Challenges in the management of hypertension and heart failure

Uitdagingen in de behandeling van hypertensie en hartfalen
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op
woensdag 22 februari 2017 des middags te 2.30 uur

door

Martine Maria Anne Beeftink

geboren op 13 maart 1986
te ’s Gravenhage

Challenges in the management of hypertension and heart failure

Martine Beeftink, Utrecht, 2017

Layout: Martine Beeftink
Cover design: Martine Beeftink & Sung-Wook Noort
Cerro colorado Vinicunca, Peru
Printed by: Gildeprint - Enschede

ISBN: 987-94-6233-523-3

Financial support by the Dutch Heart Foundation, the Heart & Lung Foundation Utrecht and the Dutch Kidney Foundation for the publication of this thesis is gratefully acknowledged.

The research described in part two of this thesis was supported by the Netherlands Cardiovascular Research Initiative with support of the Dutch Heart Foundation (CVON2014-11 RECONNECT).

The DIASTOLE trial was supported by an unrestricted grant by Medtronic. Further financial support for the publication of this thesis by St. Jude Medical Nederland B.V. was gratefully acknowledged.

Promotoren: Prof. dr. P.A.F. Doevendans
Prof. dr. M.L. Bots

Copromotoren: Dr. M. Voskuil
Dr. W. Spiering

Everything will be okay in the end. If it's not okay, then it's not the end.

Paulo Coelho

Table of contents

Chapter 1-
Introduction and outline

Based on Treatment Strategies 2013;3;71-74

Martine M.A. Beeftink, Michiel Voskuil

Introduction

Denervation of the sympathetic nerves has been subject of interest as a potential interventional therapeutic target for the treatment of hypertension as early as the 1930s. Based on observations of hypertension in renal disease, Page and Heuer[1] hypothesized that nervous influences from the kidney may be causally related to essential hypertension. For this reason, they performed the first (albeit unsuccessful) bilateral renal denervation in a patient with uncomplicated essential hypertension. Despite the negative outcome, their hypothesis was further investigated in subsequent studies, which indeed provided evidence for a beneficial effect of surgical sympathetic denervation on blood pressure.[2-4] However, clinical applicability has been unattainable so far, due to the severe side-effects and complications (including perioperative mortality) of this invasive procedure.[3]
Recently, the belief in sympathetic denervation as a treatment strategy has been fueled with renewed enthusiasm by the development of a new intervention method utilizing a radiofrequency ablation catheter for endovascular denervation of the renal sympathetic nerves.[5] This invention provides a minimally-invasive approach, with promise of much lower complication rates. The first positive results of percutaneous renal denervation (RDN) in patients with resistant hypertension combined with evidence for sympathetic hyperactivity in various other diseases have generated a universal excitement about the possible applications of this novel treatment modality.
This chapter aims to discuss the rationale of renal denervation and provide an overview of its potential applications, based on the situation in 2013. It ends with an outline of the work presented in this thesis.

Sympathetic control of blood pressure

Central sympathetic activation of the kidney is conducted through the efferent sympathetic nerves that originate from the thoracic and lumbar sympathetic trunk. They terminate in the renal tubules, the juxtaglomerular apparatus and the renal blood vessels, where they regulate sodium reabsorption, renin secretion and renal vascular resistance, respectively.[6-8] Enhanced sympathetic firing rates increase sodium reabsorption, leading to an expansion of blood volume and resulting in higher arterial blood pressure levels. The activation of the Renin-Angiotensin-Aldosterone-system (RAAS) further enhances the sodium reabsorption and causes direct vasoconstriction, further elevating arterial blood pressures.[9]
The kidneys give feedback to the central nervous system through the afferent renal nerve pathway. These afferent nerves are activated through changes in the chemical environment of the renal interstitium (e.g. adenosine during renal ischemia, uremic toxins) from the renal chemoreceptors and renal hydrostatic changes from the renal baroreceptors.[7,10,11] This information is relayed to the central neuronal circuits, modulating central sympathetic tone by mediating baroreflex and vasomotor tone through circuits in the medulla, and activating vasopressinergic neurons in the hypothalamus.[6,7]
The afferent nerves also execute short feedback loops, known as the reno-renal reflexes, that relay information from the afferent fibers via the neuraxis directly back to the renal efferent fibers.[12] For instance, in high-sodium diet activation of the afferent renal nerves contributes to suppression of the efferent nerve activity, leading to accelerated sodium

excretion.[13] This mechanism allows the renal function to be self-regulated and execute rapid adjustment of urinary sodium and water excretion.[14]
This dual function of the kidney as both effector and modulator of the sympathetic nervous system, allows the kidneys to finely regulate blood pressure homeostasis, but also holds the potential of developing a vicious circle that generates a maintained sympathetic hyperactivity. In fact, experimental studies have shown that activation of the sympathetic nervous system causes hypertension.[15] Moreover, in patients with hypertension, the level of sympathetic activation relates directly to the severity of the disease and the presence of end-organ damage.[16,17] Therefore, modulating sympathetic tone is a logical therapeutic strategy, as illustrated by the longstanding and broad application of ß-adrenergic receptor blockers.

Percutaneous renal denervation (RDN) now composes a new therapeutic technique for modulating sympathetic tone. It is a minimally invasive, catheter-based technique that delivers radiofrequent energy to the wall of the renal artery, taking advantage of the fact that both the afferent and efferent sympathetic nerves travel within and closely adjacent to the renal arterial wall.[18] The radiofrequent energy causes circumscribed transmural injury that affects the nerve fascicles without clinically important damage to the vessel wall, resulting in ablation of the sympathetic nerves.[19]
This results in an effective lowering of sympathetic nervous activity as has been demonstrated by lowered muscle sympathetic nerve activity (MSNA), which is a measurement for sympathetic outflow to the periphery.[20] Also, direct measurements of catecholamines in the inferior vena cava showed attenuation of the increase in catecholamine levels that follows after electrical stimulation of the renal sympathetic nerves when the denervated renal artery was stimulated.[21]
The clinical effect on resistant hypertension was tested in a proof-of-principal trial in 45 patients with a systolic blood pressure (SBP) >160 mmHg despite being treated with at least three antihypertensive drugs.[5] After bilateral treatment of the main renal artery, office-based blood pressures were reduced by -14/-10 at 1 month and -27/-17 at 12 months. These results were confirmed in a randomized controlled trial that showed a blood pressure reduction of -32/-12 mmHg at 6 months in the RDN group, whereas blood pressure in the control group did not change.[22] The reduction of blood pressure was sustained at 12 month follow-up and control patients who crossed over to RDN showed a similar blood pressure reduction to patients in the intervention group.[23] Similar results have been achieved in a few smaller cohorts[24-27], including one in patients with milder hypertension (SBP 130-160 mmHg).[28] None of the studies reported serious long-term adverse effects. Observed perioperative complications included renal artery dissection (treated by stenting, without further sequelae or prolonged hospitalization), pseudoaneurysm of the femoral artery and hypotensive complaints.[5,23] No significant deterioration of kidney function[5,22-25,28] or renal artery stenosis[5,24,28] was found.
These results give hope for patients with resistant hypertension who are otherwise at continuous risk of developing cardiovascular complications. It also opens doors for other possible indications for RDN. Sympathetic hyperactivity can not only be found in hypertension, but is present in other diseases that involve sympathetic target-organs, such as left ventricular hypertrophy, heart failure, cardiac arrhythmias, kidney disease, diabetes mellitus and metabolic syndrome.[29]

It may be that increased sympathetic activity in these diseases is merely a reflection of disease progression. However, increasing evidence suggests that adrenergic stimulation in fact plays a major role in the pathophysiology of end-organ damage as described below.

Sympathetic control in other diseases

Heart disease

Insufficiently treated hypertension leads to cardiac remodeling, fibrosis, hypertrophy and eventually heart failure.[30] For a long time, pressure overload was considered to be the primary underlying pathophysiological cause. However, it is now recognized that direct adrenergic stimulation also largely contributes to myocardial remodeling in hypertension.
Experimental research has demonstrated that increased levels of the sympathetic neurotransmitter norepinephrine results in myocyte hypertrophy, increased apoptosis of cardiomyocytes and deficits in cardiomyocyte contractility.[30] The responsible mechanism appears to involve cardiotoxic increases in intracellular calcium and activation of local inflammatory pathways through direct effects of catecholamines[30,31] and oxidative and inflammatory stress through activation of the RAAS.[30,32] However, the exact mechanism remains to be elucidated.

Clinically, the level of sympathetic nervous activity (SNA) is correlated to the left ventricular mass in patients with essential hypertension[33] and increases further in the presence of diastolic dysfunction and heart failure.[17] It is unlikely that this is merely a reflection of disease progression, because in patients with similar blood pressure levels a higher level of SNA was found in those with diastolic dysfunction.[17] In addition, experimental adrenergic stimulation by infusion of norepinephrine causes concentric left ventricular hypertrophy in the absence of elevated blood pressure.[34,35]
Conversely, influencing the sympathetic pathways in rats resulted in improvement of left ventricular hypertrophy, left ventricular dilatation and heart failure.[36,37] This may indicate that cardiac remodeling in hypertensive patients may respond to RDN. Indeed, a small study in humans demonstrated a reduction in left ventricular mass in patients treated with RDN for resistant hypertension.[38]
These results may indicate a beneficial effect of RDN in patients with heart failure with preserved left ventricular ejection fraction (HFpEF). Initially, this condition was considered merely a transitional state from left ventricular hypertrophy to systolic heart failure. However, it is now recognized as a separate entity and recent observations indicate that it is becoming the dominant form of heart failure in the community, with increasing morbidity and mortality.[39,40] The condition is characterized by impaired ventricular relaxation and reduced compliance of the ventricles, leading to diastolic dysfunction and eventually heart failure.[41] The underlying structural changes in the myocardium include the same spectrum of changes associated with sympathetic stimulation, indicating a possible role of the sympathetic nervous system in the pathophysiology.[17] Indeed, sympathetic nerve activity is elevated in these patients and increases proportionally to severity of the disease.[17,42] Unfortunately, there is currently no successfully proven therapy for HFpEF yet. Although favorable effects would be expected from treatment with ß-adrenergic receptorblockers and RAAS-influencing drugs, the results from large clinical trials have been disappointing.[43,44]

The positive effects of RDN in hypertension provide optimism that similar results may be achieved in HFpEF. A randomized clinical trial (presented in part two of this thesis) has been undertaken to investigate whether RDN can improve cardiac function and symptoms in these patients.[45]

In systolic heart failure, the role of the sympathetic nervous system has been acknowledged for quite some time and drugs that interfere with the RAAS and ß-adrenergic pathways have been the cornerstones of treatment for decades now. Nevertheless, heart failure continues to have high morbidity and mortality rates.[46] As the doses of the pharmacological drugs are usually insufficient for complete suppression of ß-adrenergic and angiotensin II receptors, it has been hypothesized that RDN may have additional benefits when added to the pharmacological treatment.[47] These effects may reach beyond improvement of ventricular function and also reduce heart failure complications such as cardiac arrhythmias and renal failure.

The risk of sudden cardiac death in congestive heart failure is correlated to the cardiac norepinephrine spillover rate and arterial plasma norepinephrine levels, indicating potential benefits from sympathetic nervous system supression.[46] Left cardiac sympathetic denervation (LCSD) has been shown to improve arrhythmia in patients with catecholaminergic polymorphic ventricular tachycardia, long QT syndrome and other life-threatening ventricular arrhythmia.[48-50] However, this intervention still requires surgical intervention under general anesthesia. Moreover, LCSD results in localized suppression of sympathetic stimulation only. So far, there is little experience with RDN for ventricular arrhythmia, but theoretically it can combine treatment of arrhythmia with the beneficial effect of systemic suppression of sympathetic activity and the possibility to perform the intervention under local anesthesia. The first-in-man experience with RDN in two patients with therapy resistant electrical storm significantly reduced ventricular tachyarrhythmia, but further research is needed to confirm these preliminary results.[51]
The effect of RDN on supraventricular tachyarrhythmias, that occur frequently in association with hypertension and diastolic dysfunction, shows some promising results. Renal denervation has been shown to reduce heart rate and atrioventricular node conduction time.[52,53] When pulmonary vein isolation (PVI) is combined with RDN, it leads to reduced inducibility of atrial fibrillation during rapid atrial pacing and reduces atrial fibrillation recurrences compared with PVI alone.[54] Currently a double-blind randomized trial is undertaken to confirm these results.[55]

There is a strong relation between heart failure and kidney failure. Not only is renal failure a frequent complication of heart failure and hypertension, renal failure is also associated with an increased risk of cardiovascular disease. When both diseases exist concomitantly, the risk for morbidity and mortality exponentially increases.[56] Moreover, lower glomerular filtration rate is an independent predictor of mortality in heart failure and the prognostic value is increased when combined with cardiac sympathetic nervous activity.[57,58] Experimental research has demonstrated improved renal hemodynamics following RDN in swine.[59] Moreover, it improved renal function and prevented hypertension in a chronic kidney model in rats.[10,60] However, these results have not yet been confirmed in humans.

The metabolic syndrome

The metabolic syndrome is a common disorder that entails high blood pressure, impaired glucose tolerance, elevated cholesterol levels and obesity. The syndrome is associated with high prevalence of subclinical organ damage that substantially increases cardiovascular risk. This includes microalbuminuria, impaired renal function, increased left ventricular mass, diastolic dysfunction, arterial stiffening and higher incidence of large artery plaques.[61,62]

There are several hypotheses about the exact mechanism that is involved in the development of the metabolic syndrome and its complications, with the sympathetic nervous system being a prime suspect. Elevated levels of SNA are present in patients with obesity, diabetes mellitus and hypertension. In diabetes, the magnitude of SNA is related to the severity along the diabetes spectrum ranging from impaired glucose tolerance to insulin-dependent diabetes mellitus type 2.[63] This is not surprising, as sympathetic stimuli alter glucose metabolism and insulin sensitivity through local hemodynamic effects, decreasing the local availability of insulin, and increasing adipose tissue lipolysis, increasing the release of free fatty acids into the circulation.[61] The hypothesis is further supported by the observation that the organ damage seen in diabetes and obesity highly resembles the spectrum of complications seen in high sympathetic states, as described above. Moreover, in obese patients sympathetic activity is closely related to subclinical organ damage, such as impaired endothelial function, increased left ventricular mass index and impaired cardiac function.[64] Therefore, it seems reasonable to investigate the effects of sympathetic denervation in patients with the metabolic syndrome or complicated diabetes mellitus.

A retrospective study investigating these effects in patients with resistant hypertension showed significant reductions in fasting glucose and insulin levels, not correlated to the blood pressure reduction.[65] However, more research is needed to investigate whether renal denervation can indeed be used as a non-pharmaceutical approach in insulin resistance.

Interaction of disease states

Although these possible indications for RDN have been discussed separately here, the role of the sympathetic nervous system appears much more complex. Not only is there great overlap in the observed (micro-)vascular and cardiac complications, there is also great interaction between the different disease states.

Clearly, obese patients are more prone to developing diabetes. Additionally, in both obese patients and diabetics there is a higher prevalence of hypertension. There are indications that sympathetic hyperactivity is potentiated when both diseases are present in the same individual, but the evidence is not unambiguous. However, complication rates are indeed increased when patients suffer multiple disease with increased sympathetic tone.[61]

It gets even more complex when obstructive sleep apnea syndrome (OSAS) is taken into the equation. OSAS has been considered the responsible mechanism for sympathetic hyperactivity in obesity by stimulation of the sympathetic chemoreceptors. However, sympathetic hyperactivity is also present in lean patients suffering from OSAS.[66] Furthermore, OSAS has been related to hypertension, heart failure, metabolic syndrome, chronic kidney disease and cardiac arrhythmias, and concomitant presence is related to higher complication rates and adverse outcome.[67]

Conclusion

Sympathetic hyperactivity appears to play an important role in the pathophysiology of hypertension. Modulation of the sympathetic nervous system using RDN has shown beneficial effects in patients with (resistant) hypertension. This has fueled the expectation that other disease states with sympathetic hyperactivity may benefit from RDN as well. However, further research is needed to confirm these hypotheses and to unravel the intricate continuity of the sympathetic nervous system and the associated conditions. The studies in this thesis further investigated the use of renal denervation in hypertension and explored the therapeutic potential of renal denervation for HFpEF.

Outline of the thesis

The first part of this thesis focusses on hypertension. **Chapter 2** discusses the safety of temporary withdrawal of antihypertensive medication in the diagnostic work-up of patients with difficult-to-control hypertension, including patients eligible for renal denervation. **Chapter 3** describes the highly variable changes in ambulatory blood pressure that can be observed after withdrawal of antihypertensive medication, revealing several disadvantages of blood pressure as endpoint in clinic and research. In **Chapter 4**, the changes in home blood pressure during the first year after renal denervation are discussed. This chapter also compares the application of home measurement in renal denervation research to the more conventional office measurements and ambulatory measurements. **Chapter 5** provides insight in the influence of the spatial distribution of renal denervation ablations on the blood pressure effect, aiming to contribute to the optimization of the renal denervation procedure.

In part two of this thesis, the potential therapeutic application of renal denervation in HFpEF is investigated. **Chapter 6** discloses the results of a systematic review, describing the relation between sympathetic nervous activity and HFpEF. Based on the hypothesis that reducing sympathetic activity is beneficial for patients with HFpEF, we designed and executed a randomized controlled trial. The design and rationale of the DIASTOLE trial is provided in **Chapter 7**. In **Chapter 8** we describe the prevalence of diastolic dysfunction and HFpEF in a general cardiac outpatient population, illustrating the challenging quest for patients eligible to participate in HFpEF trials. Lastly, the results of the DIASTOLE trial are described in **Chapter 9**.

Chapter 10 contains a summary of the challenges encountered in hypertension and heart failure, discussing the main findings of the results presented in this thesis and the interpretations and recommendations derived from these results.

References

1. Page IH, Heuer GJ. The effect of renal denervation on the level of arterial blood pressure and renal function in essential hypertension. J Clin Invest 1935;14:27–30.
2. Goldblatt H, Wartman WB. Studies on experimental hypertension : VI. The effect of section of anterior spinal nerve roots on experimental hypertension due to renal ischemia. J Exp Med 1937;66:527–534.
3. Morrissey DM, Brookes VS, Cooke WT. Sympathectomy in the treatment of hypertension; review of 122 cases. Lancet 1953;1:403–408.
4. Grimson KS, Wilson H, Phemister DB. The early and remote effects of total and partial para-vertebral sympathectomy on blood pressure: an experimental study. Ann Surg 1937;106:801–825.
5. 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;373:1275–1281.
6. DiBona GF. Neural control of the kidney: past, present, and future. Hypertens 2003;41:621–624.
7. Ciriello J, de Oliveira CVR. Renal afferents and hypertension. Curr Hypertens Rep 2002;4:136–142.
8. Sobotka PA, Mahfoud F, Schlaich MP, et al. Sympatho-renal axis in chronic disease. Clin Res Cardiol 2011;100:1049–1057.
9. Goldsmith SR, Sobotka P a., Bart B a. The Sympathorenal Axis in Hypertension and Heart Failure. J Card Fail 2010;16:369–373.
10. Campese VM, Kogosov E. Renal afferent denervation prevents hypertension in rats with chronic renal failure. Hypertens 1995;25:878–882.
11. Kopp UC. Renorenal reflexes in hypertension. J Hypertens 1993;11:765–773.
12. Kopp UC. Neural control of renal function. San Rafael; Morgan & CLaypool Life Sciences; 2011.
13. Kopp UC, Jones SY, DiBona GF. Afferent renal denervation impairs baroreflex control of efferent renal sympathetic nerve activity. Am J Physiol Regul Integr Comp Physiol 2008;295:R1882–90.
14. Phillips JK. Pathogenesis of hypertension in renal failure: role of the sympathetic nervous system and renal afferents. Clin Exp Pharmacol Physiol 2005;32:415–418.
15. Katholi RE, Whitlow PL, Hageman GR, Woods WT. Intrarenal adenosine produces hypertension by activating the sympathetic nervous system via the renal nerves in the dog. J Hypertens 1984;2:349–359.
16. Schlaich MP, Kaye DM, Lambert E, et al. Relation between cardiac sympathetic activity and hypertensive left ventricular hypertrophy. Circulation 2003;108:560–565.
17. Grassi G, Seravalle G, Quarti-Trevano F, et al. Sympathetic and baroreflex cardiovascular control in hypertension-related left ventricular dysfunction. Hypertens 2009;53:205–209.
18. Atherton DS, Deep NL, Mendelsohn FO. Micro-anatomy of the renal sympathetic nervous system: a human postmortem histologic study. Clin Anat 2012;25:628–33.
19. Steigerwald K, Titova A, Malle C, et al. Morphological assessment of renal arteries after radiofrequency catheter-based sympathetic denervation in a porcine model. J Hypertens 2012;30:2230–9.
20. Hering D, Lambert EA, Marusic P, et al. Substantial reduction in single sympathetic nerve firing after renal denervation in patients with resistant hypertension. Hypertens 2013;61:457–464.
21. Chinushi M, Izumi D, Iijima K, et al. Blood pressure and autonomic responses to electrical stimulation of the renal arterial nerves before and after ablation of the renal artery. Hypertens 2013;61:450–456.
22. 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;376:1903–1909.
23. 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;126:2976–2982.
24. Simonetti G, Spinelli A, Gandini R, et al. Endovascular radiofrequency renal denervation in treating refractory arterial hypertension: a preliminary experience. Radiol Med 2012;117:426–444.
25. Voskuil M, Verloop WL, Blankestijn PJ, et al. Percutaneous renal denervation for the treatment of resistant essential hypertension; the first Dutch experience. Neth Heart J 2011;19:319–323.
26. Zuern CS, Rizas KD, Eick C, et al. Effects of Renal Sympathetic Denervation on 24-hour Blood Pressure Variability. Front Physiol 2012;3:134.

27. Mortensen K, Franzen K, Himmel F, et al. Catheter-based renal sympathetic denervation improves central hemodynamics and arterial stiffness: a pilot study. J Clin Hypertens 2012;14:861–870.
28. Kaltenbach B, Franke J, Bertog SC, et al. Renal sympathetic denervation as second-line therapy in mild resistant hypertension: a pilot study. Catheter Cardiovasc Interv 2013;81:335–339.
29. Esler M, Jennings G, Biviano B, et al. Mechanism of elevated plasma noradrenaline in the course of essential hypertension. J Cardiovasc Pharmacol 1986;8 Suppl 5:S39–43.
30. Wright JW, Mizutani S, Harding JW. Pathways involved in the transition from hypertension to hypertrophy to heart failure. Treatment strategies. Heart Fail Rev 2008;13:367–375.
31. Communal C, Singh K, Pimentel DR, Colucci WS. Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the beta-adrenergic pathway. Circulation 1998;98:1329–1334.
32. Cacciapuoti F. Molecular mechanisms of left ventricular hypertrophy (LVH) in systemic hypertension (SH)-possible therapeutic perspectives. J Am Soc Hypertens 2011;5:449–55.
33. Burns J, Sivananthan MU, Ball SG, et al. Relationship between central sympathetic drive and magnetic resonance imaging-determined left ventricular mass in essential hypertension. Circulation 2007;115:1999–2005.
34. Patel MB, Stewart JM, Loud A V, et al. Altered function and structure of the heart in dogs with chronic elevation in plasma norepinephrine. Circulation 1991;84:2091–2100.
35. Patel MB, Loud A V, King BD, et al. Global myocardial hypertrophy in conscious dogs with chronic elevation of plasma norepinephrine levels. J Mol Cell Cardiol 1989;21 Suppl 5:49–61.
36. Jiang W, Tan L, Guo Y, et al. Effect of renal denervation procedure on left ventricular hypertrophy of hypertensive rats and its mechanisms. Acta Cir Bras 2012;27:815–820.
37. Perlini S, Ferrero I, Palladini G, et al. Survival benefits of different antiadrenergic interventions in pressure overload left ventricular hypertrophy/failure. Hypertens 2006;48:93–97.
38. Brandt MC, Reda S, Mahfoud F, et al. Effects of renal sympathetic denervation on arterial stiffness and central hemodynamics in patients with resistant hypertension. J Am Coll Cardiol 2012;60:1956–1965.
39. Edelmann F, Schmidt AG, Gelbrich G, et al. Rationale and design of the “aldosterone receptor blockade in diastolic heart failure” trial: a double-blind, randomized, placebo-controlled, parallel group study to determine the effects of spironolactone on exercise capacity and diastolic function in pa. Eur J Heart Fail 2010;12:874–882.
40. Bhatia RS, Tu J V, Lee DS, et al. Outcome of heart failure with preserved ejection fraction in a population-based study. N Engl J Med 2006;355:260–269.
41. Borlaug BA, Kass DA. Mechanisms of diastolic dysfunction in heart failure. Trends Cardiovasc Med 2006;16:273–279.
42. Sugiura M, Yamamoto K, Takeda Y, et al. The relationship between variables of 123-I-metaiodobenzylguanidine cardiac imaging and clinical status of the patients with diastolic heart failure. Int J Cardiol 2006;113:223–228.
43. Udelson JE, Feldman AM, Greenberg B, et al. Randomized, double-blind, multicenter, placebo-controlled study evaluating the effect of aldosterone antagonism with eplerenone on ventricular remodeling in patients with mild-to-moderate heart failure and left ventricular systolic dysfunction. Circ Heart Fail 2010;3:347–353.
44. Edelmann F, Wachter R, Schmidt AG, et al. Effect of spironolactone on diastolic function and exercise capacity in patients with heart failure with preserved ejection fraction: the Aldo-DHF randomized controlled trial. JAMA 2013;309:781–791.
45. Verloop WL, Beeftink MMA, Nap A, et al. Renal denervation in heart failure with normal left ventricular ejection fraction. rationale and design of the diastole (denervation of the renal sympathetic nerves in heart failure with normal lv ejection fraction) trial. Eur J Heart Fail 2013;15:1429–1437.
46. 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;26:1257–1263.
47. Sobotka PA, Krum H, Bohm M, et al. The role of renal denervation in the treatment of heart failure. Curr Cardiol Rep 2012;14:285–292.
48. Odero A, Bozzani A, De Ferrari GM, Schwartz PJ. Left cardiac sympathetic denervation for the prevention of life-threatening arrhythmias: the surgical supraclavicular approach to cervicothoracic sympathectomy. Heart Rhythm 2010;7:1161–1165.
49. Schneider HE, Steinmetz M, Krause U, et al. Left cardiac sympathetic denervation for

the management of life-threatening ventricular tachyarrhythmias in young patients with catecholaminergic polymorphic ventricular tachycardia and long QT syndrome. Clin Res Cardiol 2013;102:33–42.
50. Coleman MA, Bos JM, Johnson JN, et al. Videoscopic left cardiac sympathetic denervation for patients with recurrent ventricular fibrillation/malignant ventricular arrhythmia syndromes besides congenital long-QT syndrome. Circ Arrhythm Electrophysiol 2012;5:782–788.
51. Ukena C, Bauer A, Mahfoud F, et al. Renal sympathetic denervation for treatment of electrical storm: first-in-man experience. Clin Res Cardiol 2012;101:63–67.
52. Ukena C, Mahfoud F, Spies A, et al. Effects of renal sympathetic denervation on heart rate and atrioventricular conduction in patients with resistant hypertension. Int J Cardiol 2013;167:2846–2851.
53. 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. Hypertens 2013;61:225–231.
54. 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;60:1163–1170.
55. Ahmed H, Miller MA, Dukkipati SR, et al. Adjunctive renal sympathetic denervation to modify hypertension as upstream therapy in the treatment of atrial fibrillation (H-FIB) study: clinical background and study design. J Cardiovasc Electrophysiol 2013;24:503–509.
56. Palazzuoli A, Ronco C. Cardio-renal syndrome: an entity cardiologists and nephrologists should be dealing with collegially. Heart Fail Rev 2011;16:503–508.
57. Doi T, Nakata T, Hashimoto A, et al. Cardiac mortality assessment improved by evaluation of cardiac sympathetic nerve activity in combination with hemoglobin and kidney function in chronic heart failure patients. J Nucl Med 2012;53:731–740.
58. Matsushita K, van der Velde M, Astor BC, 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;375:2073–2081.
59. Tsioufis C, Papademetriou V, Dimitriadis K, et al. Catheter-based renal sympathetic denervation exerts acute and chronic effects on renal hemodynamics in swine. Int J Cardiol 2013;168:987–992.
60. Campese VM, Kogosov E, Koss M. Renal afferent denervation prevents the progression of renal disease in the renal ablation model of chronic renal failure in the rat. Am J Kidney Dis 1995;26:861–865.
61. Mancia G, Bousquet P, Elghozi JL, et al. The sympathetic nervous system and the metabolic syndrome. J Hypertens 2007;25:909–920.
62. Capra A, Galderisi M, Giannattasio C, et al. Early alterations in left ventricular diastolic function in normotensive diabetic patients. Blood Press 2012;21:110–115.
63. Straznicky NE, Grima MT, Sari CI, et al. Neuroadrenergic dysfunction along the diabetes continuum: a comparative study in obese metabolic syndrome subjects. Diabetes 2012;61:2506–2516.
64. Lambert E, Sari CI, Dawood T, et al. Sympathetic nervous system activity is associated with obesity-induced subclinical organ damage in young adults. Hypertens 2010;56:351–358.
65. 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;123:1940–1946.
66. Grassi G, Facchini A, Trevano FQ, et al. Obstructive sleep apnea-dependent and -independe F-Variations nt adrenergic activation in obesity. Hypertens 2005;46:321–325.
67. Kasai T, Bradley TD. Obstructive sleep apnea and heart failure: pathophysiologic and therapeutic implications. J Am Coll Cardiol 2011;57:119–127.

Part ONE -
Advanced diagnostic and therapeutic strategies in hypertension

Chapter 2 -
The safety of temporary discontinuation of antihypertensive medication in a population with difficult-to-control hypertension

Submitted

Martine M.A. Beeftink, Nicolette G.C. van der Sande, Michiel L. Bots, Pieter A. Doevendans, Peter J. Blankestijn, Frank L.J. Visseren, Michiel Voskuil, Wilko Spiering

Abstract

Objective
To investigate the safety of temporary discontinuation of antihypertensive medication for diagnostic work-up in patients with difficult-to-control hypertension.

Background
Successful control of blood pressure relies on identification of secondary causes and contributing factors of hypertension. As antihypertensive medication can interfere with diagnostic investigations, temporary discontinuation of medication is advised. However, there are concerns about the safety of temporary discontinuation of antihypertensive medication in patients with difficult-to-control hypertension.

Methods
We assessed the occurrence of adverse cardiovascular and cerebrovascular events potentially attributable to temporary discontinuation of antihypertensive medication between February 2010 and March 2016 (n=604) in our Analysis of Complicated Hypertension (ACH) screening program. A reference group (n=604) was extracted from the Second Manifestations of ARTerial disease (SMART) study cohort (comprising a similar cohort at our hospital in whom medication was not stopped) and individually matched for blood pressure, age, sex and history of cardiovascular disease.

Results
Discontinuation of medication was well tolerated; 62% reported no complaints, 24% had mild discomfort that could be left untreated and 14% experienced complaints that required prescription of antihypertensive escape medication. Three major adverse events were observed in the ACH group between discontinuation of medication and 30 days after restart of medication (event rate = 31.2 events per 1000 patient years). In the reference cohort, five cardiovascular events were observed during a similar follow-up period (event rate = 51.2 events per 1000 patient years).

Conclusion
Temporary discontinuation of antihypertensive medication for diagnostic purposes is safe and generally well tolerated by patients with difficult-to-control hypertension.

Introduction

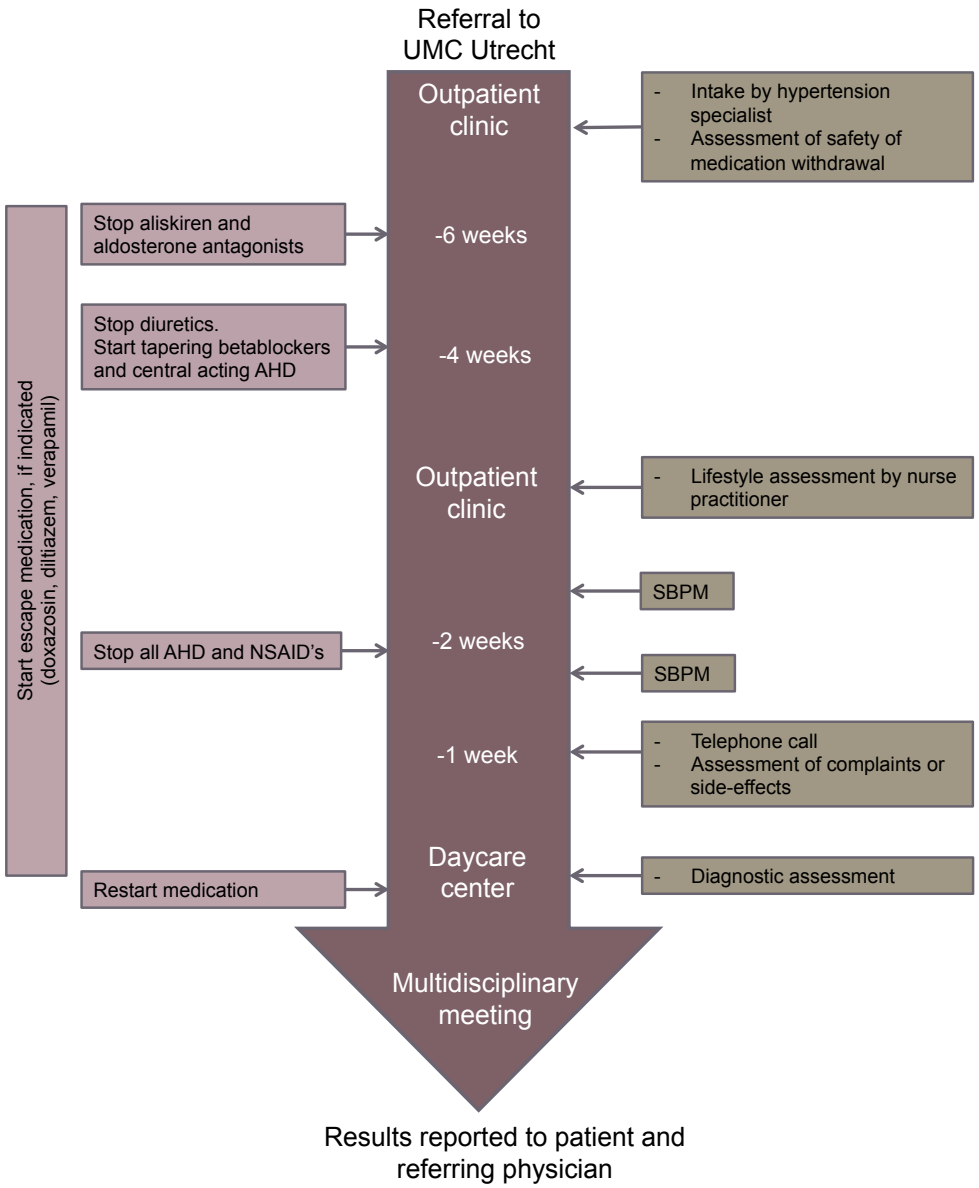
The increased risk of stroke, coronary heart disease and vascular mortality related to hypertension can be significantly reduced by long-term control of blood pressure (BP).[1,2] Yet, control rates of hypertension are still disappointingly low.[3] Patients with difficult-to-control hypertension form a special subgroup of patients with uncontrolled BP, as secondary causes are more prevalent in this population.[4] These patients may particularly benefit from an extensive diagnostic work-up for identification of potential secondary causes, non-adherence and contributing factors.
Concurrent use of antihypertensive drugs (AHD) impedes the establishment of secondary causes for hypertension, because they can interfere with the (biochemical) diagnostic tests. Particularly the renin-angiotensin-aldosterone-system (RAAS) axis is influenced by various antihypertensive drugs, including ACE-inhibitors, angiotensin receptor blockers, aldosterone antagonists, and betablockers.[5-8] For example, the prevalence of primary hyperaldosteronism ranges from 6% in an unselected hypertensive population to 11% in resistant hypertension, increasing up to 18% when interfering medication is stopped prior to the investigations.[9-12] It is therefore advised to temporarily discontinue antihypertensive medication prior to the diagnostic investigations.[13] Yet, the scientific evidence that temporary discontinuation of AHD is safe in this high risk population is still lacking. In the majority of patients, discontinuation of AHD will result in an asymptomatic return of BP to pre-treatment levels only. A small minority may experience a syndrome known as 'withdrawal syndrome' or 'discontinuation syndrome'. This syndrome is characterized by rapid return or overshoot of blood pressure with signs and symptoms of sympathetic overactivity, and is associated with an increased acute risk of cardiovascular and cerebrovascular events.[14] The extent of this risk during temporary discontinuation of AHD in a high risk population has not yet been well researched.
Therefore, we aimed to evaluate the safety of temporary discontinuation of antihypertensive medication as part of a highly standardized diagnostic protocol in patients with difficult-to-control hypertension.

Methods

This study analyzed the electronic record data of patients undergoing the 'Analysis of Complicated Hypertension' (ACH) at the University Medical Center Utrecht in Utrecht, the Netherlands. A reference group was extracted from the SMART (Second Manifestations of ARTerial disease) cohort.[15,16] All data were de-identified for research purposes, according to the Dutch Medical Research involving Human Subjects Act (WMO) and the Dutch Personal Data Protection Act (WBP).
The study was approved by the medical ethics committee of the University Medical Center Utrecht and executed in accordance with the Good Clinical Practice guidelines. The need for written informed consent was waived by the Medical Ethics Committee.

Patient population
The patient population consisted of all patients who were referred for difficult-to-control hypertension and who entered the ACH program between February 2010 and March 2016. The ACH is a highly standardized diagnostic program designed to identify secondary causes of

Figure 1. Overview of the diagnostic work-up for difficult-to-control hypertension.



Overview of the diagnostic work-up with temporary medication withdrawal for patients with difficult-to-control hypertension. The arrow represents the timeline of the diagnostic program. The schedule for the withdrawal of antihypertensive medication is depicted on the left hand side of the arrow, the appointments and assessments during the program are depicted on the right hand side.

hypertension, identify contributing factors of hypertension and assess overall cardiovascular risk profile in patients with difficult-to-control hypertension (i.e. persistent hypertension despite optimal treatment according to the current guidelines, and/or presence of end-organ damage or vascular complications). A schematic overview is provided in figure 1. Patients who were not taking any antihypertensive drugs at the time of referral as well as patients in whom all medication was continued during the screening program were excluded from analysis.

Discontinuation of antihypertensive medication

At the outpatient clinic, a hypertension specialist assessed whether it was deemed safe to discontinue medication. Cardiovascular events (i.e. myocardial infarction, stroke or transient ischemic attack; TIA) within 6 months before the ACH program were an absolute contra-indication for discontinuation of AHD. Relative contraindications included other cardiovascular comorbidities (e.g. stable coronary artery disease) or severe hypertension (defined as a treated blood pressure above 180/110 mmHg or history of hypertensive urgency requiring hospital admission). Antihypertensive medication was stopped in a protocolled staged fashion (figure 1). Betablockers and central acting AHD were tapered down in two weeks to avoid medication withdrawal syndrome.

During the screening period, protocolled dosages of diltiazem, doxazosin or verapamil were available as escape medication, as they do not interfere with the biochemical evaluation of the aldosterone-renin-ratio. Escape medication could be prescribed at the start of the screening (preventive, for example in case of a relative contra-indication) or at any time during the screening if deemed necessary by the treating hypertension specialist or (if unavailable) the specialist on call. Indications to start escape medication during the screening included a dangerous increase in BP occurred (generally >180/110 mmHg) or if the patient experienced severe anxiety or complaints.

Safety monitoring during medication withdrawal

All patients received a personalized withdrawal schedule and were instructed to contact the hospital 24/7 if BP increased above 180 mmHg systolic or 110 mmHg diastolic on home blood pressure monitoring, if alarm symptoms occurred, or if the patient felt insecure or had any questions. Blood pressure was monitored during the medication withdrawal by home blood pressure measurements (HBPM) that were uploaded to a secure internet site and visible to the appropriate staff at the hospital. One week after the last antihypertensive drug was stopped, patients received a scripted telephone call by the nurse practitioner to identify possible side effects and potential complications. During the telephone call, the HBPM levels were evaluated and patients were specifically asked for complaints of headache, dizziness, visual complaints and chest pain.

Reference group

We used the SMART (Second Manifestations of ARTerial disease) cohort to construct a reference group to compare the occurrence of adverse events to a similar population that did not discontinue medication. The SMART study is an ongoing single-center prospective cohort study of patients newly referred to our hospital with clinically manifest atherosclerotic vessel disease or marked risk factors for atherosclerosis.[15,17] Patients who were included in both the ACH and SMART cohort, were filtered from the SMART cohort before each ACH

patient was matched 1-to-1 for SBP and 10-year risk of cardiovascular events to a SMART subject, with maximally tolerated differences of 15 mmHg or 3%, respectively.

Endpoints

The primary endpoint was defined as the occurrence of major adverse cardio- and cerebrovascular events (MACE) between the start of medication withdrawal until 30 days after restart of medication. MACE was defined as (1) cardiovascular death, (2) non-fatal acute coronary syndrome (ACS), (3) acute heart failure or (4) non-fatal stroke (including subarachnoid hemorrhage and TIA). Since the reference group had no medication withdrawn, a surrogate time window of 59 days (the mean follow-up in the ACH group) after inclusion was used. The endpoints defined in SMART were similar to the endpoints defined for the ACH, but did not include TIA.

As secondary outcome for the ACH group, we assessed the occurrence of blood pressure-related complaints and visits to the emergency department during medication withdrawal.

Data analysis

Results are presented as mean ± standard deviation, median (interquartile rage, IQR) or as an absolute number with percentages unless otherwise specified. Dosages of AHD were converted into defined daily doses (DDD) using conversion factors provided by the World Health Organization (WHO) Collaborating Centre for Drug Statistics Methodology.[18] The 10-year risk for cardiovascular disease was calculated for each individual using the Framingham score[19] for individuals without prior cardiovascular disease and the SMART risk score[16] for patients with a history of cardiovascular events.

Between-group differences for continuous variables were analyzed using the independent samples T-test or Mann-Whitney U-test, as appropriate. For categorical variables the Chi-square test was used. Life tables were used to analyze event-free survival and expressed as event rate per 1000 patient years. Results were considered statistically significant if the 95% confidence interval (CI) did not include 0 or if the two-tailed probability value (p-value) did not exceed 0.05. All analyses were performed with SPSS statistical software version 22 (IBM SPSS Data Collection, Chicago, Illinois, USA).

Results

Between 2010 and 2016, 692 patients entered the ACH program. Eighty-eight patients were excluded from analysis; 72 patients were not taking any AHD at the time of referral, two patients continued all medication during the ACH, for eleven patients the program was put on hold prior to medication withdrawal, and three patients decided against the screening program. The remaining 604 patients were included in the current analysis and could be individually matched to a reference subject. The patient characteristics of both groups are depicted in table 1.

In the ACH group, 457 patients (76%) completely discontinued all medication throughout the screening program, while 147 patients (24%) had escape medication prescribed. Escape medication was prescribed as an intervention for (complaints of) high BP in 82 patients (see also below), as prevention for relative contra-indications in 64 patients, and one patient accidentally continued an antihypertensive drug during the screening.

Table 1. Patient characteristics.

	Study cohort (n=604)	Reference cohort (n=604)
Age, years	54 ± 13	50 ± 15
Sex, male	292 (48%)	290 (48%)
Office SBP (treated), mmHg	172 ± 26	165 ± 27
Office DBP (treated), mmHg	98 ± 14	94 ± 16
Office SBP (untreated), mmHg	167 ± 23	N/A
Office DBP (untreated), mmHg	98 ± 14	N/A
24-hr SBP (untreated), mmHg	153 ± 19	N/A
24-hr DBP (untreated), mmHg	93 ± 12	N/A
CVD		
Cardiac	68 (11%)	30 (5%)
Cerebral	59 (10%)	30 (5%)
AAA	3 (<1%)	7 (1%)
PAD	17 (3%)	19 (3%)
BMI, kg/m^2	28.2 ± 4.9	27.1 ± 5.3
Total cholesterol, mmol/L	5.3 ± 1.1	5.6 ± 1.6
HDL-c, mmol/L	1.3 ± 0.4	1.3 ± 0.4
LDL-c, mmol/L	3.2 ± 1.0	3.5 ± 1.3
Triglycerides	1.6 ± 1.1	2.1 ± 4.1
Diabetes Mellitus	71 (12%)	168 (27.8%)
eGFR, mL/min/1.73m^2	82 ± 25	82 ± 22
Smoking		
current	70 (12%)	175 (29%)
stopped	263 (44%)	161 (28%)
never	261 (44%)	268 (44%)
pack-years	17 ± 17	11 ± 17
10-yrs CV risk, %	9.3 ± 8.3	9,8 ± 10.9

Patient characteristics of the study population (medication withdrawal) and reference cohort. Reference cohort was matched for SBP, age, sex and history of cardiovascular disease. eGFR was calculated using the CKD-EPI formula. Untreated BP represents office BP after discontinuation of AHD. Abbreviations: AAA = abdominal aorta aneurysm, BMI = body mass index, CVD = cardiovascular disease, DBP = diastolic blood pressure, eGFR = estimated glomerular filtration rate, HDL-c = high-density lipoprotein, LDL-c = low density lipoprotein, PAD = peripheral artery disease, SBP = systolic blood pressure.

Major adverse cardio- and cerebrovascular events

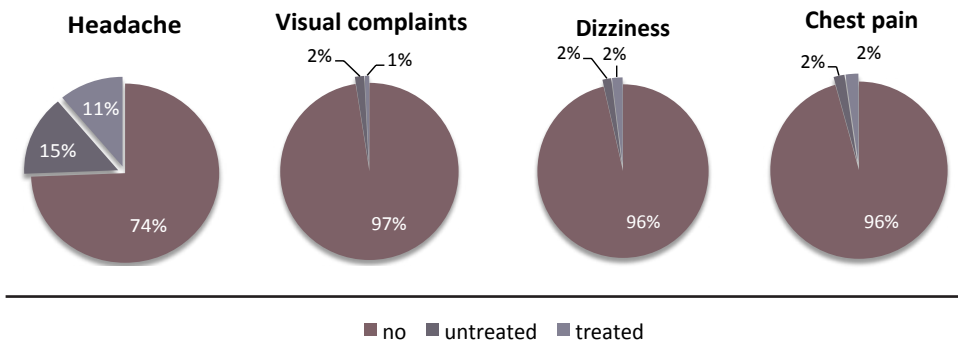
In the ACH group, three major adverse events occurred during a mean follow-up of 59 days (ranging from 42 to 72 days), corresponding to an event rate of 31.2 events per 1000 patient years (95% CI 6.4 to 91.2).

In short, a 68-year old woman with a history of cardiac complaints experienced a mild

non-ST elevation myocardial infarction (NSTEMI) three days after complete withdrawal of medication. She was treated with staged percutaneous coronary intervention for three-vessel disease. A 66-year old man with a history of atrial fibrillation and a NSTEMI was diagnosed with TIA's in the posterior circulation two days after restart of medication. Lastly, a 63-year old man with a history of peripheral and coronary arterial disease was diagnosed with a sick sinus syndrome and transient unstable angina pectoris during atrial fibrillation 28 days after restart of medication, for which he received a scheduled PCI. All three patients had above-average baseline office blood pressure (respectively 180/96 mmHg, 198/108 mmHg and 190/100 mmHg, versus 172/98 mmHg).

In the reference group, three major adverse events occurred during follow-up (59 days for all patients), corresponding to an event rate of 30.7 events per 1000 patient years (95% CI 7.8 to 84.2). A 70-year old man experienced an ischaemic stroke, a 53-year old woman experienced a cardiac event requiring coronary bypass surgery and a 51-year old man required percutaneous coronary intervention for a cardiac event.

Figure 2. Complaints during medication withdrawal and need for treatment.



Tolerability of medication withdrawal in ACH

Figure 2 depicts the occurrence of the most common complaints and the need for treatment during the ACH program. The majority of patients completed the ACH program and medication withdrawal without any side effects (n=373, 62%) or with mild discomfort (e.g. slight headache or tiredness) that did not require escape medication (n-149, 24%). Eighty-two patients (14%) reported complaints that led to the prescription of escape medication. Twenty-six ACH patients (4%) visited the emergency department during or after medication withdrawal. In 23 cases of them, no adverse event could be demonstrated and the ACH could continue without any further obstacles. Two patients experienced complaints that may be attributable to the discontinuation of medication for the ACH program. One patient was admitted for severe hypertension (199/133 mmHg) while not taking the prescribed escape medication (diltiazem). Another patient developed atrial fibrillation during an episode of fever due to laryngitis while he was tapering his betablocker, for which the ACH was postponed. The third patient experienced dizziness possibly related to a hypokalemia of 2.7 mmol/L (reference value 3.8-5.0) and dislocated her shoulder in a fall (not attributable

to discontinuation of the medication). The ACH analysis later demonstrated primary hyperaldosteronism based on an adrenal adenoma causing the hypokalemia.

Discussion

We reported our six years experience with temporary discontinuation of antihypertensive drugs in the context of the diagnostic work-up of patients with difficult-to-control hypertension. When performed in a highly structured program with careful patient selection and protocol for monitoring patient safety, short-term withdrawal of antihypertensive medication does not seem to increase the acute risk of major vascular events. In addition, temporary discontinuation of medication was well-tolerated by the vast majority of patients, as 86% of patients experienced no complaints or only experienced mild discomfort that required no intervention.

Our findings may have important clinical implications for the diagnostic evaluation of difficult-to-control hypertension. As mentioned in the introduction, temporary withdrawal of antihypertensive medication can improve the accuracy of diagnostic tests for secondary hypertension. However, temporary discontinuation of antihypertensive medication is not without controversy in this population with difficult-to-control hypertension and subsequent high cardiovascular risk. Physicians may be reluctant to interrupt treatment in this population, fearing major cardiovascular complications with potential irreversible damage. Our results indicate that temporary withdrawal of medication does not increase the acute risk of major adverse cardio- and cerebrovascular events when performed in a well-controlled setting. We demonstrated an equal number of events in the study group, compared to the

Table 2. Event rates in different arms of hypertension trials.

Study	Age	Baseline BP	ΔBP	Events /1000 PY	Intervention
SHEP[23] (1991)	72 ± 7	170/77	-27/-9	18.7	Chloortalidon ± atenolol
	72 ± 7	170/77	-15/-5	27.1	Placebo
SYST-EUR[24] (1997)	70 ± 7	174/86	-23/-7	23.3	Nitrendipine
		174/91	-13/-2	33.9	Placebo
LIFE[25] (2002)	70 ± 7	174/98	-30/-17	23.8	Losartan
		175/98	-29/-17	27.9	Atenolol
VALUE[26] (2004)	67 ± 8	154/87	-15/-8	25.5	Valsartan
		155/88	-17/-10	24.7	Amlodipine
FEVER[27] (2005)	52 ± 7	154/91	-17/-8	26.4	Felodipine
		154/91	-11/-6	37.6	Placebo
HYVET[28] (2008)	84 ± 3	173/91	-15/-19	33.7	Indapamide ± Perindopril
		173/91	-7/-11	50.6	Placebo
Present study	54 ± 13	172/98	-6/0	31.2	Cessation of AHD

Table depicts main study characteristics for the present study and recent large hypertension trials that provided event rates for major adverse cardiovascular events. Age was measured in years, blood pressure was measured in mmHg. Abbreviations: AHD = antihypertensive drugs, BP = blood pressure, PY = patient years.

reference group with a similar cardiovascular risk that did not discontinue medication. The event rate in our cohort was lower compared to event rates reported in large randomized-controlled pharmacological hypertension trials, and similar to the study arms receiving treatment (table 2).[23-28] However, there are two major differences between these trials and our study that need to be taken into account. First, we included TIA's and unstable angina pectoris in our definition of MACE to have a low threshold for adverse events. These events are generally not included in the endpoint definition of the abovementioned hypertension trials, resulting in a relative overestimation of events in our study. Secondly, in our study medication was discontinued for a limited amount of time only, resulting in wide confidence intervals compared to hypertension trials where patients are assigned to placebo for several months to years.

Studies investigating the short-term effect of antihypertensive medication compared to placebo are scarce. We know of two meta-analyses who investigated short-term placebo-controlled trials, one of which assessed (mostly unpublished) data submitted to the Food and Drug Administration. Both demonstrated no differences in event rates between intervention and placebo.[20,21] When defining adverse events as a composite endpoint quite similar to ours (death, non-fatal stroke, TIA, non-fatal congestive heart failure, non-fatal myocardial infarction and angina pectoris), the event rate in the meta-analysis of DeFelice et al. was 29.2 per 1000 patient years for placebo arms compared to 31.2 per 1000 patient years in our study.[21] These findings further support our conclusion that short-term interruption of treatment does not seem to affect the acute risk of major adverse events and irreversible harm.

Our results may not only benefit the routine clinical care of hypertension, but may also be of specific interest to device-based intervention trials. The research involving renal denervation treatment for hypertension has uncovered some important shortcomings and confounders in various trials, most importantly the influence of AHD on blood pressure outcomes. To overcome these issues, several trials have been announced to involve washout of AHD and medication-free endpoint measurement (such as the Spyral HTN OFF-MED[22], the Vessix REINFORCE (NCT02392351) and the Paradise RADIANCE-HTN (NCT02649426) trials). Our results may guide researchers in the design of their protocol and diminish ethical objections when temporary discontinuation is required for study entry or endpoint measurement. Yet, our results may not apply to prolonged AHD cessation throughout several months of follow-up or to patients with controlled hypertension if BP increases in response to AHD discontinuation.

When interpreting our results, one should be aware of the limitations to our reference group. The ACH program has been common clinical practice in our hospital since 2010. All patients referred for the analysis of difficult-to-control hypertension are subjected to the same program, including the withdrawal of antihypertensive medication. Therefore, no control group could be included that underwent the ACH program with continuation of medication. We tried to overcome this by creating a reference group from the SMART study, which population closely resembles the ACH population. Furthermore, patients were individually matched for blood pressure, age, sex and cardiovascular history to optimize comparability of both populations. Although this approach is suboptimal compared to a true control group (preferably with a randomized design), the reference group provided the best available opportunity to place our results into perspective.

Perspectives

Our results indicate that the temporary (up to six weeks) discontinuation of antihypertensive medication for the diagnostic evaluation of hypertension does not increase the acute risk of cardiovascular events, provided it is performed in a well-controlled setting in specialized hospitals with appropriate protocols for monitoring safety. Our institutionally devised protocol with extensive safety monitoring does not reflect the conditions of routine medical care and similar protocols should only be applied in tertiary health care facilities with expert knowledge on the management of patients with difficult-to-control hypertension. Special attention should be given to careful patient selection, as patients who carry increased risk (e.g. those with high baseline blood pressure levels and/or a history of cardiovascular disease) may require continuation of medication or preventive escape medication. When performed with proper regard for patient selection and safety, diagnostic screening programs including a strategy for safe discontinuation of medication will likely increase the diagnosis of secondary causes of hypertension and reduce bias in hypertension research.

References

1. Collins R, Peto R, MacMahon S, Godwin J, Qizilbash N, Collins R, et al. Blood pressure, stroke, and coronary heart disease. Part 2, short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. Lancet 1990; 335:827–838.
2. Psaty BM, Lumley T, Furberg CD, Schellenbaum G, Pahor M, Alderman MH, et al. Health outcomes associated with various antihypertensive therapies used as first-line agents: a network meta-analysis. JAMA 2003; 289:2534–2544.
3. Chow CK, Teo KK, Rangarajan S, Islam S, Gupta R, Avezum A, et al. Prevalence, awareness, treatment, and control of hypertension in rural and urban communities in high-, middle-, and low-income countries. JAMA 2013; 310:959–68.
4. Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, et al. Resistant Hypertension: Diagnosis, Evaluation, and Treatment. Hypertension 2008; 51:1403–1419.
5. Bauer JH, Sunderrajan S, Reams G. Effects of calcium entry blockers on renin-angiotensin-aldosterone system, renal function and hemodynamics, salt and water excretion and body fluid composition. Am J Cardiol 1985; 56.
6. Colantonio D, Casale R, Desiati P, Giandomenico G, Bucci V, Pasqualetti P. Short-term effects of atenolol and nifedipine on atrial natriuretic peptide, plasma renin activity, and plasma aldosterone in patients with essential hypertension. J Clin Pharmacol 1991; 31:238–242.
7. Bauer IH, Reams GP, Wu Z, Lau-Sieckman A. Effects of losartan on the renin-angiotensin-aldosterone axis in essential hypertension. J Hum Hypertens 1995; 9:237–243.
8. Stowasser M, Ahmed AH, Pimenta E, Taylor PJ, Gordon RD. Factors affecting the aldosterone/renin ratio. Horm. Metab. Res. 2012; 44:170–176.
9. Fogari R, Preti P, Zoppi A, Rinaldi A, Fogari E, Mugellini A. Prevalence of primary aldosteronism among unselected hypertensive patients: a prospective study based on the use of an aldosterone/renin ratio above 25 as a screening test. Hypertens Res 2007; 30:111–7.
10. Douma S, Petidis K, Doumas M, Papaefthimiou P, Triantafyllou A, Kartali N, et al. Prevalence of primary hyperaldosteronism in resistant hypertension: a retrospective observational study. Lancet 2008; 371:1921–1926.
11. Verloop WL, Vink EE, Voskuil M, Vonken E-J, Rookmaaker MB, Bots ML, et al. Eligibility for percutaneous renal denervation: the importance of a systematic screening. J Hypertens 2013; 31:1662–1668.
12. Stowasser M, Gordon RD, Gunasekera TG, Cowley DC, Ward G, Archibald C, et al. High rate of detection of primary aldosteronism, including surgically treatable forms, after “non-selective” screening of hypertensive patients. J Hypertens 2003; 21:2149–57.
13. Stowasser M, Taylor PJ, Pimenta E, Ahmed AHA-A, Gordon RD. Laboratory investigation of primary aldosteronism. Clin Biochem Rev 2010; 31:39–56.
14. Houston MC. Abrupt cessation of treatment in hypertension: Consideration of clinical features, mechanisms, prevention and management of the discontinuation syndrome. Am Heart J 1981; 102:415–430.
15. Simons PCG, Algra A, Van De Laak MF, Grobbee DE, Van Der Graaf Y. Second manifestations of ARTerial disease (SMART) study: Rationale and design. Eur J Epidemiol 1999; 15:773–781.
16. Dorresteijn J a, Visseren FL, Wassink a M, Gondrie MJ, Steyerberg EW, Ridker PM, et al. Development and validation of a prediction rule for recurrent vascular events based on a cohort study of patients with arterial disease: the SMART risk score. Heart 2013; 99:866–872.
17. Kaasenbrood L, Boekholdt SM, van der Graaf Y, Ray KK, Peters RJG, Kastelein JJP, et al. Distribution of Estimated 10-Year Risk of Recurrent Vascular Events and Residual Risk in a Secondary Prevention Population. Circulation 2016.; 134:1419-1429.
18. WHO Collaborating Centre for Drug Statistics Methodology. Guidelines for ATC classification and DDD assignment 2015. 18th ed. ; 2014. http://www.whocc.no/ddd/definition_and_general_considera/
19. D’Agostino RB, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General Cardiovascular Risk Profile for Use in Primary Care: The Framingham Heart Study. Circulation 2008; 117:743–753.
20. Al-Khatib SM, Califf RM, Hasselblad V, Alexander JH, McCrory DC, Sugarman J. Medicine. Placebo-controls in short-term clinical trials of hypertension. Science 2001; 292:2013–5.
21. DeFelice a, Willard J, Lawrence J, Hung J, Gordon M a, Karkowsky a, et al. The risks associated with short-term placebo-controlled antihypertensive clinical trials: a descriptive meta-analysis. J Hum Hypertens 2008; 22:659–668.
22. Kandzari DE, Kario K, Mahfoud F, Cohen SA, Pilcher G, Pocock S, 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. Am Heart J 2016; 171:82–91.
23. SHEP Cooperative Research Group. Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension. Final results of the Systolic Hypertension in the Elderly Program (SHEP). JAMA 1991; 265:3255–3264.
24. Staessen JA, Fagard R, Thijs L, Celis H, Arabidze GG, Birkenhäger WH, et al. Randomised double-blind comparison of placebo and active treatment for older patients with isolated systolic hypertension. The Systolic Hypertension in Europe (Syst-Eur) Trial Investigators. Lancet 1997; 350:757–64.
25. Dahlof B, Devereux BR, Kjeldsen SE. Cardiovascular morbidity and mortality in the losartan intervention for end point reduction in hypertension study (LIFE): a randomised trial against atenolol. ACC Curr J Rev 2002; 11:26.
26. Julius S, Kjeldsen SE, Weber M, Brunner HR, Ekman S, Hansson L, et al. Outcomes in hypertensive patients at high cardiovascular risk treated with regimens based on valsartan or amlodipine: The VALUE randomised trial. Lancet 2004; 363:2022–2031.
27. Liu L, Zhang Y, Liu G, Li W, Zhang X, Zanchetti A. The Felodipine Event Reduction (FEVER) Study: a randomized long-term placebo-controlled trial in Chinese hypertensive patients. J Hypertens 2005; 23:2157–72.
28. Peters R, Beckett N, Forette F, Tuomilehto J, Clarke R, Ritchie C, et al. Incident dementia and blood pressure lowering in the Hypertension in the Very Elderly Trial cognitive function assessment (HYVET-COG): a double-blind, placebo controlled trial. Lancet Neurol 2008; 7:683–689.

Chapter 3 –
Cessation of antihypertensive medication results in a highly variable blood pressure response between subjects with difficult-to-control hypertension

Submitted

Martine M.A. Beeftink, Margreet F. Sanders, Michiel L. Bots, Jaap A. Joles, Peter J. Blankestijn, Michiel Voskuil, Wilko Spiering

Abstract

Background
Because antihypertensive drugs interfere with the diagnostic work-up for secondary hypertension, temporary withdrawal is recommended when an underlying endocrine disorder is suspected. We aimed to investigate the effects of temporary withdrawal of antihypertensive medication on blood pressure levels.

Methods
The electronic record data of patients undergoing the Analysis of Complicated Hypertension (ACH) at the University Medical Center Utrecht were analyzed. Ambulatory blood pressure measurements were evaluated before and after withdrawal of antihypertensive medication. Change in mean 24-hour systolic blood pressure was stratified in ascenders (>5 mmHg BP increase), descenders (>5 mmHg BP decrease) and equals (≤5 mmHg BP change).

Results
On average, blood pressure increased by 9/6 mmHg (SD 16/9) after cessation of 4 ± 2 standard doses of antihypertensive medication (n=153). Ninety-two patients (60%) experienced an increase in BP >5 mmHg after withdrawing a mean of 4.5 ± 2.1 daily defined doses (DDD) of medication. Thirty-five patients (23%) had a stable blood pressure after cessation of medication (3.6 ± 1.7 DDD), while in twenty-six patients (17%) blood pressure decreased >5 mmHg despite withdrawal of 3.3 ± 2.3 DDD. Lower baseline blood pressure was observed in ascenders, but no significant differences were found in renal function and important lifestyle factors such as urinary sodium excretion, body mass index and physical inactivity.

Conclusions
Ambulatory blood pressure does not increase after withdrawal of prescribed antihypertensive medication in 40% of patients with difficult-to-control hypertension. These findings emphasize the difficulty in the evaluation of blood pressure endpoints, particularly in interventional hypertension research.

Introduction

Hypertension is a common clinical problem that poses a therapeutic problem for physicians worldwide: blood pressure control among treated patients is poor, varying from 10 to 32,5%.[1-3] Approximately 5 to 30% of hypertensive patients is believed to suffer from apparent resistant hypertension, which is defined as blood pressure (BP) above treatment goals despite a specific pharmacological therapy.[4] Most likely, this number is overestimated as patients may be falsely diagnosed with apparent resistant hypertension due to factors such as unrecognized secondary causes, white coat hypertension and non-adherence to antihypertensive treatment.
The American Heart Association (AHA) and the European Society of Hypertension (ESH) recommend a thorough work-up for the evaluation of resistant hypertension.[4,5] This work-up includes exclusion of pseudo-resistance, identification of contributing lifestyle factors, discontinuation of interfering substances, screening for secondary causes and optimizing pharmacologic treatment. Because antihypertensive drugs (AHD) interfere with the diagnostic work-up for secondary causes (particularly the renin-aldosterone-system axis), temporary withdrawal of AHD is advisable during these investigations.[6,7]
Implementing the evaluation of secondary causes and the exclusion of white-coat hypertension in one diagnostic program is logistically convenient and allows the evaluation of untreated BP. It may also be of value for interventional trials, as off-medication BP measurements are unhindered by non-adherence which is a known confounding factor in hypertension research. Yet, the effects of AHD withdrawal on ABPM levels have not been well researched.
Therefore, we analyzed repeated ABPMs performed before and after AHD withdrawal during the routine screening program for difficult-to-control hypertension at our facility. The obvious hypothesis was that withdrawal of (high doses of) antihypertensive medication would result in a sizable increase in blood pressure.

Methods

We analyzed electronic patient record data of an ongoing single-center prospective cohort of patients undergoing the Analysis of Complicated Hypertension (ACH) at the University Medical Center Utrecht, Utrecht, the Netherlands. The ACH is a highly standardized diagnostic program for all patients referred for difficult-to-control hypertension, defined as persistent hypertension despite optimal treatment according to the current guidelines, and/or presence of end-organ damage or vascular complications of hypertension. The program includes temporary withdrawal of AHD to avoid interference with the diagnostic investigations. Data were de-identified for research purposes, according to the Dutch Medical Research involving Human Subjects Act and the Dutch Personal Data Protection Act. The study was approved by the Medical Ethics Committee of the University Medical Center Utrecht and executed in accordance with the Good Clinical Practice guidelines. The necessity to obtain written informed consent was waived by the Ethics Committee.

Study population
All patients in whom two ABPMs were performed during the ACH screening program between February 2010 and May 2016 were included in the current analysis. Patients were

only included if the first ABPM was performed while patients were taking their routine antihypertensive medication and the second ABPM was performed after withdrawal of their medication (figure 1). Patients in whom less than 1 standard dose of AHD (defined daily dose, see below) was withdrawn were excluded from analysis.

Blood pressure measurements

The outline of the screening program is depicted in figure 1. All ambulatory blood pressure measurements were performed using the WatchBP® 03 ambulatory BP monitor (Microlife AG, Widnau, Switzerland) and in accordance to the European Society of Hypertension (ESH) recommendations.[8] Readings were taken at least every 30 minutes during the day and every 60 minutes during the night. True night times were based on diary entries and mean values for systolic (SBP) and diastolic (DBP) blood pressure were calculated. ABPMs with less than 70% successful measurements were considered unreliable and excluded from analysis.

Withdrawal of antihypertensive medication

Antihypertensive medication was withdrawn in a stepwise fashion depending on the duration of action (figure 1) and treatment was restarted immediately after the investigations at the daycare center. If complete withdrawal of AHD was deemed unsafe because of cardiovascular history or high baseline blood pressure, if BP increased above 180/110 mmHg of if the patient experienced severe complaints or anxiety, so called “escape medication” could be prescribed that presumably does not influence the diagnostic investigations (diltiazem, doxazosin or verapamil).

Other study measurements

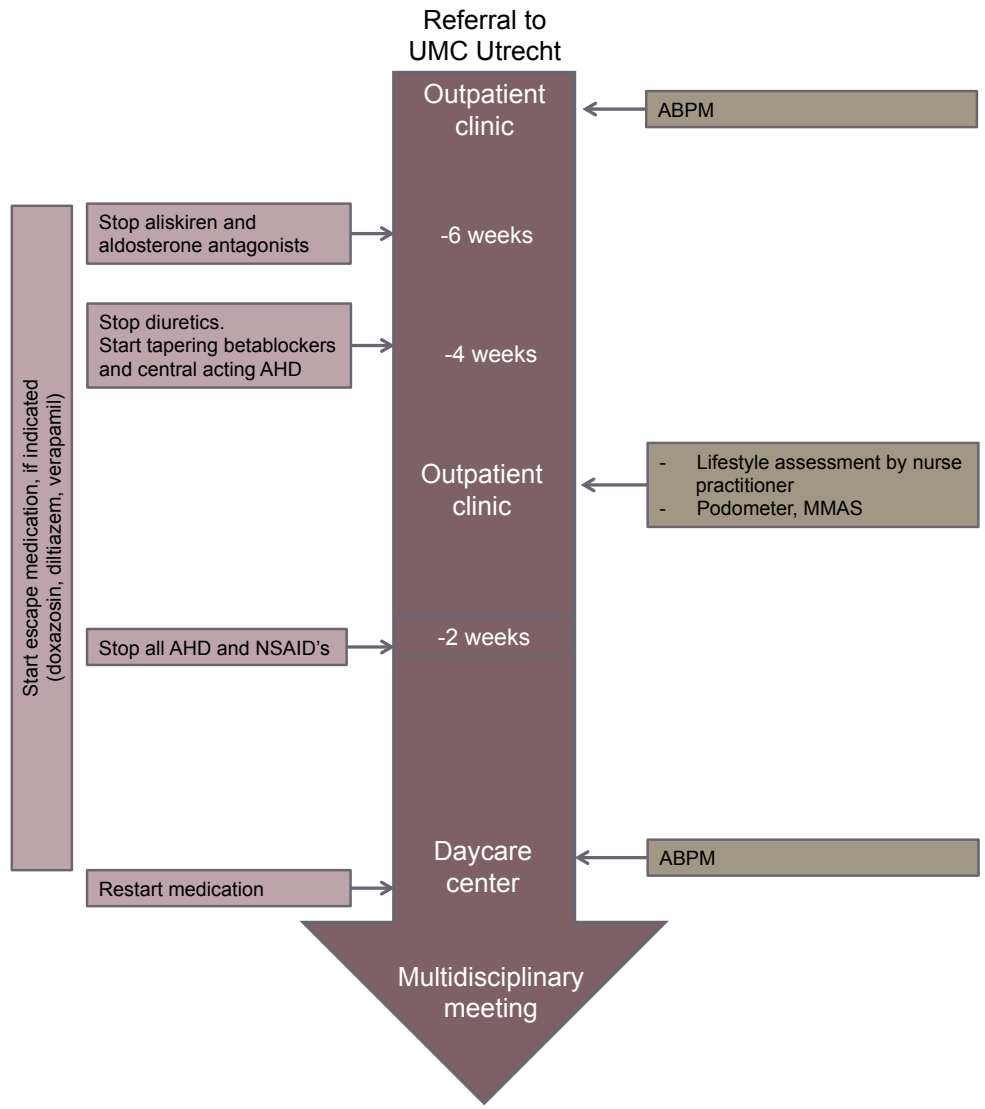
Previous medical history was verified at the first visit to the outpatient clinic. Adherence to AHD was assessed by means of the Morisky Medication Adherence Scale (MMAS). Non-adherence was suspected if patients scored <6 on the MMAS. Physical inactivity was estimated according to the Dutch healthy exercise norm (NNGB): at least 30 minutes of moderately intensive exercise on at least five days a week (summer and winter). The day before the visit to the daycare center, patients were instructed to collect 24-hour urine while maintaining a stable diet for measurement of 24-hour urinary sodium excretion.

Data analysis

Dosages of AHD were converted into defined daily doses (DDD) using conversion factors provided by the World Health Organization (WHO) Collaborating Centre for Drug Statistics Methodology.[9] The change in 24-hour BP was calculated by subtracting the mean ABPM with medication from the ABPM without medication. Thus, a negative number represents a decrease in BP.

The change in SBP was stratified in predefined categories: ascenders (>5 mmHg BP increase), descenders (>5 mmHg BP decrease) and equals (≤5 mmHg BP change). The cut-off of 5 mmHg was chosen based on a review of available literature: among articles studying the reproducibility of ABPMs, the largest change in systolic blood pressure was 5 mmHg with a mean of -0.9 mmHg (supplementary table S1). In addition, this cut-off is below the mean change in 24-hour SBP in the placebo arm of a large antihypertensive efficacy trial.[10]

Figure 1. Overview of the diagnostic work-up for difficult-to-control hypertension.



Overview of the hypertension screening program including the medication withdrawal. The arrow represents a time-line. The boxes on the right hand side depict the timing of both ABPMs and other investigations. The boxes on the left hand side depict the schedule for the withdrawal of antihypertensive medication. Medication was withdrawn in a step-wise fashion depending on a drug’s action duration. Escape medication could be prescribed during any time of the program, if indicated. Medication was restarted immediately after the medication-free ABPM. Abbreviations: ABPM = ambulatory blood pressure monitoring, AHD = antihypertensive drugs, BP = blood pressure, HBPM = home blood pressure monitoring, MMAS = Morisky medication adherence scale, NSAID = non-steroid anti-inflammatory drug, HBPM = home blood pressure monitoring.

Statistical analysis

Baseline characteristics are presented as mean ± standard deviation, median (range) or number (percentage), unless otherwise specified. Between-group differences (>2 groups) in continuous variables were assessed using the one-way ANOVA analysis with Hochberg GT2 post-hoc analysis for unequal sample sizes, when indicated. If the assumptions for ANOVA were not met and could not be resolved with appropriate transformation of the data, the Kruskal-Wallis test was performed as non-parametric alternative. Differences in continuous variables between two independent groups were assessed by the Student's t-test or the Mann Whitney-U test, when appropriate. Categorical variables were analyzed using the Chi-square test or Fisher's exact test, when appropriate. Results were considered statistically significant if the 95% confidence interval (CI) did not include 0 or if the two-tailed probability value (p-value) did not exceed 0.05. All analyses were performed using SPSS statistical software version 22 (IBM SPSS Data Collection, Chicago, Illinois, USA).

Results

Population

One hundred and ninety-eight patients performed both an ABPM with medication and an ABPM without medication during the ACH screening program. Fifteen patients withdrew less

Table 1. Patient characteristics.

	n=153
Sex, male	71 (46%)
Age, yrs	57 ± 12
BMI, kg/m²	28 ± 5
eGFR, mL/min/1.73m²	81 ± 13
24-hr sodium excretion, mmol/24h	144 ± 71
Treated 24-hr SBP, mmHg	146 ± 16
Treated 24-hr DBP, mmHg	86 ± 11
Amount of AHD, DDD	4,4 ± 2
Number of AHD pills, median (min-max)	3 (1-7)
diuretics	97 (63%)
aldosterone antagonist	28 (18%)
RAAS-inhibitor	142 (93%)
ß-blocker	80 (52%)
α-blocker	18 (12%)
calcium-channel blocker	95 (62%)
centrally acting AHD	8 (5%)
direct vasodilators	1 (1%)
Escape medication	42 (27%)

Abbreviations: BMI = body mass index, eGFR = estimated glomerular filtration rate, BP = blood pressure, AHD = antihypertensive drugs, DDD = daily defined dose.

than 1 DDD of antihypertensive medication and were excluded from analysis. Twenty-two patients were excluded because of <70% successful measurements on either one or both of the ABPMs, and eight patients were excluded because they did not use any medication due to intolerance and performed both ABPMs without medication. The patient characteristics of the remaining 153 patients from whom the data were analyzed are presented in table 1. All medication was withdrawn in 111 patients, whereas 42 used escape medication during the second ABPM. On average, patients had a treated blood pressure of 146/86 mmHg (SD 16/11), while taking 3 antihypertensive drugs with an average daily defined dose of 4.4 (indicating medication was used in above-average dosages).

BP response to medication withdrawal

The mean time lapse between both ABPMs was 2.8 months. Patients discontinued their medication for a maximum of 6 weeks, with a mean of 4.1 weeks. After cessation of medication, 24-hour blood pressure increased by 9/6 mmHg (SD 16/9) while the amount of withdrawn medication was -4.1 DDD (SD 2.1).

Figure 2 demonstrates the individual 24-hour systolic blood pressure changes after withdrawal of antihypertensive medication. Ninety-two patients (60%) experienced an increase in BP greater than 5 mmHg ('ascenders') while withdrawing a mean of 4.5 ± 2.1 DDD. Thirty-five patients (23%) had a stable blood pressure ('equals') after cessation of medication (3.6 ± 1.7 DDD), while twenty-six patients (17%) had a decrease in BP greater than 5 mmHg ('descenders') despite withdrawing a mean of 3.3 ± 2.3 DDD (p=0.01 for between group differences in DDD). When stratification was based on day-time SBP, the distribution of ascenders (n=91), equals (n=36) and descenders (n=26) was similar. When analyzing night-time SBP, however, there were slightly more descenders (n=31, 20%), while the number of ascenders decreased (n=86, 56%) and the number of equals remained stable (n=36, 24%).

BP response and potential associated factors

Between-group differences in several factors are depicted in figures 3A-3C and table 2. A significant difference in the amount of withdrawn medication was seen between patients with an increase in BP and patients with a decrease (mean difference 1.2 DDD, p=0.005, figure 3A). There was a significant difference in baseline systolic ABPM (i.e. ABPM with medication) between descenders (159 ± 16 mmHg), equals (149 ± 15 mmHg) and ascenders (141 ± 14 mmHg, p < 0.001, figure 3B). Ascenders had a significantly higher BMI compared to equals (mean difference 2.3 kg/m², p=0.04), but not compared to descenders (mean difference 1.1kg/m², p=0.59, figure 3C). There were no significant differences in renal function, urinary sodium excretion (as a measure of dietary sodium intake), physical inactivity, use of BP-elevating drugs, non-adherence to AHD, and baseline office blood pressure (table 2). The change in BP was not correlated to the percentage of valid measurements on either ABPM or the duration of treatment for hypertension.

Complete medication withdrawal versus escape medication

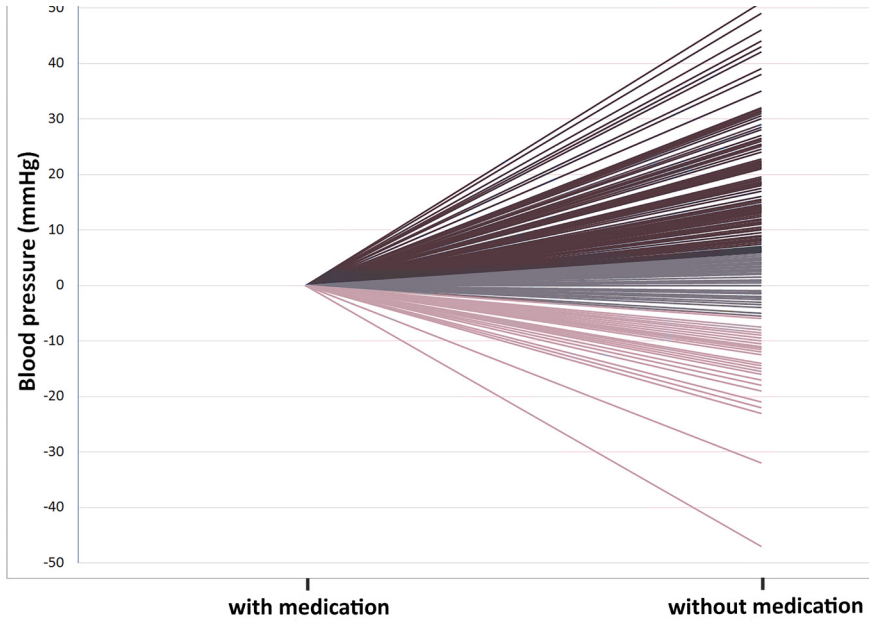
One hundred eleven patients (73%) were able to completely stop all AHD without the need to use escape medication. The change in blood pressure (+ 9/5 mmHg, SD 16/9) and the amount of withdrawn medication (4.1 DDD, SD 2.1) in this group was similar to the complete cohort. The distribution of patients between ascenders (n=62, 56%), equals (n=29, 26%) and descenders (n=20, 18%) for patients who completely stopped medication was not statistically

different from patients who needed to use escape medication (p=0.18), as was the average change in SBP (11 ± 17 mmHg versus 9 ± 16 mmHg, p=0.52).

Discussion

We report a remarkable observation in ambulatory blood pressure change after cessation of antihypertensive medication. First, we observed only a modest increase in blood pressure after cessation of more than three standard doses of AHD among patients receiving long-term treatment for difficult-to-control hypertension. Secondly, in 40% of the patients BP did not change or even decreased after cessation of medication.
This is an interesting observation, as the general hypothesis is that blood pressure will increase in response to the cessation of (highly dosed) antihypertensive medication. Indeed, previous research reports a rapid return to pretreatment levels or overshoot hypertension with signs of sympathetic overdrive in the vast majority of cases.[11,12] Most studies that demonstrated stable blood pressure after discontinuation of medication, investigated the success of discontinuation of unnecessary antihypertensive drug treatment in mild hypertension treated with long-term monotherapy[11,13,14], which is a very different population compared

Figure 2. Individual changes in blood pressure after withdrawal of antihypertensive medication.



Individual changes in 24-hour systolic blood pressure after withdrawal of antihypertensive medication. Patients are stratified by individual change in blood pressure. The dark lines represent patients with an increase >5 mmHg (ascenders), the light lines represent patients with a decrease <5 mmHg (descenders) and the intermediate lines represent patients with less than 5 mmHg change (equals).

Table 2. Between-group differences in potential related factors.

	ascenders (n=92)	unchanged (n=35)	descenders (n=26)	p =
Sex, male	39 (42%)	18 (51%)	14 (54%)	0.47
Age, yrs	58 ± 11	54 ± 13	55 ± 13	0.35
Sodium excretion, mmol/24h	151 ± 77	126 ± 44	148 ± 73	0.33
eGFR, ml/min/1.73m²	70 ± 16	72 ± 14	73 ± 14	0.57
Renal impairment, eGFR <60	1 (4%)	1 (3%)	12 (14%)	0.12
BMI, kg/m²	28 ± 5	26 ± 4	27 ± 4	0.04
Physical inactivity	12 (46%)	13 (37%)	34 (37%)	0.68
Non-adherence (MMAS)	3 (3%)	1 (3%)	1 (4%)	0.29
BP elevating drugs	4 (15%)	4 (11%)	10 (11%)	0.82
Family history of HTN	61 (66%)	25 (71%)	14 (54%)	0.35

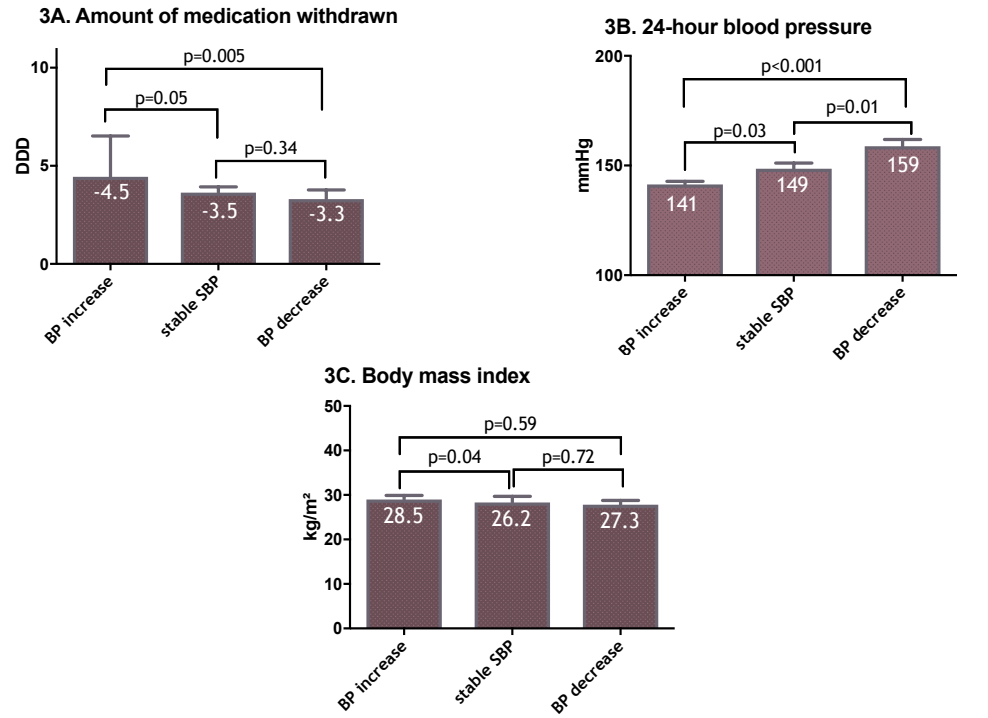
Abbreviations: BMI = body mass index, DBP = diastolic blood pressure, eGFR = estimated glomerular filtration rate, HTN = hypertension, MMAS = Morisky medication adherence scale, SBP = systolic blood pressure.

to our cohort with difficult-to-treat hypertension despite comprehensive treatment. When speculating about the possible explanations for the lack of BP increase in 40% of our cohort, we can formulate several possible hypotheses.

First, non-adherence to antihypertensive medication is a well-known problem in both hypertension research and treatment. This may be a plausible explanation for the patients whose BP remained stable or only slightly increased. But for those patients whose BP declined, it would mean that patients who were previously not taking their medication started doing so after being told to stop their medication, which seems rather counterintuitive. In addition, poor adherence is more prevalent in patients with more extensive medication regimens (i.e. more pills) and higher dosages[15], whereas in our cohort the most extensive medication regimens were used by the patients with an increase in BP (figure 1A). Although non-adherence appeared very low in our study (only 3% was identified as non-adherent), this number is likely underestimated due to limited accuracy of the MMAS.[16] Previous research in similar populations have reported non-adherence in up to 50% of patients.[17,18]

Secondly, a placebo effect or Hawthorne effect may have occurred. Merely participating in a trial (or a screening program) has been shown to have a beneficial effect on BP. A meta-analysis revealed a mean decrease of 5.9 mmHg in systolic office BP in the placebo arms of hypertension trials.[19] When selecting only studies that included patients with resistant hypertension, this increased to 8.8 mmHg. However, this effect appears to mainly affect the white coat component and, thus, is more pronounced in office BP than ABPM.[20] In ABPM, it appears to be limited to a 3 mmHg decrease in 24-hour systolic BP.[10,20,21]
Third, regression to the mean is a well-known factor that occurs frequently in hypertension research. This statistical phenomenon occurs in repeated measurements when relative high or low measurements are followed by less extreme measurements that are closer to the true mean and is particularly distinct when patients are included or excluded based on these

Figure 3. Between-group differences in potential related factors.



Between-group differences in the amount of medication withdrawn (figure 3A), baseline 24-hour blood pressure (with medication, figure 3B) and body mass index (figure 3C) for patients with, respectively, an increase in BP (ascenders), a stable BP (equals) or a decrease in BP (descenders) after cessation of antihypertensive medication.

outliers.[22] The influence of regression to the mean may have been amplified in our study by stratifying patients according to the individual's change in BP (figure 2). This is supported by the fact that baseline BP (i.e. the ABPM with medication) was significantly higher in the descenders compared to the equals while ascenders had a significantly lower baseline BP. However, this does not completely explain why at group level the BP increased less than would be expected after cessation of high dosages of AHD. The average BP reduction that may be expected from starting 3 antihypertensive drugs at half the standard dose is approximately 20 mmHg[23], while we observed an increase of only 8 mmHg after cessation of more than 4 standard doses of AHD. In addition, because ABPM uses the mean value of many measurements throughout 24 hours, ABPM is believed to be less susceptible to regression to the mean.[24]

Lastly, long-term treatment of hypertension may result in chronic resetting of the baroreceptor and vascular remodeling, which may also influence BP responsiveness to stimuli. Baroreceptors play an essential role in maintaining mean arterial pressure at normal levels by altering sympathetic nervous outflow.[25] In baroreceptor resetting, the threshold to activate this negative feedback loop is adjusted to the prevailing blood pressure.[26] In long-term treatment of hypertension, the threshold is adjusted to the lower

blood pressure achieved by the medication. In the acute phase after medication withdrawal blood pressure rises, but the baroreceptor feedback loop counteracts by lowering blood pressure to the threshold that has been set while the patient was still using medication. Vascular remodeling (including arteriolar hypertrophy, decreased vasodilation reserve, increased intima thickness and endothelial dysfunction) occurs in long-term hypertension and causes a structural elevation of resistance and reduced vascular compliance.[27] Adequate treatment of hypertension can (partially) reverse vascular remodeling, thus increasing vascular compliance and BP responsiveness to stimuli, the effects of which may continue after withdrawal of treatment.

Most likely, a combination of these explanations have contributed to our results. Although improvements in lifestyle can have impressive results on blood pressure, the time lapse during our screening program (2.8 months) seems insufficient for substantial lifestyle changes. More importantly, the screening program is merely a baseline assessment of lifestyle factors and lifestyle intervention is not performed until after the second ABPM. Nonetheless, patients may have modified their behavior in response to their participation in the screening program, and this may have contributed to our results.

<u>Perspectives</u>

Our findings emphasize the difficulties encountered in hypertension management and research. Hypertension trials are often challenged by factors influencing BP measurements, such as non-adherence or changes in pharmacological treatment, regression to the mean, patient selection, white coat effects and variations in disease severity.[28-30] The use of ABPM has been increasingly implemented in hypertension research as well as the routine medical care of hypertensive patients, because it is less susceptible to some of these factors.[29] Some studies have even announced OFF-med ambulatory measurements to minimize interference of confounding factors.

Our findings illustrate that researchers and physicians should be aware that ABPM is still not completely exempted from these confounders (even when performed off medication) and that ambulatory BP values should be interpreted accordingly.

Furthermore, our findings underline the disadvantages of BP as an endpoint in clinical research. Particularly the 'responder' classification, in which BP reduction is stratified below or above a pre-specified threshold, may substantially bias the results of hypertension trials. For ABPM, the most commonly used threshold to classify a patient as a 'responder' is 5 mmHg, which would likely result in considerable misclassification especially when this endpoint is measured at a single point in time. Both in the clinic and in research, repeated ABPMs may be needed to avoid mislabeling.

<u>Strengths and limitations</u>

Our study was conducted under a highly standardized screening protocol for difficult-to-control hypertension that has been developed in accordance to the AHA and ESH guidelines and has been routine medical care at our facility for over five years. All BP measurements were performed using validated ambulatory devices and only readings with sufficient reliability (>70% successful measurements) were included in the analysis. Furthermore, ABPM has higher reproducibility compared to office blood pressure and is considered less susceptible to the usual factors confounding BP measurements.[10,31,32] All measurements were

performed on ABPM devices of the same manufacturer and type, to avoid information bias. We did not include a control group in this study. Therefore, we were unable to compare our results to patients who underwent the screening program without any change in medication. Our study population only included eight patients who did not use any medication throughout the screening program, a number too small for proper comparison. Furthermore, we did not perform drug metabolite analysis to confirm adherence to the pharmacological regimen and to the withdrawal schedule. Although we believe the study interval is too short (2.8 months) for BP to be affected by significant lifestyle changes, we did not reevaluate lifestyle factors after the second ABPM.
Lastly, this report described repeated ABPM in our six years experience with medication withdrawal in the diagnostic work-up of hypertension as part of routine medical care for patients with difficult-to-control hypertension. As such, we were unable to perform a prior power calculation. Although the cohort is of reasonable size, the analysis for between-group differences may have suffered from limited sample size.

Conclusion

Cessation of considerable dosages of antihypertensive drugs resulted in only a modest increase in 24-hour blood pressure, driven by a lack of increase (or even a decrease) in blood pressure in almost half of the patients. These findings emphasize the difficulties encountered in research involving patients with (resistant) hypertension, particularly when an intervention is involved. Possible explanations for this observation include presence of regression to the mean, placebo effects, non-adherence and/or resetting of the baroreceptor threshold. These influencing factors should be taken into account when interpreting blood pressure endpoints in clinical research. Repeated ABPMs may be indicated in the clinic as well as in research to avoid mislabeling based on a single reading.

References

1. Chow CK, Teo KK, Rangarajan S, Islam S, Gupta R, Avezum A, et al. Prevalence, awareness, treatment, and control of hypertension in rural and urban communities in high-, middle-, and low-income countries. JAMA 2013; 310:959–68.
2. Basu S, Millett C. Social epidemiology of hypertension in middle-income countries: Determinants of prevalence, diagnosis, treatment, and control in the WHO SAGE study. Hypertension 2013; 62:18–26.
3. Pereira M, Lunet N, Azevedo A, Barros H. Differences in prevalence, awareness, treatment and control of hypertension between developing and developed countries. J Hypertens 2009; 27:963–975.
4. ESH/ESC Task Force for the Management of Arterial Hypertension, Society ES of H (ESH) and of the ESC. 2013 ESH/ESC Guidelines for the management of arterial hypertension. Eur Heart J 2013; 34:2159–2219.
5. Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, 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–e526.
6. Stowasser M, Ahmed AH, Pimenta E, Taylor PJ, Gordon RD. Factors affecting the aldosterone/renin ratio. Horm. Metab. Res. 2012; 44:170–176.
7. Bauer IH, Reams GP, Wu Z, Lau-Sieckman A. Effects of losartan on the renin-angiotensin-aldosterone axis in essential hypertension. J Hum Hypertens 1995; 9:237–243.
8. O'Brien E, Asmar R, Beilin L, Imai Y, Mallion JM, Mancia G, et al. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. J Hypertens 2003; 21:821–848.
9. WHO Collaborating Centre for Drug Statistics Methodology. Guidelines for ATC classification and DDD assignment 2015. 18th ed. ; 2014. http://www.whocc.no/ddd/definition_and_general_considera/
10. Staessen JA, Thijs L, Bieniaszewski L, O'Brien ET, Palatini P, Davidson C, et al. Ambulatory monitoring uncorrected for placebo overestimates long-term antihypertensive action. Systolic Hypertension in Europe (SYST-EUR) Trial Investigators. Hypertension 1996; 27:414–20.
11. Beltman FW, Heesen WF, Kok RH, Smit AJ, May JF, de Graeff PA, et al. Predictive value of ambulatory blood pressure shortly after withdrawal of antihypertensive drugs in primary care patients. Br Med J 1996; 313:404–406.
12. Houston MC. Abrupt cessation of treatment in hypertension: Consideration of clinical features, mechanisms, prevention and management of the discontinuation syndrome. Am Heart J 1981; 102:415–430.
13. Nelson MR, Reid CM, Krum H, Ryan P, Wing LMH, McNeil JJ. Short-term predictors of maintenance of normotension after withdrawal of antihypertensive drugs in the Second Australian National Blood Pressure Study (ANBP2). Am J Hypertens 2003; 16:39–45.
14. Sieg-Dobrescu D, Burnier M, Hayoz D, Brunner HR, Waeber B. The return of increased blood pressure after discontinuation of antihypertensive treatment is associated with an impaired post-ischemic skin blood flow response. J Hypertens 2001; 19:1387–1392.
15. Osterberg L, Blaschke T. Adherence to medication. N Engl Med 2005; 353:487–497.
16. Pandey A, Raza F, Velasco A, Brinker S, Ayers C, Das SR, et al. Comparison of Morisky Medication Adherence Scale with therapeutic drug monitoring in apparent treatment-resistant hypertension. J Am Soc Hypertens 2015; 9:420–426e2.
17. Azizi M, Pereira H, Hamdidouche I, Gosse P, Monge M, Bobrie G, et al. Adherence to Antihypertensive Treatment and the Blood Pressure Lowering Effects of Renal Denervation in the Renal Denervation for Hypertension (DENERHTN) Trial. Circulation 2016; 134:847–857.
18. Jung O, Gechter JL, Wunder C, Paulke A, Bartel C, Geiger H, et al. Resistant hypertension? Assessment of adherence by toxicological urine analysis. J Hypertens 2013; 31:766–774.
19. Patel HC, Hayward C, Ozdemir BA, Rosen SD, Krum H, Lyon AR, et al. Magnitude of Blood Pressure Reduction in the Placebo Arms of Modern Hypertension Trials: Implications for Trials of Renal Denervation. Hypertension 2015; 65:401–406.
20. Mancia G, Omboni S, Parati G, Ravogli A, Villani A, Zanchetti A. Lack of placebo effect on ambulatory blood pressure. Am J Hypertens 1995; 8:311–315.
21. Omboni S, Zanchetti A. Antihypertensive efficacy of lercanidipine at 2.5, 5 and 10 mg in mild to

moderate essential hypertensives assessed by clinic and ambulatory blood pressure measurements. Multicenter Study Investigators. J Hypertens 1998; 16:1831–1838.
22. Barnett AG. Regression to the mean: what it is and how to deal with it. Int J Epidemiol 2004; 34:215–220.
23. Law MR. Value of low dose combination treatment with blood pressure lowering drugs: analysis of 354 randomised trials. Bmj 2003; 326:1427.
24. Rümke CL. [regression to the mean]. Ned Tijdsch Geneeskd 184AD; 128:158.
25. Lovic D, Manolis A, Lovic B, Stojanov V, Lovic M, Pittaras A, et al. The Pathophysiological Basis of Carotid Baroreceptor Stimulation for the Treatment of Resistant Hypertension. Curr Vasc Pharmacol 2014; 12:16–22.
26. Kougias P, Weakley SM, Yao Q, Lin PH, Chen C. Arterial baroreceptors in the management of systemic hypertension. Med Sci Monit 2010; 16:RA1-A8.
27. Laurent S, Boutouyrie P. The Structural Factor of Hypertension: Large and Small Artery Alterations. Circ Res 2015; 116:1007–1021.
28. Mahfoud F, Böhm M, Azizi M, Pathak A, Durand Zaleski I, Ewen S, et al. Proceedings from the European clinical consensus conference for renal denervation: Considerations on future clinical trial design. Eur Heart J 2015; 36:2219–2227.
29. O'Brien E. Twenty-four-hour ambulatory blood pressure measurement in clinical practice and research: a critical review of a technique in need of implementation. J Intern Med 2011; 269:478–495.
30. Davis EM, Appel LJ, Wang X, Greene T, Astor BC, Rahman M, et al. Limitations of analyses based on achieved blood pressure: Lessons from the African American Study of Kidney Disease and Hypertension Trial. Hypertension 2011; 57:1061–1068.
31. Wendelin-Saarenhovi M, Isoaho R, Hartiala J, Helenius H, Kivelä SL, Hietanen E. Long-term reproducibility of ambulatory blood pressure in unselected elderly subjects. Clin Physiol 2001; 21:316–322.
32. Eguchi K, Hoshide S, Hoshide Y, Ishikawa S, Shimada K, Kario K. Reproducibility of ambulatory blood pressure in treated and untreated hypertensive patients. J Hypertens 2010; 28:918–924.

Chapter 4 -

Renal denervation in a real life setting: a gradual decrease in home blood pressure.

PLOS One 2016; 11(9); e0162251

Martine M.A. Beeftink, Wilko Spiering , Michiel L. Bots, Willemien L. Verloop, Rosa L. de Jager, Margreet F. Sanders, Evert-Jan Vonken, Peter J. Blankestijn, Michiel Voskuil

Abstract

Objectives
To investigate the blood pressure dynamics after renal denervation through monthly home blood pressure measurements throughout the first 12 months.

Background
Renal denervation is a relatively new treatment for hypertension and has shown mixed effects for effectivity. It is unknown, however, whether blood pressure acutely decreases shortly after the procedure, or more gradually over the course of several months.

Methods
A cohort of 70 patients performed highly standardized monthly home blood pressure monitoring during the first year after denervation according to the European Society of Hypertension guidelines. At baseline and 12 months follow-up, office and ambulatory blood pressure as well as routine physical and laboratory assessment was performed.

Results
Home blood pressure decreased with a rate of 0.53 mmHg/month (95% CI 0.20 to 0.86) systolic and 0.26 mmHg/month (95% CI 0.08 to 0.44) diastolic throughout 12 months of follow-up, while the use of antihypertensive medication remained stable (+0.03 daily defined doses/month, 95% CI -0.01 to 0.08). On average, a 12 month reduction of 8.1 mmHg (95% CI 4.2 to 12.0) was achieved in home systolic blood pressure, 9.3 mmHg (95% CI -14.2 to -4.4) as measured by 24-hour ambulatory blood pressure monitoring and 15.9 mmHg (95% CI -23.8 to -7.9) on office measurements.

Conclusion
Blood pressure reduction after renal denervation occurs as a gradual decrease that extends to at least one-year follow-up. Home monitoring seems a suitable alternative for ambulatory blood pressure monitoring after renal denervation.

Introduction

Hypertension is common in the western society and the risk of vascular complications is strongly related to blood pressure levels.[1] As the greatest contributor to cardiovascular morbidity and mortality hypertension is associated with 10.4 million premature deaths annually.[2] Despite a wealth of treatment options, blood pressure control is limited: only a third of patients receiving antihypertensive drugs are adequately controlled.[3]
In 2009, catheter-based renal denervation (RDN) was introduced as a new, promising treatment for patients with persistent hypertension despite comprehensive pharmacological treatment.[4] Initially RDN showed impressive results, mostly in cohort studies and some small randomized trials,[4-7] but more recent studies have shown mixed results for efficacy.[8-11] In the discussion following these results many gaps in the knowledge of RDN were identified, including issues concerning study design, patient selection, medication adherence as effect modifiers, the optimal procedural approach, anatomical variation and the lack of a reliable marker of procedural success.[12,13]
Among these issues is the uncertainty when to expect a response of RDN on blood pressure. It is unknown whether BP acutely decreases shortly after the intervention or more gradually over the course of several months. Therefore, we investigated home blood pressure measurements (HBPM) throughout the first year after RDN treatment to elucidate the dynamics of BP following RDN.

Methods

This study was conducted at the University Medical Centre Utrecht and is part of the Dutch National Renal Denervation Registry (NCT02482103) that was approved by the Medical Ethics Committee of the UMC Utrecht.[14] The registry contains screening, procedural and follow-up data of all patients treated with RDN in the Netherlands. The requirement to obtain informed consent for the registry was waived by the Medical Ethics Committee. All patients provided written informed consent for the original RDN study they participated in, or provided verbal informed consent if the RDN procedure was performed as routine medical care. The study was conducted in accordance with the Declaration of Helsinki[15] and the Dutch Medical Research Involving Human Subjects Act (WMO).

Study population
For the current analysis, we studied a cohort of consecutive patients that performed HBPM throughout the first year after RDN for resistant hypertension (an office systolic BP ≥160 mmHg and/or a 24-hour SBP ≥135 mmHg, despite the use of at least 3 antihypertensive drugs at maximally tolerated doses) or the inability to be adequately treated for hypertension due to recorded intolerance for antihypertensive drugs (non-resistant hypertension). Before intervention, all patients were subjected to a thorough screening procedure including 24-hour ambulatory blood pressure monitoring (ABPM), to exclude pseudo-resistant hypertension, significant white coat effect and secondary causes, as previously described.[16] This screening includes temporary cessation of all antihypertensive drugs, if deemed safe, to avoid interference with the investigations and to obtain unconfounded BP measurements. Immediately after these investigations, BP medication was restarted at once. Physicians were asked not to change the antihypertensive medication unless absolutely necessary.

The final decision for eligibility for RDN was made by a multidisciplinary team, consisting of a vascular medicine specialist (WS), a nephrologist (PB), an interventional cardiologist (MV) and an interventional radiologist (EJV). Major exclusion criteria included ineligible renal artery anatomy, an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m^2, severe co-morbidity and patient refusal.[17]
The RDN procedure was performed via transfemoral approach according to the respective instructions for use of the device. The choice for the type of RDN catheter was left to the discretion of the interventionalist.

Home blood pressure monitoring (HBPM)
To perform HBPM, patients received an automated WatchBP Home device (Microlife Inc., Widnau, Switzerland). Patients were instructed according to the European Society of Hypertension recommendations[18,19] to perform HBPM every month for a total of 12 months, starting one week after RDN. The HBPM routine was started one week after RDN and each following measurement week was scheduled to start on the same day of the next month. Additionally, HBPM was performed during the medication-free screening prior to RDN.
HBPM measurements were to be taken in a seated position after five minutes of rest, two times in the morning (6-9AM) and two times in the evening (6-9PM) during seven consecutive days. All measurements were automatically stored to the device and uploaded to a secure internet site (BP@home, MobiHealth B.V., the Netherlands). Patients were unable to add, delete or change any measurements on the device or on the BP@home server. In accordance to the guidelines[18,19] measurements taken on the first day of every week were discarded to avoid non-representative measurements due to anxiety with the technique and weeks with less than 12 readings were excluded for analysis to secure the quality of the measurements. The BP measurements from each HBPM period of seven days were used to calculate the mean systolic (SBP) and diastolic (DBP) home BP for each month. Medication use for each period was recorded based on prescription history and detailed history taking. Prescribed dosages of antihypertensive drugs for each time point were converted into defined daily doses (DDD) using conversion factors provided by the World Health Organization (WHO) Drug Classification.[20] The cumulative daily intake of antihypertensive drugs was calculated for each patient using the sum of all DDDs. No toxicological urine or blood analyses to confirm medication adherence was performed.

Routine follow-up data
Office BP, laboratory results, medical history and physical examination were registered during screening and at six and 12 months follow-up. Ambulatory BP monitoring was performed during screening and 12 months follow-up. A subgroup of patients repeated the medication-free period at 12 months follow-up as part of a different study protocol.[21] All BP measurements were performed on Microlife WatchBP 03 devices (Microlife Inc., Windau, Switzerland) in accordance to the ESH guidelines.[22] Readings for ABPM were taken at least every 30 minutes during day and night using appropriate cuff sizes and repeated if more than 30% of measurements failed. Serum creatinine was used to estimate renal function (eGFR) using the CKD-EPI formula.[23]

Statistical analysis
Results are presented as mean ± standard deviation or as absolute number with percentages, unless otherwise specified.
Multilevel linear mixed effect models were used for analysis of BP over time. This model

Table 1: Patient characteristics

	n=70
Age, years	59 ± 9
Gender, (male)	43 (63%)
Caucasian	67 (96%)
Office SBP, mmHg	191 ± 31
Office DBP, mmHg	104 ± 16
BMI, kg/m^2	29 ± 5
eGFR, mL/min/1.73m^2	80 ± 18
CVD	26 (37%)
Dyslipidemia	35 (50%)
DM type 2*	22 (31%)
No. of AHD, median (min-max)	3 (0-6)
Total DDD (sum of all AHD)	5,4 ± 3,6
Type of antihypertensive medication used	
Diuretic	54 (77%)
RAAS inhibitor[$]	60 (86%)
Aldosterone antagonist	20 (29%)
Beta-blocker	42 (60%)
Alpha-blocker	12 (17%)
Calcium-channel blocker	46 (66%)
Central acting antihypertensive	2 (3%)
Direct vasodilating drug	3 (4%)
No. of HBPM weeks, mean (min.-max.)	11 (3-12)
RDN device used	
Medtronic Symplicity	66 (94%)
St Jude Enlightn II	3 (4%)
Covidien OneShot	1 (1%)
Indication for RDN	
Resistant hypertension	52 (74%)
Non-resistant hypertension	18 (26%)

Data is presented as absolute number (percentage) or as mean ± standard deviation, unless otherwise specified. *DM type 1 was exclusion criterion for RDN treatment. [$]RAAS inhibitors composed of angiotensin converting enzyme inhibitors, angiotensin receptor antagonists and direct renin inhibitors. Abbreviations: SBP systolic blood pressure, DBP diastolic blood pressure, BMI body mass index, eGFR estimated glomerular filtration rate, CVD cardiovascular disease, DM diabetes mellitus, AHD antihypertensive drugs, DDD daily defined dose, RAAS Renin-Angiotensin-Aldosterone System, HBPM home blood pressure monitoring, RDN renal denervation.

has several advantages in modelling changes over time over a repeated measures ANOVA. In particular the model is not hampered by missing data and it provides estimation of effect size and precision.[24] Time in months was entered as a continuous variable, and random intercepts as well as random slopes were allowed in the model as appropriate guided by the -2 Log Likelihood statistic. Only post-RDN measurements were entered into the model, to avoid interference of an artificial increase in medication caused by the medication-free screening period. Pre-selected variables were added to the unconditional model (model I) as fixed effects, resulting in model II (age, gender, antihypertensive medication) and model III (age, gender, antihypertensive medication, body mass index (BMI), estimated glomerular filtration rate (eGFR), smoking status and baseline office BP) to correct for their possible influence on BP slope. Daily use of medication (DDD) was entered into the model as time-varying variable, while baseline variables were added as time-independent variables. The HBPM changes over time were subsequently modelled in pre-specified subgroups of risk factors and potentially confounding factors. For continuous variables, stratification was performed below or above the median for the study population. Interaction terms for each variable were added to the model to test for significant differences between subgroups.
For BMI, eGFR, office BP and ABPM, the change between baseline and 12 months follow-up was assessed by means of the paired samples T-test, or Wilcoxon signed rank test when appropriate.
Results were considered statistically significant if the 95% confidence interval (CI) did not include 0 or if the two-tailed probability value (p-value) did not exceed 0.05. All analysis was performed with SPSS statistical software version 22 (IBM SPSS Data Collection, Chicago, Illinois, USA).

Table 2. Change in home blood pressure over time after renal denervation.

	estimate slope (mmHg/month)	95% CI
Model I (unconditional)		
Mean SBP	-0.56	-0.89;-0.24
Mean DBP	-0.27	-0.46;-0.07
Mean heart rate	-0.09	-0.18;-0.001
Model II (adjusted for age, sex and AHD)		
Mean SBP	-0.51	-0.84;-0.18
Mean DBP	-0.25	-0.44;-0.07
Mean heart rate	-0.09	-0.23;0.05
Model III (full model)		
Mean SBP	-0.53	-0.86;-0.20
Mean DBP	-0.26	-0.44;-0.08
Mean heart rate	-0.09	-0.23;0.04

The estimated slope represents the change in home blood pressure per month. Multivariable analysis in model III (full model) was adjusted for daily use of medication, age, sex, body mass index, estimated glomerular filtration rate, baseline office blood pressure and smoking. Abbreviations: SBP = systolic blood pressure, DBP = diastolic blood pressure, AHD = antihypertensive drugs (sum of all daily defined doses).

Results

Our registry included 118 patients who were treated with RDN between June 2010 (the start of RDN at our facility) and May 2014. As of May 2011, all patients (n=90) consecutively received an automated device to perform HBPM. Sixteen patients refused HBPM or did not have access to internet, resulting in 74 patients of whom HBPM was available. Four patients only performed measurements during the screening procedure and were excluded from analysis.
Characteristics of the remaining 70 patients are shown in table 1. They performed a total of 756 HBPM periods after RDN (83% success rate). Five HBPM periods were discarded due to an insufficient number of measurements, all from a different subject. The average number of measuring periods was 11, with only three patients accomplishing less than six HBPM periods. The number of measuring periods contributing to each of the monthly averages is

Table 3. Change in home blood pressure over time after renal denervation in various strata of baseline patient characteristics.

	n=	estimate slope (mmHg/month)	95% CI	p=
Resistant hypertension	52	-0.72	-1.11 ; -0.33	0.06
Medication intolerance	18	0.11	-0.41 ; 0.64	
Spironolactone at baseline = yes	19	-0.77	-1.62 ; 0.07	0.61
Spironolactone at baseline = no	51	-0.52	-0.91 ; -0.13	
Systolic office BP <188mmHg	35	-0.29	-0.74 ; 0.16	0.16
Systolic office BP ≥188mmHg	35	-0.72	-1.22 ; -0.23	
Isolated systolic HTN = yes	13	-0.44	-0.93 ; -0.05	0.81
Isolated systolic HTN = no	57	-0.58	-0.96 ; -0.19	
BMI <28.5kg/m	34	-0.22	-0.71 ; 0.26	0.15
BMI ≥28.5kg/m	36	-0.73	-1.20 ; -0.27	
Baseline eGFR <82ml/min/1.73m^2	35	-0.74	-1.23 ; -0.26	0.05
Baseline eGFR ≥82ml/min/1.73m^2	35	-0.15	-0.62 ; 0.32	
Sodium excretion <162mmol/24h	34	-0.49	-0.90 ; -0.08	0.72
Sodium excretion ≥162mmol/24h	36	-0.60	-1.11 ; -0.09	
Smoking = no	60	-0.64	-0.98 ; -0.29	0.09
Smoking = yes	10	0.29	-1.03 ; 1.61	

Estimated slope represents the estimated change in home blood pressure per month. P-value represents significance level for interaction. Abbreviations: BP = blood pressure, BMI = body mass index, eGFR = estimated glomerular filtration rate, HTN = hypertension.

Figure 2. Changes in blood pressure measured by various modalities and antihypertensive medication over time.

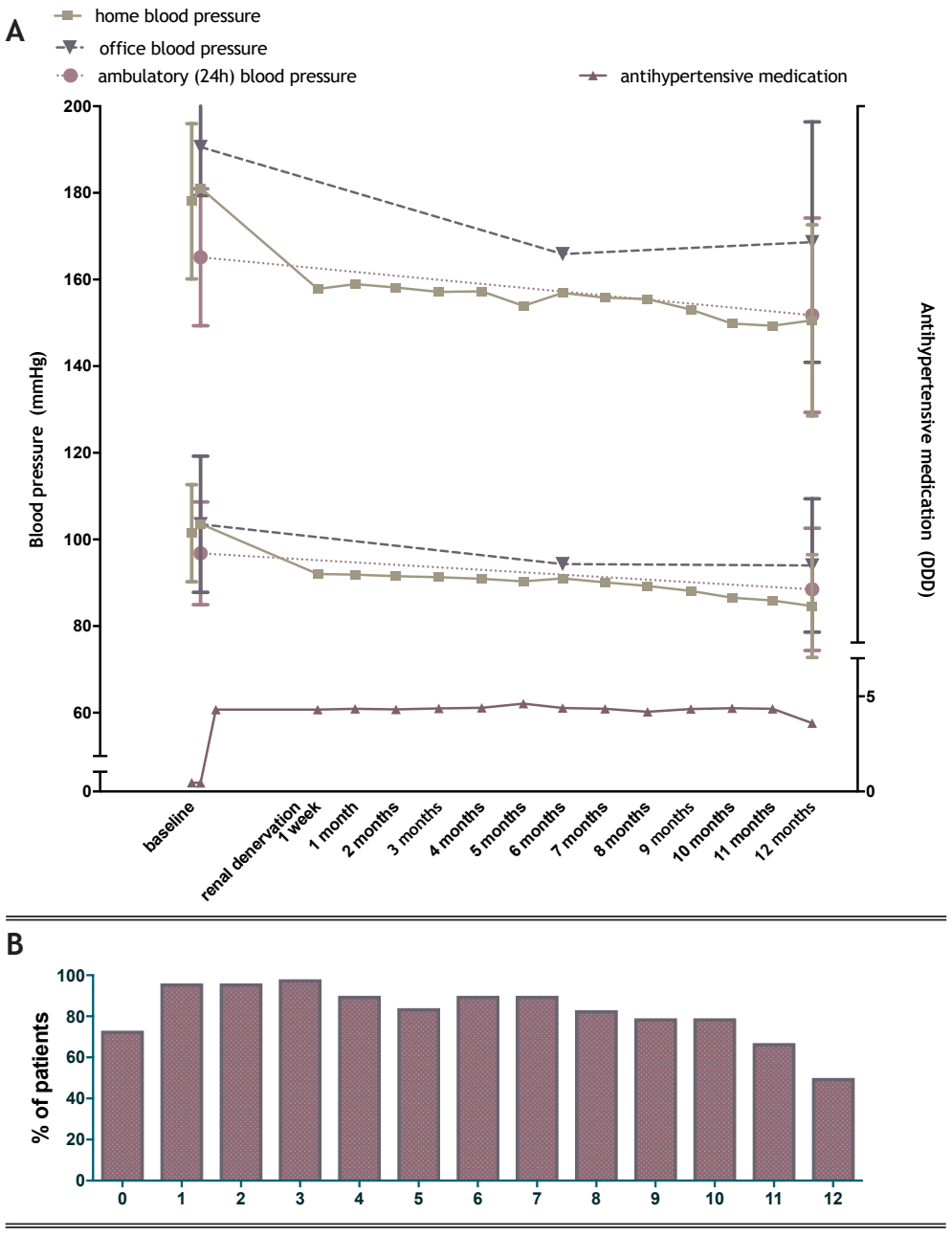


Figure A depicts the change in blood pressure measured by various modalities (displayed on left axis) and antihypertensive medication (displayed on right axis). SD bars for BP overlapped for both baseline and 12-month follow-up, indicating lack of statistical significance, but were omitted in the figure for clarity. Figure B depicts the percentage of patients completing home blood pressure measurements (HBPM) for each measuring period.

depicted in figure 1. Fifty-two patients were treated with RDN for resistant hypertension, while eighteen patients were included with an inability to tolerate optimal pharmacological treatment due to documented intolerance to antihypertensive drugs. The mean amount of prescribed medication at baseline was 6.7 ± 3 for patients with resistant hypertension and 1.7 ± 1 for patients with intolerance to AHD.
Overall, the amount of antihypertensive medication prescribed decreased from 5.8±3.8 DDD at baseline to 5.3±3.6 DDD at 12 months with a mean difference of 0.5 (95% CI -1.3 to 0.2). There was no change in eGFR (Δ 0.33 ml/min/1.73m², 95% CI -2.1 to 2.8) or BMI (Δ 0.14 kg/m², 95% CI -0.33 to 0.6) between baseline and 12 months follow-up. In addition, there was no change in urinary sodium excretion (Δ 18 mmol/24hrs, 95% CI -34 to 70), although follow-up urine analysis was only available for 29 subjects.

Table 4. Rate of change in blood pressure measured by various modalities.

	SBPM		ABPM		OBPM	
	delta	95% CI	delta	95% CI	delta	95% CI
systolic BP	-8,1	(-12,0 to -4,2)	-9,3	(-14,2 to -4,4)	-15,9	(-23,8 to -7,9)
diastolic BP	-3,8	(-6,1 to -1,6)	-5,5	(-8,0 to -3,0)	-6,6	(-10,1 to -3,0)

Rate of change calculated as absolute reduction in mmHg per year. SBPM = self-blood pressure measurements, ABPM = ambulatory blood pressure measurements, OBPM = office blood pressure measurements.

Home blood pressure measurements

Mean HBPM values decreased from 181/104 mmHg (SD 19/13) during the medication-free screening period before RDN to 158/92 mmHg (SD 17/13) one week after RDN and 152/87 mmHg (SD 21/13) at one year follow-up. Due to the medication-free baseline screening, the DDD differed from the baseline measurement to 1 week after renal denervation (0.5 versus 4.3), but remained stable from week 1 through month 12 (estimated slope 0.03 DDD/month, 95% CI -0.01 to 0.08).

The unconditional mixed model (model I) showed a significant change in BP of -0.56 mmHg/month after RDN (95% CI -0.89 to -0.24) for SBP and -0.27 mmHg/month for DBP (95% CI -0.46 to -0.07) during the 12 months after treatment (Table 2). In the full model adjusting for pre-specified variables (model III), BP change remained statistically significant at -0.53 mmHg/month (95%CI -0.86 to -0.20) for SBP and -0.26 mmHg/month (95% CI -0.44 to -0.08) for DBP.
Heart rate changed significantly in the unconditional model, but this change was not clinically relevant (-0.09 bpm per month) nor statistically significant after correction for pre-specified variables. Results for the different subgroups are shown in table 3.

Figure 2 shows the change of SBP measured by various modalities. Office BP decreased from 191/104 mmHg (SD 31/16) at baseline to 169/94 mmHg (SD 28/15) at twelve months follow-up, while mean 24-hour BP decreased from 165/97 mmHg (SD 16/16) to 152/89 mmHg (SD 22/14), levelling up with HBPM levels at 12 months follow-up. The rates of change for the various modalities are presented in table 4.

Discussion

Our results show a number of important observations. First, a gradual decrease in BP following RDN was observed over the course of 12 months. Second, the observed decrease did not reach an obvious plateau during our follow-up and was unaffected by changes in the amount of antihypertensive medication. Lastly, the rate of change in home BP measurements and 24-hour ambulatory BP measurements was comparable, while office measurements appeared to be higher throughout the study.

These results provide an answer in the debate concerning the timing of the effect of RDN. It has been stated by some that RDN has a rapid effect during the first trimester, while others have advocated that the effect may take several months to occur.[25,26] In previous studies, the time intervals between BP measurements were too large to investigate the exact timing of effects. We performed monthly home BP measurements (HBPM), enabling us to investigate the dynamics of BP more accurately and to determine the timing of BP changes after RDN. Our results show that BP decreased in a gradual fashion without an obvious dip. This may indicate that BP changes after RDN indeed do not occur as an acute drop but simply as a gradual change over a long period of time, which is in line with previous studies that demonstrated further BP reductions at a similar rate at 24 and 36 months compared to 1 year follow-up.[27,28] That concept is further supported by the results of the DENERHTN trial[8] demonstrating a gradual decrease on HBPM throughout 6 months quite similar to ours, although the absolute decrease in HBPM was higher. The latter may be explained by the uptitration of antihypertensive medication based on the HBPM readings in the DENERHTN trial. The observed gradual decrease in both studies may also be due to considerable interpersonal variety in the occurrence of BP effects that are levelled off at group level. The observations would imply that BP management can benefit greatly from implementing HBPM in the routine follow-up after RDN. Frequent HBPM allows for quick detection of BP changes and subsequent adjustment of antihypertensive treatment that may be delayed in the case of occasional office or ambulatory measurements.

An interesting finding in our study is that, in contrast to patients with resistant hypertension, patients with medication intolerance did not show a decrease (if anything, a slight increase) in blood pressure. This is in contrast to the findings of De Jager et al.[29], who demonstrated significant decreases in office and ambulatory blood pressure in a small cohort of patients not taking AHD. Obtaining measurements unbiased by antihypertensive medication is important, since changes in adherence may influence the results after RDN in opposing ways: decreased adherence may mask the effects of renal denervation, while increased adherence may induce an apparent RDN effect that is not really there. Therefore, further research involving a not treated hypertensive population will be of special interest.[30]
HBPM also has additional value in hypertension research. Previous studies in the field of RDN have mostly used office BP measurements as an endpoint. However, it is known that office BP measurements are subjected to several disadvantages, such as observer bias and white coat effect. Even when performed under ideal circumstances, such as proper positioning of the patient, well-trained personnel and selection of the correct cuff size, office BP readings have poor reliability and tend to overestimate true BP.[31-33] This is also reflected by the higher baseline and follow-up values for office BP in our analysis. ABPM and HBPM provide

better reproducibility, are more accurately related to the real BP of daily life and are not subjected to the white coat effect.[19] In turn, HBPM has the advantage over ABPM that it is cheaper, more convenient for the patient and allows multiple measurements over longer periods of time.[34,35]
Randomized sham-controlled trials, such as the HTN-3 trial[10] and the study by Desch et al.,[11] are of superior methodology to assess therapeutic efficacy and have failed to demonstrate a statistically significant benefit of RDN compared to a sham procedure. Still, these studies have investigated the patient under highly controlled circumstances, whereas our patients were studied in a setting that reflects real life.

Strengths and limitations
We studied blood pressure changes after RDN using HBPM, which is considered more reliable than office measurements and has not yet been widely used in RDN research. Using HBPM also minimized the influence of regression-to-the-mean in our analysis, because the effect of this statistical phenomenon quickly diminishes after a few readings[36] and HBPM uses the mean of many BP measurements. Furthermore, HBPM also allowed us to investigate BP changes in a real life setting, as opposed to the highly controlled hospital setting of clinical trials.
As stated in the introduction, recent trials raised several issues concerning the effectiveness of RDN, including technical aspects, anatomical issues, patient selection, study design and timing of the BP response. In the current analysis we were able to address the latter, but other aspects remained unaddressed. As long as a quantitative measure for the extent of nerve damage effectuated by the RDN procedure is lacking, any statements concerning causality are highly speculative. Therefore, we can make a statement regarding when BP reduction occurs after RDN, but we cannot provide an answer in the discussion whether the observed effect is caused by the intervention. Although we did observe an apparent drop in BP between baseline and the first measurement after RDN (one week post-RDN), this observation is biased by an artificial increase in antihypertensive medication caused by the medication free screening period and therefore not included in the LMM analysis. Therefore, we can neither rule out nor demonstrate the coexistence of an acute drop in BP during the first days.
Lastly, we did not include a control group and therefore cannot compare the BP effect in our intervention group to BP control measured by HBPM in a hypertensive population without intervention. The observed BP effect in our study was modest and the use of HBPM may have contributed to the BP reduction as it may not only be used as a diagnostic, but also as an educational tool. Especially when combined with telemonitoring, HBPM can contribute to better BP control and the need for less antihypertensive medication.[37-39] However, this effect, if present, is likely to be small: in a meta-analysis of 37 studies with a duration up to 36 months, the average effect of HBPM and telemonitoring on BP was less than 3 mmHg systolic.[40]

Conclusion

In this study we have evaluated BP dynamics the first year after RDN. Using frequent home BP monitoring in a real life setting we demonstrated a gradual decrease over time after RDN. Future research needs to distinguish whether this decrease represents a true effect

of RDN, or whether it is effectuated through other factors as discussed above. Particularly in hypertension research, the use of a randomized sham-controlled design and reliable BP measurements is key. For outcome measurement in these studies, HBPM may be a more informative and convenient alternative to ABPM. However, it is important to realize that any statements concerning causality between the RDN procedure and the observed effect on BP are highly speculative as long as a quantitative measure for the extent of nerve damage is lacking.

References

1. 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):1903-1913.
2. World Health Day. A global brief on Hypertension World Health Day 2013. 2013:1-40.
3. Chow CK, Teo KK, Rangarajan S, et al. Prevalence, awareness, treatment, and control of hypertension in rural and urban communities in high-, middle-, and low-income countries. JAMA. 2013;310(9):959-968.
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):1275-1281.
5. 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(25):2976-2982.
6. 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):2132-2140.
7. Hering D, Marusic P, Walton AS, et al. Sustained sympathetic and blood pressure reduction 1 year after renal denervation in patients with resistant hypertension. Hypertension. 2014;64(1):118-124.
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):1957-1965.
9. Rosa J, Widimsky P, Tou ek 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. 2014;65(2):407-413.
10. 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):1393-1401.
11. Desch S, Okon T, Heinemann D, et al. Randomized sham-controlled trial of renal sympathetic denervation in mild resistant hypertension. Hypertension. 2015;65(6):1202-1208.
12. Persu A, Jin Y, Fadl Elmula FEM, Jacobs L, Renkin J, Kjeldsen S. Renal denervation after symplicity HTN-3: An update. Curr Hypertens Rep. 2014;16(8).
13. Tzafriri AR, Mahfoud F, Keating JH, et al. Innervation patterns may limit response to endovascular renal denervation. J Am Coll Cardiol. 2014;64(11):1079-1087.
14. Sanders MF, Blankestijn PJ, Voskuil M, et al. Safety and long-term effects of renal denervation: Rationale and design of the Dutch registry. Neth J Med. 2016;74(1):5-15.
15. World Medical Association. WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. 2013;(June 1964):1-8.
16. Vink EE, Verloop WL, Bost RBC, et al. The blood pressure-lowering effect of renal denervation is inversely related to kidney function. J Hypertens. 2014;32:2045-2053.
17. Verloop WL, Vink EE, Voskuil M, et al. Eligibility for percutaneous renal denervation: the importance of a systematic screening. J Hypertens. 2013;31(8):1662-1668.
18. Parati G, Stergiou GS, Asmar R, et al. European Society of Hypertension practice guidelines for home blood pressure monitoring. J Hum Hypertens. 2010;24(12):779-785.
19. O'Brien E, Asmar R, Beilin L, et al. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. J Hypertens. 2003;21(5):821-848.
20. WHO Collaborating Centre for Drug Statistics Methodology. Guidelines for ATC Classification and DDD Assignment 2015. 18th ed.; 2014. http://www.whocc.no/ddd/definition_and_general_considera/.
21. 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(5):558-567.
22. ESH/ESC Task Force for the Management of Arterial Hypertension. 2013 Practice guidelines for the management of arterial hypertension of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC): ESH/ESC Task Force for the Management of Arterial Hypertension. J Hypertens. 2013;31(10):1925-1938.
23. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-612.

24. Krueger C, Tian L. A comparison of the general linear mixed model and repeated measures ANOVA using a dataset with multiple missing data points. Biol Res Nurs. 2004;6(2):151-157.
25. Kaiser L, Beister T, Wiese A, et al. Results of the ALSTER BP real-world registry on renal denervation employing the Symplicity system. EuroIntervention. 2014;10(1):157-165.
26. Symplicity HTN-1 investigators. Catheter-Based Renal Sympathetic Denervation for Resistant Hypertension: Durability of Blood Pressure Reduction Out to 24 Months. Hypertension. 2011;57(5):911-917.
27. Esler MD, Böhm M, Sievert H, et al. Catheter-based renal denervation for treatment of patients with treatment-resistant hypertension: 36 month results from the symplicity HTN-2 randomized clinical trial. Eur Heart J. 2014;35(26):1752-1759.
28. Krum H, Schlaich MP, Sobotka P a., et al. Percutaneous renal denervation in patients with treatment-resistant hypertension: Final 3-year report of the Symplicity HTN-1 study. Lancet. 2014;383(9917):622-629.
29. de Jager RL, Sanders MF, Bots ML, et al. Renal denervation in hypertensive patients not on blood pressure lowering drugs. Clin Res Cardiol. 2016.
30. Mahfoud F, Böhm M, Azizi M, et al. Proceedings from the European clinical consensus conference for renal denervation: Considerations on future clinical trial design. Eur Heart J. 2015;36(33):2219-2227.
31. Ali S, Rouse a. Practice audits: reliability of sphygmomanometers and blood pressure recording bias. J Hum Hypertens. 2002;16(5):359-361.
32. Reeves RA. The rational clinical examination. Does this patient have hypertension? How to measure blood pressure. JAMA. 1995;273(15):1211-1218.
33. Pickering TG, Hall JE, Appel LJ, et al. Recommendations for blood pressure measurement in humans and experimental animals. Part 1: Blood pressure measurement in humans: A statement for professionals from the subcommittee of professional and public education of the American Heart Association cou. Hypertension. 2005;45(1):142-161.
34. Pickering TG, White WB, Giles TD, et al. When and how to use self (home) and ambulatory blood pressure monitoring. J Am Soc Hypertens. 4(2):56-61.
35. Head G a, McGrath BP, Mihailidou AS, et al. Ambulatory blood pressure monitoring in Australia: 2011 consensus position statement. J Hypertens. 2012;30(2):253-266.
36. Rümke CL. [regression to the mean]. Ned Tijdsch Geneeskd. 184AD;128(4):158.
37. Margolis KL, Asche SE, Bergdall AR, et al. Effect of home blood pressure telemonitoring and pharmacist management on blood pressure control: a cluster randomized clinical trial. Jama. 2013;310(1):46-56.
38. Mc Namara KP, Versace VL, Marriott JL, Dunbar J a. Patient engagement strategies used for hypertension and their influence on self-management attributes. Fam Pract. 2014;31(4):437-444.
39. Verberk WJ, Kroon A a., Lenders JWM, et al. Self-measurement of blood pressure at home reduces the need for antihypertensive drugs: A randomized, controlled trial. Hypertension. 2007;50(6):1019-1025.
40. Agarwal R, Bills JE, Hecht TJW, Light RP. Role of home blood pressure monitoring in overcoming therapeutic inertia and improving hypertension control: A systematic review and meta-analysis. Hypertension. 2011;57(1):29-38.

Chapter 5 -
Renal denervation beyond the bifurcation: the effect of distal ablation placement on safety and blood pressure.

Accepted for publication in J Clin Hyp

Martine M.A. Beeftink, Wilko Spiering, Mark R. de Jong, Pieter A. Doevendans, Peter J. Blankestijn, Arif Elvan, Jan-Evert Heeg, Michiel L. Bots, Michiel Voskuil

Abstract

Objectives
This analysis aimed to investigate the effect and safety of the spatial distribution of renal denervation radiofrequency applications on blood pressure reduction.

Background
The blood pressure-lowering effects of renal denervation vary considerably. Incomplete targeting of the renal sympathetic nerves may play a role herein. Anatomical studies have demonstrated that the nerves are located closer to the renal artery lumen in the distal segments, indicating that distal denervation may be more effective.

Methods
The angiography images of 97 patients who underwent renal denervation for hypertension were reviewed and categorized according to the location of the radiofrequency ablations: proximal to the bifurcation (group 1, n=39), or beyond the bifurcation in one artery (group 2, n=34) or in both arteries (group 3, n=24). Between-group differences in outcome and safety were assessed.

Results
Office blood pressure at 12 months decreased by 16/5 mmHg (SE 5/2) in bilateral proximal denervation, 13/11 mmHg (SE 4/2) in unilateral distal denervation and 11/6 mmHg (SE 6/3) in bilateral distal denervation (p = 0.15/0.72). The reduction in 24-hour blood pressure was 3/3 mmHg (SE 3/2) for bilateral proximal, 8/5 mmHg (SE 3/2) for unilateral distal and 10/6 mmHg (SE 4/2) for bilateral distal treatment (p = 0.17/0.26). No differences in adverse events were observed.

Conclusion
Renal denervation distal to the bifurcation is safe, but the current analysis does not provide solid evidence for improved efficacy. Further (preclinical) studies are needed to determine the optimal procedural approach and establish the full potential of renal denervation.

Introduction

Renal denervation (RDN) is developed as a non-pharmacologic treatment option for hypertension, particularly for those patients who fail to reach target blood pressure (BP) despite adequate antihypertensive medication.[1] RDN is performed by delivering radiofrequency (RF) energy to the renal artery lumen to damage the adjacent sympathetic nerves, thus reducing the efferent and afferent sympathetic signaling to and from the kidney. However, the effectiveness of RDN for lowering blood pressure varies greatly among studies and patients.[2-7]
This variability in treatment effect has been attributed partly to incomplete interruption of the renal sympathetic nerves due to insufficient penetration of the radiofrequency energy. We previously published a case-report that demonstrated histological damage restricted to maximum 2 mm from the luminal surface, leaving a large part of the nerves unaffected.[8] Recently, this observation was confirmed in a porcine model, with only 14% of nerves located within the lesion areas.[9] Thus, optimization of the RDN procedure is needed to ensure successful disruption of the sympathetic signaling. Several anatomical studies have investigated the anatomical distribution of the renal nerves and consistently demonstrated that the distance between the renal artery lumen and the renal sympathetic nerves decreases in the distal segments of the artery.[10-13] Therefore, it is to be expected that more successful disruption of the sympathetic nerves and subsequently more consistent treatment effects can be achieved through distal delivery of RF applications. However, in fear of excess complications due to smaller vessel diameters, RDN has been most commonly performed in the main renal artery only, where the distance between renal artery lumen and the sympathetic nerves is at its greatest.
Therefore, we studied a cohort of patients treated with renal denervation in two expert hospitals in the Netherlands to assess whether renal denervation beyond the bifurcation may be safe and whether differences could be observed in BP effects compared to proximal denervation.

Methods

This study is part of the Dutch National Renal Denervation Registry (NCT02482103) that was approved by the Medical Ethics Committee of the University Medical Center Utrecht. The study was conducted in accordance with the Declaration of Helsinki[14] and the Dutch Medical Research Involving Human Subjects Act. The registry holds clinical and procedural data of patients that are treated with RDN in the Netherlands.

Study population
Subjects were screened and treated according to the Dutch consensus on RDN for the treatment of hypertension.[15] Patients were eligible for renal denervation when suffering from resistant hypertension, defined as an office systolic BP ≥160 mmHg and/or a 24-hour systolic BP ≥135 mmHg, despite the use of at least 3 antihypertensive drugs from different classes at optimal doses, preferably including a diuretic.[16] Patients were also eligible if blood pressure remained above the same thresholds, but intolerance to antihypertensive drugs prevented adequate pharmacological treatment. The presence of pseudo-resistant hypertension and significant white coat effect was excluded by ambulatory blood pressure monitoring (i.e. mean 24-hour systolic BP ≥135 mmHg). Secondary causes of hypertension and pseudo-resistance were excluded by a hypertension specialist according to the international guidelines.[17] Exclusion of other contra-indications and the final decision for eligibility was made in a multidisciplinary team according to the consensus documents. Presence of obstructive sleep apnea syndrome was not considered a contra-indication if

patients were treated appropriately. In contrast to the consensus document, presence of multiple renal arteries was not considered a contra-indication for RDN based on advancing insights.

Location of renal denervation

For the current analysis, we reviewed cineangiographic images of all RDN procedures performed at the departments of cardiology of the University Medical Center Utrecht, Utrecht, and the Isala Hospital, Zwolle, between July 2010 and December 2014. Both centers recorded angiography images of the renal arteries at the start and the end of the procedure, as well as images of all individual ablations. To avoid inter-observer variability, all cineangiographies were evaluated by a single researcher who was blinded for clinical outcome. The location of each individual RF application in relation to the renal artery bifurcation was determined. Subsequently, patients were categorized in one of three groups: (1) bilateral proximal, if all ablations in both artery were performed proximal to the bifurcation; (2) unilateral distal, if only one artery was ablated proximal to the bifurcation and the contralateral artery was treated distally; and (3) bilateral distal, if in both arteries one or more ablations were performed beyond the bifurcation. The total number of ablations beyond the bifurcation for each artery and for each patient was determined, as well as the overall number of ablations per patient.

Renal denervation

All RDN procedures were performed via transfemoral approach according to the respective instructions for use of the device. The choice for the type of RDN catheter, the total number of RF applications, the location of the ablations, as well as whether or not to ablate beyond the bifurcation was at the discretion of the interventionalist. In practice, ablations were placed as distally as possible regardless of the presence of any branching, as long as the diameter of the vessel or branch was sufficient (i.e. ≥ 4 mm). The procedures

Table 1. Baseline characteristics.

	n=97
Sex, male	62 (64%)
Age	62 ± 10
BMI, kg/m^2	29 ± 5
eGFR, mL/min/1.73m^2	76 ± 16
Cerebrovascular history	14 (14%)
Cardiovascular history	19 (20%)
Periferal arterial disease	12 (12%)
Diabetes mellitus	20 (21%)
Dyslipidemia	39 (40%)
OSAS	5 (5%)
Office BP, mmHg	175/97 ± 26/15
24hour BP, mmHg	156/91 ± 19/14
Amount of AHD, DDD	4,9 ± 2,8
Number of AHD pills	4 (0-6)

BMI = body mass index, eGFR = estimated glomerular filtration rate, OSAS = obstructive sleep apnea syndrome, BP = blood pressure, AHD = antihypertensive drugs, DDD= daily defined dose.

Table 2. Procedural aspects.

	n=97
Device	
Medtronic Symplicity flex	79 (81%)
Medtronic Spyral	10 (10%)
St Jude EnligHTN	8 (8%)
Total ablations	13 (3-24)
Location of RDN	
Bilateral proximal	39 (40%)
Unilateral distal	34 (35%)
Bilateral distal	24 (25%)
Contrast administered, mL	174 ± 66

Procedural aspects of the renal denervation procedure. Abbreviations: RDN = renal denervation.

were performed by three experienced interventionalists (two electrophysiologists and an interventional cardiologist) with extensive experience with the procedure.

Measurements

Medical history, prescribed antihypertensive medication, lifestyle factors, physical examination, basic laboratory and blood pressure measurements were recorded at baseline and at 12 months follow-up. Ambulatory 24-hour (ABPM) and office BP measurements were performed in accordance with the international guidelines using validated BP monitors.[18]
Prescribed dosages of antihypertensive drugs for each time point were converted into defined daily doses (DDD) using conversion factors provided by the World Health Organization Drug Classification.[19] The cumulative daily intake of antihypertensive drugs was calculated for each patient using the sum of all DDD's.
Creatinine levels were recorded at baseline, preprocedural, postprocedural and at 12 months follow-up. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI method.[20]

Statistical analysis

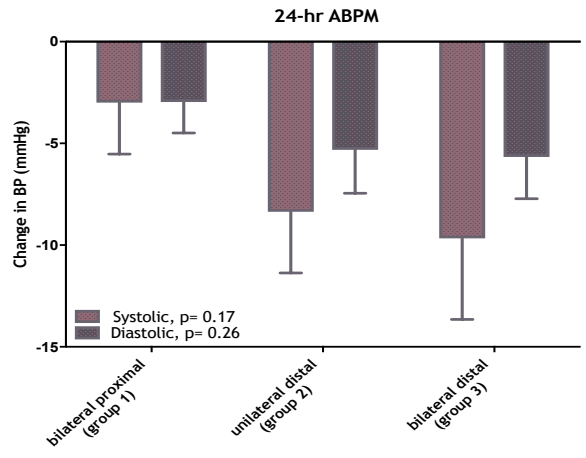
The difference between mean values of baseline and 12 months follow-up measurements (e.g. office BP, ABPM, BMI, eGFR) were calculated. Thus, a negative value represents a decrease at 12 months after RDN. Results are presented as mean ± standard deviation, mean (min.-max.) or as an absolute number with percentages, unless otherwise specified.
For paired samples analysis, the paired student t-test or Wilcoxon signed rank test was used when appropriate. Differences in blood pressure reduction between the three categories of RDN ablation patterns (i.e. (1) bilateral proximal, (2) unilateral distal, (3) bilateral distal) were assessed using the independent samples Jonckheere-Terpstra test for ordered alternatives. The Jonckheere-Terpstra test is a non-parametric method for testing differences between treatments. The advantage of the Jonckheere-Terpstra test over the Kruskal-Wallis test is that is more powerful when a particular direction in group medians is expected (i.e. bilateral proximal < unilateral distal < bilateral distal).[21]
Differences in blood pressure reduction between patients with proximal ablations only (group 1) and patients with distal ablation (group 2 and 3 combined) were assessed using the independent samples Mann Whitney U test. The relation between blood pressure changes

and the categories of RDN ablation placement were further assessed using multivariable linear regression models to correct for possible confounding factors by entering preselected variables (age, sex, eGFR, BMI, prescribed dosages of antihypertensive drugs and RDN device) into the model. The rule of thumb of ten cases per variable in multivariable analysis was applied to avoid over-fitted models.[22] Age and sex were entered into the crude model (model I) to create model II, and model III was composed of all abovementioned variables. The B coefficient for each model represents the change in blood pressure for each additional artery that is treated distal to the bifurcation.
Linear regression was also performed to assess the relation between the change in blood pressure and the total number of ablations, and the number of distal ablations. Results were considered statistically significant if the 95% confidence interval (CI) did not include 0 or if the two-tailed probability value (p-value) did not exceed 0.05. All analyses were performed with SPSS statistical software version 22 (IBM SPSS Data Collection, Chicago, Illinois, USA).

Results

Between July 2010 and December 2014, 123 patients were treated with renal denervation for hypertension at the University Medical Center Utrecht and the Isala Hospital Zwolle in the Netherlands. Ten patients were excluded from analysis because of unavailable or incomplete angiography data, and sixteen patients had no clinical follow-up available. The baseline characteristics of the remaining 97 patients are provided in table 1.
During 12-months follow-up, no significant changes occurred in BMI (Δ -0.1 ± 1.7 kg/m², p = 0.97), eGFR (Δ 1.5 ± 10 mL/min/1.73m², p = 0.50) or the amount of prescribed antihypertensive drugs (Δ -0.3 ± 2.7 DDD, p = 0.18).
Table 2 shows the procedure-related characteristics. Thirty-nine patients were ablated only

Figure 1. Change in 24-hour ambulatory blood pressure for different RDN locations.



This figure shows the change in 24-hour systolic (light purple) and diastolic (dark purple) ambulatory blood pressure for the different categories of RDN location. Error bars represent the standard error of the mean. The p-value represents the probability value for between-groups differences obtained by the Jonckheere-Terpstra test. ABPM = ambulatory blood pressure monitoring, BP = blood pressure.

Table 3. Absolute reductions in office and ambulatory blood pressure at 12 months after renal denervation in each treatment group.

	bilateral proximal	unilateral distal	bilateral distal	p-value*
Office blood pressure				
SBP	-16.0 ± 4,8	-13.0 ± 3.5	-10.7 ± 6.2	0.15
DBP	-5.4 ± 2.5	-10.6 ± 2.3	-6.0 ± 3.2	0.72
24-hour ambulatory blood pressure				
SBP	-3.0 ± 2.5	-8.4 ± 3.0	-9.7 ± 4.0	0.17
DBP	-3.0 ± 1.5	-5.3 ± 2.1	-5.7 ± 2.1	0.26

Table shows the change in office and 24-hour mean blood pressure from baseline to 12 month follow-up for each of the three treatment groups. Values are depicted as absolute changes ± standard error of the mean. *p-value for between-group differences. SBP = systolic blood pressure, DBP = diastolic blood pressure

proximal to the renal artery bifurcation (group 1), 34 patients were treated distal to the bifurcation in one of the renal arteries (group 2) and 24 patients were treated distally in both renal arteries (group 3). An average of 13 ± 4 (bilateral proximal), 13 ± 3 (unilateral distal) and 14 ± 3 (bilateral distal) RF applications were delivered in each patient (p = 0.2). Previous vascular disease (defined as coronary artery disease, cerebrovascular disease and/or peripheral coronary artery disease), as an approximation for more advanced arterial disease, was not statistically different among the three treatment groups (p = 0.52).

<u>Effects on blood pressure</u>
Overall, office blood pressure decreased from 175/97 mmHg (SD 26/15) at baseline to 161/89 mmHg (SD 25/15) at 12 months after RDN (p <0.001 for both systolic and diastolic BP). An absolute reduction of 14/8 mmHg (SE 3/2) was achieved for office BP. The changes in office and 24-hour mean BP in the different treatment groups are depicted in table 3.
Ambulatory BP measurements both before and 12 months after RDN were available for 70 patients. Unavailability of ABPM was mostly due to patient refusal. In the total patient group, mean 24-hour BP decreased from 156/91 mmHg (SD 19/14) at baseline to 146/84 mmHg (SD 19/13) after RDN (absolute reduction 7/4 mmHg, SE 2/1, p = 0.002 / < 0.001, figure 1). The changes in office and 24-hour BP between baseline and 12 months follow-up for the three groups were not significantly different.
In multivariable linear regression, the relation between BP effect and location of the ablations remained non-significant after correction for pre-specified variables (table 4). When comparing patients with proximal ablations only (group 1) to patients with distal ablations (group 2 and 3 combined), no significant differences in systolic office and ambulatory BP reduction was observed (p = 0.25 for office BP, p=0.15 for ambulatory BP). There was no relation between the change in systolic ambulatory BP (-0.3 mmHg for each ablation, 95% CI -1.3 to 0.7) and the total number of ablations. However, the change in systolic ambulatory BP was significantly related to the number of distal ablations (-1.7 mmHg for each distal ablation, 95% CI -3.1 to 0.4).

<u>Procedure related complications</u>
Ablation notches or spasm occurred in nineteen patients and required treatment with intra-arterial nitroglycerin in thirteen cases. In all patients the spasm resolved without any

clinically relevant sequelae (in three patients a non-significant stenosis remained without consequences for renal function). The occurrence of spasm was not related to the location of the ablations (p = 0,58). No other periprocedural complications occurred. Four post-procedural complications were recorded; three groin haematomas and 1 femoral pseudo-aneurysm, all of which resolved without sequelae. Peri-procedural renal function remained stable (73 ± 17 mL/min/1.73m^2 before the procedure, 72 ± 15 mL/min/1.73m^2 directly after the procedure (p = 0.87) and 71 ± 18 mL/min/1.73m^2 at 4 weeks after RDN (p = 0.98)).

Discussion

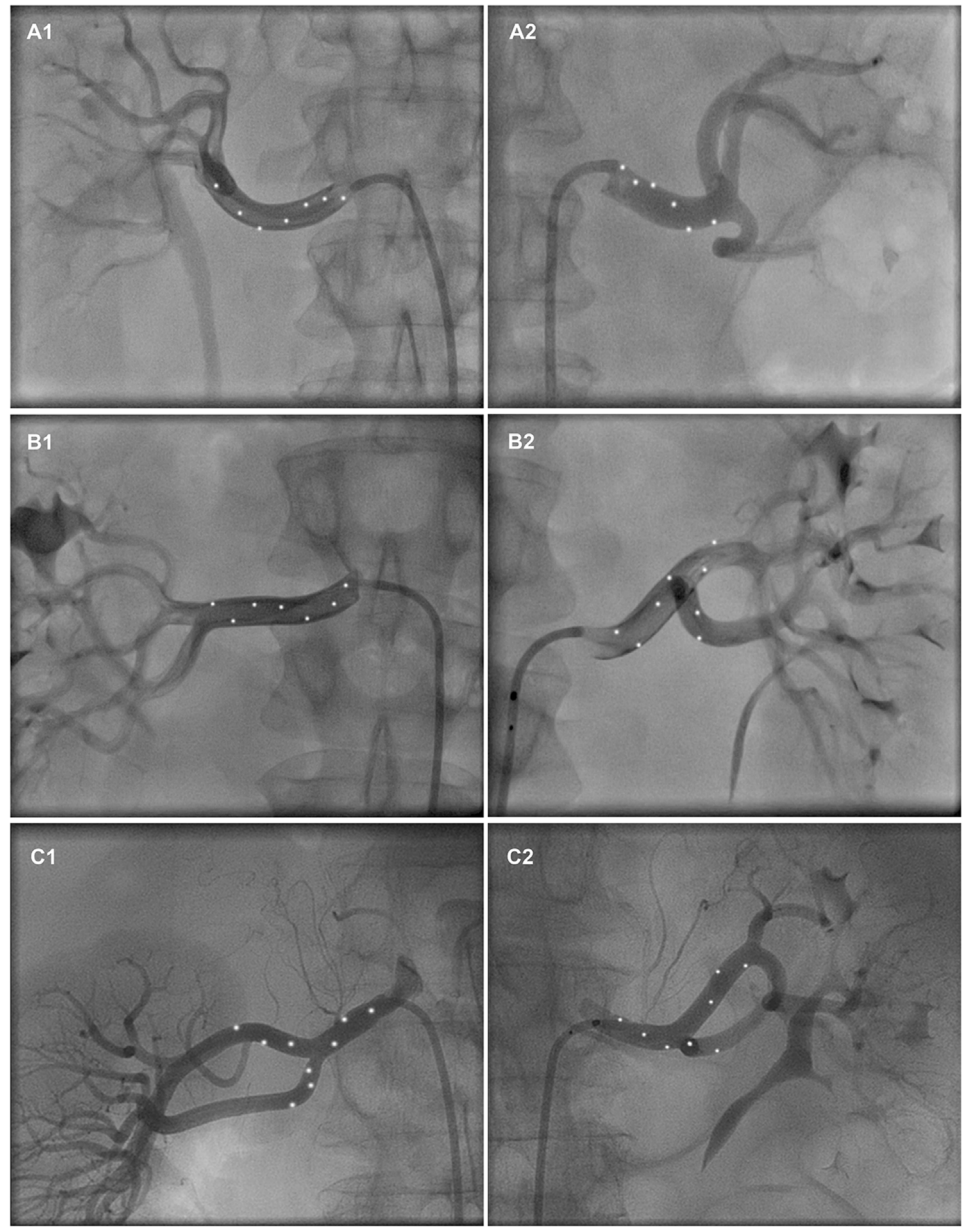
In the current analysis we demonstrated that RDN distal to the renal artery bifurcation may be feasible and safe. Despite the smaller diameter of renal artery branches, the occurrence of vascular spasm was similarly low in patients with distal ablations compared to proximal ablations, nor did we find an adverse effect on renal function. Yet, we did not demonstrate that denervation beyond the bifurcation is more potent, i.e. results in greater blood pressure reductions.
Recently, the focus of RDN research has shifted towards identification of predictors of success and optimization of the procedural approach in an attempt to explain and counteract the variation in blood pressure reductions among clinical trials and between patients within trials.[23] After the neutral results of the sham-controlled HTN-3 trial[4], experts from the US and Europe published important considerations for future studies, including procedural

Table 4. Univariable and multivariable regression analysis of denervation location and change in blood pressure.

	systolic BP			diastolic BP		
	n=	B	95% CI	n=	B	95% CI
Office blood pressure						
Model I (univariate)	93	2.7	(-4.2 to 9.6)	93	-0.7	(-4.4 to 3.1)
Model II (corrected for age, sex)	93	1.2	(-5.8 to 8.3)	93	-0.9	(-4.8 to 3.0)
Model III (corrected for age, sex, eGFR, BMI, DDD and device)	93	2.5	(-5.3 to 10.3)	93	-0.3	(-4.7 to 4.1)
24-hr ambulatory blood pressure						
Model I (univariate)	71	-3.5	(-7.9 to 0.9)	71	-1.4	(-4.2 to 1.3)
Model II (corrected for age, sex)	71	-3.1	(-7.8 to 1.6)	71	-1.2	(-4.1 to 1.7)
Model III (corrected for age, sex, eGFR, BMI, DDD and device)	71	-3.4	(-8.6 to 1.9)	71	-1,8	(-5.0 to 1.3)

Univariable and multivariable analysis of the relation between location of denervation and the change in blood pressure. The B coefficient represents the additional change in blood pressure for each artery that is ablated beyond the bifurcation. BP = blood pressure eGFR = estimated glomerular filtration rate, BMI = body mass index, DDD = daily defined dose of antihypertensive medication

Figure 2. Angiography examples of different RDN locations.



Angiography images of the right (A1,B1,C1) and left (A2,B2,C2) renal artery of three patients, demonstrating the different locations of the renal denervation procedures. White dots represent the location of the ablation points. Patient A was treated proximal to the renal artery bifurcation only (group 1), patient B was treated distal to the bifurcation in one renal artery (group 2) and patient C was treated distal to the bifurcation in both arteries (group 3).

aspects, trial design, patient selection, outcome measurements and preclinical studies.[24,25] The possible improvement of the procedural aspects relies for a large part on a better understanding of the depth, location and distribution of the renal sympathetic nerves, as well as the type and extend of tissue damage that is induced by RDN. In the last two years, several histological studies have been performed to fill these gaps in our knowledge.[10,12,13,26,27] These studies have uniformly demonstrated that the nerves surrounding the distal segments of the renal artery (containing the renal artery bifurcation) are smaller in number and located in closer proximity to the arterial lumen. This provides a foundation for the hypothesis that distal ablations are more effective: the RF energy could more easily reach the renal artery nerves in the perivascular tissue and fewer nerves need to be adequately targeted to disrupt the sympathetic pathway to and from the kidney. This hypothesis has been supported by preclinical research in canine and porcine models that have demonstrate greater reductions in renal norepinephrine (NE) levels when renal denervation was performed distal to the renal artery bifurcation.[11,28] Our results could not demonstrate solid data substantiating a clinical benefit from distal denervation. In linear regression, there was a relation between change in ambulatory BP and the absolute number of distal ablations, but we were unable to demonstrated significant differences in both office and ambulatory BP between patients treated bilateral proximal, unilateral distal or bilateral distal to the bifurcation.

There are several possible explanations for our findings. First, it is possible that, although the nerves are located closer to the renal artery lumen in the distal segments, they were still located out of reach for the RF energy preventing successful disruption of the sympathetic signaling to and from the kidney. The extent of nerve damage caused by RDN is poorly studied, partly because a functional test to assess the successful destruction of the renal sympathetic nerves in vivo is lacking. Electrical stimulation as used by Gal et al[29] is a promising technique to verify nerve destruction intra-operatively, but has not yet been validated. The currently available histological evidence indicates that the current catheters may have insufficient tissue penetration to adequately target the perivascular nerves in all patients. Vink et al. reported on the limited destruction of renal nerves after RDN in a case study of a human subject.[8] Subsequent animal studies have confirmed that the average depth of RDN lesions are confined to a maximum of 2.2 mm for the currently available catheters.[9,28] Given the fact that the average distance to the renal nerves ranges from 3.4 – 4.3 mm proximally to 2.0 - 2.6 mm in the distal segment, a large proportion of nerves is located out of reach, even if RDN is performed distal to the bifurcation.[10,12] Henegar et al. demonstrated that in the distal segment of the renal artery 96% of the nerves are located within 3.0 mm from the lumen-intimal transition, but RDN resulted in damage of only 50% of the nerves in that segment.[11] Moreover, deeper penetration does not necessarily lead to increased nerve damage. A study involving a prototype catheter achieved lesions up to 3.8 mm, but affected ≤20% of the nerves.[27]

This observation raises another critical issue. Thus far, instructions on the location, duration, intensity and circumference of the ablations have been based on expert knowledge aiming to achieve sufficient damage to the renal sympathetic nerves. However, compelling scientific evidence to support these recommendations is lacking. More importantly, it is unknown what percentage of damaged nerves is necessary to interrupt the sympathetic signaling to and from the kidneys. Several preclinical animal studies have demonstrated that the mean percentage of injured nerves does not exceed 50%, with percentages as low as 14%.[9,11,27,30,31] Whether these percentages are sufficient to achieve blood pressure reduction in humans is unknown, but the results of Tzafriri et al. suggest that higher percentages are needed: renal NE levels remained stable in seven out of eight treated renal arteries and were only significantly reduced in an artery with more than 60% nerve damage.[27]

Lastly, the translation from preclinical research to clinical practice may be too difficult. The majority of animal studies utilize a measurement of sympathetic nervous activity, such as renal tissue norepinephrine (NE) levels. However, direct measurement of sympathetic nervous activity in human subjects is not available and indirect measurements, such as muscle sympathetic nerve activity, are cumbersome. Although it is known that renal NE levels correspond to sympathetic nerve activity, the relation between renal NE levels and blood pressure has not been quantified yet. Henegar et al. previously demonstrated a correlation between blood pressure change and renal tissue NE levels in a hypertensive canine model, but these correlations were not statistically significant.[32] This may explain why we were unable to reproduce the results in the studies by Mahfoud et al.[28] and Henegar et al..[11]

Strengths and limitations

There are some limitations to our study. When interpreting the results of our study, it is important to realize that our study was a retrospective analysis and did not have the appropriate design to test for superiority. It was mainly meant as a hypothesis generating analysis. Further research, preferably in a randomized design, is needed to determine whether renal denervation distal to the bifurcation is an improvement of the current procedural technique. Furthermore, the lack of randomization may have introduced other potentially confounding factors to our study. The location of the denervation as well as the choice for RDN device was unprotocolized and left at the discretion of the interventionalist. This may have introduced bias, because the reasons to choose for a certain device or the location of the ablations are unknown. Systematic differences in patient sickness and arterial disease, among others, could have biased our results. Also, the difference in ablation location between study groups 1, 2 and 3 is less pronounced that it would have been in a randomized setting.[28] Further research in which the location of the ablations is predetermined should address these issues.

Since the current research question was derived from advancing insights and was not a premeditated analysis of the Dutch National Renal Denervation Registry, we were unable to do a power analysis. Our sample size is relatively small and may suffer from limited power after further separation into three groups. The sample size also prohibited proper subgroup analysis of a potential bias caused by the use of three different RDN catheter in our cohort. We did perform a sensitivity analysis including only the patients with the most used device (81%), which yielded similar results to the complete cohort. Lastly, we did not perform toxicological analysis to confirm adherence to medication.

Despite these limitations, this study adds to the current knowledge on RDN. We were among the first to analyze the effects of RDN beyond the bifurcation in human subjects. To this day, many clinical trials have restricted RDN to the renal artery segments proximal to the renal artery bifurcation out of safety concerns. However, we found no reason to believe that distal denervation poses an impermissible additional risk over the currently advised approach of proximal denervation that may obstruct prospective studies.

Conclusion

Renal denervation still is a relatively new field of research, with many gaps in our knowledge to be filled. The current study aimed to provide some insight in the effects and side effects of ablations in the distal segments in human subjects. Low incidence of vascular spasm and other adverse events in both proximal and distal denervation strategies were noted. This finding may facilitate the design and planning of future studies aimed to identify a superior treatment strategy for this procedure. Although we found a trend towards a dose-response relation between distal ablation placement and ABPM, we were unable to provide solid

evidence and further research, preferably in a randomized design, is needed to determine whether renal denervation distal to the renal artery bifurcation will improve the blood pressure lowering effect of the renal denervation procedure. Yet, many other gaps in our knowledge remain, including the achieved lesion depth, the achieved nerve damage and the threshold nerve damage that is needed for effective blood pressure reduction. A technique that could be used as readout of the procedure may further increase the success rate of renal denervation. In addition, a better understanding of the relation between outcomes in preclinical research (e.g. renal NE levels) and clinical outcomes used in human research (e.g. blood pressure reduction) will contribute to a better translation from bench to bedside. The field of renal denervation is likely to benefit greatly from (pre)clinical studies aimed to elucidate these issues.

References

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):1275-1281.
2. 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(9756):1903-1909.
3. Esler MD, Krum H, Schlaich M, Schmieder RE, Böhm M, Sobotka P a. Renal sympathetic denervation for treatment of drug-resistant hypertension: One-year results from the symplicity htn-2 randomized, controlled trial. Circulation. 2012;126(25):2976-2982.
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):1393-1401.
5. Desch S, Okon T, Heinemann D, et al. Randomized sham-controlled trial of renal sympathetic denervation in mild resistant hypertension. Hypertension. 2015;65(6):1202-1208.
6. 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):1957-1965.
7. Rosa J, Widimsky P, Tou ek 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. 2014;65(2):407-413.
8. Vink EE, Goldschmeding R, Vink A, Weggemans C, Bleijs RL a W, Blankestijn PJ. Limited destruction of renal nerves after catheter-based renal denervation: Results of a human case study. Nephrol Dial Transplant. 2014;29(8):1608-1610.
9. Verloop WL, Hubens LEG, Spiering W, et al. The Effects of Renal Denervation on Renal Hemodynamics and Renal Vasculature in a Porcine Model. PLoS One. 2015;10(11):e0141609.
10. Amsterdam WA. van, Blankestijn PJ, Goldschmeding R, Bleys RLAW. The morphological substrate for Renal Denervation: nerve distribution patterns and parasympathetic nerves. A post-mortem histological study. Ann Anat - Anat Anzeiger. 2015;204:71-79.
11. Henegar JR, Zhang Y, Hata C, Narciso I, Hall ME, Hall JE. Catheter-Based Radiofrequency Renal Denervation: Location Effects on Renal Norepinephrine. Am J Hypertens. 2015.
12. Sakakura K, Ladich E, Cheng Q, et al. Anatomic assessment of sympathetic peri-arterial renal nerves in man. J Am Coll Cardiol. 2014;64(7):635-643.
13. Tellez A, Rousselle S, Palmieri T, et al. Renal artery nerve distribution and density in the porcine model: biologic implications for the development of radiofrequency ablation therapies. Transl Res. 2013;162(6):381-389.
14. World Medical Association. WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. 2013;(June 1964):1-8.
15. Verloop WL, Agema WRP, Allaart CP, et al. Renal denervation for the treatment of hypertension: the Dutch consensus. Neth J Med. 2014;72(9):449-454.
16. 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(25):e510-e526.
17. ESH/ESC Task Force for the Management of Arterial Hypertension. 2013 Practice guidelines for the management of arterial hypertension of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC): ESH/ESC Task Force for the Management of Arterial Hypertension. J Hypertens. 2013;31(10):1925-1938.
18. O'Brien E, Asmar R, Beilin L, et al. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. J Hypertens. 2003;21(5):821-848.
19. WHO Collaborating Centre for Drug Statistics Methodology. Guidelines for ATC Classification and DDD Assignment 2015. 18th ed.; 2014. http://www.whocc.no/ddd/definition_and_general_considera/.
20. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-612.
21. Bewick V, Cheek L, Ball J. Statistics review 10: Further nonparametric methods. Crit Care. 2004;8(3):196-199.

22. Moons KGM, Royston P, Vergouwe Y, Grobbee DE, Altman DG. Prognosis and prognostic research: what, why, and how? BMJ. 2009;338:b375.
23. Persu A, Kjeldsen S, Staessen J a, Azizi M. Renal Denervation for Treatment of Hypertension: a Second Start and New Challenges. Curr Hypertens Rep. 2016;18(1):6.
24. Mahfoud F, Böhm M, Azizi M, et al. Proceedings from the European clinical consensus conference for renal denervation: considerations on future clinical trial design. Eur Heart J. 2015;36(33):2219-2227.
25. White WB, Galis ZS, Henegar J, et al. Renal denervation therapy for hypertension: pathways for moving development forward. J Am Soc Hypertens. 2015;9(5):341-350.
26. Roy AK, Fabre A, Cunningham M, Buckley U, Crotty T, Keane D. Post mortem study of the depth and circumferential location of sympathetic nerves in human renal arteries--implications for renal denervation catheter design. Catheter Cardiovasc Interv. 2015;86(2):E32-E37.
27. Tzafriri AR, Mahfoud F, Keating JH, et al. Innervation patterns may limit response to endovascular renal denervation. J Am Coll Cardiol. 2014;64(11):1079-1087.
28. Mahfoud F, Tunev S, Ewen S, et al. Impact of Lesion Placement on Efficacy and Safety of Catheter-Based Radiofrequency Renal Denervation. J Am Coll Cardiol. 2015;66(16):1766-1775.
29. Gal P, de Jong MR, Smit JJJ, Adiyaman A, Staessen J a, Elvan A. Blood pressure response to renal nerve stimulation in patients undergoing renal denervation: a feasibility study. J Hum Hypertens. 2015;29(5):292-295.
30. Steigerwald K, Titova A, Malle C, et al. Morphological assessment of renal arteries after radiofrequency catheter-based sympathetic denervation in a porcine model. J Hypertens. 2012;30(11):2230-2239.
31. Tzafriri AR, Keating JH, Markham PM, et al. Arterial microanatomy determines the success of energy-based renal denervation in controlling hypertension. Sci Transl Med. 2015;7(285):285ra65-ra285ra65.
32. Henegar JR, Zhang Y, Rama R De, Hata C, Hall ME, Hall JE. Catheter-Based Radiorefrequency Renal Denervation Lowers Blood Pressure in Obese Hypertensive Dogs. Am J Hypertens. 2014;27(October):1-8.

Part TWO:
Sympathetic hyperactivity and heart failure with preserved ejection fraction.

Chapter 6 -

A systematic review concerning the relation between the sympathetic nervous system and heart failure with preserved left ventricular ejection fraction

PLoS One 2015;10(2);e0117332

Willemien L. Verloop, Martine M.A. Beeftink, Bernadet T. Santema, Michiel L. Bots, Peter J. Blankestijn, Maarten J. Cramer, Pieter A. Doevendans, Michiel Voskuil

Abstract

Objective
This review summarizes the available literature regarding the relation between HFpEF and SNA.

Background
Heart failure with preserved left ventricular ejection fraction (HFpEF) affects about half of all patients diagnosed with heart failure. The pathophysiological aspect of this complex disease state has been extensively explored, yet it is still not fully understood. Since the sympathetic nervous system is related to the development of systolic HF, we hypothesized that an increased sympathetic nerve activation (SNA) is also related to the development of HFpEF.

Methods
Electronic databases and reference lists through April 2014 were searched resulting in 7722 unique articles. Three authors independently evaluated citation titles and abstracts, resulting in 77 articles reporting about the role of the sympathetic nervous system and HFpEF. Of these 77 articles, fifteen were included for critical appraisal: six animal and nine human studies.

Results
Based on the critical appraisal, we selected nine articles (three animal studies and six human studies) for further analysis. In all the animal studies, isoproterenol was administered to mimic an increased sympathetic activity. In human studies, different modalities for assessment of sympathetic activity were used. The studies selected for further evaluation reported a clear relation between HFpEF and SNA.

Conclusion
Current literature confirms a relation between increased SNA and HFpEF. However, current literature is not able to distinguish whether enhanced SNA results in HFpEF, or HFpEF results in enhanced SNA. The most likely setting is a vicious circle in which HFpEF and SNA sustain each other.

Introduction

Heart failure with preserved left ventricular ejection fraction (HFpEF) affects about half of all patients with a clinical presentation of heart failure (HF).[1,2] There is no consensus concerning the definition of HFpEF. The European guidelines define HFpEF as a clinical syndrome in which classical HF symptoms are present, accompanied by a normal or only mildly reduced left ventricular (LV) systolic function.[3] Using this definition, HFpEF becomes a mixed collection of different underlying causes of HF. The American Heart Association (AHA) guidelines and the consensus statement of the European Society of Cardiology (ESC) define HFpEF as a clinical HF state, which is accompanied by objective evidence of diastolic dysfunction (DD).[4,5]
Irrespective of the definition, we still have much to learn about HFpEF. This is all the more important since no successful treatment is available yet.[3,4] A number of studies have been conducted investigating different pharmacological treatment strategies for HFpEF. Unfortunately, these studies failed to provide unambiguous results.[6-10] Even though HFpEF has been the focus of various mechanistic studies, the exact pathophysiology is still unknown.[11]
It is generally accepted that HFpEF is characterized by prolonged isovolumic LV relaxation, slow LV filling, and an increased diastolic LV stiffness.[12] The consequent impairment of diastolic filling leads to an inappropriate pressure increase after volume load.[13] Eventually, this may lead to heart failure.[14,15]
The sympathetic nervous system (SNS) may play an important role in the genesis of HFpEF when accompanied by DD.[16] The underlying structural changes in the myocardium seen in HFpEF include the same spectrum of changes associated with catecholamine-induced cardiomyopathies.[17,18] However, while the role of the increased sympathetic nerve activity (SNA) in the development and progression of HF with reduced ejection fraction (HFrEF) is well established[19,20], to our knowledge, no systematic review has yet evaluated the relationship between SNA and HFpEF. Therefore, the objective was to systematically evaluate the role of SNA in HFpEF. In this respect, only HFpEF in combination with DD is taken into account.
The activity of the SNS can be measured in different ways. Examples are measurement of plasma or urinary norepinephrine (NE) level, assessment of local NE spillover, muscle sympathetic nerve activity (MSNA), iodine 123-metaiodobenzylguanidine (MIBG), or heart rate variability (HRV).

Methods

Search strategy
This systematic review was conducted and reported in accordance with the “preferred reporting items for systematic reviews and meta-analyses” (PRISMA) statement.[21]
We conducted a systematic review to determine if there is a relationship between the sympathetic nervous system and heart failure with preserved LVEF. All available literature in the PubMed, Embase and Cochrane databases was searched using a pre-defined search strategy. A librarian checked the syntax before the search was conducted.
The titles and abstracts of the retrieved articles were reviewed by three authors (WLV, MMAB, BTS). Full-text papers were retrieved from abstracts selected for further review. The references of these papers were also reviewed to identify relevant articles that may have been missed by the search strategy, e.g. studies that were not found due to negative results. If necessary, individual researchers were contacted by e-mail to obtain the full text, or to enquire about unpublished or unreported results.
All full-text articles were reviewed by three authors (WLV, MMAB, BTS) using pre-defined inclusion/exclusion criteria (Table 1). Articles were only included when the inclusion criteria of HFpEF were clearly defined. Only articles that included DD in the definition of HFpEF

were included, studies about HFpEF based on valvular dysfunction or other disease entities were excluded. Citations from journals in languages other than English were not included. No pre-specified limitations were placed regarding species (human or animal) or NYHA functional class. Individual case reports, editorials, expert opinions, and review articles were excluded, as were studies regarding the diagnosis or treatment of HFpEF. Studies investigating the prognosis of patients with HFpEF were also excluded. To minimize the risk of multiple publication bias, we only included publications with a pathophysiological objective when they contained original data. We pre-specified the data to be extracted from the included studies before reading the articles. Two authors (WLV, MMAB) independently extracted these data and listed them in a table.

Critical appraisal and analysis

Critical appraisal was independently performed using pre-set criteria. These criteria are outlined in table 2 and table 3. In advance we decided only to include an article in the final analysis if it scored at least half of the maximum available points. Since we expected that there would be a large diversity in outcome measures, no pre-defined principal summary measures were composed. Studies were graded for the modality used to measure sympathetic activity, as reliability of these modalities differ.[22,23] Local NE spillover, MSNA, and MIBG are considered as the best and most direct measures of sympathetic activity.[24,25] Serum levels of NE and HRV are an indirect measure of SNA and are considered as less qualitative measures of sympathetic activity.[23-25]

Results

The search was conducted on October 31st, 2013 and identified 7722 unique articles. The search was updated on April 10th, 2014. A flowchart of the search is depicted in figure 1. After screening the titles and abstracts 77 articles remained that met the criteria for full text review. Fifteen full-text articles (6 animal studies; 9 human) were considered relevant to the study and were included in the critical appraisal.[26-40]

Table 1. Pre-set inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
1. Investigating the relationship between HFpEF and the sympathetic nervous system.	1. Investigating systolic heart failure.
2. Investigating the relationship between diastolic dysfunction and the sympathetic nervous system.	2. Only investigating LVH without giving information about diastolic dysfunction.
	3. No original data (i.e. review, expert opinion).
	4. Study does not investigate relation SNA and HF.
	5. Only abstract.
	6. Full text in language other than English.
	7. Therapeutic study.
	8. HFpEF based on valve dysfunction, myocardial ischemia or hypertrophic cardiomyopathy.
	9. Prognostic study.

Animal studies

The animal studies that met the criteria for critical appraisal investigated whether a change in activity of the sympathetic nervous system might induce diastolic dysfunction. All animal studies used a model of isoproterenol (ISO) to mimic an increased SNA. Isoproterenol is a non-selective ß-adrenergic agonist structurally similar to epinephrine. The assumed increase in SNA was not measured in any of the animal studies.

Three animal studies scored high (4 points or more) in the critical appraisal and were therefore further evaluated.[26,27,31] Rejected studies scored less than half of the available points mainly because the study-aim was insufficiently focused on DD or HFpEF.

In brief, the three selected animal studies demonstrated that administration of a ß-adrenergic agonist established diastolic dysfunction in an experimental setting: the first study is the study from Grimm et al. Authors observed that dosages up to 150 mg/kg ISO led to diastolic dysfunction in mice as evaluated by echocardiography.[27] Dosages higher than 150 mg/kg led to systolic heart failure or death.[27] In the second study: the study of Brooks et al. an abnormal LV diastolic pressure-volume (PV) relationship and an increased myocardial stiffness in mice treated with 10 mg/kg ISO for five days was observed without impairment of systolic function.[26] The third study: the study of Yoshikawa et al. treated rats with 2.4 mg/kg/day ISO for seven days and observed a significant increase in fibrosis, accompanied by an increase in LV hypertrophy by histology and a decrease in diastolic function by echocardiography.[31]

A major drawback of these three studies is that the percentage of animals in which ISO administration did or did not lead to DD or HFpEF ('responder rate') was not clearly reported. Impairment of DD was already observed after a dosage of 2.4 mg/kg/day ISO for seven days. A single dosage of 150 mg/kg ISO also induced DD. Higher dosages led to systolic heart failure.

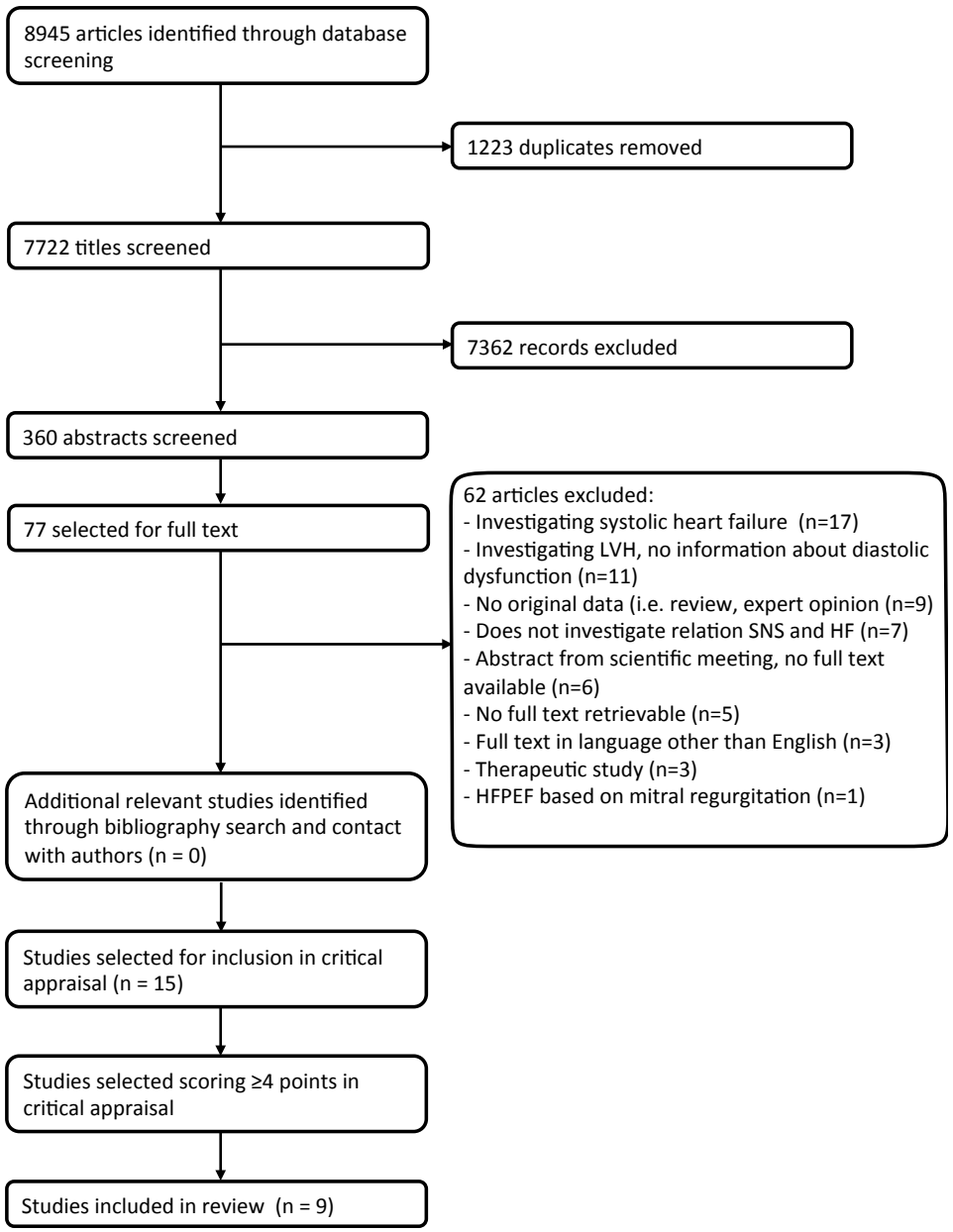
Human studies

No studies could be found that prospectively studied a human cohort whether an increased SNA leads to HFpEF during follow-up. In most human studies evaluated by the critical appraisal, patients were included that already had DD based on echocardiographic data. Therefore, the natural progression to HFpEF could not be studied.

All studies were hindered by a small sample size of patients (max. 34 patients). Another limitation is that not all studies reported the diastolic parameters: Sugiura and Arora et al. included HF-patients with a good ventricular function (LVEF>45%) irrespective of diastolic parameters.[32,38] Echocardiographic examination was often limited to E/A ratio and deceleration time, despite that E/e' is nowadays considered a more reliable parameter.[5] An explanation may be the evolving guidelines on assessment of diastolic function that did not involve E/e' at the time the first studies were conducted. For the assessment of SNA, different entities were used in the human studies (i.e. heart rate variability (HRV), MSNA, MIBG, or norepinephrine levels). Six out of nine human studies scored 4 points or more in the critical appraisal and were therefore further evaluated.

In brief; the six human studies selected for further evaluation confirmed a relationship between SNA and HFpEF. Arora et al. observed that patients with HFpEF exhibit a reduction in HRV compared to control subjects.[32] Patients with HFrEF had more decreased HRV values compared to patients with HFpEF. In the study of Grassi et al. abnormal baroreflex modulation and increased MSNA-levels were observed in hypertensive patients with DD compared to hypertensives without DD and normal controls.[33] Nixdorff et al. observed an increase of peak early (E-wave) and late (A-wave) diastolic filling velocities and a shortening of deceleration time after administration of even the lowest dose of ISO (0.1 ug/min).[35] Piccirillo et al. performed HRV and observed that hypertensives with diastolic dysfunction have a higher sympathetic and lower vagal modulation of the sinus node compared to hypertensives

Figure 1. Flowchart of the search.



without DD and normotensive controls.[36] deSouza et al. observed that patients with HFpEF had higher MSNA values compared to hypertensive patients with normal diastolic function although HRV values were similar among the groups.[37] In the study of Sugiura et al. it was concluded that cardiac SNA as assessed by MIBG increases proportionally with severity of HFpEF.[38]

Discussion

To our knowledge, this is the first systematic review investigating the relationship between sympathetic nerve activity and HFpEF in combination with DD. Based on the animal studies we concluded that administration of ISO and therefore increased SNA is related to HFpEF in an experimental setting. Based on the human studies, we concluded that an increased SNA - irrespective of the method of assessment - is indeed related to diastolic dysfunction and/or true HFpEF. However, the available literature about this topic is very scarce, let alone that results could be pooled. Moreover, administration of ISO in animals is not exactly the same as the increase in SNA in humans. However, no animal model to induce HFpEF has been accepted so far and we consider ISO administration the best available at present.

As mentioned above, different definitions are used for HFpEF.[3,5] In the current review, the recommendations from the AHA guidelines and the ESC consensus document were followed, thereby excluding HFpEF based on hypertrophic cardiomyopathy among others.[4,5] We chose to follow this line in order to obtain a set of studies with a more or less homogeneous patient population.
In the human studies, different ways of assessing sympathetic activity were used. The most straightforward method to measure SNS is measurement of plasma or urinary (NE) level. Plasma NE concentrations however are a resultant of removal rates and not selectively release rates.[41] Also, the precise origin of urinary NE levels is a matter of debate. Assessment of local NE spillover by a radiotracer technology displays the rate at which NE is released from the sympathetic nerves into the circulation. This is quantified by intravenous infusion of titrated NE combined with regional sampling. MSNA is a real-time measure of sympathetic nerve activity. Multiunit recordings of efferent postganglionic MSNA are obtained with a tungsten microelectrode into a muscle fascicle of the peroneal nerve.[42] MIBG imaging uses a norepinephrine analogue labeled with a radioactive isotope to image adrenergic receptors in many organs, including the heart.[43] MIBG imaging has been shown a very reliable marker of sympathetic activity in both HFpEF and HFrEF disease states.[44] Moreover, Nakata et al demonstrated the long-term prognostic value of altered cardiac sympathetic function as assessed by MIBG imaging in HF patients.[45] HRV displays the variability of the resting heart rate and is a measure of the balance between the sympathetic- and parasympathetic nervous system.[46] HRV is linked through the baroreceptor reflex and a more indirect way to measure SNA.[23] Not all ways of SNA measurement are even reliable. This supposed difference in reliability was taken into account in the critical appraisal. Although NE-spillover and MSNA are among the most reliable ways to measure SNA, they are semi-invasive and time-consuming. Moreover, MSNA is hard to obtain in small animals like mice.[25] MIBG washout rate has a strong correlation with MSNA and therefore allows non-invasive assessment of general sympathetic nerve activity.[24] In the studies selected for critical appraisal, local NE spillover was not used as a method to quantify sympathetic activity.

The study of Grassi et al. showed conflicting results; no difference in plasma NE-concentration was observed whereas MSNA values were altered in the patients with DD.[33] In our analysis, the results of MSNA were taken into account whereas the NE-results were not due to the limited sensitivity of these markers of sympathetic tone. In the studies of Arora et al. and

Table 2. Critical appraisal of animal studies.

First author, year	Study aim	Clearly defined hypothesis	Model to induce HFPEF	Assess-ment of diastolic dysfunc-tion	Assess-ment of sympa-thetic activity	Clear report of findings	Value of study	Score
Grimm[27], 1998	+	+/-	+	+	+/-	+/-	+	4
Krishnamurthy[28], 2007	-	+/-	+	+	+/-	+/-	+/-	1
LaCroix[29], 2008	-	+	+	+	+/-	+	-	2
Brooks[26], 2009	+	+/-	+	+	+/-	+	+/-	4
Ma[30], 2011	-	+/-	+	+	+/-	+/-	+/-	1
Yoshikawa[31], 2012	+	+	+	+	+/-	+/-	+/-	4

Study aim:+: study is focused on interpreting the relation between sympathetic activity (SNA) and diastolic dysfunction (DD); -: study is not focused on interpreting the relation between SNA and DD . Clearly defined hypothesis: +: hypothesis clearly defined; +/-: aim of study clearly defined, no hypothesis formulated; -: no clear aim nor hypothesis. Model to induce HFPEF: +: ISO infusion; -: transaortic constriction. Assessment of diastolic dysfunction: +: invasive measurement of LV diastolic filling pressures or echocardiographic evaluation of DD according to latest ESC guidelines; +/-: echocardiographic evaluation without use of E/E'; -: confirmation of normal LVEF only. Assessment of sympathetic activity: +: yes; +/-: no. Clear report of findings: +: results clearly described AND critical about own research; +/-: results clearly described OR critical about own research; -: results not clearly described AND not critical about own research. Value of study: To what extent is the study relevant to answering the current question.
The score displayed in the right column is the sum of scores: "+" accounts for 1 point; "+/-" for 0 points; "-" for -1 point.

Table 3. Critical appraisal of human studies.

First author, year	Study design	Number of patients	Study aim	Clearly defined study aim	Patient selection	Assess-ment of diastolic dysfunc-tion	Assess-ment of sympa-thetic activity	Clear report of findings	Value of study	Score
Nixdorff[35], 1997	Cohort	10	++	+	N/A	+/-	+/-	+	+/-	4
Hirono[34], 2001	Cohort	26	-	-	+	+/-	+/-	+	-	-1
Vinch[40], 2003	Cross-sectional	14	-	+	+	+/-	-	+/-	-	-1
Arora[32], 2004	Cross-sectional	19	++	+	+	-	+/-	+	+	5
Piccirillo[36], 2006	Cross-sectional	30	++	+	+	+/-	+/-	+	+	6
Sugiura[38], 2006	Cohort	34	+	+	+	+/-	+	+	+	6
Tsuchida[39], 2007	Cross-sectional	8	-	+	+	-	+	+	-	1
Grassi[33], 2009	Cross-sectional	17	+	+	+	+/-	+	+	+	6
deSouza[37], 2013	Cross-sectional	15	+	+	+	+	+	+	+	7

Number of patients =: Number of patients with diastolic dysfunction/HFpEF. Study aim: ++: study is focused on interpreting the relation between SNA and DD AND patient selection was clearly explained (diastolic dysfunction defined and not just distinction between LVEF </> 45%) AND data collection was clear. +: study is focused on interpreting the relation between SNA and DD AND patient selection was clearly explained OR data collection was clear; -: study is focused on interpreting the relation between SNA and DD OR patient selection was clearly explained OR data collection was clear; --: study is not focused on interpreting the relation between SNA and DD AND/OR patient selection was not clearly explained AND/OR data collection was not clear. Patient selection: +: Sole HFpEF or clear distinction between HFpEF and HFrEF; -: no clear distinction between HFpEF and HFrEF. Assessment of diastolic dysfunction: +: invasive measurement of LV diastolic filling pressures OR echocardiographic evaluation of DD according to latest ESC guidelines; +/-: echocardiographic evaluation without use of E/E'; -: confirmation of normal LVEF only. Evaluation of sympathetic activity: ++: NE-spillover locally measured; +: MSNA OR MIBG; +/-: HRV or adrenergic stimulation; -: plasma NE concentration. Clear report of findings: +: results clearly described AND critical about own research; +/-: results clearly described OR critical about own research; -: results not clearly described AND not critical about own research. Value of study: To what extent is the study relevant to answering the current question.
The score displayed in the right column is the sum of scores: "+" accounts for 1 point; "+/-" for 0 points; "-" for -1 point.

Piccirillo et al., HRV was assessed to obtain information about the parasympathetic nervous system.[32,36] In the critical appraisal these studies scored less due to the use of HRV. Since HRV is an indirect measurement of SNA, it is not known whether the results of Arora et al. may lead to an over- or underestimation of the relation between HFpEF and SNA.[23]

Can the results be explained by more diseased states?

From a critical point of view, some experts argued that the increased SNA in patients with DD can be attributed to the higher BP since that is often present in patients with DD.[47] To respond to this criticism, Grassi et al. and Piccirillo et al. only included patients with similar blood pressure (BP) levels.[33,36] Based on these two studies, we argue that increased sympathetic activation seen in DD is not attributable to a more diseased hypertensive state.[33,36] As left ventricular hypertrophy (LVH) is also related to an increased SNA and often present in DD, the presence of LVH may have caused us to overestimate the increased SNA.[48] Yet, Grassi et al. found similar LV masses among both hypertensive groups (with and without DD).[33]

Since ß-adrenergic stimulation often causes both HFpEF and HFrEF, SNS-induced HFpEF could be a precursor state of HFrEF or both diseases could be entities resulting from increased SNA.[49] The study of Grimm et al. contributes to this discussion by showing systolic HF after administration of higher dosages of ISO.[27] Seeland et al. described comparable results in a mouse model undergoing adrenergic stimulation: five months after stimulation LVH was observed in all mice.[50] However, 12 months after stimulation ventricular dilatation and accompanying systolic dysfunction was observed in all mice.[50] More recent studies, however, have made clear that the two diseases are indeed two separate entities.[49,51]

Diastolic dysfunction and SNA; the chicken or the egg?

As Rosendorff previously discussed, it is not yet clear whether DD potentiates the sympathetic activation or whether the increased SNA causes DD.[47] Based on their results, Grassi et al. concluded that DD enhances the already elevated MSNA.[33] However, the authors admitted that their data did not allow them to determine whether the greater SNA observed in patients with DD is the cause or the consequence of the cardiac alteration.[33] Other studies included in the current review concluded that it should be the other way around.[26,35] Moreover, sympathico-inhibition has shown to delay the progression of DD.[52-55] Finally, Leite-Moreira et al. showed that ß-adrenergic stimulation influences cyclic AMP, resulting in a changed diastolic relaxation.[56] Rosendorff set out two plausible ways that increased SNA causes DD: an indirect and a direct way. In the indirect way SNA induces hypertension, which imposes a mechanical load on the LV and consequent stiffening of the ventricles.[47] In the direct way, SNA has a direct effect on both hypertension and diastolic dysfunction.[47] Increased SNA activity plays a role in cardiac remodeling.[57] This is illustrated by the fact that sympathetic stimulation can induce pro-inflammatory cytokine expression[58] and can induce alterations in the sarcoplasmic reticulum, plasma membrane, and cytoskeletal proteins.[12,56] However, one crucial question cannot be answered with the above theories: what is the trigger for an increased SNA? A mechanism is needed that is responsible for SNS stimulation. In HFrEF an ischemic model can be the precursor of increased SNA.[59] However, in HFpEF ischemia is often not present as a precursor of SNA.[4]

With the current available evidence, we cannot simply state that one causes the other. Paulus et al. has clearly set out that different comorbidities contribute to a systemic inflammatory state, which induces oxidative stress in the coronary microvascular endothelium.[60] The presence of reactive oxygen species (ROS) is important in the paradigm proposed by Paulus et al.[60] ROS are strongly believed to be related to increased SNA.[61,62] We believe that a vicious circle is present in which HFpEF and SNA sustain each other. It is highly likely that other factors like the metabolic syndrome or renal ischemia are triggers for this circle.[63]

The effect of sympathico-inhibition on HFpEF and DD

Until now, no treatment has yet convincingly shown to improve clinical status, morbidity and mortality in HFpEF.[3,6] It is of relevance whether therapies targeting the sympathetic nervous system are successful in HFpEF. Based on their sympatholytic effect, beta-blockers may be useful in HFpEF. The SENIORS trial suggested that nebivolol may be beneficial in elderly patients with HFpEF.[8] As a derivative of SNA, angiotensin II may be a target for treatment. While RAS inhibition has been shown to reduce SNA,[64] the CHARM study found no clear benefit in patients with HFpEF treated by RAS inhibition.[65] Varying results were reported in other studies investigating the effect of RAS-inhibition in patients with HFpEF: in a meta-analysis, RAS-inhibition was not associated with consistent reduction in HF hospitalization or mortality in HFpEF-patients.[66]

Echocardiography is a reliable tool to objectively assess diastolic function. The effects on echocardiography should be taken into account when conducting a therapeutic study in a HFpEF population. Echocardiographic parameters have been used in some studies investigating aldosterone antagonists, beta-blockade, exercise training, and RAS-inhibition. Although some studies reported an improvement in clinical state, no clear effects on echocardiographic parameters were observed.[10,67,68] Based on our results, it is plausible that modulation of SNA can improve the clinical status of patients with HFpEF. Unfortunately, no studies investigating a treatment for HFpEF have evaluated SNA after treatment. One study of interest is that of Brandt et al. that recently showed an improvement in diastolic function after renal denervation.[69] The authors established a decrease in sympathetic activity by renal denervation and consequently observed improvement in echocardiographic parameters.[69] Therefore, renal denervation may be an attractive option for the treatment of HFpEF by disrupting the vicious circle between HFpEF and hyperactive SNA.

Limitations of the current review

First of all, the available evidence is limited and heterogeneous in design. This heterogeneity may have influenced our results. We tried to uniform the different studies by using the critical appraisal.

Although our search strategy was extensive and also focused on studies that showed HFpEF and SNA to be unrelated, it is possible that our search resulted in a relative over-representation of positive studies. By checking references of selected articles we tried to obtain studies that reported negative findings about the suggested relation. This search did not result in any relevant full text articles.

In the human studies, we could not report about medical history, medication use and other determinants for heart failure because these determinants were not clearly reported. It should, however, be taken into account that most patients studied were treated with drugs that affect sympathetic activity. Since most antihypertensive drugs (indirectly) lower SNA[70], the currently observed relation between SNA and HFpEF may be an underestimation. Potentially, an even stronger association between SNA and HFpEF does exist.

Not all human studies included patients with HFpEF; half of the human papers studied patients with DD instead of HFpEF. However, DD is an important, if not the main, precursor of HFpEF. Moreover, Sugiura et al. showed a higher cardiac sympathetic activity in HFpEF patients with a higher NYHA class.[38] Though, at this point we should keep in mind that the clinical symptoms in HFpEF are not solely explained by DD, but can also be explained by reduced chronotropic, vasodilator, and cardiac output reserve during exercise.[71]

A possible confounding factor in this review is that HFpEF-patients are more often older. Age itself is related to sympathetic activity and may be a confounder in the available literature.[4] Selected studies did not report whether they corrected for the baseline characteristics.

To complicate matters, HFpEF has only recently been recognized as an important clinical problem and preserved ejection fraction was previously often considered as a diagnosis of exclusion.[16] Therefore the available literature is less extensive compared to HFrEF.

Conclusion

Based on our results, we conclude that current literature confirms a relation between increased SNA and HFpEF. However, current literature is not able to distinguish whether enhanced SNA results in HFpEF, or HFpEF results in enhanced SNA. The most likely setting is a vicious circle in which HFpEF and SNA sustain each other. Disruption of this vicious circle may be an attractive treatment option.

Acknowledgements

We gratefully acknowledge dr. ir. Kramer for her assistance in the search strategy of this review.

References

1. Mahadevan G, Dwivedi G, Williams L, et al. Epidemiology and diagnosis of heart failure with preserved left ventricular ejection fraction: rationale and design of the study. Eur J Heart Fail 2012;14:106–112.
2. Mosterd A, Hoes AW. Clinical epidemiology of heart failure. Heart 2007;93:1137–1146.
3. Mcmurray JJ V, Adamopoulos S, Anker SD, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012. Eur J Heart Fail 2012;14:803–869.
4. Yancy CW, Jessup M, Bozkurt B, et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2013;62:e147–239.
5. Paulus WJ, Tschope C, Sanderson JE, et al. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction. Eur Heart J 2007;28:2539–2550.
6. Borlaug BA, Paulus WJ. Heart failure with preserved ejection fraction: pathophysiology, diagnosis, and treatment. Eur Heart J 2011;32:670–679.
7. Daniel KR, Wells G, Stewart K, et al. Effect of aldosterone antagonism on exercise tolerance, doppler diastolic function, and quality of life in older women with diastolic heart failure. Congest Hear Fail 2009;15:68–74.
8. Van Veldhuisen DJ, Cohen-Solal A, B??hm M, et al. Beta-Blockade With Nebivolol in Elderly Heart Failure Patients With Impaired and Preserved Left Ventricular Ejection Fraction. Data From SENIORS (Study of Effects of Nebivolol Intervention on Outcomes and Rehospitalization in Seniors With Heart Failure). J Am Coll Cardiol 2009;53:2150–2158.
9. Yusuf S, Pfeffer M a, Swedberg K, et al. Effects of candesartan in patients with chronic heart failure and preserved left-ventricular ejection fraction: The CHARM-preserved trial. Lancet 2003;362:777–781.
10. Edelmann F, Wachter R, Schmidt AG, et al. Effect of spironolactone on diastolic function and exercise capacity in patients with heart failure with preserved ejection fraction: the Aldo-DHF randomized controlled trial. JAMA 2013;309:781–791.
11. Van Kraaij DJW, Van Pol PEJ, Ruiters AW, et al. Diagnosing diastolic heart failure. Eur J Heart Fail 2002;4:419–430.
12. Zile MR, Baicu CF, Gaasch WH. Diastolic heart failure--abnormalities in active relaxation and passive stiffness of the left ventricle. N Engl J Med 2004;350:1953–1959.
13. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function. Circulation 2002;105:1387–1393.
14. Mandinov L, Eberli FR, Seiler C, Hess OM. Diastolic heart failure. Cardiovasc Res 2000;45:813–825.
15. Wachter R, Schmidt-Schweda S, Westermann D, et al. Blunted frequency-dependent upregulation of cardiac output is related to impaired relaxation in diastolic heart failure. Eur Heart J 2009;30:3027–3036.
16. Hogg K, McMurray J. Neurohumoral pathways in heart failure with preserved systolic function. Prog Cardiovasc Dis 2005;47:357–366.
17. Wright JW, Mizutani S, Harding JW. Pathways involved in the transition from hypertension to hypertrophy to heart failure. Treatment strategies. Heart Fail Rev 2008;13:367–375.
18. Cacciapuoti F. Molecular mechanisms of left ventricular hypertrophy (LVH) in systemic hypertension (SH)-possible therapeutic perspectives. J Am Soc Hypertens 2011;5:449–55.
19. Grassi G, Seravalle G, Cattaneo BM, et al. Sympathetic activation and loss of reflex sympathetic control in mild congestive heart failure. Circulation 1995;92:3206–3211.
20. Eisenhofer G, Friberg P, Rundqvist B, et al. Cardiac sympathetic nerve function in congestive heart failure. Circulation 1996;93:1667–1676.
21. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med 2009;6:e1000100.
22. Esler M, Jennings G, Lambert G, et al. Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. Physiol Rev 1990;70:963–985.
23. Vink EE, de Jager RL, Blankestijn PJ. Sympathetic hyperactivity in chronic kidney disease: pathophysiology and (new) treatment options. Curr Hypertens Rep 2013;15:95–101.
24. Yuyama R, Yuasa F, Hikosaka M, et al. Iodine 123-metaiodobenzylguanidine imaging reflect

generalized sympathetic activation in patients with left ventricular dysfunction. Clin Physiol Funct Imaging 2005;25:34–39.
25. Hilz MJ, Dutsch M. Quantitative studies of autonomic function. Muscle Nerve 2006;33:6–20.
26. Brooks WW, Conrad CH. Isoproterenol-induced myocardial injury and diastolic dysfunction in mice: structural and functional correlates. Comp Med 2009;59:339–343.
27. Grimm D, Elsner D, Schunkert H, et al. Development of heart failure following isoproterenol administration in the rat: role of the renin-angiotensin system. Cardiovasc Res 1998;37:91–100.
28. Krishnamurthy P, Subramanian V, Singh M, Singh K. Beta1 integrins modulate beta-adrenergic receptor-stimulated cardiac myocyte apoptosis and myocardial remodeling. Hypertens 2007;49:865–872.
29. LaCroix C, Freeling J, Giles A, et al. Deficiency of M2 muscarinic acetylcholine receptors increases susceptibility of ventricular function to chronic adrenergic stress. Am J Physiol Heart Circ Physiol 2008;294:H810–20.
30. Ma X, Song Y, Chen C, et al. Distinct actions of intermittent and sustained beta-adrenoceptor stimulation on cardiac remodeling. Sci China Life Sci 2011;54:493–501.
31. Sumita Yoshikawa W, Nakamura K, Miura D, et al. Increased passive stiffness of cardiomyocytes in the transverse direction and residual actin and myosin cross-bridge formation in hypertrophied rat hearts induced by chronic beta-adrenergic stimulation. Circ J 2013;77:741–748.
32. Arora R, Krummerman A, Vijayaraman P, et al. Heart rate variability and diastolic heart failure. Pacing Clin Electrophysiol 2004;27:299–303.
33. Grassi G, Seravalle G, Quarti-Trevano F, et al. Sympathetic and baroreflex cardiovascular control in hypertension-related left ventricular dysfunction. Hypertens 2009;53:205–209.
34. Hirono O, Kubota I, Minamihaba O, et al. Left ventricular diastolic dysfunction in patients with bronchial asthma with long-term oral beta2-adrenoceptor agonists. Am Heart J 2001;142:E11.
35. Nixdorff U, de Mey C, Belz GG, et al. Beta-adrenergic stimulation enhances left ventricular diastolic performance in normal subjects. J Cardiovasc Pharmacol 1997;29:476–484.
36. Piccirillo G, Germano G, Vitarelli A, et al. Autonomic cardiovascular control and diastolic dysfunction in hypertensive subjects. Int J Cardiol 2006;110:160–166.
37. De Souza SBC, Rocha JA, Cuoco MAR, et al. High muscle sympathetic nerve activity is associated with LV dysfunction in treated hypertensive patients. Am J Hypertens 2013;26:912–917.
38. Sugiura M, Yamamoto K, Takeda Y, et al. The relationship between variables of 123-I-metaiodobenzylguanidine cardiac imaging and clinical status of the patients with diastolic heart failure. Int J Cardiol 2006;113:223–228.
39. Tsuchida T, Fukuma N, Oikawa K, et al. Relationship between plasma norepinephrine at peak exercise and 123I-MIBG imaging of the heart and lower limbs in heart failure. J Nippon Med Sch 2007;74:114–122.
40. Vinch CS, Aurigemma GP, Hill JC, et al. Usefulness of clinical variables, echocardiography, and levels of brain natriuretic peptide and norepinephrine to distinguish systolic and diastolic causes of acute heart failure. Am J Cardiol 2003;91:1140–1143.
41. Malpas SC. Sympathetic nervous system overactivity and its role in the development of cardiovascular disease. Physiol Rev 2010;90:513–557.
42. Fagius J, Wallin BG. Long-term variability and reproducibility of resting human muscle nerve sympathetic activity at rest, as reassessed after a decade. Clin Auton Res 1993;3:201–205.
43. Patel AD, Iskandrian AE. MIBG imaging. J Nucl Cardiol 2002;9:75–94.
44. Verschure DO, Veltman CE, Manrique A, et al. For what endpoint does myocardial 123I-MIBG scintigraphy have the greatest prognostic value in patients with chronic heart failure? Results of a pooled individual patient data meta-analysis. Eur Heart J Cardiovasc Imaging 2014;15:996–1003.
45. Nakata T, Nakajima K, Yamashina S, et al. A pooled analysis of multicenter cohort studies of (123) I-mIBG imaging of sympathetic innervation for assessment of long-term prognosis in heart failure. JACC Cardiovasc Imaging 2013;6:772–784.
46. De la Sierra A, Calhoun DA, Vinyoles E, et al. Heart rate and heart rate variability in resistant versus controlled hypertension and in true versus white-coat resistance. J Hum Hypertens 2014;28:416–420.
47. Rosendorff C. The chicken and the egg: sympathetic nervous system activity and left ventricular diastolic dysfunction. Hypertens 2009;53:108–109.
48. Kelm M, Schafer S, Mingers S, et al. Left ventricular mass is linked to cardiac noradrenaline in normotensive and hypertensive patients. J Hypertens 1996;14:1357–1364.

49. Borlaug BA, Redfield MM. Diastolic and systolic heart failure are distinct phenotypes within the heart failure spectrum. Circulation 2011;123:2006–2013.
50. Seeland U, Selejan S, Engelhardt S, et al. Interstitial remodeling in beta1-adrenergic receptor transgenic mice. Basic Res Cardiol 2007;102:183–193.
51. Bronzwaer JGF, Paulus WJ. Diastolic and systolic heart failure: different stages or distinct phenotypes of the heart failure syndrome? Curr Heart Fail Rep 2009;6:281–286.
52. Marmor A, Green T, Krakuer J, et al. A single dose of cilazapril improves diastolic function in hypertensive patients. Am J Med 1989;87:61S–63S.
53. Martin-Fernandez B, de las Heras N, Miana M, et al. Spironolactone prevents alterations associated with cardiac hypertrophy produced by isoproterenol in rats: involvement of serum- and glucocorticoid-regulated kinase type 1. Exp Physiol 2012;97:710–718.
54. Miyoshi T, Nakamura K, Yoshida M, et al. Effect of vildagliptin, a dipeptidyl peptidase 4 inhibitor, on cardiac hypertrophy induced by chronic beta-adrenergic stimulation in rats. Cardiovasc Diabetol 2014;13:43.
55. Buss SJ, Backs J, Kreusser MM, et al. Spironolactone preserves cardiac norepinephrine reuptake in salt-sensitive Dahl rats. Endocrinology 2006;147:2526–2534.
56. Leite-Moreira AF. Current perspectives in diastolic dysfunction and diastolic heart failure. Heart 2006;92:712–718.
57. Levick SP, Murray DB, Janicki JS, Brower GL. Sympathetic nervous system modulation of inflammation and remodeling in the hypertensive heart. Hypertens 2010;55:270–276.
58. Murray DR, Prabhu SD, Chandrasekar B. Chronic beta-adrenergic stimulation induces myocardial proinflammatory cytokine expression. Circulation 2000;101:2338–2341.
59. Spyrou N, Rosen SD, Fath-Ordoubadi F, et al. Myocardial beta-adrenoceptor density one month after acute myocardial infarction predicts left ventricular volumes at six months. J Am Coll Cardiol 2002;40:1216–1224.
60. Paulus WJ, Tschöpe C. A Novel Paradigm for Heart Failure With Preserved Ejection Fraction. J Am Coll Cardiol 2013;62:263–271.
61. Grande MT, Pascual G, Riolobos AS, et al. Increased oxidative stress, the renin-angiotensin system, and sympathetic overactivation induce hypertension in kidney androgen-regulated protein transgenic mice. Free Radic Biol Med 2011;51:1831–1841.
62. Hubens LEG, Verloop WL, Joles JA, et al. Ischemia and reactive oxygen species in sympathetic hyperactivity states: a vicious cycle that can be interrupted by renal denervation? Curr Hypertens Rep 2013;15:313–320.
63. Rutten FH, Cramer M-JM, Paulus WJ. [Heart failure with preserved ejection fraction: Diastolic heart failure]. Ned Tijdschr Geneeskd 2012;156:A5315.
64. Klein IHHT, Ligtenberg G, Oey PL, et al. Enalapril and losartan reduce sympathetic hyperactivity in patients with chronic renal failure. J Am Soc Nephrol 2003;14:425–430.
65. Persson H, Lonn E, Edner M, et al. Diastolic dysfunction in heart failure with preserved systolic function: need for objective evidence:results from the CHARM Echocardiographic Substudy-CHARMES. J Am Coll Cardiol 2007;49:687–694.
66. Shah R V, Desai AS, Givertz MM. The effect of renin-angiotensin system inhibitors on mortality and heart failure hospitalization in patients with heart failure and preserved ejection fraction: a systematic review and meta-analysis. J Card Fail 2010;16:260–267.
67. Edelmann F, Gelbrich G, Dungen H-D, et al. Exercise training improves exercise capacity and diastolic function in patients with heart failure with preserved ejection fraction: results of the Ex-DHF (Exercise training in Diastolic Heart Failure) pilot study. J Am Coll Cardiol 2011;58:1780–1791.
68. Conraads VM, Metra M, Kamp O, et al. Effects of the long-term administration of nebivolol on the clinical symptoms, exercise capacity, and left ventricular function of patients with diastolic dysfunction: results of the ELANDD study. Eur J Heart Fail 2012;14:219–225.
69. 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;59:901–909.
70. Funder JW, Carey RM, Fardella C, et al. Case detection, diagnosis, and treatment of patients with primary aldosteronism. J Clin Endocrinol Metab 2008;93:3266–3281.
71. Borlaug BA, Melenovsky V, Russell SD, et al. Impaired chronotropic and vasodilator reserves limit exercise capacity in patients with heart failure and a preserved ejection fraction. Circulation 2006;114:2138–2147.

Chapter 7 -
Renal denervation in heart failure with normal left ventricular ejection fraction. Rationale and design of the DIASTOLE trial.

Eur J Heart Failure 2013;15(12);p1429-1437

Willemien L.Verloop, Martine M.A. Beeftink, Alex Nap, Michiel L. Bots, Birgitta K. Velthuis, Yolande E. Appelman, Maarten-Jan Cramer, Willem R.P. Agema, Asbjørn M. Scholtens, Pieter A. Doevendans, Cor P. Allaart, Michiel Voskuil

Abstract

Objective
The DIASTOLE trial will investigate whether renal sympathetic denervation influences parameters of HFpEF.

Background
Increasing evidence suggests an important role of hyperactivation of the sympathetic nervous system (SNS) in the clinical phenomena of heart failure with normal left ventricular ejection fraction (HFpEF) and in hypertension. Moreover, the level of renal sympathetic activation is directly related to the severity of heart failure. Since percutaneous renal denervation (RDN), has been shown to be effective in modulating elevated SNS activity in patients with hypertension, it can be hypothesized that RDN has a positive effect on HFpEF.

Methods
DIASTOLE (NCT01583881) is a multicenter, randomized controlled trial. Sixty patients, diagnosed with HFpEF and treated for hypertension, will be randomly allocated in a one-to-one ratio to undergo renal denervation on top of medical treatment (n=30) or to maintain medical treatment alone (n=30). Primary objective is to investigate efficacy of RDN by means of pulsed wave Doppler echocardiographic parameters. Secondary objectives include safety of RDN and a comparison of changes in the following parameters after RDN: left ventricular (LV) mass, LV volume, LV ejection fraction and left atrium volume as determined with magnetic resonance imaging. Also, MIBG-uptake and -washout, BNP levels, blood pressure, heart rate variability, exercise capacity and quality of life will be assessed.

Conclusion
DIASTOLE is a randomized controlled trial evaluating renal denervation as a treatment option for HFpEF. The results of the current trial will provide important information regarding the treatment of HFpEF, and therefore may have major impact on future therapeutic strategies.

Introduction

Globally, heart failure is a major and increasing clinical problem with a prevalence of 1 to 2% in the adult population of developed countries.[1] Among persons of 70 years or older, this prevalence rises to ≥10%.[1] In earlier reports, diastolic heart failure (= 'heart failure with normal left ventricular ejection fraction' or 'heart failure with preserved ejection fraction'; HFpEF) was considered to be more benign than systolic heart failure with a lower mortality and morbidity rate. Contrary to this belief, recent observations show that diastolic dysfunction has become the dominant form of heart failure (HF) in the community. With worsening morbidity and mortality[2-4] HFpEF leads to substantial economic costs, while we still lack knowledge regarding its etiology. In addition, no treatment has yet convincingly shown reduction in morbidity and mortality in patients with HFpEF.[5]
The European Society of Cardiology (ESC) guidelines state that diuretics can be used as in heart failure with reduced left ventricular ejection fraction (HFrEF), and that treatment of hypertension and myocardial ischemia is considered to be important.[5] Functionally, HFpEF is characterized by impaired left ventricular (LV) relaxation and reduced compliance of the ventricles. The resulting impairment of diastolic filling leads to inappropriate pressure increases after physiological volume loads, which in time may lead to congestive heart failure.[2,6] Hypertension is a major factor contributing to diastolic dysfunction by impairing left ventricular diastolic relaxation and increasing cardiac mass in response to chronic pressure overload. The sympathetic nervous system (SNS) is known to play an important role in the development and sustainability of an increased blood pressure (BP).[7] Moreover, it is also known that SNS plays an important role in the development and prognosis of HFrEF.[8-14] Both preclinical and clinical studies have shown a relationship between an elevated SNS and the development of diastolic dysfunction or HFpEF, irrespective of the presence of hypertension.[15-17] Based on the relationship between the SNS and HFpEF, it has been suggested that use of beta blockers may be useful in HFpEF. Beta-blockers have been investigated in the large randomized controlled SENIORS trial. This trial suggests that nebivolol may be beneficial in patients with HFpEF.[18]
Renal denervation is a percutaneous catheter based approach to disrupt renal sympathetic nerves. The first studies in patients with resistant hypertension showed this technique to be effective in modulation of the SNS[19] and safe, illustrated by a lack of (long-term) vascular or renal injury.[20] Furthermore, Brandt et al. showed in a retrospective analysis an improvement in both HFrEF and HFpEF parameters using echocardiography after renal denervation.[21]
In the light of these findings, the Denervation of the renAl Sympathetic nerves in hearT failure with nOrmal Left ventricular Ejection fraction (DIASTOLE)-trial (NCT01583881) has been designed. Objective of this prospective randomized controlled trial is to investigate whether renal denervation is an effective means to modulate the detrimental effects of the sympathetic nervous system in patients with heart failure with normal LV ejection fraction and hypertension.

Study design

DIASTOLE is a multicenter randomized controlled trial. Participating trial centers will screen all consecutive outpatients and inpatients that fulfil the pre-screening criteria, i.e. patients with HFpEF and (a history of) hypertension, who are on a stable drug regimen.

Patients who fulfil all criteria for entry into the study and give informed consent, will be randomized to undergo RDN on top of medical treatment, or to medical treatment only throughout the study period. Randomization will be in a 1:1 ratio, stratified per center using a computerized randomization facility. During the study period, no changes in drug regimen will be scheduled, unless deemed necessary by the treating physician. Over an 18-month period, we plan to include 60 patients at three Dutch trial sites. A study flow chart is shown in the online supplement.
DIASTOLE is an investigator-initiated trial that was designed, and will be conducted and analyzed, independently of the financial contributors. Study data will be collected and retained by the investigators and will not be available for the financial contributors.

Study objectives
The primary objective of the DIASTOLE trial is to determine whether RDN on top of medical treatment[5] is superior to medical treatment only in improving echocardiographic parameters of diastolic function in patients with HFpEF. Also of particular interest is to prove the safety of this novel therapeutic modality in this patient population.

Endpoints
To investigate the study objectives listed above, the following primary endpoint was chosen:

Change in E/e´, the ratio of peak early transmitral ventricular filling velocity to early diastolic tissue Doppler velocity, which is a non-invasive indicator of left ventricular end-diastolic pressure (LVEDP), at 12 months compared with baseline.

The parameter of E/e' was chosen since it has an excellent accuracy to predict diastolic dysfunction[22]; the measurement yields a high sensitivity and specificity.[23] Moreover, this parameter is widely used in clinical practice and is superior as a predictor of prognosis to other echocardiographic parameters.[24]
Furthermore, DIASTOLE will investigate the safety of the intervention assessing renal function and adverse clinical events. Besides changes in E/e', Ard-Ad and left atrial volume index will be compared before and after renal denervation, and the rate of change between the treatment groups will be compared. Other secondary endpoints include an evaluation of effects of RDN on magnetic resonance imaging (MRI) parameters, systolic function, neurohumoral activation, quality of life, and exercise capacity.
Primary, secondary, and safety endpoints are summarized in table 1.

Inclusion and exclusion criteria
The study inclusion and exclusion criteria are listed in Table 2. Men or women over 18 years of age who meet the inclusion/exclusion criteria and provide written consent to participate will be screened further for inclusion in the study. A major inclusion criterion is the diagnosis of HFpEF. A flow chart, published as a consensus document in 2007 by Paulus et al., will be used to confirm the diagnosis of HFpEF (figure 1, page 117).[24] Preferably, only patients with sinus rhythm (SR) are included in this trial. However, also patients with accepted atrial fibrillation (AF) with well-regulated ventricular response can be included. A well-regulated ventricular response is defined as AF with a ventricular rate of less than 100 beats per minute.[25] Echocardiography has been shown to be reliable to diagnose HFpEF in patients

Table 1. Primary, safety and secondary endpoints in the DIASTOLE trial.

Primary endpoint
Change in E/E' at 12 months.
Safety endpoint
Occurrence of hositalisation due to a complication of renal denervation
Change in cardiac systolic function during follow-up
A composite cardiovascular endpoint of myocardial infarction, sudden cardiac death, stroke, aortic or lower limb revascularization procedure, lower limb amputation, death from aortic or peripheral arterial dialysis, death because of renal failure, or hospital admission for hypertensive emergency unrelated to non-adherence or non-persistence with drugs
All-cause mortality
Secondary endpoints
Change in primary endpoint after 6 months
Change in any echocardiographic diastolic parameter at 12 months
Change in LV mass, LV volume, LA volume, LVEF, and RVEF assessed by MRI
Change in LVEDP and LV relaxation in cardiac catheterization at 12 months
Change in neurohumoral activation (natriuretic peptides) at 12 months
Change in 6 min walking distance at 12 months
Change in quality of life (MInnesota Living with Heart Failure Questionnaire) at 12 months
Change in occurrence of hospitalization for clinical heart failure at 12 months
Change in office-based BP and 24h ambulatory BP at 12 months
Change in R-R interval at 12 months
Change in (myocardial) MIBG uptake and washout at 12 months

Abbreviations: NBP = blood pressure, LA = left atrium, LV = left ventricle, LVEF = left ventricular ejection fraction, LVEDP = left ventricular end-diastolic pressure, MIBG = metaiodobenzylguanidine, MRI = magnetic resonance imaging, RV = left ventricle, RVEF = right ventricular ejection fraction.

with AF.[26] For a good quality of magnetic resonance imaging, R-waves should be identifiable during MRI. A screening failure is defined as a patient that does not meet all of the criteria for the study. The patient may meet the initial criteria, but may be excluded after baseline diagnostics have been performed (including angiography). Screening failure at any point prior to use of the renal denervation device (including at the time of angiography), will result in disqualification from study participation. Patients who are disqualified up to the moment of renal denervation will be replaced to a maximum of 60 subjects.

Duration of the trial
The planned recruitment period is 18 months. After treatment, patients will be followed for 12 months. Therefore, the total duration of the study will be 30 months.

Study intervention
Renal denervation in addition to current medical treatment will be compared with current medical treatment only. Renal denervation will be performed using the Medtronic Symplicity™ Renal Denervation System. This is a 6Fr compatible catheter, equipped with a

Table 2. Inclusion and exclusion criteria

Inclusion criteria
Individual is diagnosed with heart failure with a normal LV ejection fraction. • signs and symptoms of heart failure; • normal or mildly abnormal systolic LV function (LVEF ≥50%); • evidence of diastolic LV dysfunction.
Individual should fulfil the diagnostic WHO criteria for hypertension: SBP >140 mmHg and/or DBP >90mmHg, while treated with ≥2 antihypertensive drugs. This treatment is expected to be maintained for at least 6 months. Using this regimen, the BP should be adequately controlled (<140/90 mmHg by 24 hour ambulatory BP measurement).
Individual adheres to a stable drug regimen for HFpEF, with no changes for a minimum of 2 weeks prior to enrolment, and which is expected to be maintained for at least 6 months.
Individual is ≥18 years of age.
Individual agrees to have all study procedures performed and is competent and willing to provide written informed consent to participate in this clinical study.
Exclusion criteria
Known myocardial infarction as a cause of HFpEF.
Renal artery anatomy ineligible for treatment.
eGFR <30 mL/min/1.73m^2 (MDRD calculation).
Known secondary cause of hypertension.
Known other cause (e.g. COPD) of respiratory dysfunction.
A myocardial infarction, unstable angina pectoris, or a cerebrovascular accident in the last 6 months.
Diabetes mellitus type 1.
Scheduled or planned surgery of cardiovascular intervention in the next 6 months.
Any serious medical condition which may adversely affect the safety and/or effectiveness of the participant or the study.
Pregnant,nursing or planned pregnancy.
An unresolved history of drug use or alcohol dependency.
Cannot comprehend or follow instructions, or is unlikely to comply with study follow-up.
Current enrolment in another investigational drug or device trial.
Any contraindication for MRI.

Abbreviations: BP = blood pressure, COPD = chronic obstructive pulmonary disease, eGFR = estimated glomerular filtration rate, HFpEF = heart failure with preserved ejection fraction, MDRD = modification of Diet in Renal Disease, MRI = magnetic resonance imaging, WHO = world health organization.

single platinum RF electrode. The catheter is used in conjunction with a standard dispersive electrode. The catheter is introduced via a standard percutaneous technique in the femoral artery. The catheter is positioned in contact with the vessel wall of the renal artery at the desired location. Consequently, the catheter is connected to the radiofrequency (RF) generator. Multiple treatments at positions along the renal artery are performed, aiming at at least 4-6 ablation points per renal artery. This is done from distal to proximal in the artery with 5 mm interspaces in a spiral manner. The RF generator automatically delivers controlled RF energy at specific power, temperature, and time settings. The intended treatment involves the delivery of a relatively low power RF burst (up to 8W compared to

other cardiac RF devices that generally operate in excess of 30W) for a 2-minute period per ablation point. The treatment will be performed bilaterally.

Key measurements

In table 3 an overview of the different examinations at each follow-up time point is given.

Echocardiography

The primary endpoint is to investigate the efficacy of RDN by means of pulsed wave Doppler echocardiographic parameters in patients diagnosed with HFpEF and hypertension.
Echocardiography plays a critical diagnostic role in patients with heart failure and Doppler echocardiography, which measures the velocity of intracardiac blood flow, can be helpful in the assessment of diastolic function.[27] The parameters of greatest value to diagnose HFpEF are the lateral E/e', the left atrial volume index (LAVI), and the Ard-Ad.[22,23] Alterations in the pattern of these velocities give insight into left ventricular diastolic function and prognosis.[28,29] Pulsed wave Doppler echocardiography will be performed by trained echocardiographists according to a standardized operating procedure (SOP) at baseline and during follow-up 6 and 12 months after RDN.

The variables determined are:

- The change in the ratio of mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (e') (E/e'), determined by pulsed wave Doppler.
- The change in duration of reversed pulmonary vein arterial systolic flow (Ard) minus the duration of the mitral valve atrial wave flow (Ad) (Ard-Ad), determined by pulsed wave Doppler and expressed in milliseconds.
- The change in left atrial volume index (LA volume indexed for body surface area), determined by pulsed wave Doppler and expressed in milliliters per square meter.
- Dyssynchrony will be evaluated at baseline and during follow-up.

To ensure the high quality and validity of echocardiographic data obtained, and to exclude interobserver variability, an echo coordinator will act as blinded reference for all aspects related to echocardiography. The UMC Utrecht is set as core lab for this trial. In collaboration with the study investigators, the echo coordinator has prepared a detailed standard operating procedure for obtaining all echocardiographic parameters needed for the DIASTOLE.

Safety

Investigating the safety of RDN in patients with HFpEF and hypertension is one of the main endpoints of this study. Events (in terms of major and minor adverse events, see table 1) and possible complications will be documented during the follow-up. All events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.
Renal function will be assessed before and after treatment to investigate whether RDN influences renal function in this specific population. Renal function will be calculated on basis of Modification of Diet in Renal Disease (MDRD) Study criteria.[30]
In terms of safety, in the Netherlands all patients treated with renal denervation are included in a nationwide web-based registry. The patients in this trial randomized to treatment arm will therefore also be included in this database. This registry has a follow-up of 5 years.

Table 3. Different examinations at each time point

	V1 week -3	V2[a] week 0	TC week 1	V3 6 months	V4 12 months
Visit window permitted			±18 d	±14 d	±14 d
Signing informed consent	x				
Medical history	x				
Medication review	x		x	x	x
Safety monitoring[b]		x[a]	x	x	x
Echocardiography	x			x	x
BP measurements[c]	x	x		x	x
Electrocardiography	x			x	x
24h holter and HRV	x				x
MRI	x			x	x
MRA (aorta + renal arteries)	x				
Nuclear imaging	x				x
Pressure-volume loops[d]	x				x
Laboratory tests	x			x	x
MLWHFQ	x			x	x
6-min walking test	x			x	x
NYHA status	x			x	x

a) V2 will only take place in the patients randomized to the intervention group.
b) Safety is investigated by means of minor and major adverse events, characterized by periprocedural complications, occurence of hospitalizations for clinical heart failure, change in systolic function, acute myocardial infarction, cerebrovascular accident, or all-cause mortality.
c) Blood pressure measurements; both office and 24h ambulatory measurements.
d) Pressure-volume loops are only performed in a subpopulation (± 10 patients).
Abreviations:BP = blood pressure, HRV = heart rate variability, MLHFQ = Minnesota Living with heart failure questionnaire, MRI = magnetic resonance imaging, MRA = magnetic resonance angiography, NYHA = New York Health Association, TC = telephone call.

Magnetic resonance imaging

HFpEF is characterized by several structural cardiac abnormalities; both ventricular (LV end diastolic volume (LVEDV) in particular) en left atrial (LA) volume are increased in patients with HFpEF. The increase in atrial volume is supposed to play a pivotal role in the development of atrial fibrillation in patients with HFpEF.[22] Both echocardiography and cardiac magnetic resonance imaging (cMRI) can give information about diastolic dysfunction.[31] However, echocardiography has important disadvantages, including limited field of view and calculation errors relative to flow direction. Small changes in LA or LV volumes can reliably be detected by cMRI as opposed to echocardiography; these small changes may be important when evaluating therapy response. Due to its high spatial and temporal resolution, cMRI is more suitable to quantify volumes and function.[32] We aim to quantify the effect of RDN on LVEDV and LA volume. Additional to the cMRI, a magnetic resonance angiography of the renal arteries is performed to check whether renal anatomy is eligible for treatment. Eligible anatomy is defined as at least one main renal artery with a diameter ≥4mm and a length ≥20mm bilateral.

Laboratory investigations

The influence of neurohumoral agents on the diastolic function has been intensively investigated. The adrenergic system is the best described neurohumoral mechanism capable of acutely modulating both systolic and diastolic functions.[33,34] Peptides, such as endothelin (ET) and angiotensin-II (AngII), have deleterious effects on systolic and diastolic function, contributing to ventricular remodeling with myocardial hypertrophy and fibrosis. In the past years increasing evidence has shown linkage of several neurohumoral mediators to the acute modulation of diastolic function, with special emphasis on the active myocardial relaxation and passive properties of the cardiac muscle. The neurohumoral factor studied most in relation to HFpEF is the brain natriuretic peptide (BNP).[35,36] To obtain a complete overview of neurohumoral factors, we will also determine the concentrations of endothelin, aldosterone, angiotensin-II, plasma renin activity (PRA), and atrial natriuretic peptide (ANP). Laboratory investigations will be performed in fasting condition. Aldosterone, angiotensin-II and PRA will be assessed in supine, resting position.

Nuclear imaging

1. Nuclear imaging with both metabolic and molecular tracers may provide important insight into the cascade of HFpEF.[37] Moreover, this imaging modality can be used as marker to provide useful prognostic information in patients with HFpEF.[37] For a complete overview of different imaging modalities, nuclear imaging will be performed as well in the DIASTOLE trial. Nuclear imaging will consist of different subtypes. Sympathetic innervation will be measured by MIBG (Metaiodobenzylguanidine, Iobenguane), using an adrenalin-analogue. MIBG-scintigraphy and semi-quantitative measurements derived from it have been shown to correspond with heart failure prognosis in the ADMIRE-HF study.[38] Kidney disease is known to increase the risk of cardiovascular disease, and in one study in children with chronic kidney disease where cardiac SNS activity was measured by MIBG, myocardial washout was elevated before and normalized after renal transplant.[39] Also, a small number of studies have shown MIBG parameters to improve after effective medical treatment for hypertension. We will evaluate whether RDN will establish a decreased cardiac strain and normalization of sympathetic drive.[40]
2. Myocardial oxygen consumption will be quantified using [^{11}C]-acetate PET.
3. Perfusion will be measured using $H_2{}^{15}O$ PET.

Volume pressure loops

In a subpopulation, a cardiac catheterization procedure will be performed for measurement of pressure volume loops. Invasive measurement of pressure volume loops constitutes the gold standard in studying abnormal LV relaxation, filling, diastolic distensibility, and diastolic stiffness. Invasively acquired evidence of diastolic dysfunction is considered to provide definite evidence of HFpEF.[24] The conductance catheter provides continuous online measurements of LV pressure and volume. Cardiac performance will be assessed by heart rate, stroke volume, end-diastolic volume, end-systolic volume, and cardiac output. Systolic preload-dependent LV function will be assessed by EF, end-systolic pressure, and maximal rate of the LV pressure change (dP/dtmax). The systolic preload-independent LV function will be assessed by the end-systolic PV relationship. The end-systolic PV relationship is characterized by its linear slope, by the end-systolic elastance (EES) and the ratio of the afterload to the EES. Diastolic load-dependent LV function is quantified by

LVEDP, isovolumetric relaxation (relaxation time constant, ô), and the minimal rate of LV pressure change (dP/dtmin). The end-diastolic PV relationship (dP/dV) can be calculated to determine LV functional chamber stiffness (LV stiffness, b). Furthermore, the load-independent diastolic function can be derived from the end-diastolic PV relationship with exponential fitting to obtain the chamber stiffness constant (LV stiffness constant, â).

Randomization

Randomization will be 1:1 for either RDN in addition to current medication, or medication only. Stratification will be done per center, using a computerized randomization facility. The two study groups will be studied concurrently.

Statistical analysis

Changes in all efficacy parameters within groups will be calculated, comparing the follow-up measurements with baseline values. Whether the rate of change in the efficacy measurements within the treatment groups differs from zero will be evaluated using an appropriate paired parametric or non-parametric test. Mean changes in the parameters will be presented with 95% confidence intervals (95% CI). A two-tailed p-value of less than 0.05 divided by the number of tests (Bonferroni correction) will be regarded as statistically significant.

Differences in the rate of change in efficacy parameters between groups will be evaluated using appropriate parametric or non-parametric tests. Differences in rate of change between groups will be presented with 95% CIs. A two-tailed P value of less than 0.05 divided to the number of tests will be regarded as statistically significant. To counteract the problem of multiple comparisons, the Bonferroni correction will be used.

Sample size calculation

A sample size calculation has been performed for the primary endpoint E/e' (echocardiography). To obtain an estimate of the standard deviation and expected difference in E/e' before and after treatment, a literature search was performed. In this literature search, we looked at outcome in E/e' between control groups and groups with a (pharmacological) intervention. Based on these findings, we expect a mean difference for E/e'(x – x_0) of 2.05 in the treated group, and a mean difference for E/e'(x – x_0) of -0.1 in the control group. The mean standard deviation of the difference found in the literature is 2.81.[2,41-43]

For the sample size calculation, a value of 0.80 was used for desired power, and α (type I error rate) was set at 0.05. Our sample size calculation, including the above mentioned expected effects and standard deviation gave an estimate of the sample size of 27 patients. Based on experience from the literature, a dropout rate of 10% is expected. To make sure that the study is not underpowered, a total of 30 patients will be included in every arm.

Ethical considerations

The study will be conducted according to the principles of the Declaration of Helsinki (59th amendment, Seoul 2008) and in accordance with the Dutch Medical Research Involving Human Subjects Act. The study is carried out in keeping with applicable local laws and regulations. The study protocol has been approved by the ethics committee of the UMC Utrecht. No DSMB has been constituted. We expect to include all patients before a reliable interim analysis can be performed in this limited patient cohort.

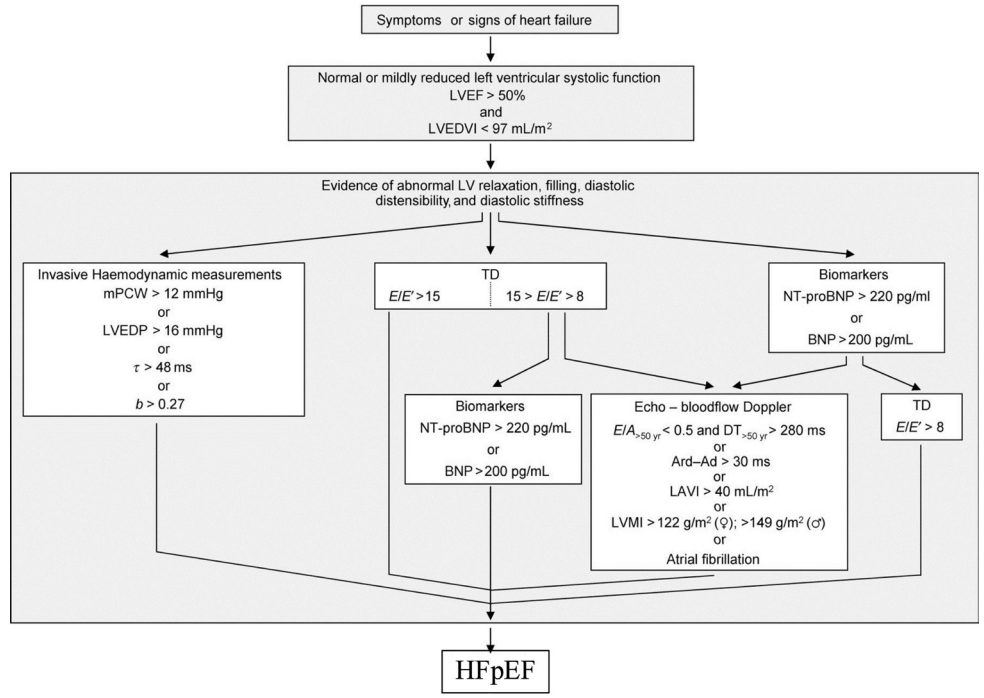
Data management

Data management will be processed by Castor® online electronic data system, part of Ciwit B.V, using a Lloyd's Register Quality Assurance (LRQA) certified server according to ISO international information security standards.

Discussion

The aim of the study is to evaluate safety and efficacy of RDN in the modulation of diastolic function. Recent studies showed that decreasing sympathetic nerve activity from and to the kidneys using RDN results in a clinically significant reduction of blood pressure in patients with so called therapy resistant hypertension.[44] Renal denervation appears to be a safe and relatively easy minimally invasive technique to interfere with this sympathetic nerve

Figure 1. Diagnostic flowchart for HFpEF.



Diagnostic flowchart on 'How to diagnose HFpEF' from the ESC consensus statement from Paulus et al. Eur Heart J 2007; 28; 2539-2550.

Abbreviations: LVEF = left ventricular ejection fraction, LVEDVI = left ventricular end-diastolic volume index, mPCW = mean pulmonary capillary wedge pressure, LVEDP = left ventricular end-diastolic pressure, t = time constant of left ventricular relaxation, b = constant of left ventricular chamber stiffness, TD = tissue Doppler, E= early mitral valve flow velocity, E' = early TD lengthening velocity, NT-proBNP = N-terminal-pro brain natriuretic peptide, BNP = brain natriuretic peptide, E/A = ratio of early (E) to late (A) mitral valve flow velocity, DT = deceleration time, Ard = duration of reverse pulmonary vain atrial systole flow, Ad = duration of mitral valve atrial wave flow, LAVI = left atrial volume index, LVMI = left ventricular mass index.

hyperactivity. The recent published expert consensus document from the European Society of Cardiology states that renal denervation can be considered as a therapeutic option in patients with resistant hypertension. Given the fact that renal denervation also reduces whole-body sympathetic nerve activity, this therapy may also be beneficial in other clinical states, characterized by sympathetic nervous system activation.[45]
It is well known that the sympathetic nervous system has a causative role in the development and prognosis of HFrEF. However, recently both preclinical and clinical studies have also shown a relationship between an elevated SNS and the development of diastolic dysfunction or HFpEF.[17] This relation was irrespective even of the presence of hypertension.[15]
In patients with a history of hypertension and diastolic dysfunction it is debatable whether a potential positive effect of RDN is due to an improved diastolic function, or due to a better-controlled BP. For this reason, only patients with an adequately controlled BP will be included. In addition, Davies et al. showed in their pilot study that renal denervation led to improved clinical symptoms and exercise capacity in patients with systolic heart failure.[46] In this normotensive population, no change in BP was observed.

In the current protocol, echocardiography has been chosen as primary endpoint. Some investigators have questioned the value of echocardiography in HFpEF.[47] However, Doppler transmitral and tissue Doppler annular velocities, which will be assessed in our protocol, have been shown to be the most reliable non-invasive assessment of diastolic function.[48] Also, echocardiography is the most broadly available diagnostic technique to assess diastolic parameters. A second concern regarding echocardiography is the existence of atrial fibrillation, often present in patients with HFpEF. To overcome such concerns, we decided only to include patients with SR or accepted AF with well-regulated ventricular response. As stated, echocardiography has been shown to be reliable to diagnose HFpEF in patients with AF.[26] As shown by Zhao et al., the occurrence of AF can be positively influenced by RDN.[49]
Furthermore, we believe that by using a broad spectrum of investigations regarding HFpEF, this protocol offers a complete assessment to evaluate the effects of RDN in HFpEF.[50] Among others, major secondary endpoints have been designed, including cardiac MRI and PV-loop assessment.

Conclusion

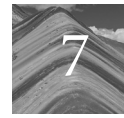
Heart failure with a preserved LV ejection fraction is a disease with high prevalence, morbidity, and mortality. Up to date, no established effective treatment strategies are known. Partly, this can be explained by a lack of knowledge regarding etiology. However, as previous studies have shown a relationship between elevated SNS and HFpEF, modulation of SNS by RDN may result in an improvement of the clinical status of patients with HFpEF. The current randomized controlled trial will provide important information regarding the treatment of HFpEF, and therefore may have major impact on future therapeutic strategies.

References

1. Mosterd A, Hoes AW. Clinical epidemiology of heart failure. Heart 2007;93:1137–1146.
2. Edelmann F, Schmidt AG, Gelbrich G, et al. Rationale and design of the “aldosterone receptor blockade in diastolic heart failure” trial: a double-blind, randomized, placebo-controlled, parallel group study to determine the effects of spironolactone on exercise capacity and diastolic function in pa. Eur J Heart Fail 2010;12:874–882.
3. Bhatia RS, Tu J V, Lee DS, et al. Outcome of heart failure with preserved ejection fraction in a population-based study. N Engl J Med 2006;355:260–269.
4. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function. Circulation 2002;105:1387–1393.
5. Mcmurray JJ V, Adamopoulos S, Anker SD, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012. Eur J Heart Fail 2012;14:803–869.
6. Wachter R, Schmidt-Schweda S, Westermann D, et al. Blunted frequency-dependent upregulation of cardiac output is related to impaired relaxation in diastolic heart failure. Eur Heart J 2009;30:3027–3036.
7. Schlaich MP, Sobotka PA, Krum H, et al. Renal denervation as a therapeutic approach for hypertension: novel implications for an old concept. Hypertens 2009;54:1195–1201.
8. Grassi G, Seravalle G, Cattaneo BM, et al. Sympathetic activation and loss of reflex sympathetic control in mild congestive heart failure. Circulation 1995;92:3206–3211.
9. 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;73:615–621.
10. Kaye DM, Lambert GW, Lefkovits J, et al. Neurochemical evidence of cardiac sympathetic activation and increased central nervous system norepinephrine turnover in severe congestive heart failure. J Am Coll Cardiol 1994;23:570–578.
11. Sobotka PA, Krum H, Bohm M, et al. The role of renal denervation in the treatment of heart failure. Curr Cardiol Rep 2012;14:285–292.
12. Rundqvist B, Elam M, Bergmann-Sverrisdottir Y, et al. Increased cardiac adrenergic drive precedes generalized sympathetic activation in human heart failure. Circulation 1997;95:169–175.
13. Packer M. The neurohormonal hypothesis: a theory to explain the mechanism of disease progression in heart failure. J Am Coll Cardiol 1992;20:248–254.
14. Eisenhofer G, Friberg P, Rundqvist B, et al. Cardiac sympathetic nerve function in congestive heart failure. Circulation 1996;93:1667–1676.
15. Grassi G, Seravalle G, Quarti-Trevano F, et al. Sympathetic and baroreflex cardiovascular control in hypertension-related left ventricular dysfunction. Hypertens 2009;53:205–209.
16. Somsen GA, Dubois EA, Brandsma K, et al. Cardiac sympathetic neuronal function in left ventricular volume and pressure overload. Cardiovasc Res 1996;31:132–138.
17. Piccirillo G, Germano G, Vitarelli A, et al. Autonomic cardiovascular control and diastolic dysfunction in hypertensive subjects. Int J Cardiol 2006;110:160–166.
18. Van Veldhuisen DJ, Cohen-Solal A, B??hm M, et al. Beta-Blockade With Nebivolol in Elderly Heart Failure Patients With Impaired and Preserved Left Ventricular Ejection Fraction. Data From SENIORS (Study of Effects of Nebivolol Intervention on Outcomes and Rehospitalization in Seniors With Heart Failure). J Am Coll Cardiol 2009;53:2150–2158.
19. Hering D, Lambert EA, Marusic P, et al. Substantial reduction in single sympathetic nerve firing after renal denervation in patients with resistant hypertension. Hypertens 2013;61:457–464.
20. Verloop WL, Voskuil M, Doevendans PA. Renal denervation: a new treatment option in resistant arterial hypertension. Neth Heart J 2013;21:95–98.
21. Brandt MC, Reda S, Mahfoud F, et al. Effects of renal sympathetic denervation on arterial stiffness and central hemodynamics in patients with resistant hypertension. J Am Coll Cardiol 2012;60:1956–1965.
22. Osranek M, Seward JB, Buschenreithner B, et al. Diastolic function assessment in clinical practice: the value of 2-dimensional echocardiography. Am Heart J 2007;154:130–136.
23. Shuai X-X, Chen Y-Y, Lu Y-X, et al. Diagnosis of heart failure with preserved ejection fraction: which parameters and diagnostic strategies are more valuable? Eur J Heart Fail 2011;13:737–745.
24. Paulus WJ, Tschope C, Sanderson JE, et al. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the

Heart Failure and Echocardiography Associations of the European Society of Cardiology. Eur Heart J 2007;28:2539–2550.
25. Groenveld HF, Tijssen JGP, Crijns HJGM, et al. Rate control efficacy in permanent atrial fibrillation: successful and failed strict rate control against a background of lenient rate control: data from RACE II (Rate Control Efficacy in Permanent Atrial Fibrillation). J Am Coll Cardiol 2013;61:741–748.
26. Arques S, Roux E, Sbragia P, et al. Usefulness of bedside tissue Doppler echocardiography and B-type natriuretic peptide (BNP) in differentiating congestive heart failure from noncardiac cause of acute dyspnea in elderly patients with a normal left ventricular ejection fraction and permanent, nonvalvular atrial fibrillation: insights from a prospective, monocenter study. Echocardiography 2007;24:499–507.
27. Persson H, Lonn E, Edner M, et al. Diastolic dysfunction in heart failure with preserved systolic function: need for objective evidence:results from the CHARM Echocardiographic Substudy-CHARMES. J Am Coll Cardiol 2007;49:687–694.
28. Galderisi M. Diastolic dysfunction and diastolic heart failure: diagnostic, prognostic and therapeutic aspects. Cardiovasc Ultrasound 2005;3:9.
29. Khouri SJ, Maly GT, Suh DD, Walsh TE. A practical approach to the echocardiographic evaluation of diastolic function. J Am Soc Echocardiogr 2004;17:290–297.
30. Levey AS, Bosch JP, Lewis JB, et al. 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 1999;130:461–470.
31. Daneshvar D, Wei J, Tolstrup K, et al. Diastolic dysfunction: improved understanding using emerging imaging techniques. Am Heart J 2010;160:394–404.
32. Bellenger NG, Burgess MI, Ray SG, et al. Comparison of left ventricular ejection fraction and volumes in heart failure by echocardiography, radionuclide ventriculography and cardiovascular magnetic resonance; are they interchangeable? Eur Heart J 2000;21:1387–1396.
33. Edelmann F, Stahrenberg R, Polzin F, et al. Impaired physical quality of life in patients with diastolic dysfunction associates more strongly with neurohumoral activation than with echocardiographic parameters: quality of life in diastolic dysfunction. Am Heart J 2011;161:797–804.
34. Grassi G, Bolla G, Quarti-Trevano F, et al. Sympathetic activation in congestive heart failure: reproducibility of neuroadrenergic markers. Eur J Heart Fail 2008;10:1186–1191.
35. Grewal J, McKelvie RS, Persson H, et al. Usefulness of N-terminal pro-brain natriuretic Peptide and brain natriuretic peptide to predict cardiovascular outcomes in patients with heart failure and preserved left ventricular ejection fraction. Am J Cardiol 2008;102:733–737.
36. Mak GS, DeMaria A, Clopton P, Maisel AS. Utility of B-natriuretic peptide in the evaluation of left ventricular diastolic function: comparison with tissue Doppler imaging recordings. Am Heart J 2004;148:895–902.
37. Katoh S, Shishido T, Kutsuzawa D, et al. Iodine-123-metaiodobenzylguanidine imaging can predict future cardiac events in heart failure patients with preserved ejection fraction. Ann Nucl Med 2010;24:679–686.
38. Jacobson AF, Senior R, Cerqueira MD, et al. Myocardial iodine-123 meta-iodobenzylguanidine imaging and cardiac events in heart failure. Results of the prospective ADMIRE-HF (AdreView Myocardial Imaging for Risk Evaluation in Heart Failure) study. J Am Coll Cardiol 2010;55:2212–2221.
39. Parisotto V, Lima EM, Silva JMP, et al. Cardiac sympathetic dysautonomia in children with chronic kidney disease. J Nucl Cardiol 2008;15:246–254.
40. Kosaka T, Nakagawa M, Ishida M, et al. Cardioprotective effect of an L/N-type calcium channel blocker in patients with hypertensive heart disease. J Cardiol 2009;54:262–272.
41. Daniel KR, Wells G, Stewart K, et al. Effect of aldosterone antagonism on exercise tolerance, doppler diastolic function, and quality of life in older women with diastolic heart failure. Congest Hear Fail 2009;15:68–74.
42. Yip GWK, Wang M, Wang T, et al. The Hong Kong diastolic heart failure study: a randomised controlled trial of diuretics, irbesartan and ramipril on quality of life, exercise capacity, left ventricular global and regional function in heart failure with a normal ejection fraction. Heart 2008;94:573–80.
43. Kosmala W, Przewlocka-Kosmala M, Szczepanik-Osadnik H, et al. A randomized study of the beneficial effects of aldosterone antagonism on lv function, structure, and fibrosis markers in metabolic syndrome. JACC Cardiovasc Imaging 2011;4:1239–1249.

44. Schlaich MP, Sobotka PA, Krum H, et al. Renal sympathetic-nerve ablation for uncontrolled hypertension. N Engl J Med 2009;361:932–934.
45. Mahfoud F, Luscher TF, Andersson B, et al. Expert consensus document from the European Society of Cardiology on catheter-based renal denervation. Eur Heart J 2013;34:2149–2157.
46. 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;162:189–192.
47. Mahadevan G, Dwivedi G, Williams L, et al. Epidemiology and diagnosis of heart failure with preserved left ventricular ejection fraction: rationale and design of the study. Eur J Heart Fail 2012;14:106–112.
48. De Boeck BW l, Cramer M-JM, Oh JK, et al. Spectral pulsed tissue Doppler imaging in diastole: a tool to increase our insight in and assessment of diastolic relaxation of the left ventricle. Am Heart J 2003;146:411–419.
49. Zhao Q, Yu S, Huang H, et al. Effects of renal sympathetic denervation on the development of atrial fibrillation substrates in dogs with pacing-induced heart failure. Int J Cardiol 2013;168:1672–1673.
50. Van Kraaij DJW, Van Pol PEJ, Ruiters AW, et al. Diagnosing diastolic heart failure. Eur J Heart Fail 2002;4:419–430.



Chapter 8 -
The quest for patients with heart failure with preserved ejection fraction; an onerous task.

Submitted

M.M.A. Beeftink , A. Nap, M.J.M. Cramer, W.R.P. Agema, A.M.W. Alings, P.A. Doevendans, M Voskuil

Abstract

Background
The pathophysiology of heart failure with preserved ejection fraction (HFpEF) is insufficiently understood. Although 50% of heart failure patients is said to have a preserved ejection fraction, many HFpEF studies struggle to identify eligible patients.

Methods
We performed a structured search of the echocardiography database of the University Medical Center Utrecht for patients who fulfill the criteria for HFpEF. All echocardiographic measurements between July and December 2014 were digitally extracted and assessed for presence of diastolic dysfunction in conjunction with a preserved ejection fraction. Subsequently, electronic medical records were examined for presence of signs and symptoms of heart failure.

Results
Out of the total of 3194 echocardiograms identified, diastolic parameters were assessed in 1534 (48%). Definite diastolic dysfunction was established in 326 patients (21%). Based on echocardiographic criteria and anamnesis, 70 of 326 patients (21%) fulfilled the European criteria for HFpEF. The treating cardiologist considered the diagnosis of HFpEF in 14 of these 70 patients (20%) and eventually confirmed the diagnosis HFpEF in only 6 patients (9%).

Conclusion
The presence of diastolic dysfunction and HFpEF is relatively low in the hospital setting, and, if present, is not always recognized by the treating physician.

Background

In recent decades, heart failure with preserved ejection fraction (HFpEF) is subject of interest of many scientific research and clinical guidelines. Although the short-term mortality is lower than in heart failure patients with a reduced ejection fraction, long-term mortality and recurrent hospitalization rates are similar.[1,2] Yet, a proven effective treatment is lacking and further research is therefore needed.[3]
Although it has been stated that about half of patients with heart failure have a preserved ejection fraction[4], many clinical trials on HFpEF have encountered difficulties in the recruitment of subjects (e.g. Clinicaltrial.gov identifiers NCT 00670111, NCT00840463, NCT01735916, NCT01872234, NCT02041130; the PEP-CHF trial[5], the RDT-PEF trial[6] and the DIASTOLE trial[7]). We report the results of a structured echocardiography-based screening of our cardiology outpatient clinic for patients eligible for participation in a HFpEF trial (NCT01583881).[7]

Aims

To assess the prevalence of diastolic dysfunction and isolated HFpEF in the echocardiography database of our hospital; to assess whether diastolic dysfunction and/or HFpEF was mentioned in the patient record; and to assess the number of patients eligible for participation in the DIASTOLE trial, investigating the effects of renal denervation in patients with HFpEF.[7]

Methods

We analyzed the electronic database containing all echocardiography performed at the University Medical Center Utrecht (the Netherlands). All echocardiography is performed and interpreted by sonographers and supervised by cardiologists specialized in cardiac imaging. For the current analysis, we extracted all routine echocardiography measurements performed between July and December 2014. Presence of HFpEF was assessed according to the echocardiographic criteria formulated in the European consensus document.[8] These criteria require (a) signs and symptoms of heart failure; (b) a normal or mildly reduced left ventricular (LV) ejection fraction; and (c) evidence of abnormal LV relaxation. For practical reasons, these requirements were assessed in reverse order in our analysis.

First, presence of diastolic dysfunction was assessed. In accordance to the consensus document, diastolic dysfunction was defined as a ratio of the mitral inflow velocity over mitral annular velocity (E/e') >15, or >8 with additional signs of abnormal left ventricular (LV) relaxation. These additional criteria for LV relaxation are shown in figure 1.

Secondly, LV ejection fraction (LVEF) was assessed. LVEF was considered to be preserved is the measured LVEF was >50%. If poor image quality prevented accurate measurement, LVEF was considered preserved if LV systolic function was judged as 'normal' by the sonographer. Lastly, we assessed whether signs or symptoms of heart failure were present. Data from the corresponding visit to the outpatient clinic were retrieved from the medical records. Presence and severity of heart failure symptoms were recorded using to the New York Heart Association (NYHA) classification. In addition, we assessed whether the presence of diastolic dysfunction was mentioned in the physician's notes and whether the diagnosis of HFpEF was considered.

Results

A total of 3194 patients underwent echocardiography between July and December 2014 (figure 1). In almost half of the patients, spectral tissue Doppler echocardiography of the left ventricular walls was performed (n=1534, 48%). Evidence of abnormal left ventricular diastolic function according to the European consensus document was present in 326 patients; 123 (38%) had a reduced LVEF and 203 (62%) had a preserved LVEF. The majority (n=215, 66%) had grade 1 diastolic dysfunction (impaired relaxation), grade 2 (pseudonormal) was present in 75 patients (23%) and 36 (11%) had grade 3 (restrictive diastolic dysfunction).

Among the 203 patients with diastolic dysfunction and preserved LVEF, 134 patients (66%) were asymptomatic (NYHA functional class 1), 66 patients (32%) had signs and symptoms of heart failure corresponding to NYHA function class 2 or higher and four patients (2%) were asymptomatic at the time of the echocardiography, but had a recent episode of acute decompensated heart failure.
Hence, 70 patients fulfilled the triad necessary for the diagnosis of HFpEF according to the European consensus document (symptoms for heart failure, normal LVEF and abnormal LV diastolic function). In fourteen of these patients, the diagnosis of HFpEF was considered by the treating physician in the differential diagnosis and in six patients (9%) the diagnosis was eventually established (figure 2). Twenty-one patients (30%) were diagnosed with an alternative diagnosis (n=6 cardiomyopathy, n=8 significant valvular disease, n=2 pulmonary hypertension, n=4 coronary insufficiency, n=1 cor pulmonale). In the remaining 43 patients (61%) no definite diagnosis for shortness of breath was established.

Conclusion

Our results demonstrate that diastolic dysfunction is often encountered, especially in an elderly population, but that the prevalence of true isolated HFpEF is rather low. When applying the criteria for diagnosing HFpEF according to the European consensus statement[8] to patients who underwent echocardiography at our hospital within a 5-month period, we were able to identify suspected HFpEF in 70 patients. Twenty-one patients were eventually

Figure 2. Clinical diagnosis in patients fulfilling European criteria for HFpEF (n=70)

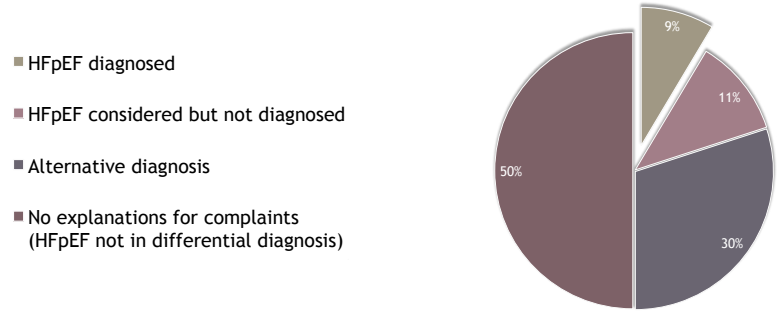
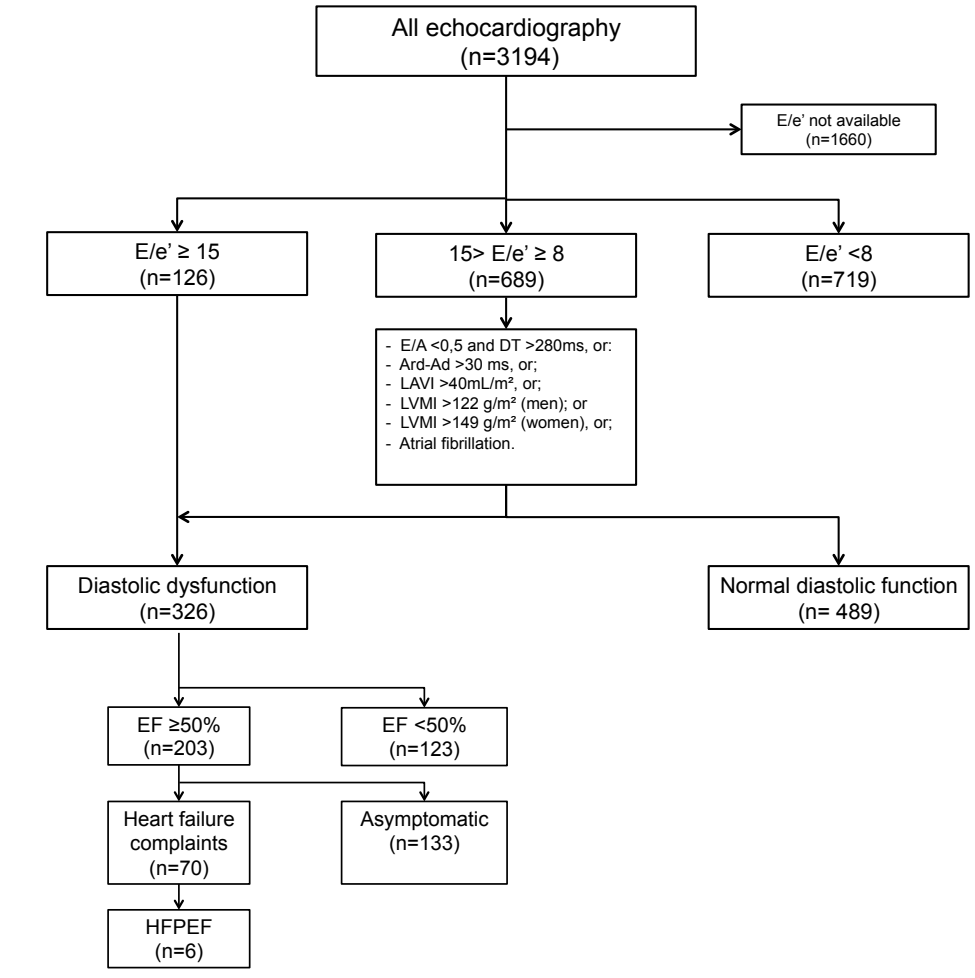


Figure shows the final diagnoses as determined by the treating physician in patients (n=70) fulfilling the European criteria for HFpEF. In 50% of patients there was no definite explanation for the heart failure complaints found by the treating physician and HFpEF was not mentioned as a possibility in the diagnostic considerations.

Figure 1. Flow chart.



Abbreviations: EF = ejection fraction, HFpEF = heart failure with preserved ejection fraction, LAVI = left atrial volume index, E= early mitral valve flow velocity, E' = early tissue Doppler lengthening velocity, E/A = ratio of early (E) to late (A) mitral valve flow velocity, DT = deceleration time, Ard = duration of reverse pulmonary vain atrial systole flow, Ad = duration of mitral valve atrial wave flow, LAVI = left atrial volume index, LVMI = left ventricular mass index.

diagnosed with an alternative diagnosis, leaving only 49 patients out of the original 3194 patients (1.5%).

HFpEF is a relatively new recognized condition for which no proven effective treatment has yet been found. As such, studies aiming to unravel the complex pathophysiology and investigating novel treatment options are mandatory. However, enrolment issues are not uncommon among HFpEF studies, although HFpEF is reported in up to 50% of the heart failure population.[4] Without consideration for additional in- and exclusion criteria for trial participation, we could identify only a limited number of patient which (suspected) isolated

HFpEF in our hospital. This number may even be lower when the customary criteria for trial participation are also applied.
A recent study demonstrated that only 0.8% of patients admitted with acute decompensated heart failure had isolated diastolic dysfunction and were eligible for participation in a HFpEF trial.[9] As patients with HFpEF are generally elderly, frail and frequently have multiple comorbidities, it is believed that a majority of HFpEF patients remain in primary care, contributing to the low prevalence observed in the hospital setting. This may pose a challenge for clinical trials, which are most often performed in academic centers and which may greatly benefit from improved collaboration with primary care centers and diagnostic echocardiography laboratories.
Yet, another important issue is that HFpEF remains a difficult diagnosis. Although the definition is evolving, it still remains mostly a diagnosis of exclusion. The "typical" symptoms of heart failure are also non-specific and may be caused by common comorbidities of HFpEF. As a result, there continues to be debate whether HFpEF truly exists, or whether these patients experience a heart failure-like clinical picture caused by an alternative diagnosis, such as chronic obstructive pulmonary disease or the proposed "EXIT" (exercise intolerance) syndrome.[10,11]

The diagnosis of HFpEF can be supported by evidence of abnormal left ventricular diastolic function. However, its implementation is hindered by the fact that access to echocardiography may be limited in primary care and the interpretation of diastolic indices is not straightforward as the reference values for the different parameters vary over different age categories. The current grading system for diastolic dysfunction leaves almost 25% of patients unclassified.[12] In addition, changes in filling status and preload can rapidly alter the transmitral filling patterns and diastolic function may appear normal on a rest examination while dysfunction may manifest during exercise.[13]
The difficult diagnosis of HFpEF is reflected in a number of papers reporting a high number of unrecognized HFpEF[14-16] as well as a high number of overdiagnosis in primary care.[16,17] Even in our cardiology department, we identified 70 patients with suspected HFpEF according to the guidelines, but the diagnosis of HFpEF was only established in six patients.
Simplified diagnostic criteria and development of improved diagnostic tests are needed to better recognize HFpEF. Optimization of the collaboration between heart failure specialists and general practitioners as well as general cardiologists may further improve the diagnosis of HFpEF in the primary care.

Our study may have limited generalizability as we assessed the prevalence of diastolic dysfunction and HFpEF in an academic setting. In addition, we included all patients who underwent echocardiography at our cardiology department, regardless of the indication for echocardiography. However, a history of ischemic heart disease is an important risk factor for HFpEF. Thus, exclusion of all patients who visit our outpatient clinic for the follow-up of ischemic heart disease may have resulted in an underestimation of HFpEF in our population. We observed a large number of cases with missing E/e' measurements, probably due to technical errors, poor image quality and specific indications for the echocardiogram ('short focused echo' e.g. for follow-up of known valvular disease). Because we used the HFpEF criteria according to the European consensus document, in which E/e' is a mandatory parameter for the diagnosis of HFpEF, we may have missed cases.
In conclusion, HFpEF is infrequently encountered in an academic setting, contributing to the difficult enrolment of HFpEF trials. Improved collaboration between research centers, primary care and diagnostic centers seems essential in future trials.

References

1. Bhatia RS, Tu J V, Lee DS, Austin PC, Fang JM, Haouzi A, Gong YY, Liu PP. Outcome of heart failure with preserved ejection fraction in a population-based study. N Engl J Med 2006;355:260–269.
2. Boonman-De Winter LJM, Hoes AW, Cramer MJ, Jongh G De, Janssen RRT, Rutten FH. Prognosis of screen-detected heart failure with reduced and preserved ejection fraction in patients with type 2 diabetes. Int. J. Cardiol. 2015;185:162–164.
3. Senni M, Paulus WJ, Gavazzi a., Fraser a. G, Diez J, Solomon SD, et al. New strategies for heart failure with preserved ejection fraction: the importance of targeted therapies for heart failure phenotypes. Eur Heart J 2014;35:2797–2815.
4. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. N Engl J Med 2006;355:251–259.
5. Cleland JGF, Tendera M, Adamus J, Freemantle N, Polonski L, Taylor J. The perindopril in elderly people with chronic heart failure (PEP-CHF) study. Eur Heart J 2006;27:2338–2345.
6. Patel HC, Rosen SD, Hayward C, Vassiliou V, Smith GC, Wage RR, et al. Renal denervation in heart failure with preserved ejection fraction (RDT-PEF): a randomized controlled trial. Eur J Heart Fail 2016;18:703-712
7. Verloop WL, Beeftink MMA, Nap A, Bots ML, Velthuis BK, Appelman YE, Cramer MJ, Agema WRP, Scholtens AM, Doevendans PA, Allaart CP, Voskuil M. Renal denervation in heart failure with normal left ventricular ejection fraction. rationale and design of the diastole (denervation of the renal sympathetic nerves in heart failure with normal lv ejection fraction) trial. Eur J Heart Fail 2013;15:1429–1437.
8. Paulus WJ, Tschope C, Sanderson JE, Rusconi C, Flachskampf F a., Rademakers FE, et al. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. Eur Heart J 2007;28:2539–2550.
9. Patel HC, Hayward C, Mario C di, Cowie MR, Lyon AR, Rosen SD. Heart failure with preserved ejection fraction: the impact of stricter definitions. Eur J Heart Fail 2014;16:767–771.
10. Henein M, Owen A. HFNEF breathlessness: Is it really heart failure? Int J Cardiol 2010;143:111–112.
11. Pérez-Calvo JI, Montero-Pérez-Barquero M, Formiga F. Diagnosis of Heart Failure With Preserved Ejection Fraction. J Am Coll Cardiol 2013;61:1748–1749.
12. Kuwaki H, Takeuchi M, Chien-Chia Wu V, Otani K, Nagata Y, Hayashi A, Iwataki M, Fukuda S, Yoshitani H, Abe H, Otsuji Y. Redefining diastolic dysfunction grading: Combination of E/A ≤0.75 and Deceleration Time >140 ms and E/ε' ≥10. JACC Cardiovasc Imaging 2014;7:749–758.
13. Møller JE, Pellikka PA, Hillis GS, Oh JK. Prognostic importance of diastolic function and filling pressure in patients with acute myocardial infarction. Circulation 2006;114:438–444.
14. Riet EES van, Hoes AW, Limburg A, Landman MAJ, Hoeven H van der, Rutten FH. Prevalence of unrecognized heart failure in older persons with shortness of breath on exertion. Eur J Heart Fail Wiley Online Library; 2014;16:772–777.
15. Rutten FH, Cramer M-JM, Grobbee DE, Sachs APE, Kirkels JH, Lammers J-WJ, Hoes AW. Unrecognized heart failure in elderly patients with stable chronic obstructive pulmonary disease. Eur Heart J 2005;26:1887 LP-1894.
16. Hancock HC, Close H, Mason JM, Murphy JJ, Fuat A, Singh R, Wood E, Belder M De, Brennan G, Hussain N, Kumar N, Wilson D, Hungin APS. High prevalence of undetected heart failure in long-term care residents: Findings from the Heart Failure in Care Homes (HFinCH) study. Eur J Heart Fail 2013;15:158–165.
17. Valk MJ, Mosterd A, Broekhuizen BDL, Zuithoff NPA, Landman MAJ, Hoes AW, Rutten FH. Overdiagnosis of heart failure in primary care: a cross-sectional study. Br J Gen Pract 2016;

Chapter 9 -
Renal denervation in heart failure with normal left ventricular ejection fraction. Results from the DIASTOLE trial.

In preparation

Martine M.A. Beeftink, Willemien L. Verloop, Alex Nap, Michiel L. Bots, Birgitta K. Velthuis, Maarten-Jan Cramer, Willem R.P. Agema, Pieter A. Doevendans, Marco A.M.W. Alings, Cor P. Allaart, Michiel Voskuil

Abstract

Objectives
To determine whether catheter-based renal denervation on top of medical treatment is superior to medical treatment alone in improving echocardiographic parameters of diastolic function. To show the safety of this novel therapeutic modality in this patient population.

Background
Heart Failure with Preserved Ejection Fraction (HFpEF) affects approximately half of the patients suffering from heart failure, particularly the elderly. Although its prevalence is increasing, no progress has been made in the treatment of HFpEF. As sympathetic hyperactivity appears to play a role in its pathophysiology, we investigated catheter-based renal denervation as a potential new treatment for HFpEF.

Methods
We performed a multi-center open-label randomized clinical trial. Patients meeting the European criteria for HFpEF were randomized in a 1:1 fashion to renal denervation plus medical treatment, or to medical treatment only. The primary endpoint was change in diastolic dysfunction, assessed as E/e' on echocardiography, at 12 months follow-up. Secondary endpoints included change in functional and neurohumoral parameters for efficacy and left ventricular systolic function, complications and all-cause mortality for safety. Sample size calculation indicated a required sample size of 54 patients to detect a clinical relevant difference in E/e' between treatment groups.

Results
Enrolment started in May 2012 and ended on October 1st, 2015. In total, fourteen patients were randomized. Median change in E/e' ratio was -2.2 (range 3.3) in the intervention group and +0.8 (range 15) in the usual care group. No procedural complications or mortality were observed. One patient, randomized to the control group, was admitted for acute decompensated heart failure.

Conclusions
The study was stopped prematurely because of difficulties in recruitment and was therefore underpowered to detect changes in the predetermined endpoints. We found no safety objections against future larger trials to further exploring the potential of catheter-based RDN in heart failure.

Introduction

Heart failure is a major clinical problem, affecting approximately 1 to 2% of the adult population and >10% of the elderly population.[1] Although it was previously believed that heart failure with preserved ejection fraction (HFpEF) was a benign syndrome representing normal aging in the heart, it is now considered to have a similar morbidity and mortality risks as heart failure with reduced ejection fraction (HFrEF).[2] However, the pharmacological treatment that is the cornerstone of treatment for HFrEF has failed to demonstrate proven effectiveness in HFpEF. The current treatment of HFpEF is mostly based on expert opinion and entails reducing volume overload as well as the detection and treatment of comorbidities and precipitating factors, such as hypertension, atrial fibrillation and myocardial infarction.[3]

In contrast to HFrEF, in HFpEF the systolic left ventricular function is unaffected while the ventricular relaxation and compliance of the ventricles are impaired. As a result, the diastolic filling is reduced and inappropriate pressure increases in the left atrium may lead to volume overload and congestive heart failure.[4,5] The exact etiology of HFpEF is not yet fully elucidated, but the current consensus is that HFpEF is a systemic syndrome rather than a specific cardiac syndrome. Systemic inflammatory responses and coronary microvascular endothelial dysfunction, induced by comorbidities such as metabolic syndrome, arterial hypertension and renal dysfunction, are thought to cause the cardiac abnormalities.[6] The sympathetic nervous system may be an important mediator in this complicated system, since it is associated with HFpEF as well as the comorbidities that are known precipitators to HFpEF, such as hypertension and metabolic syndrome.[7] Subsequently, the sympathetic nervous system (SNS) may entail a possible therapeutic target. Since pharmacological inhibition of the SNS, such as beta-receptor blocking- or centrally acting sympatholytic agents, failed to show beneficial results in HFpEF[8-10], device-based therapy may offer new opportunities. Renal denervation (RDN) targets the renal sympathetic nerves by administering radiofrequency (RF) energy to the renal artery vessel wall. Penetrating the perivascular tissue, the RF energy is aimed to damage the adjacent sympathetic nerves, subsequently disrupting the sympatho-renal axis and inhibiting sympathetic nervous activity.[11]

Therefore, we hypothesized that catheter-based RDN on top of medical treatment is superior to medical treatment alone in improving diastolic function in patients with HFpEF. We set out to conduct a randomized controlled trial to test this hypothesis and to assess the safety of catheter-based RDN, a new technique in this patient population.

Methods

The DIASTOLE trial (NCT01583881) was a multicenter prospective randomized open-label clinical trial, conducted at three clinics in the Netherlands. The study was conducted in accordance to the declaration of Helsinki[13], the Good Clinical Practice Guidelines and the Dutch Medical Research Involving Human Subjects Act. The protocol was approved by the Medical Ethics Committee of the University Medical Center Utrecht and all participants gave written informed consent.

Patient population
The design and rationale of the DIASTOLE trial has been previously published.[12] In short, patients were eligible if they fulfilled the European Consensus Statement on the diagnosis of HFpEF, defined as (1) signs and/or symptoms of heart failure, (2) normal or mildly reduced LV systolic function (EF >50%), and (3) evidence of abnormal LV relaxation, filling, diastolic distensibility and/or diastolic stiffness.[14] In addition, patients had to fulfil the

diagnostic WHO criteria for hypertension (i.e. an untreated systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg) and be adequately controlled by least two antihypertensive drugs. Major exclusion criteria included myocardial infarction as direct cause of heart failure, Hypertrophic Obstructive Cardiomyopathy (HOCM), severe chronic obstructive pulmonary disease (COPD) Gold III or higher, and hemodynamically significant valvular heart disease. Further exclusion criteria included contra-indications for RDN[15], contra-indications for magnetic resonance imaging (MRI), and any serious medical condition or frailty that may adversely affect the safety and/or effectiveness of the participant or the study.

Intervention

Patients were randomly assigned in a 1:1 fashion to either RDN plus medical treatment or to medical treatment alone (according to the 2012 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure[16]).

Catheter-based RDN was performed by an interventional cardiologist using the Medtronic Symplicity™ Renal Denervation System, a 6Fr compatible, single-use radiofrequency probe, with at least six ablation per renal artery. Information about number of (treated) renal arteries, number of ablations, ablation notches, ablation time, amount of contrast used, electrophysiological measurements (e.g. impedance and power) and peri-procedural complications was collected. Antihypertensive and heart failure medication were intended to be maintained throughout the study at baseline dose, but modifications were allowed if adjustment of medication was deemed medically necessary (e.g. due to significant BP changes, heart failure worsening or adverse events directly related to the medications).

Study measurements and follow-up

During the screening procedure, patients were subjected to a physical examination, a 24-hour holter registration, 24-hour blood pressure monitoring (ABPM), the six minute walking test[17], the Minnesota Living With Heart Failure Questionnaire (MLWHFQ)[18], echocardiography, cardiac magnetic resonance imaging (cMRI) and laboratory tests.[12] Potential adverse events were assessed by a telephone call one week after RDN (intervention group) or three weeks after randomization (control group). Baseline measurements were repeated at 6 months (physical examination, 6-minute walking test, ABPM, laboratory tests, echocardiography and optionally cMRI) and 12 months follow-up (all). For a detailed description of study measurements, we refer to the design and rationale of our study.[12] Adverse events were assessed during each follow-up visit and were defined as (1) occurrence of hospitalization due to a complication of RDN, (2) decrease in cardiac systolic function during follow-up, (3) a composite cardiovascular endpoint of myocardial infarction, sudden cardiac death, stroke, aortic or lower limb revascularization procedure, lower limb amputation, death from aortic or peripheral arterial disease, dialysis, death from renal failure, hospital admission for hypertensive emergency unrelated to non-adherence or non-persistence with drugs, or (4) all-cause mortality.

Study endpoints

The primary study endpoint was change from baseline in E/e' ratio on echocardiography at 12 months after RDN. Secondary endpoints included, among others, occurrence of minor and major adverse events as defined in the previous paragraph; left atrial volume index (LAVI) on echocardiography; change in left ventricular mass index (LVMI) and left ventricular ejection fraction (LVEF) assessed by cMRI; change in brain natriuretic peptides (BNP); change in 24-hour blood pressure; change in exercise capacity assessed by the six minute walking test (6MWT); changes in quality of life assessed by the MLWHFQ; and occurrence of hospitalization for clinical heart failure during 12 months follow-up.

Table 1. Baseline characteristics

	renal denervation (n=7)	medical treatment (n=7)
Age, years	65 (IQR 6)	67 (IQR 19)
Sex, male	3 (43%)	3 (43%)
Ethnicity, caucasian	7 (100%)	6 (86%)
History of CAD	2 (29%)	2 (29%)
History of CVD	1 (14%)	1 (14%)
Diabetes mellitus	5 (71%)	3 (43%)
Dyslipidaemia		
Current smoker	0	0
Previous smoker	4 (57%)	4 (57%)
eGFR <60 mL/min/m²	1 (14%)	0
Heart rate, bpm	75 (IQR 24)	60 (IQR 17)
Office BP, mmHg	129/77 (IQR 11/17)	153/84 (IQR 30/16)
24hour BP, mmHg	122/72 (IQR 25/16)	133/73 (IQR 9/18)
Body mass index, kg/m²	31 (IQR 6)	37 (IQR 14)
NYHA function class at enrolment		
I	1	0
II	4	5
III	2	1
IV	0	0
LVEF, %	58 (IQR 9)	60 (IQR 8)
E/e'	10 (IQR 7)	14 (IQR 2)
BP lowering medication		
Diuretic	6 (86%)	4 (57%)
RAAS-inhibitors	7 (100%)	7 (100%)
Beta-blocker	5 (71%)	5 (71%)
Alpha-blocker	1 (14%)	1 (14%)
CCB	4 (57%)	5 (71%)
DDD	3,8 (IQR 3,1)	4,0 (IQR 3,6)

Values are depicted as median (interquartile range) or absolute number (percentage). Abbreviations: BP = blood pressure, CAD = coronary artery disease, CCB = calcium channel blocker, CVD = cerebrovascular disease, DDD = daily defined dose, eGFR = estimated glomerular filtration rate, LVEF = left ventricular ejection fraction, RAAS = renin-angiotensin-aldosterone system.

Statistical analysis

Sample size calculation yielded a required sample size of 54 patients (27 in each arm) to have a 80% power to detect significant difference in the primary endpoint (E/e') of 2.1 based on a standard deviation of 2.8.[12] Due to the very slow enrolment, we were forced to terminate the study with only fourteen patients randomized. Primary efficacy analysis is based on the (modified) intention to treat population including all patients randomized with available E/e'-data at follow-up. Non-parametric tests were used for the analysis of all endpoints. Continuous variables are presented as a median (interquartile range, IQR). Categorical data are presented as counts (percentages). Between-group differences for all continuous endpoints were assessed using the independent samples Mann-Whitney, using the change at follow-up compared to baseline. Change in LVEF at 12 month follow-up compared to baseline in each study arm was assessed by the related-samples Wilcoxon signed rank test. Results were considered to be statistically significant if the two-tailed p-value was less than 0.05.

Results

Since the start of this study in May 2012, eighteen patients were included. After exclusion of four patients with screen failure (three renal artery stenoses and one secondary hypertension), fourteen patients were randomized. The study was prematurely terminated in October 2015 due to very difficult enrolment despite an extended recruitment period and the addition of extra recruiting centers. At the UMC Utrecht, 119 potentially eligible patients were asked to participate in the study, of which ten were willing to participate. The most important reasons for exclusion were patient refusal (n=41), frailty (n=25), diagnosis of HFpEF insufficiently established or patient was asymptomatic (n=20), presence of an exclusion criterion (n=18) and miscellaneous reasons (n=5). For the other two centers, screening information was not available.

The general baseline characteristics of the fourteen randomized patients are shown in table 1. In the RDN group, one patient was lost to follow-up due to an esophageal carcinoma that was diagnosed shortly after treatment.

Renal denervation procedure

Patients had a median of 8 ablations per renal artery, with a median temperature rise of 58 degrees Celsius and a median drop in impedance of -13. One patient was treated unilaterally because denervation of the left renal artery failed. No periprocedural complications related to the RDN procedure occurred.

Primary and secondary efficacy endpoint

Figure 1 shows the changes in different efficacy endpoints at 6 months and 12 months follow-up compared to baseline. The median change in E/e' ratio at 12 months follow-up was -2.2 (values ranging from -3.6 to -0.3) in the intervention group and +0.8 (values ranging from -6.9 to +5.7) in the usual care group (p=0.27). The median change in mean 24-hour systolic blood pressure (SBP) was +12 mmHg (values ranging from -1 to +15) in the RDN group and -9 mmHg (values ranging from -26 to +2) in the control group. The changes in blood pressure may be affected by changes in antihypertensive drugs, as blood pressure lowering drugs were reduced due to a low blood pressure in two RDN patients and in one control patient, whereas medication was increased in three individuals in the control group. One patient who was assigned to the control group was admitted for acute decompensated heart failure. This 68-year old man was admitted to the hospital with acute decompensated heart failure and discharged 11 days later. No heart failure related hospital admissions occurred in the RDN group.

Safety and SAE's

No relevant deterioration in left ventricular ejection fraction was observed. Median change at 12 months compared to baseline was +4.0% (values ranging from 0 to +13) in the RDN group and -2.5% (values ranging from -6 to +6) in the control group (p=0.19 for between-group difference). Nine serious adverse events occurred during the course of the study, none of which was assumed to be related to the RDN procedure. One patient was admitted for atrial fibrillation and one patient experienced an acute coronary syndrome. With the exception of the aforementioned episode of acute decompensated heart failure, the remaining events were not related to heart failure. We observed no deaths.

Discussion

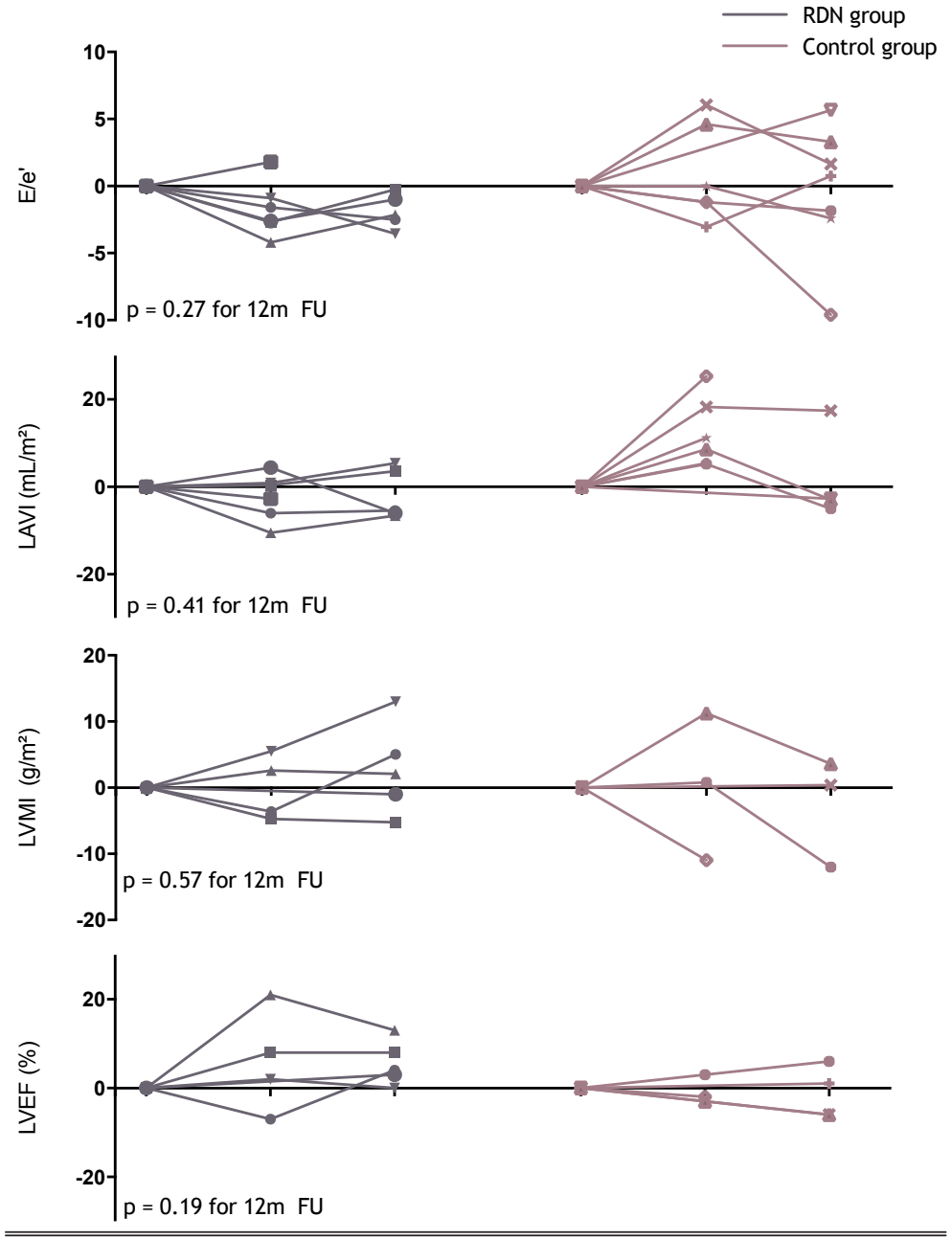
We report the results of our randomized controlled trial investigating the efficacy and safety of RDN in patients with HFpEF. No statistically significant effect on the primary efficacy endpoint, change in E/e' at 12 month follow-up, was observed. Notably, we found no evidence suggesting safety concerns for the application of RDN in this patient population. However, this study suffered from enrolment issues, as we were able to randomize only fourteen of the required 57 patients, leaving our study severely underpowered. Low power does not only reduce the probability of detecting statistically significant results, it also has a reduced likelihood that the observed results indeed reflect a true effect. As a consequence, conclusions regarding our results are limited.

Catheter-based RDN has been mostly used for the treatment of (resistant) hypertension and its application in patients with heart failure is relatively new. There was prior concern about possible adverse effects of RDN in this novel population, fearing similar problems to those encountered with the use of moxonidine, a centrally acting antihypertensive drug. The MOXCON study investigated the use of moxonidine in a population of patients with HFrEF.[10] The hypothesis was similar to our study; inhibition of the SNS results in superior treatment of heart failure. Although the moxonidine group indeed demonstrated decreases levels of plasma norepinephrine (indicating a reduced sympathetic nervous activity), it also had an increased mortality rate, mostly due to sudden cardiac death and pump failure.[10]

One of the concerns was that RDN in heart failure patients might lead to severe hypotension and related complications. Therefore, we chose to include only patients with treated hypertension in order to be able to counteract potential hypotension by withdrawing antihypertensive medication. In addition, this would prevent debate whether an observed effect would be merely due to better control of blood pressure (with subsequent improvement of diastolic function) or would truly reflect treatment of diastolic dysfunction. In our study, two patients in the RDN group and one patient in the control group had a reduction in antihypertensive drugs because of low blood pressure, but none of the patients experienced true hypotension. We also did not observe any procedure-related adverse events. Thus, our study found no support for the fear that RDN poses a risk for patients with HFpEF, although we realize our small sample size and limited follow-up. These results are in line with two small previous trials that investigated the effect of RDN in HFpEF[19] and HFrEF[20]. Therefore, there seem to be no safety objections against future larger trials to further exploring the potential of catheter-based RDN in heart failure.

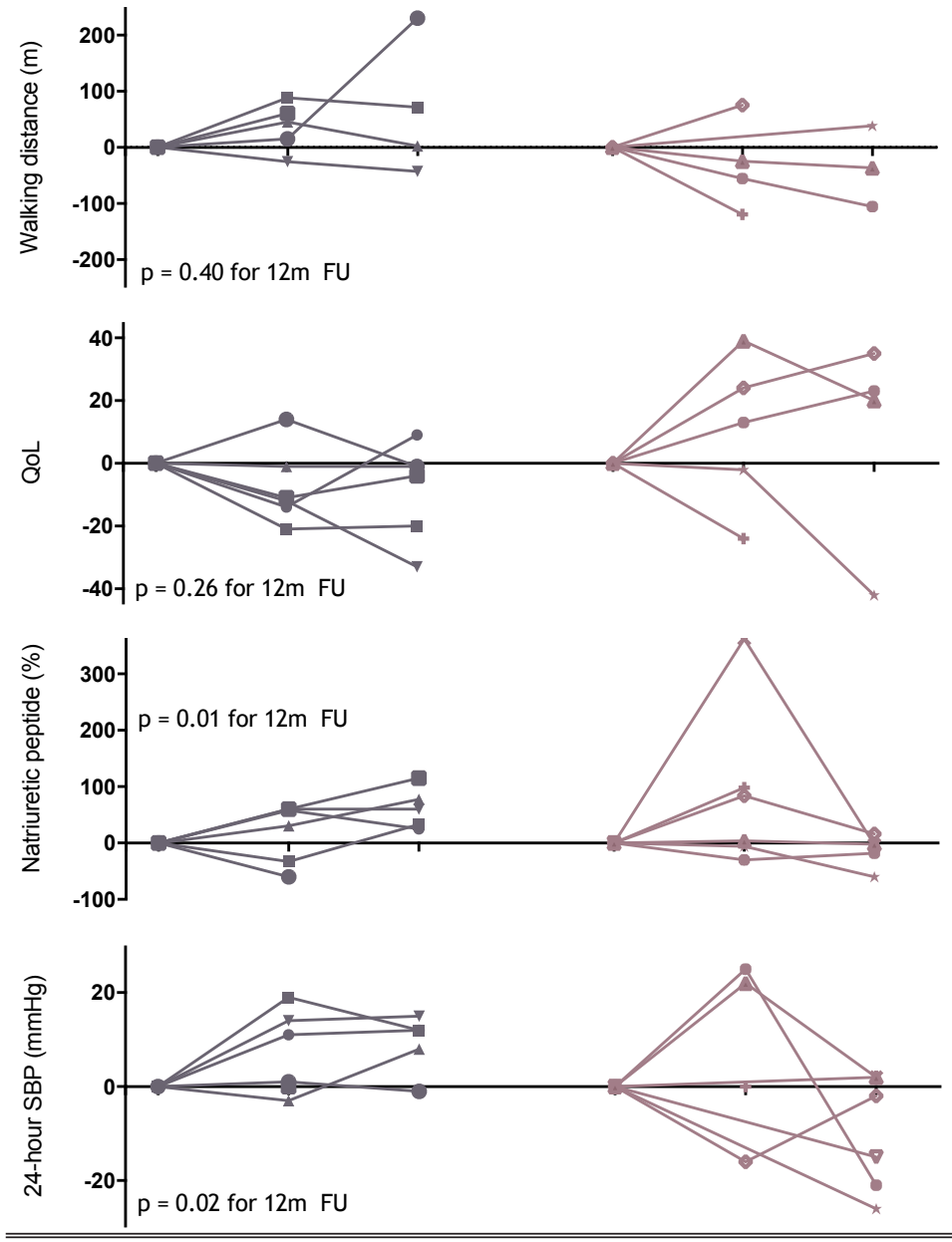
However, future trials should take into account the difficulties encountered in the enrolment of the current study. First, HFpEF is a relatively new disease and much is still unknown about its pathophysiology. As a result, there is no definite consensus on the exact characteristics of the disease. The definition and diagnostic criteria for HFpEF are continuously evolving, illustrating the difficulty of correctly diagnosing the disease.[3,16] In addition, the diagnosis of

Figure 1. Changes in efficacy endpoints at 6- and 12-month follow-up compared to baseline.



Change in efficacy endpoints at 6 months and 12 months follow-up compared to baseline. Statistical testing was performed for the 12-month follow-up only. Each line represents an individual patient. The renal denervation group is shown on the left hand side, while the control group is depicted on the right hand sight. For, LVEF and 6MWT walking distance, an increase reflects an improvement. For the remaining endpoints, a decrease is considered beneficial.

Figure 1. (continued)



Abbreviations: RDN = renal denervation; LAVI = left atrial volume index; LVMI = left ventricular mass index; LVEF = left ventricular ejection fraction; 6MWT = 6 minute walking test; QoL = quality of life as measured by the Minnesota living with heart failure questionnaire; SBP = systolic blood pressure.

HFpEF is hindered by the fact that the "typical" clinical symptoms are non-specific and co-morbidities are prevalent. As a result, the presence of HFpEF often remains unrecognized.[21-23] Furthermore, there is often debate whether suspected heart failure in the presence of a preserved LVEF should be contributed to HFpEF or to an alternative diagnosis, such as obesity, myocardial ischemia or chronic obstructive pulmonary disease (COPD).[21,24] Hence, HFpEF is a challenging diagnosis which obviously complicates the enrolment of HFpEF trials. In addition, the complexity of HFpEF may also complicate the outcomes of HFpEF trials. HFpEF is a heterogeneous disease with a multifactorial pathophysiology that might require separate treatment strategies for different HFpEF phenotypes. Subsequently, researchers will have to balance between stricter inclusion criteria for a more homogeneous population and broader criteria to facilitate enrolment.

Secondly, HFpEF patients are generally older, have more comorbidities and more often remain in the care of the general practitioner.[25] As such, they may be more likely to decide against participation in trial. In our study, 21% of eligible patients refrained from participation due to frailty. Another 34% did not want to participate for other reasons, the majority because they considered themselves "too old". In addition, it was difficult for included patients to adhere to the study protocol. Particularly the cMRI appeared to be bothersome, which regrettably resulted in missing follow-up measurements for five out of the fourteen patients. For future studies, collaboration with general practitioners, long-care facilities, and diagnostics facilities may help to reach HFpEF patients that are not referred to academic hospitals. Furthermore, researchers may consider executing study procedures in the primary care to accommodate the less mobile patients.

The current status of the RDN treatment may also have important implications for future trials. RDN has suffered a major setback after the publication of the results of the HTN-3 trial.[26] Following these disappointing results, the previous results of trials into the effect of RDN have been reviewed with a more critical eye. Several shortcomings of these trials and of the RDN procedure itself have been brought to attention.[27] Among the most important issues is the fact that RDN remains a 'black box' procedure: there is a lack of a quantitative read-out of procedural success. In addition, our knowledge on the depth of the achieved ablations, the amount of nerve damage achieved and the amount of nerve damage needed is insufficient. These issues have to be resolved before further research is conducted in the HFpEF population.

Conclusions and clinical implications

The present trial is inconclusive due to the small sample size, but its information may be of value in a collaborative action of pooling individual data from existing trials into the same topic; the RDT-PEF[19] and the RESPECT-HF (clinicaltrials.gov identifier NCT02041130) trials. At present, this collaboration has started and is expected to finish in the spring of 2017 with a total number of approximately 60 patients in all trials combined.

Acknowledgements

We acknowledge the support from the Netherlands Cardiovascular Research Initiative, an initiative with support of the Dutch Heart Foundation, CVON2014-11 RECONNECT.

References

1. Mosterd A, Hoes AW. Clinical epidemiology of heart failure. Heart 2007;93:1137–1146.
2. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. N Engl J Med 2006;355:251–259.
3. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 2016;37:2129–2200.
4. Edelmann F, Schmidt AG, Gelbrich G, Binder L, Herrmann-Lingen C, Halle M, Hasenfuss G, Wachter R, Pieske B. Rationale and design of the 'aldosterone receptor blockade in diastolic heart failure' trial: a double-blind, randomized, placebo-controlled, parallel group study to determine the effects of spironolactone on exercise capacity and diastolic function in pa. Eur J Heart Fail 2010;12:874–882.
5. Wachter R, Schmidt-Schweda S, Westermann D, Post H, Edelmann F, Kasner M, Lüers C, Steendijk P, Hasenfuß G, Tschöpe C, Pieske B. Blunted frequency-dependent upregulation of cardiac output is related to impaired relaxation in diastolic heart failure. Eur Heart J 2009;30:3027–3036.
6. Shah SJ, Kitzman DW, Borlaug BA, Heerebeek L van, Zile MR, Kass DA, Paulus WJ. Phenotype-Specific Treatment of Heart Failure With Preserved Ejection Fraction. Circulation 2016;134:73–90.
7. 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;108:227–237.
8. Veldhuisen DJ van, Cohen-Solal A, B??hm M, Anker SD, Babalis D, Roughton M, Coats AJS, Poole-Wilson PA, Flather MD. Beta-Blockade With Nebivolol in Elderly Heart Failure Patients With Impaired and Preserved Left Ventricular Ejection Fraction. Data From SENIORS (Study of Effects of Nebivolol Intervention on Outcomes and Rehospitalization in Seniors With Heart Failure). J Am Coll Cardiol 2009;53:2150–2158.
9. Hernandez AF, Hammill BG, O'Connor CM, Schulman KA, Curtis LH, Fonarow GC. Clinical Effectiveness of Beta-Blockers in Heart Failure. Findings From the OPTIMIZE-HF (Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients With Heart Failure) Registry. J Am Coll Cardiol 2009;53:184–192.
10. Cohn JN, Pfeffer MA, Rouleau J, Sharpe N, Swedberg K, Straub M, Wiltse C, Wright TJ. Adverse mortality effect of central sympathetic inhibition with sustained-release moxonidine in patients with heart failure (MOXCON). Eur J Heart Fail 2003;5:659–667.
11. Verloop WL, Voskuil M, Doevendans PA. Renal denervation: a new treatment option in resistant arterial hypertension. Neth Heart J Netherlands; 2013;21:95–98.
12. Verloop WL, Beeftink MMA, Nap A, Bots ML, Velthuis BK, Appelman YE, Cramer MJ, Agema WRP, Scholtens AM, Doevendans PA, Allaart CP, Voskuil M. Renal denervation in heart failure with normal left ventricular ejection fraction. rationale and design of the diastole (denervation of the renal sympathetic nerves in heart failure with normal lv ejection fraction) trial. Eur J Heart Fail 2013;15:1429–1437.
13. World Medical Association. WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. 2013;1–8.
14. Paulus WJ, Tschope C, Sanderson JE, Rusconi C, Flachskampf F a., Rademakers FE, et al. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. Eur Heart J 2007;28:2539–2550.
15. Verloop WL, Agema WRP, Allaart CP, Blankestijn PJ, Khan M, Meuwissen M, Muijs van de Moer WM, Rensing BJWM, Spiering W, Voskuil M, Doevendans P a. Renal denervation for the treatment of hypertension: the Dutch consensus. Neth J Med 2014;72:449–454.
16. Mcmurray JJ V, Adamopoulos S, Anker SD, Auricchio A, Böhm M, Dickstein K, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012. Eur J Heart Fail 2012;14:803–869.
17. Butland RJ, Pang J, Gross ER, Woodcock AA, Geddes DM. Two-, six-, and 12-minute walking tests in respiratory disease. Br Med J (Clin Res Ed) 1982;284:1607–1608.
18. Rector TS, Cohn JN. Assessment of patient outcome with the Minnesota Living with Heart Failure questionnaire: Reliability and validity during a randomized, double-blind, placebo-controlled trial of pimobendan. Am Heart J 1992;124:1017–1025.
19. Patel HC, Rosen SD, Hayward C, Vassiliou V, Smith GC, Wage RR, Bailey J, Rajani R, Lindsay

AC, Pennell DJ, Underwood SR, Prasad SK, Mohiaddin R, Gibbs JSR, Lyon AR, Mario C Di. Renal denervation in heart failure with preserved ejection fraction (RDT-PEF): a randomized controlled trial. Eur J Heart Fail 2016;
20. Davies JE, Manisty CH, Petraco R, Barron AJ, Unsworth B, Mayet J, Hamady M, Hughes AD, Sever PS, Sobotka PA, Francis DP. First-in-man safety evaluation of renal denervation for chronic systolic heart failure: Primary outcome from REACH-Pilot study. Int J Cardiol 2013;162:189–192.
21. Riet EES van, Hoes AW, Limburg A, Landman MAJ, Hoeven H van der, Rutten FH. Prevalence of unrecognized heart failure in older persons with shortness of breath on exertion. Eur J Heart Fail Wiley Online Library; 2014;16:772–777.
22. Rutten FH, Cramer M-JM, Grobbee DE, Sachs APE, Kirkels JH, Lammers J-WJ, Hoes AW. Unrecognized heart failure in elderly patients with stable chronic obstructive pulmonary disease. Eur Heart J 2005;26:1887 LP-1894.
23. Hancock HC, Close H, Mason JM, Murphy JJ, Fuat A, Singh R, Wood E, Belder M De, Brennan G, Hussain N, Kumar N, Wilson D, Hungin APS. High prevalence of undetected heart failure in long-term care residents: Findings from the Heart Failure in Care Homes (HFinCH) study. Eur J Heart Fail 2013;15:158–165.
24. Caruana L, Petrie MC, Davie AP, McMurray JJ. Do patients with suspected heart failure and preserved left ventricular systolic function suffer from 'diastolic heart failure' or from misdiagnosis? A prospective descriptive study. BMJ 2000;321:215–218.
25. Lam CSP, Donal E, Kraigher-Krainer E, Vasan RS. Epidemiology and clinical course of heart failure with preserved ejection fraction. Eur J Heart Fail 2011;13:18–28.
26. Bhatt DL, Kandzari DE, O'Neill WW, D'Agostino R, Flack JM, Katzen BT, Leon MB, Liu M, Mauri L, Negoita M, Cohen S a, Oparil S, Rocha-Singh K, Townsend RR, Bakris GL. A controlled trial of renal denervation for resistant hypertension. N Engl J Med 2014;370:1393–1401.
27. Mahfoud F, Böhm M, Azizi M, Pathak A, Durand Zaleski I, Ewen S, et al. Proceedings from the European clinical consensus conference for renal denervation: Considerations on future clinical trial design. Eur Heart J 2015;36:2219–2227.

Chapter 10 -
General Discussion

The purpose of the research described in this thesis was to address several challenges encountered in the management of hypertension and heart failure with preserved ejection fraction. The thesis is divided in two parts. **Part ONE** discusses the management of difficult-to-control hypertension, focusing on the influence of antihypertensive medication on the diagnostic work-up strategy of hypertension and the shortcomings of renal denervation (RDN) as a treatment option for hypertension. **Part TWO** focuses on heart failure with preserved ejection fraction (HFpEF), describing the role of the sympathetic nervous system in HFpEF. Also, the rationale and design, challenges and results of the DIASTOLE trial that investigated renal denervation as a potential treatment for HFpEF are described.

Advanced diagnostics and therapeutic strategies in hypertension

Hypertension is a major health care problem in Western society, affecting over 30% of the Dutch population aged 30 to 70 years old.[1] Approximately 54% of stroke and 47% of ischemic heart disease can be contributed to hypertension[2], accounting for approximately 10.4 million annual premature deaths globally.[3] It has been estimated that "optimal" blood pressure control (defined as office blood pressure <140/90 mmHg) could prevent up to 50% of cardiovascular events.[4] Yet, achieving optimal blood pressure control proves to be a major challenge.
A distinct subtype of uncontrolled hypertension is resistant hypertension. Resistant hypertension is defined as (1) persistent hypertension above target (usually <140/90 mmHg for office blood pressure) despite prescribed treatment with three or more antihypertensive drugs of different classes at optimal dose, ideally including a diuretic, or (2) the necessity to prescribe more than three different antihypertensive agents to achieve adequate blood pressure control.[5] This definition was formulated to identify patients who may particularly benefit from an extensive diagnostic work-up for secondary causes of their hypertension and the development of new therapeutic strategies.

The detection of secondary causes of hypertension, particularly endocrine disorders, is hampered by interference of antihypertensive medication.[6] Hence, temporary discontinuation of interfering medication is recommended to improve the performance of diagnostic tests for secondary hypertension.[7] In **chapter 2**, we report on the safety of temporary discontinuation of antihypertensive medication in our 6-year experience with an extensive diagnostic work-up program for difficult-to-control hypertension at our own institution. In addition to patients with resistant hypertension, this program also included other patients at high risk of secondary hypertension such as patients with elevated blood pressure at a young age. We hypothesized that a controlled strategy of short-term discontinuation of antihypertensive medication would not result in an increased risk of major adverse cardiovascular and cerebrovascular events. Our study revealed an event rate that was similar (or even lower) in comparison to our reference group of patients with similar blood pressure levels undergoing a vascular prevention program but whose medication was not discontinued, and to placebo-arms in long-term hypertension trials. We also demonstrated that the temporary discontinuation of antihypertensive medication was well tolerated by the majority of patients. These findings show that it is feasible and safe to implement short-term discontinuation of antihypertensive medication in the diagnostic work-up for hypertension provided it is performed in an experienced center where patients are closely monitored and special attention is given to patients at higher risk of complications.

Among the biggest challenges in hypertension research is defining a reliable, unbiased, endpoint measurement. Ultimately, treatment of hypertension is directed at reducing cardiovascular events such as stroke and myocardial infarction. Therefore, hard endpoints

such as cardiovascular morbidity and mortality are clinically more relevant, but make randomized clinical trials large and time consuming (and thereby expensive and not always realistic or needed). In addition, these clinical endpoints may not be useful in daily clinical practice for the evaluation of management strategies in an individual patient. Blood pressure reduction is generally considered a valid surrogate endpoint for cardiovascular mortality and morbidity. As a result, it is the most applied endpoint in hypertension research, particularly in the earlier stages of the evaluation of therapeutic options. Although often treated as an objective endpoint measurement, blood pressure levels are easily affected by external factors that are not related to the intervention under study, but do affect the level of blood pressure during the trial.

The analysis described in **chapter 3** illustrates the influence of external factors on blood pressure reduction in hypertension studies. In this chapter, we analyzed repeated ambulatory blood pressure monitoring (ABPM) before and after withdrawal of antihypertensive medication. The obvious hypothesis was that blood pressure would increase in response to withdrawal of treatment. However, we observed that this was not the case for 40% of patients: blood pressure did not change in 23%, and 17% of cases even showed a decrease on mean 24-hour systolic blood pressure after cessation of more than 3 standard doses of antihypertensive medication. This observation emphasizes the potential threats of external factors to the internal and external validity of hypertension studies. Without regard for potential external factors, we would be tempted to conclude that antihypertensive treatment is ineffective in almost half of the patients treated for hypertension, or even that discontinuation of antihypertensive medication causes an improvement in blood pressure in a smaller percentage of patients. However, it is much more likely that the observed effects were (in part) caused by external factors such as changes in prescribed medication, placebo or Hawthorne effects, and (changes in) adherence, or statistical phenomena such as regression to the mean.

The discussion of the different external factors in hypertension research is comprehensive and may reach beyond the scope of this thesis. Therefore, we will focus on three external factors that have been most important for the research described in this thesis: regression to the mean and changes in antihypertensive medication are discussed below, while non-adherence will be discussed in the next section.
Regression to the mean can occur when repeated measurements are made on the same subject or unit of observation.[8] Random variation of a measurement can result in an extreme outlier in one of the measurements of a certain variable. The value on the other measurement tends to be closer to the true biological average, giving the impression of an effect size. The likelihood of regression to the mean is greatest when data are analyzed on a subject level, or when the outcome measurement (e.g. the blood pressure level) is used as a threshold for enrolment. As a result, hypertension trials should always be on alert for regression to the mean, as blood pressure is often both endpoint and inclusion criterion. Furthermore, the effect of regression to the mean may be stronger when a dichotomized endpoint is used, such as the "responder" classification which stratifies to responder or non-responder based on their individual change in blood pressure above a predefined threshold. This may result in an over-classification of responders, potentially blurring the true effects of the treatment. Repeatedly measured continuous outcome measurements, when compared to responder classification, are less influenced by regression to the mean. If a responder classification is however preferred, the influence of regression to the mean on the responder classification may be reduced by requiring patients to demonstrate a certain blood pressure reduction on all follow-up visits, as opposed to a single visit.[9]

Second, changes in prescribed antihypertensive medication may obviously influence blood pressure outcomes. Hypertension trials attempt to counteract this by requiring patients to remain on their baseline medication for the duration of the trial. A run-in period, in which patients should have a stable combination of antihypertensive medication during several weeks prior to the start of the trial, can be applied to make it more likely that a patient can complete study participation with unchanged prescribed medication. However, in a study that used a run-in of stable antihypertensive medication for at least 4 weeks as inclusion criterion, only 18 out of 40 patients had unchanged medication at 6 months follow-up.[10] A useful tool for analyzing drug utilization statistics is the daily defined dose (DDD) classification, designed by the World Health Organization Collaborating Centre for Drug Statistics Methodology.[11] Unfortunately, this classification is underutilized. Because the DDD takes the prescribed dosages into account, it is a better estimate of effective daily drug consumption[12] and should replace the currently most used estimate of drug consumption, i.e. (changes in) the number of pills. Still, it is important to realize that neither tool identifies changes to a different formulary and a switch from, for example, 40 mg furosemide to 25 mg hydrochlorothiazide (both 1 DDD), may still influence blood pressure as a patient may respond better to one agent or the other.

To resolve the therapeutic impasse encountered in patients with resistant hypertension, novel therapeutic approaches to optimize blood pressure control have been of increasing interest in recent decades with a special surge in device- or procedure-based strategies targeting the sympathetic nervous system. One of these novel interventions is catheter-based RDN. This endovascular procedure aims to damage the renal sympathetic nerves by delivering radiofrequency energy to the renal artery wall, disrupting the vicious circle of sympathetic activity between both kidneys and the brain which is thought to cause and sustain hypertension.[13] The enthusiasm for RDN was thriving after the initial HTN-1 cohort study and the HTN-2 randomized controlled trial.[14,15] However, after this initial flying start RDN came to a crashing stop in 2014 with the publication of the double-blind randomized sham-controlled Symplicity HTN-3 trial, that failed to meet its primary efficacy endpoint (change between 6 months and baseline in systolic office BP between treatment groups).[16] In the reflections that followed, several remaining questions concerning the mechanism of action of RDN were formulated.[17,18] We tried to contribute to resolving two of these questions in the studies discussed in chapters 4 and 5.

In **chapter 4**, we aimed to provide more insight in blood pressure changes following RDN to breach the lack of consensus on the timing of the effect on blood pressure and, thus, on optimal follow-up. We were particularly interested whether blood pressure acutely decreases shortly after RDN or whether the procedure takes effect over the course of several months. Therefore, we performed home blood pressure monitoring (HBPM) during one week every month throughout the first year after RDN. We observed a decrease over the first year of follow-up in systolic blood pressure after adjustment for known confounders, similar to the magnitude of change in ABPM in the HTN-3 trial.[19] Yet, we were unable to identify an abrupt change in blood pressure, indicating that blood pressure may decrease in a gradual rather than an abrupt fashion. Our study design did not allow for conclusion regarding causality, because our study was designed as a non-randomized, non-controlled study. Therefore, it is not possible to precisely quantify whether the effect on blood pressure is mainly effectuated by RDN or in part through external factors, such as regression to the mean, changes in antihypertensive medication, Hawthorne effects, lifestyle changes or natural history.

The study described in **chapter 5** aimed to contribute to the optimal procedural approach for RDN. Recent anatomical studies have demonstrated that the distance between the renal artery lumen and the perivascular renal nerves is smallest in the distal segments of the renal artery.[20-22] Thus, it can be hypothesized that denervation distal in the renal artery results in more nerve damage and, consequently, a more effective procedure. Therefore, we assessed the effect of distal ablation placement on safety and blood pressure. We found no significant difference in blood pressure reduction between patients who were treated proximal to the bifurcation, compared to patients who were also treated distally. The observed relation between the absolute number of distal ablations and systolic ambulatory blood pressure may indicate a beneficial effect of distal denervation but further research is needed. At least, denervation in the distal segments did not seem to affect the safety of the procedure. These observations may be a first step towards optimization of the denervation procedure, but several other important gaps in our knowledge remain.

Most importantly, a read-out of the procedure is currently lacking. Although there are some methods available for the measurement of sympathetic outflow (e.g. plasma or urinary norepinephrine levels, muscle sympathetic nerve activity, iodine 123-metaiodobenzylguanidine (MIBG) an heart rate variability), there is no 'gold-standard' method for assessing sympathetic activity. Furthermore, no biomarkers for nerve damage following RDN have been identified yet and non-invasive imaging modalities are unable to visualize the renal sympathetic nerves to confirm successful disruption in vivo. RDN therefore remains a 'black box' procedure in which we are uncertain whether the procedure has been effective.
The lack of a read-out can be compensated for if irrefutable evidence exists for the efficacy of the procedure, i.e. histological evidence that RDN consistently results in interruption of the sympathetic nerves. Yet, the histological evidence is limited. The few studies that have investigated penetration of the radiofrequency energy into the perivascular tissue suggest that the depth of the ablation is insufficient to reach the majority of renal sympathetic nerves.[21,23-26] It has been suggested that the newer generation RDN catheters are more effective,[27] but histological evidence or direct comparison of blood pressure lowering effects between different devices to support this statement is lacking.

Lastly, there appears to have been too little attention to the possible influence of external factors in renal denervation studies. Particularly non-adherence may have adversely affected blood pressure outcomes of renal denervation trials. Adherence to prescribed medication is known to be poor in hypertension treatment; non-adherence is reported in 25% to 53% of patients.[28-30] Even among patients enrolled in a trial, ~50% of patients have been shown to be non-adherent.[31] More importantly, patients have been shown to improve, for example, their lifestyle and adherence to the prescribed medication (also referred to as the Hawthorne effect).[32] However, the reverse may also be true for trials, as patients may believe pharmacological treatment of hypertension is made redundant by the intervention. This stresses the importance of a double-blind trial design in which patients are unaware of their treatment status. Furthermore, future RDN trials should consider implementing objective measurement of adherence to antihypertensive medication (e.g. toxicological assessment) throughout follow-up to investigate the influence of changes in adherence. Some studies have attempted to eliminate the influence of non-adherence by performing toxicological analysis at baseline,[10,33] but in order to fully investigate possible influence of non-adherence toxicological analysis should be performed both at baseline and at follow-up. Another option is to perform off-med measurements, as proposed for the Spyral HTN-OFF MED study.[34] We have shown in chapter 2 that temporarily discontinuation of antihypertensive medication is feasible and safe. When sufficient time is allowed for the

effects of the drugs to wear off, this may be the most “pure” assessment of blood pressure in interventional trials.

Sympathetic hyperactivity and heart failure with preserved ejection fraction

Heart failure with preserved ejection fraction (HFpEF) has only relatively recent been established as a separate entity in the field of heart failure. It is generally agreed upon that HFpEF comprises approximately 50% of all heart failure patients and that its prognosis is similar to that of heart failure with reduced ejection fraction (HFrEF).[35] Yet, there is no definitive evidence or consensus on the etiology and pathophysiology. Also, many proven effective drugs for HFrEF have failed to show beneficial effects in HFpEF, including angiotensin converting enzyme (ACE-)inhibitors,[36,37] angiotensin receptor blockers (ARBs),[36,38,39] aldosterone antagonists[40,41] and beta-blockers.[36,42] The most supported hypothesis is that HFpEF is a systemic syndrome, rather than a specific cardiac disease, that results from a pro-inflammatory state induced by risk factors and co-morbidities such as hypertension, diabetes, obesity, chronic kidney disease and chronic obstructive pulmonary disease.[43] The common denominator in these diseases is sympathetic hyperactivity, suggesting a possible role of the sympathetic nervous system in the pathophysiology of HFpEF. In **chapter 6**, we report the results of a systematic review of both clinical and preclinical studies to investigate the potential relation between the sympathetic nervous system and HFpEF. Although the available literature was scarce and heterogeneous in design, the best available evidence indicated that increased sympathetic nervous activity was indeed related to diastolic dysfunction and HFpEF both in human studies and in preclinical research. We also found evidence that the increased sympathetic activity could not be simply attributed to the presence of hypertension in HFpEF patients, as sympathetic activity was significantly increased in hypertensive HFpEF patients compared to hypertensive controls with similar blood pressure levels.

The presence of sympathetic hyperactivity makes sympathetic blockade a potential therapeutic target in HFpEF. Therefore, we designed the DIASTOLE trial to investigate whether RDN may show benefit for patients with HFpEF (**Chapter 7**). The DIASTOLE (DenervatIon of the renAl Sympathetic nerves in heart failure with nOrmal Left ventricular Ejection fraction) trial was designed as a multicenter, prospective, randomized controlled trial. We aimed to enroll 60 patients, randomly allocated in a one-to-one ratio to undergo RDN with continuation of medical treatment or to continue medical treatment alone. The primary endpoint was defined as change in echocardiographic E/e’ ratio as a measure of diastolic dysfunction at 12 months follow-up. The safety of the procedure was also of special interest, as RDN had not yet been performed in this population. Secondary endpoint included changes in quality of life, exercise capacity, heart failure biomarkers, MRI parameters, hospitalizations for clinical heart failure, and myocardial MIBG-uptake and washout. We estimated 18 months to be required to complete enrolment and thus expected a total study duration of approximately 30 months.

Chapter 8 addresses the difficulty in enrolling patients in the DIASTOLE study. We searched for patients with isolated HFpEF in an electronic database containing all echocardiography performed at our hospital. We complemented the echocardiography data with clinical data from the patients’ medical records to identify patients who fulfill the criteria for HFpEF according to the European consensus document (i.e. signs and/or symptoms of heart failure; normal or mildly reduced left ventricular ejection fraction (LVEF); evidence of left ventricular diastolic dysfunction).[44] The echocardiographic definition for diastolic

dysfunction in this document relies for a large part on the E/e’ parameter. We found that spectral tissue Doppler of the mitral annulus (yielding E/e’) was not performed in approximately half of the patients undergoing echocardiography at our cardiac function unit (for instance because of a short focused echo on valvular disease). Out of the total of 3194 patients, 70 patients fulfilled the criteria for HFpEF. However, only six patients were indeed diagnosed with HFpEF by their treating physician. These results illustrate that HFpEF is a difficult diagnosis and is infrequently encountered in the academic setting, and even when present, remains often unrecognized.

This has also impacted the enrolment of the DIASTOLE trial, the results of which are described in **Chapter 9**. Despite modifications of the in- and exclusion criteria, addition of more participating centers and prolonging of the enrollment period we were forced to terminate the study with only 14 patients randomized. In this small sample size, no safety-issues of the RDN procedure for this particular population were encountered. Especially, no hemodynamic complications were encountered. There was a subtle difference for the primary endpoint (effect on E/e’ at 12 months) between the intervention group and the control group (median change -2.2 versus +0.8). Nevertheless, these results are insufficient with respect to statistical power to make a firm statement regarding the effectiveness of RDN in the treatment of HFpEF.
Unfortunately, enrolment issues are not uncommon in HFpEF research. Besides the above-mentioned issues, HFpEF patients are generally elderly, frail and have high rates of comorbidities. As a result, they often remain in the care of their general practitioner and are therefore infrequently encountered in the hospital setting where most research is conducted. In addition, establishment of the diagnosis is hindered by the presence of the mentioned comorbidities, lack of access to echocardiographic assessment and changing guidelines on the diagnosis of HFpEF. The current 2016 guidelines define HFpEF as (1) signs and symptoms of heart failure, (2) left ventricular ejection fraction ≥50% and (3) elevated levels of natriuretic peptides and at least one additional criterion (i.e. left ventricular hypertrophy, left atrial enlargement or diastolic dysfunction).[45] Yet, this definition contains some indistinct elements. Symptoms that are considered ‘typical’ for heart failure, such as shortness of breath, reduced exercise tolerance, fatigue and ankle swelling, are also non-specific and have a long differential diagnosis. In addition, signs and symptoms caused by fluid retention may resolve quickly after diuretic therapy. As a consequence, HFpEF often remains unrecognized, particularly when an alternate explanation for shortness of breath, such as chronic obstructive pulmonary disease (COPD), is present.[46,47]

Echocardiographic examination to assess evidence of abnormal left ventricular relaxation is essential for an adequate diagnosis of HFpEF, but access to echocardiography is limited in primary care.[48] In addition, the reference values for indices of diastolic (dys)function vary over different age categories, complicating straightforward interpretation. As a result, the echocardiographic diagnosis may be difficult to establish, illustrated by the fact that the current grading system fails to classify approximately a quarter of patients with diastolic dysfunction.[49] Thus, even in an institution where all facilities are available, such as an academic hospital, the condition is often not recognized. Improved collaboration between heart failure specialists and general practitioners as well as general cardiologists could have great benefits for both patient treatment and trial enrolment. Training of general practitioners in the current guidelines for the diagnosis and treatment of heart failure has been shown to improve the management and functional capacity of patients with heart failure.[50] Further assistance by heart failure specialists may further help improve diagnostic accuracy. In addition, co-operation may create opportunities to translocate HFpEF research from academia to local hospitals and primary care.

Conclusions

The studies discussed in this thesis contributed to a better insight in the challenges encountered in the management of hypertension and heart failure with preserved ejection fraction. Establishment of the correct diagnosis, careful definition and unbiased assessment of endpoint measurements, critical and thorough evaluation of new treatment modalities, and improved co-operation will benefit the routine clinical care and research efforts in hypertension and HFpEF.

First, establishment of the correct diagnosis is essential to prevent heterogeneous populations and misdiagnoses in research that may lead to spurious results. Since the 1970s, resistant hypertension has been subject of interest for many hypertension studies. However, recently the distinction has been made between 'true' and 'apparent' resistant hypertension after improved screening methods demonstrated high percentages of secondary causes or non-adherence in patients who were considered treatment resistant.[31] This has particularly impacted RDN research, as not truly resistant hypertensive patients are thought to have greatly influenced the reliability of trial results.[17] Likewise, HFpEF studies suffer from heterogeneous populations and misdiagnoses since its pathophysiology is still incompletely understood and the definition of the condition is still evolving. This complicates trial enrolment and may contribute to lack of effect in HFpEF studies, but is not easily resolved until the characteristics of the condition are unraveled.

Secondly, endpoint measurements should be formulated carefully and assessed with regard for possible effect modifiers. We discussed the influence of several important effect modifiers in hypertension research. Improving blood pressure measurements by performing repeated measurements (e.g. ambulatory or home monitoring), performing double-blinded studies to reduce the influence of factors such as observer bias and placebo effects and assessing non-adherence by toxicological analyses are examples of important interventions to improve the reliability of blood pressure endpoints in hypertension studies. In HFpEF, the lack of complete understanding of its pathophysiology also results in a lack of consensus on the most appropriate endpoint. As a result, many studies include different endpoint measures, such as echocardiographic parameter of diastolic dysfunction (e.g. E/e'), physical performance measurements (e.g. 6MWT or cardiopulmonary exercise testing) and biochemical parameters of acute heart failure (e.g. natriuretic peptides). A general consensus on endpoint parameters will likely improve comparability between different studies and help to accelerate the efforts into unraveling this complicated disease state.

Third, critical and thorough (preclinical) evaluation of new treatment modalities prevents premature introduction of devices in clinical treatment. After the initial trials demonstrated impressive effect, relatively little attention was given to the proof of concept of RDN. Only after the HTN-3 trial failed to meet its efficacy endpoint, did the search for preclinical and histological evidence for the working mechanism of RDN increase. Although it is easy to appoint shortcomings in retrospect, there are several lessons to be learned to improve the introduction of new medical devices in the future. The introduction of a new pharmacological agent in the health care market is bound by strict regulations, including proven effectiveness in placebo-controlled trials. Yet, these restrictions do not (yet) apply to the medical device industry. The medical community has a responsibility to define a similar set of requirements to which a novel device should comply. The history of RDN has demonstrated the indispensability of a read-out of procedural success, histological proof-of-concept and a double-blind sham-controlled randomized clinical trial in the evaluation of a new device, and these should be among the minimal requirements.

Lastly, there are many opportunities for collaboration between different health care professionals to improve routine clinical care and research. A great example is the multidisciplinary diagnostic approach to difficult-to-control hypertension, described in chapter 2, which is a collaboration between different departments (e.g. vascular medicine, nephrology, radiology, clinical epidemiology and cardiology) and different health care professionals (e.g. physicians and, nurse specialists). In a complicated diagnosis such as HFpEF, patient management may also greatly benefit from multidisciplinary approaches. As HFpEF knowledge mostly resides with the academic research facilities, while the patients mostly populate the primary care, great gains can be achieved in closer collaboration between first and tertiary care. This may not only benefit patient care, but also trial enrolment. In the Netherlands, all hospitals that perform RDN procedures participate in the Dutch National Renal Denervation Registry, which has resulted in possibly one of the largest cohorts of patients to date. Parts of this cohort have been used for the analyses presented in chapter 4 and 5. Finally, collaboration may also offer a solution to trials struggling with enrolment. Besides the DIASTOLE trial, two other studies investigating the efficacy of RDN for HFpEF (the RDT-PEF study[51] and the RESPECT-HF (NCT02041130) trials) were also forced to terminate prematurely due to slow enrolment. Separately, these studies are considerably underpowered. However, when combined in a pooled analysis these studies may jointly be able to make a substantiated statement regarding the efficacy of RDN in HFpEF. Therefore, we are currently preparing an individual patient data analysis, combining (mostly unpublished) data from all three studies. Hopefully, this collaboration is able to make a valuable contribution to the field of HFpEF and can inspire researchers who encounter similar challenges.

References

1. Blokstra A., Vissink P., Venmans LMAJ., et al. [Nederland de Maat Genomen - Monitoring van Risicofactoren in de Algemene Bevolking].; 2011.
2. Lawes CM, Hoorn S Vander, Rodgers A. Global burden of blood-pressure-related disease, 2001. Lancet. 2008;371:1513-1518.
3. World Health Day. A global brief on Hypertension World Health Day 2013. 2013:1-40.
4. Wong ND, Thakral G, Franklin SS, et al. Preventing heart disease by controlling hypertension: Impact of hypertensive subtype, stage, age, and sex. Am Heart J. 2003;145:888-895.
5. 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-e526.
6. Stowasser M, Ahmed AH, Pimenta E, Taylor PJ, Gordon RD. Factors affecting the aldosterone/renin ratio. Horm Metab Res. 2012;44:170-176.
7. Jansen PM, van den Born BJH, Frenkel WJ, et al. Test characteristics of the aldosterone-to-renin ratio as a screening test for primary aldosteronism. J Hypertens. 2014;32:115-126.
8. Barnett AG. Regression to the mean: what it is and how to deal with it. Int J Epidemiol. 2004;34:215-220.
9. Lambert T, Nahler A, Rohla M, et al. Endpoint design for future renal denervation trials — Novel implications for a new definition of treatment response to renal denervation. Int J Cardiol. 2016;220:273-278.
10. Ott C, Mahfoud F, Schmid A, et al. The effect of renal denervation in moderate treatment-resistant hypertension with confirmed medication adherence. J Hypertens. September 2016.
11. WHO Collaborating Centre for Drug Statistics Methodology. Guidelines for ATC Classification and DDD Assignment 2015. 18th ed.; 2014.
12. Modolo R, de Faria AP, Ritter AM V, Coca A, Moreno H. Defined daily dose (DDD) and its potential use in clinical trials of resistant hypertension. Int J Cardiol. 2016;202:515-516.
13. Goldsmith SR, Sobotka P a., Bart B a. The Sympathorenal Axis in Hypertension and Heart Failure. J Card Fail. 2010;16:369-373.
14. Symplicity HTN-1 investigators. Catheter-Based Renal Sympathetic Denervation for Resistant Hypertension: Durability of Blood Pressure Reduction Out to 24 Months. Hypertension. 2011;57:911-917.
15. 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;126:2976-2982.
16. Bhatt DL, Kandzari DE, O'Neill WW, et al. A controlled trial of renal denervation for resistant hypertension. N Engl J Med. 2014;370:1393-1401.
17. Mahfoud F, Böhm M, Azizi M, et al. Proceedings from the European clinical consensus conference for renal denervation: Considerations on future clinical trial design. Eur Heart J. 2015;36:2219-2227.
18. Angle JF, Prince EA, Matsumoto AH, et al. Proceedings from the Society of Interventional Radiology Foundation Research Consensus Panel on Renal Sympathetic Denervation. J Vasc Interv Radiol. 2014;25:497-509.
19. Bakris GL, Townsend RR, Flack JM, et al. 12-Month Blood Pressure Results of Catheter-Based Renal Artery Denervation for Resistant Hypertension - The SYMPLICITY HTN-3 Trial. J Am Coll Cardiol. 2015;65:1314-1321.
20. Sakakura K, Ladich E, Cheng Q, et al. Anatomic assessment of sympathetic peri-arterial renal nerves in man. J Am Coll Cardiol. 2014;64:635-643.
21. Amsterdam WA. van, Blankestijn PJ, Goldschmeding R, Bleys RLAW. The morphological substrate for Renal Denervation: nerve distribution patterns and parasympathetic nerves. A post-mortem histological study. Ann Anat - Anat Anzeiger. 2015;204:71-79.
22. Roy AK, Fabre A, Cunningham M, Buckley U, Crotty T, Keane D. Post mortem study of the depth and circumferential location of sympathetic nerves in human renal arteries--implications for renal denervation catheter design. Catheter Cardiovasc Interv. 2015;86:E32-7.
23. Verloop WL, Hubens LEG, Spiering W, et al. The Effects of Renal Denervation on Renal Hemodynamics and Renal Vasculature in a Porcine Model. PLoS One. 2015;10:e0141609.
24. Rippy MK, Zarins D, Barman NC, Wu A, Duncan KL, Zarins CK. Catheter-based renal sympathetic denervation: Chronic preclinical evidence for renal artery safety. Clin Res Cardiol. 2011;100:1095-1101.
25. Steigerwald K, Titova A, Malle C, et al. Morphological assessment of renal arteries after radiofrequency catheter-based sympathetic denervation in a porcine model. J Hypertens. 2012;30:2230-2239.
26. Vink EE, Goldschmeding R, Vink A, Weggemans C, Bleijs RL a W, Blankestijn PJ. Limited destruction of renal nerves after catheter-based renal denervation: Results of a human case study. Nephrol Dial Transplant. 2014;29:1608-1610.
27. Thorp AA, Schlaich MP. Device-based approaches for renal nerve ablation for hypertension and beyond. Front Physiol. 2015;6.
28. Jung O, Gechter JL, Wunder C, et al. Resistant hypertension? Assessment of adherence by toxicological urine analysis. J Hypertens. 2013;31:766-774.
29. Tomaszewski M, White C, Patel P, et al. High rates of non-adherence to antihypertensive treatment revealed by high-performance liquid chromatography-tandem mass spectrometry (HP LC-MS/MS) urine analysis. Heart. 2014;100:855-861.
30. Pandey A, Raza F, Velasco A, et al. Comparison of Morisky Medication Adherence Scale with therapeutic drug monitoring in apparent treatment-resistant hypertension. J Am Soc Hypertens. 2015;9:420-426e2.
31. Azizi M, Pereira H, Hamdidouche I, et al. Adherence to Antihypertensive Treatment and the Blood Pressure Lowering Effects of Renal Denervation in the Renal Denervation for Hypertension (DENERHTN) Trial. Circulation. 2016;134:847-857.
32. van Onzenoort HAW, Menger FE, Neef C, et al. Participation in a Clinical Trial Enhances Adherence and Persistence to Treatment. Hypertension. 2011;58:573-578.
33. Rosa J, Widimsky P, Tou ek 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. 2014;65:407-413.
34. 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. Am Heart J. 2016;171:82-91.
35. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. N Engl J Med. 2006;355:251-259.
36. Verbrugge FH, Duchenne J, Bertrand PB, Dupont M, Tang WHW, Mullens W. Uptitration of renin-angiotensin system blocker and beta-blocker therapy in patients hospitalized for heart failure with reduced versus preserved left ventricular ejection fractions. Am J Cardiol. 2013;112:1913-1920.
37. Cleland JGF, Tendera M, Adamus J, Freemantle N, Polonski L, Taylor J. The perindopril in elderly people with chronic heart failure (PEP-CHF) study. Eur Heart J. 2006;27:2338-2345.
38. Massie BM, Carson PE, McMurray JJ, et al. Irbesartan in Patients with Heart Failure and Preserved Ejection Fraction. N Engl J Med. 2008;359:2456-2467.
39. Yusuf S, Pfeffer M a, Swedberg K, et al. Effects of candesartan in patients with chronic heart failure and preserved left-ventricular ejection fraction: The CHARM-preserved trial. Lancet. 2003;362:777-781.
40. Pitt B, Pfeffer M a., Assmann SF, et al. Spironolactone for heart failure with preserved ejection fraction. N Engl J Med. 2014;370:1383-1392.
41. Edelmann F, Gelbrich G, Duvinage A, et al. Differential interaction of clinical characteristics with key functional parameters in heart failure with preserved ejection fraction - Results of the Aldo-DHF trial. Int J Cardiol. 2013;169:408-417.
42. Hernandez AF, Hammill BG, O'Connor CM, Schulman KA, Curtis LH, Fonarow GC. Clinical Effectiveness of Beta-Blockers in Heart Failure. Findings From the OPTIMIZE-HF (Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients With Heart Failure) Registry. J Am Coll Cardiol. 2009;53:184-192.
43. Paulus WJ, Tschöpe C. A Novel Paradigm for Heart Failure With Preserved Ejection Fraction. J Am Coll Cardiol. 2013;62:263-271.
44. Paulus WJ, Tschope C, Sanderson JE, et al. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. Eur Heart J. 2007;28:2539-2550.
45. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J. 2016;37:2129-2200.

46. Riet EES van, Hoes AW, Limburg A, Landman MAJ, Hoeven H van der, Rutten FH. Prevalence of unrecognized heart failure in older persons with shortness of breath on exertion. Eur J Heart Fail. 2014;16:772-777.
47. Rutten FH, Cramer M-JM, Grobbee DE, et al. Unrecognized heart failure in elderly patients with stable chronic obstructive pulmonary disease. Eur Heart J. 2005;26:1887 LP-1894.
48. van Mourik Y, Bertens LCM, Cramer MJM, et al. Unrecognized heart failure and chronic obstructive pulmonary disease (COPD) in frail elderly detected through a near-home targeted screening strategy. J Am Board Fam Med. 2014;27:811-821.
49. Kuwaki H, Takeuchi M, Chien-Chia Wu V, et al. Redefining diastolic dysfunction grading: Combination of E/A ≤0.75 and Deceleration Time >140 ms and E/ε′ ≥10. JACC Cardiovasc Imaging. 2014;7:749-758.
50. van Riet EES, Hoes AW, Limburg A, Landman MAJ, Zuithoff PNA, Rutten FH. Effect of training general practitioners in drug treatment of newly detected heart failure patients with reduced or preserved ejection fraction: A cluster randomized trial. Int J Cardiol. 2016;217:174-182.
51. Patel HC, Rosen SD, Hayward C, et al. Renal denervation in heart failure with preserved ejection fraction (RDT-PEF): a randomized controlled trial. Eur J Heart Fail. 2016;18:703-712.

Appendix

Nederlandse samenvatting

Dit proefschrift richt zich op verschillende uitdagingen in de diagnostiek van en behandeling van hypertensie en hartfalen met behouden ejectiefractie.
Hypertensie is de meest voorkomende risicofactor voor hart- en vaatziekten. Ongeveer 30% van de Nederlandse bevolking tussen 30 en 70 jaar oud heeft hypertensie en wereldwijd draagt het bij aan circa 10.4 vroegtijdige sterfgevallen per jaar. Adequate behandeling van hypertensie kan ongeveer de helft van alle hart- en vaatziekten voorkomen, maar het percentage patiënten waarbij voldoende controle van de bloeddruk bereikt wordt (<140/90 mmHg), is teleurstellend laag. Een speciale subgroep zijn de patiënten met zogenoemde therapieresistente hypertensie, waarbij de bloeddruk niet onder controle is ondanks ten minste drie antihypertensiva, of waarbij minstens vier antihypertensiva nodig zijn om de bloeddruk voldoende te behandelen. Deze patiënten hebben een verhoogd cardiovasculair risico en een hogere kans op een secundaire oorzaak, waardoor juist deze groep voordeel zou kunnen hebben bij een uitgebreide analyse naar de oorzaken van de hypertensie en een gespecificeerde behandeling. In deel 1 van dit proefschrift staat de behandeling van moeilijk behandelbare hypertensie centraal, met specifieke aandacht voor de invloed van medicatie op de diagnostiek en voor de tekortkomingen van renale denervatie in de behandeling van hypertensie.
Hartfalen met een behouden ejectie fractie (HFpEF) wordt pas sinds kort beschouwd als een apart ziektebeeld. In tegenstelling tot hartfalen met verminderde ejectie fractie (HFrEF) is de systolische functie van het hart intact, maar bestaat er met name een stoornis in de diastolische functie (relaxatie). Er is nog weinig bekend over de etiologie en pathofysiologie, maar in het algemeen wordt aangenomen dat HFpEF niet zozeer een aandoening van het hart alleen is, maar dat het onderdeel is van een systemische ziekte veroorzaakt door een inflammatoire reactie in het lichaam. Helaas bestaat er nog geen bewezen effectieve behandeling voor deze patiënten. De studies in deel 2 van dit proefschrift richten zich op de rol van het sympathisch zenuwstelsel in dit ziektebeeld en op renale denervatie als potentieel effectieve behandeling.

Deel 1 - vooruitstrevende diagnostische en therapeutische aanpak van hypertensie.

Het gelijktijdig gebruik van antihypertensieve medicatie kan invloed hebben op de testresultaten in de diagnostiek naar secundaire oorzaken van hypertensie. In **hoofdstuk 2** wordt de veiligheid van het tijdelijk stoppen van antihypertensieve medicatie onderzocht. De Analyse Gecompliceerde Hypertensie (AGH) is een uitgebreid screeningsprogramma voor het identificeren van secundaire oorzaken en bijdragende factoren van moeilijk behandelbare hypertensie. Hierbij wordt de medicatie tijdelijk gestaakt om te voorkomen dat deze invloed heeft op de testresultaten. We vergeleken het optreden van hart- en vaatziekten (zoals hartinfarct, TIA's en herseninfarcten of -bloedingen) tijdens en kort na de medicatiestop van patiënten in de AGH analyse met een controlegroep, bestaande uit patiënten met een vergelijkbaar cardiovasculair risico waarbij de medicamenteuze behandeling niet onderbroken werd. In beide cohorten kwamen evenveel cardiovasculaire events voor. Het tijdelijk staken van medicatie lijkt dus geen verhoogd risico met zich mee te brengen. Deze aanpak kan mogelijk zorgen voor een verbetering van de diagnostiek rondom

hypertensie, en biedt ook mogelijkheden voor betrouwbaardere meting van eindpunten in interventiestudies voor hypertensie. Hierbij is het echter wel van belang dat een dergelijke analyse wordt uitgevoerd in een ziekenhuis met de juiste expertise en dat de veiligheid van de patiënten gewaarborgd is.
Hoofdstuk 3 laat zien dat het meten van bloeddruk onderhevig is aan verschillende factoren die de meting kunnen verstoren. In deze studie werd gekeken welk effect het tijdelijk staken van de medicatie heeft op 24-uurs bloeddrukmetingen. Hoewel verwacht werd dat de bloeddruk zou stijgen in reactie op het stoppen van de medicatie, zagen we dat de bloeddruk in circa 40% van de patiënten gelijk bleef of zelfs daalde. De oorzaken van deze opmerkelijke observatie kunnen op basis van deze studie niet met zekerheid worden vastgesteld, maar waarschijnlijk zijn er meerdere factoren die hebben bijgedragen aan onze resultaten. De belangrijkste factoren lijken te zijn: het optreden van regressie naar het gemiddelde, veranderingen in de voorgeschreven medicatie en het niet juist innemen van de voorgeschreven medicatie.
Deze (en andere) factoren lijken ook een rol gespeeld te hebben in de teleurstellende resultaten van renale denervatie. Renale denervatie is een relatief nieuwe behandeling voor deze patiënten, waarbij endovasculair radiofrequente energie wordt vrijgegeven aan de wand van de nierarterie, met als doel de naastgelegen sympathische zenuwvezels te onderbreken. Aangezien het sympathische zenuwstelsel een belangrijke bijdrager is aan het ontstaan en onderhouden van hypertensie, zou dit moeten resulteren in een lagere bloeddruk. De initiële resultaten van deze nieuwe behandeling waren veelbelovend, maar een grote studie uit 2014 toonde aan dat het effect op bloeddruk niet alleen zichtbaar was bij patiënten die renale denervatie hadden ondergaan: een vergelijkbare bloeddrukdaling werd gezien in de patiënten die met een sham-procedure waren behandeld. Veel is nog onduidelijk over deze behandeling en in hoofdstuk 4 en 5 hebben we geprobeerd om meer inzicht te verkrijgen in twee specifieke vraagstellingen.
In **hoofdstuk 4** werd getracht inzicht te verkrijgen in de wijze waarop bloeddruk daalt na renale denervatie. Om dit te onderzoeken hebben de proefpersonen elke maand gedurende een week thuismetingen gedaan gedurende het eerste jaar na het onderzoek. Deze analyse toonde aan dat de bloeddrukafname niet acuut optreedt direct na de behandeling, maar dat de bloeddruk gedurende het gehele eerste jaar geleidelijk afneemt.
Hoofdstuk 5 beschrijft een onderzoek naar de optimale uitvoering van de procedure. Anatomische studies hebben aangetoond dat de sympathische zenuwvezels distaal in de nierarterie dichter bij het lumen van de nierarterie liggen dan in de proximale arterie. Zodoende zouden de zenuwen distaal beter binnen het bereik van de renale denervatie liggen en zou de behandeling daar dus mogelijk effectiever zijn. Om dit te onderzoeken, zijn alle angiografie-beelden van patiënten in het UMC Utrecht en de Isala Klinieken geanalyseerd en hebben we bloeddrukeffecten vergeleken tussen patiënten die op verschillende plaatsen in de nierarterie zijn behandeld. Hierbij werden geen significante verschillen gevonden tussen patiënten die proximaal of distaal in de nierarterie behandeld zijn. Wel was er een significante relatie tussen de bloeddrukdaling en het totale aantal ablaties dat distaal van de bifurcatie was geplaatst. Hoewel deze resultaten wellicht een eerste stap kunnen zijn in de verbetering van de procedure, blijven er veel hiaten in onze kennis omtrent de behandeling, zoals het gebrek aan parameters die tijdens de procedure kunnen aantonen of de behandeling effectief is.

Deel 2 - sympatische overactiviteit en hartfalen met behouden ejectie fractie.

Hoofdstuk 6 beschrijft de resultaten van een systematische review, waarbij gekeken is of er een relatie bestaat tussen sympathische overactiviteit en hartfalen met behouden ejectie fractie (HFpEF). Hierbij is gekeken naar zowel klinische als preklinische onderzoeken. Hoewel de beschikbare literatuur beperkt was en de opzet van de verschillende studies sterk verschilde, suggereren de resultaten dat er inderdaad een relatie is tussen verhoogde sympathische activiteit en HFpEF. De verhoogde sympathische activiteit kon bovendien niet verklaard worden door de aanwezigheid van hypertensie bij HFpEF patiënten, aangezien patiënten met zowel HFpEF als hypertensie een hogere sympathische activiteit hadden dan patiënten met alleen hypertensie van vergelijkbare ernst.
Deze bevindingen wijzen erop dat renale denervatie mogelijk een effectieve behandeling is voor HFpEF. **Hoofdstuk 7** beschrijft de opzet en rationale van de DIASTOLE studie (DenervatIon of the renAl Sympathetic nerves in hearT failure with nOrmal Left ventricular Ejection fraction). Deze prospectieve, multicenter, gerandomiseerde studie was opgezet om 60 patiënten te includeren en 1:1 te randomiseren naar behandeling met renale denervatie (naast de medicamenteuze behandeling die zij al ontvingen) of alleen medicamenteuze behandeling. Het primaire eindpunt was verandering in echocardiographische E/e' ratio, een parameter voor diastolische dysfunctie, bij 12 maanden follow-up. Aangezien renale denervatie nog nooit was toegepast in deze populatie, was ook de veiligheid van de procedure van speciaal belang. De secundaire eindpunten omvatten onder andere kwaliteit van leven, inspanningstolerantie, biomarkers, MRI-parameters en ziekenhuisopnames voor hartfalen.
De inclusie bleek echter moeilijker dan verwacht, hetgeen wordt beschreven in **hoofdstuk 8**. We hebben de elektronische database met alle echocardiografie in ons ziekenhuis doorzocht op patiënten die voldeden aan de criteria voor HFpEF. Van de 3194 patiënten voldeden 70 patiënten aan de criteria voor HFpEF. De diagnose was echter bij slechts zes patiënten daadwerkelijk gesteld. Bij 21 patiënten was er een alternatieve verklaring voor de klachten, maar bij 43 patiënten werd er geen cardiale diagnose gesteld voor de klachten en was HFpEF niet overwogen als diagnose. Deze bevindingen illustreren dat HFpEF een moeilijke diagnose is, die weinig voorkomt in een academisch centrum, en dat het mogelijk niet altijd wordt herkent door de behandelend arts.
Hoofdstuk 9 beschrijft de resultaten van de DIASTOLE studie. Door de moeilijkheden bij de inclusie is de studie vroegtijdig beëindigd met slechts veertien van de beoogde 60 gerandomiseerde patiënten, ondanks versoepeling van de in- en exclusiecriteria en het toevoegen van deelnemende centra. In dit kleine patiëntenaantal zagen we geen complicaties die de veiligheid van renale denervatie in deze populatie in twijfel trekken. Het primaire eindpunt liet een subtiel effect zien in de interventiegroep ten opzichte van de controlegroep, maar er kan uiteraard geen uitspraak gedaan worden over de effectiviteit van de behandeling voor HFpEF.

Conclusies en aanbevelingen.

De studies in dit proefschrift hebben bijgedragen aan een beter inzicht in de uitdagingen waarmee men te maken heeft in de diagnostiek, de behandeling en het wetenschappelijk

onderzoek op het gebied van hypertensie en hartfalen. Hierbij kunnen vier belangrijke punten worden benadrukt.
Ten eerste is het belangrijk om de diagnose correct te stellen. We hebben bij hypertensie gezien dat diverse factoren de metingen kunnen beïnvloeden. Wanneer hier onvoldoende aandacht voor is, kan dit resulteren in misdiagnose. Een belangrijk voorbeeld is 'resistente hypertensie' waarvan de prevalentie aanzienlijk lager is dan voorheen gedacht, voornamelijk omdat secundaire oorzaken en non-adherence vaak onopgemerkt bleken. Bij HFpEF hebben zowel patiënten als de wetenschap nadeel van het feit dat er geen eenduidige definitie van het ziektebeeld is. Verder onderzoek naar de pathofysiologie en patiëntkarakteristieken zal in de komende jaren hopelijk bijdragen aan een betere definitie en meer homogene patiëntenpopulatie.
Ten tweede kunnen deze verstorende factoren en onduidelijkheid over de diagnose een gevaar vormen voor wetenschappelijke resultaten, omdat de metingen onbetrouwbaar zijn, de patiëntenpopulatie niet goed gedefinieerd of omdat onduidelijkheid bestaat over de meest geschikte eindpuntmaat.
Ten derde zou er een meer kritische evaluatie moeten plaatsvinden van nieuwe therapieën alvorens deze worden toegelaten tot de markt. Voor medicatie bestaan al strenge toelatingseisen, maar voor nieuwe technologieën is dit minder goed vastgelegd. De medische wereld heeft een verantwoordelijkheid om de effectiviteit te bewijzen voordat een nieuwe technologie wordt goedgekeurd. In de praktijk zal dit moeten betekenen dat er minstens een dubbelblinde gerandomiseerde trial is verricht.
Tenslotte toont dit proefschrift de kracht van samenwerking aan. De analyse voor gecompliceerde hypertensie is een samenwerking tussen verschillende afdelingen en verschillende zorglijnen, waarbij er hoogwaardige zorg voor de patiënt wordt gerealiseerd. Ook voor onderzoek liggen hier unieke kansen. Zo zijn alle patiënten die in Nederland behandeld zijn met renale denervatie opgenomen in een landelijke registratie en zou samenwerking tussen verschillende zorglijnen essentieel kunnen zijn voor het doen van wetenschappelijk onderzoek op het gebied van aandoeningen als HFpEF. Teleurstellende situaties bieden soms ook mooie kansen. Momenteel wordt een publicatie voorbereid waarbij de gegevens van de DIASTOLE-studie worden gecombineerd met twee vergelijkbare studies die eveneens een problematische inclusie hadden. Hopelijk kan deze samenwerking een mooie bijdrage aan het onderzoek naar HFpEF leveren en andere onderzoekers inspireren.

List of publications

Renal denervation in the treatment of hypertension and other disease states with elevated sympathetic activity.
Beeftink MM, Voskuil M.
Treatment Strategies 2013;3(1):71-75.

Renal denervation in heart failure with normal left ventricular ejection fraction. Rationale and design of the DIASTOLE (DenervatIon of the renAl Sympathetic nerves in hearT failure with nOrmal Lv Ejection fraction) trial.
Verloop WL, Beeftink MM, Nap A, Bots ML, Velthuis BK, Appelman YE, Cramer MJ, Agema WR, Scholtens AM, Doevendans PA, Allaart CP, Voskuil M.
Eur J Heart Fail. 2013 Dec;15(12):1429-37.

A systematic review concerning the relation between the sympathetic nervous system and heart failure with preserved left ventricular ejection fraction.
Verloop WL, Beeftink MM, Santema BT, Bots ML, Blankestijn PJ, Cramer MJ, Doevendans PA, Voskuil M.
PLoS One. 2015 Feb 6;10(2):e0117332. doi: 10.1371/journal.pone.0117332.

Denervation of the renal arteries in metabolic syndrome: the DREAMS-study.
Verloop WL, Spiering W, Vink EE, Beeftink MM, Blankestijn PJ, Doevendans PA, Voskuil M.
Hypertension. 2015 Apr;65(4):751-7.

Renal denervation in hypertensive patients not on blood pressure lowering drugs.
De Jager RL, Sanders MF, Bots ML, Lobo MD, Ewen S, Beeftink MM, Böhm M, Daemen J, Dörr O, Hering D, Mahfoud F, Nef H, Ott C, Saxena M, Schmieder RE, Schlaich MP, Spiering W, Tonino PA, Verloop WL, Vink EE, Vonken EJ, Voskuil M, Worthley SG, Blankestijn PJ.
Clin Res Cardiol. 2016 Sep;105(9):755-62.

Renal Denervation in a Real Life Setting: A Gradual Decrease in Home Blood Pressure.
Beeftink MM, Spiering W, Bots ML, Verloop WL, De Jager RL, Sanders MF, Vonken EJ, Blankestijn PJ, Voskuil M.
PLoS One. 2016 Sep 15;11(9):e0162251.

Renal denervation beyond the bifurcation: the effect of distal ablation placement on safety and blood pressure.
Beeftink MM, Spiering W, de Jong MR, Doevendans PA, Blankestijn PJ, Elvan A, Heeg JE, Bots, ML, Voskuil M.
Accepted for publication in J Clin Hyp.

Cessation of antihypertensive medication results in a highly variable blood pressure response between subjects with difficult-to-control hypertension.
Beeftink MM, Sanders MF, Bots ML, Joles JA, Blankestijn PJ, Voskuil M, Spiering W.
Manuscript submitted.

The safety of temporary withdrawal of antihypertensive medication in a population with difficult-to-control hypertension.
Beeftink MM, van der Sande NG, Bots ML, Doevendans PA, Blankestijn PJ, Visseren FL, Voskuil M, Spiering W.
Manuscript submitted.

The quest for patients with heart failure with preserved ejection fraction; an onerous task.
Beeftink MM, Nap A, Cramer MJ, Agema WR, Alings AM, Doevendans PA, Voskuil M.
Manuscript submitted.

S100B as a direct marker for the success of renal denervation.
de Jager RL, Beeftink MM, Sanders MF, Vonken EJ, Bots ML, Blankestijn PJ. On behalf of the UMCU RDN study group.
Manuscript submitted.

The impact of medication adherence on the treatment effect of renal denervation versus usual care in resistant hypertensive patients. Results of a randomised controlled trial (SYMPATHY).
de Jager RL, de Beus E, Beeftink MM, Sanders MF, Vonken EJ, Voskuil M, van Maarseveen EM, Bots ML, Blankestijn PJ. On behalf of the SYMPATHY investigators.
Manuscript submitted.

Renal artery and parenchymal changes after renal denervation: assessment by magnetic resonance angiography.
Sanders MF, van Doormaal PJ, Beeftink MM, Bots ML, Fadl Elmula FE, Habets J, Hammer F, Hoffmann P, Jacobs L, Mark PB, Persu A, Renkin J, Roditi G, Spiering W, Staessen JA, Taylor AH, Verloop WL, Vink EE, Vonken EJ, Voskuil M, Leiner T, Blankestijn PJ. On behalf of the ENCOReD consortium.
Manuscript submitted.

Dankwoord

Zoals de titel van mijn proefschrift al aangeeft, gaat het doen van promotieonderzoek gepaard met diverse uitdagingen. Vele mensen hebben mij geholpen om deze uitdaging tot een succes te brengen en ik wil hierbij iedereen die een bijdrage heeft geleverd aan dit proefschrift bedanken.

Allereerst mijn dagelijks begeleiders, dr. Voskuil en dr. Spiering.
Beste Michiel, dank je wel voor het vertrouwen dat je in mij had om de DIASTOLE te gaan uitvoeren. Mede dankzij jouw onuitputtelijke optimisme is het gelukt om tot dit eindresultaat te komen. Elke keer als ik de hoop dreigde te verliezen, praatte je me weer moed in om vol te houden. Dit leverde ook wel eens frustratie op, omdat ik al klaar was voor plan B terwijl jij nog voorrang wilde geven aan de inclusie van de DIASTOLE. Ook wanneer we het niet eens konden worden over een manuscript en ik probeerde vast te houden aan mijn gelijk (ook als ik dat niet aan mijn zijde had), zal mijn eigenwijsheid je wel eens parten hebben gespeeld. Maar je hebt gelijk gekregen; het is allemaal goed gekomen. Het onvergetelijke tennispotje op het dak van het Hyatt hotel in San Diego zal me ook altijd bijblijven (en ja, jij won). Dank je wel voor je geduld, je humor, je relativeringsvermogen en je steun.
Elsbeth, achter elke hardwerkende man staat een nog harder werkende vrouw. Dank je wel voor alle tijd die Michiel aan mij kon besteden en bedankt voor de lieve sms'jes met reminders voor Michiel (bijvoorbeeld mijn verjaardag).

Beste Wilko, wat fijn dat ik jou heb mogen toevoegen aan mijn promotieteam. Je was al nauw betrokken, maar met het opstellen van plan B werd je officieel mijn tweede copromotor. Je tomeloze enthousiasme en complimenten voor het werk dat we samen deden, hebben me met name aan het einde van mijn promotietijd heel veel energie en vertrouwen gegeven. Ik heb veel geleerd van jouw integere en kritische werkwijze in het onderzoek, en de manier waarop jij patiëntenzorg levert, is een voorbeeld voor velen. Dank je wel!

Mijn promotoren, prof. Doevendans en prof. Bots.
Beste prof. Doevendans, ik vind het enorm bewonderenswaardig dat iemand die zoveel promovendi op zoveel verschillende onderwerpen heeft, elke promovendus altijd weer weet te inspireren en op weg te helpen. Bedankt voor de kritische noten, de relativering en de inspiratie. Bedankt ook voor de mogelijkheid om mee te werken aan het ICC-project, waarbij ik de lessen die we van het renale denervatie onderzoek hebben geleerd, kon omzetten in de praktijk.

Beste prof. Bots, beste Michiel, toen Michiel Voskuil mij voorzichtig polste of ik jou als tweede promotor wilde hebben, heb ik geen seconde getwijfeld. Tijdens mijn hele promotietraject was je al nauw betrokken en mocht ik altijd bij je aankloppen met vragen over de registry, epidemiologie, statistiek of iets anders. In de auto naar Leuven werd plan B gesmeed voor mijn proefschrift, waarvoor ik je enorm dankbaar ben.

Bedankt aan alle patiënten die bereid zijn geweest deel te nemen aan de studies en registry beschreven in dit proefschrift.

Leden van de beoordelingscommissie, prof. Hoes, prof. Visseren, prof. Leiner, prof. Kroon, dr. Appelman, ik vind het een eer dat u de tijd heeft willen nemen om mijn proefschrift te lezen en te beoordelen. Dr. Hollander, dank dat u bereid bent om in de oppositie plaats te nemen.

Ik ben enorm trots op het multidisciplinaire samenwerkingsverband dat wij in het UMC hebben; eerst specifiek voor renale denervatie, later wat breder voor hypertensie in het algemeen. Dr. Blankestijn, beste Peter, ook jou ben ik veel dank verschuldigd voor plan B. Dank voor je kritische blik op mijn manuscripten en de leerzame patiëntbesprekingen. Dr. Vonken, beste Evert-Jan, dank voor je droge humor en je wijze lessen over de MRI-beeldvorming. Dr. Joles, beste Jaap, dank voor je enthousiasme en kritische vragen tijdens de researchbesprekingen, en voor de naamgeving van de 'Mikado' studie.

Rosa en Margreet, mijn RenDen-buddies van de nefrologie. Samen hebben we geworsteld met de weerslag van de HTN-3 studie. Makkelijker werd het er niet op, maar met elkaar hebben we ons er goed doorheen geslagen. Rosa, knap hoe jij de Sympathy toch tot een goed einde hebt gebracht na de HTN-3 . Dank je wel voor alle gezellige momenten, de studies waar we samen aan gewerkt hebben en de schattige foto's en filmpjes van thuis. Succes bij de geriatrie en heel veel geluk samen met Viktor, Milou en Tygo. Margreet, wat een hoofdpijn heb je gehad van de registry, maar keihard doorgezet. Respect! Ons bezoekje aan Berlijn samen was geweldig. Geniet samen met Wouter van jullie mooie nieuwe huis en van alle hoogtepunten die volgen.

Willemien, pionier! Ik heb ontzettend veel bewondering voor de manier waarop jij samen met Eva het renale denervatie programma in Utrecht (en eigenlijk in Nederland) hebt weten op te zetten. Dank je wel voor je gezelligheid, de hectoliters thee, de droppot en de geweldige basis die je voor mij hebt gelegd. Je dacht de DIASTOLE als rijdende intercity aan me over te dragen, maar noemde het uiteindelijk een trage stoptrein. Zullen we het gooien op de Intercity Direct? Hij rijdt niet altijd op tijd, maar brengt je via het buitenland uiteindelijk toch waar je wilt komen…
Eva, jij hebt ons allemaal laten zien hoe het moet; promoveren op renale denervatie. Dank voor de goede basis en veel succes in de toekomst.

Nicolette, dank je wel voor de fijne samenwerking met het aanleggen van de AGH database. Samen hebben we lekker zitten beulen, piekeren en klagen, maar gelukkig ook veel gelachen. Heel veel succes met het afronden van jouw promotie.
Wietze, dank je wel voor al jouw werk met de "achterkant" van de AGH-database en je interesse in onze andere projecten. Rutger, dank je wel voor je hulp met de koppeling van SMART met de AGH- database. Die koffie krijg je nog een keer, beloofd.
Collega's van Isala Klinieken in Zwolle, dank voor jullie bijdrage aan de analyse over renale denervatie voorbij de bifurcatie. Roland, bedankt voor je hulp met het analyseren van de CAG's. Sanne, bedankt voor het opzoeken van de missende kreatinines.

Ook voor de DIASTOLE heb ik mogen samenwerken met vele mensen van verschillende afdelingen en ziekenhuizen. Mooi om te merken dat, zelfs in een groot ziekenhuis als het UMC, de lijntjes kort zijn.

Maarten-Jan, bedankt voor je oneindige interesse in mij en mijn onderzoek en je onuitputtelijke energie om mij te helpen waar je kan. We gaan nog eens gezellig bij de Koreaan eten met mijn Koreaan!
Frans Rutten, dank voor de mogelijkheid om de Julius cohorten te doorspitten voor de DIASTOLE. Jammer dat we het BNP-project niet hebben kunnen doorzetten.

Dr. Allaart, dr. Nap, beste Cor en Alex, bedankt voor jullie inzet om ook in het VUmc patienten voor de DIASTOLE te vinden. Beste Debby, dank voor al jouw werk voor de DIASTOLE. Dr. Alings, beste Marco, dank voor alle moeite die je gedaan hebt om ook het Amphia als deelnemend centrum te kunnen opstarten.
Dr. Velthuis, beste Birgitta, dank je wel voor je expertise op het gebied van de radiologie en voor de tijd die je nam aan het begin van mijn promotietraject om mij wegwijs te maken in de cardiale MRI. Anneke Hamersma, dank voor het inplannen van alle MRI's. Dr. Scholtens, beste Asbjørn, dank voor de nucleaire imaging, jammer dat het uiteindelijk te weinig cases waren voor een gedegen analyse. Adele, dank voor het analyseren van alle urines en voor de verhalen over je prachtige kleinzoon.
Beste Rebecca, wat ben ik blij dat jij bereid bent om te helpen met de statistische analyse van de meta-analyse van de DIASTOLE. Dank je wel voor je geduld en flexibiliteit!

Bedankt aan alle collega onderzoekers in het Q, de villa, de bunker, het mierennest en het farfield voor de leuke weekendjes weg, de borrels en de gezelligheid.
Marloes, wat jammer dat er aan ons kantoorhuwelijk een einde is gekomen! Succes met jouw laatste loodjes en hopelijk komen we elkaar in de kliniek nog tegen. Bart, dank je wel voor je wijze levenslessen ('wat is nou het ergste dat er kan gebeuren?') en mijn nieuwe nickname. Arjan, bedankt voor de mooie muurversieringen en voor je uitstekende capaciteiten als klankbord als ik weer eens vast zat met schrijven. Mariam en Jetske, leuk om in het Antonius weer samen te werken! Marijke, Laurens en Mark, ik hoop dat ik jullie niet teveel heb ontmoedigd; succes met jullie promotietrajecten.

Aan alle cardiologen en arts-assistenten van het UMC Utrecht: bedankt voor de samenwerking en alle patiënten die jullie hebben verwezen voor de renale denervatie. Adriaan, dank voor het opeten van al mijn baksels. Steven, dank voor je interesse en het gezellige weekend in San Diego.

Inge en Corina, dank voor alle gezellige kletspraatjes en het gebruik van de bloeddrukmeters. Niels, dank voor alle koffie (uhm.. 'werkoverleg'). Alle medewerkers van de poli cardiologie, de poli vasculaire en de hartkatheterisatiekamers, dank voor jullie gezelligheid, prettige samenwerking en ondersteuning. Speciale dank aan Herman voor alle planning en de moeilijke taak om de agenda van Michiel te beheren, en aan Mathilde Hissink voor de planning van de psedatie voor de procedures. Thomas, bedankt voor je ontembare enthousiasme voor renale denervatie. Leendert, bedankt voor de DBC-lessen. Marloes, bedankt voor je hulp bij alle financiën. Eveline, bedankt voor je hulp met de extractie van de echo-gegevens uit Xcelera. Corien en Judith, bedankt voor de samenwerking.
Tamara, Jantine, Ingrid, Natasja en Sylvia, bedankt voor al jullie (administratieve) hulp.
Lieve dames van de R&D, dank voor jullie steun, gezelligheid, kletspraatjes en alle taarten en ander lekkers.

De cardiologen van het Diakonessenhuis Utrecht; bedankt voor de kans om in mijn zesde jaar een ASAS bij jullie te lopen en vervolgens als arts-assistent verder verliefd te worden op het vak cardiologie. Bedankt voor jullie betrokkenheid bij de DIASTOLE en de mogelijkheid om ook bij jullie patiënten te includeren. Willem, speciale dank gaat uit naar jou, want zonder jou was dit proefschrift er nooit gekomen.

Oud-collega's van de neurochirurgie, dank voor het ontzettend leuke en leerzame jaar. Annick, bedankt ook voor alle koffietjes en bijkletsmomentjes in de jaren daarna. Succes met jouw promotie en opleiding (gaat helemaal goedkomen)!

Nieuwe collega's in het St. Antonius; dank voor de warme ontvangst en de collegialiteit. Arthur, ik had me geen betere mentor kunnen wensen. Dank je wel!

Aan alle vrienden en familie; heel hartelijk dank voor jullie interesse en medeleven de afgelopen jaren.
Lieve Anita, Catherine, Daniël, Doesjka, Johanna, Jurriaan en Lotte, het betekent ontzettend veel voor me dat ik jullie na al die jaren nog steeds tot mijn goede vrienden mag rekenen. Dank jullie wel voor alle steun, het luisterend oor, maar vooral voor alle leuke momenten die we samen hebben beleefd. Ik hoop dat we deze nog heel lang mogen beleven samen.
Lieve Bas en Judit, Erwin en Annemieke, Gijs en Gunhild, bijzonder hoe snel vriendschappen zo hecht kunnen worden. Bedankt voor alle medeleven, wijze raad en knuffels van de kinderen. Bedankt dat jullie deel uitmaken van mijn leven.
Lieve Anneke, de echte wetenschapper van ons tweeën. Zelfs als je naar de andere kant van de wereld verhuist, blijven we goede vriendinnen. Dank je wel voor het geven van het goede voorbeeld (ik trek alleen geen Harry Potter jurk aan), je humor en je vriendschap. Ik moet nog steeds lachen als ik aan onze 'osmotische aanvallen' terugdenk.
Lieve Willemijn, dank je wel voor alle gezellige etentjes.

Aan mijn paranifmen, dank jullie wel dat jullie me vandaag willen bijstaan.
Lieve Lilian, al vanaf dag 1 van de studie geneeskunde kan ik alles bij je kwijt. Langzaam hebben de Lili-en-Tine's-culinaire-hoogstandjes-en-film-avondjes plaatsgemaakt voor avonden met wat meer diepgang (ok, en Buffy), waarbij we alles bij bespreken, zowel de leuke dingen, als de frustraties over promoveren, opleiding en het leven. Je bent een aanwinst voor de jeugdgezondheidszorg en een verrijking van mijn leven. Ik verheug me nu al op het Buffy-dansje in het zweetkamertje.
Lieve Anita, we hebben samen al zoveel avonturen beleefd. Het begon allemaal met de Rome-reis, maar daar zijn onder andere ook al een overnachting op station Brussel-Zuid, (nét niet beroofd), "verdwalen" in Wicklow, een ondergesneeuwd Riga en het ontdekken onze Tiroler droomman bij gekomen. Ik vind het heel speciaal om deze dag daar aan toe te voegen. Dank je wel voor alle mooie momenten die we samen beleefd hebben en hopelijk nog gaan beleven. En ik ben ontzettend blij dat je Jaap hebt gevonden om nog meer mooie herinneringen mee te maken.

Lieve Wim, dank je wel voor je support, je wijze raad en je heerlijke wijnen. Lieve Sabbah, bedankt voor de heerlijke appeltaart en de mooie stokrozen.

Lieve Dave en Ria, bedankt dat jullie me zo warm hebben ontvangen in jullie gezin. Het voelt voor mij als thuiskomen bij jullie. Bedankt voor jullie attentheid, de gezellige spelletjes en alle babi ketjap die altijd speciaal voor mij gemaakt wordt (en ajam pedis, en gehaktballen, en Indische boontjes).
Lieve Ae-Ri en Micha, wat bof ik met zo'n schoonzus en zwager. Ae-Ri, je hebt me het goede voorbeeld gegeven, niet alleen van een succesvolle promotie en verdediging, maar vooral van het kiezen van je eigen pad. Ik bewonder jouw keuze om na je promotie je passie te volgen en je droom waar te maken.

Lieve Wouter, mijn grote broer, toen ik afstudeerde zei je tegen mij dat doctorandus slechts 'hij die nog doctor moet worden' betekent. Nu is het dan zover! Ik ben trots dat ik jouw kleine zusje mag zijn en altijd zal blijven. Dank je wel voor je onvoorwaardelijke interesse en steun. Lieve Srishti, wat is het mooi om te zien hoe Wouter en jij elkaar tot een beter mens maken. Dank je wel voor alles dat je voor hem betekent.

Lieve paps en mams, ik kan me geen betere ouders wensen. Jullie hebben me altijd de ruimte gegeven om te doen wat ik graag wil en gesteund waar nodig. Lieve mam, niet alleen mijn uiterlijk heb ik van jou, ook de eigenschappen waar ik het meest trots op ben, heb ik van jou geërfd. Jouw empathie en zorgzaamheid zijn mijn voorbeeld voor hoe ik als arts wil zijn. Lieve paps, jij was bijna de enige die niet verbaasd was dat ik onderzoek ging doen. Mijn analytisch vermogen heb ik dan ook van jou, denk ik, net als je ongeduldigheid. Gelukkig leerde je me ook relativeren en de gezichtsuitdrukking die wij vroeger aan de keukentafel al deelden, is tijdens mijn promotieonderzoek meer dan eens van pas gekomen. Dank jullie wel voor jullie onvoorwaardelijke steun, jullie warmte en al jullie adviezen (al dan niet toepasbaar in onderzoek).

Lieve Sung-Wook, mafstkees, mijn grote rots in de branding. Het is een cliché, maar zonder jou had ik het niet gekund. Het laatste jaar ben ik zeker niet altijd gezellig geweest, maar je bleef me altijd steunen. Dank je wel voor je begrip, je eindeloze vertrouwen, je humor, je kookkunsten en vooral je geduld. Je maakt mijn leven compleet. I love you übersmoost!

Curriculum Vitae

Martine werd geboren op 13 maart 1986 in ‘s-Gravenhage. Nadat zij haar gymnasiumdiploma had behaald aan het Christelijk Gymnasium Sorghvliet ging zij in 2004 geneeskunde studeren in Utrecht. Tijdens haar studie heeft ze onder andere een co-schap neurologie in Edinburgh en een co-schap dermatologie in Californië gelopen. Hoewel zij altijd dacht dat ze neuroloog zou worden, raakte ze in haar vijfde jaar geïnteresseerd in de cardiologie. In haar laatste jaar heeft ze bij beide specialismen semi-arts stages gelopen en heeft ze onderzoek gedaan op het raakvlak tussen de cardiologie en de neurologie. Na haar afstuderen was ze negen maanden werkzaam als arts-assistent cardiologie in het Diakonessenhuis Utrecht en aansluitend een jaar als arts-assistent neurochirurgie in het UMC Utrecht. Hierna heeft zij definitief de keuze gemaakt voor de cardiologie. In januari 2013 begon zij aan een promotieonderzoek naar renale denervatie als behandeling voor hypertensie en hartfalen met behouden ejectiefractie (promotoren prof. dr. Doevendans en prof. dr. Bots, copromotoren dr. Voskuil en dr. Spiering), wat geresulteerd heeft in dit proefschrift. Sinds 1 oktober 2016 is zij werkzaam als arts-assistent cardiologie in het St. Antonius ziekenhuis in Nieuwegein.