Novel methods to manage patients with difficult-to-control hypertension

PhD Thesis by
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Table of Contents
Acknowledgements........................................................................................................4
List of papers ...................................................................................................................6
Selected abbreviations....................................................................................................7
Introduction ......................................................................................................................8
  Hypertension and cardiovascular diseases ..................................................................8
  Uncontrolled hypertension .........................................................................................8
  Treatment-resistant hypertension .............................................................................9
  Renal sympathetic denervation .................................................................................9
  Impedance cardiography ............................................................................................10
    Background ................................................................................................................10
    Impedance cardiography with the Hemodynamic and Oxygen transport MANagement
    (HOTMAN) system: ..................................................................................................11
Aims of the thesis ............................................................................................................13
Materials and methods..................................................................................................14
  The Oslo Renal Denervation (RDN) study (Paper I-II) ............................................14
    Study design and population ....................................................................................14
    Randomization ..........................................................................................................15
    Study treatment ..........................................................................................................16
    Visits in the follow-up period .................................................................................17
  Procedures ..................................................................................................................17
  Study endpoints ...........................................................................................................19
  Statistical methods .....................................................................................................19
  Approvals and funding ...............................................................................................20
Meta-analysis of randomized controlled trials (RCTs) of renal denervation in treatment-
resistant hypertension (Paper III) ................................................................................20
  Study design and population ......................................................................................20
  Study endpoints ..........................................................................................................20
  Reporting and statistical methods: ............................................................................20
  Funding .......................................................................................................................21
The Beauty study (The BEtter control of BP in hypertensive pAtients monitored Using the
HOTMAN sYstem) (Paper VI) ......................................................................................21
  Study design and population ......................................................................................21
  Randomization ..........................................................................................................22
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Fadl El Mula M. Fadl El Mula
List of papers

   Renal sympathetic denervation in patients with treatment-resistant hypertension after witnessed intake of medication before qualifying ambulatory blood pressure.

   Adjusted drug treatment is superior to renal sympathetic denervation in patients with true treatment-resistant hypertension.

   Meta-analysis of randomized controlled trials of renal denervation in treatment-resistant hypertension.

   A Randomized and Controlled Study of Non-Invasive Hemodynamic Monitoring as a Guide to Drug Treatment of Uncontrolled Hypertensive Patients.
   *J Hypertension.* 2015 Dec;33(12):2534-45

The papers are referred to by their Roman numerals throughout the thesis.
# Selected abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABP</td>
<td>Ambulatory blood pressure</td>
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<tr>
<td>ABPM</td>
<td>Ambulatory blood pressure monitoring</td>
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<tr>
<td>ACEI</td>
<td>Angiotensin-converting enzyme inhibitor</td>
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<tr>
<td>ARB</td>
<td>Angiotensin II-receptor blocker</td>
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<tr>
<td>BP</td>
<td>Blood pressure</td>
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<tr>
<td>BSA</td>
<td>Body surface area</td>
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<tr>
<td>CCB</td>
<td>Calcium channel blocker</td>
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<tr>
<td>CI</td>
<td>Cardiac index</td>
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<td>CO</td>
<td>Cardiac output</td>
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<tr>
<td>CVDs</td>
<td>Cardiovascular diseases</td>
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<td>DBP</td>
<td>Diastolic blood pressure</td>
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<tr>
<td>eGFR</td>
<td>Estimated glomerular filtration rate</td>
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<tr>
<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
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<tr>
<td>HR</td>
<td>Heart rate</td>
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<tr>
<td>HRV</td>
<td>Heart rate variability</td>
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<tr>
<td>HT</td>
<td>Hypertension</td>
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<tr>
<td>ICG</td>
<td>Impedance cardiography</td>
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<tr>
<td>IHM</td>
<td>Integrated hemodynamic management</td>
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<tr>
<td>IRR</td>
<td>Incidence rate ratio</td>
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<tr>
<td>LVEF</td>
<td>Left ventricular ejection fraction</td>
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<tr>
<td>LVMI</td>
<td>Left ventricular mass index</td>
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<tr>
<td>LSWI</td>
<td>Left stroke work index</td>
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<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
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<tr>
<td>RDN</td>
<td>Renal sympathetic denervation</td>
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<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>TRH</td>
<td>Treatment-resistant hypertension</td>
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<tr>
<td>UCH</td>
<td>Uncontrolled hypertension</td>
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<tr>
<td>USA</td>
<td>United States of America</td>
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<tr>
<td>VAS</td>
<td>Visual analog scale</td>
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Introduction

Hypertension and cardiovascular diseases

Cardiovascular diseases (CVDs) are the number one cause of death globally, and more people die annually from CVDs than from any other causes. An estimated 17.5 million people died from CVDs in 2012, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke (1). The aging and growth of the population resulted in an increase by 41% in global cardiovascular deaths between 1990 and 2013 (2).

Hypertension is a major public health challenge worldwide with a prevalence of approximately 30% in adult population (3, 4) and it was estimated in 2000 that approximately one billion people were affected by hypertension and around 1.5 billion will be affected by 2025 (5). Hypertension is the leading single risk factor globally for CVDs and mortality, accounting for 9.4 million deaths and 7% of global disability-adjusted life years (DALYs) (6, 7). Hypertension is a well-established risk factor for morbidity and mortality associated with coronary artery disease, heart failure, chronic kidney disease, dementia, and stroke (8-16). Early blood pressure (BP) control in hypertensive patients guarantees the best prevention of cardiovascular events in the long term (17). A large meta-analysis of individual data for one million adults in 61 prospective studies showed that throughout middle and old age, BP is strongly and directly related to vascular (and overall) mortality, without any evidence of a threshold down to at least 115/75 mm Hg (18).

Uncontrolled hypertension

Despite increased awareness of hypertension in the last years, approximately 40% of patients with hypertension are untreated (19, 20). Moreover, approximately 10–20% of patients treated for hypertension remain with uncontrolled high blood pressure (BP) despite prescription of antihypertensive drugs (21, 22). This fraction of patients is approximately 7.5% if concomitantly increased ambulatory BP is also taken into account (22) and it includes patients being prescribed several antihypertensive drugs. Drug-treated, but uncontrolled hypertension (UCH) is a worldwide problem and the mechanisms explaining UCH may be multiple. In spite of education efforts and antihypertensive drugs, blood pressure control rates remain low. Patients may have truly severe hypertension and may need more or better selected drugs, a need frequently unmet because of physicians resistance to increase or change prescriptions (physician inertia) (23). Patients may also have secondary causes of hypertension interfering with the drug treatment, conditions usually treated by resolving the underlying problems (24).

A frequent reason for UCH is poor drug adherence (25-27) that may be due to side-effects induced by drugs. Side-effects, in particular, may be caused by antihypertensive drugs that have been prescribed but do not match the underlying hemodynamic cause of the high BP. In addition, important psychological aspects including doctor-patient relationship, patient’s emotion-focused coping (acceptance and disavowal) and problem-focused coping with their chronic diseases have been found positively associated with better physical and psychological self-care in patients with chronic heart failure (28). Similar mechanisms may be present in hypertension, though not well documented. Moreover, hypertension is in most cases an

8
asymptomatic health condition, and one will assume that hypertensive patients will be less prone to adapt a physical and psychological self-care and take medication that might lead to side effects. Such patients clearly need new therapeutic approaches to optimize their blood pressure control.

**Treatment resistant hypertension**

Treatment resistant hypertension (TRH) is a well-defined subgroup of UCH. TRH is a blood pressure that remains above treatment goal in spite of concomitant use of three or more antihypertensive drugs from different classes. Patients, who require more than four drug classes to have their blood pressure controlled, are also considered to have TRH. The regimen should include a diuretic and all doses should be in the highest recommended and/or maximally tolerated doses (24, 29). The exact prevalence of TRH is unknown, because it depends on the populations studied and most important the level of health care where investigations have been carried out. In USA the prevalence was estimated to approximately 8.9-16.5% (3, 30-32) of the drug-treated hypertensive population, but these estimates ignores temporal trends, possible nonadherence to medications and potential submaximal doses of medications (30, 31). After accounting for the estimated 50% of patients with pseudo-resistance, the prevalence of true resistant hypertension falls to approximately 5% of the treated hypertensive population in USA (33). Persu and colleagues estimated the prevalence of TRH to approximately 3-30% based on an experience at 11 European expert centres (34). TRH was more frequent in people who were older, obese and male. Treatment resistant patients are more likely to have albuminuria, reduced renal function, and a history of diabetes mellitus, coronary heart disease, stroke or heart failure (31). The occurrence of fatal and nonfatal cardiovascular events was found to be four times higher in patients with true TRH compared to patients that respond to treatment of HT (35).

TRH represents a huge therapeutic challenge, and recently various new approaches have been studied in order to more effectively treat these patients. Among these are renal sympathetic denervation and the use of hemodynamically adapted medical treatment. Both methods have been subjects of the current thesis.

**Renal sympathetic denervation**

The role of the sympathetic nervous system in the pathophysiology of hypertension is substantiated by a wealth of experimental and clinical arguments (36-43). Renal sympathetic efferent and afferent nerves, which lie within and immediately adjacent to the wall of the renal artery may have an important role for initiation and maintenance of systemic hypertension (44-47).

Prior to the development of the modern pharmacological treatment of hypertension, surgical sympathectomy was introduced as a treatment for hypertension. Sympathectomy had to include denervation of the abdominal organs in order to be effective, and it was thus termed splanchnicectomy. It required a prolonged hospital stay (2–4 weeks) and a long recovery period (1-2 months) and more importantly a skilled surgeon to perform it. The first surgical sympathectomy for hypertension was performed by the surgeon Fritz Bruening in 1923 (48, 49). More extensive operations were developed later. Since the end of 1930s, surgical
sympathectomy was used for the treatment of severe hypertension (50-52). Surgical sympathectomy showed to be effective in lowering blood pressure, however, these methods were associated with high perioperative morbidity and mortality and long-term complications, including bowel and bladder incontinence, erectile dysfunction, severe postural hypotension and even difficulty in walking (52-54). Due to these complications the procedure was gradually abandoned. In the meanwhile modern pharmacological antihypertensive drugs have been gradually developed, leading to significant improvement of the control of hypertension. However, the control of hypertension still remains disappointingly low, leaving uncontrolled patients at increased cardiovascular risk. Thus, the need for new therapeutic strategies to address this problem opened the door to introduce new interventional techniques.

In 2009, Henry Krum and colleagues presented a breakthrough study, namely Symplicity HTN-1 (55), where they performed a selective percutaneous renal sympathetic nerves denervation (RDN) by the radiofrequency (RF) ablation technique as an effective and safe treatment of patients with TRH. This study was followed in 2010 with the Symplicity HTN-2 study, the first and only randomized controlled study until recently, that showed RDN is feasible, effective and safe treatment for patients with TRH (56). Publication of the Symplicity studies was followed up by an unprecedented wave of enthusiasm leading to a wide use of RDN not only in patients with TRH, but also in patients with other diseases that were characterized by increased sympathetic overdrive (57-65). Many companies developed their own devices and techniques to perform RDN. However, the randomized evidence was very limited and many confounding factors had not been taken in consideration when the results of the Symplicity HTN-2 were interpreted. This study had many important limitations and potential biases (66-68); the selection of patients enrolled in the study and the evaluation of efficacy were based on office BP rather than ABPM, which is state-of-the-art (69), particularly in TRH (70). ABPM reduces patients and investigators-related biases, measurement error, minimizes the white-coat effect and has greater reproducibility, and therefore provides a better estimate of patient’s usual blood pressure and cardiovascular prognosis (71, 72). In addition, this pioneer study had put a little emphasis to exclude patients with secondary HT and most importantly to exclude those with poor drug adherences. Poor drugs adherence is known for many decades as a major problem among patients with TRH (25-27). Moreover the lack of BP decrease in the control group also raises concerns. Though Symplicity HTN-2 was a randomized study, it was not blinded and patients randomized to the RDN group received an active treatment (interventional treatment) compared to the control group that received no active treatment (maintained medical therapy, without active change during the study’s period). The difference in the treatment offered to the study populations might have led to a disappointment among controls, thus causing an imbalanced impact of the Hawthorne and placebo effects. This may explain the noticeable lack of BP decrease in control group (66, 67).

**Impedance cardiography**

**Background**

By adapting medical treatment to the hemodynamic state of the patient, a better BP control may be obtained. However, methods most often used to assess hemodynamical parameters in patients such as eccocardiography and invasive methods like thermodilution are not feasible
in general practice. Therefore, impedance cardiography (ICG) is an alternative non-invasive hemodynamic diagnostic and monitoring technology that could be considered. Impedance cardiography, also referred to as Electrical Impedance Plethysmography (EIP) or Thoracic Electrical Bioimpedance (TEB), has been based on an old principle of physics established in Russia in the 1940s and applied to the measurement of cardiac output. National Aeronautics and Space Administration (NASA) helped develop the technology in the 1960s to measure the cardiac output of astronauts in the space program researched (73).

ICG measures the total electrical conductivity of the thorax and its changes in time to process continuously a number of cardiodynamic parameters, such as stroke volume (SV), cardiac output (CO), Ventricular Ejection Time (VET), Pre-ejection Period, calculating systemic vascular resistance (SVR) and volume status. The system used to detect the impedance changes caused by a high-frequency, low magnitude current flowing through the thorax between additional two pairs of electrodes located outside of the measured segment. The sensing electrodes also detect the ECG signal, which is used as a time indicator. Different formulae for calculating the stroke volume were proposed in the mid-1960s and revised several times thereafter. Similarly, different models of the human thorax (cylinder or truncated cone) are used by software calculations. The oldest is the equation of Kubicek described in 1966 (73). More recently, Bernstein and Sramek proposed a simplified equation (74, 75), that integrated into the calculation software of multiple monitors on the market. This equation can be written as follows: \( SV = LVET \cdot k \cdot (dZ/dt)_{\text{max}} / Z_0 \), where \( SV \) is the stroke volume, \( LVET \) is the left ventricular ejection time, \( k \) is a constant estimated from a patient normogram incorporating the main morphologic characteristics (height, gender, age), \( Z_0 \) represents the baseline impedance of the thorax, and \( (dZ/dt)_{\text{max}} \) is the maximum impedance change during the cardiac cycle. ICG has demonstrated its usefulness and reproducibility during the last years in various populations, including patients with hypertension and cardiovascular diseases (76-81).

**Impedance cardiography with the Hemodynamic and Oxygen transport MANagement (HOTMAN) system:**

The HOTMAN® System (Hemo Sapiens Inc., San Ramon, California, USA), is an ICG system, validated against an invasive thermodilution approach (82). This system allows a noninvasive assessment of patient’s hemodynamics with two important improvements compared with other previous similar devices: use of a very low current (7mA, 300–400-fold lower than that used by other products, making it safer for the patient); and use of a new data signal processing and an improved mathematical algorithm. Compared with classical ‘impedance cardiography, which measures cardiac index (CI), stroke index (SI, i.e. stroke volume, SV/body surface area, BSA), thoracic fluid content (TFC) and systemic vascular resistance index (SVRI), the HOTMAN system has some different characteristics. It measures SVRI per beat, that is, SSVRI (stroke systemic vascular resistance index), an index of vasoactivity, which is not detected by classical systems (Figure 1). Furthermore, the intravascular volume is not evaluated through TFC (which is subject to an overestimation bias in chronic obstructive pulmonary disease and other patients, as it is influenced by any liquid present in the thorax), but calculated as a component of total contractility according to Frank–Starling law. More specifically, intravascular volume is calculated according to the following.
equation: total contractility \((TC) = \text{intravascular volume (IV) + inotropy (I)}\), so \(IV = TC - I\)

(wherein TC is measured through left stroke work index \((LSWI)\)) (Figure 1) (83).

Utilizing of ICG in the management of hypertension is based on the following assumption; when the pharmacological class of antihypertensive agent does not address the hemodynamic state, BP reduction may be limited, BP fall is delayed, and side effects occur more frequently. By contrast, when the pharmacological class of antihypertensive treatment is adapted to the hemodynamic state (for instance diuretics for hypervolemia, or CCB/ACEI/ARB for increased peripheral resistances), BP reduction occurs more rapidly and to a greater extent. Thus, using an integrated therapeutic approach (IHM - Integrated Hemodynamic Management) aiming at detecting vasoconstriction and/or hypervolemia and/or hyperinotrophy through ICG, by the HOTMAN® System, facilitates selection of the most appropriate antihypertensive drugs. This has been demonstrated by other ICG systems in the management of patients with TRH (84) and in patients with mild to moderate hypertension (85). However, the randomized evidence was limited, thus justifying the need for our study.

Figure 1.
A: Technique for impedance cardiography measurements by the HOTMAN® system.

B: Display page of monitoring hemodynamic parameters by HOTMAN® system
Aims of the thesis

The aims of the thesis were to assess the efficacy and safety of two novel methods; renal sympathetic denervation (RDN) and impedance cardiography (ICG) in management of patients with uncontrolled high blood pressure.

A secondary aim was to introduce these two novel methods in our hospital if they turned out to be both safe and effective.

The specific research hypotheses:

- To test the hypothesis that RDN lower blood pressure effectively in patients with treatment resistant hypertension even after excluding possible important confounding factors such as poor drug adherence (Paper I-II)
- To test the hypothesis that blood pressure lowering effect of RDN is superior to optimized intensive medical therapy guided by the hemodynamic profile obtained by ICG in patients with true treatment resistant hypertension (Paper II)
- To test the hypothesis that RDN is an effective and safe treatment modality in patients with treatment resistant hypertension compared to conventional medical therapy when assessed by a sum up of the existing randomized controlled trials (Paper III)
- To test the hypothesis that monitoring hemodynamic parameters by ICG combined with applying a predefined algorithm of drug selection (integrated hemodynamic management) is superior to classical drug selection in controlling blood pressure in patients with uncontrolled hypertension (Paper IV)
Materials and methods

The Oslo Renal Denervation (RDN) study (Paper I-II)

Study design and population
The Oslo RDN study is an investigator-initiated, prospective, single centre, open, randomized, parallel groups study [ClinicalTrials.gov Identifier: NCT01673516]. In this study we investigated for the first time the BP lowering effect of RDN versus clinically adjusted drug treatment in true treatment resistant hypertension (TRH) after excluding patients with confounding spurious and secondary hypertension and poor drug adherence. TRH was defined as uncontrolled hypertension with an office SBP >140 mm Hg, despite intake of maximally tolerated doses of ≥3 antihypertensive drugs including a diuretic. In addition, patients had to qualify by having mean ambulatory daytime SBP >135 mm Hg immediately after investigator witnessed intake of their antihypertensive morning drugs. Before the qualifying ambulatory BP measurements, drug treatment was unchanged for at least 2 weeks, and no change in medication was pre-planned for the following 6 months. Patients could be 18 to 80 years of age with normal renal arteries verified by computed tomography or MRI examination within 2 years before participation. Patients with estimated glomerular filtration rate (eGFR) <45 mL/min per 1.73 m² (MDRD formula), urine albumin/creatinine ratio >50 mg/mmol or type 1 diabetes mellitus were not included.

A total of 83 patients referred specifically to RDN, mainly from hospitals and specialist practices in Norway were thoroughly worked-up (Figure 2) in the Nephrology outpatient clinic and Section of cardiovascular and renal research at Oslo University Hospital, Ullevål. The main randomized study was preceded by a prospective, non-randomized, open pilot study in the time period from December 2011 through June 2012, using the same inclusion and exclusion criteria. In the non-randomized study (Paper I) 18 patients with apparent TRH were assessed for eligibility and only six patients were found to have true TRH and underwent RDN. In the following main randomized study (Paper II) 65 patients with apparent TRH were also thoroughly assessed for eligibility in the time period from August 2012 to June 2013 and only 19 patients were found to have a true TRH (Figure 3).
**Abbreviations:** BP: blood pressure. *BP increasing factors; using hormonal contraceptives, Drug-induced or harmful use of alcohol, NSAIDs, cyclosporine, corticosteroid, sympathomimetic and natural products, excessive intake of salt, liquorice, or caffeine. ABPM: ambulatory BP measurement. PRA: plasma renin activity. eGFR: estimated glomerular filtration rate. **Use of various pharmacological combinations including potent vasodilating agents.

**Randomization**

In the main study (Paper II) patients were randomized with a 1:1 allocation, using a permuted block randomization list through a telephone call to a hospital employee who was not involved in the study, who was uninformed about the nature of the study, and who opened a sealed envelope arranged in a fixed order and documented in writing the outcome of the randomization. We randomized 20 patients but excluded one patient post randomization because Conn’s disease was diagnosed at this stage; ten patients allocated to adjusted drug treatment (control group) and nine patients allocated and underwent renal sympathetic denervation (RDN group).
Figure 3. Patients’ flow diagram in Oslo RDN study

**Study treatment**
The control group had their antihypertensive medication adjusted at baseline, 1 month, and at 3 months according to 2007 European Society of Hypertension/European Society of Cardiology hypertension guidelines and guided by individual hemodynamic profile using a predefined management algorithm of the device; non-invasive integrated hemodynamic measurements (IHM) by ICG of the HOTMAN system. In short (described in details in the Introduction), this procedure aimed at tailoring the antihypertensive treatment to the underlying hemodynamic aberration such as further increasing the dose of diuretic in volume-overloaded patients or prescribing, or increasing the dose of vasodilatory medicines in patients with high peripheral vascular resistance, or reducing the dose of drugs that cause
negative inotropy in patients with reduced cardiac inotropy. The \textit{RDN group} underwent renal sympathetic denervation and their medication was aimed at being maintained unchanged.

\textbf{Visits in the follow-up period}
In both groups blood samples, urine samples and office BP measurements obtained at baseline, 1, 3 and 6 months while ambulatory BP measurements obtained at baseline, 3 and six months follow-up.

\textbf{Procedures}
- \textbf{Blood pressure}: Office and ambulatory BPs were measured by a validated oscillometric device (Microlife WatchBP O3; Microlife Health Management Ltd., Cambridge, UK). After relaxing in 5 minutes, office BP measurements were performed three times with the patient in a sitting position, with one-minute interval between them. The first measurement was excluded whereas the mean value of the second and third measurements was calculated as office BP. ABPM was performed throughout a 24-h period with the device programmed to inflate and record BP at a pre-specified intervals: 20 min intervals from 06.00 h to less than 22.00 h (daytime) and 30 min intervals from 22.00 h to less than 06.00 h (night-time). It was a priori decided that at least 70% valid measurements were required. During ABPM patients were asked to refrain from unusual and/or intense physical activities. Measurement and selection of the cuff size and arm have to comply with the recommendation of the ESH/ESC for conventional and ambulatory blood pressure measurement (24).
- \textbf{Witnessed intake of medication}: Patients were asked to bring their prescribed medication to the clinical visit. Medication was documented and administered by the investigator and swallowed by the patient under continuous observation, to secure the intake of prescribed medication in prescribed doses (Figure 4). Patients were then continuously under the observation by the investigator to prohibit throwing up the pills until 24-hour ambulatory BP device had been mounted and tested out in a somewhat more lengthy procedure than usually to prolong the period of observation. Patients stayed in the hospital for 2 hours to capture those with potential symptomatic hypotension caused by full intake of medication. Visits with subsequent ambulatory BP measurements were done in the morning, and further observation of patients in the hospital was done during working hours

\textbf{Figure 4.} Illustration of steps of witnessed intake of medication

\begin{itemize}
\item Patients were asked to bring their prescribed medication to the clinical visit
\item Medication was documented and administered by the investigator and swallowed by the patient under continuous observation
\item Patients were then continuously under the observation by the investigator (in order to prohibit throwing up again of the pills) until 24-hour ambulatory BP device had been mounted and tested out
\end{itemize}

\textbf{Abbreviations}: BP: blood pressure
- **Renal sympathetic denervation:** The procedure was performed using the single-electrode Symplicity Catheter System (Ardian/Medtronic, Mountain View, CA, USA) by experienced invasive radiologists. The femoral artery was accessed with the standard endovascular technique and the single-electrode Symplicity catheter was advanced into the renal artery and connected to a radiofrequency generator (Figure 5). Then in average 8 (6 to 11) discrete, low-power radiofrequency treatments were applied along the length of each renal artery, separated both longitudinally and rotationally within each renal artery (Figure 5). During ablation, the catheter system monitored tip temperature and impedance, altering radiofrequency energy delivery in response to a predetermined algorithm. Pain was treated with appropriate intravenous analgesics and anxiolytics as needed. All procedures took between 40 and 50 minutes and were considered successful by interventionists. Patients were hospitalized overnight for observation.

**Figure 5.** Illustrations of the system and method used to perform renal sympathetic denervation.

* A

Radiofrequency Generator of the Symplicity catheter system (Ardian/Medtronic, Mountain View, CA, USA)

* B

The single-electrode Symplicity radiofrequency catheter (Ardian/Medtronic, Mountain View, CA, USA)

* C

Angiography image showing the ablation catheter in the right renal artery (the arrow points to the tip of the single-electrode catheter). From the Cath laboratory at Oslo University Hospital

* D

Ablation catheter in the renal artery. Nerves are schematically illustrated. In each renal artery performed 4-8 ablations with approximately 5 mm distance symmetrically distributed around the artery circumference

* Reprinted by permission Medtronic (CA, USA). * Reprinted by permission Tidsskr Nor Laegeforen (Oslo, Norway)
- Impedance cardiography with the HOTMAN® system: Impedance cardiography recordings by the HOTMAN® System were performed with patients in the supine position, resting for at least 5 minutes before measurement and involving non-invasive measurement of thoracic bioimpedance through placement of four pairs of thoracic electrical bio-impedance specific sensors placed on the neck and lower thorax. Electrical impedance changes were digitally processed to record and calculate different hemodynamic parameters (Figure 1). At least 3 minutes were required to be recorded for each patient at any occasion. If there was visible variation in the quality of signals or in the hemodynamic parameters, we had to record for more minutes until we could see stable signals on the screen. Recording used for adjustment of treatment was always based on at least one minute of stable recording, so called representative minute with the best quality of signals. Recording with incorrect and poor signals were to be considered as missing. Hemodynamic measurements using HOTMAN® system were performed also in the RDN group with blinding of clinician for these data.

- Blood and urine sampling: Blood and urine samples collected at all visits; screening, baseline, 1, 3 and 6 months. Different measurements performed for assessment for eligibility at screening and baseline visits, while other ones were done during follow-up, mainly for safety purposes.

Study endpoints
- Efficacy endpoints: The primary endpoint of efficacy was the absolute change in office SBP at 6-month follow-up. Change of office DBP, ambulatory BPs, heart rate and the percentage of normalization of office and ambulatory SBPs at 6-month follow-up were secondary endpoints of efficacy.

- Safety endpoint: Adverse events were regularly investigated actively at each visit, by giving participants a written self-questionnaire in which common adverse events related to RDN and that related to antihypertensive drugs were proposed in a neutral order for the purpose of catching the data. In both group blood samples and urine samples were collected at each visit. Change of renal function was assessed by change in estimated glomerular filtration rate (eGFR), using MDRD formula.

Statistical methods
All statistical analyses were done with IBM SPSS Statistics 20.0 (SPSS, Chicago, IL). All variables were tested by the Kolmogorov–Smirnov test for normality, and all BP variables were normally distributed. Analysis was by intention-to-treat. We assessed continuous variables including the primary end point between groups with Student 2 sample t test. We compared categorical variables with Fisher exact test. For within-group paired data, a paired t-test was used. A 2-sided α level of 0.05 was used for all superiority testing. Continuous variables are presented as mean ± SD. Categorical variables are presented as proportions.

Power and sample size calculation: We postulated that the difference between the RDN group and the drug adjustment group would be at least 20 mmHg in office systolic BP favoring the RDN group based on the Symplicity HTN-2 study (2) in which 6-month data showed that office BP in the RDN group dropped by 32/12 mmHg. Twenty seven patients per group would be required to demonstrate a difference of 20 mmHg at an alpha risk of 5% and a beta risk of 20% in a 2-sided t-test, assuming a SD of 13 mmHg. To end up with 27 subjects per group for per protocol analysis we aimed to enroll 60 patients and randomize 30 patients.
to each group (http://www.clinicaltrials.gov). However, after protocol approval and because we did not see BP lowering effect in our pilot study of patients who had RDN in an uncontrolled design, we decided to do one interim analysis after inclusion of one third of subjects. We did a data inspection that led to the decision of performing the interim analysis. The decision was immediately reported on clinicaltrials.gov and in a notification to The National Committee for Research Ethics in Norway. On October 22, 2013 the investigators decided to stop inclusion because RDN showed inferior BP lowering effect compared to adjusted drug treatment, and it was considered unethical to continue doing RDN in hypertensive patients with uncontrolled BP and previous cardiovascular disease and/or target organ damage.

Approvals and funding
The study was approved by The National Committee for Research Ethics in Norway and by the institutional research committee at Oslo University Hospital. All patients gave written informed consent for participation in the study and publication of results before included in the study. All patients who qualified for the procedure within the 11-month time period were included. All expenses were covered by the hospital and patients were not paid.

Meta-analysis of randomized controlled trials (RCTs) of renal denervation in treatment-resistant hypertension (Paper III)

Study design and population
The study was an investigator initiated meta-analysis and a systematic literature review based on published summary statistics of randomized controlled trials of renal denervation in an attempt to sum up the randomized evidence on the efficacy and safety of RDN as treatment modality in treatment resistant hypertensive patients.

We searched the PubMed and EMBASE databases for publications in English on renal denervation since 1 January 2009, the year in which the first proof-of concept study was published (55). Finally, we examined the www.clinicaltrials.gov website for published and ongoing randomized trials of RDN in hypertensive patients. Trials qualified if the reports included sufficient information on key points required for their interpretation; randomized controlled trials that involved patients with TRH, trial should comply with the Consolidated Standards of Reporting Trials (CONSORT) quality criteria (86), the central tendency (mean) and spread (SD) of the office and ambulatory blood pressure at randomization and follow-up (6-month follow-up) and the incidence of adverse effects.

We identified eight published randomized controlled trials (56, 87-93). We excluded one study (87) because it did not meet the CONSORT quality criteria. Thus, the current meta-analysis included seven randomized controlled trials (56, 88-93).

Study endpoints
- **Efficacy endpoints**: The pooled effect of office and 24-hour BPs in RDN treated group compared to control group at 6-month follow-up.
- **Safety endpoint**: Change of renal function assessed by pooled change in the estimated glomerular filtration rate (eGFR) in RDN treated group compared to control group at 6-month
follow-up. In addition the proportion of adverse events was compared between the 2 groups at 6-month follow-up.

**Reporting and statistical methods:**
Reporting was done according to the *Preferred reporting items for systematic reviews and meta-analyses: The “PRISMA” statement* (94) for reporting systematic reviews and meta-analyses.

We used SAS software version 9.3 (SAS Institute Inc., Cary, NC) to enter the extracted data into a dedicated database for subsequent statistical analysis. We expressed the central tendency and spread of continuous variables as mean and SD, respectively. We calculated the within-study effect size by subtracting the treatment effect in the control group from that in the RDN group. Efficacy was assessed by the BP changes from baseline to 6 months. Changes in eGFR from baseline to 6 months were evaluated as a measure of safety. We assumed that the true effect sizes differed among studies. Therefore, we estimated the pooled effect size and its confidence interval (CI) from random-effects models as implemented in the PROC MIXED procedure of the SAS package. Each study was weighted by the inverse of the within- and between-study variances. The latter were estimated using an iterative maximum likelihood method. We checked the null hypothesis of homogeneity across individual studies by the Cochran’s Q and the $I^2$ tests (12). For Cochran’s Q test, the $p$ value indicating significance was set at less than 0.10. For $I^2$, values less than 25%, from 25% to 50%, and more than 50% indicated modest, moderate and substantial heterogeneity, respectively. We performed sensitivity analyses by assessing the effect on the pooled estimates after removal of one study at a time. Finally, we computed pooled odds ratios for the risk of a major adverse event from stratified 2 x 2 contingency tables.

**Funding**

**The Beauty study (The BEtter control of BP in hypertensive pAtients monitored Using the HOTMAN sYstem) (Paper VI)**

**Study design and population**
The study was an investigator initiated, designed and driven multicenter prospective randomized parallel groups controlled study with sites at five European Hypertension Excellence centers: Gdansk (Poland), Milan (Italy), Oslo (Norway), Paris (France) and Tallinn (Estonia) – [ClinicalTrials.gov Identifier: NCT01482364].

Patients aged 18–85 years of either gender were referred from general practices responding to letters of invitation or were recruited directly by newspaper advertisements or referrals to the university outpatient clinics. They were worked-up at the five participating university hospitals in the time period from October 2011 through December 2012 by physicians trained in these institutions. Drug-treated, but uncontrolled hypertension (UCH)
was defined as elevated office SBP (>140 mmHg) despite regular intake of two to four or more antihypertensive drugs (towards the end of recruitment we accepted up to seven drugs but in few patients only, which is reflected by a total average of three drugs). Additionally, patients had to qualify by also having mean ambulatory daytime SBP of at least 135 mmHg. Prior to the qualifying ABPM, drug treatment was unchanged for two weeks and no other change in medication was pre-planned for the following 6 months.

Three hundred and fifteen patients were assessed for eligibility for the study. One hundred and sixty seven patients were found eligible and randomized to either integrated hemodynamic management (IHM) adjusted drug treatment “IHM-group” (n =83) or classical clinical adjustment of medical treatment “control group” (n =84). Out of the randomized individuals, 156 completed 6-month follow-up and were analysed (intention-to-treat).

**Randomization**

Patients were randomized using a predetermined 2 x 2 randomization list through a website organized by the monitor of the study (Sintesi Research, Milan, Italy).

**Procedures**

- **Blood pressures:** Measurement of office and ambulatory BPs were performed as has been described under “Oslo RDN study” and according to recommendations of the ESH/ESC for conventional and ambulatory blood pressure measurement (83), using the same validated oscillometric device (Microlife WatchBP O3; Microlife Health Management Ltd., Cambridge, UK)

- **Impedance cardiography with the HOTMAN® (Hemodynamic & Oxygen Transport Management) System:** As has been described under “Oslo RDN study”

**Study endpoints**

- **Efficacy endpoints:** The primary endpoint of efficacy was the absolute change in daytime SBP, under ambulatory conditions (ABPM) after a 6 months follow-up. Absolute change in daytime DBP, 24-hour SBP, 24-hour DBP, night-time SBP, night-time DBP, office SBP and office DBP after a 6 months follow-up were secondary efficacy variables. Other endpoints of efficacy were percentage of normalization of SBP and DBP at daytime, 24-hour and night-time under ambulatory conditions (ABPM), normalization of office SBP and DBP, rate of fall (slope with time) in office SBP and visit-to-visit variability of office BP (SBP/DBP)

- **Safety endpoint (Adverse events and quality of life):** Adverse events were regularly investigated actively at each visit, by giving participants a written self-questionnaire in which 30 common adverse events related to antihypertensive drugs were proposed in a neutral order for the purpose of catching the data. Quality of life was assessed at each visit by a visual analog scale (VAS). Adverse event was defined as any untoward medical occurrence in a patient administered with a pharmaceutical product (both the HOTMAN monitoring and selected drugs) and which did not necessarily have a causal relationship with this product. The relationship of an adverse event to the selected drugs was graded as definite, probable, possible, unlikely, and unrelated. The severity of an adverse event was graded as mild (discomfort noted, but no disruption of normal daily activity); moderate (discomfort sufficient to reduce or affect normal daily activity), or severe (instability to work or perform normal daily activity). A serious adverse event was any untoward medical occurrence that results in death or was life-threatening, evaluated by investigators as yes or no.
**Statistical methods**

Data were recorded in an SAS database and analyzed using SAS 9.2 (SAS Institute Inc., Cary, North Carolina, USA). The primary endpoint was evaluated by two-sample t-test. A linear regression model on ambulatory SBP at month 6, adjusting for the baseline ambulatory SBP value, center, sex, age and BMI, was applied to account for potential risk factors. The secondary endpoints on efficacy were evaluated using two-sample t-test on BP changes and chi-square test with one degree of freedom for the percentage of normalization of BP. Rate of fall (slope with time) in office BP over the five visits was analysed by a longitudinal analysis. A mixed model was applied including (as response variable) all the BP measurements available from baseline to study end for each patient in the full analysis set. The visit-to-visit BP variability (VVV) was log converted before applying t-test. The distribution of type of antihypertensive drugs (classified as agents acting on the renin–angiotensin system, b-blocking agents, CCBs, diuretics and others) at the study end was compared in the two arms by a chi-square test with four degrees of freedom. The number of adverse events in each group was compared by a Poisson model, accounting for the total follow-up times in full analysis set. Incidence rate ratio (IRR) between IHM group and control group was computed on overall adverse events, serious adverse events, drug-related adverse events, endpoint of special interest and the severity of adverse events. Quality of life was compared by t-test. A 2-sided α level of 0.05 was used for all superiority testing.

The sample size was calculated to detect a different decrease of ambulatory daytime SBP from baseline to study end between IHM and classical drug selection group. One hundred and eight individuals per group were required to detect a difference of 5 mmHg with 80% power at a type one error of 5% in a two-sided t-test, assuming a SD of 13 mmHg. To end up with 108 individuals per group it was recommended to enrol 250 participants and randomize 125 participants per group.

**Approvals, funding and monitoring**

The study was approved by all relevant committees for clinical research ethics in the participating countries and by the institutional research committee of all five hospitals. All patients gave written informed consent for participation and publication of results. All patients who qualified for the procedure within the 24-month time period were included. Expenses were mostly covered by the participating hospitals and partly by grants-in-aid from one sponsor (Hemo Sapiens Inc. European Office, Bucharest, Romania), and patients were not paid. The study was monitored by Sintesi Research (Milan, Italy), an independent company with no relationship to the investigators and the sponsor.
Summary of results

Paper I
We investigated for the first time the BP lowering effect of RDN in treatment-resistant hypertension following witnessed intake of antihypertensive medication just prior to qualifying ambulatory BP. Two thirds of patients with apparent TRH who were referred by specialist (n=18), were found non-eligible for RDN for various reasons among which normal ambulatory BP being the most common. Six patients with true TRH underwent RDN. Mean office and ambulatory BPs remained unchanged up to six months after renal denervation. No major adverse events recorded at 6-month follow-up.

Paper II
We investigated in this paper for the first time, in a randomized controlled study design, the BP lowering effect of RDN versus clinically adjusted drug treatment, guided by integrated hemodynamic management (IHM) using impedance cardiography, in patients with true TRH after excluding patients with confounding poor drug adherence. Patients with apparent TRH (n=65) were referred specifically for RDN. Forty five patients were found non-eligible for various reasons with poor drug adherence being the most common reason (n=14). Patients with true TRH were randomized to either RDN (n=9) or clinically adjusted drug treatment (n=10). Office SBP and DBP were significantly lower in the drug-adjusted group at 6 months (p=0.002 and p=0.004, respectively), and absolute changes in SBP were larger in the drug-adjusted group (p=0.008). Ambulatory BPs changed in parallel to office BPs. Regarding safety, one patient in the RDN group had a myocardial infarction five months after the procedure otherwise no major adverse events related to RDN procedure were recorded at 6-month follow-up.

Paper III
In this paper we systematically reviewed and meta-analysed summary statistics of the randomized clinical trials of RDN in patients with TRH to assess the BP lowering effect and safety of RDN. Of 5652 patients screened in seven trials, 985 (17.4%) qualified and were randomized to control (n=397) or RDN with Symplicity catheters (n=588). Follow-up was 6 months. In both control and RDN patients, antihypertensive treatment was continued and/or optimized. For BP outcomes, there was heterogeneity among trials. There were no significant BP pooled effects (control minus RDN), where -4.9/-3.5 mmHg (95% confidence interval(CI), -20.9 to 11.1/-8.9 to 1.9) for office BP, while -2.8/-1.5 mmHg (CI, -6.5 to 0.8/-3.3 to 0.4) for 24-hour BP and 0.81 ml/min/1.73 m² (-1.69 to 3.30) for eGFR. Removing one trial at a time produced confirmatory results. Adverse events occurred in 7.4% and 9.9% of control and RDN patients, respectively (p=0.24).

Paper IV
We investigated whether utilizing non-invasive monitoring of hemodynamic parameters by impedance cardiography combined with a drug selection algorithm (integrated hemodynamic management – IHM) compared with conventional drug selection may improve uncontrolled
hypertension in five European Hypertension Excellence centres. One hundred and sixty seven patients randomized to IHM-guided (n=83) vs. conventional control (n=84). The average number of antihypertensive drugs increased from 3.1 to 4.1 in both groups and differed only in a rise of the use of diuretics in the IHM groups (from 13 to 31%). Ambulatory daytime SBP, defined as the primary endpoint, decreased markedly and to the same extent from baseline to 6 months in IHM (−15.8±14.8 mmHg) and control (−15.4±14.5 mmHg) groups (p=0.87), with a similar behaviour of office SBP (no between group differences, p=0.18). Average number of adverse events was a secondary endpoint and was significantly lower in IHM than in controls (p=0.008) but of a more general type and not necessarily related to drug treatment.
Discussion of materials and methods

General methodological considerations

We recruited and investigated patients with uncontrolled hypertension (UCH) in (Paper IV), where UCH was defined as an uncontrolled high BP, documented by ABPM, despite intake of 2 or more antihypertensive drugs. In (Paper I, II and III) patients with the well-defined uncontrolled hypertension, namely treatment resistant hypertension were investigated. In the latter population we have tested the efficacy and safety of RDN compared to continued and/or optimized conventional drug treatment, while in the first population we tested the efficacy and safety of using impedance cardiography in tailoring antihypertensive drug treatment compared to conventional drug selection. Though there is a difference in definition between these populations, they shared many common baseline characteristics such as a predominantly male population, high BMI, and middle age. However, they differed in number of antihypertensive drugs and slightly in average BPs (Table 1). It became clear that recruiting patients with TRH was much more difficult, only 29% (Paper I and II) and 17.4% (Paper III) were found to be eligible, compared to recruiting patients with UCH where 53% were found to fulfil inclusion criteria.

Table 1. Patients’ characteristics at baseline.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Oslo RDN</th>
<th>RDN Meta-analysis</th>
<th>Beauty</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paper I-II</td>
<td>Paper III</td>
<td>Paper IV</td>
</tr>
<tr>
<td>Nº of assessed for eligibility</td>
<td>85</td>
<td>5652</td>
<td>315</td>
</tr>
<tr>
<td>Nº of eligible (%)</td>
<td>25 (29%)</td>
<td>985 (17.4%)</td>
<td>167 (53%)</td>
</tr>
<tr>
<td>Eligible women (%)</td>
<td>8 %</td>
<td>30 %</td>
<td>39 %</td>
</tr>
<tr>
<td>Mean age (y)</td>
<td>60</td>
<td>58</td>
<td>56</td>
</tr>
<tr>
<td>Mean body mass index, kg/m²</td>
<td>29</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>Nº of drugs</td>
<td>5</td>
<td>4.7</td>
<td>3</td>
</tr>
</tbody>
</table>

Abbreviation: BP indicates blood pressure.

Specific methodological considerations

The Oslo RDN study (Paper I and II)

Eligibility: We designed our study with the intention to include only patients with true TRH, trying to avoid the important limitations and potential biases of the Symplicity HTN-2 (63-65), that were discussed earlier in the introduction. We excluded patients with the important confounding factors of poor drug adherence and secondary or spurious hypertension by
having a systematic thorough work-up at screening phase (Figure 2). Patients with secondary HT and those who achieved normal BPs after optimizing their treatment regimen were excluded. To avoid including patients with white coat hypertension and those with poor drug adherence, ABPM were measured in all patients immediately after witnessed intake of antihypertensive drugs and only those with a mean ambulatory daytime SBP > 135 mm Hg were included.

Poor drug adherence can be assessed and monitored by many different methods, indirect and direct ones (95). The indirect methods are mainly based on self-report (questionnaire, diary and interview) and pill count (manually, electronic pill box and prescription registries) and these methods may easily be manipulated by the patient. Witnessed intake of medication, also known as direct observed therapy (DOT) and therapeutic drug monitoring (TDM) are examples of direct methods (95). TDM is generally defined as the clinical laboratory measurement of a chemical parameter that, with appropriate medical interpretation, will directly influence drug prescribing procedures. TDM has been proven to be effective in unveiling poor drug adherence among hypertensive patients (27, 96-100). However, using TDM could not secure their intake of medication as needed in our study. Witnessed intake of medication was used in our study because it is the only method ensuring the administration of correct medication in correct dosage and at the correct hours, which, in combination with subsequent ambulatory BP measurement, is suitable for confirming the effect of pharmacological treatment. The strength of this method is its ability to effectively and directly reveal poor drug adherence. By applying a systematic thorough work-up and using ambulatory BP measurements in all patients immediately after witnessed intake of antihypertensive drugs, we could with high degree of assurance recruit patients with true TRH. A limitation of the method presents in patients who have not taken their prescribed medications regularly at home, thus drug persistence cannot be assured. Optimal serum concentration, and thereby full therapeutic effect, cannot be expected in these patients, with the possible intake of only one dosage (101), since many hypertensive drugs need days and weeks to reach steady state. This may have underestimated our prevalence of poor drug adherence.

There are ethical aspects regarding the use of DOT in treatment of hypertension. DOT was originally developed for the treatment of patients with tuberculosis (102). DOT can easily be justified as part of the treatment of tuberculosis because of limited duration of treatment, compared to antihypertensive treatment which most often is permanent. In addition, tuberculosis is a highly contagious disease, often with fatal outcomes if left untreated. The same arguments cannot be used for treating hypertension. However, our experiences from the Oslo-RDN study indicate an important role for DOT in the examination and evaluation of apparent TRH. The potential BP-lowering effect of DOT in the treatment of hypertension, both in short- and long-term perspectives, requires further research.

**Study Design:** We used a randomized prospectively controlled, open study design, a similar study design of that used in the Symplicity HTN-2 study. However, patients enrolled in the control group in our study were offered an active treatment (The control group had their antihypertensive medication adjusted at baseline, 1 month, and at 3 months according to 2007 European Society of Hypertension/European Society of Cardiology hypertension guidelines (103) and guided by using a non-invasive integrated hemodynamic measurements of the
impedance cardiography with the HOTMAN System). By offering an active treatment (IHM) to the control group and using ABPM, we assumed that we could balance and limit the differential impact of the Hawthorne and placebo effects between the study’s groups.

The lack of patient and investigator’s blindness in our study was one of the main limitations. However, RDN is an invasive procedure and one could not achieve blindness without using a sham procedure in the control group. Sham procedures can reduce possible placebo and Hawthorne effects; however, their use does not exclude other sources of bias such as variations in type of antihypertensive drugs and their dosages and the degree of adherence to treatment. It is worth to mention that there is a clear disagreement between the European and the American experts in hypertension regarding use of sham procedure, the latter assume that a sham procedure in the control group is mandatory to assure blinding which is very important to evaluate the real effect of RDN (104). While the European experts expressed concerns; whether a sham procedure would be necessary in a trial of resistant hypertensive on standardized treatment and if adherence variability can be minimized with ambulatory BP as the primary end point. In addition, the risk is not negligible and the use of invasive sham is possibly unethical in mild to moderate hypertensive patients (105).

Sample Size: The modest number of patients may be considered a limitation. However, the strength of our study was the careful identification of patients with true TRH compared to the first randomized controlled study, Symplicity HTN-2. In addition, our two RDN studies (Paper I and II) showed consistent results that strengthen the overall conclusions.

Procedure: Though the single-electrode Symplicity catheter system was described lately as too imprecise and operator dependent, we assumed that RDN in our study was performed successfully by two experienced and appropriately trained invasive radiologists. Moreover, a high number of radiofrequency ablations, on average 8 (range 6-11), were applied per renal artery compared to an average of 5 (range 4-6) in Symplicity HTN-2 (56). In spite of the technical limitations of Symplicity Catheter System, this was the only one that has been thoroughly documented in randomized controlled studies so far (Paper III). Many companies have developed their own RDN systems, but none of these have yet proved their efficacy in RCTs.

Meta-analysis of RCTs of RDN in treatment of TRH (Paper III)
Meta-analysis, especially, of the randomized controlled studies is a statistical procedure that integrates the results of several independent studies, plays a central role in evidence-based medicine. In fact, in the hierarchy of evidence, where clinical evidence is ranked according to the strength of the freedom from various biases that beset medical research, meta-analyses are in the top (106, 107). That is why we included only randomized controlled trials (RCTs) of RDN in our study.

The strength of this systemic review and meta-analysis was applying the standards of the Preferred Reporting Items for Systematic reviews and Meta-Analyses “PRISMA” statement (94). In addition, all included trials complied with the standards of the Consolidated Standards of Reporting Trials (CONSORT) statement of RCT (86). However, the main limitation of this study was that we performed a meta-analysis of the “aggregate data” of the included studies and not a meta-analysis of “individual participant data”, a preferred method
that considered as the gold standard method (108-111). A meta-analysis based on individual participant data offers advantages over analyses using aggregated data, including a greater ability to validate the quality of data and to conduct appropriate statistical analysis. Further, it is easier to explore differences in effect across subgroups. However various methods of analysis exist and no single method can be recommended for all scenarios (112, 113). In the beginning we tried to collect individual participant data, but we failed because investigators of some RCTs did not respond to our repeated requests. An additional limitation was differences in trial designs; while all patients allocated to RDN arms in all included studies were treated with the same RDN system, (single-electrode Symplicity RF Catheter System (Medtronic, Mountain View, CA, USA)), patients allocated to control arms received different treatment modalities. However, these limitations were to some extent balanced with the other strengths of this meta-analysis. First, this study had a small risk of publication bias, since RDN has a high profile in hypertension research, and because we carefully searched the trial registry (http://www.clinicaltrials.gov) of the National Institutes of Health for unpublished data. Secondly, we statistically used the random-effect model (114) after assessing for the homogeneity across individual studies to account for the differences in trial design, population size, and variation in effect among included RCTs. Thirdly, the results of the analyses were consistent after we performed sensitivity analyses by assessing the effect on the pooled estimates after removal of one study at a time.

The Beauty study (Paper IV)

**Eligibility:** In this study we recruited patients with drug-treated, but uncontrolled hypertension (UCH) without getting mixed up with the controversy regarding “treatment resistant hypertension”. Thus, in this study we intended to include a more common hypertensive population. We acknowledge that a frequent reason for UCH is poor drug adherence (25-27) that might be due to complexity of medication regimen (26) and side-effects. The fact that drug adherence assessment was not a part of our study design during selection of patients could be a limitation of this study. Our attention to assess properly poor drug adherence emerged later during designing our Oslo RDN study and was not part of the considerations in the Beauty study. In addition, despite that the study was a prospective randomized controlled, it was open and prone to patients and investigators-related biases. However, the primary endpoint was ambulatory daytime SBP, a blind endpoint that reduces the impact of patients and investigators-related biases and limits the differential impact of the Hawthorne and placebo effects (115) between the study’s groups and might to some degree have balanced the absence of assessment of drug adherence. Another limitation was that lead investigators were in principle blinded for the IHM (HOTMAN System) readings in patients in the control arm, but it is possible that some information might have leaked from technician or patient to physician, in as much as IHM readings were open for technician and to some degree also for patients. In this regard, the fact that the same doctor was responsible for the treatment of patients in both arms might have carried a potential bias. One more possible limitation was that the treatment of control group might have differed from one centre to another according to the local routines of selection and dose titration of the different
antihypertensive drugs at each of these five centres. However, this limitation was adjusted for in the statistical analysis.

**Sample Size:** Despite this was a multicentre study and we had less strict inclusion and exclusion criteria; we could not manage to recruit the pre-planned recommended sample size of 250 individuals. Out of 315 hypertensive patients, 167 individuals with UCH were randomized and 156 completed 6-month follow-up (intention-to-treat). Failure of recruitment was one of the main limitations in this study, though the analysed individuals at 6-month follow-up were considered enough to maintain statistical power, and we virtually had no differences in office and ABPs. However, one cannot rule out a type-2 statistical error.

**Procedure:** Impedance cardiography with the HOTMAN System is an easy method and do not need a high experienced person to apply compared to other invasive and mini-invasive hemodynamic procedures. But in spite of the technological improvement in the HOTMAN system compared to the older ICGs and of its validation against invasive hemodynamic studies (79), the noninvasive method we used was likely, somehow, affected by the known intrinsic problems of impedance cardiography, which may have limited the accuracy of patient’s hemodynamic assessment (116).
Discussion of the results

General results aspects
Renal sympathetic denervation by the radiofrequency energy using the single-electrode Symplicity catheter system did not show superior BP lowering effect compared to the conventional drug treatment, neither in the Oslo RDN study (Paper I-II) nor in the polled effect of all existing valid RCTs in RDN (Paper III). However, the overall results should be interpreted with cautions because there are limitations of each of these studies that might have affected their outcomes.

We also found that utilizing noninvasive monitoring of hemodynamic parameters by ICG, combined with a drug selection algorithm (integrated hemodynamic management – IHM) did not show superiority over conventional drug selection in treatment of patients with UCH (Paper IV). Interestingly, in Oslo RDN study (Paper II) IHM showed better BP lowering effect compared to RDN in treatment resistant hypertensive patients. The difference in outcomes between these two studies though using the same technique might be in apart explained by different study design and populations, in addition to many other specific limitations of each of these studies.

Specific results aspect
The Oslo RDN study (Paper I and II)
Among patients with apparent TRH (n = 83) referred from hospitals and specialists, approximately only one third were considered to have true TRH after thorough work-up. In the remaining two thirds, BP control was achieved or secondary causes for hypertension were discovered. The main reason for lack of BP control was poor drug adherence, in consistence with many other studies (34). In our RDN study, drug adherence was evaluated by witnessed drug intake followed by 24-h ABPM. In our opinion, this is the best method for investigating whether the prescribed drug regimen is effective or if adjustments are necessary. The strength of this method is its ability to effectively and directly reveal poor drug adherence. A theoretical limitation of the method presents in patients who have not taken their prescribed medications regularly at home. Poor drug adherence, although known for decades (25, 26), is once again actualized with the development and introduction of novel methods for treating TRH (27, 96, 97, 99). Our findings revived the fact that patients with true TRH represent a substantial minority in the hypertensive population.

The most important findings were that patients with true TRH who underwent RDN did not experience meaningful BP lowering effect, in fact, patients randomized to adjusted drug treatment guided by noninvasive hemodynamic measurements (control group) had a significant lower BP compared to RDN group (p=0.008), (Paper II) (Figure 6). We were the first ever to investigate in a prospective randomized controlled study the BP lowering effects of RDN in patients with true TRH. Our results were in a huge contrast to the first randomized study of Symplicity HTN-2 (56). Though our sample size was smaller than in Symplicity HTN-2 study, we may consider our results more reliable because we included patients with true TRH. Our findings illustrate the importance of a robust recruitment regime in which true
treatment resistant hypertensive patients are identified and confounding factors, mainly poor drug adherence and secondary HT excluded.

**Figure 6.**
Change in BPs at 3 and 6-month follow-up in the Oslo RDN study

Abbreviations: SBP: systolic blood pressure; DBP: diastolic blood pressure

Though the overall results do not support a BP lowering effect of RDN, we have to be aware of that considering individual data, possibly four patients that underwent RDN (two in Paper I and two in Paper II) could be characterized as responders. Thus, we cannot rule out that among true treatment resistant hypertensive patients there may be a few that might respond effectively to RDN. This has also been observed and reported in other trials (117). However, these hypertensive patients are still difficult to identify prior to or during the procedure of RDN.

**The meta-analysis of RCT of RDN (Paper III)**
The randomized evidence of BP lowering effect of RDN was limited until recently. After approximately four weeks of reporting the results of our Oslo RDN study, the Symplicity HTN-3 trial (89) was presented in 2014. This is till now the largest and best powered trial (n =535), applying a randomized design with a RDN arm and a sham arm (control), as requested by the Food and Drug Administration (FDA) in USA. This study failed to reach its efficacy endpoint, a reduction in office systolic BP 6 months after RDN with the single-electrode Symplicity catheter system. Even though there are some limitations of this trial, it annihilated the seemingly unlimited prospects of a large market, thereby stalling research on RDN. The Symplicity HTN-3 study followed up by other few randomized controlled studies (90-93). Interestingly, all of these studies had also used the same single-electrode of the Symplicity catheter system (Medtronic (Adrian), Mountain View, CA, USA). Seven RCTs found eligible and included in this meta-analysis (56, 88-93). It is noticeable that only 985 (17.4%) of 5652 screened patients were found eligible and were randomized in these seven trials. This finding strengthens ours (Paper I –II) where a minority of patients were found to be eligible for RDN.

The main finding of our meta-analysis was that the BP-lowering effect of RDN with the Symplicity catheter system on top of continued or optimized antihypertensive drug treatment was not superior to the treatment in the control groups. Our results were in contrast
to the previous quantitative reviews and meta-analyses published on RDN (118-120) that concluded that RDN was an effective treatment of TRH. These reviews and meta-analyses had many potential limitations and biases. First, pooled estimates in these meta-analyses combined non-randomized and randomized studies and were therefore confounded by the weaknesses of uncontrolled studies, in particular placebo and nocebo (115) effects, and inaccuracies in data extraction and reporting (121). Secondly, the earlier reviews (118, 119) included the trial conducted by Pokushalov and colleagues (87) which, for reasons explained elsewhere (122), did not meet the CONSORT criteria (86) and was therefore excluded from our meta-analysis. Finally, the previous meta-analyses included only two (118) or three (119, 120) randomized trials. The lack of updated evidence based on only randomized controlled trials in RDN justified our updating of the literature review (123, 124).

We have performed the first meta-analysis of the randomized evidence of the BP lowering effect of RDN and concluded that RDN failed to show superior effect compared to conventional treatment. However, there are some limitations that might explain some of the variable effect of RDN observed in the included trials. First, these studies have had different study designs, different work-up methods, most importantly; assessment for drug adherence. Secondly, patients remained on multiple different drug treatment with or without RDN. Thirdly, in the all reviewed trials, RDN was performed with the single-electrode Symplicity RF catheter (Medtronic, Mountain View, CA, USA), a device that may be too imprecise and operator dependent to meet the objective of effective RDN. Improved catheter designs, multielectrode or stabilized by an inflatable balloon or expandable basket, in addition to using other denervation technic; intraluminal ultrasound technic (125), cryoenergy ablation (126), transcatheter alcohol-mediated perivascular renal denervation (127), are currently available. But this new techniques must be validated in well-designed RCTs. Even though RDN by the Symplicity systems was not superior to conventional drug treatment, it was showed to be safe. The safety of the procedure will motivate further research in the field.

**The Beauty study (Paper IV)**

This was the first study to assess impedance cardiography by HOTMAN system in treatment of UCH in a randomized controlled study design. Previous studies (84, 85, 128) suggest that, when the pharmacological class of antihypertensive treatment is adapted to the hemodynamic state, BP reduction occurs more rapidly and to a greater extent. However, these studies were based on impedance cardiography devices characterized by a questionable accuracy in defining patients’ hemodynamic state (129, 130). Ours is the first study addressing this issue through the use of HOTMAN system, which was independently validated in its ability to quantify the hemodynamic status in comparison with invasive assessments based on the thermodilution technique (82).

Despite of the theoretical advantages of a drug selection and titration based on IHM, no differences in the reduction and normalization of BPs were observed between the IHM and control groups (Figure 7). Although our study cannot provide a definite explanation for this finding, it might have been influenced by the relatively moderate number of participants we were able to recruit. In addition, patient management was carried out by experienced doctors working in five European Hypertension Excellence centres. This implies that, even in the
control group, drug selection and titration was done with accuracy, which may have minimized the between group differences in achieved BP.

**Figure 7.**
Change of Ambulatory BP at 6-month follow-up

**Abbreviations:** BP: blood pressure; IHM: Integrated hemodynamic management

Though the Beauty study failed to reach its efficacy endpoint, the use of diuretic treatment was higher with concomitantly improvement of patients’ hemodynamic status. Moreover, significantly less adverse events were reported during 6-month follow-up in the IHM group (Figure 8). These secondary findings might indicate that IHM by HOTMAN system could be a useful tool in tailoring antihypertensive drugs in patients who experience adverse events. Thus, these findings warrant further investigation in properly powered trials. We have not yet analysed the data of echocardiography and arterial stiffness assessment, using applanation tonometry of SphygmoCor® system, to measure pulse wave velocity (PWV) and calculating central blood pressure (BP) and augmentation index (AIx). It is of huge interest to assess changes of these parameters and see if they can correlate to the improvement of patients’ hemodynamic in the IHM group.

**Figure 8.**
Mean number of overall adverse events

**Abbreviations:** IHM: integrated hemodynamic management, CO: control
Clinical Implication

Our studies (Paper I, II and III) showed that RDN is not an evident treatment for patients with TRH in contrast to the pioneer novel trials (55, 56) and previous systematic reviews and meta-analyses (118-120). Therefore RDN is not ready for routine clinical care and should only be offered to patients within a context of clinical research in highly skilled tertiary referral centres that share data with registries independent of the manufacturers (66).

In our randomized RDN study (Paper II), utilizing noninvasive monitoring of hemodynamic parameters by Impedance cardiography combined with IHM by the HOTMAN system had a significantly better BP lowering effect than RDN in patients with TRH. However, in our larger study (Paper IV) IHM by HOTMAN system failed to show superiority over the conventional drug selection in treatment of uncontrolled hypertension. Therefore, we do not recommend using IHM in routine clinical care of hypertension, especially in experienced hypertension centres (European Hypertension Excellence centres).

General and important clinical implication of this thesis is highlighting the importance of a systemic thorough investigation of patient with apparent TRH. It is of great importance to assess the possibilities of poor drug adherence since it is the main cause of apparent TRH. Witnessed drug intake with subsequent ambulatory blood pressure measurement was showed to be a reliable method, and we can recommend that “witnessed drug intake” to be used more in the routinely assessment of patients with apparent TRH. An additional important clinical implication is that a majority of patients with difficult-to-control hypertension can be managed after thorough selection and optimization of their antihypertensive drugs according to current guidelines (24).
Future perspective

Though BP-lowering effect of RDN with the Symplicity catheter system was not found to be superior to continued or optimized antihypertensive drug treatment, almost all experts leading the RDN trials reported that a minority of patients experienced a spectacular BP response to RDN (131, 132). In addition, RDN showed to be safe, and this may motivate further research. The future of RDN will be determined by clinical trials showing BP lowering effect that targeting never-treated patients with stage I – II hypertension (24), low risk factor profile and evidence of sympathetic overactivity. This approach would exclude comorbidities and irreversible target organ damage, including stiffening of the conduit arteries and remodelling of the microcirculation. Since guidelines propose lifestyle measures in such patients up to several months (24), patients can be kept off medications, avoiding confounding by non-adherence and changes in drug treatment (104, 105, 133). Furthermore, there is emerging need to develop markers and measures that could confirm that RDN do occur during the procedure.

Renal nerve stimulation is a promising method to ascertain the completeness of denervation (134) and may allow identification of the anatomical sites where ablations have to be made; thereby addressing the big variability in the course of the renal sympathetic nerves along the renal arteries (134). Trials designed along these lines and according to the European and American experts recommendations (104, 105, 133, 135), perhaps stratified according to the RDN system or place of energy delivery and selection of appropriate population, could definitely establish or exclude RDN as a treatment modality in hypertension. It is worth to mention that two new randomized sham-controlled studies (SPYRAL HTN-ON MED Study and SPYRAL HTN-OFF MED Study) in USA, using the new Symplicity Spyral™ multi-electrode renal denervation system (136) have started recruiting patients. These studies have been designed to avoid the limitations and biases of the previous Symplicity studies (55, 56, 89). The new studies are also funded by Medtronic as in the previous ones (55, 56, 89). It will of huge interest to follow up the results of these studies in the near future. Many experts consider these two studies to be the last serious trials by Medtronic to revive or reject RDN as a treatment modality in hypertension.

Though utilizing noninvasive monitoring of hemodynamic parameters by impedance cardiography combined with a drug selection algorithm (integrated hemodynamic management – IHM) of the HOTMAN system was not found to be better than conventional drug selection in treatment of UCH, the use of IHM was associated with a lower rate of adverse events. Thus, although our data do not support using IHM in routine clinical care, they do support the need of further research on this approach, to identify conditions where it might be clinically useful, such as in more severe HT populations (Paper II). In addition, this method was tested in European Hypertension Excellence centres, and it will be of huge interest if it can be evaluated at the primary and secondary care levels.

Lastly, despite of the huge unprecedented industrial interest in the past few years to develop new mini-invasive novel methods; RDN (55), baroreflex activation therapy (BAT) (137), carotid body ablation (138), arteriovenous fistula (139), vagal nerve stimulation (140) and baroreflex modulation using self-expanding carotid implant (141) to manage TRH, one should not forget the complexity and multifactorial character of hypertension. Previous knowledge has been
building up through clever research by generations of investigators and hypertension cannot be resolved overnight by one type treatment modality. One should remember that one size does not fit all. That is why it is of great importance, while trying to develop new mini-invasive treatment modalities, to continue the ongoing research to develop and test new antihypertensive drugs (142-156) targeting different pathophysiological mechanisms (157) despite the need for large investments (158). In addition, it is warranted to continue testing new treatment strategies, as it was shown, in a recently published paper (159) that adding spironolactone was the most effective add-on drug compared to non-spironolactone add-on drugs in treatment-resistant hypertension.
Conclusions

Paper I
Renal sympathetic denervation by single-electrode Symplicity radiofrequency catheter system did not effectively lower BP in patients with true treatment-resistant hypertension after excluding poor drug adherence and other confounding factors. Witnessed intake of antihypertensive drugs followed-up with 24-hour ambulatory BP measurements found to be applicable and reliable method to identify patients with poor drug adherence.

Paper II
Renal sympathetic denervation using single-electrode Symplicity radiofrequency catheter system was a safe procedure in the short term follow-up. However, RDN did not show meaningful BP lowering effect and it showed to be inferior to intensive optimized medical therapy guided by hemodynamic profile (integrated hemodynamic management - IHM) obtained by impedance cardiography of the HOTMAN system in patients with true treatment resistant hypertension, after excluding poor drug adherence and other confounding factors. Poor drug adherence found to be the most common cause of apparent treatment resistant hypertension.

Paper III
Systemic review and meta-analysis of the randomized controlled trials of renal sympathetic denervation in treatment of patients with treatment resistant hypertension using the single-electrode Symplicity radiofrequency catheter system was probably a safe procedure in the short term follow-up. However, RDN was not superior in lowering BPs compared to continued or optimized antihypertensive drug treatment.

Paper IV
Monitoring of hemodynamic parameters, using impedance cardiography of the HOTMAN system, and then applying a predefined algorithm of drug selection (i.e. integrated hemodynamic management - IHM) was not superior to classical drug selection in controlling BP in patients with high uncontrolled hypertension. However, the use of IHM was associated with a lower rate of adverse events.
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47


