic (%)	22 (71)
Aliskirene	4 (12.9)

Renal denervation procedure

RDN was performed using standard approved percutaneous catheter systems. All of them have similar radiofrequency applying proprietary protocols, aimed to be efficacious in the amount of energy delivered at each point and to be safe avoiding severe damage to vessel endothelium and walls.

Their difference was in the number of radiofrequency points applied simultaneously. The Symplicity® Flex system (n=25) is a single, point by point, radiofrequency applying system. The EnligHTN® (n=4) and OneShot® (n=2) catheter are simultane-

ous multipoint radiofrequency applying systems. In all of them, radiofrequency has to be applied in multiple equidistant points, in a 360° a spiral fashion, using the standard technique, as previously reported.

The median number of radiofrequency applications per artery and per patient are revealed in table 10.

Table 10: Procedure characteristics of catheter based renal denervation.

RDN Procedure	
Median number of applications right renal artery	5.1±1.3
Median number of applications left renal artery	5.7±1.1
Median number of applications per patient	10.8±2.3

Impact on blood pressure

At baseline, average office systolic BP and diastolic BP was 176±24 mmHg and 90±14 mmHg, respectively, with an average heart rate of 73±11 bpm. The ABPM measurements revealed the following average values: 150±20 mmHg for systolic BP, 83±10 mmHg for diastolic BP with an average pulse pressure of 67±18mmHg (Table 11).

Table 11: Results of blood pressure and heart rate measurements at baseline and one-year follow-up.

	Baseline	One-year	P
Office systolic BP (mmHg)	176±24	149±13	<.001
Office diastolic BP (mmHg)	90±14	79±11	<.001
Heart rate (bpm)	73±11	70±11	.261
ABPM systolic BP (mmHg)	150±20	132±14	<.001
ABPM diastolic BP (mmHg)	83±10	74±9	<.001
ABPM pulse pressure (mmHg)	67±18	58±13	.001
ABPM mean pressure (mmHg)	105±9	95.3±8.4	<.001
ABPM heart rate (bpm)	67.6±9.1	65.5±9.5	.090
ABPM systolic BP responders (%)*	-	26 (83.9)	-
Office systolic BP responders (%)**	-	22 (71)	-

Bpm: beats per minute; *ABPM systolic BP responders: a decrease of 2mmHg between baseline and one-year follow-up; **Office systolic BP responders: a decrease of 10mmHg between baseline and one-year follow-up.

Overall, at one-year follow-up, there was a statistically significant reduction on office systolic BP (176±24 to 149±13mmHg, p<0.001), on diastolic BP (90±14 to 79±11mmHg, p<0.001), on ABPM systolic BP (150±20 to 132±14 mmHg, p<0.001) and on ABPM diastolic BP (83±10 to 74±9 mmHg, p<0.01) (Fig. 8).

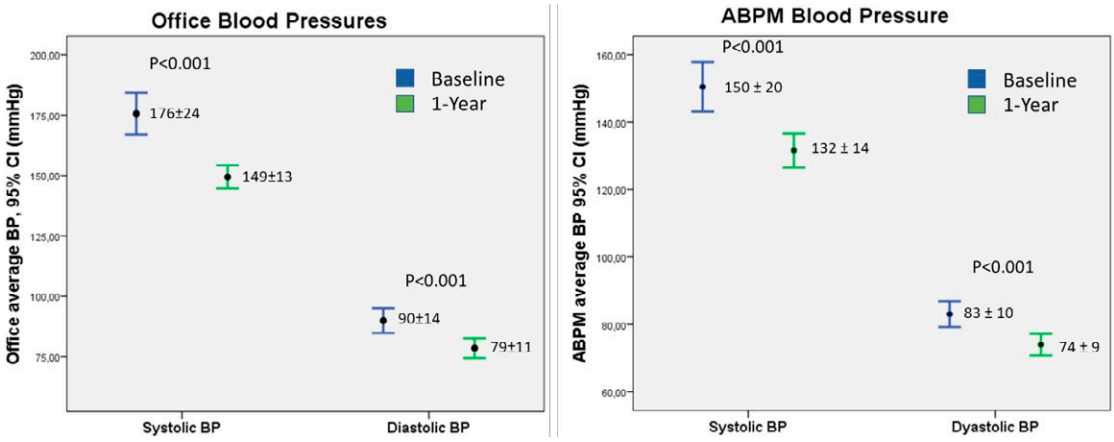


Figure 8. BP results one year after RDN. There was a statistically significant decrease in both systolic and diastolic BP, in office and ABPM measurements.

A significant decrease was also found in the average 24-hours pulse pressure, measured by ABPM, an important surrogate of TOD, that decreased on average 9 mmHg, from 67±18 to 58±13 mmHg (p= 0.001) alongside with the average 24 hours BP in ABPM, from 105±9 to 95.3±8.4 mmHg (p<0.001), (Fig. 9).

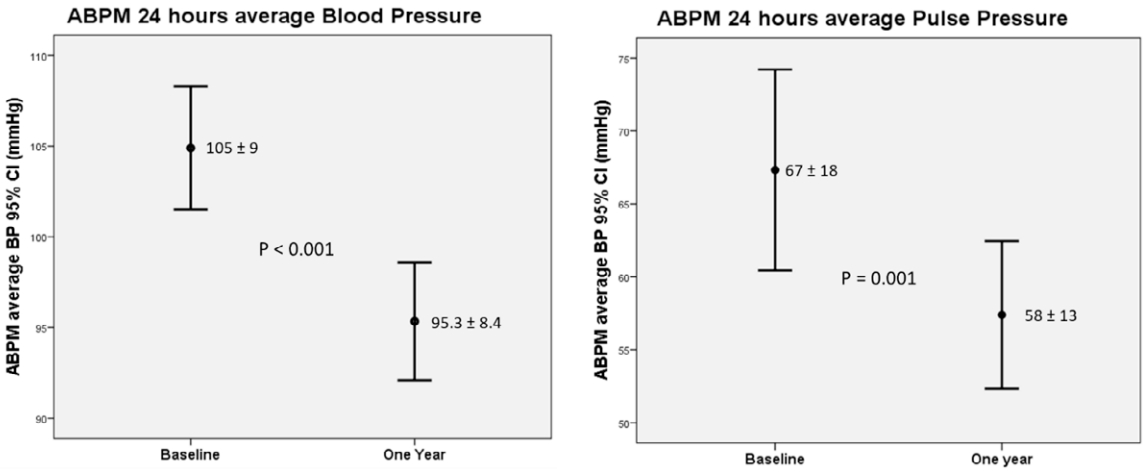


Figure 9. A statistically significant decrease on 24-hourse average Pulse Pressure and on Mean BP, measured by ABPM.

Those results were found, despite the significant reduction in the number of both antihypertensive drugs and classes in use at one year: 5.8±1.1 to 5.0±1.2 (p=0.002) and 5.5±0.9 to 4.9±1.1 (p=0.015) respectively (Table 12).

Table 12. Antihypertensive medication at baseline and at one-year follow-up.

	Baseline	One year	p
Mean number of antihypertensive drugs	5.8±1.1	5.0±1.2	0.002
Mean number of classes	5.5±0.9	4.9±1.1	0.015
ACE inhibitors	19 (61.3)	17(54.8)	0.688
ARBs (%)	19 (61.3)	18 (58.1)	1.0
Beta-blockers (%)	26 (83.9)	27 (87.1)	1.0
Calcium channel blockers (%)	30 (96.8)	21 (67.7)	0.012
Diuretics (%)	27 (87.1)	24 (77.4)	0.727
Spironolactone (%)	23 (74.2)	26 (83.9)	0.453
Sympatholytic (%)	22 (71)	19 (61.3)	0.508
Aliskirene	4 (12.9)	0	0.046

At follow-up, 22 from the total of 31 patients (71%) were considered office systolic BP responders and 26 (84%) ABPM systolic BP responders, based on a decrease of more than 10mmHg on office systolic BP and 2mmHg on ABPM systolic BP.

Impact on left ventricle structure and function

Transthoracic echocardiographic studies were performed at baseline and at one-year follow-up in all patients and stored in digital format on a dedicated workstation for off-line subsequent analyses. The exams were all performed by one of two experienced operators, while the analysis and interpretation were done by another operator, not involved in the images acquisition, all blinded to patient's BP status and sequence of tests. The echocardiographic execution and interpretation protocols are mentioned in detail in the Methods chapter.

The echocardiographic data values obtained at baseline and at one-year follow--up, for heart structure and function, are enlisted in Table 13.

Table 13. Transthoracic echocardiographic parameters at baseline and at one-year follow-up.

	Baseline	One-year	p
Heart structure			
LVEDV (mL/m2)	93.3±18,2	110.9±27.4	.004
LVESV (mL/m2)	35.8±12.6	38.2±3.1	.121
IVSTd (mm)	13.4±1.9	13.1±2.4	.616
PWTd (mm)	11.7±1.6	11.8±1.7	.620
LVEDD (mm)	48.7±5.8	47.8±5.4	.230
LVESD (mm)	28.9±5.7	27.9±6.5	.296
LV mass/BSC (g/m2)	152.3±32.4	135.7±33.9	<.001
Left atrium size/BSC (cm/m2)	32.8±8.3	34.1±6.2	.227
Function			
LVEF Simpson (%)	64.5±9.2	67.7±9.1	.001
Stroke volume (ml)	81.7±14.9	102.7±16.7	.075
Mitral valve E Vmax (cm/s)	73.6±15.2	73.2±16.4	.881
Mitral valve A Vmax (cm/s)	88.3±16.5	86.0±21	.469
Mitral valve E/A ratio	0.84±0.21	0.86±0.20	.574
Mitral valve E deceleration time (ms)	224.9±49.4	247.3±50.5	.015
Mitral valve lateral E' (cm/s)	7.2±1.8	7.3±2.1	.417
Mitral valve lateral E/e' ratio	11.0±3.3	10.5±3.5	.228

After RDN, there was a significant improvement on LV end diastolic volume index, from 93.3±18.2 at baseline to 110.9±27.4 ml/m2 at one year ($p<0.001$), and on the LV ejection fraction, from 64.5±9.2% to 67.7±9.1% ($p=0.001$), as depicted from Figure 10.

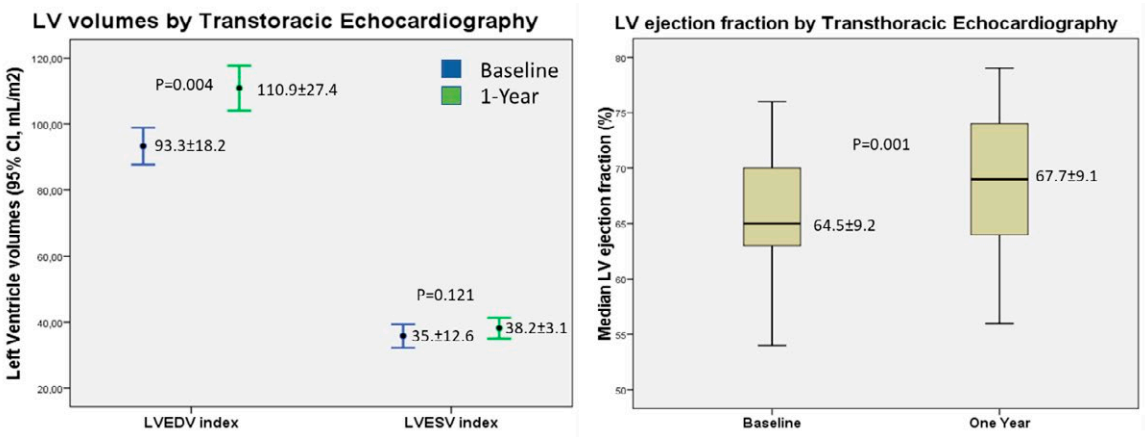


Figure 10: Statistically significant changes on LV end diastolic volume and function, one year after RDN.

Regarding LV mass index, an important endpoint of HTN related TOD, there was a significant decrease (152.3±32.4 to 135.7±33.9 g/m2, p<0.001) one year after RDN, shown in Figure 11.

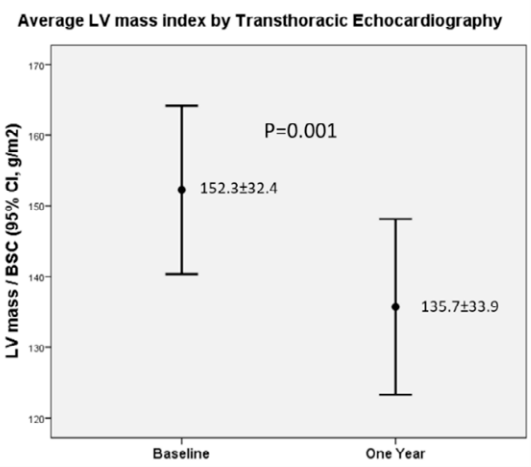


Figure 11: Statistically significant changes on LV mass index, one year after RDN.

Assuming the cutoff value of 5% decrease in LV mass at one-year after the procedure, a responder definition to RDN, 65% of patients (n=20) were considered responders to RDN.

Reduction on LV mass reached statistical significance in ABPM systolic BP responders (n=26), from 148±32 to 133±29g/m^2 (p<0.001). In non-responders (n=5),

LV mass also decreased from 166±23 to 129±15g/m^2 (p=0.05), although not reaching statistical significance certainly due to sample size. (Fig. 12)

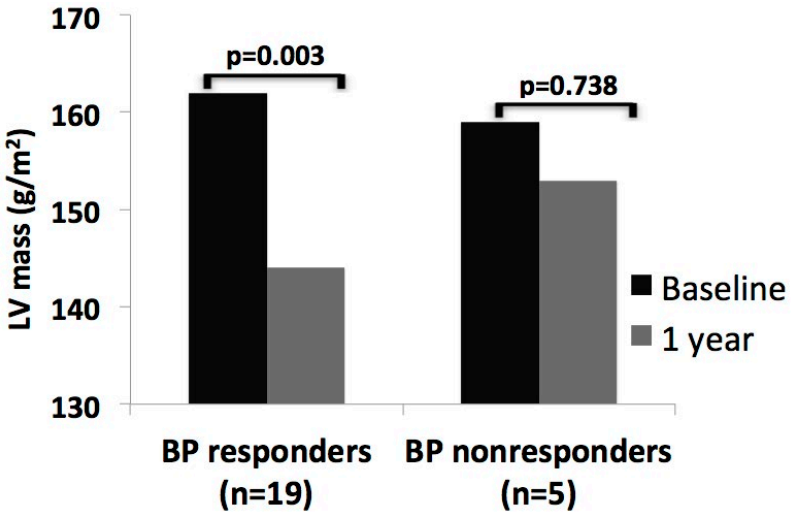


Figure 12. Comparison of LV mass changes at baseline and one-year follow-up, according to BP responders (n=19) and non-responders (n=5) to RDN.

The changes on LV mass index were in line with those observed in BP measurements, Office and on ABPM (Fig. 13).

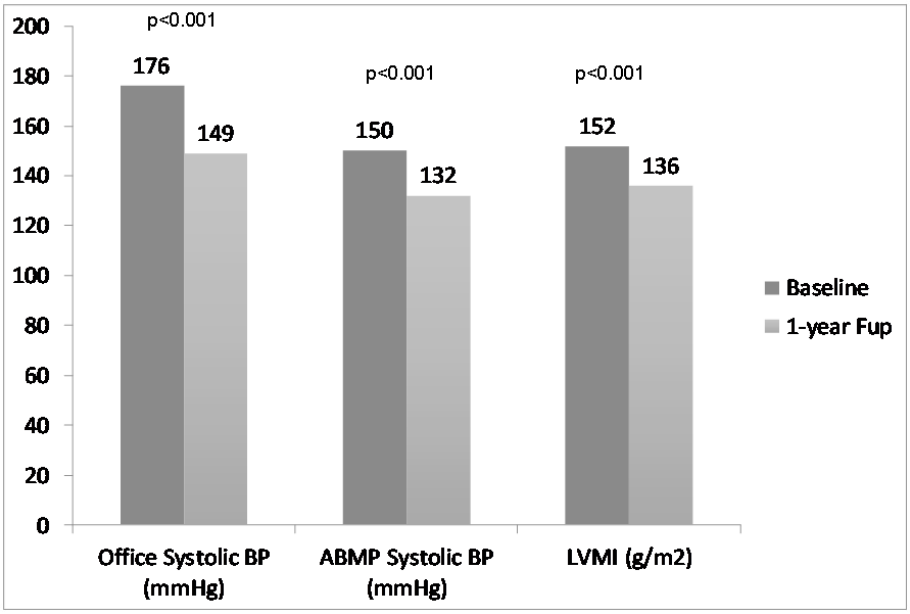


Figure 13. RDN results one year after RDN, on BP (both office and ABPM) and on LV mass index, with significant reductions in all parameters.

It seems, from the scatter-plot graphic (Fig. 14), a cross analysis relationship between changes on LV mass index and on ABPM systolic BP, one year after RDN, for the entire population, that there is not significantly linear correlation, between those two variables, as depicted by the very low r2 values obtained.

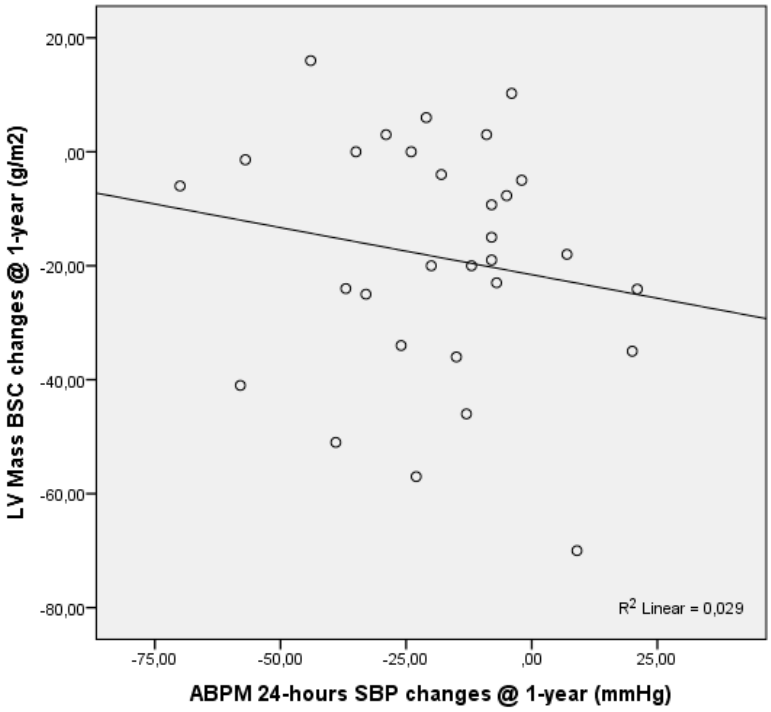


Figure 14. Cross analysis relationship between LV mass index and ABPM systolic BP changes at one-year follow-up.

However, a note should be taken, on the large variability observed in the LV mass index changes, after RDN, that may eventually account for the lack of linear correlation between changes in BP and in LV mass index. (Fig. 15)

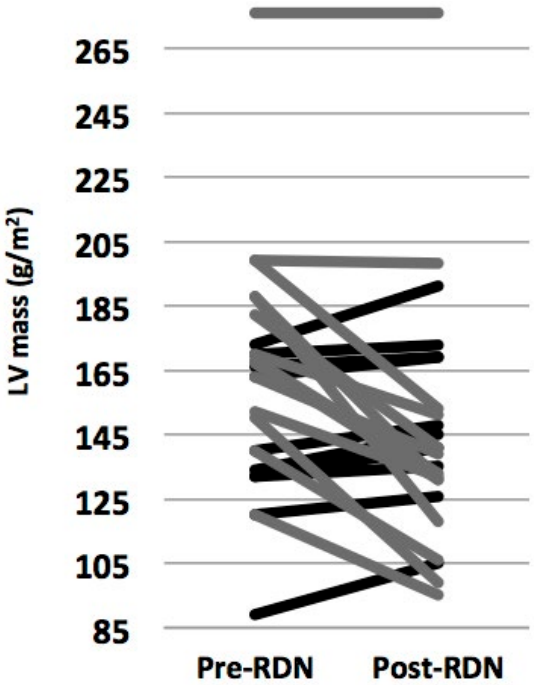


Figure 15. Left ventricle mass changes (g/m2) at baseline and one after RDN, for responders (gray line) and non-responders (dark line).

Transthoracic echocardiography at baseline revealed LV hypertrophy (LV mass index > 115 g/m2) in 27 patients (87%), with a mean LV mass of 152±32 g/m^2. According to the international standard definitions provided in the previous chapter, the large majority of patients had concentric hypertrophy (74%), with only 3% presenting a normal pattern. There were no significant changes in the geometric distributions patterns with RDN until one-year follow-up, eventually due to the small sample size or a short follow-up. The distribution among different geometric patterns is shown in Figure 16.

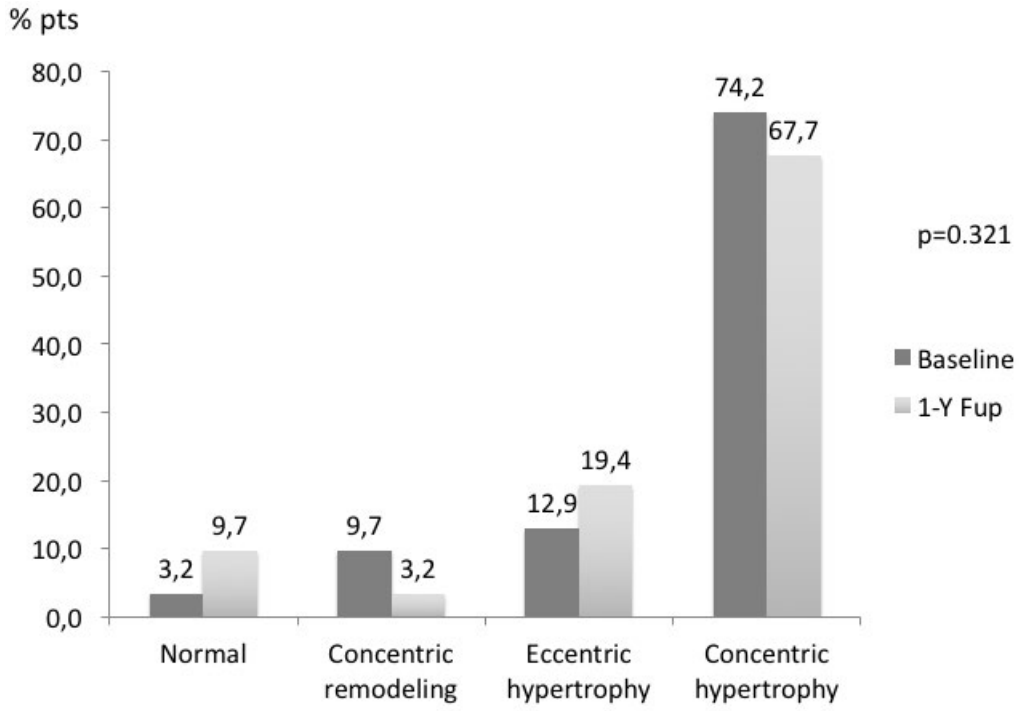


Figure 16. Comparison of different LV geometric patterns at baseline and one year after RDN.

Average left atrium size, changed after RDN, from 32.8±8.3 to 34.1±6.2cm/m^2 (p=0.227), a non-significant change, 15 patients (48.4%) had a left atrium size ≥ 34cm/m2.

For the entire population, after RDN, E/A ratio changed from 0.84±0.21 to 0.86±0.2 (p=0.574), E-wave deceleration time changed from 224.9±49.4 to 247.3±50.5ms (p=0.015) and E/e' ratio changed from a baseline of 11±3.3 to 10.5±3.5 (p=0.228) at one-year, a very mild reduction not reaching statistically significance. Overall, according to previous mentioned criteria, LV diastolic dysfunction was diagnosed in 29 patients (93.5 %), 11 of them (37.9%) had grade 1 diastolic dysfunction and 18 patients (62.1%) a pseudo-normal pattern. There were no patients with a restrictive filling pattern. Once again, there were no statistically significant changes in each diastolic function group from baseline to one year after RDN. (Fig. 17) Two patients, were in atrial fibrillation.

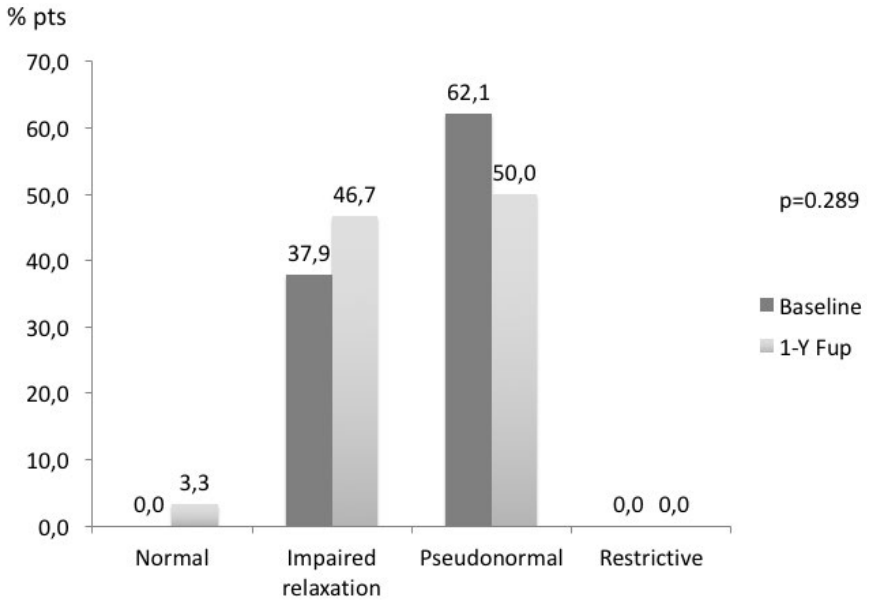


Figure 17. Analysis of LV diastolic function at baseline and one year after RDN. The percentage of patients in each diastolic function group (Normal, Impaired relaxation, pseudo normal and restrictive) is depicted.

Impact on albuminuria and renal function

All changes in renal function and ACR, from baseline to one year after RDN, can be depicted from Table 14.

Table 14: Changes in renal function one year after catheter based renal denervation.

	Baseline	One year	P
eGFR (ml/min/1,73m2)	73.6±25.1	72.5±25.1	0.711
ACR*	25.8 (9.0-574.0)	14.8 (4.5-61.0)	0.007
ACR in ABPM BP responders *	25.6 (8.7-382.8)	15.9 (4.4-55.0)	0.009
ACR in ABPM BP non-responders*	165.0 (8.8-1423.5)	13.6 (5.7-1417.0)	0.345
ACR in ABPM Dippers*	20.8 (6.8-290.0)	9.4 (3.7-41.1)	0.028
ACR in ABPM non-Dippers*	62.3 (9.1-852.3)	20.8 (9.3-197.1)	0.096
ACR in diabetic patients*	48.9 (9.1-1116.3)	23.1 (4.3-123.8)	0.028
ACR in non-diabetic patients*	25.4 (5.2-68.6)	10.9 (3.4-20.8)	0.066

* (mg/g)

There were no changes on the average eGFR from baseline (76.4 ± 24.7 mL/min/1.73m2) to one year after RDN (72.5 ± 25.1mL/min/1.73m2, p=0.711). Five patients had chronic kidney disease defined has having an eGFR < 60 mL/min/1.73m2.

Baseline median ACR was 25.8 (IQR 9.0-574.0) and 15 patients (48,4%), had an ACR > 30 mg/g. One year after RDN, ACR decreased to a median value of 14.8 mg/g (IQR 4.5-61.0 mg/g), a statistically significant decrease in the ACR one year after RDN (p=0.007), with some extreme out layers.

A note for the lower dispersion of ACR at one-year, assessed through the observed changes in the interquartile range from baseline (IQR 9.0-574.0) to one year after RDN (IQR 4.5-61.0 mg/g). (Fig. 18)

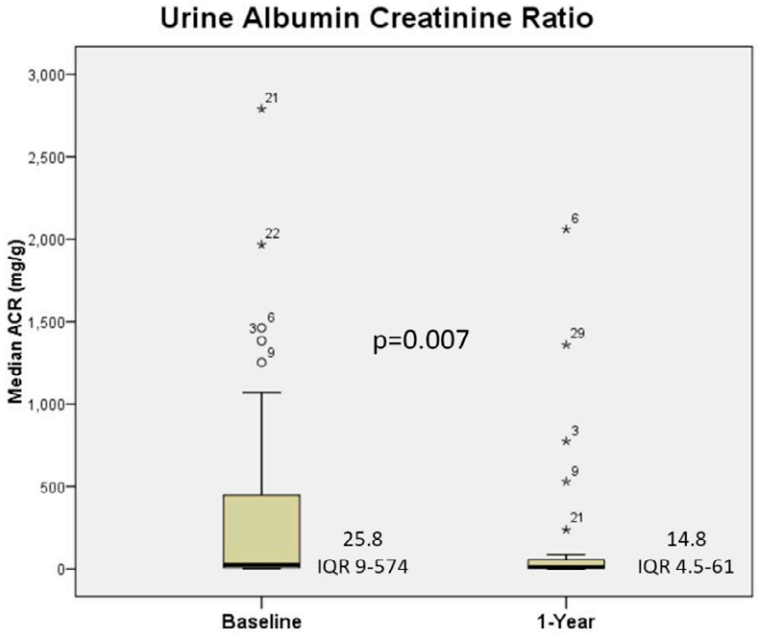


Figure 18. Decrease in the median ACR after RDN.

The distribution of patients across the different classes of urinary albumin excretion, also demonstrates a favourable effect (Fig. 19). Interestingly, a significant reduction in the percentage of patients with an ACR >300mg/g between baseline and one-year, was also found (Fig. 19).

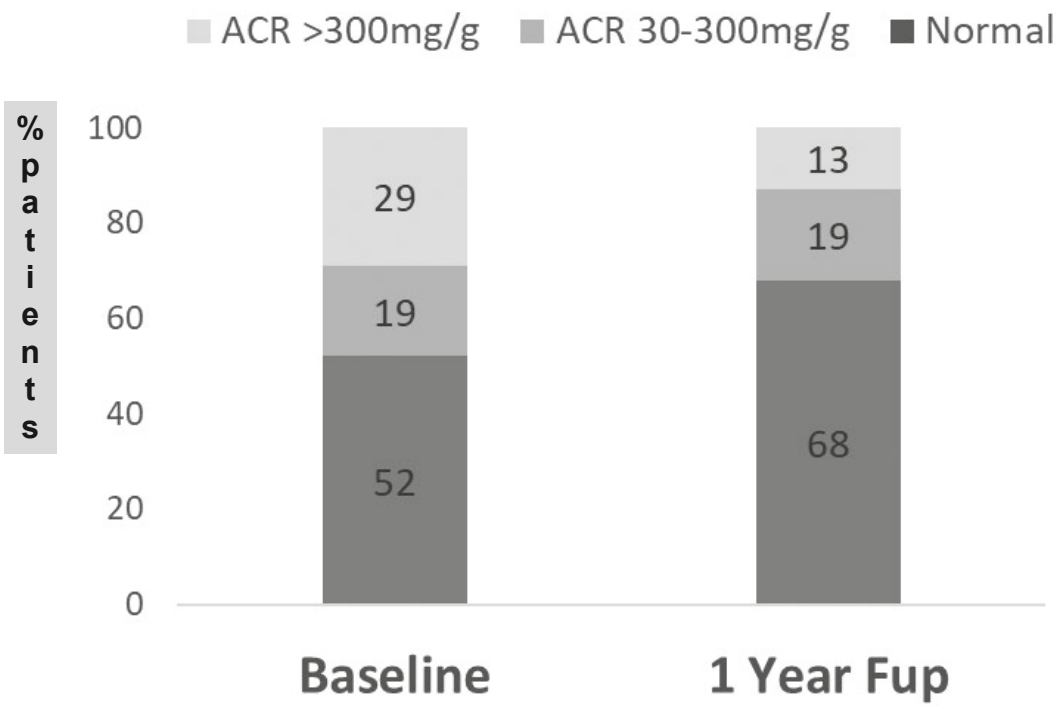


Figure 19: ACR changes after RDN, according to different ACR subgroups.
A decrease in the percentage of patients with an ACR >300mg/g and an increase in patients with normal urinary albumin excretion one year after RDN.

Considering any ACR reduction as a responder to RDN, 77.4% (n=24) of patients were responders. When the ACR results were split according to ABPM systolic BP responder status, a significant reduction in ACR [from 25.6 mg/g (IQR 8.7-382.8 mg/g) to 15.9 mg/g (IQR 4.4-55.0 mg/g), p=0.009] was found in ABPM responders group, to RDN. In the ABPM non-responders group to RDN, a trend in ACR decrease [from 165.0mg/g (IQR 8.8-1423.5 mg/g) to 13.6 mg/dl (IQR 5.7-1417.0 mg/g), p=0.345], was also found, although non-statistically significant (Fig. 20).

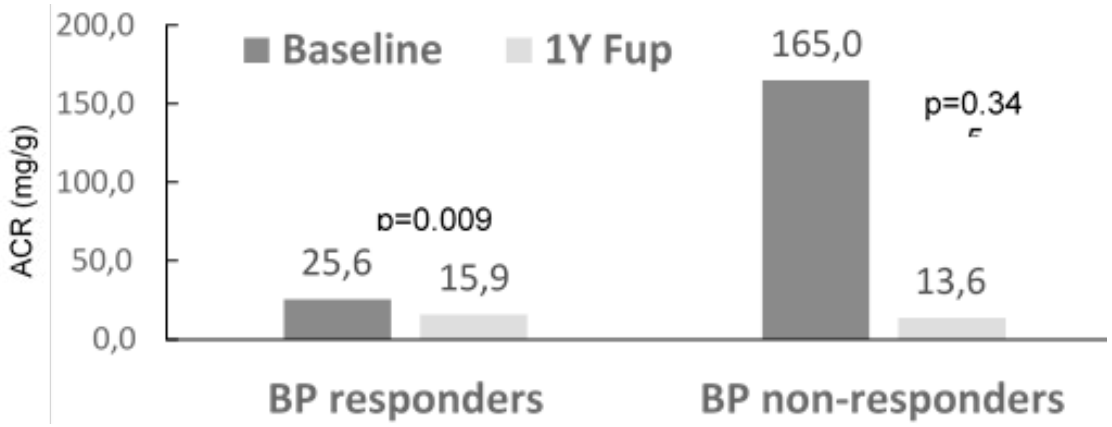


Figure 20: Results of ACR one year after RDN, according to ABPM systolic BP responder subgroups. A significant reduction in the median values of ACR on BP-responder's subgroup, and a numerically decrease also in non-responders.

The same analysis, performed regarding ABPM dipper status at baseline, obtained similar results, a statistically significant decrease in ACR, in patients with a dipper ABPM response (20.8 IQR: 6.8-290 to 9.4 IQR: 3.7-41.1mg/g, p=0.028), in line with those achieved on ABPM responder's status. (Fig. 21).

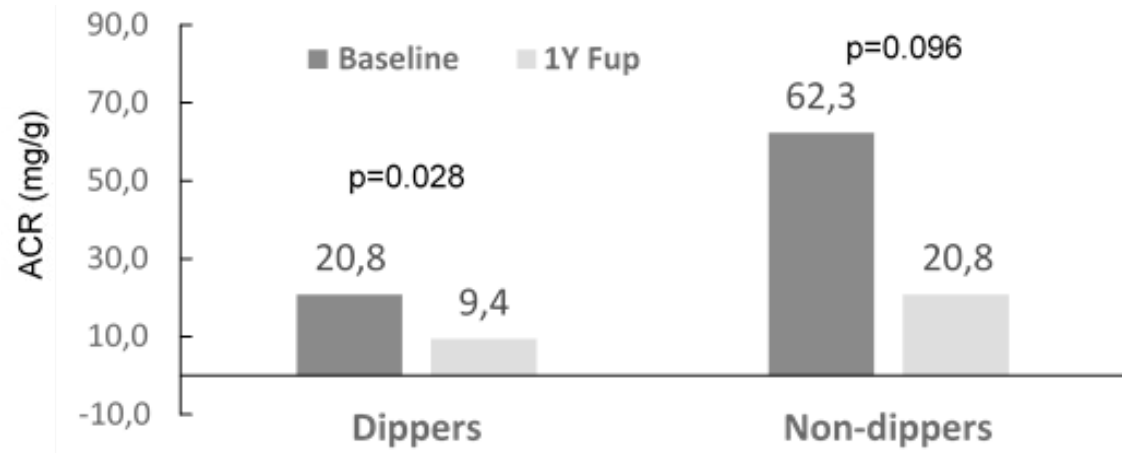


Figure 21: Results of ACR at 1 year after renal denervation according to ABPM Dipper status at baseline. There was a significant reduction in the median values of ACR in the Dippers subgroup, and a numerically decrease in non-dippers.

Patients with diabetes had a higher median baseline ACR value, with statistically significant decrease one year after RDN, from 48.9 (IQR 9.1-1116.3) to 23.1 mg/g (IQR 4.3-123.8) a p=0.028. A trend in ACR decrease was also found in non-diabetic patients, from 25.4mg/g (IQR: 5.3-68.6mg/g) to 10.9 mg/dl (IQR: 3.4-20.8 mg/g) a p=0.066, although not statistically significant, probably due to the small sample subgroup (n=9). (Fig. 22).

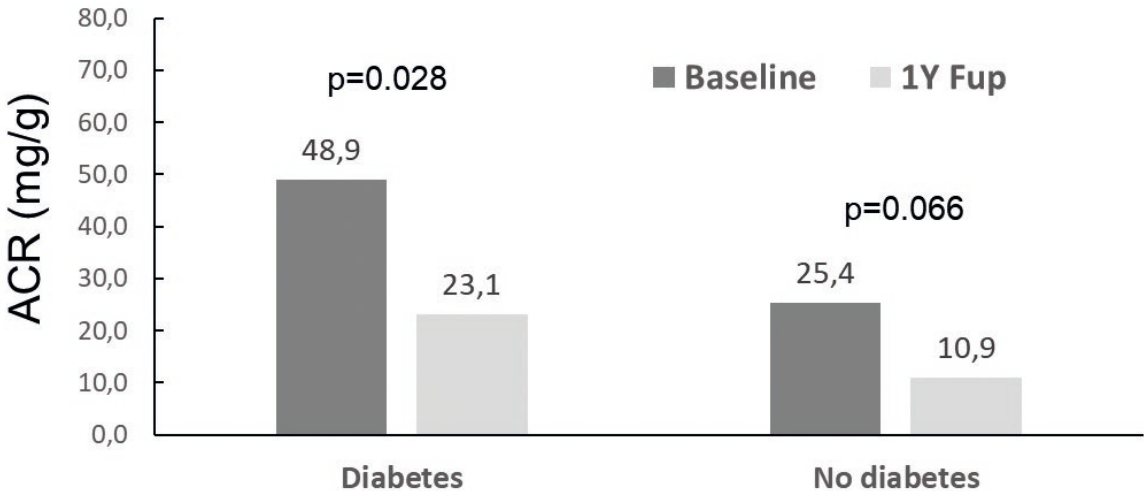


Figure 22: Results of ACR one year after RDN, according to Diabetic status.
A significant reduction in the median ACR in patients with diabetes, and a numerically decrease in the smaller subgroup of patients without diabetes.

Overall, for the entire population, there were no linear correlation relationship between ACR and ABPM systolic BP changes at one-year, as depicted by the low r2 values in the scatter-plot graphic (Fig. 23)

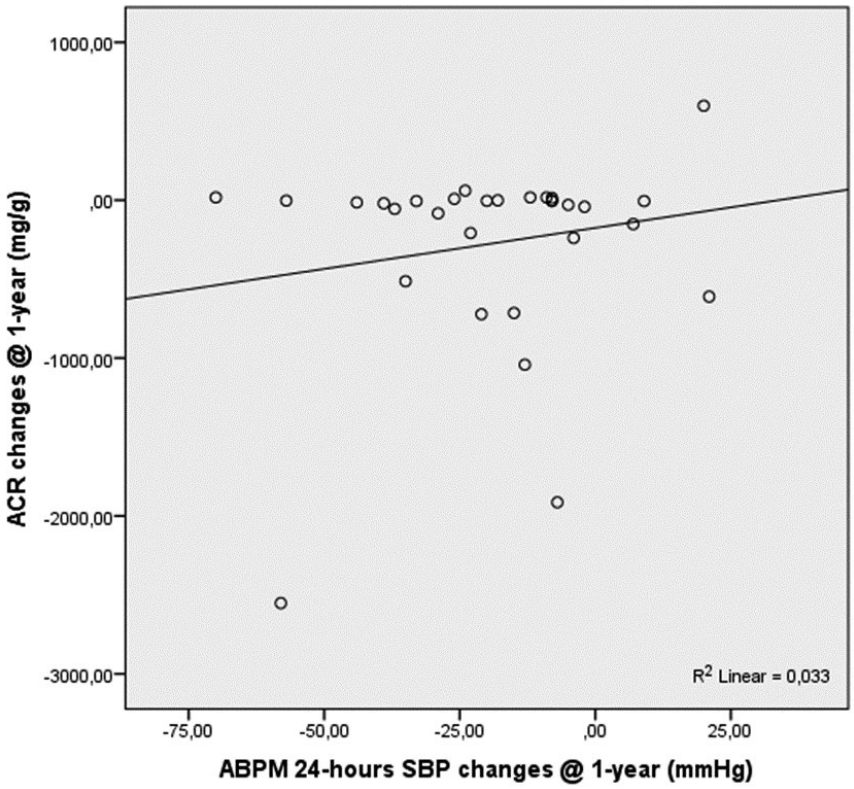


Figure 23. Crosse analysis relationships between ACR and ABPM systolic BP changes at one-year follow-up.

Relationships between ABPM blood pressure measurements, ACR and LV mass index after renal denervation

Since the beginning of modern physiology, in the twentieth century, it has been recognized a direct linear relationship between chronic higher BP and the severity of damages found in organs, known to have a special sensitivity to HTN (TOD), such as the kidneys, the heart and the arterial system. In line with such context, one of the main purposes of this study was to analyses possible linear relationships between detected changes in BP, and changes on selected surrogates of TOD, namely changes on LV mass, changes on renal function and on ACR, after RDN.

In the cross analysis combining the observed simultaneous changes on ABPM systolic BP, on LV mass index and on ACR, one year after RDN, there seems to be no linear relationship between changes either in ABPM systolic BP, LV mass index and ACR, for the entire population, as depicted by the very low r2 values obtained and the scatter plots. (Fig. 24).

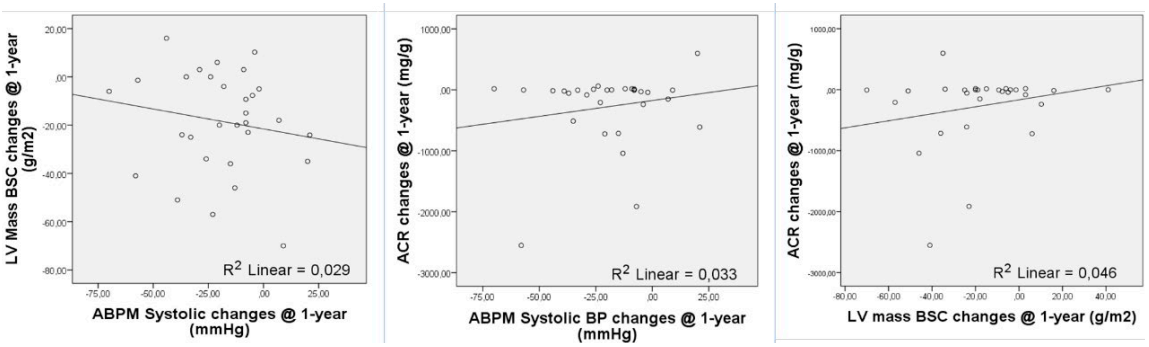
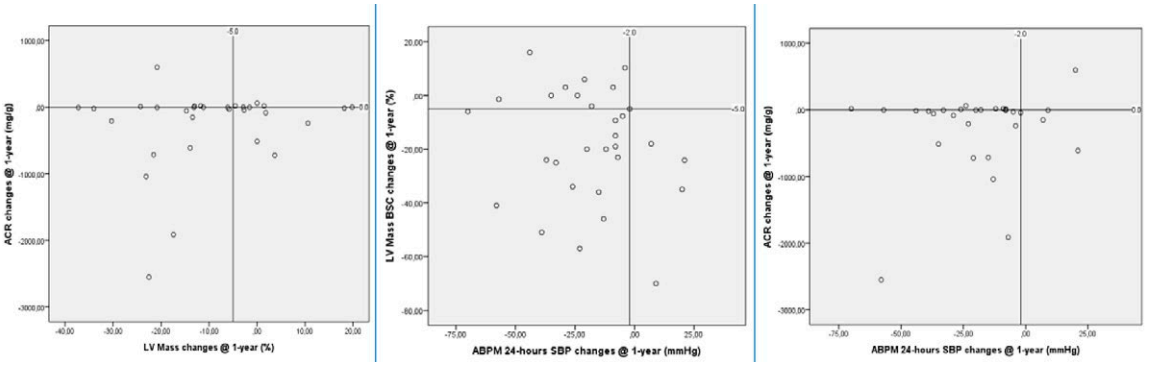


Figure 24: Cross analysis of correlation between changes in average systolic BP on ABPM, ACR and LV mass one year after RDN.

Regarding the response rate to RDN to any of the selected endpoints, in the cross analysis combining the observed simultaneous changes on ABPM systolic BP, on LV mass index and on ACR, one year after RDN, it seems uncommon to find patients that do not respond to RDN in both analysed surrogates (upper right quadrant). Frequently they will respond to both (inferior left quadrant), more evident in the ABPM-systolic BP *vs* ACR relationship (Fig. 25).



BPM SBP vertical line set at 2mmHg for responder in ABPM systolic BP reduction.
ACR horizontal line set at 0 mg/g for responder in ACR reduction.
LV Mass vertical and horizontal line set to 5% reduction in LVM.

Figure 25: Cross analysis of responders one year after RDN, to ABPM systolic BP, LV Mass and ACR.

We must consider the presence of some extreme out layers in the ACR distribution, which may bias the relationship with the other endpoints. If we exclude these ACR out layers values from the analyses, the same conclusion may be drawn, as in previous graphics, (Fig. 26).

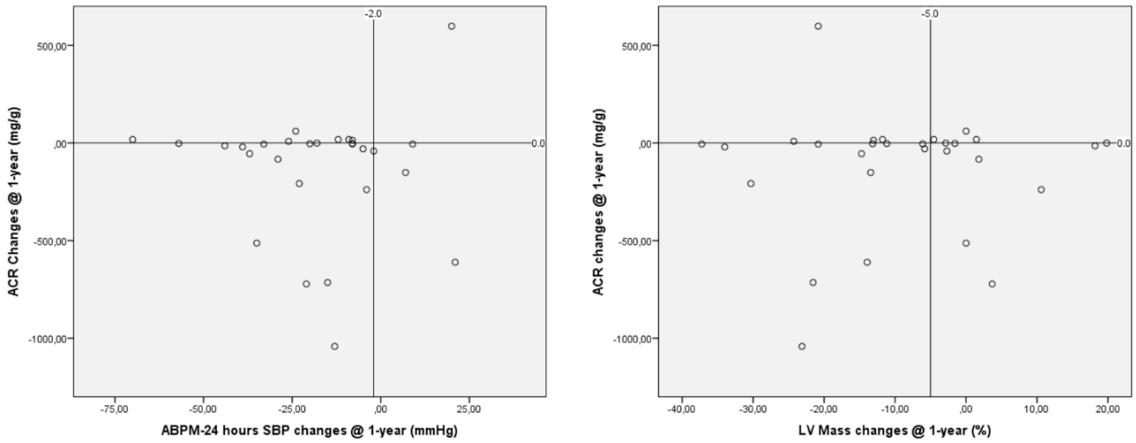


Figure 26: Cross analysis of responders one year after RDN, excluding ACR extreme out layers (those with ACR >1500).

We also looked specifically to the proportion of responders to RDN, from all patients (n=31), in any of the selected endpoints, isolated or in combination with each other (Fig. 27).

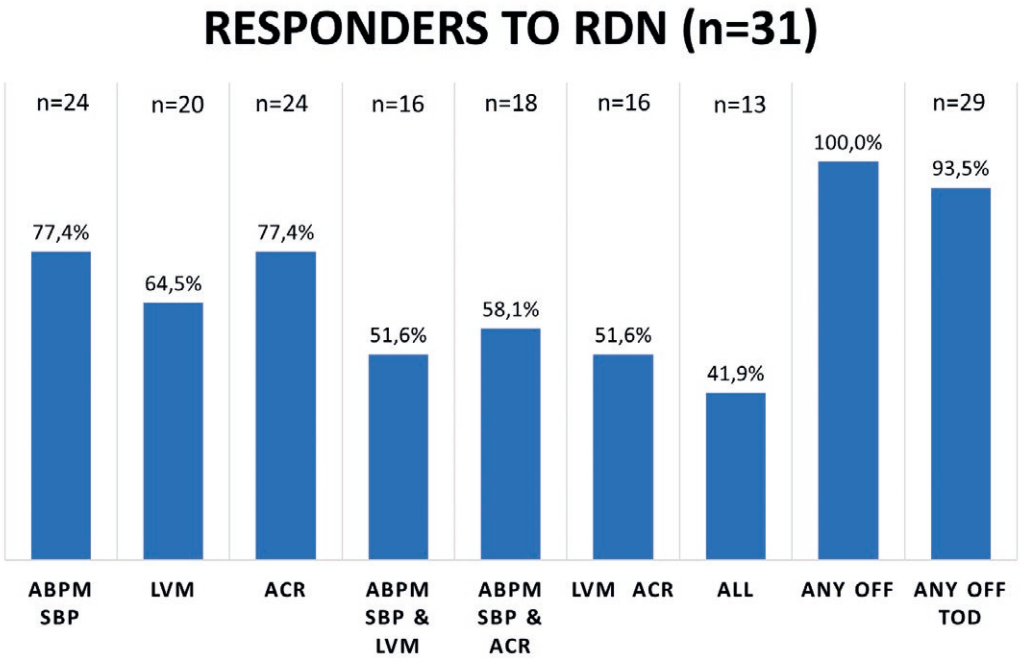


Figure 27: Rate of of responders to RDN at one year, regarding the studied endpoints, according to predefined cutoffs: >2mmHg decrease in average ABPM systolic BP, > 5% decrease in LV mass and any decrease in ACR value.

From that analysis, it seems that there is some consistency among them. All patients showed a response to RDN in at least one endpoint, according to the predefined cutoffs, either a decrease on ABPM systolic BP, or on LV mass or on ACR. Less patients had responded to RDN in all endpoints, but still a clinically significant amount, 42% (n=13),.

Looking for the proportions of responders to each isolated endpoint, the values seems to leverage around 65% to 77%, with lesser responders regarding LV mass (65%) and equal proportions in ABPM average systolic BP and ACR values (77%). Nonetheless, all patients with severe resistant HTN, once submitted to RDN, responded at least to one important known surrogate of a major clinical event, HTN, proteinuria or myocardial hypertrophy.

The small patient's sample in this study make-it impossible to achieve statistical significance

The reason for such puzzling discrepancies is presently unknow. It may represent a different sensitivity to RDN, of underlying mechanisms driving myocardial hypertrophy, BP rising or proteinuria, or eventually, it may be related to a different role of SNS activity in their underlying mechanism. But at present, it is still an open question.

Safety of renal denervation

An important concern of the RDN procedure was safety. There were no major complications events, namely death, serious bleeding, emboli or life-threatening arrhythmias.

No kidney related complications occurred during the study, such as chronic or transient loss of kidney function, kidney perforation or renal artery perforation, occlusion, dissection, stenosis or pseudoaneurysm.

Concerning the vascular site access, there were 3 mild hematomas spontaneously resolved, and 1 femoral pseudoaneurysm treated with surgery. All of them without any permanent sequelae. There were other minor events such as symptomatic hypotension (one patient), orthostatic hypotension (one patient) both easily controlled with drug adjustment. No other minor complications such as contrast related, electrolyte disturbances or with procedure related medications.

A major concern regarding the renal impact of RDN, was changes in renal function, namely GFR. There were no significant changes in creatinine as in eGFR as depicted from Figure 28.

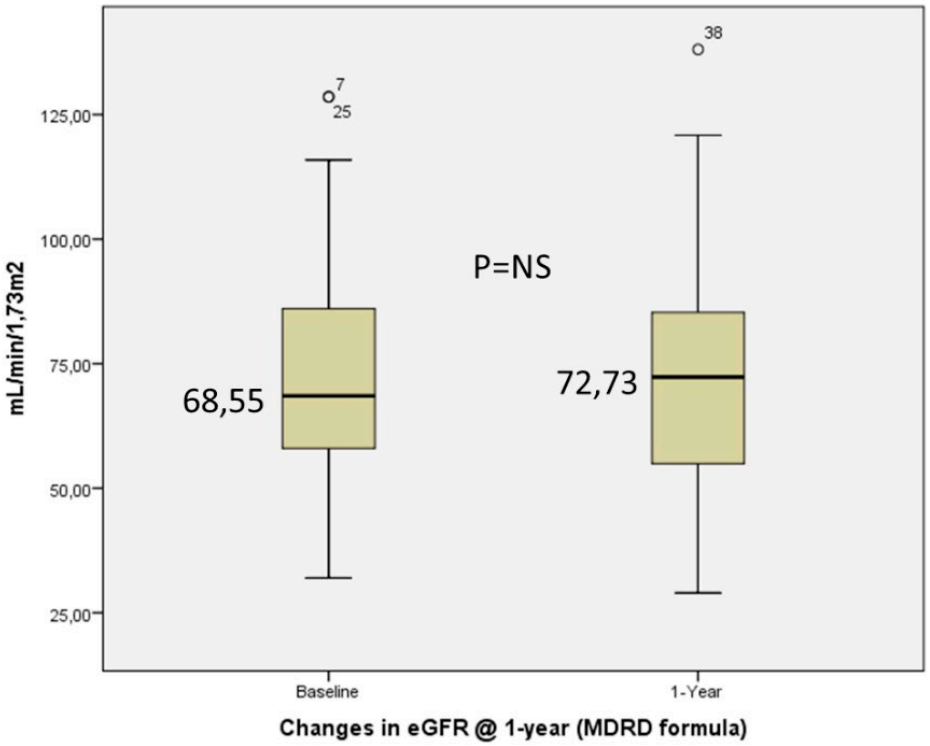


Figure 28: Changes in the median values of eGFR, between baseline and one year, after RDN.

In summary, renal denervation was a very safe procedure. Even considering, that those patients were frequently obese (68%), some of them severely obese (9 patients, 29%, with a BMI>35kg/m2), which significantly increases the risk of femoral access complications, the overall rate of clinically relevant access vascular complications, demanding special attention, was very low, 3% (1 patient), managed without any permanent sequalae.

CHAPTER 4 DISCUSSION

This chapter is partially adapted from the following articles:

Renal denervation for resistant hypertension.
M de Sousa Almeida, P de Araújo Gonçalves, E Infante de Oliveira, et al.
Rev Port Cardiol. 2015; 34 (2):125-135.

Renal Denervation in patients with resistant hypertension: six months results.
H Dores, M de Sousa Almeida, P de Araújo Gonçalves, et al.
Rev Port Cardiol. 2014 33(4): 197-204.

Impact of Renal Sympathetic Denervation on Left Ventricular Structure and Function at 1-Year Follow-Up.
M de Sousa Almeida; P Araujo Gonçalves, P Branco et al.
PLoS ONE 2016 11(3): e0149855. doi:10.1371/journal.pone.0149855

Changes in albumin-to-creatinine ratio at 12-month follow-up in patients undergoing renal denervation.
H Sousa, P Branco, M de Sousa Almeida et al.
Rev Port Cardiol. 2017 36(5): 343-351.

Sections:

Summary

In the present chapter, a comprehensive discussion of the main results of the thesis is provided. The main findings of our study, at one-year follow up, were: 1) RDN in patients with severe resistant hypertension was associated with significant reduction on both office and 24-hours ambulatory blood pressure measurements; 2) There was a significant reduction on left ventricle mass index, a recognized marker of hypertension related target organ damage; 3) There was a significant decrease in the median urinary albumin to creatinine ratio, and in the percentage of patients with an urinary albumin to creatinine ratio >30mg/g, observed both in blood pressure responders and non-responders, without changes in estimated glomerular filtration rate; 4) there were no linear relationships among the changes observed after renal denervation, in the three studied endpoints: ABPM systolic blood pressure; Left ventricle mass index and urinary albumin creatinine ratio. These 4 main findings are discussed in separate subchapters.

The initial results with RDN were very promising [1-3], but the simplicity HTN-3[4] failed to meet its primary efficacy endpoint, raising doubts about the real biological effect of this catheter based treatment on HTN. The unexpected negative results of HTN-3, were extensively discussed and many possible explanations for the results were pointed out, clinical, patient selection, extensive changes in medication during the study and procedure related, mostly regarding the low number and inadequate pattern of radiofrequency applications.[5]

The results from the recent studies, Spyral HTN off-med[6] and Radiance Solo Trial[7], helped to clarify some of the doubts raised by Symplicity HTN-3 Trial. These randomized blinded trials, with sham controlled arms, in hypertensive patients without drug treatment showed a significant decrease on systolic and diastolic ABPM BP in patients submitted to RDN when compared to the sham-controlled group, providing the scientific evidence for an isolated clinical meaningful effect of RDN on HTN.

Almost all trials performed to date used as a marker for RDN efficacy, the single endpoint of changes in BP from baseline. However, its well known in the medical community, the close relationship between HTN and damages in some specific organs – HTN target organs damages.[8-11] Such damages have a close relationship with patients 'prognosis.[12-16]

The aim of these study was to evaluate in an integrated approach, the impact of RDN in BP and in specific damages on organs known to be targets of HTN long-term harmful effect. Such impact was assessed through the simultaneous evaluation of changes on BP and on the LV structure, function and on kidney function. Up-to-date, to the best of our knowledge, it's the first time such approach has been performed.

At one year after RDN, the main findings of our study were: 1) RDN in patients with severe resistant HTN was associated with significant reduction in both office and ABPM BP; 2) There was a significant reduction in LV mass index, a recognized marker of HTN related TOD; 3) There was a significant decrease in the median ACR, and in the percentage of patients with an ACR >30mg/g at one-year follow up; observed both in BP responders and non-responders, without changes in eGFR; 4) There were no linear relationships among the changes observed after renal denervation, in the three studied endpoints: ABPM systolic blood pressure; Left ventricle mass index and urinary albumin creatinine ratio.

Each one of these results, due to their clinical relevance will be discussed in a separate subchapter.

Impact on blood pressure

Renal denervation has been associated with a significant reduction on both office and ABPM BP in many trials[1, 17, 18] and registries.[19] Our findings seems to be in line with such results, revealing a statistical and also clinical meaningful drop on office and ABPM BP at one year after RDN, with an average reduction on office systolic BP of 27 mmHg (from 176±24 to 149±13mmHg, p=<.001) and 11 mmHg on diastolic BP (from 90±14 to 79±11mmHg, p<.001). Although in a smaller degree, similar results were obtained in ABPM measurements: an average decrease of 18mmHg on systolic BP (from 150±20 to 132±14mmHg, p=<.001), 9 mmHg on diastolic BP (from 83±10 to 74±9mmHg, p<.001) and 5mmHg on average (67±18 to 58±13mmHg, p=0.001) on ABPM pulse pressure, an important clinical surrogate of HTN related TOD. This favorable impact on BP also meant a readjustment in the number of antihypertensive drugs prescribed, with a reduction in the number of drugs in use at one year after RDN,

from 5.8±1.1 to 5.0±1.2 (p=0.002) and in the number of drug classes, from 5.5 to 4.9±1.1 (p=0.015).

The rate of responders to RDN after one year, were 83.9% considering a drop of 10mmHg in office systolic BP and 71% considering a drop of 2mmHg in ABPM. Those results are all in line with most of the previous and more recently published trials and studies.[1, 6, 7, 17, 20-22]

Symplicity HTN-2 study,[17] the first randomized trial to show a BP reduction at six-month follow-up after RDN, reported an average reduction of 32 mmHg on systolic BP and 12 mmHg on diastolic BP, but other trials like Prague-15,[23] DENERHTN[22] and the more recent trials,[6, 7, 21] showed a more modest but still consistent decrease in BP, more in line with those obtained in this study.

Off notice is the low percentage of patients with true severe resistant HTN, which were considered suitable by our RDN-Team for RDN. From a total of 318 patients, with suspected resistant HTN evaluated in a dedicated HTN outpatient clinic, only 65 were considered eligible for RDN, a ratio of 5 to 1 (20%). The main reasons for exclusion were adequate BP control after drug adjustments (n=139, 44%), severe renal dysfunction with a eGFR <30mL/min (n=85, 27%), and the identification of secondary causes of HTN (n= 31, 10%), accounting together for more than 80% of the refusals.

The HTN treatment strategy adopted in this study of maximum tolerated drug treatment, before recommending RDN, may eventually had an influence in the final results. In addition, the number of patients treated with spironolactone in this study (74,2% at baseline and 83.9% at one year) was much higher in comparison with some previous published studies.[4, 17, 24] Such disparity mean that such drugs are more tolerable than previously noticed, but also, that the patients selected to RDN were most probably true resistant hypertensive. DenerHTN trial[22] used spironolactone in 79% of patients with resistant HTN submitted to RDN, similar to this study and achieved similar results on ABPM BP decrease, reinforcing the concept that RDN on top of optimal anti-hypertensive therapy decreases BP even further. Interestingly, one predictor of HTN decrease with RDN, in HTN 3 trial[5], was previous treatment with spironolactone.

The use of spironolactone may have had an influence on the final results, not only as a predictor of HTN decrease with RDN, but also by its direct effect on LV hypertrophy[25, 26] and on proteinuria,[27-29] opening an argument on the possible synergistic effect of RDN on top of optimal anti-hypertensive drug treatment including spironolactone.

A limitation of this registry was the absence of control of drug taking by the patients, by serum or urine testing. A previous study, assessing adherence to HTN drug therapy through measurement of serum antihypertensive drug levels, revealed that 34.5% had no detectable drugs in circulation and that 65.5% met criteria for nonadherence to drug treatment.[30] In the more recent Spyral HTN-ON MED trial[21], 37.5% of patients had incomplete or non-adherence to drug therapy, with a high variability along the study, even knowing that they were under close scrutiny and drug testing. Such limitation can also make a case for the synergistic effect of RDN and drug therapy, as the long-term effects of the former are not depend on patient's will and can therefore limit the negative impact of patients non-adherence on HTN treatment, with its "always-on effect".[21]

In this background, it is difficult to be certain whether the impact of RDN on BP levels is due to the intervention itself, or to a possible better compliance to therapy, or even a placebo effect, so care should be used in translating these results to clinical grounds, until newer studies and results are published.

Another important issue is that sympathetic activity may vary along the day, from patient to patient, and in different stages of HTN. It is therefore crucial to measure sympathetic activity, ideally before and after RDN, to identify objective parameters able to predict the response to RDN, to enable better selection of patients with greater potential to respond and to identify possible non-responders, avoiding unnecessary procedures and risks. Until then, the combined use of a safer technique as RDN with drug treatment, may overcome such limitation, as drugs can still exert their effect on those hypertensive patients with unidentified normal sympathetic activity.

Finally, our results come from a registry with a very rigorous selection process of patients for RDN, perceived from the high number of antihypertensive drugs (median 5.8), the wide use of spironolactone, the high baseline office and ABPM BP as depicted from the patient selection flowchart, with an almost 5 to 1 proportion between patients that were evaluated and selected. It is worth mentioning that an median of 5.8 drugs were higher than those reported by other similar studies[31, 32] (ranging from 4.3 in the study of Schirmer SH et al[31] to 4.7 in the study of Brandt MC et al.[32]).

Considered to be a more accurate evaluation of treatment impact on BP, ABPM was used in all patients.[33] Lastly, our results were evaluated one year after RDN, a significantly longer follow-up than those reported by some previous studies, who evaluated patients at 6 months follow up.[31, 32, 34]

Impact on left ventricular structure & function

Left ventricle hypertrophy is one of the most important markers of HTN related TOD and has been associated with an increased rate of cardiovascular events and death, independently of BP values[12-14]. With this rational, this study pursued to evaluate the impact of RDN on LV structure and function, at one-year follow-up.

In this study, a significant reduction on LV mass was noticed, in line with previous studies that also used transthoracic echocardiography [31, 32] or cardiac magnetic resonance [34]. Brandt MC et al[32] in a study including 46 patients, using transthoracic echocardiogram, found that RDN was associated with a significant reduction in LV mass index and improvements in mitral valve lateral E/E′, indicator of LV filling pressure. In another small study using a similar methodology, Schirmer SH et al [31] assessed the impact of RDN on LV hypertrophy by echocardiography and were able to document that in patients with resistant HTN, the observed reductions in LV mass were similar across terciles of baseline systolic BP, suggesting that the pathophysiology behind LV hypertrophy could be related to a direct effect of SNS hyperactivity, not dependent on BP or heart rate. But, it is worth mentioning that in the present study, the use of drugs, was on average 5.8, higher than 4.3, reported by Schirmer et al[31] and 4.7, reported by Brandt MC et al [32].

In this registry a linear correlation between LV mass and ABPM systolic BP changes at one-year, was not found, who suggests that LV hypertrophy reduction after RDN might be affected by other mechanisms beyond BP reduction. Previous studies[35] described that for similar BP reductions, different pharmacological agents had a different impact on LV hypertrophy. In one interesting study, for the same magnitude of BP reduction, a greater regression in LV hypertrophy was achieved with a drug combination that targeted simultaneously the neuroendocrine activity (both the RAAS and the SNS activity).[36]

A recent cardiac magnetic resonance study [37] evaluating the impact of RDN on LV structure, in hypertensive patients, observed that the reduction on LV mass, besides the reduction in myocyte hypertrophy was also associated to an absolute reduction on collagen content and diffuse interstitial myocardial fibrosis, helping to clarify the possible mechanisms underlying the LV mass reduction observed in previous studies. In another study, using cardiac magnetic resonance imaging, Mahfoud F et al [34] also verified that at 6-months follow-up, RDN was associated with a significant reduction in LV mass index, an improvement in LVEF and a reduction in LV circumferential strain, a surrogate of diastolic function.

Taken together, these studies are consistent with the favorable impact of RDN in LV mass regression and improvement in several markers of diastolic function. In our study, we also found a significant reduction in LV mass but there were no significant changes in transthoracic echocardiogram parameters of diastolic function. In addition, we didn't find any reduction in left atrial volume index. There was a small but significant increase in LVEF and LVEDV, which could be explained, at least partially, by the numerically lower heart rate at one-year follow-up documented on office and on ABPM recordings. This small increase in LVEF is in line with some [32, 34] but not all of previous studies [31].

The lack of a linear correlation found in this study, between the observed changes in BP and the observed changes in LV mass, raises some doubts on the assumption, that a sustained high systemic arterial BP and the consequential increased after-load is the main stimulus promoting concentric hypertrophy on LV cardiomyocytes.[38-40] Our results and those from previous studies are not in line with such hypothesis.

An alternative explanation, for such a lack of correlation, may rely in the fact that an increased SNS activity is responsible, in different degrees, for a rise in BP by well--known mechanisms,[41-43] and also for an increase in LV mass by either a direct stimulus on cardiac myocytes,[44] or indirectly through the activation of RAAS axis, a well-known promoter of LV hypertrophy.[45]

In such a scenario, an approach with drug-agents blocking the RAAS axis combined with RDN reducing the SNS activity, may well be much more efficacious in reducing BP and the overall impact of HTN related TOD, with a better improvement of cardiovascular risk and prognosis of hypertensive patients, instead of simply pursuing the reduction of BP either with drugs or RDN. This reinforces the concept that solo changes in BP may not be the best surrogate of efficacy, in assessing overall RDN impact on the prognosis of patients with severe resistant HTN.

Impact on renal function

Concerning the impact of RDN on renal function, the main findings of our study were: 1) a significant decrease in the median ACR and in the percentage of patients with an ACR >30mg/g, between baseline and one year after RDN; 2) a reduction in ACR observed in both BP responders and non-responders; 3) there were no changes on eGFR.

The ACR is a well-recognized marker of long term HTN damaging impact on renal function, who has been linked to unfavourable cardiovascular outcomes in

several studies.[46-48] In a recent study, Ott C et al[49] found a significant reduction on ACR at 6-months follow-up, in 59 patients with resistant HTN (average ABPM systolic BP of 156mmHg, treated with an average of 5.5 drugs). In contrast with the previous study, we also included patients with normal (<30mg/g) baseline ACR and therefore our median values are lower than those reported by Ott C et al.[49] Even with this mixed population of different ACR baseline profiles, with half of them having a normal urinary albumin excretion (51.6% with ACR <30mg/dl), a mean age of 65 years, an average baseline ABPM systolic BP of 150mmHg and a treated with median number of 5.8 drugs per patient, our results are very similar to the study of Ott C et al.[49] Even though, we included a higher percentage of patients with type 2 diabetes (71%), compared to the 51% in the study from Ott C.[49] Another single centre study, recently published, Verloop et al [50] achieved only a modest decrease in HTN after RDN, and failed to demonstrate any significant decrease in both LV mass (by cardiac magnetic resonance) and urinary albumin excretion. These results are in contrast with others in previous studies and with our owns. To understand such discrepancy, some differences in the selected population should be taken in to consideration. In the study of Verloop et al[50], the mean age was lower (58 years) and so was the median of 4 antihypertensive drugs, as opposed to 5.5 in the study of Ott C et al[49] and 5.8 in our study, so was the much lower prevalence of diabetes (only 15%) and the fact that the authors didn't excluded patients with an eGFR<45ml/min.

One interesting observation in our study is the fact that the reduction on ACR was also found in BP non-responders, although not reaching statistical significance, probably due to the small sample size of this subgroup. This raises the question of whether RDN, by reducing sympathetic hyperactivity, might have a positive direct effect on glomerular endothelium function, independently of the hemodynamic effect derived from the BP reduction, since there is a close relationship between urinary albumin excretion, glomerular endothelium dysfunction and glycocalyx damaging. [47, 51]

We may raise the hypothesis, if a modulation of SNS through RDN will positively affect endothelial dysfunction, a common denominator in cardiovascular and renal disease, normally associated to an increased SNS activity.[52] If so, such a favourable impact on endothelial dysfunction may affect, in different degrees, BP and glomerular function and ultimately have a favourable impact in the overall cardiovascular risk, which is the ultimate-goal for these patients, independently of BP changes *per se*.

These results are in line with those found for LV mass index and taken together reinforces the concept that solo changes in BP may not be the best surrogate of efficacy for the overall impact of RDN on the prognosis of patients with severe resistant HTN.

These favourable results of reduction on ACR and on LV mass index, found in this study, should be interpreted in the context of the high cardiovascular risk of these patients with resistant HTN, [11, 53] if proven to be consistent, such reductions are expected to contribute significantly to lower the overall cardiovascular risk, commonly associated to severe resistant HTN, although at the present, there is no published studies on the prognostic impact of RDN in major clinical outcomes.

Relationships between changes on blood pressure and on target organ damage

One aim of this study was to analyse in the entire population, the relationships between changes in BP and on surrogate markers of HTN related TOD, by the simultaneous evaluation of the RDN impact, at one year, on BP, LV function and structure and on renal function. To pursuit such task, a cross analysis of BP, ACR and LV mass responders to RDN was performed, looking for the presence of linear correlations and their responder status distribution in the scatter plots graphics.

The following results were found. When depicted alone, the rate of responders to each one of the selected endpoints, showed some consistency, ranging from 64% on LV mass to 77% on ABPM systolic BP and ACR. When combined, the rate of responders, decreased significantly, still, almost half of the patients (41%) responded simultaneously to all endpoints (ABPM systolic BP; LV mass and ACR) and all of them responded at least to one endpoint.

Such discrepancy between the rate of responders to a single endpoint or to a combined set, could be related to a lack of a linear relationship between them. When we analysed the relationships between changes on BP, on LV mass and on ACR, no linear correlation was found between them. Such results don't come as a surprise, as they were previously reported in other studies. [44, 54-57]

As previously mentioned, it is understood that LV and proteinuria are direct consequences of long-term chronic mechanical stress of high BP, on the LV myocardial and arterial walls, eventually causing endothelial dysfunction, and changes of glomerular permeability to proteins.[58, 59 57, 60, 61] In such model, it is perceived, that the correction of HTN, with a sustained drop on BP, will have a negative feed-back on the

HTN mechanical stress over the cardio-renal system, thus, a positive effect either on LV mass or in the ACR, which should be proportional to the degree of BP decrease. In such "mechanical" context, it is expected to find a certain linear relationship between the degree of BP decrease after RDN and the degree of improvement in the HTN related TOD, LV mass index and ACR values. That was not the case in this study with RDN.

The lack of any linear correlation between the decrease in BP, achieved after RDN, and the changes in HTN related TOD, assessed through LV mass and ACR changes, raises doubts if other factors, besides high BP mechanical trauma, are also implicated in the underlying mechanisms of HTN related TOD, such as LV hypertrophy and glomerular endothelial dysfunction.[44, 56, 62, 63] In this case, isolated changes in BP may not be sufficient to alter the stimulus (or may not be the prime stimulus) driving myocardial hypertrophy or glomerular endothelial dysfunction.

If this hypothesis is confirmed, then any HTN treatment strategy, aiming only BP decrease, may not be sufficient to achieve the ultimate goal of reducing long-term HTN--related TOD and the ultimate goal of preventing major clinical events affecting HTN patients.

A possible alternative, to be tested in future studies, is a treatment strategy aiming, in simultaneous, the sympathetic-endocrinal system (SNS and RAAS axis), known to have a pivotal role in BP control and eventually in HTN aetiology. If such approach proves to be more efficacious in the control of HTN and in the prevention of TOD, then current strategy of using only drug treatment as a first option to HTN treatment, must be changed for a combined initial approach of RDN and drug treatment.

Off course, other studies and trials are strongly needed to prove such concepts and to increase our understanding on the full scope of RDN impact in the cardiovascular and renal systems.

Procedure safety

One of the most important results of this study was procedure safety. There were no major complications, clinical or kidney related, such as chronic or transient loss of kidney function and there was no change in eGFR at one year after RDN. Some minor events, like symptomatic hypotension (one patient), orthostatic hypotension (one patient), were easily controlled with drug adjustment.

The only concern in this study were complications related to femoral access site: 3 mild hematomas spontaneously resolved, and 1 femoral pseudoaneurysm treated with surgery during the hospital stay (1 in 31 patients, 3,2%). All of them without any permanent sequelae. We should consider, however, the high-risk population for femoral access procedures. Obesity (BMI>30kg/m2) was present in 68% of patients and severe obesity (BMI>35kg/m2) in 29% (9 patients), who makes femoral access particularly risky. Overall the rate of clinically relevant, access vascular complications needing special attention, were very low, considering the single case of pseudoaneurysm successfully managed without sequalae.

In previous studies,[1, 4, 6, 7, 20, 21, 64] the rate of serious complication related to RDN were also very low, ranging from 0 to 13%, in line with this study, although in this study, the BMI was higher than in previous studies (31.8 vs 30.6kg/m2), the patients were sicker, with more antihypertensive drugs, including spironolactone, all of them with severe HTN resistant to drug treatment and 71% were diabetics, all predictors of vascular access complications.[65]

In the future, the standard use radial access, will decrease even further the rate of complications,[66] allowing the treatment of more complex and higher risk patients. Of notice is the fact that, this research group performed the first case of RDN by radial access,[67] that will became the standard access route, once dedicated radial devices become available.

Study limitations

This study has several limitations that must be acknowledge. It is a single center prospective registry, with a small sample size population, making difficult to draw definitive conclusions on RDN safety and efficacy and to identify predictors for non-responders.

Routine assessment of renal artery during follow-up, to identify local vascular complications, was not systematically performed in all patients, preventing a full assessment of long-term renal artery safety, after radiofrequency ablation.

There was no control group and no blinding for the patients and the physicians performing either the follow-up or the echocardiogram, although the important outcome measurements, ABPM and ACR values, were performed by cardiac and laboratory technicians unware of treatment status.

There were changes on antihypertensive drug therapy during follow-up, which can interfere with BP, LV mass and ACR measurements. Nevertheless, in our study the

mean number of drugs was reduced at follow-up, meaning that, the reduction obtained with RDN could have been underestimated in this real-world setting.

Patients adherence to drug therapy was assessed only by questionnaires during prescheduled clinical visits. It was not verified with validated techniques such as urine and blood testing.

Cardiac resonance was not used and could have provided a more accurate evaluation of LV mass and function changes.

Conclusions

In this single center, non-blinded, prospective registry involving patients with severe resistant hypertension, refractory to drug therapy, renal denervation by means of radiofrequency, was associated with a significant reduction in blood pressure by office and 24 hours ambulatory measurements. In addition, there was a significant decrease in left ventricular mass, evaluated by transthoracic echocardiogram, and a significant decrease in the median urinary albumin to creatinine ratio, without changes in estimated glomerular filtration rate, at 12-months follow-up, after renal denervation. There was also a reduction in the percentage of patients with a pathological urinary albumin excretion.

All those changes occurred, despite a reduction on antihypertensive drug treatment, prescribed during the 12 months of follow-up.

Interestingly, our results revealed an absence of any linear correlation between changes in blood pressure and changes in the selected surrogates of hypertension related target organ damage, namely left ventricular mass and urinary albumin to creatine ratio. In the absence of an objective measurement of sympathetic activity before and after renal denervation, it is difficult to establish a causality effect between the level of blood pressure and the level of changes in the left ventricular mass or urinary albumin to creatine ratio, an important limitation for renal denervation.

Another important result from our registry was that renal denervation proved to be a very safe procedure. Without clinically relevant complications, namely major vascular, renal or cardiovascular complications, until 12 months of follow-up.

Thus, renal denervation appears to be a valid option for patients with resistant hypertension, with important clinical benefits beyond improved blood pressure control. Nevertheless, randomized studies with larger populations samples are required to assess the impact of this intervention on major clinical events in the long-term.

Bibliography

1. Krum H, Schlaich M, Whitbourn R, et al. Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet* 2009; **373**(9671): 1275-81.
2. Schlaich MP, Sobotka PA, Krum H, Lambert E, Esler MD. Renal sympathetic-nerve ablation for uncontrolled hypertension. *N Engl J Med* 2009; **361**(9): 932-4.
3. Symplicity HTNI, Esler MD, Krum H, et al. Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet* 2010; **376**(9756): 1903-9.
4. Bhatt DL, Kandzari DE, O'Neill WW, et al. A controlled trial of renal denervation for resistant hypertension. *N Engl J Med* 2014; **370**(15): 1393-401.
5. Kandzari DE, Bhatt DL, Brar S, et al. Predictors of blood pressure response in the SYMPLICITY HTN-3 trial. *Eur Heart J* 2015; **36**(4): 219-27.
6. Townsend RR, Mahfoud F, Kandzari DE, et al. Catheter-based renal denervation in patients with uncontrolled hypertension in the absence of antihypertensive medications (SPYRAL HTN-OFF MED): a randomised, sham-controlled, proof-of-concept trial. *Lancet* 2017.
7. Azizi M, Schmieder RE, Mahfoud F, et al. Endovascular ultrasound renal denervation to treat hypertension (RADIANCE-HTN SOLO): a multicentre, international, single-blind, randomised, sham-controlled trial. *Lancet* 2018.
8. Cuspidi C, Macca G, Sampieri L, et al. High prevalence of cardiac and extracardiac target organ damage in refractory hypertension. *Journal of hypertension* 2001; **19**(11): 2063-70.
9. Cuspidi C, Vaccarella A, Negri F, Sala C. Resistant hypertension and left ventricular hypertrophy: an overview. *Journal of the American Society of Hypertension : JASH* 2010; **4**(6): 319-24.
10. de Sousa Almeida M, de Araujo Goncalves P, Branco P, et al. Impact of Renal Sympathetic Denervation on Left Ventricular Structure and Function at 1-Year Follow-Up. *PloS one* 2016; **11**(3): e0149855.
11. Muiesan ML, Salvetti M, Rizzoni D, et al. Resistant hypertension and target organ damage. *Hypertension research : official journal of the Japanese Society of Hypertension* 2013; **36**(6): 485-91.
12. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990; **322**(22): 1561-6.

13. Ruilope LM, Schmieder RE. Left ventricular hypertrophy and clinical outcomes in hypertensive patients. *Am J Hypertens* 2008; **21**(5): 500-8.
14. Bombelli M, Facchetti R, Carugo S, et al. Left ventricular hypertrophy increases cardiovascular risk independently of in-office and out-of-office blood pressure values. *Journal of hypertension* 2009; **27**(12): 2458-64.
15. Gerstein HC, Mann JF, Yi Q, et al. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA : the journal of the American Medical Association* 2001; **286**(4): 421-6.
16. Salles GF, Cardoso CR, Fiszman R, Muxfeldt ES. Prognostic importance of baseline and serial changes in microalbuminuria in patients with resistant hypertension. *Atherosclerosis* 2011; **216**(1): 199-204.
17. Esler MD, Krum H, Schlaich M, Schmieder RE, Bohm M, Sobotka PA. Renal sympathetic denervation for treatment of drug-resistant hypertension: one-year results from the Symplicity HTN-2 randomized, controlled trial. *Circulation* 2012; **126**(25): 2976-82.
18. Papademetriou V, Tsioufis CP, Sinhal A, et al. Catheter-based renal denervation for resistant hypertension: 12-month results of the EnligHTN I first-in-human study using a multielectrode ablation system. *Hypertension* 2014; **64**(3): 565-72.
19. Bohm M, Mahfoud F, Ukena C, et al. Rationale and design of a large registry on renal denervation: the Global SYMPLICITY registry. *EuroIntervention* 2013; **9**(4): 484-92.
20. Böhm M, Mahfoud F, Ukena C, et al. First Report of the Global SYMPLICITY Registry on the Effect of Renal Artery Denervation in Patients With Uncontrolled Hypertension. *Hypertension* 2015.
21. Kandzari DE, Bohm M, Mahfoud F, et al. Effect of renal denervation on blood pressure in the presence of antihypertensive drugs: 6-month efficacy and safety results from the SPYRAL HTN-ON MED proof-of-concept randomised trial. *Lancet* 2018.
22. Azizi M, Sapoval M, Gosse P, et al. Optimum and stepped care standardised antihypertensive treatment with or without renal denervation for resistant hypertension (DENERHTN): a multicentre, open-label, randomised controlled trial. *Lancet* 2015; **385**(9981): 1957-65.
23. Rosa J, Widimský P, Toušek P, et al. Randomized Comparison of Renal Denervation Versus Intensified Pharmacotherapy Including Spironolactone in True-Resistant Hypertension: Six-Month Results From the Prague-15 Study. *Hypertension* 2015; **65**(2): 407-13.
24. Worthley SG, Tsioufis CP, Worthley MI, et al. Safety and efficacy of a multi-electrode renal sympathetic denervation system in resistant hypertension: the EnligHTN I trial. *Eur Heart J* 2013; **34**(28): 2132-40.
25. Edwards NC, Steeds RP, Stewart PM, Ferro CJ, Townend JN. Effect of spironolactone on left ventricular mass and aortic stiffness in early-stage chronic kidney disease: a randomized controlled trial. *J Am Coll Cardiol* 2009; **54**(6): 505-12.

26. Sato A, Hayashi M, Saruta T. Relative Long-Term Effects of Spironolactone in Conjunction with an Angiotensin-Converting Enzyme Inhibitor on Left Ventricular Mass and Diastolic Function in Patients with Essential Hypertension. *Hypertension Research* 2002; **25**(6): 837-42.
27. Bianchi S, Bigazzi R, Campese VM. Long-term effects of spironolactone on proteinuria and kidney function in patients with chronic kidney disease. *Kidney international* 2006; **70**(12): 2116-23.
28. Saklayen MG, Gyebi LK, Tasosa J, Yap J. Effects of additive therapy with spironolactone on proteinuria in diabetic patients already on ACE inhibitor or ARB therapy: results of a randomized, placebo-controlled, double-blind, crossover trial. *Journal of investigative medicine : the official publication of the American Federation for Clinical Research* 2008; **56**(4): 714-9.
29. Chrysostomou A, Becker G. Spironolactone in Addition to ACE Inhibition to Reduce Proteinuria in Patients with Chronic Renal Disease. *New England Journal of Medicine* 2001; **345**(12): 925-6.
30. Ceral J, Habrdova V, Vorisek V, Bima M, Pelouch R, Solar M. Difficult-to-control arterial hypertension or uncooperative patients? The assessment of serum antihypertensive drug levels to differentiate non-responsiveness from non-adherence to recommended therapy. *Hypertension research : official journal of the Japanese Society of Hypertension* 2011; **34**(1): 87-90.
31. Schirmer SH, Sayed MM, Reil JC, et al. Improvements in left ventricular hypertrophy and diastolic function following renal denervation: effects beyond blood pressure and heart rate reduction. *J Am Coll Cardiol* 2014; **63**(18): 1916-23.
32. Brandt MC, Mahfoud F, Reda S, et al. Renal sympathetic denervation reduces left ventricular hypertrophy and improves cardiac function in patients with resistant hypertension. *J Am Coll Cardiol* 2012; **59**(10): 901-9.
33. O'Brien E, Parati G, Stergiou G, et al. European Society of Hypertension position paper on ambulatory blood pressure monitoring. *Journal of hypertension* 2013; **31**(9): 1731-68.
34. Mahfoud F, Urban D, Teller D, et al. Effect of renal denervation on left ventricular mass and function in patients with resistant hypertension: data from a multi-centre cardiovascular magnetic resonance imaging trial. *Eur Heart J* 2014; **35**(33): 2224-31b.
35. Devereux RB, Dahlof B, Gerdts E, et al. Regression of hypertensive left ventricular hypertrophy by losartan compared with atenolol: the Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) trial. *Circulation* 2004; **110**(11): 1456-62.
36. Burns J, Ball SG, Worthy G, Struthers AD, Mary DA, Greenwood JP. Hypertensive left ventricular hypertrophy: a mechanistic approach to optimizing regression assessed by cardiovascular magnetic resonance. *J Hypertens* 2012; **30**(10): 2039-46.
37. Doltra A, Messroghli D, Stawowy P, et al. Potential reduction of interstitial myocardial fibrosis with renal denervation. *J Am Heart Assoc* 2014; **3**(6): e001353.

38. Reant P, Metras A, Detaille D, et al. Impact of Afterload Increase on Left Ventricular Myocardial Deformation Indices. *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography* 2016; **29**(12): 1217-28.

39. Shimko VF, Claycomb WC. Effect of Mechanical Loading on Three-Dimensional Cultures of Embryonic Stem Cell-Derived Cardiomyocytes. *Tissue engineering Part A* 2008; **14**(1): 49-58.

40. Toischer K, Rokita AG, Unsöld B, et al. Differential Cardiac Remodeling in Preload Versus Afterload. *Circulation* 2010; **122**(10): 993-1003.

41. Lohmeier TE. The sympathetic nervous system and long-term blood pressure regulation. *Am J Hypertens* 2001; **14**(6 Pt 2): 147S-54S.

42. Guyenet PG. The sympathetic control of blood pressure. *Nat Rev Neurosci* 2006; **7**(5): 335-46.

43. DiBona GF. Physiology in perspective: The Wisdom of the Body. Neural control of the kidney. *American journal of physiology Regulatory, integrative and comparative physiology* 2005; **289**(3): R633-41.

44. Fisher JP, Young CN, Fadel PJ. Central Sympathetic Overactivity: Maladies and Mechanisms. *Autonomic Neuroscience* 2009; **15**(148 (1-2)): 5-15.

45. Kurdi M, Booz GW. New Take on the Role of Angiotensin II in Cardiac Hypertrophy and Fibrosis. *Hypertension* 2011; **57**(6): 1034-8.

46. Waheed S, Matsushita K, Astor BC, Hoogeveen RC, Ballantyne C, Coresh J. Combined association of creatinine, albuminuria, and cystatin C with all-cause mortality and cardiovascular and kidney outcomes. *Clin J Am Soc Nephrol* 2013; **8**(3): 434-42.

47. Satchell S. The role of the glomerular endothelium in albumin handling. *Nat Rev Nephrol* 2013; **9**(12): 717-25.

48. Chronic Kidney Disease Prognosis C, Matsushita K, van der Velde M, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet* 2010; **375**(9731): 2073-81.

49. Ott C, Schmid A, Ditting T, et al. Renal denervation in a hypertensive patient with end-stage renal disease and small arteries: a direction for future research. *Journal of clinical hypertension* 2012; **14**(11): 799-801.

50. Verloop WL, Vink EE, Spiering W, et al. Effects of renal denervation on end organ damage in hypertensive patients. *European journal of preventive cardiology* 2015; **22**(5): 558-67.

51. Salmon AH, Satchell SC. Endothelial glycocalyx dysfunction in disease: albuminuria and increased microvascular permeability. *J Pathol* 2012; **226**(4): 562-74.

52. Almeida Mde S, Goncalves Pde A, Oliveira EI, Carvalho HC. Renal denervation for resistant hypertension. *Rev Port Cardiol* 2015; **34**(2): 125-35.

53. Calhoun DA, Jones D, Textor S, et al. Resistant hypertension: diagnosis, evaluation, and treatment. A scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Hypertension* 2008; **51**(6): 1403-19.
54. Devereux RB, Pickering TG, Harshfield GA, et al. Left ventricular hypertrophy in patients with hypertension: importance of blood pressure response to regularly recurring stress. *Circulation* 1983; **68**(3): 470-6.
55. Devereux RB, Savage DD, Sachs I, Laragh JH. Relation of hemodynamic load to left ventricular hypertrophy and performance in hypertension. *The American journal of cardiology* 1983; **51**(1): 171-6.
56. Devereux RB, Drayer JI, Chien S, et al. Whole blood viscosity as a determinant of cardiac hypertrophy in systemic hypertension. *The American journal of cardiology* 1984; **54**(6): 592-5.
57. Devereux RB, Pickering TG, Alderman MH, Chien S, Borer JS, Laragh JH. Left ventricular hypertrophy in hypertension. Prevalence and relationship to pathophysiologic variables. *Hypertension* 1987; **9**(2 Pt 2): II53-60.
58. Cannon WB. The Wisdom of the Body. NY: W. W. Norton. 1932.
59. Hall J. Guyton and Hall Textbook of Medical Physiology. 13th Edition. 2015.
60. Phillips RA. Etiology, pathophysiology, and treatment of left ventricular hypertrophy: focus on severe hypertension. *Journal of cardiovascular pharmacology* 1993; **21 Suppl 2**: S55-62.
61. Lorell BH, Carabello BA. Left Ventricular Hypertrophy. *Pathogenesis, Detection, and Prognosis* 2000; **102**(4): 470-9.
62. Esler M. The 2009 Carl Ludwig Lecture: Pathophysiology of the human sympathetic nervous system in cardiovascular diseases: the transition from mechanisms to medical management. *Journal of applied physiology* 2010; **108**(2): 227-37.
63. Bruno RM, Ghiadoni L, Seravalle G, Dell'oro R, Taddei S, Grassi G. Sympathetic regulation of vascular function in health and disease. *Front Physiol* 2012; **3**: 284.
64. Esler MD, Krum H, Sobotka PA, Schlaich MP, Schmieder RE, Bohm M. Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet* 2010; **376**(9756): 1903-9.
65. Wiley JM, White CJ, Uretsky BF. Noncoronary complications of coronary intervention. *Catheterization and cardiovascular interventions : official journal of the Society for Cardiac Angiography & Interventions* 2002; **57**(2): 257-65.
66. Jolly SS, Yusuf S, Cairns J, et al. Radial versus femoral access for coronary angiography and intervention in patients with acute coronary syndromes (RIVAL): a randomised, parallel group, multicentre trial. *Lancet* 2011; **377**(9775): 1409-20.
67. de Araujo Goncalves P, Teles RC, Raposo L. Catheter-based renal denervation for resistant hypertension performed by radial access. *The Journal of invasive cardiology* 2013; **25**(3): 147-9.

CHAPTER 5 FUTURE RESEARCH OPPORTUNITIES

This chapter is partially adapted from the following articles:

Renal Denervation for Resistant Hypertension.

M de Sousa Almeida, P de Araújo Gonçalves, E Infante de Oliveira, et al.

Rev Port Cardiol. 2015; 34 (2):125-135.

Renal Denervation in patients with resistant hypertension: six months results.

H Dores, M de Sousa Almeida, P de Araujo Gonçalves, et al.

Rev Port Cardiol. 2014 33(4): 197-204.

Impact of Renal Sympathetic Denervation on Left Ventricular Structure and Function at 1-Year Follow-Up.

M de Sousa Almeida; P Araujo Gonçalves, P Branco et al.

PLoS ONE 2016 11(3): e0149855. doi:10.1371/journal.pone.0149855

Changes in albumin-to-creatinine ratio at 12-month follow-up in patients undergoing renal denervation.

H Sousa, P Branco, M de Sousa Almeida et al.

Rev Port Cardiol. 2017 36(5): 343-351.

Sections:

Summary

The arrive of newer device-based treatments, will certainly change the field of hypertension management, perhaps its diagnosis and underlying pathophysiology. Results of previous and undergoing studies and trials, had already changed clinical practice. The routine use of 24-hour ambulatory blood pressure management as standard for hypertension assessment and management is one of them. The important problem of patient adherence to treatment will become central in planning hypertension treatment strategies, especially with drugs, and probably will make device-based renal denervation, a first choice with its "always on treatment effect". Patient preference will also play a role in future clinical decision-making.

Still, there are many ongoing challenges for renal denervation. The identification of underlying mechanism beyond non-responsiveness to RDN, is a priority. It may be related to the technical procedure, a limitation that can be overcome with technological improvements, newer devices and a better knowledge on renal nerves mapping and trafficking. Proper patient selection will also be of utmost importance since patients with low or normal renal nerve trafficking, may not need the renal denervation, avoiding unnecessary risks. To overcome such limitation, a reliable technology, capable of assessing renal sympathetic activity before and after the procedure, to validate its efficacy, is much needed. Only then will be possible to better understand the underlying pathophysiology and possibly enlarge the scope of sympathetic renal denervation, to treatment of other diseases closely related to an increased sympathetic activity.

New changes in hypertension management

One of the first conclusions coming from RDN trials and studies, performed until now, was the pivotal role of ABPM measurements in HTN management, in the diagnostic phase, allowing a better definition of resistant HTN, but also in the assessment of treatment efficacy. ABPM became the central testing in HTN management overshadowing office assessment.[1]

Another issue, that surfaced with RDN studies and trials, was patient adherence to drug treatment. Even in trials were patients were strongly advised to comply with medication, and were told in advance that they were under closed surveillance with periodical drug testing for compliance, 15% failed to comply, with no reasonable justification.[2, 3] Low patient's compliance to drug treatment will certainly imply a redefinition of resistant hypertension, as the most important cause for resistant HTN may be the lack of patient's adherence to treatment.

These unresolved issues became more relevant in face of the hypothesis of a "single shot" treatment for HTN, that is "always on", and is not dependable on patient will and future compliance.

The recently published Spyral HTN-Off Med trial[2] started a new strategy for the initial treatment of HTN, a device-based strategy, instead of a drug-based treatment. This was the first time that a device-based treatment for essential HTN was tested as a first-choice strategy. The long-term success of such a strategy will depend, ultimately, on the answer to some important questions on RDN: Is BP control sustainable in the long-term? Which side effects can we expect? Are there additional pleotropic effects?

The new active role of patients, in the clinical decision process, regarding HTN management, is another important issue, raised by the Spyral HTN-Off Med Trial.[2] The patient will have the power to co-decide with his physician, witch treatment he wants as a first choice for the initial treatment of its HTN: a device based or a drug based. This new role may come along with a more prominent patient expectation on HTN treatment. Until now, with drug treatment, patients expected HTN control. With a more invasive, complex and single shot procedure, they may expect HTN cure with no additional needs for drugs and no side effects, an evidence that at the present is still lacking.

These new tools for HTN treatment, will also mean newer treatment options: HTN device-based treatment only, drug treatment only, as first choices or low dose drugs combined with device treatment.

The long-standing efficacy is still to be demonstrated. Many open question remains: will it fades with time? Will there be a place for a *redo* procedure? If so, What will be the added efficacy? What about complications or side effects of a repeated procedure. Will it maintain the very favourable safety profile of RDN as described so far?

Another pending question, is: Which patients will be non-responders to RDN? Why some patients are non-responders? Is it related to the device or to the technique? Is there a patient phenotype more adequate for a device, for a drug approach,

both or for a specific device? Their identification in advance would be very useful, not only because it will avoid unnecessary procedures and risks, but also because it will improve treatment success, patient safety and confidence, and ultimately, outcomes.

Higher efficacy in HTN treatment is expected alongside higher complexity. Until then more studies and trials are needed to provide proper scientific evidence to support such new strategies.

New side effects

Until now, all published studies and trials on RDN had in common a very high safety profile for all tested devices. Nevertheless, published results were all in the short-term.[4-10] It is possible, with longer follow-ups, that the safety profile may change, with higher or newer complications or side effects. Long-term renal artery stenosis is still a concern, although rarely reported so far. A sustainable decrease in SNS activity, after RDN, may have unpredictable and newer side effects, demanding our constant watchfulness to promptly identify them and provide the proper solution.

The more recent trials[2, 3] still reveals a high variability in HTN response to RDN. A significant proportion of patients, around 20 to 30%, on average, didn't showed a decrease in BP after RDN. They were tagged as non-responders. But, even though some of them didn't showed significant changes in BP after RDN, others, rather showed an increase in BP after RDN. Such behaviour may be related to the complex pathophysiology of HTN or to the intrinsic variability of BP response to RDN. But, it may also be a potential side effect of RDN, a paradoxal increase in HTN after RDN.

Such concern, strongly supports the need for markers of SNS activity, simple, reliable, reproductible, applicable on real time and able to verify SNS activity before and after RDN, allowing the online assessment of its efficacy.[11]

The medical community must be on continuous alert and prepared to deal properly with such unwanted events. Such environment is not totally new for interventional cardiologists, since the extensive experience in dealing with other previous devices, has teach them how to deal with such surprises and use them as an opportunity to improvement.

New indications and future clinical challenges

Since SNS has a direct interference in a broad range of systems in the human body, initiating or stopping many different actions in different tissues and organs, through distinct receptors, it is largely expectable that trafficking modulation of sympathetic renal nerves, namely the renal afferent ones may have more generalized effects besides BP changes.[12-14]

Sustained chronic change in sympathetic activity is involved in many different diseases, from metabolic to psychological disorders, including ischemic heart disease,[15] heart failure,[16, 17] kidney disease,[18] type 2 diabetes,[19, 20] obesity,[19, 20] obstructive sleep apnoea[21] and inflammatory bowel disease[22], issues previously reviewed in the introduction chapter.

Among such a *pleiades* of effects, it will not be a surprise, if newer pleotropic effects arise from sympathetic activity modulation provided by RDN. After RDN, the expected overall decrease on SNS drive, may have a significant impact in other clinical scenarios characterized by SNS hyperactivity. Small proof of concept studies, have already showed promising results.

The association between heart failure and increased sympathetic drive is well known. Interestingly, cardiac and renal spillover of norepinephrine are more closely associated with mortality than circulating catecholamine concentrations, both related to worst outcomes.[23, 24] This provides the evidence that reducing NE spillover from the kidney, by RDN, could have a beneficial symptomatic and prognostic effect.[25, 26]

In animal models, RDN after myocardial infarction showed an improvement on sodium excretion[27], increased cardiac output, improved renal blood flow[28], and a down--regulation of angiotensin AT1 receptors mediating maladaptive responses.[29] In a multicentre study on patients with resistant HTN treated by RDN, a subgroup of patients with LV dysfunction, with their anatomic and functional myocardial parameters assessed by cardiac magnetic resonance, had their LVEF and circumferential strain significantly increased.[30] The REACH pilot study, in heart failure, provided evidence that RDN was able to improve the 6 minutes walking test results, without affecting BP (average 120 mmHg at baseline).[31]

Type 2 diabetes and insulin resistance have a strong association with resistant HTN. About 50% of resistant HTN patients are considered to be insulin resistant, increasing the risk for type 2 diabetes and, since insulin resistance is dependent on sympathetic activity, it appears likely that it could also be a target for RDN.[32, 33] Small studies revealed that, along with BP reductions, RDN improved fasting glucose, sustainable decline in glycated hemoglobin concentrations, insulin and C-peptide con-

centrations, as well as insulin sensitivity indices in patients with resistant HTN and metabolic disease, suggesting that RDN might improve the diabetic status, in those patients.[34, 35]

Obstructive sleep apnea and resistant HTN are a well-known association[35], another possible target for RDN. In 2011, Witkowski et al published a pilot study on the effect of RDN in 10 patients with resistant HTN and obstructive sleep apnea. At 6-months, there was an improvement on apnea-hypopnea indexes.[35] An experimental model, revealed that RDN was able reduce the post-apneic BP rise, renal hypo perfusion during apnea and activation of the renin–angiotensin system in the kidney.[36, 37]

Regarding atrial fibrillation, an epidemic affecting millions of patients worldwide, it was shown in a recent pilot trial,[38] that patients with resistant HTN and symptomatic paroxysmal or persistent atrial fibrillation, refractory to ≥2 antiarrhythmic drugs, randomized to pulmonary vein isolation only or in association with RDN, at 12-month follow-up, 69% of patients submitted to RDN were free of atrial fibrillation, compared to 29% of patients treated with pulmonary vein isolation only, supporting a potential usefulness of RDN on AF treatment.

In ventricular arrhythmias, the scientific evidence supporting the fundamental role of sympathetic activity on their origin is overwhelming. In an animal model of ischemia/reperfusion-arrhythmias, RDN decreased the occurrence of ventricular arrhythmias/fibrillation and attenuated the rise in LV end diastolic pressure during LV ischemia, without influencing infarct size, changes in ventricular contractility, BP and reperfusion arrhythmias.[39] In other small case series, involving patients with dilated cardiomyopathy and an electrical ventricular storm, RDN was able to reduce discharges from the implantable cardioverter defibrillators and ventricular ectopias.[40] Hoffmann et al.[41] reported that RDN can be safely and effectively performed, as an adjunct to cardiac catheter ablation, in a hemodynamically unstable patient with ventricular storm after ST elevation myocardial infarction.

The value of these findings is still controversial and confirmatory studies are needed.

The impact of RDN and changes on SNS activity in other diseases, and human systems disarrangements, outside the cardiovascular field, is still to be addressed. But the underlying pathophysiological evidence supporting their deep interconnecting relationships is overwhelming.

New horizons have been opened in the understanding on how SNS disarranges may drive disease, alongside with new and better tools to track and treat those changes. Altogether, they will certainly push the development of newer collaborations

between medical groups, from different fields of knowledge, not usually involved in the endovascular field.

A future challenge, will be the establishment of RDN efficacy in the reduction of major clinical endpoints in patients with HTN (the 3rd step): death, stroke, acute coronary syndromes, heart failure and kidney dysfunction. Only then, it will be possible to safely assume that RDN is at least as efficacious as drugs in HTN treatment. If that becomes a reality, it is reasonable to assume that RDN may be the first choice for HTN initial treatment strategy. The challenges facing such endeavor, are mostly economical. The trials needed to prove such benefits, will require large numbers of patients and longer follow-ups, making them hugely expensive, but unavoidable!

Assessment of sympathetic nerve system activity

There are still a considerable number of important issues, regarding RDN, that were not properly addressed until now, or even at all. One of them is how to properly measure RDN efficacy in respect to the decrease in renal SNS activity after RDN. A methodology capable of quantifying renal SNS activity before and after RDN, able to be used in clinical practice, safe, comfortable and reliable, is still lacking.

Until now, the scientific bases validating RDN, are clinical. Meaning the favorable impact of RDN on surrogates of an increased SNS activity: HTN; myocardial hypertrophy or renal disfunction. Some small studies in animals and humans used NE renal spill-over and smooth muscle tonus, as a proof of concept that RDN decreases SNS activity. But, such tests are not feasible to a broader daily clinical use.

A marker for SNS activity, simple, reliable, reproducible, applicable on real time, leading to a better pathophysiological understanding of what is happening after RDN, is much needed.[11] It will help to explain possible side effects, the lack of efficacy in certain patients (non-responders) driving for a better patient selection, and it will track possible renal nerve regrowth in the long term. It will help to validated RDN, to understand non-responder patients, to improve the technique and devices. Most of all, it will enlarge the scope of RDN as a new treatment strategy for other diseases, known to be related to SNS hyperactivity. It will also serve as an important tool to understand the pathophysiology of other diseases, and eventually establish their relationship to SNS disarrangements, for example, the broad field of psychosomatic diseases.

Final remarks

There are urgent unmet medical needs to address by the academic, scientific and medical community. The latest published trials had shown very promising results on RDN efficacy. The enthusiasm arousing from such results may tempt the medical community to start using RDN solely based on the trials clinical indications, and skip other fundamental questions, but those answers are much needed to a better understanding of how SNS disarrangements affects human health. Such understanding will become of utmost importance and will provide the much-needed support to prevent and treat diseases at earlier stages.

Bibliography

1. O'Brien E, Parati G, Stergiou G, et al. European Society of Hypertension position paper on ambulatory blood pressure monitoring. *Journal of hypertension* 2013; **31**(9): 1731-68.
2. Townsend RR, Mahfoud F, Kandzari DE, et al. Catheter-based renal denervation in patients with uncontrolled hypertension in the absence of antihypertensive medications (SPYRAL HTN-OFF MED): a randomised, sham-controlled, proof-of-concept trial. *Lancet* 2017.
3. Kandzari DE, Bohm M, Mahfoud F, et al. Effect of renal denervation on blood pressure in the presence of antihypertensive drugs: 6-month efficacy and safety results from the SPYRAL HTN-ON MED proof-of-concept randomised trial. *Lancet* 2018.
4. Krum H, Schlaich M, Whitbourn R, et al. Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet* 2009; **373**(9671): 1275-81.
5. Esler MD, Krum H, Sobotka PA, Schlaich MP, Schmieder RE, Bohm M. Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet* 2010; **376**(9756): 1903-9.
6. Bhatt DL, Kandzari DE, O'Neill WW, et al. A controlled trial of renal denervation for resistant hypertension. *N Engl J Med* 2014; **370**(15): 1393-401.
7. Worthley SG, Tsioufis CP, Worthley MI, et al. Safety and efficacy of a multi-electrode renal sympathetic denervation system in resistant hypertension: the EnligHTN I trial. *Eur Heart J* 2013; **34**(28): 2132-40.
8. Azizi M, Sapoval M, Gosse P, et al. Optimum and stepped care standardised antihypertensive treatment with or without renal denervation for resistant hypertension (DENERHTN): a multicentre, open-label, randomised controlled trial. *Lancet* 2015; **385**(9981): 1957-65.
9. Azizi M, Schmieder RE, Mahfoud F, et al. Endovascular ultrasound renal denervation to treat hypertension (RADIANCE-HTN SOLO): a multicentre, international, single-blind, randomised, sham-controlled trial. *Lancet* 2018.
10. Fadl Elmula FE, Jin Y, Yang WY, et al. Meta-analysis of randomized controlled trials of renal denervation in treatment-resistant hypertension. *Blood pressure* 2015; **24**(5): 263-74.
11. Persu A, Kjeldsen S, Staessen JA, Azizi M. Renal Denervation for Treatment of Hypertension: a Second Start and New Challenges. *Current hypertension reports* 2016; **18**(1): 6.

12. Esler M, Kaye D. Sympathetic nervous system activation in essential hypertension, cardiac failure and psychosomatic heart disease. *Journal of cardiovascular pharmacology* 2000; **35**(7 Suppl 4): S1-7.
13. DiBona GF. Physiology in perspective: The Wisdom of the Body. Neural control of the kidney. *American journal of physiology Regulatory, integrative and comparative physiology* 2005; **289**(3): R633-41.
14. Abboud FM. The Walter B. Cannon Memorial Award Lecture, 2009. Physiology in perspective: The wisdom of the body. In search of autonomic balance: the good, the bad, and the ugly. *American journal of physiology Regulatory, integrative and comparative physiology* 2010; **298**(6): R1449-67.
15. Graham LN, Smith PA, Huggett RJ, Stoker JB, Mackintosh AF, Mary DA. Sympathetic drive in anterior and inferior uncomplicated acute myocardial infarction. *Circulation* 2004; **109**(19): 2285-9.
16. Kishi T, Hirooka Y. Central Mechanisms of Abnormal Sympathoexcitation in Chronic Heart Failure. *Cardiology Research and Practice* 2012; **2012**: 1-7.
17. Triposkiadis F, Karayannis G, Giamouzis G, Skoularigis J, Louridas G, Butler J. The Sympathetic Nervous System in Heart Failure. Physiology, Pathophysiology, and Clinical Implications. *Journal of the American College of Cardiology* 2009; **54**(19): 1747-82.
18. Neumann J, Ligtenberg G, Klein IH, et al. Sympathetic hyperactivity in hypertensive chronic kidney disease patients is reduced during standard treatment. *Hypertension* 2007; **49**(3): 506-10.
19. Esler M, Rumantir M, Wiesner G, Kaye D, Hastings J, Lambert G. Sympathetic nervous system and insulin resistance: from obesity to diabetes. *Am J Hypertens* 2001; **14**(11 Pt 2): 304S-9S.
20. Landsberg L. Insulin-mediated sympathetic stimulation: role in the pathogenesis of obesity-related hypertension (or, how insulin affects blood pressure, and why). *Journal of hypertension* 2001; **19**(3 Pt 2): 523-8.
21. Narkiewicz K, Somers VK. The sympathetic nervous system and obstructive sleep apnea: implications for hypertension. *Journal of hypertension* 1997; **15**(12 Pt 2): 1613-9.
22. Furlan R, Ardizzone S, Palazzolo L, et al. Sympathetic overactivity in active ulcerative colitis: effects of clonidine. *American journal of physiology Regulatory, integrative and comparative physiology* 2006; **290**(1): R224-32.
23. Hasking GJ, Esler MD, Jennings GL, Burton D, Johns JA, Korner PI. Norepinephrine spillover to plasma in patients with congestive heart failure: evidence of increased overall and cardiorenal sympathetic nervous activity. *Circulation* 1986; **73**(4): 615-21.
24. Petersson M, Friberg P, Eisenhofer G, Lambert G, Rundqvist B. Long-term outcome in relation to renal sympathetic activity in patients with chronic heart failure. *Eur Heart J* 2005; **26**(9): 906-13.

25. Bohm M, Linz D, Urban D, Mahfoud F, Ukena C. Renal sympathetic denervation: applications in hypertension and beyond. *Nature reviews Cardiology* 2013; **10**(8): 465-76.
26. Bohm M, Ewen S, Kindermann I, Linz D, Ukena C, Mahfoud F. Renal denervation and heart failure. *European journal of heart failure* 2014.
27. Villarreal D, Freeman RH, Johnson RA, Simmons JC. Effects of renal denervation on postprandial sodium excretion in experimental heart failure. *The American journal of physiology* 1994; **266**(5 Pt 2): R1599-604.
28. Masaki H, Imaizumi T, Harasawa Y, Takeshita A. Dynamic arterial baroreflex in rabbits with heart failure induced by rapid pacing. *The American journal of physiology* 1994; **267**(1 Pt 2): H92-9.
29. Clayton SC, Haack KK, Zucker IH. Renal denervation modulates angiotensin receptor expression in the renal cortex of rabbits with chronic heart failure. *American journal of physiology Renal physiology* 2011; **300**(1): F31-9.
30. Mahfoud F, Urban D, Teller D, et al. Effect of renal denervation on left ventricular mass and function in patients with resistant hypertension: data from a multi-centre cardiovascular magnetic resonance imaging trial. *Eur Heart J* 2014; **35**(33): 2224-31b.
31. Davies JE, Manisty CH, Petraco R, et al. First-in-man safety evaluation of renal denervation for chronic systolic heart failure: primary outcome from REACH-Pilot study. *International journal of cardiology* 2013; **162**(3): 189-92.
32. Mancia G, Bousquet P, Elghozi JL, et al. The sympathetic nervous system and the metabolic syndrome. *Journal of hypertension* 2007; **25**(5): 909-20.
33. Huggett RJ, Scott EM, Gilbey SG, Stoker JB, Mackintosh AF, Mary DA. Impact of type 2 diabetes mellitus on sympathetic neural mechanisms in hypertension. *Circulation* 2003; **108**(25): 3097-101.
34. Mahfoud F, Schlaich M, Kindermann I, et al. Effect of renal sympathetic denervation on glucose metabolism in patients with resistant hypertension: a pilot study. *Circulation* 2011; **123**(18): 1940-6.
35. Witkowski A, Prejbisz A, Florczak E, et al. Effects of renal sympathetic denervation on blood pressure, sleep apnea course, and glycemic control in patients with resistant hypertension and sleep apnea. *Hypertension* 2011; **58**(4): 559-65.
36. Linz D, Mahfoud F, Schotten U, et al. Renal sympathetic denervation suppresses postapneic blood pressure rises and atrial fibrillation in a model for sleep apnea. *Hypertension* 2012; **60**(1): 172-8.
37. Linz D, Hohl M, Nickel A, et al. Effect of renal denervation on neurohumoral activation triggering atrial fibrillation in obstructive sleep apnea. *Hypertension* 2013; **62**(4): 767-74.
38. Pokushalov E, Romanov A, Corbucci G, et al. A randomized comparison of pulmonary vein isolation with versus without concomitant renal artery denervation in

patients with refractory symptomatic atrial fibrillation and resistant hypertension. *J Am Coll Cardiol* 2012; **60**(13): 1163-70.

39. Linz D, Wirth K, Ukena C, et al. Renal denervation suppresses ventricular arrhythmias during acute ventricular ischemia in pigs. *Heart rhythm : the official journal of the Heart Rhythm Society* 2013; **10**(10): 1525-30.
40. Ukena C, Bauer A, Mahfoud F, et al. Renal sympathetic denervation for treatment of electrical storm: first-in-man experience. *Clinical research in cardiology : official journal of the German Cardiac Society* 2012; **101**(1): 63-7.
41. Hoffmann BA, Steven D, Willems S, Sydow K. Renal Sympathetic Denervation as an Adjunct to Catheter Ablation for the Treatment of Ventricular Electrical Storm in the Setting of Acute Myocardial Infarction. *Journal of cardiovascular electrophysiology* 2013.

ATTACHMENTS

LIST OF ATTACHMENTS

ATTACHMENT A

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REVIEW ARTICLE

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REVIEW ARTICLE

Renal denervation for resistant hypertension

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KEYWORDS
Hypertension;
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Abstract There is a marked contrast between the high prevalence of hypertension and the low rates of adequate control. A subset of patients with suboptimal blood pressure control have drug-resistant hypertension, in the pathophysiology of which chronic sympathetic hyperactivation is significantly involved. Sympathetic renal denervation has recently emerged as a device-based treatment for resistant hypertension. In this review, the pathophysiological mechanisms linking the sympathetic nervous system and cardiovascular disease are reviewed, focusing on resistant hypertension and the role of sympathetic renal denervation. An update on experimental and clinical results is provided, along with potential future indications for this device-based technique in other cardiovascular diseases.

PALAVRAS-CHAVE
Hipertensão arterial;
Sistema nervoso simpático;
Desnervação renal

Desnervação renal para hipertensão arterial resistente

Resumo A elevada prevalência da hipertensão está em claro contraste com a sua ainda insuficiente taxa de controlo. Um importante subgrupo destes doentes apresenta uma hipertensão resistente aos fármacos, na qual a hiperativação crónica do sistema nervoso simpático tem importantes implicações fisiopatológicas. Recentemente, a desnervação simpática renal emergiu como um tratamento de intervenção para a hipertensão arterial resistente. No presente artigo, são revistos os mecanismos fisiopatológicos subjacentes à interação entre o sistema nervoso simpático e as doenças cardiovasculares, com particular enfâse na hipertensão

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arterial resistente e no papel da desnervação simpática renal. É igualmente feita uma atualização dos resultados de estudos experimentais e clínicos, bem como de potenciais futuras indicações desta técnica de intervenção noutras doenças do foro cardiovascular.

List of abbreviations

BP	blood pressure
CI	confidence interval
eGFR	estimated glomerular filtration rate
SNS	sympathetic nervous system
RAAS	renin-angiotensin-aldosterone system
RDN	renal denervation
RF	radiofrequency

Introduction

Hypertension is the leading global risk factor for cardiovascular mortality, accounting for more than nine million deaths worldwide in 2010.[1] Its close association with myocardial infarction, heart failure, stroke, end-stage renal disease and cardiovascular death is well established, with 54% of stroke and 47% of ischemic heart disease worldwide attributable to high blood pressure (BP).[2] Effective BP lowering has consistently been shown to reduce overall cardiovascular risk,[3] but rates of adequate BP control remain suboptimal, despite the wide range of antihypertensive drugs available and strong evidence supporting their use. A recently published study confirmed that rates of BP control in European countries are low, with only 37% of treated hypertensive patients achieving recommended BP values.[4]

The blame for such low rates cannot be attributed only to poor treatment. Resistant hypertension has a prevalence ranging from 15% to 30% of treated hypertensive patients,[5] and is an important cause of failure of BP control. Most importantly, these patients exhibit a worse prognosis, with a higher risk for cardiovascular events, compared to hypertensive patients without resistant hypertension.[6]

In recent decades, the renin-angiotensin-aldosterone system (RAAS) has been the central focus of hypertension treatment and management. The availability of safe, effective and evidence-based drugs that block this system has meant that the role of other systems, particularly the autonomic nervous system, has been neglected.

The sympathetic nervous system (SNS) and its possible role in the pathogenesis of hypertension is receiving increasing attention. The aim of this review is to provide an update on the current understanding of the role of the SNS in blood pressure control and its implications for sympathetic renal denervation (RDN).

The sympathetic nervous system and cardiovascular disease

The development of open surgical sympathectomy in the 1930s highlighted the role of the SNS in severe hypertension, since it appeared to be effective in lowering high BP in patients with severe hypertension.[7,8] However, the procedure was abandoned due to its poorly tolerated side effects and high surgical risk, especially after the appearance of ganglionic blockers, the first effective antihypertensive drug class.[9]

The recent development of a new device-based approach to treat severe resistant hypertension, through RDN, focused attention on the already well-known role of the SNS in initiating and maintaining high BP in patients with essential hypertension.[10,11]

Assessment of the sympathetic nervous system in humans

The major reason that the SNS has been so neglected is not because there are doubts concerning its critical role in the pathogenesis of hypertension and other cardiovascular diseases, but because it has been difficult to study and test this relation, due to the complex and clinically impractical methods used for assessing the SNS in humans. Until the early 1970s, the most common techniques were measurements of blood levels and urine excretion rates of norepinephrine and its derivatives, which provide a gross estimate of whole-body sympathetic activity at best.[12] Since then, new methods have emerged for measuring sympathetic nerve firing rates in subcutaneous nerves and for assaying plasma concentrations of sympathetic transmitters.

Microneurography, a technique reported first by Hagbarth and Vallbo,[13] provided a tool to study nerve firing in subcutaneous sympathetic nerves in skin and skeletal muscle vessels. It is based on recording bursts of nerve activity, synchronous with the heartbeat, generated in skeletal muscle vascular efferent nerves, through tungsten electrodes inserted in the skin. It is highly reproducible and closely related to sympathetic traffic directed to other structures and can be repeated over time, allowing assessment of the effects of interventions, direct quantification of sympathetic nerve traffic regulating vasomotor tone, and study of instantaneous reactions to rapid stimuli.

The spillover technique for measurement of norepinephrine release, first applied by Esler et al,[14] is an

Table 1 Effects of increased sympathetic nerve activity.

Vascular
Smooth muscle cell hypertrophy and proliferation
Endothelial dysfunction and damage
Arterial stiffness
Impairment of postural blood pressure control and syncope
Hypertension
Atherosclerosis
Cardiac
Myocyte hypertrophy
Left ventricular hypertrophy
Arrhythmia
Psychogenic heart disease
Renal
Renal artery vasoconstriction
Sodium and fluid retention
Microalbuminuria
RAAS activation
Metabolic
Insulin resistance
Dyslipidemia

RAAS: renin-angiotensin-aldosterone system.

isotope dilution method that calculates the clearance and spillover of norepinephrine, using an intravenous infusion of tritium-labeled norepinephrine. The relationship between the sympathetic nerve fiber firing rate of an organ and the rate of norepinephrine spillover into the venous effluent of that organ provides the rationale for using measures of regional norepinephrine release as a surrogate for sympathetic tone in individual organs,[15] enabling assessment of regional sympathetic nervous function in humans.

This technique was central to the demonstration that heart failure patients had sympathetic overactivity rather than sympathetic denervation, as thought at the time, and opened the way to the routine use of beta-blockers in heart failure.[16]

Sympathetic nervous system overactivity

Besides its central role in cardiovascular homeostasis, by controlling vascular tone through vasoconstriction of small resistance arteries, the sympathetic system also affects and regulates numerous other physiological processes (Table 1). There is growing evidence that sustained chronic changes in sympathetic activity are involved in the pathogenesis of many disease states, from metabolic to psychological disorders, including ischemic heart disease,[17] chronic heart failure,[18,19] hypertension,[20–22] kidney disease,[23] type 2 diabetes,[24] obesity,[24] metabolic syndrome,[24] obstructive sleep apnea,[25] depression[26] and inflammatory bowel disease.[27] A chronically overactive sympathetic system is linked to a worse prognosis in patients with heart failure and end-stage renal disease.[28,29]

Sympathetic nervous system overactivity and cardiovascular disease

There is growing evidence that the deleterious effects on blood vessels and myocardium of an overactive SNS are independent of increased BP.[30] Chronic SNS activation without an increase in BP can cause hypertrophy and proliferation of vascular smooth muscle cells as well as having a direct trophic effect on cardiac myocytes, increasing left ventricular (LV) mass and wall thickness,[30] while BP reduction after catheter-based RDN has been shown to lead to a significant reduction in LV mass and improvement in diastolic function.[31]

These structural changes in the myocardium and the direct effects of an overactive SNS contribute to the high incidence of arrhythmias commonly seen in patients with hypertension.[32]

The link between mental stress, psychiatric illness and cardiovascular disease, although best established for heart disease consequential to acute mental stress and depressive illness, has been much more difficult to establish.[26] Acute mental stress can trigger sympathetic outflow to the heart and adrenal secretion of epinephrine. In patients with preexisting atherosclerosis, not only can increased epinephrine cause ventricular arrhythmias (especially in the presence of coronary artery stenosis), but the attendant BP surge can fissure coronary plaques and promote platelet aggregation, predisposing to thrombosis.[26] Takotsubo (stress) cardiomyopathy is a good example of extreme acute activation of cardiac sympathetic outflow to the heart,[26] as are panic attacks accompanied by coronary spasm and cardiac arrhythmias.[33] In patients with depressive illness, chronic cardiac sympathetic outflow is at almost the same level as seen in patients with heart failure, and is accepted as a primary cause for heart disease, associated with a worse prognosis.

The sympathetic nervous system and atherosclerosis

The pivotal role of endothelial impairment in the development of atherosclerosis and in future cardiovascular risk is well established. Less known is the interaction between the SNS and endothelial function. Virtually all cardiovascular risk factors and diseases in which increased adrenergic drive is involved are also characterized by endothelial dysfunction.[34] Nitric oxide (NO), one of the main mediators of endothelial function, is also an important neurotransmitter, involved in the autonomic regulation of cardiovascular function, and acts as a sympathoinhibitory substance within the central nervous system.[35] Acute and chronic increases in SNS activity, through endothelial dysfunction and endothelial cell damage, have been shown to contribute to the subsequent development of atherosclerosis.[26,30,34]

SNS overactivity has also been linked to the development of metabolic disturbances such as insulin resistance and dyslipidemia.[36] Not only can increased SNS activity in itself lead to insulin resistance, particularly in hypertensive patients,[36] but also elevated circulating insulin levels due to insulin resistance in obese patients can precipitate an increase in SNS activity, leading to hypertension.[30,36,37] There is abundant evidence that statins, through their

numerous pleiotropic effects, reduce and even normalize excessive SNS activity, improving LV function and arterial baroreflex sensitivity.[26,30,34]

Sympathetic nervous system function and heart failure

The observation that norepinephrine concentrations were reduced in the failing heart suggested the existence of sympathetic denervation,[38] despite the increased concentrations in peripheral venous plasma commonly found in patients with heart failure,[39] indicating overall increased SNS activity except in the heart. Later studies confirmed very high levels of norepinephrine spillover from the heart in heart failure, up to 50 times the normal range in untreated patients,[26] demonstrating high sympathetic tone in the failing heart. This was later explained by a reduction in the concentration of beta-1 adrenoreceptors in the failing myocardium, due to downregulation of these receptors by increased sympathetic activity in the failing heart.[26]

This increased sympathetic activity in the peripheral circulation and kidneys leads to adverse effects, causing vasoconstriction, increasing cardiac work, and promoting sodium retention and ventricular overfilling. The strong link between the level of sympathetic activity in heart failure, progressive ventricular deterioration, the development of ventricular arrhythmias, sudden death and reduced survival[26] provided the rationale for the subsequent use of beta-blockers in heart failure.[40]

Sympathetic nervous system function and essential hypertension

A well-known consequence of an overactive SNS is an increase in BP. Previous studies showed not only that sympathetic outflow to the kidneys was increased, but that the extent of the outflow was also related to the degree of essential hypertension.[20,22,26,41] Regional measurements of norepinephrine spillover to the kidneys support this concept, and indicate that more than 50% of cases of essential hypertension present significant sympathetic hyperactivation.[42] Renal sympathetic nerve activity is pivotal in the pathogenesis of essential hypertension, through its influence on renin release, sodium and water excretion, peripheral vasoconstriction, cardiac contraction and venous capacitance.[26]

The safety and efficacy of BP lowering achieved recently with RDN has led to renewed interest in the role of sympathetic activity in the pathogenesis of essential hypertension. Correct identification of sympathetic hyperactivation in patients with essential hypertension can lead to better selection of patients for RDN treatment.

Resistant hypertension

Data from the PAP study involving 5023 adult patients showed that the prevalence of hypertension in Portugal was 42%, of whom only 46.1% were aware of the fact, 39% were taking medication and only 11.2% had BP values below the recommended thresholds.[43]

Table 2 Evaluation of patients with resistant hypertension considered to be potential candidates for renal denervation.

1st step
Exclusion of pseudoresistance (by 24-hour ABPM)
Exclusion of secondary causes
Search for conditions that maintain high BP values
2nd step
Modification of antihypertensive treatment (optimization of dosages and combinations; use of aldosterone blockers if possible)
Reassessment of BP control with 24-hour ABPM
3rd step
Assessment of renal artery anatomy (CT, MRI or invasive angiography; Doppler ultrasound)
Assessment of renal function (eGFR ideally >45 ml/min/1.73 m^2)

ABPM: ambulatory blood pressure monitoring; BP: blood pressure; CT: computed tomography; eGFR: estimated glomerular filtration rate; MRI: magnetic resonance imaging.

Not all patients with uncontrolled hypertension are considered to be resistant, as there are several factors that can contribute to lack of control, including inadequate treatment regimens (type and/or dosage of drugs), poor adherence to medical therapy, and undetected secondary causes of hypertension. A diagnosis of resistant hypertension should therefore only be made after ruling out other factors.

Resistant hypertension has been defined as BP values above 140/90 mmHg (or >130/80 mmHg in patients with diabetes or chronic kidney disease) in patients treated with three or more antihypertensive drugs at appropriate doses, including if possible a diuretic.[44] In an alternative definition, patients with target BP values can be considered to have resistant hypertension if they need to take at least four different antihypertensive drugs; this is known as controlled resistant hypertension.[45] The prevalence of resistant hypertension has been reported as 5–30%,[46–48] the figure varying according to the hypertensive population being studied, with higher percentages in cohorts from centers specializing in the treatment of hypertension compared to community-based cohorts.

At the present time, and in the light of the available clinical studies, patients considered to be good candidates for RDN should have more severe treatment-resistant hypertension, defined as office systolic BP of at least 160 mmHg (150 mmHg in type 2 diabetes).[49–51]

Renal denervation

Assessment of a potential candidate for RDN should follow several steps (Table 2) designed to select candidates expected to benefit from this intervention. According to a recent European Society of Hypertension (ESH) position paper on RDN,[52] it is recommended that patients should undergo careful assessment in centers that have considerable experience in dealing with hypertension (ideally ESH excellence centers).

The first step should be to exclude pseudoresistance, secondary causes of hypertension and conditions that maintain high BP values. Pseudoresistance can be excluded by 24-hour ambulatory BP monitoring, which is recommended not only for pre-RDN assessment but as good practice in the assessment of all patients with hypertension.[5,53] In patients with severe resistant hypertension considered for RDN, the initial assessment should also include investigation of possible secondary causes of hypertension, including primary aldosteronism, renal artery stenosis, pheochromocytoma, Cushing's disease, hyperparathyroidism and aortic coarctation, although some of these are very uncommon.[44] Some conditions that can maintain high BP values should also be treated when possible, such as severe obesity, high salt and alcohol intake, concomitant use of drugs that raise BP and the presence of obstructive sleep apnea.

The second step should be the optimization of antihypertensive treatment, including the use of diuretics and aldosterone blockers, optimization of dosages and combinations, and reassessment of BP control with 24-hour ambulatory BP monitoring (ABPM).

The third step should be assessment of renal artery anatomy, as there are relative contraindications for RDN related to the number of renal arteries (multiple main arteries) and their diameter (ideally >4 mm) and length (ideally >20 mm) as well as eGFR, which should be above 45 ml/min/1.73 m^2. Some of these are considered contraindications to RDN, because they were excluded from RDN trials, but are regarded in clinical practice as relative contraindications; some of these patients have been treated with RDN and included in small studies and registries.[54–56]

Experimental studies on renal denervation

The finding that renal sympathetic activity is increased in spontaneously hypertensive rats, the animal model most often used in investigation of essential hypertension, has shone light on the role of the renal SNS in the pathogenesis of hypertension.[57] In an experimental model of hypertension and obesity in dogs subjected to a high-fat diet, RDN not only prevented the appearance of hypertension but also increased urinary sodium excretion by 50%.[58] In another animal model of chronic renal failure, sympathectomy prevented hypertension and was associated with decreased adrenergic activity in the hypothalamic nuclei.[59]

Renal lesions induced by injection of phenol cause a sustained rise in BP and norepinephrine release by the hypothalamus without changing eGFR. RDN of these animals prevented the rise in BP.[60] In different animal models, the effects of RDN have consistently shown the important role of the renal SNS in the pathophysiology of hypertension.

In humans, surgical sympathectomy lowers high BP and improves the cardiovascular prognosis of patients with severe hypertension.[7–9,61–63] Its poorly tolerated side effects, which include severe orthostatic hypotension, anhidrosis, intestinal disturbances and sexual dysfunction, and its high surgical risk, have led to the technique being abandoned. Nevertheless, it has proved the importance of the SNS in BP control beyond doubt.

Clinical studies on percutaneous sympathetic renal denervation

Symplicity HTN-1,[11] a proof-of-principle study, was the first to evaluate RDN in patients with severe resistant hypertension. One year after the procedure, mean office BP fall was 27 mmHg systolic and 17 mmHg diastolic, and was maintained until 24 months of follow-up, with 13% non-responders. A subgroup analysis assessing renal and systemic sympathetic activity showed a 47% reduction in renal norepinephrine spillover in these patients.[64]

In the Symplicity HTN-2 multicenter clinical trial,[64,65] 106 patients with severe resistant hypertension under medication were randomized to optimal antihypertensive medical therapy alone or to RDN plus optimal antihypertensive medical therapy. The primary endpoint was change in office BP at six-month follow-up. A significant fall in BP was observed in patients who underwent RDN: −32 mmHg in systolic BP and −12 mmHg in diastolic BP ($p<0.01$) compared to an increase of 1 mmHg in systolic BP and no change in diastolic BP (p=NS) in patients under optimal medical therapy alone at six months following RDN. A subgroup analysis of 24-hour ABPM data revealed a similar pattern, a fall of 11 mmHg in systolic BP and 7 mmHg in diastolic BP in the RDN group ($p<0.001$) compared to a fall of 3 mmHg and 1 mmHg on medical therapy alone (p=NS). The magnitude of the difference in BP fall between RDN and optimal medical therapy alone was maintained at 12-month follow-up.[64]

Alongside its efficacy, RDN was a safe procedure. In both studies, only minor vascular complications occurred, mainly at the puncture site: hematomas and pseudoaneurysms (four patients), one renal artery dissection during the diagnostic procedure, successfully treated with a stent, and no major complications. Regarding renal function, there were no significant changes in eGFR during follow-up.[49,66]

The recently published EnlightHTN I trial[51] also revealed a significant fall in both office BP and mean 24-hour ABPM values at six months, with a good safety profile.

The published data indicate that RDN has an excellent short-term safety profile, although data on the long-term risk of renal artery stenosis are lacking. The results of the Symplicity trials and EnlightHTN I are certainly promising, but their open design meant that bias in BP measurements, or even the extent of the placebo effect in treated patients, could not be properly addressed. Some of these limitations were addressed in the recently published randomized SYMPLICITY HTN-3 trial.[67,68] This was the first blinded sham-controlled study of RDN for treatment of resistant hypertension. The primary efficacy endpoint was the mean change in office systolic BP from baseline to six months in the RDN arm (n=364) compared to the control arm (n=171). At six-month follow-up, there was a difference of 2.39 mmHg in the change in systolic BP (−14.13±23.93 mmHg in the RDN arm vs. −11.74±25.94 mmHg in the sham procedure arm), which did not reach statistical significance (95% confidence interval [CI]: −6.89 to 2.12, p=0.26).

The secondary efficacy endpoint was the change in mean 24-hour ambulatory BP at six months. A statistically

non-significant difference of 1.96 mmHg (95% CI: −4.97 to 1.06, p=0.98) was seen at six months (−6.75±15.11 mm Hg in the RDN arm vs. −4.79±17.25 mm Hg in the control arm).

The rate of major adverse events at six months was 4% in the RDN arm vs. 5.8% in the control arm (p=0.37). The primary safety endpoint (a composite of major adverse events) rate was 1.4% in the RDN arm, less than the pre-specified objective of 9.8%, reaching statistical significance (p<0.001).

These conflicting results between Symplicity HTN-3[68] and the previous Symplicity trials, HTN-1[11] and HTN-2[65] (Table 3), may be related to a different and more rigorous design adopted in HTN-3, a different study population, more aggressive antihypertensive medication, and the requirement that no changes in antihypertensive medication could be made in the six months after the procedure. There was also potential for procedural variability due to the large number of centers involved in the HTN-3 study and a low case load per operator, each performing only three procedures on average (most performed their first and only RDN procedure for the trial). The inclusion for the first time of a large proportion of African-American patients (24.8% of the RDN arm and 29.2% of the control arm), a population known to be resistant to RAAS blockers, could have had a negative impact on the efficacy of RDN; a subgroup analysis revealed a statistically significant difference favoring the RDN arm in non-African-American patients. Overall antihypertensive medication was more intensive than in previous studies, probably reflecting the more severe hypertensive patients included. Regression to the mean may also at least partially explain the differences.[69] The presence for the first time of a sham procedure in the control arm (renal angiography was performed in all patients before randomization) may have diluted the expected placebo effect favoring the treated group.

Puzzling findings in Symplicity HTN-3 include the smaller decrease in office systolic BP from baseline to six months in the RDN arm, about half that observed in the Symplicity HTN-2 RDN group, despite similar baseline BP in the two studies, raising doubts as to whether RF energy was properly delivered. A larger decrease in BP was also observed in the HTN-3 control group compared to the much smaller decrease in the HTN-2 control group. These findings raise the question of whether a less effective denervation procedure allied to more aggressive medical therapy could have played a major role in the HTN-3 results.

The fact that there was no measurement to confirm that the renal nerves were in fact denervated by the procedure, because there is no test that can be easily performed in a large trial, is a major limitation to this and to almost all of these trials.

While the Symplicity HTN-3 follow-up will continue as planned for up to five years, the fact that many patients crossed over from the control arm to the RDN arm at six months will make it more difficult to draw significant conclusions concerning the long-term clinical results of RDN therapy and to assess the placebo effect over time.

Nevertheless, the Symplicity HTN-3 trial is a landmark in the development of RDN treatment, signaling the start of its reflection phase, in which new hypotheses generated by this trial can be addressed.

Renal denervation: new devices

The high expectations and enthusiasm created in the medical device industry has led many companies to develop new or improved technical solutions for RDN, some of which are commercially available (Table 4).

From predictable improvements of the original procedure to out-of-the-box ideas, many innovations are being integrated in the new designs. These include alternative mechanisms of action, like ultrasound catheters and balloons with microinjection systems to deliver neurotoxins. Simultaneously activated multi-electrodes not only significantly shorten procedural time but also increase reproducibility, ensuring that all quadrants are adequately denervated, while radial access reduces access site vascular complications, and manipulating renal catheters by a craniocaudal approach is generally less challenging and safer. Pain control is also a challenge, as electric current, tissue burning, and nerve damage, although essential components of the procedure, all cause discomfort. The more recent radiofrequency catheters with bipolar electrodes reportedly reduce discomfort due to a significantly smaller electric field during activation, but this is not yet clinically proven; the absence of an acute procedural efficacy endpoint is still a major limitation. Procedural success is difficult to determine and to correlate with BP response. Efforts are being made to find a biomarker or physiological test that indicates acute RDN success.

Sympathetic renal denervation: potential future indications

The overall decrease in SNS drive through RDN may be a valid alternative in clinical scenarios characterized by sympathetic hyperactivity other than resistant hypertension. A few of these alternative applications have already been explored and show promising results.

The association between heart failure and increased sympathetic drive is well known. Interestingly, cardiac and renal norepinephrine spillover is more closely associated with mortality than circulating catecholamine concentrations, although both are associated with worse outcomes.[16,70] This suggests that reducing norepinephrine spillover from the kidney could have beneficial symptomatic and prognostic effects.[71,72]

In animal models, RDN after myocardial infarction led to improvement in sodium excretion,[73] increased cardiac output, improved renal blood flow,[74] and down-regulation of angiotensin AT1 receptors mediating maladaptive responses.[75] In a multicenter study of patients with resistant hypertension treated by RDN with anatomical and functional myocardial parameters assessed by MRI, a subgroup of patients with LV dysfunction had significantly increased ejection fraction and circumferential strain.[76]

The REACH pilot study in heart failure provided evidence that RDN improved six-minute walk distances without affecting BP (mean 120 mmHg at baseline).[77] Other ongoing clinical trials will provide further evidence on the potential of RDN to influence the course and outcome of heart failure. Type 2 diabetes and insulin resistance are other conditions that have a strong association with resistant hypertension.

Table 3 Studies of renal denervation.

Trial	Symplicity HTN-1[11]	Symplicity HTN-2[65]		Symplicity HTN-3[68]		EnlightHTN-1[51]	RAPID[90]	REDUCE-HTN FIM[91]
Device	RF, single-electrode (Symplicity®)	RF, single-electrode (Symplicity®)	No RDN	RF, single-electrode (Symplicity®)	No RDN (sham)	RF, multi-electrode (EnlightHTN®)	RF, balloon (OneShot®)	RF, balloon (Vessix®)
No. of patients	47	52	54	564	171	46	50	41
Randomized	No	Yes		Yes		No	No	No
Sham control	No	-	No	-	Yes	No	No	No
Black (%)	4[a]	2[a]	4[a]			2.2[a]	NA	7.3
Mean baseline office SBP (mmHg)	177±20	178±18	178±16	179±16	180±17	176	181.6±20.8	183±18.1
Mean no. of antihypertensive drugs	4.7±1.5	5.2±1.5	5.3±1.8	5.1±1.4	5.2±1.4	4.1±0.6	4.9	5.1±1.7
Aldosterone blockers (%)	NA	17	17	22.5	28.7	13	22	26.8
Office SBP change at 6 months (mmHg)	−22	−32±23	1±21	−14.1±23.9	−11.7±25.9	−26	−20	−27.6
24-hour ABMP SBP change at 6 months (mmHg)	−11[b]	−11±15	−3±19	−6.8±15.1	−4.8±17.2	−10	−11	−8.5
Response rate (≥10 mmHg change in office SBP from baseline)	87%	84%	35%	58.3%	48.5%	80%	62	85%

ABPM: ambulatory blood pressure monitoring; NA: not available; RDN: renal denervation; RF: radiofrequency; SBP: systolic blood pressure.

[a] Described as non-white.

[b] Only nine RDN responder patients had adequate ABPM at baseline and longer than 30 days.

Table 4 Sympathetic renal denervation devices.

Company	Medtronic	Medtronic	St. Jude	Maya/Covidien	ReCor Medical	Boston Scientific	Terumo	Cordis
Product	Symplicity Flex™	Symplicity Spyral™	EnlightHTN™	OneShot™	Paradise™	Vessix V2™	Iberis™	Renlane™
Catheter size	6F	6F	8F	9F	6F	8F	6F	6F
Energy type	RF	RF	RF	RF	Ultrasound	RF	RF	RF
Catheter design	Single-electrode, monopolar	Multi-electrode (4), monopolar	Multi-electrode, monopolar	Multi-electrode, monopolar, irrigated	Ultrasound; balloon with cooling	Multi-electrode, bipolar, non-compliant balloon	Single-electrode, monopolar	Multi-electrode, monopolar
Over-the-wire	No	Yes	No	Yes	Yes	Yes	No	No
Energy delivery time	2 min	1 min	1 min	2 min	5 min	30 sec	2 min	30 sec
Total treatment time	16–24 min	2 min	4 min	4 min	Unknown	2 min	16–24 min	Unknown
Vessel obstruction	No	No	No	Yes	Yes	Yes	No	No
Trials	Symplicity HTN-1[111], Symplicity HTN-2[64], Symplicity HTN-3[68]	FIM[92]	EnlightHTN-1[51]	RHAS[90]	PARADISE[93], REALISE[94]	Reduce HTN[91]	–	RENABLATE I[95]

RF: radiofrequency.

Half of resistant hypertension patients are considered to be insulin resistant, increasing the risk for type 2 diabetes, and since insulin resistance is dependent on sympathetic activity it appears likely that it could also be a target for RDN.[78,79] In a pilot study, along with reducing BP, RDN improved fasting glucose, insulin, and C-peptide concentrations, as well as homeostasis model assessment-insulin resistance indices in patients with resistant hypertension and metabolic disease, suggesting that RDN might improve diabetic status in these patients.[80] Witkowski et al. showed a decline in glycated hemoglobin concentrations after RDN.[81]

The association between obstructive sleep apnea and resistant hypertension is well known.[81] In 2011 Witkowski et al. published a pilot study on the effect of RDN in 10 patients with resistant hypertension and obstructive sleep apnea. At six months there was an improvement in apnea-hypopnea indices.[81] In an experimental model, it has been shown that RDN reduces post-apneic BP rise, renal hypoperfusion during apnea and RAAS activation in the kidney.[82,83] The value of these findings is still controversial and confirmatory studies are needed.

In an animal model[84] of obstructive sleep apnea and induced atrial fibrillation (AF), RDN decreased the atrial refractory period and AF recurrence,[82] providing better rate control.[85] In a pilot trial, patients with resistant hypertension and symptomatic paroxysmal or persistent atrial fibrillation refractory to $\geq$2 antiarrhythmic drugs were randomized to pulmonary vein isolation alone or associated with RDN. At 12-month follow-up 69% of patients treated with RDN were AF-free, compared to 29% of those treated with pulmonary vein isolation only.[86] These experimental findings indicate the potential usefulness of RDN in AF treatment.

The evidence for the fundamental role of sympathetic activity in ventricular arrhythmias is overwhelming. In an animal model of ischemia/reperfusion arrhythmias, RDN decreased the occurrence of ventricular arrhythmias/fibrillation and attenuated the rise in LV end-diastolic pressure during LV ischemia without influencing infarct size, changes in ventricular contractility, BP or reperfusion arrhythmias.[87] In a small case series involving patients with chronic heart failure and ventricular electrical storm, RDN reduced discharges from implantable cardioverter-defibrillators and ventricular ectopies.[88] Hoffmann et al.[89] reported that RDN can be safely and effectively performed as an adjunct to cardiac catheter ablation, in a hemodynamically unstable patient with ventricular storm after ST-elevation myocardial infarction. Although these are early and preliminary findings, the underlying biological plausibility will certainly heighten interest in these potential future applications of RDN.

Conclusions

It is now accepted that an overactive sympathetic system has a pivotal role in the pathophysiology of several diseases besides essential hypertension. Related conditions like depression, mental stress, hypertension, diabetes, obesity, sleep apnea, metabolic syndrome, ischemic heart disease, heart failure and chronic renal failure, all have a common link, the often neglected hyperactive SNS. In a new era with new tools to control and treat sympathetic hyperactivity,

perhaps this system will finally receive the attention it deserves.

The inability to treat hypertension effectively is due in part to a lack of understanding of the fundamental mechanisms involved in BP control. There is a complex mixture of hormonal, neural and intrinsic factors, all acting together, over different time scales and with different feedback control pathways, and it seems unlikely that any of the current treatment approaches is actually targeting the factors that originally led to the rise in BP. Catheter-based RDN is a truly innovative approach to treat hypertension by changing sympathetic activity. In patients with resistant hypertension, the technique has significantly reduced BP as well as sympathetic nerve activity and norepinephrine spillover, with high safety levels. These achievements are well documented in several international multicenter trials and registries. Along with its proven efficacy in BP reduction, it has the potential to positively affect insulin resistance and diabetes, LV mass, proteinuria and arrhythmias, as indicated by various small proof-of-concept studies.

Nevertheless, there are still important issues that need to be addressed in the near future, like the impossibility of determining whether denervation was effective, what level of denervation is needed to achieve clinical success, which patients have an appropriate phenotype for RDN, and what endpoints should be used to define RDN success (merely BP reduction or reduction in target organ damage). Much needs to be done and will be in the coming years, but a new window has certainly been opened not only to address hypertension, but most importantly to address SNS dysfunction.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Lim SS, Vos T, Flaxman AD, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380:2224–60.
2. Lawes CM, Vander Hoorn S, Rodgers A. Global burden of blood-pressure-related disease, 2001. Lancet. 2008;371:1513–8.
3. Turnbull F. Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. Lancet. 2003;362: 1527–35.
4. Tocci G, Rosei EA, Ambrosioni E, et al. Blood pressure control in Italy: analysis of clinical data from 2005–2011 surveys on hypertension. J Hypertens. 2012;30:1065–74.
5. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens. 2013;31:1281–357.
6. Daugherty SL, Powers JD, Magid DJ, et al. Incidence and prognosis of resistant hypertension in hypertensive patients. Circulation. 2012;125:1635–42.
7. Hoobler SW, Manning JT, Paine WG, et al. The effects of splanchnicectomy on the blood pressure in hypertension; a controlled study. Circulation. 1951;4:173–83.
8. Smithwick RH, Whitelaw GP, Kinsey D. Surgical approach to the treatment of essential hypertension. Results of therapy (medical and surgical). Am J Cardiol. 1962;9:893–9.
9. Harington M, Kincaid-Smith P, McMichael MJ. Results of treatment in malignant hypertension: a seven-year experience in 94 cases. Br Med J. 1959;2:969–80.
10. DiBona GF, Esler M. Translational medicine: the antihypertensive effect of renal denervation. Am J Physiol Regul Integr Comp Physiol. 2010;298:R245–53.
11. Krum H, Schlaich M, Whitbourn R, et al. Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. Lancet. 2009;373:1275–81.
12. Von Euler US, Hellner S, Purkhold A. Excretion of noradrenaline in urine in hypertension. Scand J Clin Lab Invest. 1954;6:54–9.
13. Hagbarth KE, Vallbo AB. Pulse and respiratory grouping of sympathetic impulses in human muscle-nerves. Acta Physiol Scand. 1968;74:96–108.
14. Esler M, Jackman G, Bobik A, et al. Determination of norepinephrine apparent release rate and clearance in humans. Life Sci. 1979;25:1461–70.
15. Blombery PA, Heinzow BG. Cardiac and pulmonary norepinephrine release and removal in the dog. Circ Res. 1983;53:688–94.
16. Hasking GJ, Esler MD, Jennings GL, et al. Norepinephrine spillover to plasma in patients with congestive heart failure: evidence of increased overall and cardiorenal sympathetic nervous activity. Circulation. 1986;73:615–21.
17. Graham LN, Smith PA, Huggett RJ, et al. Sympathetic drive in anterior and inferior uncomplicated acute myocardial infarction. Circulation. 2004;109:2285–9.
18. Kishi T, Hirooka Y. Central mechanisms of abnormal sympathoexcitation in chronic heart failure. Cardiol Res Pract. 2012;2012:1–7.
19. Triposkiadis F, Karayannis G, Giamouzis G, et al. The Sympathetic nervous system in heart failure. Physiology, pathophysiology, and clinical implications. J Am Coll Cardiol. 2009;54:1747–82.
20. Esler M. The sympathetic system and hypertension. Am J Hypertens. 2000;13 6 Pt 2:99S–105S.
21. Esler M, Rumantir M, Kaye D, et al. Sympathetic nerve biology in essential hypertension. Clin Exp Pharmacol Physiol. 2001;28:986–9.
22. Esler M. The sympathetic system in essential hypertension. Rev Port Cardiol. 2000;19 Suppl 2:II9–14.
23. Neumann J, Ligtenberg G, Klein IH, et al. Sympathetic hyperactivity in hypertensive chronic kidney disease patients is reduced during standard treatment. Hypertension. 2007;49:506–10.
24. Esler M, Rumantir M, Wiesner G, et al. Sympathetic nervous system and insulin resistance: from obesity to diabetes. Am J Hypertens. 2001;14 11 Pt 2:304S–9S.
25. Narkiewicz K, Somers VK. The sympathetic nervous system and obstructive sleep apnea: implications for hypertension. J Hypertens. 1997;15 12 Pt 2:1613–9.
26. Esler M. The 2009 Carl Ludwig Lecture: pathophysiology of the human sympathetic nervous system in cardiovascular diseases: the transition from mechanisms to medical management. J Appl Physiol. 2010;108:227–37.
27. Furlan R, Ardizzone S, Palazzolo L, et al. Sympathetic overactivity in active ulcerative colitis: effects of clonidine. Am J Physiol Regul Integr Comp Physiol. 2006;290:R224–32.
28. Kaye DM, Lefkovits J, Jennings GL, et al. Adverse consequences of high sympathetic nervous activity in the failing human heart. J Am Coll Cardiol. 1995;26:1257–63.
29. Zoccali C, Mallamaci F, Parlongo S, et al. Plasma norepinephrine predicts survival and incident cardiovascular events in patients with end-stage renal disease. Circulation. 2002;105: 1354–9.

30. Fisher JP, Young CN, Fadel PJ. Central sympathetic overactivity: maladies and mechanisms. Auton Neurosci. 2009;148:5–15.
31. Schirmer SH, Sayed MM, Reil JC, et al. Improvements in left ventricular hypertrophy and diastolic function following renal denervation: effects beyond blood pressure and heart rate reduction. J Am Coll Cardiol. 2013.
32. Lown B, Verrier RL. Neural activity and ventricular fibrillation. N Engl J Med. 1976;294:1165–70.
33. Esler M, Alvarenga M, Lambert G, et al. Cardiac sympathetic nerve biology and brain monoamine turnover in panic disorder. Ann NY Acad Sci. 2004;1018:505–14.
34. Bruno RM, Ghiadoni L, Seravalle G, et al. Sympathetic regulation of vascular function in health and disease. Front Physiol. 2012;3:1–15.
35. Hirooka Y, Kishi T, Sakai K, et al. Imbalance of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension. Am J Physiol Regul Integr Comp Physiol. 2011;300:R818–26.
36. Landsberg L. Insulin-mediated sympathetic stimulation: role in the pathogenesis of obesity-related hypertension (or, how insulin affects blood pressure, and why). J Hypertens. 2001;19 3 Pt 2:523–8.
37. Grassi G, Seravalle G, Quarti-Trevano F, et al. Excessive sympathetic activation in heart failure with obesity and metabolic syndrome: characteristics and mechanisms. Hypertension. 2007;49:535–41.
38. Chidsey CA, Braunwald E, Morrow AG, et al. Myocardial norepinephrine concentration in man. Effects of reserpine and of congestive heart failure. N Engl J Med. 1963;269: 653–8.
39. Thomas JA, Marks BH. Plasma norepinephrine in congestive heart failure. Am J Cardiol. 1978;41:233–43.
40. The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II): a randomised, trial. Lancet. 1999;353:9–13.
41. Esler M. The sympathetic nervous system through the ages: from Thomas Willis to resistant hypertension. Exp Physiol. 2011;96:611–22.
42. Esler M, Lambert E, Schlaich M. Point: chronic activation of the sympathetic nervous system is the dominant contributor to systemic hypertension. J Appl Physiol. 2010;109:1996–8 [discussion 2016].
43. De Macedo ME, Lima MJ, Silva AO, et al. Prevalence, awareness, treatment and control of hypertension in Portugal: the PAP study. Rev Port Cardiol. 2007;26:21–39.
44. Calhoun DA, Jones D, Textor S, et al. Resistant hypertension: diagnosis, evaluation, and treatment: a scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. Circulation. 2008;117:e510–26.
45. Sarafidis PA, Georgianos P, Bakris GL. Resistant hypertension – its identification and epidemiology. Nat Rev Nephrol. 2013;9:51–8.
46. de la Sierra A, Segura J, Banegas JR, et al. Clinical features of 8295 patients with resistant hypertension classified on the basis of ambulatory blood pressure monitoring. Hypertension. 2011;57:898–902.
47. Egan BM, Zhao Y, Axon RN, et al. Uncontrolled and apparent treatment resistant hypertension in the United States, 1988 to 2008. Circulation. 2011;124:1046–58.
48. Prugger C, Keil U, Wellmann J, et al. Blood pressure control and knowledge of target blood pressure in coronary patients across Europe: results from the EUROASPIRE III survey. J Hypertens. 2011;29:1641–8.
49. Symplicity HTNI. Catheter-based renal sympathetic denervation for resistant hypertension: durability of blood pressure reduction out to 24 months. Hypertension. 2011;57:911–7.
50. Murray ED, Krum H, Schlaich M, et al. Renal sympathetic denervation for treatment of drug-resistant hypertension: one-year results from the Symplicity HTN-2 randomized, controlled trial. Circulation. 2012;126:2976–82.
51. Worthley SG, Tsioufis CP, Worthley MI, et al. Safety and efficacy of a multi-electrode renal sympathetic denervation system in resistant hypertension: the EnlightHTN I trial. Eur Heart J. 2013;34:2132–40.
52. Schmieder RE, Redon J, Grassi G, et al. Updated ESH position paper on interventional therapy of resistant hypertension. EuroIntervention. 2013;9(R):R58–66.
53. Hypertension: Clinical management of primary hypertension in adults. This guideline partially updates and replaces NICE clinical guideline 34; 2011. www.nice.org.uk/guidance/CG127
54. Schlaich MP, Bart B, Hering D, et al. Feasibility of catheter-based renal nerve ablation and effects on sympathetic nerve activity and blood pressure in patients with end-stage renal disease. Int J Cardiol. 2013;168:2214–20.
55. Bohm M, Mahfoud F, Ukena C, et al. Rationale and design of a large registry on renal denervation: the Global SYMPLICITY registry. EuroIntervention. 2013;9:484–92.
56. Araújo Gonçalves P, Infante de Oliveira E, Cyrne Carvalho H, et al. Desnervação Renal para HTA Resistente: Resultados Iniciais de um Registo Nacional. Rev Port Cardiol. 2013;32(Espec Congr):12–3.
57. Thorén P. Efferent renal nerve traffic in the spontaneously hypertensive rat. Clin Exp Hypertension A: Theory Pract. 1987;9 Suppl 1:259–79.
58. Kassab S, Kato T, Wilkins FC, et al. Renal denervation attenuates the sodium retention and hypertension associated with obesity. Hypertension. 1995;25 4 Pt 2:893–7.
59. Campese VM, Kogosov E. Renal afferent denervation prevents hypertension in rats with chronic renal failure. Hypertension. 1995;25 4 Pt 2:878–82.
60. Ye S, Zhong H, Yanamadala V, et al. Renal injury caused by intrarenal injection of phenol increases afferent and efferent renal sympathetic nerve activity. Am J Hypertens. 2002;15:717–24.
61. Grimson KS. The surgical treatment of hypertension. Adv Intern Med. 1947;2:173–94.
62. Hafkenschiel JH, Fitts WT Jr. The surgical treatment of hypertension with particular reference to adrenalectomy and sympathectomy. Trans Am Coll Cardiol. 1955;5:107–12.
63. Hutchison GB, Evans JA. Should sympathectomy for arterial hypertension be abandoned? Review of surgical therapy at the Lahey Clinic: 1950–1954. Surg Clin N Am. 1957;37:871–89.
64. Esler MD, Krum H, Schlaich M, et al. Renal sympathetic denervation for treatment of drug-resistant hypertension: one-year results from the Symplicity HTN-2 randomized, controlled trial. Circulation. 2012;126:2976–82.
65. Esler MD, Krum H, Sobotka PA, et al. Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. Lancet. 2010;376:1903–9.
66. Mahfoud F, Cremers B, Janker J, et al. Renal hemodynamics and renal function after catheter-based renal sympathetic denervation in patients with resistant hypertension. Hypertension. 2012;60:419–24.
67. Kandzari DE, Bhatt DL, Sobotka PA, et al. Catheter-based renal denervation for resistant hypertension: rationale and design of the SYMPLICITY HTN-3 Trial. Clin Cardiol. 2012;35:528–35.
68. Bhatt DL, Kandzari DE, O'Neill WW, et al. A controlled trial of renal denervation for resistant hypertension. N Engl J Med. 2014;370:1393–401.
69. Barnett AG, van der Pols JC, Dobson AJ. Regression to the mean: what it is and how to deal with it. Int J Epidemiol. 2005;34:215–20.
70. Petersson M, Friberg P, Eisenhofer G, et al. Long-term outcome in relation to renal sympathetic activity in patients with chronic heart failure. Eur Heart J. 2005;26:906–13.

71. Bohm M, Linz D, Urban D, et al. Renal sympathetic denervation: applications in hypertension and beyond. Nat Rev Cardiol. 2013;10:465–76.
72. Bohm M, Ewen S, Kindermann I, et al. Renal denervation and heart failure. Eur J Heart Fail. 2014.
73. Villarreal D, Freeman RH, Johnson RA, et al. Effects of renal denervation on postprandial sodium excretion in experimental heart failure. Am J Physiol. 1994;266 5 Pt 2:R1599–604.
74. Masaki H, Imaizumi T, Harasawa Y, et al. Dynamic arterial baroreflex in rabbits with heart failure induced by rapid pacing. Am J Physiol. 1994;267 1 Pt 2:H92–9.
75. Clayton SC, Haack KK, Zucker IH. Renal denervation modulates angiotensin receptor expression in the renal cortex of rabbits with chronic heart failure. Am J Physiol Renal Physiol. 2011;300:F31–9.
76. Mahfoud F, Urban D, Teller D, et al. Effect of renal denervation on left ventricular mass and function in patients with resistant hypertension: data from a multi-centre cardiovascular magnetic resonance imaging trial. Eur Heart J. 2014.
77. Davies JE, Manisty CH, Petraco R, et al. First-in-man safety evaluation of renal denervation for chronic systolic heart failure: primary outcome from REACH-Pilot study. Int J Cardiol. 2013;162:189–92.
78. Mancia G, Bousquet P, Elghozi JL, et al. The sympathetic nervous system and the metabolic syndrome. J Hypertens. 2007;25:909–20.
79. Huggett RJ, Scott EM, Gilbey SG, et al. Impact of type 2 diabetes mellitus on sympathetic neural mechanisms in hypertension. Circulation. 2003;108:3097–101.
80. Mahfoud F, Schlaich M, Kindermann I, et al. Effect of renal sympathetic denervation on glucose metabolism in patients with resistant hypertension: a pilot study. Circulation. 2011;123:1940–6.
81. Witkowski A, Prejbisz A, Florczak E, et al. Effects of renal sympathetic denervation on blood pressure, sleep apnea course, and glycemic control in patients with resistant hypertension and sleep apnea. Hypertension. 2011;58:559–65.
82. Linz D, Mahfoud F, Schotten U, et al. Renal sympathetic denervation suppresses postapneic blood pressure rises and atrial fibrillation in a model for sleep apnea. Hypertension. 2012;60:172–8.
83. Linz D, Hohl M, Nickel A, et al. Effect of renal denervation on neurohumoral activation triggering atrial fibrillation in obstructive sleep apnea. Hypertension. 2013;62:767–74.
84. Linz D, Schotten U, Neuberger HR, et al. Negative tracheal pressure during obstructive respiratory events promotes atrial fibrillation by vagal activation. Heart Rhythm. 2011;8:1436–43.
85. Linz D, Mahfoud F, Schotten U, et al. Renal sympathetic denervation provides ventricular rate control but does not prevent atrial electrical remodeling during atrial fibrillation. Hypertension. 2013;61:225–31.
86. Pokushalov E, Romanov A, Corbucci G, et al. A randomized comparison of pulmonary vein isolation with versus without concomitant renal artery denervation in patients with refractory symptomatic atrial fibrillation and resistant hypertension. J Am Coll Cardiol. 2012;60:1163–70.
87. Linz D, Wirth K, Ukena C, et al. Renal denervation suppresses ventricular arrhythmias during acute ventricular ischemia in pigs. Heart Rhythm. 2013;10:1525–30.
88. Ukena C, Bauer A, Mahfoud F, et al. Renal sympathetic denervation for treatment of electrical storm: first-in-man experience. Clin Res Cardiol. 2012;101:63–7.
89. Hoffmann BA, Steven D, Willems S, et al. Renal sympathetic denervation as an adjunct to catheter ablation for the treatment of ventricular electrical storm in the setting of acute myocardial infarction. J Cardiovasc Electrophysiol. 2013.
90. Verheye S. Rapid renal sympathetic denervation for resistant hypertension using the OneShot™ Ablation System (RAPID) Study: Primary Endpoint 6-Month Results. TCT; 2013. http://www.tctmd.com/txshow.aspx?tid=1374091&id=121514&trid=1373709
91. Schofer J. Clinical evidence in catheter-based renal sympathetic denervation – building the data. In: EuroPCR. 2013. http://www.pcronline.com/file/download/(path)/var/www/media/pcronline/diapos/EuroPCR2013/(id)/2276-20130522_1708_Room242AB_Schofer_Joachim/(ext)/pdf/.(ref)/131358
92. Whitbourne R. Early results of first-in-man simultaneous multisite renal denervation treatment for resistant hypertension; 2013. http://www.pcronline.com/Lectures/2013/Early-results-of-first-in-man-simultaneous-multisite-renal-denervation-treatment-for-resistant-hypertension
93. Bonan R. ReCor Medical Paradise: first-in-man results of a novel circumferential catheter-based ultrasound technology for renal denervation; 2012. http://wwwpcronlinecom/Lectures/2012/ReCor-Medical-Paradise-first-in-man-results-of-a-novel-circumferential-catheter-based-ultrasound-technology-for-renal-denervation
94. Realise AP. Clinical Trial: Preliminary results demonstrate catheter based ultrasound renal denervation is associated with a decrease in sympathetic activity; 2014. http://www pcronlinecom/Lectures/2014/Preliminary-results-from-the-REALISE-trial-show-endovascular-ultrasound-renal-denervation-ultrasound-associated-with-decrease-in-sympathetic-overactivity
95. Wijns W. RENABLATE I Feasibility Study: 3 months results; 2014. http://www.pcronline.com/Lectures/2014/Renablate-study-presentation

ATTACHMENT B

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Renal denervation in patients with resistant hypertension: Six-month results☆

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KEYWORDS
Resistant hypertension;
Renal denervation;
Left ventricular hypertrophy

Abstract
Introduction: Increased activation of the sympathetic nervous system plays a central role in the pathophysiology of hypertension (HTN). Catheter-based renal denervation (RDN) was recently developed for the treatment of resistant HTN.
Aim: To assess the safety and efficacy of RDN for blood pressure (BP) reduction at six months in patients with resistant HTN.
Methods: In this prospective registry of patients with essential resistant HTN who underwent RDN between July 2011 and May 2013, the efficacy of RDN was defined as ≥10 mmHg reduction in office systolic blood pressure (SBP) six months after the intervention.
Results: In a resistant HTN outpatient clinic, 177 consecutive patients were evaluated, of whom 34 underwent RDN (age 62.7±7.6 years; 50.0% male). There were no vascular complications, either at the access site or in the renal arteries. Of the 22 patients with complete six-month follow-up, the response rate was 81.8% (n=18). The mean office SBP reduction was 22 mmHg (174±23 vs. 152±22 mmHg; p<0.001) and 9 mmHg in diastolic BP (89±16 vs. 80±11 mmHg; p=0.006). The number of antihypertensive drugs (5.5±1.0 vs. 4.6±1.1; p=0.010) and pharmacological classes (5.4±0.7 vs. 4.6±1.1; p=0.009) also decreased significantly. Of the 24-hour ambulatory BP monitoring and echocardiographic parameters analyzed, there were significant reductions in diastolic load (45±29 vs. 27±26%; p=0.049) and in left ventricular mass index (174±56 vs. 158±60 g/m^2; p=0.014).

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Conclusion: In this cohort of patients with resistant HTN, RDN was safe and effective, with a significant BP reduction at six-month follow-up.

PALAVRAS-CHAVE
Hipertensão arterial resistente;
Desnervação renal;
Hipertrofia ventricular esquerda

Desnervação renal em doentes com hipertensão arterial resistente: resultados aos seis meses de seguimento

Resumo
Introdução: O aumento da atividade do sistema nervoso simpático desempenha um papel preponderante na fisiopatologia da hipertensão arterial (HTA). Recentemente foi desenvolvida uma técnica de intervenção percutânea – a desnervação renal (DNR) – para o tratamento da HTA resistente.
Objetivo: Avaliar a segurança imediata e a eficácia da DNR aos seis meses na redução da pressão arterial em doentes com HTA resistente.
Métodos: Registo prospetivo de doentes com HTA essencial resistente submetidos a DNR entre julho de 2011 e maio de 2013. A eficácia da DNR foi definida pela redução ≥10 mmHg da pressão arterial sistólica (PAS), avaliada na consulta dos seis meses de seguimento.
Resultados: Numa consulta de HTA resistente avaliaram-se 177 doentes consecutivos, dos quais 34 (idade 62,7±7,6 anos; 50,0% homens) efetuaram DNR. Não ocorreram complicações vasculares, nomeadamente no acesso ou nas artérias renais. Nos 22 doentes com seguimento completo aos seis meses, a taxa de respondedores foi 81,8% (n=18). A PAS na consulta diminuiu em média 22 mmHg (174±23 *versus* 152±22 mmHg; p<0,001) e a diastólica 9 mmHg (89±16 *versus* 80±11 mmHg; p=0,006). O número de fármacos anti-hipertensores (5,5±1,0 *versus* 4,6±1,1; p=0,010) e de classes farmacológicas (5,4±0,7 *versus* 4,6±1,1; p=0,009) também diminuíram significativamente. Dos parâmetros da monitorização ambulatória da pressão arterial de 24 h e ecocardiográficos analisados, a percentagem de cargas diastólicas (45±29 *versus* 27±26%; p=0,049) e o índice de massa ventricular esquerda (174±56 *versus* 158±60 g/m^2; p=0,014) diminuíram significativamente.
Conclusão: Na população estudada de doentes com HTA resistente submetidos a DNR, esta foi uma intervenção segura e eficaz na redução da pressão arterial aos seis meses de seguimento.

Introduction

Hypertension (HTN) is one of the main independent risk factors for global mortality.[1] Its high prevalence and increasing incidence, including among young adults, are a major public health concern.[2]

Despite the many approved and recommended therapeutic options, the rate of control of HTN is far from ideal.[3] This was demonstrated by the PAP study on the prevalence, awareness, treatment and control of HTN in Portugal,[4] which showed not only a high prevalence of HTN in individuals aged 18 and over (42.1%) but also a low rate of control (11.2%). Although various factors contribute to poor control, in a significant number of cases HTN is resistant to drug therapy and it is therefore essential to identify such patients given their high risk of cardiovascular events.[5–7] The limitations of current drug therapies probably reflect the complex pathophysiological mechanisms involved in the development and persistence of HTN.[8,9] Chronic activation of the sympathetic nervous system is an important mechanism in resistant HTN, and so a new interventional technique – renal denervation (RDN) – has been developed, consisting of endovascular application of radiofrequency energy in the renal arteries to modulate renal sympathetic activity.[10,11]

The safety and efficacy of RDN were first documented in the Symplicity HTN-1[11] and Symplicity HTN-2 trials,[12] and there is evidence that similar levels of blood pressure (BP) reduction are maintained in the medium term.[13,14] We recently published our initial experience with this technique to treat patients with resistant HTN.[15]

The aim of this study was to assess the safety and efficacy of RDN for BP reduction at six months in patients with resistant HTN.

Methods

Study design and population

In this prospective registry of 177 consecutive patients evaluated in the resistant HTN outpatient clinic of a tertiary center between July 2011 and May 2013, resistant HTN was defined as office BP of ≥140/90 mmHg despite therapy with at least three antihypertensive drugs (including a diuretic) at maximum tolerated doses.[16] Possible secondary causes of HTN were excluded in all patients. Patients were selected for RDN in joint meetings between the cardiologists and nephrologists responsible for patient assessment

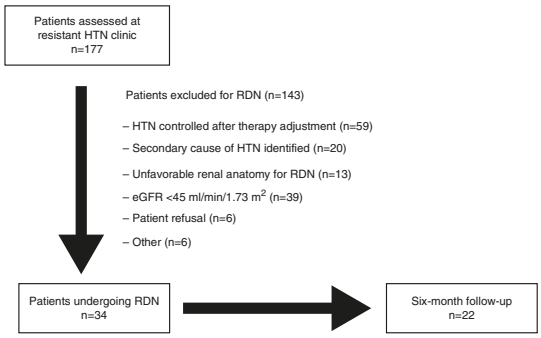

Figure 1 Patient selection. eGFR: estimated glomerular filtration rate; HTN: hypertension; RDN: renal denervation.

in the HTN clinic. The procedures were approved by the hospital's ethics committee and patients' informed consent was obtained. The study design is summarized in Figure 1. The criteria used in selecting patients for RDN were recently published by de Araújo Gonçalves et al.[15]

After clinical and laboratory assessment in accordance with the protocol, 34 patients were selected for RDN, of whom 22 completed six-month follow-up. The final analysis assessed the immediate safety of the procedure in all patients and its efficacy in the group with complete six-month follow-up.

Clinical assessment and diagnostic exams

Renal artery angiography was performed in all patients to assess anatomical suitability for RDN, and in 73.5% (n=25) of those considered eligible, noninvasive computed tomography angiography was performed prior to RDN. The anatomical criteria were renal artery diameter $\geq$4 mm and absence of significant tortuosity or >50% stenosis. Demographic variables, clinical history and anthropometric data were recorded. Baseline assessment prior to RDN included systolic (SBP) and diastolic blood pressure (DBP) at the last consultation, transthoracic echocardiography, 24-hour ambulatory BP monitoring (ABPM) and laboratory tests. Antihypertensive medication was also recorded, both the number of drugs and pharmacological classes, divided into the following categories: angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, direct renin inhibitors, aldosterone antagonists, diuretics, beta-blockers, calcium-channel blockers and alpha-blockers.

Renal denervation procedure

The procedure was performed via femoral access in all cases except one in which the left radial artery was used. After gaining vascular access, abdominal aortography and selective renal artery angiography were performed. Radiofrequency energy was applied in both renal arteries using the following systems: Symplicity® (Medtronic, USA) in 26 patients, EnligHTN® (St. Jude Medical, USA) in six, and OneShot® (Covidien, USA) in two. The device is connected to a radiofrequency generator that automatically programs and controls impedance, temperature and duration of the application, independently of the operator, on the basis of the manufacturer's protocols for each type of device. The Symplicity® system performs 4–6 applications lasting 120 s each in both renal arteries, beginning in the most distal segment of the vessel, at intervals of around 5 mm and in different quadrants of the arterial wall.[11] EnligHTN® is a multi-electrode system that provides multiple applications without the need to maneuver the device; the procedure also begins with the most distal electrode with four sequential applications lasting 90 s each, the ideal being two series of four applications in each artery.[17] The more recently approved OneShot® system uses a guidewire and a single irrigated balloon-mounted spiral electrode that applies energy for 120 s.[18] All procedures were performed under sedation with anesthesia support (propofol and remifentanil in weight-adjusted doses) and anticoagulation with unfractionated heparin for a minimum activated clotting time of 250 s. In all cases of femoral access, the access site was closed using an Angio-Seal® (St. Jude Medical, USA). There were no complications at the access site or in the renal arteries following RDN; there was one case of renal artery spasm and stenosis on final angiographic assessment, in a procedure performed on an accessory renal artery with a diameter at the lower recommended limit (4 mm).

Follow-up

To assess the efficacy of RDN at six months, we used the definition of responder used in validation studies of the technique: reduction in office SBP of $\geq$10 mmHg at follow-up. Office DBP, number of antihypertensive drugs and pharmacological classes, and 24-hour ABPM values were also assessed at follow-up, as well as the following echocardiographic parameters: left ventricular mass index (LVMI), left ventricular ejection fraction (LVEF), left atrial volume index, E/A ratio (E and A representing maximum early and late mitral flow velocities, respectively, by pulsed Doppler), E wave deceleration time, and E/e′ ratio (e′ representing mitral annular early diastolic velocity by tissue Doppler).

The immediate safety of RDN was assessed on the basis of complications related to the vascular access site (hematoma or pseudoaneurysm) or to selective renal artery catheterization or radiofrequency application (spasm, stenosis, dissection, thrombosis or perforation).

Statistical analysis

The statistical analysis was performed using Statistical Package for Social Sciences® for Windows, version 19.0 (SPSS, Inc., Chicago, IL). Categorical variables were expressed as frequencies (percentages in brackets) and compared using Fisher's exact test. Continuous variables were expressed as means $\pm$ standard deviation and compared using the Student's t test when appropriate. Results with $p<0.05$ were considered statistically significant.

Table 1 Baseline characteristics of the study population (n=34).

	n (%)
Demographic data	
Age (years)	62.7±7.6
Male	17 (50.0)
Caucasian	32 (94.1)
Cardiovascular risk factors	
BMI (kg/m^2)	30.9±5.3
Obesity (BMI ≥30.0 kg/m^2)	19 (55.9)
Diabetes	22 (64.7)
Dyslipidemia	23 (67.6)
Current smoking	1 (2.9)
Family history of CAD	2 (5.9)
Personal history	
CAD	7 (20.6)
Peripheral arterial disease	4 (11.8)
Myocardial infarction	2 (5.9)
PCI	5 (14.7)
Stroke	3 (8.8)
Chronic renal failure	6 (17.6)
Obstructive sleep apnea	3 (8.8)
Pharmacological therapy	
Number of drugs	5.8±1.0
Number of classes	5.5±0.8
Office blood pressure	
SBP (mmHg)	175±23
DBP (mmHg)	92±18
24-hour ABPM	
Mean SBP (mmHg)	151±20
Mean DBP (mmHg)	85±16
Systolic load (%)	73±25
Diastolic load (%)	45±28
Transthoracic echocardiography	
LV mass index (g/m^2)	164±48
LV ejection fraction (%)	63±9
Left atrial volume index (ml/m^2)	37±15
E/A ratio	0.9±0.4
E-wave deceleration time (ms)	239±65
E/e′ ratio	12±4
GFR (ml/min/1.73 m^2)	81.8±36.3
Serum creatinine (mg/dl)	1.1±0.4

ABPM: ambulatory blood pressure monitoring; BMI: body mass index; CAD: coronary artery disease; DBP: diastolic blood pressure; GFR: glomerular filtration rate; LV: left ventricular; PCI: percutaneous coronary intervention; SBP: systolic blood pressure.

Results

Baseline characteristics of patients

The baseline characteristics of patients undergoing RDN are shown in Table 1. Their mean age was 62.7±7.6 years, 50% were male (n=17) and most (94.1%, n=32) were Caucasian. Cardiovascular risk factors included obesity in 55.9% (mean body mass index 30.9±5.3 kg/m^2), type 2 diabetes in 64.7%, dyslipidemia in 67.6%, current smoking in 2.9%, and family history of premature coronary artery disease (CAD) in 5.9%. Personal history included vascular disease in any territory in 32.4% (n=11) – peripheral arterial disease in 11.8% (n=4), cerebrovascular disease in 8.8% (n=3), and CAD in 20.6% (5.9% with previous myocardial infarction and 14.7% with percutaneous coronary intervention). Three patients (8.8%) had concomitant obstructive sleep apnea and their elevated BP levels persisted despite home noninvasive ventilatory support. Mean estimated glomerular filtration rate (eGFR) was 81.8±36.3 ml/min/1.73 m^2; 17.6% had chronic renal failure, defined as eGFR <60 ml/min/1.73 m^2. Mean serum creatinine was 1.1±0.4 mg/dl.

At the last consultation prior to RDN, mean SBP and DBP were 175±23 mmHg and 92±18 mmHg, respectively, and mean heart rate was 71±18 bpm, while 24-hour ABPM showed the following mean values: SBP 151±20 mmHg, DBP 85±16 mmHg, mean arterial pressure 107±14 mmHg, pulse pressure 68±16 mmHg, systolic load 73±25%, diastolic load 45±28% and heart rate 69±12 bpm, with absence of circadian rhythm in 57.1% of patients. Transthoracic echocardiography revealed left ventricular (LV) hypertrophy in most patients (90.9%), with mean LVMI of 164±48 g/m^2. Mean LVEF was 66±9%, and only four patients presented reduced LVEF (<55% by Simpson's biplane method). Mean left atrial volume index was 37±15 g/m^2, E/A ratio 0.9±0.4, E-wave deceleration time 239±65 ms and E/e′ ratio 12±4. On average, patients were medicated with 5.8±1.0 antihypertensive drugs, from 5.5±0.8 pharmacological classes. The most commonly prescribed drug classes were calcium channel blockers, used in 97.1% (n=33), diuretics in 88.2% (n=30) and beta-blockers in 82.4% (n=28). Both aldosterone antagonists and alpha-blockers were prescribed in 70.6% (n=24), angiotensin receptor blockers in 61.8% (n=21), angiotensin-converting enzyme inhibitors in 52.9% (n=18) and renin inhibitors in 14.7% (n=5).

Six-month follow-up

Of the 22 patients with complete six-month follow-up, 18 (81.8%) were considered responders (Figure 2). Of the four non-responders, only one had higher BP after RDN than the baseline value, while the other three showed reductions of less than 10 mmHg. Mean office SBP decreased by 22 mmHg, a statistically significant reduction (174±23 vs. 152±22 mmHg, p<0.001), and mean DBP also fell significantly, by 9 mmHg (89±16 vs. 80±11 mmHg, p=0.006) (Figure 3). Other parameters that changed significantly six months after RDN were diastolic load on 24-hour ABPM (45±29% vs. 27±26%, p=0.049) and LVMI (174±56 vs. 158±60 g/m^2, p=0.014). The echocardiographic parameters used to assess systolic and diastolic function did not change significantly, nor did serum creatinine (1.0±0.3 vs. 1.0±0.4 mg/dl, p=0.344) (Table 2). The number of antihypertensive drugs (5.5±1.0 vs. 4.6±1.1, p=0.010) and pharmacological classes (5.4±0.7 vs. 4.6±1.1, p=0.009) also decreased significantly after RDN. It was not possible to compare the different RDN systems since patients treated by the OneShot® (n=2) and EnligHTN® (n=6) systems had not completed the six-month

Table 2 Office blood pressure, pharmacological therapy, 24-hour ambulatory blood pressure monitoring and echocardiographic parameters before and six months after renal denervation (n=22).

	Before RDN	After RDN	p
Office BP			
SBP (mmHg)	174 ± 23	152 ± 22	<0.001
DBP (mmHg)	89 ± 16	80 ± 11	0.006
Pharmacological therapy			
Number of drugs	5.5 ± 1.0	4.6 ± 1.1	0.010
Number of classes	5.4 ± 0.7	4.6 ± 1.1	0.009
24-hour ABPM			
SBP (mmHg)	146 ± 18	141 ± 17	0.279
DBP (mmHg)	79 ± 10	77 ± 14	0.459
Systolic load (%)	66 ± 28	55 ± 30	0.209
Diastolic load (%)	45 ± 29	27 ± 26	0.049
Transthoracic echocardiography			
LVMI (g/m^2)	174 ± 56	158 ± 60	0.014
LVEF (%)	63 ± 5	65 ± 8	0.139
LA volume index (ml/m^2)	35 ± 13	34 ± 12	0.470
E/A ratio	0.9 ± 0.3	0.8 ± 0.4	0.535
E-wave deceleration time (ms)	226 ± 60	217 ± 32	0.572
E/e′ ratio	11.3 ± 2.8	11.4 ± 2.7	0.923

ABPM: ambulatory blood pressure monitoring; BP: blood pressure, DBP: diastolic blood pressure; LA: left atrial; LVEF: left ventricular ejection fraction; LVMI: left ventricular mass index; RDN: renal denervation; SBP: systolic blood pressure.

follow-up period. However, their inclusion in the study did enable the baseline characteristics of patients selected for RDN to be described, and the immediate safety of the procedure to be assessed.

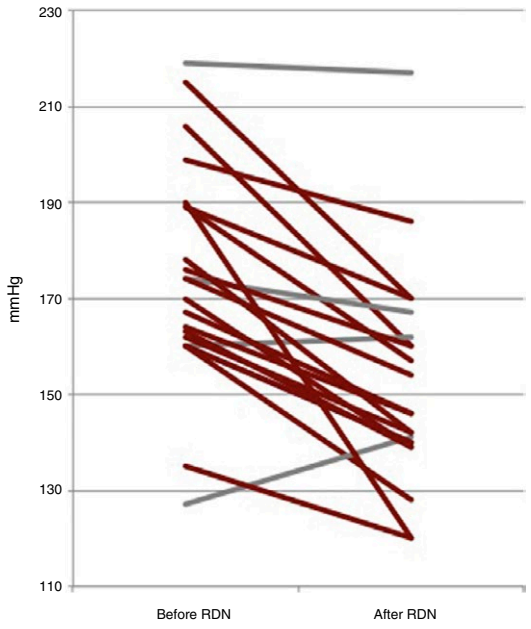

Figure 2 Systolic blood pressure before and six months after renal denervation (n=22). RDN: renal denervation.

Discussion

RDN proved to be safe in this group of patients with resistant HTN, with no serious complications. There were no access site complications such as pseudoaneurysm; one patient was the first published case of RDN via radial access.[19] There were no cases of renal artery dissection, thrombosis or rupture, and only one case of spasm and stenosis, observed at the end of the procedure performed on an accessory renal artery with a diameter at the lower recommended limit.

The safety of RDN was first demonstrated in 2009 by the Symplicity HTN-1 study,[11] which enabled the technique to be introduced into clinical practice. Nevertheless, the

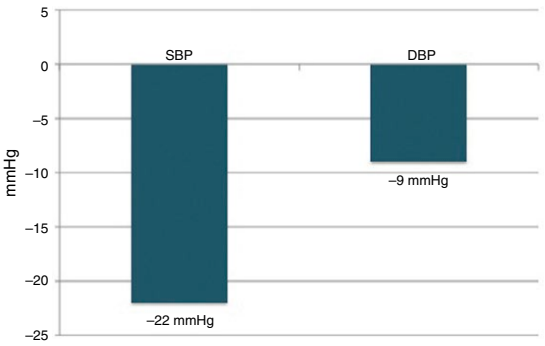

Figure 3 Mean change in office systolic and diastolic blood pressure at baseline and six months after renal denervation. DBP: diastolic blood pressure; SBP: systolic blood pressure.

studies in the literature involved small numbers of patients, and follow-up periods are still too short to draw definitive conclusions on the technique's safety in the medium to long term.

As well as demonstrating its safety, our study showed RDN to be effective in reducing office BP at six-month follow-up, with a mean reduction in SBP of 22 mmHg; there was a reduction in SBP of at least 10 mmHg in 82% of cases, as well as a significant fall in mean DBP (9 mmHg). Its impact on office BP also meant that the number of antihypertensive drugs prescribed decreased significantly.

The growing number of hypertensive patients and the morbidity and mortality associated with poor BP control, due in part to resistant HTN, point to the need for alternative therapeutic approaches. It is estimated that around seven million deaths and 64 million disability-adjusted life years each year can be attributed to poorly controlled HTN.[20] Literature reviews indicate that around 15% of hypertensive patients may have resistant HTN,[5,6] which occurs more frequently in men, those aged >55 years, blacks, and those with diabetes, obesity or chronic end-stage renal failure.[5,6,8,9]

Management of patients with resistant HTN is complex; it is essential to rule out secondary causes of HTN, optimize drug therapy and exclude white coat or other pseudo-resistant forms of HTN prior to applying advanced techniques such as RDN. The low percentage of patients in a resistant HTN outpatient clinic who were considered suitable for RDN in the present study demonstrates the complexity of patient selection for this technique, the ratio of the total number assessed to those considered eligible being approximately 5:1 (19.2%).

Pharmacological therapy in HTN is mainly based on drugs that act on the renin-angiotensin-aldosterone system, the sympathetic nervous system being considered of secondary importance. However, the role of sympathetic modulation in HTN was demonstrated more than half a century ago. An association has been shown between sympathetic nervous system activation and different forms and stages of HTN, including the earliest.[21–24] In addition, the effect of sympathectomy in reducing BP has also been demonstrated, although this technique was abandoned due to procedure-related complications and the subsequent development of antihypertensive drugs.[25–27] Recent advances in miniaturized devices for radiofrequency ablation have made percutaneous sympathetic denervation possible and have renewed interest in intervention in the relationship between sympathetic activity and HTN.

Our results are similar to those of previous studies demonstrating the efficacy of RDN in patients with resistant HTN. The Symplicity HTN-2 study,[12] the first randomized trial to show BP reduction at six-month follow-up, reported a mean reduction of 32 mmHg in SBP and 12 mmHg in DBP. In absolute terms, the reduction was greater than in our study, but comparison between the results of the two studies is difficult for various reasons: our sample was smaller (approximately half); baseline BP levels were higher in Symplicity HTN-2[12] (SBP 178 vs. 174 mmHg and DBP 97 vs. 89 mmHg), making a greater fall in BP more likely; and particularly importantly, drug therapy was not maintained throughout follow-up in our study population, which may have affected our results. The above may also explain the differences found in BP values on 24-hour ABPM: in contrast to the Symplicity HTN-2 study,[12] in which 24-hour ABPM values fell significantly six months after RDN, our study found a statistically significant reduction in diastolic load only, even though mean SBP and DBP decreased (by 5 and 3 mmHg, respectively). Besides the short-term benefits demonstrated in the present study, the long-term efficacy of RDN has been reported in up to 36 months of follow-up.[14]

With regard to echocardiographic parameters, there was a significant reduction in LVMI, in line with the results published by other groups.[28] This finding is particularly important since LV hypertrophy is a marker of subclinical target organ damage and is associated with cardiovascular events.[29] Furthermore, regression of LV hypertrophy as a result of better HTN control has been shown to improve prognosis.[30] However, unlike in previous studies,[28] our analysis found no significant improvement in systolic or diastolic function after RDN, probably due to the small sample size.

Some questions remain concerning the applicability of RDN. Careful patient selection, thorough investigation of the reasons behind nonadherence to drug therapy and exclusion of white coat HTN are essential aspects that require improvement. A recent study in 84 hypertensive patients assessing adherence to therapy through measurement of serum antihypertensive drug levels showed that 34.5% had no detectable drugs in the circulation and that 65.5% met criteria for nonadherence.[31] Against this background, it is difficult to determine whether the impact of RDN on BP levels is due to the intervention itself, possible improved compliance with therapy, or even a placebo effect, as seen in various areas of medicine. The Symplicity HTN-3 study,[32] currently in progress, one endpoint of which is ABPM assessment, will help to answer some of these questions. On the other hand, sympathetic activity may vary from patient to patient, and it is therefore crucial to identify objective parameters that will predict the response to RDN, enabling those with greater potential to respond to be selected and possible non-responders to be identified. The number of applications and the radiofrequency dose may in the future be established on an individual basis, adapted to the specific characteristics of each patient. Another question concerns sympathetic nervous system activation in the different stages of HTN. It is possible that sympathetic modulation in the early stages of HTN has a greater beneficial effect and can influence the natural history of the disease. Finally, further studies are required to determine the impact of RDN on morbidity and mortality in patients with resistant HTN, as well as to validate the cost-effectiveness of the technique, although preliminary data suggest that this is favorable.[33] The expectations surrounding RDN are reflected by the fact that several endovascular intervention systems are currently under development, clinical trials and registries of which will increase knowledge in this area and answer some of the above questions, leading to improvements in patient comfort and the procedure's safety and efficacy, which are essential for more widespread adoption of the technique.[34]

Limitations

The present study has certain limitations. Firstly, the study population was small, which means it is not possible to draw

definitive conclusions as to the efficacy and safety of RDN, make comparisons between the different RDN systems used in terms of safety, or determine the demographic and clinical profile of patients who will not respond to RDN. The fact that control renal artery angiography was not systematically performed during follow-up prevented a full assessment of the medium- to long-term safety of radiofrequency ablation. It was also not possible to compare the efficacy of the various devices used, since all the patients with complete six-month follow-up were treated with the Symplicity® system. Lastly, changes were made in drug therapy during follow-up, which may have influenced assessment of the efficacy of RDN, leading to underestimation of its effect on the various parameters studied.

Conclusion

In this cohort of patients with resistant essential HTN, RDN was safe and effective at six-month follow-up, with significant reductions in office SBP and DBP and a significant decrease in the number of antihypertensive drugs prescribed. In addition, RDN significantly reduced LVMI, a known marker of target organ damage. RDN thus appears to be a valid option for patients with resistant HTN, with benefits beyond improved BP control. Nevertheless, randomized studies with larger populations are required to assess the impact of this intervention on clinical events in the long term.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data and that all the patients included in the study received sufficient information and gave their written informed consent to participate in the study.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Lim SS, Vos T, Flaxman, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380:2224–60.
2. Dores H, Salvador F, Santos P, et al. Blood pressure in young adults. Rev Por Cardiol. 2010;29:1495–508.
3. de la Sierra A, Segura J, Banegas JR, et al. Clinical features of 8295 patients with resistant hypertension classified on the basis of ambulatory blood pressure monitoring. Hypertension. 2011;57:898–902.
4. Espiga de Macedo M, Lima MJ, Silva AO, et al. Prevalência, conhecimento, tratamento e controlo da hipertensão em Portugal. Estudo PAP. Rev Port Cardiol. 2007;26:21–39.
5. Persell SD. Prevalence of resistant hypertension in the United States, 2003–2008. Hypertension. 2011;57:1076–80.
6. Pimenta E, Calhoun DA. Resistant hypertension: incidence, prevalence and prognosis. Circulation. 2012;125: 1594–6.
7. Daugherty SL, Powers JD, Magid DJ, et al. Incidence and prognosis of resistant hypertension in hypertensive patients. Circulation. 2012;125:1635–42.
8. Sarafidis PA. Epidemiology of resistant hypertension. J Clin Hypertens (Greenwich). 2011;13:523–8.
9. Roberie DR, Elliott WJ. What is the prevalence of resistant hypertension in the United States? Curr Opin Cardiol. 2012;27:386–91.
10. Schlaich MP, Sobotka PA, Krum H, et al. Renal sympathetic nerve ablation for uncontrolled hypertension. N Engl J Med. 2009;361:932–4.
11. Krum H, Schlaich M, Whitbourn R, et al. Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. Lancet. 2009;373:1275–81.
12. Krum H, Barman N, Schlaich M, et al. Catheter-based renal sympathetic denervation for resistant hypertension: durability of blood pressure reduction out to 24 months. Hypertension. 2011;57:911–7.
13. Esler MD, Krum H, Schlaich M, et al. Renal sympathetic denervation for treatment of drug-resistant hypertension: one-year results from the Symplicity HTN-2 randomized, controlled trial. Circulation. 2012;126:2976–82.
14. Krum H, Schlaich M, Sobotka P, et al. Long-term follow-up of catheter-based renal denervation for resistant hypertension confirms durable blood pressure reduction. J Am Coll Cardiol. 2012;60(17_S).
15. de Araújo Gonçalves P, Almeida MS, Branco B, et al. Ablação da atividade simpática renal para tratamento da hipertensão arterial resistente. Rev Port Cardiol. 2012;31:671–5.
16. Sarafidis PA, Georgianos P, Bakris GL. Resistant hypertension – its identification and epidemiology. Nat Rev Nephrol. 2013;9:51–8.
17. Templin C, Jaguszewski M, Ghadri JR, et al. Vascular lesions induced by renal nerve ablation as assessed by optical coherence tomography: pre- and post-procedural comparison with the Simplicity® catheter system and the EnligHTN™ multi-electrode renal denervation catheter. Eur Heart J. 2013. Apr 25 [Epub ahead of print].
18. Ormiston JA, Watson T, van Pelt N, et al. Renal denervation for resistant hypertension using an irrigated radiofrequency balloon: 12-month results from the Renal Hypertension Ablation System (RHAS) trial. EuroIntervention. 2013;9:70–4.
19. de Araújo Gonçalves P, Teles RC, Raposo L. Catheter-based renal denervation for resistant hypertension performed by radial access. J Invasive Cardiol. 2013;25:147–9.
20. Perkovic V, Huxley R, Wu Y, et al. The burden of blood pressure-related disease: a neglected priority for global health. Hypertension. 2007;50:991–7.
21. DiBona GF, Kopp UC. Neural control of renal function. Physiol Rev. 1997;77:75–197.
22. DiBona GF, Esler M. Translational medicine: the antihypertensive effect of renal denervation. Am J Physiol Regul Integr Comp Physiol. 2010;298:245–53.
23. Esler M. The 2009 Carl Ludwig Lecture: pathophysiology of the human sympathetic nervous system in cardiovascular diseases: the transition from mechanisms to medical management. J Appl Physiol. 2010;108:227–37.

24. Bertog SC, Sobotka PA, Sievert H. Renal denervation for hypertension. JACC Cardiovasc Interv. 2012;5:249–58.
25. Grimson KS. Total thoracic and partial to total lumbar sympathectomy and celiac ganglionectomy in the treatment of hypertension. Ann Surg. 1941;114:753–75.
26. Peet M, Woods W, Braden S. The surgical treatment of hypertension: results in 350 consecutive cases treated by bilateral supradiaphragmatic splanchnicectomy and lower dorsal sympathetic ganglionectomy. Clinical lecture at New York session. JAMA. 1940;115:1875–85.
27. Smithwick RH, Thompson JE. Splanchnicectomy for essential hypertension; results in 1,266 cases. JAMA. 1953;152:1501–4.
28. Brandt MC, Mahfoud F, Reda S, et al. Renal sympathetic denervation reduces left ventricular hypertrophy and improves cardiac function in patients with resistant hypertension. J Am Coll Cardiol. 2012;59:901–9.
29. Ruilope LM, Schmieder RE. Left ventricular hypertrophy and clinical outcomes in hypertensive patients. Am J Hypertens. 2008;21:500–8.
30. Devereux RB, Wachtell K, Gerdts E, et al. Prognostic significance of left ventricular mass change during treatment of hypertension. JAMA. 2004;292:2350–6.
31. Ceral J, Habrdova V, Vorisek V, et al. Difficult-to-control arterial hypertension or uncooperative patients? The assessment of serum antihypertensive drug levels to differentiate non-responsiveness from non-adherence to recommend therapy. Hypertens Res. 2011;34:87–90.
32. Kandzari DE, Bhatt DL, Sobotka PA, et al. Catheter-based renal denervation for resistant hypertension: rationale and design of the SYMPLICITY HTN-3 Trial. Clin Cardiol. 2012;35: 528–35.
33. Geisler BP, Egan BM, Cohen JT, et al. Cost-effectiveness and clinical effectiveness of catheter-based renal denervation for resistant hypertension. J Am Coll Cardiol. 2012;60: 1271–7.
34. Bunte MC, Oliveira EI, Shishehbor MH. Endovascular treatment of resistant and uncontrolled hypertension: therapies on the horizon. J Am Coll Cardiol Intv. 2013;6:1–9.

ATTACHMENT C

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RESEARCH ARTICLE

Impact of Renal Sympathetic Denervation on Left Ventricular Structure and Function at 1-Year Follow-Up.

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RESEARCH ARTICLE

Impact of Renal Sympathetic Denervation on Left Ventricular Structure and Function at 1-Year Follow-Up

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Abstract

Background

Catheter-based sympathetic renal denervation (RDN) is a recent therapeutic option for patients with resistant hypertension. However, the impact of RDN in left ventricular (LV) mass and function is not completely established. Our aim was to evaluate the effects of RDN on LV structure and function (systolic and diastolic) in patients with resistant hypertension (HTN).

Methods and Results

From a single centre prospective registry including 65 consecutive patients with resistant HTN submitted to RDN between July-2011 and April-2015, 31 patients with baseline and 1-year follow-up echocardiogram were included in this analysis. Mean age was 65±7 years, 48% were males, 71% had type 2 diabetes. Most had hypertension lasting for more than 10 years (90%), and were being treated with a median number of 6 anti-hypertensive drugs, including 74% on spironolactone. At 1-year, there was a significant decrease both on office SBP (176±24 to 149±13mmHg, p<0.001) and DBP (90±14 to 79±11mmHg, p<0.001), and also in 24h ABPM SBP (150±20 to 132±14mmhg, p<0.001) and DBP (83±10 to 74 ±9mmHg, p<0.001). There was also a significant decrease in LV mass from 152±32 to 136 ±34g/m^2 (p<0.001), an increase in LV end diastolic volume (93±18 to 111±27 mL, p = 0.004), an increase in LV ejection fraction (65±9 to 68±9%, p = 0.001) and mitral valve E deceleration time (225±49 to 247±51ms, p = 0.015) at 1-year follow up. There were no significant changes in left atrium volume index or in the distribution of patients among the different left ventricle geometric patterns and diastolic function subgroups.

Conclusions

In this single centre registry of patients with resistant hypertension, renal denervation was associated with significant reduction in both office and ABPM blood pressure and a significant decrease in left ventricle mass evaluated by transthoracic echocardiogram at 1 year follow-up.

Introduction

Long-standing hypertension (HTN) results in cardiac remodelling including myocardial hypertrophy, diastolic dysfunction and left atrial (LA) enlargement leading to atrial and ventricular arrhythmias, heart failure and ultimately to myocardial infarction and stroke, which are the leading causes of death and morbidity in developed countries [1].

The link between chronic sympathetic hyperactivity and drug-resistant HTN is well known for several years, and is the rationale behind the development of catheter-based sympathetic renal denervation (RDN). This treatment approach for drug resistant HTN had very promising results in early non-blinded studies [2,3]. Recently, the lack of positive results on a randomized sham-controlled trial raised doubts on the efficacy and patient selection for this procedure, reinforcing the need for further research in this field [4]. Sympathetic drive is also implicated in the development of left ventricular hypertrophy (LVH) [5,6], but little is known about the impact of RDN in left ventricular performance. The aim of the present study was to evaluate the effects of RDN on LV structure and function (systolic and diastolic) in patients with resistant HTN.

Methods

Study design and population

From a single centre prospective registry including 65 consecutive patients with resistant HTN submitted to RDN between July-2011 and April-2015, 31 patients with baseline and 1-year follow-up 24h ABPM and transthoracic echocardiogram were included in this analysis. As per protocol, all patients underwent a comprehensive transthoracic echocardiogram (TTE) at baseline and at 1-year after RDN. The inclusion, exclusion criteria and clinical feature regarding this registry were previously reported [7]. The research was approved by the Ethics committee of Hospital de Santa Cruz and Nova Medical School, Lisbon, Portugal. Written informed consent was collected for all the patients. Study design is summarized in Fig 1.

In summary, the patients selected had to be older than 18 years, with an office systolic blood pressure (SBP) above 160mHg while receiving a stable antihypertensive regimen involving at least three drugs (including a diuretic). Before RDN, during pre-scheduled visits at the outpatient clinic for a period not less than 6 weeks, secondary causes for HTN were excluded, compliance to medical treatment was assured and drug therapy was adjusted until maximal tolerated regimens. Only then, if target BP values were not obtained, patients were considered for RDN. Anatomical criteria were adopted from Symplicity trials.[2,8] Demographic variables, clinical characteristics, anthropometric data, laboratory values, drug treatment and procedure details were recorded and stored in a dedicated database. Creatinine clearance was calculated using MDRD formula.[9]

Blood pressure measurement and definition of responders

Office BP readings were taken in a seated position with an oscillometric semiautomatic sphygmomanometer Omron HEM-907 monitor (Omron Healthcare, USA) after 5 min of rest

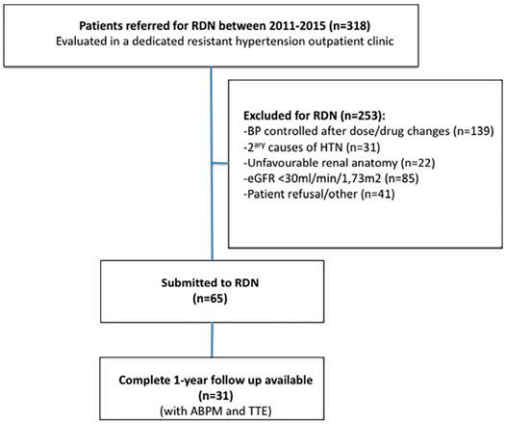

Fig 1. Flowchart with patient selection. From the total number of patients evaluated in a dedicated outpatient hypertension clinic (n = 318), 65 patients were submitted to renal denervation, after the exclusion of 253 due to several reasons listed. From these 65 patients, it was possible to obtain complete 1 year follow up with ambulatory blood pressure measurement and transthoracic echocardiogram. RDN—renal denervation; HTN—hypertension; eGFR—estimated glomerular filtration rate; ABPM –24 hours ambulatory blood pressure measurement; TTE-transthoracic echocardiogram.

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according to the European Guidelines for the management of arterial hypertension [10] At baseline, BP was measured in both arms and the arm with the higher BP was used for all subsequent readings. Averages of the triplicate measures were calculated and used for analysis.

Twenty-four hours ambulatory blood pressure measurements (ABPM) were taken with an ABM monitor (Spacelabs Healthcare, USA), according to the current European Society of Hypertension guidelines[10].

Blood pressure responders to RDN treatment were defined as those who had a reduction in office SBP of $\geq$10 mmHg at one year follow-up or a reduction of 2mmHg in ABPM 24 hours SBP according to Symplicity HTN3 trial design[11].

Renal denervation procedure

We have previously reported the details of the RDN procedure in our center [12]. Briefly, all procedures were performed under mild anaesthesia for sedation and pain control (propofol and remifentanil in weight-adjusted doses). Anticoagulation with unfractionated heparin was used in order to obtain an activated clotting time between 250–300 seconds. After gaining femoral artery access in all cases except one (where the radial artery was used), abdominal aortography and selective renal artery angiograms were performed to confirm anatomic eligibility. At the end, in cases with femoral access, the site was closed using a sealing device (Angio-Seal® -St. Jude Medical, USA).

RDN was performed using the Symplicity® (n = 25), the EnligHTN® (n = 4), OneShot® (n = 2) catheter using the standard technique, as previously reported [7,12,13].

Transthoracic echocardiography

Comprehensive two-dimensional and Doppler transthoracic echocardiographic studies were performed at baseline and at 1-year follow-up in all patients, using VIVID 7 ultrasound system (General Electric Heathcare). All echocardiographic recordings were stored in digital format

on a dedicated workstation for off-line subsequent analysis. The exams were performed by one of two experienced operators (EH and MJA), while analysed and interpreted by the non-performer operator, both blinded to patients' clinical, BP status and sequence of images.

Left ventricular size was evaluated by both linear (using M-mode 2D guided diameters obtained perpendicular to the LV long axis) and volumetric (using the biplane method of disks summation from tracings of the blood-tissue interface in the apical four- and two-chamber views), according to accepted recommendations from the American Society of Echocardiography and the European Association of Cardiovascular Imaging [14]. LV ejection fraction was calculated using the following formula: EF = (EDV − ESV)/EDV, with LV volume estimates obtained by the biplane method of disks.

Assessment of LV mass (LVM) was performed by the linear method using the cube formula (LV mass = 0.8 · 1.04 · [(IVS + LVID + PWT)3 −LVID3]+0.6g), with 2D guided M-mode measurements obtained at end-diastole from the parasternal approach perpendicular to the LV long axis measured at the level of the mitral valve leaflet tips. LV hypertrophy was considered present when LV mass exceeded 115 g/m^2 for men and 95 g/m^2 for women.

We also calculated the relative wall thickness (RWT) measured as twice the posterior wall thickness divided by left ventricular end-diastolic diameter, and determined the LV anatomical pattern in each participant. Normal LVM and RWT were defined as normal LV anatomy, normal LVM and RWT >0.42 as concentric LV remodeling, increased LVM and RWT >0.42 as concentric LVH and increased LVM in the presence of RWT <0.42 as eccentric LVH [15]. Left atrial (LA) volume measurement was done using the disk summation algorithm similar to that used to measure LV volume, when the LA chamber was at its greatest dimension (end of LV systole).

LV diastolic function was assessed by pulsed-wave Doppler examination of mitral inflow and Doppler tissue imaging of the mitral annulus. Peak velocities of early (E) and late (A) trans-mitral flow and deceleration time (DT) were determined, and the ratio E/A was calculated. Doppler tissue imaging with pulsed-wave Doppler at the level of septal and lateral mitral annulus was used to measure e' velocities. The average of septal and lateral mitral annulus e' peak velocities, were used to calculate the E/e' ratio. The Valsalva maneuver was performed to distinguish normal from pseudo-normal patterns. Spectral recordings were obtained at a sweep speed of 100 mm/s at end-expiration, and each measurement was averaged over multiple cardiac cycles to account for inter-beat variability.

Grade 1 diastolic dysfunction (impaired relaxation) was defined by the presence of an E/A ratio <0.8, a deceleration time >200 ms and E/e′ ratio <8 in the presence of an enlarged left atrium. Moderate (pseudo-normal, grade 2) diastolic dysfunction was defined as a mitral E/A ratio >0.8 and <1.5 that decreases by 50% during the Valsalva maneuver, E/e' ratio 9 to 12 and e'<8 cm/s. Finally, severe (grade 3) diastolic dysfunction corresponds to restrictive LV filling defined by E/A ratio >2, DT <160 ms, and average E/e'>13. All subjects with impaired LV relaxation, pseudo-normal or restrictive filling patterns were defined as having LVDD [16].

Statistical analyses

Continuous variables are reported as mean ± standard deviation. Normality was tested with the Kolmogorov-Smirnov test and/or Q-Q Plot visual assessment. Normally distributed variables were compared between baseline and one year follow-up using a paired Student t test or a Wilcoxon matched-pairs test if not normally distributed. Discrete variables are expressed as frequencies and percentages (in brackets). Statistical comparisons of baseline characteristics and at follow-up were performed using the chi-square test or Fisher's exact test, when appropriate, for categorical variables and the paired *t*-Student's test or the Saterwate test for

continuous variables. A two-tailed p value <0.05 is considered as statistically significant. Linear regression analyses were used to calculate the correlation between the change in blood pressure and the change in echocardiographic parameters. SPSS, Statistical Package for the Social Sciences®, V.21.0 (IBM SPSS Inc, Chicago, IL) software was used for data processing and statistical analysis.

Results

Patient characteristics

From the total number of patients evaluated in a dedicated outpatient hypertension clinic (n = 318), 65 patients were submitted to renal denervation, after the exclusion of 253 due to several reasons (listed in Fig 1). From these 65, it was possible to obtain complete 1 year follow up with ambulatory blood pressure measurement and transthoracic echocardiogram in 31 patients that were the final population included in this analysis. Mean age was 65±7 years, 48% were males (n = 15), and all were caucasians. Concerning traditional cardiovascular risk factors, obesity was present in 68% of the patients (mean body mass index 32±6 Kg/m^2), type 2 diabetes in 71%, dyslipidaemia in 68% and active smoking in one patient (3.2%). Coronary artery disease was present in 10 patients (32%) and any vascular disease in 11 (36%). Estimated mean glomerular filtration rate was 76±25mL/min/1.73m^2, with five patients having chronic kidney disease, defined as eGFR <60 ml/min/1.73 m^2. Baseline characteristics are shown in Table 1.

Table 1. Patient's baseline and RDN procedure characteristics.

Demographic and clinical variables	
Age (years)	65±7
Male (%)	15 (48.4)
Caucasians (%)	31 (100)
Weight (kg)	86±16
Height (m)	1.65±0.1
BMI (kg/m^2)	31.8±5.5
Obesity (%)	21 (67.7)
Atrial fibrillation (%)	1 (3.2)
Previous stroke (%)	2 (6.5)
Type 2 Diabetes (%)	22 (71)
Dyslipidaemia (%)	21 (67.7)
Smoking (%)	1 (3.2)
Sleep apnea (%)	5 (19.1)
eGFR (ml/min/1,73m^2)	76.4±24.7
CKD* (%)	5 (16.1)
Hypertension > 10 years (%)	28 (90.3)
Coronary artery disease (%)	10 (32.3)
Any vascular disease (%)	11 (35.5)
RDN Procedure	
Mean number of applications right renal artery	5.1±1.3
Mean number of applications left renal artery	5.7±1.1
Mean number of applications per patient	10.8±2.3

eGFR, estimated glomerular filtration rate;
CKD, *Chronic kidney disease(eGFR <60 ml/min/1,73m^2)

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The majority of patients (90%) had hypertension lasting for more than 10 years, treated with a median of 5.8 anti-hypertensive agents from a median of 5.5 different pharmacological classes. Almost all patients were treated with calcium antagonists, 96.8% (n = 30), 87% with diuretics, 74% with spironolactone, 61% with ACE inhibitors, 61% with ARB inhibitors, 84% with beta-blockers and 71% with a sympatholytic drug. (Table 2)

Blood pressure control by RDN

At baseline, mean office SBP and diastolic blood pressure (DBP) were 176±24 mmHg and 90 ±14 mmHg, respectively, and mean heart rate was 73±11 bpm. The 24-hour ABPM showed the following average values: SBP 150±20 mmHg, DBP 83±10 mmHg, pulse pressure 67 ±18mmHg (Table 3).

Overall, at one-year follow-up, there was a significant reduction in both office SBP (176±24 to 149±13mmHg, $p<0.001$) and DBP (90±14 to 79±11mmHg, $p<0.001$). On 24-hour ABPM, there was a significant drop on: SBP (150±20 to 132±14 mmHg, $p<0.001$, mean decrease of 18 mmHg), on DBP (83±10 to 74±9 mmHg, average decrease of 9 mmHg, $p<0.01$) and on pulse pressure from 67±18 to 58±13 mmHg, $p = 0.001$, a mean decrease of 5 mmHg (Fig 2).

This was found in spite of the significant reduction in the number of both antihypertensive drugs and classes in use at 1-year: 5.8±1.1 to 5.0±1.2 ($p = 0.002$) and 5.5±0.9 to 4.9±1.1 ($p = 0.015$) respectively.

At 1-year follow-up, 22 of patients (71%) were considered office SBP responders and 26 (84%) ABPM SBP responders based on a drop of more than 10mmHg on office SBP and 2mmHg on 24 hours ABPM SBP.

Echocardiographic parameters

Transthoracic echocardiography at baseline revealed LV hypertrophy in 27 patients (87%), with a mean LV mass of 152±32 g/m^2. Distribution among different geometric patterns is shown in Fig 3. The large majority had concentric hypertrophy (74%), with only 3% presenting a normal pattern. All patients had a preserved EF (>55% by Simpson's biplane method), with a mean LVEF of 65±9%. Mean LA volume was 33±8mL/m^2, and 48.4% had ≥ 34ml/ m2.

LVDD was diagnosed in 29 (93.5%) patients, 11 (37.9%) of them had grade 1 diastolic dysfunction, 18 patients a pseudo-normal pattern (62.1%); 2 patients were in atrial fibrillation and

Table 2. Antihypertensive medication.

	Baseline	One year	p
Mean number of antihypertensive drugs	5.8±1.1	5.0±1.2	0.002
Mean number of classes	5.5±0.9	4.9±1.1	0.015
ACE inhibitors	19 (61.3)	17(54.8)	0.688
ARBs (%)	19 (61.3)	18 (58.1)	1.0
Beta-blockers (%)	26 (83.9)	27 (87.1)	1.0
Calcium channel blockers (%)	30 (96.8)	21 (67.7)	0.012
Diuretics (%)	27 (87.1)	24 (77.4)	0.727
Spironolactone (%)	23 (74.2)	26 (83.9)	0.453
Sympatholytic (%)	22 (71)	19 (61.3)	0.508
Aliskirene	4 (12.9)	0	0.046

ACE, Angiotensin converting enzyme; ARB, Angiotensin receptor blockers

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Table 3. RDN results on blood pressure and heart rate.

	Baseline	One-year	P
Office systolic BP (mmHg)	176±24	149±13	< .001
Office diastolic BP (mmHg)	90±14	79±11	< .001
Heart rate (bpm)	73±11	70±11	.261
ABPM systolic BP (mmHg)	150±20	132±14	< .001
ABPM diastolic BP (mmHg)	83±10	74±9	< .001
ABPM pulse pressure (mmHg)	67±18	58±13	.001
ABPM mean pressure (mmHg)	105±9	95,3±8,4	< .001
ABPM heart rate (bpm)	67.6±9.1	65.5±9.5	.090
ABPM SBP responders* (%)	-	26 (83.9)	-
Office SBP responders** (%)	-	22 (71)	-

BP, blood pressure; bpm, beats per minute; ABPM, 24 hours ambulatory blood pressure measurement;
* ABPM SBP responders: a decrease of 2mmHg between baseline ABPM SBP and at one year;
**Office SBP responders: a decrease of 10mmHg between baseline office SBP and at one year.

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there were no patients with a restrictive filling pattern (Fig 4). For the entire population, E/A ratio was 0.8±0.2, E-wave deceleration time 225±49ms and E/e' ratio 11±3.

After one-year, there was an overall significant reduction in LV mass (152±32 to 136±34g/m^2, p<0.001—Fig 2), an increase in mitral valve deceleration time (from 225±49ms to 247±51ms, p = 0.015—Table 4). There were no significant changes in the distribution of patients among the different LV geometric patterns (Fig 3) or in the percentage of patients in each diastolic function group (Fig 4) from baseline to 1 year after renal denervation.

Relation between blood pressure reduction and echocardiographic findings

Reduction in LV mass reached statistical significance in ABPM SBP responders (n = 26): 148 ±32 to 133±29g/m^2, p<0.001. In non-responders (n = 5), LV mass also decreased: 166±23 to 129±15g/m^2, p = 0.05, although not reaching statistical significance certainly due to sample

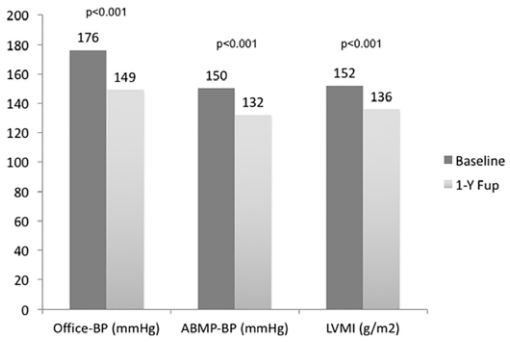

Fig 2. Results at 1 year after renal denervation (blood pressure and left ventricle mass index). Results in systolic blood pressure (both office and ABPM) and LVMI in TTE at 1-year follow-up are shown, with significant reductions in both parameters. BP- blood pressure; ABPM –24 hours ambulatory blood pressure measurement; LVMI—left ventricle mass index; TTE-transthoracic echocardiogram.

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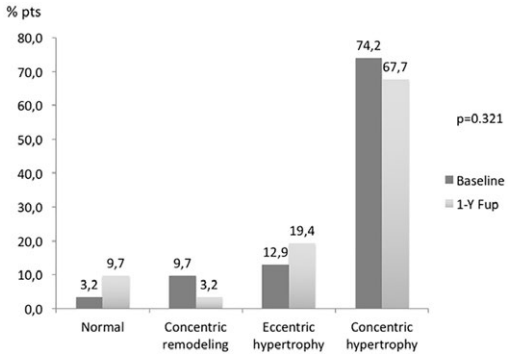

Fig 3. Comparison of different LV geometric patterns at baseline and 1 year after renal denervation. The percentage of patients in each LV geometric pattern class is depicted. Concentric remodelling was defined as relative wall thickness (RWT) of >0.42 with normal LV mass and normal geometry was defined as a RWT of ≤0.42 with normal LV mass.

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size. From the scatter-plot graphic (Fig 5) where the relationship between LV mass and ABPM SBP changes at one year for the entire population is shown, we observe that changes in SBP and LV mass are not correlated, as depicted by the very low r2 values obtained.

Safety

There were 3 mild hematomas and 1 femoral pseudoaneurysm, treated with surgery without any permanent sequelae. There were no complications related to the renal arteries, namely dissection or perforation.

Discussion

The main findings of our study were: 1) Renal denervation in patients with resistant HTN was associated with significant reduction in both office and ABPM blood pressure at 1 year follow-

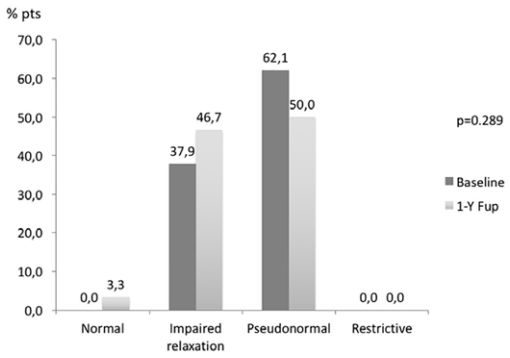

Fig 4. Comparison of LV diastolic function at baseline and 1 year after renal denervation. The percentage of patients in each diastolic function group (Normal, Impaired relaxation, pseudonormal and restrictive) is depicted.

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Table 4. Echocardiographic parameters at baseline and at one-year follow-up in patients submitted to RDN.

	Baseline	One-year	p
Anatomy			
LVEDV (mL)	93.3±18,2	110.9±27.4	.004
LVESV (mL)	35.8±12.6	38.2±3.1	.121
IVSTd (mm)	13.4±1.9	13.1±2.4	.616
PWTd (mm)	11.7±1.6	11.8±1.7	.620
LVEDD (mm)	48.7±5.8	47.8±5.4	.230
LVESD (mm)	28.9±5.7	27.9±6.5	.296
LV mass/BSC (g/m^2)	152.3±32.4	135.7±33.9	< .001
LA volume index (ml/m^2)	32.8±8.3	34.1±6.2	.227
Function			
LVEF Simpson (%)	64.5±9.2	67.7±9.1	.001
Stroke volume (ml)	81.7±14.9	102.7±16.7	.075
Mitral valve E Vmax (cm/s)	73.6±15.2	73.2±16.4	.881
Mitral valve A Vmax (cm/s)	88.3±16.5	86.0±21	.469
Mitral valve E/A ratio	0.84±0.21	0.86±0.20	.574
Mitral valve E deceleration time (ms)	224.9±49.4	247.3±50.5	.015
Mitral valve lateral E' (cm/s)	7.2±1.8	7.3±2.1	.417
Mitral valve lateral E/E'	11.0±3.3	10.5±3.5	.228

LVEDVI, left ventricle end-diastolic volume; LVESVI, left ventricle end-systolic volume; IVSTd, interventricular septum thickness on diastole; PWTd, posterior wall thickness on diastole; LVEDD, left ventricle end-diastolic diameter, LVESD, left ventricle end-systolic diameter; LV, left ventricle; BSC, body surface area; LA, left atrium; LVEF, left ventricle ejection fraction.

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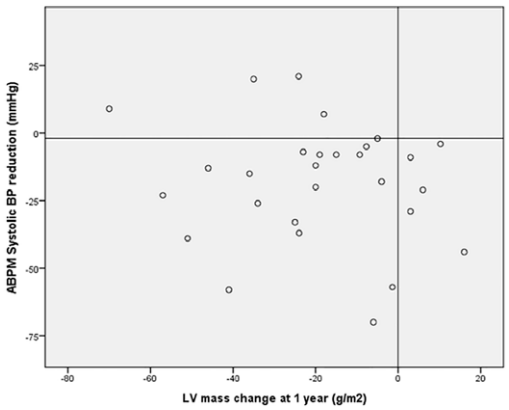

Fig 5. Relation between LV mass index and ABPM systolic BP changes at 1 year follow-up. Horizontal line set at 2mmHg for responder in ABPM systolic BP reduction. Five patients had regression in LVMI without significant (>2mmHg) reduction in ABPM systolic BP and 5 additional patients were ABPM systolic BP responders but without reduction in LVMI. BP- blood pressure; ABPM –24 hours ambulatory blood pressure measurement; LV—left ventricle.

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up; 2) There was a significant reduction in left ventricle mass index, a recognized marker of HTN target organ damage.

Renal denervation has been associated with significant reductions in both office and ABPM blood pressure in many trials[2,3,17] and registries[18]. In a recent large randomized trial the reductions in systolic blood pressure, the primary endpoint of the trial, was not significant as compared to a sham control arm, in striking contrast with previous trial.[4] Many possible confounding factors were pointed out that could explain these apparent contradictory findings, [19] but most importantly these inconsistent results of renal denervation makes a strong case for additional studies looking beyond blood pressure measurements. With this rational we sought to evaluate the impact of renal denervation in left ventricle hypertrophy, which is one of the most important markers of target organ damage of HTN and has been associated with an increased rate of cardiovascular events and death independent of BP values[20–22]. At 1 year after renal denervation, there was a significant reduction in left ventricle mass and our results are in line with previous studies using both transthoracic echocardiogram [23,24] and cardiac magnetic resonance [25].

Brandt MC et al[24] in a study including 46 patients, found that renal denervation was associated with a significant reduction in LV mass index and filling and improvements in mitral valve lateral E/E´, indicator of LV filling pressure in transthoracic echocardiogram. In another small study using similar methodology, Schirmer SH et al [23] evaluated the impact of renal denervation in left ventricle hypertrophy by echocardiogram and were able to document that in patients with resistant HTN, the observed reductions in LV mass were similar across tertiles of systolic blood pressure, suggesting that the pathophysiology could be related also to a direct effect of sympathetic hyperactivity, not dependent on blood pressure or heart rate. In our registry we didn´t found a correlation between LV mass and ABPM SBP changes at one year (Fig 5), which suggests that LV hypertrophy reduction, after RDN, might be affected by other mechanisms beyond BP reduction. This is not new in the field of HTN and it has been previously described that for similar BP reduction, different pharmacological agents could lead to different impact on LV hypertrophy [26]. In one interesting study, a greater regression in LV hypertrophy was documented for a drug combination that targeted neuroendocrine activity (both renin-angiotensine-aldosterone system and sympathetic nervous system), for the same magnitude of BP reduction [27]. Regarding the pathophysiological mechanism of the observed reduction of LV mass, it could be the result not only of a reduction in myocyte hypertrophy but also of absolute collagen content and diffuse interstitial myocardial fibrosis, as was suggested in a recent cardiac MRI study [28].

In a multicenter study including 72 patients and using cardiac magnetic resonance imaging, Mahfoud F et al [25] also demonstrated that at 6 months follow-up renal denervation was associated with a significant reduction in left ventricle mass index, an improvement in ejection fraction and a reduction in left ventricle circumferential strain, a surrogate of diastolic function. Taken together these studies are consistent in regression of LV mass and improvement in several markers of diastolic function. In our study, we also found a significant reduction in LV mass but there were no significant changes in transthoracic echocardiogram parameters of diastolic function. In addition, we didn't found any reduction in left atrial volume index. There was a small but significant increase in LV ejection fraction and LV end-diastolic volume, which could be explained at least partially by the numerically lower heart rate at 1 year follow-up documented both on office and on the average 24-hour heart rate from the ABPM recording. This small increase in EF is in line with some [24,25] but not all of the previous studies [23].

Some additional particular features of the present study should also be taken in consideration. First, our results come from a registry with a very rigorous selection process of patients for renal denervation, perceived from the high mean number (5.8) of antihypertensive drugs,

the baseline office and ABPM blood values and the patient selection flowchart presented in Fig 1, with an almost 5:1 proportion of patients evaluated/treated (only 65 patients submitted to RDN out of the 318 with resistant HTN evaluated in our outpatient clinic). It is also worth mentioning that an average of 5.8 drugs is higher than that reported by other similar studies evaluating the impact of RDN on LV mass (ranging from 4.3 in the study of Schirmer SH et al [23] to 4.7 in the study of Brandt MC et al [24]. Secondly, in our study we have a very high percentage of patients taking spironolactone on baseline (74%). This high aldosterone antagonist use is in line with the described strict selection process, and in addition it might have also contributed to explain the positive results after renal denervation, since it has been demonstrated that patients previously treated with spironolactone where better responders to this procedure. [4,19] Thirdly, we used 24-hour ABPM in all patients and this is considered to be a more accurate evaluation of the impact of treatment on blood pressure.[10] Lastly, our results both in blood pressure and LV mass were evaluated at 1 year, a significantly longer follow up than that reported by the previous studies that evaluated patients at 6 months follow up.[23–25]

Limitations

The present study has several limitations that should be acknowledged: 1) It is a single centre prospective registry with a small sample size. 2) The lack of a control group and the fact that there was no blinding either for RDN (sham not performed) or for the physicians performing the follow-up echocardiograms; 3) There were changes on antihypertensive drug therapy during the clinical follow-up which can influence the reductions in blood pressure and LV mass, although in our study the mean number of drugs was reduced. This way, the reduction obtained with renal denervation could have been underestimated in this real world setting; 4) No specific techniques were used to control for patient adherence to medication; 5) Echocardiograms were not reviewed in a core lab, which could potentially be associated with less reproducible measurements; 6) Cardiac MRI was not used and could have provided a more accurate evaluation of LV mass changes.

Conclusions

In this single centre registry of patients with resistant hypertension, renal denervation was associated with significant reduction in both office and 24h-ABPM blood pressure, and a significant decrease in left ventricle mass evaluated by transthoracic echocardiogram at 1 year follow-up. There were no significant changes in left atrium volume index or in the distribution of patients among the different left ventricle geometric patterns and diastolic function subgroups.

Author Contributions

Conceived and designed the experiments: MSA PG PB HD MSC MJA NN AA. Performed the experiments: MSA PG PB HSS AG EH MJA. Analyzed the data: MSA PG JM AG NN AA MM MJA. Contributed reagents/materials/analysis tools: PB MJA EH. Wrote the paper: MSA PG JM MSC HD NN AA MM MJA.

References

1. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, et al. (2014) 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA 311: 507–520. doi: 10.1001/jama.2013.284427 PMID: 24352797

2. Krum H, Schlaich M, Whitbourn R, Sobotka PA, Sadowski J, Bartus K, et al. (2009) Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. Lancet 373: 1275–1281. doi: 10.1016/S0140-6736(09)60566-3 PMID: 19332353

3. Esler MD, Krum H, Schlaich M, Schmieder RE, Bohm M, Sobotka PA (2012) Renal sympathetic denervation for treatment of drug-resistant hypertension: one-year results from the Symplicity HTN-2 randomized, controlled trial. Circulation 126: 2976–2982. doi: 10.1161/CIRCULATIONAHA.112.130880 PMID: 23248063
4. Bhatt DL, Kandzari DE, O'Neill WW, D'Agostino R, Flack JM, Katzen BT, et al. (2014) A controlled trial of renal denervation for resistant hypertension. N Engl J Med 370: 1393–1401. doi: 10.1056/NEJMoa1402670 PMID: 24678939
5. Corea L, Bentivoglio M, Verdecchia P (1983) Echocardiographic left ventricular hypertrophy as related to arterial pressure and plasma norepinephrine concentration in arterial hypertension. Reversal by atenolol treatment. Hypertension 5: 837–843. PMID: 6228528
6. Perlini S, Palladini G, Ferrero I, Tozzi R, Fallarini S, Facoetti A, et al. (2005) Sympathectomy or doxazosin, but not propranolol, blunt myocardial interstitial fibrosis in pressure-overload hypertrophy. Hypertension 46: 1213–1218. PMID: 16216989
7. Dores H, de Sousa Almeida M, de Araujo Goncalves P, Branco P, Gaspar A, Sousa H, et al. (2014) Renal denervation in patients with resistant hypertension: six-month results. Rev Port Cardiol 33: 197–204. doi: 10.1016/j.repc.2013.09.008 PMID: 24472425
8. Symplicity HTNI, Esler MD, Krum H, Sobotka PA, Schlaich MP, Schmieder RE, et al. (2010) Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. Lancet 376: 1903–1909. doi: 10.1016/S0140-6736(10)62039-9 PMID: 21093036
9. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 130: 461–470. PMID: 10075613
10. O'Brien E, Parati G, Stergiou G, Asmar R, Beilin L, Bilo G, et al. (2013) European Society of Hypertension position paper on ambulatory blood pressure monitoring. J Hypertens 31: 1731–1768. doi: 10.1097/HJH.0b013e328363e964 PMID: 24029863
11. Kandzari DE, Bhatt DL, Sobotka PA, O'Neill WW, Esler M, Flack JM, et al. (2012) Catheter-based renal denervation for resistant hypertension: rationale and design of the SYMPLICITY HTN-3 Trial. Clin Cardiol 35: 528–535. doi: 10.1002/clc.22008 PMID: 22573363
12. de Araujo Goncalves P, de Sousa Almeida M, Branco P, Gaspar A, Dores H, Carvalho MS, et al. (2012) [Renal sympathetic denervation for treatment of resistant hypertension]. Rev Port Cardiol 31: 671–675. doi: 10.1016/j.repc.2012.02.016 PMID: 22963861
13. Verheye S, Ormiston J, Bergmann MW, Sievert H, Schwindt A, Werner N, et al. (2015) Twelve-month results of the rapid renal sympathetic denervation for resistant hypertension using the OneShotTM ablation system (RAPID) study. EuroIntervention 10: 1221–1229. doi: 10.4244/EIJY14M12_02 PMID: 25452198
14. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. (2015) Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging 16: 233–270. doi: 10.1093/ehjci/jev014 PMID: 25712077
15. Gaasch WH, Zile MR (2011) Left ventricular structural remodeling in health and disease: with special emphasis on volume, mass, and geometry. J Am Coll Cardiol 58: 1733–1740. doi: 10.1016/j.jacc.2011.07.022 PMID: 21996383
16. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, et al. (2009) Recommendations for the evaluation of left ventricular diastolic function by echocardiography. J Am Soc Echocardiogr 22: 107–133. doi: 10.1016/j.echo.2008.11.023 PMID: 19187853
17. Papademetriou V, Tsioufis CP, Sinhal A, Chew DP, Meredith IT, Malaiapan Y, et al. (2014) Catheter-based renal denervation for resistant hypertension: 12-month results of the EnligHTN I first-in-human study using a multielectrode ablation system. Hypertension 64: 565–572. doi: 10.1161/HYPERTENSIONAHA.114.03605 PMID: 24935940
18. Bohm M, Mahfoud F, Ukena C, Bauer A, Fleck E, Hoppe UC, et al. (2013) Rationale and design of a large registry on renal denervation: the Global SYMPLICITY registry. EuroIntervention 9: 484–492. doi: 10.4244/EIJV9I4A78 PMID: 23965354
19. Kandzari DE, Bhatt DL, Brar S, Devireddy CM, Esler M, Fahy M, et al. (2015) Predictors of blood pressure response in the SYMPLICITY HTN-3 trial. Eur Heart J 36: 219–227. doi: 10.1093/eurheartj/ehu441 PMID: 25400162
20. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP (1990) Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. N Engl J Med 322: 1561–1566. PMID: 2139921

21. Ruilope LM, Schmieder RE (2008) Left ventricular hypertrophy and clinical outcomes in hypertensive patients. Am J Hypertens 21: 500–508. doi: 10.1038/ajh.2008.16 PMID: 18437140

22. Bombelli M, Facchetti R, Carugo S, Madotto F, Arenare F, Quarti-Trevano F, et al. (2009) Left ventricular hypertrophy increases cardiovascular risk independently of in-office and out-of-office blood pressure values. J Hypertens 27: 2458–2464. doi: 10.1097/HJH.0b013e328330b845 PMID: 19654559

23. Schirmer SH, Sayed MM, Reil JC, Ukena C, Linz D, Kindermann M, et al. (2014) Improvements in left ventricular hypertrophy and diastolic function following renal denervation: effects beyond blood pressure and heart rate reduction. J Am Coll Cardiol 63: 1916–1923. doi: 10.1016/j.jacc.2013.10.073 PMID: 24315919

24. Brandt MC, Mahfoud F, Reda S, Schirmer SH, Erdmann E, Bohm M, et al. (2012) Renal sympathetic denervation reduces left ventricular hypertrophy and improves cardiac function in patients with resistant hypertension. J Am Coll Cardiol 59: 901–909. doi: 10.1016/j.jacc.2011.11.034 PMID: 22381425

25. Mahfoud F, Urban D, Teller D, Linz D, Stawowy P, Hassel JH, et al. (2014) Effect of renal denervation on left ventricular mass and function in patients with resistant hypertension: data from a multi-centre cardiovascular magnetic resonance imaging trial. Eur Heart J 35: 2224–2231b. doi: 10.1093/eurheartj/ehu093 PMID: 24603307

26. Devereux RB, Dahlof B, Gerdts E, Boman K, Nieminen MS, Papademetriou V, et al. (2004) Regression of hypertensive left ventricular hypertrophy by losartan compared with atenolol: the Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) trial. Circulation 110: 1456–1462. PMID: 15326072

27. Burns J, Ball SG, Worthy G, Struthers AD, Mary DA, Greenwood JP (2012) Hypertensive left ventricular hypertrophy: a mechanistic approach to optimizing regression assessed by cardiovascular magnetic resonance. J Hypertens 30: 2039–2046. doi: 10.1097/HJH.0b013e328356b850 PMID: 22828088

28. Doltra A, Messroghli D, Stawowy P, Hassel JH, Gebker R, Leppanen O, et al. (2014) Potential reduction of interstitial myocardial fibrosis with renal denervation. J Am Heart Assoc 3: e001353. doi: 10.1161/JAHA.114.001353 PMID: 25516438

ATTACHMENT D

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ORIGINAL ARTICLE

Changes in albumin-to-creatinine ratio at 12-month follow-up in patients undergoing renal denervation.

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ORIGINAL ARTICLE

Changes in albumin-to-creatinine ratio at 12-month follow-up in patients undergoing renal denervation

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KEYWORDS
Resistant hypertension;
Renal denervation;
Albuminuria;
Blood pressure

Abstract
Introduction: Sympathetic renal denervation (RDN) was developed as a treatment for the management of patients with resistant hypertension. This procedure may have a positive impact on hypertension-related target organ damage, particularly renal disease, but the evidence is still limited.
Objective: To assess the impact of RDN on the albumin-to-creatinine ratio (ACR) at 12-month follow-up.
Methods and Results: From a single-center prospective registry including 65 patients with resistant hypertension undergoing renal denervation, 31 patients with complete baseline and 12-month follow-up blood pressure (BP) measurements (both office and 24-h ambulatory blood pressure monitoring [ABPM]) and ACR were included in the present study. Mean age was 65± 7 years, 52% were female, most (90%) had been diagnosed with hypertension for more than 10 years, 71% had type 2 diabetes and 33% had vascular disease in at least one territory. Mean estimated glomerular filtration rate was 73.6±25.1 ml/min/1.73 m^2 and 15 patients (48%) had an ACR >30 mg/g. After 12 months, 22 patients were considered BP responders (73%). ACR decreased significantly from a median of 25.8 mg/g (interquartile range [IQR] 9.0-574.0 mg/g) to 14.8 mg/g (IQR 4.5-61.0 mg/g, p=0.007). When the results were split according to systolic BP responder status on ABPM, we found a significant reduction in responders (from 25.6 mg/g [IQR 8.7-382.8 mg/g] to 15.9 mg/g [IQR 4.4-55.0 mg/g], p=0.009), and a numerical decrease in the non-responder subgroup (from 165.0 mg/g [IQR 8.8-1423.5 mg/g] to 13.6 mg/dl [IQR 5.7-1417.0 mg/g], p=0.345).

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Conclusions: Besides significant reductions in blood pressure (both office and 24-h ABPM), renal denervation was associated with a significant reduction in ACR, a recognized marker of target organ damage.

PALAVRAS-CHAVE
Hipertensão arterial resistente;
Desnervação renal;
Albuminúria;
Pressão arterial

Rácio albumina-creatinina aos 12 meses de seguimento após desnervação renal

Resumo
Introdução: A desnervação simpática renal (RDN) foi desenvolvida como uma forma de tratamento para os doentes com hipertensão arterial resistente (R-HTN). Este procedimento poderá ter um impacto favorável nas lesões de órgão alvo relacionadas com a hipertensão, nomeadamente a doença renal, no entanto, a evidência disponível ainda é escassa.
Objetivo: Avaliar o impacto da RDN no rácio albumina-creatinina (ACR) aos 12 meses de seguimento após RDN.
Métodos e resultados: Registo prospetivo de centro único incluindo 65 doentes com R-HTN submetidos a RDN, dos quais 31 doentes com avaliação basal e a um ano completa da pressão arterial (na consulta e na monitorização ambulatória [ABPM]) e da ACR foram incluídos no presente estudo. A idade média foi de 65±7 anos, 52% do sexo feminino. A maioria da população tinha diagnóstico de HTN há >10 anos, 71% tinha diabetes tipo 2 e 33% tinham doença vascular em pelo menos um território. A taxa de filtração glomerular estimada foi de 73,6± 25,1 ml/min/1,73 m^2 e 48% (15 doentes) tinham uma ACR>30 mg/g. Aos 12 meses de seguimento, 22 doentes foram considerados respondedores na pressão arterial (73%). A ACR teve uma descida significativa de uma mediana de 25,8 mg/g (IQR 9,0-574,0 mg/g) para 14,8 mg/g (IQR 4,5-61,0 mg/g, p=0,007). Quando os resultados foram divididos em subgrupos, de acordo com o estado de respondedor à pressão arterial na ABPM, verificou-se uma redução significativa nos respondedores (de 25,6 mg/g [IQR 8,7-382,8 mg/g] para 15,9 mg/g [IQR 4,4-55,0 mg/g], p=0,009), e uma tendência no subgrupo de não respondedores (de 165,0 mg/g [IQR 8,8-1423,5 mg/g] para 13,6 mg/dl [IQR 5,7-1417,0 mg/g], p=0,345).
Conclusão: Para além da descida significativa da pressão arterial (quer na consulta quer na monitorização ambulatória de 24 h), a desnervação renal associou-se a uma redução significativa da ACR, um reconhecido marcador de lesão de órgão alvo na hipertensão arterial.

Introduction

Cardiovascular disease is the leading cause of morbidity and mortality in developed countries and hypertension is one of its most important risk factors.[1] Some hypertensive patients have drug-resistant hypertension and are at a higher risk of events.[2,3] Besides clinical events, assessment of target organ damage can provide earlier insights into the biological impact of hypertension. For several years, albuminuria has been recognized as an indicator of cardiovascular risk, although the pathophysiology behind this association is still not fully understood.[4–6]

In recent years sympathetic renal denervation (RDN) has been developed as a treatment for the management of patients with resistant hypertension[7,8] and it may have a positive impact on hypertension-related target organ damage. An example is recently published reports of reductions in left ventricular hypertrophy after RDN.[9–11] The kidney is also an important organ in this context, but evidence on the effect of RDN on proteinuria is still limited and results are conflicting.[12,13] The aim of the present study was to assess the impact of RDN on the albumin-to-creatinine ratio (ACR) at 12-month follow-up.

Methods

Study design and patient population

From a single-center prospective registry including 318 patients with resistant hypertension referred for RDN between July 2011 and April 2015, we included for the purpose of the present study 31 patients with complete information on blood pressure (BP) measurements (both office and 24-h ambulatory blood pressure monitoring [ABPM]) at baseline and 12 months, transthoracic echocardiogram and renal function (creatinine clearance and ACR), out of 65 patients who were considered good candidates and underwent RDN (Figure 1).

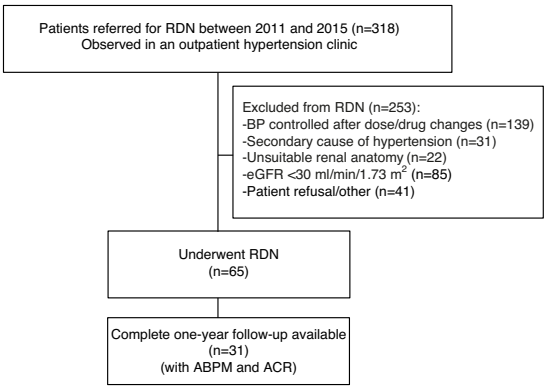

Figure 1 Flowchart of patient selection. Of the total number of patients observed in an outpatient hypertension clinic (n=318), 253 were excluded for various reasons (see main text) and 65 underwent renal denervation. Of these 65, complete 12-month follow-up with ambulatory blood pressure monitoring and transthoracic echocardiographic data were available in 31 patients. ABPM: 24-h ambulatory blood pressure monitoring; ACR: albumin-to-creatinine ratio; BP: blood pressure; eGFR: estimated glomerular filtration rate; RDN: renal denervation.

The details of this patient population have been previously described.[11,14] Briefly, all patients who underwent RDN were aged over 18 years, with persistent office systolic blood pressure (SBP) above 160 mmHg even after optimal antihypertensive therapy (at least three drugs, including a diuretic). Before RDN all patients were studied for secondary causes of hypertension and visited regularly (for at least six weeks) in order to ensure drug regime optimization and full compliance with medical treatment.

Demographic, clinical, anthropometric, laboratory and procedural data were recorded and stored in a dedicated database and written informed consent was obtained from all patients. The study was approved by the ethics committee of Hospital de Santa Cruz and Nova Medical School, Lisbon, Portugal.

Blood pressure measurement and definition of responders

Office BP readings were measured in a seated position, after a 5 min rest (in accordance with the European guidelines for the management of arterial hypertension), using an Omron HEM-907 semiautomatic oscillometric sphygmomanometer (Omron Healthcare, USA).

At baseline, before RDN, BP measurements were taken in both arms and the arm with the higher BP was selected for all subsequent readings. The mean of three measurements was used for analysis. An ABM monitor (Spacelabs Healthcare, USA) was used for 24-h ABPM assessment.

Patients with a decrease of 10 mmHg or more in office SBP or of 2 mmHg or more in 24-h ABPM SBP at 12-month follow-up were considered to be BP responders to RDN.[11,15]

Renal function and albuminuria measurement

Creatinine clearance was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. Albuminuria was obtained in spot urine and measured using the ACR, expressed in mg/g, which is equivalent to 24-h albumin excretion in mg/day. ACR values were acquired before RDN (at baseline) and at 12-month follow-up.

Renal denervation procedure

RDN procedures were performed using mild anesthesia (propofol and remifentanil) for sedation and pain control. An activated clotting time of 250-300 s was obtained with unfractionated heparin. After femoral artery access, abdominal aortography and selective renal artery angiography were performed to confirm anatomic eligibility. In all cases, the femoral access site was closed using a sealing device (Angio-Seal®, St. Jude Medical, USA).

Denervation was performed using the following radiofrequency systems: Symplicity® (n=25), EnligHTN® (n=4) and OneShot® (n=2), in accordance with standard techniques.

Statistical analysis

Continuous variables are reported as mean ± standard deviation. Normality was tested with the Kolmogorov-Smirnov test and/or visual assessment of a Q-Q plot. Normally distributed variables were compared between baseline and 12-month follow-up using a paired Student's t test, or a Wilcoxon matched-pairs test if not normally distributed. Discrete variables are expressed as frequencies and percentages. Statistical comparisons of characteristics at baseline and at follow-up were performed using the chi-square test or Fisher's exact test, as appropriate, for categorical variables and the paired Student's t test for continuous variables. A two-tailed p value <0.05 was considered as statistically significant.

SPSS® version 21.0 (IBM SPSS Inc, Chicago, IL) was used for data processing and statistical analysis.

Results

Patient characteristics

A total of 318 patients were observed in an outpatient hypertension clinic between 2011 and 2015. Of these, 253 were excluded due to: (a) BP control being achieved after dose and/or drug changes (n=139); (b) a secondary cause of hypertension (n=31); (c) renal anatomy considered unsuitable for RDN on computed tomography angiography; (d) estimated glomerular filtration rate (eGFR) <30 ml/min/1.73 m^2; (e) patient refusal after discussion of the expected benefits and risks. A total of 65 patients were considered good candidates and underwent RDN. Of these, the first 31 with complete data on blood and urine samples, office BP and 24-h ABPM, and transthoracic echocardiogram at both baseline and 12-month follow-up were included in the present analysis (Figure 1). Data on left ventricular mass and function have been reported elsewhere.[11]

Table 1 Baseline characteristics and renal denervation procedures.

Demographic and clinical variables	
Age (years)	65±7
Male (%)	15 (48.4)
Caucasian (%)	31 (100)
Weight (kg)	86±16
Height (m)	1.65±0.1
BMI (kg/m^2)	31.8±5.5
Obesity (%)	21 (67.7)
Atrial fibrillation (%)	1 (3.2)
Previous stroke (%)	2 (6.5)
Type 2 diabetes (%)	22 (71)
Dyslipidemia (%)	21 (67.7)
Smoking (%)	1 (3.2)
Sleep apnea (%)	5 (19.1)
eGFR (ml/min/1.73 m^2)	76.4±24.7
CKD (%)	5 (16.1)
Hypertension >10 years (%)	28 (90.3)
Coronary artery disease (%)	10 (32.3)
Any vascular disease (%)	11 (35.5)
RDN procedure	
Mean no. of RF applications, right renal artery	5.1±1.3
Mean no. of RF applications, left renal artery	5.7±1.1
Mean no. of RF applications per patient	10.8±2.3

BMI: body mass index; CKD: chronic kidney disease (eGFR <60 ml/min/1.73 m^2); eGFR: estimated glomerular filtration rate; RDN: renal denervation; RF: radiofrequency.

Mean age was 65±7 years, all patients were Caucasian and 48% (n=14) were male. Regarding cardiovascular risk factors, 71% had type 2 diabetes, 68% were obese (mean body mass index 32±6 kg/m^2), 68% had dyslipidemia and only one patient was an active smoker. Ten patients (33%) had manifestations of vascular disease (mainly coronary artery disease). Mean eGFR was 76.4±24.7 ml/min/1.73 m^2 and five patients had chronic kidney disease (eGFR <60 ml/min/1.73 m^2) (Table 1). At baseline, median ACR was 25.8 (interquartile range [IQR] 9.0-574.0) and 15 (48%) patients had ACR >30 mg/g.

Most patients (90%) had known hypertension for at least 10 years and were treated at baseline with a mean of 5.8 anti-hypertensive drugs, corresponding to a mean of 5.5 different drug classes. Of note, 74% were treated with spironolactone. Details on antihypertensive medication at baseline and follow-up are presented in Table 2.

Blood pressure control

Mean office SBP and diastolic BP (DBP) at baseline were 176±24 mmHg and 90±14 mmHg, respectively, and mean heart rate was 73±11 bpm. On 24-h ABPM, mean SBP and DBP were 150±20 mmHg and 83±10 mmHg, respectively (Table 3).

At 12-month follow-up mean SBP had decreased from 176±24 to 149±13 mmHg (p<0.001) and DBP from 90±14 to 79±11 mmHg (p<0.001). These changes were consistent with the results of 24-h ABPM, in which mean SBP decreased from 150±20 to 132±14 mmHg (p<0.001) and mean DBP from 83±10 to 74±9 mmHg (p=0.001). At 12-month follow-up, 71% of patients were considered office SBP responders and 84% were considered ABPM SBP responders (Table 3). During this period there was also a reduction in the number of antihypertensive drugs and classes taken; the number of drugs decreased from 5.8±1.1 to 5.0±1.2 (p=0.002) and drug classes from 5.5±0.9 to 4.9±1.1 (p=0.02) (Table 2).

Changes in albumin-to-creatinine ratio after renal denervation and relation with blood pressure control

At baseline, median ACR was 25.8 mg/g (IQR 9.0-574.0 mg/g) and 48.4% of patients (n=15) had an ACR >30 mg/g. We found a significant reduction at 12-month follow-up to a median of 14.8 mg/g (IQR 4.5-61.0 mg/g, p=0.007) (Table 3).

Interestingly, we also found a significant reduction in the percentage of patients with ACR >30 mg/g between baseline and 12-month follow-up (Figure 2). Considering patients with any ACR reduction as ACR responders, 77.4% (n=24) of patients were ACR responders. The distribution of patients across the different classes of urinary albumin excretion also demonstrated a favorable effect (Figure 3).

Table 2 Antihypertensive medication.

	Baseline	12 months	p
Mean no. of antihypertensive drugs	5.8±1.1	5.0±1.2	0.002
Mean no. of drug classes	5.5±0.9	4.9±1.1	0.015
ACE inhibitors	19 (61.3)	17 (54.8)	0.688
ARBs (%)	19 (61.3)	18 (58.1)	1.0
Beta-blockers (%)	26 (83.9)	27 (87.1)	1.0
Calcium channel blockers (%)	30 (96.8)	21 (67.7)	0.012
Diuretics (%)	27 (87.1)	24 (77.4)	0.727
Spironolactone (%)	23 (74.2)	26 (83.9)	0.453
Sympatholytics (%)	22 (71)	19 (61.3)	0.508
Aliskiren	4 (12.9)	0	0.046

ACE: angiotensin-converting enzyme; ARB: angiotensin receptor blockers.

Table 3 Blood pressure, heart rate and albumin-to-creatinine ratio before and 12 months after renal denervation.

	Baseline	12 months	p
Office SBP (mmHg)	176±24	149±13	<0.001
Office DBP (mmHg)	90±14	79±11	<0.001
Heart rate (bpm)	73±11	70±11	0.261
ABPM SBP (mmHg)	150±20	132±14	<0.001
ABPM DBP (mmHg)	83±10	74±9	<0.001
ABPM pulse pressure (mmHg)	67±18	58±13	0.001
ABPM mean BP (mmHg)	105±9	95.3±8.4	<0.001
ABPM heart rate (bpm)	67.6±9.1	65.5±9.5	0.090
ABPM SBP responders[a] (%)	-	26 (83.9)	-
Office SBP responders[b] (%)	-	22 (71)	-
eGFR (ml/min/1.73 m^2)	73.6±25.1	72.5±25.1	0.711
ACR (mg/g)	25.8 (9.0-574.0)	14.8 (4.5-61.0)	0.007
ACR in ABPM BP responders (mg/g)	25.6 (8.7-382.8)	15.9 (4.4-55.0)	0.009
ACR in ABPM BP non-responders (mg/g)	165.0 (8.8-1423.5)	13.6 (5.7-1417.0)	0.345
ACR in ABPM dippers (mg/g)	20.8 (6.8-290.0)	9.4 (3.7-41.1)	0.028
ACR in ABPM non-dippers (mg/g)	62.3 (9.1-852.3)	20.8 (9.3-197.1)	0.096
ACR in diabetic patients (mg/g)	48.9 (9.1-1116.3)	23.1 (4.3-123.8)	0.028
ACR in non-diabetic patients (mg/g)	25.4 (5.2-68.6)	10.9 (3.4-20.8)	0.066

ABPM: 24-h ambulatory blood pressure; ACR: albumin to creatinine ratio; BP: blood pressure; bpm: beats per minute; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure.
[a] Decrease of 2 mmHg between baseline ABPM SBP and at 12 months.
[b] Decrease of 10 mmHg between baseline office SBP and at 12 months.

When the results were split according to ABPM SBP responder status, we found a significant reduction in responders (from 25.6 mg/g [IQR 8.7-382.8 mg/g] to 15.9 mg/g [IQR 4.4-55.0 mg/g], p=0.009), and a numerical decrease in non-responders (from 165.0 mg/g [IQR 8.8-1423.5 mg/g] to 13.6 mg/dl [IQR 5.7-1417.0 mg/g], p=0.345), probably due to the small number of patients in this subgroup (Table 3 and Figure 4). The same analysis was performed according to dipper status and the results were similar, with a significant reduction in patients with dipper status on baseline ABPM (Table 3 and Figure 5).

With regard to diabetic status, patients with diabetes had a higher median baseline ACR and showed a statistically significant decrease (from 48.9 mg/g [IQR 9.1-1116.3 mg/g] to 23.1 mg/g [IQR 4.3-123.8 mg/g], p=0.028); there was also a numerical decrease in ACR in non-diabetic patients

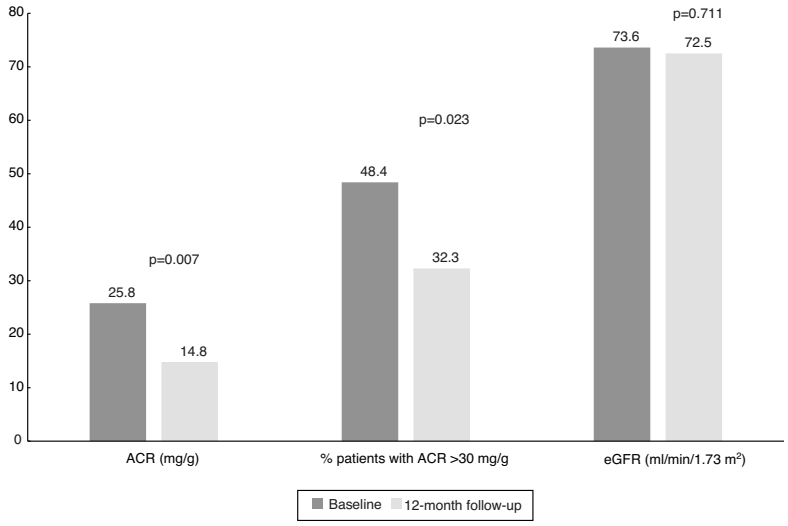

Figure 2 Values of albumin-to-creatinine ratio and estimated glomerular filtration rate at 12 months after renal denervation. There was a significant reduction in the median values of ACR and the percentage of patients with ACR >30 mg/g, without significant changes in eGFR. ACR: albumin-to-creatinine ratio; eGFR: estimated glomerular filtration rate.

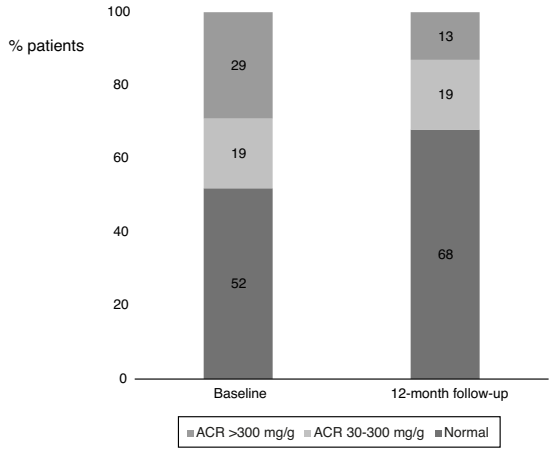

Figure 3 Percentages of patients in the different albumin-to-creatinine ratio subgroups. There was a numerical decrease in the percentage of patients with an ACR >300 mg/g and an increase in patients with normal urinary albumin excretion between baseline and 12-month follow-up. ACR: albumin-to-creatinine ratio.

(from 25.4 mg/g (IQR 5.3-68.6 mg/g) to 10.9 mg/dl (IQR 3.4-20.8 mg/g), p=0.066), probably also due to the small patient sample in this subgroup (n=9) (Table 3 and Figure 6).

Safety

There were four vascular access complications: three hematomas and one femoral pseudoaneurysm. No significant changes in eGFR were seen (Table 3 and Figure 2).

Discussion

The main findings of our study are: (1) RDN was associated with a significant BP reduction at 12-month follow-up; (2) there was a significant decrease in median ACR, without significant changes in eGFR, and a significant reduction in the percentage of patients with ACR >30 mg/g between baseline and 12-month follow-up; (3) the reduction in ACR was observed in both BP responders and non-responders.

Although the initial results with catheter-based RDN were very promising,[7,8,16] the most recent and largest randomized trial, SYMPLICITY HTN-3,[15] failed to meet its primary efficacy endpoint, raising doubts about the real biological effect of this treatment. The unexpected negative results of HTN-3 have been extensively discussed and many possible reasons have been put forward, both clinical (mainly related to patient selection) and technical (procedure-related, particularly the number and pattern of radiofrequency applications).[17] Another possible factor is how the efficacy of RDN is currently measured, and examining target organ damage may provide a better assessment than BP values. In line with this are the recent results in LV mass and function after RDN, for which several groups have published positive results at six-month follow-up based on both transthoracic echocardiography[11,18] and cardiac magnetic resonance imaging (MRI).[10] Our group recently reported a significant reduction in left ventricular mass at 12-month follow-up, without correlation with changes in systolic ABPM.[11]

Another approach to monitoring hypertension-related target organ damage is to calculate ACR, a recognized marker which has been linked to cardiovascular outcomes in several studies on hypertension.[4–6] Ott et al.[19] found a significant reduction in ACR at six-month follow-up in 59 patients with resistant hypertension (mean 24-h ABPM SBP 156 mmHg, treated with a mean of 5.5 antihypertensive

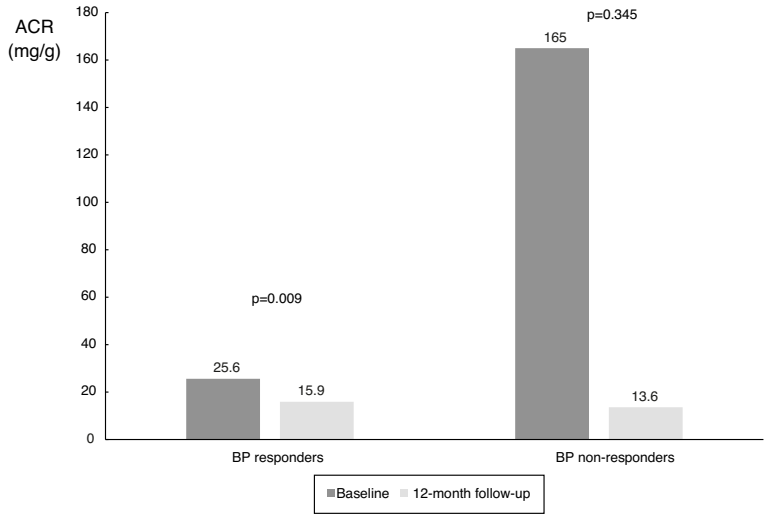

Figure 4 Values of albumin-to-creatinine ratio at 12 months after renal denervation in 24-h ambulatory blood pressure monitoring responder subgroups. There was a significant reduction in median ACR in the BP responder subgroup, and a numerical decrease in non-responders. ACR: albumin-to-creatinine ratio; BP: blood pressure.

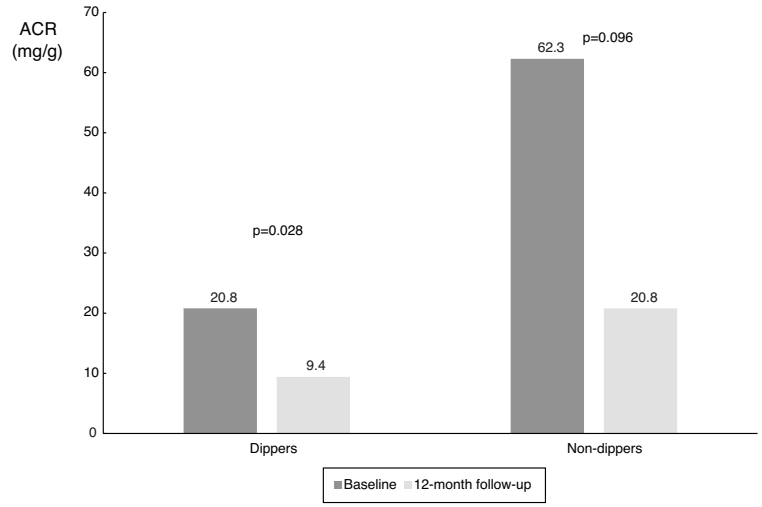

Figure 5 Values of albumin-to-creatinine ratio at 12 months after renal denervation according to dipper status on 24-h ambulatory blood pressure monitoring. There was a significant reduction in median ACR in the dipper subgroup, and a numerical decrease in non-dippers. ACR: albumin-to-creatinine ratio.

drugs). In contrast with the latter study, we also included patients with normal (<30 mg/g) baseline ACR and therefore our median values are lower that those reported by Ott et al. Of note, even among this mixed population of different ACR baseline profiles, half of whom had normal urinary albumin excretion (51.6% with ACR <30 mg/dl), the mean age (65 years), baseline ABPM SBP (150 mmHg) and mean number of drugs (5.8) were very similar to the study by Ott et al. Our study also included a higher percentage of patients with type 2 diabetes (71%, compared to 51% in Ott et al.'s study). In another recently published single-center study, Verloop et al.[13] failed to demonstrate any significant decrease in either LV mass (by cardiac MRI) or urinary albumin excretion, and found only a modest impact on blood pressure. These results are in contrast with previous studies and our results, and may have been due to differences in patient populations. In the study by Verloop et al.,[13] the mean age was lower (58 years) and so was the mean number of drugs (4, as opposed to 5.5 in Ott et al. and 5.8 in our study). Other important differences are the much lower prevalence of diabetes (only 15%) and the fact that the authors did not exclude patients with eGFR <45 ml/min/1.73 m^2.

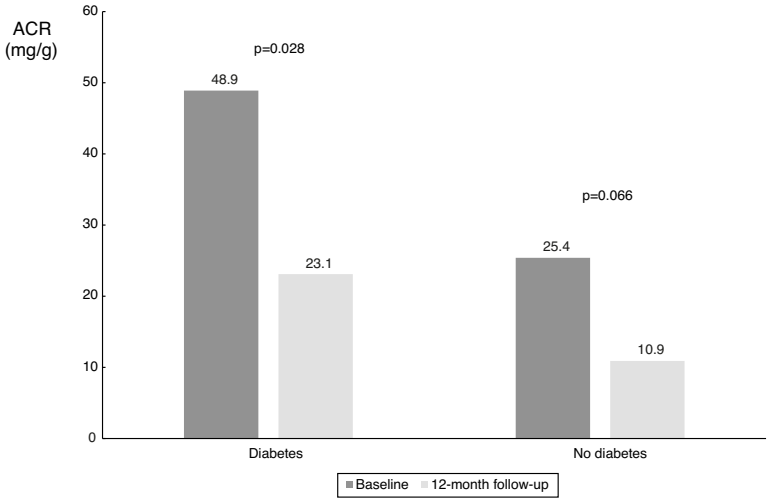

Figure 6 Values of albumin-to-creatinine ratio at 12 months after renal denervation according to diabetic status. There was a significant reduction in median ACR in patients with diabetes, and a numerical decrease in the smaller subgroup of patients without diabetes. ACR: albumin-to-creatinine ratio.

One interesting observation in our study is the fact that the reduction in ACR was also found in BP non-responders, although this did not reach statistical significance, probably due to the small size of this patient subgroup. This raises the question of whether RDN, by reducing sympathetic hyperactivity, might have a positive direct effect on glomerular endothelial function independent of the hemodynamic effect derived from blood pressure reduction, since there is a close association between urinary albumin excretion and glomerular endothelium dysfunction and glycocalyx loss.[5,20] These two factors may be modulated by autonomic nervous system tone and, in theory, this positive impact on endothelial physiology could be linked to the expected decrease in overall cardiovascular risk that is the ultimate goal of RDN. On the other hand, endothelial dysfunction is only one of several effects of increased sympathetic activity, a common denominator in cardiovascular and renal pathophysiology.[21] Finally, the ACR reduction seen in our study should be interpreted in the context of the high cardiovascular risk of patients with resistant hypertension,[2,3] and this reduction is expected to help to lower this risk, although no prospective studies have been published on the prognostic impact of RDN on clinical outcomes.

Limitations

The present study has some limitations that should be mentioned: (1) it is a single-center prospective registry with a small sample size; (2) the physicians following patients after RDN were not blinded, although the most important outcome measurements (24-h ABPM and ACR) were performed by cardiac and laboratory technicians unaware of treatment status; (3) there was no control group or sham procedure; (4) changes were made in antihypertensive therapy during follow-up, which could have influenced reductions in blood pressure and ACR (but the mean number of drugs actually decreased during follow-up, which could lead to underestimation of the benefit of RDN in this daily practice setting).

Conclusions

In this single-center unblinded study of patients with resistant hypertension undergoing RDN, we found a significant reduction in both office BP and 24-h ABPM which was associated with a significant decrease in median ACR, without significant changes in eGFR. At 12-month follow-up, there was a reduction in the percentage of patients with pathological urinary albumin excretion, and this reduction was independent of BP responder status.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens. 2013;31:1281–357.
2. Calhoun DA, Jones D, Textor S, et al. Resistant hypertension: diagnosis, evaluation, and treatment. A scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. Hypertension. 2008;51:1403–19.
3. Muiesan ML, Salvetti M, Rizzoni D, et al. Resistant hypertension and target organ damage. Hypertens Res. 2013;36:485–91.
4. Chronic Kidney Disease Prognosis ConsortiumMatsushita K, van der Velde M, Astor B, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. Lancet. 2010;375:2073–81.
5. Satchell S. The role of the glomerular endothelium in albumin handling. Nat Rev Nephrol. 2013;9:717–25.
6. Waheed S, Matsushita K, Astor BC, et al. Combined association of creatinine, albuminuria, and cystatin C with all-cause mortality and cardiovascular and kidney outcomes. Clin J Am Soc Nephrol. 2013;8:434–42.
7. Krum H, Schlaich M, Whitbourn R, et al. Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. Lancet. 2009;373:1275–81.
8. Symplicity HTN-2 InvestigatorsEsler MD, Krum H, Sobotka PA, et al. Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. Lancet. 2010;376:1903–9.
9. Schirmer SH, Sayed MM, Reil JC, et al. Improvements in left ventricular hypertrophy and diastolic function following renal denervation: effects beyond blood pressure and heart rate reduction. J Am Coll Cardiol. 2014;63:1916–23.
10. Mahfoud F, Urban D, Teller D, et al. Effect of renal denervation on left ventricular mass and function in patients with resistant hypertension: data from a multi-centre cardiovascular magnetic resonance imaging trial. Eur Heart J. 2014;35:2224–31.
11. de Sousa Almeida M, de Araujo Goncalves P, Branco P, et al. Impact of renal sympathetic denervation on left ventricular structure and function at 1-year follow-up. PLOS ONE. 2016;11:e0149855.
12. Ott C, Mahfoud F, Schmid A, et al. Improvement of albuminuria after renal denervation. Int J Cardiol. 2014;173:311–5.
13. Verloop WL, Vink EE, Spiering W, et al. Effects of renal denervation on end organ damage in hypertensive patients. Eur J Prev Cardiol. 2015;22:558–67.
14. Dores H, de Sousa Almeida M, de Araujo Goncalves P, et al. Renal denervation in patients with resistant hypertension: six-month results. Rev Port Cardiol. 2014;33:197–204.
15. Bhatt DL, Kandzari DE, O'Neill WW, et al. A controlled trial of renal denervation for resistant hypertension. N Engl J Med. 2014;370:1393–401.
16. Schlaich MP, Sobotka PA, Krum H, et al. Renal sympathetic-nerve ablation for uncontrolled hypertension. N Engl J Med. 2009;361:932–4.
17. Kandzari DE, Bhatt DL, Brar S, et al. Predictors of blood pressure response in the SYMPLICITY HTN-3 trial. Eur Heart J. 2015;36:219–27.

18. Brandt MC, Mahfoud F, Reda S, et al. Renal sympathetic denervation reduces left ventricular hypertrophy and improves cardiac function in patients with resistant hypertension. J Am Coll Cardiol. 2012;59:901–9.
19. Ott C, Schmid A, Ditting T, et al. Renal denervation in a hypertensive patient with end-stage renal disease and small arteries: a direction for future research. J Clin Hypertens (Greenwich). 2012;14:799–801.
20. Salmon AH, Satchell SC. Endothelial glycocalyx dysfunction in disease: albuminuria and increased microvascular permeability. J Pathol. 2012;226:562–74.
21. Almeida Mde S, Goncalves Pde A, Oliveira EI, et al. Renal denervation for resistant hypertension. Rev Port Cardiol. 2015;34:125–35.

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