# TREATMENT-RESISTANT HYPERTENSION: A FOCUS ON PREVALENCE OF NON-ADHERENCE, ASSOCIATED FACTORS AND RENAL SYMPATHETIC DENERVATION

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# ABSTRACT

Hypertension is a leading cause of cardiovascular and cerebrovascular morbidity and mortality worldwide. Treatment-resistant hypertension represents a cohort of patients with treated hypertension with at least 3 antihypertensives. In this thesis the important role of adherence testing, and prevalence of non-adherence is described to establish a diagnosis of true treatment-resistant hypertension. A review of renal sympathetic denervation has identified the development and shortcomings of this technique and research studies to date which has been further adapted for use by using carbon dioxide angiography in a pilot study of patients with chronic kidney disease to eliminate the risk of contrast-induced nephropathy associated with the commonly used iodinated contrast agents. A study was designed and conducted to describe the phenotypical and biochemical characteristics of patients with true treatment-resistant hypertension and to assess fluid and tissue body composition, arterial stiffness, endothelial dysfunction, inflammation and presence of obstructive sleep apnoea in these patients. The results have shown that patients with true treatment-resistant hypertension have a metabolic-syndrome-like phenotype with a high risk of future cardiovascular events, evidence of hyperaldosteronism, higher endothelial dysfunction and inflammation but similar levels of arterial stiffness when compared to non-treatment-resistant hypertension patients.

# DEDICATION

*To my beloved family*

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# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| **4-PL** | Four-parameter logistic |
| **ABP** | Ambulatory blood pressure |
| **ABPM** | Ambulatory blood pressure monitoring |
| **ACE** | Angiotensin-converting enzyme |
| **ACR** | Albumin creatinine ratio |
| **ADMA** | Asymmetric dimethyl arginine |
| **ADH** | Antidiuretic hormone |
| **AHA** | American Heart Association |
| **AHS** | Antihypertensive screen |
| **Ang** | Angiotensin |
| **ANP** | Atrial natriuretic peptide |
| **ASCOT** | Anglo-Scandinavian Cardiac Outcomes Trial |
| **ARB** | Angiotensin II receptor blocker |
| **ARR** | Aldosterone-renin ratio |
| **ATM** | Adipose tissue mass |
| **ATPase** | Adenosine triphosphatase |
| **BCM** | Body composition monitor |
| **$BH_4$** | Tetrahydrobiopterin |
| **BIHS** | British and Irish Hypertension Society |
| **BIS** | Bioimpedance spectroscopy |
| **BMI** | Body mass index |
| **BMQ** | Beliefs about medicines questionnaire |
| **BP** | Blood pressure |
| **BRS** | Baroreflex sensitivity |
| **cGMP** | Cyclic guanosine monophosphate |
| **CCB** | Calcium channel blocker |
| **cFLC** | Combined free light chains |
| **cfPWV** | Carotid-femoral pulse wave velocity |
| **CI** | Confidence interval |
| **CKD** | Chronic kidney disease |
| **CKD-EPI** | Chronic Kidney Disease Epidemiology Collaboration |
| **CRIC** | Chronic Renal Insufficiency Cohort |
| **CRP** | C-reactive protein |
| **CT** | Computed tomography |
| **DBP** | Diastolic blood pressure |
| **DEXA** | Dual-energy x-ray absorptiometry |
| **DENERHTN** | Renal Denervation for Hypertension |
| **DOT** | Directly observed therapy |
| **ECE** | Endothelin converting enzyme |
| **ECF** | Extracellular fluid |
| **ECG** | Electrocardiogram |
| **ECW** | Extracellular water |
| **eGFR** | Estimated glomerular filtration rate |
| **ELISA** | Enzyme-linked immunosorbent assay |

| | |
|---|---|
| **ESRD** | End-stage renal disease |
| **ESS** | Epworth sleepiness scale |
| **ET** | Endothelin |
| **FACT-RHY** | A study of Factors AssoCiated with Treatment-Resistant HYpertension |
| **FDC** | Fixed-dose combination |
| **FLC** | Free light chain |
| **GDNF** | Glial cell line-derived neurotrophic factor |
| **GFR** | Glomerular filtration rate |
| **HDL-C** | High density lipoprotein cholesterol |
| **HbA1c** | Haemoglobin A1c |
| **HIV** | Human immunodeficiency virus |
| **HR** | Heart rate |
| **ICAM** | Intercellular cell adhesion molecule |
| **ICW** | Intracellular water |
| **IFCC** | International Federation of Clinical Chemistry |
| **IQR** | Interquartile range |
| **ISO** | The International Organization for Standardization |
| **LC-MS** | Liquid chromatography-mass spectrometry |
| **LDL-C** | Low density lipoprotein cholesterol |
| **LnRHI** | Log natural reactive hyperaemia index |
| **LTM** | Lean tissue mass |
| **LVH** | Left ventricular hypertrophy |
| **MAP** | Mean arterial pressure |
| **MDRD** | Modification of Diet in Renal Disease |
| **MEMS** | Medication event monitoring system |
| **MI** | Myocardial infarction |
| **MMAS** | Morisky Medication Adherence Scale |
| **MRA** | Magnetic resonance renal angiography |
| **mRNA** | Messenger ribonucleic acid |
| **MSNA** | Muscle sympathetic nerve activity |
| **NADPH** | Nicotinamide adenine dinucleotide phosphate |
| **NHANES** | National Health and Nutrition Examination Survey |
| **NICE** | National Institute for Health and Care Excellence |
| **NO** | Nitric oxide |
| **NOS** | Nitric oxide synthase |
| **NT-proBNP** | N terminal pro B-type natriuretic peptide |
| **ODI** | Oxygen desaturation index |
| **OH** | Overhydration |
| **OR** | Odds ratio |
| **OSA** | Obstructive sleep apnoea |
| **PAT** | Peripheral arterial tone |
| **PPI** | Patient Public Involvement |
| **PWV** | Pulse wave velocity |
| **RAAS** | Renin-angiotensin-aldosterone system |
| **RCT** | Randomised control trial |
| **RDN** | Renal sympathetic denervation |

| | |
|---|---|
| **RHI** | Reactive hyperaemia index |
| **ROC** | Receiver operating characteristic |
| **ROS** | Reactive oxygen species |
| **SBP** | Systolic blood pressure |
| **SD** | Standard deviation |
| **sFLT-1** | Soluble receptor fms-like tyrosine kinase-1 |
| **SHR** | Spontaneously hypertensive rats |
| **SNA** | Sympathetic nerve activity |
| **SPRINT** | Systolic Blood Pressure Intervention Trial |
| **SSAHT** | Stepped-care standardised antihypertensive treatment |
| **TBW** | Total body water |
| **TIA** | Transient ischaemic attack |
| **TNFα** | Tumour necrosis factor alpha |
| **TRH** | Treatment-resistant hypertension |
| **TSH** | Thyroid stimulating hormone |
| **UKAS** | United Kingdom Accreditation Service |
| **UKRDA** | United Kingdom Renal Denervation Affiliation |
| **VCAM** | Vascular cell adhesion molecule |
| **WCE** | White coat effect |
| **WHO** | World Health Organisation |
| **WMHC** | West Midlands Hypertension Centre |

# CHAPTER 1 INTRODUCTION

## 1.1 Hypertension

Hypertension or high blood pressure (BP) is a major preventable cause of cardiovascular morbidity and mortality worldwide. Hypertension is an established risk factor for ischaemic and haemorrhagic stroke, myocardial infarction (MI), heart failure, chronic kidney disease (CKD), cognitive impairment and premature death. BP is normally distributed in the population and therefore a clear 'cut-off' is difficult to determine, although a BP of <120 mmHg systolic and <80 mmHg diastolic is often stated to be normal. Diagnosis of hypertension has been traditionally based on a cut-off of 140/90 mmHg. However, cardiovascular benefits of lower BP are present at even lower values than those considered normal. The risks of hypertension are continuous; every 2 mmHg rise in systolic BP (SBP) is associated with a 7% increased risk of mortality from ischaemic heart disease and 10% increased risk of mortality from stroke (1).

The World Health Organisation (WHO) has identified high SBP as the leading risk factor responsible for the burden of disease worldwide (2). Between 1990 and 2017, high SBP has been consistently responsible for the largest number of all-cause deaths worldwide. In 2017, 10.4 million deaths and 218 million disability-adjusted life-years were attributable to high SBP (2). Estimates show that cardiovascular disease is responsible for over a quarter of the total deaths worldwide with 15.3 million deaths in 2017, of which high SBP was an attributable

risk factor for 54.6% of the deaths (3). **Figure 1.1** shows percentage of total deaths from cardiovascular diseases worldwide, which are attributable to high SBP in 2017.

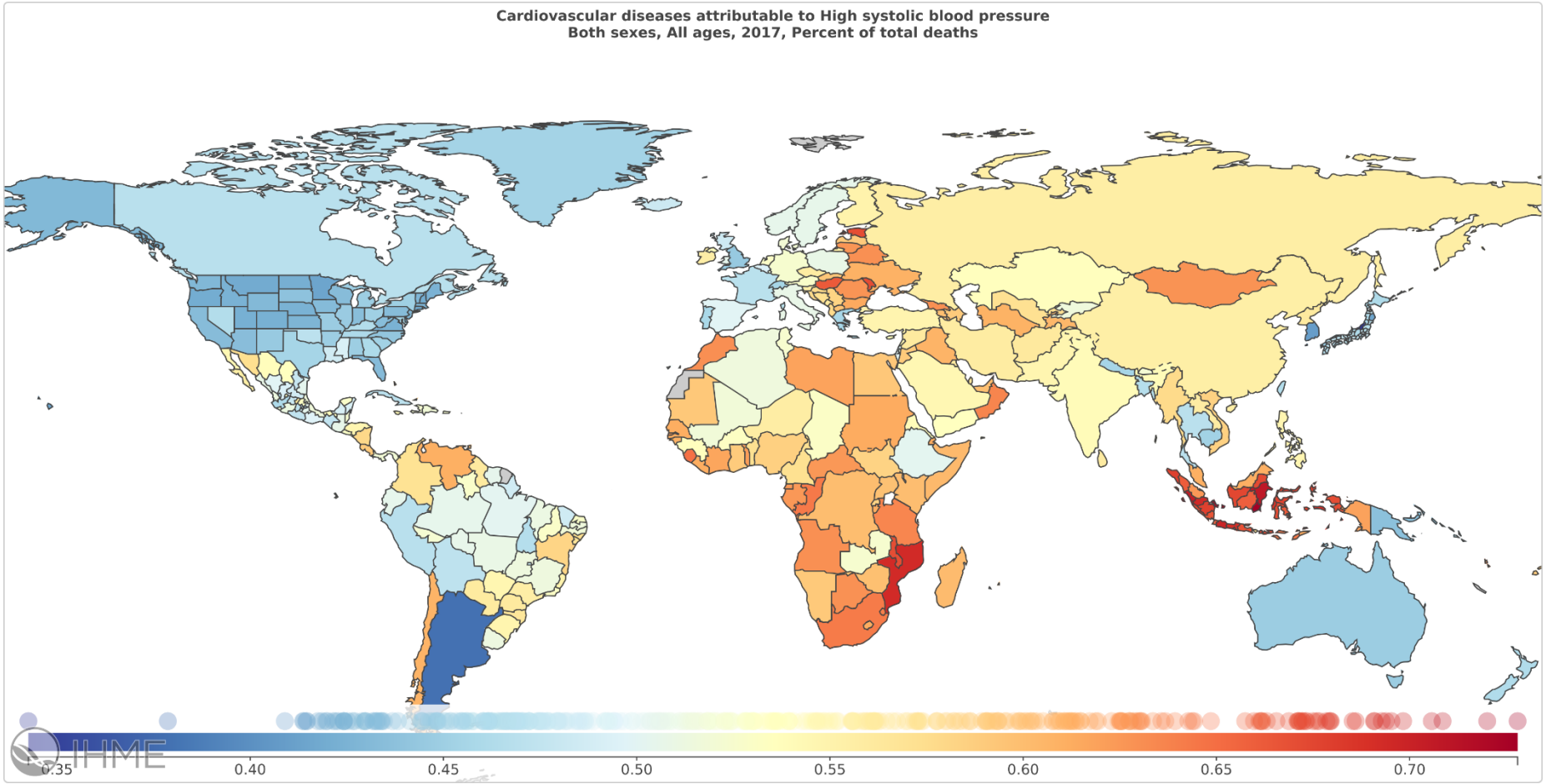



**Figure 1.1:** Percentage of total deaths from cardiovascular diseases worldwide which are attributable to high systolic blood pressure in 2017**.** Cardiovascular diseases include rheumatic heart disease, ischaemic heart disease, stroke, non-rheumatic valvular heart disease, cardiomyopathy and myocarditis, atrial fibrillation and flutter, aortic aneurysm, peripheral artery disease, endocarditis, and other cardiovascular and circulatory disease. Generated using the online Global Burden of Disease Compare visualisation tool (3).

## 1.2 Epidemiology and risks of hypertension

Prevalence of hypertension is common; in 2008, approximately 40% of the adults above the age of 25 worldwide were diagnosed with hypertension. The prevalence of hypertension varies greatly around the world; the highest prevalence is seen in the Africas Region at 46% and the lowest prevalence in the Americas at 35% (4).

In England, figures from Department of Health's 2017 Health Survey England have shown that prevalence in adults of age 16 years or older was 28% in men, 25.0% in women and 27% overall (5). From 2003 until 2014, the overall prevalence has largely remained the same at 30% which then dropped to 27% in 2017 with greatest reduction observed in men (see **Figure 1.2**). Hypertension is largely an asymptomatic condition and therefore quite often remains undiagnosed. Public Health England estimates over 5.5 million adults in England are unaware that they have hypertension (6). Diseases caused by hypertension (coronary heart disease, stroke, vascular dementia and CKD) have been estimated to cost the National Health Service over £2.1 billion a year (6).

High BP increases risks in a continuous fashion for overall mortality, mortality from cardiovascular and coronary heart disease, MI, heart failure, left ventricular hypertrophy (LVH), atrial fibrillation, stroke/transient ischaemic attack, peripheral vascular disease and end-stage renal disease (ESRD). A metanalysis of almost 1 million adults from prospective studies have shown that even down to the usual BP levels of 115/75, each difference of 20 mmHg in usual SBP is associated with about a two-fold difference in age-specific mortality rates from ischaemic heart disease (see **Figure 1.3**), stroke (see **Figure 1.4**), and other vascular causes (7).

Conversely, evidence from metanalyses of randomised clinical trials (RCTs) of antihypertensives show a significant reduction in risk even with modest reductions in BP (8, 9). Law et el. demonstrated reductions of 22% and 41% in risk of coronary heart disease events and stroke respectively for a BP reduction of 10 mmHg systolic and 5 mmHg diastolic (8). Ettehad et al. showed that a 10-mmHg reduction in SBP reduced the risk of major cardiovascular disease events by 20%, coronary heart disease by 17%, stroke by 27%, heart failure by 28%, and all-cause mortality by 13% (9).

However, despite the risks associated with high BP and the benefits of BP-lowering treatments, almost half (43%) of the hypertensive adults surveyed in England in 2017, were untreated (5). Furthermore, the percentage of patients achieving BP control was  low at only 38% of all hypertensives in 2017, however this percentage has doubled from 19% since 2003 (see **Figure 1.5**).

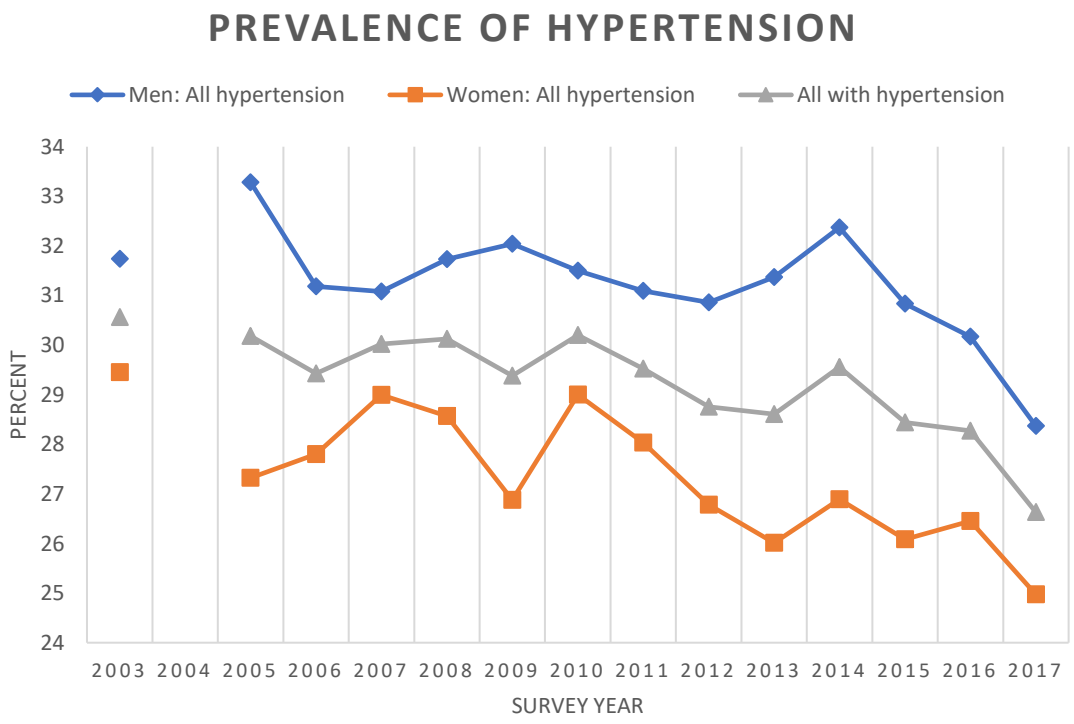



**Figure 1.2:** Prevalence of hypertension in England from 2003–2017 for men, women and overall [data derived from (5)].

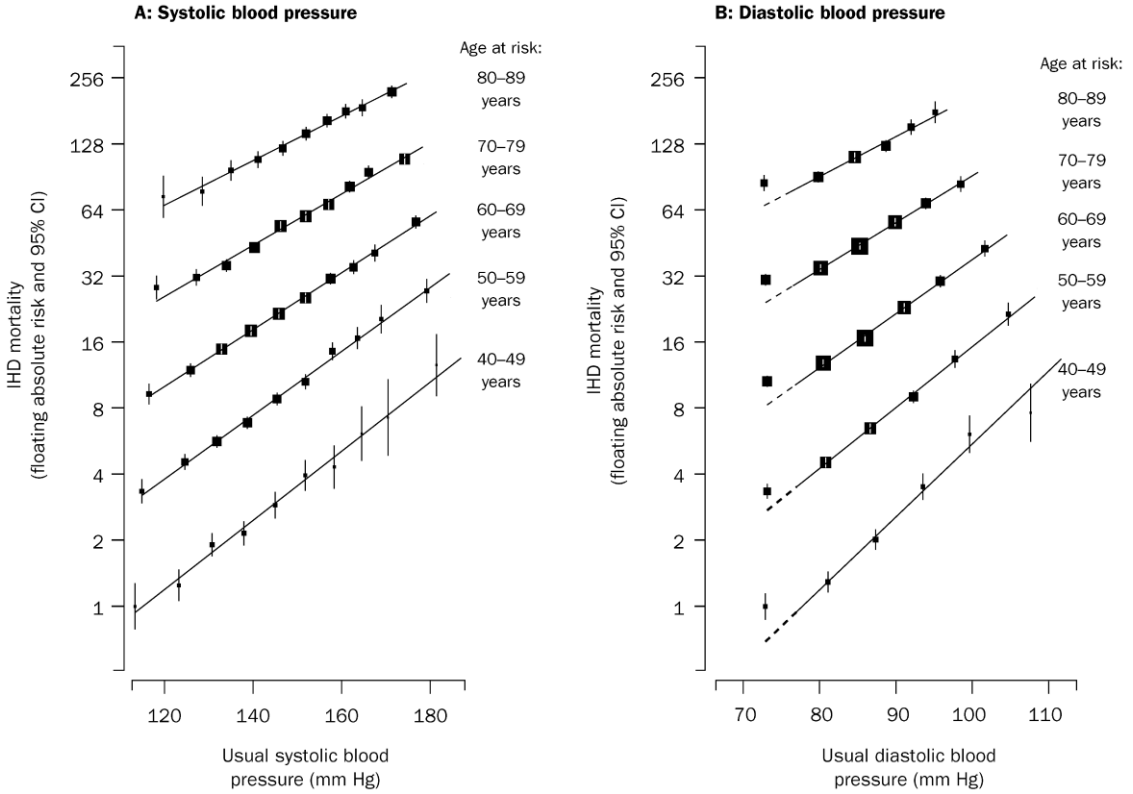



**Figure 1.3:** Ischaemic heart disease mortality rate in each decade of age versus usual blood pressure at the start of that decade (7).

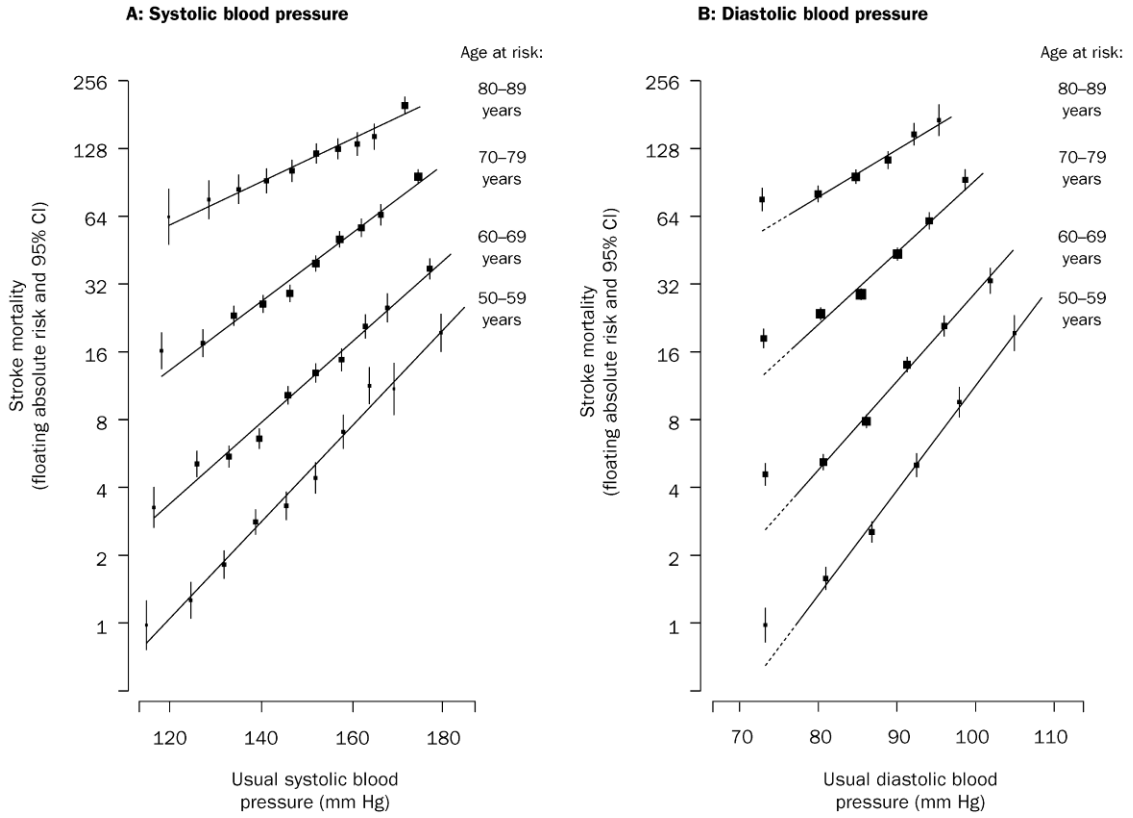



**Figure 1.4:** Stroke mortality rate in each decade of age versus usual blood pressure at the start of that decade (7).

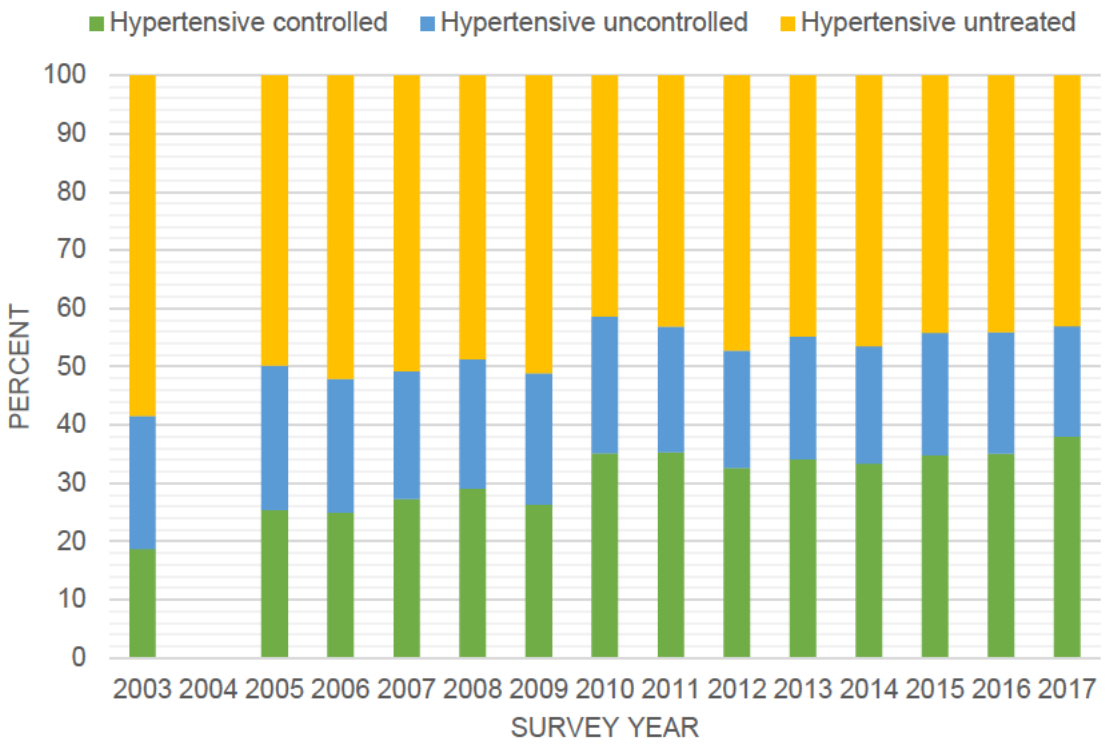



**Figure 1.5:** Proportion of controlled, uncontrolled and untreated hypertensives in England between 2003–2017 [data derived from (5)].

## 1.3 Blood pressure measurement

As has been shown that even very small rises in BP cause a significant increase in the risk of future morbidity and mortality. Since all treatment decisions in patients with hypertension are based on the non-invasively measured BP readings, it is paramount that every effort should be made to ensure that the BP measurement is as accurate as possible. Guidelines on how to measure BP accurately and minimise errors exist (10). Measurement of clinic, ambulatory or home BP has given rise to phenomenon of BP variability, white coat effect (WCE), and masked hypertension which are discussed below in further detail.

### 1.3.1 Blood pressure variability

BP is a dynamic physiological parameter which continuously fluctuates as a result of a complex interplay of external factors (e.g. environment, stress, altitude) and internal factors (posture, emotional stress, volume status) that induce BP changes and countered by cardiovascular regulatory mechanisms. BP variability can range from seconds to days. Very short-term (beat-to-beat variability) occurs as a result of autonomic modulation, short-term variability over 24 hours is affected by individual's activity and sleeping pattern, and mid-term day-to-day or long-term (visit-to-visit) variability is driven by factors such as antihypertensive therapy, errors in measurement of BP, fluctuations in adherence to treatment and seasonal changes.

Broadly, two types of variations are responsible for variations in the clinic BP – measurement and biologic. Measurement variations involve the observer and are sources of errors that can affect a BP reading. Reeves has compiled a very long list of factors which lead to measurement variation (11). Although errors arising

from these factors can be minimised through training of staff to consistently use the guidelines set out to measure BP (10). Unsurprisingly, these errors mask true changes in BP and are very common than most healthcare practitioners may appreciate (12). Biological variation which is random can be overcome simply by repeating the BP measurement, however systematic variations, such as BP changes in response to environmental temperature, can only be minimised if they are recognised and controlled. Furthermore, Clark et al. showed that the there is no significant circadian rhythm to BP beyond the effect of sleep-wakefulness cycle and that the effect of various activities was responsible for the majority of variation in BP over a 24-hour period (13).

Mancia et al. have shown that 24-hour BP variability progressively increases in normotensive to increasingly severe hypertensive individuals and is positively correlated with mean arterial pressure [MAP] (14). Further support for this observation arises from the fact that reductions in BP mean, by antihypertensive medications, are responsible for decreases in BP variability (15). Importantly, 24-hour BP variability has been shown to be an independent predictor of target organ damage, cardiovascular and stroke mortality (16, 17).

The long-term or visit-to-visit BP variability may be explained by weekly working patterns (18), seasonal temperature changes (19) or age-related arterial stiffness (20). However, understanding of visit-to-visit BP variability remains incomplete (15). Prognostic importance of long-term BP variability has been highlighted by many. In the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT), the authors have shown that visit-to-visit variability in SBP and maximum SBP are strong predictors of stroke (21) which could be explained by lower BP variability in the

amlodipine-treated group compared to atenolol-treated group (22). A metanalysis of 389 studies concluded that “drug-class effects on interindividual variation in BP can account for differences in effects of antihypertensive drugs on risk of stroke independently of effects on mean SBP” (23). The evidence supporting BP variability as an independent predictor of cardiovascular outcomes is not conclusive. Whilst some have shown increased BP variability to be associated with increased risk of stroke (21, 24) and cardiovascular mortality (25), others have found no association between BP variability and cardiovascular outcomes over and beyond mean SBP (26, 27).

### 1.3.2 Ambulatory and home blood pressure monitoring

Traditional method of measuring clinic BP involved the use of a sphygmomanometer and a stethoscope by a healthcare professional, which has been largely replaced by the more convenient oscillometric devices capable of measuring BP repeatedly to improve accuracy and reduce BP variability. Ambulatory blood pressure monitoring (ABPM) has been used in hypertension for quite some time; Perloff et al. first showed prognostic value of ABPM over 35 years ago (28). However, the use of ABPM in routine clinical practice was first recommended in the UK by National Institute of Health and Care Excellence (NICE) for the diagnosis of hypertension (29). The use of ABPM has borne out of a systematic review which showed that neither clinic nor home BP are sufficiently specific or sensitive for diagnosis of hypertension (30) and a health economic analysis concluded that ABPM is a cost effective test in routine clinic practice (31).

The consensus on diagnostic thresholds for ABPM agreed upon by the European Society of Hypertension (32) are based on the outcome-driven studies (33, 34):

- 24-hour average ≥130/80 mmHg
- Awake (daytime) average ≥135/85 mmHg
- Asleep (night-time) average ≥120/70 mmHg

ABPM has several advantages over clinic BP; it can provide information on the patterns of BP such as identifying masked, white-coat or nocturnal hypertension as well as assessing BP variability and response to antihypertensive therapy over 24 hours. Additionally, a large number of readings provide information on changes in BP in an individual's usual daily living environment and could be used to assess the effect of certain activities on the BP. Importantly, it is a stronger predictor of cardiovascular morbidity and mortality than the clinic BP (32). Conversely, the equipment and software required is expensive although the information it provides makes it cost-effective. Repeat testing with ABPM may not be universally accepted by patients as it can cause discomfort, sleep disturbance and is more burdensome (32).

Home BP monitoring is a more acceptable method for repeat measurements compared to ABPM. Like ABPM, outcome-driven diagnostic thresholds have been defined for home BP monitoring (35). The optimal home BP measuring schedule has been described as 2 readings, whilst seated in a resting position, taken in the morning and night over a week (36). Crucially, self-monitoring has been shown to lead to clinically significant BP reduction and improved adherence

(37-40). Furthermore, sphygmomanometers capable of telemonitoring may revolutionise the management of hypertension (37).

### 1.3.3 White-coat effect

The alerting response generated by either the anticipation or the act of measurement of clinic BP can cause the BP to rise and is termed as WCE (41). The rise in BP is transient and if diagnosis of hypertension is based upon this BP it could lead to significant overestimation. WCE is affected by the environment and the measurer; clinic BP is lower in a health centre compared to hospital when measured by the same physician (42) and BP measured by a nurse was lower compared to one measured by a physician (43). Recent development of a fully automated oscillometric sphygmomanometer, which takes five measurements of BP over a set interval whilst the patient is sat alone in quiet room, has been shown to virtually eliminate the WCE with accuracy approaching that of daytime ambulatory BP [ABP] (44). The same technique was used in the recent landmark Systolic Blood Pressure Intervention Trial [SPRINT] (45). It therefore seems to be a more attractive option for routine use in the clinic setting compared to repeated ABPMs.

The term white-coat hypertension may be used where an elevated clinic BP which is found to be normal (<135/85 mmHg) on daytime ABP or home BP monitoring (46, 47). Whilst WCE may be present in majority of the patients (41, 48), prevalence of white-coat hypertension ranges from 15% to 50% depending on the thresholds used (49-54). The diagnosis of white-coat hypertension needs to be confirmed on repeat ABPMs over time due to the variation in the magnitude of WCE (55). White-coat hypertension in itself is not a benign entity; up to 43%

of patients with white-coat hypertension may go on to develop persistent hypertension (56). Furthermore, a systematic review and metanalysis showed that untreated white-coat hypertension is associated with an increased risk for cardiovascular events and all-cause mortality, whereas treated WCE is not associated with elevated risk (57).

### 1.3.4 Masked hypertension

Masked hypertension is the opposite to white-coat hypertension where clinic BP is normal (<140/90 mmHg) but daytime ABP or home BP is elevated (≥135/85 mmHg). The term is only used for untreated patients, whereas the term 'masked uncontrolled hypertension' is where treated patients have elevated BP on ABPM or home BP monitoring. The main problem in clinical practice is how to identify patients with masked hypertension, a condition which affects 10% of the general population (58). A meta-analysis of prospective observational studies showed that masked hypertension and masked uncontrolled hypertension were associated with a higher risk of all-cause and cardiovascular mortality, cardiovascular events, stroke, cardiac events and renal disease events regardless of the method of out-of-clinic BP measurement used (59).

Pickering et al. in 2007 suggested that if taking the prevalence rates for white-coat hypertension and masked hypertension to be 15% and 10% respectively, a total of 40 million people in the US are misclassified on the basis of clinic BP, either because of false positive (10 million due to white-coat hypertension) or false negatives [30 million with masked hypertension] (58). Screening with either ABPM or home BP monitoring is therefore required to identify all patients with masked hypertension. Clearly this is likely to be impractical, however evidence

suggests three clinic BP measurements at two visits and three measurements in the morning and in the evening over 2 days for home BP may be sufficient (60). Instead, it may be more appropriate to focus efforts on patients with characteristics shown to be associated with masked hypertension which include diabetes mellitus (61), CKD (62-64); obstructive sleep apnoea [OSA] (65), unexplained tachycardia (66), and LVH based on electrocardiogram [ECG] (67).

## 1.4 Pathophysiology of hypertension

There is no single unifying mechanism responsible for the development of primary hypertension. Decades of research have identified a myriad of neural, renal, vascular and hormonal pathways that in conjunction with environmental and genetic factors conspire together to produce human hypertension; a model similar to the mosaic theory of hypertension first proposed by Irvine Page in 1949 (68). An overview of these mechanisms and factors are described below.

### 1.4.1 Neural mechanisms

#### 1.4.1.1 Overview of the autonomic nervous system

Autonomic nervous system plays a key role in the homeostasis of essential bodily functions and is divided into sympathetic and parasympathetic. At a very basic anatomical level it consists of a collection of afferent and efferent neurons, that connect the central nervous system to the effector organs.

The central regulation of sympathetic outflow is carried out by specialised areas both in the medulla oblongata, located in the brainstem, which include nucleus tractus solitarius, caudal ventrolateral medulla, and rostral ventrolateral medulla and the supramedullary paraventricular nucleus located in the hypothalamus. Nucleus tractus solitarius receives direct sensory input arising from the baroreceptors in the carotid sinus and aortic arch, peripheral chemoreceptors and renal afferents. Rostral ventrolateral medulla is a site of central sympathetic outflow and considered the primary regulator of excitatory sympathetic nervous outflow to the cardiovascular system and receives both inhibitory and excitatory inputs.

The efferent activity is relayed to the effector organs via preganglionic neurons originating in the central nervous system and the peripheral ganglionic neurons. The specific effector organs involved in the neural control of BP include the heart, blood vessels and kidneys and contain adrenergic receptors which interact with the noradrenaline released by the postganglionic neurons.

#### 1.4.1.2 Baroreceptor reflex

Baroreceptor reflex is an inhibitory reflex aims to dampen short-term fluctuations in the BP. Stimulation of baroreceptors, as a consequence of increased BP or increased cardiac filling pressure, sends inhibitory signals to the nucleus tractus solitarius which evokes increased parasympathetic efferent outflow and decreased sympathetic outflow resulting in bradycardia and vasodilatation to counter the effects of elevated BP. When the BP falls and the stretch-mediated stimulation of baroreceptors is reduced, resulting in increased sympathetic nerve activity (SNA) and reduced parasympathetic activity. This continuous adjustment of the opposing actions of sympathetic and parasympathetic nervous systems maintains the BP at the desired level.

#### 1.4.1.3 Excitatory neural reflexes

The main excitatory neural reflexes initiate in the carotid body chemoreceptors, kidneys and the skeletal muscles. Peripheral chemoreceptors are stimulated by hypoxia or hypercapnia and cause reflex increase in SNA resulting in peripheral vasoconstriction to increase the BP and tissue perfusion (69). In OSA, recurrent episodes of cessation of respiratory airflow and nocturnal hypoxia from airway collapse stimulate the chemoreceptor reflex to increase SNA and eventually BP (70); both of these effects of nocturnal hypoxia are attenuated by treatment with

continuous positive airway pressure [CPAP] (70). Persistent daytime increased SNA in OSA contributes to high BP and is driven by intermittent hypoxia; the carotid body chemoreceptors are sensitised to hypoxia causing tonic activation of chemoreceptor afferents despite normal blood oxygen concentration (71, 72).

Renal afferents stimulation by ischaemic metabolites (e.g. adenosine) and uraemic metabolites (e.g. urea) serve to increase the central sympathetic outflow. These pathways have been proposed to be the culprit of hypertension caused by renovascular disease and CKD. Similarly, sensory afferents arising from the skeletal muscle which are activated by mechanical and chemical changes occurring during muscle contraction and exercise evoke increased SNA resulting in increased BP, cardiac output and perfusion of the skeletal muscles.

#### 1.4.1.4 Long-term neural regulation of blood pressure

As noted above, different neural pathways are involved in the short-term regulation of BP in response to physical and emotional stimuli. Baroreceptor reflex has been implicated in the increased renal SNA and consequently long-term BP control (73). However, Osborn et al. have carried out a comprehensive review of the experimental research in this area to conclude that long-term BP control is beyond the control of arterial baroreflex (74). It is clear that increased renal SNA is the final common pathway leading to renal sodium retention consequently hypertension (75). However, exactly how the increased renal SNA is maintained in the long-term and the specific role of central nervous system in the long-term control of BP is not well understood (73).

### 1.4.2 Assessment of sympathetic nervous system

There are multiple methods of assessing SNA in the body; each with its own limitations.

#### 1.4.2.1 Urine and plasma catecholamines

Noradrenaline is the principle neurotransmitter of the sympathetic nervous system. Twenty-four-hour urinary excretion of catecholamines or their metabolites only provides an average or ‘static’ assessment of SNA and cannot provide information on acute effects of adrenergic stimuli (76). Plasma noradrenaline concentration can be measured through a simple venous blood test and provides an estimate of global SNA (77). It has been shown to correlate well with the central sympathetic outflow to the skeletal muscle (78-80). However, significant limitations remain; (76), its use is limited by the fact that circulating noradrenaline represents a small proportion of the secreted neurotransmitter from the nerve terminal. Furthermore, there is lack of reproducibility compared to microneurography (76, 81).

#### 1.4.2.2 Isotope dilution renal noradrenaline spillover

Regional noradrenaline spillover into plasma can be measured using constant rate intravenous infusion of tritiated (radiolabelled) noradrenaline (82). It overcomes the limitations of measuring plasma noradrenaline levels alone and in addition provides assessment of SNA of individual internal organs which is not possible with microneurography (76). This technique, however, remains experimental due to its invasive nature and specialist equipment required.

#### 1.4.2.3 Microneurography

Microneurography is a technique which can be used for direct measurement of SNA at the level of postganglionic nerve fibres of awake human subjects. Muscle SNA (MSNA) is known to have a close, dynamic relationship to BP and is involved in BP regulation via the arterial baroreflex (83). Therefore, it can provide an estimate of resting SNA. Microneurography has the advantages that it provides direct, continuous, highly reproducible (81) measurements which can be used to provide dynamic assessment of either skin or skeletal muscle nerve fascicles relatively safely (76). The technique, however, is invasive, requires considerable expertise, limited to a laboratory, difficult to compare with between subjects and, can only be used to assess muscle and skin regional beds which form only a fraction of the peripheral circulation and may not be representative of overall SNA (76). However, in normotensive humans, simultaneous measurement of MSNA and noradrenaline spillover in the heart (84) or the kidney (85) at rest show significant positive correlations.

### 1.4.3 Role of the sympathetic nervous system in hypertension

Roman scholar Cornelius Celsus (25 BC – 50 AD) was probably the first person to observe the relationship between sympathetic nervous system and elevated BP by noting that exercise, passion and the presence of a doctor could all cause the pulse to increase in rate and tenseness (86). The sympathetic nervous system plays an important role in maintenance of MAP by altering cardiac output, total peripheral resistance, sodium excretion and modulation of the renin-angiotensin-aldosterone system (RAAS) in response to acute changes in BP. Chronically activated sympathetic nervous system leads to increases in cardiac

output and peripheral resistance, and retention of sodium and water all of which play important roles in the development and maintenance of hypertension (87). Increase in tubular sodium reabsorption, renin release and renal vascular resistance lead to shifting of the pressure natriuresis curve to the right and thus contributing to chronic elevation of BP (**Figure 1.6**).

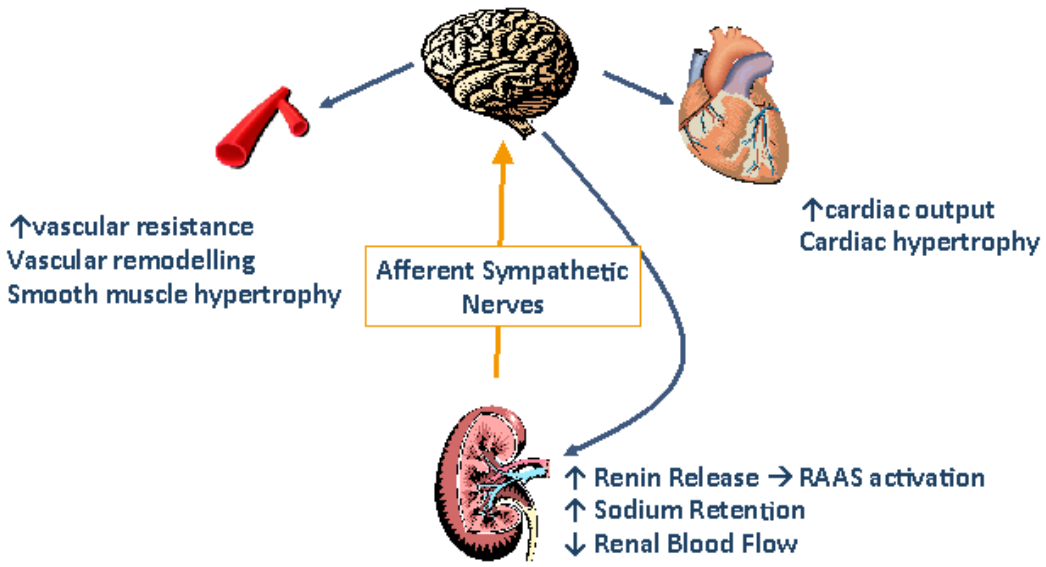



**Figure 1.6:** Effects of sympathetic activation and the role of afferent renal sympathetic nerves in the development and control of hypertension.

Increased SNA has been demonstrated in both animal models and humans with primary hypertension compared to those with normal BP (88-90). Vascular remodelling and smooth muscle hypertrophy, another consequence of increased SNA, contribute to the increased peripheral resistance (91) and play important roles in the development and maintenance of chronic hypertension. Moreover, increased SNA in hypertension contributes to end-organ damage as suggested by the correlation between left ventricular mass and reduced vascular compliance and elevated circulating noradrenaline (92, 93). SNA overactivity may also be responsible for diastolic hypertension in young as indicated by the correlation between heart rate (HR) and diastolic BP suggesting autonomic nervous system

imbalance in the form of relative SNA overactivity (94). SNA overactivity has also been implicated in obesity related hypertension. Both the renal and cardiac sympathetic activities have been demonstrated to be increased in this situation (95).

#### 1.4.3.1 Sympathetic nerve activity in primary hypertension

There is overwhelming evidence supporting that SNA is a major factor in the pathogenesis and pathophysiology of primary hypertension (96). Early stages of hypertension are characterised by increased HR, cardiac output, plasma noradrenaline (97), regional noradrenaline spillover (98, 99) and MSNA (90, 100, 101).

Noradrenaline spillover in the heart and kidneys of hypertensive patients have consistently been shown to be elevated (98, 99). Excess cardiac noradrenaline spillover has been shown to be responsible for promotion of LVH in the hypertensive patients (102, 103). The effects of increased renal SNA are realised through three main mechanisms; increased renin secretion rate from the juxtaglomerular cell via stimulation of $\beta_1$-adrenoreceptor, increased sodium reabsorption and decreased urinary sodium excretion at the renal tubular epithelial cells via stimulation of the $\alpha_{1B}$-adrenorecptor, and renal vasoconstriction of the renal vasculature resulting in decreased renal blood flow via simulation of $\alpha_{1A}$-adrenoreceptor (104).

Many studies have demonstrated an augmentation of MSNA in patients with essential hypertension (90, 100, 101). Indeed, MSNA has been shown to increase progressively from normotension to moderate and severe essential hypertension, paralleling the progressive increase in BP values (101).

#### 1.4.3.2 Mechanism of sympathetic stimulation in hypertension

The mechanism of sympathetic stimulation in hypertension is complex but perhaps involves both baroreceptor and chemoreceptor pathways. Both central and peripheral baroreceptors are reset at higher BPs in hypertension; and return to normal as BP is lowered with treatment (105-107). Other factors implicated in sympathetic stimulation include abnormal sympathetic innervation and renal morphogenesis, oxidative stress, endothelial dysfunction involving endothelin and nitric oxide (NO) pathways, and angiotensin (Ang) II; these are discussed in more detail later.

#### 1.4.3.3 Role of renal sympathetic nerves in hypertension

Renal nerves are the ‘communication link between the central nervous system and the kidney’. The major structural and functional components of the kidney, the vessels, glomeruli, and tubules are innervated. In response to various stimuli, both mechanical and chemical, the efferent renal sympathetic nerves are activated. Activation of the efferent renal sympathetic nerves leads to reduction in renal blood flow and glomerular filtration rate (GFR), increase in renal tubular sodium and water reabsorption, and increase in renin release. Inhibition of these nerves has the functionally opposite effects. The afferent renal sympathetic nerves generally, in response to stretch, exert an inhibitory effect through facilitating sodium and water excretion. This reno-renal reflex plays an important role in maintaining sodium and water homeostasis in various physiological and pathological states. However, in states of kidney disease and injury the excitatory fibres in the afferent sympathetic nerves are stimulated which leads to increased peripheral and renal SNA leading to peripheral vasoconstriction, increased cardiac output, renal vasoconstriction, increased renin release and tubular salt

retention all of which contribute to elevation of systemic BP. It is postulated that it is the activation of these fibres that contribute to initiation and maintenance of essential hypertension (108-110).

The evidence for the important role that the renal sympathetic nerves play in the pathogenesis and maintenance of hypertension come from animal experiments. There is a suggestion that the association between the sympathetic nervous system and the kidney starts in utero. Glial-cell-line-derived neurotrophic factor (GDNF) has been shown to play a significant role in the development of metanephric kidneys in particular the normal development of renal sympathetic innervation and glomerular morphogenesis (111). GDNF heterozygous mice have abnormal renal morphogenesis; their kidneys are approximately 25% smaller with up to 30% fewer nephrons and these mice have an 18mmHg higher MAP compared to GDNF homozygous mice (112). In spontaneously hypertensive rats (SHR), there is greater sympathetic innervation of blood vessels (113) and renal SNA is elevated (110, 114, 115). Studies of neonatal sympathectomy have shown a long-term reduction in arterial pressure reduces BP long-term (116-118). This is further confirmed when kidneys from neonatally sympathectomised SHR are transplanted into untreated SHR. MAP is lower in these animals compared to the control group in which kidneys from hydralazine-treated animals are transplanted into untreated SHR. This suggests that neonatal sympathectomy induces chronic changes to SHR kidney function leading to reduction of MAP even when extrarenal sympathetic tone is restored (119). It is suggested that it is the impairment of the reno-renal reflex mentioned above that contributes to the development of hypertension in SHR. Furthermore, there is a lot of evidence demonstrating renal sympathetic denervation (RDN) can delay

development and attenuate several different types of experimental hypertension (120).

Experiments in both rat models and humans have demonstrated that the sympathetic nervous system exhibits preferential activation of renal sympathetic fibres in response to baroreceptor unloading (121, 122). In the human experiment enalapril, an angiotensin-converting enzyme (ACE) inhibitor, was administered to healthy individuals. There was a minor drop in MAP, but renal noradrenaline spillover increased to 26% and 49% in response to low and high dose enalapril respectively, whilst the cardiac and total body noradrenaline spillover remained constant. These findings suggest that in healthy individuals without activated RAAS, there is selective stimulation of renal SNA in response to baroreceptor unloading (122). Purported mediators of baroreflex resetting include Ang II and oxygen free radicals (123, 124).

### 1.4.4 Renal Mechanisms

#### 1.4.4.1 Excess sodium intake

Through evolution, the human kidneys have developed effective transport mechanisms to retain the filtered sodium in the face of scarcity of salt in the diet of prehistoric humans. Over the past 100 years, salt intake has increased from less than 0.25g/day in our prehistoric ancestors to an average of 9-12g/day today in most countries around the world (125). Our kidneys' ability to handle the excessive sodium load is severely challenged and leads to development of hypertension through expansion of plasma volume, increased cardiac output and triggers autoregulatory responses to increase total peripheral resistance.

A large body of evidence from animal, epidemiological, migration, population-based intervention, and human genetic studies confirm the significant role salt plays in the development of hypertension.

The best evidence from animal studies comes from studies of chimpanzees (126, 127); progressive addition of salt to their diet of over 20 months led to development of hypertension which reversed on cessation of salt loading. The dietary salt load was within the human dietetic range.

Numerous epidemiological studies have examined groups of undeveloped communities some with access to salt in one form or the other but otherwise no differences in other aspects of lifestyle and diet of other undeveloped communities with no access to salt (128-130). The results of these studies consistently showed elevated BP to be associated with increased salt intake. The Intersalt study, carried out at 52 places around the world, was highly influential in showing correlation between salt intake and hypertension (131).

Studies of migration from low-salt rural areas to high-salt urbanised areas showed higher prevalence of hypertension in migrants (132, 133).

Population-based interventions which have been successful in reducing the salt intake have accompanied significant reduction in the population's average BP (134-136) and mortality from coronary heart disease and stroke (137, 138).

Molecular genetic studies of rare Mendelian forms of hypertension and hypotension in humans have shown the mutated gene products in all cases act in the same physiologic pathway in the kidney; altering net renal salt reabsorption (139).

#### 1.4.4.2 Pressure natriuresis

Experimental studies have shown that magnitude of urinary sodium excretion is dependent upon the perfusion pressure of the kidneys (140). Therefore, in normotensive individuals, a rise in BP causes increased urinary sodium and water excretion to return BP to normal (**Figure 1.7**). This concept is termed pressure-natriuresis and is a consequence of increased renal perfusion pressure causing an increase in the hydrostatic pressure of the capillaries surrounding the medullary thick ascending loop of Henle (141, 142), where 33% of the sodium is reabsorbed, resulting in fluid accumulation in the peritubular interstitial fluid and reduction in sodium reabsorption (143, 144). Accordingly, in hypertensive patients, a persistently raised BP should theoretically lead to profound volume depletion via pressure natriuresis (**Figure 1.7**). However, as this does not occur suggesting that the pressure natriuresis curve has reset at a higher arterial pressure, such that a higher perfusion pressure is required to achieve any given level of natriuresis (**Figure 1.8**). As well as shifting the pressure natriuresis to the right the only other way of increasing arterial pressure is through a high salt intake (**Figure 1.8**). In a steady state, renal excretion would match intake but at the expense of hypertension.

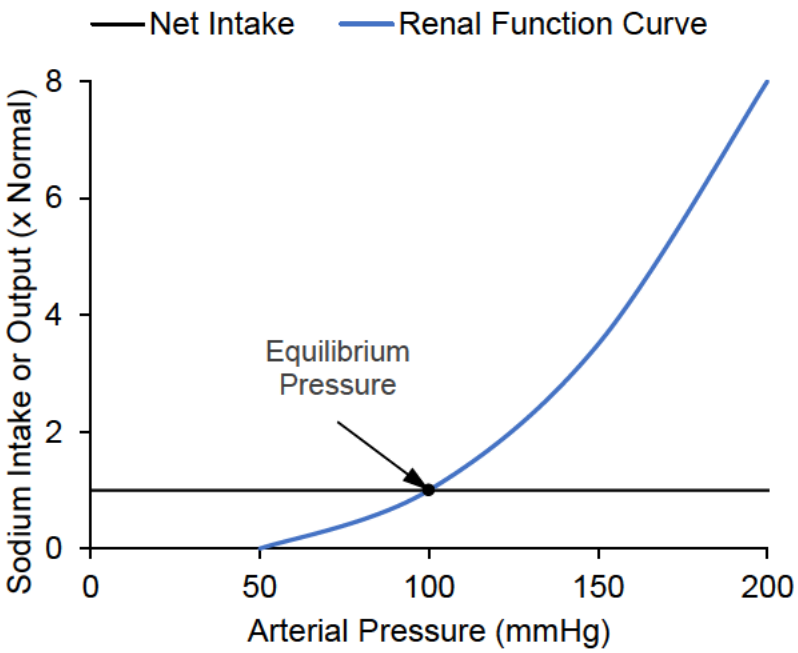



**Figure 1.7:** Renal function curve showing arterial pressure regulation by the kidney-fluid volume pressure control system. In a steady state, sodium and water intake (Net Intake) matches output (Renal Function Curve). Equilibrium pressure is where these two curves intersect and is the unique level at which arterial pressure will be regulated. A rise in arterial pressure causes increase sodium and water excretion to bring arterial pressure back down to equilibrium level. A drop in arterial pressure causes opposite changes in sodium and water excretion to restore equilibrium pressure.

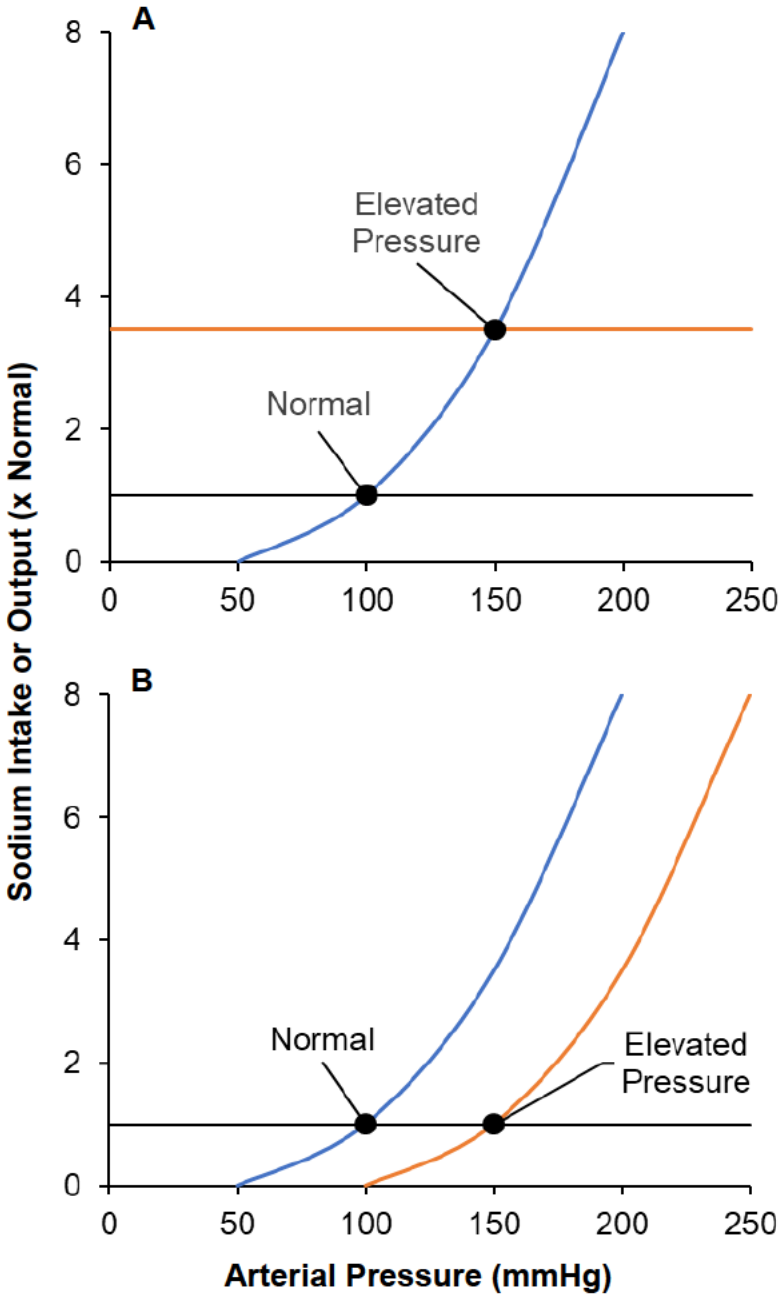



**Figure 1.8:** Two ways in which arterial pressure can be increased. If net sodium (salt) and water intake is increased to a higher level (A) or by shifting the renal function to the right (B).

#### 1.4.4.3 Pressure Natriuresis in Hypertension

Unlike in the monogenic forms of hypertension, only a portion of the adults in the general population develop hypertension by the age of 55 years despite most adults having eaten a salt rich diet since childhood. This observation alongside results of animal studies (121) point to a variable degree of BP sensitivity to salt. As explained above, patients with hypertension have a reset (or right-shift) of their pressure natriuresis curve to maintain normal balance but at the expense of higher arterial pressures. A varying sensitivity to salt means that salt-resistant hypertension is characterised by a parallel shift of the pressure-natriuresis curve whereas in salt-sensitive hypertension the shift is also accompanied by a change in the slope (**Figure 1.9**).

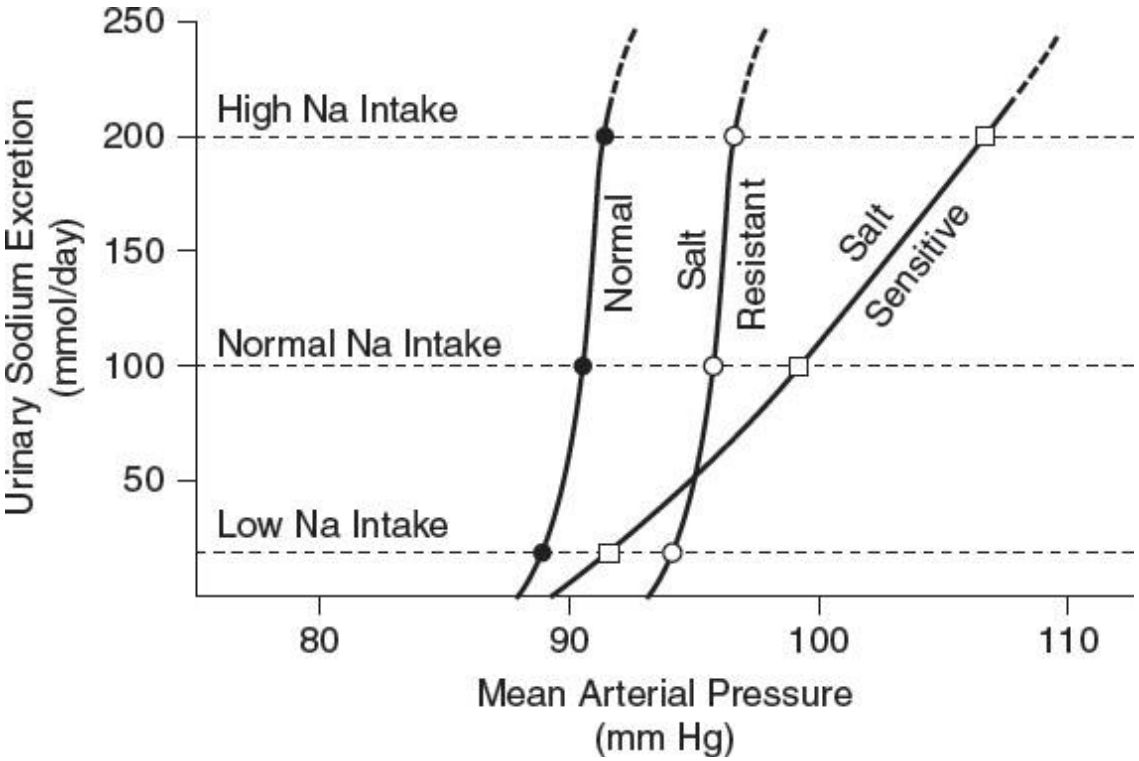



**Figure 1.9:** Schematic renal function curves in essential hypertension according to varying sensitivity to salt. In the normal individual, the slope of the curve is very steep so that even a high salt intake will not raise the arterial pressure by much. In salt-resistant hypertension the renal function curve is shifted to the right in parallel to the normal curve. The effect of this shift though raises the arterial pressure, further increases due to high salt intake are smaller in magnitude due to preserved steep slope of the renal function curve. In contrast, in salt-sensitive hypertension the right shift of the curve is accompanied by a flattening of the slope which means that not only the arterial pressure is set a higher level, it is more sensitive to changes in salt intake – a high salt intake will cause a large increases in arterial pressure and conversely, unlike salt-resistant hypertension, reducing the salt intake will significantly lower the arterial pressure. Image from (145).

#### 1.4.4.4 Causes of impaired natriuresis

There are numerous pathophysiological mechanisms which can cause impaired natriuresis (146), some of which are listed below:

- Hyperinsulinaemia is present in metabolic syndrome and can reduce urinary excretion of sodium (147).

- Acquired tubulointerstitial disease has also been implicated in the pathogenesis of salt-sensitive hypertension (148). According to this theory, salt-sensitive hypertension occurs in two phases. In the first phase, episodic BP elevations are caused by hyperactivity of sympathetic nervous system and RAAS and a second phase characterised by persistently raised BP. The transition to second phase occurs as a consequence of catecholamine-induced elevations in BP that preferentially damage juxtamedullary and medullary regions of the kidney that do not autoregulate well to sudden changes in renal perfusion (148). Tubulointerstitial injury occurs when the episodic pressor response causes an increase in peritubular capillary pressure and a reduction of peritubular capillary blood flow resulting in ischaemia of tubules and interstitium. This local injury triggers stimulation of vasoconstrictors (Ang II, adenosine or renal sympathetic nerves) and inhibition of vasodilators (NO, dopamine or prostaglandins) which further augment local ischaemia resulting in abnormal tubuloglomerular feedback and enhanced sodium reabsorption. Peritubular capillary rarefaction increases renovascular resistance further blunting pressure natriuresis. The combination of the impaired pressure

natriuresis and enhanced tubuloglomerular feedback results in reduction in NaCl excretion.

- Reduced nephron mass has been postulated that a congenitally acquired deficit of effective nephron mass may be responsible for impaired natriuresis (149). Congenital oligonephropathy or reduced number of nephrons, as a result of intrauterine growth has been shown to be associated with hypertension (150). Other studies including a metanalysis have confirmed these findings (151-155) suggesting an average of 260,000 fewer nephrons with each kilogram decrease in birth weight.

- Increased efferent SNA activity and its effects on the kidneys have been described above.

- Abnormal regulation of the RAAS has been implicated in impaired natriuresis and the pathogenesis of salt-sensitive hypertension (156). Under normal conditions, a salt load should suppress the RAAS to allow increased excretion to maintain haemostasis. The mechanisms by which this occurs have been described in detail in the sections to follow. In patients with salt-sensitive hypertension, however, either inappropriately high or normal plasma renin activity has been demonstrated and the term “non-modulators” have been used to described them (157, 158). These abnormalities of the RAAS cause impaired renal sodium handling.

- Impairment in the production of NO has important consequences including medullary ischaemia and impaired natriuresis, the mechanisms for which have been described later.

- Endogenous dopamine is synthesised in the proximal tubule and the subsequently causes vasodilatation and inhibits sodium reabsorption, thereby opposing effects of Ang II. A defect in this mechanism has been shown to result in impaired natriuresis (159).

- Dysfunction of natriuretic peptides may also cause impaired natriuresis (160).

#### 1.4.4.5 Volume dependant mechanisms

In the presence of impaired natriuretic capacity, salt loading will result in expansion of blood and extracellular fluid (ECF) volume caused by increased sodium and water retention by the kidneys. This increase in ECF volume is further augmented for two reasons. First excess salt increases osmolality which stimulates osmoreceptors in the brain making the person feel thirsty and drink more water. Second, the same osmoreceptor stimulation activates neural pathways causing the release of antidiuretic hormone (ADH) from the hypothalamus which in turn acts on the renal collecting ducts to reabsorb water and thereby increases the ECF volume. The resultant increases in the blood volume and cardiac preload increase the cardiac output via the Frank-Starling mechanism, raising the arterial pressure. However, in chronic essential hypertension, ECF excess or increased cardiac output is difficult to demonstrate. Instead, a secondary increase in total peripheral resistance is thought to maintain a high BP in chronic essential hypertension. Experiments by Guyton and colleagues have demonstrated these changes (161) which have been summarised in **Figure 1.10**.

Two theories – endogenous ouabain-like substances and autoregulation – explain how the rise in total peripheral resistance occurs.

The ECF volume expansion has been proposed to release at least two ‘natriuretic hormones’; atrial natriuretic peptide (ANP) and another hormone from the hypothalamus. The second hormone, an endogenous ouabain-like substance can partially inhibit Na/K adenosine triphosphatase (ATPase). Actions of ANP and endogenous ouabain-like substance increase sodium and water excretion to restore the sodium balance. Inhibition of the Na/K ATPase by endogenous ouabain-like substance can also cause net rise in intracellular calcium concentration via the compensatory increased activity of sodium calcium exchanger. An increased intracellular calcium in vascular smooth muscle cells could cause vasoconstriction and, consequently, an increase in total peripheral resistance (162, 163).

Alternatively, Guyton hypothesis, postulates that long term arterial pressure rise is maintained by a process of “autoregulation of blood flow” (164). This is where an acute rise in arterial pressure and the accompanying increased perfusion of tissues, delivers too much oxygen and other nutrients which causes automatic vasoconstriction of the local blood vessels and normalises the blood flow (165, 166). The autoregulatory vasoconstriction of systemic circulation increases the total peripheral resistance. The Guyton hypothesis is summarised in **Figure 1.11**.

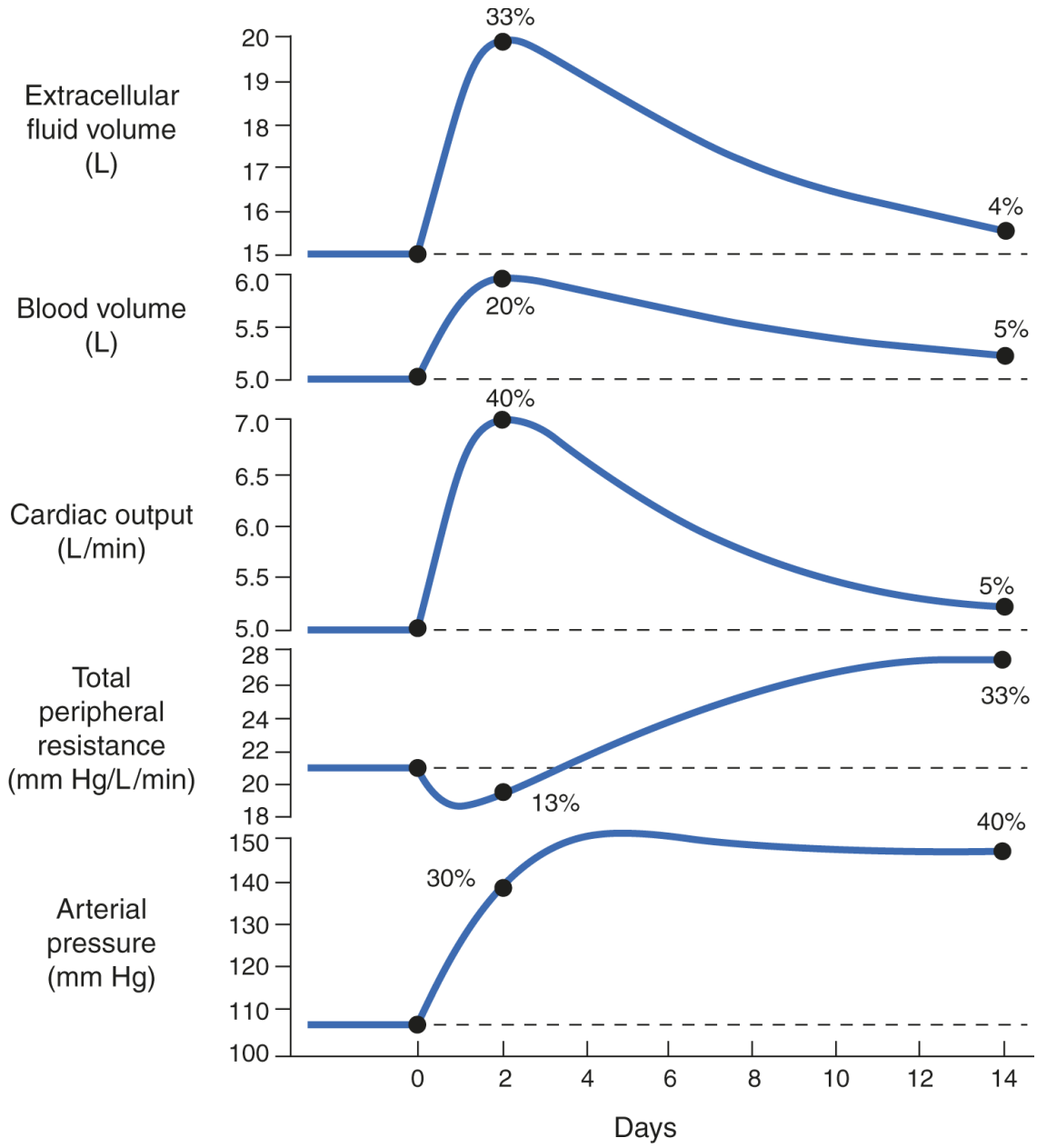



**Figure 1.10:** Sequential changes in cardiovascular haemodynamic parameters that occur over the first few weeks following short-term salt-loading. The extracellular fluid volume, blood volume and cardiac output all increases in the first few days. These changes increase the arterial pressure. Total peripheral resistance initially decreases due to activity of arterial baroreceptor reflex to try and lower the arterial pressure. Total peripheral resistance progressively increases once the baroreflex “resets” to a higher arterial pressure and explains the relatively slow rise in arterial pressure in the first few days. Although extracellular fluid volume, blood volume and cardiac output slowly return to normal, total peripheral resistance remains persistently elevated driving the high arterial pressure (161). Image from (167).

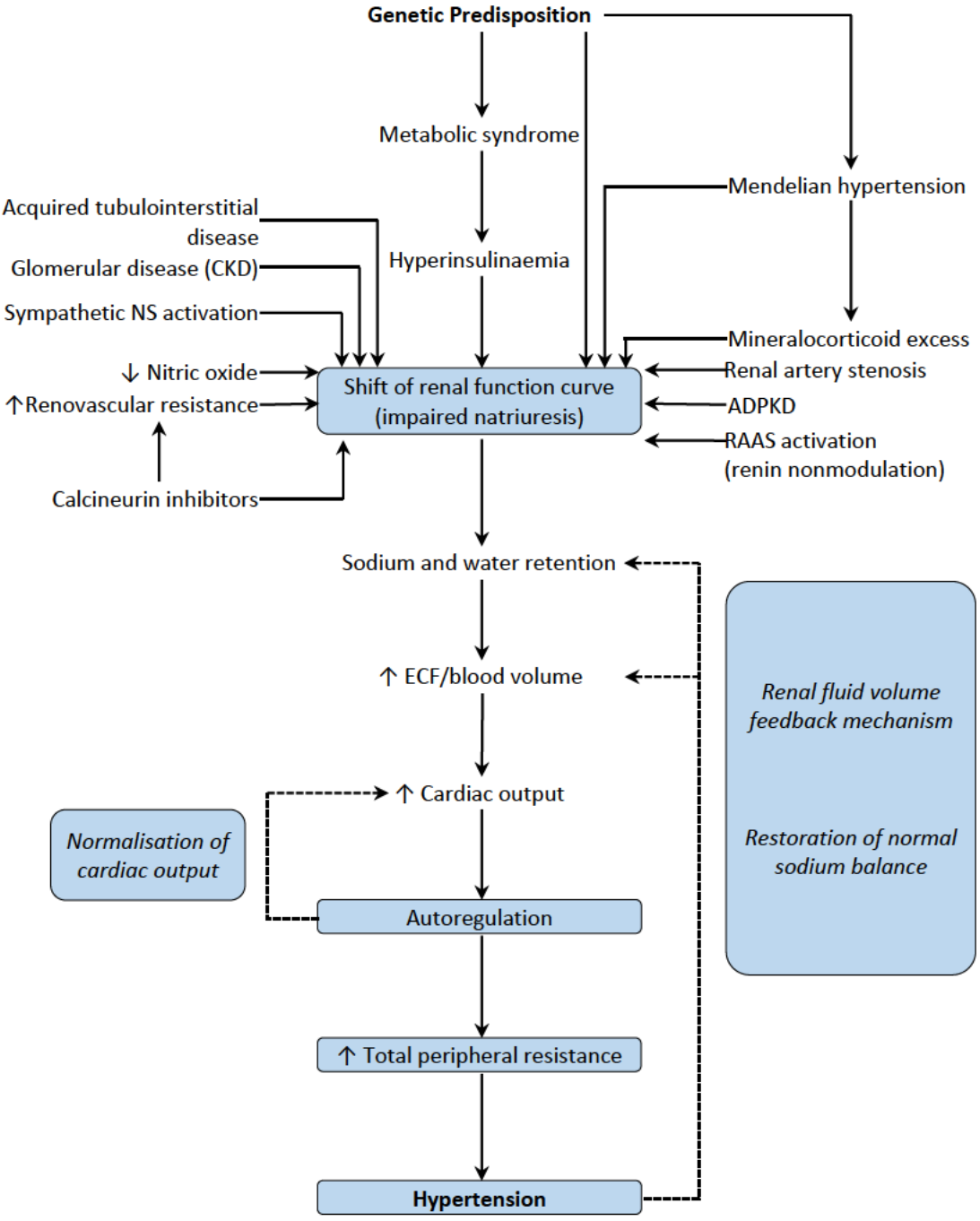



**Figure 1.11:** The Guyton hypothesis. A defect in the renal sodium handling or impaired natriuresis is the fundamental prerequisite for the development of all causes of hypertension which shifts the renal function curve to the right. An initial sodium and water retention increase the extracellular fluid (ECF) volume which raises the blood pressure as a result of volume expansion and increased cardiac output. In the long-term, autoregulation of blood flow in the systemic circulation increases total peripheral resistance and maintains systemic hypertension. The renal fluid-volume feedback mechanism restores normal sodium and fluid balance and returns the ECF volume back to normal via pressure natriuresis, albeit at the expense of hypertension. Guyton hypothesis therefore explains the paradox of systemic hypertension, in the presence of impaired natriuresis with an

increased total peripheral resistance and without a detectable rise in the ECF volume. Impaired natriuresis itself is a consequence of different genetic and pathophysiological factors highlighted in the schematic.

### 1.4.5 Hormonal mechanisms: the renin-angiotensin-aldosterone system

The RAAS is well known to play a central role in BP regulation through its action on sodium and water excretion, vascular inflammation and remodelling, and endothelial cell dysfunction. Circulating renin, an enzyme produced by the juxtaglomerular cells of the kidneys, converts hepatic-derived angiotensinogen to Ang I which is then cleaved by ACE to form Ang II. Majority of ACE is produced by the lung but is also present on the surface of vascular endothelial cells of other organs including the heart and the kidney.

In the sections that follow actions of key components of the RAAS and their role in hypertension are described.

#### 1.4.5.1 Regulation of renin secretion

The main stimulators of renin secretion include decreased renal perfusion pressure, decreased sodium chloride delivery to the macula densa, and β-adrenergic stimulation. Whereas opposite changes in renal perfusion pressure and sodium delivery to the macula densa inhibit renin secretion. Furthermore, Ang II affects renin release through a negative feedback loop independent of volume or tubular transport processes. Renal perfusion pressure is detected by the renal baroreceptors in the afferent arterioles and specialised tubular cells in the thick ascending limb of loop of Henle, an area named macula densa, detect the sodium chloride content of the filtrate. Other renin-stimulating factors include prostaglandin $E_2$, prostacyclin, dopamine, glucagon and NO. Whereas ANP, endothelin (ET), ADH and adenosine block renin secretion.

#### 1.4.5.2 Angiotensin II

Ang II is the principal effector of the RAAS and mediates its physiological actions through Ang II receptors present throughout the body. Although there are multiple subtypes of Ang II receptors, almost all of Ang II actions are mediated via the Ang II type I receptors ($AT_1$).

In the cardiovascular system, Ang II is a potent direct vasoconstrictor leading to increase in total peripheral resistance. Its action on the sympathetic nervous system increases SNA which further enhances the vasoconstrictor effects of Ang II through the release of catecholamines. It stimulates aldosterone secretion from the adrenal medulla which regulates the activity of epithelial sodium channel in the distal nephron. Aldosterone enhances sodium reabsorption and promotes potassium secretion and hence regulates ECF volume.

In the kidney it controls the GFR through regulation of vascular tone of renal arterioles. Its vasoconstrictor effects are more pronounced in the efferent arteriole, which decreases the renal blood flow and raises the hydrostatic filtration pressure in the glomerular capillaries, preserving the GFR in the face of reduced BP (168). Ang II increases the activity of $Na^+/H^+$ exchanger in the apical surface of proximal tubular epithelial cells to increase sodium reabsorption (169). Furthermore, Ang II stimulates $Na^+/K^+$ ATPase (170, 171) and $Na^+/HCO_3^-$ cotransporter (172) on the basolateral surface again enhancing sodium reabsorption.

Ang II has been shown to play a role in vascular smooth muscle cell hypertrophy, migration, differentiation, calcification, extracellular matrix proteins and inflammation, which underlie hypertension and cardiovascular disease (173). Ang

II causes endothelial dysfunction mediated through impaired synthesis of NO, a potent vasodilator. It also activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase leading to generation of reactive oxygen species (ROS). Together these changes promote atherosclerosis (173).

#### 1.4.5.3 Local renin-angiotensin-aldosterone systems

In the “traditional” RAAS, circulating Ang II is the principal effector, however emerging evidence has identified new RAAS members and a functionally-active tissue-based RAAS has been characterised (174, 175). Local RAASs have been described in numerous organ systems including the heart and the kidney (176, 177).

Intrarenal RAAS plays an important role in a process called “tubulovascular crosstalk” (178). Renal Ang II levels have been shown to be several times higher than circulating levels (179) and causes vasoconstriction. However medullary circulation has been shown to be resistant to vasoconstrictor effects of Ang II (180). Dickhout et al. have demonstrated Ang II increases [$Ca^{2+}$] in pericytes of the descending vasa recta and the tubular epithelial cells of the medullary thick ascending limb. As result, tubular epithelial cells produce NO which diffuses across into the pericytes surrounding the descending vasa recta and buffer the constricting effects of Ang II thereby setting up the “tubulovascular crosstalk”. The buffering actions of NO and other vasodilators (cyclooxygenase and prostaglandins) counteract the constricting effects of Ang II and other vasoconstrictors including ROS and therefore regulate the medullary blood flow and oxygen supply to the medulla (181).

### 1.4.6 Vascular mechanisms

Blood vessels form an integral part of the cardiovascular system and changes in their structure and function leads to hypertension. BP is a product of cardiac output and total peripheral resistance; changes in the vascular tone determine total peripheral resistance and hence BP. Calcium influx into cytosol of the vascular smooth muscle is the final common pathway leading to the vasoconstriction. Endothelial dysfunction, vascular remodelling and inflammation are hallmarks of vessel dysfunction present in hypertension. Numerus molecular pathways are involved underlying the vascular changes that characterise hypertension and are described below.

#### 1.4.6.1 Endothelial dysfunction and hypertension

Association between hypertension and endothelial dysfunction is well established (182-185); a phenotypical alteration of the vascular endothelium that precedes the development of adverse cardiovascular events and its presence predicts future events (186). Data from the Framingham Heart study suggest that the severity of hypertension is positively associated with the degree of impairment of endothelial function (187).

Endothelial dysfunction is characterised by reduction in bioavailability of endothelium-derived NO, a change that precedes any structural changes associated with atherosclerosis (188). Endothelial dysfunction can also be indicated by elevated serum concentrations of endothelial adhesion molecules, including intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 [VCAM-1] (189). These molecules accelerate atherosclerosis by promoting migration of inflammatory cells through the

endothelium. Furthermore, vascular endothelial growth factor and its soluble receptor fms-like tyrosine kinase-1 (sFLT-1) are also associated with endothelial dysfunction, vascular remodelling, and endothelial repair and regeneration mechanisms (190-192).

#### 1.4.6.2 Nitric oxide pathway

First identified in 1980 as an endothelium-dependant relaxing factor, NO is a highly reactive free radical. The endothelium of all blood vessels express the enzyme NO synthase (NOS) which uses the NADPH as an electron source to catalyse the conversion of L-arginine and oxygen to citrulline and NO. The gaseous NO diffuses to the vascular smooth muscle cells to activate soluble guanylate cyclase which in turn activates the cyclic guanosine monophosphate (cGMP) cascade resulting in a decrease of intracellular [$Ca^{2+}$] culminating in relaxation of smooth muscle cells and vasodilation. Furthermore, it inhibits platelet adhesion and aggregation and excretion of anti-inflammatory, antiproliferative, and antimigratory effects on leukocytes, endothelial cells, and vascular smooth muscle cells, thus providing protection from atherosclerosis (193). The endothelial NOS is activated by pharmacological and mechanical stimuli; acetylcholine or bradykinin bind to their respective receptors to stimulate NOS whereas the sheer stress of blood flow in the endothelium also stimulates NOS therefore protecting the endothelium from the shearing effects of hypertension. Endogenous inhibitors of NOS have been identified such as asymmetric dimethyl arginine (ADMA) and may also enhance endothelial dysfunction.

#### 1.4.6.3 Reactive oxygen species pathway

Generation of ROS such as oxygen superoxide ($O_2^-$) and hydrogen peroxide ($H_2O_2$) attenuate the protective effects of NO and is one of the principal mechanisms responsible for endothelial dysfunction. The ROS scavenge NO, thereby reduce its bioavailability. The overproduction of $O_2^-$ and $H_2O_2$ can activate mechanisms which can cause cell growth, fibrosis, inflammation and eventually vascular remodelling. The term oxidative stress is used to described chronic elevation of ROS and is associated with hypertension, atherosclerosis and diabetes.

Vascular ROS in particular $O_2^-$ is generated mainly by NADPH oxidase, a multi-subunit enzyme which has several isozymes (173). All vascular NADPH oxidases are activated by the circulating Ang II. Although NOS normally generates NO, ROS are also generated when either L-arginine or one of the cofactors (tetrahydrobiopterin or $BH_4$) required by NOS are deficient, process termed “NOS uncoupling”; oxygen is used as a substrate instead to produce $O_2^-$ (194). Additionally, ROS generated by NADPH oxidases further reduces the availability of $BH_4$ augmenting NOS uncoupling. Xanthine oxidase pathways and Ang II mediated mitochondrial dysfunction are other mechanisms of ROS generation.

The cumulative effects of activation of redox-sensitive cellular responses include migration, contraction, inflammation, extracellular matrix modulation, cell growth and apoptosis, which, if left uncontrolled, contribute to hypertensive vascular damage (195).

#### 1.4.6.4 Endothelin pathway

The first ET was discovered in the 1980s an endothelial-derived vasoconstrictor (196, 197). Several ETs have been identified with ET-1 being the most abundant form. ET-1, a 21-amino acid peptide, is produced through conversion of big ET-1 by endothelin converting enzyme (ECE) which has multiple isoforms. ET-1 acts by stimulating endothelin type A and type B ($ET_A$ and $ET_B$) receptors which are found on the vascular smooth muscle cells. $ET_A$ receptors are predominantly found in arteries whereas more $ET_B$ receptors (but still not as many as $ET_A$) are found in veins and pulmonary vasculature. Their primary action is to cause smooth muscle contraction and growth resulting in vasoconstriction, however, stimulation of $ET_B$ receptors found on the vascular endothelial cells cause the opposite effects; smooth muscle relaxation and growth inhibition. ET-1 production is regulated by different factors; NO may inhibit, whereas Ang II, ROS, adrenaline and leptin are some of the many factors that stimulate. ET-1 also contributes to oxidative stress through stimulating superoxide generation (198, 199).

In hypertension, ET-1 has been shown to cause vascular hypertrophy (200, 201). In kidneys, renal medulla contains high levels of $ET_B$ receptor and the ET-1 precursor, big ET-1, binding to the $ET_B$ vasodilates the medullary circulation (202). $ET_B$ receptors on the tubular epithelium inhibit reabsorption of sodium and thick ascending limb and collecting duct (203-205). Both of these mechanisms contribute to natriuretic effects of ET-1 and $ET_B$ receptor (206-208).

Increased production of ET-1 in the endothelium and the kidney, the increased oxidative stress, vascular remodelling and the reversal of these effects by

endothelin antagonists have shown their potentially significant role in the development of hypertension (209).

#### 1.4.6.5 Arterial stiffness

The hardening of human arteries with age and disease is well known. Increased central arterial stiffness is associated with adverse cardiovascular outcomes and is a strong predictor of future CV events and all-cause mortality (210). Changes in arterial structure can be quantified in terms of vessel stiffness, which is the pressure required to provide a unit change in volume (211).

Intermittent ventricular contraction causes large changes in BP and flow within the arteries. The peak BPs can damage the peripheral microvasculature due to barotrauma. Arteries provide a dampening function so that a steady continuous blood flow is maintained to peripheral tissues throughout systole and diastole. Arterial walls, in particular the aorta, contain predominantly elastin fibres which allow significant dilation during systole. During diastole, the arteries recoil to drive the blood along the arterial tree and augment coronary artery blood flow. The pulse wave from the arteries reflected back to the heart is called pulse wave reflection, which resists the forward travelling blood. As the aorta stiffens it is less able to accommodate the blood volume from the heart which raises the systolic pressure and left ventricular afterload. A persistently raised afterload exposes the heart to high systolic pressures which increase myocardial work consequently result in LVH. Arterial stiffness is associated with LVH in normotensive and hypertensive patients (212, 213). Reduced elastic recoil leads to a decrease in diastolic BP reducing coronary perfusion which alongside the ventricular hypertrophy results in subendocardial ischaemia (214, 215). Furthermore, LVH

attenuates the backward travelling suction wave within the coronaries that drives the majority of coronary flow (216).

Numerous studies have now established aortic stiffness as an independent predictor of cardiovascular events. Pulse wave velocity (PWV) is a measure of arterial stiffness and signifies the velocity at which the BP pulse wave propagates down a blood vessel. In a cross-sectional study, aortic PWV was shown to be associated with cardiovascular risk, as calculated from the Framingham equations (217). Furthermore, aortic stiffness is significantly associated with the risk of all-cause and cardiovascular mortality in patients with essential hypertension (218).

#### 1.4.6.6 Inflammation in hypertension

Various inflammatory mechanisms have been described in relation to hypertension (219). The acute phase protein, C-reactive protein (CRP) is produced by the liver in response to a wide range of acute or chronic inflammatory disease processes and is involved in complement activation and promoting phagocytosis. CRP is considered as the inflammatory marker with the strongest association in hypertension (219). Increased plasma CRP levels have been demonstrated in many clinical trials of hypertension (220-225). CRP further promotes inflammation through stimulating release of proinflammatory cytokines such as interleukin-6, interleukin-1 beta, and tumour necrosis factor alpha (TNF-$\alpha$) from monocytes (226) and also ICAM-1 and VCAM-1 from vascular endothelial cells (227).

Inflammation is thought to play an important role in endothelial dysfunction observed in hypertension (219). Inflammation can alter the careful balance of

synthesis and degradation of molecules involved in the regulation of vascular tone, in particular NO and its precursor NO synthase. Both CRP and TNF-$\alpha$ down-regulate NO synthase messenger ribonucleic acid (mRNA) activity leading to reduced NO levels, with a consequent increase in peripheral resistance mediated through impaired vascular relaxation and increased vascular stiffness (219).

Furthermore, oxidative stress, triggered by chronic inflammation, has been shown to be associated with hypertension and is a major cause of endothelial dysfunction which is once again mediated through bioavailability of the NO (219). Hyperaldosteronism and ageing are also known to be associated with hypertension and there is evidence to suggest their role in chronic inflammation.

Harrison et al. have proposed a paradigm for inflammation and immune cell activation in hypertension (228). They suggested that SNA originating from the central nervous system as a result of Ang II, chronic stress or salt intake initiates hypertensive process while T-cell activation in kidney and blood vessels completes the conversion of prehypertension to hypertension.

Immunoglobulins have also been found to play a role in pathogenesis of hypertension. Elevated immunoglobulin levels have been found in patients with essential hypertension when compared to normotensives (229-232). Although it is unclear whether the rise in immunoglobulins is primary or secondary, it is thought to be a consequence of vascular damage from high BP (233). More recently, serum immunoglobulin light chains, which are independent of the intact immunoglobulin molecule and are referred to as free light chains (FLC), have been associated with chronic inflammation (234). Studies have now been able to

attribute evidence of an independent association between an elevated serum FLC level and a higher mortality risk (235, 236). Furthermore, an increased serum FLC level is an independent risk factor for mortality and progression to ESRD in patients with stages 3-5 CKD (237) and also for prognosis in patients with acute heart failure secondary to atherosclerotic coronary heart disease (238). However, an association with serum FLC and hypertension has not yet been studied and given the earlier association with intact immunoglobulin levels it can be hypothesised that serum FLC may be linked with hypertension.

### 1.4.7 Obesity

The Health Survey for England has shown that 28.7% of Adults (aged 16 year or over) in England in 2017 were obese which has risen from 15% in 1993 (239). National Health Service hospital episode statistics in England has shown that in the financial year 2017/18 there were 1323 hospital admissions per 100,000 population which has more than doubled from 530 per 100,000 in 2011/12 (5). A linear relationship has been shown to exist between body mass index (BMI) and BP, and excess weight is responsible for approximately 65% to 75% of the risk for hypertension (240, 241). Haemodynamic consequences of obesity include an increase in extracellular volume and an increased blood flow which in turn increases venous return and cardiac output (242). Multiple mechanisms are involved in obesity-mediated hypertension. Increased renal sodium reabsorption and impaired pressure natriuresis are key factors responsible for a rise in BP. Compression of the kidneys by increased visceral and retroperitoneal fat, activation of RAAS and increased SNA are all implicated in the impaired natriuresis associated with weight gain (243).

Excess visceral, retroperitoneal and renal sinus fat increase intrabdominal pressure which in turn, increases the renal interstitial fluid pressure inhibiting tubular sodium reabsorption (242). The renal compression initiates renal haemodynamic mechanisms which initially result in glomerular hyperfiltration and over time reduced GFR and CKD. This pattern of fat distribution further impairs renal function through inflammation and expansion of medullary extracellular matrix. The fatty tissues additionally may have lipotoxic effects on the kidney (242).

In obesity hypertension all components of the RAAS are increased despite sodium retention and hypertension which should normally suppress Ang II formation. Renal compression and increased renal SNA contribute to the renin secretion. The role of Ang II in promoting sodium reabsorption in obesity hypertension is highlighted through experimental (244-246) and small clinical trials (247-249) that have demonstrated that angiotensin II receptor blockers (ARBs) and ACE inhibitors diminish sodium reabsorption, volume expansion and increased BP. Plasma aldosterone levels have been shown to correlate BMI and obesity hypertension (250, 251). Mineralocorticoid receptor antagonism has been shown to reduce sodium retention, high BP and glomerular hyperfiltration in dogs (252). Several mechanisms have been postulated to contribute to mineralocorticoid receptor activation, however the importance these mechanisms, as yet, remains unclear (242).

Autonomic nervous system plays a key role in development of obesity hypertension. Several lines of investigations point to an increased SNA in obesity (242): there is regional heterogeneity of SNA (253); BP-lowering effects of

pharmacological therapies aimed at either blockade of adrenergic activity (α/β-adrenergic blockers) or clonidine (a centrally-acting α-2 agonist) are greater in the obese animals and adrenergic blockade lowers ambulatory BP significantly more in human obese patients compared to lean patients (254-256) and; RDN markedly attenuates sodium retention and hypertension in obese animals and humans with treatment-resistant hypertension [TRH] (257-259).

In obesity there is not a global increase in SNA, instead the sympathetic nervous system differentially activated in different tissues of the body. For example, in dogs the increased HR, decreased HR variability and high BP observed in obesity is attributable to a decrease in the parasympathetic activity of the heart (253, 260). In humans, sympathetic outflow from the heart is reduced with noradrenaline spillover 40% - 50% less in obese individuals compared to lean individuals (89, 261). In contrast, the increased SNA is observed in the kidney and skeletal muscle (253).

Multiple mediators of increased SNA have been suggested and include:

- Impaired baroreflex activity
- Chemoreceptor activation due to intermittent hypoxia in sleep apnoea
- Hyperinsulinaemia
- Ang II
- Cytokines produced by adipocytes such as leptin, interleukin-6 and tumour necrosis factor α
- Central nervous system proopiomelanocortin pathway

### 1.4.8 Genetic and environmental factors

Hypertension is a complex trait and is a result of interaction between genetic predisposition and environmental factors. Family history has long been known to be associated with risk development of hypertension (262, 263). Genetic influences on BP have been demonstrated by family studies (262, 264-266). Parental history of hypertension is associated with high lifetime risk of incident hypertension (267). Variation in BP attributable to all genetic factors varies from 25% in pedigree studies to 65% in twin studies (268, 269). Except for the rare monogenic causes of hypertension (139) and despite decades of research little is known about specific genetic factors responsible for essential hypertension.

Advances in medicine and technology have allowed genome sequencing which have helped to develop targeted treatments at genetic defects identified, especially in the case of many cancers. Such genome wide association studies have been carried out for chronic illnesses including hypertension (270-274). Although these studies have identified multiple genetic loci associated with BP, surprisingly they only account for around 1% of the variation (271, 272). This incongruity between the estimates of heritability of BP from family studies of around 30-40% (275) and the genome wide association studies is termed “missing heritability” and is not unique to hypertension (276). When considering the complexity of the plethora of mechanisms implicated in the development of hypertension, the lack of a predominant single genetic locus does not seem so surprising. It has been hypothesised that epigenetics may be responsible for the missing heritability (275).

Genetic predisposition alone is not sufficient for development and persistence of hypertension, a bidirectional interaction between genetic factors and environmental exposures including lifestyle factors is necessary. Unlike genetic factors, more is known about the association of environmental and lifestyle factors with hypertension. A range of environmental exposures have been shown to increase BP. The role of salt and obesity has already been discussed in detail and the association of hypertension with other major environmental exposures is briefly described below.

- Cigarette smoking is an established major risk factor for coronary artery disease and raises BP through increased SNA (277, 278). It also contributes to endothelial dysfunction through impaired NO-mediated vasodilatation (279).

- Caffeine has been long known to cause an acute rise in BP which is mediated through antagonism of vasodilatory adenosine receptors (280) and increased SNA (281). A metanalysis of studies examining the effect of coffee, a common source of ingested caffeine, in hypertensive individuals concluded that beyond the acute BP rise lasting ≥3 hours, no association between longer-term coffee consumption and increased BP (282). However, caffeine consumed in soft drinks has been shown to be associated with an increased risk of hypertension (283).

- Alcohol consumption have been shown to elevate BP and associated with higher prevalence of hypertension (284-286). Alcohol ingestion is closely followed by an initial vasodilation-mediated drop in BP followed by a subsequent rise (287-289). There are several possible mechanisms

through which alcohol can raise BP (290); increased SNA is the principal mechanism for the rise in BP (291). Systematic reviews of alcohol intake have shown small but clinically significant rise in BP (292) and a reciprocal drop in BP with reduction in the alcohol intake (293). Epidemiological studies have suggested a possible beneficial effect of modest alcohol intake especially with red wine consumption. However a RCT comparing abstinence, alcohol-free red wine, red wine and beer intake showed that two types of alcohol were associated with similar level of BP rise, and the red wine with or without alcohol content was not associated with improvement in endothelial function that had been hypothesised to improve BP (294).

- Cold weather climates (19) and higher altitude (295, 296) have both been shown to increase BP via increased SNA.

- Low vitamin D in cohort studies has been shown to be associated with an increased risk of incident hypertension (297-299). However, metanalyses of several RCTs have concluded that vitamin D supplementation is ineffective in lowering BP (300, 301).

- Potassium is a major intracellular cation in the body and is required for vascular smooth muscle relaxation thereby lowering BP (302). Unlike sodium intake, modern day dietary potassium ingestion has declined compared to our prehistoric ancestors. Potassium has been shown to have a negative relationship with BP (131). Metanalyses have shown that increased potassium intake reduces BP in people with hypertension and is associated reduced risk of stroke (303, 304).

## 1.5 Treatment-resistant hypertension

### 1.5.1 Definition

Treatment-resistant hypertension (TRH) is defined as an uncontrolled BP with a clinic SBP ≥ 140 mmHg and/or a diastolic BP (DBP) ≥ 90 mmHg despite the use of maximally tolerated doses of 3 or more antihypertensive medications of different classes including a diuretic (1, 305). This group of patients may be referred to as having uncontrolled TRH. The American Heart Association (AHA) guidelines also classes anyone with BP controlled by 4 or more antihypertensive medications as TRH and the term controlled TRH is used to describe these patients. Since the inception of this thesis, publication of the SPRINT trial has challenged the well-established target BP for diagnosis and treatment of hypertension (45). In light of its findings, the American College of Cardiology/AHA have updated their hypertension guidelines with a radical change in the BP targets used for diagnosis and treatment of hypertension; lowering the level of BP to 130/80 mmHg which also affects the definition of TRH (306). The American definition has retained the second element of the definition which includes patients with controlled BP taking 4 or more antihypertensive medications. This significant change will no doubt increase the prevalence of hypertension and, consequently, TRH. However, for the purpose of this thesis a BP cut-off of 140/90 mmHg is used to define both hypertension and TRH.

Suboptimal BP control is well known to be associated with an increased risk of cardiovascular and cerebrovascular events and is consequently ranked highest amongst the list of risk factors attributable to burden of disease worldwide (307). A linear relationship has been shown to exist between level of BP control and risk

of cardiovascular events (7, 308, 309). Unsurprisingly the risks known to be associated with hypertension are even greater in patients with TRH. Some estimates suggest that patients with TRH are twice as likely to experience a cardiovascular event as compared to patients without TRH (310).

### 1.5.2 Prevalence and incidence of treatment-resistant hypertension

The prevalence of true TRH is difficult to determine due to several different reasons. Prevalence of true TRH is affected by inadequacies in clinic BP measurements, presence of white-coat hypertension, non-adherence to antihypertensives, use of nonoptimal classes of antihypertensives, or non-adequate dosing. Inability to exclude the impact of these factors has led to the use of terms “apparent TRH” or “pseudo-resistance”. Therefore, the prevalence of true TRH will clearly be less than the reported prevalence estimates which are likely to represent apparent TRH.

The definition of uncontrolled TRH is widely used to estimate the prevalence of TRH with reported estimates ranging from 5% to 30% (311-316). Prevalence of TRH is higher when RCTs are used; a metanalysis by Achelrod et al. has reported the pooled estimate of prevalence of TRH at 13.72% (95% confidence interval [CI] = 11.19%–16.24%) for observational studies and 16.32% (95% CI = 10.68%–21.95%) for the RCTs (317). Hayek et al. have illustrated that prevalence of TRH can easily be manipulated depending on the stringency of the definition used (318). They showed that in the same population the prevalence dropped from 30.9% (SBP >140 mmHg and ≥3 medications or controlled on 4 medications) to 3.4% (ensuring at least 3 medications maximally dosed and one must be diuretic).

In a larger cohort of 468,877 hypertensive patients, 9.5% had uncontrolled TRH and only 4.7% were considered to be receiving optimal antihypertensive combination at their maximally tolerated doses (319).

Unlike prevalence, studies reporting the incidence of TRH are scarce. A retrospective cohort study of 205,750 incident patients with hypertension, 1.9% developed TRH within a median of 1.5 years from initial treatment (310)

### 1.5.3 Prognosis of treatment-resistant hypertension

The importance of accurately identifying patients with true TRH cannot be underestimated; there is growing evidence to suggest that these patients are at a much higher risk of cardiovascular events, CKD and death (310). Daugherty et al. have shown that, after adjustment, the hazard ratio for developing a cardiovascular event in this population is 1.47 [95% CI: 1.33 – 1.62 ] (310). Salles et al. found that over a five-year follow-up period, patients with true TRH had a 2.4-fold (95% CI, 1.0- to 5.8-fold) increased risk of having a fatal or nonfatal cardiovascular event compared with white-coat TRH patients (320). In a cohort of 60,327 patients with TRH, comprising patients with both uncontrolled and controlled TRH, from a single healthcare system the risks of TRH were compared to 410,059 patients with non-TRH (321); adjusted hazard ratios for ischaemic heart event, congestive heart failure, cerebrovascular event, ESRD and all-cause mortality were all significantly increased for patients with TRH (see **Figure 1.12**). Interestingly, these analyses clearly show that the hazard ratios were significantly higher across all except cerebrovascular event for any type of TRH (uncontrolled or controlled). The results from another observational cohort study of patients with hypertension show that apparent TRH was associated with an increased risk

for coronary heart disease and all-cause mortality but not stroke (322). Although patients with uncontrolled TRH had a more than a two-fold higher risk of coronary heart disease events, there was no corresponding increase in risk for patients with controlled TRH when compared to patients without TRH. The results from these studies confirm the increased morbidity and mortality associated with TRH. Furthermore, the results suggest that the controlled TRH may also be associated by with increased risks albeit to a lesser degree than uncontrolled TRH.

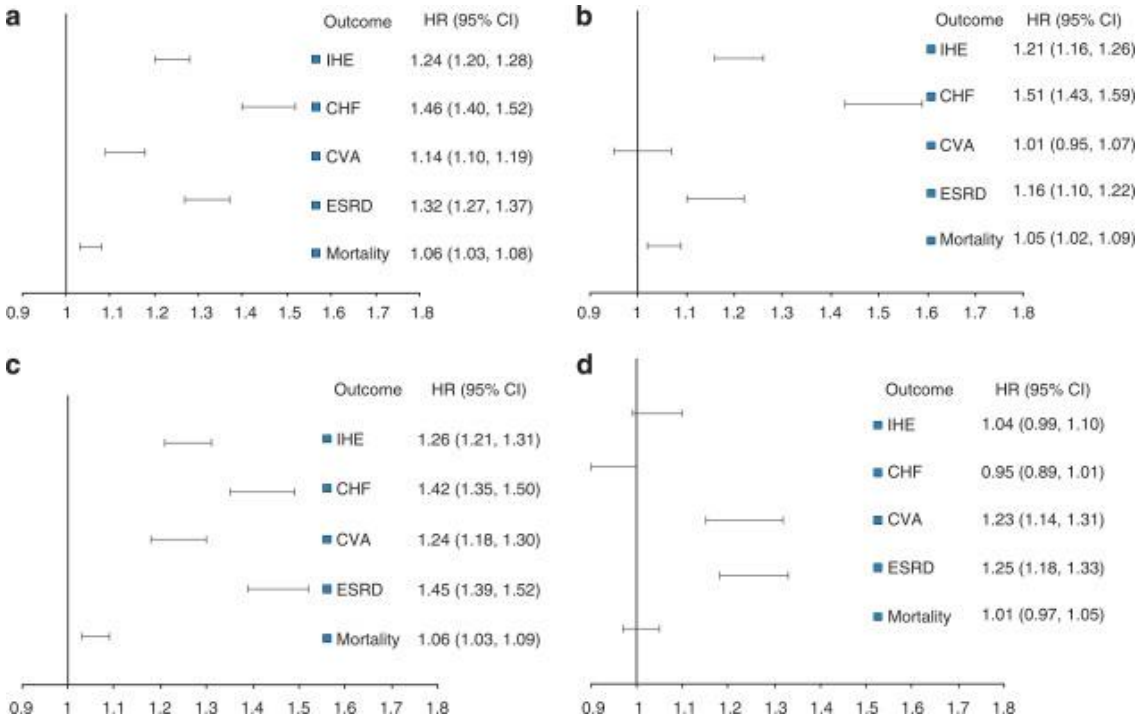



**Figure 1.12:** Risk comparisons for outcomes among hypertension categories. Multivariable adjusted hazard ratio (95% confidence interval) for ischemic heart event (IHE), congestive heart failure (CHF), cerebrovascular accident (CVA), end-stage renal disease (ESRD), and all-cause mortality in subjects with (a) RH (cRH+uRH) in comparison with those with non-RH, (b) cRH vs. non-RH, (c) uRH vs. non-RH, and (d) uRH vs. cRH. cRH, controlled resistant hypertension; non-RH, non-resistant hypertension; uRH, uncontrolled resistant hypertension. Figure from (321).

### 1.5.4 Causes of pseudo-resistance

The wide range of prevalence figures reflect the challenges in separating the true resistance from the pseudo-resistance. Non-adherence to antihypertensives, WCE, and inadequate BP measurement significantly contribute to presence of pseudo-resistance. BP measurement and WCE has been discussed in detail (see section 1.3); relevance of WCE and non-adherence in TRH is discussed below.

#### 1.5.4.1 White-coat effect in treatment-resistant hypertension

The ABPM has been the gold standard for diagnosis of hypertension as it helps to exclude patients who have white-coat hypertension. In treated hypertensive patients the term WCE is used instead. WCE is one of the main reasons for the widely variable reported prevalence rates of TRH. Clinic or office BP readings have been used in studies to estimate prevalence of TRH until recently. With the increasing use of ABPM in clinical practice, it is now clear that WCE is common in treated patients with hypertension (323). Furthermore, a positive correlation exists between clinic BP and the magnitude of WCE suggesting that patients with the highest clinic BP have the largest WCE (323, 324). It is therefore not surprising that a large proportion of patients with TRH have been found to have significant WCE (315, 320). De la Sierra et al. found that in a large Spanish registry of 68045 patients with hypertension, 8295 (12.2%) had TRH, of which 3113 (37.5%) had WCE such that their ABPM results classified them as having controlled BP instead (315).

### 1.5.5 Non-adherence and its significance in treatment-resistant hypertension

The treatment of most chronic illnesses is often characterised by long-term pharmacological interventions, which have been shown to be effective through series of rigorous clinical trials. These pharmacological interventions are only effective if patients follow medical advice on the prescribed treatment regimen. Suboptimal or non-adherence is common; on average around 50% of all prescribed medications for chronic conditions are not taken as prescribed (325, 326). Non-adherence has serious health economic implications on a societal level and a source of physical and emotional distress for patients.

Adherence to medications including placebo is associated with improved health benefits supporting the idea of “healthy adherer effect”, which is where patients’ adherence to their prescribed medication may be a reflection of their overall healthy behaviour. A meta-analysis has shown that adherence to prescribed beneficial medication, including a placebo, is associated with significantly lower mortality compared with suboptimal adherence (327).

Non-adherence puts an enormous cost burden on the health service through medication wastage. A report of a study commissioned by the Department of Health, UK in 2010 estimated the cost of National Health Service primary and community care prescription medicines wastage in England to be £300 million per year and that for antihypertensive medication to be at least £100 million a year (328).

In this section, medication non-adherence in chronic health conditions with particular reference to TRH have been reviewed.

#### 1.5.5.1 Medication adherence in clinical trials

Achievement of optimal adherence in an RCT is important. Level of adherence influences the magnitude of observed treatment effect; greater adherence increases the effect size and poor adherence may fail to distinguish the two treatments. Therefore, it is crucial in estimation of true treatment effect and consequently the power of the trial. Adherence also affects the incidence of adverse events; non-adherence to a treatment with worse adverse effects profile may falsely prove to be of similar safety when compared to treatment with a favourable adverse effects profile. Adherence is also an important indicator of how readily a treatment is accepted by the patients.

Despite its importance, adherence is under-reported in clinical trials; with only 33% to 46% of published RCTs reporting adherence rates (329, 330). On the other hand, reported rates of adherence are often remarkably high in these RCTs resulting in potential overestimation of adherence due to under-reporting (329-333). This may be related to extra attention received by the study patients, patient selection and the observer effect altering patient behaviour.

#### 1.5.5.2 Adherence vs compliance in relation to doctor-patient relationship

Adherence and compliance are both terms used, quite often interchangeably, in medical literature to describe patients' medication-taking behaviour. Adherence implies an agreement between the patient and the clinician on the proposed treatment plan and is reflected in the Oxford Dictionary's definition; *'believe in and follow the practices of*'. Compliance, conversely, is a passive act of following

a clinician's recommendations which is echoed in the Oxford Dictionary: *'the action or fact of complying with a wish or command'*.

The use of the two terms, adherence and compliance, closely tracks the evolution of doctor-patient relationship throughout the ages (334). Traditionally the role of the doctor has been that of someone who uses his/her knowledge, skills and expertise to choose the interventions he/she felt were in the best interest of the patient. In this paternalistic model of doctor-patient relationship the patient is merely a passive observer with little or no input in the decision-making process and is required to co-operate with the doctor's orders. Compliance can be seen to align more closely with this traditional model of medical practice.

The doctor-patient relationship has evolved into that of patient-centred approach where patients' ideas, concerns and expectations form the core of a clinical encounter. Patients take an active role and responsibility of their health and treatment. The doctor works in mutual partnership to form a therapeutic alliance with the patient creating an environment in which the patient feels comfortable to be involved in treatment decisions. The process of information sharing, communicating available treatment options and explanation of risks and benefits associated with these treatments aids both parties to take steps to build a plan about the preferred treatment and reach agreement on the treatment to implement. This change in the psychology of the doctor-patient relationship could be partly explained by the rise in prevalence in chronic illnesses and the advances in healthcare in managing these conditions which often require long-term therapy to reduce the risk of morbidity and mortality associated with them. The emphasis on shared decision-making has been driven by poor compliance

or adherence to long-term therapies, with major reasons being that patients do not share the doctors' view that a particular medication may be appropriate for them or are worried about side-effects or possible long-term harmful effects of the medications. It is, unsurprising, that adherence is now the more preferred term and the World Health Organisation citing the following definition (335):

> *'the extent to which a person's behaviour – taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider'*

#### 1.5.5.3 Threshold to define adherence in different chronic conditions

There is no consistency on how medication adherence is reported in the literature (329). Adherence can be reported qualitatively as patient being adherent or non-adherent which will depend on the method used to assess adherence. Medication adherence percentage is a common quantitative measure used which is defined as the percentage of number of pills taken during a certain period divided by the number of pills prescribed by a clinician during the same period (336):

$$Medication\ adherence\ percentage = \frac{number\ of\ pills\ taken\ during\ time\ X}{number\ of\ pills\ prescribed\ during\ time\ X} \times 100$$

There is no consensus on the defined threshold to define adherence and non-adherence. Traditionally a cut-off value of 80% has been used to dichotomise adherence. It has been repeatedly noted that healthcare usage and costs in many chronic conditions including hypertension, diabetes, hyperlipidaemia, schizophrenia, are reduced in patients where medication adherence exceeds 80% (337-341). Caution is advised when converting a continuous adherence

measure into a dichotomous or categorical measure unless it has been validated for use (342, 343). In many conditions a validated cut-off for adherence is either not present or a surrogate such as hospitalisation is used (341). There are a few examples where either drug- or condition-specific cut-offs are defined empirically. In hypertension, consumption of >80% of the prescribed medications has been shown to maintain BP control (344). In patients taking anti-retroviral for infection with Human Immunodeficiency Virus (HIV) 79% of patients who took at least 90% of their medication achieved virologic suppression, compared with approximately 50% of those whose medication adherence percentage was 50% to 89% (345). The success rate of Helicobacter pylori eradication therapy has been shown to be 96% for patients who took greater than 60% of the prescribed medications and 69% for patients who took less (346). These examples highlight that it is pertinent to avoid using a universal cut-off for adherence as clearly the threshold used should correlate with the desired therapeutic outcome for that condition. Conversely, it is also possible that in some circumstances consumption of less than the recommended amount of medication may be enough to achieve a desired therapeutic effect (338).

#### 1.5.5.4 Assessment of medication adherence

Numerous methods to assess patients' medication taking behaviour exist and they are broadly divided into direct and indirect methods (see **Table 1.1**). Direct methods include direct observation of medication being ingested by the patient, detection of a drug or its metabolite in the blood or urine, detection of a biologic marker that is included in the drug formulation, and more recently automatic electronic monitoring of drug ingestion by incorporating a specially designed microchip within each dose of a drug (347). The direct methods provide proof that

medication has been taken by the patient and are the most accurate measure of adherence. These methods have many drawbacks; they require expensive quantitative assays, are intrusive to the patient requiring collection of bodily fluids and provide dichotomous outcome of Yes/No without revealing pattern of adherence. These approaches assess point adherence and may not reflect long-term adherence and may lead to white-coat adherence (348). This is where patients' adherence improves leading up to a measurement or clinic visit. Also, differences in metabolism of drugs in individual patients may impact on the use of chemical markers to assess adherence.

Directly observed therapy is burdensome to the healthcare provider as patients often need to be inpatient and professionals are required to monitor the process. Furthermore, there is still room for patient to deceive by hiding the pills in the mouth until they can discard them afterwards. Due to these impracticalities, direct observation is more suitable where patients are on a single-dose therapy and can be observed in an outpatient setting or where patients are already hospitalised.

Indirect measures of adherence have been the most widely used methods in research. Patient-reported measures include patient interview where patients are asked about their experience of taking their medicines. Validated questionnaires can be used to eliminate some of the subjectivity of patient interviews. Asking patients to keep medication diaries can overcome the recall bias which may be associated with interviews and questionnaires. In certain populations, such as children and patients with cognitive impairment it may be necessary to involve the parents or caregiver. These methods are all easy to use but can easily be distorted by the patients to appease their clinician. Assessment of a clinical

response or measurement of physiological marker (e.g. measuring the HR in patients taking beta-blockers) by a clinician are often used to as surrogates for adherence.

Pill counting has been the commonest method measuring adherence in clinical trials. This is where the number of pills remaining with the patient are counted between two scheduled clinic visits and compared with the actual number of pills that should have consumed over the same period to derive an adherence ratio (349). An important assumption is made with pill counting is that all the unreturned pills have been taken by the patient. Some other drawbacks of this method include that prior to clinic visits patients may lose or drop some pills or they may also systematically discard their pills to give an impression of enhanced adherence which is reflected in adherence exceeding 100% (350). Furthermore, patients may transfer drugs from the original container/packaging to new containers and also may fill their containers by ordering their prescriptions in advance which is common in patients with chronic illness which can underestimate adherence (349). A pill count does not generate a medication-taking pattern and similarly taking the correct number of pills does not provide any information on whether the patient follows the dosing regimen consistently (351, 352).

Rates of prescription refill is another objective measure of assessing adherence which can be utilised where data from pharmacy systems can be derived. Like pill counting, this method assumes that refill rates correspond to actual medication-taking behaviour. In practice this can be time consuming as patients often attend different pharmacies for their prescriptions, therefore it is more suited

to a centralised pharmacy system. Even then the accuracy of any outcome measure depends on the reliably collected complete data.

Electronic monitoring which involves medication packaging devices which are capable of recording bottle opening and time stamping the event have been in use for almost last 30 years (353). A bottle opening is assumed to correlate with ingestion of medication by the patient. Electronic monitoring has a clear advantage over other methods in that it provides detailed information on individual patients' medication-taking behaviours compared to just providing an average over a period of time. Its use in clinical trials and comparison with other adherence measures has advanced our knowledge and understating of patients' medication-taking behaviour (354). Costs associated with electronic monitoring may prove to be a hindrance to its use in large clinical trials. Due to the accuracy of this technique, it is often used as a reference for other adherence measures, however evidence of opening a bottle still does not equate to ingestion of a tablet.

The various measures of adherence described have their strengths and weakness and may be appropriate in different situations (see **Table 1.1**). It is therefore reasonable to state that no single measure of adherence can be classed as the gold standard and a combination of measures may be used to maximise the accuracy (355, 356).

**Table 1.1:** Different measures of adherence and their respective advantages and disadvantages

| Measure | Advantages | Disadvantages |
|---|---|---|
| **Direct** | | |
| Directly observed therapy | Most accurate<br>Able to assess side-effects as they appear | Resource intensive<br>Patients may still hide the medications in their mouth before discarding them<br>Provides Yes/No information on adherence |
| Measurement of medication or its metabolite in urine or serum | Objective | Expensive assays required<br>May only be qualitative, i.e. Yes/No<br>Susceptible to abuse, i.e. mixing medications in urine sample<br>Adherence only tested at a single point in time<br>White-coat adherence occurs when patients know the schedule of tests<br>Variations in medication metabolism |
| Measurement of biological marker | Objective | Expensive<br>Intrusive - requires collection of bodily specimens |
| **Indirect** | | |
| Electronic medication monitors | Provides detailed information on pattern and level of adherence<br>Highly accurate<br>Can identify partial adherence<br>Often used as a standard to compare other measure against | Expensive<br>Requires technical support<br>Overestimation can occur where patients accidently or intentionally open the container<br>It requires medications to be removed from their original packaging |

| Measure | Advantages | Disadvantages |
|---|---|---|
| Prescription refill rates | Can identify patients at risk for treatment failure<br>Provide medication-refilling pattern<br>Can provide accurate information in some healthcare systems that require verification for insurance purposes. | Records may be incomplete or difficult to access<br>Assumptions are made about medication-taking behaviours<br>Unable to identify partial adherence |
| Pill count | Objective<br>Provides information on long-term adherence | Patients can hoard or keep back medications to appear to be adherent or they simple forget to bring some or all the medications<br>Can be time-consuming if taking numerous medications<br>Arbitrary cut-off value used to define non-adherence<br>Cannot identify medication-taking pattern |
| Measurement of clinical response or physiological markers | Can be simple, quick and generally easily to perform such as measuring heart rate in patients taking beta-blockers | Other factors may affect the clinical response or the physiological marker |
| Patient questionnaires | Simple, inexpensive<br>Provide real-time feedback<br>Identify belief and barriers to adherence<br>Validated for use | Affected by patient recall<br>Patients can easily distort the results<br>Relatively poor sensitivity and specificity<br>Affected by language barriers and the questions in the questionnaire |
| Patient Diaries | Simple<br>Can overcome poor recall<br>Can be used for children or patients with learning difficulties by their parents or carers etc. | Open to manipulation by patients or their carers |

#### 1.5.5.5 Medication non-adherence and apparent TRH

In medicine the term ‘resistant’ implies a condition which fails to respond to usual medical therapy. In hypertension there are eight different classes of antihypertensive medications available – namely thiazide/ thiazide like diuretics, RAAS inhibitors, calcium channel blockers (CCBs), alpha-adrenergic receptor antagonists, beta-adrenergic receptor antagonists, central vasodilators, aldosterone receptor antagonists and miscellaneous. The recommended three first line antihypertensive agents include CCBs, RAAS inhibitors, and thiazide diuretics. There is emerging evidence that the preferred fourth-line antihypertensive agents are aldosterone receptor antagonists when compared with beta adrenergic receptor blockers and alpha-adrenergic receptor blockers (357).

Patients need to be sufficiently adherent to the prescribed therapy for it to be considered to have failed. Therefore, assessment of adherence is a crucial aspect of management of patients with chronic conditions such as hypertension. Furthermore, increasing the number of antihypertensive medications for patients may lead to increased risk of adverse effects and possible drug interactions. Medication adverse effects negatively impact patients’ adherence and uncertainty on the part of the physician as to whether or not to intensify a patient’s treatment. This ‘clinical inertia’, is detrimental to patients with true TRH given the high risks of morbidity and mortality associated with uncontrolled BP. Assessment of adherence may help overcome this inertia and help guide management plan particularly in those who are truly resistant. Studies reporting rates of non-adherence in patients with TRH and the methods used to assess adherence are summarised in **Table 1.2**.

Observational cohort or cross-sectional reports from specialist hypertension centres or general hospitals form the mainstay of studies describing the prevalence of non-adherence among TRH patients (358). A varied range of adherence measures have been used with the direct testing of the drug or its metabolite in serum or urine being the commonest in recent years (**Table 1.2**). This choice is likely a manifestation of the healthcare setting where these studies have been carried out; specialist hypertension centres with access to sophisticated medical laboratories that are able to perform these tests. These centres will have a larger proportion of patients with TRH and therefore developing an assay to detect antihypertensives and their metabolites is cost effective in the long run.

Ceral et al. used liquid chromatography-mass spectrometry (LC-MS) to detect antihypertensives in sera of 84 patients with apparent TRH (359). All of the evaluated antihypertensives were present in 29 (34.5%) patients, no drugs detected in the same number and the remaining 26 (31%) had some of their antihypertensives in their sera. Jung et al. concluded that low adherence was the commonest cause of poor BP control among 375 patients referred with uncontrolled BP (360). After excluding WCE, secondary causes of hypertension and optimisation of antihypertensive therapy, 76 patients remained in whom LC-MS was carried out on urine samples. They found that 36 (47%) were adherent and 40 (53%) were non-adherent, of which 12 (30%) had complete non-adherence.

Direct observation, where patients are observed taking their medication in an ambulatory setting or as an inpatient followed by ABPM or regular BP monitoring,

has been used as a surrogate marker for adherence (361, 362). A sustained reduction in BP following observed ingestion of tablets indicates prior non-adherence although any BP reduction observed could be attributable to BP variability, WCE or regression of BP to the mean. Furthermore, it has been demonstrated that patients presenting with very high BPs in emergency department often drop their BP without any intervention. Grassi et al. have shown that 32% of patients presenting to emergency department with a systolic BP >180mmHg and/or diastolic BP >110 mmHg had a drop of least a 20mmHg in basal systolic BP and/or a 10mmHg reduction in basal diastolic BP after a 30-minute period rest where patients were seated in a comfortable and quiet room without talking or active listening (363). Some of these limitations can be minimised by using standardised and guideline-recommended BP measurements and ABPM prior to commencing the DOT to exclude WCE, and reduce visit-to-visit BP variability and regression to the mean (362).

The author has shown that in patients with TRH after optimisation of antihypertensive therapy, exclusion of secondary causes of hypertension and WCE, only half of the patients were truly treatment-resistant (362). In non-adherent patients, the mean 24-hr ABP drop observed was 19.5/9.4mmHg when compared to ABPM carried out preceding the DOT. Although, this method makes intuitive sense in patients with TRH, it is resource and time intensive. Patients require close monitoring as dramatic drop in BP can often lead to symptomatic hypotension in patients.

Population studies have been able to study adherence rates in much larger populations (310, 364). These studies used proportion of days covered by

antihypertensives based on pharmacy refill data and a cut-off of 80% to dichotomise adherence. Sim et al., using databases of Kaiser Permanente healthcare system in the US, found that of the 60,327 patients with TRH 7% were non-adherent (364). Daugherty et al looked at 205,750 incident patients diagnosed with hypertension and found that after excluding non-adherent patients, 3,960 patients went on to develop TRH during a median 1.5 years from initial treatment (310). Of the 3,548 with TRH who had adherence assessed by prescription refill rate, 1505 patients (42.4%) were non-adherent (365). Interestingly, they reported that treatment intensification but not medication adherence was significantly associated with 1-year BP control (365). The treatment intensification was carried out in only 21.6% of the visits with elevated BP. A number of possible explanations have been suggested by the authors including, clinical inertia, co-morbid conditions and lack of evidence to support use of ≥3 antihypertensives at the time. It is possible that clinicians may be reluctant to intensify treatment if they are uncertain about a patient's adherence; Rose et al. showed that adherent patients were more likely to receive treatment intensification which improved BP control (366). This effect was similar in size regardless of the level of adherence.

Medication event monitoring system (MEMS), a type of electronic adherence measure where a microchip integrated in the cap of the medication packaging records the date and time whenever it is opened, has been used to assess adherence in TRH patients (367, 368). Grigoryan et al showed that pseudo-resistance (non-adherence and WCE) was present in half of the patients with apparent TRH; non-adherence was present in 20 of the 69 (29%) patients (367). Burnier et al. highlight the superiority of electronic devices in adherence

monitoring in that the continuous monitoring itself can improve adherence and BP control as well as providing the clinicians with detailed and objective information on patients' medication-taking behaviours (368).

A systematic review by Durand et al. summarizes 24 published studies reporting adherence in TRH patients (358). There were an average of 86 patients from 21 studies, the largest of which included 339 patients. Three population studies include a total of 66,529 patients. They report a pooled mean non-adherence rate of 31.2%; with highest rates reported in studies that used direct methods testing patients' sera or urine, followed by studies that used DOT, 47.9% and 44.6%, respectively. The lowest rate of non-adherence was found in a single study reporting on medication possession ratio at 3.3%.

Therefore, WCE and suboptimal adherence are common causes of apparent TRH. Published research in this field often fails to exclude either one or both of these factors when reporting on prevalence of TRH. Estimated prevalence of apparent TRH of 13.7% will drop taking into account the non-adherence rates and WCE each estimated to be 31.2% and 37.5% respectively. Only one very recent study systematically tested and excluded pseudo resistance and showed a true TRH prevalence of 3.3% [95% CI = 3.0 to 4.0] (369). Even a conservative estimate of 50% pseudo resistance will bring the prevalence of true TRH to around 6% - 7% of total hypertensive population. The recent change in the American guideline will, however, increase this estimate.

Despite the evidence of suggesting high rates of non-adherence in TRH patients there is very little in the literature reporting on reasons of non-adherence (358). Baseline BP, age, gender, ethnicity, income and other socioeconomic indicators

have been linked to non-adherence in TRH. It has been shown that pill burden is associated with non-adherence (370, 371). Reasons for non-adherence are rarely attributable to one single factor and are instead complex and multifactorial. A WHO-proposed model suggests the factors responsible for medication non-adherence can be categorised into five domains – social- and economic-related factors; health system/healthcare team-related factors; therapy-related factors; condition-related factors; and patient-related factors – which apply to the hypertensive population as well (335).

**Table 1.2:** Summary of studies reporting rates of non-adherence and the methods used to measure adherence.

| Study | Definition of TRH | Adherence method | Sample size | Number non-adherent (%) |
|---|---|---|---|---|
| Daugherty et al. **(365)** | AHA/ESH/ESC | Prescription refill rate – non-adherent if proportion of days covered was <80% | 3548 | 1504 (42.4) |
| Sim et al. **(364)** | AHA/ESH/ESC | Prescription refill rate – non-adherent if proportion of days covered was <80% | 60327 | 4223 (7.0) |
| Burnier et al. **(368)** | SBP ≥140 or DBP ≥90 on ≥3 AHTs on two consecutive visits ≥1 month apart | MEMS – non-adherent if <80% of days covered | 41 | 21 (51.2) |
| Grigoryan et al. **(367)** | ABPM ≥135/85 or (≥125/75 if diabetic) on ≥3 AHTs | MEMS – non-adherent if taking <80% of all prescribed doses | 69 | 20 (29.0) |
| Garg et al. **(372)** | SBP ≥140 or DBP≥ 90 on ≥3 AHTs | Patient interview – physician determines if patient is non-adherent | 141 | 23 (16.0) |
| Yakovlevich et al. **(373)** | SBP ≥140 or DBP≥ 90 on ≥3 AHTs | Patient interview – physician determines if patient is non-adherent | 91 | 9 (9.9) |
| Massierer et al. **(374)** | BP ≥140/90 on ≥3 AHTs incl. a diuretic | Self-reported questionnaire – non-adherent if score ≥3 on 4-item Morisky Medication Adherence Scale (MMAS-4) | 86 | 21 (24.4) |

| Study | Definition of TRH | Adherence method | Sample size | Number non-adherent (%) |
|---|---|---|---|---|
| Hameed et al. **(362)** | SBP ≥140 or DBP ≥ 90 on ≥3 AHTs | Directly observed therapy (DOT) – ≥ 5mmHg reduction in mean 24-hr ambulatory SBP between Pre-DOT and DOT measurements was used to indicate nonadherence | 48 | 24 (50) |
| Brinker et al. **(375)** | AHA/ESH/ESC | Therapeutic drug monitoring – non-adherence defined if levels of ≥1 AHTs below minimal detection limit | 56 | 30 (53.6) |
| Ceral et al. **(359)** | SBP ≥150 or DBP ≥95 on ≥3 AHTs | Serum drug level – non-adherence defined if levels of ≥1 AHTs below minimal detection limit | 84 | 55 (65.5) |
| Ewen et al. **(376)** | SBP ≥140 on ≥3 AHTs including a diuretic at highest or maximally tolerated dose | Direct testing of plasma and/or urine – non-adherence defined if levels of ≥1 AHTs below minimal detection limit | 100 | 48 (48.0) |
| Jung et al. **(360)** | Clinic BP ≥140/90 or ABPM ≥130/80 on ≥4 AHTs | Direct testing of urine – non-adherence defined if levels of ≥1 AHTs below minimal detection limit | 76 | 40 (52.6) |
| Rosa et al. **(377)** | Clinic BP ≥140/90 and ABPM ≥130/80 on ≥3 AHTs | Direct testing of blood – non-adherence defined if levels of ≥1 AHTs below minimal detection limit | 72 | 27 (37.5) |

| Study | Definition of TRH | Adherence method | Sample size | Number non-adherent (%) |
|---|---|---|---|---|
| Strauch et al. **(378)** | Clinic BP ≥140/90 and ABPM ≥130/80 on ≥3 AHTs | Direct testing of blood – non-adherence defined if levels of ≥1 AHTs below minimal detection limit | 339 | 110 (32.4) |
| Beaussier et al. **(379)** | SBP ≥140 or DBP≥ 90 on ≥3 AHTs incl. a diuretic | Combination of plasma test, urine test, pill count and patient interview – each element was given a score and a score of <2 defined non-adherence | 164 | 30 (18.3) |

**Abbreviations:** TRH, treatment-resistant hypertension; AHA, American Heart Association, European Society of hypertension, European Society of Cardiology; SBP, systolic blood pressure, DBP, diastolic blood pressure; AHTs, antihypertensives; MEMS, medication event monitoring system; ABPM, ambulatory blood pressure monitoring; BP, blood pressure.

#### 1.5.5.6 Management of non-adherence to antihypertensive medication

There is no proven intervention that has been shown to significantly improve adherence and a recent Cochrane Review concluded that (325):

> *"Across the body of evidence, effects were inconsistent from study to study, and only a minority of lowest risk of bias RCTs improved both adherence and clinical outcomes. Current methods of improving medication adherence for chronic health problems are mostly complex and not very effective, so that the full benefits of treatment cannot be realized."*

It is important to have a careful consultation with the patient to identify and address the potential causes of suboptimal adherence described by the WHO and one such approach is described by the author (380).

Up to 75% of patients with hypertension require more than 1 antihypertensive agent to achieve BP control (381). Pill burden is a well-established reason for non-adherence in hypertension. Single-pill fixed-dose combinations (FDC) have been recommended, for patients with hypertension requiring more than one antihypertensive agent, to help improve adherence and consequently BP control (306, 382). A meta-analysis has shown that FDCs significantly improve adherence and persistence in hypertensive patients with non-significant beneficial trends in BP and adverse effects compared with free drug combinations (383). A cohort study of 13,350 patients comparing fixed-drug with free-pill combinations also showed that the FDC group had superior adherence rates of 70% compared to 42% and a significantly lower risk of composite clinical outcomes including death or hospitalization for acute MI, heart failure, or stroke

(384). More recently, low-dose fixed triple drug combination antihypertensive pill has been used to show improved BP control compared to usual care in patients with mild to moderate hypertension (385). This low-dose FDC is being suggested as the initial therapy compared to currently accepted practice of monotherapy. Apart from reducing pill burden it may be associated with reduced adverse effects and consequently increased acceptability by the patients due to the lower doses of individual agents used. Furthermore, targeting different pathways by different antihypertensive agents may improve efficacy. A pilot study has shown that a single-pill fixed triple drug combination achieved a mean reduction of 22.8/13.6 mmHg in clinic BP and 9.3 mmHg reduction in 24-hr mean arterial pressure after 18 weeks in 13 patients with TRH (386). Further larger studies are warranted in patients with TRH to assess the effectiveness of FDC and their impact on patients' medication-taking behaviour.

Self-monitoring of BP, where patients monitor their own BP at home, has been used as an intervention to show improvements in BP (38, 39) and adherence (40). Patients self-monitoring their BP at home consulted less often with their primary care physician which helps to bring the costs of self-monitoring on par with usual care (387). Self-monitoring on its own, however, may not be enough to improve BP control (388). Complex interventions including systematic medication titration by doctors, pharmacists, or patients; education; or lifestyle counselling in conjunction with self-monitoring lead to clinically significant BP reduction which persists for at least 12 months (388). A recent RCT has shown that self-monitoring, with or without telemonitoring, when used by primary care physicians to titrate antihypertensive treatment in individuals with uncontrolled hypertension, significantly lowers BP compared with titration guided by clinic

readings (37). However, the efficacy of self-monitoring of BP in lowering BP in individuals with TRH has not yet been demonstrated.

Motivational Interviewing has a robust evidence base to increase motivation and facilitate change across a range of health-related behaviours. A meta-analysis of hypertension studies (389), involving seven underpowered RCTs shows that motivational interviewing has a significant effect on systolic BP both after intervention and at follow-up. However, most studies had a small sample size limiting statistical power, and motivational interviewing was often used as one component of multiple interventions. Although there is lack of robust evidence for its efficacy in apparent TRH, it is a low cost, easy-to-administer intervention that may be tried in this situation.

A recent study suggests that repeated biochemical urine and serum analyses for antihypertensive agents may be used as a therapeutic approach to improve BP control in nonadherent hypertensive patients. In this study from two hypertension centres in Europe (UK and Czech Republic), discussing results of urine (UK) and serum (Czech Republic) antihypertensive assays with non-adherent patients resulted in improvements in adherence and BP control – an average reduction of 19.5/7.5 mmHg in one centre and 32.6/17.4 mmHg in the other (390). However, this was a retrospective study with unclear follow-up period, and, by authors' own admission, white coat adherence effect could not be ruled out.

Finally, a recent RCT tested if a smartphone app to increase patient engagement would improve medication adherence and BP control in 411 patients with uncontrolled hypertension. There was a small improvement in self-reported

adherence in the intervention group, but there was no difference in BP control between the intervention and control groups (391).

#### 1.5.5.7 Conclusion

A large proportion of patients with apparent TRH are non-adherent to prescribed treatment. Availability of urine assays for antihypertensive drugs and metabolites in the recent years has made it easier to identify nonadherence which has significant detrimental consequences. However, no single management strategy has been shown to be effective in improving adherence in apparent TRH. Future research should focus on identifying interventions that will improve adherence in this group of patients.

### 1.5.6 Pathophysiological factors

The pathophysiological mechanisms of TRH not different to those of essential hypertension which have already been discussed in detail (See section 1.4). There are however specific factors that contribute to TRH and are discussed below.

#### 1.5.6.1 Genetics

Large scale studies have been carried out to identify association of genetic polymorphisms with hypertension (270, 271). Similar studies have been conducted to discover any link between genetic polymorphisms and responses to antihypertensive drugs (392, 393). The majority of genetic studies of TRH have thus far tried to identify candidate genes with less than adequate sample size. These have focussed on specific genes and pathways that targets of the currently used antihypertensives, albeit with modest sample sizes and P values to allow robust associations (394, 395). Recently relatively larger studies have also tried to establish an attributable genetic component specifically to TRH (396, 397).

Genetics of Hypertension Associated Treatment (GenHAT) study utilises the clinical data collected by the Antihypertensive and Lipid lowering Treatment to Prevent Heart Attack Trial (ALLHAT) to assess association of 78 candidate genetic polymorphisms implicated in the development of hypertension and cardiovascular disease (396). The findings of this study show that two genetic variants in the angiotensinogen gene (rs699, rs5051) were significantly associated with TRH among white participants when compared to treatment responsive controls.

Similarly another genetic study utilised data collected as part of INVEST-GENES (the INnternational VErapamil-SR Trandolapril STudy—GENEtic Substudy) and WISE (Women's Ischemia Syndrome Evaluation) to show that the ATP2B1 rs12817819 A allele is associated with increased risk for TRH in hypertensive participants with documented coronary artery disease or suspected ischemic heart disease (397). The ATP2B1 gene encodes the plasma membrane calcium ATPase which transports intracellular calcium ions against very large concentration gradients and plays a critical role in intracellular calcium homeostasis. Since calcium is involved in smooth muscle and cardiac contraction, a disequilibrium in calcium concentration may predispose to TRH.

It is however currently unclear how these genetic variations alter response to drugs that target them. A recent scientific statement by AHA on TRH concluded that "much larger studies of well-characterized individuals with treatment RH and of individuals before and after treatment with specific antihypertensive therapies will be needed to better define the role of common and rare genetic variation in causing resistant hypertension and modulation of drug response" (398).

#### 1.5.6.2 Obesity

Obesity has been identified as an important risk factor for the pathogenesis of hypertension (399). The mechanisms associated with obesity hypertension have been discussed already (see section 1.4.7). As highlighted earlier, obesity is associated hyperaldosteronism and in obese TRH patients mineralocorticoid receptor antagonists have been shown to significantly lower BP (400, 401). Furthermore, the reduction in BP of obese patients with TRH occurred despite the concurrent use of ACE inhibitors or ARBs suggesting that mineralocorticoid

receptor activity in obesity is independent to Ang II mediated aldosterone secretion.

Evidence from population studies further supports a direct role of obesity in TRH (313, 364, 402). The results of 13,775 hypertensive patients from the US National Health and Nutrition Examination Survey (NHANES) show that BMI >30 kg/$m^2$ is an independent risk factor for apparent TRH; the odds ratio (95% CI) of apparent TRH as compared to all patients with uncontrolled hypertension persistently increased from 1.78 (1.20 – 2.64) during 1988 – 1994 to 2.04 (1.17 – 3.53) during 2005 – 2008 (313). In the Spanish ABPM registry, which included 14,461 patients with TRH, the risk of TRH was significantly higher in patients with a BMI >30kg/$m^2$ [Odds Ratio (OR): 1.62, 95% CI: 1.32 – 1.99] (402). Results from another large database of 470,386 patients with hypertension and of whom 60,327 were resistant also show an increased risk of TRH in patients who had BMI >30kg/$m^2$ [OR: 1.46, 95% CI: 1.42 – 1.51] (364). Together these results and the pathophysiological mechanisms of obesity hypertension suggest a prominent role of obesity in TRH.

#### 1.5.6.3 Salt intake

Dietary sodium plays a significant role in development and maintenance of hypertension as already highlighted (see section 1.4.4) by plethora of confirmatory evidence. Therefore, logic would state that in severe hypertension such as TRH excess dietary sodium intake is likely to be a significant contributor to uncontrolled BP. However, unlike in hypertension, the large-scale studies assessing impact of dietary sodium intake on BP are lacking in TRH populations. In a prospective study of 204 patients with true TRH a third of the patients were

found to have a high dietary sodium intake (403). Another of study characterising 279 patients with TRH, there was no significant difference in 24-hour urinary sodium excretion when compared with controls, however patients with TRH had significantly higher brain natriuretic peptide, a marker of intravascular volume expansion (404). Further evidence of role of excess dietary sodium ingestion contributing to TRH comes from interventional studies which have shown that a reduction in dietary salt intake is associated with substantially reductions in BP (405, 406).

#### 1.5.6.4 Obstructive sleep apnoea

The prevalence of OSA is very high in patients with TRH; it has been shown to be present in as many as 71% to 90% and is often severe (403, 407-414). The mechanism by which OSA causes high BP through activation of the SNA is described earlier (see section 1.4.1.3). Furthermore, increased fluid retention due to mineralocorticoid excess and high dietary sodium ingestion which characterises TRH causes the oedema of upper airways and may therefore be contributing to the increased prevalence of OSA (414-418). The severity of OSA has been shown to be reduced by treatment with mineralocorticoid receptor antagonists (419-421).

The treatment of OSA with CPAP has been shown to reduce 24-hour MAP by 2 – 3 mmHg (422). In patients with TRH, similar reductions were observed in 24-hour MAP and more a higher percentage of patients reverted to nocturnal dipper pattern of BP with CPAP (423). The BP reductions were even better when the adherence to CPAP was taken into account (423). However, when assessing CPAP for the prevention of cardiovascular events in OSA, CPAP plus usual care

compared with usual care did not prevent cardiovascular events with moderate-to-severe OSA. (424). Multiple meta-analyses of CPAP for OSA have confirmed only a modest reduction of BP by about 3/2 mmHg (425-428).

Patients with TRH are clearly at a higher risk of OSA and are likely to need to continue with multiple antihypertensives in addition to CPAP to achieve BP control. It is therefore important to regularly screen for symptoms suggestive of OSA and to consider prompt investigations to confirm the diagnosis.

### 1.5.7 Medications contributing to treatment-resistant hypertension

Medications prescribed for conditions other than hypertension can either directly increase BP or interact with pharmacokinetic and/or pharmacodynamic characteristics of antihypertensives reducing their BP-lowering efficacy. Although the prevalence of drug-induced hypertension is unknown a variety of medications and substances raise BP via several different mechanisms which are discussed below.

#### 1.5.7.1 Mineralocorticoid excess and activation of renin-angiotensin-aldosterone system

Synthetic glucocorticoids, regardless of route of administration, can increase BP which is mediated through the mineralocorticoid effects. Steroids cause sodium and water retention and consequently an increase in cardiac output results in high BP (429). Glycyrrhizin acid, the active ingredient in liquorice, inhibits 11-β-hydroxysteroid dehydrogenase enzyme which is responsible for converting cortisol into inactive cortisone. Inhibition of this enzyme by liquorice results in excess endogenous cortisol which binds to the mineralocorticoid receptors

causing sodium and water retention, potassium loss, and a decrease in plasma renin and aldosterone levels (430).

The CYP17A1 inhibitor abiraterone acetate (an androgen used for prostate cancer) and antifungals (ketoconazole and itraconazole) can also increase BP by interfering with steroid synthesis via the counter-regulatory stimulation of adrenocorticotrophic hormone release which in turn leads to synthesis of aldosterone and 11-deoxycorticosterone (429, 431, 432). The resulting apparent mineralocorticoid excess causes a rise in BP.

A moderate increase in BP results from contraceptives containing combination of oestrogen and progestogen through increasing angiotensinogen synthesis and activation of the RAAS (433). The progestogen-only contraceptives (434) and hormone replacement therapy (435) have not been shown to increase BP.

#### 1.5.7.2 Increased sympathetic nerve activity

Several antidepressants can increase BP through direct activation of SNA and include venlafaxine (a serotonin and noradrenaline reuptake inhibitor), monoamine oxidase inhibitors and tricyclic antidepressants. Similarly, nasal and ophthalmic decongestants that contain adrenaline or phenylephrine and illicit drugs, cocaine and amphetamines, can also activate the sympathetic nervous system causing the BP to rise.

#### 1.5.7.3 Direct vasoconstrictors

Immunosuppressive agents which include calcineurin inhibitors such as ciclosporin and tacrolimus increase the BP through systemic and renal vasoconstriction; increased ET-1 production, decreased NO availability and activation of RAAS have all been implicated (436). Similar mechanisms and an

increased plasma viscosity induce high BP caused by the use of recombinant human erythropoietin used to treat anaemia (429).

Vascular endothelial growth factor inhibitors that include monoclonal antibodies and tyrosine kinase inhibitors are increasingly being used for the treatment of malignancies and are associated with development of hypertension (437). These agents increase BP through an imbalance of NO and ET-1 pathways, and capillary rarefaction.

#### 1.5.7.4 Diverse mechanisms

Non-steroidal anti-inflammatory drugs can cause variable increases in BP mediated through diverse mechanisms including increased sodium retention, inhibition of renal vasodilation through decreased prostaglandin production, and reducing the BP-lowering efficacy of diuretics, ACE inhibitors, ARBs and β-blockers (438, 439). Antiretrovirals (lopinavir and titonavir) have been shown to increase BP through an increase in BMI (440).

### 1.5.8 Secondary causes of treatment-resistant hypertension

#### 1.5.8.1 Primary aldosteronism

Hyperaldosteronism is relatively uncommon in the general population with prevalence of around 1% (441) which rises to approximately 8% in patients with primary hypertension (442). The estimates of prevalence in hypertensive patients vary from 8% to 32% (441, 443-447). The prevalence of hyperaldosteronism has been estimated to be higher in patients with TRH at approximately 20% (398). The higher prevalence of hyperaldosteronism has been further substantiated by the results of the PATHWAY-2 study; an RCT comparing the antihypertensive benefit of placebo, spironolactone, bisoprolol and doxazosin in patients with

confirmed TRH (357). Spironolactone was found to be superior to all other agents in achieving the largest BP reduction and BP control. In the first a series of sub studies of the PATHWAY-2 trial, antihypertensive response correlated with baseline plasma renin levels, plasma aldosterone levels and aldosterone-renin ratio (ARR); a high ARR and low renin concentration both strongly predicted a large BP response to spironolactone (448). A second sub study showed that the superior reduction of BP achieved with spironolactone is associated with elimination of thoracic volume excess (448). These results confirm the widely suspected significant pathophysiological role of salt and water retention driving TRH.

#### 1.5.8.2 Renal parenchymal disease

Majority, if not all, forms of renal parenchymal disease can cause hypertension especially in the presence of impaired renal function and the prevalence of hypertension increases with progression decline in kidney function. Hypertension is a risk factor for cardiovascular disease and CKD, if remains uncontrolled can lead to rapid progression of CKD, and is a leading cause of ESRD. On the other hand, CKD itself can exacerbate hypertension, often necessitating the use of a combination of antihypertensive drugs of different classes to achieve BP control.

In the 2001–2010 NHANES participants majority of the CKD patients (54.4% overall and increasing from 30.2% in stage 1 CKD to 78.9% in stage 4 CKD) require antihypertensives (449). The proportion of patients with a target BP of 130/80mmHg in CKD also decreases with progressive decline in kidney function (44.6% overall and decreasing from 49.5% in stage 1 CKD to 30.2% in stage 4 CKD) and increasing the threshold for target BP to 140/90 mmHg increased the

proportion of CKD patients BP control to 66.5%. These results have been replicated in the Chronic Renal Insufficiency Cohort (CRIC), where 67.1% and 46.1% had their hypertension controlled to <140/90 mmHg and <130/80 mmHg, respectively (450). Compared with people without CKD prevalence of TRH is reported to be twice as high in patients with CKD and in the CRIC study participants with CKD, 40% had apparent TRH (450, 451). The prevalence of apparent TRH increased with worsening renal function (22.3% in those with estimated GFR (eGFR) >60ml/min/1.73m$^2$ and progressively increasing to 54.2% in patients with eGFR <30ml/min/1.73m$^2$) and the presence of apparent TRH was an independent predictor for a higher risk of mortality and adverse cardiovascular and renal outcomes (451). These mechanisms have already been described in detail earlier (see section 1.4). In CKD, however, the predominant role of sodium and fluid retention is worth emphasizing. An impaired natriuresis underpins the Guyton hypothesis and is a prerequisite for salt sensitive hypertension (**Figure 1.11**); in CKD the salt sensitivity of the BP increases exponentially with a decrease of overall kidney function and consequently a larger increase in intravascular volume (452). The fluid and sodium excess that characterises CKD limits the efficacy of BP-lowering medications that lack a natriuretic effect and patients often require multiple antihypertensives to achieve BP control and promote TRH.

#### 1.5.8.3 Renal artery stenosis

Renovascular hypertension caused by renal artery stenosis is major cause of secondary hypertension. Renovascular disease has been shown to be present in 5 – 7% of population, which increases with age (453, 454), However, the prevalence in TRH has been shown to be higher at 24.2% (455). Atherosclerosis

is by far the main cause of renal artery stenosis accounting for up to 90 percent of cases of renal artery stenosis (456). Other less common causes include fibromuscular dysplasia, renal artery dissection or infarction, Takayasu arteritis, radiation fibrosis, and obstruction caused by aortic endovascular stents.

Pathophysiologically, unilateral renal artery stenosis causes reduced renal perfusion, usually occurs with narrowing of renal artery lumen by 70% or more, activating the RAAS leading to release of renin and Ang II which causes the BP to rise through impaired natriuresis, increase SNA and endothelial dysfunction. Importantly, in the acute phase the contralateral kidney undergoes hyperperfusion and glomerular hyperfiltration with associated RAAS activation from the stenotic kidney (457). Persistently elevated BP can then lead to atherosclerotic lesions and parenchymal injury in the contralateral kidney with associated proteinuria shown to be originating from the non-stenotic kidney (457).

Logically, based on the success of interventional techniques for atherosclerotic lesions in other organs, such techniques have been applied to patients with renovascular hypertension. However, major RCTs of revascularisation in renal artery stenosis to date have failed to show a substantial improvements in renal function, BP and, adverse cardiovascular and renal events at the expense of a higher risk of serious complications associated with the intervention (458-460). Therefore, currently optimal medical therapy remains the treatment of choice in such patients.

#### 1.5.8.4 Phaeochromocytoma

Catecholamine-producing tumours can arise from the chromaffin cells of the adrenal medulla or the sympathetic ganglia and are labelled

phaeochromocytomas and paragangliomas respectively. Both present with a similar clinical features including paroxysmal hypertension, episodic headaches, sweating and tachycardia. The tumours are rare, occurring in less than 0.2% of the patients with hypertension (461, 462), but prevalence may be higher in patients investigated for TRH (463). Overproduction of catecholamines (adrenaline, noradrenaline and dopamine) by chromaffin cells increase BP by inducing arteriolar vasoconstriction and inotropic increase of cardiac output (464). BP changes include the characteristic paroxysmal increase, sustained hypertension, orthostatic hypotension, consistently normal or even hypotension in some (464).

#### 1.5.8.5 Cushing syndrome

Cushing syndrome is result of excess cortisol due to overproduction of adrenocorticotrophic hormone either from a pituitary adenoma or ectopic secretion from a non-pituitary tumour. The excess cortisol overwhelms the ability of 11-β-hydroxysteroid dehydrogenase to convert cortisol to cortisone. The unconverted cortisol can act on the mineralocorticoid receptors promoting sodium and fluid retention and consequently hypertension. Cushing syndrome itself is a relatively rare and the role of Cushing syndrome in TRH remains uncertain (398).

## 1.6 Role of renal denervation in treatment-resistant hypertension

### 1.6.1 Historical treatments for hypertension – modulation of sympathetic activity

On the basis of the vasoconstrictor and cardioacceleratory properties of the sympathetic nervous system, a number of therapies have been developed in an attempt to modify the effect of the sympathetic nervous system on BP control. Efficacy of these therapies further support the important role the SNA plays in hypertension. In 1923, Fritz Bruening performed the first surgical sympathectomy for hypertension based on the hypothesis that reduction of sympathetic outflow will lead to reduction in BP (465). This original concept was further developed by other surgeons such as Peet and Smithwick (466, 467) who performed more extensive operations termed splanchnicectomy which was offered to those with severe hypertension with evidence of cardiovascular damage. These procedures resulted in impressive reduction in BP but were associated with significant surgical morbidities and complicated by orthostatic symptoms. With the availability of effective medical therapy these procedures were abandoned. However, the success of surgical sympathectomy in reducing high BP led to the development of drugs producing chemical sympathectomy. A number of ganglion-blocking agents were developed to treat hypertension like hexamethonium and tetraethylammonium chloride (468, 469). These agents were the first pharmacological agents to block sympathetic outflow from the ganglion and also one of the earliest medical therapies for hypertension. Alpha-

methyldopa a centrally acting anti-hypertensive agent which acts by inhibiting sympathetic outflow through binding to $\alpha_2$-adrenoreceptors became available in the 1960s (470). Use of this drug is uncommon nowadays except in the context of hypertension in pregnancy. The role of sympathetic nervous system in the pathogenesis of hypertension is mediated by the alpha- and beta-adrenergic receptors. Beta-adrenergic receptor blockers were shown to be beneficial in the management of hypertension in 1964 (471). The alpha-1 receptors present in the blood vessels are responsible for peripheral resistance. Selective alpha-1 receptor blockers were developed with a view to treating hypertension by reducing elevated total peripheral resistance (472). However, in practice these agents have been found to have very modest BP lowering effect (473).

Another example of sympathetic modulation to achieve BP control is bilateral nephrectomy that used to be carried out in dialysis patients with refractory hypertension (474). The improvement in BP control in this situation is likely to be due to removal of the sympatho-excitatory effects of the damaged kidneys.

### 1.6.2 Catheter-based renal denervation in TRH

A greater understanding of the role of the sympathetic nervous system in the pathogenesis and maintenance of primary hypertension and the lessons learnt from modulation of sympathetic activity in hypertension control has led to a number of novel device-based therapies of which catheter-based RDN is the foremost. Initial encouraging results from clinical studies led to more than 10,000 procedures being carried out in TRH across the world in the last few years.

#### 1.6.2.1 The procedure

The renal nerves arise from T10 to L2 spinal segments, arborise around the renal artery and primarily lie within the adventitia of the artery. This presents an opportunity to ablate the renal sympathetic nerves percutaneously via the lumen of the main renal artery using a catheter connected to a radiofrequency generator. The access is gained through the common femoral artery which is punctured, under conscious sedation and local anaesthetic injection to the groin, using ultrasound guidance. A guide sheath is introduced using a standard Seldinger technique (**Figure 1.13**). A renal angiogram is performed using a non-ionic contrast agent to delineate the renal artery, ostia, bifurcation and accessory arteries. If the renal artery anatomy is suitable, a RDN catheter is positioned in the renal artery under fluoroscopic guidance at an initial position proximal to bifurcation of the renal artery (**Figure 1.14**). The early RDN catheters developed were unipolar with a flexible tip fitted with an electrode that can discharge radiofrequency waves when connected with a radiofrequency generator. More recently multipolar catheters of various designs have been developed. These are fitted with multiple electrodes capable of delivering multiple ablations simultaneously.

The treatment involves the delivery of relatively low power and precisely focused radiofrequency bursts through the wall of the renal artery to disrupt the surrounding renal nerves lying in the adventitia. Depending on the type of the catheter being used, unipolar or multipolar, it is repositioned after each treatment to ablate the sympathetic nerves in each quadrant of the renal artery in a helical pattern. Catheter tip temperature and impedance are constantly monitored during ablation, and radiofrequency energy delivery is regulated according to a

predetermined algorithm. After a satisfactory treatment, a repeat selective renal arteriogram is taken to ensure patency of the renal artery post-ablation. The process is then repeated in the other renal artery and/or any accessory renal arteries. Following successful completion of the procedure a vascular closure device is deployed.

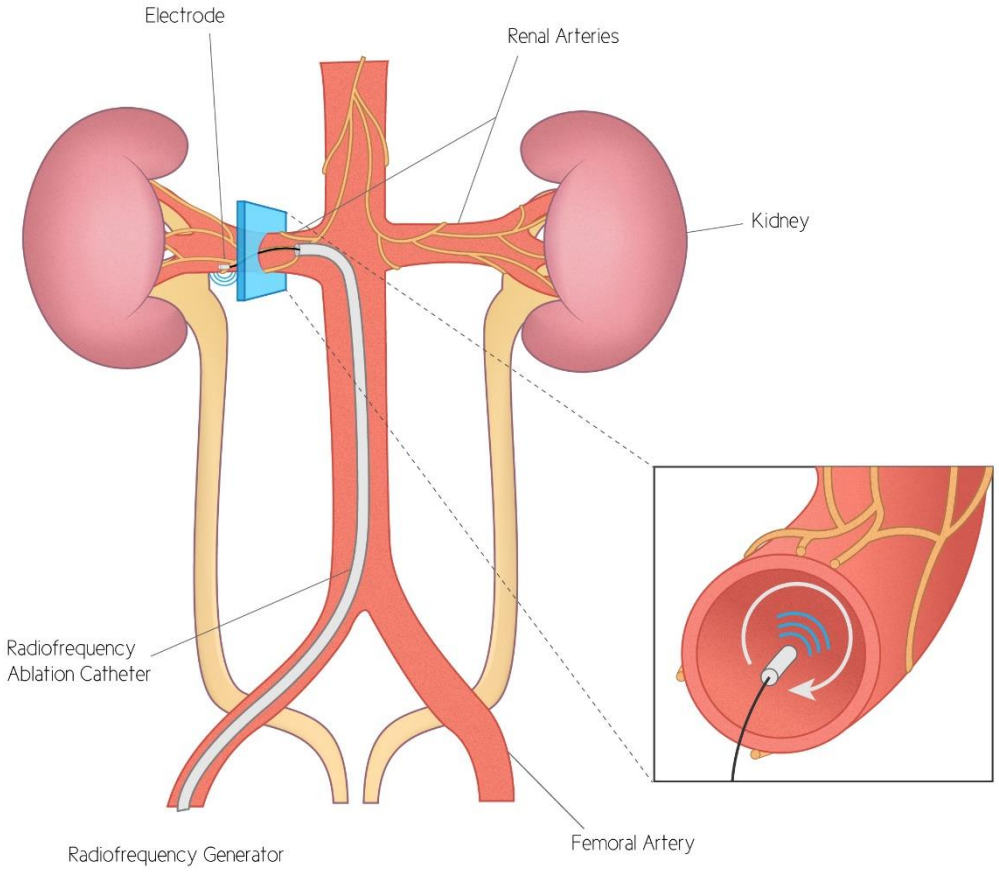



**Figure 1.13:** Diagram demonstrating the radiofrequency renal denervation procedure.

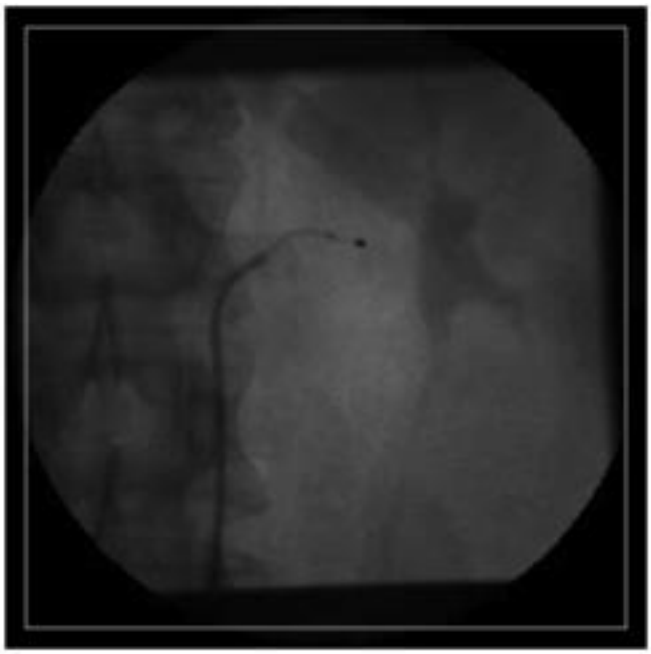


**Figure 1.14:** Image showing renal denervation catheter in the left renal artery at the point of delivering radio-frequency ablation.

#### 1.6.2.2 Pre-clinical and proof-of-principle studies of renal denervation

The preclinical studies of catheter-based RDN were carried out in juvenile swine model. It was shown to be comparable to direct surgical RDN via renal artery transection and re-anastomosis. As proof of success, noradrenaline spillover rates were reduced by more than 85% after denervation (475). In humans, bilateral RDN was demonstrated to reduce noradrenaline spillover, decrease renin activity, and increase renal plasma flow at 30 days after the procedure (476).

The proof-of-concept trial of RDN in humans (SYMPLICITY HTN-1), included 45 patients with TRH with a clinic SBP ≥160 mmHg who underwent RDN using the single electrode catheter [Symplicity catheter system, Ardian Inc. Mountain View, CA, USA; Medtronic Inc., Santa Rosa, CA, USA] (475). All patients were taking

at least three antihypertensive agents of different classes including a diuretic. Significant reductions in office BP readings compared to baseline were observed, -22/-11 mmHg and -27/-17 mmHg at 6 months and 12 months after RDN respectively.

Serial eGFR, renal angiography and magnetic resonance renal angiography (MRA) were used to assess safety of the procedure. The procedure was found to be safe with no significant change in renal function and no incidence of renal artery stenosis on the MRA at 6 months. Two patients experienced adverse events; one had renal artery dissection which occurred before any radiofrequency ablation could be applied to the artery and the second had a pseudoaneurysm at the femoral access site.

To assess physiological response of RDN, efferent SNA at the level of the kidneys was assessed by isotope dilution renal noradrenaline spillover testing in a subgroup of 10 patients. A mean reduction of noradrenaline spillover of 47% (95% confidence interval: 28–65%) was observed 1 month after RDN. These observations, alongside the substantial reductions in clinic BP suggested successful targeting of efferent renal nerves. Therefore, this initial proof-of-concept study in human subjects demonstrated that the procedure was safe and was associated with a significant BP reduction at one year.

Furthermore, BP reduction following RDN was sustained. One hundred and fifty-three patients undergoing open-label RDN, including the cohort of the proof of principle study, were followed up for 3 years. There was a mean reduction of 32/14 mmHg in office BP at 3 years with 93% of the patients having a reduction in SBP of greater than ≥10 mmHg (477). The average number of BP-lowering

medications prescribed at baseline and 36 months were similar. Sustained BP reduction suggested that re-growth of efferent nerve fibres had not occurred over time.

#### 1.6.2.3 Further clinical studies demonstrating efficacy of catheter based renal denervation

An open-label, multicentre, RCT of RDN versus usual care in patients with TRH further supported the efficacy and safety of the procedure (478). In this open labelled RCT, efficacy of RDN was compared against usual care; 106 patients were randomised to either RDN or a control arm. At six months, there was a 32/12 mmHg reduction in office BP in the RDN group, but no difference in office BP in the control arm, when compared with baseline BP. No procedural or device-based adverse events were observed.

Following these encouraging results, a number of new catheter systems were developed and the efficacy tested in clinical trials. A meta-analysis of the early studies of RDN further supported the BP lowering benefits of this therapy (479). In the RCTs, there were reductions in mean office BP at 6- and 12-months of 28.9/11.0 mmHg and 25.4/10.0 mmHg respectively with RDN compared with medical therapy. Similar lowering in office BP was observed in uncontrolled studies; reductions of 25.0/10.0 mmHg and 22.8/10.6 mmHg respectively at 6- and 12-months post RDN compared with baseline.

#### 1.6.2.4 Pitfalls of early studies of renal denervation

There were two main criticisms of early studies of catheter-based RDN. ABP readings were infrequently used for primary assessment of BP response. The few studies, which reported on the ABP, showed much lower BP reductions with a

mean of 13.2/7.3 mmHg at 6 months after procedure compared with baseline (479). Secondly, there was absence of blinding due to the invasive nature of the procedure. Therefore, the placebo effect could not be excluded and the medication taking behaviour of the patient may have been altered by inclusion in a device-based trial.

#### 1.6.2.5 The sham-controlled trial of renal denervation

Sham controlled trials are considered to be the most rigorous way of assessing efficacy of new surgical techniques or device-based interventions. As such, RDN was compared against a sham procedure where the patients randomised to the sham arm received a renal angiogram only [SYMPLICITY HTN-3] (480). This RCT included 535 patients with TRH across 88 centres in the USA and was designed to address the issues raised regarding the design of prior open-label RDN studies. The mean changes in office SBP after 6 months were -14.1 mmHg and -11.7 mmHg respectively in the denervation and sham arms, an absolute difference of 2.4 mmHg between the groups. The primary efficacy endpoint, a difference of 5 mmHg between the groups in change of office SBP from baseline to 6 months, was not met. The secondary efficacy endpoint of a 2-mmHg difference between groups in change in 24-hour ambulatory systolic BP was also not met; there was a reduction of 6.75 mmHg obtained in the denervation group and 4.79 mm Hg in the sham-procedure group. However, there was no difference between the groups in major safety endpoints, 1.4% in denervation group versus 0.6% in the sham group. Therefore, the sham-controlled study suggests that catheter-based RDN is a safe procedure but does not lower BP effectively in patients with TRH.

***Critical analysis of the sham-controlled study***

Critical analysis of this study by the investigators and others suggest a number of factors which may have been associated with better BP response in some patients within this trial (481, 482). Factors which have been shown to be associated with favourable BP response within this trial include:

a) Number of ablations: Consistently greater reductions in BP were observed in patients who received a higher number of renal artery ablations in the treatment group**.** An increased difference in office and ABPs with an increasing number of ablations was observed after patients in the two groups were propensity score matched for the baseline characteristics. This trend was shown to be statistically significant. Twelve or more ablations in both renal arteries were found to be predictive of a greater change in ambulatory systolic BP. There was no increase in safety events corresponding to the increasing number of renal artery ablations.

b) Pattern of ablation: There was a trend towards an increasing BP response with delivery of ablations in a four-quadrant pattern to neither, one or both renal arteries. However, this was not statistically significant. In the treatment arm, only 19 (5%) patients received four-quadrant ablations in both renal arteries.

c) Younger age: The subgroup of patients <65 years in age was associated with office SBP change in the RDN group on univariate analysis but not in the multivariable model.

d) Ethnicity: A significant difference in office SBP was observed in the non-African-American subgroup as compared with the African-American subgroup, but there was no significant difference observed in mean 24-hour ABP or home BP.

e) Higher baseline clinic SBP: Multivariable analysis of the overall group identified baseline office SBP ≥180 mmHg was a positive predictor of 6-month response of office BP reduction.

f) Type of antihypertensive medications used: Multivariable analysis showed use of aldosterone antagonist to positively predict an increased 6 month change in office SBP. Conversely, use of vasodilators was found to be a negative predictor for change in office SBP at 6 months.

g) Higher baseline eGFR: Baseline eGFR ≥60 mL/min/1.73m$^2$ was shown to be an independent predictor of a greater change in ABP at 6 months.

Factors that may have contributed to the apparent ineffectiveness of RDN in the sham-controlled study are operator inexperience and medication adherence. The procedure-related predictors observed, four-quadrant ablations and total number of ablations in both renal arteries, could be a consequence of operator inexperience of a relatively new procedure. In 88 centres in the US, where the study was conducted, 364 RDN procedures were carried out. One hundred and eleven interventionists performed at least one procedure, with approximately one third doing one procedure and only 26 operators doing more than five procedures suggesting the majority of interventionists were relatively inexperienced with the RDN procedure (482). The procedure had been unlicensed in the US prior to this trial so the learning curve of the operators fell within the period of the trial.

Adherence to prescribed antihypertensive medication was assessed by self-reported diary completed by the patient. This is an indirect method of assessing mediation adherence which does not prove actual ingestion, is open to manipulation and is likely to over-estimate adherence. It is important to assess adherence to prescribed antihypertensive medication in patients with TRH objectively as up to half may be completely or partially non-adherent (362, 377). Inclusion in a clinical trial may result in a change in the subjects' pill-taking behaviour due to their awareness of being observed. This phenomenon is termed 'the Hawthorne effect'.

#### 1.6.2.6 Results of studies published after the sham-controlled trial

Since the publication of SYMPLICITY HTN-3, a number of studies have been published which help us understand the role of RDN in the management of TRH and give us a future direction. Some of the main studies are described here.

A prospective, open-label, multicentre registry was set up to assess the safety and efficacy of RDN (483). The results of nearly 1000 patients undergoing RDN around the world confirm the favourable safety profile of the procedure. The mean 6-month reduction in office and 24-hour ambulatory SBP were 11.6 mmHg and 6.6 mmHg respectively. This was a real-world registry with more lenient inclusion criteria. The greatest reduction in clinic and 24-hour ambulatory SBPs (20.3 mmHg and 8.9 mmHg respectively) were seen in patients with severe hypertension at baseline: office SBP ≥160 mmHg with a 24-hour ambulatory SBP ≥135 mmHg and taking ≥3 antihypertensive medication.

The results of an open-label, multicentre RCT of stepped-care standardised antihypertensive treatment (SSAHT) with or without RDN for TRH was published

(484). This was a rigorously designed trial which randomised patients to either RDN or to continue on SSAHT after confirmation of TRH by ABPM four weeks after having been commenced on SSAHT. There were 48 patients in the RDN group and 53 patients in the control group. Adherence to antihypertensive medications was assessed using a validated 8-item Morisky Medication Adherence Scale [MMAS-8] and 24-hour ABPM was used to measure the primary efficacy of RDN in this trial.

The results showed a reduction of 15.4/9.7 mmHg and 9.5/6.6 mmHg in the 24-hour ABPM for RDN and control groups respectively with a difference of 5.9/3.1 mmHg between the groups. There was a statistically significant reduction in 24-hour ambulatory SBP in patients with proven TRH receiving RDN in addition to the standardised antihypertensive regimens as recommended by European and UK hypertension guidelines. Therefore, although this was an open-label study, this study addressed the two of the factors that may have affected the results of the sham-controlled study, i.e. use of ABP as primary measure of efficacy and more formal adherence testing.

However, another RCT compared RDN to intensified pharmacotherapy including spironolactone showed no significant difference in BP reductions (ambulatory and office) in the two groups (485). This was the first RCT to include patients with true TRH by excluding non-adherence using quantitative plasma drugs level measurements, white-coat hypertension, and secondary causes of hypertension. Unlike the previous RCT (484), the antihypertensive regimen here was not standardised and spironolactone was added in the intensified pharmacotherapy arm of the study.

Another real-world study (UK Renal Denervation Affiliation, UKRDA) involving 253 patients with true TRH reported a drop of 22/9 mmHg and 12/7 mmHg in clinic and ambulatory BP respectively at 11 months, which was independent of medication changes during follow-up and use of aldosterone antagonists (486). Furthermore, patients in the two highest quartiles of daytime ambulatory SBP at baseline (mean systolic ABP at baseline of 176 and 199 mmHg respectively) exhibited significant ABP reductions (22 and 14 mmHg respectively), whilst those in the lowest quartile exhibited little response. **Table 1.3** summarises the major clinical studies of RDN to date, including their strengths and weaknesses.

**Table 1.3:** A table showing studies of renal denervation including their methods, primary outcomes, strengths and weaknesses

| Study | Method | Number of patients | Primary outcome | Strengths | Weaknesses |
|---|---|---|---|---|---|
| SYMPLICITY HTN-1 **(477)** | Single-arm, open-label, case-series | 153 | Change in clinic BP at 6 months post-RDN: -22/-10 mmHg (150)<br>Change in clinic BP at 3 years post-RDN: -32/-14 mmHg (88) | • First proof-of-concept study demonstrating safety and efficacy of RDN<br>• Patients followed up for 3 years<br>• Assessment of noradrenaline spillover<br>• RDN shown to be safe | • No ABP measurements<br>• Lack of a comparator group |
| SYMPLICITY HTN-2 **(478)** | Open label, RCT, RDN vs usual treatment | 106 (52 RDN; 56 control) | Difference in mean clinic BP at 6 months post-RDN between RDN and usual treatment: -33/-12 mmHg | • Randomised controlled trial<br>• Statistically powered to detect a difference between RDN and usual treatment.<br>• RDN shown to be safe | • Un-blinded |
| SYMPLICITY HTN-3 **(480)** | Single blinded, RCT, RDN vs sham procedure | 535 (364 RDN; 171 Sham) | Difference in mean clinic SBP at 6 months post-RDN between RDN and Sham: -2.4 mmHg | • Largest RCT of RDN<br>• Sham comparator group<br>• Confirms safety of RDN | • Change in ABP not a primary endpoint.<br>• lack of operator experience resulting in inadequate ablation<br>• self-reported medication adherence assessment |
| Global SYMPLICITY Registry **(483)** | Single-arm, open-label, case series | 998 | Change in clinic and 24-hour mean SBP at 6 months: -11.5 and -6.6 respectively. | • Real-life use of RDN<br>• Analysis shows severe hypertension more likely to be responders | • Heterogonous inclusion criteria<br>• SYMPLICITY catheter only |

| Study | Method | Number of patients | Primary outcome | Strengths | Weaknesses |
|---|---|---|---|---|---|
| DENERHTN **(484)** | Open label, RCT, RDN vs stepped-care standardised antihypertensive treatment (SSAHT) | 106 (53 RDN, 53 SSAHT) | Difference in mean 24-hour ABP at 6 months between RDN and SSAHT: -5.9/-3.1 mmHg | • Change in ABP is the primary outcome<br>• Experienced operators<br>• Standardised antihypertensive regimens used<br>• Validated questionnaire used for adherence assessment | • Non-use of aldosterone antagonists<br>• Unblinded (No sham procedure) |
| PRAGUE-15 **(485)** | Open label, RCT, RDN vs intensified pharmacotherapy (PHAR) | 106 (52 RDN; 54 PHAR) | Difference in mean 24-hour ambulatory BP at 6 months between RDN and PHAR: -0.5/-1.1 mmHg | • True TRH confirmed.<br>• Adherence confirmed using plasma antihypertensive drug level<br>• Use of aldosterone<br>• Change in ABPM is the primary outcome | • Unblinded (No sham procedure) |
| UK renal denervation affiliation report **(486)** | Single-arm, open-label, case series | 253 | Change in mean daytime ABP after an average of 8.5 months following RDN: -12/-7 mmHg | • Well characterised group of patients<br>• High percentage of patients with ABP results<br>• Real-life use of RDN | • No control/sham group for comparison<br>• Retrospective registry |
| EnligHTN I **(487)** | Single-arm, open label, series | 46 | Change in clinic BP at 6 months post-RDN: -26/-10 mmHg | • Multi-electrode catheter | • Observational study |
| EnligHTN II **(488)** | Single-arm, open label, series | 103 | Change in clinic BP at 6 months post-RDN: -18/-8 mmHg | • Multi-electrode catheter | • Observational study |
| EnligHTN III **(489)** | Single-arm, open label, series | 39 | Change in clinic BP at 6 months post-RDN: -25/-7 mmHg | • Multi-electrode catheter | • Observational study |

| Study | Method | Number of patients | Primary outcome | Strengths | Weaknesses |
|---|---|---|---|---|---|
| SPYRAL HTN-OFF MED **(490)** | Single blinded, RCT, RDN vs sham procedure | 80 (38 RDN; 42 Sham) | Change in mean 24-hr ABP at 3 months: RDN -5.5/-4.8 mmHg vs sham -0.5/-0.4 mmHg | • Sham comparator group<br>• Multi-electrode catheter<br>• Single experienced operator per centre<br>• Objective adherence testing<br>• Use of ABP as primary outcome | • Unpowered<br>• Short follow-up |
| SPYRAL HTN-OFF MED **(491)** | Single blinded, RCT, RDN vs sham procedure | 80 (38 RDN; 42 Sham) | Change in mean 24-hr ABP at 6 months: RDN -9.0/-6.0 mmHg vs sham -1.6/-1.9 mmHg | • Sham comparator group<br>• Multi-electrode catheter<br>• Single experienced operator per centre<br>• Objective adherence testing<br>• Use of ABP as primary outcome | • Unpowered<br>• Short follow-up |
| RADIANCE-HTN SOLO **(484, 492)** | Single blinded, RCT, RDN vs sham procedure | 146 (74 RDN; 72 Sham) | Change in mean daytime ABP at 2 months: RDN -8.5/-5.1 mmHg vs sham -2.2/-2.6 mmHg | • Sham comparator group<br>• Powered for primary end-point<br>• Use of ABP as primary outcome | • Adherence not assessed<br>• Short follow up<br>• Did not include patients with TRH |

**Abbreviations:** RCT, randomised controlled trial; RDN, renal sympathetic denervation; BP, blood pressure; SBP, systolic blood pressure; ABP, ambulatory blood pressure; SBP, systolic BP; TRH, treatment-resistant hypertension.

### 1.6.2.7 Future direction

Although sound physiological principles and surgical precedent underpin RDN as a therapy for TRH, clinical studies so far have produced conflicting results. Further research is required before its implementation in routine clinical management of TRH. Lessons learnt from these studies are listed below which should be considered in future clinical research.

***Appropriate patient selection***

The results the clinical trials of RDN suggest younger patients (<65 years), those with normal kidney function, and those with higher baseline office BP (systolic ≥180 mmHg) respond best to RDN. Patients should have a thorough assessment to confirm the presence of true TRH with exclusion of WCE, non-adherence and secondary causes of hypertension. Reliable methods are now available to test adherence to antihypertensives using urine samples (493, 494) which have confirmed that up to 50% of patients with TRH are partially or completely non-adherent (360).

***Medication optimisation***

Patients with TRH in the trials of RDN were on 4-5 antihypertensive agents on average. There is now good evidence from trials of pharmacological intervention to recommend the addition of an aldosterone receptor antagonist to the routine combination of a thiazide diuretic, a CCB and a renin angiotensin system blocking agents to treat TRH (357, 495). Post hoc analysis of SYMPLICITY HTN-3 also shows higher response rate to denervation in patients taking aldosterone receptor antagonists (481). Any trials designed to test the efficacy of RDN should consider all patients to be started on a standardised regimen of

antihypertensives, as used in the DENERHTN trial, with 6-8 weeks of time to allow the medication to take their full affect before RDN is attempted. Adherence assessment, using a direct method such as urine drug analysis, should be made at each post RDN visit to allow the researchers to attribute any BP changes observed directly to RDN.

***Appropriate efficacy assessment***

Studies of denervation should be powered for difference in ABPM, as it is less sensitive to regression to the mean (32, 496), rather than office BP.

***Procedural development***

Failure of SYMPLICITY HTN-3 to meet its primary efficacy may be in part due to operator inexperience in RDN as highlighted above. Recently, Sakakura and colleagues have shown from autopsy examination of renal arteries of 20 patients with hypertension (497) that the distance from renal artery lumen to nerve location is shortest (mean 2.6 mm) in the distal arterial segments compared with the proximal (mean 3.4 mm) and middle segments (mean 3.1 mm), although the volume of renal nerves is higher in the proximal and mid-vessel. As radio-frequency waves do not penetrate deeper than 3mm, it is possible that the variable responses seen between variable open-label studies and sham controlled study of RDN is related to the mechanism and technique by which ablations were delivered. In future studies, efforts should be made to deliver radiofrequency energies distally in the renal artery with a view to achieving more complete denervation of the kidney.

Multi-electrode catheters have now been developed to deliver radio-frequency ablations simultaneously to multiple points around the renal artery. Use of these catheters will help deliver four quadrant ablations and increase the number of ablations producing more complete denervation.

***Need for a test to confirm adequacy of renal denervation***

Currently there is no simple way of assessing whether adequate denervation has taken place. Regional noradrenaline spillover and peroneal MSNA are two methods which have been used to assess the effect of RDN on sympathetic activity (498-500). However, due to the invasive nature of these techniques and the expertise required to perform these tests, there is limited uptake of these tests in clinical trials let alone in routine clinical practice. Therefore, there is a need for a test which will allow confirmation of successful denervation, ideally whilst the patient is still on the table to allow the operator to make adjustments as necessary.

***Identifying predictors of response***

There is heterogeneity in the level of BP-response to RDN. It will be helpful to be able to predict which patients with TRH are more likely to respond to RDN. This will allow appropriate patient selection for selective use of the technique with greater success. Post hoc-analyses of clinical trials of RDN, Global Symplicity Registry and UKRDA have highlighted patient- and procedure-related predictors of response. Other studies have focussed upon physiological testing and biochemical markers of response to RDN. In a clinical trial of RDN the use of baroreflex sensitivity (BRS) has been tested as a predictive marker of response to RDN (501). The results of this study show that cardiac BRS is a strong and

independent predictor of response to RDN in patients with TRH. Another study examined the effect of RDN on angiogenic markers, and found ICAM-1 and VCAM-1 and sFLT-1 predict response to RDN (502).

### 1.6.3 Ultrasound renal sympathetic denervation

High frequency ultrasound RDN is a non-invasive form of RDN which is undergoing clinical trial at present. It is based on delivery of externally focused ultrasound to the renal nerves using doppler-based ultrasound image guidance to track and automatically correct for renal artery motion during treatment. The novel applicator used incorporates an anatomically customized phased-array ultrasound transducer to generate and focus therapeutic energy to the depth of the renal artery, and an ultrasound imaging transducer to facilitate locating, targeting and tracking the renal artery for treatment.

Following preliminary feasibility studies in an open animal model, studies were performed using the external applicator system in Yorkshire swine. There was a 71% reduction in norepinephrine levels at 1 week following RDN which persisted for 6 weeks demonstrating the feasibility of targeted external ultrasound for RDN in an animal model and supported further investigation in patients with hypertension (503).

The proof-of-principle study of twenty-four patients with refractory hypertension demonstrated a 27mmHg reduction in SBP at 6 months after denervation treatment. This initial technique utilised external focused ultrasound navigated by a targeting catheter in the renal artery. In the second phase of the study, 13 patients with TRH underwent bilateral externally focused ultrasound utilizing a 5F intravascular catheter for targeting and tracking. The procedure was well

tolerated except one patient complained of back pain lasting 24 hours. At 6 week follow up an 18 mmHg reduction in office SBP was noted (504).

In the next stage, a fully non-invasive ultrasound RDN was studied on 23 patients with TRH with office SBP greater than 160 mmHg taking more than 3 antihypertensive medications. At 6 weeks the investigators reported an average reduction of office SBP of 23 mmHg. Six patients experienced back pain resolving within 24 hours of the procedure (505).

This technology appears promising. Currently, an international multicentre phase 3 RCT, in patients with TRH, is underway, comparing targeted ultrasound RDN with sham treatment. If this study shows significant BP reduction in the treatment arm, it may prove to be a useful non-invasive device-based therapy for treatment of TRH. Factors to be considered for future research in RDN for TRH are listed below:

- Appropriate patient selection
    - age (<65 years)
    - baseline office systolic BP at high end (≥180 mmHg)
    - exclusion of WCE, secondary hypertension and nonadherence to antihypertensive medication
- Medication optimisation
    - standardised regimen of medication for at least 6 weeks before procedure
    - aldosterone antagonists
    - adherence testing at every visit following RDN using direct method, e.g. urine assay

- Use of 24-hour ABPM to assess efficacy
- Improvement in procedural factors
    - use of multipolar catheters to achieve maximum number of ablations (≥12)
    - distal ablations
- Need for a test to confirm adequacy of denervation at the time of procedure
- Identification of predictors of response to improve patient selection
    - clinical markers
    - BRS
    - angiogenic markers

### 1.6.4 Latest trials of renal sympathetic denervation

Since the publication of this review as a book chapter, three further sham-controlled RCTs of RDN have been published and also summarised in Table 4.1; SPYRAL HTN-OFF MED (490), SPYRAL HTN-ON MED (491), and RADIANCE-HTN SOLO (492). The two SPYRAL studies used the newer multi-electrode SPYRAL radiofrequency catheter with 4 electrodes arranged in a spiral to ensure 4-quadrant ablation of renal sympathetic nerves. The SPYRAL trials included patients with clinic SBP between 150 and 180 mmHg, a DBP ≥90 mmHg and a mean 24-hr ambulatory SBP between 140 and 170 mmHg. Patients included in the OFF MED trial were either antihypertensive naïve or in whom all their antihypertensives were stopped. Whereas patients taking up to 3 standardised medications were included in the ON MED trial with adherence to antihypertensives tested using serum and urinary antihypertensive assays based on LC-MS which was supplemented by directly observed administration of

antihypertensives when performing an ABPM. Both of these studies were proof-of-concept studies with no prespecified efficacy or safety endpoints.

The two SPYRAL studies randomised 80 patients to receive RDN (n=38) and sham procedure (renal angiography alone, n= 42). In the OFF MED trial, the 24-hour BP significantly decreased from baseline to 3 months in the RDN group; 24-hr SBP -5·5 mmHg (95% CI -9·1 to -2·0; p=0·003), 24-hr DBP -4·8 mmHg (-7·0 to -2·6; p<0·0001) There was no significant corresponding drop in the sham-control group; 24-hr SBP -0·5 mmHg (95% CI -3·9 to 2·9; p=0·764) and 24-hr DBP -0·4 mm Hg (-2·2 to 1·4; p=0·645). In the ON MED trial, significant reduction in 24-hr ABP was observed from baseline to 6 months in the RDN group; SBP -9.0 mmHg (95% CI -12.7 to -5.3; p<0.0001) and DBP -6.0 mmHg (-8.5 to -3.5; p<0.0001). No significant change in 24-hr ABP was present in the sham-control group; SBP -1.6 mmHg (95% CI -5.2 to 2.0, p=0365) and DBP -1.9 mmHg (-4.7 to 0.9; p=0.172). Furthermore, 60% of the patients were found to be adherent and no major adverse events were recorded.

The RADIANCE-HTN SOLO used catheter-based ultrasound RDN and a similar study design to OFF MED. However, there was no formal objective assessment of adherence and only patients taking either none or up to two antihypertensives were included. Unlike the SPYRAL trials, RADIANCE SOLO was powered to detect a reduction of 6 mmHg in the daytime ABP and 146 patients with moderate hypertension (daytime ABP 135-170/85-105 mmHg) were randomised to RDN (n=74) and sham-group (n=72). At 2 months the reduction in daytime ambulatory SBP was greater with RDN (-8·5 mmHg, SD 9·3) than with the sham procedure (-2·2 mmHg, SD 10·0; baseline-adjusted difference between groups: -6·3 mmHg,

95% CI -9·4 to –3·1, p=0·0001). There were no adverse safety events in either group.

SPYRAL OFF MED and RADIANCE SOLO, by removing the effect of antihypertensive medications, provide the proof that by employing improved technique in well-designed and well-conducted sham-controlled studies, RDN is is biologically effective in lowering BP. Furthermore, SPYRAL ON MED proves the efficacy of RDN despite the prescription of medical treatment.

Significant changes to trial design and conduct include objective assessment of adherence (SPYRAL studies only), exclusion of isolated systolic hypertension, primary endpoint based on ABP, standardised medications, use of multi-electrode catheter, ablative treatment extended into distal renal artery branches with higher number of ablations, and limiting to a single experienced proceduralist per centre

Some limitations, however, remain; the SPYRAL studies are proof-of-concept only and not powered for safety and efficacy endpoints, the follow-up period is relatively short, not all patients responded to RDN, the results of these studies are not applicable to patients with TRH and there was no measure of effective renal nerve ablation.

### 1.6.5 Conclusion

Hypertension is one of the most preventable causes of premature morbidity and mortality in the world. TRH is a major clinical problem that requires further treatment options. Catheter-based RDN is a technology borne out of sound basic science and clinical precedent. Clinical studies of this technology in TRH so far have produced conflicting results. Critical analysis of these studies suggests that there are a number of patient-related and procedural factors which will need to be addressed in future studies before implementation of catheter-based RDN in routine clinical practice. Recent well-designed and conducted studies of RDN have addressed some of the shortcomings of earlier studies leading to evolution of this treatment modality confirming its biological efficacy in lowering BP and providing the basis for the design of larger RCTs which are needed to prove the efficacy and safety of RDN.

## 1.7 Summary and scope of Thesis

Hypertension has been widely accepted as a significant public health issue, responsible for significant morbidity and mortality and incurs substantial costs to the healthcare systems worldwide. Despite seemingly small increases of BP associated with significant increases in risk of mortality from cardiovascular disease and stroke, inaccuracies in measurement of BP account for large variations in BP. Use of home and ambulatory BP monitoring has improved diagnosis and management of hypertension but has also unveiled masked and white-coat hypertension, both of which are also associated with increased risks.

The pathophysiology of hypertension is complex and multiple mechanisms have been shown to contribute to development and persistence of increased BP which is reflected in the range of therapeutic treatments available. Despite advances in pharmacological and procedural interventions to lower BP, a large proportion of patients have persistently uncontrolled BP.

A specific subgroup of patients with persistent uncontrolled hypertension despite optimal pharmacological treatment with three antihypertensives are labelled as TRH. These patients have been shown to have even greater risk of complications associated with hypertension. The mechanisms responsible for TRH remain unclear; non-adherence to antihypertensives and WCE commonly inflate the prevalence of TRH whereas other medications, OSA, obesity and secondary causes of hypertension further contribute to increased prevalence TRH. Recent enthusiasm in RDN had offered a promising therapeutic option for this cohort of patients, however this technique is still in its infancy and the mixed results

suggest that hypertension in these patients is not primarily driven by sympathetic overactivity and other mechanisms and factor may be equally as important.

This thesis therefore consists of three broad aims:

1. Assess prevalence of non-adherence and predictors of non-adherence in patients with apparent TRH using directly observed therapy (DOT) and urine antihypertensive screen (AHS).

2. Review the use of RDN for patients with TRH in the published literature and report on the safety and efficacy of RDN in patients with significant renal impairment (eGFR 15 - 45 ml/min/1.73 $m^2$) performed using $CO_2$ as the sole contrast agent.

3. Explore the factors associated with associated with true TRH in a study of Factors AssoCiated with Treatment-Resistant Hypertension (FACT-RHY). The objectives for this prospective cohort study in UK patients with non-TRH and true TRH include:

    a. Describe phenotypical and biochemical characteristics of patients with true TRH.

    b. Investigate whether any meaningful pathophysiological differences exist between patients with true TRH and non-TRH. The proposed mechanisms investigated will include arterial stiffness, endothelial dysfunction, inflammation, body composition, and OSA.

    c. Investigate association between non-adherence and patients' beliefs and attitudes towards their medicines.

### 1.7.1 Structure of thesis

This thesis began with an introduction chapter which outlined the definition, epidemiology and risks associated with hypertension. The introduction emphasised the importance of accurate and consistent measurement technique to eliminate BP variability and highlighted the use of ABPM and home BP monitoring to distinguish between different phenotypes of hypertension which was followed by a detailed overview of the pathophysiology of hypertension. Lastly definition, prevalence, prognosis and pathophysiology of TRH is described.

Within chapter 1 the definition of medication non-adherence in chronic health conditions with particular reference to TRH is reviewed. The methods and techniques used for the assessment of non-adherence and their respective strengths and weaknesses are discussed. prevalence of non-adherence in TRH is also reviewed and the strategies that can be used to improve adherence. Furthermore, a narrative review of role of RDN in patients with TRH is discussed in detail in the introductory chapter.

Chapter 2 reports the outcomes from a retrospective study of DOT and urine AHS in patients with TRH attending a specialist secondary care hypertension clinic.

Chapter 3 describes the methods and results of a pilot study on the safety and efficacy of RDN in patients with significant renal impairment performed using $CO_2$ angiography.

Chapter 4 introduces the FACT-RHY study followed by detailing the methods and results of the FACT-RHY study. A discussion of the results follows in chapter 5

and lastly the main findings of the thesis are summarised and concluded in chapter 6.

# CHAPTER 2 PREVALENCE OF NON-ADHERENCE IN PATIENTS WITH TRH – A RETROSPECTIVE STUDY OF PATIENTS UNDERGOING ADHERENCE TESTING

## 2.1 Introduction

Adherence, or lack thereof, including its clinical significance, how it is defined and the various methods available to assess it have already been discussed (see section 1.5.5). The reported prevalence of TRH is extremely variable and is strongly affected by presence of non-adherence. Prevalence of TRH and non-adherence in this group of patients has been in discussed in detail in sections 1.5.2 and 1.5.5.5 respectively.

The aim of this retrospective study is to report the outcomes of two direct methods of adherence testing, namely DOT and urine AHS, in patients attending a specialist secondary care hypertension clinic. The prevalence of non-adherence in patients with apparent TRH and factors which may predict non-adherence are examined in this study.

## 2.2 Methods

All patients who underwent formal adherence testing either through attending a DOT clinic or by the means of a urine AHS were retrospectively selected for this study. These are patients who were referred to the specialist hypertension clinic at Birmingham Heartlands Hospital by both primary care and secondary care physicians for the assessment and management of uncontrolled hypertension.

### 2.2.1 Standardised protocol

On initial presentation all patients were assessed using a standardised protocol, which included complete medical history, clinical examination, clinic BP, lipid profile, urea and electrolytes, 24-hour ABPM, electrocardiogram and a targeted echocardiogram to examine for LVH. Serum renin and aldosterone, urine catecholamines and metanephrines, serum or urine cortisol, and computed tomography (CT) renal angiogram were done where secondary hypertension was suspected and in patients with apparent TRH, after ruling out significant WCE by ABPM. Patients were then managed with lifestyle modification advice, and adjustment and addition of antihypertensive medications taking into account drug intolerances or adverse drug reactions.

### 2.2.2 Clinical pharmacist review

All patients undergoing any assessment for adherence to antihypertensive medications were seen by a clinical pharmacist who undertook a medicines use review process to identify and address any adherence issues with antihypertensive medications.

This review covered any practical difficulties that the patient may have opening the pill packaging, ordering of medications, side effects, their health beliefs of hypertension and antihypertensives, and any intentional or unintentional reasons for omitting medications. The pharmacist also contacted the primary care physician to assess prescription refill frequency. Wherever possible, the patient's usual pharmacy was also contacted by the clinical pharmacist to check if medications were being collected by the patients in a timely manner.

### 2.2.3 Directly Observed Therapy clinic

If despite these measures patients' BP remained uncontrolled, they were referred to the DOT clinic, led by the clinical pharmacist and run by a Specialist Hypertension Nurse, for formal assessment of their adherence to prescribed antihypertensive medications.

Patients were advised not to take any of their antihypertensive medication on the day of the DOT clinic. The clinical pharmacist prescribed the patients' usual antihypertensive medications on a drug chart which were dispensed from the hospital pharmacy. Most patients were on single dose regime for all antihypertensive agents. For the few patients taking twice daily or thrice daily medications, the clinical pharmacist converted the dose to an appropriate level for a single dose for the DOT clinic. Patients were asked to bring all of their prescribed medications at the DOT clinic visit at which point the hypertension nurse compared the patients' drugs against the dispensed list prior to administering them. If patients were taking any branded medications, they were given their dose of the branded medication from their own supply instead of the generic medication supplied by the hospital pharmacy.

At the DOT clinic visit, patients were fitted with a second 24-hour ABPM prior to receiving any medications, and patients were not informed of BP readings during their DOT clinic visit. Each prescribed drug was administered at its current dose by the nurse, under the guidance of the clinical pharmacist; the first drug one hour after arrival and thereafter at 60-minute intervals. Patients were directly observed by the nurse for 7 hours and all symptoms were recorded. The results of the ABPM were compared with that carried out prior to the DOT clinic visit. The pre-DOT clinic ABPM used was the one carried out when the patient first visited the clinic with apparent TRH, which was repeated immediately prior to the DOT clinic if this was more than 6 months old.

### 2.2.4 Urine Antihypertensive Screen

From November 2014 onwards, after the availability of an AHS, developed locally at Birmingham Heartlands Hospital pathology laboratory, DOT was replaced with the urine AHS. The specific methods of how the urine sample is analysed are described in detail elsewhere, but briefly urine samples were collected from patients at their routine clinic appointment and transported to the laboratory at room temperature and analysed using LC-MS (493). A total of 23 commonly prescribed antihypertensive medications can be tested using this method.

### 2.2.5 Data collection

The data were collected retrospectively on all the patients who attended the DOT clinic and those patients who had the urine AHS. Data were recorded from clinical case notes and the hypertension clinic database in which data were collected prospectively.

Data was recorded for all patients who attended the DOT clinic from August 2007 to March 2014 with a gap of 18 months when no trained hypertension nurse was available. All patients who had a urine AHS between October 2014 and November 2016 were included.

Data recorded included basic demographics, co-morbidities, smoking status, number of medications, clinic BP, side-effects suggesting hypotension, pre-DOT and DOT ABPM results, and antihypertensives prescribed and present in the urine.

## 2.2.6 Definition of Adherence

### 2.2.6.1 Directly observed therapy

The mean 24-hour ABP readings before DOT and at the DOT clinic were compared to identify the patients with true TRH. In the absence of a defined 24-hour ABP cut-off to diagnose non-adherence in the literature, an arbitrary ≥5 mmHg reduction in mean 24-hour systolic ABP between Pre-DOT and DOT measurements was used to indicate non-adherence. ABP has greater reproducibility when compared to office BP and shows very little intra-patient variability (506, 507). A reduction in SBP of 5 mmHg has been shown to result in significantly reduced risk of cardiovascular morbidity and mortality with pharmacological therapy (508) and has also been used as a significant difference in mean 24-hour ABP in RDN studies (480).

### 2.2.6.2 Urine AHS

Any medications detected in the urine AHS were then compared to patient's usual prescription to assess adherence, expressed as a percentage of number of antihypertensive medications detected out of total number of antihypertensive

medications prescribed. Patients were labelled as fully adherent if all the prescribed antihypertensives that are detectable were present in the urine; non-adherent if no drugs were present; and partially adherent if only some were present.

### 2.2.7 Statistical Analysis

All data were anonymised before analysis. As this was an audit of retrospective data carried out with the aim of service improvement, the local ethics committee felt that no formal ethics approval was required.

Data were recorded and analysed using Microsoft Excel, IBM SPSS Statistics for Windows 24.0 (IBM Corp., Armonk, N.Y., USA) and Stata Statistical Software; Release 13 (StataCorp LP, College Station, TX, USA). The Shapiro–Wilk test was applied to test for normality of distribution of data. Normally distributed data are presented as mean and standard deviation (SD) and non-normal data as median and inter-quartile range (IQR). Both confidence interval and p-value were used to express statistical significance and a p-value of $<0.05$ was considered statistically significant.

#### 2.2.7.1 Binary logistic regression

Binary logistic regression was used to generate prediction model for overall non-adherence. Adherence status was used as the binary dependant variable, where non-adherence was defined as absence of at least 1 antihypertensive in the urine AHS. The variables entered in the model included age, gender, ethnicity (after transformation into a Caucasian and non-Caucasian binary variable), number of comorbidities (cumulative of diabetes mellitus, ischaemic heart disease, and cerebrovascular disease), BP indices (SBP and DBP for clinic and 24hr), total

number of antihypertensives prescribed, prescription to RAAS inhibitors, CCBs and diuretics.

The backward elimination was performed manually, individually removing any variable in the model with the highest p-value greater than the significance level of 0.05. Age and gender were kept in the models regardless of their significance level. By rerunning the model with each deletion of the of a variable, each iteration of the model has a larger sample size as the missing data on the excluded variables will no longer result in missing cases in the multivariable model. The final model contained 129 patients and 1 excluded due to missing data.

The continuous variables (clinic SBP, age and the total number of antihypertensives prescribed) included in the final model were individually examined and found to have no outlying values. Furthermore, presence of outliers within the model itself was assessed by examining the standardised residuals. There was one standardized residual with a value of -3.268 SD. There was no significant change in the p-values and odds ratios for the independent variables when the model was re-run with this case excluded, therefore it was kept in the analysis. Multicollinearity was not present in the three continuous variables of the final model.

Linearity of the continuous variables with respect to the logit of the dependent variable was assessed via the Box-Tidwell procedure (509). A Bonferroni correction was applied using all 9 terms in the model resulting in statistical significance being accepted when $p < 0.00556$ (510). Based on this assessment, all continuous independent variables were found to be linearly related to the logit of the dependent variable.

The binary logistic regression model's ability to discriminate between patients who were adherent and those who were non-adherent was evaluated using receiver operating characteristic (ROC) curve to estimate the area under the curve. Nagelkerke pseudo $R^2$ was used to summarise the proportion of variation in the dependant variable associated with the predictor or independent variables.

## 2.3 Results

### 2.3.1 Baseline demographics

Fifty-six patients attended the DOT clinic of whom 50 patients had the complete ABP readings. For two of the six patients it was not possible to fit a BP monitor at the DOT clinic, and four did not have any ABP readings prior to the DOT clinic. These 6 patients have been excluded from further analysis in this study.

Of the remaining 50 clinic attendances, two patients attended twice, hence their demographic data was only included once in the final analysis.

A total of 130 patients underwent 145 urine AHSs up until the end of November 2016. The baseline demographic data for the patients who had undergone repeated urine AHS measurements were included only once.

The baseline characteristics of the two study cohorts were generally similar. There were almost equal number of males and females in the DOT cohort, 47.9% and 52.1% respectively. In the AHS cohort, there were relatively higher proportion of males at 56.2% (n=73). The mean (SD) age was 62 (11.0) and 60.3 years (12.6) in the DOT and AHS cohorts respectively. The median (IQR) duration of hypertension was available for the DOT cohort (n=41, for whom it was recorded in their clinical notes) and was 12.0 (7.5 – 22.0) years. The median (IQR) number of antihypertensive medications taken by the DOT cohort was 5 (4 – 5) compared to 4 (3 – 5) in the AHS cohort. The number of patients with significant comorbidities including diabetes mellitus, cerebrovascular disease, ischaemic heart disease and peripheral vascular disease was similar in the two study populations. The baseline characteristics are summarised in **Table 2.1**. Data on

smoking status duration of hypertension was not available for the urine AHS cohort whereas eGFR data was not available for the DOT cohort.

**Table 2.1:** Baseline demographic and clinical characteristics of directly observed therapy and urine antihypertensive screen cohorts

| | **DOT (n=48)** | **Urine AHS (n=130)** |
|---|---|---|
| **Age, years (mean±SD)** | 62.0 (11.0) | 60.3 (12.6) |
| **Gender, n (%)** | | |
| Male | 23 (47.9) | 73 (56.2) |
| Female | 25 (52.1) | 57 (43.8) |
| **Ethnicity, n (%)** | | |
| Caucasian | 26 (54.2) | 79 (60.8) |
| African / Caribbean | 9 (18.8) | 16 (12.3) |
| South Asian | 13 (27.1) | 21 (16.2) |
| Other | 0 | 4 (3.1) |
| **Smoking Status, n (n=35, %)** | | |
| Non-smoker | 20 (41.7) | |
| Smoker | 8 (16.7) | N/A |
| Ex-smoker | 7 (14.6) | |
| **Body mass index, kg/m$^2$ (n=34, mean±SD)** | 32.0 (5.5) | 32.1 (5.9) |
| **Duration of Hypertension, years, (n=41, median, IQR)** | 12.0 (7.5 - 22.0) | N/A |
| **Number of antihypertensives (median, IQR)** | 5 (4 - 5) | 4 (3 - 5) |
| **eGFR, ml/min/1.73m$^2$ (mean±SD)** | 69.8 (21.4) | 66.8 (20.3) |
| **Comorbidities, n (%)** | | |
| Diabetes mellitus | 15 (31.3) | 39 (30.0) |
| Cerebrovascular disease | 10 (20.8) | 21 (16.2) |
| Ischaemic heart disease | 15 (31.3) | 26 (20.0) |
| Peripheral vascular disease | 3 (6.3) | 5 (3.8) |

**Abbreviations:** DOT, directly observed therapy; AHS, antihypertensive screen; SD, Standard deviation; IQR, interquartile range; eGFR; estimated glomerular filtration rate.

### 2.3.2 Baseline BPs

In the urine AHS cohort, BP measurements correspond to the clinic attendance when the urine sample was collected. The mean (SD) clinic BP was 178.0/94.9 (22.7/ 19.2) mmHg. The mean (SD) BP reading taken in the outpatient clinic prior to the DOT clinic was 187.5/99.5 (25.3/17.6) mmHg and the first BP reading on the day of the DOT clinic prior to taking any medications was 184.1/102.5 (23.9/21.4) mmHg.

ABPM carried out closest in date to the day of clinic attendance is reported in the urine AHS cohort. The mean (SD) values for the ABPM for the two study cohorts are listed in **Table 2.2**. In the DOT cohort, there was no difference in either mean 24-hour or daytime ABP between pre-DOT and DOT clinic readings. There was, however, a significant drop in the night ABP readings with a mean drop of 8.4 mmHg (p=0.016) in SBP and 5.6 mmHg (p=0.003) in DBP.

**Table 2.2:** Mean (standard deviation) clinic and ambulatory blood pressure (mmHg) readings in the two cohort groups

| | Urine AHS | | DOT | | | |
|---|---|---|---|---|---|---|
| | n | BP | n | Pre-DOT | DOT | P value |
| **Clinic SBP** | 129 | 178.0 (22.7) | 47 | 187.5 (25.3) | 184.1 (23.9) | 0.27 |
| **Clinic DBP** | 129 | 94.9 (19.2) | 47 | 99.5 (17.6) | 102.5 (21.4) | 0.17 |
| **24-hour systolic ABP** | 117 | 160.2 (19.7) | 50 | 160.7 (21.5) | 156.5 (23.4) | 0.15 |
| **24-hour diastolic ABP** | 117 | 88.0 (14.0) | 50 | 88.0 (15.0) | 85.8 (15.9) | 0.16 |
| **Day systolic ABP** | 117 | 162.3 (19.3) | 50 | 164.7 (21.2) | 161.2 (22.3) | 0.23 |
| **Day diastolic ABP** | 117 | 89.9 (14.4) | 50 | 91.0 (15.5) | 89.5 (16.3) | 0.34 |
| **Night systolic ABP** | 109 | 152.1 (24.4) | 46 | 151.5 (23.1) | 143.1 (29.5) | 0.02 |
| **Night diastolic ABP** | 109 | 82.3 (13.9) | 46 | 81.1 (14.6) | 75.5 (15.7) | 0.003 |

No statistical comparison has been carried out between urine AHS and DOT cohorts; the p-values relate to paired sample t-test done on the pre- and post-DOT BPs within the DOT cohort. **Abbreviations:** AHS, antihypertensive screen; DOT, directly observed therapy; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; ABP, ambulatory blood pressure.

### 2.3.3 Adherence in the DOT cohort

In the DOT cohort, there was a mean reduction of 4.3 mmHg (95% CI, -10.1 to 1.6) in the DOT clinic 24-hour ambulatory SBP when compared to pre-DOT clinic ABPM. **Figure 2.1** shows change in the 24-hour ambulatory SBP in this cohort. The graph shows two distinct groups of patients; those with significant reduction in their ambulatory SBP and those in whom the ambulatory SBP showed a rise or no significant change (<5 mmHg 24-hour ambulatory SBP). **Figure 2.2** shows the change in 24-hour ambulatory SBP in these two groups.

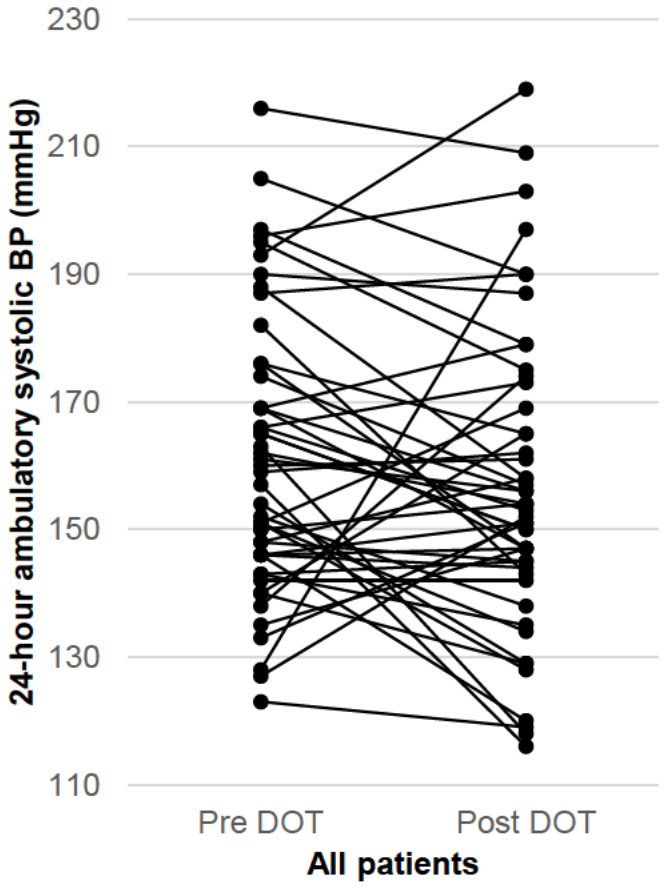



**Figure 2.1:** Individual 24-hour ambulatory systolic BP profiles for all patients at pre- and post-DOT clinic

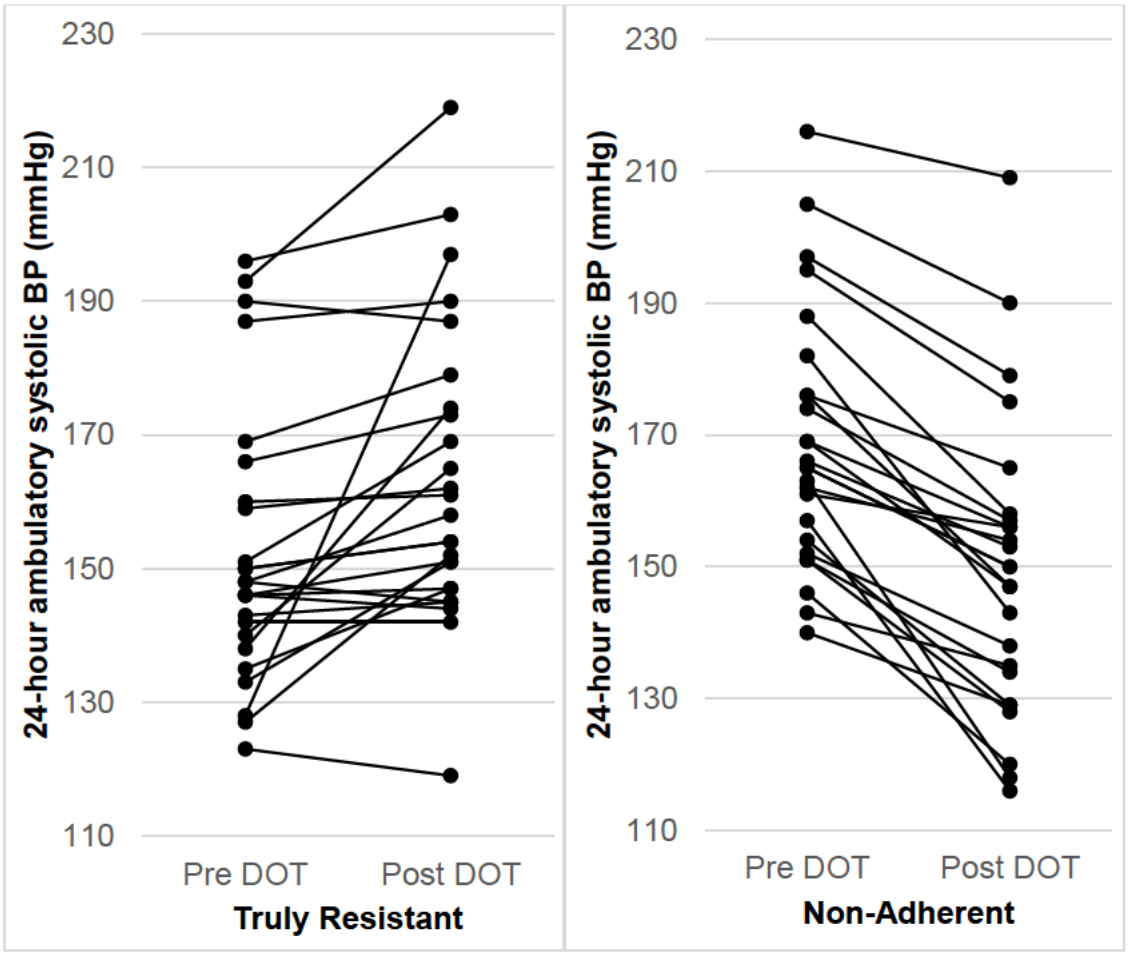



**Figure 2.2**: Individual 24-hour ambulatory systolic BP profiles for non-adherent (left) and truly resistant (right) hypertensive patients at pre- and post- DOT clinic.

In the DOT cohort, SBP control (daytime ambulatory SBP <135 mmHg) was achieved in 5 (10%) and DBP control (daytime ambulatory DBP <85 mmHg) in 7 (14%) patients. The proportion achieving night-time SBP or DBP control (ABP <120/75 mmHg) was 15% and 21% respectively.

Nine (18.8%) patients complained of symptoms suggestive of hypotension at the DOT clinic; of whom 7 were found to be non-adherent. Although there was no significant drop in the average 24-hour ABP of the other two patients, the symptoms of hypotension coincided with periods of relative hypotension throughout the day. Twelve (25.0%) patients had at least one medication omitted

on the day of the DOT clinic either in response to symptoms associated with observed hypotension or to prevent onset of symptoms if there was a substantial drop in the BP. Of these patients three patients had three medications and four patients had 2 medications omitted.

Patients were tested for any possible associations between their non-adherence status, using the aforementioned criteria; gender, ethnicity, smoking status, and comorbid conditions. There was no significant association between non-adherence and these parameters.

### 2.3.4 Adherence in the Urine AHS cohort

Sixty (46.2%) patients in the urine AHS cohort were fully adherent to their prescribed antihypertensive medications (**Figure 2.3**). Of the remaining 70 patients, 22 (16.9%) were non-adherent with no antihypertensive medications detected in the urine and 48 (36.9%) were partially adherent with at least one antihypertensive medication undetected in the urine.

A binary logistic regression was performed which showed gender, age, clinic SBP, number of all prescribed antihypertensive medications and a prescription of CCB to predict non-adherence to antihypertensive medications (**Table 2.3**). When compared to a model with constant being the only variable, the logistic regression model was statistically significant indicating a relationship between the predictors and the outcome; $\chi^2(5) = 35.66$, $p<0.001$. The model explained 32.3% (Nagelkerke pseudo $R^2$) of the variance in non-adherence and correctly classified 72.9% of cases. The area under the curve for this logistic regression model was 0.787 (0.708, 0.866; $p<0.001$) suggesting a fair level of discrimination based on the five simple clinical parameters used (**Figure 2.4**).

In this model, increasing age was associated with reduced OR of non-adherence and a higher clinic SBP, increasing the number of antihypertensives and a prescription of CCBs were all associated with a higher OR of non-adherence in the urine AHS cohort. The probabilities of overall nonadherence obtained from this model are shown in **Figure 2.5**.

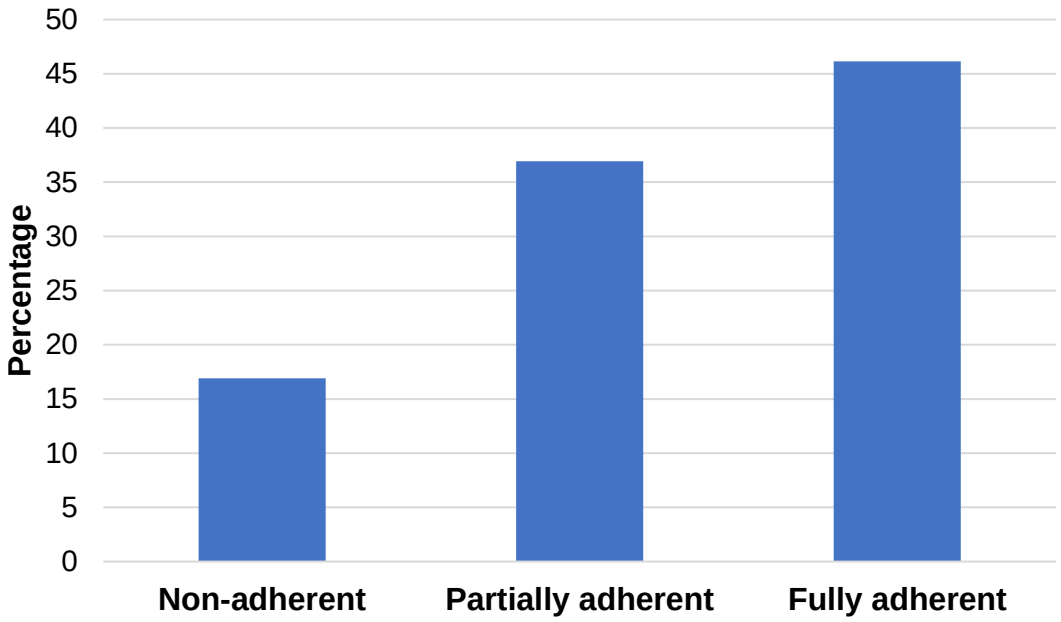



**Figure 2.3:** Percentage of adherent, non-adherent and partially adherent patients in the urine antihypertensive screen cohort.

**Table 2.3:** Univariable and multivariable logistic regression of gender, age, clinic SBP, number of all prescribed antihypertensives and prescribed CCB on non-adherence

| Variable | Unadjusted | | | Adjusted | | |
|---|---|---|---|---|---|---|
| | Odds Ratio | 95% CI | *P* | Odds Ratio | 95% CI | P |
| Female | 1.727 | 0.855, 3.491 | 0.128 | 2.516 | 1.088, 5.818 | 0.031 |
| Age | 0.965 | 0.937, 0.993 | 0.016 | 0.942 | 0.908, 0.977 | 0.001 |
| Clinic SBP | 1.017 | 1.001, 1.033 | 0.040 | 1.029 | 1.009, 1.050 | 0.005 |
| No. of all prescribed antihypertensives | 1.395 | 1.033, 1.884 | 0.030 | 1.567 | 1.082, 2.270 | 0.018 |
| Prescribed CCB | 6.000 | 2.881, 19.143 | 0.002 | 6.023 | 1.658, 21.887 | 0.006 |
| Nagelkerke pseudo $R^2$ | | | | 32.3% | | |
| Chi-square | | | | 35.66, df=5, p<0.001 | | |

**Abbreviations:** SBP, systolic blood pressure; CCBs, calcium channel blockers.

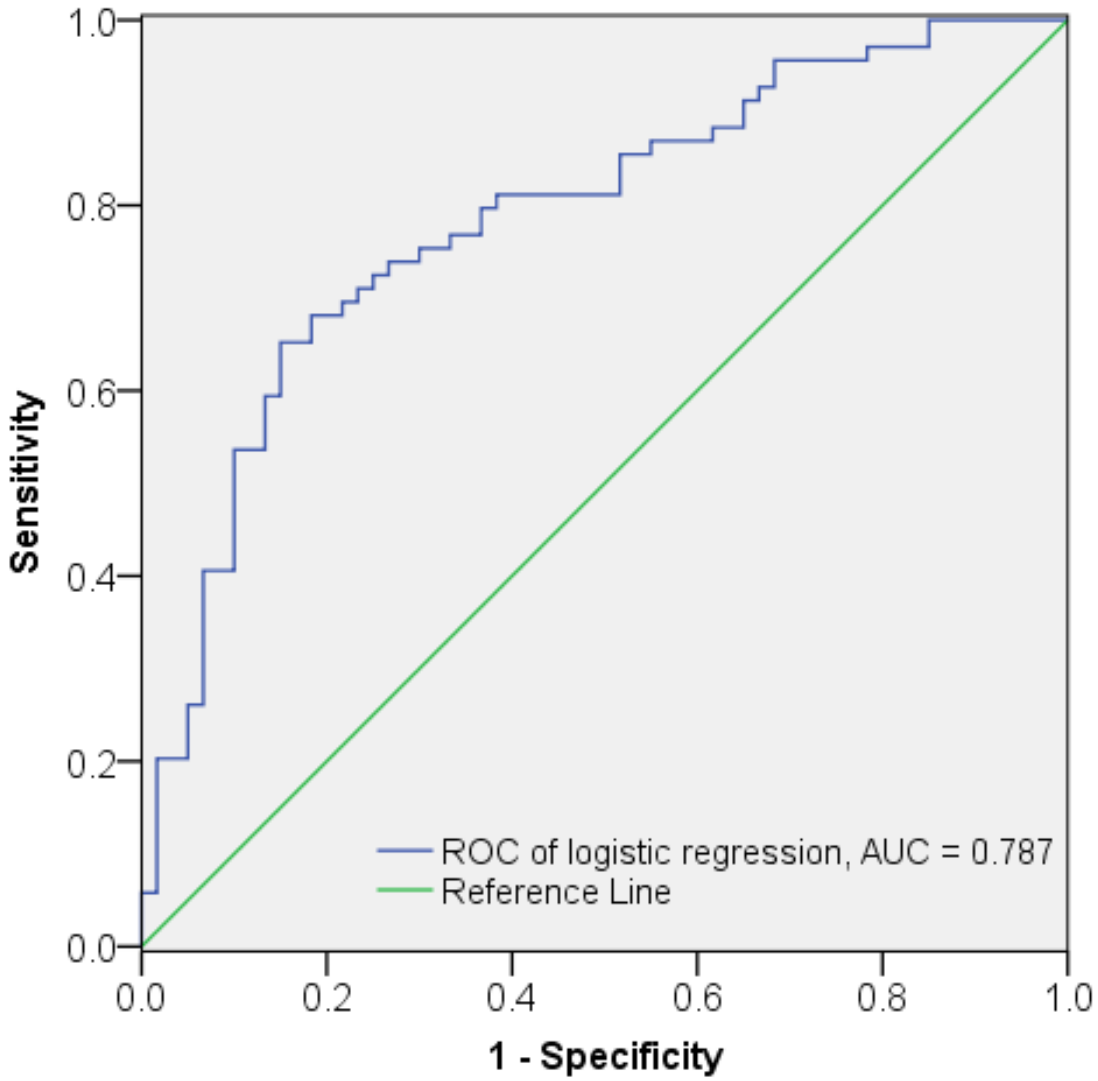



**Figure 2.4:** Receiver operator characteristic (ROC) curve of logistic regression model

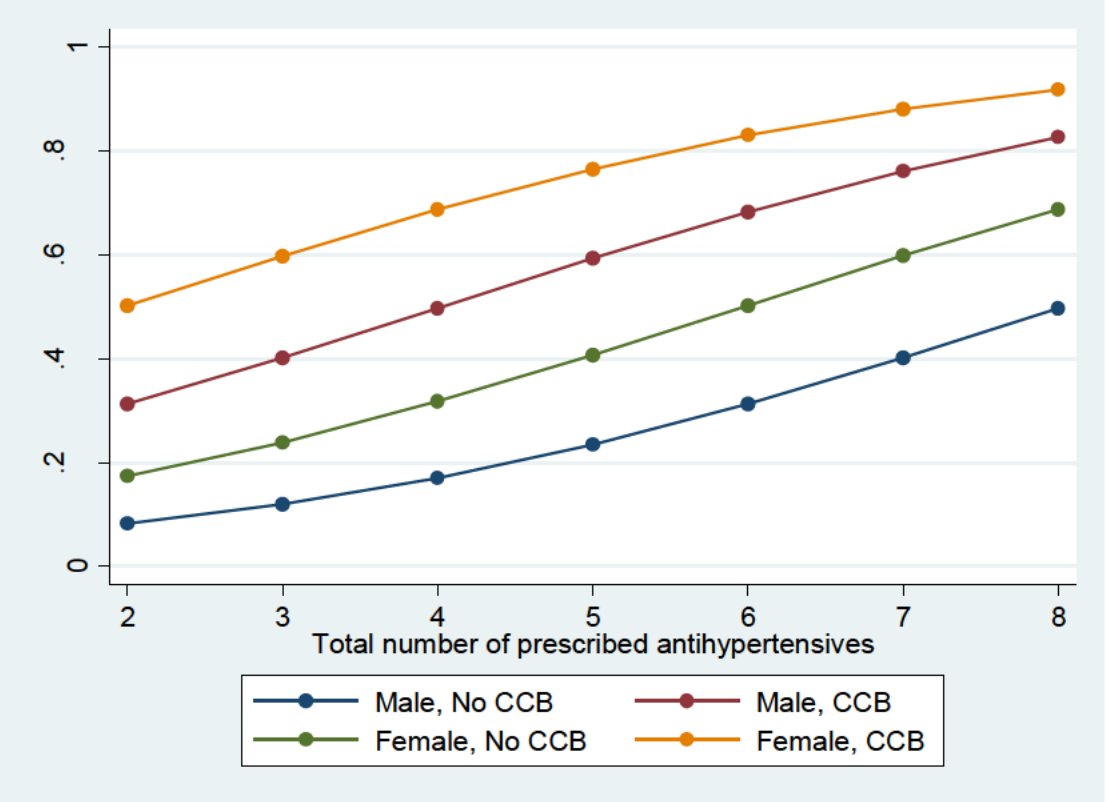



**Figure 2.5:** Predicted probability of nonadherence by gender, prescription of calcium channel blockers (CCB) and total number of prescribed antihypertensives.

### 2.3.5 Medications in the study population

Number and class of antihypertensive medications prescribed to patients in both cohorts was available, however the number of any prescribed medications was only available for the urine AHS cohort. Both cohorts were prescribed a similar number of antihypertensives; the median (IQR) number in DOT and urine AHS cohorts was 5 (4-5) and 4 (3-5) respectively. The distribution of number of antihypertensives prescribed is shown in **Figure 2.6**.

The class of antihypertensive prescribed was generally similar in both groups; angiotensin II receptor blockers, beta-adrenergic receptor antagonists and loop diuretics were more commonly prescribed in the DOT cohort whilst thiazide diuretics were more commonly prescribed in the urine AHS cohort. The

distribution of different classes prescribed in the two cohorts is shown in **Figure 2.7**.

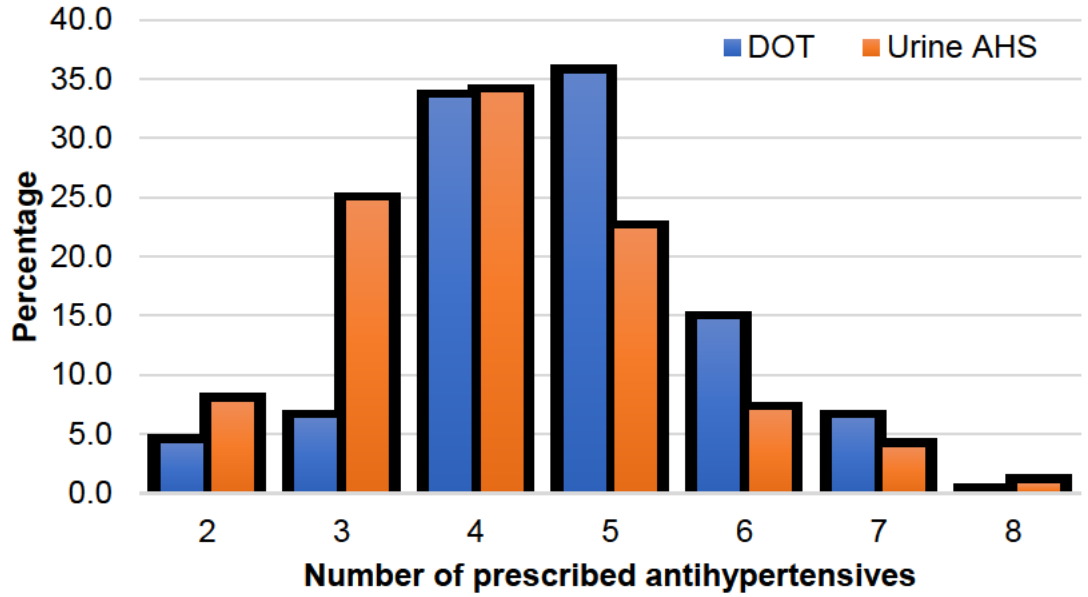



**Figure 2.6:** Number of antihypertensives prescribed in directly observed therapy (DOT) and urine antihypertensive screen (AHS) cohorts.

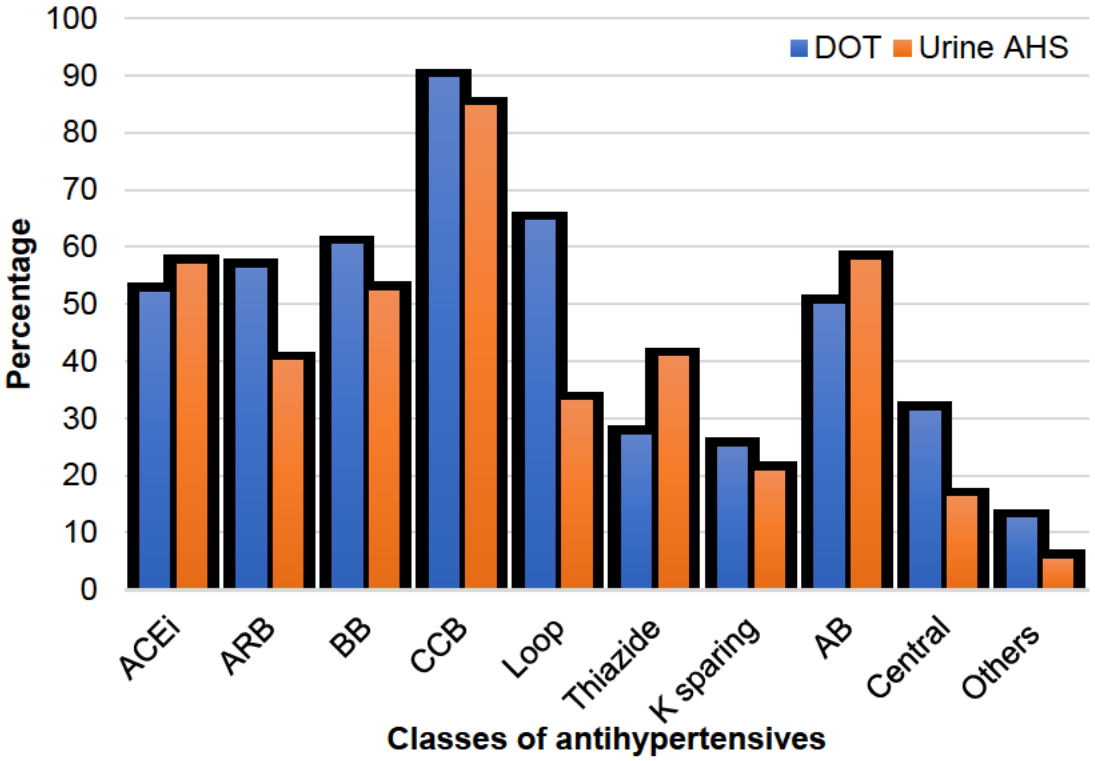



**Figure 2.7:** Classes of antihypertensives prescribed in directly observed therapy (DOT) and urine antihypertensive screen (AHS) cohorts.

**Figure 2.8** shows the total number of prescriptions to each class of antihypertensive and the total number detected on urine AHS. Calcium channel blockers had the highest level of nonadherence at 45.5% whereas beta-adrenergic blockers had the lowest rate of non-adherence at 28.3%. Non-adherence rate for RAS inhibitors, diuretics and others were 38.7%, 37.8%, and 31.2% respectively.

Further analysis of pattern of antihypertensives was performed. Number of prescribed antihypertensives divided in tertiles were compared for any associations across age in quartiles, gender, number of comorbidities and individual comorbidities including diabetes mellitus, cerebrovascular disease and ischaemic heart disease (see **Table 2.4**). A statistically significant association was present with number of co-morbidities (p=0.005), diabetes mellitus (p<0.001) and ischaemic heart disease.

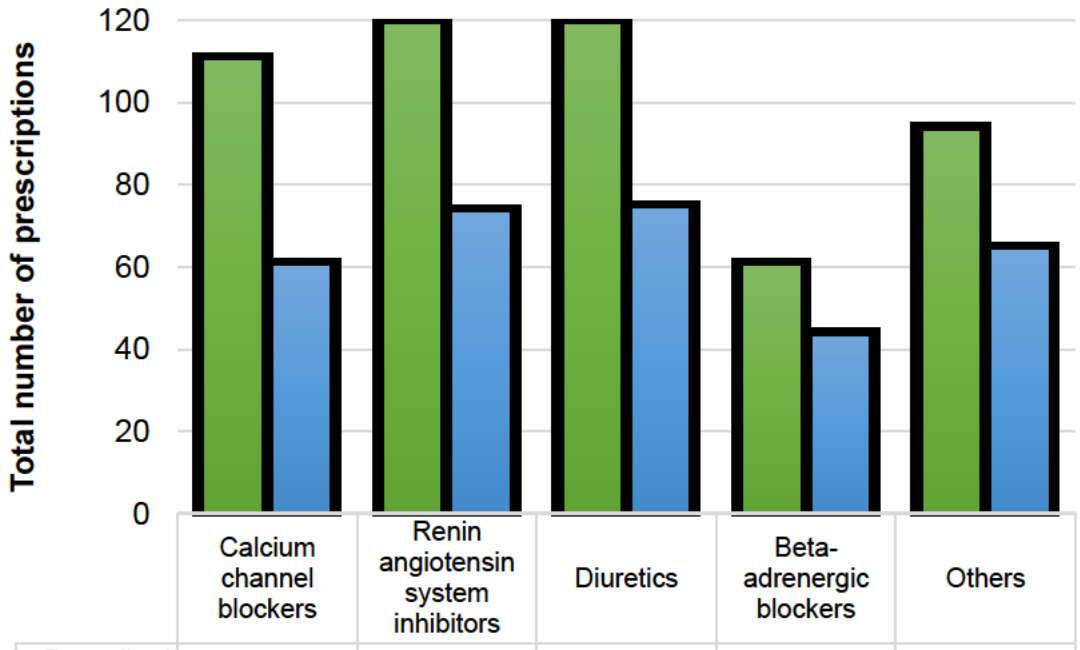



**Figure 2.8** A graph showing the number of medications in each antihypertensive class prescribed and detected in urine.

**Table 2.4** Association of number of antihypertensives prescribed with age, gender and comorbidities.

| n (%) | Number of AHTs in tertiles | | | p-value |
|---|---|---|---|---|
| | 1 – 3 | 4 | 5-8 | |
| **Total** | 42 | 44 | 44 | |
| **Age quartiles, years** | | | | |
| 18 – 50 | 16 (38) | 9 (20) | 7 (16) | |
| 51 – 60 | 8 (19) | 13 (30) | 12 (27) | 0.189 |
| 61 – 70 | 11 (26) | 12 (27) | 10 (23) | |
| >71 | 7 (17) | 10 (23) | 15 (34) | |
| **Gender** | | | | |
| Male | 21 (50) | 26 (59) | 26 (59) | 0.621 |
| Female | 21 (50) | 18 (41) | 18 (41) | |
| **Number of comorbidities** | | | | |
| 0 | 29 (69) | 21 (48) | 17 (39) | 0.005 |
| 1 | 10 (24) | 18 (41) | 13 (29) | |
| 2 – 3 | 3 (7) | 5 (11) | 14 (32) | |
| **Comorbidities** | | | | |
| Diabetes mellitus | 3 (7) | 15 (34) | 21 (48) | <0.001 |
| Cerebrovascular disease | 8 (20) | 6 (14) | 7 (16) | 0.762 |
| Ischaemic heart disease | 3 (7) | 8 (18) | 15 (34) | 0.008 |

All data is presented as counts (percentage is expressed as a proportion of the corresponding column total).

Association between class of antihypertensive prescribed (RAS inhibitors, beta adrenergic receptor blockers, CCB, diuretics and others) was also examined across the same parameters and are described in **Table 2.5**. A statistically significant association was only present between a prescription to diuretics and age in quartiles (p=0.009) as well as between a prescription to a RAS inhibitor and a previous history of cerebrovascular disease (p=0.038).

A prescription to a CCB was explored further in detail due to it being a strong predictor of non-adherence in the binary logistic model (see **Table 2.3**). The distribution of different CCBs prescribed is laid out in **Figure 2.9**. Amlodipine was by far the commonest CCB prescribed in this cohort at 59% (n=65). Further

sensitivity analysis suggested that patients with a prescription of a dihydropyridine CCB were more likely to be associated with non-adherence than the non-dihydropyridine CCB (**Table 2.6**). In addition, a prescription of amlodipine showed no significant association to adherence status (**Table 2.6**).

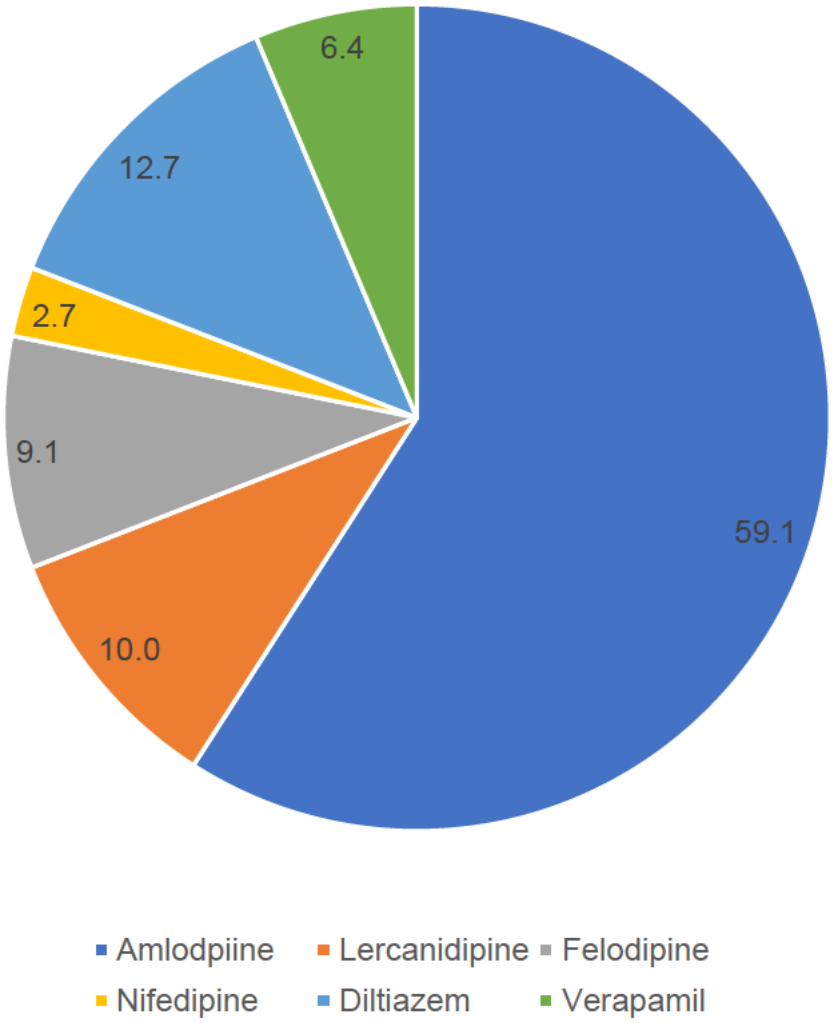



**Figure 2.9** Percentage of different calcium channel blockers prescribed in the urine antihypertensive screen cohort.

**Table 2.5** A table showing association of prescription of different classes of antihypertensives with age, gender and co-morbidities

| n (%) | Total | Renin angiotensin system inhibitors | Beta-adrenergic blockers | Calcium channel blockers | Diuretics | Others |
|---|---|---|---|---|---|---|
| **Age quartiles, years** | | | | | | |
| 18 – 50 | 32 | 32 (100) | 13 (41) | 30 (94) | 18 (56) | 18 (56) |
| 51 – 60 | 33 | 31 (94) | 20 (61) | 30 (91) | 29 (88) | 20 (61) |
| 61 – 70 | 33 | 28 (85) | 16 (49) | 25 (76) | 27 (82) | 23 (70) |
| >71 | 32 | 30 (94) | 19 (59) | 25 (78) | 27 (84) | 24 (75) |
| **Gender** | | | | | | |
| Male | 73 | 68 (93) | 36 (49) | 65 (89) | 56 (77) | 50 (69) |
| Female | 57 | 53 (93) | 32 (56) | 45 (79) | 45 (79) | 35 (61) |
| **Number of comorbidities** | | | | | | |
| 0 | 67 | 63 (94) | 30 (45) | 57 (85) | 47 (70) | 39 (58) |
| 1 | 41 | 37 (90) | 24 (59) | 35 (85) | 35 (85) | 29 (71) |
| 2-3 | 22 | 21 (96) | 14 (64) | 18 (82) | 19 (86) | 17 (77) |
| **Comorbidities** | | | | | | |
| Diabetes mellitus | 39 | 39 (100) | 22 (56) | 33 (85) | 35 (90) | 31 (80) |
| CVA | 21 | 17 (81) | 11 (52) | 19 (91) | 17 (81) | 13 (62) |
| IHD | 26 | 24 (92) | 18 (69) | 20 (77) | 23 (89) | 21 (81) |

All data is presented as counts (percentage is expressed as a proportion of the corresponding row total). Statistical significance was only present between a prescription to diuretics and age (P=0.009) and between prescription to a renin angiotensin system inhibitor and previous history of CVA (P=0.038). Abbreviations: CVA, cerebrovascular disease; IHD, ischaemic heart disease.

**Table 2.6** Association between adherence status and type of calcium channel blocker prescribed

| n (%) | Total | Adherent (n=60) | Non-adherent (n=70) | p-value |
|---|---|---|---|---|
| **Prescription to dihydropyridine CCB** | | | | |
| Yes | 89 | 35 (39) | 54 (77) | 0.035 |
| No | 41 | 25 (61) | 16 (23) | |
| **Prescription to non-dihydropyridine CCB** | | | | |
| Yes | 21 | 9 (15) | 12 (17) | 0.927 |
| No | 109 | 51 (85) | 58 (83) | |
| **Prescription to amlodipine** | | | | |
| Yes | 65 | 26 (43) | 39 (56) | 0.218 |
| No | 65 | 34 (57) | 31 (44) | |

All data is presented as counts (percentage is expressed as a proportion of the corresponding column total). **Abbreviation:** CCB, calcium channel blocker.

## 2.4 Discussion

In this study, two different direct methods to assess adherence have been able to show that in a cohort of patients with apparent TRH, who were on 3 or more antihypertensive agents, at least half had suboptimal adherence.

In the DOT cohort, only half (50%) had true TRH. The other half were non-adherent to prescribed antihypertensive medication with a mean drop in 24-hour ABP of 19/9 mmHg after supervised administration of antihypertensive medications; in 25% patients at least one medication had to be omitted for symptomatic hypotension. There was no association between true TRH and demographic and other parameters.

In the urine AHS cohort, 60 (46.2%) patients took all of their antihypertensives medications as prescribed and hence had true TRH. Unlike DOT, urine AHS can provide direct quantitative assessment of degree of non-adherence, showing that 22 (16.9%) patients were completely non-adherent.

Binary logistic regression modelling, in the AHS cohort, has shown that gender, age, clinic SBP, number of all prescribed antihypertensive medications, and prescription of a CCB were all significantly associated with non-adherence (**Figure 2.5**).

In this study females were more likely to be non-adherent. The effect of gender on non-adherence has been described in the literature, where either no association between gender and non-adherence (511, 512) or higher likelihood of non-adherence in females (371, 513, 514) has been described in hypertensive populations. Although the underlying mechanism for this association is unclear,

there are likely to be other gender-specific factors which may be responsible for such observations. Different behavioural attitudes have been observed in males and female with more men admitting to forgetting, changing the dosage and that they had recovered, compared to more women reporting filling the prescription but not taking the drug and reporting the development of adverse drug reactions as a reason for non-adherence (515). In hypertensive men, factors associated with low adherence include sexual dysfunction and BMI ≥25 kg/$m^2$. In contrast, dissatisfaction with communication with their healthcare provider and depressive symptoms were associated with low adherence in women (516).

Like gender, reported evidence examining the association between age and non-adherence is inconsistent. Non-adherence has been shown to be associated with younger age (371, 512, 514, 517), older age (513) and no association at all (511, 518). Younger patients are likely to be working with busier lifestyles and may have higher tendency of unintentional non-adherence. Lifelong treatment for a chronic illness which is largely asymptomatic may also be a contributing factor to non-adherence in the young. Conversely, it can be argued that the older patients, with greater severity of illness, are more motivated to adhere. The very old patients may have carers who help to ensure optimal adherence. Furthermore, these patients may represent a survivor cohort effect, implying successful medication-taking behaviour.

Higher SBP was associated with increased non-adherence in this study. The published evidence has shown that uncontrolled BP (519-521) and an elevated SBP (522, 523) is associated with non-adherence. McNaughton et el. showed that SBP in patients prescribed ≥3 antihypertensives was 20.8 mmHg higher in

those who were non-adherent when compared to those who were adherent (523). These findings are consistent with non-adherence and highlight the importance of assessing adherence in treated hypertensive patients with uncontrolled BP.

Association of non-adherence with increasing the number of antihypertensive medications is an important finding which has been echoed in the published literature (371, 383, 518, 524). It makes common sense that polypharmacy increases risk of non-adherence. Additionally, intentional non-adherence may promote overprescribing exacerbating the problem. A direct and objective adherence method such as the urine AHS can identify intentional non-adherence in this case. It is then, however, of no surprise that in patients with TRH, often requiring in excess of three antihypertensives, non-adherence is highly prevalent prompting the need to consider single-pill FDCs in such patients. The benefits and evidence to support use of single-pill FDCs have already been in discussed in detail earlier in section 1.5.5.6.

Previous finding of highest risk of nonadherence with diuretics (371, 525), when compared to other classes of antihypertensives, is not replicated in our logistic regression model. Instead, prescription to a CCB was predictive of non-adherence. The reason for this discrepancy is unclear, but two major differences exist between our study and a metanalysis examining the impact of drug class in non-adherence to antihypertensives (525). Firstly, patients in the urine AHS cohort were taking at least 3 or more antihypertensives of different classes whereas the included studies in a meta-analysis included patients with any number of antihypertensives; the authors admitting that their finding of diuretics as being the most common class of antihypertensive associated with non-

adherence cannot be generalised to those taking ≥1 antihypertensive. Second, this meta-analysis excluded studies reporting cross-sectional studies reporting adherence at a single point in time, therefore direct comparison with our results is inappropriate.

Exploratory analysis of number and class of antihypertensives prescribed in the AHS cohort suggested that patients with diabetes mellitus and ischaemic heart disease were more likely to be prescribed a higher number of antihypertensive medications. This is not a surprising finding given that some of the common antihypertensives can be used for multiple indications in these conditions.

Further subgroup analysis of CCBs showed that a prescription to dihydropyridines was most likely to be associated with nonadherence; however, amlodipine which accounted for 73% of the dihydropyridine prescriptions was not associated with non-adherence. It is possible that failure of the urinary assay to adequately detect some or all of the CCBs could explain the high level of non-adherence. Whilst it has been shown that some CCBs such as nifedipine can be unstable in urine but the assay was still sensitive enough to detect it for a qualitative assessment of adherence (493). The commonest prescribed CCB, amlodipine, has an elimination half-life of around 36-48 hours (526). The urine AHS has a lower limit of detection of 1ng/mL and the median (IQR) of detected levels present in patients' urine have been shown to be 445.2 (316) µg/L (493). Although, a long half-life means that it will take a few days to reach a steady state, the assay is sensitive enough to detect the drug taken at least 12 hours ago and more importantly an undetectable level is suggestive of nonadherence which is

more than just forgetting to take a single dose of the drug the day before the before the test.

When samples of the AHS cohort were combined with samples sent to the BHH laboratories from 8 other regional hypertension centres; CCBs remained a strong predictor of non-adherence in the combined sample of 300 patients (527). Possible explanation for this association could be explained by adverse effects associated with dihydropyridines particularly ankle swelling. A recent study has demonstrated that an independent association of non-adherence between a diuretic, hydroclorothiazide, and a CCB, nifedipine, is attributable to the adverse effects of these medications (528). These findings are further supported by a recently published study with the aim to assess pattern of prescribing following initiation of a CCB (529). Savage et al. showed that older adults with hypertension who are newly dispensed a CCB subsequently receive a loop diuretic presumably to counteract the commonly observed side effect of peripheral oedema associated with a CCB (529). Nevertheless, this finding should prompt the clinicians to assess impact of side-effects and pharmacological interactions of commonly prescribed antihypertensives.

Oral medication, like intravenous dosing of drugs, provides a steady state blood level after five half-lives (530). In the DOT clinic, only a single dose of every prescribed medication was administered in patients who might not have taken their drug(s) over a long period. So, a single dose of a drug after a period of non-concordance would not be expected to produce a therapeutic steady state level. This might be expected to often lead to a less than optimal BP response especially in completely non-adherent patients. However, the directly observed

administration of multiple different drugs in non-adherent patients to some extent overcomes this ‘pharmacokinetic handicap.’ Some antihypertensives are known to cause a first-dose phenomenon which is where a sudden, often severe, BP drop occurs after the first dose or resuming the drug after a long period of abstinence. Alpha-adrenergic antagonists can commonly cause first-dose hypotension; the effect seems to be pronounced with concomitant use of beta-adrenergic antagonists (531). Similarly, ACE inhibitors exhibit this phenomenon particularly in patients with volume contraction and in those whose BP is Ang II dependant, such as congestive cardiac failure (532). However, unlike the alpha-adrenergic antagonists, with ACE inhibitors the first-dose hypotensive effect is differential across the class (533). Nevertheless, the varying potency and half-lives of the drugs administered means that the BP response rate of 50% may be an underestimate of true non-adherence in the DOT cohort.

In the DOT cohort, an arbitrarily definition of a drop in average 24-hour systolic ABP of ≥5 mmHg between Pre-DOT and Post-DOT measurements in an individual patient was used as an indication of non-adherence in the absence of a clear definition in the literature. ABP has greater reproducibility when compared to office BP and shows very little intra-patient variability (506, 507). In the group deemed to be truly treatment-resistant there was a mean rise in average 24-hour ABP of 11/5 mmHg, while in the those deemed non-adherent there was a mean reduction of 19/9 mmHg between pre-DOT and Post-DOT ABP ($p<0.001$). Larger studies will need to be conducted in the future to clarify the best BP cut point to indicate non-adherence. Interestingly, the level of suboptimal adherence estimated in this study through detection of antihypertensives in the urine is

broadly similar to DOT suggesting a degree of agreement in both methods of non-adherence.

Non-adherence rate of at least 50% in our study is consistent with the results of a UK study showing non-adherence rate of 60% in people referred to a hypertension clinic with uncontrolled hypertension. This study also administered antihypertensive medication under supervision in a clinic setting. However, this study had a smaller sample size and BP control was assessed mainly by office readings (361). Moreover, directly observed administration of antihypertensive medications has been used in assessment of adherence in patients with apparent TRH before RDN (534, 535).

The findings of our study also echo the results of a study assessing adherence to antihypertensive medications in patients with apparent TRH by means of toxicological urine screening, with a reported non-adherence rate of 53% (536). Another study assessing the adherence to antihypertensive medications through toxicological urine screening reported a non-adherence rate of 25% (494). However, the majority (60%) of these patients in this study were new referrals from primary care and the authors did not specify whether the remaining had TRH (494). Serum toxicological analysis of antihypertensive drug levels has also been used in patients with apparent TRH to differentiate non-responsiveness from non-adherence to recommended therapy (359, 378).

Non-adherence is common among people with chronic diseases. A meta-analysis involving 376,162 patients from 20 studies assessing adherence to 7 drug classes that prevent cardiovascular disease (aspirin, statins and 5 classes of antihypertensive drugs) showed similar results (326). The estimate for

adherence across all studies was 57% with a considerably higher rate of adherence to medication for secondary versus primary prevention: 66% versus 50% respectively (326). However, the studies included in the meta-analysis used prescription refill frequency to estimate adherence which tends to overestimate adherence as prescription refill is used as a surrogate for the patient actually taking the medication in question.

It has been demonstrated that only about 10% to 15% of the patients with treated hypertension are ‘still engaged’ with their antihypertensive drug regimens at 5 or more years after the initiation of treatment (537). In this study, the duration of hypertension was only available for the DOT cohort. The median duration of hypertension in this cohort was 12 years which may explain the level of non-adherence in our study population.

Although adherence is quite commonly quantified, aiming to achieve a certain level of adherence is only useful in conjunction with the pharmacokinetics of medications and observed effects of intended therapy. For instance, a certain drug may still achieve its intended effect even if the patient takes 80% of the prescribed doses depending on the pharmacokinetic profile of the drug in question. It has been demonstrated that patients with adherence of less than 90% to their prescribed antihypertensive therapy start to experience a rise in their BP (368). Therefore, in the urine AHS cohort patients were only deemed to be adherent, if all their usual prescribed antihypertensives that are detectable were present in the urine.

The study population is representative of patients with TRH as it included patients who meet criteria for the diagnosis of TRH with a mean office BP of ≥178/94

mmHg and a median antihypertensive medication burden of ≥4 agents across the two cohorts. Ten (7.7%) patients in urine AHS cohort and 2 (4.2%) patients in the DOT were taking 2 medications for the treatment of their hypertension as they had intolerances to multiple antihypertensive agents whereas the rest were all taking at least three medications (**Figure 2.6**). As many as 81.5% of all the patients were taking at least a single diuretic, 91.0% were on a renin-angiotensin system blocking agent and 79.8% were on a CCB as part of their antihypertensive regime. These results highlight that the study population are a highly specific subgroup of hypertensive patients with severe hypertension despite aggressive pharmacological management of their hypertension and counselling including that for adherence.

Not all of the patients identified as being non-adherent achieved target BP control after administration of medication in direct supervision. There was, however, a trend towards a greater proportion achieving the target for the night-time ABP; it is likely that the medications are producing the greatest effect at night due to the staggered nature of dosing at the DOT clinic. As discussed before, a drug takes up to 5 half-lives to achieve a steady-state plasma level (530) and hence in completely non-adherent patients the greatest effect of their medications on their BP will not be apparent after a single dose at the DOT clinic. This is even more likely to be the case in the DOT cohort as they had uncontrolled grade III hypertension (pre-DOT clinic BP of 185.8/99.3 mmHg). Furthermore, almost a quarter of the patients had at least one medication omitted at the DOT clinic to prevent significant hypotension or for symptoms of hypotension and in some patients up to 3 medications. Omitting medications at the DOT clinic will have no doubt underestimated the true BP reduction and hence fewer patients achieved

the ambulatory BP targets. In a small proportion of the patients' drug-attributable side-effects were observed by the hypertension specialist nurse (data not shown). This may suggest that non-adherence in these patients may have been because of side effects.

The strengths and weakness of different methods of assessing adherence have been discussed earlier in section 1.5.5.4. The main advantage of administering the prescribed antihypertensive regime under direct observation, in contrast to other methods of assessing adherence including urine AHS, is that the resultant effect of the treatment is immediately apparent by monitoring the patient's BP. Comparison of ABPMs allows the clinician to demonstrate to patient that their BP is responsive to medications and patients can be directly observed for any unreported side-effects attributable to their medications which may have been affecting their adherence. This method, however, is resource intensive; although patients are not required to be admitted to hospital hence saving bed days, healthcare professional(s) and the patient are still required to be present for direct observation of medication ingestion and monitoring for any potential side-effects of significant hypotension.

There is no agreed definition to define non-adherence in response to observed antihypertensive treatment. The cut-off of 5 mmHg 24-hour SBP in this study was based on what was considered a clinically meaningful BP reduction. Decreases in cardiovascular morbidity and mortality have been observed with small reductions in SBP (2 to 5 mm Hg) with antihypertensive drug therapy (508, 538). Furthermore, a 5-mmHg reduction in systolic ABP has been used in clinical trials as an end point (480, 486, 490). However, the BP reductions observed in this

study could be coincidental. These could be explained by various phenomena including regression to the mean, usual BP variation and adaptation.

BP exhibits regression to the mean where long-term BP is less extreme than baseline (539-541). Indeed, studies with higher baseline BPs are likely to have a greater ‘drop’ on subsequent measurements in the placebo groups (541). Recently Moore et al. have shown for the first time that ABPM is also susceptible to regression to the mean (542). Therefore, it is possible that a reduction of 5mmHg or more in some of the patients in the DOT cohort is simply a statistical consequence of regression to the mean and not an indicator of actual non-adherence. Conversely, it is worthwhile noting that these patients had multiple attendances at the hypertension clinic before assessment of adherence was carried out. A persistently uncontrolled BP was a mandatory requirement for assessment of adherence and may have minimised the effect of regression to the mean.

Secondly, BP is a dynamic physiological parameter and shows variability throughout the day and has been discussed in **section 1.3.1**. The indices used to assess short-term BP variability include 24-hour SD, 24-hour weighted SD, coefficient of variation, and average real variability (543). All the raw BP data and/or 24-hour SDs are needed to evaluate BP variability and in this study, it was not possible to obtain this information; only the average BPs could be found from clinic letters or the hypertension database. It was therefore not possible to assess the usual BP variability. However as mentioned earlier, BP variability is positively correlated with MAP (14) and in this cohort of poorly controlled hypertensives with

high baseline BPs the BP variability is likely to be high and could easily account for a 5mmHg cut-off.

These statistical indices of BP variability are far from ideal (544); 24-hour SDs and coefficient of variance are poorly reproducible(544) and average real variability requires up to 48 BP readings over 24 hours without meaningful loss of prognostic information (545). The current NICE Hypertension guidelines recommend BP measurements should be programmed to repeat at least every 30 minutes during the day and hourly overnight and a minimum of 14 daytime readings needed for diagnosis (546). This protocol was followed for all patients undergoing ABPM at the WMHC and it would only lead to a maximum of 40 readings if 100% of all the measurements yielded a BP reading. In reality, this is often not the case due to poor compliance to instructions by the patient and other technical issues. It is possible to program to repeat BP measurements every 20 or even 10 minutes which will yield more usable BP readings at the expense of tolerability of the procedure. For the same reasons relating to lack of availability of raw data, the adequacy of the ABPM could not be confirmed. However, NICE guidelines for measurement of ABPM were followed for all patients where only patients with at least 14 daytime measurements are accepted as having an adequate ABPM (546).

Although the SDs reported in **Table 2.2** are high, these do not represent intra-patient variability and only indicate the variation observed within the average ABP readings of the entire DOT cohort. As discussed, the intra-patient BP variability could not be examined.

Thirdly, adaptation which is where BP decreases in the first 2 hours of recording, has been shown to in ABPM and is reduced on subsequent measurements in both normotensive (547) and hypertensive individuals (548). This phenomenon may also explain some or all the BP reduction observed in the DOT cohort.

However, allowing for the aforementioned limitations, if higher cut-offs of 10, 15 or 20 mmHg BP drop are considered then the non-adherence rate falls to 42%, 32% or 20% respectively.

A percentage reduction in BP from baseline is an alternative method that could have been used. This method could counter some of the effects of regression to the mean and adaptation by taking into account the baseline ABP. However, an arbitrary cut-off will again have to be used given that most clinical trials and clinical guidelines of hypertension focus on actual BP change rather than a percentage change. When analysed, the mean (SD) percentage change in average 24-hour SBP and DBP was -2.0% (13.8) and -3.7% (13.4) respectively.

Though chemical markers of antihypertensive drugs in either urine or serum unequivocally establish whether the drugs have been ingested by the patients this method doesn't rule out pharmacological non-response to a particular drug. Differences in metabolism of drugs in individual patients may also impact on the use of chemical markers to assess adherence. In addition, random sampling alone does not confirm adherence or lack thereof as it cannot provide information on when the doses were taken or omitted. Furthermore, there is the ethical issue of informing patients of the proposed test. Prior knowledge of testing schedule may promote white-coat adherence (348). However, a small retrospective study has shown that serum therapeutic drug monitoring, when combined with

counselling, for patients with apparent TRH found to be non-adherent, resulted in markedly improved BP control without increasing the treatment intensity (375). This method may have a role in serial monitoring of adherence at each clinic visit which may help to improve adherence, however the cost of repeated measure may be prohibitive to this approach.

Although this study shows a direct effect of adherence on BP control, this effect has been difficult to establish in the past as highlighted in a systematic review carried out to establish whether poor compliance leads to inadequate BP control (549). Two common reasons are: firstly, only patients with high drug adherence agree to take part in clinical trials assessing their adherence leading to selection bias; and secondly the act of assessing their adherence may lead to actual adherence in all groups being tested, including the control group, negating any change that may have been observed resulting in the absence of any statistically significant differences in BP. Indeed, some studies have used the principle that actively measuring adherence may lead to significant improvement in BP control and prevents unnecessary treatment escalation (368, 550).

Due to the relatively high resource requirements of DOT, adherence assessment was carried in patients suspected of TRH after WCH and secondary causes of TRH had been systematically excluded and patients had seen a clinical pharmacist for treatment optimisation. Therefore, the DOT cohort represent a highly selective cohort of patients meaning that the results of this cohort cannot be generalised to the wider population with hypertension but remain relevant to patients with suspected TRH. Conversely, due to the relatively reduced patient burden and the cost-efficiency of urine AHS, this test was carried out concurrently

with other investigations in all patients suspected of TRH. The results were used to then optimise antihypertensive management in these patients. Comparatively, therefore the results from AHS cohort are more widely applicable to patients suspected of TRH but will still only be relevant to a small proportion of patients overall in the general hypertensive population.

The main limitation of this study, in addition to the retrospective nature, is that both methods only tests adherence at a single point in time. The lack of any significant association between non-adherence and baseline characteristics in the DOT cohort may be due to lack of power in a study with a small sample size. Given the limitations, this study suggests that a substantial percentage of apparent TRH patients are non-adherent to prescribed treatment; with females, younger age, clinic SBP, number of prescribed antihypertensives and prescription of a CCB shown to be associated with non-adherence. However, the reasons for non-adherence are likely to be complex and multifactorial. Psychological or psychosocial assessment of the non-adherent individuals may shed some light on the underlying factors driving non-adherence. This study is also unable to suggest possible solutions to the problem; further research is required to explore effective interventions to improve non-adherence and ultimately disease control. One can hypothesise that psychological counselling or motivational interviewing (551) may help, as may single-pill FDC therapy (552).

# CHAPTER 3 RENAL SYMPATHETIC DENERVATION IN MODERATE-TO- SEVERE CHRONIC KIDNEY DISEASE USING A NOVEL NON-IODINATED CONTRAST FREE PROTOCOL

## 3.1 Background

Hypertension is a risk factor for cardiovascular disease and CKD, if remains uncontrolled can lead to rapid progression of CKD, and is a leading cause of ESRD. On the other hand, CKD itself can exacerbate hypertension, often necessitating the use of a combination of antihypertensive drugs of different classes to bring raised BP under control. Compared with people without CKD prevalence of TRH is reported to be twice as high in patients with CKD and in the 3,612 CRIC study participants with CKD, 42% had apparent TRH (450).

Patients with CKD have a much higher risk of cardiovascular disease when compared to patients without CKD; meta-analyses show impaired renal function to be an independent risk factor for cardiovascular disease (553). Vascular calcifications are more pronounced in CKD and may be partly responsible for the excess cardiovascular morbidity and mortality observed in CKD patients (554). Moreover, improvement in BP control is known to slow the decline of renal function in patients with CKD (555).

The pathophysiology of hypertension in CKD is multifactorial with sodium retention and excessive activation of the RAAS being most important. Sympathetic nervous system is also overactive and has been implicated to play role in the progression of CKD (556). In ESRD, SNA is substantially increased and elevated noradrenaline plasma levels have been linked with poor cardiovascular outcomes (476, 557). Recently, procedure-based approaches have been investigated in patients with TRH to achieve BP control. Percutaneous catheter-based radiofrequency ablation of afferent and efferent sympathetic nerves of renal arteries is one such technique and is the most widely researched to date. Due to the risk of contrast-induced nephropathy associated with the use of iodinated contrast materials used, patients with eGFR <45 ml/min/1.73m$^2$ have been excluded from major trials of RDN. Only a handful of pilot studies thus far have investigated the use of RDN in patients with moderate to severe (eGFR 15-44 ml/min/1.73m$^2$) renal impairment (558-562).

Carbon dioxide ($CO_2$) angiography is a well-recognised and safe alternative to iodine-based contrast material in patients with CKD or iodinated contrast agent allergy (563-565). A negative contrast image with digital subtraction angiography is produced when the directly injected $CO_2$ displaces blood intravascularly. $CO_2$ angiography has been shown to prevent deterioration of renal function in patients with known renal impairment and has been successfully used in such patients for various diagnostic and therapeutic indications (558, 564-566).

In this pilot study, the feasibility of RDN is examined in patients with significant renal impairment (eGFR 15 - 45 ml/min/1.73 m$^2$) performed using $CO_2$ as the sole contrast agent. The primary aim of the study of renal denervation using carbon

dioxide angiography was to assess the safety of RDN using CO2 angiography (a novel contrast agent for renal denervation) in patients with severe CKD. The study was conceived and started before the publication of the Symplicity HTN-3 study with a view to design a substantive trial RDN in patients with severe CKD.

## 3.2 Methods

### 3.2.1 Patient selection

All patients, aged 18-75 years, were recruited from screening patients attending the outpatient nephrology and specialist hypertension clinics at Heart of England NHS Foundation Trust in Birmingham. Patients were screened based on their outpatient clinic SBP, number of antihypertensive medications used, and outpatient eGFR. Only patients with TRH, clinic SBP ≥140 mmHg, taking at least three antihypertensive medications, and an eGFR between 15-44 ml/min/1.73m$^2$ were selected for RDN. Patients with eGFR ≥45 ml/min/1.73m$^2$ or <15 ml/min/1.73m$^2$, diagnosis of type I diabetes mellitus, substantial stenotic valvular heart disease, pregnancy or planned pregnancy during the study and, a history of MI, unstable angina, or cerebrovascular accident in the previous 6 months were excluded (see appendix I for study protocol).

### 3.2.2 Clinic visits

At baseline visit, clinic BP was assessed in a standardised manner following the British and Irish Hypertension Society (BIHS) guidelines. Briefly, an average of at least 2 SBPs was used to confirm systolic hypertension. BP was taken with patients resting in a chair for 5 minutes and a minute's interval between the end of one measurement and the start of the next measurement. All patients had a baseline serum electrolytes, urea and creatinine, urinary albumin-creatinine ratio

(ACR) and 24-hour ABP measured. Serum creatinine was used to calculate eGFR using the Modification of Diet in Renal Disease (MDRD) Study equation to confirm that the baseline eGFR met the inclusion criteria. Anatomical suitability for RDN was confirmed using non-contrast CT reconstruction of renal arteries. Baseline data recorded include basic demographics, comorbidities, and number and name of antihypertensive medications.

All patients were followed up at 7 days, 1, 3, and 6 months post-procedure to assess renal function, BP response and monitor for any potential procedural complications. At each visit medication review, clinic BP and urinary ACR was carried out. A repeat ABPM was performed at the 6-month follow-up visit to assess clinical response. There was no a priori restriction on changes to anti-hypertensive medications post RDN.

### 3.2.3 Carbon dioxide angiography and renal sympathetic denervation

Renal angiography was performed utilising $CO_2$ as the sole contrast agent to confirm the renal anatomy prior to performing RDN (567). The procedure was carried out, as a day-case, by two interventional radiologists in all patients both with considerable experience of both $CO_2$ angiography and RDN.

$CO_2$ requires specialist equipment to eliminate air contamination and excess compression whilst ensuring safe delivery. A home-made $CO_2$ delivery system (**Figure 5.1**), similar to Kawasaki et al (568), was used. The sealed system consisted of a gas cylinder, a 3-way stopcock, a $CO_2$ bag and a 20-ml Luer lock syringe. The source of $CO_2$ must be confirmed as a closed system circuit, free from air contamination. The closed system was filled with $CO_2$ and the gas purged

four times to minimize contamination. When the $CO_2$ bag was sufficiently filled, the $CO_2$ cylinder was disconnected to control volume injection and avoid excess delivery. Approximately 50 ml was then discarded from the system to reduce air contamination from the connective tubing. Prior to direct injection of $CO_2$ contrast agent, the syringe, $CO_2$ bag and sheath were connected via a 3-way stopcock. The RDN technique used is based on that described in the Symplicity HTN-2 trial and previous publications (478, 564, 569).

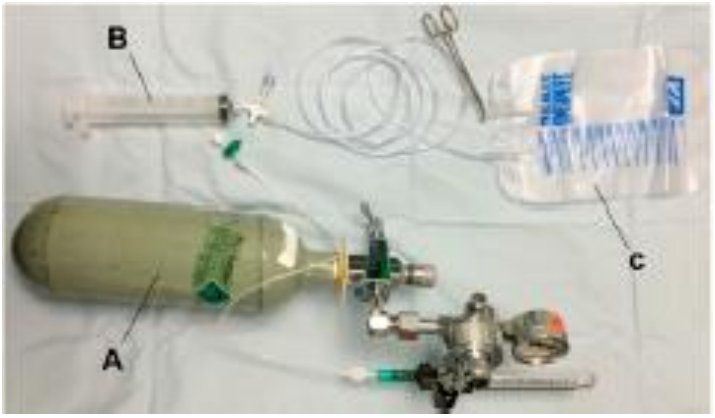



**Figure 3.1:** A home-made carbon dioxide delivery system: (A) a pressurized $CO_2$ cylinder with filter, (B) a 50-ml syringe, attached to a one-way valve, and (C) a Merit disposal depot reservoir bag with removable scissor seal.

Each patient was consented for the procedure and potential complications including abdominal pain, bleeding from puncture site, pseudoaneurysm, renal artery dissection, renal artery thrombosis and failure of procedure. Patient monitoring including oxygen saturation, electrocardiogram and BP was used to identify acute deteriorations.

Midazolam and fentanyl were used to provide sedation and analgesia throughout the procedure and lignocaine was used as a local anaesthetic, injected into the right groin. Heparin was used to manage coagulation and Isoket, an intra-arterial vasodilator, was used to prevent vasospasm.

The patient was positioned to elevate the target site, ideally by 45º, to utilize gaseous reflux to reduce incomplete vasculature filling. The common femoral artery was punctured using ultrasound guidance and a 6-Fr guide sheath introduced using a standard Seldinger technique. After the initial puncture, a 5-Fr pigtail catheter over a standard J-wire is introduced. Using the first lumbar vertebra as the central anatomical landmark, a digital subtraction aortogram was taken using $CO_2$ as the contrast agent. To reduce air contamination, a small volume, approximately 5 ml, was initially expelled when the sheath was attached to the closed $CO_2$ circuit. $CO_2$ was slowly injected by hand via the delivery syringe. The rate of $CO_2$ injection should be faster than the rate of blood flow to facilitate complete filling; aiming for an injection rate of 10–20ml/s. To allow for full elimination, injections of $CO_2$ contrast were separated by 2–3min. Theoretically, owing to rapid first-pass elimination in the lungs, there is no limit to the volume of $CO_2$ that can be used. However, 100 ml is reported as the maximum recommended amount (570). High-rate frames and image stacking improve the quality of the digital subtraction angiography as they reduce the effects of gas diffusion. The renal artery was accessed using a 5-Fr Cobra catheter™ (Boston Scientific, Malborough MA 01752). A selective angiogram was performed using $CO_2$ to demarcate the renal artery, ostia, bifurcation and accessory arteries.

The Symplicity Flex™ catheter (Medtronic) was inserted at an initial position of 5mm proximal to bifurcation of the renal artery at a posteroinferior angle. The new multielectrode 4-Fr Symplicity Spyral catheter (Medtronic) was also used; it allows for simultaneous ablations, thus reducing ablation time as only one burn cycle is required. The vessel resistance was checked to ensure it is >300 Ω.

Additional analgesia, fentanyl 50 mcg i.v., was administered at this stage to pre-empt pain related to radiofrequency ablation delivery. The foot pedal was used for a 2-min burn cycle when radiofrequency ablation occurs via the Symplicity Flex catheter tip electrode. The second burn is completed 5mm proximal with a rotation of 45$^{o}$. Repeating this burn cycle will treat the full circumference of the artery, effectively targeting the sympathetic nerve fibres. This helical burn cycle technique is continued up to 5mm from the renal ostium at the superior surface of the renal artery. The above process is simplified when using the Symplicity Spyral catheter; the helical shape simultaneously provides four-quadrant burns in a 1-min burn cycle. The Symplicity catheter is removed and cleaned; the guide catheter is aspirated and discarded. A repeat selective right renal arteriogram is taken to ensure patency post ablation. A selective left renal arteriogram is performed, and the process is repeated for the left-hand side. **Figure 5.2** demonstrates images obtained from the above protocol. Following successful completion of the procedure, a 6-Fr Angioseal™ (St Jude Medical, Minnetonka MN, USA) vascular closure device was deployed.

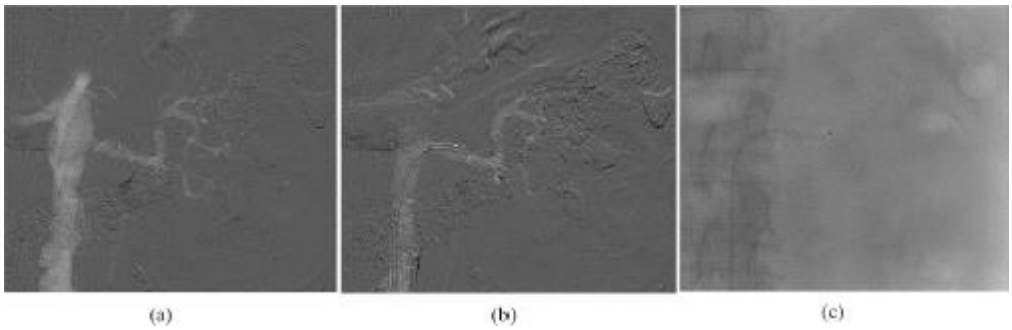



**Figure 3.2:** Carbon dioxide angiography: (a) Baseline selective left renal artery carbon dioxide ($CO_2$) angiogram to assess dimensions of the main renal artery segment prior to renal sympathetic denervation (RDN); (b) post-RDN selective renal artery CO2 angiogram; (c) a Simplicity™ (Medtronic) device within the left renal artery during RDN procedure.

### 3.2.4 Statistical analysis

Statistical analysis was performed using SPSS Statistics 22 (IBM, Armonk, NY). The Shapiro-Wilk test was applied to test for normality of distribution of data. Median and interquartile range has been used to report the non-normally distributed parameters. Related-Samples Wilcoxon Signed Rank Test was used to compare baseline creatinine, eGFR and, clinic and ABPM BP readings with follow-up to detect any statistically significant changes. A two-sided P value of <0.05 was considered statistically significant. Written informed consent was obtained from all patients (see appendix II for the study patient information sheet and appendix III for study consent form). The study was reviewed by the local research ethics committee (NRES Committee West Midlands – Edgbaston) and granted approval. The study was registered with ClinicalTrials.Gov (NCT02863510).

## 3.3 Results

Eleven patients with CKD underwent RDN. The baseline characteristics for the patients are summarized in **Table 3.1**. There were 8 (72.7%) men and the median (IQR) age was 57 (49–66) years. Seven (63.6%) patients were Caucasian, 2 (18.2%) African Caribbean and 2 (18.2%) South Asian.

**Table 3.1** Baseline characteristics of the study population

| **Baseline Characteristics** | |
|---|---|
| **Age, years – median (IQR)** | 57.0 (49.0 – 66.0) |
| **Gender – n (%)**<br>Male<br>Female | <br>8 (72.7)<br>3 (27.3) |
| **Ethnicity – n (%)**<br>Caucasian<br>African-Caribbean<br>Asian | <br>7 (63.6)<br>2 (18.2)<br>2 (18.2) |
| **BMI, kg/m$^2$ – median (IQR)** | 32.8 (25.7 – 38.7) |
| **Co-morbidities – n (%)**<br>Type II diabetes mellitus<br>Cerebrovascular disease | <br>5 (45.5)<br>2 (18.2) |
| **Antihypertensive medication – n (%)**<br>ACE inhibitor<br>Angiotensin receptor blocker<br>Aldosterone antagonist<br>α-adrenergic blocker<br>β-adrenergic blocker<br>Calcium channel blocker<br>Loop diuretic<br>Thiazide/Thiazide-like diuretic | <br>4 (36.4)<br>5 (45.5)<br>1 (9.1)<br>5 (45.5)<br>4 (36.4)<br>11 (100)<br>5 (45.5)<br>2 (18.2) |

**Abbreviations:** IQR, interquartile range; BMI, body mass index; ACE, angiotensin-converting enzyme.

The median (IQR) number of antihypertensive medications taken by the study population at baseline was 4 (3–4). All patients were taking a CCB, 9 (81.8%) were taking either an ACE inhibitor or an ARB and 7 (63.6%) were taking at least one diuretic (loop, thiazide/thiazide-like, or potassium-sparing). At six months most patients (n=9, 81.8%) were taking the same number of anti-hypertensive medications, and 2 patients had at least one anti-hypertensive medication stopped.

**Table 3.2** summarises the results of renal function, albuminuria and BP parameters at baseline and follow-up visits. Baseline median (IQR) serum creatinine was 2.26 (1.66 – 3.47) mg/dL with a median (IQR) eGFR of 29 (18 – 41) ml/min/1.73 $m^2$. The median (IQR) of differences between serum creatinine at baseline and 6 months post-RDN was 0.25 (0.09 – 0.53) mg/dL which was statistically significant (p=0.008). The median of differences between serum creatinine at baseline and the earlier follow up visits were not statistically significant. Similarly, median of differences between eGFR at baseline and 6-month follow up visit was statistically significant with a median (IQR) difference of -4 (-11 – -1) ml/min/1.73 $m^2$, (p= 0.012). Median albuminuria, measured as urine ACR, of the study population showed an improving trend (**Table 3.2**); however, the median of differences in ACR at baseline and follow-up visits was not statistically significant.

**Table 3.2:** Study Parameters at baseline and serial follow-up

| Median (IQR) | Baseline (n=11) | Week 1 (n=11) | Week 4 (n=11) | Week 12 (n=10) | Week 26 (n=11) |
|---|---|---|---|---|---|
| Creatinine, mg/dL | 2.26 (1.66-3.47) | 2.02 (1.91-3.45) | 2.27 (1.75-3.35) | 2.21 (1.75-4.07) | 2.24 (1.71-3.81) |
| eGFR, ml/min/1.73m$^2$ | 29 (18-41) | 33 (19-36) | 29 (20-38) | 28 (16-38) | 25 (17-34) |
| Urine ACR, mg/mmol | 203 (63-412) | 231 (55-283) | 136 (23-386) | 146 (15-382) | 137 (6-370) |
| Clinic SBP, mmHg | 170 (158-180) | 169 (158-175) | 157 (135-170) | 159 (152-177) | 164 (149-174) |
| Clinic DBP, mmHg | 89 (75-95) | 87 (78-87) | 83 (74-91) | 86 (71-103) | 86 (74-94) |
| 24-hour ambulatory SBP, mmHg | 155 (149-161) | | | | 161 (149-168) |
| 24-hour ambulatory DBP, mmHg | 86 (73-94) | | | | 86 (84-101) |
| Day ambulatory SBP, mmHg | 159 (149-164) | | | | 163 (152-167) |
| Day ambulatory DBP, mmHg | 89 (75-95) | | | | 88 (84-100) |
| Night ambulatory SBP, mmHg | 147 (141-163) | | | | 155 (144-169) |
| Night ambulatory DBP, mmHg | 82 (73-86) | | | | 83 (74-90) |

**Abbreviations:** IQR, interquartile range; eGFR, estimated glomerular filtration rate; ACR, albumin-creatinine ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure. Note: conversion factor for creatinine in mg/dL to µmol/L, ×88.4.

At baseline median (IQR) clinic BP was 170 (158 – 180) mmHg systolic and 89 (74 – 99) mmHg diastolic. The median clinic SBP was lower at six months but the median of difference, -14 (-24 – 5) mmHg was not statistically significant. The apparent reduction in median clinic BP was not accompanied by a corresponding reduction in ABP parameters where the median SBP was higher at six months overall and when separated into day and night (Table 5.2). Statistically, however, the median (IQR) of difference was only found to be significant for daytime SBP which was 7 (-2 – 12), mmHg (p=0.045). The change in all others ABP was not statistically significant.

Mean total number of radiofrequency ablations delivered during the RDN procedure was 11.3 (standard deviation, 2.4). There were no minor atherosclerotic findings, which could have limited the radiofrequency ablation probe contact with the true wall of the artery, in any of the patients who underwent RDN. Patients with significant renal artery stenosis due to any atherosclerotic lesions were excluded at the screening stage by CT renal angiogram. Procedure-related complications observed include; one patient had flank pain which was managed with simple analgesics, another patient had groin pain which did not require any analgesics and another patient had groin haematoma requiring overnight admission but no intervention. Two patients progressed to ESRD requiring dialysis; one patient commenced on peritoneal dialysis at 6 months and ten days after the procedure and the other required haemodialysis at 5 months and 27 days.

## 3.4 Discussion

This is the first reported study to perform RDN with the sole use of $CO_2$ as a contrast agent. This pilot study shows that $CO_2$ renal angiography can be used safely to perform RDN in patients with significant renal impairment where there is a risk of contrast-induced nephropathy commonly associated with iodinated contrast agents. In this study, there was no significant change in median serum creatinine and eGFR at 7 days after the procedure. There was no significant improvement in BP control but a trend towards an improvement in albuminuria at 6 months.

The primary aim of this study was whether the use of iodinated contrast agents could be avoided in patients with significant renal impairment and uncontrolled hypertension and successfully perform RDN using $CO_2$ angiography. $CO_2$ angiography is not completely risk-free, however the adverse events associated with $CO_2$ angiography are uncommon and are usually limited to the organs being injected (571). In particular, a vapour-lock phenomenon can occur where the large doses of $CO_2$ can cause obstruction to blood flow and hence potentially causing a vapour lock and ischaemia downstream if unresolved. Contamination of $CO_2$ with room air can result in air embolus. Neurotoxicity, pain, nausea, vomiting and urge to defecate are amongst the other possible complications. The risk of these rare complications can be reduced through understanding the unique properties of $CO_2$ and ensuring appropriate precautions are observed when preparing and administering it (571, 572). No patients in this study cohort suffered any adverse events that could be attributable to $CO_2$ angiography and the only observed adverse events were secondary to puncture site complications which are common to any percutaneous angiographic procedure. $CO_2$ digital

subtraction angiography allowed adequate placement of the RDN catheter for the radiofrequency ablation which was confirmed by the impedance drops observed for every ablation.

It is well known that the risk of developing contrast-induced nephropathy is associated with the volume of contrast used. $CO_2$ renal artery angiography has been used to reduce, but not completely omit, the amount of iodinated contrast given when carrying out intravascular procedures in individuals with significant renal function impairment (558). In our study the use of iodinated contrast agents has been wholly replaced by $CO_2$, hence completely negating the risk of developing contrast-induced nephropathy. There are no reported studies comparing safety of $CO_2$ versus radio-iodine contrast with pre-procedural hydration in patients undergoing RDN. However, a recent meta-analysis comparing the incidence of acute kidney injury (AKI) with $CO_2$ versus iodinated contrast media reported that $CO_2$ use is associated with a modestly reduced rate of AKI despite the patients with iodinated contrast media being optimised with pre-hydration (573). Furthermore, a recent RCT trial of prophylactic hydration to protect renal function from intravascular iodinated contrast material in patients at high risk of contrast-induced nephropathy showed no prophylaxis to be non-inferior and cost-saving in preventing contrast-induced nephropathy compared with intravenous hydration (574). Patients with CKD are at a higher risk of developing contrast-induced nephropathy and any measures which help reduce the risk of contrast-induced nephropathy should be considered for patients undergoing interventional procedures.

Unlike other studies of RDN (480, 484), only a modest reduction in clinic SBP was observed at six months after RDN. Ambulatory SBP readings conversely were higher at six months. The most likely explanation for this observed rise in ambulatory SBP could be progression of CKD and the consequent sodium and water retention. In 9 (82%) of the patients' serum creatinine was higher, and accordingly a lower eGFR, at 6 months post RDN when compared with baseline values.

Other potential reason for non-response could be explained by inadequate denervation of renal arteries as majority, 8 (73%), of patients underwent RDN using the first generation, single electrode, Symplicity catheter with which an overall mean of 10 ablations were performed. Kandzari and colleagues' post hoc analysis of SYMPLICITY HTN-3 data, the largest trial of RDN to date, showed that the number of ablations delivered were predictive of successful BP response in their study population (481). Specifically, patients who received a total of ≥14 ablations to their renal arteries were more likely to achieve a significant reduction in SBP; clinic -14.1 mmHg and ambulatory -6.8 mmHg. Following these findings, a decision was made to switch over to the multi-electrode Symplicity Spyral catheters which were used to deliver radiofrequency ablation to the last 3 patients with an average of 14 ablations. Of these 3 patients, 2 achieved reductions of 5 and 4 mmHg in their ambulatory SBP and the other patient had a rise of 8 mmHg

The median serum creatinine at 7 days post RDN showed no significant decline and, if anything, a trend towards improved creatinine. However, by 6 months the decline in the kidney function was statistically significant suggesting a continued progression of CKD. Given the small number of patients in the study a detailed

analysis of whether RDN affected the rate of decline in kidney function was not performed. A recent study in 46 patients with CKD (baseline eGFR ≤60 mL/min/1.73m$^2$) has provided some evidence that RDN can slow further deterioration of renal function irrespective of BP-lowering effects (562).These results build upon similar findings reported by others (561, 575).

There is some evidence to suggest that RDN may have beneficial effect in reducing urinary albumin excretion which may be unrelated to improvement in BP control (575). In this study, a non-significant trend towards the improved urinary excretion appeared at 4 weeks post procedure which was maintained at subsequent follow-up periods until 6 months.

For clinical efficacy, in a 1:1 sham-RCT of RDN for patients with CKD uncontrolled hypertension, a sample size of 227 per group would be needed with an 80% power and a two-sided significance of $p<0.05$, taking into account a 10% attrition rate. This number would increase to 304 for a desired power of 90%. This calculation is based on assuming (a) a clinically significant 5 mmHg difference between treatment groups (in favour of RDN) with respect to the average change in 24-hour ambulatory SBP from baseline to 6 months; (b) a 18 mmHg SD of the SBP change per group (576, 577).

## 3.5 Conclusion

This is the first study of RDN in patients with moderate-to-severe CKD which has avoided the use of traditional iodinated contrast altogether by replacing them with $CO_2$ angiography. However, this was a pilot single-arm study with no sham comparator and was not powered to detect a meaningful reduction in clinic or ABPs. The results have shown that there were no adverse procedural events associated with $CO_2$ angiography and any future trials of RDN should not exclude patients with moderate-to-severe CKD on the basis of risk of contrast-induced nephropathy associated with the conventional methods. Further research, with a larger study population, is needed to investigate whether RDN can achieve substantial reductions in BP and albuminuria, and any effect on the rate of decline of CKD.

# CHAPTER 4 A STUDY OF FACTORS ASSOCIATED WITH TREATMENT-RESISTANT HYPERTENSION (FACT-RHY)

## 4.1 Introduction

Understanding of pathophysiology of TRH is limited by challenges in correctly diagnosing patients with true TRH and isolating them from patients with apparent or pseudo-TRH and those with secondary causes of TRH. As has already been shown that non-adherence is a major contributor to pseudo-resistance along with white-coat hypertension. Furthermore, ambivalent results of RDN in patients with TRH and use of mineralocorticoid receptor antagonists in these patients allude to a heterogeneous pathophysiological mechanisms contributing to development of TRH. At the inception of this thesis, there were no cohort studies that had examined patients with true TRH after systematically excluding non-adherence, WCE and secondary causes of hypertension to understand the pathophysiological characteristics of these patients.

A study of factors associated with treatment-resistant hypertension (FACT-RHY) was designed as a cohort study which proposed to comprehensively characterise a group of hypertensive subjects referred to a tertiary hypertension clinic for uncontrolled BP, with the aim of understanding factors associated with true treatment resistance, once secondary causes, non-adherence and under-

treatment have been excluded as the cause of treatment failure. A detailed phenotypic analysis will be performed, with the aim of identifying markers that may have a causal role in resistance to treatment. Specific objectives of the study that will be explored in this thesis include:

i. Assess association with vascular markers including arterial stiffness and endothelial function

ii. Assess association between body composition and hypertension as measured using bioimpedance spectroscopy

iii. Evaluate level of sympathetic nervous system activity in the study patients

iv. Assess prevalence of undiagnosed obstructive sleep apnoea in the study population

v. Explore patients' personal views about medicines prescribed for hypertension and medicines in general.

## 4.2 Methods

### 4.2.1 Study design

The original study was designed as a cohort study of patients with hypertension presenting to the West Midlands Hypertension Centre (WMHC), a tertiary hypertension clinic based at Birmingham Heartlands Hospital. In this thesis, results of a cross-section of the patients recruited for this study from June 2017 to June 2018 are presented. This study proposed to comprehensively characterise a group of hypertensive subjects referred to a tertiary hypertension clinic for uncontrolled BP, with the aim of understanding factors associated with true treatment resistance, once secondary causes, WCE, non-adherence and under-treatment have been excluded as the cause of treatment failure. A detailed phenotypic analysis was performed, with the aim of identifying markers that may have a causal role in resistance to treatment. The study protocol is detailed in appendix IV.

### 4.2.2 Patient recruitment

All patients with treated hypertension, between the ages of 18 and 80 years of age, an eGFR (MDRD equation) ≥30ml/min/1.73m$^2$ without any previously confirmed cause of secondary hypertension including renal artery stenosis, phaeochromocytoma, primary hyperaldosteronism, Cushing's disease or OSA were eligible to take part in this study.

Patients due to attend clinic each week were screened, where possible, prior to their attendance. All patients deemed eligible were approached on the day of the clinic, where the purpose of the study was explained, a patient information sheet provided (see appendix V), and any questions answered. Patients were able to

provide written consent (see appendix V) on the day of the clinic visit or at a subsequent visit. A follow-up phone call was made to patients to confirm consent and arrange an appointment for the study visit.

The study was reviewed by national research ethics committee (South East Scotland REC 01, REC reference: 16/SS/0162) and granted approval. The study was conducted in accordance with the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects and guideline on Good Clinical Practice.

### 4.2.3 Study Visit

#### 4.2.3.1 Clinical History

At the study visit, consent was re-confirmed before any measurements were made. Basic patient demographics were recorded including age, gender and ethnicity (Caucasian, African-Caribbean, Asian or other). Clinical history was taken to record duration of hypertension in years (<1, 1-5, 6-10, 11-15, 16-20, >20). A comprehensive medication history was taken to record the names all medications taken by the patient. Dosage and frequency of all antihypertensive medications were also documented. Smoking status was recorded as never, current and ex, and for the latter 2 responses smoking pack years were calculated by multiplying the number of pack of cigarettes smoked per day by the number of years the person had smoked. Patients were asked about their average weekly alcohol intake which was recorded as number of units. Past medical history of angina, previous MI, heart failure, dyslipidaemia, diabetes mellitus, stroke, transient ischaemic attack (TIA), CKD and peripheral vascular disease was recorded and, where necessary, validated using patient's electronic medical

records. Family history was taken to record number of first-degree relatives with hypertension, cerebrovascular disease (stroke or TIA) and ischaemic heart disease (defined as angina or MI in a first degree relative younger than 60 years of age).

#### 4.2.3.2 Clinical Examination

***Body mass index***

Patients' height and weight were measured to calculate their BMI, expressed as $kg/m^2$. Patients were asked to take off shoes and height was measured in centimetres, to the nearest 0.5 cm using the Seca 222 telescopic height measuring rod stadiometer (Seca, Hamburg, Germany). Weight was measured to the nearest 0.1 kg using the Seca 703 digital column scales (Seca, Hamburg, Germany). Patients were weighed with a single layer of clothing and asked to remove any heavy items from their pockets.

***Waist-to-hip ratio***

Waist-to-hip ratio was calculated by measuring the circumferences of waist and hip. Patients were asked to stand up straight with arms hanging loosely by their sides, feet together and looking straight ahead. For the waist circumference, a measuring tape was placed horizontally midpoint between lower costal margin and top of the iliac crest. Hip circumference was measured at the widest part of the hips by placing the tape measure horizontally across the greater trochanters. Whilst measuring patient was asked to breathe out gently, the measuring tape was pulled taut and a measurement to the nearest 5mm was taken at the end of a normal expiration for waist and mid-expiration for hip. A repeat measurement was taken for both waist and hip and the average of two values was used to

calculate the waist-to-hip ratio. A Seca 201 Ergonomic circumference measuring tape (Seca, Hamburg, Germany) was used to for all patients.

***Clinic blood pressure – BpTRU***

Clinic BP measurement was performed using the BpTRU-100 (BpTRU Medical Devices, Coquitlam, BC, Canada). The BpTRU device is a fully automated sphygmomanometer using oscillometric technique to measure BP which is a method commonly used by most BP monitoring devices. The BpTRU automatically measures BP taking six BP readings with a programmable time interval between each reading; it discards the first reading and calculates the average of the last 5 measurements. It has been clinically validated for use against the standards set out by the BIHS, achieving the highest A/A grade for accuracy (578). ABPM is considered a 'gold standard' for the diagnosis of hypertension and the assessment of whether target has been achieved. The BpTRU readings have been shown to accurately reflect ABPM readings rather than routine single clinic BP readings (579-582). Although ABPM has been shown to be cost-effective for diagnosis of hypertension (31), it is neither cost effective nor practical as a routine method of BP monitoring and is the least favourable method among patients due to the discomfort and sleep disturbance experienced (583).

The BP was measured in accordance with the recommendations set out by the BIHS (584). Patients were seated in a relaxed position for five minutes in a quiet room without talking. Mid-arm circumference was measured to select the BP-cuff recommended by the manufacturer, which was placed on the upper arm at the level of the heart. The arm was supported on the arm rest with the patient seated

on a chair, back supported and both feet on the floor. One measurement was taken on each arm and recorded; the arm with the highest BP reading was used for all subsequent readings. The BpTru was programmed to take measurements at 2-minute intervals (from the start of one reading to the start of the next). All 6 BpTRU BP readings were taken with the operator present, with no interaction between the patient and the operator. The last 5 measurements including the BpTRU average were recorded.

***Electrocardiogram***

During the study visit, a 12-lead ECG was performed on all patients using GE MAC 1600 (GE Healthcare, Chicago, IL). The limb and chest electrodes were positioned in accordance with guidelines set out by the Society for Cardiological Science & Technology (585).

The Cornell voltage (586) was calculated as the sum of amplitudes of R-wave in lead aVL and S wave in lead V3. The Cornell voltage was multiplied with QRS duration to compute the Cornell product (587), which if ≥2440 mm x ms was used to define LVH.

#### 4.2.3.3 Body composition monitoring

Whole-body bioimpedance spectroscopy (BIS) body composition monitoring was performed in all patients at the clinic visit using Fresenius body composition monitor (BCM; Fresenius Medical Care, Bad Homburg, Germany). BIS involves measurement of impedance to low voltage alternating current of different frequencies passing through the body. Tissues have varying levels of electrical conductivity depending on their composition. While high-frequency current passes through the total body water (TBW), low-frequency current cannot

penetrate cell membranes and thus flows exclusively through the extracellular water (ECW). The BCM is able to measure impedance at 50 discrete frequencies over a range from 5 kHz to 1 MHz to determine the electrical resistances of the TBW and the ECW using Cole modelling and the equations by Moissl et al. (588). These two parameters can then be used to calculate intracellular water (ICW) using the fluid volume model. Ratio of ECW and ICW and ratio of ECW and TBW (589, 590) have been used as a measure of fluid status in studies using bioimpedance.

A large diversity exists in the relative distribution of intracellular and extracellular water distribution in the main tissues comprising the human body. In a normal hydration healthy person, lean tissue (mainly muscle) consists of 70% water which is predominantly intracellular. Conversely adipose tissue (mainly lipids) consists of 20% water which is predominantly extracellular (591). Therefore, diversity in the relative distribution of lean and adipose tissue mass in healthy individuals will significantly alter the ratio extracellular to intracellular water.

Chamney et el. have a developed a body composition model consisting of three principal body compartments; normally hydrated lean tissue, normally hydrated adipose tissue and excess fluid (591). This model is able to account for the different ratios of ECW to ICW in the lean and adipose tissues over a wide range of body compositions to calculate excess fluid. Excess fluid or overhydration is almost exclusively ECW and healthy individuals are considered to have normal hydration with no over- or under-hydration. In conditions where excess fluid accumulates, it represents an expansion in ECW whereas the ICW remains unchanged. This excess fluid may accumulate within either the lean or adipose

tissues, existing as oedema, or as a separate compartment such as ascites or pleural effusion. Overhydration (OH) is calculated from the difference between the normal expected ECW and actual measured ECW. Normal OH ranges from -1.1 to +1.1 L (10th to 90th percentile) in healthy normal population (592).

The Fresenius BCM uses the 3-compartment body composition model and the BIS-derived volumes of TBW, ECW and ICW to separate body weight into normally hydrated lean tissue mass (LTM), normally hydrated adipose tissue mass (ATM) and OH. Its measurements have been validated against gold-standard references (e.g. bromide, deuterium, dual-energy X-ray absorptiometry [DEXA], air displacement plethysmography, clinical assessment) in over 1000 healthy individuals and patients (593).

Patients were asked to lie down in a relaxed supine position on the examination table. Patients were positioned to ensure legs were kept apart and arms were not in contact with the trunk. Two electrodes each were placed 5 cm apart on the right wrist and right ankle after the patient had been in recumbent position for at least 5 min. Each electrode was connected to the appropriate lead on the BCM. Patients' age, gender, height, weight and BP was inputted into the BCM and the measurement was taken. Output indices recorded include OH, lean tissue index, fat tissue index, TBW, ECW, ICW, ECW/ICW, LTM, fat tissue mass and ATM. Since the inter- and intra-observer reproducibility is good, only a single BCM measurement was performed for each individual patient (594).

#### 4.2.3.4 EndoPAT

Functional assessment of endothelial function was carried out using EndoPAT 2000 (Itamar Medical, Israel). It uses a trademarked peripheral arterial tone (PAT)

technology to non-invasively measure arterial tone changes in the peripheral arterial beds. The PAT signal is measured from the fingertip by recording finger arterial pulsatile volume changes.

Patient preparation involved ensuring patients were fasting for at least 4 hours prior to testing including abstaining from caffeine. Any jewellery, watches or rings were removed prior to testing. Testing was carried out with the patient lying supine on an examination table, resting for at least 15 minutes in a quiet, dimly lit and temperature-controlled room.

The EndoPAT system was prepared by entering patient's age, gender, height weight and BP. Two disposable PAT probes were connected to the pneumo-electric tubing which together were placed in the PAT probe slots on the arm supports. The probes were deflated before asking the patient to insert one finger, preferably the index finger, from both hands into each of the two PAT probes, ensuring the study fingers were placed all the way into the end of the probes. Patients were asked to trim their nails where their lengths prevented their fingers reaching the end of the probes. The PAT probes were then inflated, foam anchor rings were placed on the finger adjacent to the study finger and the pneumo-electric tubing was looped and clipped to the foam anchor rings ensuring these rings and the tubing do not come in contact with the probes. Patients' forearms were then rested on the arm supports with the fingers dangling over the edge of the supports, again ensuring the probes are not touching any other object. A 10 cm wide BP cuff connected to a manual sphygmomanometer was placed over the upper arm on the test arm.

Patients were advised to keep quiet and refrain from moving their study fingers throughout the entire procedure. Signals from the two PAT channels were visualised on the laptop connected to the EndoPAT 2000. Adjustments to the signal gains/scaling were made to ensure adequate visualisation of the signals over a period of one minute. If the signal showed any artefacts, position of patients' fingers and probes were re-adjusted to ensure that the PAT probes were not in touch with anything at all.

Once stable signals were visualised, baseline recording was started for at least 5 minutes. As per manufacturer guidance, the timescale for viewing the trace was changed from 1 minute to 15 seconds and the signal gain of the arm to be occluded was amplified to 20,000. At this point, patients were reminded to keep their fingers still and warned that they might feel paraesthesiae in their occluded arm, hands or fingers.

The BP cuff was rapidly inflated to a supra-systolic level, the recommended pressure of at least 60mmHg above SBP and no less than 200mmHg. Total cessation of blood flow to the hand was verified by total absence of PAT signal from the occluded hand. If the appearance of any PAT signal was noted, cuff pressure was increase by an additional 50 mmHg and up to maximum of 300mmHg. The arterial occlusion was maintained for exactly five minutes – periodically checking the pressure in the occluding cuff to ensure proper inflation; increasing pressure if required. After five minutes of occlusion, the BP cuff was completely and rapidly deflated, signal gains and timescale were readjusted to baseline values, post occlusion phase recording for another five minutes before

stopping the test. The PAT probes were deflated, removed from patients' fingers and discarded alongside the anchor rings.

The EndoPAT 2000 software automatically analyses the recorded waveforms by identifying and marking the occlusion borders. It assesses digital flow-mediated dilatation during reactive hyperaemia using measurements from both arms. The reactive hyperaemia index (RHI) is the post-to-pre occlusion PAT signal ratio in the occluded arm, relative to the same ratio in the control arm, corrected for baseline vascular tone of the occluded arm. It also calculates a log natural transformation of RHI (LnRHI), which the manufacturers claim to provide better double-sided distribution than RHI that is closer to normal distributionand able to offer better separation between disease states. A normal result is RHI > 1.67 or LnRHI > 0.51 and abnormal result is RHI ≤ 1.67 or LnRHI ≤ 0.51.

#### 4.2.3.5 Arterial Stiffness

Pulse wave analysis and pulse wave velocity was measured in all the patients at the study visit using the Vicorder system (Skidmore Medical, Bristol, UK). It uses BP cuffs and a volume displacement technique to measure simultaneous pressure waveforms of arteries at different sites. Patients were in supine position for 15 minutes in a temperature-controlled room before the measurement was started.

Pulse wave analysis was performed first by placing a 10 cm wide BP cuff over the brachial artery. A brachial BP was measured by a digital oscillometric technique using the Vicorder system for calibrating peripheral waveforms. Immediately afterward, the BP cuff was then statically re-inflated to 70 mmHg to obtain a digitally computed brachial pressure wave trace. The Vicorder system

software applied a previously described brachial-to-aortic transfer function (595) to convert the brachial waveform trace to derive a waveform and values for central BP. The augmentation index which is defined as the difference between the first and second systolic peaks of the central BP waveform, expressed as a percentage of the pulse pressure. The Vicorder system software was able to automatically identify the first and second central systolic peaks to calculate the augmentation index. The BP and the brachial waveform were recorded three times to calculate an average augmentation index.

Pulse wave velocity was measured second; a 10 cm wide BP cuff was placed around the upper thigh, as close to the groin as possible to obtain a femoral artery waveform. The carotid pulse on the same side was manually palpated using the anatomical landmarks and a 3 cm wide partial cuff was placed over the carotid pulse position with the cuff taut around the neck. The path length was measured in cm, in accordance with the manufacturer guidance, from the tip of the shoulder in line with supra sternal notch and a fixed spot on the thigh-placed BP cuff. The Vicorder system simultaneously inflated both cuffs to a static pressure of 65 mmHg to obtain and record two waveforms using volume displacement. The waveforms were captured over a period of 10 seconds. The Vicorder software automatically measures the foot-to-foot transit time which corresponds to the delay in arrival of the foot of the waveform from the carotid to the femoral artery. The Vicorder software uses an in-built cross-correlation algorithm around the peak of the second derivative of pressure. The transit time and the distance from the shoulder tip to the femoral cuff recorded earlier was used by the Vicorder system to automatically calculate the PWV. Three separate traces were captured to obtain three PWVs and the average of three was used for further analysis.

#### 4.2.3.6 Blood testing

Blood samples were taken from all patients towards the end of the study visit after they had been lying in a supine position for at least 30 minutes or more. Blood samples were taken for urea and electrolytes profile, lipid profile, bone profile, CRP, thyroid stimulating hormone (TSH), Cortisol, Vitamin D, haemoglobin A1c (HbA1c), serum FLC, renin, aldosterone, high sensitivity troponin I and N terminal pro B type natriuretic peptide (NT-proBNP). These tests were all routinely carried out at the local pathology laboratories of Birmingham Heartlands Hospital using validated techniques. The laboratories themselves were awarded UKAS (United Kingdom Accreditation Service) accreditation to the internationally recognised ISO 15189 standard.

Where any parameter values were reported as below the level of quantification, the lower limit of quantification divided by 2 was used to impute a value for any such instance (596, 597).

#### 4.2.3.7 Biomarkers of endothelial dysfunction

Specific biomarkers of endothelial dysfunction were evaluated using commercially available enzyme-linked immunosorbent assay (ELISA) kits for sFLT-1 (human VEGF1), ET-1, endoglin, ICAM-1 and VCAM-1.

The serum which was collected at the study visit in a serum separator tube, allowed to clot for 30 minutes at room temperature before centrifugation for 30 minutes and 1000g and stored at -80 $^{o}$C until required. Samples were gently thawed using dry ice to room temperature. All the reagents including wash buffer, substrate solution, calibrator diluent and standard were prepared as per supplied manufacturer's instructions.

Assay diluent was added to each microplate well, followed by addition of standard, control and sample solution per well before covering with an adhesive strip and incubating for the recommended duration on a horizontal orbital microplate shaker as specified. At the end of incubation, each well was decanted and then washed by filling each well with wash buffer before complete removal of liquid. The wash process was repeated three times.

After washing the conjugate solution was added to each well before covering with a new adhesive strip and re-incubating on the shaker as specified. The wash process was repeated after second period of incubation. Substrate solution was then added to each well and allowed to incubate, protected from light, on the benchtop or the shaker as specified. Stop solution is then added to the each well to stop the reaction producing a colour change from blue to yellow. The micro plate was gently tapped to ensure thorough mixing and allow a uniform colour change.

The optical density of each well was determined within 30 minutes using a Spark 10M microplate reader (Tecan Group Ltd, Männedorf, Switzerland) at 450nm and 540nm wavelengths. The readings at 540 nm were subtracted from the readings at 450nm to correct for optical imperfections in the plate.

A preliminary ELISA was performed for each biomarker by sampling different dilutions of serum from both groups of patients to determine the optimum dilutions to use for the entire sample population.

***Calculation of results***

The zero standard optical density was subtracted from wavelength-corrected readings for each standard and sample. A standard curve was created for standards for each microplate by generating a four-parameter logistic (4-PL) curve-fit for all biomarkers except VCAM-1 for which log/log curve-fit was used, as recommended by the manufacturer. SigmaPlot for Windows version 14.0 (Systat Software Inc., San Jose, California, US) was used to generate the curve-fit graphs (see appendices VI – X). The equations for each curve-fit or line of best fit was used to determine the concentrations of biomarker for the corrected optical density readings for each sample. If the samples were diluted, the concentrations obtained using the standard curve were multiplied by the dilution factor.

#### 4.2.3.8 Spot urine testing

At the end of the study visit all patients were asked to provide a midstream urine which was analysed at Birmingham Heartlands Hospital laboratories for quantification of spot urine ACR. The urine sample was also used to carry out urine antihypertensive assay which was able to detect presence of 23 commonly prescribed antihypertensive drugs or their metabolites (**Table 4.1**).

**Table 4.1:** List of antihypertensives detectable by the urine antihypertensive assay.

| Class of drug | Antihypertensives detectable |
|---|---|
| Angiotensin-converting enzyme inhibitors | Lisinopril, perindopril, ramipril, enalapril |
| Angiotensin receptor blockers | Losartan, irbesartan, candesartan |
| Beta-adrenoreceptor blockers | Atenolol, bisoprolol, metoprolol, labetalol |
| Calcium channel blockers | Amlodipine, diltiazem, felodipine, verapamil, nifedipine |
| Diuretics | Indapamide, bendroflumethiazide, hydrochlorothiazide, furosemide, spironolactone |
| Others | Doxazosin, moxonidine |

#### 4.2.3.9 24-hour urinary collection

At the study clinic, all the patients were provided with two different collection bottles (one plain and the other acidified) for 24-hour urinary collection to be completed at home. Patients were instructed to collect the urine on a day of their choice within 2 weeks of the study visit ensuring all the urine they passed over a 24-hour period was collected in the urine bottles and return the sample bottles within 24 hours of collection. They were advised to a collect a separate 24-hour urine in each of the two different collection bottles.

The urine collected in the acidified bottle was used to carry out catecholamines (noradrenaline, adrenaline and dopamine) and metadrenalines (normetadrenaline, metadrenaline and 3-methoxytyramine). The plain bottle was used to test for urinary electrolytes including sodium and potassium. The concentrations of all the tested were converted from per litre to per day by multiplying the volume of urine collected, in litres over 24 hours. It was assumed

that all sodium was ingested in the form of sodium chloride and daily salt intake was calculated by multiplying 24-hour urinary sodium (mmol/day) with molecular weight of sodium chloride (58.5 g/mol) and dividing by 1000 to convert it to g/day.

#### 4.2.3.10 Overnight pulse oximetry

Overnight pulse oximetry was carried out to screen for presence of obstructive sleep apnoea. Where available, Pulsox-300i (Konica Minolta Sensing, Osaka Japan) and SoftTip pulse oximetry sensor (EnviteC, Wismar, Germany) was used to carry out overnight pulse oximetry. Patients were given instructions and a practical demonstration of using the device at the study visit. Patients were advised to wear the Pulseox-300i on their wrist like a wristwatch using the adjustable Velcro straps. They were told to place the SoftTip oxygen probe on any finger, preferably the index, of the same hand ensuring correct orientation and fit of the probe. They were advised to start recording once going to sleep and turning off the device when awake ensuring at least 4 hours of sleep was captured on the device. The data was downloaded and analysed using Visi-Download (Stowood Scientific Instruments, Oxford, UK) software by a trained respiratory physiologist.

Oxygen desaturation index (ODI) was used to assess likelihood of a diagnosis of OSA. A desaturation event is described as a fall of haemoglobin oxygen saturation by a certain percentage of the baseline oxygen saturation. The ODI is the number of desaturation events that occur per hour for a given percentage drop in oxygen saturation. Three levels of desaturation events were reported; >2%, >3% and >4%. An ODI of ≥5 desaturations events per hour at >3% level was defined as abnormal (598). Any patient meeting this criterion was referred to

specialist sleep clinic at Birmingham Heartlands hospital for further evaluation of possible OSA.

#### 4.2.3.11 Epworth Sleepiness Scale

All patients attending the study visit were asked to complete an Epworth Sleepiness Scale (ESS) questionnaire (see appendix XI). The ESS is an 8-item questionnaire that measures subjective sleepiness (599). Patients are given a choice of 4 options to rate how likely they are to fall asleep in 8 different situations. The four options (no chance, slight chance, moderate chance, high chance) are scored 0 to 3, giving a maximum possible score of 24 which represents a high chance of falling asleep in all 8 situations. The ESS final score was dichotomized into < 11 (low risk for sleepiness) and ≥ 11 (high risk).

#### 4.2.3.12 Beliefs about medicines questionnaire

All patients were asked to complete beliefs about medicines questionnaire (BMQ) at their study visit (see appendix XII). The BMQ comprises 18 statements each with a 5-point Likert scale response ranging from strongly disagree to strongly agree. The BMQ is composed of two sections; specific and general. The specific section evaluates patients' beliefs about their medication about a specific condition; it includes two scales comprising 5 question each assessing patients' beliefs about the necessity of taking medications for their illness and the level of concern about the potential adverse effects of taking their current condition-specific medications. The general section focuses on patients' beliefs about medicines in general and it also comprises of two scales of 4 questions each. The general-overuse scale evaluates patients' views about how doctors use

medications and the general-harm scale explores their views about the extent to which they perceive medications as harmful.

Each of the 18 statements are rated on a five-point Likert scale (strongly disagree, disagree, uncertain, agree and strongly disagree). The five Likert scale responses were scored from 1 (strongly disagree) to 5 (strongly agree). Scores from the statements for each of the four scales are added up to give an overall scale score with the highest score indicating stronger beliefs in the concepts of the scale. A necessity-concern differential was calculated as the difference between the score of specific-necessity and specific-concerns subscale a resulting high positive value indicates a strong belief in the necessity of medications that outweighs the concerns about their undesirable effects.

### 4.2.4 Statistical Analysis

Data was recorded in a Microsoft Access database designed specifically for the FACT-RHY study. Statistical analysis was carried using IBM SPSS Statistics for Windows 25.0 (IBM Corp., Armonk, N.Y., USA). Mean and standard deviation were reported for normally distributed data. Visual inspection of histogram, normal probability plot and Shapiro-Wilk test were used to assess for the normality of the data. Log-transformation was used where non-parametric continuous variables could be converted into log-transformed parametric variables. Log-transformed data were reported as un-transformed mean and 95% confidence intervals of the mean. Non-parametric data was expressed as median and IQR. Parametric continuous variables were compared using Student's t-test and non-parametric continuous data were compared using Mann-Whitney U test.

Levene's test for Equality of Variances was used to select the appropriate t-test value.

Categorical variables were compared using the chi-square test and Yates' Correction for Continuity was reported where both variables have only two categories to compensate for the over-estimate of chi-square value when used with a 2-by-2 table. If the percentage of cells with an expected count of less than 5 was found to be more than 20%, Fisher's Exact Test was reported where the two variables had only 2 categories and Likelihood Ratio was reported where more than two categories were present. Pairwise exclusion method was used where data contained missing values.

Pearson's bivariate correlation analysis was used to examine the strength of relationship between two continuous variables and reported as Pearson product-moment correlation coefficient or 'r'. Both confidence interval and p-value were used to express statistical significance and a two-sided p-value of <0.05 was considered statistically significant.

Binary logistic regression analysis used for chosen dependant categorical variables with two categories.

#### 4.2.4.1 Multiple linear regression

Multiple linear regression analysis was performed to assess the effect of independent variables on the chosen dependant continuous variable. Preliminary analyses, including correlation matrix of all eligible variables and variance inflation factor were used to assess the strength of multicollinearity between the variables. Variance inflation factor of greater than 10 was used to define multicollinearity (600) and if present, was minimised by removing the variable

contributing to collinearity. Linearity was assessed by partial regression plots and a plot of studentized residuals against the unstandardised predicted values. Homoscedasticity was assessed by visual inspection of a plot of studentized residuals versus unstandardized predicted values. Assumption of normality of the residuals was tested by visual inspection of histogram of residuals with superimposed normal curve and a P-P plot. Outliers were detected where a case's standardised residual is greater than ±3 SDs. Leverage values were used to determine whether any cases exhibit high leverage; leverage values less than 0.2 were considered as safe, 0.2 to less than 0.5 as risky, and values of 0.5 and above as dangerous (601). Influential points were examined where value for Cook's distance exceeded 1 (602).

For both multiple linear and binary logistic regression analyses backward elimination was performed manually, individually removing any variable in the model with the highest p-value greater than the significance level of 0.05. By rerunning the model with each deletion of a variable, each iteration of the model has a larger sample size as the missing data on the excluded variables will no longer result in missing cases in the multivariable model. Any cases with missing data were excluded from the regression models.

#### 4.2.4.2 Analysis of variance

A two-way analysis of variance (ANOVA) was performed where an interaction between two categorical variables was suspected to be associated with a continuous variable. Residual analysis was performed to test for the assumptions of the two-way ANOVA. Outliers were assessed by inspection of a boxplot,

normality was assessed using Shapiro-Wilk's normality test for each cell of the design and homogeneity of variances was assessed by Levene's test.

## 4.3 Results

### 4.3.1 Patient recruitment

In total 417 consecutive patients were assessed for eligibility between 30th January 2017 to 29th January 2018 (see **Figure 4.1**). Ninety-one patients were excluded; of the 326 eligible patients, 236 patients were approached in the hypertension clinic. Of the 91 excluded patients; 25 either had no hypertension or controlled with lifestyle measures alone, 22 were either <18 or >80 years of age, 21 had a secondary cause of hypertension, 16 had an eGFR <30 ml/min/1.73m$^2$, and 7 for other reasons. Patient information sheet was provided to 196 patients. Forty patients refused to take part at the outset with a further 36 refused after receiving the patient information sheet. One hundred and twenty-six patients provided written consent and 100 patients attended the study visit.

Sixty-three patients were taking three or more antihypertensives of which 41 were classed as TRH based on their BP and adherence to their pharmacological therapy (see **Figure 4.2**). TRH group comprised of 13 patients prescribed four or more antihypertensives with controlled BP and 28 patients prescribed three or more antihypertensives with uncontrolled BP. All 41 patients were shown to have at least three or more antihypertensives in their urine proving their adherence. Any patient prescribed <3 antihypertensives (n=37) and those prescribed ≥3 antihypertensives found not to have TRH (n=22) were classified as the non-TRH group. There were 19 patients taking at least 3 antihypertensive but found to be non-adherent and labelled as having pseudo-TRH and there were 3 patients who were taking 3 antihypertensives with confirmed adherence and controlled BP and labelled as controlled HTN (see **Figure 4.2**).

The baseline demographics of patients comprising the non-TRH group (<3 antihypertensive and pseudo-TRH / controlled HTN) were compared and listed in **Table 4.2**. Generally, the two groups were similar across gender, ethnicity, BP, smoking status, duration of hypertension, comorbidities (except for ischaemic heart disease) and family history. Patients taking less than 3 antihypertensives were significantly younger than those with pseudo-TRH; mean ± SD 41.3 ± 13.2 and 52.8 ± 11.3 (p=0.001) respectively. Higher proportion of patients labelled pseudo-TRH were found to have ischaemic heart disease, 5 (22.7%) versus 1 (2.7%) in those taking <3 antihypertensives (p=0.023).

The two groups were combined to form a non-TRH comparator group. Ideally, the analysis would be best carried out comparing those with TRH to individual subgroups given the significant difference in age and prevalence of ischaemic heart disease in these two subgroups. However, due to relatively small numbers these analyses may not yield much meaningful comparisons. Age and, where appropriate, co-morbidities will be included any multivariable analysis to overcome this limitation.

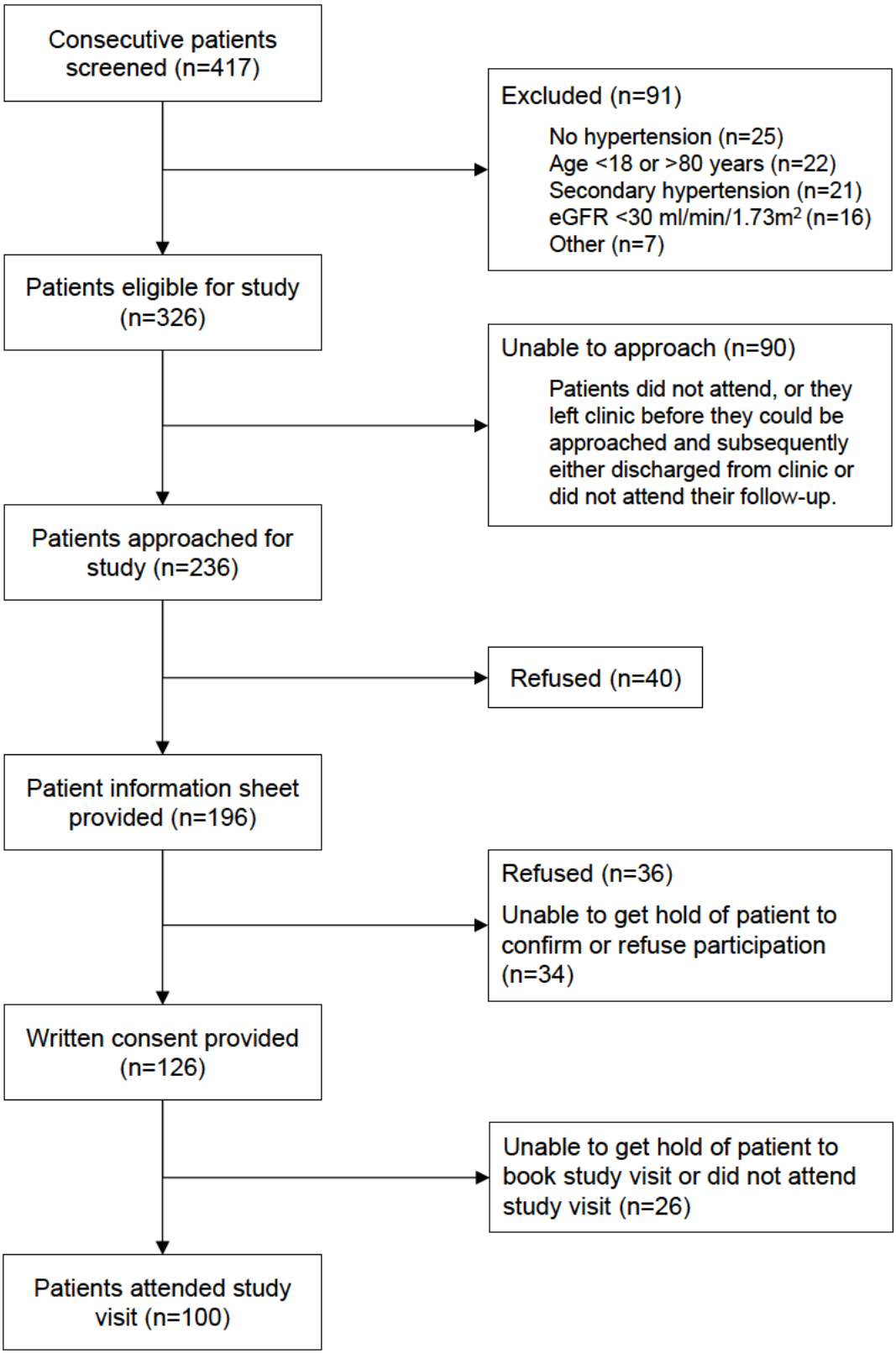



**Figure 4.1:** Flow diagram of patient recruitment in the FACT-RHY study

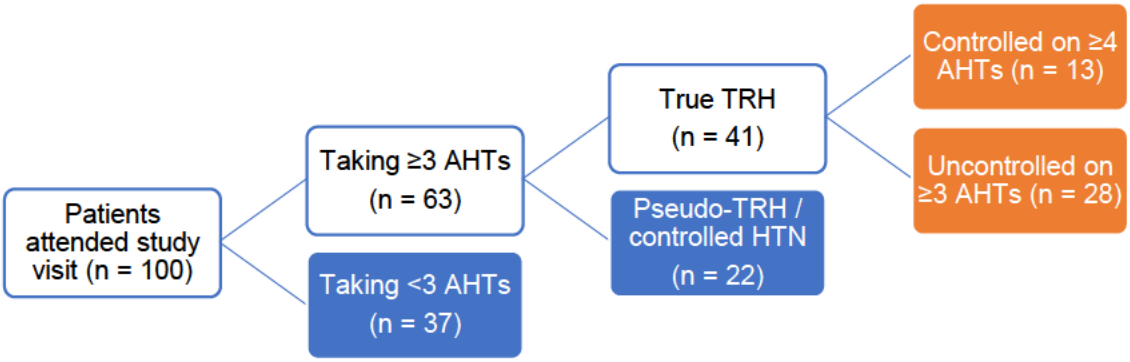



**Figure 4.2:** Allocation of patients into groups - treatment-resistant hypertension (orange) and non-TRH (blue). Abbreviations: AHTs, antihypertensives; TRH, treatment-resistant hypertension.

**Table 4.2** Comparison of baseline demographics of two patients taking<3 AHTs and those with psuedo-TRH / controlled HTN

| | <3 AHTs | Pseudo-TRH / controlled HTN | P value |
|---|---|---|---|
| N | 37 | 22 | |
| Age§, years | 41.4 ± 13.2 | 52.8 ± 11.3 | 0.001 |
| **Gender** | | | 1.000 |
| Male | 16 (43.2) | 9 (40.9) | |
| Female | 21 (56.8) | 13 (59.1) | |
| **Ethnicity** | | | 0.232 |
| Caucasian | 20 (54.1) | 12 (54.5) | |
| African-Caribbean | 7 (18.9) | 3 (13.6) | |
| Asian | 10 (27.0) | 5 (22.7) | |
| Mixed | 0 (0) | 2 (9.1) | |
| Systolic BP, mmHg | 138 (127 – 157) | 143 (136 – 163) | 0.256 |
| Diastolic BP, mmHg | 94 ± 13 | 98 ± 14 | 0.274 |
| **Smoking Status** | | | 0.939 |
| Never | 19 (46.3) | 33 (55.9) | |
| Ex | 13 (31.7) | 14 (23.7) | |
| Current | 9 (22.0) | 12 (20.3) | |
| **Duration of hypertension** | | | 0.619 |
| <1-5 years | 20 (54.1) | 9 (40.9) | |
| 6-15 years | 13 (35.1) | 10 (45.5) | |
| >15 years | 4 (10.8) | 3 (13.6) | |
| **Comorbidities** | | | |
| Ischaemic heart disease | 1 (2.7) | 5 (22.7) | 0.023 |
| Diabetes Mellitus | 1 (2.7) | 4 (18.2) | 0.059 |
| Cerebrovascular disease | 2 (5.4) | 2 (9.1) | 0.624 |
| Chronic kidney disease | 1 (2.7) | 0 (0) | 1.000 |
| Peripheral vascular disease | 1 (2.7) | 0 (0) | 1.000 |
| **Family History** | | | |
| Hypertension | 28 (75.7) | 20 (90.9) | 0.184 |
| Ischaemic heart disease | 9 (24.3) | 4 (18.2) | 0.749 |
| Cerebrovascular disease | 6 (16.2) | 5 (22.7) | 0.731 |

Data presented as frequencies (percentage), mean ± standard deviation, and median (interquartile range). Abbreviations: AHTs, antihypertensives; TRH, treatment-resistant hypertension; HTN, hypertension; BP, blood pressure.

### 4.3.2 Baseline demographics

One hundred patients attended the study visit with almost equal number of males and females. The ethnic make-up of the study population reflects the catchment population of Birmingham Heartlands Hospital with a majority Caucasian followed by Asian and African-Caribbean; 51%, 24% and 22% respectively. The prevalence of TRH was similar in males and females (p=0.311) and did not differ significantly with ethnicity (p = 0.546). Baseline demographics are summarised in **Table 4.3**.

The mean BMI was similar in TRH and control patients; 32.5 kg/m$^2$ and 31.4 kg/m$^2$ respectively. The waist-to-hip and waist-to-height ratios were both significantly higher in the TRH group when compared to the control group; with a mean difference of 0.05 (95% CI: 0.008 – 0.098) and 0.04 (95% CI: 0.003 – 0.068) respectively.

Mean (SD) SBP in the TRH group was 149 (27) mmHg in the TRH group compared to 145 (25) mmHg in the control group (p=0.491). The mean diastolic BP was 89 (14) mmHg and 95 (13) mmHg respectively in the two groups (p = 0.034). LVH which was assessed from the ECG using the Cornell product criteria (587) was present in 21 patients of whom 14 (34.1%) had TRH (p = 0.007).

Fifty-two patients in the entire cohort never smoked, and the remaining 48 patients were either ex-smokers (n=27) or current smokers (n=21). The median (IQR) smoking pack years was 16 (5 – 35) which was relatively higher in the TRH group compared to the control group. More than three quarters of all study patients reported drinking no regular alcohol. The mean (SD) alcohol intake in the 19 remaining patients was 11 (6) units per week with a higher alcohol

consumption in the TRH group than the control group. The differences observed for smoking and alcohol consumption between the two study groups were, however, statistically non-significant.

Self-reported duration of hypertension was significantly higher in the TRH group ($p<0.001$) with 83% (n=34) of patients with TRH reported being treated for hypertension for more than 5 years compared to 49% (n=29) non-TRH patients with a treatment duration of 5 years or less. The prevalence of TRH did not differ significantly in patients with other co-morbidities including ischaemic heart disease, cerebrovascular disease (stroke or TIA), CKD and peripheral vascular disease. Only presence of diabetes mellitus was significantly associated with TRH; 27% of patients with TRH had diabetes compared to 9% in controls ($p=0.024$). Family history of hypertension, ischaemic heart disease or cerebrovascular disease did not differ significantly in TRH and control groups.

**Table 4.3:** Baseline demographic and clinical characteristics of all study patients

| | All Patients | TRH | Non-TRH | P value |
|---|---|---|---|---|
| N | 100 | 41 | 59 | |
| Age§, years | 48 (46 – 51) | 57 (53 – 60) | 44 (40 – 47) | **<0.001** |
| **Gender** | | | | 0.364 |
| Male | 47 | 22 (53.7) | 25 (42.4) | |
| Female | 53 | 19 (46.3) | 34 (57.6) | |
| **Ethnicity** | | | | 0.546 |
| Caucasian | 51 | 19 (46.3) | 32 (54.2) | |
| African-Caribbean | 22 | 12 (29.3) | 10 (16.9) | |
| Asian | 24 | 9 (22.0) | 15 (25.4) | |
| Mixed | 3 | 1 (2.4) | 2 (3.4) | |
| Body Mass Index, kg/m$^2$ | 31.9 ± 6.0 | 32.5 ± 4.9 | 31.4 ± 6.6 | 0.406 |
| Waist to Hip ratio§ | 0.92 (0.90 – 0.95) | 0.96 (0.92 – 0.99) | 0.90 (0.87 – 0.93) | **0.017** |
| Waist to Height ratio | 0.61 ± 0.09 | 0.63 ± 0.07 | 0.59 ± 0.10 | **0.031** |
| Systolic BP, mmHg§ | 145 (140 – 149) | 147 (139 – 155) | 143 (137 – 149) | 0.501 |
| Diastolic BP, mmHg | 93 ± 14 | 89 ± 14 | 95 ± 13 | **0.034** |
| Left ventricular hypertrophy | 21 | 14 (34.1) | 7 (11.9) | **0.015** |
| **Smoking Status** | | | | 0.598 |
| Never | 52 | 19 (46.3) | 33 (55.9) | |
| Ex | 27 | 13 (31.7) | 14 (23.7) | |
| Current | 21 | 9 (22.0) | 12 (20.3) | |
| Smoking pack years§ (n=44) | 14 (10 – 20) | 20 (12 – 32) | 11 (7 – 17) | 0.057 |
| Alcohol intake, units per week (n=19) | 11 ± 6 | 14 ± 7 | 9 ± 5 | 0.082 |
| **Duration of hypertension** | | | | **<0.001** |
| <1-5 years | 36 | 7 (17.1) | 29 (49.2) | |
| 6-15 years | 33 | 10 (24.4) | 23 (39.0) | |
| >15 years | 31 | 24 (58.5) | 7 (11.9) | |
| **Comorbidities** | | | | |
| Ischaemic heart disease | 13 | 7 (17.1) | 6 (10.2) | 0.479 |
| Diabetes Mellitus | 16 | 11 (26.8) | 5 (8.5) | **0.029** |
| Cerebrovascular disease | 9 | 5 (12.2) | 4 (6.8) | 0.481 |
| Chronic kidney disease | 4 | 3 (7.3) | 1 (1.7) | 0.302 |
| Peripheral vascular disease | 4 | 3 (7.3) | 1 (1.7) | 0.302 |
| **Family History** | | | | |
| Hypertension | 78 | 30 (73.2) | 48 (81.2) | 0.468 |
| Ischaemic heart disease | 21 | 8 (19.5) | 13 (22.0) | 0.956 |
| Cerebrovascular disease | 23 | 12 (29.3) | 11 (18.6) | 0.317 |
| Number of antihypertensives | 3 (2 – 4) | 4 (3 – 5) | 2 (1 – 3) | **<0.001** |
| Total number of medications | 5 (3 – 8) | 7 (5 – 10) | 3 (2 – 6) | **<0.001** |

Data presented as frequencies (percentage), mean ± standard deviation, and median (interquartile range). Log transformed variables are reported as mean (95% confidence interval) and indicated by §. Parametric data were analysed using independent-samples t-test. Non-parametric data were analysed using Mann-Whitney U test. Pearson's $\chi^2$ test was used for counts.

### 4.3.3 Adherence and medications

All prescribed medications were recorded from the study population. The median (IQR) number of antihypertensives prescribed to the entire study population was 3 (2 – 4); 4 (3 – 5) in TRH group and 2 (1 – 3) in the control group. The median for total number of all prescribed medications was 5 (3 – 8) in all patients, 7 (5 – 10) in TRH group and 3 (2 – 6) in the control group ($p<0.001$). Lipid-lowering pharmacological therapy was prescribed to 49% (n=20) patients with TRH and 19% of control patients ($p=0.002$).

The proportions of patients prescribed individual class antihypertensive pharmacological agents are detailed in **Table 4.4**. CCBs, RAAS inhibitors and diuretics were the commonest classes of antihypertensives prescribed overall and in both groups. All classes of antihypertensives were more commonly prescribed in TRH patients.

Adherence to antihypertensive medications was categorised into three groups; adherent, partially-adherent and non-adherent. In adherent patients all the prescribed antihypertensives medications were present in their urine. In partially-adherent patients at least one or more of their prescribed antihypertensives were not detected in their urine. Non-adherent patients had none of their prescribed antihypertensives in the urine. Fifty-eight patients were fully adherent, 29 partially-adherent and 13 non-adherent as shown in **Figure 4.3**. Patients were assumed to be adherent to any antihypertensive that the AHS had not been optimised to detect which occurred in 20 individual prescriptions out of a total of 300 antihypertensives prescribed to the total study population equating to 6.7%.

Adherence to each class of antihypertensives was assessed as the proportion of all antihypertensives belonging to a particular class that were prescribed in the study population to examine any differences present by type of antihypertensive. Similar rates of adherence were observed across all the classes of antihypertensives and, in particular, the three most commonly used groups including CCBs, RAAS inhibitors and all diuretics (see **Figure 4.4**).

**Table 4.4:** Number of prescribed medications and class of antihypertensives

| | All Patients | TRH | non-TRH | P value |
|---|---|---|---|---|
| Number of antihypertensives | 3 (2 – 4) | 4 (3 – 5) | 2 (1 – 3) | <0.001 |
| Total number of medications | 5 (3 – 8) | 7 (5 – 10) | 3 (2 – 6) | <0.001 |
| Lipid lowering therapy | 31 | 20 (48.8) | 11 (18.6) | 0.003 |
| **Class of prescribed antihypertensives** | | | | |
| Calcium channel blocker | 72 | 34 (82.9) | 38 (64.4) | 0.072 |
| Renin angiotensin aldosterone system inhibitors | 87 | 40 (97.6) | 47 (79.7) | 0.021 |
| Diuretic | 57 | 33 (80.7) | 24 (40.7) | <0.001 |
| Thiazide/thiazide-like | 33 | 17 (41.5) | 16 (27.1) | 0.199 |
| Loop | 20 | 12 (29.3) | 8 (13.6) | 0.093 |
| Potassium-sparing | 22 | 18 (43.9) | 4 (6.8) | <0.001 |
| Beta-adrenergic receptor blocker | 29 | 20 (48.8) | 9 (15.3) | 0.001 |
| Alpha-adrenergic receptor blocker | 27 | 20 (48.8) | 7 (11.9) | <0.001 |
| Others | 9 | 8 (19.5) | 1 (1.7) | 0.003 |

Data on number of antihypertensives and total number of medications are presented as median (IQR). All other data are presented as counts (percentage is expressed as a proportion of the corresponding column total).

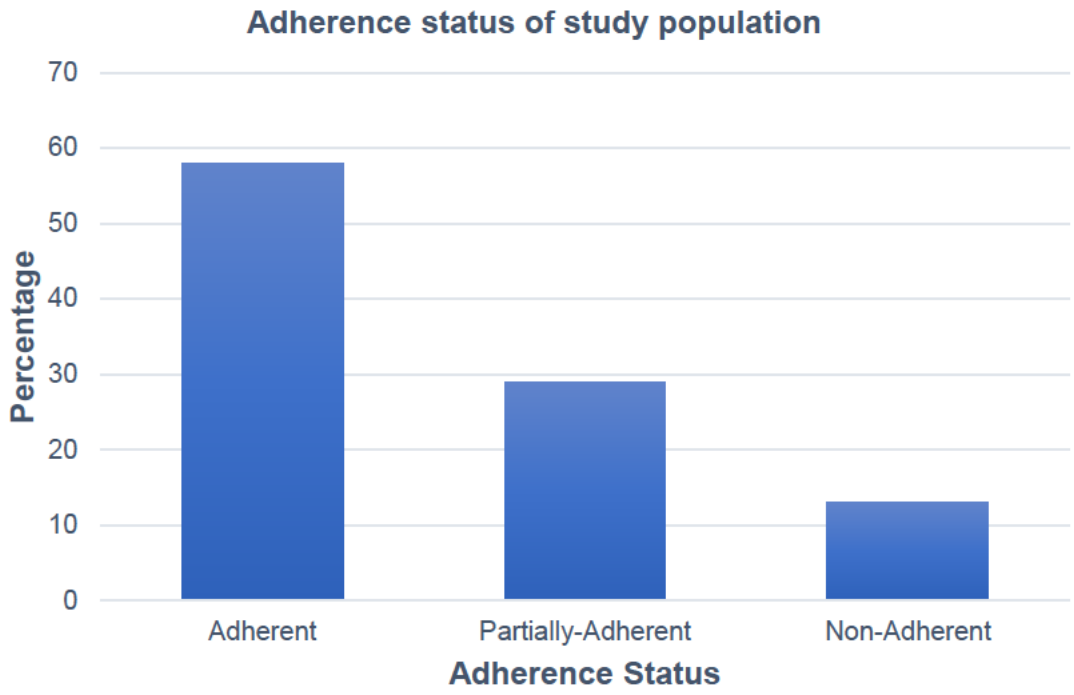



**Figure 4.3:** Adherence status of the study population.

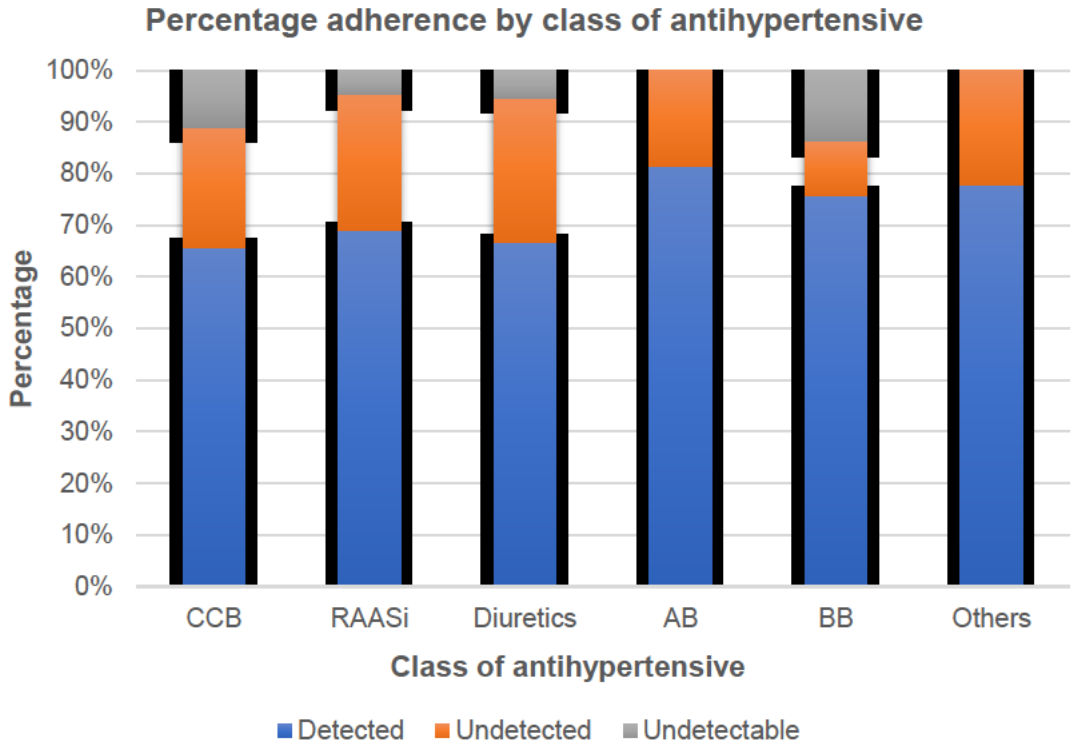



**Figure 4.4:** Percentage of prescribed antihypertensives detected, undetected or undetectable by class. **Abbreviations:** CCB, calcium channel blocker; RAASi, renin-angiotensin-aldosterone inhibitor; AB, alpha-adrenergic receptor blocker; BB, beta-adrenergic receptor blocker.

### 4.3.4 Biochemical characteristics

Results of the blood tests carried out the study population including TRH and control groups are summarised in **Table 4.5**. The kidney function derived using the measured serum creatinine to calculate eGFR (using either the Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] or the MDRD equations) was significantly lower in the TRH group when compared to the control group; 76 (21) ml/min/1.73m$^2$ and 97 (20) ml/min/1.73m$^2$ respectively ($p<0.001$). In the liver function profile, serum albumin was significantly lower in patients with TRH. There was no significant difference in any of the indices measured in the lipid profile between both groups of patients. Similarly, levels of TSH, serum cortisol, CRP and Vitamin D were similar across the two group of patients.

HbA1c was significantly higher in patients with TRH when compared to controls; 42 (38 – 47) mmol/mol and 38 (35 – 41) mmol/mol respectively ($p= 0.001$). NT-proBNP and high-sensitivity troponin I were both significantly elevated in patients with TRH. Median (IQR) NT-pro-BNP was 126 (72 – 390) ng/L in TRH patients compared to 54 (27 – 91) ng/L in the control patients (<0.001). ARR was also found to be significantly raised in patients with TRH; 21 (6 – 51) pmol/mUL compared to 8 (2 – 19) pmol/mUL ($p=0.012$).

**Table 4.5:** Biochemical characteristics of the study population including TRH and control groups.

| | N | All Patients | TRH | non-TRH | P value |
|---|---|---|---|---|---|
| Sodium, mmol/L | 100 | 139 (138 – 141) | 139 (138 – 141) | 139 (138 – 141) | 0.989 |
| Potassium, mmol/L | 100 | 4.0 ± 0.4 | 3.9 ± 0.4 | 4.1 ± 0.4 | **0.038** |
| Urea, mmol/L | 100 | 5.7 ± 1.8 | 6.6 ± 1.9 | 5.1 ± 1.4 | **<0.001** |
| Serum creatinine, µmol/l | 100 | 76 (66 – 95) | 88 (72 – 110) | 69 (62 – 88) | **<0.001** |
| eGFR CKD-EPI, ml/min/1.73m$^2$ | 100 | 89 ± 23 | 76 ± 21 | 97 ± 20 | **<0.001** |
| Phosphate, mmol/L | 100 | 1.05 ± 0.19 | 1.06 ±0.18 | 1.06 ±0.19 | 0.981 |
| Alkaline Phosphatase§, IU/L | 100 | 77 (72 – 82) | 81 (73 – 89) | 74 (67 – 82) | 0.231 |
| Total Protein§, g/L | 100 | 72 (71 – 73) | 71 (69 – 73) | 72 (71 – 74) | 0.311 |
| Albumin (g/L) | 100 | 37 ± 3.0 | 36 ± 2.7 | 38 ± 3.0 | **0.009** |
| Corrected calcium, mmol/L | 100 | 2.44 (2.39 – 2.50) | 2.46 (2.40 – 2.54) | 2.44 (2.39 – 2.50) | 0.470 |
| CRP, mg/L | 100 | 3 (2 – 7) | 4 (2 – 8) | 3 (1 – 6) | 0.096 |
| Glucose, mmol/L | | 5.2 (4.7 – 5.9) | 5.4 (4.8 – 6.7) | 5.1 (4.6 – 5.5) | 0.134 |
| Cholesterol§, mmol/l | 100 | 4.9 (4.7 – 5.2) | 4.8 (4.4 – 5.2) | 5.0 (4.8 – 5.3) | 0.223 |
| Triglycerides, mmol/L | 100 | 1.4 (0.9 – 2.2) | 1.6 (1.1 – 2.2) | 1.3 (0.9 – 2.2) | 0.491 |
| HDL-C§, mmol/L | 100 | 1.17 (1.11 – 1.23) | 1.14 (1.04 – 1.26) | 1.19 (1.12 – 1.26) | 0.522 |
| LDL-C, mmol/L | 95 | 3.04 (2.37 – 3.68) | 2.95 (2.06 – 3.35) | 3.19 (2.45 – 3.89) | 0.119 |
| Cholesterol / HDL-C§ ratio | 100 | 4.2 (3.9 – 4.5) | 4.2 (3.8 – 4.6) | 4.2 (3.9 – 4.6) | 0.742 |
| TSH, mU/L | 99 | 1.10 (0.78 – 1.60) | 0.98 (0.77 – 1.65) | 1.10 (0.80 – 1.50) | 0.828 |
| Cortisol§, nmol/L | 99 | 178 (162 – 195) | 188 (165 – 214) | 171 (149 – 196) | 0.333 |
| Total Vitamin D§, nmol/L | 94 | 34.7 (30.7 – 39.3) | 35.6 (29.2 – 43.5) | 34.2 (29.0 – 40.2) | 0.742 |
| HbA1c – IFCC, mmol/mol | 100 | 40 (36 – 44) | 42 (38 – 47) | 38 (35 – 41) | **0.001** |
| Kappa/Lambda ratio§ | 100 | 1.24 (1.17 – 1.31) | 1.33 (1.22 – 1.45) | 1.17 (1.09 – 1.26) | **0.025** |
| Combined free light chains§ | 100 | 35.9 (33.2 – 38.9) | 41.2 (36.7 – 46.4) | 32.6 (29.5 – 36.1) | **0.006** |
| NT-proBNP§, ng/L | 82 | 84 (64 – 110) | 163 (108 – 247) | 52 (38 – 69) | **<0.001** |
| hs-Troponin I, ng/L | 100 | 3 (3 – 7) | 5 (3 – 18) | 3 (3 – 3) | **<0.001** |
| Aldosterone / Renin ratio§, pmol/mUL | 88 | 12 (9 – 15) | 18 (11 – 28) | 9 (7 – 13) | **0.016** |

Log transformed variables are reported as mean (95% confidence interval) and indicated by §. **Abbreviations:** eGFR: estimated glomerular filtration rate, CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration, CRP: c-reactive protein, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, TSH: thyroid stimulating hormone, HbA1c: haemoglobin A1c, IFCC: International Federation of Clinical Chemistry and Laboratory Medicine, NT-proBNP: N terminal pro B type natriuretic peptide.

### 4.3.5 Spot and 24-hr urine tests

The results of all tests conducted on urinary samples provided by the study population are summarised in **Table 4.6**. Urine sample collected at the study visit was analysed for microalbuminuria. Median (IQR) albumin-to-creatinine ratio, was higher in the TRH group compared to the control group; 2.8 (1.1 – 9.2) mg/mmol and 1.4 (0.7 – 7.7) mg/mmol but it did not meet the significance cut-off (p=0.077). Adequate 24-hour urinary collection was possible in 60% of the study population. The proportion of satisfactory acidified 24-hour urine collection for urinary free catecholamines and metadrenalines was available for 25 (61%) TRH and 35 (59%) control patients. Similar proportions were observed for plain 24-hour urine collection used for estimation of daily salt intake; 27 (66%) and 33 (56%) respectively.

None of the urinary free catecholeamines – noradrenaline, adrenaline and dopamine – and urinary free metadrenalines – normetadrenaline, metadrenaline, and 3-methoxytyramine – were significantly different in the two groups of patients. The median (IQR) daily salt intake, estimated by using the measured urinary sodium output, was 6.9 (4.7 – 10.9) g/day in the TRH patients and was similar to the control patients who consumed a median of 6.6 (4.6 – 10.0) g of salt per day (p=0.608).

**Table 4.6:** Spot and 24-hour urine tests in the study population including TRH and control groups

| | N | All Patients | TRH | non-TRH | P value |
|---|---|---|---|---|---|
| Albumin / Creatinine ratio, mg/mmol | 99 | 1.6 (0.8 – 8.6) | 2.8 (1.1 – 9.2) | 1.4 (0.7 – 7.7) | 0.077 |
| UF Noradrenaline output§, nmol/day | 58 | 288 (248 – 336) | 291 (219 – 387) | 286 (240 – 342) | 0.916 |
| UF Adrenaline output§, nmol/day | 59 | 18 (14 – 23) | 16 (11 – 23) | 20 (15 – 27) | 0.309 |
| UF Dopamine output, nmol/day | 60 | 1978 (1231 – 2500) | 1507 (981 – 2419) | 1996 (1455 – 2527) | 0.258 |
| UF Normetadrenaline output§, µmol/day | 59 | 1.80 (1.56 – 2.08) | 1.90 (1.47 – 2.45) | 1.74 (1.45 – 2.07) | 0.551 |
| UF Metadrenaline output§, µmol/day | 59 | 0.62 (0.54 – 0.71) | 0.59 (0.48 – 0.74) | 0.64 (0.53 – 0.77) | 0.621 |
| UF 3-methoxytyramine output§, µmol/day | 56 | 0.81 (0.71 – 0.93) | 0.80 (0.63 – 1.02) | 0.82 (0.69 – 0.97) | 0.904 |
| Daily salt intake§, g/day | 60 | 6.6 (5.8 – 7.7) | 7.2 (5.9 – 8.6) | 6.3 (5.1 – 7.7) | 0.344 |

Log transformed variables are reported as mean (95% confidence interval) and indicated by §.
**Abbreviations**: UF, urinary free.

### 4.3.6 Body Composition

Body composition monitoring using BIS was carried out in all 100 patients attending the study visit. The comparison of baseline body composition parameters of the TRH and control groups is summarised in **Table 4.7**. OH is presented as absolute values in litres and as a percentage of ECW. Absolute OH is divided into three groups; normal (-1.1L to +1.1L), overhydrated (> +1.1L) and, underhydration (< -1.1L). The mean absolute OH and percentage OH were higher in the TRH compared to control but did not reach statistical significance. The BCM identified only 5 patients with absolute overhydration overall, 3 of whom had TRH. Interestingly, however, 47% were found to be underhydrated with an absolute OH less than -1.1 litres with the remaining 48% normally hydrated. The distribution of OH in the study population is shown in **Figure 4.5**.

The mean TBW and ECW, although higher in the patients with TRH when compared to the controls, did not reach statistical significance. The mean ICW was similar in the two study groups. The mean ECW-to-ICW ratio was higher in the TRH group compared to controls; 0.86 (0.09) and 0.82 (0.07) respectively (p=0.013). Similarly, mean ECW-to-TBW ratio was higher in the TRH group comparatively; 0.46 (0.03) and 0.45 (0.02) respectively (p=0.016). However, the magnitude of the mean difference between the two groups is small.

Diuretics alter the body's composition of fluid compartments through increasing sodium and water loss at the kidney. Therefore, a prescription to a diuretic may significantly affect the results observed in the two study groups, especially in the TRH group who had a statistically significant higher rate of prescription to a diuretic (see **Table 4.4**). Therefore, a two-way ANOVA was conducted to explore

whether a prescription of diuretic affected the indices of fluid compartments across the two study groups. A separate ANOVA was carried for OH, OH expressed as percentage of ECW, TBW, ECW, ICW, ECW-to-ICW ratio and, ECW-to-TBW ratio. The interaction effect between TRH status and a prescription to diuretic was not statistically significant in any of the 7 separate two-way ANOVA analyses (see **Table 4.8**).

When comparing the various indices of body tissue composition as measured by the Fresenius BCM the two groups appear similar (**Table 4.7**). There is no significant difference in mean lean tissue index and mean fat tissue index across the two groups. Likewise, lean and fat tissue expressed as a percentage of body weight was similar for TRH and control patients.

Correlations between BCM parameters of water distribution and biochemical parameters in the entire study population are listed in **Table 4.8** and the statistically significant correlations of OH are graphically presented in **Figure 4.6**. OH was negatively associated with serum albumin in the overall sample level ($r = -0.215$, $p = 0.032$). Serum albumin was associated with other measures of fluid excess, with the magnitude of correlation coefficient greatest with ECW-to-ICW ratio ($r = -0.361$, $p<0.001$). This observed association was stronger in the TRH cohort compared to the controls (**Figure 4.6**a). OH also correlated positively with aldosterone-to-renin ratio ($r = 0.250$, $p = 0.019$; **Figure 4.6**b), NT-proBNP ($r = 0.201$, $p = 0.070$), and albumin-to-creatinine ratio ($r = 0.281$, $p = 0.005$; **Figure 4.6**c) and correlated negatively with daily salt intake ($r = - 0.265$, $p = 0.041$; **Figure 4.6**d). No association between OH and eGFR (CKD-EPI) was found, eGFR was, however, negatively associated with ECW-to-ICW ratio ($r = -0.239$, $p = 0.017$).

**Table 4.7:** Comparison of body composition parameters in TRH and control patients

| | All Patients | TRH | non-TRH | P value |
|---|---|---|---|---|
| Overhydration, L | -1.0 ± 1.2 | -0.7 ± 1.4 | -1.1 ± 1.0 | 0.075 |
| Overhydration, % of ECW | -5.9 ± 6.9 | -4.5 ± 7.3 | -6.9 ± 6.4 | 0.085 |
| **Overhydration status** | | | | 0.341 |
| Normal | 48 (48.0) | 22 (53.7) | 26 (44.1) | |
| Overhydrated | 5 (5.0) | 3 (7.3) | 2 (3.4) | |
| Underhydrated | 47 (47.0) | 16 (39) | 31 (52.5) | |
| Total body water, L | 38.9 ± 9.0 | 39.6 ± 8.7 | 38.4 ± 9.2 | 0.530 |
| Extracellular water, L | 17.7 ± 4.2 | 18.2 ± 4.0 | 17.3 ± 4.3 | 0.286 |
| Intracellular water, L | 21.2 ± 5.0 | 21.4 ± 5.0 | 21.1 ± 5.1 | 0.814 |
| ECW / ICW ratio | 0.83 ± 0.08 | 0.86 ± 0.09 | 0.82 ± 0.07 | **0.013** |
| ECW / TBW ratio | 0.45 ± 0.02 | 0.46 ± 0.03 | 0.45 ± 0.02 | **0.016** |
| Lean tissue index, kg/m$^2$ | 14.0 ± 2.8 | 14.3 ± 3.1 | 13.8 ± 2.5 | 0.428 |
| Fat tissue index kg/m$^2$ | 18.2 ± 6.1 | 18.4 ± 5.5 | 18.1 ± 6.6 | 0.816 |
| Lean tissue mass, % of body weight | 45.1 ± 10.7 | 44.6 ± 10.5 | 45.5 ± 10.9 | 0.708 |
| Fat tissue mass, % of body weight | 41.0 ± 7.9 | 41.0 ± 7.9 | 41.0 ± 8.0 | 0.970 |
| Adipose tissue mass, kg | 51.9 ± 18.2 | 51.9 ± 16.4 | 51.8 ± 19.5 | 0.981 |
| Body cell mas, kg | 22.5 ± 7.2 | 22.8 ± 7.8 | 22.3 ± 6.8 | 0.708 |

**Abbreviations:** ECW, extracellular water; ICW, intracellular water; TBW, Total Body water.

**Table 4.8:** Interaction between use of diuretics and TRH status for indices of bodily fluid compartments

| | F | P value | Partial Eta squared |
|---|---|---|---|
| Overhydration | 0.200 | 0.656 | 0.002 |
| Overhydration % of ECW | 0.258 | 0.612 | 0.003 |
| Total body water | 0.033 | 0.856 | 0.0003 |
| Extracellular water | 0.047 | 0.829 | 0.0005 |
| Intracellular water | 0.254 | 0.615 | 0.003 |
| ECW / ICW ratio | 3.226 | 0.076 | 0.033 |
| ECW / TBW ratio | 3.669 | 0.058 | 0.037 |

**Abbreviations:** ECW, extracellular water; ICW, intracellular water; TBW, Total Body water.

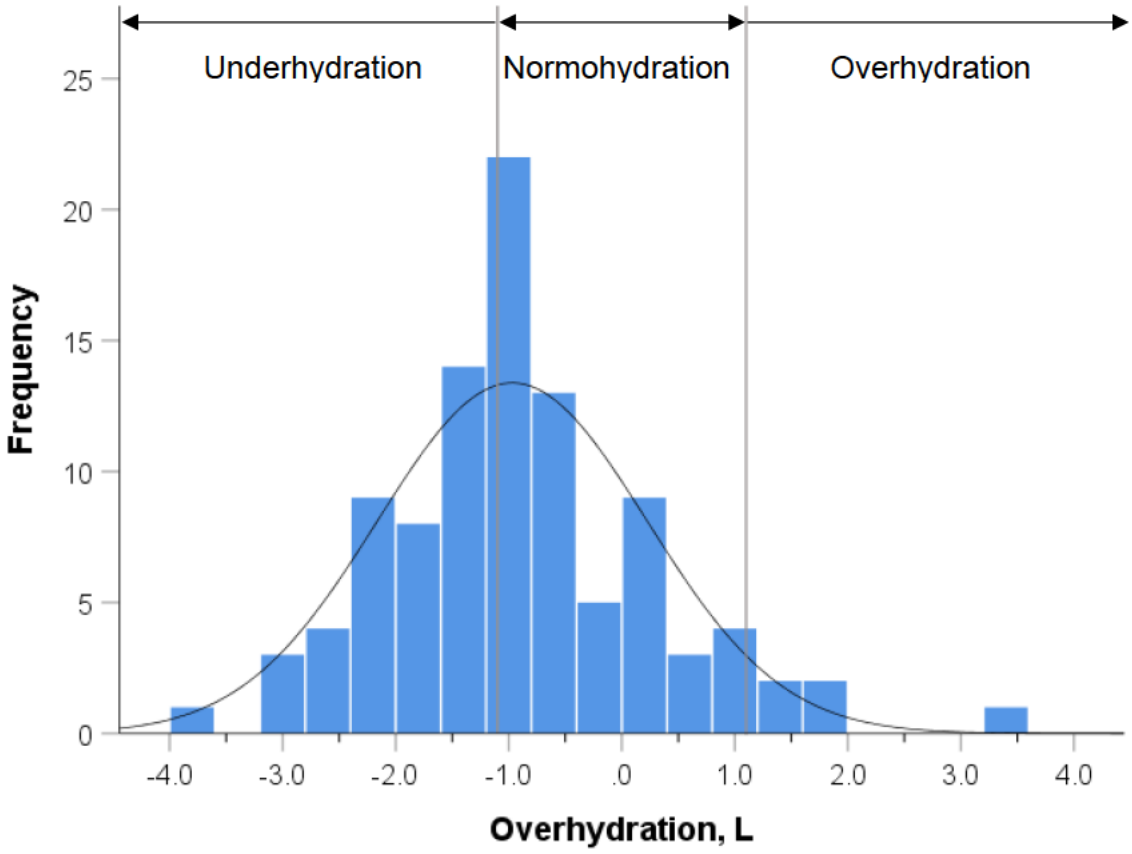



**Figure 4.5:** The distribution of overhydration (OH) in 100 patients with hypertension, ranging between -3.8L to 3.4L.

**Table 4.9:** Correlation matrix of BCM parameters of water distribution and biochemical parameters in the entire study population.

| | | OH, L | OH, %ECW | TBW, L | ECW, L | ICW, L | ECW/ ICW |
|---|---|---|---|---|---|---|---|
| **Serum sodium, mmol/L (n=100)** | r | 0.009 | 0.023 | 0.236 | 0.255 | 0.209 | 0.143 |
| | p | 0.930 | 0.819 | 0.018 | 0.011 | 0.037 | 0.155 |
| **eGFR CKD-EPI, ml/min/1.73m$^2$ (n=100)** | r | -0.080 | -0.064 | -0.052 | -0.097 | -0.012 | -0.239 |
| | p | 0.428 | 0.526 | 0.608 | 0.339 | 0.909 | 0.017 |
| **Serum albumin, g/L (n=100)** | r | -0.215 | -0.280 | -0.147 | -0.219 | -0.081 | -0.361 |
| | p | 0.032 | 0.005 | 0.144 | 0.029 | 0.421 | <0.001 |
| **Aldosterone/Renin ratio, pmol/mU (n=88)** | r | 0.250 | 0.227 | 0.044 | 0.090 | 0.004 | 0.256 |
| | p | 0.019 | 0.033 | 0.681 | 0.403 | 0.969 | 0.016 |
| **NT-proBNP, ng/L (n=82)** | r | 0.201 | 0.181 | 0.105 | 0.124 | 0.085 | 0.102 |
| | p | 0.070 | 0.104 | 0.350 | 0.268 | 0.450 | 0.362 |
| **Albumin/Creatinine ratio, mg/mmol (n=99)** | r | 0.281 | 0.222 | -0.051 | -0.017 | -0.075 | 0.121 |
| | p | 0.005 | 0.027 | 0.615 | 0.864 | 0.463 | 0.231 |
| **Daily Salt Intake, g/day (n=60)** | r | -0.265 | -0.177 | 0.328 | 0.307 | 0.333 | -0.066 |
| | p | 0.041 | 0.177 | 0.010 | 0.017 | 0.009 | 0.614 |

**Abbreviations:** OH, overhydration; ECW, extracellular water; TBW, total body water; ICW, intracellular water; eGFR, estimated glomerular filtration rate; CKD-EPI, chronic kidney disease – epidemiology collaboration; NT-proBNP; N-terminal pro B-type natriuretic peptide; r, Pearson's correlation coefficient; p, significance value.

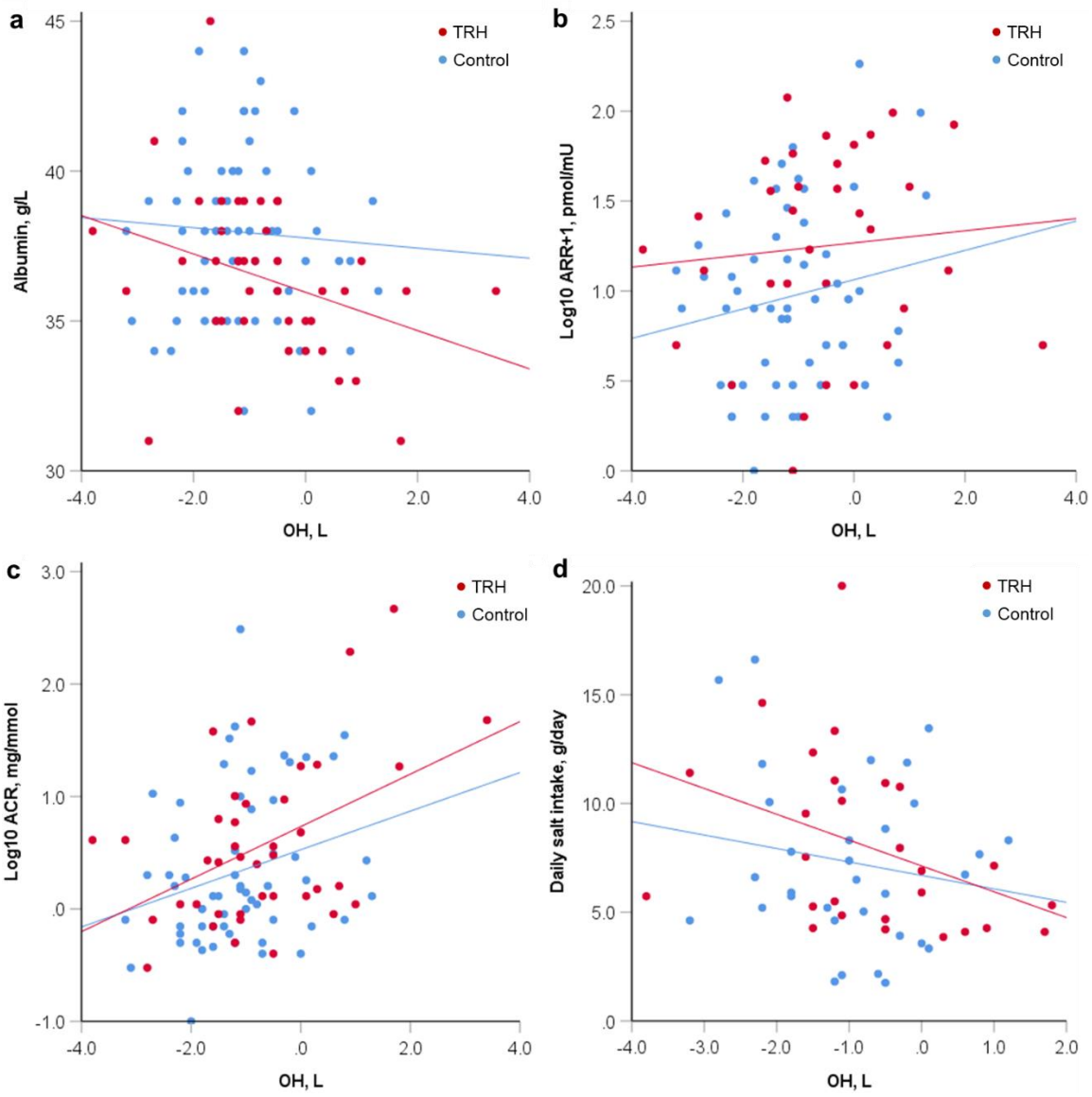



**Figure 4.6:** Factors associated with overhydration (OH) Univariate analysis of the correlations of OH with the (a) albumin, (b) $Log_{10}$ aldosterone-renin ratio (ARR) – 1, (c) $log_{10}$ urine albumin-creatinine ratio (ACR), and (d) daily salt intake.

### 4.3.7 Arterial stiffness

Pulse wave analysis and PWV measurements were carried out in all patients using the Vicorder and are shown in **Table 4.10**. Mean peripheral SBP as measured by the Vicorder was higher in the TRH patients compared to controls, 162 ± 22 mmHg and 153 ± 23 mmHg respectively, following the same pattern as the mean SBP measured by the BpTRU device. The DBP measured by the Vicorder on the other hand was the same in the two groups; BpTRU-determined DBP was shown to be much higher in control patients (**Table 4.3**). Central or aortic SBP determined by pulse wave analysis was similar in the two groups of patients. Both central and peripheral pulse pressures were significantly higher in the patients with TRH compared to controls. Augmentation index, either alone or adjusted to a HR of 75 beats per minute, was not found be significantly different in the two groups of patients. Mean carotid-femoral PWV (cfPWV) was, however, significantly higher in patients with TRH when compared to controls; 10.0 ± 2.2 m/s and 8.8 ± 2.1 m/s respectively ($p = 0.008$). Except stroke volume, which was also higher in the TRH cohort, estimates of other haemodynamic parameters calculated by pulse wave analysis did not differ significantly between TRH patients and controls (**Table 4.9**).

Univariate correlations of other study parameters with cfPWV are shown in **Table 4.10**. There was a strong positive correlation between increasing age and cfPWV ($r = 0.527$ $p<0.001$). Gender, ethnicity, BMI and smoking status had no significant correlation with cfPWV, whilst smoking pack years, a longer duration of hypertension and presence of diabetes mellitus had significant positive correlation with cfPWV. Numbers of total prescribed medications and antihypertensives, and prescription to a lipid lowering agent had a modest

positive correlation with cfPWV. Use of a CCB and beta-adrenergic receptor blocker were only weakly correlated with cfPWV.

Amongst the biochemical markers eGFR (CKD-EPI) had the strongest correlation with cfPWV; a negative correlation with increasing eGFR ($r = -0.373$, $p<0.001$). C-reactive protein, HbA1c, $\log_{10}$ (aldosterone-renin ratio +1) and $\log_{10}$ (albumin-creatinine ratio) correlated positively whilst serum albumin correlated negatively with cfPWV. Body composition parameters including ECW-ICW ratio, lean tissue index and fat tissue index correlated with cfPWV. Except peripheral DBP (measured using BpTRU) and HR all other haemodynamic parameters correlated with cfPWV with both peripheral and central SBPs exhibiting the strongest of correlations; $r = 0.508$ and 0.510 respectively ($p<0.001$ for both). Calculated Qrisk also showed a positive correlation with cfPWV.

**Table 4.10:** Haemodynamic parameters of arterial pulse wave analysis in the entire study population stratified according to patient groups.

| | All Patients | TRH | non-TRH | P value |
|---|---|---|---|---|
| cfPWV§, m/s | 9.1 (8.7 – 9.5) | 9.8 (9.2 – 10.5) | 8.6 (8.1 – 9.1) | **0.005** |
| AIx, % | 26.9 ± 8.7 | 27.4 ± 8.2 | 26.5 ± 9.1 | 0.605 |
| $AIx_{75}$, (%) | 24.5 (20.7 – 31.6) | 24.6 (20.7 – 31.2) | 24.5 (20.6 – 31.7) | 0.952 |
| Heart rate, bpm | 68 ± 12 | 62 ± 12 | 71 ± 12 | **<0.001** |
| Peripheral SBP§, mmHg | 155 (151 – 159) | 160 (153 – 167) | 152 (146 – 157) | 0.055 |
| Peripheral DBP§, mmHg | 82 (80 – 84) | 82 (79 – 85) | 82 (80 – 85) | 0.946 |
| Peripheral PP, mmHg | 71 (59 – 84) | 77 (64 – 89) | 68 (57 – 77) | **0.012** |
| Central SBP§, mmHg | 151 (147 – 156) | 156 (150 – 163) | 148 (143 – 153) | 0.054 |
| Central PP, mmHg | 68 (56 – 80) | 74 (62 – 84) | 64 (55 – 74) | **0.013** |
| MAP, mmHg | 113 ± 14 | 114 ± 14) | 113 ± 13 | 0.711 |
| Cardiac output, L/min | 6.9 (6.0 – 8.5) | 6.6 (5.9 – 8.3) | 6.9 (6.0 – 8.3) | 0.823 |
| Total peripheral resistance, $mmHg.min.mL^{-1}$ | 0.99 ± 0.22 | 0.99 ± 0.24 | 0.98 ± 0.20 | 0.897 |
| Stroke volume§, mL | 106 (100 – 113) | 116 (105 – 127) | 100 (93 – 108) | **0.016** |

Log transformed variables are reported as mean (95% confidence interval) and indicated by §.
**Abbreviations:** TRH, treatment-resistant hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; AIx, augmentation index; AIx75, augmentation index corrected for heart rate of 75 beats per minute; bpm, beats per minute; cfPWV, carotid-femoral pulse wave velocity.

**Table 4.11:** Univariate analyses of carotid-femoral pulse wave velocity as the dependant outcome variable

| | Correlation coefficient | P value |
|---|---|---|
| **Demographics** | | |
| Age, years | 0.527 | **<0.001** |
| Gender (Male) | -0.058 | 0.563 |
| Ethnicity | | |
| African-Caribbean | 0.043 | 0.672 |
| Asian | 0.013 | 0.895 |
| Body mass index, kg/m$^2$ | 0.135 | 0.181 |
| Waist to Hip ratio | 0.174 | 0.083 |
| Waist to Height ratio | 0.233 | **0.020** |
| Smoking pack years* (n=44) | 0.398 | **0.007** |
| Duration of hypertension | | |
| <1 – 5 years | -0.222 | 0.027 |
| 6 – 15 years | -0.062 | 0.543 |
| >15 years | 0.293 | 0.003 |
| Presence of diabetes mellitus | 0.209 | 0.037 |
| **Medications** | | |
| Number of antihypertensives | 0.305 | **0.002** |
| Total number of medications | 0.326 | **0.001** |
| Calcium channel blocker use | 0.265 | **0.008** |
| Renin angiotensin system inhibitors use | 0.012 | 0.906 |
| Diuretic use | 0.107 | 0.287 |
| Beta adrenergic receptor blocker use | 0.254 | **0.011** |
| Lipid lowering therapy use | 0.348 | **<0.001** |
| **Biochemical markers** | | |
| eGFR, ml/min/1.73m$^2$ | -0.373 | **<0.001** |
| Albumin | -0.239 | **0.017** |
| C-reactive protein, mg/L | 0.253 | **0.011** |
| Kappa-Lambda ratio | 0.168 | 0.095 |
| HbA1c – IFCC, mmol/mol | 0.237 | **0.017** |
| Cholesterol/HDL ratio | 0.102 | 0.314 |
| hs-Troponin I, ng/L | -0.076 | 0.455 |
| NT-proBNP, ng/L | 0.202 | **0.069** |
| $Log_{10}$ (aldosterone-renin ratio +1), | 0.303 | **0.004** |
| $Log_{10}$ (albumin-creatinine ratio), | 0.227 | **0.024** |
| Daily salt intake, g/day | -0.108 | 0.410 |
| **Body composition** | | |
| Overhydration (OH), L | -0.067 | 0.509 |
| Extracellular water to intracellular water ratio | 0.403 | **<0.001** |
| Lean tissue index, kg/m$^2$ | -0.284 | **0.004** |
| Fat tissue index, kg/m$^2$ | 0.249 | **0.012** |
| **Haemodynamics** | | |
| BpTRU peripheral systolic blood pressure, mmHg | 0.418 | **<0.001** |
| BpTRU peripheral diastolic blood pressure, mmHg | 0.188 | 0.062 |
| Peripheral systolic blood pressure, mmHg | 0.508 | **<0.001** |
| Peripheral diastolic blood pressure, mmHg | 0.229 | **0.022** |
| Peripheral pulse pressure, mmHg | 0.477 | **<0.001** |
| Central systolic blood pressure, mmHg | 0.510 | **<0.001** |
| Central pulse pressure, mmHg | 0.481 | **<0.001** |
| Mean arterial pressure, mmHg | 0.420 | **<0.001** |
| Augmentation index, % | 0.292 | **0.003** |
| Augmentation index (heart rate corrected), % | 0.317 | **0.001** |
| Heart rate, bpm | 0.113 | 0.262 |

| | Correlation coefficient | P value |
|---|---|---|
| **Risk assessment** | | |
| QRisk | 0.425 | **<0.001** |
| Absolute risk difference | 0.296 | **0.003** |
| Relative risk | -0.085 | 0.409 |

**Abbreviations:** eGFR, estimated glomerular filtration rate; HbA1c, haemoglobin A1c; IFCC, International Federation of Clinical Chemistry; HDL, high density lipoprotein; NT-proBNP, N terminal pro B-type natriuretic peptide.

Age and BP have been shown to be major determinants of cfPWV (20), and the AHA recommends taking the effect of HR into consideration when determining arterial stiffness (603). Hierarchical multiple regression analysis was carried out to assess whether the univariate statistically significant association between TRH and cfPWV persisted after controlling for the influence of age, MAP and HR. Preliminary analyses were conducted to ensure no violation of the assumptions of normality, linearity, multicollinearity and homoscedasticity. Four cases were excluded from the final model; 1 for missing data and the other three were treated as potential outliers based on their standardised residuals. Age, MAP and HR were entered at step 1, explaining 61.3% of the variance in cfPWV. After entry of TRH status at step 2 the total variance explained by the model as a whole was 62.2%, F (4,91) = 37.48, p<0.001. The TRH status explained an additional, statistically insignificant, 0.9% (p=0.147) of the variance in cfPWV, after controlling for age, MAP and HR. In the final model, age, MAP and HR were statistically significant with age scoring highest beta value (beta = 0.66, p<0.001) compared to MAP (beta=0.31, p<0.001) and HR (beta=0.28, p<0.001). The beta value for TRH status, however, was not found to be statistically significant (beta=0.11, p=0.147). The results of this model are summarised in **Table 4.11**.

A further hierarchical multiple linear regression analysis was performed to assess if any other factors are predictive of cfPWV after controlling for the impact of age,

HR and MAP. Preliminary analyses were conducted to ensure no violation of the assumptions of normality, linearity, multicollinearity and homoscedasticity. Twenty cases were excluded from the final model; 18 for missing data and the other two were treated as potential outliers based on their standardised residuals. Age, MAP and HR were entered at step 1, explaining 56.1% of the variance in cfPWV. At step 2, variables known to be risk factors for cardiovascular and cerebrovascular disease, and variables which were shown to be correlated with cfPWV at a p-value <0.1, were added all at once. Variables exhibiting multi-collinearity defined as variance inflation factor >10 were removed one at a time until none of the variables had a variance inflation factor >10. Any variables with p-value of >0.05 were then removed from the model individually until the remaining variables were statistically significant. When manually removing variables, any variable with the highest p-value was removed first. In the final model, non-Caucasian ethnicity, waist-to-hip ratio, history of ischaemic heart disease, , prescription of a diuretic, lean tissue index, and serum NT-pro BNP were all significantly associated with cfPWV after controlling for the effect of age, HR and MAP. After entry of the aforementioned variables at step 2 the total variance explained by the model as a whole was 78.2%, F (10,69) = 24.69, p<0.001. These variables explained an additional 22.1% (p<0.001) of the variance in cfPWV, after controlling for age, MAP and HR. In the final model, all the variables entered were statistically significant, their coefficients and beta are summarised in **Table 4.12**.

**Table 4.12:** Linear regression model of TRH status on carotid-femoral pulse wave velocity

| | Mean cfPWV change, m/s | 95% CI | | Std. Beta | P value | $R^2$ | $\Delta R^2$ |
|---|---|---|---|---|---|---|---|
| | | Lower | Upper | | | | |
| **Step 1** | | | | | | 0.613 | 0.601 |
| Constant | -3.656 | -6.193 | -1.119 | | 0.005 | | |
| Age, years | 0.098 | 0.078 | 0.117 | 0.699 | <0.001 | | |
| Heart rate, bpm | 0.045 | 0.026 | 0.065 | 0.315 | <0.001 | | |
| MAP, mmHg | 0.051 | 0.018 | 0.063 | 0.255 | 0.001 | | |
| **Step 2** | | | | | | 0.622 | 0.606 |
| Constant | -4.146 | -6.754 | -1.538 | | 0.002 | | |
| Age, years | 0.093 | 0.072 | 0.113 | 0.662 | <0.001 | | |
| Heart rate, bpm | 0.045 | 0.021 | 0.068 | 0.283 | <0.001 | | |
| MAP, mmHg | 0.044 | 0.024 | 0.063 | 0.305 | <0.001 | | |
| TRH Status | 0.430 | -0.153 | 1.014 | 0.108 | 0.147 | | |

**Abbreviations:** cfPWV, carotid-femoral pulse wave velocity; MAP, mean arterial pressure; TRH, treatment-resistant hypertension.

**Table 4.13:** Linear regression model of predictors of carotid-femoral pulse wave velocity

| | Mean cfPWV change | 95% CI | | Std. Beta | P value | $R^2$ | $\Delta R^2$ |
|---|---|---|---|---|---|---|---|
| | | Lower | Upper | | | | |
| **Step 1** | | | | | | 0.561 | 0.544 |
| Constant | -3.729 | -6.641 | -0.818 | | 0.013 | | |
| Age, years | 0.092 | 0.068 | 0.117 | 0.622 | <0.001 | | |
| Heart rate, bpm | 0.052 | 0.025 | 0.078 | 0.336 | <0.001 | | |
| MAP, mmHg | 0.042 | 0.017 | 0.066 | 0.280 | 0.001 | | |
| **Step 2** | | | | | | 0.782 | 0.750 |
| Constant | -6.342 | -9.472 | -3.213 | | <0.001 | | |
| Age, years | 0.076 | 0.054 | 0.097 | 0.509 | <0.001 | | |
| Heart rate, bpm | 0.052 | 0.031 | 0.073 | 0.337 | <0.001 | | |
| MAP, mmHg | 0.051 | 0.033 | 0.070 | 0.345 | <0.001 | | |
| African-Caribbean | 0.941 | 0.366 | 1.516 | 0.202 | 0.002 | | |
| Asian | 0.756 | 0.178 | 1.334 | 0.162 | 0.011 | | |
| Waist-Hip Ratio | 5.108 | 2.693 | 7.523 | 0.269 | <0.001 | | |
| History of IHD | 1.367 | 0.628 | 2.106 | 0.222 | <0.001 | | |
| Use of diuretics | -0.928 | -1.431 | -0.425 | -0.234 | <0.001 | | |
| LTI | -0.187 | -0.279 | -0.094 | -0.263 | <0.001 | | |
| NT-pro BNP | 0.001 | 0.001 | 0.002 | 0.276 | <0.001 | | |

**Abbreviations:** cfPWV, carotid-femoral pulse wave velocity; MAP, mean arterial pressure; IHD, ischaemic heart disease; LTI, lean tissue index; NT-pro BNP, N terminal pro B type natriuretic peptide.

### 4.3.8 Endothelial dysfunction

Endothelial dysfunction was assessed by two different methods; measurement of specific biomarkers associated with endothelial dysfunction and assessment of RHI using EndoPAT. The results of 5 biomarkers and EndoPAT evaluation are summarised in **Table 4.14**. EndoPAT was performed in 45 patients due to a delay in acquiring the equipment required to carry out the assessment. Two patients were excluded as they did not complete the full EndoPAT assessment due to failure to tolerate the full 5-minute brachial occlusion. The EndoPAT RHI or LnRHI was not significantly different in the two groups of patients. Similarly, when patients were divided into normal or abnormal groups based on the LnRHI (normal LnRHI >0.51; abnormal LnRHI ≤0.51), there was no significant difference in the proportion of patients with abnormal endothelial dysfunction in patients with TRH compared to controls; 7 (41.2%) vs 8 (30.7%) respectively (p=0.528).

The ELISA for the five biomarkers was carried out in all patients. In one patient the endoglin level was below the detectable limit and in another 3 patients ICAM-1 level was below the detectable limit and hence these patients were excluded. Median serum ET-1 concentration was significantly higher in patients with TRH compared to control group of patients; 1.98 (1.61 – 2.38) pg/mL and 1.81 (1.40 – 2.26) pg/mL (p=0.001). Similarly, a higher median concentration of VCAM-1 was observed in the TRH group at 1136.9 (1024.0 – 1229.7) ng/mL when compared to the median concentration of the control group at 1049.0 (956.3 – 1131.6) ng/mL (p=0.017). There was no significant difference in serum concentrations of sFLT-1, endoglin, and ICAM-1 in the two groups of patients.

All parameters of endothelial dysfunction were assessed for any possible bivariate correlations; serum VCAM-1 was found to positively correlate with ICAM-1 ($r=0.439$, $p<0.001$) and serum ET-1 was found to positively correlate with sFLT-1 ($r=0.209$, $p=0.037$). There were no significant correlations between the EndoPAT indices and the measured biomarkers.

Exploratory multivariable linear regression was carried out for each of the five biomarkers to assess whether any of the age, gender, ethnicity, comorbidities, BMI, BP and TRH status were predictive of the biomarkers. After testing the assumptions of linear regression, the final models predictive of sFLT-1, Endoglin, ET-1, VCAM-1 and ICAM-1 are presented in **Table 4.15**.

**Table 4.14:** Assessment of endothelial dysfunction: EndoPAT measured reactive hyperaemia index and ELISA measured biomarkers.

| | N | All Patients | TRH | non-TRH | P value |
|---|---|---|---|---|---|
| Reactive hyperaemia index | 43 | 1.88 (1.59 – 2.49) | 1.86 (1.56 – 2.34) | 1.92 (1.61 – 2.59) | 0.358 |
| LnRHI | 43 | 0.70 ± 0.28 | 0.64 ± 0.25 | 0.73 ± 0.29 | 0.321 |
| EndoPAT Endothelial function | | | | | 0.709 |
| Normal | | 28 (65.1) | 10 (58.8) | 18 (69.2) | |
| Abnormal | | 15 (34.9) | 7 (41.2) | 8 (30.8) | |
| sFLT-1, pg/mL | 100 | 276.22 (238.72 – 309.08) | 273.73 (237.09 – 306.86) | 278.13 (237.98 – 315.79) | 0.825 |
| Endoglin, ng/mL | 99 | 4.56 ± 1.16 | 4.44 ± 1.20 | 4.65 ± 1.06 | 0.362 |
| Endothelin-1, pg/mL | 100 | 1.99 (1.87 – 2.11) | 2.25 (2.07 – 2.46) | 1.82 (1.69 – 1.97) | **<0.001** |
| VCAM-1, ng/mL | 100 | 1077.2 (973.4 – 1176.4) | 1136.9 (1024.0 – 1229.7) | 1049.0 (956.3 – 1131.6) | **0.017** |
| ICAM-1, ng/mL | 97 | 92.28 (81.41 – 108.85) | 95.71 (73.99 – 111.03) | 90.77 (83.51 – 108.36) | 0.901 |

**Abbreviations:** LnRHI, Log natural reactive hyperaemia index; sFLT-1, soluble fms-like tyrosine kinase-1; VCAM-1, vascular cell adhesion molecule-1; ICAM-1 intercellular adhesion molecule-1.

**Table 4.15** Linear regression model of predictors of biomarkers of endothelial dysfunction

| | Mean change | 95% CI | | Std. Beta | P value | $R^2$ | $\Delta R^2$ |
|---|---|---|---|---|---|---|---|
| | | Lower | Upper | | | | |
| **sFLT-1 (n=100)** | F (2,97) = 6.518; p = 0.002 | | | | | 0.118 | 0.100 |
| Constant | 258.904 | 244.863 | 272.946 | | <0.001 | | |
| Ex smoker | 33.706 | 8.465 | 58.948 | 0.256 | 0.009 | | |
| History of IHD | 48.062 | 14.74 | 81.383 | 0.277 | 0.005 | | |
| **Endoglin (n=99)*** | F (3,95) = 4.560; p = 0.005 | | | | | 0.126 | 0.098 |
| Constant | 6.622 | 5.229 | 8.016 | | <0.001 | | |
| Age, years | -0.017 | -0.033 | -0.001 | -0.208 | 0.038 | | |
| BMI, kg/m$^2$ | -0.042 | -0.078 | -0.006 | -0.224 | 0.024 | | |
| History of IHD | 0.936 | 0.28 | 1.593 | 0.285 | 0.006 | | |
| **Endothelin-1 (n=100)** | F (2,97) = 8.607; p<0.001 | | | | | 0.151 | 0.133 |
| Constant | 1.073 | 0.568 | 1.577 | | <0.001 | | |
| TRH status | 0.404 | 0.148 | 0.661 | 0.295 | 0.002 | | |
| Gender | 0.3 | 0.048 | 0.553 | 0.222 | 0.02 | | |
| **VCAM-1 (n=100)** | F (3,96) = 5.876; p=0.001 | | | | | 0.155 | 0.129 |
| Constant | 904.219 | 609.384 | 1199.054 | | <0.001 | | |
| BMI, kg/m$^2$ | 5.916 | 0.247 | 11.584 | 0.195 | 0.041 | | |
| Systolic BP, mmHg | 2.870 | 1.154 | 4.585 | 0.404 | 0.001 | | |
| Diastolic BP, mmHg | -4.752 | -7.885 | -1.618 | -0.366 | 0.003 | | |
| **ICAM-1 (n=97)*** | F (3,93) = 7.954; p<0.001 | | | | | 0.204 | 0.179 |
| Constant | 97.380 | 91.065 | 103.696 | | <0.001 | | |
| African Caribbean | -21.169 | -32.878 | -9.460 | -0.346 | 0.001 | | |
| Asian | -11.482 | -22.313 | -0.652 | -0.204 | 0.038 | | |
| Current smoker | 17.488 | 6.389 | 28.587 | 0.292 | 0.002 | | |

Excluded cases because of missing data are indicated by *. Abbreviations: sFLT-1, soluble receptor fms-like tyrosine kinase-1; IHD, ischaemic heart disease, BMI, body mass index; TRH, treatment-resistant hypertension; VCAM-1, vascular cell adhesion molecule-1; BP, blood pressure; ICAM. intercellular adhesion molecule-1.

### 4.3.9 Epworth sleepiness score and overnight pulse oximetry

All patients completed the ESS with no missing data. The median (IQR) score in the entire study population was 6 (3 – 12) with median scores of 6 (4 – 13) and 7 (3 – 11) in the TRH and control groups respectively. The total score was dichotomised into low and high risk for sleepiness as previously described. There was no significant difference in the proportion of patients at high risk of sleepiness when comparing the TRH group with the control group. The median of ESS and the proportion of patients at risk of sleepiness are summarised in **Table 4.16**.

A total of 40 patients completed an overnight pulse oximetry measurement. Five patients' results were excluded as their total time analysed was significantly shorter than the required 4 hours for an adequately reliable analysis. Two patients had a total analysis time of within 10 minutes of completing 4 hours and were included in the statistical analysis. An ODI≥5 dips per hour was used to categorise patients with likelihood of presence of OSA. Twenty-two (63%) of 35 patients had an abnormally high ODI; 8 (89%) patients with TRH and 14 (54%) control patients had potentially undiagnosed OSA (p=0.109). There was no significant difference in the ODI, proportion of time with oxygen saturations less than 90% and nocturnal nadir oxygen saturation in the two groups of patients in this study. The parameters for overnight pulse oximetry are detailed in **Table 4.16**.

A bivariate correlation analysis was carried out to identify factors with any significant correlations with ODI and are listed in **Table 4.17**. Statistically significant positive correlations were present with males, BMI, waist-to-hip ratio, waist-to-height ratio and lean tissue index. There was no correlation between

ESS and ODI. Relationship between gender and ODI was further examined to show that males had a significantly higher median (IQR) ODI compared to females; 12.1 (4.7 – 32.9) and 5.5 (2.8 – 8.7) respectively (p=0.016).

**Table 4.16:** Epworth sleepiness scale and overnight pulse oximetry parameters according to TRH status.

| Subscale | All Patients | TRH | non-TRH | P value |
|---|---|---|---|---|
| ESS | 6 (3 – 12) | 6 (4 – 13) | 7 (3 – 11) | 0.910 |
| Sleepiness risk | | | | 1.000 |
| Low (ESS < 10) | 70 | 29 (71) | 41 (70) | |
| High (ESS ≥ 11) | 30 | 12 (29) | 18 (30) | |
| Presence of OSA | | | | 0.109 |
| Normal (ODI < 5) | 13 (37) | 1 (11) | 12 (46) | |
| Abnormal (ODI ≥ 5) | 22 (63) | 8 (89) | 14 (54) | |
| Oxygen desaturation index, dips/hour | 6.5 (3.0 – 12.1) | 10.0 (6.0 – 22.8) | 5.6 (2.9 – 9.8) | 0.138 |
| Proportion of time with oxygen saturation <90%, % | 0.3 (0.1 – 1.3) | 1.0 (0.2 – 3.5) | 0.2 (0.1 – 1.0) | 0.119 |
| Nocturnal nadir oxygen saturation, % | 91.3 (90.1 – 91.8) | 91.1 (88.8 – 91.7) | 91.4 (90.0 – 91.9) | 0.565 |

**Abbreviations**: ESS, Epworth sleepiness scale; OSA, obstructive sleep apnoea; ODI, oxygen desaturation index.

**Table 4.17:** Factors correlated with oxygen desaturation index.

| | Correlation coefficient | P value |
|---|---|---|
| Age, years | -0.019 | 0.913 |
| Gender (Male) | 0.451 | 0.007 |
| Ethnicity | | |
| African-Caribbean | -0.118 | 0.499 |
| Asian | 0.068 | 0.699 |
| Duration of hypertension | | |
| 6 – 15 years | -0.081 | 0.645 |
| >15 years | -0.038 | 0.826 |
| Presence of diabetes mellitus | -0.144 | 0.408 |
| BpTRU peripheral SBP, mmHg | -0.005 | 0.975 |
| BpTRU peripheral DBP, mmHg | 0.082 | 0.640 |
| Body mass index, $kg/m^2$ | 0.421 | 0.012 |
| Waist to Hip ratio | 0.344 | 0.043 |
| Waist to Height ratio | 0.344 | 0.011 |
| Lean tissue index, $kg/m^2$ | 0.359 | 0.034 |
| Fat tissue index, $kg/m^2$ | 0.217 | 0.210 |
| Epworth sleepiness score | 0.099 | 0.573 |

**Abbreviations:** SBP, systolic blood pressure; DBP, diastolic blood pressure.

### 4.3.10 Beliefs about medicines questionnaire

All patients attending the study visit completed the BMQ. Only 7 (0.4%) out of a total possible 1800 responses from the study population were missing. The Cronbach's alpha for specific-necessity, specific-concerns, general-overuse and general-harm subscales were 0.83, 0.71, 0.77 and 0.68 respectively. These results of the 4 subscales have relatively high internal consistency and therefore the responses to the BMQ items can be considered reliable. The computed mean scores for the BMQ specific and general scales are shown in **Table 4.18**, alongside their Cronbach's alpha and the percentage of patients with a score above the scale midpoint.

Majority of the patients (75%) reported a strong belief in the necessity of their antihypertensives medication for maintaining health as indicated by a mean score greater than the scale midpoint. However, a larger proportion of patients (62%) also had strong concerns about the potential adverse effects of their antihypertensives on their health (mean score greater than scale midpoint).

In unadjusted analysis, there was no significant difference in the mean of any of the 4 subscales when comparing non-adherent patients to adherent patients. Similarly, there was no significant difference in the necessity–concerns differential between the adherent and non-adherent patients. The mean subscale scores and the necessity–concerns differential stratified according to adherence status are listed in **Table 4.19**.

When comparing to controls, patients with TRH reported higher mean scores for their beliefs in both the necessity of their antihypertensive medications (19.9 ± 3.2 vs 17.0 ± 3.9, $p<0.001$) and their concerns about the potential adverse effects

associated with these medications (17.6 ± 3.0 vs 15.0 ± 4.3, p<0.001). However, there was no significant difference in the necessity–concerns differential in the two groups. Patients with TRH also reported significantly higher scores for their beliefs about overuse of medicines (12.6 ± 3.3 vs 10.7 ± 3.4, p=0.006) but no difference in the score in their beliefs about harm caused by medicines in general. The mean subscale scores and the necessity–concerns differential stratified according to TRH status are listed in **Table 4.20**.

A binary logistic regression analysis was performed with adherence status (adherent or non-adherent) as the dependant variable to test whether beliefs about medicines were able to predict measured adherence when controlling for demographic factors (age, gender and index of multiple deprivation decile). Subscale scores for necessity, concerns, necessity-concerns differential, overuse and harm were added to the model and were sequentially removed if any of these variables had a p-value of >0.05 until the remaining variables were statistically significant. The full model, containing age, gender, index of multiple deprivation decile, overuse and harm, was statistically significant, $\chi^2$ (5, N=100) = 12.96, p=0.024, indicating that the model was able to distinguish between patients who were adherent or non-adherent. The model as a whole explained between 12.2% (Cox and Snell R square) and 16.4 (Nagelkerke R square) of the variance in adherence status, and correctly classified 71.0% of the cases. As shown in **Table 4.21**, only two of the independent variables made a unique statistically significant contribution to the model (overuse and harm). The strongest predictor of non-adherence was a firm belief that medications cause harm with an odds ratio of 1.28 (95% CI: 1.05 – 1.56). This indicated that patients who hold a stronger general belief that medications are associated with harm

were 28% more likely to be non-adherent, controlling for all other factors in the model. The odds ratio of 0.80 for the general-overuse scale was less than 1, indicating that for every additional increase in score of this scale patients were 20% less likely to be non-adherent. Addition of other demographic (ethnicity) and clinical (duration of hypertension and number of antihypertensive medications prescribed) factors did not explain a significant amount of variance in reported adherence.

**Table 4.18:** Mean scores and percentage of patients with scores above the scale midpoint on BMQ

| Subscale | Mean ± s.d. | Percentage of patients with scores above the scale midpoint | Cronbach's alpha |
|---|---|---|---|
| Specific - necessity | 18.2 ± 3.9 | 75 | 0.83 |
| Specific - concerns | 16.1 ± 4.0 | 62 | 0.71 |
| General - overuse | 11.5 ± 3.5 | 39 | 0.77 |
| General - harm | 9.8 ± 3.2 | 21 | 0.68 |

**Table 4.19:** Mean subscale scores and necessity-concerns differential for BMQ according to adherence status.

| Subscale | All Patients | Adherent | Non-adherent | P value |
|---|---|---|---|---|
| Specific – necessity | 18.2 ± 3.9 | 18.3 ± 4.0 | 18.2 ± 3.7 | 0.901 |
| Specific – concerns | 16.1 ± 4.0 | 16.1 ± 3.9 | 16.1 ± 4.3 | 0.968 |
| Necessity–concerns differential | 2.1 ± 4.4 | 2.2 ± 4.6 | 2.1 ± 4.2 | 0.884 |
| General – overuse | 11.5 ± 3.5 | 11.8 ± 3.5 | 11.1 ± 3.3 | 0.347 |
| General – harm | 9.8 ± 3.2 | 9.4 ± 2.9 | 10.5 ± 3.4 | 0.087 |

**Table 4.20:** Mean subscale scores and necessity-concerns differential for BMQ according to TRH status

| Subscale | All Patients | TRH | non-TRH | P value |
|---|---|---|---|---|
| Specific - necessity | 18.2 ± 3.9 | 19.9 ± 3.2 | 17.0 ± 3.9 | <0.001 |
| Specific - concerns | 16.1 ± 4.0 | 17.6 ± 3.0 | 15.0 ± 4.3 | <0.001 |
| Necessity–concerns differential | 2.1 ± 4.4 | 2.3 ± 4.0 | 2.0 ± 4.8 | 0.762 |
| General - overuse | 11.5 ± 3.5 | 12.6 ± 3.3 | 10.7 ± 3.4 | 0.006 |
| General - harm | 9.8 ± 3.2 | 10.3 ± 3.1 | 9.5 ± 3.2 | 0.209 |

**Abbreviations**: TRH, treatment-resistant hypertension.

**Table 4.21:** Binary logistic regression predicting likelihood of non-adherence

| | B | S.E. | Odds Ratio | 95% C.I. for Odds Ratio | | P value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| Age | -0.007 | 0.016 | 0.99 | 0.96 | 1.03 | 0.689 |
| Female | 0.399 | 0.444 | 1.49 | 0.62 | 3.56 | 0.369 |
| Index of Multiple Deprivation Decile | -0.113 | 0.083 | 0.89 | 0.76 | 1.05 | 0.174 |
| Overuse | -0.224 | 0.088 | 0.80 | 0.67 | 0.95 | 0.011 |
| Harm | 0.247 | 0.099 | 1.28 | 1.05 | 1.56 | 0.013 |
| Constant | 0.191 | 1.221 | 1.21 | | | 0.876 |

# CHAPTER 5 DISCUSSION

The purpose of this observational study was to comprehensively describe demographic, biochemical and physiological characteristics of patients with true TRH. The results of the study are divided into sections and are discussed below.

## 5.1 Baseline demographics

In this prospectively recruited cohort, patients with adherence-proven TRH were older with longer duration of hypertension, had higher waist-to-hip and waist-to-height ratios, and had a higher prevalence of diabetes mellitus and LVH when compared to a control group of patients with treated hypertension. These findings are echoed by earlier retrospective observational studies examining the prevalence of TRH based on data from large disease registries and databases such as NHANESs (313, 314, 402).

A major drawback of the studies based on NHANESs is that they did not systematically exclude WCE when defining patients with uncontrolled TRH and thus therefore overestimating the prevalence of TRH (313, 314). The study by de la Sierra et al. has clearly shown that as many as 40% of the patients labelled as having TRH based on clinic BP alone have normal BP when measured using ABPM (402). Furthermore, none of these studies have assessed level of non-adherence present in their respective cohorts, which has been shown to be significantly high in patients with TRH. In the FACT-RHY study, both WCE and

non-adherence have been systematically excluded to define a cohort of true TRH. Despite the differences in criteria to define TRH, similar characteristics have been observed to be associated with TRH suggesting that WCE and non-adherence may not impact on the phenotype of patients with TRH.

Earlier studies have found a high BMI to be associated with TRH (313, 314, 402). When comparing the quantitative differences in the mean BMIs for the TRH and the comparator groups, it is evident that these differences are not large and interestingly both the TRH and the comparator groups collectively had means in the 'overweight' category (604). In the FACT-RHY study, although the mean BMI was higher in the TRH group, it was statistically non-significant. The mean BMI is, however, not too dissimilar to that observed in earlier studies. In the FACT-RHY study, interestingly, significantly higher waist-to-height and waist-to-hip ratios were observed in TRH patients, suggesting distribution of adipose tissue around the waist in these patients.

A large collaborative analysis of data from almost 900,000 adults in 57 prospective studies has shown that every 5 $kg/m^2$ increase in BMI is associated with at least 5 mm Hg higher SBP (605). BMI is strongly correlated with measures of central obesity including waist circumference and waist-to-hip ratio (606, 607). There is evidence to suggest that both waist circumference and waist-to-hip ratio are much more strongly associated with BP than BMI (608-614). Conversely, others have shown that there is no difference between BMI and measures of central adiposity (615-617) or that BMI may even be more strongly correlated with BP (618-621). A recent analysis of data from DEXA from two large-scale studies of whole-body imaging further quantifies the association between SBP and

regional fat mass (622). Furthermore, obesity has been shown to be an independent risk factor for the occurrence of TRH (623).

Malden et al. have shown that it is the visceral android (or abdominal) fat which was associated positively with SBP and not subcutaneous android or gynoid fat. They further state that correlations between high SBP and the commonly used anthropometric measures of central adiposity are explained by their correlation with visceral fat (622). The measures of adiposity themselves are not directly causal of morbidity and mortality associated with obesity, instead it is their interaction with BP, lipids and insulin resistance which is responsible for the burden of disease associated with obesity.

There is no single mechanism which is solely responsible for high BP associated with obesity. The postulated pathophysiological mechanisms involve a complex interplay of overactivation of the sympathetic nervous system, endothelial dysfunction, activation of the RAAS and physical compression of the kidneys and are discussed in section 1.4.7 (624, 625). Obesity-induced increase in leptin release from adipocytes, hyperinsulinaemia resulting from insulin resistance and high level of free fatty acids and adipokines are some of the stimulatory factors implicated in driving the aforementioned mechanisms (624, 625).

A significantly high proportion of patients with TRH had co-existing diagnosis of diabetes mellitus compared to non-TRH patients. Data on the duration of diabetes mellitus in individual patients was not recorded, therefore it is not possible to elucidate whether TRH preceded development of diabetes mellitus or vice versa. The high prevalence of diabetes in TRH patients in the FACT-RHY study is consistent with what others have found (313, 314, 402). Diabetes and

hypertension often co-exist together; Emdin et al. showed that in an analysis of 4.1 million individuals free from diabetes and cardiovascular disease, a 20 mmHg higher SBP and a 10 mmHg higher DBP were respectively associated with a 58% and a 52% higher risk of new-onset diabetes (626). Although observational studies cannot prove causality, there is indirect evidence from a meta-analysis of RCTs of antihypertensive medications that ACE inhibitors and ARBs are associated with a reduced risk of incident diabetes (627). These results suggest instead of BP, RAAS activation may be responsible for development of diabetes in hypertensive patients. However, other classes of antihypertensives have off-target effects which may explain the lack of reduction in risk of incident diabetes with lowering BP (628). Pathophysiological pathways including sympathetic overactivity, insulin resistance, RAAS activation, inflammation and oxidative stress are common in both hypertension and diabetes a complex interaction of these pathways is likely to be responsible for the co-existence of these two conditions (629).

Over a third of the patients with TRH had LVH in the present study, which is lower than the echocardiographic-proven LVH reported in a meta-analysis of prevalence of LVH in TRH (630). Cuspidi et al. found that in a meta-analysis of 11 studies prevalence rates of echocardiographic LVH ranged from 55% to 75% of patients with TRH (630). Identification of LVH in the present study was based on the Cornell product criteria which, although has high specificity, has low sensitivity when compared to echocardiography and will result in high false negative rate. This limitation may be the reason for the relatively low observed prevalence of LVH in this study. Association between LVH and cardiovascular morbidity and mortality is well established and is an independent risk factor for

cardiovascular outcomes emphasising the clinical importance of its detection (631). Furthermore, regression of LVH by antihypertensive treatment has been shown improve to prognosis and reduce risk of adverse cardiovascular outcomes (630, 632-635).

## 5.2 Biochemical characteristics

The results of laboratory investigations from the FACT-RHY study show that patients with TRH have a significantly higher serum creatinine, aldosterone/renin ratio, HbA1c, kappa/lambda ratio, NT-proBNP and troponin I and significantly lower serum potassium and eGFR when compared to non-TRH patients. Furthermore, there was no significant difference in the urinary catecholamines, metadrenalines, urine ACR and estimated daily salt intake between the two groups of patients.

Hypertension is the second leading cause of CKD and progression to ESRD. Autoregulation of renal blood flow and intraglomerular hydrostatic pressure through pre-glomerular vasoconstriction of renal blood vessels attenuates barotrauma to glomeruli and microvasculature from high BP. Excessively high BPs can still cause renal injury despite normal autoregulatory mechanisms resulting in glomerulosclerosis and can eventually compromise the preventative autoregulatory mechanisms amplifying the renal damage (636). Patients with such renal damage are at a greater of risk of development of progressive renal impairment and CKD. Pathophysiological sequalae of CKD namely impaired natriuresis and subsequent sodium and water retention; increased activity of the RAAS; sympathetic overactivity; secondary hyperparathyroidism raising intracellular calcium concentration; treatment with erythropoietin; and endothelial

dysfunction, can all potentiate high BP thus setting up a vicious cycle of hypertension which is difficult to control.

A high serum creatinine, and consequently a low eGFR, is a marker of renal impairment which itself can be a cause or a consequence of prolonged uncontrolled hypertension. Renal parenchymal disease is the commonest identifiable cause of TRH and must be investigated in any patient with TRH (637). In the present study, any patient with moderate or severe renal impairment (CKD 4 or 5) was excluded. Only 3 patients in the TRH and 1 patient in the non-TRH group had a confirmed diagnosis of CKD. A mean difference of 20 ml/min in the eGFR was present between the two groups of patients which may be a consequence of uncontrolled BP in the TRH group. Urine ACR, another marker of renal damage, was found to be higher in the TRH but it did not reach statistical significance is another indicator that patients with TRH may have developed target organ damage. However, it is equally plausible to attribute the albuminuria and to a lesser extent the lower eGFR to higher prevalence of diabetes mellitus in the TRH cohort. In a cross-sectional study such as this, it is difficult to elucidate which is responsible for the pattern of results observed. Furthermore, it is also important to recognise the inherent limitations of using serum creatinine for estimation of the GFR (638). The commonly used MDRD equation often underestimates the true GFR. Estimated GFR calculated using the CKD-EPI provides a more accurate assessment of true GFR with individuals with normal or slightly low GFR but still carries wide confidence intervals.

Previous studies have also shown that TRH is associated with reduced eGFR and albuminuria (402, 604, 639). Almost all patients with TRH in the FACT-RHY

study were prescribed a RAAS-blocking antihypertensive which also reduce the amount of protein leak from the kidneys and therefore may be responsible for the comparatively low levels of albuminuria observed.

Patients with true TRH in the FACT-RHY cohort had significantly higher plasma ARR, a finding supported by previous researchers (404, 640). This group of patients also had a significantly lower serum potassium level compared to the non-TRH, although the absolute difference between the groups is small. Interestingly, the lower level of serum potassium was present despite a significantly higher prescription of mineralocorticoid receptor antagonists, which often increase the serum potassium levels. It could be argued that the prescription to other diuretics (loop and/or thiazide/thiazide-like) may have counteracted the hyperkalaemic effect of the mineralocorticoid receptor antagonists. However, there was no significant difference between the use of loop and thiazide/thiazide-like diuretics in the two groups of patients. The lower serum potassium level observed in the true TRH group may therefore represent an underestimate in the presence of mineralocorticoid receptor antagonist use. The combination of high plasma ARR and the low serum potassium is most probably a consequence of primary hyperaldosteronism usually found in hypertension (640, 641).

Patients with confirmed primary hyperaldosteronism with prescribed often respond well to use of a mineralocorticoid receptor antagonist (642); mean reductions of 25/10 mmHg have been reported with doses of 75mg to 225mg of spironolactone, the more potent of the two mineralocorticoid receptor antagonists (643). In two small studies, BP control has been shown to be achieved in around

48 to 76% of patients with primary hyperaldosteronism taking a mineralocorticoid receptor antagonists (644, 645). However over long-term, BP control may deteriorate either as a proportion of patients develop essential hypertension or as a consequence of vascular remodelling caused by the long-standing hyperaldosteronism (646). In the FACT-RHY study, 15 patients with true TRH were prescribed a mineralocorticoid receptor antagonist and a further sub analysis showed no statistically significant mean difference in BP and serum potassium between patients with or without the use of a mineralocorticoid receptor antagonist.

Limitations of both serum potassium level and ARR should be borne in mind when interpreting the present results. Although a widely taught fact that a low serum potassium level may well be the first clue to an underlying diagnosis of hyperaldosteronism, recent evidence suggests that only a minority of patients with hyperaldosteronism have hypokalaemia (647, 648). The screening for hyperaldosteronism is most commonly carried out using the ARR. This test however is not completely accurate, as both plasma renin activity and aldosterone concentration are susceptible to manipulation in either direction by a range of antihypertensives, dietary sodium and serum potassium level (442). For optimum specificity and sensitivity, these medications should either be stopped or substituted with alternative antihypertensives which do not affect the levels of these assays, and potassium levels controlled prior to performing ARR. However, discontinuation of antihypertensives for a prolonged period is often impractical and even dangerous especially in patients with TRH. There is evidence suggesting that ARR is a fairly accurate test and concomitant antihypertensive drug therapy or variation in dietary sodium balance does not adversely affect the

test accuracy (447, 649). Guidelines for diagnosis of hyperaldosteronism accept ARR as a suitable screening test even with continued use of antihypertensive provided its limitations are acknowledged (442). Based on these reasons, patients in the present study were not asked to change their sodium intake or stop their antihypertensives prior to venepuncture and sampling.

Patients with TRH were found to have significantly higher levels of cardiac biomarkers troponin I and NT-proBNP in the present study. Troponin I, a highly specific biomarker of myocardial injury, is used for diagnosis of MI and acute coronary syndromes. Importantly, a high troponin I level only signifies myocardial necrosis and not the cause of cause of myocardial cell death. Development of increasingly sensitive troponin assays have allowed elevations from causes other than acute coronary syndromes to be detected. Wallace et al. have shown that troponin levels to be minimally increased in the general population without acute coronary syndromes and commonly associated congestive heart failure, CKD, LVH or diabetes mellitus (650). Regardless of the cause of elevation, a high troponin without definite acute coronary syndrome represents an increased risk for future cardiovascular events (651).

In hypertensive patients an elevated troponin I may indicate presence of LVH or congestive heart failure. Further analysis of the FACT-RHY cohort shows that patients with LVH had statistically higher troponin I compared to patients with non LVH; median (IQR) 13 (4 – 23) ng/L and 3 (3 – 3) ng/L respectively ($p<0.001$). In the entire FACT-RHY cohort, none of the patients had a coexisting history of congestive heart failure and only 4 patients had CKD, therefore it is highly likely that the rise in troponin I observed is related to LVH.

NT-proBNP is released by the ventricles of the heart as a response to the volume and pressure overload. It has been shown to be elevated in heart failure and is used as a biomarker for screening of heart failure and left ventricular systolic dysfunction in the primary care (652-655). NT-proBNP levels have also been shown to be elevated in patients with LVH (656, 657), which itself can be a precursor to development of left ventricular systolic dysfunction and congestive heart failure. Since none of the patients in the FACT-RHY cohort had confirmed diagnosis of congestive heart failure, an elevated NT-proBNP is likely to represent presence of LVH. Indeed, analysis of all patients with LVH in the study cohort showed that patients with LVH had a significantly higher mean (95% CI) NT-proBNP level 226.1 (130.9 – 390.7) ng/L compared to patients without LVH, 61.3 (46.7 – 80.5) ng/L, $p<0.001$.

Although the TRH group have a higher mean NT-proBNP compared to the non-TRH group it is still within the normal limits. Hypertension is the most common co-morbidity in patients who have heart failure with preserved ejection fracture (658). In heart failure with preserved ejection fraction, on average values of NT-proBNP are lower than that for heart failure with reduced ejection fraction (659, 660), therefore it is feasible that the ‘normal’ NT-proBNP levels observed in some TRH patients may represent underlying heart failure with preserved ejection fraction.

Furthermore, Levels of NT-proBNP have been shown to be higher in patients with renal impairment, which may be a consequence of fluid excess or that its clearance is reduced with a reduction in eGFR (661, 662). Therefore, higher

levels observed in the TRH group may simply be a result of reduced eGFR in this group.

The relatively high level of glycaemia observed in the TRH patients in the FACT-RHY study could be explained by the higher prevalence of diabetes mellitus in this group of patients compared to the non-TRH group. However, others have also found similar observations; perhaps suggesting a significant relationship between insulin resistance and TRH (463, 663).

Interestingly, the lipid profile in the two groups was similar with no statistically significant differences. One possible explanation of this observation includes a statistically higher proportion of patients with TRH were prescribed a lipid-lowering medication and given their adherence to antihypertensive medications; one can assume that they exhibit a similar behaviour across all their medications and therefore an improved lipid profile.

Free light chains are a marker of B cell activation and are raised in a multitude of inflammatory conditions (234). Combined FLCs (cFLCs) have been shown to be an independent risk factor for mortality in the general population (235, 236), mortality and progression to ESRD in patients with CKD (237), and mortality in patients with acute decompensated heart failure (238). In FACT-RHY study, patients with TRH, for the first time, have been shown to have a significantly elevated cFLC and kappa to lambda free light chain ratio. The cause of raised serum cFLCs is unknown in hypertension, although it has been proposed that a rise in immunoglobulins in hypertension maybe secondary to vascular damage from high BP (233). The elevated cFLCs may simply represent a generalised immune system activation. FLCs can be elevated in renal impairment and may

therefore reflect renal clearance which has been shown to be decreased in TRH patients. Correlation with high-sensitivity CRP, another marker of chronic inflammation, has not been possible at the time of this thesis.

Lack of significant association between urinary catecholamines and metadrenalines with TRH was surprising. However, 24-hour collection of urine is often cumbersome with different levels of adherence to collection instructions. In the FACT-RHY cohort only 60% of the patients provided an adequate sample for analysis which may not have been enough for statistical significance. Furthermore, urinary levels of catecholamines and metadrenalines do not adequately estimate the total sympathetic activity in the body and are often susceptible to manipulation to extrinsic factors including exercise, diet and certain medications. Similarly, a lack of difference in dietary salt intake, estimated from 24-hour urinary sodium output, in the two groups may reflect a small sample size.

## 5.3 Body composition

The results of the BIS analysis of body composition show that patients with true TRH had significant expansion in ECW (relative to either ICW or TBW) compared to patients without TRH. The absolute differences observed between the two groups were, however, small. Prescription of diuretics in the study population may also explain the lack of fluid excess detected, particularly in the TRH group. However, despite a higher proportion of patients with TRH prescribed a diuretic, ANOVA analysis has shown that when taking into account the prescription to a diuretic the differences in expansion of ECW observed remained significant. Although an expansion in ECF volume plays a key role in development of hypertension (664), it is difficult to demonstrate ECF excess. Different

hypotheses, described in detail in section 1.4.4 and summarised below, have been suggested for this observation.

An increase in ECF volume caused by either increased sodium and water retention by the kidneys leads to an increase in the circulating blood volume which consequently should increase stroke volume and cardiac output via the Frank-Starling mechanism and result in hypertension. However, in chronic essential hypertension, ECF excess or increased cardiac output is difficult to demonstrate. Instead, an acute rise in cardiac output mediated through ECF expansion causes compensatory haemodynamic mechanisms which restore sodium balance and thereby normalise ECF volume and cardiac output. It has been demonstrated that impaired natriuresis at the level of the kidney, mediated by number of different mechanisms, is the central process driving essential hypertension. The transient ECF volume expansion has been shown to inhibit Na/K ATPase which causes intracellular sodium levels to rise which is compensated through increase in Na/Ca exchange resulting increased intracellular calcium. An increased intracellular calcium in vascular smooth muscle cells could cause vasoconstriction and resultant increase in total peripheral resistance. Therefore, an increase in total peripheral resistance maintains a high BP in chronic essential hypertension. Alternatively, according to Guyton hypothesis (see **Figure 1.11**), postulates that autoregulatory vasoconstriction of systemic circulation in response to an expansion in ECF volume returns the cardiac output to normal but does so at the expense of systemic hypertension.

In the present study serum albumin, a protein predominantly present in plasma, correlated negatively ECW/ICW ratio. Water retention causes ECF volume expansion distributes equally across the two main extracellular compartments; plasma and interstitial fluid. A low serum albumin concentration is often seen in oedema states, a result of reduced plasma oncotic pressure leading to expansion in ECF. Low serum albumin can also be caused by malabsorption, malnutrition, liver disease, nephrotic syndrome, burns, protein-losing enteropathy, malignancy and pregnancy. Patients in the present study were asked about all past medical history at the study visit and did not have any of the conditions associated with hypoalbuminaemia.

Unsurprisingly, low eGFR and high ARR were also associated with a raised ECW/ICW ratio. Both renal impairment and hyperaldosteronism cause sodium and water retention in the body, thereby resulting in hypertension. Interestingly, there was no significant difference in the lean tissue and fat tissue mass in the two groups of patients. This difference reflects the non-difference of BMI in the two groups. As discussed previously, the distribution of fat tissue may be a more important than the quantity itself. Although BIS is able to determine the contribution of individual types of tissue in the body composition, it cannot describe their anatomical distribution.

Clinically, BIS is used routinely to guide fluid and dry weight assessment in patients with ESRD on haemodialysis and peritoneal dialysis. Unlike essential hypertension, ESRD is associated with significantly higher levels of fluid retention which make BIS an ideal tool for routine and repeated measurements of fluid and nutritional status and guide therapy decisions on fluid removal on either mode of

dialysis. In the FACT-RHY study, BIS was used to investigate whether either fluid excess or body composition had an association with TRH. BIS is not routinely used in essential hypertension; however, arguments have been made for its use in patients with difficult to control hypertension or TRH (665, 666). There are a handful of studies which have used a technique called impedance cardiography, which allows measurement of a patient's haemodynamic phenotypic profile which can then be used to tailor individualised antihypertensive therapeutic choices to maximise the BP lowering response of the given therapeutic selection (666). Impedance cardiography uses same principles as BIS to calculate various haemodynamic measures as well as thoracic fluid content; the latter is used to guide decisions on diuretic therapy. In the FACT-RHY study, whole body fluid content was estimated using whole-body BIS whereas impedance cardiography uses segmental BIS to calculate the thoracic fluid content. A meta-analysis of five studies using impedance-cardiography-derived haemodynamic data as an adjunct to therapeutic decision-making in the treatment of hypertension showed that patients were more than twice as likely to achieve BP control when using impedance cardiography to direct treatment choices (666). Furthermore, this technique has been shown to be cost-effective in both the short and long-term (667).

Use of BIS is not without its limitations; in routine clinical practice it is contraindicated in pregnant women and individuals with pacemakers. Ideally, patients should be adequately prepared, avoiding excessive physical activity, avoidance of alcohol and water intake for up to 6 hours prior to testing. These guidelines, however, are often difficult follow in routine clinical practice. The technique in itself has a basic limitation that it assumes that the whole body is

cylinder with a uniform conductivity and affects the accuracy of the measurements. The circumference of the trunk is greater than the limbs which means that the whole-body resistance is predominantly (up to 80%) determined by the resistance in arms and legs which happen to also contain the least amount of water (668). Therefore, its plausible that in the present study the lack of overall fluid excess in the two groups is a consequence of this limitation. Although segmental measurements of the body can overcome this limitation, by providing more accurate precise description of body composition, there is no evidence to suggest that it translates into better hard clinical outcomes (668).

## 5.4 Arterial stiffness

This observational study suggests that there is no significant difference in cfPWV between patients with true TRH from those with non-TRH, after controlling cfPWV for age, MAP and HR. Increased aortic stiffness is a hallmark of the ageing process and is associated with many diseases including atherosclerosis, hypertension, CKD and diabetes mellitus. Arterial stiffness is a well-recognised risk factor for increased cardiovascular disease including MI, heart failure, and mortality as well as stroke, dementia and CKD (217, 669-676). Increased cfPWV causes earlier return of wave reflections in systole leading higher to higher systolic, lower diastolic pressures and greater pressure fluctuations. These changes lead to ventricular-arterial uncoupling and increased afterload which results in LVH and remodelling. A combination of genetic influences and alterations in the structural and functional characteristics of the arterial wall are responsible for arterial stiffness. Structural changes are mediated through vascular calcification, alterations in extracellular matrix by chronic inflammation and accumulation of advanced glycation end products whereas functional effects

are mediated via endothelial cell signalling and vascular smooth muscle tone; a consequence of endothelial dysfunction, activation of RAAS, and dietary salt intake (677).

A limited number of studies of arterial stiffness in TRH have been reported with mixed results (678-682). Arterial stiffness has been shown to be increased in three studies comparing patients with TRH with hypertensive controls (678-680). However, these results were based on relatively small number of patients, the largest of which included 79 patients (when comparing TRH to hypertensive controls) (680), and one study compared patients with a complete failure to control BP termed as refractory hypertension (678). Importantly, only one of the these studies controlled for the well-established effect of BP on arterial stiffness (680), and none took the effects of age or HR into consideration in their analysis of PWV. Brandt et al. showed age and MAP controlled PWV to be no different in 110 patients with TRH; their control group, however, comprised only of 10 patients (681). Lastly, Sabbatini et al. also showed that in a relatively large number of patients with TRH (n=116), no significant difference in PWV was detected when compared to equal number of hypertensive controls (682). Again, they failed to control for the known influences of age, HR and MAP on PWV. The sample size in the FACT-RHY study is comparable to the larger of the studies of arterial stiffness in TRH and, importantly, controls for the effects of age, HR and MAP on PWV alongside a direct and objective assessment of adherence, which none of the other reported studies have done.

Pulse pressure and augmentation index are other markers of arterial stiffness. In the current study both central and peripheral pulse pressures were elevated in

patients with TRH. However, neither the HR-corrected nor uncorrected augmentation indexes were significantly raised. Since PWV is considered gold standard measure of arterial stiffness, further analyses of augmentation index or pulse pressure to assess the impact of other known influences, such as age were not carried out.

A possible explanation of lack of difference observed in the arterial stiffness between TRH and non-TRH is the higher number of antihypertensives used to attempt to control BP for patients with TRH. Antihypertensives have been shown to reduce arterial stiffness (677). Diuretics, nitrates, CCBs and RAAS inhibitors are the most commonly used pharmacological therapy used for reduction of vascular stiffness (677). Use of diuretics and CCBs in isolated systolic hypertension, a hallmark of ageing and arterial stiffness, has long been shown to improve clinical outcomes (683-685). There is however a lack of large RCTs designed specifically to improve arterial stiffness and any associated improvement in cardiovascular outcomes; one such trial is now underway (686). Importantly, most antihypertensives are directed at the dynamic vasoconstrictive component of arterial stiffness and are not effective at targeting the underlying structural abnormalities and changes in vascular signalling that occur in stiffening (677).

Conversely, it could be argued that patients in the non-TRH group were on the same classes of antihypertensives as the TRH group and thereby any effect of antihypertensive-mediated vascular destiffening should be similar across the cohort. However, as has been shown that non-adherence to antihypertensive was present in the patients in the non-TRH group, therefore these patients may

not have benefited from the vascular de-stiffening effects of the said antihypertensives.

## 5.5 Endothelial dysfunction

The ELISA analysis has shown significantly elevated levels of serum ET-1 and serum VCAM-1, suggesting presence of endothelial dysfunction in patients with TRH compared to non-TRH patients.

ET-1, a potent vasoconstrictor, is a 21 amino acid peptide synthesised and released continuously by the vascular endothelial cells (687). It is produced by all types of blood vessels in the body and acts mainly via a G protein-coupled receptor, selective $ET_A$ receptor, present primarily in the smooth muscle cells comprising the vascular medial layer. Endothelin receptors mediate contraction, proliferation and hypertrophy of the vascular smooth muscle cells (688). ET-1 has a long-lasting and potent vasoconstrictor effect throughout the human cardiovascular system and plays a role in perfusion of all organs of the human body. Increased levels of ET-1 have been found in patients with hypertension (689). In the present work, significantly higher levels of ET-1 in patients with true TRH compared to non-TRH patients suggest that ET-1-mediated vasoconstriction may be augmenting elevated BP. Evidence from clinical trials of a selective antagonist of $ET_A$ receptor, darusentan, in patients with TRH support the finding of the FACT-RHY study. These RCTs have shown that daursentan is effective in lowering BP in patients with TRH (690-692). However, the uptake of $ET_A$ receptor antagonists into routine clinical practice for patients with TRH has not happened thus far. Furthermore, $ET_A$ receptor antagonists have also been

shown to regress vascular hypertrophy suggesting a direct effect of ET-1 on vascular growth (693, 694).

Cell adhesion molecules, VCAM-1 and ICAM-1, found on the endothelial cell surface and mediate an inflammatory response by recruiting leukocytes to sites of injury. VCAM-1 which is upregulated by pro-inflammatory cytokines mediates adhesion to circulating leukocytes and their transendothelial migration across the endothelial cells. Low-grade inflammation and endothelial dysfunction precede development of arterial atherosclerosis; the role of ICAM-1and VCAM-1 in the pathogenesis of atherosclerosis is well-established (695-697). Elevated levels of soluble ICAM-1 and VCAM-1 have been demonstrated in patients with essential hypertension (698-703). In the present study, elevated levels of VCAM-1 but not ICAM-1 were present in patients with true TRH compared with non-TRH patients. There was, however, a positive correlation between the levels of VCAM-1 and ICAM-1 overall.

The mechanisms that cause a rise in the circulating levels of cell adhesion molecules in hypertension are not yet fully understood. Elevated circulating levels of cell adhesion molecules maybe a result of inflammation and free radical generation caused by sheer stress in response to high BP. Alternatively, ET-1 has also been shown to stimulate the expression of VCAM-1 in animal models of mineralocorticoid hypertension (704). In the present work, no correlation between levels of ET-1 and VCAM-1 was detected.

Interestingly, functional assessment of endothelial dysfunction using PAT was found to be similar in the two study groups. These results seem to contradict the findings of the biomarkers of endothelial dysfunction described earlier. This lack

of agreement can be explained by the different pathophysiological pathways involved; while ET-1 causes local vasoconstriction, PAT assesses NO-mediated vasodilatation in response to reactive hyperaemia. Therefore, an impairment in one pathway will not necessarily be expected to lead to altered function of the other.

In the present work, the lack of difference in the PAT of the two groups could represent a true indifference. There may be many other possible explanations for the observed results of PAT. Firstly, ACE inhibitors, ARBs and aldosterone antagonists have been shown to improve endothelial function by increasing the bioavailability of NO (705-708). In the FACT-RHY study, almost all patients with TRH were taking a RAAS-blocking antihypertensive which may explain the observed lack of difference in RHI. Secondly, although PAT is more operator friendly compared to the gold standard method of brachial artery ultrasound flow mediated dilatation, there is a question mark on whether both techniques measure the same thing. A number of studies have compared endothelial response to reactive hyperaemia using both techniques (709-718). The agreement between the two techniques is quite variable; six studies have demonstrated a significant positive correlation between flow mediated dilatation and PAT with correlation coefficients ranging from 0.17 to 0.57 (709-711, 713, 716, 717), whereas four studies have reported no significant correlation between the two techniques (712, 714, 715, 718). There is, however, more direct evidence that NO makes significant but incomplete contribution to reactive hyperaemia with PAT (719). Lastly the results in the present work may have been limited by the smaller number of patients who underwent PAT assessment due to logistical reasons.

## 5.6 Epworth sleepiness score and overnight pulse oximetry

There was no significant difference in ODI and ESS between the study groups of the FACT-RHY study. However, unlike the ESS questionnaire, which was completed by all the study patients, satisfactory overnight pulse oximetry was completed in only 40 patients. Although polysomnography is the 'gold standard' investigation of choice to diagnose OSA, overnight pulse oximetry is often used as a screening tool for OSA (720-723).

Despite being non-significant almost all patients with true TRH had abnormally high ODI suggesting presence of underlying OSA. All patients with an abnormally high ODI were referred to a specialist sleep clinic for further assessment, the results of which are not yet available. Therefore, the true prevalence of OSA in the FACT-RHY cohort cannot be confirmed. Sensitivity and specificity of overnight pulse oximetry is variable, but it has substantial accuracy and is a cost effective tool for the screening of OSA (724). However, if the results of the overnight pulse oximetry were to translate into actual diagnosis of OSA, a predicted prevalence of 89% in patients with TRH is consistent with previous reports of prevalence of OSA ranging from 71% to 90% (403, 407-414). At a pathophysiological level, OSA drives the increase in BP through activation of sympathetic nervous system (725-727).

In the present study, a significant proportion of the non-TRH patients (54%) had an abnormal ODI score suggesting a higher prevalence of OSA. When comparing to reported prevalence of OSA in the general population, 22% of men and 17% of women have OSA (728). A high level of obesity is equally prevalent in both the

study groups and a significant correlation between ODI and BMI was observed which may also explain the increased ODI present in the non-TRH group. Nevertheless, based on the limited sample size in the present study no firm conclusions can be drawn.

Interestingly, despite all study participants completing the ESS questionnaire, the ESS scores were very similar between the two groups of patients. Indeed, there was no significant correlation between the ESS and ODI. This finding is, however, not unique to the present study; a study characterising 204 patients with TRH found that 72.1% had OSA and that ESS score was not a predictor of OSA, except in those with severe OSA (403). The ESS is designed to detect propensity of daytime somnolence, which are usually only present in patients with OSA with moderate to severe severity. Furthermore, daytime somnolence may be present in other sleep disorders such as narcolepsy and, therefore, not specific to OSA alone.

## 5.7 Beliefs about medicines questionnaire

The results from BMQ in the FACT-RHY cohort show that patients' belief that doctors overuse or put too much trust in medications and their views about the extent to which they perceive medications as harmful are associated with non-adherence. In multivariate analysis a higher score in either the general-overuse subscale or the general-harm subscale was an independent predictor of non-adherence after controlling for effects of age, gender and index of multiple deprivation.

The finding that there are no significant differences in the two hypertension-specific subscales of the BMQ is surprising. Other studies using the BMQ to

assess the differences in beliefs of adherent and non-adherent patients have shown that adherent patients are more likely to have a strong necessity to take their illness-specific medications or that their concerns about medications outweigh the necessity of taking the medications (729-731). A metanalysis of 94 studies using BMQ have shown that the necessity-concerns framework is a valid and useful model for understanding patients' evaluations of prescribed medicines (731). The metanalysis includes studies with a wide range of chronic illnesses including hypertension, however most (88.3%) of these studies used a self-reported method to assess adherence and only a small proportion (11.7%) used a more objective measure (731). Interestingly, however, strong beliefs in either that physicians overprescribe medications or that medications are harmful contribute substantially to reduced medication adherence.

Possible factors contributing to the discordant results could include different cultural beliefs and practices in the study cohort given that almost half of the patients were from an ethnic minority. English may be their second language and may only have limited grasp of English language. Although all patients were offered to fill the questionnaire; however, they were advised that it was not compulsory if they did not want to complete the questionnaire for any reason. Almost half (43%) of the study patients were from 10 per cent of neighbourhoods that are most deprived nationally according to the Index of Multiple Deprivation; the level of understanding among patients about hypertension and the medications may be therefore be poor. Patients' understanding of hypertension, however, was not formally assessed.

## 5.8 Strengths and limitations of the FACT-RHY study

The strength of the FACT-RHY study is that it is a prospective cross-sectional study of a cohort of patients presenting to the specialist hypertension clinic. Uniquely, all patients had objective testing of adherence using the urine AHS. Although, all patients were provided with the study patient information sheet (see appendix V) that detailed the physical specimens required for the study and written consent was obtained from all patients taking part in the study, adherence testing was not explained specifically in the patient information sheet to prevent the phenomenon of 'white-coat adherence'. However, all patients were recruited from the specialist hypertension clinic and majority of them would have previously undergone routine formal adherence testing by AHS. As such, all patients were asked, with their permission, to provide a urine sample and written results of their AHS were sent to them and their general practitioners. Regardless, given the close agreement in the prevalence of non-adherence to DOT from the same clinic and urine/serum testing from other centres described earlier in this thesis, the effect of 'white-coat adherence' was probably minimal.

Another strength of this study is that 'pseudo-TRH' was systematically excluded when classifying the study cohort. All patients undergo ABPM prior to attending the specialist hypertension clinic to exclude white coat hypertension; any patient with confirmed white coat hypertension are discharged back to primary care and therefore would not have been recruited. ABPM can be uncomfortable and often patients do not like it to be repeated and to reduce the burden of tests performed on the patients at the study visit, a decision to not to repeat it. Secondly the use of BpTRU further minimised the effect of WCE at the study visit to ensure accurate classification of patients into respective groups for statistical analysis.

Furthermore, any patients with previously confirmed diagnosis of secondary hypertension were excluded and all patients underwent complete screening for secondary hypertension in the FACT-RHY study. Therefore, after excluding WCE, non-adherence and secondary hypertension the TRH group in FACT-RHY study represents a truly resistant cohort of patients which is a major strength of this study.

A single cohort of patients underwent all the testing to characterise phenotype of patients TRH and to assess the impact of various pathophysiological factors on TRH. To the author's knowledge this is the first study to comprehensively describe a group of patients with true TRH.

The main limitation of this study is that it is an observational, cross-sectional analysis of patients with TRH and all the associations described here do not imply causality. Also, the role of the factors being assessed in the development of TRH cannot be measured. Ideally a prospective, cohort study with matched controls and normotensive patients, in which assessment of patients at multiple time points was carried out, would be needed to answer that question. Given the relatively low prevalence of true TRH and the duration of hypertension before treatment resistance develops, such a study would need to be very long in duration with large numbers of participants, consequently, require considerable amount of resources and may therefore be impractical. However, the observations described here are consistent with other published works including experimental and observational research in both animal models and humans.

Another limitation is the relatively small sample size of the TRH cohort. One reason for this is that the TRH status could only be confirmed after a patient has

had their BP measured using BpTRU (to exclude WCE) and non-adherence, therefore only prevalence of apparent TRH could be used for sample size collection. The aim was to recruit 120 to 140 patients which may result in half of the patients with true TRH. However, the overall sample size and prevalence of true TRH fell short of the predicted percentage. Importantly, only less than a quarter of those screened and less than a third of those eligible took part in the study. This fact highlights the difficulty of recruitment to clinical studies, in particular, observational research in which there is no apparent direct benefit to the participants.

Another limitation is that all patients were recruited from a specialist hypertension clinic which may introduce selection bias and the findings reported here may not be generalisable to wider populations. Hypertension is mainly manged by primary care healthcare professionals and commonest reasons for referral to specialist services include difficult to treat hypertension and investigation of secondary cause of hypertension. Therefore, any hypertension research focussing on the patients in the specialist hospital clinics is likely to be non-representative of the general hypertensive population. Conversely, to recruit a reasonably large sample of patients with a true TRH would require recruitment at multiple general practices and require a very large screening sample given the low prevalence of true TRH in the hypertensive population. Patients with hypertension treated with less than three antihypertensives could have been selected from the local general practices, however due to the excess requirement of staff, their training and ethical approval procedures it was decided to select patients from a single-centre despite the shortcoming of potential selection bias that accompanies this approach.

## 5.9 Study execution

Prof. Indranil Dasgupta and the author conceived, designed and prepared the proposal of the FACT-RHY study. The author completed and submitted the ethical approval application. The author was responsible for the study administration, correspondence with patients and procurement of key equipment for the study. The author performed all of the recruitment and organised appointments for patients' study visits. The author took the clinical history and performed all the clinical examinations including anthropometric measurements, BpTRU measurements and electrocardiograms. The author performed the measurements for body composition, arterial stiffness and RHI. Venepuncture for blood sample collection, centrifugation and storing of supernatant for analysis of biomarkers of endothelial dysfunction was carried out by the author. The ELISA was performed by Sarah Hopkins at University of Aston. All the routine blood and urine analysis was carried out by the clinical laboratories at the Birmingham Heartlands Hospital. The author designed the study database, carried out data collection and analysis.

As discussed earlier, the sample size fell short of what had been hoped to achieve, the reasons for which are multifactorial:

- Initially there was a delay in the submission of ethical approval for the study which was due to the changes required in the study proposal following external feedback. More time was then needed to incorporate the suggestions made by external peer review.

- There was a delay in procurement of key equipment required for overnight pulse oximetry and RHI (EndoPAT) which were beyond the author's

control. To prevent further delay, recruitment was started without the EndoPAT device which meant that in more than half of all patients RHI was unavailable.

- One of the major obstacles to initial slow recruitment to the study was patients failing to turn up to the appointments for the study visit. Just over 200 appointments were made to complete 100 study visits which equates to more than 50% missed appointments. This resulted in a lot of wasted time preparing clinic rooms with all the test equipment and, when the patients failed to turn up, clearing up. These were all patients who had either given written or verbal consent to take part in the study. As the only researcher on this study, appointments could not be overbooked in case all the patients turned up and they would have to wait for in excess of 2 hours which was required to complete all the tests in a single visit. The choice of a single visit was decided after feedback from a group of patients at a Patient Public Involvement (PPI) meeting during the design process of the study. The PPI group preferred a single long visit compared to multiple short visits citing personal commitments, travel and parking difficulties. After the first couple of months of lacklustre performance, several measures were suggested and put in place to help reduce the rate of missed appointments. Patients were offered a refund of costs of their parking or travel by public transport; telephone and text message reminders were sent to alert participants about the upcoming appointments and substituting their routine appointments in the hypertension clinic with study visit.

- Patients also failed to return the research equipment loaned to them; in particular, the overnight pulse oximetry monitors were often returned weeks after the study visit. Although all patients signed a loan agreement at the study and despite written, text and verbal reminders the delay resulted in a significant proportion of participants missing out on this test. In hindsight this problem could have been overcome by buying extra monitors. Similarly, a large proportion of the patients either did not return or returned incomplete 24-hour urine collections which impacted on the results. To overcome this problem, sometimes the overnight pulse oximetry monitors and urine samples were picked up from participants' residences.

# CHAPTER 6 SUMMARY

Hypertension is a leading cause of burden of disease worldwide, responsible for 10.4 million deaths and 218 million disability-adjusted life-years in 2017 (2). Despite decades of experimental and clinical research, no single pathophysiological pathway has been identified that is solely responsible for the development of high BP. Instead a complex interaction between genetic predisposition, lifestyle and environmental factors culminate in hypertension.

Despite a high number of patients with uncontrolled hypertension and a similarly high prevalence of patients with apparent TRH, only a smaller percentage of patients have true TRH owing to factors contributing to ‘pseudo-resistance’. Non-adherence is one of these factors which affects many chronic conditions and hypertension much more so given that it is predominantly asymptomatic. Various methods exist to test non-adherence in patients each with their own pros and cons. In recent years the drive to identify patients with true TRH, especially for the purpose for assessing suitability for device-based therapies of hypertension, has led to development of biochemical assays to detect presence of antihypertensive medications, or their metabolites, in the urine or serum. In this thesis, the results of adherence testing using DOT and urine AHS found rate of non-adherence to be 50% and 54% respectively in a cohort of patients with apparent TRH in a specialist hypertension clinic. Furthermore, females, younger patients, number of prescribed antihypertensives and prescription to a CCB were

shown to be predictors of non-adherence. There are several important clinical implications of these results; firstly, it highlights the importance of adherence testing when assessing patients with apparent TRH to target efforts towards tackling the underlying reasons for non-adherence and hence improving BP control. Second, the two methods both have equivalent efficacy in detecting non-adherence which means that resource intensive DOT can be effectively replaced by urine AHS, although DOT is able to provide direct evidence of improved adherence on BP control and identifying any potential side-effects of antihypertensives and therefore may still be useful in some patients particularly where white-coat adherence is suspected. Thirdly, these results imply that increasing the burden of antihypertensives may in fact have the opposite effect on BP control. Therefore, future research should consider use of single-pill FDCs to assess their efficacy not only on BP control but the rate of non-adherence; it could be hypothesised that optimal adherence to lower number of antihypertensives will have a greater effect on BP control than amplifying the pharmacological therapy.

Device-based therapy, especially RDN, had become increasingly popular method of reducing BP in patients with TRH. This initial enthusiasm was primarily based on impressive BP reductions from small observational or non-sham RCT studies. However, the results of SYMPLICTY HTN-3 and the studies that followed have highlighted shortcomings in the technique itself, appropriate selection of patients, medication optimisation, marker of successful denervation at the time of procedure and identification of predictors of response to therapy. Until some or all these factors are addressed, the use of this technique may not be incorporated into routine clinical practice. The use of $CO_2$ angiography in patients with

moderate-to-severe CKD has the potential to open this technique to a wider cohort of patients with hypertension. The simple inexpensive change of imaging modality used in experience hands has been shown in this thesis to be feasible and safe in a small pilot study. Again, larger studies will be required to confirm the safety and efficacy of using $CO_2$ angiography in patients with CKD.

The principle behind the use of RDN in patients with TRH was based on the hypothesis that overactivity of the sympathetic nervous system may significantly be contributing to uncontrolled BP. However, the mixed results of RDN studies may point to the fact that SNA may not be the predominant pathophysiological factor. There have been very few research studies examining the various well-established pathophysiological factors of hypertension in patients with TRH and none in patients with true TRH. Therefore, the aim of FACT-RHY study was to describe the clinical and biochemical phenotype of patients with true TRH; and assess body composition, arterial stiffness, endothelial dysfunction, inflammation and OSA in these patients to provide an insight into the probable causes of TRH.

The cross-sectional analysis of the first 100 patients recruited to FACT-RHY has shown that older patients with longer duration of hypertension, central obesity and diabetes mellitus are more likely to have true TRH. The constellation of central obesity alongside high BP and diabetes contribute to metabolic syndrome, the prevalence of which is rising around the world.(732). The association with phenotype of metabolic syndrome culminates in a higher than normal risk of future cardiovascular and cerebrovascular disease which has also been highlighted by an almost 10% excess absolute risk which is approximately double that of patients without TRH. It is therefore important to accurately identify

patients with true TRH so that appropriate treatments can be initiated to minimise that risk.

The biochemical characteristics of patients with true TRH have also shown the increased prevalence of target organ damage associated with hypertension as highlighted by the lower eGFR and cardiac biomarkers. Furthermore, presence of secondary hypertension based on biochemical screening remains very low in this study cohort.

The biochemical and physiological assessment of body composition, arterial stiffness, RAAS and endothelial dysfunction has provided an insight to possible mechanisms that could be responsible for hypertension in patients with true TRH. The higher ARR alongside and expansion of extracellular fluid compartment are highly suggestive of hyperaldosteronism in TRH. Clinically, these support the use of spironolactone in patients with TRH which has been shown in recent large RCT of patients treated with the mineralocorticoid receptor antagonist (357, 448).

Patients with TRH were also shown to have increased ET-1 and VCAM-1, which are biomarkers of endothelial dysfunction. Vasoconstriction mediated by ET-1 may be responsible for higher BP whereas VCAM-1 adds to the increasing evidence of role of inflammatory pathways in development and maintenance of hypertension and may signify presence of underlying atherosclerosis. A higher cFLC observed in this study also indicate a role of inflammation which may be higher in patients with TRH.

Arterial stiffness has been shown to be increased in TRH. However, in this study, after controlling for the known effect of age, HR and MAP, there was no difference in the two study groups. Lastly, the results of overnight sleep oximetry from a

smaller proportion of the study cohort are suggestive of higher prevalence of OSA in patients with TRH.

The results of FACT-RHY have indicated a possible role for a multitude of mechanisms present more predominantly in TRH and warrant further investigations to prove or disprove the findings from this exploratory analysis which is limited by the lack of causality that is associated with cross-sectional studies. However, the results of FACT-RHY study has made important observations which have implications on clinical practice and have the potential to be explored further in future research. Firstly, the role of screening all patients with suspected TRH for OSA with overnight sleep oximetry is crucial as the ESS alone may not be predictive. Second, the use of bioimpedance spectroscopy could be explored to assess fluid compartments of the body and tailor appropriate antihypertensive use in patients with TRH. Lastly further research could investigate the effects of selective inhibitors of ET receptors in patients with TRH.

In summary, evidence reviewed in this thesis and the results of the studies highlight the importance of comprehensive assessment, including adherence, of patients with TRH to identify patients with true resistance. The number of factors associated with TRH discussed in this thesis emphasise the complexity of pathophysiology of TRH and leads to the conclusion that instead of a targeting a single unifying mechanism, the future of treatment of TRH lies instead in multifaceted assessment of patients to tailor therapeutic interventions at a personalised level to control BP and its comorbidities.

# LIST OF PUBLICATIONS

**Publications arising from the thesis:**

Lawson AJ, **Hameed MA**, Brown R, Cappuccio FP, George S, Hinton T, et al. Nonadherence to antihypertensive medications is related to pill burden in apparent treatment-resistant hypertensive individuals. Journal of Hypertension. 2020;38(6):1165-73.

**Hameed MA**, Dasgupta I. Medication adherence and treatment-resistant hypertension: a review. Drugs Context. 2019 Feb 4;8:212560.

**Hameed MA**, Freedman JS, Watkin R, Ganeshan A, Dasgupta I. Renal denervation using carbon dioxide renal angiography in patients with uncontrolled hypertension and moderate to severe chronic kidney disease. Clin Kidney J. 2017 Dec;10(6):778-782.

Renton M, **Hameed MA**, Dasgupta I, Hoey ET, Freedman J, Ganeshan A. The use of carbon dioxide angiography for renal sympathetic denervation: a technical report. Br J Radiol. 2016 Dec;89(1068):20160311.

**Hameed MA**, Dasgupta I. Renal Denervation. Adv Exp Med Biol. 2017;956:261-277.

**Hameed MA**, Gill P, Dasgupta I. 10-minute consultation: Poor adherence with anti-hypertensives. BMJ 2016;354:i3268.

**Other Publications:**

Sharp A, Davies J, Lobo M, Bent C, Mark P, Burchell A, Thackray S, Martin U, McKane W, Gerber R, Wilkinson J, Antonios T, Doulton T, Patterson T, Clifford P, Lindsay A, Houston G, Freedman J, Das N, Belli A, Faris M, Cleveland T, Nightingale A, **Hameed A**, Mahadevan K, Finegold J, Mather A, Levy T, D'Souza R, Riley P, Moss J, Di Mario C, Redwood S, Baumbach A, Caulfield M, Dasgupta I. Renal artery sympathetic denervation: observations from the UK experience. Clin Res Cardiol. 2016: 1-9. DOI: 10.1007/s00392-015-0959-4

**Hameed MA**, Pucci M, Martin U, Watkin R, Doshi SN, Freedman JS, Riley P, Townend JN, Crowe P, Lipkin GW, Dasgupta I. Renal denervation in patients with uncontrolled hypertension and confirmed adherence to antihypertensive medications. J Clin Hypertens (Greenwich). 2015:1–7. DOI: 10.1111/jch.12713.

**Hameed MA**, Tebbit L, Jacques N, Thomas M, Dasgupta I. Non-adherence to antihypertensive medication is very common among resistant hypertensives: results of a directly observed therapy clinic. J Hum Hypertens 2016;30:83-89. DOI:10.1038/jhh.2015.38

**Abstracts arising from this thesis:**

**Hameed MA,** Haque S, Gill P, Dasgupta I. Evaluation of patients' beliefs and perceptions of their antihypertensive medications and their association with adherence and presence of treatment-resistant hypertension using beliefs about medicines questionnaire. J Hum Hypertens 2019;33:20-21.

**Hameed MA,** Haque S, Ferro C, Gill P, Dasgupta I. Comparison of carotid-to-femoral pulse wave velocity as a maker of arterial stiffness in patients with true treatment-resistant hypertension and non-resistant treated patients with hypertension. J Hum Hypertens 2019;33:20.

**Hameed MA,** Haque S, Gill P, Dasgupta I. Assessment of fluid excess and body composition using bioimpedance spectroscopy in patients with adherence-proven treatment-resistant hypertension. J Hum Hypertens 2019;33:19-20.

**Hameed MA**, Hopkins S, Ahmed S, Haque S, Ahmed A, Gill P, Dasgupta I. Evaluation of vascular endothelial dysfunction through measurement of serological biomarkers and reactive hyperaemia index in patients with true-treatment resistant hypertension. J Hum Hypertens 2019;33:19.

**Hameed MA**, Horton L, Haque S, Gill P, Dasgupta I. A study of Factors AssoCiated with Treatment Resistant HYpertension (FACT-RHY): description of phenotypical and biochemical characteristics of patients with true treatment-resistant hypertension compared to treated patients with hypertension. J Hum Hypertens 2019;33:18-19.

Lawson A, **Hameed A**, Dasgupta I. Implementation of an objective test of medication adherence in a hypertensive population. Future Healthc J. 2019 Mar;6 (Suppl 1):87.

**Hameed M**, Cappuccio F, Padmanabhan S, Dasgupta I. Current practice for the assessment and management of non-adherence in patients with resistant hypertension: survey of British Hypertension Society members. J Hum Hypertens 2016;30:633–656.

**Hameed M**, Tebbit L, Freedman J, Ganeshan A, Watkin R, Dasgupta I. Renal sympathetic denervation in moderate to severe chronic kidney disease using carbon dioxide angiography. J Hum Hypertens 2016;30:633–656.

**Oral Presentations:**

**2019 UK Kidney Week –** Results of a study of Factors AssoCiated with Treatment Resistant HYpertension (FACT-RHY)

**2018 UK Kidney Week -** Non-adherence to Antihypertensive Medications in patients with apparent resistant hypertension.

**2015 British Hypertension Society Annual Meeting –** Results of Non-adherence survey**.**

# APPENDICES

**Appendix I:** Study protocol for Renal sympathetic denervation in moderate to severe chronic kidney disease (CKD stages 3B and 4) using a novel noniodinated contrast-free protocol.

**Renal sympathetic denervation in moderate to severe chronic kidney disease (CKD stages 3B and 4) using a novel noniodinated contrast-free protocol.**

CI: Dr Indranil Dasgupta, Consultant Nephrologist, Heartlands Hospital,
Heart of England NHS Foundation Trust, Bordesley Green East, Birmingham, B9 5SS.

Sponsor: Heart of England NHS Foundation Trust, Bordesley Green East, Birmingham, B9 5SS.

**Introduction:**

Hypertension is the most common chronic disease, affecting more than 25% of the adult population in the Western World[1]. It contributes to 62% of all strokes and 49% heart disease. It contributes to an estimated 7.1 million deaths a year worldwide (13%)[2]. Resistant hypertension is defined as raised, seated, office blood pressure (>140/90 mmHg) despite treatment with 3 or more antihypertensive agents at optimal or maximal tolerated doses[3]. The prevalence of resistant hypertension is estimated to be 10 to 20% of the general hypertensive population[4]. Chronic kidney disease (CKD) is the commonest cause of secondary hypertension. About 80% of patients with CKD have hypertension[5,6]. More than 50% patients with CKD have uncontrolled hypertension[6].

Recently, renal sympathetic hypertension (RSD) has been shown to significantly lower blood pressure (BP) in people with resistant hypertension. In the Simplicity HTN 1 observational study, in a cohort of 153 patients, there were sustained lowering of BP of 32/14 at 2 years[7,8]. The mean BP at baseline was 176/98 mmHg on a mean number 5 antihypertensive agents. In the randomised controlled study, Simplicity HTN 2, there was a mean BP reduction of 32/12 mmHg in the renal denervation group at 6 months from a base line of 178/97 mmHg (on 5 agents mean)[9]. In both of these studies, patients with eGFR <45 ml/min/1.73m$^2$ were excluded. One of the reasons for excluding this group of patients is the risk of contrast nephropathy.

Sympathetic over activity is considered to one of the main reasons for difficulty in controlling BP in CKD patients[10]. Moreover, sympathetic over activity is also thought to have a role in proteinuria and progression of renal disease[11]. Therefore, patients with resistant hypertension and moderate to severe CKD are more likely to benefit from RSD than those with no or less severe renal impairment.

**Study rationale:**

Renal sympathetic denervation has been shown to be safe and effective in patients with uncontrolled hypertension and eGFR>45mL/min per 1·73 m². However, the safety and

efficacy of this has not been studied in patients with more severe renal impairment. We aim to examine safety and efficacy of RDN in patients with eGFR between 44 and 15 ml/min/1.73 $m^2$ (CKD 3B & 4) in a pilot study which may be a precursor of a large observational study in the future. Moreover, the current imaging protocol and procedure protocol for RSD requires the use of iodinated contrast, which can have deleterious effects on renal function. Our centre has a proven track record for the use of $CO_2$ angiography in renal artery intervention. We would like to use $CO_2$ angiography in this study to minimize contrast induced deterioration in renal function in this cohort which may again be a precursor of a farther larger study.

**Number of subjects:**

This is single centre study of 10 patients.

**Study duration and dates:**

Estimated start of study: 01.02.2013
Estimated end of study: 31.08.2013

**Indication:**

RDN in patients with moderate to severe CKD – eGFR <45 - >15 ml/min/1.73$m^2$

**Study intervention:**

Renal sympathetic denervation using $CO_2$ angiography.

**Study objectives:**

Primary: safety and efficacy of RDN in CKD stages 3b and 4

Secondary: improvement in BP control, eGFR and proteinuria

**Primary endpoints:**

- Change in eGFR from baseline to 7 days and 1 month as measure of safety in terms of kidney function.
- Change in office BP from baseline to 1, 3, and 6 months for efficacy.

**Secondary endpoints:**

- Change in kidney function from baseline to 7 days, 1, 3 and 6 months.
- Change in proteinuria (albumin:creatinine ratio) from baseline to 1, 3 and 6 months.
- Change in mean daytime BP on 24 hour ambulatory monitoring from baseline to 6 months.

**Inclusion Criteria:**

- Patients aged 18–75years with a clinic systolic blood pressure of 140 mm Hg or more despite compliance with three or more antihypertensive drugs,
- eGFR<45 and >15mL/min per 1·73 $m^2$.

**Exclusion Criteria:**

- eGFR <15 mL/min per 1.73$m^2$,
- Type 1 diabetes,
- Substantial stenotic valvular heart disease,
- Pregnancy or planned pregnancy during the study,
- A history of myocardial infarction, unstable angina, or cerebrovascular accident in the previous 6 months.

**Study Design:**

Observational – Prospective

**Biostatistics and Data Analysis:**

Changes in office BP from baseline to 1, 3, and 6 months will be analysed using ANOVA test, a two tailed p value of <0.05 will be taken as significant.

**Study Schedule:**

| **Visit** | **Visit 1** | **Visit 2** | **Visit 3** | **Visit 4** | **Visit 5** | **Visit 6** |
|---|---|---|---|---|---|---|
| **Week (approximately)** | **2 – 4 weeks before RDN** | **0 – RDN** | **1** | **4** | **12** | **24** |
| Informed consent | X | | | | | |
| Physical examination | X | | | X | X | X |
| 24 Hour Blood Pressure Monitoring | X | | | | | X |
| Clinic Blood pressure | X | X | X | X | X | X |
| Blood test – U&E | X | X | X | X | X | X |
| Urine Test – Protein Creatinine Ratio | X | X | X | X | X | X |
| CT Renal Angiogram | X | | | | | |
| Renal Denervation | | X | | | | |

## References


1. Wolf-Maier K, Cooper RS, Banegas JR, et al. Hypertension prevalence and blood pressure levels in 6 European countries, Canada, and United States. JAMA 2003;289:2363-9.
2. World Health Organisation. Global status report on noncommunicable diseases 2010. WHO, 2011.
3. NICE Clinical Guidelines 127. Hypertension – The clinical management of primary hypertension in adults, 2011.
4. Pimenta E, Calhoun DA. Resistant hypertension: incidence, prevalence and prognosis. Circulation 2012;125:1635-42.
5. Vardaman M. Buckalew Jr, Richard L. Berg, Shin-Ru Wang, et al. Prevalence of hypertension in 1,795 subjects with chronic renal disease: The modification of diet in renal disease study baseline cohor. Am J Kid Dis 1996; 28:811-21.
6. Dasgupta I, Savill J, Pringle MAL, et al. Management of hypertension in patients developing end-stage renal failure. QJM 1999; 92: 519 – 525.
7. Krum H, Schlaich M, Whitbourn R, et al. Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet.* Apr 11 2009;373(9671):1275-1281.
8. Catheter-based renal sympathetic denervation for resistant hypertension: durability of blood pressure reduction out to 24 months. *Hypertension.* May 2011;57(5):911-917.
9. Esler MD, Krum H, Sobotka PA, Schlaich MP, Schmieder RE, Bohm M. Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet.* Dec 4 2010;376(9756):1903-1909.
10. Grisk O, Rettig R. Interactions between the sympathetic nervous system and the kidneys in arterial hypertension. Cardiovascular Research 61 (2004) 238– 246.
11. Park J. Cardiovascular Risk in Chronic Kidney Disease: Role of the Sympathetic Nervous System. Cardiology Research and Practice 2012. doi:10.1155/2012/319432.

**Appendix II:** Patient information sheet for renal sympathetic denervation in moderate to severe chronic kidney disease using a novel non-iodinated contrast free protocol

Birmingham Heartlands Hospital
Renal Unit
Bordesley Green East
Birmingham
B9 5SS

Tel: 0121 424 2158
Fax: 0121 424 1159

## Patient Information Sheet

**Title:** Renal sympathetic denervation in moderate to severe chronic kidney disease using a novel non-iodinated contrast free protocol

**Short Title:** Renal denervation in moderate to severe CKD using non-iodine contrast

**Part 1**

### 1 Introduction

You are being invited to take part in the above-mentioned study, sponsored by Heart of England NHS Foundation Trust (HEFT). Before you decide, it is important for you to understand why this research is being done and what it will involve for you. Please read the following information carefully. One of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish and do not hesitate to ask us if something is unclear.

### 2 What is the purpose of the study?

Renal Denervation (RDN) is a minimally invasive procedure performed under local anaesthesia which aims to reduce blood pressure in patients for whom medication alone has failed. It is performed by guiding a device (treatment catheter) into the arteries of the kidneys using X-ray guidance. Once in place, the device produces high frequency radio-waves. This permanently interrupts abnormal nerve signals from the kidney to the heart, brain and blood vessels, responsible for high blood pressure (hypertension).

RDN has been shown to be safe and effective in patients with uncontrolled high blood pressure and mild to moderate kidney function impairment or kidney damage. However, the safety and efficacy of this has not been studied in patients with more severe kidney impairment. This study is aiming to examine the safety and efficacy of RDN in this group of patients.

The RDN procedure usually requires the use of iodine containing dye (iodinated contrast) to show the doctor where the kidney arteries are. This dye can have a harmful effect on kidney function. In our hospital we have been using Carbon Dioxide ($CO_2$), a gas, for a few years to perform procedures involving kidney arteries ($CO_2$ angiogram). $CO_2$ does not have harmful effect on kidney function.

**3 Why have I been chosen?**

You have been chosen to potentially take part in this study as, based on your current medical history from our Chronic Kidney Disease (CKD) database, you meet the requirements for the study. 10 patients, aged 18-65 years, undergoing treatment at HEFT, with a medical history like yours, will be asked to take part.

**4 Do I have to take part?**

No, taking part in this study is voluntary. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and given time to review the information. You can have as much time as you need to read and think about this information and discuss with others (e.g. family, friends, GP) if you wish before deciding whether or not to take part in this study. Ask us if there is anything that is not clear or if you would like more information.

If you decide not to take part, you do not have to give a reason. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care received. If you do withdraw from the study, no new information about you will be collected, although information about you that has already been collected may continue to be used in order to preserve the value of the study. If you have any questions or concerns about this, please ask.

**5 What will happen to me if I take part?**

If you decide to take part in this study, you will be asked to sign an informed consent form. This confirms that all the study information has been clearly explained to you, including the risks and benefits of taking part, any procedures and tests to be performed, any questions have been answered and that you have been given enough time to consider whether you wish to take part in this study. You will be given a copy of this information sheet to keep, along with a copy of your consent form. Your doctor will also sign a copy of the consent form.

Your involvement will last around 24 weeks (6 months) and you will visit the hospital 6 times during the study and the following tests performed:

| **Visit** | **Visit 1** | **Visit 2** | **Visit 3** | **Visit 4** | **Visit 5** | **Visit 6** |
|---|---|---|---|---|---|---|
| **Week (approximately)** | **2 – 4 weeks before RDN** | **0 – RDN procedure** | **1** | **4** | **12** | **24** |
| Informed consent | X | | | | | |
| 24 Hour Blood Pressure Monitoring | X | | | | | X |
| Blood pressure | X | X | X | X | X | X |
| Blood test | X | X | X | X | X | X |
| Urine Test | X | X | X | X | X | X |
| CT Scan of the kidney | X | | | | | |
| Renal Denervation | | X | | | | |

Before having the RDN procedure, the research team will ask you to complete some standard medical tests, some of which are similar to the tests you have normally as part of your routine care. The tests are 24 hour blood pressure monitoring, a blood and urine test and a CT scan of your kidney arteries.

**RDN Procedure:**
Once the research team has confirmed you are suitable for the RDN procedure, you will be given an appointment in the X-ray department. RDN is performed by an Interventional Radiologist and Interventional Cardiologist, specialist doctors who are used to performing a range of minimally invasive procedures under X-ray guidance. We have a separate information sheet which will explain the RDN procedure to you in more detail.

**What happens after the RDN procedure?**

Following the procedure, you will be reviewed at your routine clinic appointment at 7 days, 1 month, 3 months and 6 months. Each visit will last approximately 30 minutes and like your normal hospital visits, your blood pressure will be measured along with urine and blood test for kidney function. At the 6 month visit, you will also be asked to have your blood pressure measured for 24 hours, like at the start of the study. Your involvement in the study is then over and you will continue to be followed up as per your normal clinical appointments.

## 6 What do I have to do?

If you decide to take part in this study you will be asked to attend the hospital for each of the visits as described in section 5.

You will be asked to wear a blood pressure device for 24 hours at home at the start and end of the study.

You must contact us at any point during the study should you feel unwell.

## 7 What are the alternative treatments?

Surgically removing the nerves that are attached to the kidneys can effectively lower blood pressure. This type of surgery, called non-selective sympathectomy, was used in the past but caused significant complications. This treatment became less common as antihypertensive drugs became more affordable and accessible. Alternatively, more blood pressure tablets will be added to your medication which may or may not lower blood pressure adequately.

## 8 What are the possible benefits of taking part?

This is still a new technique but results from Europe and the USA show consistent improvement in the blood pressure of those patients treated. This could reduce the risks of developing blood pressure related conditions such as Stroke, heart and vascular disease and slow/prevent progression of kidney disease.

## 9 What are the possible risks of taking part?

With any medical procedure there may be complications and it is important you know about these. RDN has a small risk of complications but the procedure may or may not improve blood pressure.

The primary risks of the procedure are similar to the risks of all diagnostic procedures requiring catheterization of the arteries of your body. They include: low or high blood pressure, heart rhythm disturbances, death, cardiac arrest, blood clot, injury to the artery that may require surgery, complications related to the pain and anti-anxiety medications given. The RDN research studies carried out so far have shown these complications to be extremely rare.

The most frequent complication is bruising of the groin at the site of insertion of the tube. This usually settles down but occasionally needs a blood transfusion or a further treatment to repair the artery. It is very important that if after discharge from the hospital you develop pain or a swelling at the puncture site you seek medical advice immediately.

$CO_2$ (carbon dioxide) has very few risks when used for X-ray procedures but may not be suitable in patients with advanced lung diseases such as COPD. The radiation dose is very low and does not differ significantly between the standard and research protocol.

As RDN is undertaken using x-rays you will receive a dose of radiation from the procedure. The amount of radiation received varies from patient to patient depending

on the patient size and the complexity of the procedure. However, you will receive no more dose than if you we having the RDN outside of the trial. Any radiation dose results in a risk of developing a cancer in the future. The risk increases as the dose increases. The risk from the RDN procedure is typically 1 in 250 (4 in 1000). This can be compared with the natural risk of 1 in 4 (250 per 1000) we all have of developing a fatal cancer.

If you are pregnant or likely to be pregnant then you must inform the doctors as radiation exposure can potentially harm your unborn baby.

There are additional risks that could possibly be associated with the RDN procedure. They include: kidney damage, injury to the artery, reduction of blood pressure too far or too fast that leads to complications, skin burn, blood or protein in urine, electrolyte changes.

It is important to balance these risks with the potential benefits of the procedure. Your doctor will discuss with you whether having RDN to improve your blood pressure would make these risks worthwhile.

**If there is any complication following the procedure, you will be able to ring one of the investigators on the number provided below:**

**Dr Indranil Dasgupta Tel: 01214242158**

**Dr Jonathan Freedman**

**Tel: 01214249931**

**Dr Arul Ganeshan**

**Tel: 01214243280**

## 10 What happens when the research study stops?

You will continue to be monitored at the hospital for your kidney condition every 3 months as per your routine hospital appointments.

**Part 2**

**11 What if relevant new information becomes available?**

Sometimes we get new information about the treatment being studied. If this happens, your research doctor will tell you and discuss with you, whether you should continue in the study or not. If you decide not to carry on, your research doctors will make arrangements for your care to continue. If you decide to continue in the study they may ask you to sign a further consent form confirming your decision.

**12 What will happen if I don't want to carry on with the study?**

If you decide you no longer wish to continue in the study this will not affect the standard of care you will receive in the future. Your research doctor will make arrangements for your future care to continue. If you do withdraw from the study, no new information about you will be collected, although information about you that has already been collected may continue to be used in order to preserve the value of the study. If you have any questions or concerns about this, please ask.

**13 What if something goes wrong?**

In the event that something does go wrong and you are harmed during this research and this is due to someone's negligence then you may have grounds for a legal action for compensation against Heart of England NHS Foundation Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

If you have a concern about any aspect of this study, you should ask to speak to the researchers (Dr I Dasgupta, Dr J Freedman, Dr A Ganeshan) who will do their best to answer your questions.

If you remain unhappy and wish to complain formally, you can do this by writing to Heart of England NHS Foundation Trust, Complaints Department, Birmingham Heartlands Hospital, Bordesley Green East, Birmingham, B9 5SS to telephone 0121424 3615

<u>For any concerns about your rights as a participant or any complaints please contact:</u>

<u>Patient Services: 0121 424 0808</u>

**14 Will my taking part in this study be kept confidential?**

If you join the study the research team involved in this study may need access to your medical history, including your past medical records and test results, for the purposes of this study. You will not be named in any publication or report generated as a result of participating in this study. You will be given a unique patient identification number which the medical research team will use to identify you.

In addition, some parts of your medical records and the data collected will be looked at by authorised persons from Heart of England NHS Foundation Trust and/or by representatives of the UK regulatory authorities to check that the study is being

carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

With your consent, your General Practitioner (GP) will be told that you have decided to take part in this study. They will be sent a copy of this information sheet, along with details of the procedure.

**15 What will happen to the results of the study?**

The results of the study will be analysed and published in medical publications and at conferences. You will not be named or identified in any of these publications. You will be seen in a routine outpatient clinic after your participation in the study has ended where you will be informed of the procedure and the results of the study (once available).

**16 Who is organising and funding the study?**

This study is being run by Heart of England NHS Foundation Trust. The Trust has received funding from Medtronic International Trading Sari to run the study. The results of the study will be presented in scientific meetings and published in scientific journals.

**17 Who has reviewed this study?**

All research in the NHS is looked at by an independent group of people called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable opinion by Edgbaston Research Ethics Committee. The project has also been reviewed and approved by Heart of England NHS Foundation Trust Research and Development Department who ensure that the research project meets the requirements of the Department of Health's research governance framework.

**18 Contact names and telephone numbers for further information**

For any concerns or other questions about this study, or any questions about a study related injury please contact:

Dr Indranil Dasgupta

Tel: [redacted]

Dr Jonathan Freedman

Tel: [redacted]

Dr Arul Ganeshan

Tel: [redacted]

In an emergency, please contact: Dr Indranil Dasgupta

Tel: [redacted] or [redacted]

**Thank you for taking the time to read this information sheet.**

**Appendix III:** Consent form for renal sympathetic denervation in moderate to severe chronic kidney disease using a novel non-iodinated contrast free protocol

**INFORMED CONSENT FORM**

**Patient Identification Number for this trial:**

**Title of Project:** Renal sympathetic denervation in moderate to severe chronic kidney disease using a novel non-iodinated contrast free protocol

**Name of Researcher:** Dr Indranil Dasgupta

**Please initial box**

1. I confirm that I have read and understand this information sheet version 7 dated 24 July 2013 for the above study. I have had the opportunity to ask questions and received satisfactory answers and have received a copy of the information for my reference. I am over 18 years of age (aged 18 or above). ☐
2. I understand that my participation is voluntary and that I am free to withdraw without giving any reason, without my medical care or legal rights being affected. If I decide to withdraw, any data (information) collected up to the point I withdraw may be used in order to preserve the value of the study, however no further information will be collected. ☐
3. I understand that some parts of my medical records and data collected during the study may be looked at by authorised persons from regulatory authorities or from the NHS Trust where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records even if I withdraw and I understand that my records will only be reviewed for information related to my participation in the study. ☐
4. I agree that my GP will be informed that I am taking part in this study. ☐
5. I agree to the use of my data or results which arise from my participation in this study and understand that my data or results will be limited to the use described in the information sheet. ☐
6. I agree to take part in this study. ☐

| Name of Participant | Date | Signature |
|---|---|---|
| Name of Person taking consent | Date | Signature |

# A study of Factors AssoCiated with true Treatment-Resistant HYpertension (FACT-RHY)

## Clinical Investigation Plan

### Version 1.6

### 27 March 2017

## Table of Contents

## Summary

**Title:** A study of factors associated with treatment resistant hypertension

**Design:** A single-centre, prospective, observational study

**Study objectives**

We propose to investigate whether several factors will associate with treatment resistance.

**Primary objective** is to assess association with vascular markers including arterial stiffness and endothelial function

**Secondary objectives:**

i. Assess association between body composition and hypertension as measured using bioimpedance spectroscopy
ii. Evaluate level of sympathetic nervous system activity in the study patients
iii. Assess prevalence of undiagnosed obstructive sleep apnoea in the study population
iv. Explore patients' personal views about medicines prescribed for hypertension and medicines in general.
v. Describe the risk of developing complications associated with hypertension in this cohort. These will include ischaemic heart disease, heart failure, stroke, renal failure and death.

**Subject Population:** Potentially eligible patients will be screened and recruited from the hypertension clinic based at the Heartlands Hospital.

**Time course:** Commencement of enrolment in November 2016 with anticipated enrolment completion by October 2017.

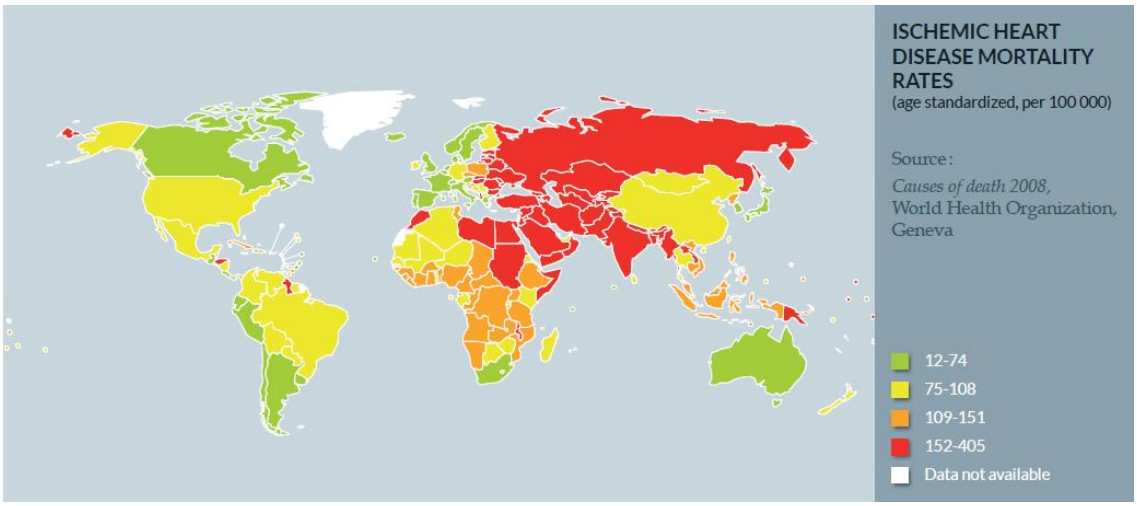
**Follow-up Duration:** 5 years (electronic only)

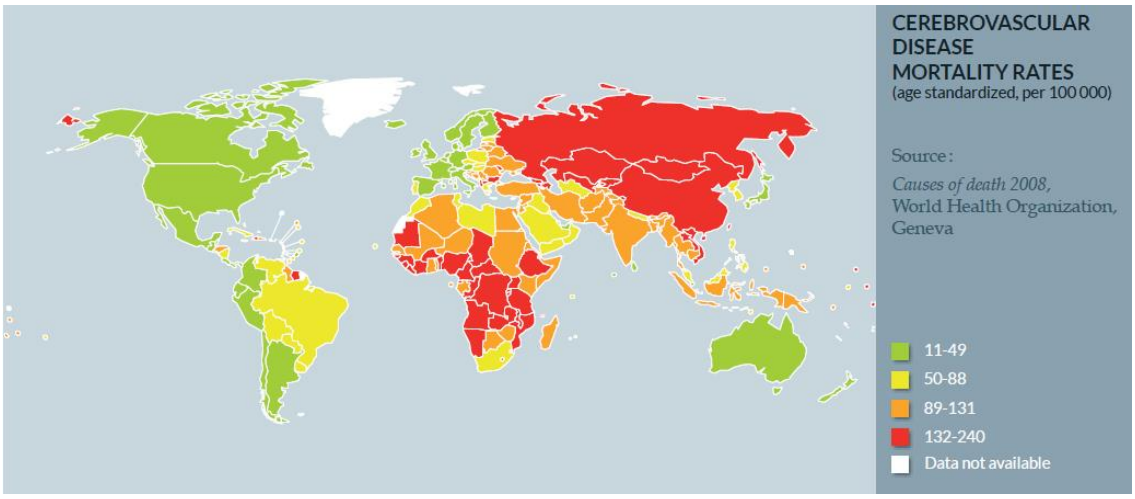
**Key Data Collection:** Medical history, physical examination including clinic blood pressure measurements, blood and urine tests, 24 hour ambulatory blood pressure monitoring, arterial pulse wave velocity, body composition monitoring, flow mediated dilatation.

# Introduction

## Hypertension

Hypertension or high blood pressure is a major preventable cause of cardiovascular morbidity and mortality worldwide. Hypertension is an established risk factor for ischaemic and haemorrhagic stroke, myocardial infarction, heart failure, chronic kidney disease, cognitive impairment and premature death. Blood pressure is normally distributed in the population and therefore a clear 'cut-off' is difficult to determine, although a blood pressure of <120 mmHg systolic and <80 mmHg diastolic is often stated to be normal. However, cardiovascular benefits of lower blood pressure are present at even lower values than those considered normal. The risks of hypertension are continuous; every 2 mmHg rise in systolic blood pressure is associated with a 7% increased risk of mortality from ischaemic heart disease and 10% increased risk of mortality from stroke. The World Health Organisation ranks hypertension as the highest amongst a list of risk factor attributable to burden of disease worldwide (1). Its estimates show that cardiovascular disease is responsible for a third of the total deaths worldwide with 17 million deaths a year (2), of which hypertension is responsible for 9.4 million deaths a year (3). Figures 1 and 2 show global total mortality rates from ischaemic heart disease and cerebrovascular disease respectively.



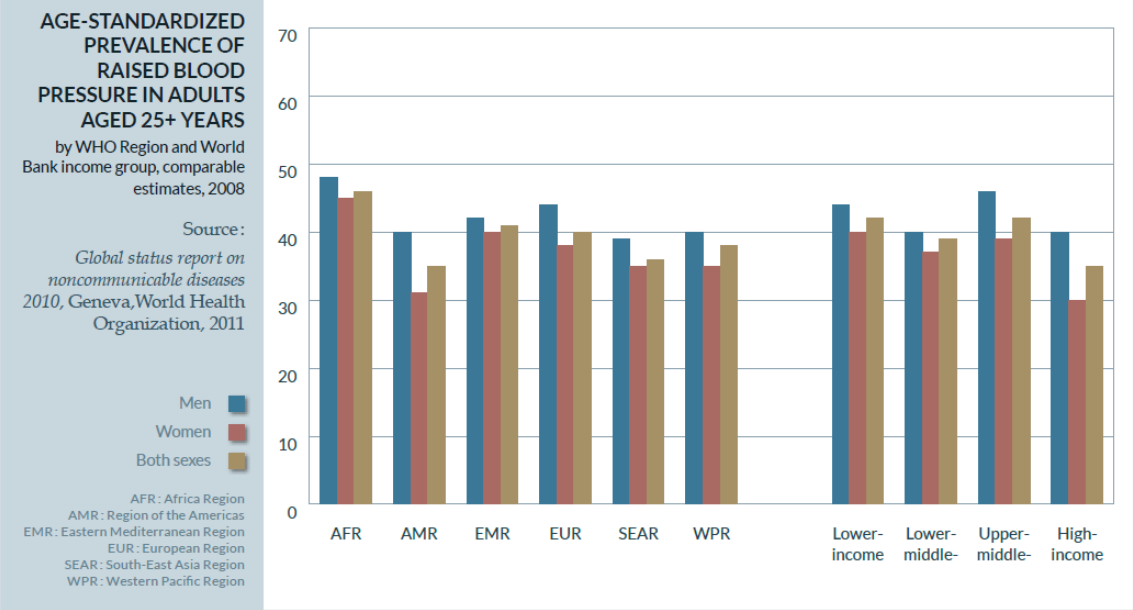



**Treatment Resistant Hypertension:**

Treatment-resistant hypertension (TRH) is defined as uncontrolled blood pressure (BP) with a clinic systolic BP ≥ 140 mmHg and/or a diastolic BP ≥ 90 mmHg despite the use of maximally tolerated doses of 3 or more antihypertensive medications of different classes including a diuretic (4,5). Suboptimal BP control is well known to be associated with an increased risk of cardiovascular and cerebrovascular events and is consequently ranked highest amongst the list of risk factors attributable to burden of disease worldwide (1). A linear relationship has been shown to exist between level of BP control and risk of cardiovascular events (6-8). Unsurprisingly the risks known to be associated with hypertension are even greater in patients with TRH. Some estimates suggest that patients with TRH are twice as likely to experience a cardiovascular event as compared to patients without TRH (9).

**Epidemiology:**

Prevalence of hypertension is common; in 2008, approximately 40% of the adults above the age of 25 worldwide were diagnosed with hypertension. The prevalence of hypertension varies greatly around the world; the highest prevalence is seen in the Africas Region at 46% of adults above the age of 25 years and lowest prevalence in the Americas at 35% as shown in Figure 3 (2).




In the UK, figures from Department of Health's 2013 Health Survey England have shown that prevalence in adults of age 16 years or older was 31.4% in men, 26.0% in women and 28.6% overall. Hypertension is largely an asymptomatic condition and therefore quite often remains undiagnosed. Public Health England estimates over 5 million adults in the UK are unaware that they have hypertension. Diseases caused by hypertension have been estimated to cost the National Health Service over £2 billion a year.

In most studies, prevalence of TRH has been reported to be 12% to 15% of the total hypertensive population (10-13) with some evidence suggesting a recent increase in the prevalence to 20% (14). The prevalence figures reported in the literature vary due to the different interpretations

of the term TRH; some studies include hypertensive patients with controlled BP on ≥4 medications (controlled TRH) and also fail to distinguish the proportion of patients who have apparent TRH due to presence of white-coat effect. True TRH is a more useful term which includes patients who have uncontrolled BP with white coat hypertension excluded and patient's medication adherence confirmed. The prevalence of true TRH has not been directly measured in large trials; however, it has been estimated to be present in half of all the patients labelled with a diagnosis of TRH (15,16).

## Pathophysiology:

Cross sectional studies have advanced age, female sex, black race, obesity, diabetes mellitus, sleep apnoea and chronic kidney disease (CKD) to be associated with TRH (17,18). A common pathophysiology implicated in some of these factors has been over-activity of the sympathetic nervous system which has led to emergence of percutaneous renal artery sympathetic denervation as a technique to control BP in patients with TRH. Though the initial results from clinical trials of renal denervation promised a significant improvement of BP control, more recent studies have shown less impressive changes in BP control. These findings suggest that the underlying mechanisms promoting TRH are likely to be more complex in nature and are probably due to a composition of different pathophysiological factors.

The results from the PATHWAY-2, a randomised double-blind, crossover trial to assess efficacy of spironolactone versus placebo, bisoprolol, and doxazosin to determine optimal treatment for patients with TRH, have suggested a predominant role of sodium retention in the pathophysiology of TRH despite use of existing diuretic therapy (19).

Thus far there are no published prospective studies examining the various pathophysiological tests being proposed in our study on a single cohort of patients with true TRH. Identification of factors predisposing to TRH may highlight potential mechanism(s) responsible for development of treatment resistance.

## Rationale for Study Parameters

### Sympathetic overactivity

Over-activation of the sympathetic nervous system is associated with chronic diseases in particular hypertension, heart failure and chronic kidney disease. Renal noradrenaline spillover rates have been shown to be significantly elevated in patients with hypertension during assessment of regional overflow of noradrenaline from the kidneys to plasma (20). Furthermore, increased noradrenaline spillover has been shown to be associated with left ventricular hypertrophy as a consequence of hypertension (21,22). Similarly, cardiorenal sympathetic nerve activity is greatly enhanced in patients with heart failure (23). End-stage renal disease (ESRD) is another condition which is characterised by substantially increased levels of sympathetic nerve activity and elevated noradrenaline plasma levels have been linked with poor cardiovascular outcomes (24,25). There is now substantial evidence implicating a specific role of sympathetic activation in the progression of CKD (26). Accordingly, targeting the sympathetic nervous system directly appears to be a logical therapeutic approach not only for the treatment of hypertension but perhaps also for mitigation of additional adverse consequences associated with sympathetic activation.

### Arterial stiffness

The hardening of human arteries with age and disease is well known. Increased central arterial stiffness is associated with adverse cardiovascular outcomes and is a strong predictor of future CV events and all-cause mortality (27). Changes in arterial structure can be quantified in terms of vessel stiffness, which is the pressure required to provide a unit change in volume (28).

Intermittent ventricular contraction causes large changes in BP and flow within the arteries. The peak BPs can damage the peripheral microvasculature due to barotrauma. Arteries provide a dampening function so that a steady continuous blood flow is maintained to peripheral tissues throughout systole and diastole. Arterial walls, in particular the aorta, contain predominantly elastin fibres which allow significant dilation during systole. During diastole, the arteries recoil to drive the blood along the arterial tree and augment coronary artery blood flow. The pulse wave from the arteries reflected back to the heart is called pulse wave reflection which resists the forward travelling blood. As the aorta stiffens it is less able to accommodate the blood volume from the heart which raises the systolic pressure and left ventricular afterload. A persistently raised afterload exposes the heart to high systolic pressures which increase myocardial work consequently result in left ventricular hypertrophy. Arterial stiffness is associated with left ventricular hypertrophy in normotensive and hypertensive patients (29,30). Reduced elastic recoil leads to a decrease in diastolic BP reducing coronary perfusion which alongside the ventricular hypertrophy results in subendocardial ischaemia (31,32). Furthermore, left ventricular hypertrophy attenuates the backward travelling suction wave within the coronaries that drives the majority of coronary flow (33).

Numerous studies have now established aortic stiffness as an independent predictor of cardiovascular events. In a cross-sectional study, aortic pulse-wave velocity (PWV) was shown to be associated with cardiovascular risk, as calculated from the Framingham equations (34).

Furthermore, aortic stiffness is significantly associated with the risk of all-cause and cardiovascular mortality in patients with essential hypertension (35). Recent studies have shown an elevated arterial stiffness in patients with TRH (36,37). Furthermore, it is worth highlighting that all that characteristics associated with TRH including old age, female sex, black race, obesity, sleep apnoea, diabetes, chronic kidney disease and left ventricular hypertrophy are also independently associated with elevated arterial stiffness (38).

## Endothelial dysfunction and hypertension

Association between hypertension and endothelial dysfunction is well established (39-42); a phenotypical alteration of the vascular endothelium that precedes the development of adverse cardiovascular events and its presence predicts future events (43). Data from the Framingham Heart study suggest that the severity of hypertension is positively associated with the degree of impairment of endothelial function (44).

Endothelial dysfunction is characterised by reduction in bioavailability of endothelium-derived nitric oxide (NO), a change that precedes any structural changes associated with atherosclerosis (45). Nitric oxide is released from the endothelium in response to sheer stress, bradykinin and acetylcholine with the aim of maintaining low arterial tone at rest (46). Flow-mediated dilatation (FMD) is widely accepted as the functional bioassay for the endothelium-derived NO and hence has become synonymous with endothelial dysfunction in cardiovascular literature (47). Briefly, diameter of the brachial artery is measured using high frequency ultrasound before and after application of a dilated BP cuff placed around the upper arm to occlude the blood flow for five minutes (48). The principle based behind the test is that NO is released as a direct response to increased blood flow occurring immediately after relaxation of the cuff; release of NO leads to brachial artery dilatation.

Endothelial dysfunction can also be indicated by elevated serum concentrations of endothelial adhesion molecules, including intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 [VCAM-1] (49). These molecules accelerate atherosclerosis by promoting migration of inflammatory cells through the endothelium. Furthermore, vascular endothelial growth factor and its soluble receptor fms-like tyrosine kinase-1 (sFLT-1) are also associated with endothelial dysfunction, vascular remodelling, and endothelial repair and regeneration mechanisms (50-52).

Although endothelial dysfunction has been widely investigated in hypertensive subjects there are only a handful of relatively small studies carried out in resistant hypertensives. These studies have shown the presence of a higher degree of endothelial dysfunction in TRH as compared to controlled hypertension (53,54).

## Inflammation in hypertension

Various inflammatory mechanisms have been described in relation to hypertension (55). The acute phase protein, C - reactive protein (CRP) is produced by the liver in response to a wide range of acute or chronic inflammatory disease processes and is involved in complement activation and promoting phagocytosis. CRP is considered as the inflammatory marker with the

strongest association in hypertension (55). Increased plasma CRP levels have been demonstrated in many clinical trials of hypertension (56-61). CRP further promotes inflammation through stimulating release of proinflammatory cytokines such as interleukin-6 (IL-6), interleukin-1 beta (IL-1$\beta$), and tumour necrosis factor alpha (TNF-$\alpha$) from monocytes (62) and also intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 from vascular endothelial cells (63).

Inflammation is thought to play an important role in endothelial dysfunction observed in hypertension (55). Inflammation can alter the careful balance of synthesis and degradation of molecules involved in the regulation of vascular tone, in particular NO and its precursor NO synthase. Both CRP and TNF down-regulate NO synthase mRNA activity leading to reduced NO levels, with a consequent increase in peripheral resistance mediated through impaired vascular relaxation and increased vascular stiffness (55).

Furthermore, oxidative stress, triggered by chronic inflammation, has been shown to be associated with hypertension and is a major cause of endothelial dysfunction which is once again mediated through bioavailability of the NO (55). Hyperaldosteronism and ageing are also known to be associated with hypertension and there is evidence to suggest their role in chronic inflammation.

Immunoglobulins have also been found to play a role in pathogenesis of hypertension. Elevated immunoglobulin levels have been found in patients with essential hypertension when compared to normotensives (64-67). Although it is unclear whether the rise in immunoglobulins is primary or secondary, it is thought to be a consequence of vascular damage from high BP (68). More recently, serum immunoglobulin light chains, which are independent of the intact immunoglobulin molecule and are referred to as free light chains (FLC), have been associated with chronic inflammation (69). Studies have now been able to attribute evidence of an independent association between an elevated serum FLC level and a higher mortality risk (70,71). Furthermore, an increased serum FLC level is an independent risk factor for mortality and progression to end stage renal disease in patients with stages 3-5 CKD (72) and also for prognosis in patients with acute heart failure secondary to atherosclerotic coronary heart disease (73). However, an association with serum FLC and hypertension has not yet been studied and given the earlier association with intact immunoglobulin levels it can be hypothesised that serum FLC may be linked with hypertension.

### Hypertension and body composition

The relationship between obesity and hypertension is well established both in adults and children (74,75). Obese individuals exhibit higher levels of office as well as ambulatory BP from childhood to old age. Obese subjects display higher BP levels than non-obese individuals even in the normotensive range. Pathophysiological differences exist in obese and non-obese patients with essential hypertension. Normal weight essential hypertensives exhibit increased arterial stiffness and systemic vascular resistance (76), whereas obese hypertensives are characterised by increased cardiac output and total plasma volume (77).

There is evidence suggesting increased sympathetic nervous system activity in obese individuals when compared to lean individuals with a similar BP, suggesting a direct relationship between obesity and elevated sympathetic nervous activity (78). Although sympathetic nervous activity has been shown to be elevated in both hypertension and obesity, the mode of sympathetic excitation in normal-weight essential hypertension differed markedly from that in obesity-related hypertension (79).

Furthermore, arterial-pressure control mechanism of diuresis and natriuresis, according to the principle of infinite feedback gain, seems to be shifted toward higher BP levels in obese individuals (80). Obese individuals have increased sodium retention, extracellular fluid and plasma volumes (81). The volume overload in obese individuals is due to increased renal tubular reabsorption in the early stages prior to development of glomerular injury and loss of nephron function (81).

Studies of body composition in patients with obesity and hypertension elucidate that fat mass, as opposed to body mass, per se, may be an important aetiological component in elevated BP in adults (82). In a longitudinal cohort study, where patients were classified into different groups based on their baseline BP, subjects were followed up for 10 years (83). The results show that an increase in body weight and fat mass was a risk factor for the development of sustained hypertension, whereas a decrease was predictive of a decrease in BP.

Bioimpedance spectroscopy is a simple and effective approach for the assessment of body composition and fluid status (84,85). The accuracy of fluid status and body composition measurements has been validated against available gold standard reference methods (86,87), and this technique has been used to monitor patients with ESRF receiving haemodialysis (88,89) or peritoneal dialysis (90,91).

### Genetic polymorphisms in TRH

Large scale studies have been carried out to identify association of genetic polymorphisms with hypertension (92,93). Similar studies have been conducted to discover any link between genetic polymorphisms and responses to antihypertensive drugs (94,95). Recently, relatively large studies have also been trying to establish an attributable genetic component specifically to TRH (96,97).

Genetics of Hypertension Associated Treatment (GenHAT) study utilises the clinical data collected by the Antihypertensive and Lipid lowering Treatment to Prevent Heart Attack Trial (ALLHAT) to assess association of 78 candidate genetic polymorphisms implicated in the development of hypertension and CVD (96). The findings of this study show that two genetic variants in the angiotensinogen (AGT) gene (rs699, rs5051) were significantly associated with TRH among white participants when compared to treatment responsive controls.

Similarly, another genetic study utilised data collected as part of INVEST-GENES (the INnternational VErapamil-SR Trandolapril STudy—GENEtic Substudy) and WISE (Women's Ischemia Syndrome Evaluation) to show that the *ATP2B1 rs12817819 A* allele is associated with increased risk for TRH in hypertensive participants with documented CAD or suspected ischemic heart disease (97). The ATP2B1 gene encodes the plasma membrane calcium ATPase and

function to transport intracellular calcium ions against very large concentration gradients and play a critical role in intracellular calcium homeostasis. Since calcium is involved in smooth muscle and cardiac contraction, a disequilibrium in calcium concentration may predispose to TRH.

# Study Design

## Overview

This study proposes to comprehensively characterise a group of hypertensive subjects referred to a tertiary hypertension clinic for uncontrolled blood pressure, with the aim of understanding factors associated with true treatment resistance, once secondary causes, non-compliance and under-treatment have been excluded as the cause of treatment failure. A detailed phenotypic analysis will be performed, with the aim of identifying markers that may have a causal role in resistance to treatment.

In this study, all patients presenting to West Midlands Hypertension Centre (WMHC) who meet the eligibility criteria will be approached. Patients who provide a written informed consent will undergo various tests. Upon completion of recruitment, patients will be stratified into different groups based upon their blood pressure control and presence or absence of true TRH. The results will then be compared to assess whether any pathophysiological differences exist in these groups. These groups will include:

**True treatment resistant hypertension** – patients with average daytime ambulatory systolic BP ≥135 mmHg, secondary causes of hypertension excluded and, adherence conformed on urine antihypertensive screen

**Uncontrolled BP due to non-adherence** – patients with average daytime ambulatory systolic BP ≥135mmHg and non-adherence confirmed on urine antihypertensive screen

**Controlled hypertension** – patients with average daytime ambulatory systolic BP <135 mmHg.

**Study aim:** To comprehensively characterise a group of hypertensive subjects referred to a tertiary hypertension clinic for uncontrolled blood pressure.

**Study objectives:**

We propose to investigate whether several factors will associate with treatment resistance.

**Primary objective** is to assess association with vascular markers including arterial stiffness and endothelial function

**Secondary objectives**

i. Assess association between body composition and hypertension as measured using bioimpedance spectroscopy
ii. Evaluate level of sympathetic nervous system activity in the study patients
iii. Assess prevalence of undiagnosed obstructive sleep apnoea in the study population
iv. Explore patients' personal views about medicines prescribed for hypertension and medicines in general.
v. Describe the risk of developing complications associated with hypertension in this cohort. These will include ischaemic heart disease, heart failure, stroke, renal failure and death.

**Study population:** Potentially eligible patients will be screened and recruited from the hypertension clinic based at the Heartlands Hospital. Only patients who meet the selection criteria will be approached to gain informed consent prior to carrying out the baseline assessments.

## Selection criteria

Participants shall be selected based on the following inclusion and exclusion criteria. An answer of "NO" to any inclusion criterion disqualifies a participant from further screening and from participation in the study.

**Inclusion:**

1. Individual is aged ≥18 and ≤80 years of age.
2. Individual agrees to all aspects of the study including the tests involved, and gives an informed signed consent.

**Exclusion:**

1. Individual has confirmed secondary hypertension including, renal artery stenosis, phaeochromocytoma, primary hyperaldosteronism, Cushing's disease or obstructive sleep apnoea.
2. Individual has an estimated glomerular filtration rate (MDRD equation) <30ml/min.

## Study methods

### Subject recruitment

All prospective, consecutive new patients presenting at the hypertension clinic will be approached to take part in this study. All new patients undergo 24-hour ambulatory blood pressure monitoring as a routine to rule out white coat hypertension and confirm diagnosis of hypertension. Any patients found to have uncontrolled BP on ≥3 antihypertensives have a routine urine antihypertensive screen to confirm adherence before secondary causes of hypertension are systemically excluded. All patients will be included to allow comparison of patients with TRH to be made with patients varying degree of severity of hypertension.

### Informed consent

Once an individual has been identified as suitable for inclusion as described above in the selection criteria, the individual can than then be approached and given relevant information about the study to make an informed decision about participation in the study. The individual will be given adequate time to discuss participation with family/friends with opportunity provided to answer any questions they may have before confirming their consent.

If after understanding and evaluating the purpose, the potential benefits and harms, requirements of the study, as well as their rights as a research participant, the individual agrees to take part, a written informed consent will be obtained which will be documented in the patient's medical notes as well.

### Study visit

Once consented, subject(s) will be invited to the study visit. At this visit, study-specific history, examination and investigations will be carried out to evaluate possible factors associated with TRH. These will include:

1) **Medical history:** to document duration of hypertension, medication history and past medical history.
2) **Physical exam:** to check and record patient's clinic BP, height, weight and, waist and hip circumferences.
3) **Laboratory testing:**
    a. **Blood test – Serum creatinine,** to calculate eGFR a factor of baseline kidney function. eGFR should be calculated using the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formulae.
    b. **Blood test – Haemoglobin A1c (HbA1c),** to evaluate glucose control.
    c. **Blood test – high sensitivity C-reactive protein (hs-CRP) and serum Free Light Chain Assays (FLC),** to evaluate a subject's inflammatory status
    d. **Blood test – vitamin D, B-type Natriuretic Peptide (BNP) lipid panel (HDL, LDL, triglycerides), and high sensitivity troponin,** to evaluate a subject's cardiovascular risk profile.
    e. **Blood test – TSH and calcium –** to complete a secondary screen
    f. **Blood test – renin and aldosterone,** to exclude hyperaldosteronism.

g. **Blood test – Angiogenic factors: soluble fms-like tyrosine kinase-1 (sFLT-1), intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), endothelin 1, and endoglin,** to assess endothelial dysfunction.
h. **Blood test – genetic analysis,** to identify any polymorphisms associated with TRH
i. **Spot urine albumin and creatinine,** to evaluate the albumin to creatinine ratio (uACR) as a factor of baseline kidney function.
j. **Spot urine antihypertensive screen,** to assess adherence to prescribed antihypertensives.
k. **24-hour urinary sodium,** to evaluate a subject's salt intake.
l. **Serum catecholamines and 24-hour urinary catecholamines and metadrenalines,** to assess sympathetic activity.

4) **12-lead ECG,** to assess cardiac rhythm and left ventricular hypertrophy
5) **Overnight pulse oximetry,** to evaluate presence of obstructive sleep apnoea (OSA).
6) **Arterial stiffness,** to determine the degree of arterial stiffness present in the study subjects. It will be measured as pulse wave velocity (PWV) using a Vicorder which uses automated cuffs around the arm and mid-thigh and a senor on the neck to obtain the aortic PWV.
7) **Body composition monitoring (BCM),** to determine a subject's fluid status in terms of total body water and its distribution in intracellular and extracellular compartments; and to determine a subject's lean and adipose tissue mass. Measurements will be performed using a portable whole-body bioimpedance spectroscopy device. The BCM measures the electrical responses at 50 different frequencies between 5 and 1000 kHz. Input variables include the patient's height, weight, age, and sex. Electrodes attached to the hand and foot on the non-dominant side of the body.
8) **Flow mediated dilatation (FMD),** to determine endothelial dysfunction present in the subjects. An acute rise in blood flow in the brachial artery will be induced following a circulatory arrest through application of sphygmomanometer cuff placed around the subject's arm for five minutes. Diameter of the brachial artery is measured using ultrasound before and after application of dilated cuff placed around the arm (48).
9) **Epworth Sleepiness Scale:** to assess likelihood of underlying OSA
10) **Beliefs about Medicines Questionnaire:** to assess patients' beliefs and attitudes towards their medications and health in general.

### Follow-up:

The follow-up will involve long-term data collection over a period of 5 years from GP, hospital and government databases to allow us to compare the natural course of TRH. Patients' clinical information will be gathered as detailed below. Informed consent will be obtained from the patients to give us their permission to access their medical records. The specific methods used to gather this information are described and currently being implemented in another on-going research study (Acute Care QUAlity in chronic Kidney Disease) at HEFT (98). The databases accessed will include:

- Interface with primary care database to record clinic BP, BP medications, bloods (eGFR) and urine (ACR) in addition to record any secondary complications of hypertension.
- Interface with secondary care databases to record hypertension-associated morbidity
- Interface with Office of National Statistics database to record hypertension-associated mortality

## Schedule of testing (both groups):

| Factor being assessed | Baseline visit | GP, HES and ONS Follow-up |
|---|---|---|
| **History** | | |
| Duration of hypertension | x | |
| Drug History | x | x |
| Co-morbidities | x | x |
| **Examination** | | |
| Clinic BP (BPTru) | x | x |
| BMI | x | |
| Waist and hip circumference | x | |
| **Blood Tests** | | |
| U&Es, inc eGFR (MDRD & CKD-EPI) | x | x |
| HbA1c, and lipids | x | |
| hsCRP | x | |
| Serum free light chain assay | x | |
| hsTropI & pro-BNP | x | |
| Vitamin D, calcium and TSH | x | |
| Renin and aldosterone | x | |
| Angiogenic markers | x | |
| Serum catecholamines | x | |
| Genetic testing | x | |
| **Urine Tests** | | |
| Albumin-Creatinine Ratio (ACR) | x | x |
| Antihypertensive screen for adherence | x | |
| 24 hr urinary sodium | x | |
| 24 hr urinary catecholamines & metadrenalines | x | |
| **Non-Invasive Investigations** | | |
| ECG | x | |
| Pulse Wave Velocity using Vicorder | x | |
| Body composition monitoring | x | |
| Flow mediated dilatation or EndoPat | x | |
| Overnight pulse oximetry | x | |
| **Questionnaires** | | |
| Epworth Sleepiness Scale | x | |
| Beliefs about Medicines Questionnaire | x | |
| **Risk Scores** | | |
| QRisk2 | x | |

***Table 1: Schedule of testing***

## Data management

### Subject numbers

All eligible patients attending the WMHC will be approached. Recruitment will last a total of 1 year. No formal sample size calculation was performed. The aim of this observational study is to characterise patients with TRH and there is limited data available to perform formal sample size calculation. Instead our estimate is based on the census of all patients recorded in the hypertension database who attended the clinic in 2015.

In 2015 a total of 282 patients were seen in the hypertension clinic at Heartlands Hospital. Of these 118 (42%) met the definition of TRH with clinic BP ≥140/90 mmHg and 3 antihypertensive medications. It was not possible to determine what percentage of these had true TRH after excluding white –coat effect, non-adherence and secondary hypertension. However, from previous published evidence it is likely to be between 50-60%, so in our population estimated 60-70 patients are likely to be truly resistant. The numbers indicated is a conservative estimate due to missing data as a small proportion of patients were not recorded in the hypertension database from which this census was performed.

We therefore expect to recruit 130 -140 patients taking into account patient choice and the selection criteria outlined above.

### Statistical analysis:

- Assess correlation of primary and secondary outcomes with treatment-resistant hypertension.
- Compare the three groups of patients to assess any significant differences between them
- Compare long term morbidity and mortality in the groups of patients.

Normally distributed continuous data will be presented as mean±SD. Shapiro-Wilk test will be carried out to test for normal distribution. Non-normally distributed data will be presented as mean (inter-quartile range). Categorical data will be presented as count (percentage) and analysed using $\chi^2$ test. Comparison of the means will be made using *t*-test if the means are normally distributed, otherwise non-parametric tests such as Mann-Whitney U test. Furthermore, multivariate linear regression analysis will be performed to adjust for any known covariables which may have a potential confounding effect.

## References

(1) Murray CJL, Lopez AD. Measuring the Global Burden of Disease. New England Journal of Medicine 2013;369(5):448-457.

(2) World Health Organisation. A global brief on hypertension: Silent killer, global public health crisis. Geneva: World Health Organisation; 2013.

(3) Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. The Lancet 2012;380(9859):2224-2260.

(4) National Institute for Health and Clinical Excellence. Hypertension: clinical management of primary hypertension in adults. CG 127 ed. London: National Institute for Health and Clinical Excellence; 2011.

(5) Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Journal of Hypertension 2013;31(7):1281-1357.

(6) Turnbull F, Neal B, Ninomiya T, Algert C, Arima H, Barzi F, et al. Effects of different regimens to lower blood pressure on major cardiovascular events in older and younger adults: meta-analysis of randomised trials. BMJ 2008;336(7653):1121-1123.

(7) Lewington S, Clarke R, Qizilbash N, Peto R, Collins R, Prospective Studies Collaboration. 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 Dec 14;360(9349):1903-1913.

(8) Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR, et al. Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney Int 2003 Jan;63(1):225-232.

(9) Daugherty SL, Powers JD, Magid DJ, Tavel HM, Masoudi FA, Margolis KL, et al. Incidence and Prognosis of Resistant Hypertension in Hypertensive Patients. Circulation 2012;125(13):1635-1642.

(10) de la Sierra A, Segura J, Banegas JR, Gorostidi M, de la Cruz JJ, Armario P, et al. Clinical Features of 8295 Patients With Resistant Hypertension Classified on the Basis of Ambulatory Blood Pressure Monitoring. Hypertension 2011;57(5):898-902.

(11) Persell SD. Prevalence of Resistant Hypertension in the United States, 2003–2008. Hypertension 2011;57(6):1076-1080.

(12) Egan BM, Zhao Y, Axon RN, Brzezinski WA, Ferdinand KC. Uncontrolled and Apparent Treatment Resistant Hypertension in the United States, 1988 to 2008. Circulation 2011;124(9):1046-1058.

(13) Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, et al. Heart Disease and Stroke Statistics—2011 Update: A Report From the American Heart Association. Circulation 2011;123(4):e18-e209.

(14) Roberie DR, Elliott WJ. What is the prevalence of resistant hypertension in the United States? Curr Opin Cardiol 2012 Jul;27(4):386-391.

(15) Judd E, Calhoun DA. Apparent and true resistant hypertension: definition, prevalence and outcomes. J Hum Hypertens 2014 Aug;28(8):463-468.

(16) Hameed MA, Tebbit L, Jacques N, Thomas M, Dasgupta I. Non-adherence to antihypertensive medication is very common among resistant hypertensives: results of a directly observed therapy clinic. J Hum Hypertens 2015.

(17) 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. Hypertension 2008 June 01;51(6):1403-1419.

(18) Sarafidis PA, Bakris GL. Resistant Hypertension. J Am Coll Cardiol 2008;52(22):1749 1757.

(19) Williams B, MacDonald TM, Morant S, Webb DJ, Sever P, McInnes G, et al. Spironolactone versus placebo, bisoprolol, and doxazosin to determine the optimal treatment for drug-resistant hypertension (PATHWAY-2): a randomised, double-blind, crossover trial. The Lancet 2015.

(20) Schlaich MP, Lambert E, Kaye DM, Krozowski Z, Campbell DJ, Lambert G, et al. Sympathetic Augmentation in Hypertension: Role of Nerve Firing, Norepinephrine Reuptake, and Angiotensin Neuromodulation. Hypertension 2004 February 01;43(2):169-175.

(21) Schlaich MP, Kaye DM, Lambert E, Sommerville M, Socratous F, Esler MD. Relation Between Cardiac Sympathetic Activity and Hypertensive Left Ventricular Hypertrophy. Circulation 2003 August 05;108(5):560-565.

(22) Burns J, Sivananthan MU, Ball SG, Mackintosh AF, Mary DASG, Greenwood JP. Relationship Between Central Sympathetic Drive and Magnetic Resonance Imaging–Determined Left Ventricular Mass in Essential Hypertension. Circulation 2007 April 17;115(15):1999-2005.

(23) Kaye DM, Lefkovits J, Jennings GL, Bergin P, Broughton A, Esler MD. Adverse consequences of high sympathetic nervous activity in the failing human heart. J Am Coll Cardiol 1995 11/1;26(5):1257-1263.

(24) Zoccali C, Mallamaci F, Parlongo S, Cutrupi S, Benedetto FA, Tripepi G, et al. Plasma Norepinephrine Predicts Survival and Incident Cardiovascular Events in Patients With End-Stage Renal Disease. Circulation 2002 March 19;105(11):1354-1359.

(25) Schlaich MP, Socratous F, Hennebry S, Eikelis N, Lambert EA, Straznicky N, et al. Sympathetic Activation in Chronic Renal Failure. Journal of the American Society of Nephrology 2009 May 01;20(5):933-939.

(26) Grassi G, Quarti-Trevano F, Seravalle G, Arenare F, Volpe M, Furiani S, et al. Early Sympathetic Activation in the Initial Clinical Stages of Chronic Renal Failure. Hypertension 2011 April 01;57(4):846-851.

(27) Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of Cardiovascular Events and All-Cause Mortality With Arterial Stiffness: A Systematic Review and Meta-Analysis. J Am Coll Cardiol 2010 3/30;55(13):1318-1327.

(28) Tomlinson LA. Methods for assessing arterial stiffness: technical considerations. Curr Opin Nephrol Hypertens 2012 Nov;21(6):655-660.

(29) Darne B, Girerd X, Safar M, Cambien F, Guize L. Pulsatile versus steady component of blood pressure: a cross-sectional analysis and a prospective analysis on cardiovascular mortality. Hypertension 1989 April 01;13(4):392-400.

(30) Girerd X, Laurent S, Pannier B, Asmar R, Safar M. Arterial distensibility and left ventricular hypertrophy in patients with sustained essential hypertension. Am Heart J 1991 10;122(4, Part 2):1210-1214.

(31) Nichols WW, O'Rourke M. McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles. 3rd ed. London, England: Oxford University Press; 1990:77–142,216–269, 283–359,398–37.; 1990. p. 77–142, 216–269, 283–359, 398–37.

(32) Watanabe H, Ohtsuka S, Kakihana M, Sugishita Y. Coronary circulation in dogs with an experimental decrease in aortic compliance. J Am Coll Cardiol 1993 5;21(6):1497-1506.

(33) Davies JE, Whinnett ZI, Francis DP, Manisty CH, Aguado-Sierra J, Willson K, et al. Evidence of a Dominant Backward-Propagating "Suction" Wave Responsible for Diastolic Coronary Filling in Humans, Attenuated in Left Ventricular Hypertrophy. Circulation 2006 April 11;113(14):1768-1778.

(34) Blacher J, Asmar R, Djane S, London GM, Safar ME. Aortic Pulse Wave Velocity as a Marker of Cardiovascular Risk in Hypertensive Patients. Hypertension 1999 May 01;33(5):1111-1117.

(35) Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, et al. Aortic Stiffness Is an Independent Predictor of All-Cause and Cardiovascular Mortality in Hypertensive Patients. Hypertension 2001 May 01;37(5):1236-1241.

(36) Pabuccu T, Baris N, Ozpelit E, Akdeniz B, Guneri S. The Relationship between Resistant Hypertension and Arterial Stiffness. Clin Exp Hypertens 2012 02/01; 2015/05;34(1):57-62.

(37) Chung CM, Cheng HW, Chang JJ, Lin YS, Hsiao JF, Chang ST, et al. Relationship between resistant hypertension and arterial stiffness assessed by brachial-ankle pulse wave velocity in the older patient. Clin Interv Aging 2014 Sep 5;9:1495-1502.

(38) Pickering TG. Arterial Stiffness as a Cause of Resistant Hypertension? The Journal of Clinical Hypertension 2007;9(5):390-395.

(39) Panza JA, Quyyumi AA, Brush JE, Epstein SE. Abnormal Endothelium-Dependent Vascular Relaxation in Patients with Essential Hypertension. N Engl J Med 1990 07/05; 2015/01;323(1):22-27.

(40) Treasure CB, Manoukian SV, Klein JL, Vita JA, Nabel EG, Renwick GH, et al. Epicardial coronary artery responses to acetylcholine are impaired in hypertensive patients. Circulation Research 1992 October 01;71(4):776-781.

(41) Panza JA, Casino PR, Kilcoyne CM, Quyyumi AA. Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. Circulation 1993 May 01;87(5):1468-1474.

(42) Panza JA, García CE, Kilcoyne CM, Quyyumi AA, Cannon RO. Impaired Endothelium-Dependent Vasodilation in Patients With Essential Hypertension: Evidence That Nitric Oxide Abnormality Is Not Localized to a Single Signal Transduction Pathway. Circulation 1995 March 15;91(6):1732-1738.

(43) Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Lerman A. Long-Term Follow-Up of Patients With Mild Coronary Artery Disease and Endothelial Dysfunction. Circulation 2000 March 07;101(9):948-954.

(44) Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF, et al. Clinical Correlates and Heritability of Flow-Mediated Dilation in the Community: The Framingham Heart Study. Circulation 2004 February 10;109(5):613-619.

(45) Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992 Nov 7;340(8828):1111-1115.

(46) Celermajer DS. Endothelial Dysfunction: Does It Matter? Is It Reversible? J Am Coll Cardiol 1997 8;30(2):325-333.

(47) Green D. Point: Flow-mediated dilation does reflect nitric oxide-mediated endothelial function. J Appl Physiol 2005;99(3):1233 1234.

(48) Harris RA, Nishiyama SK, Wray DW, Richardson RS. Ultrasound Assessment of Flow-Mediated Dilation. Hypertension 2010 May 01;55(5):1075-1085.

(49) Mulvihill N. Prediction of cardiovascular risk using soluble cell adhesion molecules. Eur Heart J 2002;23(20):1569-1574.

(50) Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev 1997 Feb;18(1):4-25.

(51) Belgore FM, Blann AD, Li-Saw-Hee FL, Beevers DG, Lip GY. Plasma levels of vascular endothelial growth factor and its soluble receptor (SFlt-1) in essential hypertension. Am J Cardiol 2001 Mar 15;87(6):805-7, A9.

(52) Bates DO, Hillman NJ, Williams B, Neal CR, Pocock TM. Regulation of microvascular permeability by vascular endothelial growth factors*. J Anat 2002;200(6):581-597.

(53) Figueiredo VN, Yugar-Toledo JC, Martins LC, Martins LB, de Faria AP, de Haro Moraes C, et al. Vascular stiffness and endothelial dysfunction: Correlations at different levels of blood pressure. Blood Press 2012 Feb;21(1):31-38.

(54) de Faria APC, Fontana V, Modolo R, Barbaro NR, Sabbatini AR, Pansani IF, et al. Plasma 8-isoprostane levels are associated with endothelial dysfunction in resistant hypertension. Clinica Chimica Acta 2014 6/10;433(0):179-183.

(55) Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. Biomed Res Int 2014;2014:406960.

(56) Stumpf C, Jukic J, Yilmaz A, Raaz D, Schmieder RE, Daniel WG, et al. Elevated VEGF-plasma levels in young patients with mild essential hypertension. Eur J Clin Invest 2009;39(1):31-36.

(57) Bautista LE, Atwood JE, O'Malley PG, Taylor AJ. Association between C-reactive protein and hypertension in healthy middle-aged men and women. Coron Artery Dis 2004 Sep;15(6):331-336.

(58) Blake GJ, Rifai N, Buring JE, Ridker PM. Blood Pressure, C-Reactive Protein, and Risk of Future Cardiovascular Events. Circulation 2003 December 16;108(24):2993-2999.

(59) Stuveling EM, Bakker SJL, Hillege HL, Burgerhof JGM, de Jong PE, Gans ROB, et al. C-Reactive Protein Modifies the Relationship Between Blood Pressure and Microalbuminuria. Hypertension 2004 April 01;43(4):791-796.

(60) Chul Sung K, Suh JY, Kim BS, Kang JH, Kim H, Lee MH, et al. High sensitivity c-reactive protein as an independent risk factor for essential hypertension. American Journal of Hypertension 2003 June 01;16(6):429-433.

(61) Xu G, Baines R, Westacott R, Selby N, Carr S. An educational approach to improve outcomes in acute kidney injury (AKI): report of a quality improvement project. BMJ Open 2014 March 01;4(3).

(62) Ballou SP, Lozanski G. Induction of inflammatory cytokine release from cultured human monocytes by C-reactive protein. Cytokine 1992;4(5):361-368.

(63) Pasceri V, Willerson JT, Yeh ETH. Direct Proinflammatory Effect of C-Reactive Protein on Human Endothelial Cells. Circulation 2000 October 31;102(18):2165-2168.

(64) Gudbrandsson T, Herlitz H, Hansson L, Lindholm L, Åke Nilsson L. IMMUNOLOGICAL CHANGES IN PATIENTS WITH PREVIOUS MALIGNANT ESSENTIAL HYPERTENSION. The Lancet 1981 2/21;317(8217):406-408.

(65) Ebringer A, Doyle AE. Raised serum IgG levels in hypertension. Br Med J 1970 Apr 18;2(5702):146-148.

(66) Kristensen Bø. Increased Serum Levels of Immunoglobulins in Untreated and Treated Essential Hypertension. Acta Med Scand 1978;203(1-6):49-54.

(67) Olsen F, Hilden M, Ibsen H. Raised levels of immunoglobulins in serum of hypertensive patients. Acta Pathol Microbiol Scand B Microbiol Immunol 1973;81(6):775-778.

(68) Kristensen BØ, Sølling K. Serum Concentrations of Immunoglobulins and Free Light Chains before and after Vascular Events in Essential Hypertension. Acta Med Scand 1983;213(1):15-20.

(69) Brebner JA, Stockley RA. Polyclonal free light chains: a biomarker of inflammatory disease or treatment target? F1000 Med Rep 2013;5(4).

(70) Anandram S, Assi LK, Lovatt T, Parkes J, Taylor J, Macwhannell A, et al. Elevated, combined serum free light chain levels and increased mortality: a 5-year follow-up, UK study. Journal of Clinical Pathology 2012 November 01;65(11):1036-1042.

(71) Dispenzieri A, Katzmann JA, Kyle RA, Larson DR, Therneau TM, Colby CL, et al. Use of Nonclonal Serum Immunoglobulin Free Light Chains to Predict Overall Survival in the General Population. Mayo Clin Proc 2012 6;87(6):517-523.

(72) Ritchie J, Assi LK, Burmeister A, Hoefield R, Cockwell P, Kalra PA. Association of Serum Ig Free Light Chains with Mortality and ESRD among Patients with Nondialysis-Dependent CKD. Clinical Journal of the American Society of Nephrology 2015 May 07;10(5):740-749.

(73) Shantsila E, Wrigley B, Lip GYH. Free Light Chains in Patients With Acute Heart Failure Secondary to Atherosclerotic Coronary Artery Disease. Am J Cardiol 2014;114(8):1243-1248.

(74) Kotsis V, Stabouli S, Bouldin M, Low A, Toumanidis S, Zakopoulos N. Impact of Obesity on 24-Hour Ambulatory Blood Pressure and Hypertension. Hypertension 2005 April 01;45(4):602-607.

(75) Stabouli S, Kotsis V, Papamichael C, Constantopoulos A, Zakopoulos N. Adolescent Obesity is Associated with High Ambulatory Blood Pressure and Increased Carotid Intimal-Medial Thickness. J Pediatr 2005 11;147(5):651-656.

(76) Weber MA, Neutel JM, Smith DHG. Contrasting clinical properties and exercise responses in obese and lean hypertensive patients. J Am Coll Cardiol 2001 1;37(1):169-174.

(77) Licata G, Scaglione R, Capuana G, Parrinello G, Di Vincenzo D, Mazzola G. Hypertension in obese subjects: distinct hypertensive subgroup. J Hum Hypertens 1990 Feb;4(1):37-41.

(78) Grassi G, Seravalle G, Cattaneo BM, Bolla GB, Lanfranchi A, Colombo M, et al. Sympathetic Activation in Obese Normotensive Subjects. Hypertension 1995 April 01;25(4):560-563.

(79) Lambert E, Straznicky N, Schlaich M, Esler M, Dawood T, Hotchkin E, et al. Differing Pattern of Sympathoexcitation in Normal-Weight and Obesity-Related Hypertension. Hypertension 2007 November 01;50(5):862-868.

(80) Kotsis V, Stabouli S, Papakatsika S, Rizos Z, Parati G. Mechanisms of obesity-induced hypertension. Hypertension Research 2010;33(5):386 393.

(81) Hall JE, Brands MW, Hildebrandt DA, Kuo J, Fitzgerald S. Role of sympathetic nervous system and neuropeptides in obesity hypertension. Brazilian Journal of Medical and Biological Research 2000;33(6).

(82) Siervogel RM, Roche AF, Chumlea WC, Morris JG, Webb P, Knittle JL. Blood pressure, body composition, and fat tissue cellularity in adults. Hypertension 1982 May 01;4(3):382-386.

(83) Markus MRP, Stritzke J, Siewert U, Lieb W, Luchner A, Döring A, et al. Variation in Body Composition Determines Long-Term Blood Pressure Changes in Pre-Hypertension: The MONICA/KORA (Monitoring Trends and Determinants on Cardiovascular Diseases/Cooperative Research in the Region of Augsburg) Cohort Study. J Am Coll Cardiol 2010 6/29;56(1):65-76.

(84) Matthie JR. Bioimpedance measurements of human body composition: critical analysis and outlook. Expert Rev Med Devices 2008 Mar;5(2):239-261.

(85) Jaffrin MY, Morel H. Body fluid volumes measurements by impedance: A review of bioimpedance spectroscopy (BIS) and bioimpedance analysis (BIA) methods. Med Eng Phys 2008 12;30(10):1257-1269.

(86) Moissl UM, Wabel P, Chamney PW, Bosaeus I, Levin NW, Bosy-Westphal A, et al. Body fluid volume determination via body composition spectroscopy in health and disease. Physiol Meas 2006;27(9):921-933.

(87) Wabel P, Chamney P, Moissl U, Jirka T. Importance of whole-body bioimpedance spectroscopy for the management of fluid balance. Blood Purif 2009;27(1):75-80.

(88) Wizemann V, Wabel P, Chamney P, Zaluska W, Moissl U, Rode C, et al. The mortality risk of overhydration in haemodialysis patients. Nephrology Dialysis Transplantation 2009 May 01;24(5):1574-1579.

(89) Machek P, Jirka T, Moissl U, Chamney P, Wabel P. Guided optimization of fluid status in haemodialysis patients. Nephrology Dialysis Transplantation 2010 February 01;25(2):538-544.

(90) Devolder I, Verleysen A, Vijt D, Vanholder R, Van Biesen W. Body composition, hydration, and related parameters in hemodialysis versus peritoneal dialysis patients. Peritoneal Dialysis International 2010 March 01;30(2):208-214.

(91) Van Biesen W, Williams JD, Covic AC, Fan S, Claes K, Lichodziejewska-Niemierko M, et al. Fluid status in peritoneal dialysis patients: the European Body Composition Monitoring (EuroBCM) study cohort. PLoS One 2011 Feb 24;6(2):e17148.

(92) Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, et al. Genome-wide association study of blood pressure and hypertension. Nat Genet 2009 Jun;41(6):677-687.

(93) Ganesh SK, Tragante V, Guo W, Guo Y, Lanktree MB, Smith EN, et al. Loci influencing blood pressure identified using a cardiovascular gene-centric array. Human Molecular Genetics 2013 April 15;22(8):1663-1678.

(94) Gong Y, McDonough CW, Wang Z, Hou W, Cooper-DeHoff RM, Langaee TY, et al. Hypertension Susceptibility Loci and Blood Pressure Response to Antihypertensives - Results from the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) Study. Circulation: Cardiovascular Genetics 2012 October 19.

(95) Silva PS, Fontana V, Luizon MR, Lacchini R, Silva WA,Jr, Biagi C, et al. eNOS and BDKRB2 genotypes affect the antihypertensive responses to enalapril. Eur J Clin Pharmacol 2013 Feb;69(2):167-177.

(96) Lynch AI, Irvin MR, Davis BR, Ford CE, Eckfeldt JH, Arnett DK. Genetic and Adverse Health Outcome Associations with Treatment Resistant Hypertension in GenHAT. Int J Hypertens 2013;2013:578578.

(97) Fontana V, McDonough CW, Gong Y, El Rouby NM, Sá ACC, Taylor KD, et al. Large-Scale Gene-Centric Analysis Identifies Polymorphisms for Resistant Hypertension. Journal of the American Heart Association 2014 December 19;3(6).

(98) Arnold JJ, Hayer M, Sharif A, Begaj I, Tabriez M, Bagnall D, et al. Acute Care QUAliTy in chronic Kidney disease (ACQUATIK): a prospective cohort study exploring outcomes of patients with chronic kidney disease. BMJ Open 2015 May 01;5(4).

**Appendix V:** Patient Information Sheet and Consent Form for FACT-RHY

Birmingham Heartlands Hospital

Bordesley Green East
Birmingham B9 5SS

Tel: 0121 424 2000
Fax: 0121 424 2200

# Patient Information Sheet

## A study of Factors AssoCiated with true Treatment-Resistant HYpertension (FACT-RHY)

### Introduction

We invite you to take part in our research study. Before you decide, it is important for you to understand why this research is being done and what it will involve for you. Please read the following information carefully. Talk to others about the study if you wish and do not hesitate to ask us if something is unclear.

### What is the purpose of the study?

In this study, we are aiming to understand why some patients' blood pressure (BP) does not improve despite the use of three or more medications to lower their BP. Medications are the mainstay of treatment in patients with hypertension (high BP). Only about one-half of all patients treated are able to have their BP controlled to target levels with medications. Patients whose BP remains high despite taking at least three medications are diagnosed as having treatment-resistant hypertension. The risk of heart attack, kidney disease and stroke is even higher in these patients compared to patients with well controlled BP.

Therefore, this research is designed to compare patients whose BP is controlled against patients whose BP remains uncontrolled to understand the differences between the groups. By analysing answers, measurements and samples collected from people who participate in this study, we may be able to work out why some people with hypertension have uncontrolled BP and more long-term health problems than others. It will also allow us to assess what factors are important for worse health outcomes in people with treatment-resistant hypertension and how treatments can best be used to help improve outcomes.

**Why have I been invited?**

You have been invited to take part in this study because our records show that you are receiving treatment for hypertension and attend the hypertension clinic at Heartlands Hospital.

**Do I have to take part?**

It is up to you to decide whether or not to take part. You will be given as much time as you require to consider taking part, but you can of course take less time if you wish. If you decide to participate you will be asked to sign a Consent Form, but you are still free to change your mind and leave the study at any time without giving a reason. If you choose not to participate or to leave the study, your future medical treatment and normal standard of care will not be affected in any way.

**What will happen to me if I take part?**

If you decide to take part in this study, you will be asked to sign the study Consent Form. You will then be asked to attend the hospital on one morning where you will have to attend having fasted overnight. You will be seen by a doctor who will collect the following information and undergo the following tests:

- Contact and basic demographic information will be collected, including your ethnic group.
- Your medical and family history will be taken. Your full medication history will be confirmed including non-prescription and complimentary medicines.
- We will record your smoking status/history and current alcohol intake.
- A physical examination will be performed. The physical examination will include BP, heart rate, ECG, height, weight, body mass index (BMI), waist and hip circumference (using a standard tape measure).
- We will ask you to provide us with a urine sample during your visit.
- With your permission we will take a sample of blood from your arm.
- You will be given two short questionnaires to complete. The first will be about your sleeping patterns and second will ask about your health and medicines. These will not have you name on it, only your unique study number and you will be asked to complete it in a private room to maintain confidentiality. There will be help available to complete this form if needed.
- We will also take some measurements of the health of your blood vessels; these will be done using non-invasive methods which will be harmless. One of these tests will involve putting a blood pressure cuff around your forearm for 5 minutes which may cause slight discomfort whilst the cuff is inflated.
- Finally, we will provide you with simple to use device which will be used to record your oxygen levels when you sleep. The device has an oxygen sensor that fits on your finger and connects to small device worn on your wrist. You will be shown how to use it.

Your visit will take up to 2 hours in total. As you will have to attend for your visit having fasted overnight, we will provide you with a breakfast of toast / cereal and a hot/cold drink when your blood tests have been completed.

You will not be required to make any further visits to hospital for this research study. Instead the researchers will aim to follow your BP, blood and urine tests remotely by accessing this information kept in your medical records at your general practice or hospital. Any new medical illnesses you may develop and any visits to hospital related to high BP will be looked at as well. Furthermore, the researchers will also request the recorded cause of death from the records kept in the Office of National Statistics database. We propose to follow any patient who agrees for this study for a period of five years.

**What will happen to the blood sample I give?**

- Your blood sample will be used to carry out routine blood tests which will be reported on the hospital systems in the usual manner. If we find anything abnormal in your routine blood results, we will ensure that you and your GP are informed and organise further testing if required.
- In addition to routine blood tests we will obtain extra four teaspoons (20 mls) of blood to carry out non-routine blood tests including some tests which are purely for research purposes. These tests will be analysed in an anonymous manner.
- The blood collected will also be used to gain your DNA to carry out genetic testing. The genetic testing will help identify specific markers, which predict individual patients' likelihood of responding to particular antihypertensive (blood pressure lowering) drugs. Your DNA will be used to carry out specific tests related to hypertension only and therefore there will not be any unrelated genetic findings from this DNA. These tests will again be carried out in an anonymous manner to preserve your confidentiality. The results of these investigations are unlikely to have any implications for you personally.
- Your blood samples will be stored for the duration of the study. The blood samples will not have your name on them and will be labelled with your unique study number.
- If after carrying out all the tests required for this study if there is any blood sample left, we would also like to keep the left-over blood samples for:
    - Analysis of other markers related to high blood pressure that may become available in the future. Your samples will be stored without your personal information and will only be used in research which has been approved by an ethics committee.

**What are the possible benefits of taking part?**

We will provide you with all your results and explanations so you will have more information about your health and the results obtained will have substantial future benefits for the hypertension community. Your results can help educate and encourage you to make changes to your lifestyle. We will have more information about your health to allow us to be more responsive to your clinical needs and tailor your treatment more effectively. We will also have more information to carry out research on the factors contributing to treatment-resistant hypertension and the development and progression of hypertension-related diseases, allowing us to improve on the treatments available.

**What are the risks/inconveniences of taking part?**

The risks associated are minimal and include discomfort whilst having a blood test done the application of a blood pressure cuff on your forearm during testing of health of your arteries. Although most of these do not present any significant risks, all these tests will take up some of your time. We will try to minimise the time commitment so that we can schedule your visits at a time convenient to you and the study team wherever possible.

**Will my details be kept confidential if I take part in the study?**

All procedures for handling, processing, storage and destruction of data will be compliant with the Data Protection Act 1998.

Your clinical notes will only be accessed by doctors and nurses involved in your care; this is the same as if you were not in the study. Any blood or urine results will only be available with your study number on and any information you give in the questionnaire will also be treated confidentially with only your study number on it to identify you. We will only use information collected about you for the purposes of this research as described in the information sheet.

If you join the study, some parts of your medical records and the data collected for the study may be looked at by authorised persons from the organisation sponsoring and/or running the research. They may also be looked at by people from the regulatory authorities and by authorised people from the NHS to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.

**What will happen to the results of the research study?**

The results of the study will be available on the study website and will include reports intended for reading by lay-people. We will prepare a summary of the results for the lay people which we will distribute to all study participants. The results of the study will be analysed and published in medical publications and at conferences. You will not be named or identified in any of these publications.

**Will my GP be told that I am in a study?**

Yes, with your permission we will write to your GP to let them know.

**What if there is a problem during the study?**

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. If you have any concerns about any aspect of this study, you should speak to your study doctor or nurse who will do their best to answer your questions.

In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence, then you may have grounds for a legal action for compensation against the sponsor but you may have to pay your legal costs.

**What will happen if I don't want to carry on with the study?**

Your participation in this study is voluntary. You are free to come out of this study at any time without giving a reason and without affecting your future care or medical treatment by contacting the study co-ordinator on the number below. Any information we have already collected will continue to be used in the study. No further information will be collected.

**What if something goes wrong?**

Medical research is covered for mishaps in the same way as for patients undergoing treatment in the NHS i.e. compensation is only available if negligence occurs.

**Who has reviewed this study?**

All research in the NHS is looked at by an independent group of people called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable opinion by South East Scotland Research Ethics Committee.

**Contact names and telephone numbers for further information**

For any concerns or other questions about this study, or any questions about a study related injury please contact:

Dr Indranil Dasgupta

Tel: [redacted]

Dr Mohammed Awais Hameed (study co-ordinator)

Tel: [redacted]

**Thank you for taking the time to read this information sheet.**

**INFORMED CONSENT FORM**

**Patient Identification Number for this trial:**

**Title of study:** A study of Factors AssoCiated with true Treatment-Resistant HYpertension (FACT-RHY)

**Name of Researcher:** Dr. Indranil Dasgupta

**Please initial box**

1. I confirm that I have read and understand this information sheet version 1.5 27 March 2017 for the above study. I have had the opportunity to ask questions and received satisfactory answers and have received a copy of the information for my reference. I am over 18 years of age. ☐

2. I understand that my participation is voluntary and that I am free to withdraw without giving any reason, without my medical care or legal rights being affected. If I decide to withdraw, any data (information) collected up to the point I withdraw may be used in order to preserve the value of the study, however no further information will be collected. ☐

3. I understand that some parts of my medical records and data collected during the study may be looked at by authorised persons from regulatory authorities or from the NHS Trust where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records even if I withdraw and I understand that my records will only be reviewed for information related to my participation in the study. ☐

4. I understand that the researchers will access my medical records kept at my GP, hospital or Office of National Statistics. I understand that any data accessed will be stored in an anonymised manner. I give my permission for the researchers to have access to my medical records as described above. ☐

5. I agree to give a sample of blood for research in this project. I understand how the sample will be collected, that giving a sample for this research is voluntary and that I am free to withdraw my approval for use of the sample at any time without giving a reason and without my medical treatment or legal rights being affected. ☐

6. I understand that the project will use my DNA from the blood sample to carry out genetic research aimed at understanding the genetic markers the predict the likelihood of responding to particular antihypertensive (blood pressure lowering) drugs and that the results of these investigations are unlikely to have any implications for me personally. ☐

7. I understand that any leftover blood sample will be stored without my personal details and the researchers may use the blood sample for future research which has been approved by an ethics committee. ☐

8. I agree that my GP will be informed that I am taking part in this study. ☐

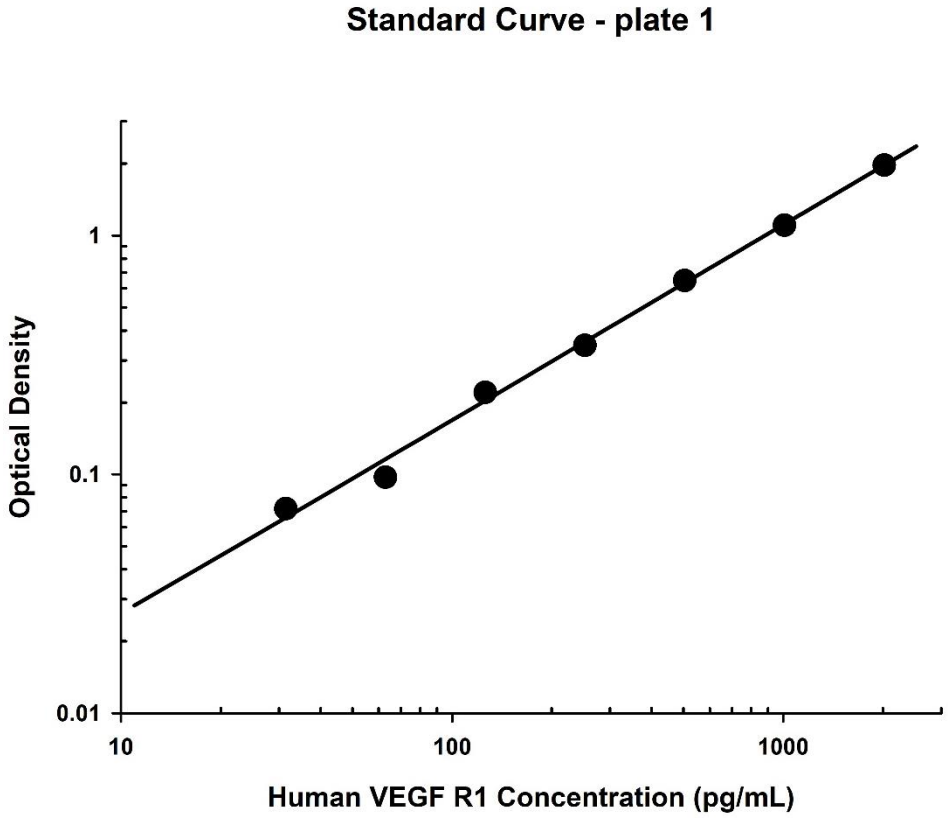
9. I agree that anonymised data collected during the study may be used by other researchers after the study has completed. They will not have access to any personal identifiable information about you. ☐

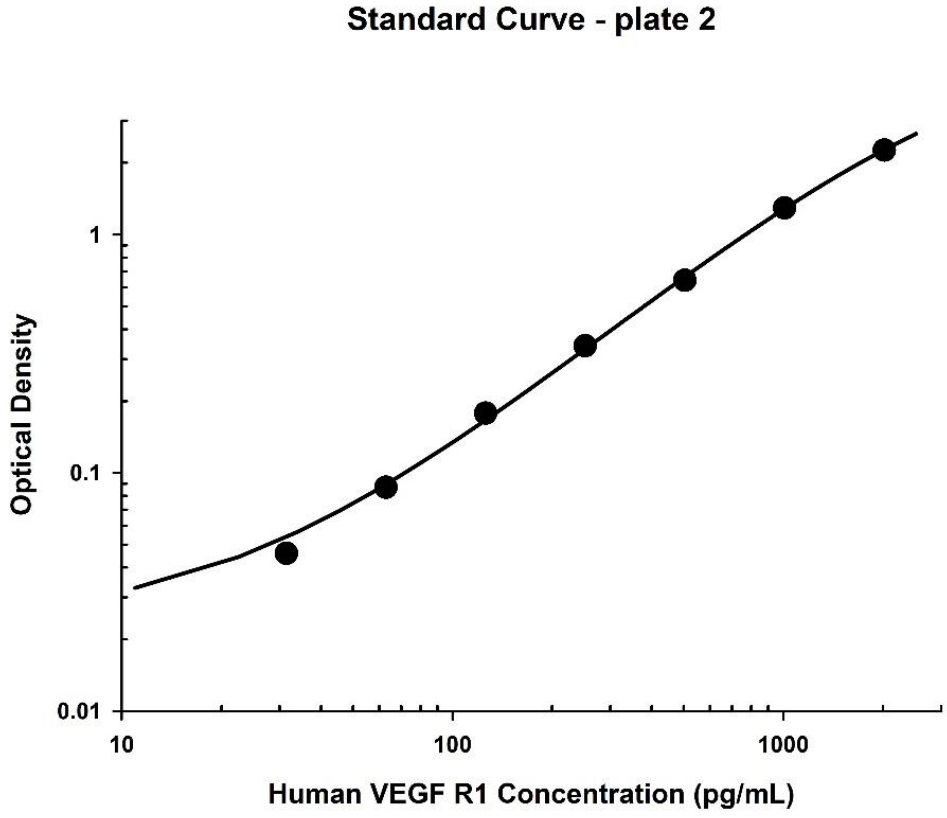
10. I agree to take part in this study. ☐

| _____ | ___/___/____ | _____ |
|---|---|---|
| Name of Participant | Date (DD/MMM/YYYY) | Signature |

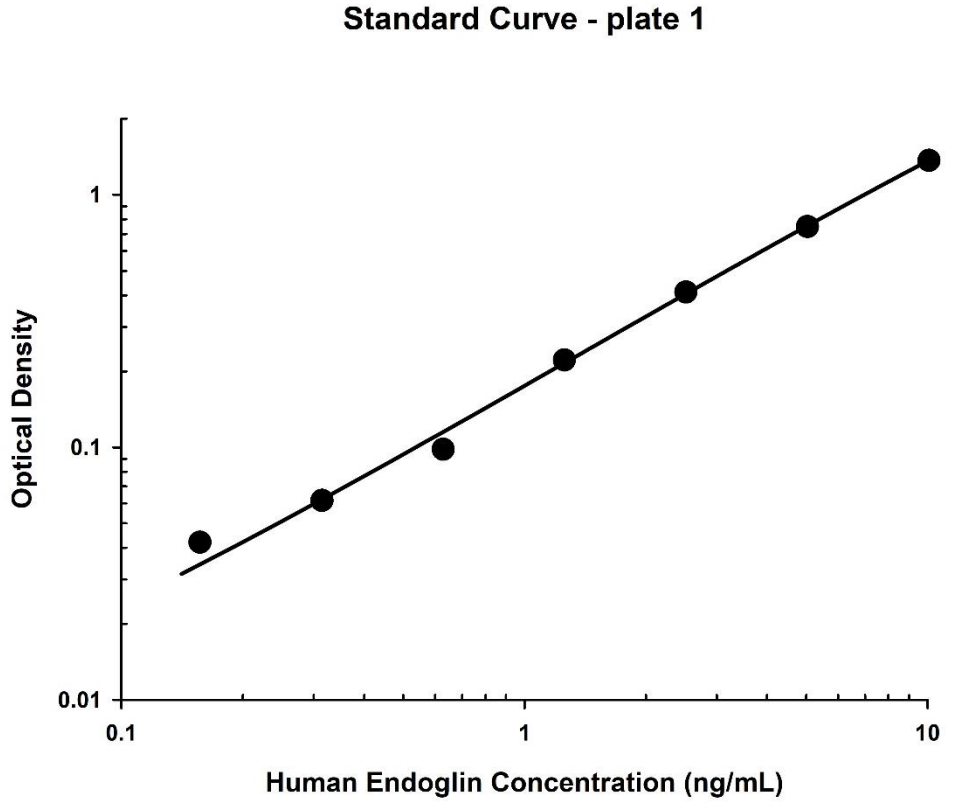
| _____ | ___/___/____ | _____ |
|---|---|---|
| Name of Person taking consent | Date (DD/MMM/YYYY) | Signature |

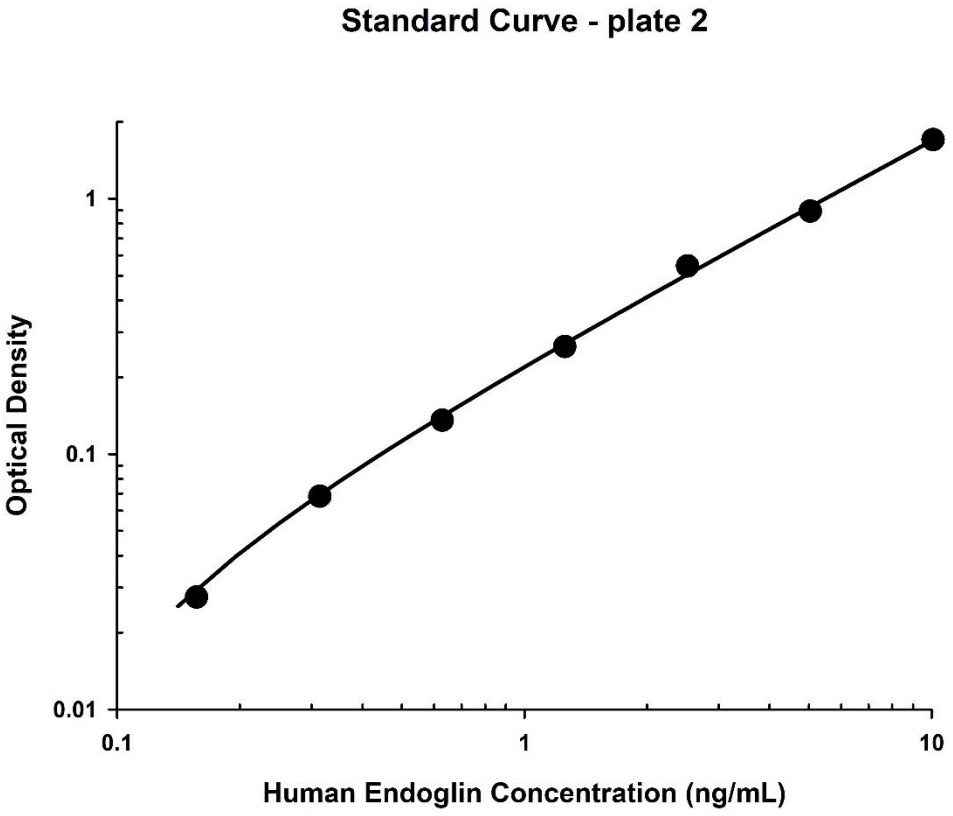
**Appendix VI:** Standard curve graphs for sFLT1 (VEGF R1)



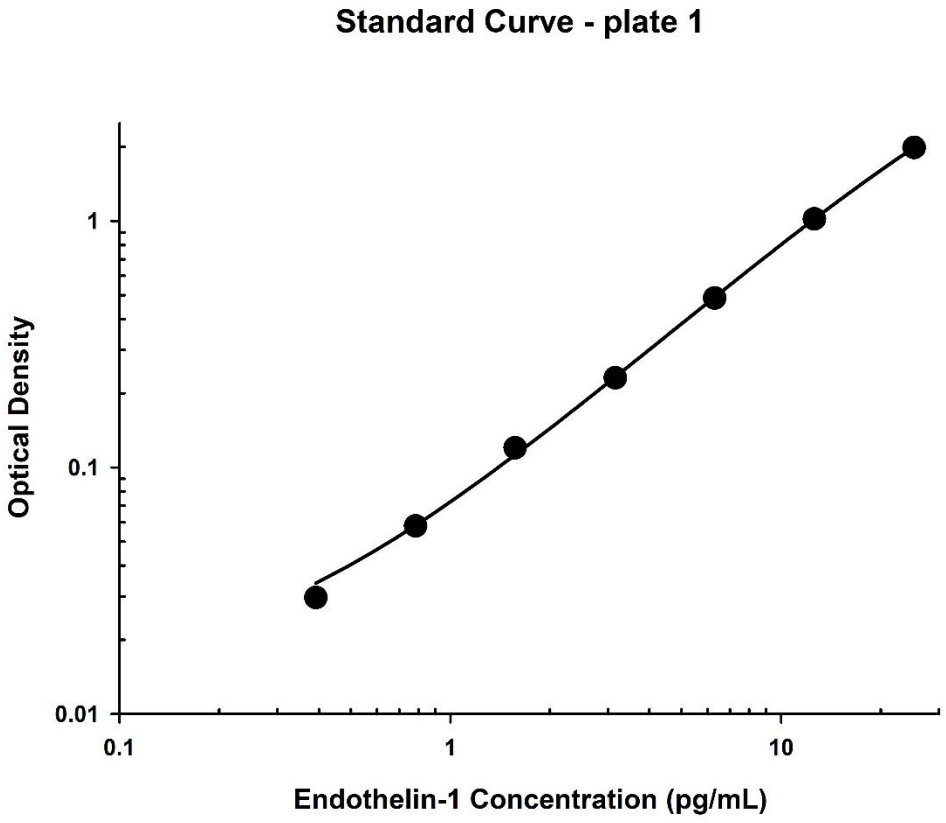



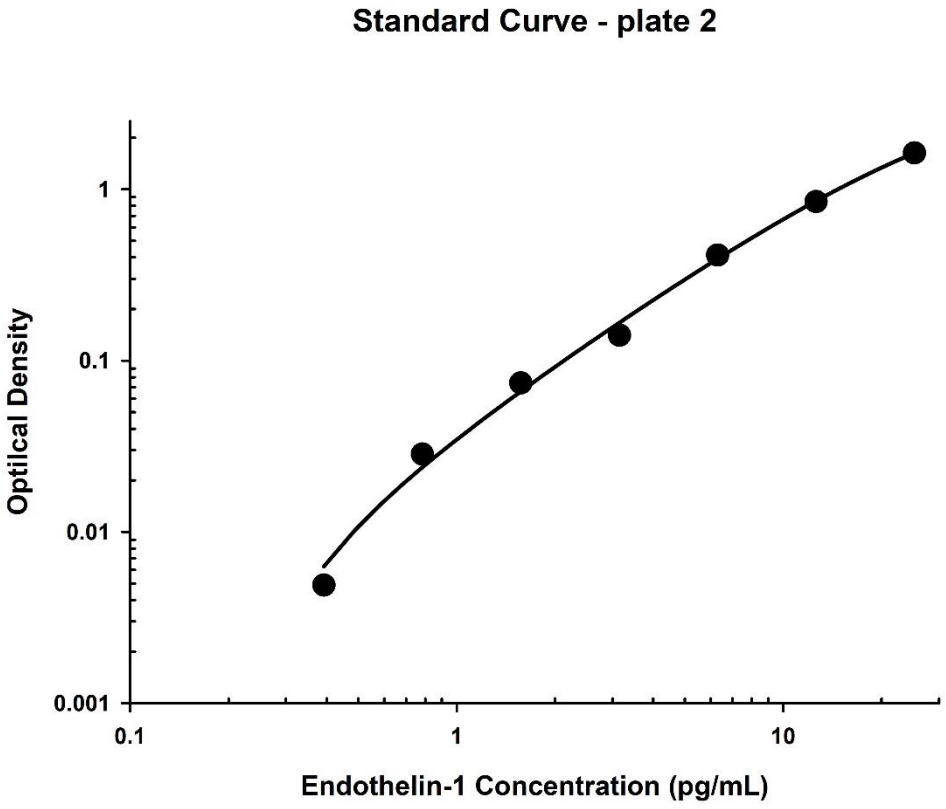
**Appendix VII:** Standard curve graphs for endoglin.



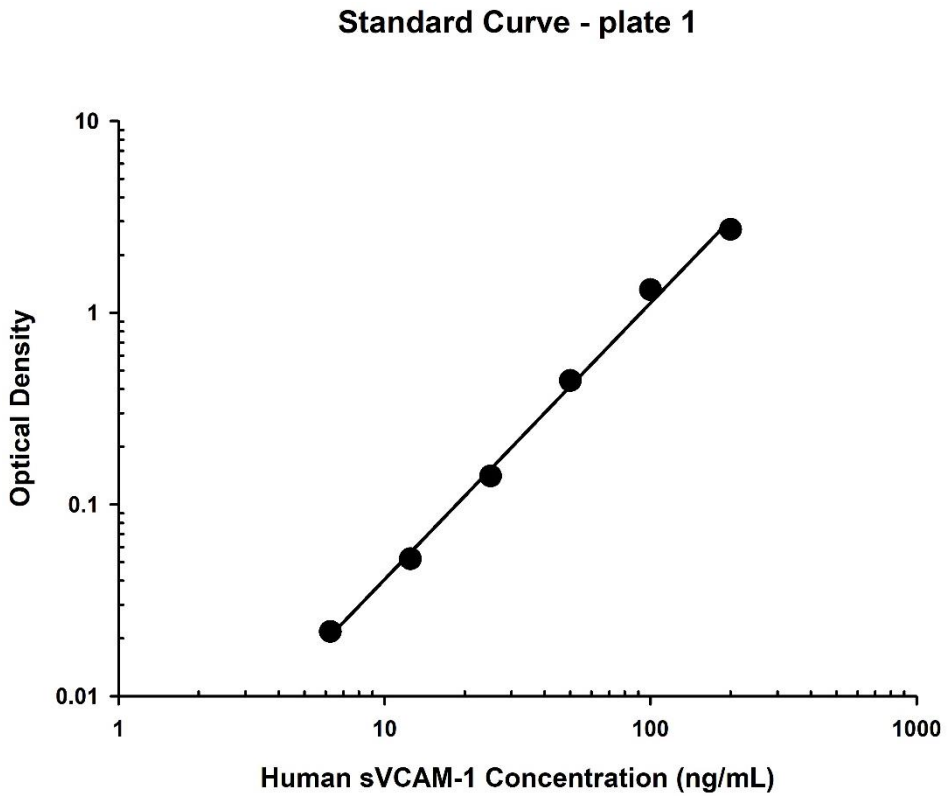



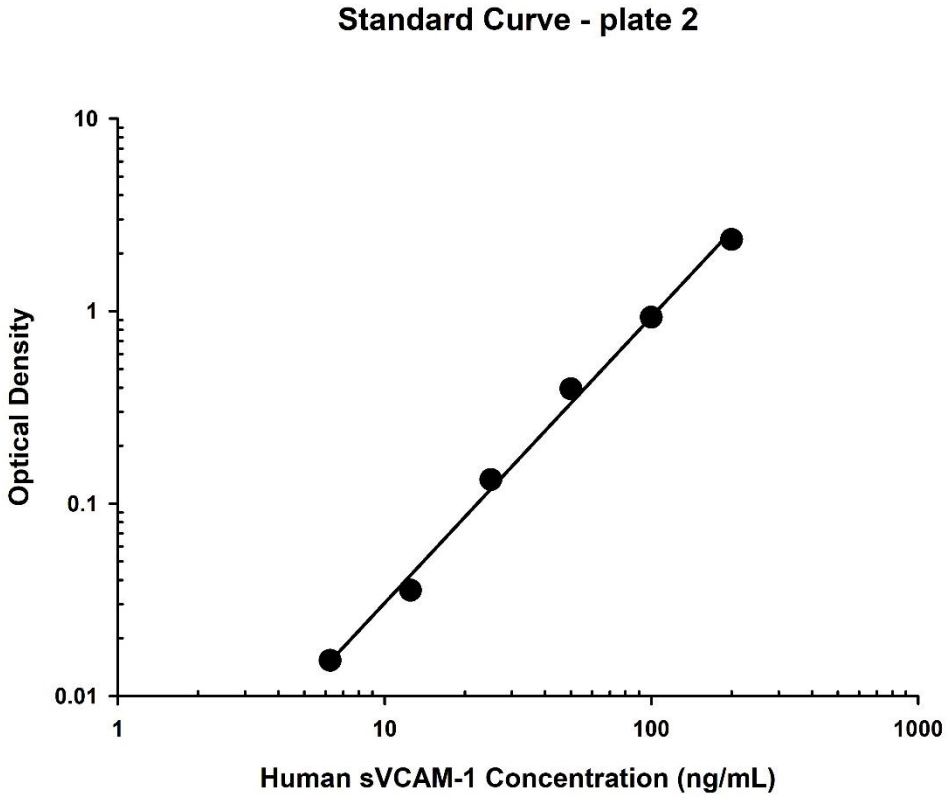
**Appendix VIII:** Standard curve graphs for endothelin-1.



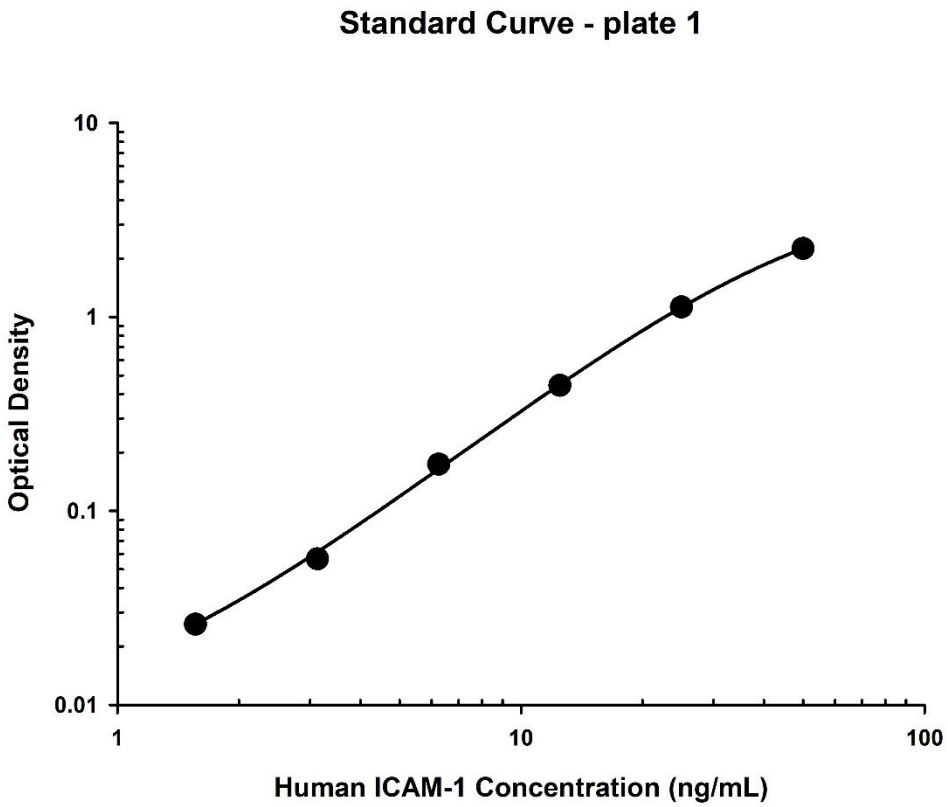



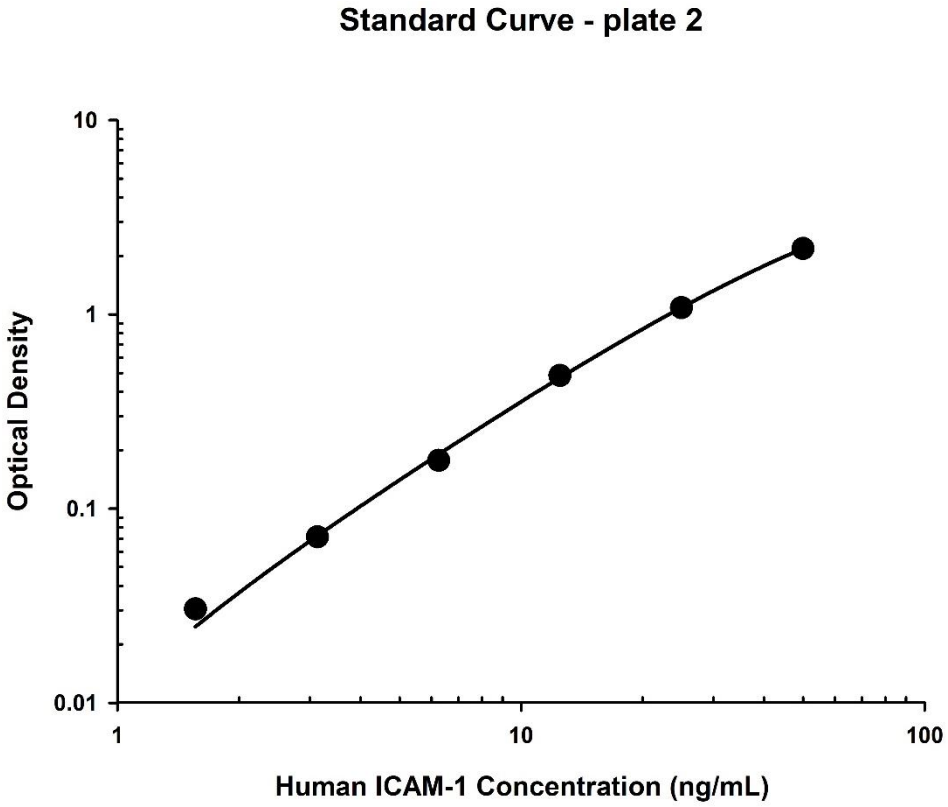
**Appendix IX:** Standard curve graphs for VCAM-1.



**Appendix X:** Standard curve graphs for ICAM-1.



**Appendix XI:** The Epworth Sleepiness Scale.

# The Epworth Sleepiness Scale

The Epworth Sleepiness Scale is widely used in the field of sleep medicine as a subjective measure of a patient's sleepiness. The test is a list of eight situations in which you rate your tendency to become sleepy on a scale of 0, no chance of dozing, to 3, high chance of dozing. The scale estimates whether you are experiencing excessive sleepiness that possibly requires medical attention.

**How Sleepy Are You?**
How likely are you to doze off or fall asleep in the following situations? You should rate your chances of dozing off, not just feeling tired. Even if you have not done some of these things recently try to determine how they would have affected you. For each situation, decide whether or not you would have:

- No chance of dozing =0
- Slight chance of dozing =1
- Moderate chance of dozing =2
- High chance of dozing =3

Please tick the box corresponding to your choice in the following table.

| **Situation** | **Chance of Dozing** | | | |
|---|---|---|---|---|
| | **0 - No chance** | **1 - Slight chance** | **2 - Moderate chance** | **3 - High Chance** |
| Sitting and reading | | | | |
| Watching TV | | | | |
| Sitting inactive in a public place (e.g., a theater or a meeting) | | | | |
| As a passenger in a car for an hour without a break | | | | |
| Lying down to rest in the afternoon when circumstances permit | | | | |
| Sitting and talking to someone | | | | |
| Sitting quietly after a lunch without alcohol | | | | |
| In a car, while stopped for a few minutes in traffic | | | | |

**Appendix XII:** Beliefs about medicines questionnaire.

# Beliefs about medicines questionnaire (BMQ)

*BMQ –Specific*
*Your views about medicines prescribed to you.*

- I would like to ask you about your personal views about medicines prescribed for your hypertension (high blood pressure).
- These are statements other people have made about their hypertension medication.
- Please indicate the extent to which you agree or disagree with them by placing a cross in the appropriate box.
- There are no right or wrong answers. I am interested in your personal views.
- Please only cross one box per question.

| Statement | Strongly Agree | Agree | Uncertain | Disagree | Strongly Disagree |
|---|---|---|---|---|---|
| My health, at present, depends on my hypertension medicines | | | | | |
| Having to take hypertension medicines worries me | | | | | |
| My life would be impossible without my hypertension medicines | | | | | |
| Without my hypertension medicines I would be very ill | | | | | |
| I sometimes worry about the long-term effects of my hypertension medicines | | | | | |
| My hypertension medicines are a mystery to me | | | | | |
| My health in the future will depend on my hypertension medicines | | | | | |
| My hypertension medicines disrupt my life | | | | | |
| I sometimes worry about becoming too dependent on my hypertension medicines | | | | | |
| My hypertension medicines protect me from becoming worse | | | | | |

*BMQ-General*

- I would like to ask you about your personal views about medicines in general.
- These are statements other people have made about medicines in general.
- Please indicate the extent to which you agree or disagree with them by ticking the appropriate box.
- There are no right or wrong answers. I am interested in your personal views.
- Please only tick one box per question.

| Statement | Strongly Agree | Agree | Uncertain | Disagree | Strongly Disagree |
|---|---|---|---|---|---|
| Doctors use too many medicines | | | | | |
| People who take medicines should stop their treatment for a while every now and again | | | | | |
| Most medicines are addictive | | | | | |
| Natural remedies are safer than medicines | | | | | |
| Medicines do more harm than good | | | | | |
| All medicines are poisons | | | | | |
| Doctors place too much trust on medicines | | | | | |
| If doctors had more time with patients they would prescribe fewer medicines | | | | | |

# REFERENCES


1. National Institute for Health and Care Excellence. Hypertension: clinical management of primary hypertension in adults. CG 127 ed. London: National Institute for Health and Care Excellence; 2011.
2. Stanaway JD, Afshin A, Gakidou E, Lim SS, Abate D, Abate KH, et al. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet. 2018;392(10159):1923-94.
3. Institute for Health Metrics and Evaluation (IHME). GBD Compare Seattle, WA: IHME, University of Washington; 2017 [Available from: http://vizhub.healthdata.org/gbd-compare.
4. World Health Organisation. A global brief on hypertension: Silent killer, global public health crisis. Geneva: World Health Organisation; 2013. Contract No.: Book, Whole.
5. NHS Digital. Health Survey for England 2017: NHS Digital; 2018 [Available from: https://digital.nhs.uk/data-and-information/publications/statistical/health-survey-for-england/2017.
6. Public Health England. Health matters: combating high blood pressure 2017 [Available from: https://www.gov.uk/government/publications/health-matters-combating-high-blood-pressure/health-matters-combating-high-blood-pressure.
7. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R, Prospective Studies C. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. The Lancet. 2002;360(9349):1903-13.
8. Law MR, Morris JK, Wald NJ. Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. BMJ (Clinical research ed). 2009;338:b1665.
9. Ettehad D, Emdin CA, Kiran A, Anderson SG, Callender T, Emberson J, et al. Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. The Lancet. 2015.
10. Muntner P, Shimbo D, Carey RM, Charleston JB, Gaillard T, Misra S, et al. Measurement of blood pressure in humans: a scientific statement from the American Heart Association. Hypertension. 2019;73(5):e35-e66.
11. Reeves RA. Does this patient have hypertension? JAMA. 1995;273(15):1211.
12. Keenan K, Hayen A, Neal BC, Irwig L. Long term monitoring in patients receiving treatment to lower blood pressure: analysis of data from placebo controlled randomised controlled trial. BMJ. 2009;338:b1492.

13. Clark LA, Denby L, Pregibon D, Harshfield GA, Pickering TG, Blank S, et al. A quantitative analysis of the effects of activity and time of day on the diurnal variations of blood pressure. Journal of Chronic Diseases. 1987;40(7):671-81.

14. Mancia G, Ferrari A, Gregorini L, Parati G, Pomidossi G, Bertinieri G, et al. Blood pressure and heart rate variabilities in normotensive and hypertensive human beings. Circulation Research. 1983;53(1):96-104.

15. Mancia G. Short- and long-term blood pressure variability. Hypertension. 2012;60(2):512-7.

16. Parati G, Pomidossi G, Albini F, Malaspina D, Mancia G. Relationship of 24-hour blood pressure mean and variability to severity of target-organ damage in hypertension. J Hypertens. 1987;5(1):93-8.

17. Kikuya M, Ohkubo T, Metoki H, Asayama K, Hara A, Obara T, et al. Day-by-day variability of blood pressure and heart rate at home as a novel predictor of prognosis. Hypertension. 2008;52(6):1045-50.

18. Murakami S, Otsuka K, Kubo Y, Shinagawa M, Matsuoka O, Yamanaka T, et al. Weekly variation of home and ambulatory blood pressure and relation between arterial stiffness and blood pressure measurements in community-dwelling hypertensives. Clinical and Experimental Hypertension. 2005;27(2-3):231-9.

19. Modesti PA, Morabito M, Bertolozzi I, Massetti L, Panci G, Lumachi C, et al. Weather-related changes in 24-hour blood pressure profile. Hypertension. 2006;47(2):155-61.

20. The Reference Values for Arterial Stiffness Collaboration. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: ‘establishing normal and reference values’. European Heart Journal. 2010;31(19):2338-50.

21. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, et al. Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. The Lancet. 2010;375(9718):895-905.

22. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, et al. Effects of β blockers and calcium-channel blockers on within-individual variability in blood pressure and risk of stroke. The Lancet Neurology. 2010;9(5):469-80.

23. Webb AJ, Fischer U, Mehta Z, Rothwell PM. Effects of antihypertensive-drug class on interindividual variation in blood pressure and risk of stroke: a systematic review and meta-analysis. The Lancet. 2010;375(9718):906-15.

24. Shimbo D, Newman JD, Aragaki AK, Lamonte MJ, Bavry AA, Allison M, et al. Association between annual visit-to-visit blood pressure variability and stroke in postmenopausal women. Hypertension. 2012;60(3):625-30.

25. Hastie CE, Jeemon P, Coleman H, McCallum L, Patel R, Dawson J, et al. Long-term and ultra long–term blood pressure variability during follow-up

and mortality in 14 522 patients with hypertension. Hypertension. 2013;62(4):698-705.

26. Asayama K, Kikuya M, Schutte R, Thijs L, Hosaka M, Satoh M, et al. Home blood pressure variability as cardiovascular risk factor in the population of Ohasama. Hypertension. 2013;61(1):61-9.

27. Schutte R, Thijs L, Liu Y-P, Asayama K, Jin Y, Odili A, et al. Within-subject blood pressure level—not variability—predicts fatal and nonfatal outcomes in a general population. Hypertension. 2012;60(5):1138-47.

28. Perloff D, Sokolow M, Cowan R. The prognostic value of ambulatory blood pressures. JAMA. 1983;249(20):2792-8.

29. Ritchie LD, Campbell NC, Murchie P. New NICE guidelines for hypertension. BMJ. 2011;343:d5644.

30. Hodgkinson J, Mant J, Martin U, Guo B, Hobbs FD, Deeks JJ, et al. Relative effectiveness of clinic and home blood pressure monitoring compared with ambulatory blood pressure monitoring in diagnosis of hypertension: systematic review. BMJ. 2011;342:d3621.

31. Lovibond K, Jowett S, Barton P, Caulfield M, Heneghan C, Hobbs FDR, et al. Cost-effectiveness of options for the diagnosis of high blood pressure in primary care: a modelling study. The Lancet. 2011;378(9798):1219-30.

32. O'Brien E, Parati G, Stergiou G, Asmar R, Beilin L, Bilo G, et al. European Society of Hypertension position paper on ambulatory blood pressure monitoring. Journal of Hypertension. 2013;31(9):1731-68.

33. Kikuya M, Hansen TW, Thijs L, BjöRklund-BodegåRd K, Kuznetsova T, Ohkubo T, et al. Diagnostic thresholds for ambulatory blood pressure monitoring based on 10-year cardiovascular risk. Circulation. 2007;115(16):2145-52.

34. Ohkubo T, Imai Y, Tsuji I, Nagai K, Ito S, Satoh H, et al. Reference values for 24-hour ambulatory blood pressure monitoring based on a prognostic criterion. Hypertension. 1998;32(2):255-9.

35. Niiranen TJ, Asayama K, Thijs L, Johansson JK, Ohkubo T, Kikuya M, et al. Outcome-driven thresholds for home blood pressure measurement. Hypertension. 2013;61(1):27-34.

36. Niiranen TJ, Johansson JK, Reunanen A, Jula AM. Optimal schedule for home blood pressure measurement based on prognostic data. Hypertension. 2011;57(6):1081-6.

37. McManus RJ, Mant J, Franssen M, Nickless A, Schwartz C, Hodgkinson J, et al. Efficacy of self-monitored blood pressure, with or without telemonitoring, for titration of antihypertensive medication (TASMINH4): an unmasked randomised controlled trial. The Lancet. 2018;391(10124):949-59.

38. Fletcher BR, Hartmann-Boyce J, Hinton L, McManus RJ. The effect of self-monitoring of blood pressure on medication adherence and lifestyle factors: a systematic review and meta-analysis. American Journal of Hypertension. 2015;28(10):1209-21.

39. Uhlig K, Patel K, Ip S, Kitsios GD, Balk EM. Self-measured blood pressure monitoring in the management of hypertension: A systematic review and meta-analysis. Annals of Internal Medicine. 2013;159(3):185-94.

40. Ogedegbe G, Schoenthaler A. A systematic review of the effects of home blood pressure monitoring on medication adherence. The Journal of Clinical Hypertension. 2006;8(3):174-80.

41. Mancia G, Grassi G, Pomidossi G, Gregorini L, Bertinieri G, Parati G, et al. Effects of blood-pressure measurement by the doctor on patient's blood pressure and heart rate. The Lancet. 1983;322(8352):695-8.

42. Enström I, Pennert K, Lindholm LH. Difference in blood pressure, but not in heart rate, between measurements performed at a health centre and at a hospital by one and the same physician. Journal of Human Hypertension. 2000;14(6):355-8.

43. Mancia G, Parati G, Pomidossi G, Grassi G, Casadei R, Zanchetti A. Alerting reaction and rise in blood pressure during measurement by physician and nurse. Hypertension. 1987;9(2):209-15.

44. Myers MG. The great myth of office blood pressure measurement. Journal of Hypertension. 2012;30(10):1894-8.

45. The SPRINT Research Group, Wright JT, Jr., Williamson JD, Whelton PK, Snyder JK, Sink KM, et al. A randomized trial of intensive versus standard blood-pressure control. New England Journal of Medicine. 2015;373(22):2103-16.

46. Verdecchia P, Staessen JA, White WB, Imai Y, O'Brien ET. Properly defining white coat hypertension. European Heart Journal. 2002;23(2):106-9.

47. O'Brien E, Asmar R, Beilin L, Imai Y, Mallion J-M, Mancia G, et al. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. Journal of Hypertension. 2003;21(5):821-48.

48. Pickering TG. The ninth Sir George Pickering memorial lecture Ambulatory monitoring and the definition of hypertension. Journal of Hypertension. 1992;10(5):401-9.

49. Pickering TG, James GD, Boddie C, Harshfield GA, Blank S, Laragh JH. How common Is white coat hypertension? JAMA. 1988;259(2):225-8.

50. Dolan E, Stanton A, Atkins N, Den Hond E, Thijs L, McCormack P, et al. Determinants of white-coat hypertension. Blood pressure monitoring. 2004;9(6):307-9.

51. Khattar RS, Senior R, Lahiri A. Cardiovascular outcome in white-coat versus sustained mild hypertension. Circulation. 1998;98(18):1892-7.

52. Verdecchia P, Schillaci G, Borgioni C, Ciucci A, Porcellati C. White-coat hypertension. The Lancet. 1996;348(9039):1444-5.

53. Martínez MaA, García-Puig J, Martín JC, Guallar-Castillón P, Aguirre de Cárcer A, Torre A, et al. Frequency and determinants of white coat

hypertension in mild to moderate hypertension: a primary care-based study. American Journal of Hypertension. 1999;12(3):251-9.

54. Verdecchia P, Porcellati C, Schillaci G, Borgioni C, Ciucci A, Battistelli M, et al. Ambulatory blood pressure. An independent predictor of prognosis in essential hypertension. Hypertension. 1994;24(6):793-801.

55. Muxfeldt ES, Fiszman R, De Souza F, Viegas B, Oliveira FC, Salles GF. Appropriate time interval to repeat ambulatory blood pressure monitoring in patients with white-coat resistant hypertension. Hypertension. 2012;59(2):384-9.

56. Mancia G, Bombelli M, Facchetti R, Madotto F, Quarti-Trevano F, Friz HP, et al. Long-term risk of sustained hypertension in white-coat or masked hypertension. Hypertension. 2009;54(2):226-32.

57. Cohen JB, Lotito MJ, Trivedi UK, Denker MG, Cohen DL, Townsend RR. Cardiovascular events and mortality in white coat hypertension. Annals of Internal Medicine. 2019;170(12):853.

58. Pickering TG, Eguchi K, Kario K. Masked hypertension: a review. Hypertension Research. 2007;30(6):479-88.

59. Zhang D-Y, Guo Q-H, An D-W, Li Y, Wang J-G. A comparative meta-analysis of prospective observational studies on masked hypertension and masked uncontrolled hypertension defined by ambulatory and home blood pressure. Journal of Hypertension. 2019;37(9):1775-85.

60. Mallion JM, Genès N, Vaur L, Clerson P, Vaïsse B, Bobrie G, et al. Detection of masked hypertension by home blood pressure measurement: is the number of measurements an important issue? Blood pressure monitoring. 2004;9(6):301-5.

61. Franklin SS, Thijs L, Li Y, Hansen TW, Boggia J, Liu Y, et al. Masked hypertension in diabetes mellitus: treatment implications for clinical practice. Hypertension. 2013;61(5):964-71.

62. Bangash F, Agarwal R. Masked hypertension and white-coat hypertension in chronic kidney disease: a meta-analysis. Clin J Am Soc Nephrol. 2009;4(3):656-64.

63. Parati G, Ochoa JE, Bilo G, Agarwal R, Covic A, Dekker FW, et al. Hypertension in Chronic Kidney Disease Part 1: Out-of-Office Blood Pressure Monitoring: Methods, Thresholds, and Patterns. Hypertension. 2016;67(6):1093-101.

64. Agarwal R, Pappas MK, Sinha AD. Masked Uncontrolled Hypertension in CKD. J Am Soc Nephrol. 2016;27(3):924-32.

65. Baguet J-P, Lévy P, Barone-Rochette G, Tamisier R, Pierre H, Peeters M, et al. Masked hypertension in obstructive sleep apnea syndrome. Journal of Hypertension. 2008;26(5):885-92.

66. Grassi G, Seravalle G, Trevano FQ, Dell'Oro R, Bolla G, Cuspidi C, et al. Neurogenic abnormalities in masked hypertension. Hypertension. 2007;50(3):537-42.

67. Hänninen M-RA, Niiranen TJ, Puukka PJ, Kesäniemi YA, Kähönen M, Jula AM. Target organ damage and masked hypertension in the general population. Journal of Hypertension. 2013;31(6):1136-43.

68. Page IH. Pathogenesis of arterial hypertension. JAMA. 1949;140(5):451-8.

69. Guyenet PG. Neural structures that mediate sympathoexcitation during hypoxia. Respiration Physiology. 2000;121(2-3):147-62.

70. Somers VK, Dyken ME, Clary MP, Abboud FM. Sympathetic neural mechanisms in obstructive sleep apnea. Journal of Clinical Investigation. 1995;96(4):1897-904.

71. Wolk R, Shamsuzzaman AS, Somers VK. Obesity, sleep apnea, and hypertension. Hypertension. 2003;42(6):1067-74.

72. Prabhakar NR, Peng YJ, Jacono FJ, Kumar GK, Dick TE. Cardiovascular alterations by chronic intermittent hypoxia: importance of carotid body chemoreflexes. Clin Exp Pharmacol Physiol. 2005;32(5-6):447-9.

73. Guyenet PG. The sympathetic control of blood pressure. Nature Reviews Neuroscience. 2006;7(5):335-46.

74. Osborn JW, Jacob F, Guzman P. A neural set point for the long-term control of arterial pressure: beyond the arterial baroreceptor reflex. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2005;288(4):R846-R55.

75. Dibona GF. Physiology in perspective: the wisdom of the body. Neural control of the kidney. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2005;289(3):R633-R41.

76. Grassi G, Esler M. How to assess sympathetic activity in humans. Journal of Hypertension. 1999;17(6):719-34.

77. Goldstein DS, McCarty R, Polinsky RJ, Kopin IJ. Relationship between plasma norepinephrine and sympathetic neural activity. Hypertension. 1983;5(4):552-9.

78. Wallin BG, Sundlöf G, Eriksson B-M, Dominiak P, Grobecker H, Lindblad LE. Plasma noradrenaline correlates to sympathetic muscle nerve activity in normotensive man. Acta Physiologica Scandinavica. 1981;111(1):69-73.

79. Mörlin C, Wallin BG, Eriksson BM. Muscle sympathetic activity and plasma noradrenaline in normotensive and hypertensive man. Acta Physiologica Scandinavica. 1983;119(2):117-21.

80. Wallin BG, Mörlin C, Hjemdahl P. Muscle sympathetic activity and venous plasma noradrenaline concentrations during static exercise in normotensive and hypertensive subjects. Acta Physiologica Scandinavica. 1987;129(4):489-97.

81. Grassi G, Bolla G, Seravalle G, Turri C, Lanfranchi A, Mancia G. Comparison between reproducibility and sensitivity of muscle sympathetic nerve traffic and plasma noradrenaline in man. Clinical Science. 1997;92(3):285-9.

82. Esler M, Jennings G, Korner P, Willett I, Dudley F, Hasking G, et al. Assessment of human sympathetic nervous system activity from measurements of norepinephrine turnover. Hypertension. 1988;11(1):3-20.

83. Wallin BG, Charkoudian N. Sympathetic neural control of integrated cardiovascular function: Insights from measurement of human sympathetic nerve activity. Muscle & Nerve. 2007;36(5):595-614.

84. Wallin BG, Esler M, Dorward P, Eisenhofer G, Ferrier C, Westerman R, et al. Simultaneous measurements of cardiac noradrenaline spillover and sympathetic outflow to skeletal muscle in humans. The Journal of Physiology. 1992;453(1):45-58.

85. Wallin BG, Thompson JM, Jennings GL, Esler MD. Renal noradrenaline spillover correlates with muscle sympathetic activity in humans. The Journal of physiology. 1996;491(3):881-7.

86. Freis ED. Historical development of antihypertensive treatment. In: Laragh GH, Brenner BM, editors. Hypertension: Pathophysiology, Diagnosis, and Management. Second edition ed. New York: Raven Press; 1995. p. 2741-51.

87. Mark AL. The sympathetic nervous system in hypertension: a potential long-term regulator of arterial pressure. Journal of hypertension Supplement : official journal of the International Society of Hypertension. 1996;14(5):S159-65.

88. Esler M, Lambert G, Brunner-La Rocca HP, Vaddadi G, Kaye D. Sympathetic nerve activity and neurotransmitter release in humans: translation from pathophysiology into clinical practice. Acta Physiologica Scandinavica. 2003;177(3):275-84.

89. Grassi G, Colombo M, Seravalle G, Spaziani D, Mancia G. Dissociation between muscle and skin sympathetic nerve activity in essential hypertension, obesity, and congestive heart failure. Hypertension. 1998;31(1):64-7.

90. Yamada Y, Miyajima E, Tochikubo O, Matsukawa T, Ishii M. Age-related changes in muscle sympathetic nerve activity in essential hypertension. Hypertension. 1989;13(6 Pt 2):870-7.

91. Oparil S, Zaman M, Calhoun DA. Pathogenesis of hypertension. Annals of Internal Medicine. 2003;139(9):761-76.

92. Marcus R, Krause L, Weder AB, Dominguez-Meja A, Schork NJ, Julius S. Sex-specific determinants of increased left ventricular mass in the Tecumseh Blood Pressure Study. Circulation. 1994;90(2):928-36.

93. Grassi G, Giannattasio C, Failla M, Pesenti A, Peretti G, Marinoni E, et al. Sympathetic modulation of radial artery compliance in congestive heart failure. Hypertension. 1995;26(2):348-54.

94. Kim JR, Kiefe CI, Liu K, Williams OD, Jacobs DR, Jr., Oberman A. Heart rate and subsequent blood pressure in young adults: the CARDIA study. Hypertension. 1999;33(2):640-6.

95. Esler M. The sympathetic system and hypertension. Am J Hypertens. 2000;13(6 Pt 2):99s-105s.

96. Martin EA, Victor RG. Premise, promise, and potential limitations of invasive devices to treat hypertension. Current Cardiology Reports. 2011;13(1):86-92.

97. Julius S, Krause L, Schork NJ, Mejia AD, Jones KA, van de Ven C, et al. Hyperkinetic borderline hypertension in Tecumseh, Michigan. J Hypertens. 1991;9(1):77-84.

98. Esler M, Jennings G, Biviano B, Lambert G, Hasking G. Mechanism of elevated plasma noradrenaline in the course of essential hypertension. Journal of cardiovascular pharmacology. 1986;8 Suppl 5:S39-43.

99. Schlaich MP, Lambert E, Kaye DM, Krozowski Z, Campbell DJ, Lambert G, et al. Sympathetic augmentation in hypertension: role of nerve firing, norepinephrine reuptake, and angiotensin neuromodulation. Hypertension. 2004;43(2):169-75.

100. Floras JS, Hara K. Sympathoneural and haemodynamic characteristics of young subjects with mild essential hypertension. Journal of hypertension. 1993;11(6):647-55.

101. Grassi G, Cattaneo BM, Seravalle G, Lanfranchi A, Mancia G. Baroreflex control of sympathetic nerve activity in essential and secondary hypertension. Hypertension. 1998;31(1):68-72.

102. Schlaich MP, Kaye DM, Lambert E, Hastings J, Campbell DJ, Lambert G, et al. Angiotensin II and norepinephrine release: interaction and effects on the heart. Journal of Hypertension. 2005;23(5):1077-82.

103. Schlaich MP, Kaye DM, Lambert E, Sommerville M, Socratous F, Esler MD. Relation between cardiac sympathetic activity and hypertensive left ventricular hypertrophy. Circulation. 2003;108(5):560-5.

104. Dibona GF, Esler M. Translational medicine: the antihypertensive effect of renal denervation. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2010;298(2):R245-R53.

105. Chapleau MW, Hajduczok G, Abboud FM. Mechanisms of resetting of arterial baroreceptors: an overview. The American Journal of the Medical Sciences. 1988;295(4):327-34.

106. Guo GB, Thames MD, Abboud FM. Arterial baroreflexes in renal hypertensive rabbits. Selectivity and redundancy of baroreceptor influence on heart rate, vascular resistance, and lumbar sympathetic nerve activity. Circ Res. 1983;53(2):223-34.

107. Xie PL, Chapleau MW, McDowell TS, Hajduczok G, Abboud FM. Mechanism of decreased baroreceptor activity in chronic hypertensive rabbits. Role of endogenous prostanoids. The Journal of Clinical Investigation. 1990;86(2):625-30.

108. Dibona GF, Sawin LL. Functional significance of the pattern of renal sympathetic nerve activation. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 1999;277(2):R346-R53.

109. DiBona GF, Kopp UC. Neural control of renal function: role in human hypertension. In: Laragh GH, Brenner BM, editors. Hypertension: Pathophysiology, Diagnosis, and Management. Second ed. New York: Raven Press; 1995. p. 1349-58.

110. DiBona GF, Kopp UC. Neural control of renal function. Physiological Reviews. 1997;77(1):75-197.

111. Sainio K, Suvanto P, Davies J, Wartiovaara J, Wartiovaara K, Saarma M, et al. Glial-cell-line-derived neurotrophic factor is required for bud initiation from ureteric epithelium. Development. 1997;124(20):4077-87.

112. Cullen-McEwen LA, Kett MM, Dowling J, Anderson WP, Bertram JF. Nephron number, renal function, and arterial pressure in aged GDNF heterozygous mice. Hypertension. 2003;41(2):335-40.

113. Head RJ. Hypernoradrenergic innervation: its relationship to functional and hyperplastic changes in the vasculature of the spontaneously hypertensive rat. Blood vessels. 1989;26(1):1-20.

114. Judy WV, Watanabe AM, Murphy WR, Aprison BS, Yu PL. Sympathetic nerve activity and blood pressure in normotensive backcross rats genetically related to the spontaneously hypertensive rat. Hypertension. 1979;1(6):598-604.

115. Lundin S, Ricksten SE, Thoren P. Interaction between "mental stress" and baroreceptor reflexes concerning effects on heart rate, mean arterial pressure and renal sympathetic activity in conscious spontaneously hypertensive rats. Acta Physiol Scand. 1984;120(2):273-81.

116. Folkow B, Hallback M, Lundgren Y, Weiss L. The effects of "immunosympathectomy" on blood pressure and vascular "reactivity" in normal and spontaneously hypertensive rats. Acta Physiol Scand. 1972;84(4):512-23.

117. Korner P, Bobik A, Oddie C, Friberg P. Sympathoadrenal system is critical for structural changes in genetic hypertension. Hypertension. 1993;22(2):243-52.

118. Lee RM, Borkowski KR, Leenen FH, Tsoporis J, Coughlin M. Combined effect of neonatal sympathectomy and adrenal demedullation on blood pressure and vascular changes in spontaneously hypertensive rats. Circ Res. 1991;69(3):714-21.

119. Grisk O, Rose H-J, Lorenz G, Rettig R. Sympathetic-renal interaction in chronic arterial pressure control. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2002;283(2):R441-R50.

120. Stella A, Zanchetti A. Functional role of renal afferents. Physiological Reviews. 1991;71(3):659-82.

121. Scislo TJ, Augustyniak RA, O'Leary DS. Differential arterial baroreflex regulation of renal, lumbar, and adrenal sympathetic nerve activity in the rat. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 1998;275(4):R995-R1002.

122. Johansson M, Rundqvist B, Petersson M, Lambert G, Friberg P. Regional norepinephrine spillover in response to angiotensin-converting enzyme inhibition in healthy subjects. J Hypertens. 2003;21(7):1371-5.

123. Abboud FM. Effects of sodium, angiotensin, and steroids on vascular reactivity in man. Federation proceedings. 1974;33(2):143-9.

124. Li Z, Mao HZ, Abboud FM, Chapleau MW. Oxygen-derived free radicals contribute to baroreceptor dysfunction in atherosclerotic rabbits. Circ Res. 1996;79(4):802-11.

125. He FJ, Macgregor GA. Reducing population salt intake worldwide: from evidence to implementation. Progress in Cardiovascular Diseases. 2010;52(5):363-82.

126. Denton D, Weisinger R, Mundy NI, Wickings EJ, Dixson A, Moisson P, et al. The effect of increased salt intake on blood pressure of chimpanzees. Nature Medicine. 1995;1(10):1009-16.

127. Elliott P, Walker LL, Little MP, Blair-West JR, Shade RE, Lee DR, et al. Change in salt intake affects blood pressure of chimpanzees. Circulation. 2007;116(14):1563-8.

128. Page LB, Damon A, Moellering RC. Antecedents of cardiovascular disease in six Solomon Islands societies. Circulation. 1974;49(6):1132-46.

129. Page LB, Vandevert DE, Nader K, Lubin NK, Page JR. Blood pressure of Qash'qai pastoral nomads In Iran in relation to culture, diet, and body form. The American Journal of Clinical Nutrition. 1981;34(4):527-38.

130. Uzodike VO. Epidemiological studies of arterial blood pressure and hypertension in relation to electrolyte excretion in three Igbo communities in Nigeria.: University of London; 1993.

131. Intersalt Cooperative Researh Group. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. Intersalt Cooperative Research Group. BMJ. 1988;297(6644):319-28.

132. He J, Klag MJ, Whelton PK, Chen J-Y, Mo J-P, Qian M-C, et al. Migration, blood pressure pattern, and hypertension: the Yi Migrant Study. American Journal of Epidemiology. 1991;134(10):1085-101.

133. Poulter NR, Khaw KT, Hopwood BE, Mugambi M, Peart WS, Rose G, et al. The Kenyan Luo migration study: observations on the initiation of a rise in blood pressure. BMJ. 1990;300(6730):967-72.

134. Forte JG, Miguel JM, Miguel MJ, de Padua F, Rose G. Salt and blood pressure: a community trial. J Hum Hypertens. 1989;3(3):179-84.

135. Tian HG, Guo ZY, Hu G, Yu SJ, Sun W, Pietinen P, et al. Changes in sodium intake and blood pressure in a community-based intervention project in China. J Hum Hypertens. 1995;9(12):959-68.

136. Takahashi Y, Sasaki S, Okubo S, Hayashi M, Tsugane S. Blood pressure change in a free-living population-based dietary modification study in Japan. Journal of Hypertension. 2006;24(3):451-8.

137. Karppanen H, Mervaala E. Sodium intake and hypertension. Progress in Cardiovascular Diseases. 2006;49(2):59-75.

138. He FJ, Pombo-Rodrigues S, Macgregor GA. Salt reduction in England from 2003 to 2011: its relationship to blood pressure, stroke and ischaemic heart disease mortality. BMJ Open. 2014;4(4):e004549-e.

139. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. Cell. 2001;104(4):545-56.

140. Selkurt EE. Effect of pulse pressure and mean arterial pressure modification on renal hemodynamics and electrolyte and water excretion. Circulation. 1951;4(4):541-51.

141. Garcia-Estan J, Roman RJ. Role of renal interstitial hydrostatic pressure in the pressure diuresis response. Am J Physiol. 1989;256(1 Pt 2):F63-70.

142. Granger JP. Pressure natriuresis. Role of renal interstitial hydrostatic pressure. Hypertension. 1992;19(1 Suppl):I9-17.

143. Haas JA, Granger JP, Knox FG. Effect of renal perfusion pressure on sodium reabsorption from proximal tubules of superficial and deep nephrons. Am J Physiol. 1986;250(3 Pt 2):F425-9.

144. Chou CL, Marsh DJ. Role of proximal convoluted tubule in pressure diuresis in the rat. Am J Physiol. 1986;251(2 Pt 2):F283-9.

145. Schrier RW. Renal and electrolyte disorders. 8th ed. Philadelphia, PY: Wolters Kluwer; 2018.

146. Rodriguez-Iturbe B, Romero F, Johnson RJ. Pathophysiological mechanisms of salt-dependent hypertension. American Journal of Kidney Diseases. 2007;50(4):655-72.

147. DeFronzo RA. The effect of insulin on renal sodium metabolism. A review with clinical implications. Diabetologia. 1981;21(3):165-71.

148. Johnson RJ, Schreiner GF. Hypothesis: the role of acquired tubulointerstitial disease in the pathogenesis of salt-dependent hypertension. Kidney Int. 1997;52(5):1169-79.

149. Brenner BM, Garcia DL, Anderson S. Glomeruli and blood pressure: less of one, more the other? American Journal of Hypertension. 1988;1(4 Pt 1):335-47.

150. Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ. 1989;298(6673):564-7.

151. Hinchliffe SA, Lynch MR, Sargent PH, Howard CV, Van Velzen D. The effect of intrauterine growth retardation on the development of renal nephrons. Br J Obstet Gynaecol. 1992;99(4):296-301.

152. Hughson MD, Gobe GC, Hoy WE, Manning RD, Douglas-Denton R, Bertram JF. Associations of glomerular number and birth weight with clinicopathological features of African Americans and Whites. Am J Kidney Dis. 2008;52(1):18-28.

153. Konje JC, Bell SC, Morton JJ, de Chazal R, Taylor DJ. Human fetal kidney morphometry during gestation and the relationship between weight, kidney morphometry and plasma active renin concentration at birth. Clin Sci (Lond). 1996;91(2):169-75.

154. Mañalich R, Reyes L, Herrera M, Melendi C, Fundora I. Relationship between weight at birth and the number and size of renal glomeruli in humans: a histomorphometric study. Kidney International. 2000;58(2):770-3.

155. Mu M, Wang S-F, Sheng J, Zhao Y, Li H-Z, Hu C-L, et al. Birth weight and subsequent blood pressure: a meta-analysis. Archives of Cardiovascular Diseases. 2012;105(2):99-113.

156. Elijovich F, Weinberger MH, Anderson CAM, Appel LJ, Bursztyn M, Cook NR, et al. Salt sensitivity of blood pressure. Hypertension. 2016;68(3):e7-e46.

157. Rydstedt LL, Williams GH, Hollenberg NK. Renal and endocrine response to saline infusion in essential hypertension. Hypertension. 1986;8(3):217-22.

158. Hollenberg NK, Moore T, Shoback D, Redgrave J, Rabinowe S, Williams GH. Abnormal renal sodium handling in essential hypertension. The American Journal of Medicine. 1986;81(3):412-8.

159. Carey RM. The intrarenal renin-angiotensin and dopaminergic systems. Hypertension. 2013;61(3):673-80.

160. Dries DL, Victor RG, Rame JE, Cooper RS, Wu X, Zhu X, et al. Corin gene minor allele defined by 2 missense mutations is common in blacks and associated with high blood pressure and hypertension. Circulation. 2005;112(16):2403-10.

161. Guyton AC, Coleman TG, Young DB, Lohmeier TE, Declue JW. Salt balance and long-term blood pressure control. Annual Review of Medicine. 1980;31(1):15-27.

162. Haddy FJ, Overbeck HW. The role of humoral agents in volume expanded hypertension. Life Sciences. 1976;19(7):935-47.

163. Blaustein MP. Sodium ions, calcium ions, blood pressure regulation, and hypertension: a reassessment and a hypothesis. American Journal of Physiology-Cell Physiology. 1977;232(5):C165-C73.

164. Guyton AC. Blood pressure control-special role of the kidneys and body fluids. Science (New York, NY). 1991;252(5014):1813-6.

165. Coleman TG, Guyton AC. Hypertension caused by salt loading in the dog. Circulation Research. 1969;25(2):153-60.

166. Granger HJ, Guyton AC. Autoregulation of the total systemic circulation following destruction of the central nervous system in the dog. Circulation Research. 1969;25(4):379-88.

167. Kaplan NM, Victor RG. Kaplan's Clinical Hypertension. 11th ed. Philadelphia, PY: Wolters Kluwer; 2014.

168. Ichikawa I, Harris RC. Angiotensin actions in the kidney: renewed insight into the old hormone. Kidney International. 1991;40(4):583-96.

169. Wang T, Chan YL. Mechanism of angiotensin II action on proximal tubular transport. Journal of Pharmacology and Experimental Therapeutics. 1990;252(2):689-95.

170. Garvin JL. Angiotensin stimulates bicarbonate transport and Na+/K+ ATPase in rat proximal straight tubules. Journal of the American Society of Nephrology. 1991;1(10):1146-52.

171. Yingst DR, Massey KJ, Rossi NF, Mohanty MJ, Mattingly RR. Angiotensin II directly stimulates activity and alters the phosphorylation of Na-K-ATPase in rat proximal tubule with a rapid time course. American Journal of Physiology-Renal Physiology. 2004;287(4):F713-F21.

172. Ruiz OS, Qiu YY, Wang LJ, Arruda JA. Regulation of the renal Na-HCO3 cotransporter: IV. Mechanisms of the stimulatory effect of angiotensin II. Journal of the American Society of Nephrology. 1995;6(4):1202-8.

173. Montezano AC, Nguyen Dinh Cat A, Rios FJ, Touyz RM. Angiotensin II and vascular injury. Current Hypertension Reports. 2014;16(6).

174. Miyazaki M, Takai S. Tissue angiotensin II generating system by angiotensin-converting enzyme and chymase. Journal of Pharmacological Sciences. 2006;100(5):391-7.

175. Reudelhuber TL. Prorenin, renin, and their receptor. Hypertension. 2010;55(5):1071-4.

176. Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin-angiotensin systems. Physiological Reviews. 2006;86(3):747-803.

177. Kobori H, Nangaku M, Navar LG, Nishiyama A. The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. Pharmacol Rev. 2007;59(3):251-87.

178. Dickhout JG, Mori T, Cowley AW. Tubulovascular nitric oxide crosstalk. Circulation Research. 2002;91(6):487-93.

179. Navar LG. Intrarenal renin–angiotensin system in regulation of glomerular function. Current Opinion in Nephrology and Hypertension. 2014;23(1):38-45.

180. Mattson DL, Raff H, Roman RJ. Influence of angiotensin II on pressure natriuresis and renal hemodynamics in volume-expanded rats. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 1991;260(6):R1200-R9.

181. Cowley AW. Renal medullary oxidative stress, pressure-natriuresis, and hypertension. Hypertension. 2008;52(5):777-86.

182. Panza JA, Quyyumi AA, Brush JE, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med. 1990;323(1):22-7.

183. Panza JA, García CE, Kilcoyne CM, Quyyumi AA, Cannon RO. Impaired endothelium-dependent vasodilation in patients with essential

hypertension: evidence that nitric oxide abnormality is not localized to a single signal transduction pathway. Circulation. 1995;91(6):1732-8.

184. Panza JA, Casino PR, Kilcoyne CM, Quyyumi AA. Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. Circulation. 1993;87(5):1468-74.

185. Treasure CB, Manoukian SV, Klein JL, Vita JA, Nabel EG, Renwick GH, et al. Epicardial coronary artery responses to acetylcholine are impaired in hypertensive patients. Circulation research. 1992;71(4):776-81.

186. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation. 2000;101(9):948-54.

187. Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF, et al. Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. Circulation. 2004;109(5):613-9.

188. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. The Lancet. 1992;340(8828):1111-5.

189. Mulvihill N. Prediction of cardiovascular risk using soluble cell adhesion molecules. European Heart Journal. 2002;23(20):1569-74.

190. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocrine reviews. 1997;18(1):4-25.

191. Belgore FM, Blann AD, Li-Saw-Hee FL, Beevers DG, Lip GY. Plasma levels of vascular endothelial growth factor and its soluble receptor (SFlt-1) in essential hypertension. The American Journal of Cardiology. 2001;87(6):805-7, A9.

192. Bates DO, Hillman NJ, Williams B, Neal CR, Pocock TM. Regulation of microvascular permeability by vascular endothelial growth factors*. Journal of anatomy. 2002;200(6):581-97.

193. Hermann M, Flammer A, Lscher TF. Nitric oxide in hypertension. The Journal of Clinical Hypertension. 2006;8:17-29.

194. Mueller CFH, Laude K, McNally JS, Harrison DG. Redox mechanisms in blood vessels. Arteriosclerosis, Thrombosis, and Vascular Biology. 2005;25(2):274-8.

195. Paravicini T, Touyz R. Redox signaling in hypertension. Cardiovascular Research. 2006;71(2):247-58.

196. Hickey KA, Rubanyi G, Paul RJ, Highsmith RF. Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. American Journal of Physiology-Cell Physiology. 1985;248(5):C550-C6.

197. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature. 1988;332(6163):411-5.

198. Callera GE, Touyz RM, Teixeira SA, Muscara MN, Carvalho MHC, Fortes ZB, et al. ET A receptor blockade decreases vascular superoxide generation in DOCA-salt hypertension. Hypertension. 2003;42(4):811-7.

199. Li L, Fink GD, Watts SW, Northcott CA, Galligan JJ, Pagano PJ, et al. Endothelin-1 increases vascular superoxide via endothelin A –NADPH oxidase pathway in low-renin hypertension. Circulation. 2003;107(7):1053-8.

200. Schiffrin EL. Endothelin: potential role in hypertension and vascular hypertrophy. Hypertension. 1995;25(6):1135-43.

201. Li JS, Larivière R, Schiffrin EL. Effect of a nonselective endothelin antagonist on vascular remodeling in deoxycorticosterone acetate-salt hypertensive rats. Evidence for a role of endothelin in vascular hypertrophy. Hypertension. 1994;24(2):183-8.

202. Vassileva I, Mountain C, Pollock DM. Functional role of ETB receptors in the renal medulla. Hypertension. 2003;41(6):1359-63.

203. Plato CF, Pollock DM, Garvin JL. Endothelin inhibits thick ascending limb chloride flux via ETB receptor-mediated NO release. American Journal of Physiology-Renal Physiology. 2000;279(2):F326-F33.

204. Gallego MS, Ling BN. Regulation of amiloride-sensitive Na+ channels by endothelin-1 in distal nephron cells. Am J Physiol. 1996;271(2 Pt 2):F451-60.

205. Zeidel ML, Brady HR, Kone BC, Gullans SR, Brenner BM. Endothelin, a peptide inhibitor of Na(+)-K(+)-ATPase in intact renaltubular epithelial cells. American Journal of Physiology-Cell Physiology. 1989;257(6):C1101-C7.

206. Gurbanov K, Rubinstein I, Hoffman A, Abassi Z, Better OS, Winaver J. Differential regulation of renal regional blood flow by endothelin-1. Am J Physiol. 1996;271(6 Pt 2):F1166-72.

207. Harris PJ, Zhuo J, Mendelsohn FA, Skinner SL. Haemodynamic and renal tubular effects of low doses of endothelin in anaesthetized rats. The Journal of physiology. 1991;433:25-39.

208. Schnermann J, Lorenz JN, Briggs JP, Keiser JA. Induction of water diuresis by endothelin in rats. American Journal of Physiology-Renal Physiology. 1992;263(3):F516-F26.

209. Schiffrin EL. Vascular endothelin in hypertension. Vascular Pharmacology. 2005;43(1):19-29.

210. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. Journal of the American College of Cardiology. 2010;55(13):1318-27.

211. Tomlinson LA. Methods for assessing arterial stiffness: technical considerations. Current opinion in nephrology and hypertension. 2012;21(6):655-60.

212. Darne B, Girerd X, Safar M, Cambien F, Guize L. Pulsatile versus steady component of blood pressure: a cross-sectional analysis and a prospective analysis on cardiovascular mortality. Hypertension. 1989;13(4):392-400.

213. Girerd X, Laurent S, Pannier B, Asmar R, Safar M. Arterial distensibility and left ventricular hypertrophy in patients with sustained essential hypertension. American Heart Journal. 1991;122(4, Part 2):1210-4.

214. Nichols WW, O'Rourke M. McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles. 3rd ed. London, England: Oxford University Press; 1990.; 1990. p. 77–142, 216–69, 83–359, 98–37.

215. Watanabe H, Ohtsuka S, Kakihana M, Sugishita Y. Coronary circulation in dogs with an experimental decrease in aortic compliance. Journal of the American College of Cardiology. 1993;21(6):1497-506.

216. Davies JE, Whinnett ZI, Francis DP, Manisty CH, Aguado-Sierra J, Willson K, et al. Evidence of a dominant backward-propagating "suction" wave responsible for diastolic coronary filling in humans, attenuated in left ventricular hypertrophy. Circulation. 2006;113(14):1768-78.

217. Blacher J, Asmar R, Djane S, London GM, Safar ME. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. Hypertension. 1999;33(5):1111-7.

218. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension. 2001;37(5):1236-41.

219. Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. BioMed research international. 2014;2014:406960.

220. Stumpf C, Jukic J, Yilmaz A, Raaz D, Schmieder RE, Daniel WG, et al. Elevated VEGF-plasma levels in young patients with mild essential hypertension. European journal of clinical investigation. 2009;39(1):31-6.

221. Bautista LE, Atwood JE, O'Malley PG, Taylor AJ. Association between C-reactive protein and hypertension in healthy middle-aged men and women. Coronary artery disease. 2004;15(6):331-6.

222. Blake GJ, Rifai N, Buring JE, Ridker PM. Blood pressure, C-reactive protein, and risk of future cardiovascular events. Circulation. 2003;108(24):2993-9.

223. Stuveling EM, Bakker SJL, Hillege HL, Burgerhof JGM, de Jong PE, Gans ROB, et al. C-reactive protein modifies the relationship between blood pressure and microalbuminuria. Hypertension. 2004;43(4):791-6.

224. Chul Sung K, Suh JY, Kim BS, Kang JH, Kim H, Lee MH, et al. High sensitivity C-reactive protein as an independent risk factor for essential hypertension. American Journal of Hypertension. 2003;16(6):429-33.

225. Xu T, Ju Z, Tong W, Hu W, Liu Y, Zhao L, et al. Relationship of C-reactive protein with hypertension and interactions between increased C-reactive protein and other risk factors on hypertension in Mongolian people, China.

Circulation journal : official journal of the Japanese Circulation Society. 2008;72(8):1324-8.

226. Ballou SP, Lozanski G. Induction of inflammatory cytokine release from cultured human monocytes by C-reactive protein. Cytokine. 1992;4(5):361-8.

227. Pasceri V, Willerson JT, Yeh ETH. Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation. 2000;102(18):2165-8.

228. Harrison DG, Marvar PJ, Titze JM. Vascular inflammatory cells in hypertension. Frontiers in Physiology. 2012;3.

229. Gudbrandsson T, Herlitz H, Hansson L, Lindholm L, Åke Nilsson L. Immunological changes in patients with previous malignant essential hypertension. The Lancet. 1981;317(8217):406-8.

230. Ebringer A, Doyle AE. Raised serum IgG levels in hypertension. BMJ. 1970;2(5702):146-8.

231. Kristensen Bø. Increased serum levels of immunoglobulins in untreated and treated essential hypertension. Acta Medica Scandinavica. 1978;203(1-6):49-54.

232. Olsen F, Hilden M, Ibsen H. Raised levels of immunoglobulins in serum of hypertensive patients. Acta Pathologica Microbiologica Scandinavica Section B Microbiology and Immunology. 1973;81B(6):775-8.

233. Kristensen BØ, Sølling K. Serum concentrations of immunoglobulins and free light chains before and after vascular events in essential hypertension. Acta Medica Scandinavica. 1983;213(1):15-20.

234. Brebner JA, Stockley RA. Polyclonal free light chains: a biomarker of inflammatory disease or treatment target? F1000 medicine reports. 2013;5(4).

235. Anandram S, Assi LK, Lovatt T, Parkes J, Taylor J, Macwhannell A, et al. Elevated, combined serum free light chain levels and increased mortality: a 5-year follow-up, UK study. Journal of clinical pathology. 2012;65(11):1036-42.

236. Dispenzieri A, Katzmann JA, Kyle RA, Larson DR, Therneau TM, Colby CL, et al. Use of nonclonal serum immunoglobulin free light chains to predict overall survival in the general population. Mayo Clinic proceedings. 2012;87(6):517-23.

237. Ritchie J, Assi LK, Burmeister A, Hoefield R, Cockwell P, Kalra PA. Association of serum Ig free light chains with mortality and ESRD among patients with nondialysis-dependent CKD. Clinical Journal of the American Society of Nephrology. 2015;10(5):740-9.

238. Shantsila E, Wrigley B, Lip GYH. Free light chains in patients with acute heart failure secondary to atherosclerotic coronary artery disease. The American Journal of Cardiology. 2014;114(8):1243-8.

239. NHS Digital. Statistics on Obesity, Physical Activity and Diet, England, 2019. NHS Digital; 2019.

240. Garrison RJ, Kannel WB, Stokes J, 3rd, Castelli WP. Incidence and precursors of hypertension in young adults: the Framingham Offspring Study. Prev Med. 1987;16(2):235-51.

241. Jones DW, Kim JS, Andrew ME, Kim SJ, Hong YP. Body mass index and blood pressure in Korean men and women: the Korean National Blood Pressure Survey. J Hypertens. 1994;12(12):1433-7.

242. Hall JE, do Carmo JM, da Silva AA, Wang Z, Hall ME. Obesity-induced hypertension: interaction of neurohumoral and renal mechanisms. Circ Res. 2015;116(6):991-1006.

243. Hall J. Mechanisms of abnormal renal sodium handling in obesity hypertension. American Journal of Hypertension. 1997;10(12):49S-55S.

244. Boustany CM, Brown DR, Randall DC, Cassis LA. AT1-receptor antagonism reverses the blood pressure elevation associated with diet-induced obesity. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2005;289(1):R181-R6.

245. Robles RG, Villa E, Santirso R, Martinez J, Ruilope LM, Cuesta C, et al. Effects of captopril on sympathetic activity, lipid and carbohydrate metabolism in a model of obesity-induced hypertension in dogs. Am J Hypertens. 1993;6(12):1009-15.

246. Alonso-Galicia M, Brands MW, Zappe DH, Hall JE. Hypertension in obese Zucker rats. Role of angiotensin II and adrenergic activity. Hypertension. 1996;28(6):1047-54.

247. Grassi G, Seravalle G, Dell'Oro R, Trevano FQ, Bombelli M, Scopelliti F, et al. Comparative effects of candesartan and hydrochlorothiazide on blood pressure, insulin sensitivity, and sympathetic drive in obese hypertensive individuals: results of the CROSS study. J Hypertens. 2003;21(9):1761-9.

248. Reisin E, Weir MR, Falkner B, Hutchinson HG, Anzalone DA, Tuck ML. Lisinopril versus hydrochlorothiazide in obese hypertensive patients: a multicenter placebo-controlled trial. Treatment in Obese Patients With Hypertension (TROPHY) Study Group. Hypertension. 1997;30(1):140-5.

249. Dorresteijn JA, Schrover IM, Visseren FL, Scheffer PG, Oey PL, Danser AH, et al. Differential effects of renin-angiotensin-aldosterone system inhibition, sympathoinhibition and diuretic therapy on endothelial function and blood pressure in obesity-related hypertension: a double-blind, placebo-controlled cross-over trial. J Hypertens. 2013;31(2):393-403.

250. Mule G, Nardi E, Cusimano P, Cottone S, Seddio G, Geraci C, et al. Plasma aldosterone and its relationships with left ventricular mass in essential hypertensive patients with the metabolic syndrome. Am J Hypertens. 2008;21(9):1055-61.

251. Rossi GP, Belfiore A, Bernini G, Fabris B, Caridi G, Ferri C, et al. Body mass index predicts plasma aldosterone concentrations in overweight-obese primary hypertensive patients. J Clin Endocrinol Metab. 2008;93(7):2566-71.

252. de Paula RB, da Silva AA, Hall JE. Aldosterone antagonism attenuates obesity-induced hypertension and glomerular hyperfiltration. Hypertension. 2004;43(1):41-7.

253. Esler M, Straznicky N, Eikelis N, Masuo K, Lambert G, Lambert E. Mechanisms of sympathetic activation in obesity-related hypertension. Hypertension. 2006;48(5):787-96.

254. Wofford MR, Anderson DC, Jr., Brown CA, Jones DW, Miller ME, Hall JE. Antihypertensive effect of alpha- and beta-adrenergic blockade in obese and lean hypertensive subjects. Am J Hypertens. 2001;14(7 Pt 1):694-8.

255. Rocchini AP, Mao HZ, Babu K, Marker P, Rocchini AJ. Clonidine prevents insulin resistance and hypertension in obese dogs. Hypertension. 1999;33(1):548-53.

256. Carlyle M, Jones OB, Kuo JJ, Hall JE. Chronic cardiovascular and renal actions of leptin: role of adrenergic activity. Hypertension. 2002;39(2 Pt 2):496-501.

257. Hering D, Marusic P, Walton AS, Lambert EA, Krum H, Narkiewicz K, et al. Sustained sympathetic and blood pressure reduction 1 year after renal denervation in patients with resistant hypertension. Hypertension. 2014;64(1):118-24.

258. 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;28(7):909-14.

259. Lohmeier TE, Iliescu R, Liu B, Henegar JR, Maric-Bilkan C, Irwin ED. Systemic and renal-specific sympathoinhibition in obesity hypertension. Hypertension. 2012;59(2):331-8.

260. Van Vliet BN, Hall JE, Mizelle HL, Montani JP, Smith MJ, Jr. Reduced parasympathetic control of heart rate in obese dogs. Am J Physiol. 1995;269(2):H629-37.

261. Vaz M, Jennings G, Turner A, Cox H, Lambert G, Esler M. Regional sympathetic nervous activity and oxygen consumption in obese normotensive human subjects. Circulation. 1997;96(10):3423-9.

262. Havlik RJ, Garrison RJ, Feinleib M, Kannel WB, Castelli WP, McNamara PM. Blood pressure aggregation in families. Am J Epidemiol. 1979;110(3):304-12.

263. Stamler R, Stamler J, Riedlinger WF, Algera G, Roberts RH. Family (parental) history and prevalence of hypertension: results of a nationwide screening program. JAMA. 1979;241(1):43-6.

264. Miall WE, Heneage P, Khosla T, Lovell HG, Moore F. Factors influencing the degree of resemblance in arterial pressure of close relatives. Clin Sci. 1967;33(2):271-83.

265. Longini IM, Jr., Higgins MW, Hinton PC, Moll PP, Keller JB. Environmental and genetic sources of familial aggregation of blood pressure in Tecumseh, Michigan. Am J Epidemiol. 1984;120(1):131-44.

266. Hayes CG, Tyroler HA, Cassel JC. Family aggregation of blood pressure in Evans County, Georgia. JAMA Internal Medicine. 1971;128(6):965-75.

267. Wang NY, Young JH, Meoni LA, Ford DE, Erlinger TP, Klag MJ. Blood pressure change and risk of hypertension associated with parental hypertension: the Johns Hopkins Precursors Study. Arch Intern Med. 2008;168(6):643-8.

268. Carretero OA, Oparil S. Essential hypertension. Circulation. 2000;101(3):329-35.

269. Kupper N, Willemsen G, Riese H, Posthuma D, Boomsma DI, de Geus EJ. Heritability of daytime ambulatory blood pressure in an extended twin design. Hypertension. 2005;45(1):80-5.

270. Ganesh SK, Tragante V, Guo W, Guo Y, Lanktree MB, Smith EN, et al. Loci influencing blood pressure identified using a cardiovascular gene-centric array. Human molecular genetics. 2013;22(8):1663-78.

271. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, et al. Genome-wide association study of blood pressure and hypertension. Nature Genetics. 2009;41(6):677-87.

272. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, et al. Genome-wide association study identifies eight loci associated with blood pressure. Nature Genetics. 2009;41(6):666-76.

273. The International Consortium for Blood Pressure Genome-Wide Association Studies. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011;478(7367):103-9.

274. Wang Y, O'Connell JR, McArdle PF, Wade JB, Dorff SE, Shah SJ, et al. Whole-genome association study identifies STK39 as a hypertension susceptibility gene. Proceedings of the National Academy of Sciences. 2009;106(1):226-31.

275. Cowley AW, Jr., Nadeau JH, Baccarelli A, Berecek K, Fornage M, Gibbons GH, et al. Report of the National Heart, Lung, and Blood Institute Working Group on epigenetics and hypertension. Hypertension. 2012;59(5):899-905.

276. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature. 2009;461(7265):747-53.

277. Shinozaki N, Yuasa T, Takata S. Cigarette smoking augments sympathetic nerve activity in patients with coronary heart disease. Int Heart J. 2008;49(3):261-72.

278. Fitzgerald PJ. Elevated norepinephrine may be a unifying etiological factor in the abuse of a broad range of substances: alcohol, nicotine, marijuana, heroin, cocaine, and caffeine. Substance Abuse: Research and Treatment. 2013;7:SART.S13019.

279. Lekakis J, Papamichael C, Vemmos C, Nanas J, Kontoyannis D, Stamatelopoulos S, et al. Effect of acute cigarette smoking on endothelium-dependent brachial artery dilatation in healthy individuals. Am J Cardiol. 1997;79(4):529-31.

280. Riksen NP, Smits P, Rongen GA. The cardiovascular effects of methylxanthines. Methylxanthines. Berlin, Heidelberg: Springer Berlin Heidelberg; 2011. p. 413-37.

281. Robertson D, Frolich JC, Carr RK, Watson JT, Hollifield JW, Shand DG, et al. Effects of caffeine on plasma renin activity, catecholamines and blood pressure. N Engl J Med. 1978;298(4):181-6.

282. Mesas AE, Leon-Muñoz LM, Rodriguez-Artalejo F, Lopez-Garcia E. The effect of coffee on blood pressure and cardiovascular disease in hypertensive individuals: a systematic review and meta-analysis. The American Journal of Clinical Nutrition. 2011;94(4):1113-26.

283. Winkelmayer WC. Habitual caffeine intake and the risk of hypertension in women. JAMA. 2005;294(18):2330.

284. Klatsky AL, Friedman GD, Siegelaub AB, Gerard MJ. Alcohol consumption and blood pressure. Kaiser-Permanente Multiphasic Health Examination data. N Engl J Med. 1977;296(21):1194-200.

285. Macmahon S. Alcohol consumption and hypertension. Hypertension. 1987;9(2):111-21.

286. Klag MJ, He J, Whelton PK, Chen JY, Qian MC, He GQ. Alcohol use and blood pressure in an unacculturated society. Hypertension. 1993;22(3):365-70.

287. Moreira LB, Fuchs FD, Moraes RS, Bredemeier M, Duncan BB. Alcohol intake and blood pressure: the importance of time elapsed since last drink. Journal of Hypertension. 1998;16(2):175-80.

288. Abe H, Kawano Y, Kojima S, Ashida T, Kuramochi M, Matsuoka H, et al. Biphasic effects of repeated alcohol intake on 24-hour blood pressure in hypertensive patients. Circulation. 1994;89(6):2626-33.

289. Aranha Rosito G. Dose-dependent biphasic effect of ethanol on 24-h blood pressure in normotensive subjects. American Journal of Hypertension. 1999;12(2):236-40.

290. Husain K, Ansari RA, Ferder L. Alcohol-induced hypertension: Mechanism and prevention. World journal of cardiology. 2014;6(5):245-52.

291. Randin D, Vollenweider P, Tappy L, Jequier E, Nicod P, Scherrer U. Suppression of alcohol-induced hypertension by dexamethasone. N Engl J Med. 1995;332(26):1733-7.

292. McFadden CB, Brensinger CM, Berlin JA, Townsend RR. Systematic review of the effect of daily alcohol intake on blood pressure. Am J Hypertens. 2005;18(2 Pt 1):276-86.

293. Xin X, He J, Frontini MG, Ogden LG, Motsamai OI, Whelton PK. Effects of alcohol reduction on blood pressure. Hypertension. 2001;38(5):1112-7.

294. Zilkens RR, Burke V, Hodgson JM, Barden A, Beilin LJ, Puddey IB. Red Wine and Beer Elevate Blood Pressure in Normotensive Men. Hypertension. 2005;45(5):874-9.

295. Wolfel EE, Selland MA, Mazzeo RS, Reeves JT. Systemic hypertension at 4,300 m is related to sympathoadrenal activity. Journal of Applied Physiology. 1994;76(4):1643-50.

296. Khalid ME, Ali ME, Ahmed EK, Elkarib AO. Pattern of blood pressures among high and low altitude residents of southern Saudi Arabia. J Hum Hypertens. 1994;8(10):765-9.

297. Forman JP, Curhan GC, Taylor EN. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension among young women. Hypertension. 2008;52(5):828-32.

298. Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, et al. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension. 2007;49(5):1063-9.

299. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, et al. Vitamin D deficiency and risk of cardiovascular disease. Circulation. 2008;117(4):503-11.

300. Qi D, Nie X, Cai J. The effect of vitamin D supplementation on hypertension in non-CKD populations: A systemic review and meta-analysis. Int J Cardiol. 2017;227:177-86.

301. Beveridge LA, Struthers AD, Khan F, Jorde R, Scragg R, Macdonald HM, et al. Effect of vitamin D Supplementation on blood pressure: a systematic review and meta-analysis incorporating individual patient data. JAMA Intern Med. 2015;175(5):745-54.

302. Stolarz-Skrzypek K, Bednarski A, Czarnecka D, Kawecka-Jaszcz K, Staessen JA. Sodium and Potassium and the Pathogenesis of Hypertension. Current Hypertension Reports. 2013;15(2):122-30.

303. Aaron KJ, Sanders PW. Role of dietary salt and potassium intake in cardiovascular health and disease: a review of the evidence. Mayo Clin Proc. 2013;88(9):987-95.

304. Aburto NJ, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP. Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. BMJ. 2013;346(apr03 3):f1378.

305. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Journal of Hypertension. 2013;31(7):1281-357.

306. Whelton PK, Carey RM, Aronow WS, Casey DE, Collins KJ, Dennison Himmelfarb C, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. J Am Coll Cardiol. 2018;71(19):e127-e248.

307. Murray CJL, Lopez AD. Measuring the global burden of disease. N Engl J Med. 2013;369(5):448-57.

308. Turnbull F, Neal B, Ninomiya T, Algert C, Arima H, Barzi F, et al. Effects of different regimens to lower blood pressure on major cardiovascular events in older and younger adults: meta-analysis of randomised trials. BMJ (Clinical research ed). 2008;336(7653):1121-3.

309. Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR, et al. Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney international. 2003;63(1):225-32.

310. Daugherty SL, Powers JD, Magid DJ, Tavel HM, Masoudi FA, Margolis KL, et al. Incidence and prognosis of resistant hypertension in hypertensive patients. Circulation. 2012;125(13):1635-42.

311. Hajjar I, Kotchen TA. Trends in prevalence, awareness, treatment, and control of hypertension in the united states, 1988-2000. JAMA. 2003;290(2):199-206.

312. Cushman WC, Ford CE, Cutler JA, Margolis KL, Davis BR, Grimm RH, et al. Success and predictors of blood pressure control in diverse North American settings: the Antihypertensive and Lipid-Lowering treatment to prevent Heart Attack Trial (ALLHAT). The Journal of Clinical Hypertension. 2002;4(6):393-404.

313. Egan BM, Zhao Y, Axon RN, Brzezinski WA, Ferdinand KC. Uncontrolled and apparent treatment resistant hypertension in the United States, 1988 to 2008. Circulation. 2011;124(9):1046-58.

314. Persell SD. Prevalence of resistant hypertension in the United States, 2003–2008. Hypertension. 2011;57(6):1076-80.

315. de la Sierra A, Segura J, Banegas JR, Gorostidi M, de la Cruz JJ, Armario P, et al. Clinical features of 8295 patients with resistant hypertension classified on the basis of ambulatory blood pressure monitoring. Hypertension. 2011;57(5):898-902.

316. Hanselin MR, Saseen JJ, Allen RR, Marrs JC, Nair KV. Description of antihypertensive use in patients with resistant hypertension prescribed four or more agents. Hypertension. 2011;58(6):1008-13.

317. Achelrod D, Wenzel U, Frey S. Systematic review and meta-analysis of the prevalence of resistant hypertension in treated hypertensive populations. American Journal of Hypertension. 2015;28(3):355-61.

318. Hayek SS, Abdou MH, Demoss BD, Legaspi JMO, Veledar E, Deka A, et al. Prevalence of resistant hypertension and eligibility for catheter-based renal denervation in hypertensive outpatients. American Journal of Hypertension. 2013;26(12):1452-8.

319. Egan BM, Zhao Y, Li J, Brzezinski WA, Todoran TM, Brook RD, et al. Prevalence of optimal treatment regimens in patients with apparent treatment-resistant hypertension based on office blood pressure in a community-based practice network. Hypertension. 2013;62(4):691-7.

320. Salles GF, Cardoso CR, Muxfeldt ES. Prognostic influence of office and ambulatory blood pressures in resistant hypertension. Archives of Internal Medicine. 2008;168(21):2340-6.

321. Sim JJ, Bhandari SK, Shi J, Reynolds K, Calhoun DA, Kalantar-Zadeh K, et al. Comparative risk of renal, cardiovascular, and mortality outcomes in controlled, uncontrolled resistant, and nonresistant hypertension. Kidney International. 2015;88(3):622-32.

322. Irvin MR, Booth JN, Shimbo D, Lackland DT, Oparil S, Howard G, et al. Apparent treatment-resistant hypertension and risk for stroke, coronary heart disease, and all-cause mortality. Journal of the American Society of Hypertension. 2014;8(6):405-13.

323. Thomas O, Shipman KE, Day K, Thomas M, Martin U, Dasgupta I. Prevalence and determinants of white coat effect in a large UK hypertension clinic population. Journal Of Human Hypertension. 2015;30:386.

324. Head GA, Mihailidou AS, Duggan KA, Beilin LJ, Berry N, Brown MA, et al. Definition of ambulatory blood pressure targets for diagnosis and treatment of hypertension in relation to clinic blood pressure: prospective cohort study. BMJ. 2010;340:c1104.

325. Nieuwlaat R, Wilczynski N, Navarro T, Hobson N, Jeffery R, Keepanasseril A, et al. Interventions for enhancing medication adherence. The Cochrane database of systematic reviews. 2014(11):Cd000011.

326. Naderi SH, Bestwick JP, Wald DS. Adherence to drugs that prevent cardiovascular disease: meta-analysis on 376,162 patients. The American Journal of Medicine. 2012;125(9):882-7.

327. Simpson SH, Eurich DT, Majumdar SR, Padwal RS, Tsuyuki RT, Varney J, et al. A meta-analysis of the association between adherence to drug therapy and mortality. BMJ. 2006;333(7557):15.

328. Trueman P, Taylor DG, Lowson K, Blighe A, Meszaros A, Wright D, et al. Evaluation of the scale, causes and costs of waste medicines. Report. York: University of York; 2010.

329. Gossec L, Dougados M, Tubach F, Ravaud P. Reporting of adherence to medication in recent randomized controlled trials of 6 chronic diseases: a systematic literature review. The American Journal of the Medical Sciences. 2007;334(4):248-54.

330. Zhang Z, Peluso MJ, Gross CP, Viscoli CM, Kernan WN. Adherence reporting in randomized controlled trials. Clinical Trials. 2014;11(2):195-204.

331. Waeber B, Leonetti G, Kolloch R, McInnes GT, for the HOT study group. Compliance with aspirin or placebo in the hypertension optimal treatment (HOT) study. Journal of Hypertension. 1999;17(7):1041-5.

332. Claxton AJ, Cramer J, Pierce C. A systematic review of the associations between dose regimens and medication compliance. Clinical Therapeutics. 2001;23(8):1296-310.

333. Cramer J, Rosenheck R, Kirk G, Krol W, Krystal J. Medication compliance feedback and monitoring in a clinical trial: predictors and outcomes. Value in Health. 2003;6(5):566-73.

334. Kaba R, Sooriakumaran P. The evolution of the doctor-patient relationship. International Journal of Surgery. 2007;5(1):57-65.

335. Sabaté E. Adherence to long-term therapies: evidence for action. Geneva, Switzerland: World Health Organisation; 2003.

336. Brown MT, Bussell JK. Medication adherence: WHO cares? Mayo Clinic Proceedings. 2011;86(4):304-14.

337. Lau DT, Nau DP. Oral antihyperglycemic medication nonadherence and subsequent hospitalization among individuals with type 2 diabetes. Diabetes Care. 2004;27(9):2149-53.

338. Steiner JF, Earnest MA. The language of medication-taking. Annals of Internal Medicine. 2000;132(11):926-30.

339. Benner JS, Pollack MF, Smith TW, Bullano MF, Willey VJ, Williams SA. Association between short-term effectiveness of statins and long-term adherence to lipid-lowering therapy. American Journal of Health-System Pharmacy. 2005;62(14):1468-75.

340. Woltmann EM, Valenstein M, Welsh DE, Lee TA, Wolschon PA, Grabowski J, et al. Using pharmacy data on partial adherence to inform clinical care of patients with serious mental illness. Psychiatric Services. 2007;58(6):864-7.

341. Karve S, Cleves MA, Helm M, Hudson TJ, West DS, Martin BC. Good and poor adherence: optimal cut-point for adherence measures using administrative claims data. Current Medical Research and Opinion. 2009;25(9):2303-10.

342. Steiner JF, Prochazka AV. The assessment of refill compliance using pharmacy records: methods, validity, and applications. Journal of Clinical Epidemiology. 1997;50(1):105-16.

343. Grymonpre R, Cheang M, Fraser M, Metge C, Sitar DS. Validity of a prescription claims database to estimate medication adherence in older persons. Medical Care. 2006;44(5):471-7.

344. Rudnick KV, Sackett DL, Hirst S, Holmes C. Hypertension in a family practice. Canadian Medical Association journal. 1977;117(5):492-7.

345. Arnsten JH, Demas PA, Farzadegan H, Grant RW, Gourevitch MN, Chang C-J, et al. Antiretroviral therapy adherence and viral suppression in HIV-infected drug users: comparison of self-report and electronic monitoring. Clinical infectious diseases. 2001;33(8):1417-23.

346. Graham DY, Lew GM, Malaty HM, Evans DG, Evans DJ, Klein PD, et al. Factors influencing the eradication of Helicobacter pylori with triple therapy. Gastroenterology. 1992;102(2):493-6.

347. Belknap R, Weis S, Brookens A, Au-Yeung KY, Moon G, DiCarlo L, et al. Feasibility of an ingestible sensor-based system for monitoring adherence to tuberculosis therapy. PloS one. 2013;8(1):e53373.

348. Feinstein AR. On white-coat effects and the electronic monitoring of compliance. Archives of Internal Medicine. 1990;150(7):1377-8.

349. Vik SA, Maxwell CJ, Hogan DB. Measurement, correlates, and health outcomes of medication adherence among seniors. Annals of Pharmacotherapy. 2004;38(2):303-12.

350. Rudd P, Byyny RL, Zachary V, LoVerde ME, Titus C, Mitchell WD, et al. The natural history of medication compliance in a drug trial: Limitations of pill counts. Clinical Pharmacology & Therapeutics. 1989;46(2):169-76.

351. Lee JY, Kusek JW, Greene PG, Bernhard S, Norris K, Smith D, et al. Assessing medication adherence by pill count and electronic monitoring in the African American Study of Kdney disease and hypertension (AASK) pilot study. American Journal of Hypertension. 1996;9(8):719-25.

352. van Onzenoort HAW, Verberk WJ, Kessels AGH, Kroon AA, Neef C, van der Kuy P-HM, et al. Assessing medication adherence simultaneously by electronic monitoring and pill count in patients with mild-to-moderate hypertension. American Journal of Hypertension. 2010;23(2):149-54.

353. Cramer JA, Mattson RH, Prevey ML, Scheyer RD, Ouellette VL. How often is medication taken as prescribed? A novel assessment technique. JAMA. 1989;261(22):3273-7.

354. Urquhart J. The electronic medication event monitor. Clinical Pharmacokinetics. 1997;32(5):345-56.

355. Liu H, Golin CE, Miller LG, Hays RD, Beck CK, Sanandaji S, et al. A comparison study of multiple measures of adherence to HIV protease inhibitors. Annals of Internal Medicine. 2001;134(10):968-77.

356. Turner BJ, Hecht FM. Improving on a coin toss to predict patient adherence to medications. Annals of Internal Medicine. 2001;134(10):1004-6.

357. Williams B, MacDonald TM, Morant S, Webb DJ, Sever P, McInnes G, et al. Spironolactone versus placebo, bisoprolol, and doxazosin to determine the optimal treatment for drug-resistant hypertension (PATHWAY-2): a randomised, double-blind, crossover trial. The Lancet. 2015.

358. Durand H, Hayes P, Morrissey EC, Newell J, Casey M, Murphy AW, et al. Medication adherence among patients with apparent treatment-resistant hypertension: systematic review and meta-analysis. Journal of Hypertension. 2017;35(12):2346-57.

359. Ceral J, Habrdova V, Vorisek V, Bima M, Pelouch R, Solar M. Difficult-to-control arterial hypertension or uncooperative patients? The assessment of serum antihypertensive drug levels to differentiate non-responsiveness from non-adherence to recommended therapy. Hypertension research : official journal of the Japanese Society of Hypertension. 2011;34(1):87-90.

360. Jung O, Gechter JL, Wunder C, Paulke A, Bartel C, Geiger H, et al. Resistant hypertension? Assessment of adherence by toxicological urine analysis. Journal of hypertension. 2013;31(4):766-74.

361. Bunker J, Callister W, Chang CL, Sever PS. How common is true resistant hypertension? Journal of human hypertension. 2011;25(2):137-40.

362. Hameed MA, Tebbit L, Jacques N, Thomas M, Dasgupta I. Non-adherence to antihypertensive medication is very common among resistant hypertensives: results of a directly observed therapy clinic. Journal of human hypertension. 2015.

363. Grassi D, O'Flaherty M, Pellizzari M, Bendersky M, Rodriguez P, Turri D, et al. Hypertensive urgencies in the emergency department: evaluating blood pressure response to rest and to antihypertensive drugs with different profiles. J Clin Hypertens (Greenwich). 2008;10(9):662-7.

364. Sim JJ, Bhandari SK, Shi J, Liu ILA, Calhoun DA, McGlynn EA, et al. Characteristics of resistant hypertension in a large, ethnically diverse hypertension population of an integrated health system. Mayo Clinic Proceedings. 2013;88(10):1099-107.

365. Daugherty SL, Powers JD, Magid DJ, Masoudi FA, Margolis KL, O'Connor PJ, et al. The association between medication adherence and treatment intensification with blood pressure control in resistant hypertension. Hypertension. 2012;60(2):303-9.

366. Rose AJ, Berlowitz DR, Manze M, Orner MB, Kressin NR. Intensifying therapy for hypertension despite suboptimal adherence. Hypertension. 2009;54(3):524-9.

367. Grigoryan L, Pavlik VN, Hyman DJ. Characteristics, drug combinations and dosages of primary care patients with uncontrolled ambulatory blood pressure and high medication adherence. Journal of the American Society of Hypertension. 2013;7(6):471-6.

368. Burnier M, Schneider MP, Chiolero A, Stubi CL, Brunner HR. Electronic compliance monitoring in resistant hypertension: the basis for rational therapeutic decisions. Journal of hypertension. 2001;19(2):335-41.

369. Hayes P, Casey M, Glynn LG, Molloy GJ, Durand H, O'Brien E, et al. Prevalence of treatment-resistant hypertension after considering pseudo-resistance and morbidity: a cross-sectional study in Irish primary care. British Journal of General Practice. 2018;68(671):e394-e400.

370. Lawson A, Brown R, P Cappuccio F, George S, Hinton T, Kapil V, et al. p-37 Non-adherence to antihypertensive medications is related to total pill burden. Journal of Human Hypertension. 2017;31(10):682 (abstract).

371. Gupta P, Patel P, Strauch B, Lai FY, Akbarov A, Maresova V, et al. Risk factors for nonadherence to antihypertensive treatment. Hypertension. 2017;69(6):1113-20.

372. Garg JP, Elliott WJ, Folker A, Izhar M, Black HR. Resistant hypertension revisited: a comparison of two university-based cohorts. American Journal of Hypertension. 2005;18(5):619-26.

373. Yakovlevitch M, Black HR. Resistant hypertension in a tertiary care clinic. Archives of Internal Medicine. 1991;151(9):1786-92.

374. Massierer D, Oliveira ACT, Steinhorst AM, Gus M, Ascoli AM, Gonçalves SC, et al. Prevalence of resistant hypertension in non-elderly adults: prospective study in a clinical setting. Arquivos Brasileiros de Cardiologia. 2012;99:630-5.

375. Brinker S, Pandey A, Ayers C, Price A, Raheja P, Arbique D, et al. Therapeutic drug monitoring facilitates blood pressure control in resistant hypertension. Journal of the American College of Cardiology. 2014;63(8):834-5.

376. Ewen S, Meyer MR, Cremers B, Laufs U, Helfer AG, Linz D, et al. Blood pressure reductions following catheter-based renal denervation are not related to improvements in adherence to antihypertensive drugs measured by urine/plasma toxicological analysis. Clinical Research in Cardiology. 2015;104(12):1097-105.

377. Rosa J, Zelinka T, Petrak O, Strauch B, Somloova Z, Indra T, et al. Importance of thorough investigation of resistant hypertension before renal denervation: should compliance to treatment be evaluated systematically? Journal of human hypertension. 2014;28(11):684-8.

378. Strauch B, Petrak O, Zelinka T, Rosa J, Somloova Z, Indra T, et al. Precise assessment of noncompliance with the antihypertensive therapy in patients with resistant hypertension using toxicological serum analysis. Journal of hypertension. 2013;31(12):2455-61.

379. Beaussier H, Boutouyrie P, Bobrie G, Frank M, Laurent S, Coudoré F, et al. True antihypertensive efficacy of sequential nephron blockade in patients with resistant hypertension and confirmed medication adherence. Journal of Hypertension. 2015;33(12):2526-33.

380. Hameed MA, Dasgupta I, Gill P. Poor adherence to antihypertensive drugs. BMJ. 2016;354:i3268.

381. Gradman AH, Basile JN, Carter BL, Bakris GL. Combination therapy in hypertension. Journal of the American Society of Hypertension. 2010;4(2):90-8.

382. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). European Heart Journal. 2013;34(28):2159-219.

383. Gupta AK, Arshad S, Poulter NR. Compliance, safety, and effectiveness of fixed-dose combinations of antihypertensive agents: a meta-analysis. Hypertension. 2010;55(2):399-407.

384. Verma AA, Khuu W, Tadrous M, Gomes T, Mamdani MM. Fixed-dose combination antihypertensive medications, adherence, and clinical outcomes: a population-based retrospective cohort study. PLOS Medicine. 2018;15(6):e1002584.

385. Webster R, Salam A, de Silva H, et al. Fixed low-dose triple combination antihypertensive medication vs usual care for blood pressure control in patients with mild to moderate hypertension in sri lanka: A randomized clinical trial. JAMA. 2018;320(6):566-79.

386. Reinhold K, Jurgen S, Antonios D. Single-pill triple fixed dose combination therapy with single component drug monitoring in treatment-resistant

hypertension: a pilot study. Current Vascular Pharmacology. 2018;16(2):197-203.

387. McManus RJ, Mant J, Bray EP, Holder R, Jones MI, Greenfield S, et al. Telemonitoring and self-management in the control of hypertension (TASMINH2): a randomised controlled trial. The Lancet. 2010;376(9736):163-72.

388. Tucker KL, Sheppard JP, Stevens R, Bosworth HB, Bove A, Bray EP, et al. Self-monitoring of blood pressure in hypertension: A systematic review and individual patient data meta-analysis. PLOS Medicine. 2017;14(9):e1002389.

389. Ren Y, Yang H, Browning C, Thomas S, Liu M. Therapeutic effects of motivational interviewing on blood pressure control: A meta-analysis of randomized controlled trials. International journal of cardiology. 2014;172(2):509-11.

390. Gupta P, Patel P, Strauch B, Lai FY, Akbarov A, Gulsin GS, et al. Biochemical screening for nonadherence Is associated with blood pressure reduction and improvement in adherence. Hypertension. 2017;70(5):1042-8.

391. Morawski K, Ghazinouri R, Krumme A, et al. Association of a smartphone application with medication adherence and blood pressure control: The medisafe-bp randomized clinical trial. JAMA Internal Medicine. 2018;178(6):802-9.

392. Gong Y, McDonough CW, Wang Z, Hou W, Cooper-DeHoff RM, Langaee TY, et al. Hypertension susceptibility loci and blood pressure response to antihypertensives: results from the pharmacogenomic evaluation of antihypertensive responses study. Circ Cardiovasc Genet. 2012;5(6):686-91.

393. Silva PS, Fontana V, Luizon MR, Lacchini R, Silva WA, Jr., Biagi C, et al. eNOS and BDKRB2 genotypes affect the antihypertensive responses to enalapril. European journal of clinical pharmacology. 2013;69(2):167-77.

394. Yugar-Toledo JC, Martin JF, Krieger JE, Pereira AC, Demacq C, Coelho OR, et al. Gene variation in resistant hypertension: multilocus analysis of the angiotensin 1-converting enzyme, angiotensinogen, and endothelial nitric oxide synthase genes. DNA Cell Biol. 2011;30(8):555-64.

395. Oliveira-Paula GH, Lacchini R, Coeli-Lacchini FB, Junior HM, Tanus-Santos JE. Inducible nitric oxide synthase haplotype associated with hypertension and responsiveness to antihypertensive drug therapy. Gene. 2013;515(2):391-5.

396. Lynch AI, Irvin MR, Davis BR, Ford CE, Eckfeldt JH, Arnett DK. Genetic and Adverse Health Outcome Associations with Treatment Resistant Hypertension in GenHAT. International journal of hypertension. 2013;2013:578578.

397. Fontana V, McDonough CW, Gong Y, El Rouby NM, Sá ACC, Taylor KD, et al. Large-Scale Gene-Centric Analysis Identifies Polymorphisms for

Resistant Hypertension. Journal of the American Heart Association. 2014;3(6).

398. Carey RM, Calhoun DA, Bakris GL, Brook RD, Daugherty SL, Dennison-Himmelfarb CR, et al. Resistant hypertension: detection, evaluation, and management: a scientific statement from the American Heart Association. Hypertension. 2018;72(5):e53-e90.

399. Landsberg L, Aronne LJ, Beilin LJ, Burke V, Igel LI, Lloyd-Jones D, et al. Obesity-related hypertension: pathogenesis, cardiovascular risk, and treatment: a position paper of The Obesity Society and the American Society of Hypertension. J Clin Hypertens (Greenwich). 2013;15(1):14-33.

400. de Souza F, Muxfeldt E, Fiszman R, Salles G. Efficacy of spironolactone therapy in patients with true resistant hypertension. Hypertension. 2010;55(1):147-52.

401. Calhoun DA. Hyperaldosteronism as a common cause of resistant hypertension. Annual Review of Medicine. 2013;64(1):233-47.

402. de la Sierra A, Banegas JR, Oliveras A, Gorostidi M, Segura J, de la Cruz JJ, et al. Clinical differences between resistant hypertensives and patients treated and controlled with three or less drugs. J Hypertens. 2012;30(6):1211-6.

403. Florczak E, Prejbisz A, Szwench-Pietrasz E, Sliwinski P, Bielen P, Klisiewicz A, et al. Clinical characteristics of patients with resistant hypertension: the RESIST-POL study. J Hum Hypertens. 2013;27(11):678-85.

404. Gaddam KK, Nishizaka MK, Pratt-Ubunama MN, Pimenta E, Aban I, Oparil S, et al. Characterization of resistant hypertension: association between resistant hypertension, aldosterone, and persistent intravascular volume expansion. Arch Intern Med. 2008;168(11):1159-64.

405. Pimenta E, Gaddam KK, Oparil S, Aban I, Husain S, Dell'Italia LJ, et al. Effects of dietary sodium reduction on blood pressure in subjects with resistant hypertension: results from a randomized trial. Hypertension. 2009;54(3):475-81.

406. McMahon EJ, Bauer JD, Hawley CM, Isbel NM, Stowasser M, Johnson DW, et al. A randomized trial of dietary sodium restriction in CKD. J Am Soc Nephrol. 2013;24(12):2096-103.

407. Lloberes P, Lozano L, Sampol G, Romero O, Jurado MJ, Rios J, et al. Obstructive sleep apnoea and 24-h blood pressure in patients with resistant hypertension. J Sleep Res. 2010;19(4):597-602.

408. Logan AG, Perlikowski SM, Mente A, Tisler A, Tkacova R, Niroumand M, et al. High prevalence of unrecognized sleep apnoea in drug-resistant hypertension. Journal of Hypertension. 2001;19(12):2271-7.

409. Logan AG, Tkacova R, Perlikowski SM, Leung RS, Tisler A, Floras JS, et al. Refractory hypertension and sleep apnoea: effect of CPAP on blood pressure and baroreflex. Eur Respir J. 2003;21(2):241-7.

410. Muxfeldt ES, Margallo VS, Guimarães GM, Salles GF. Prevalence and associated factors of obstructive sleep apnea in patients with resistant hypertension. American Journal of Hypertension. 2014;27(8):1069-78.

411. Min HJ, Cho YJ, Kim CH, Kim DH, Kim HY, Choi JI, et al. Clinical features of obstructive sleep apnea that determine its high prevalence in resistant hypertension. Yonsei Med J. 2015;56(5):1258-65.

412. Walia HK, Li H, Rueschman M, Bhatt DL, Patel SR, Quan SF, et al. Association of severe obstructive sleep apnea and elevated blood pressure despite antihypertensive medication use. J Clin Sleep Med. 2014;10(8):835-43.

413. Gonçalves SC, Martinez D, Gus M, de Abreu-Silva EO, Bertoluci C, Dutra I, et al. Obstructive sleep apnea and resistant hypertension: a case-control study. Chest. 2007;132(6):1858-62.

414. Pratt-Ubunama MN, Nishizaka MK, Boedefeld RL, Cofield SS, Harding SM, Calhoun DA. Plasma aldosterone is related to severity of obstructive sleep apnea in subjects with resistant hypertension. Chest. 2007;131(2):453-9.

415. Prejbisz A, Florczak E, Klisiewicz A, Dobrowolski P, Janaszek-Sitkowska H, Bieleń P, et al. Relationship between primary aldosteronism and obstructive sleep apnoea, metabolic abnormalities and cardiac structure in patients with resistant hypertension. Endokrynologia Polska. 2013;64(5):363-7.

416. Di Murro A, Petramala L, Cotesta D, Zinnamosca L, Crescenzi E, Marinelli C, et al. Renin-angiotensin-aldosterone system in patients with sleep apnoea: prevalence of primary aldosteronism. Journal of the Renin-Angiotensin-Aldosterone System. 2010;11(3):165-72.

417. Pimenta E, Stowasser M, Gordon RD, Harding SM, Batlouni M, Zhang B, et al. Increased dietary sodium is related to severity of obstructive sleep apnea in patients with resistant hypertension and hyperaldosteronism. Chest. 2013;143(4):978-83.

418. Gonzaga CC, Gaddam KK, Ahmed MI, Pimenta E, Thomas SJ, Harding SM, et al. Severity of obstructive sleep apnea is related to aldosterone status in subjects with resistant hypertension. Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine. 2010;6(4):363-8.

419. Yang L, Zhang H, Cai M, Zou Y, Jiang X, Song L, et al. Effect of spironolactone on patients with resistant hypertension and obstructive sleep apnea. Clinical and Experimental Hypertension. 2016;38(5):464-8.

420. Gaddam K, Pimenta E, Thomas SJ, Cofield SS, Oparil S, Harding SM, et al. Spironolactone reduces severity of obstructive sleep apnoea in patients with resistant hypertension: a preliminary report. Journal of Human Hypertension. 2010;24(8):532-7.

421. Krasinska B, Miazga A, Cofta S, Szczepaniak-Chichel L, Trafas T, Krasinski Z, et al. Effect of eplerenone on the severity of obstructive sleep

apnea and arterial stiffness in patients with resistant arterial hypertension. Pol Arch Med Wewn. 2016;126(5):330-9.

422. Gottlieb DJ, Punjabi NM, Mehra R, Patel SR, Quan SF, Babineau DC, et al. CPAP versus oxygen in obstructive sleep apnea. N Engl J Med. 2014;370(24):2276-85.

423. Martinez-Garcia MA, Capote F, Campos-Rodriguez F, Lloberes P, Diaz de Atauri MJ, Somoza M, et al. Effect of CPAP on blood pressure in patients with obstructive sleep apnea and resistant hypertension: the HIPARCO randomized clinical trial. JAMA. 2013;310(22):2407-15.

424. McEvoy RD, Antic NA, Heeley E, Luo Y, Ou Q, Zhang X, et al. CPAP for prevention of cardiovascular events in obstructive sleep apnea. N Engl J Med. 2016;375(10):919-31.

425. Montesi SB, Edwards BA, Malhotra A, Bakker JP. The effect of continuous positive airway pressure treatment on blood pressure: a systematic review and meta-analysis of randomized controlled trials. Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine. 2012;8(5):587-96.

426. Alajmi M, Mulgrew AT, Fox J, Davidson W, Schulzer M, Mak E, et al. Impact of continuous positive airway pressure therapy on blood pressure in patients with obstructive sleep apnea hypopnea: a meta-analysis of randomized controlled trials. Lung. 2007;185(2):67-72.

427. Bazzano LA, Khan Z, Reynolds K, He J. Effect of nocturnal nasal continuous positive airway pressure on blood pressure in obstructive sleep apnea. Hypertension. 2007;50(2):417-23.

428. Haentjens P, Van Meerhaeghe A, Moscariello A, De Weerdt S, Poppe K, Dupont A, et al. The impact of continuous positive airway pressure on blood pressure in patients with obstructive sleep apnea syndrome: evidence from a meta-analysis of placebo-controlled randomized trials. Arch Intern Med. 2007;167(8):757-64.

429. Grossman A, Messerli FH, Grossman E. Drug induced hypertension – An unappreciated cause of secondary hypertension. European Journal of Pharmacology. 2015;763:15-22.

430. Bisogni V, Rossi GP, Calo LA. Apparent mineralcorticoid excess syndrome, an often forgotten or unrecognized cause of hypokalemia and hypertension: case report and appraisal of the pathophysiology. Blood Press. 2014;23(3):189-92.

431. Denolle T, Azizi M, Massart C, Zennaro MC. [Itraconazole: a new drug-related cause of hypertension]. Ann Cardiol Angeiol (Paris). 2014;63(3):213-5.

432. Attard G, Reid AH, Auchus RJ, Hughes BA, Cassidy AM, Thompson E, et al. Clinical and biochemical consequences of CYP17A1 inhibition with abiraterone given with and without exogenous glucocorticoids in castrate men with advanced prostate cancer. J Clin Endocrinol Metab. 2012;97(2):507-16.

433. Pechere-Bertschi A, Maillard M, Stalder H, Bischof P, Fathi M, Brunner HR, et al. Renal hemodynamic and tubular responses to salt in women using oral contraceptives. Kidney Int. 2003;64(4):1374-80.

434. Hussain SF. Progestogen-only pills and high blood pressure: is there an association? A literature review. Contraception. 2004;69(2):89-97.

435. Affinito P, Palomba S, Bonifacio M, Fontana D, Izzo R, Trimarco B, et al. Effects of hormonal replacement therapy in postmenopausal hypertensive patients. Maturitas. 2001;40(1):75-83.

436. Hoorn EJ, Walsh SB, McCormick JA, Zietse R, Unwin RJ, Ellison DH. Pathogenesis of calcineurin inhibitor-induced hypertension. Journal of nephrology. 2012;25(3):269-75.

437. Milan A, Puglisi E, Ferrari L, Bruno G, Losano I, Veglio F. Arterial hypertension and cancer. Int J Cancer. 2014;134(10):2269-77.

438. Armstrong EP, Malone DC. The impact of nonsteroidal anti-inflammatory drugs on blood pressure, with an emphasis on newer agents. Clin Ther. 2003;25(1):1-18.

439. Sowers JR, White WB, Pitt B, Whelton A, Simon LS, Winer N, et al. The Effects of cyclooxygenase-2 inhibitors and nonsteroidal anti-inflammatory therapy on 24-hour blood pressure in patients with hypertension, osteoarthritis, and type 2 diabetes mellitus. Arch Intern Med. 2005;165(2):161-8.

440. Crane HM, Van Rompaey SE, Kitahata MM. Antiretroviral medications associated with elevated blood pressure among patients receiving highly active antiretroviral therapy. AIDS. 2006;20(7):1019-26.

441. 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. The Lancet. 2008;371(9628):1921-6.

442. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The management of primary aldosteronism: case detection, diagnosis, and treatment: an Endocrine Society clinical practice guideline. The Journal of Clinical Endocrinology & Metabolism. 2016;101(5):1889-916.

443. Lim PO, Dow E, Brennan G, Jung RT, MacDonald TM. High prevalence of primary aldosteronism in the Tayside hypertension clinic population. J Hum Hypertens. 2000;14(5):311-5.

444. Loh K-C, Koay ES, Khaw M-C, Emmanuel SC, Young WF, Jr. Prevalence of primary aldosteronism among Asian hypertensive patients in Singapore. The Journal of Clinical Endocrinology & Metabolism. 2000;85(8):2854-9.

445. Gordon RD, Stowasser M, Tunny TJ, Klemm SA, Rutherford JC. High incidence of primary aldosteronism in 199 patients referred with hypertension. Clin Exp Pharmacol Physiol. 1994;21(4):315-8.

446. Rayner BL, Opie LH, Davidson JS. The aldosterone/renin ratio as a screening test for primary aldosteronism. South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde. 2000;90(4):394-400.

447. Gallay BJ, Ahmad S, Xu L, Toivola B, Davidson RC. Screening for primary aldosteronism without discontinuing hypertensive medications: Plasma aldosterone-renin ratio. American Journal of Kidney Diseases. 2001;37(4):699-705.

448. Williams B, MacDonald TM, Morant SV, Webb DJ, Sever P, McInnes GT, et al. Endocrine and haemodynamic changes in resistant hypertension, and blood pressure responses to spironolactone or amiloride: the PATHWAY-2 mechanisms substudies. The Lancet Diabetes & Endocrinology. 2018;6(6):464-75.

449. Kuznik A, Mardekian J, Tarasenko L. Evaluation of cardiovascular disease burden and therapeutic goal attainment in US adults with chronic kidney disease: an analysis of national health and nutritional examination survey data, 2001–2010. BMC Nephrology. 2013;14(1):132.

450. Muntner P, Anderson A, Charleston J, Chen Z, Ford V, Makos G, et al. Hypertension awareness, treatment, and control in adults with CKD: results from the Chronic Renal Insufficiency Cohort (CRIC) study. American Journal of Kidney Diseases. 2010;55(3):441-51.

451. Thomas G, Xie D, Chen HY, Anderson AH, Appel LJ, Bodana S, et al. Prevalence and prognostic significance of apparent treatment resistant hypertension in chronic kidney disease: report from the Chronic Renal Insufficiency Cohort Study. Hypertension. 2016;67(2):387-96.

452. Koomans HA, Roos JC, Boer P, Geyskes GG, Mees EJ. Salt sensitivity of blood pressure in chronic renal failure. Evidence for renal control of body fluid distribution in man. Hypertension. 1982;4(2):190-7.

453. Lorenz EC, Vrtiska TJ, Lieske JC, Dillon JJ, Stegall MD, Li X, et al. Prevalence of renal artery and kidney abnormalities by computed tomography among healthy adults. Clin J Am Soc Nephrol. 2010;5(3):431-8.

454. Hansen KJ, Edwards MS, Craven TE, Cherr GS, Jackson SA, Appel RG, et al. Prevalence of renovascular disease in the elderly: a population-based study. J Vasc Surg. 2002;36(3):443-51.

455. Benjamin MM, Fazel P, Filardo G, Choi JW, Stoler RC. Prevalence of and risk factors of renal artery stenosis in patients with resistant hypertension. Am J Cardiol. 2014;113(4):687-90.

456. Safian RD, Textor SC. Renal-artery stenosis. New England Journal of Medicine. 2001;344(6):431-42.

457. Herrmann SM, Textor SC. Current concepts in the treatment of renovascular hypertension. Am J Hypertens. 2018;31(2):139-49.

458. Astral Investigators, Wheatley K, Ives N, Gray R, Kalra PA, Moss JG, et al. Revascularization versus medical therapy for renal-artery stenosis. N Engl J Med. 2009;361(20):1953-62.

459. Bax L, Woittiez AJ, Kouwenberg HJ, Mali WP, Buskens E, Beek FJ, et al. Stent placement in patients with atherosclerotic renal artery stenosis and impaired renal function: a randomized trial. Ann Intern Med. 2009;150(12):840-8, W150-1.

460. Cooper CJ, Murphy TP, Cutlip DE, Jamerson K, Henrich W, Reid DM, et al. Stenting and medical therapy for atherosclerotic renal-artery stenosis. N Engl J Med. 2014;370(1):13-22.

461. Stein PP, Black HR. A simplified diagnostic approach to pheochromocytoma. A review of the literature and report of one institution's experience. Medicine (Baltimore). 1991;70(1):46-66.

462. Pacak K, Linehan WM, Eisenhofer G, Walther MM, Goldstein DS. Recent advances in genetics, diagnosis, localization, and treatment of pheochromocytoma. Ann Intern Med. 2001;134(4):315-29.

463. Martell N, Rodriguez-Cerrillo M, Grobbee DE, LÓPez-Eady MD, FernÁndez-Pinilla C, Avila M, et al. High prevalence of secondary hypertension and insulin resistance in patients with refractory hypertension. Blood Pressure. 2003;12(3):149-54.

464. Lenders JWM, Eisenhofer G. Pathophysiology and Diagnosis of Disorders of the Adrenal Medulla: Focus on Pheochromocytoma. In: Terjung R, editor. Comprehensive Physiology. 4. Hoboken, NJ: John Wiley & Sons, Inc.; 2014. p. 691-713.

465. Freis ED. Origins and development of antihypertensive treatment. In: Laragh GH, Brenner BM, editors. Hypertension: Pathophysiology, Diagnosis, and Management. Second ed. New York: Raven Press; 1990. p. 2093-4.

466. Peet MM. Results of bilateral supradiaphragmatic splanchnicectomy for arterial hypertension. New England Journal of Medicine. 1947;236(8):270-7.

467. Smithwick RH, Thompson JE. Splanchnicectomy for essential hypertension; results in 1,266 cases. Journal of the American Medical Association. 1953;152(16):1501-4.

468. Lyons RH, Moe GK, Neligh RB, Hoobler SW, Campbell KN, Berry RL, et al. The effects of blockade of the autonomic ganglia in man with tetraethylammonium. Preliminary observations on its clinical application. Am J M Sc. 1947;213:315-23.

469. Paton WDM, Zaimis EJ. Clinical potentialities of certain bisquaternary salts causing neuromuscular and ganglionic block. Nature. 1948;162:810.

470. Oates JA, Gillespie L, Udenfriend S, Sjoerdsma A. Decarboxylase inhibition and blood pressure reduction by alpha-methyl-3,4-dihydroxy-DL-phenylalanine. Science (New York, NY). 1960;131(3417):1890-1.

471. Prichard BN, Gillam PM. Use of propranolol (Inderal) in treatment of hypertension. BMJ. 1964;2(5411):725-7.

472. Nash DT. Alpha-adrenergic blockers: mechanism of action, blood pressure control, and effects on lipoprotein metabolism. Clinical Cardiology. 1990;13(11):764-72.

473. Heran BS, Galm BP, Wright JM. Blood pressure lowering efficacy of alpha blockers for primary hypertension. The Cochrane database of systematic reviews. 2012(8):Cd004643.

474. Zazgornik J, Biesenbach G, Janko O, Gross C, Mair R, Brucke P, et al. Bilateral nephrectomy: the best, but often overlooked, treatment for refractory hypertension in hemodialysis patients. Am J Hypertens. 1998;11(11 Pt 1):1364-70.

475. Krum H, Schlaich M, Whitbourn R, Sobotka PA, Sadowski J, Bartus K, et al. Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. The Lancet. 2009;373(9671):1275-81.

476. Schlaich MP, Sobotka PA, Krum H, Lambert E, Esler MD. Renal sympathetic-nerve ablation for uncontrolled hypertension. N Engl J Med. 2009;361(9):932-4.

477. Krum H, Schlaich MP, Sobotka PA, Böhm M, Mahfoud F, Rocha-Singh K, et al. Percutaneous renal denervation in patients with treatment-resistant hypertension: final 3-year report of the Symplicity HTN-1 study. The Lancet. 2014;383(9917):622-9.

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

479. Davis MI, Filion KB, Zhang D, Eisenberg MJ, Afilalo J, Schiffrin EL, et al. Effectiveness of renal denervation therapy for resistant hypertension: a systematic review and meta-analysis. Journal of the American College of Cardiology. 2013;62(3):231-41.

480. Bhatt DL, Kandzari DE, O'Neill WW, D'Agostino R, Flack JM, Katzen BT, et al. A controlled trial of renal denervation for resistant hypertension. N Engl J Med. 2014;370(15):1393-401.

481. Kandzari DE, Bhatt DL, Brar S, Devireddy CM, Esler M, Fahy M, et al. Predictors of blood pressure response in the SYMPLICITY HTN-3 trial. European Heart Journal. 2015;36(4):219-27.

482. Lobo MD, de Belder MA, Cleveland T, Collier D, Dasgupta I, Deanfield J, et al. Joint UK societies' 2014 consensus statement on renal denervation for resistant hypertension. Heart. 2015;101(1):10-6.

483. Böhm M, Mahfoud F, Ukena C, Hoppe UC, Narkiewicz K, Negoita M, et al. First report of the global SYMPLICITY registry on the effect of renal artery denervation in patients with uncontrolled hypertension. Hypertension. 2015;65(4):766-74.

484. Azizi M, Sapoval M, Gosse P, Monge M, Bobrie G, Delsart 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. The Lancet. 2015;385(9981):1957-65.

485. Rosa J, Widimský P, Toušek P, Petrák O, Curila K, Waldauf P, et al. Randomized comparison of renal denervation versus intensified pharmacotherapy including spironolactone in true-resistant hypertension:

six-month results from the Prague-15 study. Hypertension. 2015;65(2):407-13.

486. Sharp AS, Davies JE, Lobo MD, Bent CL, Mark PB, Burchell AE, et al. Renal artery sympathetic denervation: observations from the UK experience. Clinical research in cardiology : official journal of the German Cardiac Society. 2016;105(6):544-52.

487. Worthley SG, Tsioufis CP, Worthley MI, Sinhal A, Chew DP, Meredith IT, et al. Safety and efficacy of a multi-electrode renal sympathetic denervation system in resistant hypertension: the EnligHTN I trial. European Heart Journal. 2013;34(28):2132-40.

488. Worthley SG, Wilkins GT, Webster MW, Montarello JK, Antonis PR, Whitbourn RJ, et al. Safety and performance of the next generation EnligHTN™ renal denervation system in patients with drug-resistant, uncontrolled hypertension: The EnligHTN III first-in-human multicentre study. Clinical Trials and Regulatory Science in Cardiology. 2015;8:4-10.

489. Lobo M, Saxena M, Jain AJ, Walters D, Pincus M, Montarello J, et al. 4a.09: Safety and performance of the ENLIGHTN renal denervation system in patients with severe uncontrolled hypertension: 12 month results from the ENLIGHTN II Study. Journal of hypertension. 2015;33 Suppl 1:e51.

490. Townsend RR, Mahfoud F, Kandzari DE, Kario K, Pocock S, Weber MA, et al. Catheter-based renal denervation in patients with uncontrolled hypertension in the absence of antihypertensive medications (SPYRAL HTN-OFF MED): a randomised, sham-controlled, proof-of-concept trial. The Lancet. 2017;390(10108):2160-70.

491. Kandzari DE, Böhm M, Mahfoud F, Townsend RR, Weber MA, Pocock S, et al. Effect of renal denervation on blood pressure in the presence of antihypertensive drugs: 6-month efficacy and safety results from the SPYRAL HTN-ON MED proof-of-concept randomised trial. The Lancet. 2018;391(10137):2346-55.

492. Azizi M, Schmieder RE, Mahfoud F, Weber MA, Daemen J, Davies J, et al. Endovascular ultrasound renal denervation to treat hypertension (RADIANCE-HTN SOLO): a multicentre, international, single-blind, randomised, sham-controlled trial. The Lancet. 2018;391(10137):2335-45.

493. Lawson AJ, Shipman KE, George S, Dasgupta I. A novel 'dilute-and-shoot' liquid chromatography - tandem mass spectrometry method for the screening of antihypertensive drugs in urine. Journal of analytical toxicology. 2016;40(1):17-27.

494. Tomaszewski M, White C, Patel P, Masca N, Damani R, Hepworth J, 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(11):855-61.

495. Dahal K, Kunwar S, Rijal J, Alqatahni F, Panta R, Ishak N, et al. The effects of aldosterone antagonists in patients with resistant hypertension: a meta-analysis of randomized and nonrandomized studies. American Journal of Hypertension. 2015;28(11):1376-85.

496. Warren RE, Marshall T, Padfield PL, Chrubasik S. Variability of office, 24-hour ambulatory, and self-monitored blood pressure measurements. The British journal of general practice : the journal of the Royal College of General Practitioners. 2010;60(578):675-80.

497. Sakakura K, Ladich E, Cheng Q, Otsuka F, Yahagi K, Fowler DR, et al. Anatomic assessment of sympathetic peri-arterial renal nerves in man. Journal of the American College of Cardiology. 2014;64(7):635-43.

498. Esler M, Jennings G, Korner P, Blombery P, Sacharias N, Leonard P. Measurement of total and organ-specific norepinephrine kinetics in humans. American Journal of Physiology-Endocrinology and Metabolism. 1984;247(1):E21-E8.

499. Esler M, Jennings G, Leonard P, Sacharias N, Burke F, Johns J, et al. Contribution of individual organs to total noradrenaline release in humans. Acta Physiol Scand Suppl. 1984;527:11-6.

500. Vallbo AB, Hagbarth KE, Torebjork HE, Wallin BG. Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. Physiological Reviews. 1979;59(4):919-57.

501. Zuern CS, Eick C, Rizas KD, Bauer S, Langer H, Gawaz M, et al. Impaired cardiac baroreflex sensitivity predicts response to renal sympathetic denervation in patients with resistant hypertension. Journal of the American College of Cardiology. 2013;62(22):2124-30.

502. Dorr O, Liebetrau C, Mollmann H, Gaede L, Troidl C, Rixe J, et al. Soluble fms-like tyrosine kinase-1 and endothelial adhesion molecules (intercellular cell adhesion molecule-1 and vascular cell adhesion molecule-1) as predictive markers for blood pressure reduction after renal sympathetic denervation. Hypertension. 2014;63(5):984-90.

503. Brinton TJ, Anderson T, Zhang J, Gertner ME. Ultrasound mediated renal sympathetic denervation. Circulation. 2011;124:A12272 (Abstract).

504. Neuzil P, Whitbourn RJ, Starek Z, Esler MD, Brinton T, Gertner M. TCT-61 Optimized external focused ultrasound for renal sympathetic denervation - WAVE II trial. Journal of the American College of Cardiology. 2013;62(18 Supplement 1):B20.

505. Ormiston JA, Anderson T, Brinton TJ, Dawood O, Gertner M, Kay P, et al. TCT-412 Non-invasive renal denervation using externally delivered focused ultrasound: early experience using doppler based imaging tracking and targeting for treatment. Journal of the American College of Cardiology. 2014;64(11 Supplement):B121.

506. Trazzi S, Mutti E, Frattola A, Imholz B, Parati G, Mancia G. Reproducibility of non-invasive and intra-arterial blood pressure monitoring: implications for studies on antihypertensive treatment. Journal of hypertension. 1991;9(2):115-9.

507. Prisant LM, Carr AA, Bottini PB, Thompson WO, Rhoades RB. Repeatability of automated ambulatory blood pressure measurements. The Journal of family practice. 1992;34(5):569-74.

508. Staessen JA, Wang J-G, Thijs L. Cardiovascular protection and blood pressure reduction: a meta-analysis. The Lancet. 2001;358(9290):1305-15.

509. Box GEP, Tidwell PW. Transformation of the Independent Variables. Technometrics. 1962;4(4):531-50.

510. Tabachnick BG, Fidell LS. Using Multivariate Statistics. 6th ed. New York: Pearson; 2013.

511. Swain S, Hariharan M, Rana S, Chivukula U, Thomas M. Doctor-patient communication: impact on adherence and prognosis among patients with primary hypertension. Psychological Studies. 2015;60(1):25-32.

512. Monane M, Bohn RL, Gurwitz JH, Glynn RJ, Levin R, Avorn J. Compliance with antihypertensive therapy among elderly Medicaid enrollees: the roles of age, gender, and race. Am J Public Health. 1996;86(12):1805-8.

513. Degli Esposti L, Saragoni S, Benemei S, Batacchi P, Geppetti P, Di Bari M, et al. Adherence to antihypertensive medications and health outcomes among newly treated hypertensive patients. ClinicoEconomics and Outcomes Research. 2011;3:47-54.

514. Ross S, Walker A, MacLeod MJ. Patient compliance in hypertension: role of illness perceptions and treatment beliefs. Journal Of Human Hypertension. 2004;18:607.

515. Thunander Sundbom L, Bingefors K. Women and men report different behaviours in, and reasons for medication non-adherence: a nationwide Swedish survey. Pharmacy practice. 2012;10(4):207-21.

516. Holt E, Joyce C, Dornelles A, Morisky D, Webber LS, Muntner P, et al. Sex differences in barriers to antihypertensive medication adherence: findings from the cohort study of medication adherence among older adults. J Am Geriatr Soc. 2013;61(4):558-64.

517. Maguire LK, Hughes CM, McElnay JC. Exploring the impact of depressive symptoms and medication beliefs on medication adherence in hypertension—A primary care study. Patient Education and Counseling. 2008;73(2):371-6.

518. Morrison VL, Holmes EAF, Parveen S, Plumpton CO, Clyne W, De Geest S, et al. Predictors of self-reported adherence to antihypertensive medicines: a multinational, cross-sectional survey. Value in Health. 2015;18(2):206-16.

519. Yassine M, Al-Hajje A, Awada S, Rachidi S, Zein S, Bawab W, et al. Evaluation of medication adherence in Lebanese hypertensive patients. Journal of Epidemiology and Global Health. 2016;6(3):157-67.

520. Kubo MN, Kayima JK, Were AJ, McLigeyo SO, Ogola EN. Factors associated with uncontrolled hypertension among renal transplant recipients attending nephrology clinics in Nairobi, Kenya. Journal of transplantation. 2015;2015:746563-.

521. Ramli A, Ahmad NS, Paraidathathu T. Medication adherence among hypertensive patients of primary health clinics in Malaysia. Patient preference and adherence. 2012;6:613-22.

522. Cummings DM, Wu J-R, Cene C, Halladay J, Donahue KE, Hinderliter A, et al. Perceived social standing, medication nonadherence, and systolic blood pressure in the rural south. The Journal of Rural Health. 2016;32(2):156-63.

523. McNaughton CD, Brown NJ, Rothman RL, Liu D, Kabagambe EK, Levy PD, et al. Systolic blood pressure and biochemical assessment of adherence: a cross-sectional analysis in the emergency department. Hypertension. 2017;70(2):307-14.

524. Schroeder K, Fahey T, Ebrahim S. Interventions for improving adherence to treatment in patients with high blood pressure in ambulatory settings. The Cochrane database of systematic reviews. 2004(2):CD004804.

525. Kronish IM, Woodward M, Sergie Z, Ogedegbe G, Falzon L, Mann DM. Meta-analysis: impact of drug class on adherence to antihypertensives. Circulation. 2011;123(15):1611-21.

526. Meredith PA, Elliott HL. Clinical pharmacokinetics of amlodipine. Clin Pharmacokinet. 1992;22(1):22-31.

527. Lawson AJ, Hameed MA, Brown R, Cappuccio FP, George S, Hinton T, et al. Nonadherence to antihypertensive medications is related to pill burden in apparent treatment-resistant hypertensive individuals. Journal of Hypertension. 2020;38(6):1165-73.

528. Gebreyohannes EA, Bhagavathula AS, Abebe TB, Tefera YG, Abegaz TM. Adverse effects and non-adherence to antihypertensive medications in University of Gondar Comprehensive Specialized Hospital. Clinical Hypertension. 2019;25(1):1.

529. Savage RD, Visentin JD, Bronskill SE, Wang X, Gruneir A, Giannakeas V, et al. Evaluation of a common prescribing cascade of calcium channel blockers and diuretics in older adults with hypertension. JAMA Internal Medicine. 2020;180(5):643-51.

530. Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. Rang & Dale's Pharmacology. 7 ed. Edinburgh: Churchill Livingstone; 2012.

531. Seideman P, Grahnén A, Haglund K, Lindström B, Von Bahr C. Prazosin first dose phenomenon during combined treatment with a beta-adrenoceptor blocker in hypertensive patients. Br J Clin Pharmacol. 1982;13(6):865-70.

532. Sica DA. Dosage considerations with perindopril for systemic hypertension. The American Journal of Cardiology. 2001;88(7, Supplement 1):13-8.

533. Vítovec J, Špinar J, Czech, Group SI. First-dose hypotension after angiotensin-converting enzyme (ACE) inhibitors in chronic heart failure: a comparison of enalapril and perindopril. European Journal of Heart Failure. 2000;2(3):299-304.

534. Fadl Elmula FEM, Hoffmann P, Fossum E, Brekke M, Gjønnæss E, Hjørnholm U, et al. Renal sympathetic denervation in patients with treatment-resistant hypertension after witnessed intake of medication

before qualifying ambulatory blood pressure. Hypertension. 2013;62(3):526-32.

535. Fadl Elmula FEM, Hoffmann P, Larstorp AC, Fossum E, Brekke M, Kjeldsen SE, et al. Adjusted drug treatment is superior to renal sympathetic denervation in patients with true treatment-resistant hypertension. Hypertension. 2014;63(5):991-9.

536. Burnier M, Wuerzner G, Struijker-Boudier H, Urquhart J. Measuring, analyzing, and managing drug adherence in resistant hypertension. Hypertension. 2013;62(2):218-25.

537. Christensen A, Osterberg LG, Hansen EH. Electronic monitoring of patient adherence to oral antihypertensive medical treatment: a systematic review. Journal of hypertension. 2009;27(8):1540-51.

538. Whelton PK, He J, Appel LJ, Cutler JA, Havas S, Kotchen TA, et al. Primary prevention of hypertension: clinical and public health advisory from The National High Blood Pressure Education Program. JAMA. 2002;288(15):1882-8.

539. MacMahon S, Peto R, Collins R, Godwin J, MacMahon S, Cutler J, et al. Blood pressure, stroke, and coronary heart disease: Part 1, prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. The Lancet. 1990;335(8692):765-74.

540. Morton V, Torgerson DJ. Effect of regression to the mean on decision making in health care. BMJ. 2003;326(7398):1083-4.

541. 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. Hypertension. 2015;65(2):401-6.

542. Moore MN, Atkins ER, Salam A, Callisaya ML, Hare JL, Marwick TH, et al. Regression to the mean of repeated ambulatory blood pressure monitoring in five studies. Journal of Hypertension. 2019;37(1):24-9.

543. Chadachan VM, Ye MT, Tay JC, Subramaniam K, Setia S. Understanding short-term blood-pressure-variability phenotypes: from concept to clinical practice. Int J Gen Med. 2018;11:241-54.

544. James GD. The adaptive value and clinical significance of allostatic blood pressure variation. Curr Hypertens Rev. 2019;15(2):93-104.

545. Mena LJ, Maestre GE, Hansen TW, Thijs L, Liu Y, Boggia J, et al. How many measurements are needed to estimate blood pressure variability without loss of prognostic information? American journal of hypertension. 2014;27(1):46-55.

546. National Institute for Health and Care Excellence. Hypertension in adults: diagnosis and management [internet] London: NICE; 2019 [Available from: https://www.nice.org.uk/guidance/ng136.

547. Musso NR, Vergassola C, Barone C, Lotti G. Ambulatory blood pressure monitoring: how reproducible is it? American Journal of Hypertension. 1997;10(8):936-9.

548. Prasad N, MacFadyen RJ, Ogston SA, MacDonald TM. Elevated blood pressure during the first two hours of ambulatory blood pressure monitoring: a study comparing consecutive twenty-four-hour monitoring periods. J Hypertens. 1995;13(3):291-5.

549. Wetzels GE, Nelemans P, Schouten JS, Prins MH. Facts and fiction of poor compliance as a cause of inadequate blood pressure control: a systematic review. Journal of hypertension. 2004;22(10):1849-55.

550. Wetzels GEC, Nelemans PJ, Schouten JSAG, Dirksen CD, van der Weijden T, Stoffers HEJH, et al. Electronic monitoring of adherence as a tool to improve blood pressure control: a randomized controlled trial. American Journal of Hypertension. 2007;20(2):119-25.

551. Easthall C, Song F, Bhattacharya D. A meta-analysis of cognitive-based behaviour change techniques as interventions to improve medication adherence. BMJ Open. 2013;3(Generic):e002749.

552. Weir MR, Hsueh WA, Nesbitt SD, Littlejohn TJ, Graff A, Shojaee A, et al. A titrate-to-goal study of switching patients uncontrolled on antihypertensive monotherapy to fixed-dose combinations of amlodipine and olmesartan medoxomil ± hydrochlorothiazide. The Journal of Clinical Hypertension. 2011;13(6):404-12.

553. Mahmoodi BK, Matsushita K, Woodward M, Blankestijn PJ, Cirillo M, Ohkubo T, et al. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without hypertension: a meta-analysis. The Lancet. 2012;380(9854):1649-61.

554. Sag AA, Covic A, London G, Vervloet M, Goldsmith D, Gorriz JL, et al. Clinical imaging of vascular disease in chronic kidney disease. International urology and nephrology. 2016;48(6):827-37.

555. Bakris GL, Williams M, Dworkin L, Elliott WJ, Epstein M, Toto R, et al. Preserving renal function in adults with hypertension and diabetes: a consensus approach. American Journal of Kidney Diseases. 2000;36(3):646-61.

556. Grassi G, Quarti-Trevano F, Seravalle G, Arenare F, Volpe M, Furiani S, et al. Early sympathetic activation in the initial clinical stages of chronic renal failure. Hypertension. 2011;57(4):846-51.

557. Zoccali C, Mallamaci F, Parlongo S, Cutrupi S, Benedetto FA, Tripepi G, et al. Plasma norepinephrine predicts survival and incident cardiovascular events in patients with end-stage renal disease. Circulation. 2002;105(11):1354-9.

558. Hering D, Mahfoud F, Walton AS, Krum H, Lambert GW, Lambert EA, et al. Renal denervation in moderate to severe CKD. Journal of the American Society of Nephrology. 2012;23(7):1250-7.

559. Kiuchi MG, Maia GL, de Queiroz Carreira MA, Kiuchi T, Chen S, Andrea BR, et al. Effects of renal denervation with a standard irrigated cardiac ablation catheter on blood pressure and renal function in patients with chronic kidney disease and resistant hypertension. European Heart Journal. 2013;34(28):2114-21.

560. Kiuchi MG, Chen S, Andrea BR, Kiuchi T, Carreira MA, Graciano ML, et al. Renal sympathetic denervation in patients with hypertension and chronic kidney disease: does improvement in renal function follow blood pressure control? The Journal of Clinical Hypertension. 2014;16(11):794-800.

561. Ott C, Mahfoud F, Schmid A, Ewen S, Toennes SW, Meyer MR, et al. The effect of renal denervation in moderate treatment-resistant hypertension with confirmed medication adherence. Journal of hypertension. 2016;34(12):2475-9.

562. Hering D, Marusic P, Duval J, Sata Y, Head GA, Denton KM, et al. Effect of renal denervation on kidney function in patients with chronic kidney disease. International journal of cardiology. 2017;232:93-7.

563. Moos JM, Ham SW, Han SM, Lew WK, Hua HT, Hood DB, et al. Safety of carbon dioxide digital subtraction angiography. Archives of surgery (Chicago, Ill: 1960). 2011;146(12):1428-32.

564. Kawasaki D, Fujii K, Fukunaga M, Fukuda N, Masuyama T, Ohkubo N, et al. Safety and efficacy of carbon dioxide and intravascular ultrasound-guided stenting for renal artery stenosis in patients with chronic renal insufficiency. Angiology. 2015;66(3):231-6.

565. Fujihara M, Kawasaki D, Shintani Y, Fukunaga M, Nakama T, Koshida R, et al. Endovascular therapy by CO2 angiography to prevent contrast-induced nephropathy in patients with chronic kidney disease: a prospective multicenter trial of CO2 angiography registry. Catheterization and cardiovascular interventions : official journal of the Society for Cardiac Angiography & Interventions. 2015;85(5):870-7.

566. Schreier DZ, Weaver FA, Frankhouse J, Papanicolaou G, Shore E, Yellin AE, et al. A prospective study of carbon dioxide-digital subtraction vs standard contrast arteriography in the evaluation of the renal arteries. Arch Surg. 1996;131(5):503-7.

567. Renton M, Hameed MA, Dasgupta I, Hoey ET, Freedman J, Ganeshan A. The use of carbon dioxide angiography for renal sympathetic denervation: a technical report. The British journal of radiology. 2016;89(1068):20160311.

568. Kawasaki D, Fujii K, Fukunaga M, Masutani M, Nakata A, Masuyama T. Safety and efficacy of endovascular therapy with a simple homemade carbon dioxide delivery system in patients with ileofemoral artery diseases. Circulation Journal. 2012;76(7):1722-8.

569. Kyung Jae C. Carbon dioxide angiography: scientific principles and practice. Vascular Specialist International. 2015;31(3):67-80.

570. Kerns SR, Hawkins IF. Carbon dioxide digital subtraction angiography: expanding applications and technical evolution. American Journal of Roentgenology. 1995;164(3):735-41.

571. Caridi JG, Hawkins Jr IF. CO2 digital subtraction angiography: potential complications and their prevention. Journal of Vascular and Interventional Radiology. 1997;8(3):383-91.

572. Hawkins IF, Caridi JG. Carbon dioxide ($CO_2$) digital subtraction angiography: 26-year experience at the University of Florida. European radiology. 1998;8(3):391-402.

573. Ghumman SS, Weinerman J, Khan A, Cheema MS, Garcia M, Levin D, et al. Contrast induced-acute kidney injury following peripheral angiography with carbon dioxide versus iodinated contrast media: A meta-analysis and systematic review of current literature. Catheter Cardiovasc Interv. 2017;90(3):437-48.

574. Nijssen EC, Rennenberg RJ, Nelemans PJ, Essers BA, Janssen MM, Vermeeren MA, et al. Prophylactic hydration to protect renal function from intravascular iodinated contrast material in patients at high risk of contrast-induced nephropathy (AMACING): a prospective, randomised, phase 3, controlled, open-label, non-inferiority trial. The Lancet. 2017;389(10076):1312-22.

575. Kiuchi MG, Graciano ML, Carreira MA, Kiuchi T, Chen S, Lugon JR. Long-term effects of renal sympathetic denervation on hypertensive patients with mild to moderate chronic kidney disease. Journal of clinical hypertension (Greenwich, Conn). 2016;18(3):190-6.

576. Kandzari DE, Bhatt DL, Sobotka PA, O'Neill WW, Esler M, Flack JM, et al. Catheter-based renal denervation for resistant hypertension: rationale and design of the SYMPLICITY HTN-3 Trial. Clin Cardiol. 2012;35(9):528-35.

577. Mojón A, Ayala DE, Piñeiro L, Otero A, Crespo JJ, Moyá A, et al. Comparison of ambulatory blood pressure parameters of hypertensive patients with and without chronic kidney disease. Chronobiology International. 2013;30(1-2):145-58.

578. Wright JM, Mattu GS, Perry TL, Jr., Gelferc ME, Strange KD, Zorn A, et al. Validation of a new algorithm for the BPM-100 electronic oscillometric office blood pressure monitor. Blood pressure monitoring. 2001;6(3):161-5.

579. Beckett L, Godwin M. The BpTRU automatic blood pressure monitor compared to 24 hour ambulatory blood pressure monitoring in the assessment of blood pressure in patients with hypertension. BMC Cardiovascular Disorders. 2005;5(1):18.

580. Myers MG, Valdivieso M, Chessman M, Kiss A. Can sphygmomanometers designed for self-measurement of blood pressure in the home be used in office practice? Blood pressure monitoring. 2010;15(6):300-4.

581. Godwin M, Birtwhistle R, Delva D, Lam M, Casson I, MacDonald S, et al. Manual and automated office measurements in relation to awake ambulatory blood pressure monitoring. Family Practice. 2011;28(1):110-7.

582. Myers MG, Godwin M, Dawes M, Kiss A, Tobe SW, Grant FC, et al. Conventional versus automated measurement of blood pressure in primary care patients with systolic hypertension: randomised parallel design controlled trial. BMJ. 2011;342:d286.

583. Little P, Barnett J, Barnsley L, Marjoram J, Fitzgerald-Barron A, Mant D. Comparison of acceptability of and preferences for different methods of measuring blood pressure in primary care. BMJ. 2002;325(7358):258-9.

584. British and Irish Hypertension Society. Blood pressure measurement: using automated blood pressure monitors 2017 [Available from: https://bihsoc.org/wp-content/uploads/2017/11/BP-Measurement-Poster-Automated-2017.pdf.

585. Campbell BR, D., Ross C, Eggett CJ. Clinical guidelines by consensus: recording a standard 12-lead electrocardiogram. an approved method by the Society for Cardiological Science and Technology (SCST) 2017. Available from: http://www.scst.org.uk/resources/SCST_ECG_Recording_Guidelines_2017.

586. Casale PN, Devereux RB, Alonso DR, Campo E, Kligfield P. Improved sex-specific criteria of left ventricular hypertrophy for clinical and computer interpretation of electrocardiograms: validation with autopsy findings. Circulation. 1987;75(3):565-72.

587. Molloy TJ, Okin PM, Devereux RB, Kligfield P. Electrocardiographic detection of left ventricular hypertrophy by the simple QRS voltage-duration product. J Am Coll Cardiol. 1992;20(5):1180-6.

588. Moissl UM, Wabel P, Chamney PW, Bosaeus I, Levin NW, Bosy-Westphal A, et al. Body fluid volume determination via body composition spectroscopy in health and disease. Physiological Measurement. 2006;27(9):921-33.

589. Lopot F, Nejedlý B, Novotná H, Macková M, Sulková S. Age-related extracellular to total body water volume ratio (ECV/TBW) - can it be used for “dry weight” determination in dialysis patients? application of multifrequency bioimpedance measurement. The International Journal of Artificial Organs. 2002;25(8):762-9.

590. Ohashi Y, Otani T, Tai R, Tanaka Y, Sakai K, Aikawa A. Assessment of body composition using dry mass index and ratio of total body water to estimated volume based on bioelectrical impedance analysis in chronic kidney disease patients. Journal of Renal Nutrition. 2013;23(1):28-36.

591. Chamney PW, Wabel P, Moissl UM, Müller MJ, Bosy-Westphal A, Fuller NJ, et al. A whole-body model to distinguish excess fluid from the hydration of major body tissues. The American Journal of Clinical Nutrition. 2007;85(1):80-9.

592. Passauer J, Petrov H, Schleser A, Leicht J, Pucalka K. Evaluation of clinical dry weight assessment in haemodialysis patients using bioimpedance spectroscopy: a cross-sectional study. Nephrology Dialysis Transplantation. 2009;25(2):545-51.

593. Wabel P, Chamney P, Moissl U, Jirka T. Importance of whole-body bioimpedance spectroscopy for the management of fluid balance. Blood purification. 2009;27(1):75-80.

594. Wabel P, Chamney PW, Moissl U, Mesch A, Millan-Galante M, Wiesen G, et al. Reproducibility of bioimpedance spectroscopy (BIS) for the assessment of body composition and dry weight. Journal of the American Society of Nephrology. 2007(18):255 A.

595. O'Rourke MF. Influence of ventricular ejection on the relationship between central aortic and brachial pressure pulse in man. Cardiovascular Research. 1970;4(3):291-300.

596. Senn S, Holford N, Hockey H. The ghosts of departed quantities: approaches to dealing with observations below the limit of quantitation. Stat Med. 2012;31(30):4280-95.

597. Keizer RJ, Jansen RS, Rosing H, Thijssen B, Beijnen JH, Schellens JH, et al. Incorporation of concentration data below the limit of quantification in population pharmacokinetic analyses. Pharmacol Res Perspect. 2015;3(2):e00131.

598. Ling IT, James AL, Hillman DR. Interrelationships between body mass, oxygen desaturation, and apnea-hypopnea indices in a sleep clinic population. Sleep. 2012;35(1):89-96.

599. Johns MW. A new method for measuring daytime sleepiness: the Epworth Sleepiness Scale. Sleep. 1991;14(6):540-5.

600. Hair Jr JF, Black WC, Babin BJ, Anderson RE. Multivariate Data Analysis. 7th ed. Harlow, England: Pearson; 2014.

601. Huber PJ. Robust Statistics. New York: John Wiley & Sons; 1981.

602. Cook RD, Weisberg S. Residuals and influence in regression. New York: Chapman & Hall; 1982.

603. Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, et al. Recommendations for improving and standardizing vascular research on arterial stiffness: a scientific statement from the American Heart Association. Hypertension. 2015;66(3):698-722.

604. Oliveras A, de la Sierra A. Resistant hypertension: patient characteristics, risk factors, co-morbidities and outcomes. Journal Of Human Hypertension. 2013;28:213.

605. Prospective Studies Collaboration, Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, et al. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. The Lancet. 2009;373(9669):1083-96.

606. Pischon T, Boeing H, Hoffmann K, Bergmann M, Schulze MB, Overvad K, et al. General and abdominal adiposity and risk of death in Europe. New England Journal of Medicine. 2008;359(20):2105-20.

607. Rexrode KM, Carey VJ, Hennekens CH, Walters EE, Colditz GA, Stampfer MJ, et al. Abdominal adiposity and coronary heart disease in women. JAMA. 1998;280(21):1843-8.

608. Ko GT, Chan JC, Cockram CS, Woo J. Prediction of hypertension, diabetes, dyslipidaemia or albuminuria using simple anthropometric indexes in Hong Kong Chinese. International journal of obesity and related

metabolic disorders : journal of the International Association for the Study of Obesity. 1999;23(11):1136-42.

609. Dobbelsteyn CJ, Joffres MR, MacLean DR, Flowerdew G. A comparative evaluation of waist circumference, waist-to-hip ratio and body mass index as indicators of cardiovascular risk factors. The Canadian Heart Health Surveys. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2001;25(5):652-61.

610. Zhu S, Wang Z, Heshka S, Heo M, Faith MS, Heymsfield SB. Waist circumference and obesity-associated risk factors among whites in the third National Health and Nutrition Examination Survey: clinical action thresholds. The American Journal of Clinical Nutrition. 2002;76(4):743-.

611. Ho S-Y, Lam T-H, Janus ED. Waist to stature ratio is more strongly associated with cardiovascular risk factors than other simple anthropometric indices. Annals of Epidemiology. 2003;13(10):683-91.

612. Ito H, Nakasuga K, Ohshima A, Maruyama T, Kaji Y, Harada M, et al. Detection of cardiovascular risk factors by indices of obesity obtained from anthropometry and dual-energy X-ray absorptiometry in Japanese individuals. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2003;27(2):232-7.

613. Janssen I, Katzmarzyk PT, Ross R. Waist circumference and not body mass index explains obesity-related health risk. The American Journal of Clinical Nutrition. 2004;79(3):379-84.

614. Lee CMY, Huxley RR, Wildman RP, Woodward M. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. Journal of Clinical Epidemiology. 2008;61(7):646-53.

615. Huxley R, James WPT, Barzi F, Patel JV, Lear SA, Suriyawongpaisal P, et al. Ethnic comparisons of the cross-sectional relationships between measures of body size with diabetes and hypertension. Obesity reviews. 2008;9 Suppl 1(s1):53-61.

616. Tuan NT, Adair LS, Stevens J, Popkin BM. Prediction of hypertension by different anthropometric indices in adults: the change in estimate approach. Public Health Nutrition. 2010;13(5):639-46.

617. Dong X, Liu Y, Yang J, Sun Y, Chen L. Efficiency of anthropometric indicators of obesity for identifying cardiovascular risk factors in a Chinese population. Postgraduate Medical Journal. 2011;87(1026):251-6.

618. Ho SC, Chen YM, Woo JL, Leung SS, Lam TH, Janus ED. Association between simple anthropometric indices and cardiovascular risk factors. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2001;25(11):1689-97.

619. Kristjansson K, Sigurdsson JA, Lissner L, Sundh V, Bengtsson C. Blood pressure and pulse pressure development in a population sample of women with special reference to basal body mass and distribution of body

fat and their changes during 24 years. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2003;27(1):128-33.

620. Wildman RP, Gu D, Reynolds K, Duan X, Wu X, He J. Are waist circumference and body mass index independently associated with cardiovascular disease risk in Chinese adults? The American Journal of Clinical Nutrition. 2005;82(6):1195-202.

621. Ishizaka N, Ishizaka Y, Toda EI, Koike K, Yamakado M, Nagai R. Impacts of changes in obesity parameters for the prediction of blood pressure change in Japanese individuals. Kidney and Blood Pressure Research. 2009;32(6):421-7.

622. Malden D, Lacey B, Emberson J, Karpe F, Allen N, Bennett D, et al. Body fat distribution and systolic blood pressure in 10,000 adults with whole-body imaging: UK Biobank and Oxford BioBank. Obesity. 2019.

623. Holecki M, Duława J, Chudek J. Resistant hypertension in visceral obesity. European Journal of Internal Medicine. 2012;23(7):643-8.

624. Montani JP, Antic V, Yang Z, Dulloo A. Pathways from obesity to hypertension: from the perspective of a vicious triangle. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2002;26 Suppl 2:S28-38.

625. Wofford MR, Hall JE. Pathophysiology and treatment of obesity hypertension. Current Pharmaceutical Design. 2004;10(29):3621-37.

626. Emdin CA, Anderson SG, Woodward M, Rahimi K. Usual blood pressure and risk of new-onset diabetes: Evidence from 4.1 million adults and a meta-analysis of prospective studies. Journal of the American College of Cardiology. 2015;66(14):1552-62.

627. Elliott WJ, Meyer PM. Incident diabetes in clinical trials of antihypertensive drugs: a network meta-analysis. The Lancet. 2007;369(9557):201-7.

628. Carter BL, Einhorn PT, Brands M, He J, Cutler JA, Whelton PK, et al. Thiazide-induced dysglycemia. Hypertension. 2008;52(1):30-6.

629. Cheung BMY, Li C. Diabetes and hypertension: is there a common metabolic pathway? Curr Atheroscler Rep. 2012;14(2):160-6.

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

631. Vakili BA, Okin PM, Devereux RB. Prognostic implications of left ventricular hypertrophy. American Heart Journal. 2001;141(3):334-41.

632. Verdecchia P, Angeli F, Borgioni C, Gattobigio R, de Simone G, Devereux RB, et al. Changes in cardiovascular risk by reduction of left ventricular mass in hypertension: a meta-analysis. American Journal of Hypertension. 2003;16(11):895-9.

633. Schillaci G, Pirro M, Mannarino E. Left ventricular hypertrophy reversal and prevention of diabetes. Hypertension. 2007;50(5):851-3.

634. Gaddam K, Corros C, Pimenta E, Ahmed M, Denney T, Aban I, et al. Rapid reversal of left ventricular hypertrophy and intracardiac volume overload in patients with resistant hypertension and hyperaldosteronism. Hypertension. 2010;55(5):1137-42.

635. Pierdomenico SD, Cuccurullo F. Risk reduction after regression of echocardiographic left ventricular hypertrophy in hypertension: a meta-analysis. American Journal of Hypertension. 2010;23(8):876-81.

636. Bidani AK, Griffin KA. Pathophysiology of hypertensive renal damage. Hypertension. 2004;44(5):595-601.

637. Moser M, Setaro JF. Resistant or difficult-to-control hypertension. New England Journal of Medicine. 2006;355(4):385-92.

638. Badrick T, Turner P. The uncertainty of the eGFR. Indian J Clin Biochem. 2013;28(3):242-7.

639. Oliveras A, Armario P, Hernández-del Rey R, Arroyo JA, Poch E, Larrousse M, et al. Urinary albumin excretion is associated with true resistant hypertension. Journal Of Human Hypertension. 2009;24:27.

640. Calhoun David A, Nishizaka Mari K, Zaman Mohammad A, Thakkar Roopal B, Weissmann P. Hyperaldosteronism among Black and White subjects with resistant hypertension. Hypertension. 2002;40(6):892-6.

641. Calhoun DA, Zaman MA, Nishizaka MK. Resistant hypertension. J Current Hypertension Reports. 2002;4(3):221-8.

642. Rossi GP, Cesari M, Cuspidi C, Maiolino G, Cicala MV, Bisogni V, et al. Long-term control of arterial hypertension and regression of left ventricular hypertrophy with treatment of primary aldosteronism. Hypertension. 2013;62(1):62-9.

643. Parthasarathy HK, Ménard J, White WB, Young WF, Williams GH, Williams B, et al. A double-blind, randomized study comparing the antihypertensive effect of eplerenone and spironolactone in patients with hypertension and evidence of primary aldosteronism. Journal of Hypertension. 2011;29(5):980-90.

644. Lim PO, Jung RT, Macdonald TM. Raised aldosterone to renin ratio predicts antihypertensive efficacy of spironolactone: a prospective cohort follow-up study. Br J Clin Pharmacol. 2001;48(5):756-60.

645. Karagiannis A, Tziomalos K, Papageorgiou A, Kakafika AI, Pagourelias ED, Anagnostis P, et al. Spironolactone versus eplerenone for the treatment of idiopathic hyperaldosteronism. Expert Opinion on Pharmacotherapy. 2008;9(4):509-15.

646. Rossi GP, Bolognesi M, Rizzoni D, Seccia TM, Piva A, Porteri E, et al. Vascular remodeling and duration of hypertension predict outcome of adrenalectomy in primary aldosteronism patients. Hypertension. 2008;51(5):1366-71.

647. Rossi GP, Bernini G, Caliumi C, Desideri G, Fabris B, Ferri C, et al. A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. Journal of the American College of Cardiology. 2006;48(11):2293-300.

648. Mulatero P, Stowasser M, Loh K-C, Fardella CE, Gordon RD, Mosso L, et al. Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. The Journal of Clinical Endocrinology & Metabolism. 2004;89(3):1045-50.

649. Schwartz GL, Turner ST. Screening for primary aldosteronism in essential hypertension: diagnostic accuracy of the ratio of plasma aldosterone concentration to plasma renin activity. Clinical Chemistry. 2005;51(2):386-94.

650. Wallace TW, Abdullah SM, Drazner MH, Das SR, Khera A, McGuire DK, et al. Prevalence and determinants of troponin T elevation in the general population. Circulation. 2006;113(16):1958-65.

651. Pham MX, Whooley MA, Evans GT, Liu C, Emadi H, Tong W, et al. Prognostic value of low-level cardiac troponin-I elevations in patients without definite acute coronary syndromes. American Heart Journal. 2004;148(5):776-82.

652. Hobbs FD, Davis RC, Roalfe AK, Hare R, Davies MK. Reliability of N-terminal proBNP assay in diagnosis of left ventricular systolic dysfunction within representative and high risk populations. Heart. 2004;90(8):866-70.

653. Cowie MR, Struthers AD, Wood DA, Coats AJS, Thompson SG, Poole-Wilson PA, et al. Value of natriuretic peptides in assessment of patients with possible new heart failure in primary care. The Lancet. 1997;350(9088):1349-53.

654. Mant J, Doust J, Roalfe A, Barton P, Cowie MR, Glasziou P, et al. Systematic review and individual patient data meta-analysis of diagnosis of heart failure, with modelling of implications of different diagnostic strategies in primary care. Health technology assessment (Winchester, England). 2009;13(32):1-207, iii.

655. Taylor CJ, Roalfe AK, Iles R, Hobbs FDR. The potential role of NT-proBNP in screening for and predicting prognosis in heart failure: a survival analysis. BMJ Open. 2014;4(4):e004675.

656. Andrade H, Morillas P, Castillo J, Roldán J, Mateo I, Agudo P, et al. Diagnostic accuracy of NT-proBNP compared with electrocardiography in detecting left ventricular hypertrophy of hypertensive origin. Revista Española de Cardiología (English Edition). 2011;64(10):939-41.

657. Morillas P, Castillo J, Quiles J, Nuñez D, Guillén S, Maceira A, et al. Usefulness of NT-proBNP level for diagnosing left ventricular hypertrophy in hypertensive patients. A cardiac magnetic resonance study. Revista Española de Cardiología (English Edition). 2008;61(9):972-5.

658. Tsioufis C, Georgiopoulos G, Oikonomou D, Thomopoulos C, Katsiki N, Kasiakogias A, et al. Hypertension and heart failure with preserved ejection fraction: connecting the dots. Curr Vasc Pharmacol. 2017;16(1):15-22.

659. 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. European Journal of Heart Failure. 2016;18(8):891-975.

660. Brunner-La Rocca H-P, Sanders-Van Wijk S. Natriuretic peptides in chronic heart failure. Cardiac Failure Review. 2019;5(1):44.

661. Vickery S, Price CP, John RI, Abbas NA, Webb MC, Kempson ME, et al. B-type natriuretic peptide (BNP) and amino-terminal proBNP in patients with CKD: relationship to renal function and left ventricular hypertrophy. Am J Kidney Dis. 2005;46(4):610-20.

662. Luchner A, Hengstenberg C, LöWel H, Riegger GNAJ, Schunkert H, Holmer S. Effect of compensated renal dysfunction on approved heart failure markers. Hypertension. 2005;46(1):118-23.

663. Muxfeldt ES, Bloch KV, da Rocha Nogueira A, Salles GF. True resistant hypertension: is it possible to be recognized in the office? American Journal of Hypertension. 2005;18(12):1534-40.

664. Guyton AC, Manning RD, Jr., Hall JE, Norman RA, Jr., Young DB, Pan YJ. The pathogenic role of the kidney. Journal of cardiovascular pharmacology. 1984;6 Suppl 1:S151-61.

665. Covic A, Voroneanu L, Goldsmith D. Routine bioimpedance-derived volume assessment for all hypertensives: a new paradigm. American Journal of Nephrology. 2014;40(5):434-40.

666. Ferrario CM, Flack JM, Strobeck JE, Smits G, Peters C. Individualizing hypertension treatment with impedance cardiography: a meta-analysis of published trials. Ther Adv Cardiovasc Dis. 2010;4(1):5-16.

667. Ferrario CM, Smith RD, for the CONTROL Trial Investigators. Cost-effectiveness of impedance cardiography testing in uncontrolled hypertension. American Heart Hospital Journal. 2006;4(4):279-89.

668. Davies SJ, Davenport A. The role of bioimpedance and biomarkers in helping to aid clinical decision-making of volume assessments in dialysis patients. Kidney International. 2014;86(3):489-96.

669. Chae CU, Pfeffer MA, Glynn RJ, Mitchell GF, Taylor JO, Hennekens CH. Increased pulse pressure and risk of heart failure in the elderly. JAMA. 1999;281(7):634-43.

670. Franklin SS, Larson MG, Khan SA, Wong ND, Leip EP, Kannel WB, et al. Does the relation of blood pressure to coronary heart disease risk change with aging? The Framingham Heart Study. Circulation. 2001;103(9):1245-9.

671. Mitchell GF, Moyé LA, Braunwald E, Rouleau J-L, Bernstein V, Geltman EM, et al. Sphygmomanometrically determined pulse pressure is a powerful independent predictor of recurrent events after myocardial infarction in patients with impaired left ventricular function. Circulation. 1997;96(12):4254-60.

672. Vaccarino V, Berger AK, Abramson J, Black HR, Setaro JF, Davey JA, et al. Pulse pressure and risk of cardiovascular events in the systolic hypertension in the elderly program. The American Journal of Cardiology. 2001;88(9):980-6.

673. Kostis JB, Lawrence-Nelson J, Ranjan R, Wilson AC, Kostis WJ, Lacy CR. Association of increased pulse pressure with the development of heart failure in SHEP. American Journal of Hypertension. 2001;14(8):798-803.

674. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness on survival in end-stage renal disease. Circulation. 1999;99(18):2434-9.

675. Forette F, Seux M-L, Staessen JA, Thijs L, Birkenhäger WH, Babarskiene M-R, et al. Prevention of dementia in randomised double-blind placebo-controlled Systolic Hypertension in Europe (Syst-Eur) trial. The Lancet. 1998;352(9137):1347-51.

676. Benetos A, Rudnichi A, Safar M, Guize L. Pulse pressure and cardiovascular mortality in normotensive and hypertensive subjects. Hypertension. 1998;32(3):560-4.

677. Zieman SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. Arteriosclerosis, Thrombosis, and Vascular Biology. 2005;25(5):932-43.

678. Dudenbostel T, Acelajado MC, Pisoni R, Li P, Oparil S, Calhoun DA. Refractory hypertension: evidence of heightened sympathetic activity as a cause of antihypertensive treatment failure. Hypertension. 2015;66(1):126-33.

679. Barbaro NR, Fontana V, Modolo R, De Faria AP, Sabbatini AR, Fonseca FH, et al. Increased arterial stiffness in resistant hypertension is associated with inflammatory biomarkers. Blood Press. 2015;24(1):7-13.

680. Figueiredo VN, Yugar-Toledo JC, Martins LC, Martins LB, de Faria AP, de Haro Moraes C, et al. Vascular stiffness and endothelial dysfunction: correlations at different levels of blood pressure. Blood pressure. 2012;21(1):31-8.

681. Brandt MC, Reda S, Mahfoud F, Lenski M, Böhm M, Hoppe UC. Effects of renal sympathetic denervation on arterial stiffness and central hemodynamics in patients with resistant hypertension. Journal of the American College of Cardiology. 2012;60(19):1956-65.

682. Sabbatini AR, Barbaro NR, de Faria AP, Modolo R, Ritter AMV, Pinho C, et al. Increased circulating tissue inhibitor of metalloproteinase-2 is associated with resistant hypertension. The Journal of Clinical Hypertension. 2016;18(10):969-75.

683. Cushman WC, Materson BJ, Williams DW, Reda DJ. Pulse pressure changes with six classes of antihypertensive agents in a randomized, controlled trial. Hypertension. 2001;38(4):953-7.

684. 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 Lancet. 1997;350(9080):757-64.

685. 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(24):3255-64.

686. Sharman JE, Boutouyrie P, Laurent S. Arterial (aortic) stiffness in patients with resistant hypertension: from assessment to treatment. J Current Hypertension Reports. 2017;19(1):2.

687. Davenport AP, Hyndman KA, Dhaun N, Southan C, Kohan DE, Pollock JS, et al. Endothelin. Pharmacol Rev. 2016;68(2):357-418.

688. de Nucci G, Thomas R, D'Orleans-Juste P, Antunes E, Walder C, Warner TD, et al. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. Proc Natl Acad Sci U S A. 1988;85(24):9797-800.

689. Murray CJ. Increased plasma endothelin level in patients with essential hypertension. N Engl J Med. 1990;322(3):205-.

690. Black HR, Bakris GL, Weber MA, Weiss R, Shahawy ME, Marple R, et al. Efficacy and safety of darusentan in patients with resistant hypertension: results from a randomized, double-blind, placebo-controlled dose-ranging study. J Clin Hypertens (Greenwich). 2007;9(10):760-9.

691. Bakris GL, Lindholm LH, Black HR, Krum H, Linas S, Linseman JV, et al. Divergent results using clinic and ambulatory blood pressures: report of a darusentan-resistant hypertension trial. Hypertension. 2010;56(5):824-30.

692. Weber MA, Black H, Bakris G, Krum H, Linas S, Weiss R, et al. A selective endothelin-receptor antagonist to reduce blood pressure in patients with treatment-resistant hypertension: a randomised, double-blind, placebo-controlled trial. The Lancet. 2009;374(9699):1423-31.

693. Park JB, Schiffrin EL. ET(A) receptor antagonist prevents blood pressure elevation and vascular remodeling in aldosterone-infused rats. Hypertension. 2001;37(6):1444-9.

694. Schiffrin EL, Larivière R, Li JS, Sventek P. Enhanced expression of the endothelin-1 gene in blood vessels of DOCA-salt hypertensive rats: correlation with vascular structure. Journal of Vascular Research. 1996;33(3):235-48.

695. Gómez Rosso L, Benítez MB, Fornari MC, Berardi V, Lynch S, Schreier L, et al. Alterations in cell adhesion molecules and other biomarkers of cardiovascular disease in patients with metabolic syndrome. Atherosclerosis. 2008;199(2):415-23.

696. Poston RN, Haskard DO, Coucher JR, Gall NP, Johnson-Tidey RR. Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. American Journal of Pathology. 1992;140(3):665-73.

697. Varona JF, Ortiz-Regalón R, Sánchez-Vera I, López-Melgar B, García-Durango C, Castellano Vázquez JM, et al. Soluble ICAM 1 and VCAM 1 blood levels alert on subclinical atherosclerosis in non smokers with asymptomatic metabolic syndrome. Archives of Medical Research. 2019;50(2):20-8.

698. Shalia KK, Mashru MR, Vasvani JB, Mokal RA, Mithbawkar SM, Thakur PK. Circulating levels of cell adhesion molecules in hypertension. Indian J Clin Biochem. 2009;24(4):388-97.

699. Palomo I, Marin P, Alarcon M, Gubelin G, Vinambre X, Mora E, et al. Patients with essential hypertension present higher levels of sE-selectin and sVCAM-1 than normotensive volunteers. Clin Exp Hypertens. 2003;25(8):517-23.

700. Madej A, Okopien B, Kowalski J, Haberka M, Herman ZS. Plasma concentrations of adhesion molecules and chemokines in patients with essential hypertension. Pharmacological reports : PR. 2005;57(6):878-81.

701. DeSouza CA, Dengel DR, Macko RF, Cox K, Seals DR. Elevated levels of circulating cell adhesion molecules in uncomplicated essential hypertension. American Journal of Hypertension. 1997;10(12):1335-41.

702. Cottone S, Mule G, Amato F, Riccobene R, Vadala A, Lorito MC, et al. Amplified biochemical activation of endothelial function in hypertension associated with moderate to severe renal failure. Journal of nephrology. 2002;15(6):643-8.

703. Ferri C, Desideri G, Baldoncini R, Bellini C, De Angelis C, Mazzocchi C, et al. Early activation of vascular endothelium in nonobese, nondiabetic essential hypertensive patients with multiple metabolic abnormalities. Diabetes. 1998;47(4):660-7.

704. Li L, Chu Y, Fink GD, Engelhardt JF, Heistad DD, Chen AF. Endothelin-1 stimulates arterial VCAM-1 expression via NADPH oxidase-derived superoxide in mineralocorticoid hypertension. Hypertension. 2003;42(5):997-1003.

705. Prasad A, Husain S, Quyyumi AA. Abnormal flow-mediated epicardial vasomotion in human coronary arteries is improved by angiotensin-converting enzyme inhibition: a potential role of bradykinin. Journal of the American College of Cardiology. 1999;33(3):796-804.

706. Prasad A, Tupas-Habib T, Schenke WH, Mincemoyer R, Panza JA, Waclawin MA, et al. Acute and chronic angiotensin-1 receptor antagonism reverses endothelial dysfunction in atherosclerosis. Circulation. 2000;101(20):2349-54.

707. Mancini GB, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, et al. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND (Trial on Reversing ENdothelial Dysfunction) Study. Circulation. 1996;94(3):258-65.

708. Higashi Y, Sasaki S, Nakagawa K, Ueda T, Yoshimizu A, Kurisu S, et al. A comparison of angiotensin-converting enzyme inhibitors, calcium antagonists, beta-blockers and diuretic agents on reactive hyperemia in patients with essential hypertension: a multicenter study. J Am Coll Cardiol. 2000;35(2):284-91.

709. Kuvin JT, Patel AR, Sliney KA, Pandian NG, Sheffy J, Schnall RP, et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. American Heart Journal. 2003;146(1):168-74.

710. Kuvin JT, Mammen A, Mooney P, Alsheikh-Ali AA, Karas RH. Assessment of peripheral vascular endothelial function in the ambulatory setting. Vascular Medicine. 2007;12(1):13-6.

711. Dhindsa M, Sommerlad SM, DeVan AE, Barnes JN, Sugawara J, Ley O, et al. Interrelationships among noninvasive measures of postischemic macro- and microvascular reactivity. J Appl Physiol (1985). 2008;105(2):427-32.

712. Aizer J, Karlson E, Chibnik L, Costenbader K, Post D, Liang M, et al. A controlled comparison of brachial artery flow mediated dilation (FMD) and digital pulse amplitude tonometry (PAT) in the assessment of endothelial function in systemic lupus erythematosus. Lupus. 2009;18(3):235-42.

713. Heffernan KS, Karas RH, Mooney PJ, Patel AR, Kuvin JT. Pulse wave amplitude is associated with brachial artery diameter: implications for gender differences in microvascular function. Vascular Medicine. 2010;15(1):39-45.

714. Dickinson KM, Clifton PM, Keogh JB. Endothelial function is impaired after a high-salt meal in healthy subjects. American Journal of Clinical Nutrition. 2011;93(3):500-5.

715. Hamburg NM, Palmisano J, Larson MG, Sullivan LM, Lehman BT, Vasan RS, et al. Relation of brachial and digital measures of vascular function in the community. Hypertension. 2011;57(3):390-6.

716. Schnabel RB, Schulz A, Wild PS, Sinning CR, Wilde S, Eleftheriadis M, et al. Noninvasive vascular function measurement in the community. Circulation: Cardiovascular Imaging. 2011;4(4):371-80.

717. Onkelinx S, Cornelissen V, Goetschalckx K, Thomaes T, Verhamme P, Vanhees L. Reproducibility of different methods to measure the endothelial function. Vascular Medicine. 2012;17(2):79-84.

718. Allan RB, Delaney CL, Miller MD, Spark JI. A comparison of flow-mediated dilatation and peripheral artery tonometry for measurement of endothelial function in healthy individuals and patients with peripheral arterial disease. European Journal of Vascular and Endovascular Surgery. 2013;45(3):263-9.

719. Nohria A, Gerhard-Herman M, Creager MA, Hurley S, Mitra D, Ganz P. Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. Journal of Applied Physiology. 2006;101(2):545-8.

720. Bennett JA, Kinnear WJ. Sleep on the cheap: the role of overnight oximetry in the diagnosis of sleep apnoea hypopnoea syndrome. Thorax. 1999;54(11):958-9.

721. Levy P, Pepin JL, Deschaux-Blanc C, Paramelle B, Brambilla C. Accuracy of oximetry for detection of respiratory disturbances in sleep apnea syndrome. Chest. 1996;109(2):395-9.

722. Nair AR, Ahmed S, Kunoor A, Salam B, Ponneduthamkuzhi JP, Ajith AM. Reliability of overnight desaturation index (ODI) in suspected obstructive sleep apnea. European Respiratory Journal. 2017;50(suppl 61):PA2306.

723. Suliman L, Shalabi N, Saad A. Validity of overnight pulse oximetry as a screening tool of obstructive sleep apnea. European Respiratory Journal. 2016;48(suppl 60):PA2316.

724. Netzer N, Eliasson AH, Netzer C, Kristo DA. Overnight pulse oximetry for sleep-disordered breathing in adults: a review. Chest. 2001;120(2):625-33.

725. Abboud F, Kumar R. Obstructive sleep apnea and insight into mechanisms of sympathetic overactivity. The Journal of Clinical Investigation. 2014;124(4):1454-7.

726. Mansukhani MP, Kara T, Caples SM, Somers VK. Chemoreflexes, sleep apnea, and sympathetic dysregulation. Curr Hypertens Rep. 2014;16(9):476.

727. Eckert DJ, Malhotra A. Pathophysiology of adult obstructive sleep apnea. Proc Am Thorac Soc. 2008;5(2):144-53.

728. Franklin KA, Lindberg E. Obstructive sleep apnea is a common disorder in the population—a review on the epidemiology of sleep apnea. Journal of Thoracic Disease. 2015;7(8):1311-22.

729. Rajpura J, Nayak R. Medication adherence in a sample of elderly suffering from hypertension: evaluating the influence of illness perceptions, treatment beliefs, and illness burden. J Manag Care Pharm. 2014;20(1):58-65.

730. Qvarnstrom M, Kahan T, Kieler H, Brandt L, Hasselstrom J, Wettermark B. Medication persistence to antihypertensive drug treatment - a cross-sectional study of attitudes towards hypertension and medication in persistent and non-persistent patients. Blood Press. 2019;28(5):309-16.

731. Horne R, Chapman SC, Parham R, Freemantle N, Forbes A, Cooper V. Understanding patients' adherence-related beliefs about medicines prescribed for long-term conditions: a meta-analytic review of the Necessity-Concerns Framework. PLoS One. 2013;8(12):e80633.

732. Saklayen MG. The Global Epidemic of the Metabolic Syndrome. Curr Hypertens Rep. 2018;20(2):12.