CARDIOVASCULAR HAEMODYNAMICS AND AUTONOMIC FUNCTION IN RESISTANT HYPERTENSION: RELATION TO BLOOD PRESSURE CONTROL

by

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Abstract

Introduction: Hypertension is one of the major risk factors for cardiovascular and cerebrovascular diseases such as myocardial infarction (MI) and stroke. Patients with resistant and malignant hypertension have poor prognosis and high rate of complications. Understanding the pathophysiological mechanisms underlying the severe forms of hypertension and their relationship to cardiac and endothelial function, and autonomic dysregulation is crucial to the management of blood pressure (BP). Furthermore, increased arterial stiffness, impaired cardiac function and endothelial dysfunction all act as indicators and predictors of cardiovascular events in patients with hypertension. There are little or no data on the relationship between cardiac mechanics, autonomic function and vascular function in patients with malignant and resistant hypertension. It is unknown if optimisation of antihypertensive therapy in resistant hypertension can improve cardiac mechanics, vascular and autonomic function.

Aims: To assess left ventricle (LV), vascular, and autonomic function in patients with hypertension (resistant and malignant) and in a normotensive control (NC). Also, to assess the efficacy of intensified antihypertensive treatment on myocardial mechanics, vascular and autonomic function and blood pressure variability (BPV) in patients with resistant hypertension (RH).

Methods: Cardiac haemodynamics, strain function, vascular and autonomic function were evaluated in patients with malignant hypertension (MHT), RH and in normotensive group.

Results: Patients with MHT and true RH had persistently abnormal cardiac remodelling, even after long-term intensive antihypertensive treatment, and irrespective of LV

ejection fraction (EF). Stable patients with MHT and good long-term BP control still had worse cardiac remodelling compared to those with RH suggesting that impaired global longitudinal strain (GLS) was not only influenced by afterload and could be related to more prevalent myocardial fibrosis in MHT. Patients with MHT also showed low subendocardial viability ratio (SEVR) and total peripheral resistance (TPR) compared to control subjects, indicating the presence of insufficient oxygen supply and low myocardial oxygen consumption.

Endothelial dysfunction and elevated arterial stiffness were found in patients with MHT and RH; however, autonomic function was preserved in all groups.

After 8 weeks of antihypertensive treatment, cardiac strain function, endothelial function and BPV significantly improved compared with baseline in patients with true RH; however, arterial stiffness and heart rate variability (HRV) did not change.

Conclusion: This is the first detailed cardiovascular and autonomic evaluation of two extreme phenotypes of hypertension (patients with treated MHT and patients with true RH). The study demonstrates the ability and sensitivity of advanced strain imaging to unmask differential cardiac remodelling responses in patients with MHT and RH. The study also provides findings that may potentially imply that MHT has different abnormalities. Intensive antihypertensive treatment reduces office and 24-hour BP in patients with RH and has a favourable impact on cardiac and endothelial function but had no effect on HRV or arterial stiffness.

Dedications

I dedicate this thesis to the loving memory of my father

You have successfully made me the person I am becoming. You will always be remembered.

To my mother

Who have always loved me unconditionally.

To my husband

Who has been a constant source of support and encouragement during all the challenges.

I am truly thankful for having you in my life.

To my daughters: Tala and Sara

You have made me stronger, better and more fulfilled than I could have ever imagined.

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List of abbreviations

2D - two-dimensional

4C - four-chamber

5C - five-chamber

A2C - apical two-chamber

A4C - apical four-chamber

ABPM - ambulatory blood pressure monitoring

ACEIs - angiotensin-converting enzyme inhibitors

Ach - acetylcholine

aCMQ - automated cardiac motion quantification software

AD - aortic distensibility

AF - atrial fibrillation

AHA - American Heart Association

AIx – augmentation index

ANS - autonomic nervous system

ARBs - angiotensin-receptor blockers

ASE - American Society of Echocardiography

AV - atrioventricular

AVC - aortic valve closure

baPWV – brachial-ankle pulse wave velocity

BMI - body mass index

BP - blood pressure

BPV - blood pressure variability

CA - calcium antagonists

CAD - coronary artery disease

CBP - central blood pressure

CCA - common carotid artery

CCB - calcium channel blockers

CCDd - common carotid artery diameter at diastole

CCDs - common carotid artery diameter at systole

cfPWV - carotid femoral pulse wave velocity

cGMP - cyclic guanosine monophosphate

CHD - coronary heart disease

CKD - chronic kidney disease

CMR - cardiac magnetic resonance

CO - cardiac output

CV - coefficient of variation

CVD - cardiovascular disease

DBP - diastolic blood pressure

CCDd - common carotid artery at diastole

CCDs - common carotid artery at systole

Dd - diastolic diameter

DD - diastolic dysfunction

DM - diabetes mellitus

DT - deceleration time

EACVI - European Association of Cardiovascular Imaging

ECG - electrocardiogram

ECM - extracellular matrix

EDHF - endothelium-derived hyperpolarizing factor

EDV - end diastolic volume

EF - ejection fraction

eGFR - estimated glomerular filtration rate

eNOS - endothelial nitric oxide synthase

EPCs - endothelial progenitor cells

ESC - European Society of Cardiology

ESH - European Society of Hypertension

ESRD - end stage renal disease

ESV - end systolic volume

ET-1- endothelin-1

ESV - end systolic volume

FMD - flow mediated dilatation

FMDr - flow mediated dilatation in respect to recovery diameter

FR - frame rate

GCS - global circumferential strain

GLS - global longitudinal strain

HF - high frequency

HFpF - heart failure with preserved function

HFrF - heart failure with reduced function

HR - heart rate

HRV - heart rate variability

HTN - hypertension

IVS - interventricular septum

IVSd - interventricular septal thickness at end diastole.

LA - left atrium

LAV - left atrium volume

LAVI - left atrial volume index

LF – low frequency

LS - longitudinal strain

LV - left ventricle

LVH - left ventricular hypertrophy

LVIDd - left ventricular diameter at end diastole

LVM - Left ventricular mass

LVMI - left ventricular mass index

MACE - major adverse cardiac events

MAP - mean arterial pressure

MHT - malignant hypertension

MI - myocardial infarction

M-mode - motion mode

MMP-1 - matrix metalloproteinase-1

MV - mitral valve

NADPH - nicotinamide adenine dinucleotide phosphate

NC - normotensive controls

NLR - Neutrophil to Lymphocyte ratio

NO - nitric oxide

NOS - nitric oxide synthase

PAD - peripheral artery disease

PHT - Prehypertension

PLAX - parasternal long axis view

pNN50 - percentage of adjacent NN intervals that differ from each other by more than 50 ms

PNS - parasympathetic nervous system

PP - pulse pressure

PWd - posterior wall thickness at end diastole

PWV - pulse wave velocity

RAAS - renin-angiotensin-aldosterone system

RH - resistant hypertension

rMSSD - root means successive square difference

ROI - region of interest

ROS - reactive oxygen species

RSA - respiratory rate

RWT - relative wall thickness

SA - sinoatrial

SBP - systolic blood pressure

SD - standard deviation

SDNN - standard deviation of NN Intervals

SEVR - subendocardial viability ratio

SNS - sympathetic nervous system

SOP - Standard operating procedure

SR - Strain rate

STE - speckle tracking Echocardiography

SV - stroke volume

TDI - tissue Doppler imaging

TIA - Transient ischaemic attack

TNF-α - tumour necrosis factor-α

TOD - target organ damage

TPR - total peripheral resistance

TSH - thyroid stimulating hormone

TXA_2 - thromboxane

US - ultrasound

VLF - very low frequency

CHAPTER I. INTRODUCTION

Hypertension is an established risk factor for cardiovascular diseases (CVD) such as stroke, myocardial infarction (MI) and heart failure with preserved ejection fraction (EF) (1, 2, 3, 4, 5, 6, 7). Hypertension is one of the world's predominant chronic diseases globally, affecting approximately 1 billion people worldwide and expected to increase by 20% by 2025 (8, 9). Despite advances in hypertension diagnosis and treatment strategies, malignant hypertension (MHT) and resistant hypertension (RH) remain challenging clinical problems. RH is diagnosed when office systolic and diastolic blood pressure (BP) are above 140 mmHg and 90 mmHg, respectively, (confirmed by 24-hour BP and home BP), and after confirmed adherence to concomitant treatment with three or more antihypertensive medications including a diuretic (8). MHT is another severe form of hypertension, characterised by severe increases in BP (diastolic blood pressure (DBP) >120 mmHg and out of range systolic blood pressure (SBP)), with bilateral retinal haemorrhages and/or exudates, with or without papilloedema (10). These poorly controlled forms of hypertension adversely affect cardiac mechanics, autonomic function, and endothelial function, and commonly result in elevated arterial stiffness (11, 12, 13, 14).

The mechanisms underlying the association between hypertension and cardiac remodelling are not fully established (15). Left ventricular (LV) dysfunction, left ventricular hypertrophy (LVH) and myocardial fibrosis are present in patients with long standing hypertension (16, 17, 18). Conventional echocardiography is a reliable, non-invasive method that is commonly used to evaluate LV function and to assess the

presence and the degree of LVH in hypertension (19, 20, 21). However, impaired LV systolic strain function, observed in asymptomatic patients with preserved EF with and without LVH, is undetectable by conventional echocardiography, suggesting that LVH may be preceded by LV deformation (22, 23, 24). Speckle tracking echocardiography (STE) or strain imaging was introduced in the early 2000s and has proven to be effective, non-invasive and sensitive method to detect early regional and global myocardial dysfunction before conventional echocardiography (25, 26).

The presence of fibrosis is closely associated with impaired myocardial strain (23, 27, 28, 29). During cardiac remodelling process in hypertension, the production of collagen types I and III become imbalanced. As a result, collagen fibres are deposited excessively in fibroblasts, which then transdifferentiate into myofibroblasts and accelerate myocardial fibrosis heterogeneously (30, 31). In addition, elevated matrix metalloproteinase-1 (MMP-1) turnover decreased collagen I and III degradation and led to subendocardial myocardial fibrosis. Consequently, based on Kang et al. (23) and Martinez et al. (27), it has been proposed that impaired global longitudinal strain (GLS) is linked with unbalanced collagen production and myocardial fibrosis in hypertension, which eventually leads to early systolic dysfunction (23, 27, 28, 29).

The autonomic nervous system plays a significant role in controlling BP in hypertension (32). Hypertension is associated with an imbalance of sympathetic and parasympathetic activity (33). Patients with hypertension (controlled and uncontrolled) with impaired cardiac function and autonomic function have been shown to have higher mortality rates (34). Heart rate variability (HRV) analysis is a simple, quick and non-invasive

method for assessing cardiac autonomic function and exploring the complex interaction between autonomic function and cardiovascular system (35, 36).

The onset of essential hypertension and endothelial dysfunction are closely related and may be more prominent in MHT and RH (37, 38, 39, 40, 41). Impaired endothelial function can be assessed by brachial flow-mediated dilatation (FMD) (42). In accordance with the expert consensus guidelines, FMD is a valid, non-invasive and recommended method for assessing vasodilatation of the brachial artery in response to reactive hyperaemia (43, 44). A strong association between the severity of hypertension and the degree of impaired endothelial function was described in data from the Framingham Heart Study, with each 20-mmHg increase in SBP was associated with a 0.62% decline in FMD% (45).

Endothelial dysfunction in human hypertension was first described in 1990 (46). Previously, impaired FMD was observed in hypertension group compared to the normal group (47). Imbalanced production of vasodilatory and vasoconstrictory products are typical abnormal observations in hypertension (48). However, it is still unclear whether endothelial dysfunction is a cause or a consequence of hypertension. The development of essential hypertension and impaired endothelial function are closely related (37, 38, 39, 40). A complex bidirectional association between endothelial dysfunction and hypertension has been also reported (49). Endothelial dysfunction is likely to be found in patients with RH (40, 50, 51, 52).

Hypertension is the second most significant risk factor for increased arterial stiffness after ageing (53). Arterial alterations take place as a result of structural, mechanical and

functional changes, which eventually induce arterial stiffening. Recent studies have shown that arterial stiffness and RH are closely related and can be measured by pulse wave velocity (PWV) and augmentation index (AIx) (50, 51, 54, 55, 56, 57, 58, 59). Waveform analysis of central arterial pressure and PWV has been established as a non-invasive gold standard technique for assessing arterial stiffness (60).

Vascular remodelling reflected by abnormal PWV, AIx, subendocardial viability ratio (SEVR), endothelium-dependent dilatation and carotid artery distensibility have been associated with hypertension (46, 61, 62). Assessment of arterial stiffness, carotid artery distensibility, and endothelium-dependent dilatation are safe, reliable and beneficial in providing adequate information for hypertension management (8, 14, 63). The concurrent evaluation of these indices in RH and MHT has not been investigated. In light of this background, I aimed to assess vascular function in MHT and RH.

Understanding the pathophysiological mechanism(s) of RH and its association with cardiac, vascular, and autonomic function is an essential element in treating and minimising complications. Reducing SBP by 10 mmHg, decreases the risk of coronary artery disease (CAD) by 20%, stroke by 35%, heart failure by 40% and all-cause mortality by 10-15% (64, 65, 66).

Blood pressure variability (BPV) and HRV are useful assessments of sympathetic and parasympathetic activity. Reduced HRV and elevated BPV in hypertension are directly related to target organ damage (TOD) and cardiovascular risks (67, 68, 69, 70). Nevertheless, it is poorly understood whether optimised antihypertensive therapy in RH population is linked with enhancement of cardiac autonomic function and BPV.

CHAPTER II. LITERATURE REVIEW

The purpose of this literature review is to summarise the available data on resistant and malignant hypertension and to provide a summary of the non-invasive assessments of arterial stiffness, endothelial function, cardiac remodelling and autonomic function in MHT and RH populations.

The role of impaired endothelial function in RH has been discussed; particularly the importance of FMD measurement to assess endothelial function in RH. This has been followed by an information on the non-invasive methods of evaluation of arterial stiffness; specifically, PWV and AIx tests.

I have also demonstrated the potential benefits of using STE as non-invasive imaging technique in the assessment of cardiac remodelling in patients with hypertension and specifically in uncontrolled and RH population. I have also briefly discussed the assessment of autonomic function using HRV in hypertension population.

In this literature review, PUBMED database searches were used to identify published data. Only publications in English language were selected. The following key words were used in the search: Resistant hypertension, malignant hypertension, high blood pressure, uncontrolled hypertension, vascular function, arterial stiffness, pulse wave velocity, augmentation index, vascular remodelling, endothelial function, flow-mediated dilation, speckle tracking, echocardiography, deformation imaging, systolic dysfunction.

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2.1 Hypertension

Patients with hypertension and impaired cardiac autonomic function have higher mortality rates (34). Although hypertension has been diagnosed and treated more effectively in recent years, uncontrolled hypertension remains a challenge problem, accounting for about 7.5 million deaths worldwide per year (73). A patients with RH has office systolic and diastolic BP over 140 mmHg and 90 mmHg respectively, (confirmed by 24-hour and home BP) and despite the confirmed adherence to concomitant use of three or more antihypertensive agents, including at least one diuretic (8). Based on the European Society of Hypertension and the European Society of Cardiology (ESH/ESC) guidelines for the management of arterial hypertension, RH affects up to 10% of the essential hypertension population (8). There is another severe form of hypertension known as MHT, which involves severe elevation in BP (DBP>120 mmHg and out of range SBP), with bilateral retinal haemorrhages and/or exudates, with or without papilloedema (10).

Compared to controlled essential hypertension, RH is estimated to have 50% higher cardiovascular morbidity and mortality, higher risk of target organ damage (TOD) (74, 75, 76). Patients with MHT are strongly associated with severe TOD and poor outcomes, with 80% mortality rate after 24 months, without treatment (77, 78).

There are little or no data on the relationship between cardiac mechanics, autonomic function and vascular function in patients with malignant and resistant hypertension. It is unknown if intensive therapy in RH can modify cardiac mechanics, vascular and autonomic function.

2.2 Endothelial function in hypertension

2.2.1. Endothelial structure and function

Endothelium is a thin flat single squamous mesodermal layer formed by vascular endothelial cells. Endothelial cells are located in the innermost layer of a blood vessel, acting as a barrier between blood and tissues. It lines the entire circulatory system such as the heart, arteries, veins, and small capillaries (79).

Under normal conditions, the endothelium plays a crucial role in different mechanisms within the circulatory system. This includes fluid filtration, hormone trafficking, vascular tone regulation and haemostatic balance (80). The endothelium is also maintaining blood fluidity and controls thrombosis, platelet and leukocyte (80). Endothelial cells continuously release balanced vasoactive factors. These factors are either vasodilators or vasoconstrictive substances that regulate the vascular tone. Vasodilators are nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarising factor (EDHF) and vasoconstrictive factors such as thromboxane (TXA_2) and endothelin-1 (ET-1) (81, 82). Imbalance production of these vasoactive factors lead to damage of endothelium and impaired endothelial function and is involved in many disease processes.

2.2.2. Endothelial pathophysiology

Several mechanisms can induce endothelial dysfunction. Reduced NO bioavailability is one of the keys to endothelial dysfunction and is involved in the pathogenesis of several vascular diseases. NO is a known substance that modulates vascular tone and was first identified in 1980 (83). It acts as a signalling protective molecule which is produced by the NO synthase (NOS) enzyme; specifically, by endothelial NOS (eNOS) (84, 85). eNOS

is located in caveolae and is held in inactive state by binding to caveolin-1 (86, 87). This binding of eNOS to caveolin-1 scaffold domain inhibits production of NO (87). NO is released when eNOS detaches from caveolin. This occurs when intracellular levels of Ca^{2+} are increased (87). The release of NO leads to smooth muscle relaxation and vasodilatation, which then improves the blood flow (Figure 2.1) (82). Vasodilatation occurs by stimulating soluble guanylyl cyclase and increasing cyclic guanosine monophosphate (cGMP) in smooth muscle cells (88). Impairment of eNOS production contributes to oxidative stress and impaired vascular function.

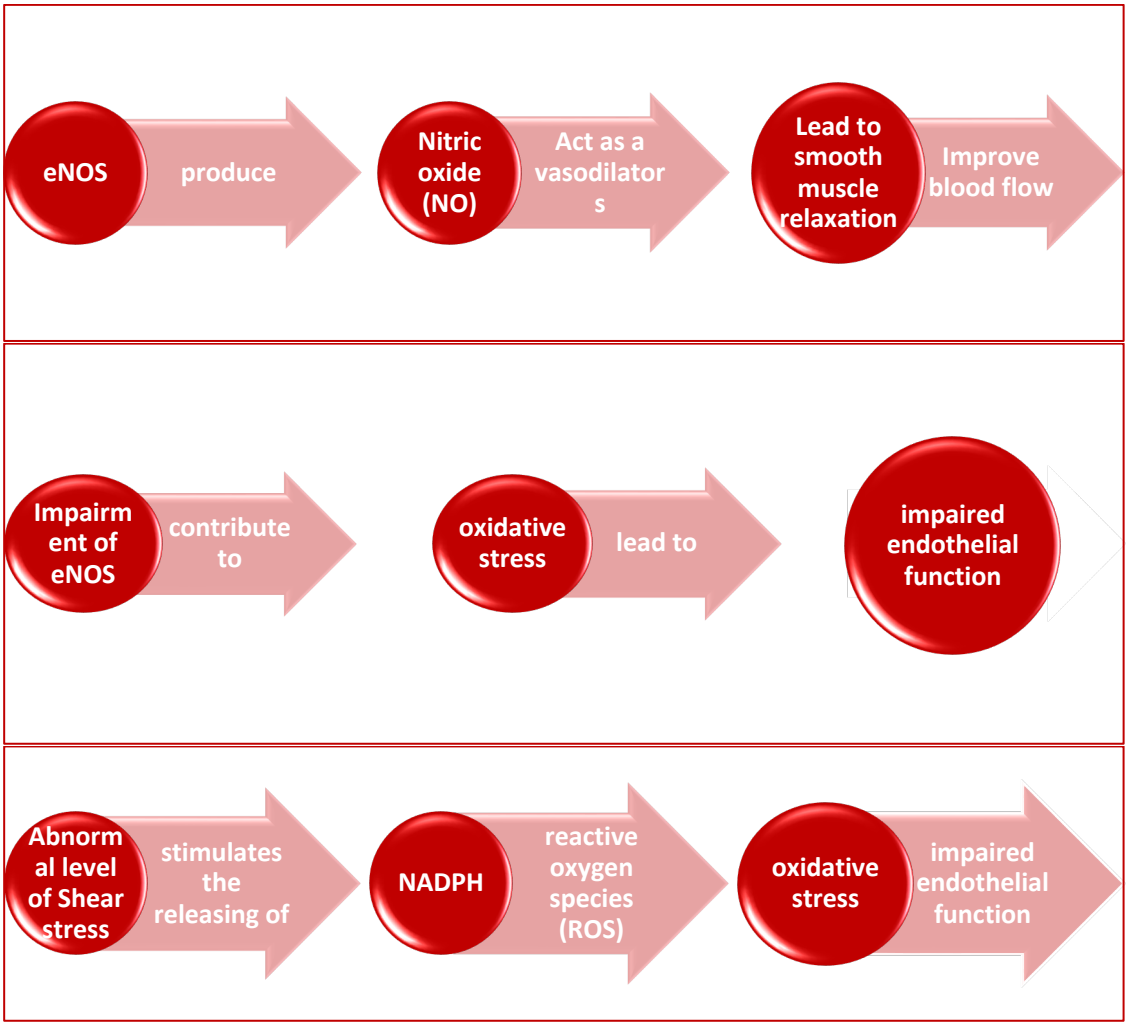


Figure 2.1 Mechanisms involved in endothelial dysfunction. eNOS: Endothelial nitric oxide synthases; NADPH: nicotinamide adenine dinucleotide phosphate.

Alteration of shear stress is another significant factor of endothelial dysfunction (84, 89). Shear stress is a tangential force of blood flow, on the endothelial surface. High BP stimulates the releasing of nicotinamide adenine dinucleotide phosphate (NADPH) which is a source of oxidases of reactive oxygen species (ROS). Shear stress generated on the endothelial cells controls both NADPH oxidase and eNOS production, imbalance of which will cause vascular oxidative stress and lead to impaired endothelial function (44, 90, 91).

2.2.3. Assessment of flow mediated dilatation

Endothelial dysfunction is involved in the pathophysiology of CVD and is associated with many cardiovascular risk factors (Table 2.1) (92). Many techniques have been used to assess endothelial function. These techniques are either invasive or non-invasive assessments. Non-invasive techniques include administration of nitrate to evaluate endothelium-independent vasodilatation, intra-arterial injection of acetylcholine or sodium nitroprusside (46, 93, 94, 95).

Endothelial function can be assessed non-invasively using the FMD technique. In most studies, 7.1% is considered the cut-off value for normal endothelial function assessed by FMD (96, 97, 98). The technique was introduced in 1992 using high resolution B-mode ultrasound (99). FMD is widely used to examine the ability of brachial artery to response to the pressure induced by the cuff inflation for 5 minutes (44). Reactive hyperaemia is the increase in blood flow post cuff release. Brachial arteries respond to the increase in blood flow and change in shear stress by dilating. Cuff deflation would increase blood flow and thereby increase shear stress and lead to brachial artery dilatation. Shear stress is related inversely to the diameter of the artery. FMD is estimated by calculating the following equation (44):

$$FMD(\%) = \frac{(Peak\ Diameter\ -\ Baseline\ Diameter)}{Baseline\ Diameter}$$

Several factors have both direct and indirect effects on FMD response in similar populations. Technical considerations such as occlusion duration and site are factors that may affect FMD response (97, 100). Reactive hyperaemia produces a greater FMD

response when the cuff is applied to the upper arm compared with that positioned on the forearm (101, 102). However, no consensus exists regarding which technique is more accurate (44). Duration of cuff inflation affects the brachial diameter post deflation. Typically, 5 minutes of forearm arterial occlusion is typically applied to provoke maximum response (44, 103, 104).

Previous studies measured FMD on different time during the day and reported diurnal variations of FMD (105, 106, 107, 108). Several studies observed markedly decrease of FMD in early morning (between 6-10 a.m.) and recovered FMD in late morning (after 10 a.m.) (105, 107). Conversely, Shaw et al. observed increased endothelium-dependent vasodilatation in the morning (108). However, Bau et al. reported no differences in FMD during the day (106).

Ageing has a significant impact on FMD (45, 47, 109, 110). Lower FMD were reported in older population. The average FMD in subjects ≥60 years was 4.5%, in subjects aged 50 to 59 years was 5.9%, in aged 40 -49 years was 7.1%, and 8.9% in <40 years (96).

Gender is another factor associated with differences in FMD response. According to a large Japanese multicentre study, the cut-off value for normal FMD response in their normotensive population (without cardiovascular risk factors or CVD) was 7.2% in men and 6.2% in women (47, 96). Furthermore, impact of the menstrual phase in females has been documented (111, 112). Several medications have influenced FMD specifically, such as drugs that target the cardiovascular system including β-blockers, nitrates, and calcium channel blockers (CCB) (44).

It has been reported that smoking, consuming caffeine, high-fat and high-carbohydrate meals are other factors that contribute to attenuated FMD (113, 114, 115, 116, 117). Therefore, it is recommended to avoid caffeine for 12 h and high-fat food and smoking for 6 h before FMD assessment (43).

It has been reported that the baseline brachial artery diameter is a potential factor affecting FMD.(47) Based on the definition of FMD, there is an inverse relationship between baseline diameter and FMD.(118, 119) Therefore, getting an accurate measurement of the baseline brachial artery diameter is essential to get true FMD.(44)

2.2.4. Endothelial dysfunction in hypertension

The first description of impaired endothelial function in human hypertension was reported in 1990 (46). Current evidence shows that endothelial dysfunction and hypertension may have a complex bidirectional relationship (49). Previous study showed a significantly lower FMD in the prehypertension group and hypertension group compared to normal BP group (47).

Imbalance of vasodilatation and vasoconstriction factors are common abnormal findings in hypertension (48). Increased vasoconstrictive factors such as plasma ET-1 has been also found in both congestive heart failure and hypertension and is associated with impaired endothelial function (120). A strong association was found between the severity of hypertension and the degree of endothelial dysfunction in the Framingham study (45).

Other studies found that different treatments of hypertension may have different effects on endothelial function. Some types of antihypertensive treatment such as β-

blockers (atenolol) do not modify endothelial function. On the other hand, angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin-receptor blockers (ARBs) could restore endothelial function to normal (121). Increased oxidative stress in hypertension caused by overproduction of angiotensin-II (122) will induce NADPH oxidase to produce ROS, leading to vascular inflammation (122, 123). By blocking their overproduction, ACEIs and ARBs increase vasorelaxation in patients with hypertension. Impaired endothelial function has also been associated with several factors and comorbidities predisposed to treatment resistance, including older age, obesity (124), obstructive sleep apnoea (124), insulin resistance (125), or hyperaldosteronism (126).

Several studies have shown that endothelial dysfunction is present in RH (40, 50, 51, 52). Significant impairment of FMD was evident in uncontrolled RH group compared to patients with prior history of RH (5.9% vs. 7.1%, $p<0.001$) (40). Another study observed significantly lower FMD in RH group (5.5±0.8%) compared to controlled hypertension group (9.2±1.4; $p<0.001$) and in healthy controls (10.1±1.1%; $p<0.001$) (52). Another study also showed that levels of 8-isoprostane were predictors of endothelial dysfunction and were significantly higher in RH compared to controlled hypertension group (50). Finally, Figueiredo et al. also showed impaired FMD in RH group compared to well controlled hypertension patients (8.3±4.7% and 10.1±5.9%) and 12.3±6.3% in normal subjects ($p<0.05$) (51).

2.2.5. Conclusion

Endothelial function is impaired in hypertension. Reduced NO bioavailability and alteration of shear stress are contributing mechanisms leading to endothelial dysfunction. Lower FMD is considered a marker of endothelial dysfunction and is

associated with high BP levels. RH showed markedly impaired FMD compared to well controlled hypertension.

Future studies are needed as it may highlight new insights into the pathophysiology and therapeutic strategies of hypertension to minimise further complications, particularly in RH.

Table 2.1 Flow mediated dilatation assessment in different populations

Author/ Year	Patients population/sample size	Results of FMD	Main findings
General population			
Maruhashi et al. 2013 (47)	• General adult population/5314	• Normal BP: FMD (6.75±3.33%) • PHT: FMD (5.96±3.15%) • Stage 1 HTN: FMD (5.56±3.07%) • Stage 3 HTN: FMD (5.07±3.07%)	• ↓ FMD in PHT group compared to normal BP. • ↓ FMD in the CVD group compared to that in the no-risk group or at-risk group
Shechter et al. 2014 (127)	• NC with no apparent heart disease/618	• More common CV event seen in FMD ≤11.3%	• In a mean clinical follow-up of 4.6 – 1.8 years, the composite CV events were significantly more common in subjects with ↓ FMD
Buchanan et al. 2017 (128)	• Sedentary individuals who exercise ≤1/9 • Exercise-trained individuals who exercise ≥3 times a week performed leg press exercise to maximal exertion on two separate occasions/6	• Before weightlifting: FMD (9.0% ± 1.2%) • After weightlifting: FMD (6.6% ± 0.8%)	• ↓ FMD in sedentary individuals after weightlifting. • Unchanged FMD in exercise-trained individuals after weightlifting. With the protective cuff: • ↑ FMD in sedentary individuals after weightlifting. • ↑ FMD in exercise-trained individuals after weightlifting

Author/ Year	Patients population/sample size	Results of FMD	Main findings
Lambiase et al. 2014 (129)	• Adolescents/45	• ↓FMD was significantly associated with a ↓DBP (r = 0.37, p = 0.01) and higher PP (r = −0.38, p = 0.01) in unadjusted models. • ↓ FMD was significantly associated with both DBP (B=6.5, SE=2.6, p=0.02) and PP (B=−12.4, SE=4.9, p=0.02) when adjusting for age, gender, fitness, and resting BP.	• ↓ FMD in lower DBP and greater PP during graded submaximal treadmill test.
Hypertension population			
Magen et al. 2010 (52)	• RH/20 • Controlled HTN/20 • NC/17	• RH: FMD (5.5 ± 0.8%) • Controlled HTN: FMD (9.2 ± 1.4%) • NC (10.1 ± 1.1%)	• ↓ FMD in RH compared to the other groups
Quinaglia et al. 2011 (40)	• Uncontrolled RH/26 • Controlled RH/40 • NC/25	• Uncontrolled RH: FMD (5.9±2.3%) • Controlled RH: FMD (7.1±5.1%) • NC: FMD (12.2±6.3 %)	• ↓ FMD in RH compared to the other groups
Figueiredo et al. 2012 (51)	• RH/44 • Controlled HTN/35 • NC/25	• RH: FMD (8.3±4.7%) • Controlled HTN: FMD (10.1±5.9%) • NC: FMD (12.3±6.3%)	• ↓ FMD in RH compared to healthy control • ↑ PWV in RH compared to the other groups

Author/ Year	Patients population/sample size	Results of FMD	Main findings
Faria et al. 2014 (50)	• RH/94 • Controlled HTN/55	• plasma 8-isoprostane levels were inversely associated with FMD in RH group (r = −0.35; p = 0.001)	• ↑ Plasma 8 isoprostane in RH • Plasma 8-isoprostane levels were inversely associated with FMD in RH group
Khan et al. 2020 (130)	• AF+HTN/ 61 • AF+HTN subgroups: - permanent AF (30) - paroxysmal AF (31) • HTN control/33	• AF+HTN: FMD (4.6%, 95% CI [2.6–5.9%]) • HTN control: FMD (2.6%, 95% CI [1.9–5.3%]) • Permanent AF: FMD (3.1%, 95% CI [2.3–4.8%]) • Paroxysmal AF: FMD (5.9%, 95% CI [4.0–8.1%])	• No difference in FMD between AF+HTN group and HTN control group. • ↓ FMD in permanent AF compared to paroxysmal AF groups

Author/ Year	Patients population/sample size	Results of FMD	Main findings
Souza-Barbosa et al. 2006 (131)	• HTN/63, divided into 4 groups: - Hydrochlorothiazide 25 mg/d - Irbesartan [IRBE] 150 mg/d - Quinapril [QUIN] 20 mg/d - IRBE 150 mg/d + QUIN 20 mg/d) • Normotensive/25	FMD at week 0 vs. week 12: • Hydrochlorothiazide: 7.3%±2 vs. 12.8±3.1 • Irbesartan:7.1%±2.8 vs. 13%±2.9 • Quinapril:7.2%±2.8 vs. 13.2%±2.1 • IRBE + QUIN: 7.5%±1.9 vs. 12.8%±3 • NC: 11.5%±2.4 vs. 13.5%±2	↑ FMD in HTN group after 12 weeks of antihypertensive therapy.
Brevetti et al. 2003 (132)	• Patients with PAD/131	• Patients with an event: FMD (5.8%) • patients without an event: FMD (7.6%)	↓ FMD in patients with cardiovascular events during follow-up compared to those without events
Fontes-Guerra et al. 2015 (133)	• RH/280	• FMD was 0.75% (−0.6 to +4.4%)	NTG but not FMD was associated with elevated night-time BP and non-dipping pattern.
Shantsila et al. 2011 (41)	• MHT/15 • HTN/40 • NC/40	• MHT: FMD (8.23%±3.82) • HTN: FMD (7.02%±4.33) • NC: FMD (12.9%±7.40)	• ↓ FMD in MHT & HTN compared to NC. • Similar FMD in MHT & HTN

Author/ Year	Patients population/sample size	Results of FMD	Main findings
Diabetes population			
Ito et al. 2015 (134)	• Type 2 DM without and with CHD /480 • Nondiabetic without and with CHD /240	• Nondiabetic with CHD: FMD (5.4 ± 3.2%) • Nondiabetic without CHD: FMD (6.9 ± 3.5%) • DM with CHD: FMD (5.6 ± 2.8%) DM without CHD: FMD (6.1 ± 3.3%)	• ↓ FMD in nondiabetic with CHD compared to nondiabetic without CHD • ↓ FMD in DM with CHD and without compared to those without both diabetes and CHD.
Meyer et al. 2008 (135)	• Type 2 DM /63 • Non diabetes control/44	• DM patients: FMD (3.8±0.8%) • Non diabetes control: FMD (6.9±0.9%)	• ↓ FMD in DM patients compared to non-diabetes.
Lockhart et al. 2011 (136)	• Type 1 DM /40 • Controls/32	• DM patients: FMD (3.95%) • Controls: FMD (7.75%)	• No difference in baseline brachial artery diameter was evident between the groups • ↓ FMD in patients with Type 1 DM compared to controls.
• **Other cardiovascular diseases**			
Suessenbacher et al. 2006 (137)	• Patients with CAD/68	Non-improved FMD (baseline vs. follow-up) • 8.81±3.9 vs. 7.71±2.9 Improved FMD (baseline vs follow-up)	• ↑ Cardiovascular events in non-improved FMD after 12 months of follow-up

Author/ Year	Patients population/sample size	Results of FMD	Main findings
		• 7.3±4 vs. 13.3±4.3	
O'Neal et al. 2014 (138)	• Multi-Ethnic Study of Atherosclerosis/2936	FMD below the sex-specific median value (median FMD; men, 3.6%; women, 4.2%; incidence rate per 1000 person-years, 7.3; 95% CI, 5.9–9.0) were more likely to develop AF.	• ↓ FMD associated with higher incidence of AF
Akar et al. 2008 (139)	In patients undergoing CRT/33 • Baseline preimplant • 90 days postimplant	• Responder: FMD at baseline (4.6%±4.5%) • Non-responders: FMD at baseline (8.6%±4.2%)	• ↓ baseline FMD in responders compared to non-responders • Improved endothelial function following CRT was observed in responders but did not reach statistical significance due to the sample size, as this study was not powered to detect those changes

Author/ Year	Patients population/sample size	Results of FMD	Main findings
Allan et al. 2013 (140)	• Patients with PAD/26 • NC/25	• Patients with PAD: FMD (2.43%) • NC: FMD (5.80)	• ↓ FMD in patients with PAD compared to NC.
Naidu et al. 2011 (141)	• Cardiac syndrome X/30 • NC /30	• Cardiac syndrome X: FMD (9.42 ± 7.20) • NC: (21.11 ± 9.16)	• ↓ FMD in cardiac syndrome X patients compared to NC.

AF: atrial fibrillation; BP: blood pressure; CAD: coronary artery disease; CHD: coronary heart disease; CHT: controlled hypertension; DBP: diastolic blood pressure; DM: diabetes mellitus; CI: confidence interval; CRT: Cardiac resynchronisation therapy; FMD: flow-mediated dilatation; NC: normotensive control; HTN: hypertension; NTG: nitroglycerine; PAD: peripheral arterial disease; PHT: prehypertension; PP: pulse pressure; PWV: pulse wave velocity; RH: resistant hypertension.

2.3. Arterial stiffness in hypertension

2.3.1. Basic principles of arterial stiffness

Arteries have thick walls to accommodate blood flow and its great pressure. The structure of the arterial wall consists of three different layers: intima, media and adventitia. The intima (inner layer) is composed of endothelial cells and connective tissue while the middle layer, which is known as tunica media, contains elastic tissue and a thick layer of smooth muscles. The outer layer, commonly known as adventitia, consists of fibrous connective tissue (142).

One of the primary functions of arteries includes blood flow transit and helps supply tissues with oxygen and nutrients (i.e., a conduit function). The other function is to dampen and smooth the flow pulsations (cushioning function) which can be compromised when the artery becomes stiffer (143, 144). During systole, blood is ejected from the LV through the aorta to the arterial system (143, 144). As a result of blood flow movement within the aorta and then arteries, a pressure wave is generated and transmitted through the arteries. When a pressure wave arrives at arterial bifurcations, a reflected wave is generated, which interacts with the pressure wave producing the aortic pulse wave and shaping the arterial pulse. The ventricular function and aortic elasticity have an effect on the transmitted wave. On the other hand, the reflected wave is influenced by a number of factors; the elasticity of the entire arterial circulation, PWV, and the reflection site distance from the heart (143, 144).

The pathophysiological mechanism of arterial stiffness can be defined as the changes in the properties of the arterial wall (145, 146). Arterial stiffness plays an important role in

BP regulation and cardiovascular function (147). Arterial remodelling occurs as a result of complex modifications including structural, mechanical and functional alterations, which eventually lead to rigidity of the artery. Arterial stiffening is associated with expanding and recoiling of arterial wall per heartbeat. The degree to which stiffness occurs can be different from one artery to another according to the elastic properties of each artery (145). Indeed, elastic properties in the tunica media of each artery determine the ability of the artery to recoil back when the pressure returns to its relaxed status. Expanding of the aortic wall in systole is determined by two factors, SBP (which is controlled by blood volume ejected in the systole) and the accumulating elastic energy of the vascular wall. The stored elastic energy acts in diastole phase to maintain continues perfusion and pressure in the aorta and arteries (142).

As mentioned above, arterial stiffening is associated with alteration of vascular smooth muscle cells (VSMC) and endothelial cell's function. VSMC are affected and stimulated by several factors, which include mechanical cell stretching, fluctuations in calcium signalling, angiotensin II, endothelin, oxidative stress and NO (148, 149, 150, 151). On the other hand, endothelial cell impairment produces an imbalance in the production and breakdown of vasodilator and vasoconstrictor substances, particularly in the production of NO and angiotensin II (152). NO is produced by endothelial cell and acts as a signalling molecule. Release of NO leads to VSMC relaxation and has a vasodilatation effect to improve the blood flow. Impairment of NO production contributes to oxidative stress and leads to impaired endothelium-dependent dilation which increases arterial stiffness (145).

2.3.2. Arterial stiffness assessments

Several methods are used to quantify arterial stiffness, including cardiac magnetic resonance (CMR), cardio ankle vascular index (CAVI), central blood pressure (CBP), pulse pressure (PP), AIx, and PWV. Of the various methods proposed, carotid-femoral PWV (cfPWV) and AIx are widely considered as the least invasive, safest, and most reliable in terms of accuracy, as recommended by the ESH in 2018 (8), and earlier by expert consensus document of 2006 (63). PWV is estimated noninvasively by measuring the distance of arterial pulse between two superficial arterial sites (e.g. carotid artery and femoral artery) and the travel time taken (153). PWV is inversely correlated to arterial compliance, therefore in a stiff artery, the reflected waves arrive at the heart earlier due to high PWV and this leads to increased pressure and decreased flow in late systole. This causes an elevated central PP, ventricular load, low EF, and high myocardial oxygen consumption (154).

A scientific statement in 2015 published by American Heart Association (AHA) recommended that measurement of arterial stiffness can help to predict cardiovascular events and it is an effective factor for risk stratification in relation to high BP treatments (11, 145, 155). In addition, increased arterial stiffness may be an independent prognostic factor for the occurrence of cardiovascular events in patients with arterial hypertension, such as coronary heart disease (CHD), congestive heart failure and stroke (156, 157, 158, 159).

The second recommended assessment of arterial stiffness is AIx. AIx is mathematically derived quantification which describes the association between the CBP and the arterial

pressure wave, including the forward and the reflected waves. The heart rate, travel time of the reflected wave, PWV, LV ejection, structure of the artery at reflection sites and certainly BP level are factors that determine this index (160, 161). AIx is estimated by calculating the following equation:

$$AIx = \frac{Ps-Pi}{Ps-Pd} \times 100$$

Where Ps: initial systolic pressure; Pi: pressure at inflection point; Pd: diastolic pressure; (Ps – Pi) refers to the augmentation pressure; (Ps – Pd) refers to PP (162).

2.3.3. Arterial stiffness and resistant hypertension

Hypertension is the second most important risk factor of increased arterial stiffness after ageing (53). In 1808, Young et al. was one of the first to emphasis the association between BP and what we now know as PWV (163).

Recent studies closely linked the presence of increased arterial stiffness and RH, assessed by PWV and AIx (Table 2.2) (41, 50, 51, 54, 55, 56, 57, 58, 59). Shantsila et al. evaluated PWV in different groups of hypertension including MHT and significantly higher PWV was observed in all groups (41). Chung et al. evaluated 142 of RH patients aged above 65 years and showed that PWV was significantly associated with the incidence of RH (P = 0.015); however, this finding could be related to the presence of some comorbidities such as diabetes mellitus (DM) in RH group compared to the controlled group which can be behind the progression of arterial stiffness (54). Pabuccu et al. showed the same possible linking of impaired AIx and PWV to resistant group, which were markedly elevated compared to the controlled group (P=0.03 and P<0.01) (55). Faria et al. evaluated an RH group and showed significantly elevated oxidative

stress determined by 8-isoprostane, suggesting some contribution of oxidative stress to endothelial dysfunction in patients with RH (50). Nevertheless, this is a cross-sectional study and any causality cannot be concluded. Also, Barbaro et al. reported that when compared to healthy control groups, patients with RH had higher PWV in association with elevated tumour necrosis factor-α (TNF-α) levels in RH and inflammatory cytokines (57, 58).

Conversely, a longitudinal study from the Framingham Heart Study demonstrated sustained arterial stiffening in both groups of hypertension (controlled and uncontrolled treated) irrespective of the BP level achieved at the end of the follow-up (56). These findings give an important insight into the relationship between elevated PWV and residual CVD risk in patient with hypertension, whether it is well controlled or resistant to treatment. However, the study group was defined as 'uncontrolled hypertension' which may be different than RH per se. Long standing duration of hypertension is likely the reason of irreversible arterial changes despite better control of BP.

Haemodynamically all above findings are linked to the fact that as hypertension progressed and becomes sustained, there is some degree of vascular remodelling. In patients with hypertension, the main direct structural alteration of the arterial wall is hypertrophy of tunica media (164). SBP is directly correlated to the degree of the aortic stiffness (142).

Hypertension is a complex of alterations in multiple systems that naturally regulate normal pressure (164). These systems included, renin-angiotensin-aldosterone system

(RAAS), renal system, and the sympathetic nervous system (SNS), all have an indirect effect on VSMS function and arterial remodelling. For example, high activation of RAAS has a significant effect on the progression of the increased stiffness in hypertension population, because angiotensin II causes VSMC hypertrophy and collagen accumulation, while aldosterone activates growth of extracellular matrix (ECM) by fibroblasts. Both changes have an adverse effect on functional properties of arteries (164). Genetic predisposition is another mechanism leading to stiff arteries in individuals with hypertension (165).

Finally, the majority of studies included patients who were diagnosed as 'true' RH, according to ESH/ESC guidelines or AHA statement with the exception of two studies (54, 166). Only three studies assessed drug adherence to confirm true RH, (Table 2.3) (50, 58, 166).

2.3.4. Arterial stiffness and target organ damage

The CBP is the pressure reflecting the perfusion pressure of the heart, brain and kidney. Therefore, elevation of CBP has consequences that impact almost the entire body systems. Indeed, cfPWV measurement was an independent indicator of the degree of vascular damage and TOD in hypertension (8, 167, 168, 169). Worsening of arterial stiffness is also linked to the increased risk of stroke and kidney failure due to the damage of the brain and renal vessels (154). Increased arterial stiffness assessed by PWV has been shown to be an independent predictor of all-cause mortality in end-stage kidney disease and in hypertension (156, 170). In the hypertension patient population

with no history of CVD, increased PWV may act as an independent predictor of patients who are at high risk of cardiovascular events (171).

A meta-analysis of 17 studies included 16,000 participants who were observed for 7.7 years showed that each 1 m/sec augmentation of PWV increase the rate of cardiovascular morbidity/mortality, and all-cause mortality by 15% (172). Moreover, Zuo et al. concluded that with each 1 SD increase in central augmented pressure, the risk of cardiovascular events or death from CVD in the older patients rose by 1.4 fold (158).

2.3.5. Impact of comorbidities and age on arterial stiffness

The progression of arterial stiffness exacerbated by the presence of comorbidities, and by ageing as demonstrated in Figure 2.2 Arterial stiffness and hypertension share a number of risks, including chronic kidney disease (CKD), DM, obesity, female sex (59), black race and old age (Figure 2.3) (167, 173).

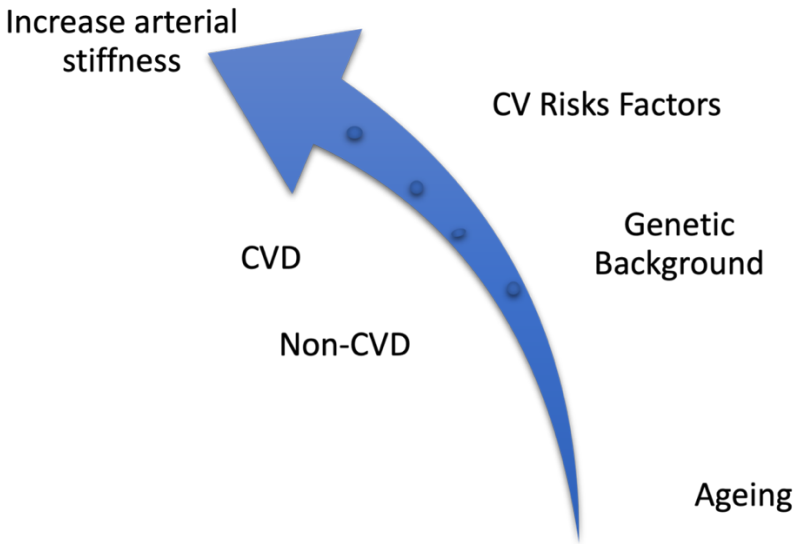


Figure 2.2 The progression of arterial stiffness exacerbated by the presence of co-morbidities. CV: Cardiovascular; CVD: Cardiovascular diseases.

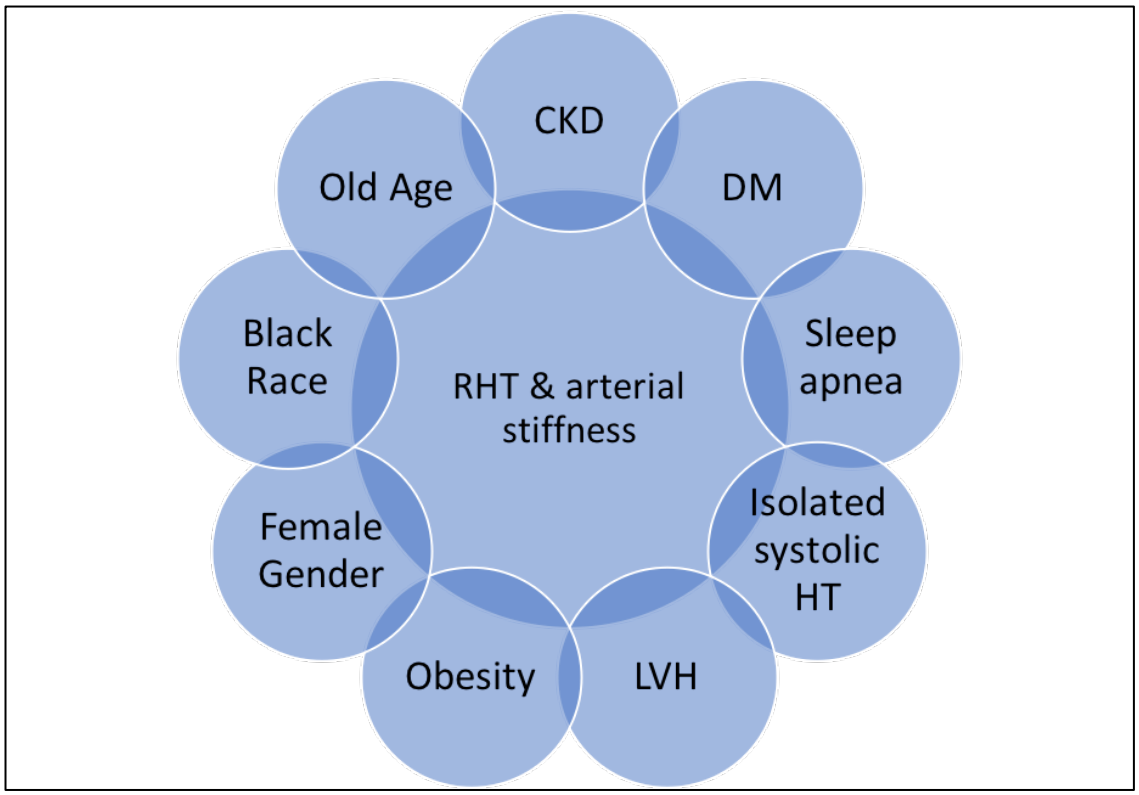


Figure 2.3 Risk factors of resistant hypertension and arterial stiffness. CKD: chronic kidney diseases; DM: diabetes mi; HT: Hypertension; LVH: Left ventricular hypertrophy; RHT: resistant hypertension.

2.3.5.1. Age and acceleration of PWV

As people age, arterial stiffness increases independently of BP elevation (174, 175). Lajemi et al. (174) and Benetos et al. (175) have linked older age to the progression of PWV in normal populations and in controlled hypertension populations who are ≥50 years old. Both results are expected because age is a contributing factor leading to the progression of arterial remodelling and PWV acceleration. Several mechanisms are implicated in this progression: elastin fragmentation, increased elastase activity, high collagen production by VSMC, elevated cross-linking of collage, altered growth factor regulation/tissue repair mechanisms, tunica media calcification, low NO production, high production of ECM of the media and adventitia and wider PP as a result of low

compliance. All these factors make the arteries stiffer and less resilient, independent of BP elevation (164, 166).

2.3.5.2. Impact of hypertension and diabetes mellitus

Concurrent of hypertension and DM is highly prevalent, and the frequency of hypertension rates is nearly twice in patients with DM compared to the normal population (176). It is expected that the risk of CAD, stroke, nephropathy and retinopathy is higher in both hypertension and DM populations. The common mechanisms of association between those two conditions are involving elevated BP, imbalance of the RAAS and vascular disorders (176).

Tedesco et al. has examined the effect of concomitant DM and hypertension on arterial stiffness changes, using PWV assessment (177). An elevated PWV was found among those who had hypertension and DM compared to those who had DM or hypertension alone, and when compared to control group with no hypertension or DM. Elevated arterial stiffness in DM was associated with increased glucose levels, which augment the production of non-enzymatic glycation and high collagen accumulation which changes the mechanical characteristics of the arterial wall. Moreover, low insulin sensitivity is associated with a decline in vascular compliance (178). For these reasons, it is expected that combination of hypertension and DM results in a degree of arterial stiffness that is markedly elevated, as opposed to the extent seen in hypertension or DM alone.

2.3.5.3. Impact of hypertension and kidney disease

Concomitant uncontrolled hypertension and renal dysfunction are common, and hypertension is considered one of the most significant causes of kidney impairment after

DM (8). Arterial stiffness and vascular dysfunction increase progressively as kidney function deteriorates. Elevated proteinuria and high salt consumption are independently linked with both CKD and RH (179, 180). At the same time, proteinuria and high salt consumption are closely associated with impaired endothelial function and increased arterial stiffness (180, 181). In fact, proteinuria has a strong predictive value for the presence of vascular dysfunction in patients with CKD (182). Increased total body sodium may also lead to arterial stiffening, which is reflected by high PP with renal impairment (183). Vascular dysfunction in renal disease population is also associated with low glomerular filtration rate, as well as dilated vessel diameter with preserved wall thickness, resulting in increased wall stress (184, 185). cfPWV is found to be high in CKD population compared to hypertension and healthy subjects, indicating that the severity of the arterial stiffening progresses more in the CKD population (185, 186, 187).

2.3.5.4. Impact of hypertension and heart failure

Elevated BP is common in patients with heart failure contributes to a worse outcome (188). Development of arterial stiffness is closely linked with impaired systolic and diastolic LV function (189). The impact of elevated arterial stiffness on the risk of developing heart failure are not well-known. On the other hand, patients with heart failure have increased arterial stiffness with both preserved function (HFpF) or reduced function (HFrF) (190, 191).

In the longitudinal Framingham Heart Study, 2539 participants without clinical heart failure were observed for 10 years and examined every 2 years (192). Central PP, AIx and cfPWV were evaluated. A total of 170 participants developed heart failure during the follow-up, and HFpF occurred in (43%) and HFrF occurred in (34%), while in 23% of

patients, the diagnosis was unclassified. High PWV was associated with an increasing risk of having heart failure of both subtypes.

One possible haemodynamic mechanism of HF development is that high arterial stiffness leads to increased LV and cardiac load (189). Also, the imbalance between myocardial oxygen supply and demand may occur due to LVH and reduced diastolic BP (frequently associated with abnormal arterial stiffness), resulting in low myocardial perfusion and subendocardial ischaemia (193). Furthermore, high arterial stiffness may lead to impairment of the intima by increase blood flow shear stress, thus contributing to atherogenesis (194).

2.3.5.5. Impact of hypertension and atrial fibrillation

Hypertension increases the risk of developing atrial fibrillation (AF), and increasing arterial stiffness is a contributing factor to incident AF (195, 196, 197, 198). For example, the Framingham study linked aortic stiffness PP to higher AF occurrence and recurrence rates (196). Each 10 mmHg increase in PP leads to the increased risk of developing AF by 12%. Lee et al. investigated the association between AF and arterial stiffness, using PWV assessment (197). The study demonstrated that presence of AF results in elevated arterial stiffness, independent of age or BP in the hypertensive population. All above findings can be explained by the following pathophysiological changes: aortic stiffness reflected by high PP may contribute to the increase cardiac load causing ventricular hypertrophy that results in ventricular diastolic dysfunction and remodelling (dilated atrial and high atrial pressure) (199, 200, 201, 202). All these would lead to electrical changes in the atrium contributing to increase risk of developing AF (198).

2.3.5.6. Impact of antihypertensive drugs

High BP is associated with both high arterial stiffness and low compliance. Thus, in order to reduce SBP, arterial stiffness and PWV need to be lowered. Several cardiovascular agents have different impacts on the structural and functional properties of the artery. However, the effects of antihypertensive medications on arterial stiffness can be direct or indirect. Many antihypertensive medications reduce arterial stiffening by lowering mean arterial pressure, reducing wave reflection and increasing the compliance. While others could cause further functional changes of arterial properties leading to arterial stiffness improvement.

ACEIs (203, 204, 205), β-blockers (10), diuretics (206), calcium antagonists and ARBs (204, 207) all showed therapeutic effect on arterial stiffness, to varying degrees, regardless of the effect on brachial BP. The reduction occurred either acutely or over a long period of follow-up. Diuretics and β-blockers lower BP but have minimal impact than all the other antihypertensive agents in decreasing arterial stiffness (10).

In addition, aldosterone blockers reduce cfPWV and AIx by enhancing NO bioactivity and improving endothelial vasodilator function (208, 209, 210). Another small study showed spironolactone was efficient at lowering BP and improving arterial stiffness in patients with hypertension and DM (206).

The ACEIs, ARBs, and CCB are the most widely used vasodilator agents and showed direct effect on arterial stiffness independent of BP reduction (211, 212). Herata et al. evaluated the effect of ramipril and atenolol in participants who have one or more

coronary risk factors, and found that the ramipril group showed significant decline of the central pressure by 5.2 mmHg (Table 2.4) (213). This finding seems to be consistent with another study, which showed an improvement of arterial stiffness after using ramipril in patients with PAD, where there was an improved aortic compliance by (0.10 ±0.02 mL/mm Hg) and decreased PWV by (1.7±0.2 m/s) (203). Furthermore, AIx decreased by (4.1±0.3%) and SBP reduced by (5±1 mm Hg) ($p<0.001$) after 24 weeks of treatment. London et al. investigated the effect of another ACEIs (quinapril) on 12 patients with hypertension and end stage renal disease (ESRD), whereby quinapril therapy caused sustained reduction in PWV, but was dependent on parallel BP reduction (205). Hence, this effect could be due to the PP reduction and improved aortic distensibility caused by reduced BP.

ARBs show an improvement of arterial stiffness according to Klemsdal et al. (207). The result demonstrated that PWV declined from 9.3 m/sec to 8.7 m/sec ($p=0.05$) after 4 weeks of treatment with losartan to 16 patients, which can be explained by the direct effect occurs as a result of vasodilatation due to smooth muscle relaxation.

Williams et al. (214), Boutouyrie et al. (215) and Asmar et al. (216) investigated combination therapy in three large longitudinal studies with long-term follow-up. The Conduit Artery Function Evaluation (CAFE) study investigated the effect of two combinations of (atenolol with bendroflumethiazide based treatment) and (amlodipine perindopril-based treatment) on the central pressure and stiffness (214). 2199 patients enrolled in five centres were followed up over 4 years. Office BP readings were the same among both groups, whereas significantly greater reduction in central pressures was

observed in the amlodipine/perindopril combination group. The EXPLORE study also compared two groups of drugs combination: amlodipine with valsartan and amlodipine with atenolol (215). Amlodipine with valsartan showed greater reduction of central pressure than amlodipine with atenolol. In the REASON trial, on subjects with hypertension, small dose combination therapy of indapamide (0.625 mg) and perindopril (2 mg) was compared to the effect of 50 mg of atenolol (216). After 12 months of follow-up, the combination dose significantly reduced brachial SBP (-6.02 mmHg; 95% CI, -8.90 to -3.14) and PP (-5.57; 95% CI, -7.70 to -3.44) compared to atenolol.

There is no certain therapy to specifically reduce arterial stiffness. However, antihypertensive medications, especially those with a vasodilatation effect, are likely to be more effective in lowering PWV.

2.3.6. Conclusion

It has been shown that there is an acceleration of arterial stiffening in patients with RH. However, the exact mechanisms of this process are not yet fully understood. It has been established that abnormal cfPWV and AIx can serve as markers of TOD and they may help predicting adverse cardiac events. These measures are useful tools for risk stratification in hypertension, particularly in its form resistant to treatment. PWV accelerates with age and with the increasing number of cardiovascular factors present in a particular patient. For this reason, this index provides a cumulative characteristic reflective of both physiological age-related risk and an individual chronic exposure to all cardiovascular risk factors. This possibly explains the prominent progression of PWV in

RH. Overall, assessment of arterial stiffness provides valuable insight into pathophysiology and prognostication in RH. It may also have a potential as a separate therapeutic target, although this possibility needs further exploration.

Table 2.2. PWV assessments in resistant hypertension population

Author/Year	Study Design	Methods	Population	Mean age/number	Main Findings in RHT
Vamsi et al. 2018 (59)	Prospective single-centre cohort study	• PWV	• RH	58.8*/80	↑ PWV (females vs. males)
Niiranen et al. 2016 (56)	Cross-sectional study	• cfPWV	• Uncontrolled treated HTN • Treated HTN	60/2127	↑ PWV in 60% of treated HTN ↑ PWV in 90% of uncontrolled treated HTN
Barbaro et al. 2015 (58)	Cross-sectional study	• cfPWV • Inflammatory b/m	• RH • Mild HTN • NC	54.7/72	↑ PWV ↑ inflammatory cytokines ↑ TNF-α No differences in IL-6
Barbaro et al. 2015 (57)	Cross-sectional study	• cfPWV • TNF-α	• RH • NC	52/51	↑ PWV ↑ TNF-α
Chung et al. 2014 (54)	Observational study	baPWV	• RH • Controlled BP • NC	65/1620	↑ baPWV
Faria et al. 2014 (50)	Cross-sectional study	• FMD • cfPWV • Plasma 8-isoprostane	• RH • Controlled HTN	57/149	↑ Plasma 8 isoprostane ↓ FMD ↑ PWV

Author/Year	Study Design	Methods	Population	Mean age/number	Main Findings in RHT
Pabuccu et al. 2012 (55)	Observational study	• cfPWV • AIx • Aortic strain/US • AD/US	• RH • CHT • NC	54.7/87	↑ AIx ↑ PWV ↓ aortic strain ↓ AD
Figueiredo et al. 2012 (51)	Observational study	• cfPWV • FMD	• RH • Controlled HTN • NC	52.6/139	↓ FMD ↑ PWV

*: median age; AD: aortic distensibility ; AIx: augmentation index; baPWV: brachial-ankle pulse wave velocity; B/M: biomarker; BP: blood pressure; cfPWV: carotid-femoral pulse wave velocity; CAVI: cardio ankle vascular index; CBP: central blood pressure; CHT: controlled hypertension; FMD: flow-mediated dilatation; HTN: hypertension; IL-6:interleukin-6; MAP: mean arterial pressure; NC: normotensive control; PP: pulse pressure; PWV: Pulse Wave Velocity; RD: renal denervation; RH: Resistant Hypertension; TNF: tumour necrosis factor- α; US: Ultrasound;

Table 2.3 Resistant hypertension studies and their fulfilment of criteria to define resistant hypertension as per European Society of Cardiology (ESC) and European Society of Hypertension (ESH) guidelines, 2018

Author/ Year	Region	Adherence assessment	Definition assessment as per (ESH/ESC)
Vamsi et al. 2018 (59)	**Croatia**	__	√
Barbaro et al. 2015 (58)	**Brazil**	√	√
Barbaro et al. 2015 (57)	**Brazil**	__	√
Chung et al. 2014 (54)	**China**	__	__
Faria et al. 2014 (50)	**Brazil**	√	√
Figueiredo et al. 2012 (51)	**Brazil**	__	√
Pabuccu et al. 2012 (55)	**Germany**	__	√

Table 2.4 Impact of antihypertensive drugs on arterial stiffness

Author/Year	Treatments	population	Sample size	Follow-up	Effect on brachial SBP	Effect on PWV	Effect on central SBP	Effect on AIx
Asmar et al. 2001 (216)	Ind/Per vs. Atenolol	HTN	471	12 M	↓ Ind/Per	similar reduction	↓ Ind/Per	↓ Ind/Per
Hirata et al. 2005 (213)	Ramipril vs. Atenolol vs. Placebo	Coronary risk factors	30	Measurements repeated every 30-60 min/5h on 3 separate days within ≥ 7 days	↓ Ramipril	similar reduction	↓ Ramipril	↓ Ramipril
London et al. 1996 (205)	Quinapril vs. Placebo	HTN+ESRD	12	After 127h of quinapril administration	↓	↓	↓	↓
Klemsdal et al. 1999 (207)	Losartan vs. Placebo	HTN	16	4 W	↓	↓	↓	-
Davies et al. 2005 (206)	Spironolactone vs. Placebo	HTN+DM	10	4 M	↓	↓	-	-

Author/Year	Treatments	population	Sample size	Follow-up	Effect on brachial SBP	Effect on PWV	Effect on central SBP	Effect on AIx
Williams et al. 2006 (214)	Aten/Thiaz vs. Aml/Per	HTN	2119	4 Y	similar reduction	similar reduction	↓ Aml/Per	↓Aml/Per
Boutouyrie et al. 2010 (215)	Aml/Vals vs. Aml/Aten	HTN	393	24 W	similar reduction	similar reduction	↓Aml/Vals	↓Aml/Vals

Aml/Aten: amlodipine and atenolol combination; Aml/Per: amlodipine and perindopril combination; Aml/Vals: amlodipine and valsartan combination; Aten/Thiaz: atenolol and thiazide combination; ESRD: end stage renal disease; HTN: hypertension; Ind/Per: indapamide and perindopril combination; PAD: peripheral artery diseases; DM: diabetes mellitus; ↓: Significant reduction

2.4. Cardiac haemodynamics

2.4.1. Principle of left ventricular function quantification by speckle tracking echocardiography

Myocardial strain refers to the percentage deformation of the myocardium during the cardiac cycle. It represents the extent of regional myocardial deformation in a specified period of time in three orthogonal directions (longitudinal, radial and circumferential). All determined by length, thickness and shortening, using the formula: $\varepsilon = (L-Lo)/ Lo$, where ε indicates strain (has a unit of %), L indicates length after deformation, and Lo indicates baseline length. Strain rate (SR) refers to the speed at which the myocardium deforms (velocity changes/ distance) (26).

Initially, two techniques were introduced to assess myocardial strain: (i) CMR in the late 1980s (217); and (ii) tissue Doppler imaging (TDI) in the 1990s (218). While TDI is considered a feasible and reliable technique, it has several limitations that still remain unresolved. TDI is highly angle dependent, is constrained to longitudinal cardiac deformation and suffers from poor signal to noise ratio (219). STE is a promising technique which was introduced in the early 2000s (25), and has been validated against sonomicrometry (which involves the implantation of piezoelectric crystals and measures of the changes in distance between embedded crystals, due to the myocardium movement) and tagged CMR (220, 221). STE is used to assess myocardial function and it overcomes the limitations of TDI (222).

The main advantage of STE is its ability to reflect active contraction within each segment, avoiding tethering effect, which makes it less influenced by artefacts. STE can measure three directions of cardiac motion and can track the speckle in any 2D direction making it less angle dependent, (Figure 2.4).

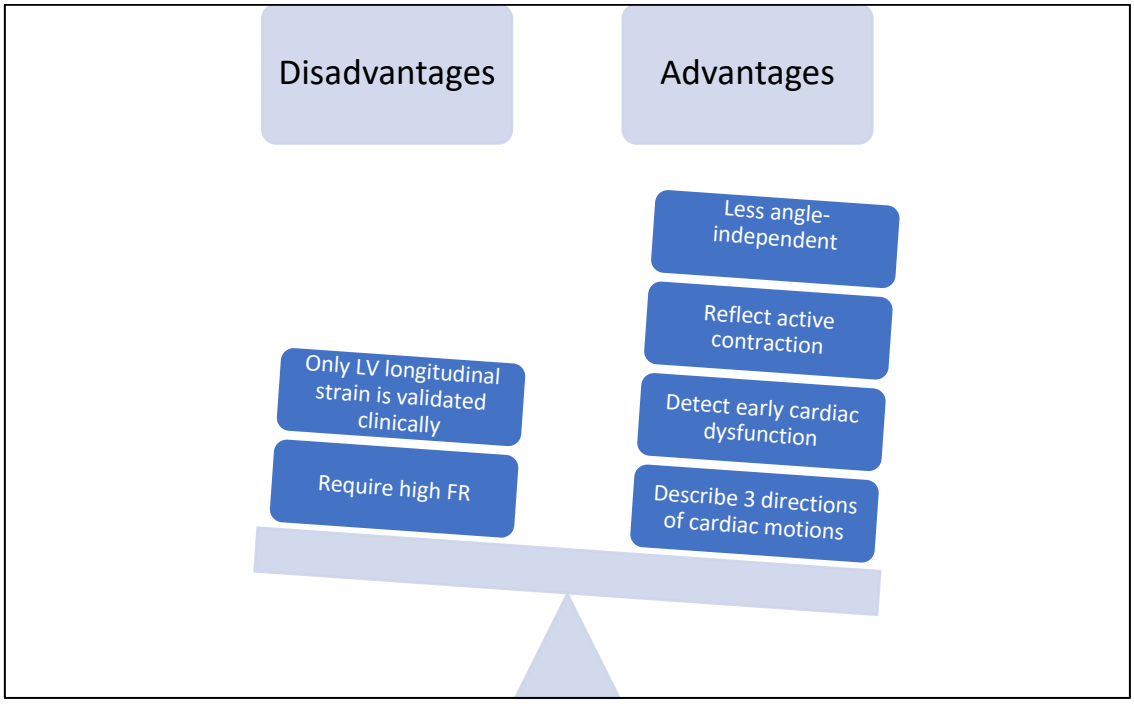


Figure 2.4 Speckle tracking echocardiography advantages. FR: Frame rate; LV: Left ventricular.

Heterogeneous ultrasound-myocardial tissue interactions produce an interference pattern, which is identified as a unique stable set of speckles (223). STE modality identifies speckles based on echocardiographic images and tracks them between consecutive frames. It includes evaluation of myocardial strain, SR, and rotational deformation, which all are obtained by using specific software (224).

Myocardial strain derived from STE can be measured in 3 planes. Circumferential and longitudinal strain represent a shortening of the LV cavity, and both have negative values

(Figure 2.5). Radial strain represents myocardial thickening of the LV in systole (secondary to the conservation of mass from longitudinal and circumferential shortening) and is denoted as a positive value. All strain parameters can be evaluated globally or regionally. GLS, global circumferential strain (GCS), and global radial strain (GRS) are calculated as an average of segmental regional strain. The average normal GLS is −19.7% with a borderline level of −18% (225, 226). Normal GCS is considered to be between −20.9% to −27.8% and average GRS is between 35.1% to 59.0% (226). STE also provides the capacity to measure twist and torsion which are parameters determine deformation of LV (25).

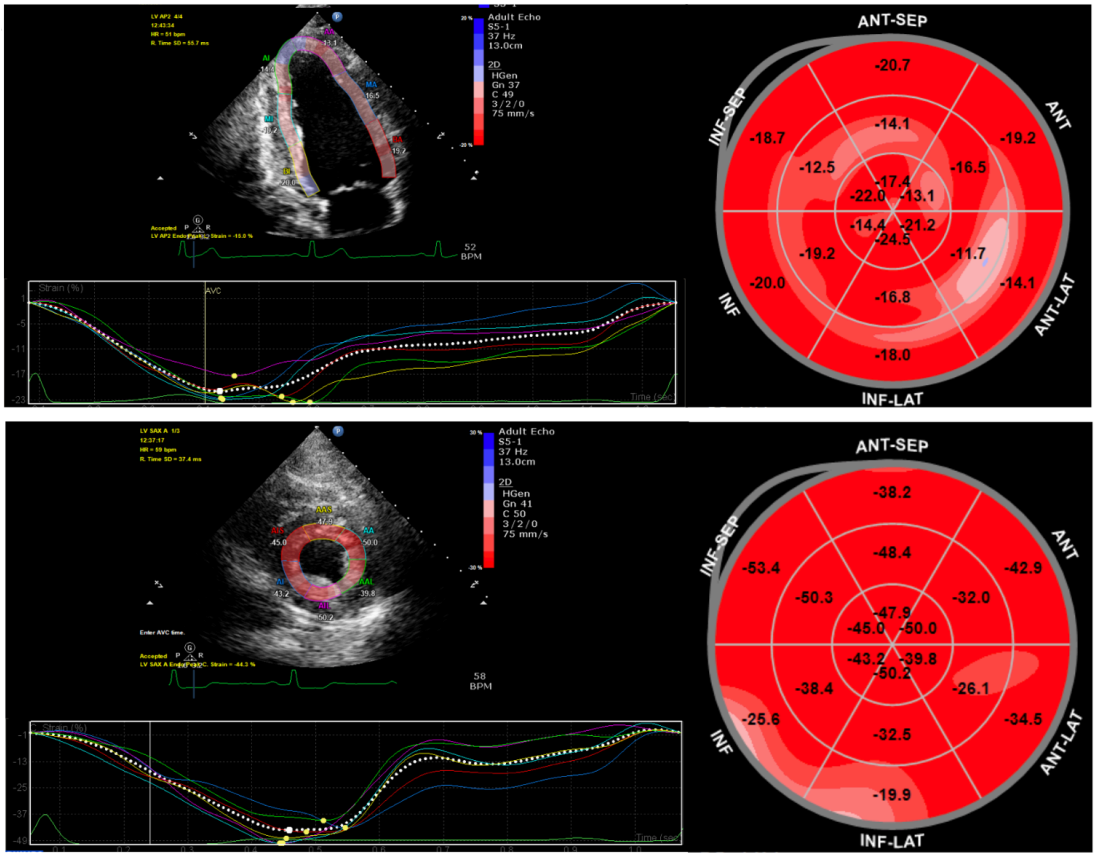


Figure 2.5 Example of global longitudinal strain (upper) and global circumferential strain (lower) of left ventricle.

2.4.2. The Role of strain in predicting early damage in hypertension

Conventional echocardiography is a reliable method widely used to detect impaired LV systolic and diastolic function in hypertension. It is also used to calculate LV mass and determine the presence and the degree of LVH, a predictor of morbidity and mortality in hypertension (19, 20). However, hypertension is associated with reduction in LV systolic strain in asymptomatic patients with normal EF with and without LVH, suggesting that LV mechanical abnormalities precede the development of LVH (22, 23, 24).

2.4.2.1. Decreased longitudinal function in hypertension

Normal myocardium consists of cardiac myocytes (30-40%) and non-myocytes components (60-70%) (227). Myocardial fibres in the subendocardial layer are oriented in a longitudinal direction which then gradually change to a transverse direction in the middle layer and revert to longitudinal in the subepicardial layer (227).

Recent studies have closely linked the presence of fibrosis to attenuated myocardial strain (23, 27, 28, 29). Cardiac remodelling in hypertension involves an imbalance in the production of collagen types I and III (these subtypes are the major stress-bearing element within the ECM). This leads to an excessive deposition of collagen fibres in fibroblasts which transdifferentiate into myofibroblasts leading to heterogeneous acceleration of myocardial fibrosis (30, 31). Moreover, increased MMP-1 turnover led to reduced collagen I and III degradation and development of subendocardial myocardial fibrosis. This implies that irregular collagen production and myocardial fibrosis are associated with reduced GLS in hypertension and hypertrophic cardiomyopathy, and eventually lead to early impairment of systolic function (23, 27, 28, 29). Another pathway leading to activation of subendocardial production of collagen in hypertension is pressure overload and high-end systolic wall stress. The process involves collagen network thickening and fibrosis build up primarily in the subendocardial layer (228).

Furthermore, fibrosis may have a possible direct effect on the rearrangement of myocardial sheets in subendocardial layers where maximum shearing deformation

occurs, compared to the other layers (227, 229, 230, 231). There is limited information available linking cardiac shear motion and systolic function.

2.4.2.2. The additive value of global longitudinal strain

Longitudinal, circumferential, and radial dysfunction do not occur in tandem with longitudinal subendocardial fibres being prone to being compromised first in several pathologies (232, 233). GLS is the most widely used clinical application of STE. It has been recommended by the American Society of Echocardiography (ASE) for evaluation of global LV systolic function and has been widely validated (26, 234, 235). GLS is considered as a strong indicator of an early phase of myocardial impairment in hypertension (Table 2.5) (24, 236, 237, 238, 239, 240, 241, 242, 243). It has been shown in some studies that the prevalence percentage of impaired GLS in hypertensive population vary between 15% to 42% (237, 244, 245, 246), suggesting for the influence of other related factors such as age, gender, ethnicity, duration of the hypertension, uncontrolled hypertension, DM and obesity (237, 244, 245, 246, 247, 248).

Studies have shown that GLS might be beneficial as an independent predictor of cardiovascular outcomes in general population (249, 250, 251), and in a population with a wide range of EF (252). GLS is a strong predictor of major adverse cardiac events (MACE) including heart failure, stroke, MI and all-cause mortality (238, 253, 254). In the Copenhagen City Heart Study, which includes 1296 of participants from general population, who underwent STE assessment between 2001 to 2003 and were followed until 2013 (254). GLS was an independent predictor of cardiovascular death and

morbidity, including HF and MI with a hazard ratio of 1.12 [1.08–1.17], p<0.001 per 1% decrease. This association persisted after multivariable adjustment for the following parameters: (age, sex, heart rate, hypertension, SBP, LVEF, LV mass index (LVMI), LV dimension, deceleration time, LA dimension and E/e' (254). Similarly, Saito et al. retrospectively collected data on MACE (all-cause death and admission because of heart failure, MI, and strokes) with (median follow-up 4 years) in asymptomatic non-ischaemic subjects with high BP (238). It has been shown that MACE occurrence was independently associated with greater incidence of concentric hypertrophy and reduced GLS (both, p<0.01).

Cheng et al. examined whether systolic dysfunction assessed by STE improved by intensive antihypertensive treatment in 182 patients with uncontrolled hypertension (241). The study assessed GLS before and after 24 weeks of antihypertensive treatment and showed an improvement in GLS in response to the treatment was independent of changes in BP and associated with increased dose. This is more likely to occur when afterload reducing treatment is used, which improves LV function independent of BP readings (255). Moreover, GLS improved by -1.4% more in uncontrolled hypertension patients not meeting RH criteria females compared to uncontrolled males with hypertension (p=0.003). This difference in the responses between the two genders could be due to the differences in GLS baseline values, where females had higher GLS compared to males. In addition, the association between female sex and improvement in GLS is unclear and has yet to be examined in the general population to confirm sex

differences associated with LV function. Another observation found an improvement in GLS by -0.46% for every 5 kg/m^2 reduction in body mass index (BMI) (p=0.015).

Similar findings have been reported by other studies which links attenuated GLS with metabolic disorders and obesity (256, 257). The RESOLVE trial examined participants with metabolic syndrome and showed reduced GLS compared to a control group (256). Wong et al. showed that insulin and BMI were significantly and independently associated with strain function in obese population (257). However, in the study by Crendal et al., 78% of participants had hypertension which may consider as confounding factor and could mask the actual association (256). In addition, 17% of participants were treated with β-blockers, which have an established effect on LV remodelling.

2.4.2.3. Circumferential and radial function

Notably, the mid-myocardial layer may remain unchanged or even increased compared to the longitudinal function, which probably explain the well-preserved function reflected by EF (24, 236, 243, 258, 259, 260). Preserved radial and circumferential function at early stages of hypertension linked to the cross-fibre shortening phenomenon from hypertension-related ventricular remodelling, where mid-wall myocardial fibres are not compromised and consequently circumferential and radial function are preserved (246). Although this explanation has received reasonable attention, other theories suggest that reduced longitudinal and circumferential strain exists with preserved EF secondary to increased LV wall thickness (260).

However, longitudinal function is not always the earliest predictor in all circumstances. Previous studies have reported that all three planes of function (longitudinal, radial and circumferential) may decline in heart failure, signifying a decompensation mechanism of the LV and impaired myocardial layers as a response to increase myocardial wall stress and disease progress (258, 261, 262). Because impaired longitudinal function has occurred in earlier phase, following decreased of radial and circumferential function which was associated with further LV dilatation leading to heart failure (263, 264, 265).

2.4.2.4. Twist and torsion deformation

Rotation, twist and torsion are several terms to describe additional deformation of the LV caused by the helical arrangement of myocardial fibres. LV rotation is defined as an apical counter-clockwise movement and basal clockwise movement in systole. During systole, the LV stores potential energy, which is subsequently released in early diastole. Twist and untwist play an important role by storing and releasing this energy which leads to LV diastolic relaxation and early diastolic filling. Twist / untwist (°) and rate (°/s) are calculated as the net difference between basal clockwise and apical anticlockwise rotation and rotation rate (25). Torsion is calculated by dividing the twist angle by apical-basal distance and measured in (°/cm) (25). In a non-diseased population, LV twist is approximately 15° with apical rotation being between 5° to 10° (counter-clockwise) and basal rotation between -4° to -7° (clockwise) as observed in studies by CMR tagging (266). A study by Dong et al. showed that as with other indices of cardiac function, rotation is affected by loading condition (preload and afterload) of LV (267). Rotation increases with increased preload (end-diastolic volumes) and decreased afterload (end-

systolic volumes) (267). Reduced LV untwisting, elevated torsion and twist have been observed in patients with hypertension (17, 268, 269, 270) and in various CVD (271, 272). Alterations of myocardial twist are also linked to ageing. Previous studies have demonstrated decreased diastolic untwisting, increased LV rotation and twist with age in a normal population (273).

2.4.3. Conclusion:

Myocardial fibre orientation is a fundamental feature of the myocardium and it has substantial role in systolic function. STE imaging is a new non-invasive cardiovascular imaging modality that can be used in clinical practice to understand the mechanism of cardiac deformation, particularly in patients with early compensation of myocardial function and in patients with RH. Using STE also offers comprehensive evaluation to detect the underlying impaired systolic function in several pathologies, including hypertension, to deliver optimal management plan. Furthermore, this powerful and valuable technique provides accurate and objective measures on global/regional contractile function.

Table 2.5 Summary of studies using 2-dimension speckle tracking analysis in hypertensive populations

Author/year	Methods	Patients population	Sample size	STE software/ Echo machine	STE parameters	Follow-up duration	Results
Bendiab et al. 2017 (237)	2D STE	HTN/Overweight HTN/DM HTN/Dyslipidemia Uncontrolled HTN	200	EchoPAC, GE	GLS	1 Y	↓GLS in uncontrolled HTN ↓GLS in long lasting HTN (>10 years)
Saito et al. 2016 (238)	2D STE	HTN without ischaemic heart disease	388	TomTec, GE	GLS	4 Y	↓GLS predicts MACE
Lee et al. 2016 (239)	2D STE	HTN	95	EchoPAC, GE	Subendocardial LS Subepicardial LS	7 Y	↓ subepicardial LS Preserved subendocardial LS
Chen et al. 2016 (240)	2D STE	Controlled HTN (group 1) Uncontrolled HTN (group2)	361	QLAB, Philips	cEss MWFs LS CS	3 M	↓ myocardial Function in group 2 vs. group 1 & 3

Author/year	Methods	Patients population	Sample size	STE software/ Echo machine	STE parameters	Follow-up duration	Results
		NC (group 3)			RS		
Cheng et al. 2014 (241)	2D STE	Intensive treatment with SBP target <130mmHg (group 1) Standard treatment with SBP target < 140 mmHg (group 2)	182	TomTec	GLS	24 W	After therapy: ↑ GLS in group 1 ↑ GLS in lower BMI ↑ GLS in women

Author/year	Methods	Patients population	Sample size	STE software/ Echo machine	STE parameters	Follow-up duration	Results
Dobrowolski et al. 2014 (242)	2D STE	RH OSA^{-}/MS^{-} (group 1) OSA^{+}/MS^{-} (group 2) OSA^{-}/ MS^{+} (group 3) OSA^{+}/MS^{+} (group 4)	155	EchoPAC, GE	GLS	-	↓ GLS in group 4 vs. group 1,2,3
Imbalzano et al. 2011 (17)	2D STE	HTN/LVH (group 1) HTN/no LVH (group 2) NC (group 3)	102	EchoPAC, GE	GLS GCS GRS	-	↓ GLS in group 1 & 2 vs. group 3

Author/year	Methods	Patients population	Sample size	STE software/ Echo machine	STE parameters	Follow-up duration	Results
Tadic et al. 2021 (274)	2D STE	NC Well-controlled HTN Uncontrolled HTN RH	45 70 58 31	EchoPAC, GE	GLS	-.	↓ GLS in RH compared to NC, and well controlled HTN
Gosse et al. 2010 (275)	2D STE	MHT NC	25 25	EchoPAC, GE	GLS GCS	11M	After therapy: ↑ GLS

2D STE: two dimensional speckle tracking echocardiography; AFI: automatic function imaging; BMI: body mass index; cESS: circumferential end-systolic wall stress; CS: circumferential strain; ; DM: diabetes mellitus; EF: ejection fraction; GCS: global circumferential strain; GE: general electric; GLS: global longitudinal strain; GRS: global radial strain; IVSDd: interventricular septal diastolic diameter; LS: longitudinal strain; LVH: left ventricle hypertrophy; MACE: major adverse cardiac events; MHT: malignant hypertension; MWFS: mid-wall fraction shortening; MS–: without metabolic syndrome; NC: normotensive control; MS+: with metabolic syndrome; OSA–: without obstructive sleep apnoea; OSA+: with obstructive sleep apnoea; PWDd: posterior wall diastolic diameter; RDN: renal denervation; RH: resistant hypertension; RS: radial strain; RWT: relative wall thickness; ↓: significant reduction; ↑: significant increase

2.5. Autonomic nervous system

2.5.1. Basic principles: The autonomic nervous system

The autonomic nervous system (ANS) controls different physiological actions such as, cardiac muscles contraction, smooth muscles, respiratory rate and BP. The ANS consists of sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). When the SNS is activated, it creates a stress responses known as the “fight or flight” response in which rise the heart rate, myocardial contractility and BP, glycogenolysis occurs, and gastrointestinal peristalsis terminates (276). When the PNS is activated, it creates relaxing responses and triggers the “rest and digest” responses; heart rate and BP drop, and gastrointestinal peristalsis and digestion resume.

There are certain transmitters in the ANS, mainly acetylcholine (ACh), noradrenaline and adrenaline. There are also two neurone chain, which is classified into preganglionic and postganglionic. noradrenaline is the predominant neurotransmitter released by the SNS and cause vasoconstriction effect, while ACh is released by the PNS and cause vasodilatation effect (277).

Cardiac energy demand and heart rate increased when noradrenaline binds to adrenoreceptors on cardiomyocytes (278). The PSN regulates heart rate and contractility by the activation of ACh (279). The vagus nerve controls heart rate primarily through the sinoatrial (SA) and atrioventricular (AV) nodes and is controlled by ACh. Ach caused elevation of potassium ion efflux hyperpolarise the pacemaker cells, causing reduction in threshold and reducing of heart rate.

2.5.2. Assessment of autonomic nervous system: heart rate variability

HRV is a simple non-invasive method to assess complex interaction between autonomic function, sympathetic nerve activity and cardiovascular system (35, 36). HRV is the fluctuation in time interval in heart rate (280, 281). It is affected by the ANS including SNS and PNS and reflects the balance between the SNS and the PNS. HRV is determined by the continuous interaction between SA node spontaneous activity, sympathetic and vagal efferent nerve activity. Reduced HRV is a strong predictor higher rate of cardiac morbidity, all-cause of mortality, hypertension, DM, patients with MI and congestive heart failure (34, 35, 280, 282, 283, 284, 285, 286). There are also associations between decreased HRV and stress, anxiety, and panic disorder (287). Additionally, HRV decreases with age and increases with regular physical activity (288, 289).

2.5.3. Measurements of heart rate variability

HRV can be assessed using time domain and frequency domain indices (282). Initially, HRV was calculated manually by using time domain methods and short periods of electrocardiogram (ECG) readings. Currently, HRV can be evaluated by using both time domain and frequency domain methods using either short-term (several minutes) or long-term (24-hour) recordings (282).

2.5.3.1. Time domain methods

Using time domain method is simple since it depends on common statistical analysis. ECG record detects each QRS complex and determines the R-R interval. Time domain includes: the standard deviation of NN Intervals (SDNN), root means successive square difference (rMSSD), and the percentage of adjacent NN intervals that differ from each

other by more than 50 ms (pNN50). (282, 290) Both rMSSD and pNN50% can be indexes of PNS (282).

2.5.3.2. Frequency domain methods

Frequency domain methods is a complex technique used to distinguish the influence of the PNS and SNS on HRV. It can be obtained from spectral analysis and determine the power of different frequencies such as low frequency (LF), high frequency (HF), total Power, very low frequency (VLF) and the ratio LF/HF (282, 291). HF represents PNS activity and is affected by the respiratory rate, whereas the LF is an index of SNS activity and it may reflect PNS activity (292, 293, 294, 295, 296).

The LF/HF ratio may be a reflective of the global sympatho-vagal balance while VLF is likely a reflection of the renin–angiotensin system and the SNS. However, interpretation of the VLF is less reliable particularly when shorter recording is used (e.g., 5 minutes) (282, 292, 297, 298).

2.5.4. Autonomic dysfunction and heart rate variability in hypertension

Hypertension has complicated pathophysiology and multifactorial pathways (299, 300, 301). Several studies reported that ANS was found to be involved in the pathophysiology of early stages of hypertension and continuing until the condition progressed to complex stages (301, 302, 303, 304).

SNS and RAAS plays important role to maintain cardiovascular homeostasis and regulate BP (305). RAAS dysregulation may result in hypertension or heart failure (306, 307).

SNS and PNS affect the cardiac muscles in antagonistic ways. Stimulation of the SNS causes vasoconstriction and increases BP rapidly by increasing cardiac contractility, and heart rate (308, 309). Furthermore, the SNS is responsible for long-term regulation of BP, and hypertension (310, 311, 312).

Lower HRV is associated with hypertension in several studies (Table 2.6) (283, 313, 314, 315, 316). Yu et al. derived HRV of 24 hour ECG and compared between age-matched NC and hypertension (controlled and uncontrolled) (313). The study revealed that HRV was significantly reduced in the hypertension group compared to NC and in uncontrolled hypertension compared to controlled hypertension. It was also showed that based on multiple regression analysis, impaired HRV indices were predictors for uncontrolled BP. Huikuri et al. evaluated HRV of 45 minutes of ECG recording and compared between age-matched subjects with hypertension and NC (283). It was observed that all indices of HRV, excluding HF, were significantly reduced in hypertension compared to NC. Multiple regression analysis revealed that BP is strong predictor of SDNN in hypertension and NC. The HRV parameters did not differ between groups after adjusting for baseline differences in SBP and BMI. Another study evaluated HRV in an older population including NC (67 years) and hypertension (68 years) (314). Reduced HRV was observed in a hypertension group compared to NC. The Framingham Heart Study compared HRV indices between hypertension and NC groups (35). Two hours of ambulatory ECG recording were used to assess HRV. It was observed that all HRV parameters, excluding LF/HF, were significantly decreased in patients with hypertension compared to NC. Reduced HRV increased the risk of developing hypertension (317, 318).

In the Framingham Heart Study, the association between HRV and new-onset hypertension was assessed after 4 years of follow-up (35). During the follow-up period, 119 male and 125 female developed hypertension. There was an association between new-onset of hypertension and LF in male subjects. Furthermore, male subjects in NC group who have lower HRV are more likely to develop hypertension. Hoshi et al. observed the association between reduced HRV and incidence of hypertension (317). According to the study, low HRV was associated with a 40-80% increase in the risk of having hypertension after four years of follow-up.

Schroeder et al. also investigated the link between hypertension and low HRV in general population aged 45 to 54 years at baseline and found that low HRV was predictive of higher risk of incident hypertension after 9 years of follow-up (318). The study also evaluated HRV profile in subjects with and without hypertension. Interestingly, HRV did not differ significantly between subjects with and without hypertension over 9 years. According to these findings, the ANS plays a role in the developing of hypertension. However, also imply the autonomic profiles of patients with hypertension and normotensive subjects become similar over time.

Higher LF and lower HF in hypertension can be explained by increased cardiac sympathetic activity and decreased parasympathetic activity (319, 320). The LF/HF ratio indicates the level of sympatho-vagal balance. Elevated LF/HF indicates high sympathetic activity and/or low parasympathetic activity (321, 322, 323, 324). It is noted that interpretating HRV indices can be complex, giving the current debates on what LF and HF mean physiologically (325, 326).

Table 2.6 Summary of studies using heart rate variability in hypertension population

Author	Year	Population/Age	FU	Results
Hoshi et al. (317)	2021	• 5153 NC • 2980 PHT	4Y	• ↓ HRV associated with incident of HTN
Maciorowska et al. (327)	2020	• 70 uncontrolled HTN+MetS/48 • 40 uncontrolled HTN no MetS/44	12M	• ↑ HRV in MetS patients after 12M of treatment
Yu et al. (313)	2018	• 120 HTN/58 • 80 NC/56	–	• ↓ HRV in HTN vs. NC • ↓ HRV in uncontrolled HTN vs controlled HTN
Daniele et al. (328)	2018	• 342 patients /61	–	• ↓ HRV associated with PP in obese
Andrade et al. (314)	2017	• 40 NC elderlies/ • 40 HTN elderlies/	–	• ↓ HRV in HTN vs. NC
Fricke et al. (329)	2017	• 12 RH &↓ HRV /64 • 9 RH & normal HRV/60	3M	• ↓ HRV associated with poorer response to RDN.
Tadic et al. (315)	2015	• 55 untreated HTN	–	• ↓ HRV in HTN

Author	Year	Population/Age	FU	Results
		• 40 NC		
Mori et al. (330)	2014	• 1418 men/ 63 • 2040 women/ 64	–	• ↓HRV associated with ↑DBP • ↑ LF/HF associated with ↑DBP
Yue et al. (316)	2014	• 36 MH/62 • 48 NC/63 • 40 HTN/62	–	• ↓ HRV in HTN and MH. • No HRV differences in HTN & MH.
Pavithran et al. (331)	2010	• 150 HTN/ 48 divided into 5 groups: • 30+amlodipine • 30+atenolol • 30+enalapril • 30+ hydrochlorothiazide • 30 amlodipine+atenolol	–	• ↑ RR intervals & ↑ HF in the amlodipine + atenolol-treated group
Fagard et al. (320)	2007	• 146 WH/50 • 176 MH/40 • 143 SHT/48 • 1020 NC/ 36	–	• ↑LF/HF in WH • No significantly different between NC, MH and SHT

Author	Year	Population/Age	FU	Results
Bilge at al. (332)	2005	• 27 untreated HTN/48	6M	• No change in HRV after amlodipine and fosinopril
Menezes et al. (333)	2004	• Group A - DBP<90 mmHg • Group C - DBP 100-109 mmHg	–	• ↓ HRV in group C
Menezes et al. (333)	2004	• Group C - DBP 100-109 mmHg	3M	• Recovery of HRV after treatment with ACEI
Schroeder et al. (318)	2003	• 11061 general population/	9Y	• ↓ HRV in HTN at baseline • ↓ HRV in individuals without HTN predicted incident of HTN • Over 9 years, there was no difference in HRV among those with and without HTN
Virtanen et al. (334)	2003	• 109 HTN men/46 • 49 NC men/44 • 82 HTN women/46 • 56 HTN women/44	–	• ↓ HRV in HTN • ↓ HRV was associated with ↑ HR & ↑ age

Author	Year	Population/Age	FU	Results
Gerritsen et al. (34)	2001	• 605 general population/66	9Y	• ↓ HRV is associated with all-cause and cardiovascular mortality
Sevre et al. (335)	2001	• 41 HTN/53 • 34 NC/53	4W	• ↓ HRV in HTN vs. NC
Singh et al. (35)	1998	• 245 HTN men/57 • 227 HTN women/62 • 686 NC men/48 • 884 NC women/49	4Y	• ↓ HRV in HTN • LF was associated with incident HTN in men in NC • SDNN, HF, and LF/HF were not associated with HTN in NC.
Huikuri et al. (283)	1996	• 168 HTN • 188 NC	–	• ↓ HRV except HF in HTN

ACEI: angiotensin-converting-enzyme inhibitors; DBP: diastolic blood pressure; HF: high frequency; HRV: heart rate variability; HR: heart rate; HTN: hypertension; LF: low frequency; MetS: metabolic syndrome; MH: masked hypertension; NC: normotensive control; PHT: Prehypertension; PP: pulse pressure; RDN: renal denervation; RH: resistant hypertension; SHT: sustained hypertension; WH: white hypertension.

Chapter III. METHODS

3.1. Introduction

This chapter contains descriptions of the study methods including the aim, the hypothesis of the study and the research design. It describes the target population, the preparation, baseline procedures, and the instruments used to assess cardiac, vascular and autonomic parameters. It also includes reproducibility analysis, details of statistical power and how the data were analysed.

3.2. Hypothesis and aims

The primary hypothesis tested was that patients with MHT would have worse measures of cardiac mechanics, vascular and autonomic function compared to patients with RH and both would have worse measures compared to the NC. I aimed to investigate the LV mechanics, vascular function and autonomic function in three groups; normotensives group and two groups of patients with hypertension: patients with a history of malignant phase hypertension and patients with RH.

The second hypothesis tested that 8-week of intensive antihypertensive treatment in RH would improve GLS by at least 1 standard deviation (SD). I aimed to assess the impacts of intensified antihypertensive treatment on myocardial mechanics, vascular function and autonomic function in the RH group.

3.3. Study design

To meet our hypotheses and aims, the study designed as a prospective observational study. The first arm of the study was cross-sectional and the second arm was a longitudinal observational study.

3.4. Study population

3.4.1. Inclusion criteria

Patients over the age of 18 years with the ability to give informed consent and a diagnosis of either MHT or RH were included. All participants had a diagnosis of malignant and resistant hypertension based on their medical records. Participants with normal BP were included as a NC.

3.4.2. Exclusion criteria

Participants with the following conditions were excluded: BMI ≥35 kg/m^2, moderate-severe valvular heart disease, previous MI or current symptomatic CAD, AF, recent (<6 months) cerebrovascular events, active infections or pyrexia illness, active chronic and systemic illnesses (e.g., respiratory diseases, renal or liver failure, neurological disease) and pregnancy.

3.5. Study design

The study protocol was approved by the West Midlands-South Birmingham Research Ethics Committee (REC reference: 18/WM/0168). Two local approvals were obtained from the Research Ethics Committee of Sandwell and West Birmingham Hospitals NHS Trust and from Liverpool Heart and Chest Hospital. The study was conducted in

accordance with the Declaration of Helsinki of the World Medical Association. All procedures were performed by a single operator at the research clinic.

The participant information sheet was provided to all participants before the study day. Written informed consent was given to all subjects.

3.5.1. Cross-sectional comparison of three groups

Two groups of patients with hypertension were recruited from the hypertension clinics at City Hospital and at Liverpool Heart and Chest Hospital: the first group included patients with a history of malignant phase hypertension and the second group included patients with RH. These two groups were compared to NC group. The NC group included participants with no history of hypertension but with other cardiovascular risk factors. They were recruited from the patients' family members and the surrounding communities using posters and advertisements on the University of Birmingham's internal website.

A total of 64 participants were recruited during the period from December 2018 to March 2020. Further recruitment has been halted due to the COVID-19 pandemic.

3.5.2. Longitudinal observational study of resistant hypertension

Patients with RH were followed up for eight weeks after the optimisation of medical treatment initiated by expert clinician at the hypertension clinic and were therefore seen on two occasions. The treatment optimisation included either increasing the dosage of current antihypertensive agents or adding a new medication.

A total of 17 participants were followed during the period from December 2018 to March 2020. Further recruitment had to stop because of the COVID-19 pandemic.

3.6. Preparation and baseline procedures

The study procedures were conducted in a quiet, temperature-controlled room (20-22ºC). In preparation for the study, participants were required to fast for 12 hours (overnight) prior to their study visit. Alcohol abstinence was required for at least 6 hours and caffeine abstinence for at least 12 hours prior to the study. Patients with hypertension were abstained from taking their antihypertensive medications on the morning of the study day in order to reduce its impact on the study results. Patients were also advised to bring their morning dose of their antihypertensive medications to their research appointment so that they could take in late morning after the study was completed.

Before the enrolment, all procedures were discussed with the participant and all questions were answered.

Medical history and current cardiovascular medication data were collected along with anthropometric measurements (height, weight, BMI and waist to hip ratio). BMI was derived by dividing weight by height squared and expressed in kg/m^2. To measure waist to hip ratio, participant stood with feet apart. The ratio was calculated as waist circumference (cm) divided by hip circumference (cm). Waist circumference was measured midway between iliac crest and lowest rib. Hip circumference was measured at the widest point between the waist and groin.

Office BP was measured three times using a validated digital BP monitor (Omron HEM-705CP, Omron Healthcare (UK) Ltd, Milton Keynes). Participants were in a seated position with their backs supported, legs uncrossed and feet flat on the floor. An appropriate cuff size was placed around a bare midpoint of the upper arm. The arm was

placed on a flat table with the cuff at the level of the participant's heart and the participant rested quietly for 5 minutes before three readings were measured and then averaged. The participants then lay supine on a couch in a comfortable position and a supported pillow and rested for 5 minutes before the procedures started, (Figure 3.1).

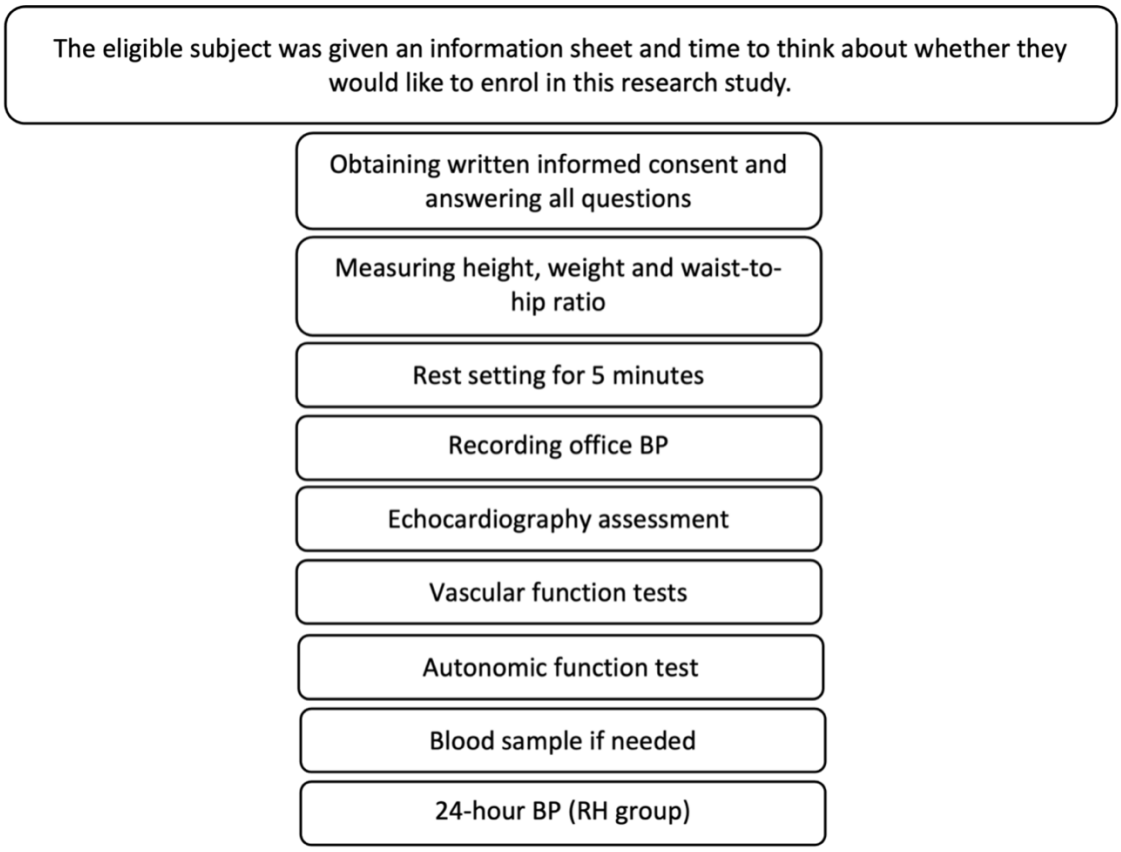


Figure 3.1 Experimental protocol. BP: blood pressure; RH: resistant hypertension

3.6.1. Transthoracic echocardiography imaging

Cardiac function and structure were assessed using Philips' ultrasound machine (CX50, Philips Healthcare, (Bothell, WA, USA) and S5-1 phased array sector ultrasound transducer. All participants had standard 2D, and Doppler echocardiography performed in accordance with the ASE chamber quantification guidelines and the European Association of Cardiovascular Imaging (EACVI).(26, 336) All images were acquired with

normal breathing and participants were in the left lateral decubitus position. Two consecutive clips were obtained for the assessment of each index except for the strain parameters where three consecutive cardiac clips were recorded. All images were transferred to automated cardiac motion quantification software (aCMQ, Phillips Healthcare) for offline analysis. Echocardiographic parameters and views acquired are shown in Table 3.1.

Table 3.1 Cardiac parameters by echocardiography

Parameters	Views
LA diameter	PLAX
LA area	Apical 4-CH/2-CH
LAV/ LAVI	Apical 4-CH/2-CH
IVS	PLAX
LVIDd	PLAX
PWd	PLAX
LVH	PLAX/ Apical 4-CH
LV mass	PLAX
LVMI	PLAX
RWT	PLAX
LV volume	Apical 4-CH/2-CH
LV EF (Simpson method)	Apical 4-CH/2-CH
GLS	Apical 4-CH/3-CH/2-CH
GCS	PSAX
Apical rotation	PSAX
Basal rotation	PSAX
R-AVC	PLAX/PSAX
Twist	PSAX
Torsion	PSAX
E	Apical 4-CH
A	Apical 4-CH
E/A ratio	Apical 4-CH
DT	Apical 4-CH
e´ (septal and lateral)	Apical 4-CH
a´ (septal and lateral)	Apical 4-CH
e´/a´ (septal and lateral)	Apical 4-CH
E/e´ (septal and lateral)	Apical 4-CH

2-CH: two chambers; 3-CH: three chambers; 4-CH: four chambers; A: peak velocity of late diastolic trans-mitral flow; a´: peak velocity of late diastolic mitral annular motions; DT: deceleration time; E: peak velocity of early diastolic trans-mitral flow; E/A: peak early filling (E-wave) and late diastolic filling (A-wave) velocities; e´: peak velocity of early diastolic mitral annular motions; EF: ejection fraction; GCS: global circumferential strain; GLS: global longitudinal strain; IVS: interventricular septum; LA: left atrium; LAV: left atrial volume; LAVI: left atrial volume index; LV: left ventricle; LVH: left ventricular hypertrophy; LVIDd: left ventricular internal diameter at end diastole; LVMI: left ventricular mass index; PLAX: parasternal long-axis; PSAX: parasternal short axis; PWd: Posterior wall thickness at end diastole; R-AVC: R wave to aortic valve closure; RWT: relative wall thickness.

3.6.1.1. Left ventricular quantifications

According to the ASE/EACVI recommendations, LV quantifications were assessed (26). Four views were obtained to assess LV chamber. Parasternal long axis view (PLAX), four chamber (4-CH), three (3-CH), and two chamber (2-CH) apical views. End of diastole was marked by mitral valve closure and end of systole was marked as aortic valve closure. Papillary muscles and trabeculae were excluded during any tracing.

LV cavity size and wall thickness were measured in parasternal window, long axis view at end-diastole. LV volume and EF were estimated with the biplane modified Simpson's mode in apical 4-CH and 2-CH views at end diastole and end systole. The largest dimension of LV was represented as end-diastole, and the smallest dimension of LV was represented as end-systole.

Measurements of interventricular septum (IVS), LV diameter and posterior wall thickness were carried out in PLAX view during diastole. Calliper was positioned perpendicular to the LV wall and cavity.

Devereux's method was used to estimate Left ventricular mass (LVM): LVM = 0.8 x (1.04[(LVIDd + PWd + $IVSd^3$– ($LVIDdd^3$) + 0.6 Where LVIDd is the left ventricular diameter at end diastole; PWd is the posterior wall thickness at end diastole; and IVSd is the interventricular septal thickness at end diastole. Relative wall thickness (RWT) was calculated as follows: (2 x PWd)/LVIDd. LVMI indexed to BSA and LV hypertrophy was identified as LVMI >95 g/m2 in female and 115 g/m^2 in male (26). It was classified as:

- Concentric hypertrophy if RWT was >0.42
- Eccentric hypertrophy if RWT <0.42)
- Concentric remodelling if LVMI was normal and RWT >0.42.

The LV EF was calculated automatically from diastolic and systolic volumes.

3.6.1.2. Diastolic function assessment

Mitral inflow Doppler and mitral annular tissue Doppler were applied to assess diastolic function. Mitral valve (MV) peak wave velocity of early trans-mitral diastole (E), MV peak velocity of late diastole (A) and E/A ratio were assessed. The deceleration time (DT) was the time interval from peak E-wave along the slope of LV filling to baseline (337).

3.6.1.3. Mitral inflow Doppler

In apical window 4-CH view, pulsed-wave Doppler was used, and Doppler sample volume was aligned parallel to the mitral inflow between the leaflets' tips. The following parameters were assessed: peak velocity of early diastolic trans-mitral flow (E), peak velocity of late diastolic trans-mitral flow (A), E/A ratio and early velocity decline of E slope and DT.

3.6.1.4. Mitral annular tissue Doppler

TDI was used to measure mitral septal and lateral annular velocities. In 4-CH view, the sample volume was placed at lateral and septal of the insertion of the mitral valve leaflets. Early (e´) and late (a´) of septal and lateral velocities were measured. To assess diastolic dysfunction, the following parameters were measured: E/A ratio, DT, average e´/a´, and average septal- lateral E/e'. Normal diastolic function parameters were defined as E/A: 1-2, DT: 150-200, average e´/a´:1-2, septal E/e': <8, lateral E/e': <10.

3.6.1.5. Left atrial size and volume

LA Anterior-Posterior dimension was measured at end-systole from inner-to-inner edge on 2D parasternal long axis view. LA inner border was traced at its largest size to measure LA area. LA inner border was traced in 4-CH and 2-CH apical views. Pulmonary veins, and LA appendage were excluded. LA Volume was calculated by modified biplane technique.

3.6.2. Speckle tracking echocardiography

All images were recorded for three cardiac cycles and then saved for off-line analysis. The updated offline Philips software 'Automated cardiac motion quantification' (aCMQ) was used to evaluate myocardium deformation and function. A good quality 2D image with high frame rate (70-100 Hz) was acquired to trace the LV endocardium at end-diastole. The region of interest (ROI) width was adjusted, if necessary, for optimal tracing of the myocardium. The aCMQ software defined and tracked the movement of LV myocardium in accordance with recommendation of the consensus document of the EACVI/ASE/Industry Task Force (338).

3.6.2.1. Left ventricular global longitudinal strain

GLS was derived from apical window (4-CH, 3-CH and 2-CH views). Apical views were divided into 6 segments: anterior, anterolateral, anteroseptal, inferior, inferoseptal, and inferolateral, (Figure 3.2).

3.6.2.2. Left ventricular global circumferential strain

GCS was averaged from three parasternal short-axis views: at level of MV basal level, at level of papillary muscles and at apical level. The aCMQ software divided the obtained

images into 6 segments: anterior, anterolateral, anteroseptal, inferior, inferoseptal, and inferolateral, (Figure 3.2).

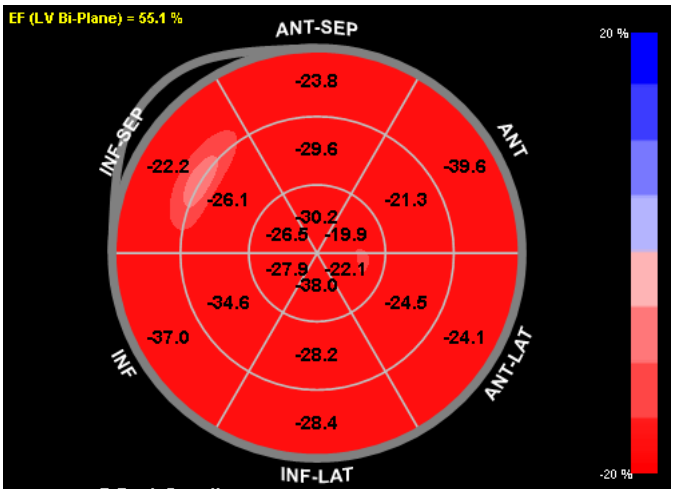


Figure 3.2 Example of circumferential strain 'bull's eye view showing 6 segments of left ventricle.

3.6.2.3. Left ventricular twist and torsion

Cardiac twist and torsion were calculated from apical and basal rotation values in parasternal short axis views. Apical counter-clockwise rotation is positive value and basal clockwise rotation is a negative value. The difference between peak apical rotation and basal rotation was calculated to estimate twist (in degrees). Apical and basal rotation at aortic valve closure were calculated to estimate net twist angle. It was defined as the difference between apical and basal rotation at aortic valve closure. Torsion /cm =net twist angle/ LV diameter from base and apex in diastole.

For further details about the STE procedure and parameters, please see Appendix 1.

3.6.3. Measurements of STE parameters for reproducibility analysis

Reproducibility of GLS and GCS were calculated as shown in table 3.2 and table 3.3. Intra-observer variability was reported as coefficient of variation (CV), where CV = (SD/mean) *100 and expressed as percentage. I measured GLS on 9 participants and repeated the measurements on a different day. The reproducibility of GLS in 9 consecutive participants was 5.29%. I also measured GCS on 6 participants and repeated the measurements on a different day. The average intra-observer CV for GCS in 6 studies was 3.05 %.

Table 3.2 Intra-observer variability measurement of global longitudinal strain

Participants	GLS-1	GLS-2	Mean_GLS	SD_GLS	CV_GLS
P1	-17.7	-19.9	-18.80	1.56	8.27
P2	-21.5	-18.2	-19.85	2.33	11.76
P3	-19.2	-19.8	-19.5	0.42	2.18
P4	-18	-21.4	-19.7	2.40	12.20
P5	-22	-20.2	-21.1	1.27	6.03
P6	-17	-16.3	-16.65	0.49	2.97
P7	-16.7	-16.8	-16.75	0.07	0.42
P8	-16.4	-16.3	-16.35	0.07	0.43
P9	-16.5	-17.3	-16.9	0.57	3.35
				Average CV	5.29

CV: coefficient of variability; GLS: global longitudinal strain; SD: standard deviation.

Table 3.3 Intra-observer variability measurement of global circumferential strain

Participants	GCS-1	GCS-2	Mean_GCS	SD_GCS	CV_GCS
P1	-30	-33.6	-31.80	2.55	8.00
P2	-28.3	-27.4	-27.85	0.64	2.29
P3	-23.8	-24.3	-24.05	0.35	1.47
P4	-36	-36.4	-36.20	0.28	0.78
P5	-27.8	-28.5	-28.15	0.49	1.76
P6	-35	-33.8	-34.40	0.85	2.47
				Average CV	3.05

CV: coefficient of variability; GCS: global circumferential strain; SD: standard deviation.

3.7. Vascular assessment

3.7.1. Arterial stiffness

cfPWV and AIx are widely considered as the least invasive, safest, and more reliable in terms of accuracy, as recommended by the ESH in 2018 (8), and earlier by expert consensus document of 2006 (63). cfPWV was estimated noninvasively by measuring the distance of arterial pulse between two superficial arterial sites (e.g. carotid artery and femoral artery) and the travel time taken (153).

VICORDER® software (Smart medical, UK) used the gold standard assessment of cfPWV between the carotid and femoral arteries and it was validated in several studies and was used to estimate cfPWV, central pressure and AIx (339, 340, 341, 342). It is safe, non-invasive, portable, easy to perform by single operator and operator independent.

The subject was positioned supine and rested for 5 minutes before the test. The following parameters are obtained from VICORDER® software: cfPWV, peripheral BP, heart rate, CBP, mean arterial pressure (MAP), stroke volume (SV), cardiac output (CO), SEVR, total peripheral resistance (TPR), augmentation pressure, and AIx.

To calculate cfPWV, the participant was in semi-prone position, resting comfortably on the bed. The neck pad was placed around the participant's neck while the pressure pad (inflatable sensor) was placed around the right side of the neck (right carotid area). The pressure cuff was placed on the upper right thigh to record femoral artery pulse. The length between the cuff and the sensor was measured to estimate the aortic pathway length. Pulse wave transit time and cfPWV were calculated.

Pulse wave analysis (PWA) was used to record and displayed brachial and central aortic BP, AIx, SEVR, TPR, CO, SV and augmentation PP. The BP cuff (adult size) was applied to the right arm and then inflated. After deflating the cuff, real time waveform was shown on the screen and results were appeared.

For further details about cfPWV/PWA procedures and parameters, please see Appendix 2.

3.7.2. Measurements of arterial stiffness for reproducibility analysis

Reproducibility of cfPWV and AIx were calculated as shown in table 3.4 and table 3.5. I performed the measurements of cfPWV and AIx on 7 participants and repeated the measurements on a different day. The average intra-observer CV for cfPWV in 7 studies was 7.71% and the average CV for AIx was 1.27%.

Table 3.4 Intra-observer variability measurement of carotid-femoral pulse wave velocity

Participants	cfPWV-1	cfPWV-2	Mean_cfPWV	SD_cfPWV	CV_cfPWV
P1	6.8	7.8	7.3	0.71	9.69
P2	5	5.1	5.05	0.07	1.40
P3	7.5	5.1	6.3	1.70	26.94
P4	7.7	7.1	7.4	0.42	5.73
P5	6.2	5.6	5.9	0.42	7.19
P6	7	7.2	7.1	0.14	1.99
P7	6.6	6.7	6.65	0.07	1.06
				Average CV	7.71

cfPWV: carotid-femoral pulse wave velocity; CV: coefficient of variability; SD: standard deviation.

Table 3.5 Intra-observer variability measurement of augmentation index

Participants	AIx-1	AIx-2	Mean_AI	SD_AI	CV_AI
P1	26	26	26.00	0.00	0.00
P2	19	20	19.50	0.71	3.63
P3	26	26	26.00	0.00	0.00
P4	12	12	12.00	0.00	0.00
P5	26	28	27.00	1.41	5.24
P6	16	16	16.00	0.00	0.00
P7	14	14	14.00	0.00	0.00
				Average CV	1.27

AIx: augmentation index; CV: coefficient of variability; SD: standard deviation.

3.7.3. Carotid artery distensibility

Elasticity of the right common carotid artery (CCA) was estimated using a Philips ultrasound machine (CX50, Philips Healthcare, (Bothell, WA, USA) and L12-3 linear array ultrasound transducer. Participants were in supine position with the neck extended. The transducer was placed on the right side of the neck to scan the right CCA. 2D and motion mode (M-mode) were used to measure systolic and diastolic diameters. To assess the elasticity, the following equation was used: distensibility = (2ΔD/Dd)/ΔP, where ΔD is

the difference in diameter between systole and diastole, Dd is the diastolic diameter, and ΔP is the PP.

3.7.4. Flow mediated dilatation

Endothelial function was assessed using Cardiovascular Suite software (version 3.4.1; FMD Studio, Quipu srl, Pisa, Italy), Phillips CX50 ultrasound machine and L12-3 linear array ultrasound probe transducer. Cardiovascular Suite software uses real-time automated edge detection and wall tracking techniques for the analysis. The software process series of ultrasound images and give automatic measurements of brachial artery diameter, and automatic analyses of the Doppler signal in order to calculate the value of instantaneous shear rate.

FMD procedure was done according to the guideline's recommendations (44). Participants was positioned supine on a couch and the right arm was extended and positioned comfortably using supported cushion. A manual sphygmomanometer cuff (5cm width, Hokanson, Bellevue, WA) was applied to the right forearm. The ultrasound probe was placed above the antecubital fossa to scan the brachial artery and held in place for the whole duration of the procedure.

Baseline images were recorded for 1 minute before the cuff occlusion. To create the occlusion, the cuff was inflated to 50 mmHg above the subject's SBP and left for 5 minutes. Following this, cuff was deflated rapidly, and continuous measurements were taken for up to 2 minutes to assess the hyperaemic response.

The software calculated the following data:

- Baseline diameter and baseline shear rate.

- Vasodilatation maximum diameter and maximum shear rate.
- Recovery diameter (mean diameter of the last 30 seconds in vasodilatation time).
- The area under the curve of the shear-rate in vasodilatation.
- FMD which was expressed as a percentage change in brachial artery diameter in respect to baseline diameter (%).

  FMD= (maximum diameter- baseline diameter)/ baseline diameter.
- FMDr which was expressed as a percentage change in brachial artery diameter in respect to recovery diameter (%).

  FMDr= (maximum diameter- baseline diameter)/ recovery diameter.

For further details about FMD procedure, please see Appendix 3.

3.7.5. Measurements of endothelial function for reproducibility analysis

Reproducibility of FMD was calculated as shown in table 6. I performed FMD on 7 participants and repeated the measurements on a different day. The average intra-observer CV of FMD in 7 studies was 10.3%.

Table 3.6 Intra-observer variability measurement of flow mediated dilatation

Participants	FMD-1 (%)	FMD-2 (%)	Mean_FMD	SD_FMD	CV_FMD
1	7.1	9.0	8.1	1.34	16.6
2	3.6	3.6	3.6	0.00	0.0
3	8.1	7.8	8.0	0.21	2.7
4	7.3	7.5	7.4	0.14	1.9
5	8.4	11.0	9.7	1.84	19.0
6	9.8	6.3	8.1	2.47	30.6
7	8.3	8.1	8.2	0.14	1.7
				Average CV	10.3

CV: coefficient of variability; FMD: flow mediated dilation; SD: standard deviation.

3.8. Heart rate variability

The autonomic function was assessed using time and frequency domain indices of the HRV analysis derived from ECG sensor (eMotion Faros, Bittium Biosignals Ltd, Kuopio, Finland). HRV parameters were calculated as recommended by the taskforce recommendations (282). The participant was positioned supine while the heart rate is monitored for 5 minutes with a small portable eMotion Faros sensor attached to 3 ECG leads and placed on the chest. The sensor was linked by Bluetooth to a laptop with the Cardiscope™ ANALYTICS program (SMART Medical Ltd, Moreton in Marsh, UK). The real-time analysis program calculated the differences between successive R-R intervals and assessed different parameters of HRV as seen in Table 7.

Table 3.7 Heart rate variability parameters

Parameters	Definitions
Time domain indices	
SDNN	Standard deviation of the normal-to-normal intervals
rMSSD	Sum of successive differences in normal-to-normal interval
pNN50	Proportion of the number of successive normal-to-normal intervals that differ by more than 50 ms
Frequency domain indices	
HF	High frequency
LF	Low frequency
LF/HF	Low frequency to high frequency ratio

For further details about HRV procedure, please see Appendix 4

3.9. Ambulatory blood pressure monitoring

24-hour ambulatory blood pressure monitoring (ABPM) was carried out on all participants in the RH group using an ABPM device (7100/WelchAllyn, USA) to assess their average BP. The pressure cuff was placed on participant's upper arm. The carrying pouch was positioned on the right side of the participant and the pouch strap worn around the hips or around the shoulders (depending on patient preferences). The readings were taken every 30 min during the day and every 60 min at night. BPV was calculated using the SD of the average of 24-hour BP readings.

3.10. Laboratory test

Blood samples were obtained from participants from their left antecubital fossa on their study visit by a trained phlebotomist in the research clinic (if they had no blood test

taken within the last six months). Blood tests requested include (full blood count, renal function, liver function, fasting glucose, lipid profile, thyroid function).

Most participants had blood tests taken within the last six months. Blood tests were taken from different sites including primary care centres, Sandwell Hospital, City Hospital and Liverpool Heart and Chest Hospital, (following trust guidelines).

3.11. Statistical power and data analyses

Power calculations were based on two previously published studies (270, 275) and the primary parameter was GLS.

The research study hypothesises that patients with MHT will have worse measures of cardiac mechanics compared to patients with RH and both will have worse measures compared to the normotensive controls. It hypothesises that primary parameter (GLS) will be reduced 1 SD in malignant hypertension compared to resistant hypertension.

In order to achieve differences between the three groups in the cross-sectional study in variance at $1-\beta=0.8$ and $p<0.05$ (ANOVA F statistic approximately 10), 21 subjects per group were demanded, (chapter 4 & 5).

I also hypothesise that 8-week intensive antihypertensive treatment in RH will improve the parameter by at least 1 SD. In order to observe differences post 8-week optimised antihypertensive treatment in RH in variance at $1-\beta=0.8$ and $p<0.05$ (paired T-test), 20 subjects are required to complete follow-up, (chapter 6 & 7).

Statistical analysis was performed using statistical analysis software (Stata/IC), 16.1 for Mac. Continuous data were subjected to the Shapiro-Wilk test to determine the nature of its distribution.

3.11.1. Cross-sectional comparison of the three groups

Normally distributed data were analysed by ANOVA with Tukey's post hoc test and were expressed as mean and SD, unless otherwise stated. Non-normally data distributed were analysed by nonparametric pairwise multiple comparisons using Dunn's test and were presented as median with interquartile range. Categorical data were compared Kruskal-Wallis H test and Dunn's post hoc test and were presented as number and percentages, (n (%)).

In the cross-sectional study, simple univariable analysis was used to investigate independent determinants of strain function (GLS), endothelial function (FMD), arterial stiffness (PWV) and autonomic function (HRV).

Multivariable linear stepwise regression model with backward selection was performed to test the potential confounders as a cause of differences between the NC, RH and MHT groups. The main outcome variables included GLS, PWV, FMD, baseline brachial diameter, LF/HF. and carotid artery distensibility. The potential confounders included presence of hypertension, duration of hypertension, age, hypercholesterolaemia, BMI eGFR, SBP, DBP and use of statin. To avoid collinearity and to select variables with a total of 6 degrees of freedom, two models were built for multivariable regression. In the first model, I included age, presence of hypertension, duration of hypertension, BMI and

eGFR. The only exception is the model for brachial artery diameter, which does not include eGFR as an independent variable.

In the second model, I included presence of hypertension, SBP, presence of hypercholesterolaemia, DBP, and statin. Afterward, I performed all the analyses above using GLS, GCS, PWV, FMD, basal brachial diameter, carotid distensibility and LF/HF, as the main outcome variable (separate models each).

3.11.2. Longitudinal observational study of resistant hypertension

Continuous variables were tested for normality using the Shapiro–Wilk test. Continuous data and normally distributed were analysed by paired t-test to determine change over time. Not normally distributed data were analysed using Wilcoxon signed rank. Chi-squared test were used to test categorical data. All findings were regarded statistically significant when p value less than 0.05. The differences (Δ) between the baseline and follow-up of all the assessed parameters were calculated.

In the longitudinal study, univariable analysis and multivariable linear stepwise regression model with backward selection were performed. I calculated the change between baseline and follow up for the dependent variable and I used baseline variables as a predictors. This is to determines whether the change between baseline and follow up depends on another variable. This adjusts for the baseline value because it is part of the outcome.

There were four separate models in which GLS, PWV, FMD and LH/LF were the dependent variables. Age, office SBP and central PP, were included in as independent variables.

It is important to note that when univariable analyses are performed, this increases the chance of making a Type 1 error. This occurs when the null hypothesis is correct but is rejected by the statistical analysis. When each analysis is performed with a 0.05 level of significance, if the null hypotheses for the analyses are true, each individual analysis has a 5% chance of resulting in a Type 1 error; however, the probability that at least one of the analyses will falsely reject the null hypothesis becomes greater than 5% in combination. Therefore, conclusions about statistical significance of the analyses should be made with caution.

It is also important to note that when sample sizes are small, if the effects that exist are not large, then the power to detect these effects can be low. This translates to a low probability of achieving statistically significant p values even when the null hypothesis should be rejected. This is referred to as a Type 2 error, and this should be considered with regard to the analyses especially when group sizes are small.

Chapter IV. CARDIAC HAEMODYNAMIC AND AUTONOMIC FUNCTION IN TWO GROUPS OF HYPERTENSIVES: RESISTANT HYPERTENSION AND MALIGNANT HYPERTENSION

4.1. Introduction

Cardiovascular haemodynamic changes and autonomic dysfunction considered a known pathological complication associated with hypertension as it was discussed in the literature review chapter (Chapter-02) (4, 5, 6, 7, 312, 343). Impaired LV function, LVH and myocardial fibrosis are recognised markers of TOD, compromised in patients with long standing hypertension (16, 17, 18). Hypertension is also associated with an imbalanced autonomic nervous system with elevated sympathetic activity and reduced parasympathetic tone (33).

Two forms of severe hypertension, MHT and RH are associated with poor prognosis. The underlying mechanisms in these groups have not been well elucidated. Subclinical adverse LV remodelling and autonomic function imbalance may be one of the underlying mechanisms.

4.2. Hypothesis and aims

I hypothesised that there are significant differences in myocardial deformation between MHT and RH and that patients with MHT would have worse cardiac and autonomic function compared to patients with RH and that both would have worse measures compared to the NC.

My study aimed to investigate autonomic function and cardiac mechanics (LV strain, twist and torsion) in two groups of patients with hypertension: patients with a history

of malignant phase hypertension and the patients with RH. In addition, the association between cardiovascular determinants and indices of LV strain and autonomic function was assessed.

4.3. Methods

4.3.1. Study design

The study protocol was approved by West Midlands-South Birmingham Research Ethics Committee (REC reference: 18/WM/0168). Two local site approvals were obtained by the research ethics committee at Sandwell and West Birmingham Hospitals NHS Trust and at Liverpool Heart and Chest Hospital. The study was conducted in accordance with the Declaration of Helsinki of the World Medical Association. All procedures were performed by a single operator.

Two groups of patients with hypertension were recruited from two different sites: 1) Hypertension clinic at City Hospital and 2) Hypertension clinic at Liverpool Heart and Chest Hospital. The first group were patients with a history of malignant phase hypertension and the second group those with RH. The NC group were participants with no history of hypertension. They were recruited from the family members of the patients and the surrounding communities using posters, and advertisement on the University of Birmingham internal website.

Participant information sheet was provided to all participants before the study day. Written informed consent was obtained from all subjects.

4.3.1.1. Inclusion criteria

Patients over the age of 18 years with the ability to provide informed consent, and a diagnosis of either MHT or RHT were included. All participants had a diagnosis of malignant and resistant hypertension based on their medical records. Participants with normal BP were included as a NC.

4.3.1.2. Exclusion criteria

Participants with the following conditions were excluded: BMI ≥35 kg/m^2, moderate-severe valvular heart disease, previous MI or current symptomatic CAD, AF, recent (< 6 months) cerebrovascular events, active infections or pyrexia illness, active chronic and systemic illnesses (e.g., respiratory diseases, renal or liver failure, neurological disease) and pregnancy.

4.3.2. Study population

A total of 64 participants were recruited in the study. The RH group comprised 23 patients (mean±SD: 57±11 y), and they were compared to 18 patients of treated MHT (54±13 y), and 23 participants as NC (50±5 y). All patients had MHT and RH were clinically confirmed diagnoses in hypertension clinics as per guidelines (8).

4.3.3. Procedures

The participants had three office BP readings measured while sitting at rest in the research clinic, and the average reading was used for analysis. After the participant was positioned in a supine position and rested for 5 minutes in a quiet room, the following procedures were obtained: standard echocardiography, strain imaging and HRV.

All procedures were assessed according to the guidelines and were explained in detail in methods chapter (Chapter-03). STE and HRV techniques were explained also in standard operating procedure (SOP), (Appendix 1 and 4).

4.3.4. Statistical analysis

Statistical analysis was performed using statistical analysis software (Stata/IC), 16.1 for Mac. Continuous data were subjected to the Shapiro-Wilk test to determine the nature of its distribution. Normally distributed data were analysed by ANOVA with Tukey's post hoc test and were expressed as mean±SD. Non-normally distributed data were analysed by nonparametric pairwise multiple comparisons using Kruskal-Wallis H test and Dunn's post hoc test and were presented as median with interquartile range. Categorical data were compared using the chi-squared test and are expressed as numbers and percentages.

Univariable analysis and multivariable linear stepwise regression model with backward selection were performed to determine the potential impact of clinical, demographic, haemodynamic, and laboratory indices on dependent variables such as GLS, GCS, twist and LF/HF.

It is important to note that when multiple analyses are performed, this increases the chance of making a Type 1 error. This occurs when the null hypothesis is correct, but is rejected by the statistical analysis. When each analysis is performed with a 0.05 level of significance, if the null hypotheses for the analyses are true, each individual analysis has a 5% chance of resulting in a Type 1 error; however, the probability that at least one of the analyses will falsely reject the null hypothesis becomes greater than 5% in combination.

It is also important to note that when sample sizes are small, if the effects that exist are not large, then the power to detect these effects can be low. This translates to a low probability of achieving statistically significant p values even when the null hypothesis should be rejected. This is referred to as a Type 2 error, and this should be considered with regard to the analyses especially when group sizes are small.

Multivariable regression analysis models include the following variables:

Model 1: Age, presence of hypertension, duration of hypertension, BMI, eGFR.

Model 2: Presence of hypertension, presence of hypercholesterolaemia, SBP, DBP, and statin. Then I performed all the analyses above using GLS, GCS, PWV, FMD, basal brachial diameter and carotid distensibility, as the main outcome variable (separate models each).

4.4. Results

4.4.1. Demographic and clinical characteristics

There were no statistically significant differences between age, sex, height, weight, BSA, waist to hip ratio, alcohol intake and smoking status whereas BMI was higher in hypertension groups (MHT and RH) (Table 4.1 and Table 4.2). The presence of DM was comparable between the three groups (p=0.27), whereas the presence of hypercholesterolaemia and CKD were higher in MHT group (67% and 33% respectively) (Table 4.2).

Table 4.1 Demographics characteristics of study population

Demographics characteristics		**NC(n=23)**	**MHT(n=18)**	**RH(n=23)**	**P**
Age, years		50±5	54±13	57±11	0.07
Sex (n) (male: female)		18:5	16:2	17:6	0.49
Ethnicity	Caucasians, n (%)	1(4%)	7(39%)	11(48%)	**<0.001**
	Asians, n (%)	22(96%)	8(44%)	4(17%)	
	Blacks, n (%)	0(0%)	3(17%)	7(30%)	
	Mixed, n (%)	0(0%)	0(0%)	1(4%)	
Height, cm		174±5	172±8	170±10	0.32
Weight, kg		81[71-94]	97[79-105]	92[80-110]	0.13
BMI, kg/m^2		27±4	31±4*	31±5*	**0.004**
BSA, m^2		1.96±0.2	2.04±0.2	1.99±0.2	0.53
Waist to hip ratio		0.93[0.89-0.97]	0.96[0.93-1.06]	0.96[0.9-1.03]	0.15

Normally distributed data are expressed as (mean ±SD). Non-normally distributed data are displayed as median with interquartile ranges. Categorical data are expressed as numbers n (%). *p<0.05 versus normal group. BMI: body mass index; BSA: body surface area; MHT: malignant hypertension; NC: normotensives controls; RH: resistant hypertension.

Table 4.2 Clinical characteristics and blood pressure indices in normotensives, malignant hypertension and resistant hypertension

Clinical characteristics		**NC(n=23)**	**MHT(n=18)**	**RH(n=23)**	**P**
Smoking	Never smoked, n (%)	16(70%)	13(72%)	18(78%)	0.31
	Current, n (%)	7(30%)	5(28%)	3(13%)	
	Ex-smoker, n (%)	0(0%)	0(0%)	2(9%)	
Alcohol, n (%)		7(30%)	6(33%)	8(35%)	0.95
Alcohol units		0[0-2.1]	0[0-3]	0[0-2]	1
Duration of HTN, years		-	7±2	8±3	-
Hypercholesterolaemia, n (%)		7(30%)	12(67%)*	8(35%)†	**0.04**
CKD, n (%)		0(0%)	6(33%)*	1(4%)†	**<0.001**
DM, n (%)		5(22%)	5(28%)	10(43%)	0.27
Office SBP, mmHg		120±7	166±32*	163±21*	**<0.001**
Office DBP, mmHg		78±8	97±17*	95±16*	**<0.001**
Heart rate, bpm		67±10	68±8	66±11	0.46

Normally distributed data are expressed as (mean ±SD). Non-normally distributed data are displayed as median with interquartile ranges. Categorical data are expressed as numbers n (%). *p<0.05 versus normal group, †p<0.05 versus malignant group. CKD: chronic kidney disease; DBP: diastolic blood pressure; DM: diabetes mellitus; HTN: hypertension; MHT: malignant hypertension; NC: normotensives controls; RH: resistant hypertension; SBP: systolic blood pressure.

By definition, none of the NC group had hypertension. Duration of hypertension in both hypertensive groups was similar with mean of (8 years±3). As expected, antihypertensive medications used were similar between MHT and RH, except for the higher use of diuretics in RH group (100% vs. 67%) (Table 4.3). There were no differences between the three groups regarding the use of other medications (Table 4.4), except for statins which were used more in patients with MHT. The average office systolic and diastolic BP were similar between MHT and RH (166/97 mmHg vs. 163 /95 mmHg,

p>0.05). There were no significant differences in mean heart rate between the three groups.

Table 4.3 Antihypertensives drugs use in hypertension groups

Antihypertensives treatment	MHT(n=18)	RH(n=23)
CCB, n (%)	13(72)	18(78)
Alpha blockers, n (%)	12(67)	16(70)
ACEI/ARBs, n (%)	12(67)	22(96)
Beta blockers, n (%)	12(67)	11(48)
Vasodilators, n (%)	5(28)	6(26)
Diuretics, n (%)	12(67)	23(100)

Categorical data are expressed as numbers n (%). ACEI: angiotensin-converting-enzyme inhibitors; ARBs: angiotensin receptor blockers; CCB: calcium channel blockers; MHT: malignant hypertension; RH: resistant hypertension.

Table 4.4 Current use of other medications in all groups

Medications	NC(n=23)	MHT(n=18)	RH(n=23)	P
Statin, n (%)	7(30)	14(78)*	7(30)†	**0.002**
Aspirin, n (%)	0(0)	3(17)	2(9)	0.14
Anticoagulant, n (%)	0(0)	1(6)	0(0)	0.28
Antipsychotic, n (%)	0(0)	1(6)	1(4)	0.60
Antidepressants, n (%)	0(0)	2(11)	4(17)	0.12

Categorical data are expressed as numbers n (%). *p<0.05 versus normal group, †p<0.05 versus malignant group. MHT: malignant hypertension; NC: normotensives controls; RH: resistant hypertension.

4.4.2. Laboratory data of all participants

Laboratory data of participants are summarised in Table 4.5. There were no significant differences in sodium, potassium levels and glycaemia control (HBA1c) between the groups. Both hypertensive groups had similar lower estimated glomerular filtration rate (eGFR) and higher creatinine and urea levels compared to control group (p=0.02). Both hypertension groups had higher neutrophils to lymphocytes ratio (NLR) compared to control group (p=0.003). However, no differences were found between MHT and RH.

Table 4.5 Laboratory data of the study participants

Lab tests	NC(n=23)	MHT(n=18)	RH(n=23)	P
Haemoglobin, g/L	146±15	137±22	136±15	0.11
Haematocrit, L/L	0.45±0.05	0.42±0.06	0.41±0.05	0.08
Mean cellular volume, fL	87±5	85±15	88±6	0.56
White cell account, 10*9/L	7[6-9]	8[6-8]	7[6-8]	0.52
Neutrophils, 10*9/L	4±1	4±1	4±1	0.12
Lymphocytes, 10*9/L	2.6±1	2.2±1	2±0.5	0.05
NLR	1.4[1.1-1.7]	1.8[1.5-2.7]*	2[1.6-2.6]*	**0.003**
Monocytes, 10*9/L	0.56[0.49-0.75]	0.55[0.45-0.6]	0.52[0.4-0.59]	0.25
Platelets, 10*9/L	271±69	257±68	265±66	0.79
HBA1c, mmol/mol	44±9	41±5	45±9	0.41
Sodium, mmol/L	139[138-140]	141[137-142]	140[138-142]	0.44
Potassium, mmol/L	4.4±0.4	4.3±0.5	4.3±0.5	0.57
Urea, mmol/L	4.5[4-6]	6[5.2-7]*	6.4[4.5-7]*	**0.02**
Creatinine, umol/L	84[68-89]	89[79-131]*	96[75-118]*	**0.02**
eGFR, mL/min/1.73 m^2	84[78-90]	78[55-88]*	70[58-85]*	**0.02**
TSH, mU/L	1.2[0.81-1.63]	1.57[0.88-1.83]	1.08[0.84-1.5]	0.66

Normally distributed data are expressed as (mean ±SD). Non-normally distributed data are displayed as median with interquartile ranges. *p<0.05 versus normal group. eGFR: estimated glomerular filtration rate; MHT: malignant hypertension; NC: normotensives controls; NLR: neutrophils to lymphocytes ration; RH: resistant hypertension; TSH: thyroid stimulating hormone.

4.4.3. Echocardiography characteristics

Standard echocardiography measurements were performed in all participants, who were enrolled in the study (Table 4.6). Both hypertension groups showed higher LV wall thickness including IVSD, PWD and RWT compared to NC. There was also a significant increase in LV mass and LVMI in both hypertension groups compared to NC with LV mass and LVMI being greater in MHT compared to RH (p=0.03). No LVH was detected in the normotensive group whereas the hypertensive groups exhibited evidence of LVH including concentric remodelling (33% in MHT, 17% in RH) and concentric hypertrophy

(39% in MHN, 26% in RH)]. The frequencies of normal patterns are distributed as follows: NC (100%), MHT (28%) and RH (57%).

In comparison with the normotensive group, the hypertensive groups showed increased LA volume and higher left atrial volume index (LAVI) ($p<0.001$). The LV end diastolic volume (EDV) and end systolic volume (ESV) were increased in MHT compared to NC only ($p=0.04$, $p=0.002$, respectively). The SV and CO did not differ significantly between the three groups ($p<0.05$). Systolic function calculated by Simpson showed normal EF (> 55%) in all groups However, compared to NC, MHT showed significant reduction ($p=0.004$).

By tissue Doppler, lateral E', septal and lateral S' were decreased in both hypertension groups compared to normotensives ($p=0.002$, $p<0.001$, respectively) whereas no differences in septal E' was observed between the three groups ($p=0.08$). Lateral and septal E/E' ratio were significantly higher in the hypertension groups ($p<0.001$); however, no difference in E/A ratio was found between the three groups. DT was increased in RH group compared to MHT and NC ($p<0.001$). Diastolic dysfunction was more prevalent in patients with hypertension (MHT 72%, RH 83% vs. NC 17%, $p<0.001$), with no differences between MHT and RH. Different patterns of diastolic dysfunction were observed in the study groups. In MHT, 33% of patients had impaired relaxation (mild dysfunction) and 33% of patients had pseudo-normal pattern (moderate dysfunction) and 6% had restrictive pattern (severe dysfunction). In RH, 48% of patients had impaired relaxation and 35% of patients had pseudo-normal pattern and none had restrictive pattern. Normotensive group had only 17% with impaired relaxation.

Table 4.6 Standard two-dimensional echocardiography characteristics among the studied groups

Echocardiography characteristics		NC(n=23)	MHT(n=18)	RH(n=23)	P
LA diameter, cm		3.4±0.3	3.8±0.5*	3.8±0.5*	**<0.001**
LA area, cm^2		16±3	21±4*	20±6*	**<0.001**
LA volume, ml		34[24-43]	63[36-70]*	48[36-65]*	**<0.001**
LAVI, mL/m^2		17[14-22]	29[22-36]*	24[18-31]*	**<0.001**
IVSD, cm		0.8±0.2	1.2±0.3*	1.1±0.3*	**<0.001**
PWD, cm		0.8[0.7-0.8]	1.2[1-1.3]*	1[0.9-1.1]*	**<0.001**
RWT		0.33[0.31-0.39]	0.49[0.42-0.54]*	0.41[0.35-0.57]*	**<0.001**
LVIDd, cm		4.5±0.5	4.9±0.5*	4.8±0.6	0.05
LVH, n (%)		0(0)	13(72)*	10(43)*	**<0.001**
LV geometry, n (%)					**<0.001**
	Normal	23(100)	5(28) *	13(57) *	
	Concentric remodelling	0	6(33) *	4(17) *	
	Concentric hypertrophy	0	7(39) *	6(26) *	
LV mass, g		113±30	236±68*	175±17*,†	**<0.001**
LVMI, $g.m^2$		58±13	112±27*	93±29*,†	**<0.001**
Abnormal LVMI		0(0)	7(39)*	6(26)	**0.008**
E, cm/s		64±11	78±21*	77±22*	**0.04**
A, cm/s		55±11	67±18*	75±18*	**<0.001**
E/A		1.2±0.2	1.2±0.4	1.1±0.3	0.28
DT, ms		180±33	186±29	225±36*,†	**<0.001**
E' lateral, cm/s		11±3	9±2*	8±2*	**0.001**
A' Lateral, cm/s		12±3	9±3*	11±3	**0.01**

Echocardiography characteristics		NC(n=23)	MHT(n=18)	RH(n=23)	P
E'/A' lateral		0.93[0.61-1.2]	1[0.67-1.4]	0.7[0.62-0.78]	0.22
E' septal, cm/s		8±2	7±2	7±2	0.08
A' septal, cm/s		11±2	9±2*	9±2*	**0.003**
E'/A' septal		0.71[0.56-0.89]	0.73[0.67-0.85]	0.68[0.58-0.78]	0.79
Average E'/A'		0.9[0.6-1]	0.9[0.6-1.1]	0.7[0.6-0.8]	0.23
E/E' septal		8.6±2	12±3*	12±4*	**<0.001**
E/E' lateral		5.6[5.1-6.6]	8.4[6.4-11]*	8.6[7-10.3]*	**<0.001**
s' septal		8[7-9]	7[6-8]*	7[5-7]*	**0.002**
s' lateral		10±2.1	8±2.2*	7±2*	**<0.001**
DD, n (%)		4(17)	13(72)*	19(83)*	**<0.001**
DD patterns	Normal	19(83)	5(28) *	4(17) *	**<0.001**
	Impaired relaxation	4(17)	6(33) *	11(48) *	
	Pseudo-normal	0(0)	6(33) *	8(35) *	
	Restrictive pattern	0(0)	1(6)	0(0)	
EF (%)		63±5	56±4*	59±6	**0.004**
EDV, ml		87±12	100±17*	93±19	**0.04**
ESV, ml		33±8	44±10*	38±11	**0.002**
SV, ml		54±5	55±10	56±12	0.80
CO, L/min		4±0.6	4±0.7	4±0.8	0.87

Normally distributed data are expressed as (mean ±SD). Non-normally distributed data are displayed as median with interquartile ranges. Categorical data are expressed as numbers n (%) $^{*}p<0.05$ versus normal group, $^{\dagger}p<0.05$ versus malignant group. CO: cardiac output; DD: diastolic dysfunction; DT: deceleration time; EDV: end-diastolic volume; EF: ejection fraction; ESV: end-systolic volume; IVSD: interventricular septum at diastole; LA: left atrial; LAVI: left atrial volume index; LV: left ventricle; LVH: left ventricular hypertrophy; LVIDD: Left ventricle internal diameter at diastole; LVMI: left ventricular mass index; MHT: malignant hypertension; NC: normotensives controls; PWD: posterior wall thickness at diastole; RH: resistant hypertension; RWT: relative wall thickness; SV: stroke volume.

4.4.4. Speckle tracking characteristics

Strain imaging assessment parameters of LV are summarised in Table 4.7. Both hypertensive groups showed markedly reduced GLS compared to NC (MHT -15.6%, RH -17.7% vs. NC -25%, all p<0.001) with GLS being lower in MHT compared to RH (p<0.05) (Figure 4.1). Both hypertensive groups also showed preserved but lower GCS vs. NC (MHT -30.7% ±4.7, RH -30% ±4.3 vs. NC -34.3% ±4.5, p=0.004 and p=0.03, respectively). All groups had normal LV twist pattern (apical counter-clockwise movement and basal clockwise movement) (Figure 4.2 and Figure 4.3). There were no significant differences in basal rotation, apical rotation, torsion and twist between the three groups.

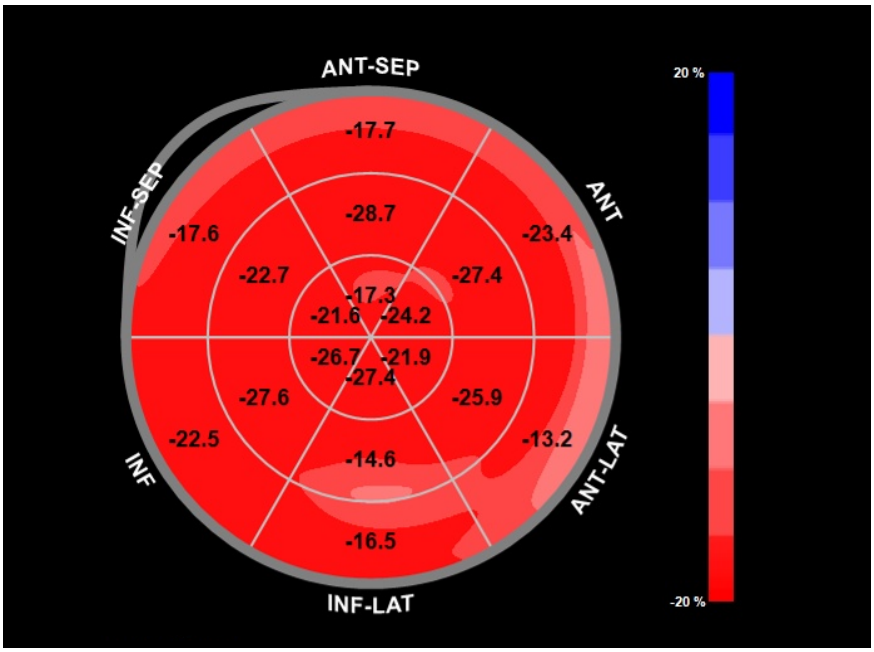


Figure 4.1 Bull's eye display of global longitudinal strain in resistant hypertensive patient

Table 4.7 Speckle tracking echocardiography characteristics

STE characteristics	NC(n=23)	MHT(n=18)	RH(n=23)	P
GLS, %	-25[-28 – -22]	-15.6[-17 – -15]*	-17.7[-20 – -16]*,†	**<0.001**
GCS, %	-34.3±4.5	-30.7±4.7*	-30±4.3*	**0.003**
Global apical rotation, °	2.6±0.92	2.7±1.5	1.9±1.4	0.12
Global basal rotation, °	-2.9±2.6	-2.8±1.8	-3.2±1.8	0.84
R-AVC time, ms	334[310-375]	377[325-423]	399[322-438]	0.12
Peak Apical rotation, °	6±2	7±3	5±3	0.19
Time to peak apical rotation, ms	432±100	463±78	455±74	0.48
Peak Basal rotation, °	-5[-9 – -4]	-5[-9.2 – -3.5]	-8[-10 – -6]	0.62
Time to peak basal rotation, ms	466±87	411±68	437±79	0.09
Peak Twist, °	12[9-16]	14[8-18]	13[11 - 15]	0.85
Apical rotation at AVC, °	4.8±2.1	4.5±2.5	3.5±2.6	0.15
Basal rotation at AVC, °	-4.7±4	-4.5±4	-5.9±3.1	0.37
Net twist AVC, °	9.6[5.8-11.8]	8[4.4-14.7]	10.9[7.2-11.6]	0.85
Torsion, °/cm	2.1±0.93	1.8±1.1	2.02±0.83	0.68

Normally distributed data are expressed as (mean ±SD). Non-normally distributed data are displayed as median with interquartile ranges. *p<0.05 versus normal group, †p<0.05 versus malignant group. AVC: aortic valve closure. GCS: global circumferential strain; GLS: global longitudinal strain; MHT: malignant hypertension; NC: normotensives controls; R-AVC: R wave to aortic valve closure; RH: resistant hypertension; STE: speckle tracking echocardiography.

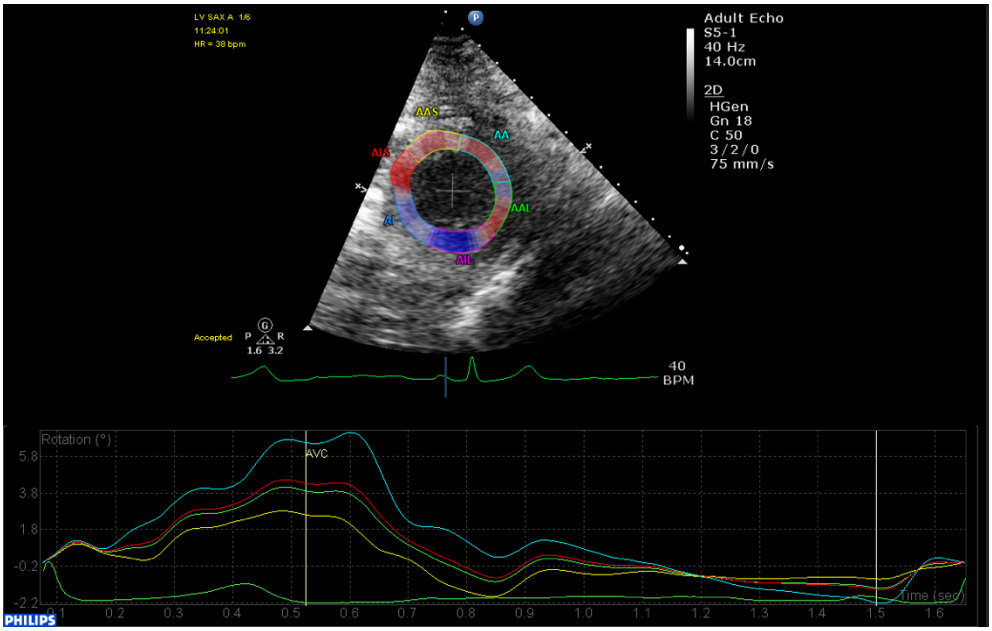


Figure 4.2 Example of apical rotation in patient with resistant hypertension

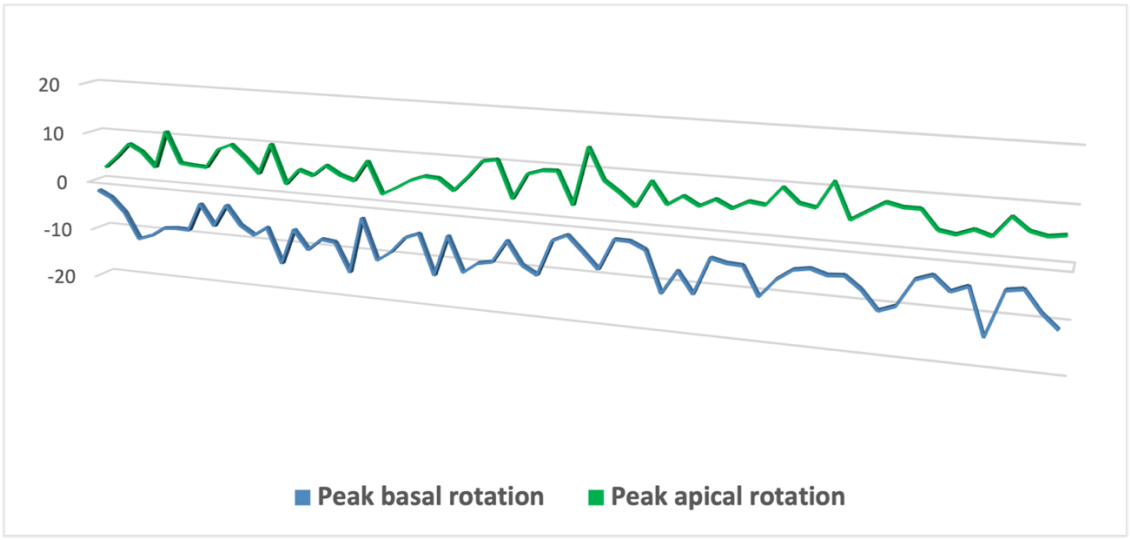


Figure 4.3 Normal LV twist pattern in all study groups. Apical counter-clockwise movement (above) and basal clockwise movement (below).

4.4.5. Associations between strain parameter and clinical, demographic, haemodynamic, and laboratory indices

Univariable analysis and multivariable stepwise linear regression analysis with backward selection were performed to detect independent determinants of GLS. Univariable associations with GLS are summarised in Table 4.8.

Table 4.8 Univariable regression analysis to determine association of global longitudinal strain with clinical, demographic, haemodynamic, and laboratory indices

Variables	Coefficient	R^2	P	95% CI
Presence of MHT	-9.6	0.70	<0.001	-11.38 – -7.89
Presence of RH	-7.5	0.70	<0.001	-9.14 – -5.88
Duration of HTN, years	-0.77	0.44	<0.001	-1.00 – - -0.55
BMI, kg/m^2	-0.36	0.11	0.009	-0.63 – -0.095
Office SBP, mmHg	-0.09	0.34	<0.001	-0.13 – - -0.06
Office DBP, mmHg	-0.16	0.29	<0.001	-0.23 – -0.10
eGFR, mL/min/1.73 m^2	0.11	0.16	0.001	0.04 – 0.18
IVSD, cm	-8.68	0.29	<0.001	-12.04 – -5.31
PWD, cm	-8.22	0.26	<0.001	-11.78 – -4.66
RWT	-13.07	0.16	0.001	-20.73 – -5.42
LV mass, g	-0.03	0.31	<0.001	-0.04 – -0.02
EF, %	0.25	0.09	0.01	0.05 – 0.45
EDV, ml	-0.07	0.07	0.03	-0.15 – -0.004
ESV, ml	-0.14	0.10	0.01	-0.26 – -0.03

BMI: body mass index; DBP: diastolic blood pressure; EDV: end-diastolic volume; EF: ejection fraction; eGFR: estimated glomerular filtration rate; ESV: end-systolic volume; HTN: hypertension; IVSD: interventricular septum at diastole; LAVI: left atrial volume index; LV: left ventricle; MHT: malignant hypertension; PWD: posterior wall thickness at diastole; RH: resistant hypertension; RWT: relative wall thickness; SBP: systolic blood pressure.

Two multivariable linear regression models were created. The multivariable linear regression models are shown in Table 4.9. The first model includes groups of participants (MHT, RH and control), duration of hypertension, age, BMI, and eGFR as independent variables. Presence of MHT (β=-0.42, p<0.001), presence of RH (β=-0.29, p<0.001) and age (β=-0.005), p=0.01) showed significant negative association with GLS whereas eGFR showed significant positive association with GLS (β=0.002, p=0.03). The second model includes groups of participants (MHT, RH and control), hypercholesterolaemia (No=0 Yes=1), office SBP, office DBP, and statin. Presence of MHT (β=-0.47, p<0.001), presence of RH (β=-0.34, p<0.001) and office DBP (β=-0.003, p=0.02) showed significant negative association with GLS.

Table 4.9 Stepwise multiple linear regression analysis of global longitudinal strain

Global longitudinal strain	Coefficient	P	95% CI
Model 1 R^2=0.77, p<0.001			
Presence of MHT	-0.42	**<0.001**	-0.54 – -0.29
Presence of RH	-0.29	**<0.001**	-0.44 – -0.12
eGFR	0.002	**0.03**	0.001 – 0.004
Age	-0.005	**0.01**	-0.009– -0.001
Model 2 R^2=0.76, p<0.001			
Presence of MHT	-0.47	**<0.001**	-0.57 – -0.37
Presence of RH	-0.34	**<0.001**	-0.43 – -0.24
Office DBP	-0.003	**0.02**	-0.006 – -0.001

Global longitudinal strain has been analysed as positive value to help interpretation of regression analysis.
DBP: diastolic blood pressure; MHT: malignant hypertension; RH: resistant hypertension.

4.4.6. Heart rate variability

Time domain and frequency domain variables of HRV were assessed and no significant differences were observed between the three groups ($p>0.05$ for all), (Table 4.10).

Table 4.10 Time and frequency domain characteristics

	NC(n=23)	MHT(n=18)	RH(n=23)	P
Heart rate, bpm	65±10	66±10	64±11	0.93
Max heart rate, bpm	76±10	75±11	73±11	0.67
Min heart rate, bpm	59±10	60±9	58±10	0.86
SDNN, ms	48 [30-81]	47 [34-64]	51 [26-63]	0.96
rMSSD, ms	33 [25-51]	30 [23-50]	28 [16-37]	0.31
HRV index	11[8-17]	9[8-11]	9[7-14]	0.28
pNN50, %	9 [3 - 30]	6 [3 - 18]	7 [1 - 15]	0.51
Total power	3291[1179 - 8217]	3335[1275 - 4826]	4661[2242 - 7122]	0.85
RSA	14±3	14±3.2	14±2	0.89
LF, ms^2	559 [195-1849]	585 [265-1006]	409 [1681126]	0.15
HF, ms^2	388 [185-834]	231 [117-378]	165 [80-457]	0.10
LF, n.u	66±18.3	67±15.7	64±22	0.91
HF, n.u	34±18.3	33.1±16	36±22	0.37
LF/HF	1.9±0.4	2.02±0.3	1.77±0.5	0.97

Normally distributed data are expressed as (mean ±SD). Non-normally distributed data are displayed as median with interquartile ranges. HF: high frequency; HRV: heart rate variability; LF: low frequency; MHT: malignant hypertension; NC: normotensives controls; pNN50: The percentage of adjacent NN intervals that differ from each other by more than 50 ms; RH: resistant hypertension; rMSSD: root mean square of successive differences; RSA: respiratory rate; SDNN: the standard deviation of NN.

No variables were identified on multivariable analysis as independent predictors of LF/HF.

4.5. Discussion

This is one of the first studies that has evaluated cardiac remodelling and autonomic changes in two populations, both characterised by severe hypertension and poor prognosis (treated MHT and RH).

4.5.1. Impact of hypertension on cardiac deformation and haemodynamic

Regarding cardiac structures and function, no previous study has evaluated cardiac changes using a robust sensitive technique, strain assessment (in addition to conventional echocardiography) and compared between treated MHT and RH. Preserved systolic function evaluated by conventional EF is frequently seen in hypertension (240). Reduced strain function, presence of LVH and myocardial fibrosis are present in patients with long lasting hypertension (17, 18). I showed that all groups had preserved EF as evaluated by conventional biplane Simpson method despite an average of 8- year history of hypertension yet MHT showed significant trend for a lower EF compared to NC group only. Importantly, subclinical reduction of systolic function was evident in patients with RH and MHT, as evaluated by reduced GLS. Moreover, in multivariable regression model, lower GLS was associated with presence of MHT and RH after adjustment for other variables.

Consistent with these findings, lower GLS was observed in hypertension compared to the normotensive group, and in uncontrolled compared to controlled hypertension

(237, 240, 242, 344). The findings of my study are also consistent with Tadic et al. who displayed reduced GLS in RH compared to controls, and well controlled hypertension and in uncontrolled hypertension (274). It also consistent with Rhea et al. who reported negative association between elevated BP there and GLS (345). Decreased longitudinal strain was also observed when EF was still preserved and became even more prominent in the presence of LVH (24, 346). Furthermore, it was previously reported that reduced GLS in hypertension was associated with hypertension severity (17, 244, 245, 246).

Moreover, I revealed negative association between office DBP and GLS. Similar to this finding, one study reported that abnormal GLS was associated with more resistant DBP in uncontrolled hypertension (241).

Although long-term cardiovascular complications associated with MHT are relatively uncommon (Heart failure is around 8-11% (78, 347), whereas MI is 4%) (348), cardiac complications have been evident in MHT population. I showed that despite the long treatment of MHT, LV dysfunction is still existent, and GLS were deteriorated to a larger extent in those with MHT compared to RH group with similar level of BP readings. This suggests that MHT might have different underlying pathophysiology features.

To date, there have been few studies investigating cardiac changes in MHT population (12, 275, 349, 350, 351, 352, 353). The majority of studies used conventional echocardiography to evaluate LV function in MHT and reported preserved EF in MHT population (12, 349, 351, 352). Except one study by Nadar et al. who displayed reduction of conventional EF, possibly as a result of very high BP levels (mean 222/136 mmHg) in

the acute malignant phase (350). Shapiro et al. evaluated prolonged effect of treated MHT on cardiovascular system and reported the presence of cardiomyopathy features in MHT (351). However, the previous studies did not use STE which is a sensitive indicator to assess early-stage impaired LV function related to myocardial fibrosis over conventional 2D echocardiography (26). The endocardial layer assessed by GLS is most susceptible to compromised due to interstitial fibrosis and hypoperfusion (27).

Several studies reported an inverse relationship between afterload and GLS and showed that GLS is influenced by loading conditions (higher afterload led to deterioration of GLS) (354, 355). Increased afterload result in an increased ESV and a reduced SV. My study showed increased ESV observed in MHT compared to NC with no differences found between MHT and RH or between NC and RH. However, similar SV observed in all the three groups. This may indicate that impaired GLS was not only influenced by afterload and could be related to more prevalent myocardial fibrosis in MHT population where there is significant reduction in GLS compared to RH. Compromised longitudinal strain was linked to serum tissue inhibitor of matrix metalloproteinase and altered collagen turnover causing myocardial fibrosis (23). Additionally, I showed an association between impaired GLS and reduced eGFR. It was previously shown that GLS has a superior prognostic value in predicting cardiovascular and all-cause mortality in different populations including patients with heart failure and patients with CKD (356, 357, 358, 359).

Previous study showed that in stable haemodialysis patients with preserved LVEF, impaired GLS was associated with poor prognosis (356). Recently, GLS was

demonstrated as a significant predictor of all-cause mortality in a large cohort with a wide range of eGFR (360).

Both hypertension groups had higher NLR compared to control group. However, no differences were found between MHT and RH. Similarly, a study showed that NLR and neutrophil count were found to be higher in the RH group than in the controlled hypertension and NC groups (361). According to the results of a previous studies, NLRs were significantly associated with incident hypertension, especially in the elderly or male Taiwanese population(362), and a high neutrophil count is a risk factor for developing hypertension (363). There was also a significant correlation between blood pressure regulation, high neutrophil counts, and low lymphocyte counts in a study of African Americans (364). The findings may indicate that NLR, as inflammation marker, plays a role in regulating blood pressure. Neutrophils were considered to be mediators that regulate inflammatory processes and are involved in releasing ROS, NO which may lead to impaired vascular endothelium and potentially hypertension (365, 366).

Both hypertensive groups also showed preserved GCS but still lower than in the NC. While previous findings obtained with 2D STE and 3D STE report preserved circumferential strain at early stages of hypertension, limited information is available about circumferential strain at severe stages of hypertension such as MHT and RH (346, 367).

Preserved circumferential strain in early stages of hypertension has been associated with the phenomenon of cross-fibre shortening due to hypertensive ventricular

remodelling, whereas mid-wall myocardial fibres were unaffected and therefore circumferential function is preserved (246). This may also explain the well-preserved function of EF (24, 236, 243, 258, 259, 260).

Indeed, these findings offer early evidence of systolic dysfunction and closely linked myocardial fibrosis to impaired myocardial strain (23, 27, 28, 29). These myocardial changes suggest a mechanism of LV decompensation and damaged myocardial layers in response to elevated myocardial wall stress and disease progression (258, 261, 262). Increased myocardial wall stress create subendocardial ischaemia, consequently increasing myocardial stiffness and decreasing myocardial strain.

Despite extensive studies of LV function, no comprehensive assessment of twist deformation in hypertension has been reported in hypertension population. Assessment of twist and torsion in CVD populations shows different and unpredictable responses of LV twist and torsion in clinical studies. To my knowledge, no previous study assessed twist and torsion using STE imaging in MHT and RH. Of note, despite decreased GLS and GCS, the current study showed preserved basal rotation, apical rotation, twist and torsion with no significant differences between the three groups reported.

As discussed earlier, LV longitudinal contractility is the earliest to be compromised in strain function (236). LV twist and torsion may remain preserved to compensate for the reduction in GLS and eventually result in preserved LV EF (236, 258, 368). Similarly, Imbalzano et al. showed that twist is preserved in patients with hypertension (17). Galderisi et al. also showed that LV torsion was not significantly different between

sedentary controls, top-level rowers, and young newly diagnosed patients with hypertension, (never treated) (346). Preserved torsion and decreased GLS have been found in patients with diastolic heart failure and preserved EF (369).

Several studies showed positive correlation between EF and Twist (272, 370, 371, 372, 373). Assuming that twist remain preserved in order to compensate for the decreased longitudinal contraction aiming to preserve normal LV function (374).

Conversely, Mizuguchi et al. reported normalisation of impaired twist in patients with hypertension after 12 months of ARBs treatment (375). It was also observed that BP and EF were normal when twist was assessed. While the present study measured twist when target BP level was not achieved.

On the other hand, some studies have reported increased twist and torsion using STE in hypertension compared to normotensives controls and in patients with concentric hypertrophy (246, 376). In addition, other previous studies used cardiac MRI and reported elevated LV twist and torsion in hypertension group compared to normotensives control (371, 377) and in patients with aortic valve stenosis and preserved EF (378), and in hypertension with concentric remodelling (379). Previous studies have reported increased LV twist in hypertension patients with normal EF, and decreased twist in hypertension patients with reduced EF (370). They also reported more collagen degradation in hypertension patients with reduced EF. Another study evaluated African patients with hypertension with low EF and showed normal twist pattern in 68% and showed reduction of LV twist using STE (372).

Park et al. reported different responses of LV twist depended on the stage of diastolic dysfunction in hypertension, and that torsion tends to normalise in moderate and severe diastolic dysfunction whereas significant impaired torsion occurred only in mild diastolic dysfunction compared to normal controls (380).

Imbalance of myocardial fibres orientations determine the changes in twist and rotation (227). In subendocardial myocardial, fibres are oriented as a right-handed helix, while the subepicardial fibres are arranged as a left-handed helix (227). As demonstrated above, subendocardial function is expected to be impaired in hypertension. Thus, the subepicardial fibres are fundamental factor in affecting LV twist. In patients with MHT and RHT, preserved twist may serve as a compensatory mechanism to maintain preserved EF.

Impaired LV diastolic function is a known cardiac complication of hypertension and is an independent predictor of CVD and all-cause mortality (381, 382). Therefore, it was expected to observe more prevalent of diastolic dysfunction indices in both hypertensives group compared to NC. However, no differences were observed between MHT and RH. These findings are consistent with previous studies (12, 275, 350, 351, 383). In the present study, hypertensive groups showed increased LA volume and higher LAVI compared to NC which reflects the severity of high LA pressure and indicates progression of worse diastolic function. This is also consistent with previous studies (12, 384).

There was a significant increase in LV mass and LVMI in both hypertension groups compared to NC with LV mass and LVMI being greater in MHT vs. RH. Similar findings have been reported by Nadar et al. (350) Previous studies showed that reduction of GLS could be related to higher LVMI and LV mass (385, 386).

4.5.2. Effect of hypertension on autonomic nervous system

Several studies have evaluated autonomic function using HRV in different types of hypertension, but to date none has examined and compared between NC, MHT and RH (35, 283, 319, 387, 388, 389, 390). Despite a trend toward decreased HRV in patients with hypertension, in the present study, no differences were detected in HRV parameters between the three groups. Similarly, Bilge et al. found no change in HRV indices among patients with mild to moderate hypertension (332).

There are potentially several possible explanations for these findings. These can be supported by the hypothesis of potential restoration of sympatho-vagal balance after prolonged hypertension exposure. This view is supported by Schroeder et al. who found that subjects with hypertension had reduced HRV at baseline which suggest the involvement of autonomic dysregulation in the development of hypertension (318). However, after 9 years of follow-up, no significant difference was detected in HRV compared to those without hypertension. These findings further support the thesis of ‘’blood pressure seeking behaviour of the central nervous system’’. Julius et al. proposed this hypothesis and suggested that sympathetic tone will tend to decline after the prolong exposure of BP elevation (33, 391). Long-term high BP associated with overactivity of sympathetic tone and reduction of parasympathetic activity led to

reversible mechanism of normalisation of CO and eventually balanced of sympathetic and parasympathetic activities. This would indicate the possibility that HRV parameters in the three groups (MHT, RH and NC) converge with time.

Another possible explanation could be that autonomic function tends to improve after treatment with ACEIs (333) or antihypertensive combination therapy (Metoprolol|+felodipine or enalaril+hydrochlorothiazide) (392). In hypertension subjects, ACEIs was associated with elevated HF, implying enhancement of parasympathetic tone (393). On the contrary, another study found that lower HRV was associated with patients who used β-blockers and diuretics (394). Another study reported reduction of HRV in patients with hypertension compared to normotensive DM (395).

4.6. Conclusion

My study examined cardiac and autonomic changes in two complex types of hypertensives patients. The results showed that patients with MHT and RH, even with long and intensive antihypertensive treatment, had persistent impaired cardiac remodelling independent of preserved EF. The use of advanced strain imaging modality unmasked differential cardiac remodelling responses in patients with MHT compared to RH. Patients in stable phase of MHT with good long-term BP control still have significantly lower GLS and greater LV mass and LVMI. LV twist, torsion and GCS are preserved in patients with MHT and RH and appear to contribute to preserved EF. In contrast, autonomic function was normalised and preserved in both hypertensives

groups and no changes were observed between hypertensives groups and normotensives controls.

Chapter V. ASSESSMENT OF VASCULAR FUNCTION IN TWO GROUPS OF HYPERTENSIVES: RESISTANT HYPERTENSION AND MALIGNANT HYPERTENSION

5.1. Introduction

Impaired vascular function is observed in hypertension. Increased cfPWV, high AIx, low subendocardial viability and decreased FMD have been associated with hypertension (46, 61). Moreover, reduction in carotid artery distensibility is also associated with hypertension (62). The concomitant assessment of these parameters in patients with RH and MHT has been rarely studied.

5.2. Hypothesis and aims

I hypothesised that patients with MHT have worse measures of vascular function compared to patients with RH and both have worse measures compared to NC.

I aimed to investigate vascular function in two groups of hypertensive patients: The first group with a history of malignant phase hypertension and the second group with RH.

5.3. Methods

5.3.1. Study design

The study protocol was approved by the West Midlands-South Birmingham Research Ethics Committee (REC reference: 18/WM/0168). Two local approvals were obtained from the Research Ethics Committee of Sandwell and West Birmingham Hospitals NHS Trust and Liverpool Heart and Chest Hospital. The study was conducted following the Declaration of Helsinki of the World Medical Association. All the procedures and experimental work were performed my myself.

Two groups of hypertensive patients were recruited from two sites: 1) the hypertension clinics at City Hospital and 2) Liverpool Heart and Chest Hospital. The first group included

patients with a history of malignant phase hypertension and the second group included those with RH. The NC group included participants with no history of hypertension. Recruiting of normotensive group was done by using posters, and advertisements on the University of Birmingham internal website and from patients' family members and the surrounding communities

Participant information sheet was provided to all participants before the study day. Written informed consent was obtained from all subjects.

5.3.1.1. Inclusion criteria

Patients older than 18 years with the ability to five informed consents, and a diagnosis of either MHT or RH were included. All participants had a diagnosis of malignant and resistant hypertension based on their medical records. Participants with normal BP were included as NC.

5.3.1.2. Exclusion criteria

Participants with the following conditions were excluded: BMI ≥35 kg/m^2, moderate-severe valvular heart disease, previous MI or current symptomatic CAD, AF, recent (<6 months) cerebrovascular events, active infections or pyrexia illness, active chronic and systemic illnesses (e.g., respiratory diseases, renal or liver failure, neurological disease) and pregnancy.

5.3.2. Study population

A total of 64 participants were recruited into the study during the period from December 2018 to March 2020. Further recruitment has been stopped because of the COVID-19 pandemic.

Twenty-three patients with RH (mean±SD: 57±11 y), were compared to 18 patients with treated MHT (54±13 y), and 23 NC (50±5 y). MHT and RH diagnoses were clinically confirmed in hypertension clinic following the current guidelines (8).

5.3.3. Procedures

While participant was in a supine position, the following procedures were obtained: FMD assessment, arterial stiffness test and carotid artery distensibility. All procedures were assessed according to the guidelines and were explained in detail in methods chapter (Chapter-03; 3.6 and 3.7). Arterial stiffness and FMD were explained also in SOP, (Appendix 2 and 3).

5.3.4. Statistical analysis

Statistical analysis was performed using statistical analysis software (Stata/IC), 16.1 for Mac. Continuous data were subjected to the Shapiro-Wilk test to determine the nature of its distribution. Normally distributed data were analysed by ANOVA with Tukey's post hoc test and were expressed as mean±SD. Non-normally distributed data were analysed by nonparametric pairwise multiple comparisons using Kruskal-Wallis H test and Dunn's post hoc test and were presented as median with interquartile range. Categorical data were compared using the chi-squared test and are expressed as numbers and percentages. A p value of <0.05 was considered statistically significant.

Univariable analysis and stepwise multivariable linear regression model with backward selection were constructed to determine clinical and demographic factors potentially influencing FMD, brachial artery diameter and cfPWV.

It is important to note that when multiple analyses are performed, this increases the chance of making a Type 1 error. This occurs when the null hypothesis is correct, but is rejected by the statistical analysis. When each analysis is performed with a 0.05 level of significance, if the null hypotheses for the analyses are true, each individual analysis has a 5% chance of resulting in a Type 1 error; however, the probability that at least one of the analyses will falsely reject the null hypothesis becomes greater than 5% in combination.

It is also important to note that when sample sizes are small, if the effects that exist are not large, then the power to detect these effects can be low. This translates to a low probability of achieving statistically significant p values even when the null hypothesis should be rejected. This is referred to as a Type 2 error, and this should be considered with regard to the analyses especially when group sizes are small.

Multivariable regression analysis models include the following variables:

Model 1: Age, presence of hypertension, duration of hypertension, BMI, eGFR. The only exception is the model for brachial artery diameter, which does not include eGFR as a predictor.

Model 2: Presence of hypertension, presence of hypercholesterolaemia, SBP, DBP, and statin.

5.4. Results

5.4.1. Demographic and clinical characteristics

I am dealing with the same participants as in the previous chapter (chapter 4). Same demographic and clinical characteristics are described previously in Table 4.1 and Table 4.2.

History of other medications was similar between the three study groups except for the higher use of statins in MHT. The concurrent medications of the study groups are summarised in Table 4.3 and Table 4.4.

Laboratory data from all three study groups are summarised in Table 4.5. There were no significant differences in glycaemia control (HBA1c), sodium and potassium levels between the groups. Both hypertensive groups had lower eGFR and higher creatinine level and urea than those in the control group (p=0.02). There were no significant differences in eGFR and creatinine between MHT and RH.

5.4.2. Flow mediated dilatation characteristics

FMD assessment was performed in the morning after an overnight fasting. The data from the FMD parameters are summarised in Table 5.1.

Baseline diameter of brachial artery increased more in MHT than NC (p=0.006). However, there were no significant differences in baseline diameter between RH and NC or between MHT and RH. Following 5 minutes of cuff occlusion, FMD percentage was markedly attenuated in both hypertensive groups (RH: 5.5±2.6% and MHT: 5.9±2.6% vs. NC: 9.9±2.5%, p<0.001) (Figure 5.1). FMD values were not statistically different between MHT and RH, (p=0.8). Maximum diameter was significantly larger in MHT compared to NC (p=0.009). There were no significant differences in maximum diameter between RH and NC or between RH and MHT. There were significant increases in recovery diameter in MHT group compared to NC (p<0.001), but no differences were found between RH and MHT or between RH and NC. There were no significant differences in shear rate

parameters between the three groups (p>0.05), except for lower positive shear rate maximum in RH compared to NC, (RH 255[183-533] vs. NC 502[358-762], p=0.02).

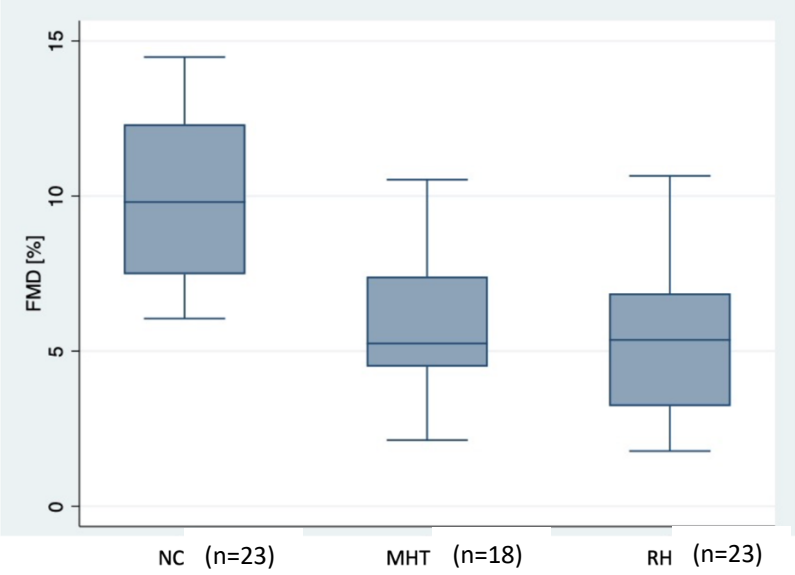


Figure 5.1 Comparison of flow mediated dilatation in hypertensive and normotensive subjects. FMD: flow mediated dilatation; MHT: malignant hypertension; NC: normotensives controls; RH: resistant hypertension

.

Table 5.1 Endothelial function Characteristics

Endothelial function characteristics	NC(n=23)	MHT(n=18)	RH(n=23)	P
FMD, %	9.9±2.6	5.9±2.6*	5.5±2.6*	**<0.001**
FMDr, %	3.7[2.2 – 14]	3.3[2.5 – - 6.1]	2.4[1.6 – 4]*	**0.03**
Baseline diameter, mm	4[3.6 – 4.6]	5[4.7 – 5.6]*	4.6[4 – 5.6]	**0.01**
Maximum diameter, mm	4.5[4 – 5]	5.5[5 – - 6]*	5[4 – 6]	**0.02**
Maximum diameter time, sec	418[376 – 458]	405[370 – 452]	440[393 – 464]	0.18
Recovery diameter, mm	4.3[3.6 – 4.8]	5.3[4.7 – 5.7]*	4.8[4.1 – 5.6]	**0.005**
Positive shear rate baseline, [sec.-1]	102[73 – 156]	88[5 – 119]	73[54 – 154]	0.48
Positive shear rate maximum, [sec.-1]	502[358 – 762]	345[210 – 590]	255[183 – 533]*	**0.02**
Positive shear rate area, [sec.-1]	5920[3428 – 14345]	5225[3133 – 10691]	6194 [2900 – 15477]	0.55
Shear rate (Positive shear rate area to maximum), [sec.-1]	4179[3046 – 8182]	3127[33 – 7404]	3839[2009 – 11543]	0.26
Negative shear rate baseline, [sec.-1]	-31[-44 – -14]	-19[-30 – -12]	-23[-29 – -12]	0.31

Normally distributed data are expressed as (mean±SD). Non-normally distributed data are displayed as median with interquartile ranges. *p<0.05 versus normal group, †p<0.05 versus malignant group. FMD: flow mediated dilatation; FMDr: flow mediated dilatation with respect to recovery diameter; MHT: malignant hypertension; NC: normotensives controls; RH: resistant hypertension.

5.4.3. Factors influencing endothelial function in resistant and malignant hypertension

Univariable analysis and stepwise multivariable linear regression model with backward selection were performed among potential confounders for FMD and brachial artery diameter. Determinants of FMD in the univariable regression analysis are summarised in Table 5.2.

Table 5.2 Univariable regression analysis to determine association of flow mediated dilatation with clinical, demographic, haemodynamic, and laboratory indices

Variables	Coefficient	R^2	P	95% CI
Presence of MHT	-3.99	0.40	<0.001	-5.61 – -2.37
Presence of RH	-4.51	0.40	<0.001	-6.03 – -2.98
Duration of HTN, years	-0.42	0.31	<0.001	-0.59 – -0.26
BMI, kg/m^2	-0.21	0.09	0.02	-0.39 – -0.04
eGFR, mL/min/1.73 m^2	0.04	0.06	0.04	0.001 – 0.09
Office SBP, mmHg	-0.04	0.15	<0.001	-0.07 – -0.02
Office DBP, mmHg	-0.06	0.10	0.01	-0.11 – -0.01
Peripheral SBP, mmHg	-0.04	0.19	<0.001	-0.07 – -0.02
Peripheral DBP, mmHg	-0.10	0.18	0.001	-0.15 – -0.04
Peripheral PP, mmHg	-0.04	0.09	0.02	-0.07 – -0.01
Central SBP, mmHg	-0.05	0.20	<0.001	-0.07 – -0.02
Central DBP, mmHg	-0.09	0.16	0.001	-0.15 – -0.04
Central PP, mmHg	-0.04	0.10	0.01	-0.07 – -0.01
cfPWV, m/s	-0.42	0.07	0.04	-0.82 – -0.02
MAP, mmHg	-0.08	0.22	<0.001	-0.12 – -0.04
Baseline brachial diameter, mm	-1.09	0.14	0.002	-1.78 – -0.41
Use of CCBs	-1.96	0.09	0.01	-3.54 – -0.38
Use of alpha blockers	-2.01	0.09	0.01	-3.59 – -0.42
Use of diuretics	-3.86	0.35	<0.001	-5.20 – -2.52
Use of ACEI/ARBs	-3.18	0.24	<0.001	-4.62 – -1.73
Use of beta blockers	-3.14	0.22	<0.001	-4.67 – -1.62

ACEIs: angiotensin-converting-enzyme inhibitors; ARBs: angiotensin receptor blockers; BMI: body mass index; CCBs: calcium channel blockers; cfPWV: carotid-femoral pulse wave velocity; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; HTN: hypertension; MAP: mean arterial pressure; MHT: malignant hypertension; NC: normotensives controls; PP: pulse pressure; RH: resistant hypertension; SBP: systolic blood pressure.

Multivariable analysis was performed and summarised in Table 5.3. The first model includes groups of participants (MHT, RH and control), duration of hypertension, age, BMI, and eGFR as independent variables. The second model includes groups of participants (MHT, RH and control), hypercholesterolaemia (No=0 Yes=1), office SBP, office DBP, and statin. Only presence of MHT and presence of RH maintained significant negative association with FMD in both models, (Table 5.3).

Table 5.3 Stepwise multivariable linear regression analysis of flow mediated dilatation

FMD	Coefficient	P	95% CI
Model 1 R^2=41, p<0.001			
Presence of MHT	-3.63	**0.01**	-6.49 – -0.77
Presence of RH	-4.06	**0.01**	-7.11 – -1.01
Model 2 R^2=41, p<0.001			
Presence of MHT	-3.96	**<0.001**	-6.21 – -1.72
Presence of RH	-4.59	**<0.001**	-6.69 – -2.49

MHT: malignant hypertension; RH: resistant hypertension.

Univariable analysis showed that dilated brachial artery diameter at baseline was positively associated with the presence of MHT (R^2=0.08, p=0.03), duration of hypertension (R^2=0.10, p=0.009), advanced age (R^2=0.22, p<0.001), high office SBP (R^2=0.09, p=0.02), creatinine (R^2=0.09, p=0.02), cfPWV (R^2=0.15, p=0.001), central SBP (R^2=0.12, p=0.006), central PP (R^2=0.12, p=0.005), (Table 5.4.).

Univariable analysis also showed that baseline brachial artery diameter was inversely related to eGFR (R^2=0.11; p=0.008) and FMD (R^2=0.14; p=0.002), (Table 5.4).

Table 5.4 Univariable analysis to determine association of baseline brachial artery diameter with clinical, demographic, haemodynamic, and laboratory indices

Variables	Coefficient	R^2	P	95% CI
Presence of MHT	0.78	0.08	0.03	0.09 – 1.47
Duration of HTN	0.08	0.10	0.009	0.02 – 0.14
Advanced age, years	0.04	0.22	<0.001	0.02 – 0.07
Office SBP, mmHg	0.01	0.09	0.02	0.002 – 0.02
Creatinine, umol/L	0.01	0.09	0.02	0.002 – 0.01
cfPWV, m/s	0.21	0.15	0.001	0.08 – 0.35
Central SBP, mmHg	0.01	0.12	0.006	0.004 – 0.02
Central PP, mmHg	0.01	0.12	0.005	0.005 – 0.02
Heart rate, bpm	-0.03	0.11	0.007	-0.06 – -0.01
eGFR, mL/min	-0.02	0.11	0.008	-0.03 – -0.005
FMD, %	-0.12	0.14	0.002	-0.21 – -0.04

cfPWV: carotid-femoral pulse wave velocity; eGFR: estimated glomerular filtration rate; FMD: flow mediated dilatation; HTN: hypertension; MHT: malignant hypertension; PP: pulse pressure; SBP: systolic blood pressure.

The first multivariable regression model includes groups of participants (MHT, RH and control), duration of hypertension, age and BMI as independent variables, (Table 5.5). Age was the only variable identified in this model as independent predictors of brachial baseline diameter, (β=0.03, p=0.02).

The second model includes groups of participants (MHT, RH and control), hypercholesterolaemia (No=0 Yes=1), office SBP, office DBP, and statin. Only office SBP was positively associated with brachial baseline diameter, (β=0.14, p=0.04).

Table 5.5 Stepwise multivariable regression analysis of variables related to baseline brachial artery diameter as the dependent variable.

Brachial baseline diameter	Coefficient	P	95% CI
Model 1 R^2=37, p<0.001			
Age	0.03	**0.02**	0.006 – 0.06
Model 2 R^2=24, p=0.04			
Office SBP	0.14	0.04	0.001 – 0.03

SBP: systolic blood pressure.

5.4.4. Arterial stiffness characteristics

Peripheral (supine) and central blood pressures were significantly elevated in MHT and RH groups compared to NC (p<0.05) with no differences between MHT and RH. Peripheral and central PP were also higher in both hypertension groups compared to NC (p<0.001). There were also no differences in heart rate between the three groups (p=0.71).

cfPWV was increased in both hypertensives compared to NC (p<0.05); however, no difference was observed in peripheral AIx and central AIx between the groups (p=0.90 and p=0.20, respectively). SEVR was decreased in MHT compared to NC (p=0.01). MAP,

SV and CO were significantly higher in MHT and RH compared to NC ($p<0.001$). TPR also found to be lower in MHT group compared to NC ($p=0.002$). However, no differences found between MHT and RH or between RH and NC. Augmentation pressure was increased significantly in MHT vs. NC ($p=0.02$) and in RH vs. NC ($p=0.0007$); however, no differences found between MHT and RH, (Table 5.6).

Table 5.6 Arterial stiffness characteristics

Arterial stiffness Characteristics	NC(n=23)	MHT(n=18)	RH(n=23)	P
cfPWV, m/s	8±0.7	10±2*	9±2*	**<0.001**
Peripheral SBP, mmHg	127±9	171±27*	173±23*	**<0.001**
Peripheral DBP, mmHg	70±5	84±14*	84±15*	**<0.001**
Peripheral PP, mmHg	57±9	87±23*	89±22*	**<0.001**
Heart rate, (bpm)	64±10	65±10	63±12	0.71
MAP, mmHg	94±6	120±19*	120±14*	**<0.001**
SV, ml	93±13	141±36*	134±31*	**<0.001**
CO, L/min	6±1	9±2*	8±2*	**<0.001**
SEVR, %	168±30	135±41*	153±36	**0.01**
TPR, PRU	0.93[0.87-1.1]	0.81[0.73-0.89]*	0.86[0.79-0.99]	**0.006**
Central SBP, mmHg	124±9	167±26*	170±23*	**<0.001**
Central DBP, mmHg	70±5	85±14*	85±15*	**<0.001**
Central PP, mmHg	54±9	83±2*	86±22*	**<0.001**
Augmentation pressure	11[8-18]	19[14-25]*	22[16-38]*	**0.001**
Central AIx, %	25±10	25±7	30±13	0.20
Peripheral AIx, %	96[92-99]	97[93-99]	96[95-99]	0.90

Normally distributed data are expressed as (mean±SD). Non-normally distributed data are displayed as median with interquartile ranges. *p<0.05 versus normal group, †p<0.05 versus malignant group. AIx: augmentation index; cfPWV: carotid-femoral pulse wave velocity; CO: cardiac output; DBP: diastolic blood pressure; MAP: mean arterial pressure; MHT: malignant hypertension; NC: normotensives controls; PP: pulse pressure; RH: resistant hypertension; SBP: systolic blood pressure; SEVR: subendocardial viability ratio; SV: stroke volume; TPR: total peripheral resistance.

5.4.5. Factors affecting arterial stiffness

Several variables were identified as independent predictors of increased cfPWV on univariable analysis, (Table 5.7). Multivariable analysis was performed using stepwise linear regression, (Table 5.8). Groups of participants (MHT, RH and control), duration of hypertension, age, BMI and eGFR were entered in the first model as independent

variables. cfPWV was independently associated with age (β=0.007, p=0.002) and presence of MHT (β=0.19, p=0.02) The second model includes groups of participants (MHT, RH and control), hypercholesterolaemia (No=0 Yes=1), office SBP, office DBP, and statin. cfPWV was independently associated with office SBP, (β=0.005, p<0.001).

Table 5.7 Univariable analysis to determine association of pulse wave velocity with clinical, demographic, haemodynamic, and laboratory indices

cfPWV	R^2	Coefficient	P	95% CI
Presence of MHT	0.30	2.72	<0.001	1.65 – 3.78
Presence of RH	0.30	1.48	0.004	0.48 – 2.48
Duration of HTN, years	0.31	0.26	<0.001	0.16 – 0.36
Advanced age, years	0.25	0.09	<0.001	0.05 – 0.13
Hypercholesterolaemia	0.09	1.16	0.02	0.19 – 2.14
CKD	0.11	2.09	0.008	0.56 – 3.61
Office SBP, mmHg	0.44	0.04	<0.001	0.03 – 0.05
Office DBP, mmHg	0.12	0.04	0.005	0.01 – 0.07
Creatinine level	0.12	0.02	0.005	0.006 – 0.03
Central SBP, mmHg	0.53	0.04	<0.001	0.03 – 0.06
Central DBP, mmHg	0.24	0.07	<0.001	0.03 – 0.10
Central PP, mmHg	0.40	0.05	<0.001	0.03 – 0.07
eGFR, mL/min/1.73 m^2	0.22	-0.05	<0.001	-0.08 – -0.02

cfPWV: carotid-femoral pulse wave velocity; CKD: chronic kidney disease; DBP: diastolic blood pressure; eGFR*:* estimated glomerular filtration rate; HTN: hypertension; MHT: malignant hypertension; PP: pulse pressure; RH: resistant hypertension; SBP: systolic blood pressure.

Table 5.8 Stepwise multivariable linear regression analysis of pulse wave velocity

cfPWV	Coefficient	P	95% CI
Model 1 R^2=0.54, p<0.001			
Age, years	0.006	**0.008**	0.002 – 0.01
Presence of MHT	0.19	**0.02**	0.04 – 0.36
Model 2 R^2=0.53, p<0.001			
Office SBP	0.005	**<0.001**	0.003 – 0.008

MHT: malignant hypertension.

5.4.6. Carotid artery distensibility

Right CCA parameters of all the three groups are displayed in Table 5.9. The diameter of the right CCA in systole and diastole were increased in MHT and RH compared to NC (p=0.004 and p=0.002, respectively). However, no significant difference was found between diameters of systole and diastole in MHT and RH. Carotid distensibility was significantly decreased in hypertension groups compared to NC (p=0.03), with no significant difference between MHT and RH.

Table 5.9 Carotid artery characteristics

Carotid artery Characteristics	NC(n=23)	MHT(n=18)	RH(n=23)	P
Right CCDs, cm	0.67±0.07	0.77±0.1*	0.74±0.1*	**0.004**
Right CCDd, cm	0.61±0.07	0.70±0.1*	0.67±0.1*	**0.002**
Distensibility	0.03[0.02-0.05]	0.02[0.01-0.03]*	0.03[01-0.03]*	**0.03**

Normally distributed data are expressed as (mean±SD). Non-normally distributed data are displayed as median with interquartile ranges. *p<0.05 versus normal group. CCDd: common carotid artery at diastole; CCDs: common carotid artery at systole; MHT: malignant hypertension; NC: normotensives control; RH: resistant hypertension.

On univariable regression, several variables showed significant association with carotid distensibility, (Table 5.10). On multivariable regression analysis, the first model includes groups of participants (MHT, RH and control), duration of hypertension, age, BMI, and eGFR as independent variables. No variables were identified as independent predictors of carotid distensibility. The second model includes groups of participants (MHT, RH and control), hypercholesterolaemia (No=0 Yes=1), office SBP, office DBP, and statin.

Office SBP (β=-0.009, p=0.02) showed significant association with carotid distensibility, (Table 5.11).

Table 5.10 Univariable analysis to determine association of carotid distensibility with several indices

Carotid Distensibility	Coefficient	R^2	P	95% CI
Presence of MHT	-0.01	0.14	0.01	-0.02 – -0.003
Presence of RH	-0.01	0.14	0.007	-0.02 – -0.003
Duration of HTN, years	-0.001	0.13	0.004	-0.002 – -0.0005
Age, years	-0.0004	0.08	0.02	-0.0008 – -0.00007
Office SBP, mmHg	-0.0002	0.25	0.000	-0.0003 – -0.0001
Office DBP, mmHg	-0.0003	0.15	0.002	-0.0006 – -0.0001
CCBs	-0.008	0.07	0.03	-0.01 – -0.0006
Alpha blockers	-0.008	0.07	0.03	-0.01 – -0.0004
Diuretics	-0.01	0.14	0.003	-0.01 – -0.004
Vasodilator	-0.01	0.14	0.002	-0.02 – -0.006
Statin	-0.01	0.13	0.004	-0.01 – -0.003
cfPWV, m/s	-0.002	0.09	0.01	-0.004 – -0.0006
Peripheral SBP, mmHg	-0.0002	0.28	<0.001	-0.0004 – -0.0001
Peripheral PP, mmHg	-0.0003	0.28	<0.001	-0.0005 – -0.0002
MAP, mmHg	-0.0003	0.18	<0.001	-0.0005 – -0.0001
Central SBP, mmHg	-0.0002	0.27	<0.001	-0.0004 – -0.0001
Central DBP, mmHg	-0.0003	0.07	0.04	-0.0005 – -0.00002
Central PP, mmHg	-0.0003	0.26	<0.001	-0.0005 – -0.0002

cfPWV: carotid-femoral pulse wave velocity; DBP: diastolic blood pressure; HTN: hypertension; MAP: mean arterial pressure; MHN: malignant hypertension; PP: pulse pressure; RH: resistant hypertension; SBP: systolic blood pressure.

Table 5.11 Stepwise multivariable linear regression analysis of carotid distensibility

Carotid distensibility	Coefficient	P	95% CI
R^2=0.36, p<0.001			
Office SBP, mmHg	-0.009	**0.02**	-0.02 – -0.001

SBP: systolic blood pressure.

5.5. Discussion

This study carried out for the first-time assessment of vascular function in two high risk groups of hypertension (MHT and RH). Vascular function assessments include endothelial function, arterial stiffness and carotid artery distensibility.

5.5.1. Endothelial function assessment

Endothelial dysfunction is closely associated with hypertension (47, 396, 397). However, there are only a very limited number of studies that have evaluated endothelial function using FMD in MHT and RH population. I showed that endothelial function is impaired as assessed by FMD in both hypertensive groups compared to control group (endothelial dysfunction was defined as FMD of <6.0%). The average FMD in subjects with cardiovascular risk factor or cardiovascular diseases, aged 50 to 59 years was 5.9%, (96). Additionally, on multivariable regression, presence of hypertension (MHT and RH) correlated negatively with FMD. This is in agreement with previous studies, reported significantly impaired FMD in severe hypertension groups compared to well controlled hypertension and normal subjects (38, 40, 51, 52). Moreover, my study showed no significant changes in FMD between MHT and RH. Similarly, Shantsila et al. found no differences in FMD between MHT and treated “high-risk” hypertension (41). This is also supported by an observation of John et al. who showed no association between the degree of impaired endothelial function and BP levels (398).

I also showed enlargement of brachial artery at baseline, maximum and recovery diameter in MHT compared to NC. However, there was no significant difference between MHT and RH or between RH and NC. Larger baseline brachial artery diameter has been significantly associated with the higher cardiovascular risk factors (45, 399, 400).

Previous findings reported that FMD and brachial baseline diameter are strongly and inversely related (47, 99, 110, 118, 401). In addition, Maruhashi et al. revealed a strong inverse correlation between FMD and brachial artery (47). Silber et al. reported that FMD after ischaemic hyperaemia is proportional to hyperaemic systolic shear stress (118). This inverse association may be explained by the fact that small artery has higher shear stress created during reactive hyperaemia. This is due to the relationship between systolic flow after ischaemic hyperaemia and arterial radius, result in higher FMD in smaller arteries.

Multivariable regression analysis revealed positive association between SBP and baseline brachial artery diameter, indicating that higher SBP will lead to larger artery and eventually impaired FMD

5.5.2. Arterial stiffness

Elevated arterial stiffness is known pathological findings in hypertension (14). I showed that both hypertensive groups had higher cfPWV than normal subjects. This finding was consistent with several previous studies reported markedly elevated cfPWV in RH group compared to the controlled group (55, 57, 58). Shantsila et al. also reported significant

increase of cfPWV in MHT group compared to healthy subjects and patients with high-risk hypertension (41). There was no difference found in cfPWV between MHT and RH. This is consistent with previous study observed no difference in cfPWV between RH and non-resistant hypertension (402).

In stepwise multivariable regression analysis, advanced age, increased office SBP and presence of MHT were found to be independent predictors for elevated cfPWV. This result is similar to that of the study by Diaz et al. who showed that increased cfPWV is closely associated with ageing and BP (403).

Central haemodynamic parameters such as subendocardial viability was also assessed in the present study. The Buckberg index or SEVR is an index to evaluate subendocardial viability by calculating myocardial oxygen supply and demand using pulse wave analysis (404). It has been shown that a hypertension population had 11% lower SEVR than other disease groups (angina and CHD) (405). It has been also reported that hypertension is expected to be associated with impaired coronary flow reserve even with the presence of normal coronary arteries by angiography and no LVH (406). Amah et al. reported that treated patients with hypertension had significantly decreased SEVR in extreme dippers than in dippers (407). Another finding showed that SEVR is decreased in untreated patients with hypertension and low coronary flow reserve compared to untreated patients with hypertension and normal coronary flow (408). However, no prior studies have been investigated SEVR determined by the Buckberg index in patients with MHT and RH. In the present study, only MHT patients showed decreased SEVR compared to normal subjects.

These finding indicates impaired subendocardial flow and it also indicates the presence of insufficient oxygen supply and low myocardial oxygen consumption in MHT patient (409). This impairment of SEVR may be because of structural and functional coronary microvascular remodelling. This is due to perivascular fibrosis of intramural arterioles that causes a reduction in vessels density in the coronary microvasculature and myocardial ischaemia (406).

In my study, no difference was observed in AIx between the groups. Similarly, Shantsila et al. observed no differences in AIx between three study groups (MHT, NC and high-risk hypertension) (41). The reason for this could be because 96% of the study control group were south Asian. It was previously reported that healthy South Asians population had an elevated AIx compared to other ethnicity groups (410). Moreover, the majority of previous studies considering normal references of AIx were based on white populations (411). Another possible explanation is that central AIx considers a less sensitive marker of arterial stiffness in older population (>50 years) and the mean age of the hypertension groups were 56 years. The same study suggests that cfPWV is a better marker in people older than 50 years (412).

TPR also found to be lower in MHT group compared to NC. However, no differences found between MHT and RH or between RH and NC. There is an association between vasodilatation and reduction of TPR (413, 414). It was reported in our study that baseline, maximum and recovery diameter of brachial artery were increased more in MHT compared to NC.

5.5.3. Carotid artery distensibility

A prior study showed carotid artery distensibility declined as BP was elevated (62). However, carotid artery distensibility has been sparsely investigated in patients with MHT and RH. Our study showed that carotid distensibility was significantly decreased in hypertension groups compared to NC ($p=0.03$), with no significant difference between MHT and RH. Multivariable regression analysis showed a negative association between carotid distensibility and SBP. Similar findings were observed by previous studies who reported associations between reduced distensibility and hypertension (415, 416, 417). However, in contrast to previous studies, the present study compared MHT and RH population, which has not been addressed previously.

Moreover, carotid artery diameter is also known to be affected by BP in patients with hypertension (49, 418, 419, 420). According to the results of my study, the diameter of CCA in systole and diastole were increased in MHT and RH compared to NC. However, no significant difference was found between MHT and RH. These are also consistent with previous studies that observed that carotid diastolic diameter was significantly greater in untreated hypertensive group compared to control subjects (421, 422). This vasodilatation process is adaptive behaviour responding to the increased afterload in hypertension (49, 423).

5.6. Conclusion

My study is the first vascular assessment of two extreme phenotypes of hypertension (patients with treated MHT and patients with RH). I showed that impaired endothelial function and elevated arterial stiffness are present in malignant and resistant

hypertension. Patients with RH and MHT appeared to have reduced FMD and increased cfPWV.

The findings also demonstrate that patients with treated MHT compared to the normotensive controls have different features of abnormalities including the following: 1) lower TPR, 2) decreased SEVR, 3) increased baseline brachial artery diameter; 4) increased maximum brachial artery diameter, and 5) increased recovery brachial artery diameter.

Chapter VI. CARDIOVASCULAR AND HAEMODYNAMIC CHANGES IN RESISTANT HYPERTENSION: RELATION TO BLOOD PRESSURE CONTROL. A LONGITUDINAL STUDY

6.1. Introduction

Cardiac deformation, structural and functional alterations are exist in hypertension (16, 17, 18). The effect of optimised antihypertensive treatment on cardiac and on vascular system is well documented in essential hypertension. However, less is known whether treatment of hypertension is associated with improved cardiac deformation and vascular function in RH population. Moreover, most of the studies exploring cardiac function over time was using conventional echocardiography parameters such as EF which has several limitations compared to strain imaging.

6.2. Hypothesis and aims

The study hypothesised that 8-week of optimised antihypertensive treatment in RH would improve the cardiac function, endothelial function, arterial stiffness and carotid distensibility.

The aim of the present study is to assess the efficacy of optimised antihypertensive treatment on cardiovascular function in the RH group.

6.3. Methods

6.3.1. Study design

The study protocol was approved by West Midlands-South Birmingham Research Ethics Committee (REC reference: 18/WM/0168). Two local approvals were obtained by the

research ethics committee at Sandwell and West Birmingham Hospitals NHS Trust and at Liverpool Heart and Chest Hospital. The study was conducted in accordance with the Declaration of Helsinki of the World Medical Association.

This is a quasi-experimental study. The study design includes measurements taken on each participant at two timepoints, one before and one eight weeks after the blood pressure optimisation treatment began. Although there was an intervention (blood pressure control), patients were not randomly selected to receive this treatment, and therefore may not represent the larger population of patients. Additionally, there was no control group of patients who did not receive blood pressure control, and therefore there may be factors outside of the scope of the study related to the passage of time (e.g., season variation, increasing age, etc.) that cannot be adjusted for. Due to the non-experimental nature of the study, causal conclusions about the effect of blood pressure treatment cannot be inferred.

Alternative study designs that could address some of these weaknesses would include designing an experiment, in which some patients are randomly assigned to receive the treatment and some are assigned to a control group that does not receive the treatment. Comparing the outcomes over time for these two groups would therefore be adjusted for time-variant factors outside of the researcher's control and allow for causal conclusions to be made. Another option to improve the design would be to repeat the measurements multiple times before and after intervention , to account for measurements error. This would allow for more precise estimation of the change between pre- and post-implementation.

All procedures were performed by a single operator. Written informed consent was given to all subjects. Patients with RH were recruited across two sites: 1) the hypertension clinics at City Hospital and 2) Liverpool Heart and Chest Hospital. A participant information sheet was provided to all participants before the study day. Written informed consent was obtained from all subjects. Inclusion criteria for the patients were being over 18 years with the ability to provide informed consent, and a diagnosis of RH. All participants had a diagnosis of RH based on their medical records and following the current guidelines (8). Exclusion criteria for my study were participants with the following conditions: BMI ≥35 kg/m^2, moderate-severe valvular heart disease, previous MI or current symptomatic CAD, AF, recent (< 6 months) cerebrovascular events, active infections or pyrexia illness, active chronic and systemic illnesses (e.g., respiratory diseases, renal or liver failure, neurological disease) and pregnancy.

6.3.2. Study population

The cohort included 17 patients with RH. Patients were recruited during the period from December 2018 to March 2020 and followed up for eight weeks, following the optimisation of the antihypertensive treatment initiated in the hypertension clinic. Antihypertensive treatment was initiated by expert clinician at the hypertension clinic and optimised by either increasing dosage or using different agents.

Further recruitment was stopped because of the COVID-19 pandemic.

6.4. Procedures

All patients who were included in this study underwent 2 separate cardiac and vascular examinations. Participants were rested in a supine position in quite room. The following

procedures were obtained: echocardiography, strain imaging, FMD, cfPWV, carotid artery distensibility. An expanded procedures section can be found in methods chapter (CH-03). Strain imaging, arterial stiffness and FMD were also explained in SOP (Appendix 1, 2 and 3).

6.5. Statistical analysis

Statistical analysis was performed using statistical analysis software (Stata/IC), 16.1 for Mac. Continuous variables were tested for normality using the Shapiro–Wilk test. Continuous data and normally distributed were analysed by paired t-test to determine change over time. Not normally distributed data were analysed using Wilcoxon signed rank. Chi-squared test was used to test categorical data. All findings were regarded as statistically significant when p value was less than 0.05. The differences (Δ) between the baseline and follow-up of all the assessed parameters were calculated.

Univariable and multivariable linear regression analysis were used to determine whether the change between baseline and follow up (Δ) depends on another variable (baseline variables)

There were three different models in which GLS, PWV, and FMD were the dependent variables. Office SBP, central PP and age were included as independent variables. GLS were analysed as positive value to homogenise interpretation of regression analysis. Regression model is limited to two independent variables because of the low number of degrees of freedom.

6.6. Results

6.6.1. Demographic and clinical characteristics of the study population

In this longitudinal study, a total of 17 patients with RH were included. Mean age of the study cohort was 58 years with average duration of 8 years of hypertension, 65% were male and 82% were smokers (Table 6.1). The percentage of participants who consumed alcohol within the recommended level was 29%, while 71% reported no alcohol consumption. The prevalence of hypercholesterolaemia was 47%, while 41% were diabetic (Table 6.2).

Table 6.1 Baseline demographic characteristics

Study sample (n=17)		
Age, years		58±11
Sex, n (%)	Male	11(65)
	Female	6(35)
Ethnicity, n (%)	White	7(41)
	Asian	3(18)
	Black	6(35)
	Mixed	1(6)
Smoking, n (%)	Current	14(82)
	Never	2 (12)
	Ex-smoker	1 (6)
Alcohol units/week		0[0-1.5]
Alcohol intake, n (%)	No consumption	12(71)
	Recommended consumption	5(29)
	Heavy consumption	0 (0)
Height, cm		170±10
Weight, kg		91±20
BMI, kg/m^2		32±5
BSA, m^2		2±0.3

Data are expressed as mean ± SD for continuous data or number n (%) for categorical data. BMI: body mass index; BSA: body surface area.

Table 6.2 Baseline clinical characteristics of the study population

Study sample (n=17)	
Duration of HTN, years	8±3
Hypercholesterolaemia, n (%)	8 (47)
DM, n (%)	7(41)
Asthma, n (%)	2(12)
Arthritis, n (%)	4(24)
Anaemia, n (%)	1 (6)
TIA, n (%)	1 (6)

Normally distributed data are expressed as (mean ±SD). Categorical data are expressed as numbers n (%). DM: diabetes mellitus; HTN: hypertension; TIA: Transient ischaemic attack.

A routine blood test was done at the time of initial screening for all patients, the mean creatinine levels were (102±27), the mean eGFR was (66±18) and the mean urea level was (6[5-7]), (Table 6.3).

Table 6.3 Baseline laboratory tests of the study population

Study sample (n=17)	
Haemoglobin, g/L	137±15
Haematocrit, L/L	0.42±0.04
Mean cellular volume, fL	89±6
White cell account, 10*9/L	6.2[5.5-7]
Neutrophils, 10*9/L	4±1
Lymphocytes, 10*9/L	2±0.5
Monocytes, 10*9/L	0.51[0.42-0.56]
Platelets, 10*9/L	250±39
HBA1c, mmol/mol	46±11
Sodium, mmol/L	140±3
Potassium, mmol/L	4±0.5
Urea, mmol/L	6[5-7]
Creatinine, umol/L	102±27
eGFR, mL/min/1.73 m^2	66±18
TSH, mU/L	1[0.85-2]
T4, mU/L	13±0.8

Normally distributed data are expressed as (mean ±SD). Non-normally distributed data are displayed as median with interquartile ranges. eGFR: estimated glomerular filtration rate; TSH: thyroid stimulating hormone.

At time of first assessment, 71% of participants were receiving CCB, 65% alpha blockers, 94% ACEIs/ARBs, 53% β-blockers, 29% vasodilators, 100% diuretics, 12% aspirin, 12% antidepressants, 6% anticoagulant, and 35% statin, (Table 6.4).

Table 6.4 Distribution of medication classes used by the study population

	Baseline visit	Follow-up visit
CCB, n (%)	12 (71)	13 (76)
Alpha blockers, n (%)	11 (65)	11 (65)
Diuretics, n (%)	17 (100)	17 (100)
ACEIs/ARBs, n (%)	16 (94)	15 (88)
β-blockers, n (%)	9 (53)	9 (53)
Vasodilators, n (%)	5 (29)	5 (29)
Aspirin, n (%)	2 (12)	2 (12)
Antidepressants, n (%)	2 (12)	2 (12)
Anticoagulant, n (%)	1 (6)	1 (6)
Statin, n (%)	6 (35)	6 (35)

Data are expressed as numbers n (%). ACEIs: Angiotensin-converting-enzyme inhibitors; ARBs: angiotensin receptor blockers; CCB: calcium channel blockers.

Following eight weeks of optimisation of antihypertensives treatment, patients with RH showed significant reduction in office SBP (159 mmHg [154-181] (baseline) to 155 mmHg [136-168] (follow-up), p=0.03), and in office DBP (94 mmHg [84-100] (baseline) to 84 mmHg [79-100] (follow-up), p=0.03). The average decrease in SBP was 2.52% and in DBP was 10.64%. There were no significant differences in heart rate at baseline and follow-up, (65±12 (baseline) vs. 67±15 (follow-up), p=0.41) (Table 6.5).

6.6.2. Echocardiography characteristics

Two standard echocardiography examinations were performed. The first exam at baseline and the second one following eight weeks of optimised antihypertensives treatment. Majority of cardiac parameters assessed by conventional echocardiography did not differ between the baseline and follow-up including EF (52% ±8 (baseline) vs. (58% ±11 (follow-up), p=0.09) (Table 6.5).

The present study showed borderline increase in LVM and LVMI, despite BP reduction (LV mass 171 g [164-186] (baseline) vs. 181 g [164-254] (follow-up), p=0.04), (LVMI 82 $g.m^2$ [78-95] (baseline) vs. 95 $g.m^2$ [77-115] (follow-up), p=0.03). At baseline LVH was present in 8 (47%) of the study cohort and in 9 (53%) at follow-up.

Diastolic dysfunction was observed in 14 (82%) at baseline and 15 (88%) at follow-up. There were no significant differences in DT (223 ms$\pm$34 vs. 208 ms$\pm$24, p=0.07), E/A (1.1$\pm$0.3 vs. 1$\pm$0.3, p=0.84), E/E' lateral (8[7-13] vs. 12[8-14], p=0.16) and E/E' septal (12[9-14] vs. 14[10-17], p=0.5).

There were no statistically significant differences in LA parameters (LA diameter, LA area, LA volume and LAVI). There were also no statistically significant differences in LV dimension, wall thickness and volume (IVSD, LVIDD, PWD, RWT, ESV, EDV and SV) (Table 6.5).

Table 6.5 Changes of clinical and standard echocardiography characteristics before and after optimised antihypertensives treatment

Study sample (n=17)	Baseline visit	Follow-up visit	P
Office SBP, mmHg	159[154-181]	155[136-168]	**0.03**
Office DBP, mmHg	94[84-100]	84[79-100]	**0.03**
Office heart rate, bpm	65±12	67±15	0.41
LA diameter, cm	4±0.5	4±0.5	0.91
LA area, cm^2	20.1±6	18.9±3	0.28
LA volume, ml	53±21	47±14	0.16
LAVI, ml/m^2	27±10	24±6	0.18
IVSD, cm	1.02[0.82-1.39]	1.1[0.95-1.3]	0.09
LVIDD, cm	4.6±0.6	4.8±0.6	0.33
PWD, cm	1.05[0.87-1.11]	0.95[0.91-1.32]	0.55
LV mass, g	171[164-186]	181[164-254]	**0.04**
LVMI, g/m^2	82[78-95]	95[77-115]	**0.03**
Abnormal LVMI	4(24)	5(29)	0.05
RWT	0.42[0.38-0.57]	0.43[0.36-0.48]	0.91
LVH, n (%)	8(47)	9(53)	0.08
E, cm/s	80±25	82±19	0.56
A, cm/s	77±19	81±18	0.18
E/A ratio	1.1±0.3	1±0.3	0.84
DT, ms	223±34	208±24	0.07
E/E' septal	12[9-14]	14[10-17]	0.05
E/E' lateral	8[7-13]	12[8-14]	0.16
E' lateral, cm/s	8.1[7-9]	7.8[6-8]	0.43
A' Lateral, cm/s	10±3	12±3	0.07
E' septal, cm/s	7±2	6±1	0.17
A' septal, cm/s	9±3	10±2	0.13
E'/A' septal ratio	0.68[0.58-0.78]	0.61[0.55-0.68]	0.09
E'/A' lateral ratio	0.71[0.65-0.96]	0.69[0.54-0.72]	0.11
Average E'/A' ratio	0.7[0.6-0.8]	0.7[0.6-0.7]	0.07
s’ septal, cm/s	6.8±2	6.5±2	0.35
s’ lateral, cm/s	7±2	8±2	0.16
EF, %	52±8	58±11	0.09
EDV, ml	81±27	83±24	0.64
ESV, ml	39±16	35±15	0.17
SV, ml	42±14	48±16	0.21

Normally distributed data are expressed as (mean ±SD). Non-normally distributed data are displayed as median with interquartile ranges. DT: deceleration time; EDV: end-diastolic volume; EF: ejection fraction; ESV: end-systolic volume; IVSD: interventricular septum at diastole; LA: left atrial; LAVI: left atrial volume index; LV: left ventricle; LVH: left ventricular hypertrophy; LVIDD: LV internal diameter at diastole; LVMI: left ventricle mass index; PWD: posterior wall thickness at diastole; RWT: relative wall thickness; SV: stroke volume.

6.6.3. Speckle tracking characteristics

After 8 weeks of follow-up, significant improvement in GLS was observed, (-17%±1.5 (baseline) vs. -19%±2.8 (follow-up), p=0.002). Additionally, GCS showed improvement following eight weeks of optimised treatment, (-29.7%±4.3 (baseline) vs. -31.1%±5.4 (follow-up), p=0.04). There was significant reduction in global basal rotation, (-3.6[-4.4 - -2.3] (baseline) vs. -1.7[-4 - -0.7] (follow-up), p=0.03), and peak basal rotation, (-8±3 (baseline) vs. -6±4 (follow-up), p=0.04), (Table 6.6).

There was no significant difference between baseline and follow-up in global apical rotation, peak apical rotation, peak twist, apical rotation at AVC, basal rotation at AVC, net twist AVC and torsion, (all p>0.05).

Table 6.6 Speckle tracking echocardiography characteristics

Study sample (n=17)	Baseline visit	Follow-up visit	P
GLS, %	-17±1.5	-19±2.8	**0.002**
GCS, %	-29.7±4.3	-31.1±5.4	**0.04**
Global apical rotation, °	2±1	3±2	0.38
Global basal rotation, °	-3.6[-4.4 - -2.3]	-1.7[-4 - -0.7]	**0.03**
R-AVC time, ms	381±81	367±84	0.56
Time to peak apical rotation, ms	479[418-525]	441[409-514]	0.60
Time to peak basal rotation, ms	439[399-467]	428[380-462]	0.83
Peak apical rotation, °	5[4-7]	8[4-12]	0.13
Peak basal rotation, °	-8±3	-6±4	**0.04**
Peak twist, °	13.8±3	13.4±5	0.82
Apical rotation at AVC, °	4[3-5]	7[3-8]	0.12
Basal rotation at AVC, °	-7±3	-5±4	0.08
Net twist AVC, °	11[9-12]	14[8-15]	0.57
Torsion, °/cm	2.3±0.7	2.4±1	0.61

Normally distributed data are expressed as (mean ±SD). Non-normally distributed data are displayed as median with interquartile ranges. AVC: aortic valve closure. GCS: global circumferential strain; GLS: global longitudinal strain.

6.6.3.1. Global longitudinal strain in patients with resistant hypertension

No variables were identified on univariable and multivariable analysis as independent predictors of ΔGLS. Age and office SBP were considered independent variables in the multivariable analysis because both have a significant impact on GLS.

6.6.4. Arterial stiffness characteristics

Following eight weeks of antihypertensives treatment, CBP showed reduction, but it did not achieve statistical significance, (Table 6.7). Central SBP, (177 mmHg [159-193] (baseline) vs. 154 mmHg [139-193] (follow-up), p=0.11), central DBP (87 mmHg ±15

(baseline) vs. 82 mmHg ±20 (follow-up), p=0.28), central PP (89 mmHg ±19 (baseline) vs. 84±23 (follow-up), p=0.23), and MAP (124 mmHg ±14 (baseline) vs. 116 mmHg±21 (follow-up), p=0.19).

Arterial stiffness parameters included: cfPWV (9.8 m/s [8-11] (baseline) vs. 9.3 m/s [8-11] (follow-up), p=0.81), augmentation pressure (24[16-38] (baseline) vs. 24[13-32] (follow-up), p=0.06), and AIx (30% ± 10 (baseline) vs. 28% ±10 (follow-up), p=0.59).

No differences between baseline and follow-up were also found with respect to TPR (0.84[0.81-0.99] (baseline) vs. 0.93[0.86-1.07] (follow-up), p=0.95), and SEVR (154%±28 (baseline) vs. 160%±30 (follow-up), p=0.42), (Table 6.7).

Table 6.7 Arterial stiffness characteristics

	Baseline visit	**Follow-up visit**	**P**
cfPWV, m/s	9.8[8-11]	9.3[8-11]	0.81
MAP, mmHg	124±14	116±21	0.19
SEVR, %	154±28	160±30	0.42
TPR, PRU	0.84[0.81-0.99]	0.93[0.86-1.07]	0.95
Central SBP, mmHg	177[159-193]	154[139-193]	0.11
Central DBP, mmHg	87±15	82±20	0.28
Central PP, mmHg	89±19	84±23	0.23
Augmentation pressure, mmHg	24[16-38]	24[13-32]	0.06
AIx, %	30±10	28±13	0.59

Normally distributed data are expressed as (mean±SD). Non-normally distributed data are displayed as median with interquartile ranges. AIx: augmentation index; cfPWV: carotid-femoral pulse wave velocity; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure; SBP: systolic blood pressure; SEVR: Subendocardial viability ratio; TPR: total peripheral resistance.

No variables were identified on univariable and stepwise multivariable analysis as independent predictors of Δ cfPWV. Multivariable analysis included age and central PP as independent variables because of their significant effects on PWV.

6.6.5. Flow mediated dilatation characteristics

After 8 weeks of antihypertensive treatment, FMD showed significant improvement compared with baseline (5.3%±3 (baseline) vs. 6.7%±3 (follow-up), p=0.04). FMDr also showed significant improvement (3 % [2-4] (baseline) vs. 5% [3-8] (follow-up), p=0.04). No significant change was found in brachial baseline diameter, maximum diameter or recovery diameter, p>0.05). There were no significant differences in shear rate parameters between baseline and follow-up, (all p>0.05), Table 6.8).

Table 6.8 Changes of endothelial function characteristics before and after optimised treatment

Study sample (n=17)	Baseline visit	Follow-up visit	P
FMD, %	5.3±3	6.7±3	**0.04**
FMDr, %	3[2-4]	5[3-8]	**0.04**
Baseline diameter, mm	4.8±1	4.8±1	0.71
Maximum diameter, mm	5±0.9	5±1	0.74
Recovery diameter, mm	4.9±0.9	4.8±1	0.58
Positive shear rate baseline [sec.-1]	81[55-95]	89[60-106]	0.96
Positive shear rate maximum [sec.-1]	255[198-350]	297[177-421]	0.61
Positive shear rate area[sec.-1]	6043[3480-10461]	8702[2601-29911]	0.15
Positive shear rate area to maximum[sec.-1]	3839[2009-6113]	4266[1726-9279]	0.26
Negative shear rate baseline [sec.-1]	-22[-25 – -16]	-31[-56 – -18]	0.15

Normally distributed data are expressed as (mean±SD). Non-normally distributed data are displayed as median with interquartile ranges. FMD: flow mediated dilatation; FMDr: flow mediated dilatation with respect to recovery diameter

No variables were identified on univariable and multivariable analysis as independent predictors of ΔFMD. Due to the significant influence age and SBP have on FMD, age and SBP were included as independent variables in the multivariate analysis.

6.6.6. Carotid artery distensibility characteristics

No differences were found in carotid artery distensibility (0.02[0.1-0.3] (baseline) vs. 0.02[0.01-0.04] (follow-up, p=0.90). Compared to baseline, carotid artery diameter in diastole decreased (0.69 cm ±0.1 (baseline) vs. 0.66 cm ±0.1 (follow-up), p=0.04) whereas no difference was found in carotid artery diameter in systole (0.76 cm ±0.1 (baseline) vs. 0.73 cm ±0.1 (follow-up), p=0.19), (Table 6.9).

Table 6.9 Carotid artery distensibility

Study sample (n=17)			
	Baseline visit	**Follow-up visit**	**P**
Right CCDs, cm	0.76±0.1	0.73±0.1	0.19
Right CCDd, cm	0.69±0.1	0.66±0.1	**0.04**
Distensibility	0.02[0.1-0.3]	0.02[0.01-0.04]	0.90

Normally distributed data are expressed as (mean±SD). Non-normally distributed data are displayed as median with interquartile ranges. CCDd: common carotid artery diameter at diastole; CCDs: common carotid artery diameter at systole.

6.7. Discussion

A total of 17 patients with true RH were followed for 8 weeks after introducing an appropriate antihypertensive therapy. The study evaluated the changes in the following: Office and central BP levels, diastolic function, myocardial systolic function assessed by EF, GLS, GCS, rotation and twist. Arterial stiffness and vascular function were assessed by cfPWV, FMD and carotid distensibility. I showed that office BP and cardiac systolic function improved in patients with true RH after applying intensified antihypertension medications. The study also showed improvement of endothelial function assessed by FMD; however central BP, arterial stiffness and diastolic function did not change.

6.7.1. Cardiac deformation and systolic function

Antihypertensive regimes have been shown to improve cardiac function and structure (424, 425). However, the timing of these improvements is still questionable. During antihypertensive therapy, diastolic function improved before EF and LVMI remodelling (424, 425, 426). Little is known about the timing of GLS progress. Because of the superior utility of strain imaging in identifying subclinical cardiac modifications, changes of GLS are expected to be identified before any changes of other conventional echocardiography indices (427).

Previous studies reported abnormal strain in hypertension populations independent of preserved EF (428, 429, 430). The majority of these studies observed patients with mild to moderate essential hypertension or well controlled hypertension. Neither of these studies, however, had evaluated strain changes in a RH population.

After a follow-up period of 2 months, true RH patients showed lower office BP (systolic and diastolic) compared with the baseline. It was noted that preserved EF showed no change between baseline and follow-up. However, impaired GLS and GCS were present at baseline and significantly showed improvement at follow-up. These findings appeared similar to observations of previous studies evaluated GLS changes in controlled hypertension patients (431), in newly diagnosed untreated patients with hypertension (432), in uncontrolled hypertension (241), and all showed improvement of GLS compared to baseline.

Limited studies have evaluated clinical and cardiovascular indices associated with longitudinal strain in RH population. Up to now, this is the first study to assess the association of non-improved GLS compared to improved GLS in RH population with preserved EF.

According to The Copenhagen City Heart Study, GLS was negatively associated with LVMI and older age and positively associated with EF in the general population (254). Another study has also found that older age was associated with deteriorating GLS in healthy population (433). One study showed that abnormal GLS at baseline was associated with more resistant DBP in uncontrolled hypertension patients (241).

Previous study showed a strong correlation between GLS and FMD in patients with DM and hypertension (434). The development of essential hypertension and impaired endothelial function are closely related and could be more pronounced in RH (37, 38, 39, 40). Endothelial dysfunction is a well-recognised predictor of cardiovascular outcomes (42).

As a result of NO imbalance, endothelial progenitor cells (EPCs) are affected, causing impaired endothelial regeneration and modifying matrix metalloproteinases. In addition, increased MMP-1 turnover appears to reduce collagen degradation and promote subendocardial myocardial fibrosis. This implies that irregular collagen production and myocardial fibrosis are associated with reduced GLS in hypertension and hypertrophic cardiomyopathy and eventually lead to early impairment of systolic function (23, 27, 28, 29). Changes in matrix metalloproteinases may induce excessive production and build-up of ECM structural proteins, resulting in fibrosis and increased myocardial stiffness (435). Furthermore, impaired endothelial function promotes monocytes to transmigrate to the myocardium, which results in interstitial fibrosis and diastolic dysfunction (436).

Importantly, impaired endothelial function would worsen coronary circulation among subjects with ischaemic heart failure (437). Also, impaired endothelial microvascular function and increased LV wall stress are observed in subjects with cardiac fibrosis (438).

It is important to note that cardiac fibrosis may not be the only consequence of hypertension. In addition, it is well known that hypertension causes hypertrophic

cardiac remodelling. However, the mechanisms of how endothelial cells contribute to this process is still poorly understood (439, 440). Previous study found that as a consequence of hypertension, endothelial cells transcriptionally activate genes linked with a fibrosis which suggests that endothelial cells may regulate cardiac fibrosis remodelling. A lineage tracing study has revealed that endothelial cells are transformed to fibroblasts through endothelial-to-mesenchymal transition (EndoMT), similar to the impacts of elevated cardiac load in hypertension (441). Circumferential strain measured by GCS showed improvement following eight weeks of optimised treatment.

6.7.2. Left ventricular mass and diastolic dysfunction

Impaired diastolic function and increased LVMI are known to be associated with hypertension (442, 443). Although several antihypertensive drugs are expected to induce regression of LV mass, LV remodelling is not always reversed (444, 445, 446, 447). The present study showed significant increase in LV mass and LVMI, even when BP decreased. Similar findings were previously reported from the Strong Heart Study included treated free-living participants with hypertension who also have obesity and DM (446). They observed increased LV mass during 4 years of follow-up, independent of optimal BP control. The present findings are inconsistent with the study done by Lonn et al. who showed improved LV mass and volume in patients with controlled BP and preserved EF after introducing 10 mg/day of ramipril (255). However, no improvement found when lower dose was used (2.5 mg/day) Their study has examined patients with well controlled hypertension while the present study enrolled patients with RH and difficult to control BP.

Diastolic dysfunction was present in the study population. No progression of LV diastolic function was noted even with BP reduction and GLS improvement. No association found between GLS and impaired diastolic function. Similarly, Tran et al. reported no association between GLS and diastolic dysfunction or high LVMI in patients with elevated BP (448). Another study observed that following antihypertensive therapy, GLS improved but LVMI and diastolic function did not change (449).

6.7.3. Arterial stiffness in resistant hypertension

Following eight weeks of antihypertensives treatment, the majority of arterial stiffness parameters showed reduction, but it did not achieve statistical significance.

Meta-analysis studies reported that antihypertensive treatment reduced cfPWV independently of BP reduction. However, cfPWV reduction was observed in long term treatment only (450, 451).

6.7.4. Endothelial function changes

Limited studies have evaluated the vascular changes assessed by FMD in patients with uncontrolled hypertension. In my study, I investigated endothelial function in confirmed RH population. After 8 weeks of antihypertensive treatment, FMD showed significant improvement compared with baseline. This result supports previous work showing modulation of endothelial function is possible and that endothelial dysfunction is a reversible condition (452).

Other studies found that different treatments of hypertension may have different effects on endothelial function. Some types of antihypertensive treatment such as β-blockers (atenolol) do not modify endothelial function. On the other hand, ACEIs or ARBs

could restore endothelial function to normal (121). Increased oxidative stress in hypertension caused by overproduction of angiotensin-II (122) will induce NADPH oxidase to produce ROS, leading to vascular inflammation (122, 123). By blocking angiotensin-II overproduction, ACEIs and ARBs increase vasorelaxation in patients with hypertension. Impaired endothelial function has also been associated with several factors and comorbidities predisposed to treatment resistance, including older age, obesity (124), obstructive sleep apnoea (124), insulin resistance (125), or hyperaldosteronism (126).

One study reported improvement of endothelial function in patients with mild essential hypertension after introducing low dose of doxazosin for 12 months (453). Another study also showed the positive effect of doxazosin on endothelial function (454). The association between doxazosin and recovery of endothelial function could be due to the ability of alpha blockers to decrease vascular tone by inhibiting of tissue growth in arteriolar structures and it can enhance the fibrinolytic function (455, 456, 457). It could also because of increasing (NOS) activity (454, 458, 459).

6.8. Conclusion

The true RH population tended to have several distinctive features in comparison to the essential hypertension population. In the present study, true RH patients showed preserved EF with no change between baseline and follow-up. However, impaired GLS and GCS were present at baseline and significantly showed improvement at follow-up. This demonstrates the ability of STE imaging as sensitive technique to detect subclinical LV dysfunction despite the presence of preserved EF in patients with RH. Furthermore,

improvement of GLS and GCS after introducing optimised antihypertensive treatment revealed that antihypertensive therapy has a favourable impact on LV deformation.

Patients in the current study also displayed impaired endothelial function at baseline and improved FMD at follow-up. Arterial stiffness and carotid distensibility did not show any differences in RH population after 2 months of antihypertensive treatment.

Chapter VII. ASSESSMENT OF HEART RATE VARIABILITY AND BLOOD PRESSURE VARIABILITY IN PATIENTS WITH RESISTANT HYPERTENSION

7.1. Introduction

The autonomic nervous system plays a critical role in controlling BP in hypertension (32). Hypertension is linked with cardiac autonomic abnormality reflected by increased sympathetic activity and decreased parasympathetic tone (33).

BPV and HRV are useful measure of sympathetic and parasympathetic activity and are therefore practical considerations for the evaluation and management of RH (460). Decreased HRV and increased BPV in hypertension are directly related to TOD and cardiovascular risk (67, 68, 69, 70). Little is known whether optimised antihypertensive treatment in RH population is associated with reducing BPV and improving cardiac autonomic function.

7.2. Hypothesis and aims

The study hypothesised that 8 weeks of optimised antihypertensive agents in RH would improve HRV and BPV parameters.

The purpose of the study was to monitor HRV and BPV before and after optimised antihypertensive treatment in RH group.

7.3. Methods

7.3.1. Study design

The study protocol was approved by the West Midlands-South Birmingham Research Ethics Committee (REC reference: 18/WM/0168). Two local approvals were obtained

from the Research Ethics Committee at Sandwell and West Birmingham Hospitals NHS Trust and at Liverpool Heart and Chest Hospital. The study was conducted in accordance with the Declaration of Helsinki of the World Medical Association. All procedures were performed by a single operator.

Patients with RH were recruited across two sites: 1) the hypertension clinics at City Hospital and 2) Liverpool Heart and Chest Hospital. Participant information sheet was provided to all participants before the study day. Written informed consent was obtained from all subjects. Inclusion criteria for the patients were being over 18 years with the ability to give informed consent, and a diagnosis of RH. All participants had a diagnosis of RH based on their medical records and according to the current guidelines.(8) Exclusion criteria for the present study were participants with the following medical conditions: BMI ≥35 kg/m^2, moderate to severe valvular heart disease, previous MI or current symptomatic CAD, AF, recent (< 6 months) cerebrovascular events, active infections or pyrexia illness, active chronic and systemic illnesses (e.g., respiratory diseases, renal or liver failure, neurological disease) and pregnancy.

7.3.2. Study population

A total of 17 patients with RH were included in the study cohort. Patients were recruited from the hypertension clinic and followed up for eight weeks after antihypertensive treatment optimisation was initiated.

7.3.3. Procedures

All patients enrolled in this study had two separate office BP, HRV and 24-hour BPV assessments (at baseline and after 8 weeks of optimisation of antihypertensive

treatment). Office BP was measured while patient seated in the research clinic and were averaged to obtain the BP value used for analysis.

Autonomic function was assessed using time and frequency domain indices of HRV analysis. The participant was positioned supine and rested for 5 minutes then the heart rate was monitored for 5 minutes using a small portable eMotion Faros sensor attached to 3 ECG leads and placed on the chest. The real-time analysis program calculated the differences between successive R-R intervals and assessed different parameters of HRV. All participants underwent 24-hour ABPM to assess their average BP (Figure 7.1 and 7.2). The pressure cuff was placed on participant's upper arm. The carrying pouch was positioned on the participant's right side and the pouch strap worn around the waists or around the shoulders (depending on patient preference). The readings were taken every 30 minutes during the day and every 60 minutes at night. More details about HRV and BPV can be found in methods chapter (CH-03; 3.8 and 3.9).

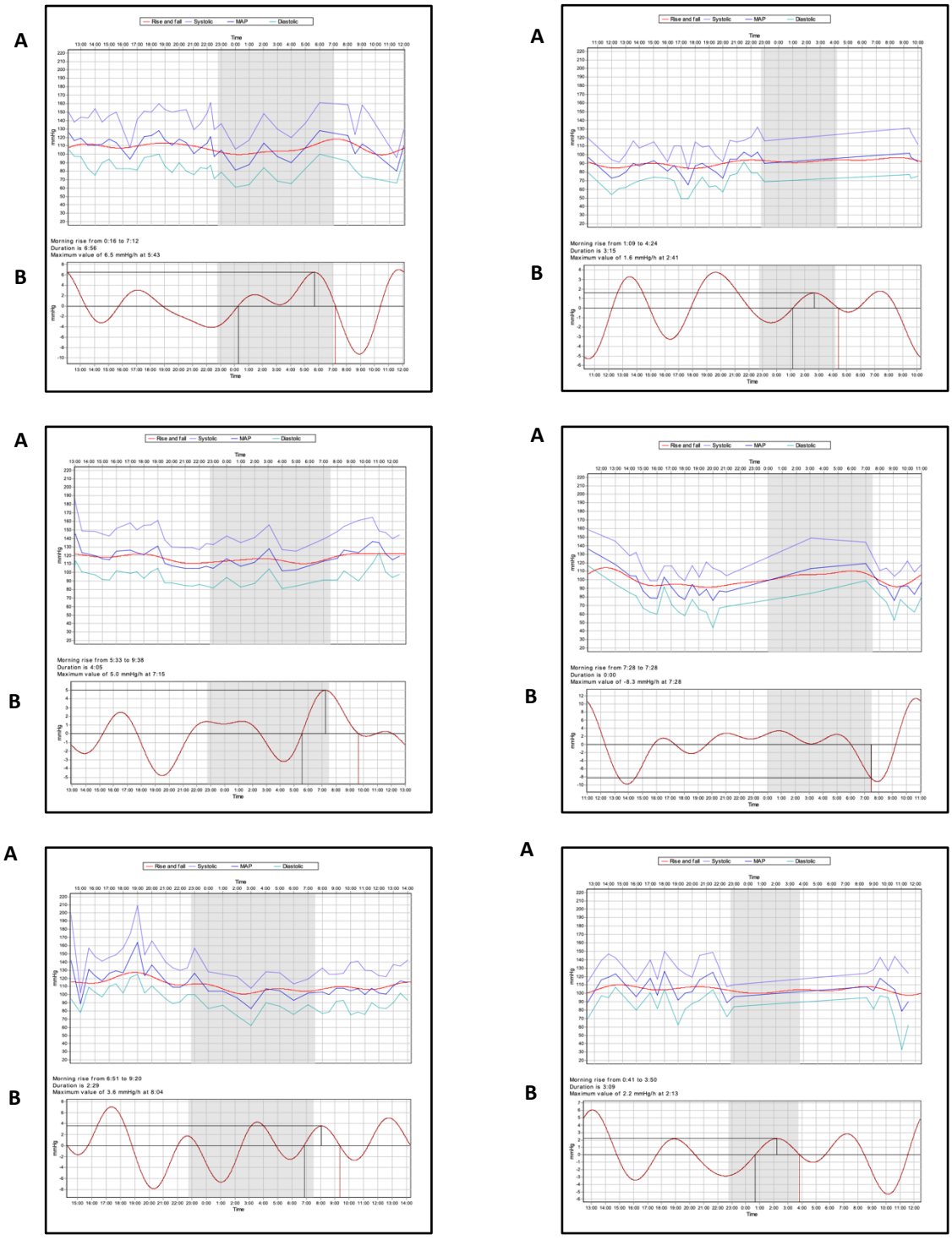


Figure 7.1 Blood pressure pattern over 24-hour of ABPM in 6 patients with resistant hypertension. Graph **A** shows rise and fall curve of SBP, DBP and MAP values. Graph **B** shows filtered MAP curve. The grey area represents blood pressure readings during the sleeping period.

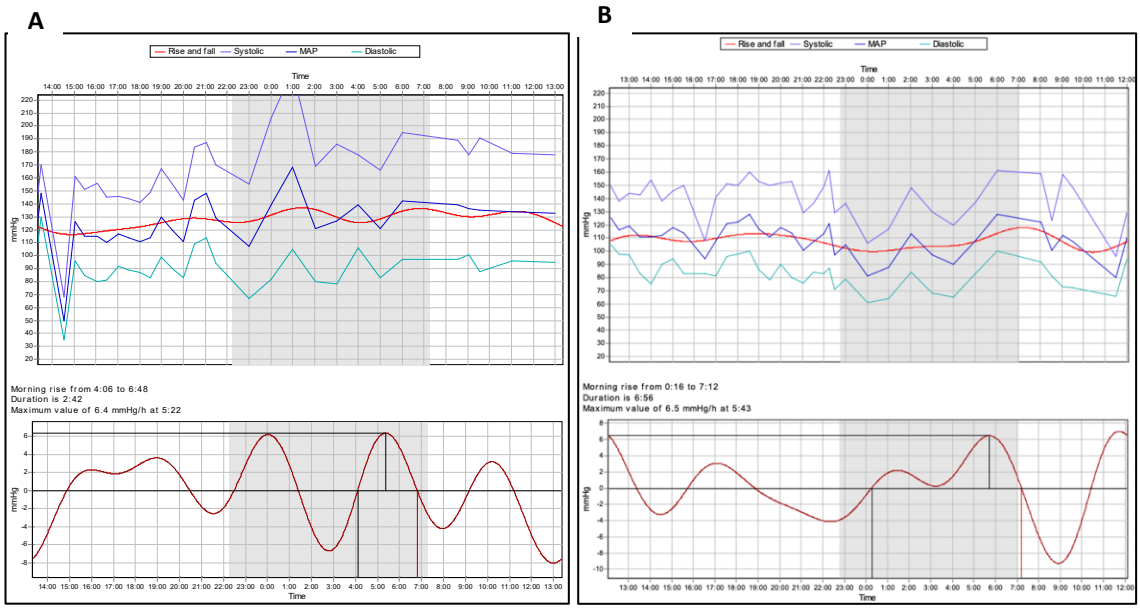


Figure 7.2 Blood pressure pattern over 24-hour of ABPM in patient with resistant hypertension. Panel (**A**) at baseline and panel (**B**) follow-up. Top graph shows rise and fall curve of SBP, DBP and MAP values. Bottom graph shows filtered MAP curve. The grey area represents blood pressure readings during the sleeping period.

7.3.4. Statistical analysis

Statistical analysis was performed using statistical analysis software (Stata/IC), 16.1 for Mac. Continuous variables were tested for normality using the Shapiro–Wilk test. Continuous data and normally distributed were analysed by paired t-test to determine change over time. Not normally distributed data were analysed using Wilcoxon signed rank. Chi-squared test were used to test categorical data. All findings were regarded statistically significant when p value less than 0.05. The differences (Δ) between the baseline and follow-up of all the assessed parameters were calculated.

Multivariable liner regression models were built to examine the factors affecting the changes of the HRV and BPV (Δ). Multivariable analysis included age and office SBP. as independent variables because of their significant effect on HRV and BPV

7.4. Results

7.4.1. Demographic and clinical characteristics

A total of 17 patients with RH were included in this longitudinal study. This is the same group of patients as in the previous chapter (Ch-06). Demographic and clinical characteristics are provided in chapter 06 (Table 6.1 and 6.2).

At initial screening, routine blood test was performed or recorded from patient's medical record if available (Table 6.3). Medication history was recorded from all patients at baseline and follow-up. (Table 6.4).

7.4.2. Heart rate variability characteristics

Time domain analysis (SDNN, rMSSD and pNN50), and frequency domain analysis (total power, LF, HF, LF/HF ratio) variables of HRV were assessed at baseline and after eight weeks of introducing optimised antihypertensive treatment. No significant changes were observed in HR (p=0.97), max HR (p=0.76), min HR (p=0.31). There was a trend towards reduction in SDNN (p=0.16), rMSSD (p=0.28), pNN50 (p=0.42), LF (p=0.32), HF (p=0.58), total power (p=0.46), HF normalised (p=0.71), and LF/HF (p=0.98). However, HRV index (p=0.08), LF normalised (p=0.71), log (LF/HF) (p=0.73) did not show any trend toward reduction, (Table 7.1).

Table 7.1 Heart rate variability characteristics

	Baseline visit	Follow-up visit	P
Heart rate, bpm	63±14	63±14	0.97
Max heart rate, bpm	71±14	70±15	0.76
Min heart rate, bpm	57±12	59±13	0.31
SDNN, ms	50[28-63]	33[25-52]	0.16
rMSSD, ms	27[17-42]	26[18-41]	0.28
HRV index	9[7-14]	9[7-11]	0.08
pNN50, %	7[0.9-20]	3[0.7-21]	0.42
LF, ms^2	386[168-683]	380[141-670]	0.32
HF, ms^2	227[109-506]	202[104-388]	0.58
Total power	4661[2711-7122]	2315[864-5793]	0.46
LF, nu	59±20	61±17	0.71
HF, nu	41±20	39±17	0.71
LF/HF	2[0.7-3]	1.7[1-2]	0.98
log(LF/HF)	0.19±0.4	0.22±0.3	0.73

Normally distributed data are expressed as (mean ±SD). Non-normally distributed data are displayed as median with interquartile ranges. HF: high frequency; LF: low frequency; pNN50: The percentage of adjacent NN intervals that differ from each other by more than 50 ms; rMSSD: root mean square of successive differences; SDNN: the standard deviation of NN.

7.4.3. Factors influencing heart rate variability in resistant hypertension

No variables were identified on univariate and multivariate analysis as independent predictors of HRV after adding age and office SBP as independent variables of multivariable model.

7.4.4. Blood pressure variability characteristics

After eight weeks of antihypertensives treatment optimisation, study cohort showed significant decline in office SBP (159 mmHg [154-181] (baseline) to 155 mmHg [136-168] (follow-up), p= 0.03), and in office DBP (94 mmHg [84-100] (baseline) to 84 mmHg [79-100] (follow-up), p=0.03). The average reduction in SBP was 2.52% and in DBP was 10.64%. There were no significant differences in heart rate at baseline and follow-up, (65 bpm ±12 (baseline) vs. 67 bpm ±15 (follow-up), p=0.41) (Table 7.2).

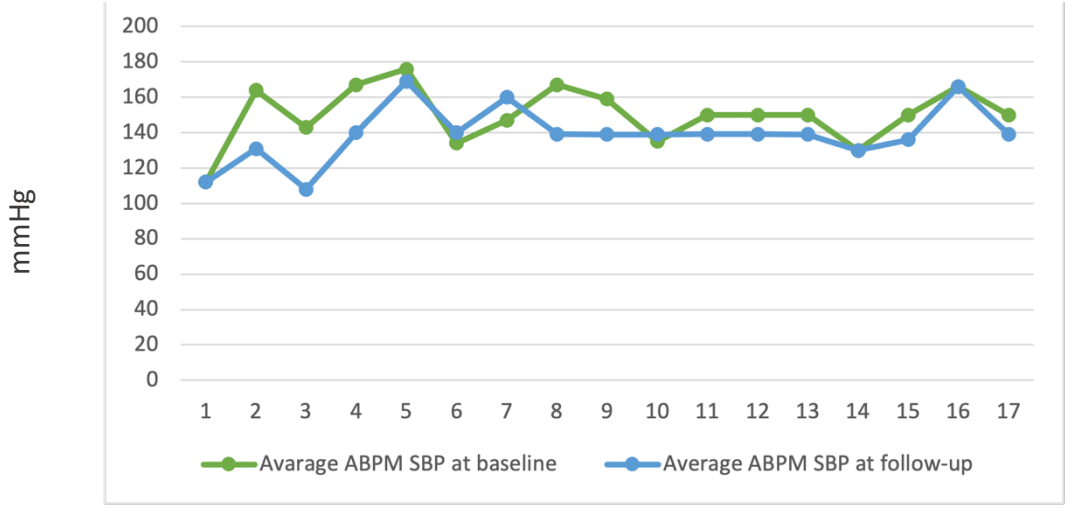
Daytime, night-time and other BPV parameters revealed no statistically significant changes compared with the baseline assessment (Table 7.2). However, lower 24-hour overall SBP and DBP were observed following eight weeks of BP optimisation (7% and 8%, respectively, Figure 7.3). In addition, a trend for reduction in DBP, SBP SD and DBP SD were observed but it did not reach statistical significance (p=0.09, p=0.09 and p=0.07 respectively). Lower values were observed during night-time versus daytime periods, SBP was 7 % lower and DBP was 10 % lower (Figure 7.4). All patients showed non dipper pattern for BP.

Table 7.2 Blood pressure variability of the study cohort

	Baseline visit	Follow-up visit	P
Office blood pressure			
Office SBP, mmHg	159[154-181]	155[136-168]	**0.03**
Office DBP, mmHg	94[84-100]	84[79-100]	**0.03**
Office heart rate, bpm	65±12	67±15	0.41
24-hour			
SBP, mmHg	149±21	140±22	0.16
DBP, mmHg	92±14	85±14	0.09
SD SBP, mmHg	24±4	24±2	0.09
SD DBP, mmHg	15[12-19]	10[9-11]	0.07
overall PP, mmHg	55±13	55±12	0.83
Daytime			
SBP, mmHg	147±20	141±18	0.35
DBP, mmHg	92±12	87±8	0.21
SBP SD, mmHg	24±8	11±6	0.08
DBP SD, mmHg	16[14-18]	8[6-10]	0.06
PP, mmHg	54±11	54±12	0.92
Night-time			
SBP, mmHg	138±32	140±23	0.89
DBP, mmHg	82±11	81±12	0.75
SBP SD, mmHg	18±9	14±7	0.50
DBP SD, mmHg	10[7-13]	10[6-12]	0.10
PP, mmHg	53[47-65]	53[48-65]	0.34
SBP daytime/night-time decrease, %	8[7.5-12]	10[8-11]	0.10
DBP daytime/night-time decrease, %	12±5	13±8	0.87

Normally distributed data are expressed as (mean ±SD). Non-normally distributed data are displayed as median with interquartile ranges. Diastolic blood pressure; PP: pulse pressure; SBP: systolic blood pressure SD: standard deviation of mean blood pressure.

A



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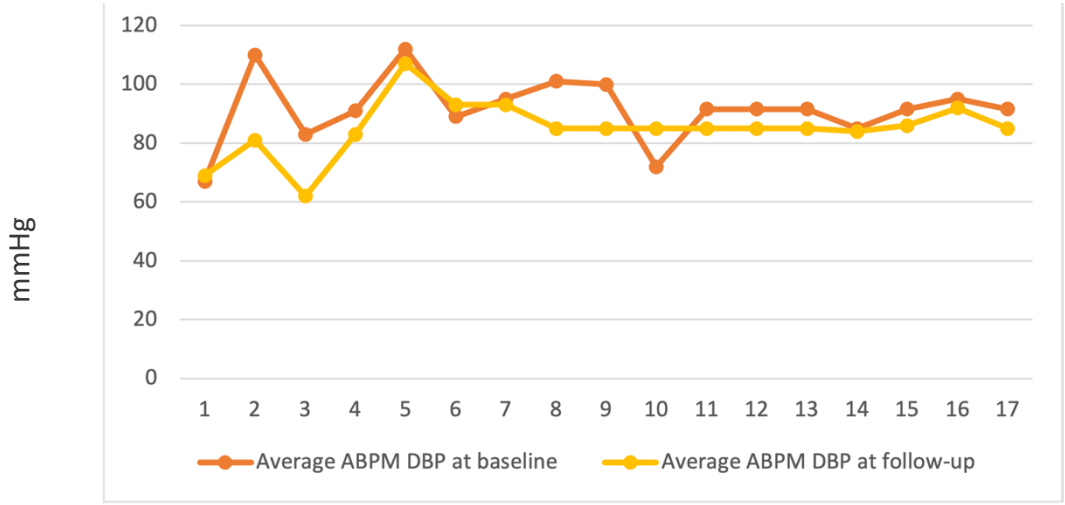


Figure 7.3 Circadian patterns of resistant hypertension patients. Lower 24-hour of overall SBP and DBP were observed following eight weeks of blood pressure optimisation (7% and 8%, respectively). Panel A represents the average ABPM SBP level at baseline (green line) and at follow-up (blue line), for each study patient. Panel B represents the average ABPM DBP level at baseline (orange line) and at follow-up (yellow line), for each study patient.

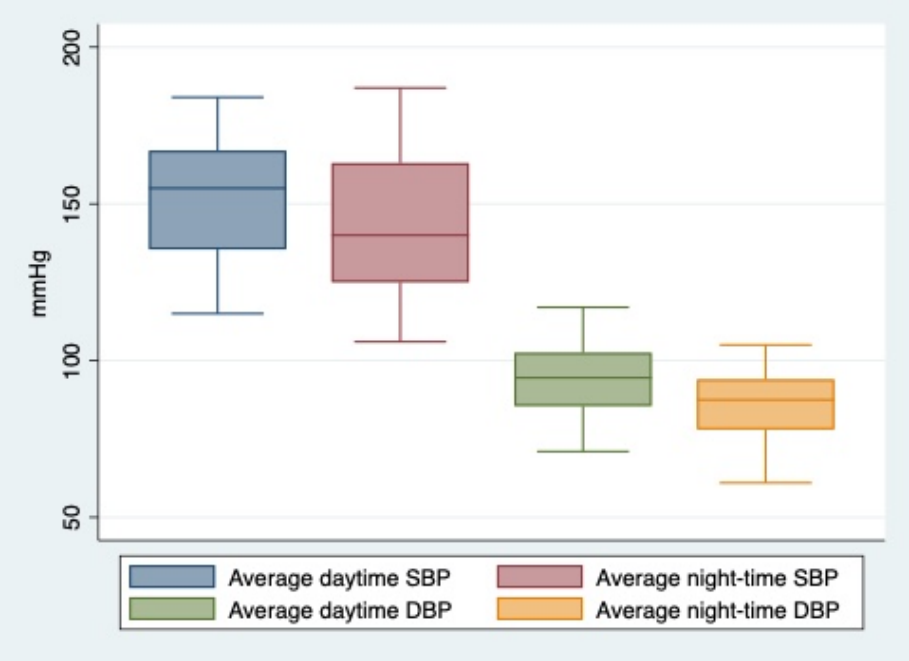


Figure 7.4 Average daytime and night-time of patients with resistant hypertension. Lower values were observed during night-time versus daytime periods, SBP was 7 % lower and DBP was 10 % lower at night-time (without imputation).

In ABPM assessment, the data were found missing completely at random which would not bias the results. Statistical analysis was done by comparing 24-hour BP with missing data and then with mean imputation technique (Table 7.3). Significant reduction was observed after mean imputation in overall BP, daytime BP and night-time DBP.

Table 7.3 Blood pressure variability before and after mean imputation

	Baseline	**Follow-up**	**P**	**Baseline**	**Follow-up**	**P**
	24-hour (before mean imputation)			**24-hour (after mean imputation)**		
SBP, mmHg	149±21	140±22	0.16	150[143-164]	139[136-140]	**0.01**
DBP, mmHg	92±14	85±14	0.09	92[89-95]	85[84-86]	**0.007**
	Daytime (before mean imputation)			**Daytime (after mean imputation)**		
SBP, mmHg	147±20	141±18	0.35	152[147-164]	141[138-140]	**0.005**
DBP, mmHg	92±12	87±8	0.21	94[92-99]	87[87-87]	**0.01**
	Night-time (before mean imputation)			**Night-time (after mean imputation)**		
SBP, mmHg	138±32	140±23	0.89	143[137-143]	141[133-141]	0.21
DBP, mmHg	82±11	81±12	0.75	86[86-88]	76[76-81]	**0.007**

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

7.4.5. Factors affecting blood pressure variability in resistant hypertension

No variables were associated with ABPM SBP on univariable analysis and multivariable analysis.

7.5. Discussion

Treatment with optimised antihypertensive agents for 8 weeks did not effectively improve cardiac autonomic function in patients with RH. In my study, I did not detect differences in HRV parameters between baseline and follow-up, despite a trend toward improving HRV after antihypertensive treatment. Several studies have assessed HRV in hypertension (35, 283, 319, 387, 388, 389, 390). In a recent study, patients with uncontrolled hypertension and metabolic syndrome experienced improved of HRV indices after 12 months of antihypertensive therapy, whereas the changes in HRV failed to reach statistical significance in patients with hypertension and with no metabolic syndrome (327). Improvement of HRV indices were also observed in another study after three months of antihypertensive treatment with ACEI (333). Majority of the previous studies evaluate patients with moderate hypertension unlike the present study where hypertension is difficult to control.

Office BP and BPV showed significant reduction after 8 weeks of antihypertensive treatment. According to several studies assessing the effect of antihypertensive agents on 24-hour ABPM, reduction of BPV is associated with the reduction of mean BP (461, 462, 463).

The effects of different antihypertensives agents on BPV was assessed previously (464). Meta-analysis showed that CCBs was the only treatment that effectively decreased BPV (464). Diuretics, and β-blockers had neutral effects when compared to placebo.

7.6. Conclusion

The study demonstrated that introducing intensive antihypertensive agents reduces office BP and therefore BPV reduction occurred in patients with RH. However, it did not contribute to improve HRV indices in this population reflecting sympathetic and parasympathetic status of these patients.

Chapter VIII. SUMMARY AND OVERALL CONCLUSION

8.1. Thesis summary

Chapter-01 (Introduction), this introductory chapter provide a general overview to the main topics of my thesis and describe the current understanding of our knowledge of the presence of malignant and resistant hypertension and their relation to cardiovascular haemodynamics, arterial stiffness mechanisms, endothelial function and autonomic regulation.

Chapter-02 (Literature review), discuss the gaps and the pathways connecting MHT and RH pathogenesis to arterial stiffness, abnormal cardiac mechanics, autonomic and endothelial dysfunction.

Chapter-03, discuss the aims and hypothesis of my thesis. Explain the research techniques used. Describes how the data collected and analysed.

In Chapter-04, the study examined cardiac and autonomic changes in two severe and (difficult to treat) forms of hypertension. The use of advanced strain imaging revealed significant impaired cardiac remodelling in both hypertension groups, even with tight antihypertensive treatment, and independent of preserved EF. Interestingly, the study showed that MHT have more severe myocardium deformation and that cardiac complications is not only affected by afterload but also related to more myocardial fibrosis. Preserved LV twist, torsion and GCS in both hypertension groups appeared to contribute to preserved EF. Autonomic function was preserved in both hypertension groups.

In Chapter-05, the relationship between arterial stiffness, endothelial function and carotid distensibility were investigated in patients with MHT and RH and were compared to NC. Endothelial dysfunction and increased arterial stiffness were observed in MHT and RH compared to control group. Low subendocardial perfusion was observed in MHT compared to NC.

In Chapter-06 and Chapter-07, RH patients were seen before and after 8 weeks of optimised antihypertensive treatment. Strain cardiac function and endothelial function revealed significant improvement compared to baseline. Conversely, arterial stiffness, BPV and HRV indices did not show any improvement.

8.2. The study strengths and limitations

There were several strengths in the study. First, the main strength of the study is that it is being conducted in two large specialist hypertension clinics that manage RH and MHT patients:

- The West Birmingham Malignant Hypertension Register and Hypertension Clinic at City Hospital, Birmingham.
- Hypertension Specialist Clinic at Liverpool Heart and Chest Hospital, Liverpool.

Second, the use of strain imaging to study cardiac deformation is another strength point in the study. The main advantage of STE is its ability to reflect active contraction within each segment, avoid tethering effect, which makes it less influenced by artefacts. STE can measure three directions of cardiac motion and can track the speckle in any 2D direction making it less angle dependent. It has high reproducibility with reduced intra-

observer and interobserver variability. Therefore, the findings support the literature and guidelines that the STE is a robust sensitive technique in detecting underlying pathology.

Lastly, all procedures were performed by single operator using the same ultrasound machine (Philips) and the same vendor (ACMQ) to avoid one of the known limitations of STE (lack of inter-vendor consistency and reproducibility of strain measurements).

The study was limited by its cross-sectional design and the small sample size, which may play a part in the lack of differences observed in HRV, BPV and arterial stiffness indices between groups. When sample sizes are small, if the effects that exist are not large, then the power to detect these effects can be low. This translates to a low probability of achieving statistically significant p values even when the null hypothesis should be rejected. This is referred to as a Type 2 error, and this should be considered with regard to the analyses especially when group sizes are small.

Although the study sample was sufficient to reveal the persistent cardiac and endothelial dysfunction, it is important to note that when statistical analyses are performed, this increases the chance of making a Type 1 error. This occurs when the null hypothesis is correct but is rejected by the statistical analysis. When each analysis is performed with a 0.05 level of significance, if the null hypotheses for the analyses are true, each individual analysis has a 5% chance of resulting in a Type 1 error; however, the probability that at least one of the analyses will falsely reject the null hypothesis becomes greater than 5% in combination. Therefore, conclusions about statistical significance of the analyses should be made with caution.

There are several reasons for the small sample size in my study:

1- Considering the low incidence of MHT population and the limited current studies on MHT.
2- Inclusion of highly selected true RH patients.
3- Exclusion of those with poor acoustic window.
4- It is also important to mention that there were no new or follow-up recruitment due to COVID-19. This had serious impacts on the progress of the recruitment and contributed to incomplete recruitment of patients and inadequately power the study.

Despite this, the study offers new and significant information and can consider as a starting point for larger studies.

Although the study showed an improvement in office BP, endothelial function, longitudinal and circumferential strain after follow-up period of 8 weeks, this period was not long enough to detect changes in HRV, BPV and arterial stiffness. Future studies may need longer follow-up to observe the effect of antihypertensive medication on autonomic function, vascular function and BPV in patients with RH.

8.3. Overall conclusion

For the first time, cardiac, vascular and autonomic function have been assessed in patients with two extreme phenotypes of hypertension (patients with treated MHT and patients with true RH). The study demonstrates that advanced strain imaging is a sensitive method to reveal differences in cardiac remodelling responses between MHT

and RH patients. I have been able to show that strain dysfunction, endothelial dysfunction and arterial stiffness are present in patients with hypertension (MHT and RH). This may explain the fact that these patients have poor prognosis. In addition, based on the study findings, MHT may have different features of cardiac abnormalities.

Despite long-term optimised antihypertensive therapy and preserved EF in stable phase MHT and true RH, cardiac remodelling remained compromised. There was worse cardiac remodelling in patients with stable MHT and good long-term BP control compared to RH. This suggests that impaired GLS may not be only related to afterload and may be associated with more prevalent myocardial fibrosis in MHT patients. Interestingly, MHT also showed poor SEVR and TPR, indicating that MHT has lower myocardial oxygen consumption and insufficient oxygen supply.

MHT and RH have abnormal endothelial function and arterial stiffness, whereas autonomic function is preserved.

The longitudinal study demonstrated that introducing intensive BP control and antihypertensive medications reduces office SBP and DBP in patients with RH. Antihypertensive therapy also has a favourable impact on LV strain deformation. However, it did not contribute to improve BPV and HRV indices.

8.4. Future research and implications for practice

Elevated BP remains one of the leading causes of death globally. Despite advances in diagnosis and treatment, the prevalence of hypertension and its cardiovascular complications are growing globally. MHT and RH are existing complex types of

hypertension population, developing higher cardiovascular risk profile and consequently have a worse prognosis.

Accurate diagnosis of MHT and RH will contribute to better management of these conditions. Further investigation into these types of hypertension would also lead to a better understanding of the underlying complex pathological processes which include cardiac mechanics alteration, vascular abnormalities and autonomic dysregulation. Accordingly, it would have been convenient if future studies compared MHT and RH with uncomplicated and well-controlled hypertension to establish the clinical cardiovascular abnormalities differences between the groups.

In the cross-sectional study, advanced strain imaging revealed significant unfavourable cardiac remodelling in both groups. These findings are present independent of preserved EF and optimised antihypertensive treatment. Therefore, it may be most beneficial in the future to include strain imaging as a routine clinical practice in echocardiography labs to provide a more comprehensive assessment of LV mechanics. Impaired endothelial function and arterial stiffness have also been observed in both groups. Therefore, it is important to focus on therapeutic interventions, targeting cardio/vascular remodelling and eventually having positive impacts on prognosis.

I also showed that patients with MHT have worse myocardium deformation and that cardiac complications are not only affected by afterload but also related to more abnormal myocardial fibrosis markers. MHT patients also showed low subendocardial viability and total peripheral resistance indicating the presence of insufficient oxygen

supply and low myocardial oxygen consumption. This impairment of SEVR may be because of structural and functional coronary microvascular remodelling. This is due to perivascular fibrosis of intramural arterioles that causes a reduction in vessels density in the coronary microvasculature and myocardial ischemia. There is still substantial uncertainty regarding how the status of myocardial fibrosis and low subendocardial viability affect the disease process and outcomes in MHT population. Ideally, these questions would be answered through more detailed assessments in prospective longitudinal studies.

In the longitudinal study, RH patients were seen before and after 8 weeks of optimised antihypertensive treatment. Strain cardiac function and endothelial function revealed significant improvement compared to baseline. In light of these findings, strain function and endothelial function may be a reversible condition if risk factors such as BP are controlled and optimised.

I did not aim to assess how the findings would affect long-term clinical outcomes. There is a need for long-term observational and randomised controlled clinical trials for assessing the efficacy of the intensified treatment plan on the long-term quality of life and outcomes.

Arterial stiffness, BPV and HRV indices did not show any improvement during the follow-up. In order to detect significant vascular and autonomic function improvements in the hypertension groups, larger sample size and longer follow-up are recommended.

APPENDICES

Appendix 1. Standard Operating Procedure 'Speckle tracking echocardiography'

Background

Speckle tracking echocardiography (STE) or strain imaging has been recognised as an effective, non-invasive and sensitive method to detect early regional and global myocardial dysfunction before conventional echocardiography.(25, 26)

The updated offline Philips software 'Automated cardiac motion quantification' (aCMQ) was used to evaluate myocardium deformation and function. The aCMQ software defined and tracked the movement of LV myocardium in accordance with recommendation of the consensus document of the European Association of Cardiovascular Imaging/ American Society of Echocardiography (EACVI/ASE) Task Force.(338)

Equipment

- Table
- Echocardiography machine
- Cardiac transducer (S5-1 phased array sector ultrasound transducer)
- aCMQ software
- Laptop
- Ultrasound gel
- Tissues
- Detergent wipes

Preparation

- Explain procedure to the patient/participant.
- Ask the patient/participant to expose his/her chest.
- Maintain privacy and dignity of the patient/participant. (Use hospital gown and bed sheet)
- Ask the patient/participant to lie on the left lateral decubitus position.

Procedure

- Turn on echocardiography machine.
- Apply a generous amount of ultrasound gel to the echocardiography probe.
- Place the probe on the chest and start scanning.
- Acquire all images with normal breathing.
- Acquire a good quality 2D image with high frame rate (70-100 Hz) to trace the LV endocardium at end-diastole.
- Obtain three consecutive clips for the assessment of each view.
- Acquire parasternal short axis (PSAX) images: Transducer 3-5 cm to the left of the left sternal border at 3rd to 5th intercostal space. Transducer indicator pointed towards patient's left (1:00 position). Transducer gradually tilted down heart axis to obtain three levels
    - PSAX at basal level
    - PSAX at mid-level
    - PSAX at apex level

- Acquire three images of apical window: Transducer placed at xiphoid level (6th intercostal space) in mid-clavicular line or nipple line.
    - 4 chamber view: Transducer indicator pointed towards patient's left (3:00 position)
    - 3 chamber view: Rotate the probe toward 1:00 position toward left shoulder.
    - 2 chamber view: Rotate the probe toward 11:00 position toward right shoulder.
- When scan is complete remove gel from the chest.
- Clean the probe and the bed according to infection control policy.

aCMQ software

- Transfer all images were to the aCMQ software for offline analysis.
- To copy the study from echo machine to CD/Flash memory:
    - Click on the study
    - File
    - Copy
    - Local
    - Browse
    - Choose CD or memory
    - Ok
- To import study from CD/flash to Q-station (aCMQ software):
    - Q-station
    - File
    - Open

- CD/flash memory
- Drag into the study (on the right)
- File
- Open
- Control+A
- Open
- Drag into the study

- To start offline analysis
  - Double click on the selected study
  - Choose the three views you want to use by pressing [ ]

(Located on the right side of image)

  - Press on [Q] (Located above the image)
  - Launch selected image in Qlab.
  - Select aCMQ
  - Loop 1, loop 2, loop 3 are my selected images
- To calculate global longitudinal strain (GLS), select apical views
  - Select 4 chamber, 3 chamber and 2 chamber
  - To confirm view: press on the selected view (ex: LVAP3)
  - Edit ED then compute
  - Edit ES then compute
  - Accept
  - Save for each loop.
- When all views are accepted, GLS will be calculated, and bull's eye will be generated.

- To calculate global circumferential strain (GCS), select PSAX views
    - Select PSAX at three levels: basal level, mid-level and apex level.
    - To confirm view: press on the selected view
    - Edit ED then compute
    - Edit ES then compute
    - Accept
    - Save for each loop.
- When all views are accepted, GCS will be calculated, and bull's eye will be generated.

Appendix 2. Standard Operating Procedure 'Arterial stiffness'

Background

Carotid-femoral pulse wave velocity (cfPWV) and augmentation index (AIx) are widely considered as the least invasive, safest, and more reliable in terms of accuracy, as recommended by the European Society of Hypertension (ESH) in 2018(8) and earlier by expert consensus document of 2006.(63) cfPWV was estimated noninvasively by measuring the distance of arterial pulse between two superficial arterial sites (e.g. carotid artery and femoral artery) and the travel time taken.(153)

VICORDER® software (Smart medical, UK) used the gold standard assessment of cfPWV between the carotid and femoral arteries and it was validated in several studies(339, 340, 341, 342) and was used to estimate cfPWV, central pressure and AIx. It is safe, non-invasive, portable, easy to perform by single operator and operator independent.

Equipment

- Couch
- Vicorder instrument
- Neckpad
- Pressure cuff

Preparation

- Explain the procedure to the participant.
- Ask the subject to lie supine and rest for 5 minutes before the test.

- Connect Vicorder device to laptop (use black cable).
- Place the arm cuff (when you use right arm, hose goes up).
- Place the thigh cuff (ensure that inflated side of the bladder is over the inside of the thigh).
- Adjust the table so the subject be in a semi-prone position with the head and shoulders raised by approximately 30 degrees, this should prevent Venous contamination of the Arterial signal.
- Open Vicorder software from the laptop.
- To create patient's name, go to new patient
- Fill 1st name, last name, ID, DOB and sex.
- Save and exit.
- Subject will be highlighted.
- Go to PWA.
- Give name to excel spreadsheet which will be generated automatically.
- Choose where to save it.
- All patients will go to the same file. (One patient, several rows).
- Place pressure cuff on the right arm.
- Click on (OSC BP) to take BP.
- Once fluctuating stopped, click on space bar.
- Click enter key to save it or click on save.
- Click ok.
- To Take central pressure, click on space bar to inflate the cuff.

- After 13 beats, press space bar or when all data has been generated.
- Click save.
- To repeat measurement, click on (Calcs).
- Close.
- To calculate cfPWV:
    - Adjust the participants' position. (Keep the participant on supine position and put Pillow under patient's shoulder to extend the neck)
    - Place the neck pad around the participant's neck the pressure pad (inflatable sensor) around the right side of the neck (right carotid area). Connect to PRESS2 on the Vicorder. (don't twist)
    - Place the pressure cuff on the upper right thigh to record femoral artery pulse. Connect to PRESS1 on the Vicorder. (don't twist)
    - Measure the distance between the Supra-Sterna Notch and the Thigh cuff in centimetres and enter for Length.
    - Go to PWV
    - Enter the measurement in the data.
    - Press space bar.
    - Cuff and sensor will start to inflate until it reaches target pressure.
    - Numbers will start to fill in.
    - Save.
    - To do it again, press space bar.

- The Systolic and Diastolic pressures may be obtained using any method and entered manually.

Appendix 3. Standard Operating Procedure 'Flow-mediated dilation'

Background

Endothelial dysfunction is involved in the pathophysiology of cardiovascular disease (CVD) and is associated with many cardiovascular risk factors.(92)Flow mediated dilatation (FMD) is widely used to assess endothelial function and examine the ability of brachial artery to response to the pressure induced by the cuff inflation.(44) A blood pressure cuff is used to temporally occlude the brachial artery. After the cuff deflated, blood flow increased in the brachial artery triggering nitric oxide (NO) release from the endothelium and consequent endothelium-dependent brachial artery vasodilatation. Cardiovascular Suite software uses real-time automated edge detection and wall tracking techniques for the analysis. The software process series of ultrasound images and give automatic measurements of brachial artery diameter, and automatic analyses of the Doppler signal in order to calculate the value of instantaneous shear rate.

Patient preparation

Several factors can affect FMD, including surrounding temperature, food intake, drugs, sympathetic stimuli, and period of menstrual circle in female. Caffeine, high-fat meal and smoking can attenuate FMD. Therefore, it is recommended that subject should fast for at least 8 to 12 h before the study. It is also recommended to conduct the study in a quiet, temperature-controlled room.

Equipment

- Couch.
- Phillips CX50 ultrasound machine.
- L12-3 linear array ultrasound transducer.
- Ultrasound gel.
- Cardiovascular Suite software (version 3.4.1; FMD Studio, Quipu srl, Pisa, Italy).
- Video grabber.
- Manual sphygmomanometer cuff.

Procedure preparation

- Ask the subject to lay supine on a couch and rest for 5 minutes.
- Explain the procedure and possibility of some discomfort during arm compression
- Extend the right arm in comfortable position using supported cushion.
- Place the manual sphygmomanometer cuff (5cm width, Hokanson, Bellevue, WA) to the right forearm.

Ultrasound machine set up

- Connect video grabber to Ultrasound machine.
- Select the probe on echocardiography machine, (L12-3 linear array ultrasound probe transducer).
- Turn on echo machine (switch on the top left of the machine).

- Enter patient data and create patient ID (top left of the keyboard) then press close.
- Press PW button and set the sample volume width. (Approximate width=1/3of the blood vessel width).
- Set the angle of the sample volume to be parallel to the vessel wall. (Ideally between 40-60 degrees).
- Placed the sample volume cursor in the centre of the vessel.
- Make sure the cursor of the sample volume is not into the region of interest (ROI) where the diameter is computed.

Cardiovascular Suite software setup

- Connect video grabber to the laptop.
- Open Cardiovascular Suite software
- Click on the Add New Patient button.
- In the new patient frame, enter the patient data.
- Click on the Save button to save the patient data.
- Click on (start the study) button.
- Time setup (top right):

  Baseline (sec)=60

  Ischemia(sec)=300

  Vasodilatation (sec)=120

  Total (sec)= 480
- Adjust calibration:

Line length, Y-line and X-line

Procedure

- Place the ultrasound probe above the antecubital fossa to scan the brachial artery.
- Identify the brachial artery.
  - By colour doppler (colour button).
  - By arterial flow pulsatile effect.
- Once brachial artery is identified, turn colour flow off.
- Held the probe in place for the whole duration of the procedure. (Marker of the probe up toward the head).
- Record the baseline images for 1 minute before the cuff occlusion.
- Inflate the cuff to 50 mmHg above the subject's SBP and leave it for 5 minutes.
- Following this, deflate the cuff rapidly.
- Record the hyperaemic response for 2 minutes.
- Remove sphygmomanometer cuff from the arm.
- Clean ultrasound gel off the subject.

The software calculated the following data:

- Baseline diameter and baseline shear rate.
- Vasodilatation maximum diameter and maximum shear rate.
- Recovery diameter (mean diameter of the last 30 seconds in vasodilatation time).
- The area under the curve of the shear-rate in vasodilatation.

- FMD which was expressed as a percentage change in brachial artery diameter in respect to baseline diameter (%).

  FMD= (maximum diameter- baseline diameter)/ baseline diameter.

- FMDr which was expressed as a percentage change in brachial artery diameter in respect to recovery diameter (%).

  FMDr= (maximum diameter- baseline diameter)/ recovery diameter.

Appendix 4. Standard Operating Procedure 'Heart rate variability'

Background

Heart rate variability (HRV) analysis is a simple, quick, validated and non-invasive method to assess cardiac autonomic function. (35, 36)

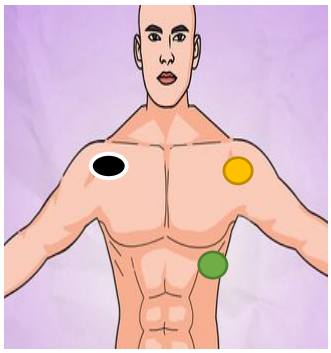
Equipment

- Couch
- Laptop
- HRV software (*Cardiscope*™ *ANALYTICS* program, SMART Medical Ltd, Moreton in Marsh, UK).
- Portable ECG sensor (eMotion Faros, Bittium Biosignals Ltd, Kuopio, Finland).
- Three electrocardiogram (ECG) leads and electrodes
- Alcohol swab
- Adhesive remover swabs

Preparation

- Explain procedure to the patient/participant.
- Ask the patient/participant to expose his/her chest.
- Maintain privacy and dignity of the patient/participant. (Use hospital gown and bed sheet)
- Ask the participant to lay supine on a couch in a comfortable position and a supported pillow.

- Prepare the skin where the electrodes will be placed by rubbing the chest with alcohol swab.
- Let the subject rested for 5 minutes before the procedures started.
- Apply the electrodes on right shoulder, left shoulder and on the anterior axillary line. (See figure below)



Procedure

- Attach the portable sensor to the ECG leads and turn it on.
- Open Bluetooth to paired laptop with ECG sensor.

http://cardiscope.com

- Open Cardioscope.
- To create new patient, go to client selection.
- Select patient.
- Go to records.

- Start live recording
- Start manoeuvre/ select your protocol.
- Set mark if there is some disturb to the patient.
- To stop recording, go to stop transmission & recording.

The real-time analysis program calculated the differences between successive R-R intervals and assessed different parameters of HRV as seen in the table below.

Table Heart rate variability parameters

Parameters	**Definitions**
Time domain indices	
SDNN	Standard deviation of the normal-to-normal intervals
rMSSD	Sum of successive differences in normal-to-normal interval
pNN50	Proportion of the number of successive normal-to-normal intervals that differ by more than 50 ms
Frequency domain indices	
HF	High frequency
LF	Low frequency
LF/HF	Low frequency to high frequency ratio

Appendix 5. List of the study publications

Reviews:

Ahsan A. Khan, Rehan T. Junejo, Reem Alsharari, G. Neil Thomas, James P. Fisher, Gregory Y. H. Lip. A greater burden of atrial fibrillation is associated with worse endothelial dysfunction in hypertension. Journal of Human Hypertension, Volume 35, Issue 8, Pages 667-677, 2021.

Reem Alsharari, David Oxborough, Gregory Y. H. Lip, Alena Shantsila. Myocardial Strain Imaging in Resistant Hypertension. Current Hypertension Reports, Volume 23, Issue 5, Pages 24, 2021.

Reem Alsharari, Gregory Y. H. Lip, Alena Shantsila. Assessment of Arterial Stiffness in Patients with Resistant Hypertension: Additional Insights into the Pathophysiology of this condition. American Journal of Hypertension, Volume 33, Issue 2, Pages 107–115, 2020.

Abstracts presentation:

Reem Alsharari, G.Neil Thomas, Gregory Y. H. Lip, Alena Shantsila. Autonomic function in resistant and malignant hypertension. British Cardiovascular Society Annual Conference, 7–10 June 2021, Manchester Central, Manchester, UK.

Ahsan Khan, Reem Alsharari, Rehan Junejo, Neil Thomas, James Fisher, G. Lip. Autonomic and vascular function characteristics in patients with atrial high rate

episodes. British Cardiovascular Society Annual Conference, 7–10 June 2021, Manchester Central, Manchester, UK. The best of the best clinical abstract winner in the category of Cardiac Rhythm Management.

Moderator poster presentation:

Reem Alsharari, G.Neil Thomas, Gregory Y. H. Lip, Alena Shantsila. Vascular function in resistant and malignant hypertension. The British and Irish Hypertension 2021 Annual Scientific Meeting 13-15 September 2021, Brighton, UK

Editorial

Reem Alsharari, Eduard Shantsila, Gregory Y. H. Lip, Alena Shantsila. 'Revisiting the diagnosis of 'resistant hypertension': what should we do nowadays'. Journal of Human Hypertension, 2021.

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