“Metabolomic investigation of antihypertensive drug response. Studies of antihypertensive treatment adherence using targeted/untargeted drug screening in tertiary care hypertension patients.”

PhD in Cardiovascular Sciences

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Submitted in fulfilment of requirements for the degree of Doctor of Philosophy (Ph.D.) in the Faculty of Medicine, University of Glasgow
December 2019

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Abstract

Hypertension is a major risk factor for several cardiovascular CV diseases. The estimated prevalence of HTN in Scotland in the adult population from 2014 to 2017 for all age groups in both sexes was 58.7%. Medication adherence is assessed using 2 different methods, either indirect or direct methods each has its own advantages and disadvantages. Non-adherence to therapy can lead to uncontrolled blood pressure (BP), deterioration in health and progression of disease state. It can also increase the cost burden on the health care system.

The main aim of this study was to assess adherence by indirect methods using the self-reported Morrisby Medication Adherence Scale (MMAS-8) and direct method by untargeted and targeted drug screening in urine samples of hypertensive patients attending Glasgow Blood Pressure clinic (GBPC). Drug screening was analysed using 3 assays: First, Birmingham heartland laboratory BIR using HP LC-MS/MS. Second, Glasgow Polynomic POL using untargeted mass spectrometry data-dependent fragmentation spectra and molecular approach (based on Hydrophilic interaction liquid chromatography. Finally, Glasgow toxicology GLA using Hollow-fibre liquid-phase microextraction followed by LC-MS/MS for 10 antihypertensive drugs.

348 patients completed Morrisby questionnaire and showed that 62.1% of patients had high adherence, while 26.7% of patients had medium adherence and only 11.2 had low adherence. Despite the high adherence detected, the level of BP control was low. Only 35% of patient who reported that they were adherent had controlled SBP.

79 urine samples were sent to Birmingham heartland laboratory and the assay was able to detect complete presence of antihypertensive medication in 49 (62%) of the urine samples. Only 6 (7.6%) samples were found to be completely absent of any medication and the remaining (30.4%) detected at least one of the prescribed antihypertensive medications (partial). No drugs were detected in patients who weren’t prescribed them.

100 urine samples were sent to Glasgow Polyomics and was able to detect complete presence of antihypertensive medication in for most drugs it tested of

the urine samples. 12 (12%) were completely absent of any medication. There was one false positive result.

Out 173 urine samples sent to Glasgow toxicology 152 samples were tested for their prescribed drugs. Results showed only 6 (3.9%) patients weren't detected for any medication in the sample, while 137 (89.5%) detected all the medication they were tested for and 9 (5.9%) had some their prescribed drugs detected (partial adherence). There was one false positive result.

57 samples were shared between the 3 assays for 4 antihypertensive drugs (Amlodipine, Atenolol, Losartan and Ramipril). Losartan was detected for all patient. Birmingham was the lowest method to detect amlodipine and atenolol. However, for Ramipril Birmingham identified more than the others. Glasgow Polyomics and Glasgow toxicology had high sensitivity for drugs around (80%). Glasgow Polyomics misidentified 1 patient that was prescribed metoprolol for atenolol.

79 patients were shared between Birmingham and Glasgow toxicology and were compared. Both methods agreed for most of the patients except GLA detected more for amlodipine and atenolol while Birmingham detected 1 more patient for Spironolactone. Analyses of the concentrations found by GLA doesn't suggest those detected by GLA but not detected by BIR had lower concentrations.

My study clearly shows no correlation between the Morisky score and adherence based on urine drug assays. The relationship between medication adherence and BP control is difficult to demonstrate. Adherence is an important and complex area of research that is essential to improve hypertension management and decrease the global burden of hypertension.

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Acknowledgement

I wish to express my sincerest gratitude to Allah for providing me with health, commitment and patience for completing my Thesis. I am deeply thankful to my supervisors, Prof. Sandosh Padmanabhan and Dr. John McClure for their continuous support, encouragement and guidance throughout my PhD studies.

I offer my sincere gratitude to my parents for their emotional support and prayers all this time. I would like to thank Justin, Rafael and all the staff at the Glasgow blood pressure clinic, Birmingham Heartlands hospital laboratory, Glasgow Polyomic Facility, Glasgow Forensic Toxicology Department and BHF for helping me with my thesis. I would like also to thank all my colleagues at the department: Linsay McCallum, Aisha Che Roos, Mohammed Bazuhair, Safaa Alsanosi, Abdulaziz Alqadi, Jahad, Alghamdi. I also thank my sponsors King Abdulaziz University and the Saudi Arabian Cultural Bureau. Finally, thank you Glasgow city for providing me with so many memories during these past years.

Author's Declaration

I declare that this thesis represents my own work. I was responsible for the analysis and interpretation of the results. The work represented in my thesis has not been previously submitted for any degree to the University of Glasgow or any other institutions.

December 2019

Definitions/Abbreviations

AA	Amino acid
ABCD	The Appropriate Blood Pressure Control in Diabetes
ABPM	Ambulatory blood pressure monitoring
ACEI	Angiotensin converting-enzyme inhibitor
ALERT	Assessment of Lescol in Renal Transplantation
ALLHAT	Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
APQ	Aging perceptions questionnaire
ARB	Angiotensin II receptor blockers
ARIC	Atherosclerosis Risk in Communities
ARMS	Adherence to Refills and Medications Scale
AST	Aspartate transaminase
AUC	Area under curve
AVG	Average
BB	Beta-blocker
BHF	British heart foundation
BHS	British Irish hypertension society
BIR	Birmingham hospital laboratory
BIR0-GLA0	Not detected in both BIR and GLA
BIR0-GLA1	Not detected in BIR but detected in GLA
BIR1-GLA0	Detected in BIR but not detected in GLA
BIR1-GLA1	Detected in both BIR and GLA
BMI	Body mass index
BMQ	Brief Medication Questionnaire
BP	Blood pressure
CCB	Calcium channel blocker
Chol	Cholesterol
CI	Confidence interval
CKD	Chronic kidney disease
CL	Chloride
CMCS	Chinese Multi-provincial Cohort Study
CV	Cardiovascular
CVD	Cardiovascular disease
DASH	Dietary approaches to stop hypertension
DBP	Diastolic blood pressures
DENERHTN	The Renal Denervation for Hypertension (DENERHTN) trial
eGFR	Estimate glomerular filtration rate
EPIC	European Prospective Investigation into Cancer and Nutrition
ESC	European Society of Cardiology
ESH	European Society of Hypertension
GBPC	Glasgow blood pressure clinic
GC	Gas chromatography
GENRES	Genetics of Drug Responsiveness in Essential Hypertension study).
GLA	Glasgow toxicology Laboratory

Hb	Haemoglobin
HBPM	Home blood pressure monitoring
HCL	Hydrochlorothiazide
HDL	High-density lipoprotein
HF-LPME	Hollow-fibre liquid-phase microextraction
HILIC	Hydrophilic interaction liquid chromatography
HIV	Human immunodeficiency viruses
HMOD	Hypertension mediated organ damage
HOPE	Heart Outcomes Prevention Evaluation
HPLC-MS	High performance liquid chromatography mass spectrometry
HTN	Hypertension
INTERMAP	International standardized population-based epidemiological study
ISH	Isolated systolic hypertension
JNC	Joint National Committee
K	Potassium
LC	Liquid chromatography
LC MS	Liquid chromatography mass spectrometry
LDL	Low-density lipoprotein
LFT	Liver function test
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
LV	Left ventricle
LVH	Left ventricular hypertrophy
MAP	Mean arterial pressure
MEMS	Medication event monitoring system
MPR	Medication possession ratio
MRI	Magnetic resonance imaging
MS	Mass spectrometry
Na	Sodium
NHS	National Health Service
NHWA	Non-Hispanic White Americans
NICE	National Health and Nutrition Examination Survey
NMR	Nuclear magnetic resonance
PAD	Peripheral artery disease
PCR	Polymerase chain reaction
PDC	Proportion of days covered
PEAR	Pharmacogenomic Evaluation of Antihypertensive Responses
POL	Glasgow Polyomics laboratory
PPt	Protein precipitation
PWV	Pulse wave velocity
RAS	Renin–angiotensin system
SABPA	Sympathetic activity and Ambulatory Blood Pressure in Africans
SAFHS	San Antonio Family Heart Study
SAM	S–adenosylmethionine
SBP	Systolic blood pressure
SEAMS	Self-efficacy for Appropriate Medication Use
SPE	Solid-phase extraction
TB	Total bilirubin

TDM	Therapeutic drug monitoring
TGL	Triglycerides
TRH	Treatment-resistant hypertension
U&E	Urea and electrolyte
VAS	Visual analogue rating scale
WBC	White blood cell
WCH	White coat hypertension
WHO	World Health Organization

1 Introduction

1.1 Hypertension definitions, measurement

1.1.1 Blood pressure, systolic and diastolic

Blood pressure is defined as the pressure of circulating blood on the walls of major arterial system of the body. The blood pressure is measured in millimetre mercury (mmHg) and it is composed of systolic pressure and diastolic pressure. Systolic blood pressure SBP is the maximum blood pressure during contraction of the ventricles while Diastolic blood pressure DBP is the minimum pressure before the next contraction (1).

1.1.2 Development of hypertension definition

The term 'Hypertension' has been quite challenging to describe and the level of BP at which an individual is considered hypertensive or not, has been a matter of debate for a long time. In the mid-20th century, two pioneers in the field, Pickering and Platt argued on the same concept of Hypertension. Platt argued that hypertension was an inherited disease with dominant type transmission with a specific (though unfamiliar) defect, with a discrete lesion and a known natural history. On the contrary, Pickering argued that hypertension was the upper end of a continuous distribution curve of blood pressure values and that blood pressure is a multiple-gene inheritance (2). The blood pressure threshold level which is used to define hypertension, is the blood pressure level that should be reduced to the point at which the level is more beneficial than harmful (3). For most of the 20th century, there was a general agreement to use DBP as the basis of diagnosis and treatment for hypertension. It arose due to the general belief that DBP contributed more to CV risk than SBP. However, it was recognized that systolic pressure is more important as a risk indicator and that isolated systolic hypertension is itself an essential entity (4). Accordingly, over the last 20 years there has been a decline in the threshold of bp from 160/100 to 140/90 (5).

1.1.3 Primary and secondary hypertension

Hypertension is mainly classified as primary hypertension and secondary hypertension. Primary hypertension also known as essential hypertension or

idiopathic hypertension, is defined as high blood pressure with no identifiable cause. It is the most prevalent condition which affects around 95% of hypertensive patients (6). While secondary hypertension refers to the high blood pressure which is caused identifiable underlying primary cause. (e.g. another medical condition); it is rare and affects around 5%. Various examples of causes responsible for secondary hypertension are polycystic kidney disease, hyperaldosteronism (Conn's syndrome) and Cushing's syndrome (7-9).

1.1.4 **Genetic in hypertension**

In patients with hypertension, positive family history is considered as a common feature. In the majority of the studies, heritability is estimated to vary between 35 and 50% (10). In addition, the meta-analyses on genome-wide association studies have identified 120 loci that are associated with BP regulation; however, they merely explain only 3.5% of the trait variance (11). On the other hand, there are various monogenic forms of hypertension which are caused by single gene mutation such as Liddle’s syndrome, glucocorticoid-remediable aldosteronism. Additionally, there are inherited forms such as pheochromocytoma and paraganglioma that are considered as rare causes for hypertension (12, 13).

1.1.5 **Prevalence of hypertension**

1.1.5.1 Hypertension prevalence in the worldwide

According to Global Health observatory data presented by WHO (2008), the overall prevalence of elevated blood pressure in adults (aged 25 and above) was recorded globally which estimated at around 40%. Due to ageing and population growth, the number of individuals with uncontrolled hypertension increased from 600 million in 1980 to about 1 billion in 2008. According to the report, across all of the WHO regions, it was observed that Males had a slightly higher prevalence of hypertension as compared to females; however, this difference was statistically significant only for Americans and Europe regions. According to statistics, hypertension is estimated to cause 7.5 million deaths which are around 12.8% of the total of all deaths. As a result, it is responsible for 57 million disability-adjusted life years (DALYS) (14). Further, the global prevalence of hypertension in 2015 was estimated to be 1.13 billion according to office BP

with a global age standardized prevalence of 24 and 20% in men and women, respectively (15). High prevalence of hypertension is consistent worldwide, regardless of income status, i.e. in countries with lower, middle and higher incomes. With an increase in age, hypertension becomes progressively more common with a prevalence of >60% in people aged >60 years (16).

1.1.5.2 Prevalence of hypertension in Scotland

According to the Scottish Health Survey, the estimated prevalence of hypertension in the adult population from 2014 to 2017 for all age groups in both sexes was 58.7%. As recorded, females had a higher prevalence compared to males for all age groups. In addition, the prevalence of hypertension in females and males for all age groups was 62.5% and 55.1% respectively (17). Figure 1-1 illustrates the prevalence of hypertension across different age group between males and females. Blood pressure measurements

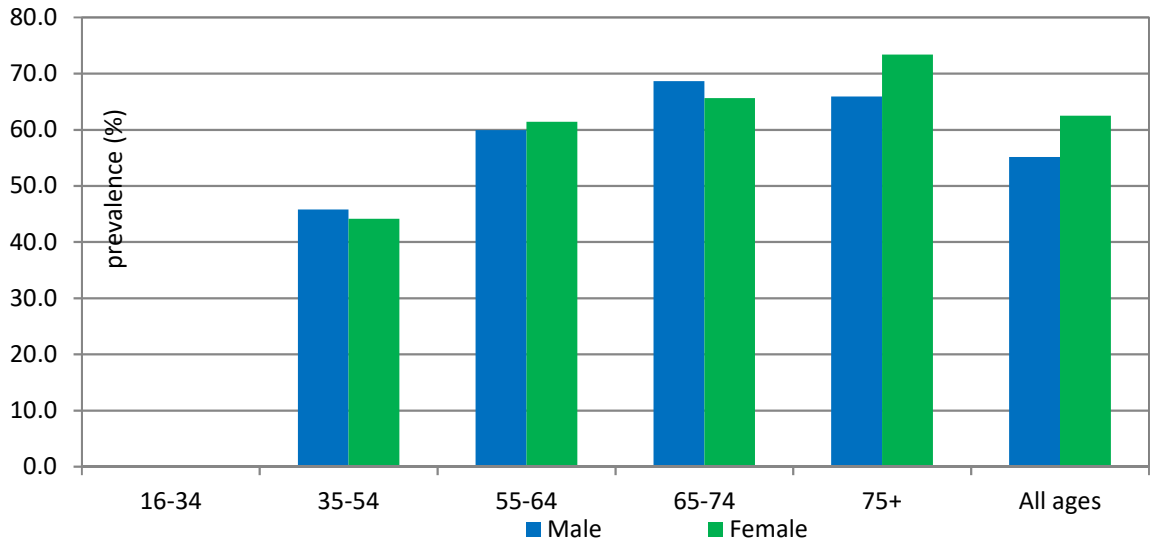


Figure 1-1 High Blood Pressure prevalence in Scotland. Source: Scottish Health Survey(17)

1.1.5.3 Office BP measurement

The British Irish hypertension society BHS recommend that for adults blood pressure should be measured at least every 5 years until the age of 80 years and annual measurement for people with high blood pressure reading at any time previously or with high normal blood pressure (systolic blood pressure 130-139 mm Hg or diastolic blood pressure 85-89 mm Hg) (18). In general, seated blood pressure is sufficient. However, standing blood pressure should be used in patients who are diabetic or elderly to exclude orthostatic hypotension. For each visit, the average of two readings should be taken to guide the treatment. For this purpose, automated or semi-automated devices are the preferred method for home or ambulatory blood pressure measurement (18).Table 1-1 demonstrates the BHS protocol to measure blood pressure.

Table 1-1 BHS protocol of blood pressure measurement using standard mercury sphygmomanometer or semiautomated device

• Use a properly maintained, calibrated, and validated device • Measure sitting blood pressure routinely: standing blood pressure should be recorded at least at the initial estimation in elderly or diabetic patients • Remove tight clothing, support arm at heart level, ensure arm relaxed and avoid talking during the measurement procedure • Use cuff of appropriate size (see box 3 in the full guidelines,3 www.bhsoc.org) • Lower mercury column slowly (2 mm per second) • Read blood pressure to the nearest 2 mm Hg • Measure diastolic blood pressure as disappearance of sounds (phase V) • Take the mean of at least two readings, more recordings are needed if marked differences between initial measurements are found • Do not treat on the basis of an isolated reading
Table is reproduced from (18)

1.1.5.4 Home blood pressure monitoring HBPM

HBPM is the average of all BP readings that are recorded by a semi-automatic, validated BP monitor for a minimum of 3 days and ideally for 6 to 7 days before visiting the clinic. Readings are recorded twice a day, in morning and evening, in a quiet room after resting for 5 minutes. The patient should be seated supporting their back and arms. For each session, two measurements are taken 1 to 2 min apart. Studies have shown that self-monitoring can have a positive effect on medication adherence and BP control (19, 20).

1.1.5.5 Ambulatory Blood pressure monitoring ABPM

ABPM provides the average of BP measurements over a defined period which is usually 24 hrs. The monitor is programmed to take a reading at 15 to 30 min intervals and produce the average BP reading for daytime, night-time and 24 hrs. For a valid ABPM session, at least 70% of normal BP readings are required. There are several indices which are derived ABPM recordings that have several prognostic values. This include including 24 h BP variability (21), morning BP surge (22) and the ambulatory arterial stiffness index (23). However, their predictive value is not clear and should be considered as a research tool. Currently, there are no indications for clinical application.

1.1.5.6 Guidelines recommendation for ABPM and home BP

Table 1-2 illustrates the criteria to diagnose HTN for ABPM and HBPM according to ESH guideline. The recent NICE guideline recommends out-of-office such as ABPM or home BP for the diagnosis of HTN. The NICE guidelines recommend the use of ABPM for patient's diagnosis as a first choice and HBPM as a second choice if a patient isn't able to tolerate ABPM. The recommendation is based on the results obtained from several studies and health economic evaluation. The results showed that ABPM is superior to clinic BP in many features. These studies conclude that: first, ABPM is the best way to measure blood pressure in predicting the development of cardiovascular events. Second, APBM is the best measurement for diagnosing hypertension followed by home BP. Third, ABPM is the most cost-effective method to establish the diagnosis of hypertension by avoiding misdiagnosis of individuals treated by unnecessary treatment (24).

Table 1-2 ESH Definitions of hypertension according to office, ABPM and HBPM levels

Category	SBP (mmHg)		DBP (mmHg)
Office BP	>_140	and/or	>_90
Ambulatory BP			
Daytime (or awake) mean	>_135	and/or	>_85
Night-time (or asleep) mean	>_120	and/or	>_70
24 h mean	>_130	and/or	>_80
Home BP mean	>_135	and/or	>_85

Table is reproduce from (25)

1.1.5.7 Advantages and disadvantages of ambulatory blood and home blood pressure monitoring

Table 1-3 summarises the pros and cons of ABPM and HBPM. There are several advantages of home BP monitoring over clinic measurement. These advantages include the ability to record multiple readings while the patient is awake for a duration, which are taken over many days and may lower the effect of white coat hypertension. Home measurement readings are usually lower than clinic readings (26). Additionally, ABPM has potential advantages of home BP monitoring and clinic readings. It provides more information by producing ABPM profile reports that contain information regarding mean daytime and night-time readings, and blood pressure variability. Also, there is evidence that ABPM values are a better predictor of cardio vascular disease CVD risk and target organ damage (27, 28). Therefore, it is considered a better method for assessing treatment effects on BP. As ABPM provides multiple measurements that are taken over a 24-h duration. During a single 24-h period, it can estimate more than 70 BP reading which minimises the white coat effect. Similar to home readings, ABPM readings are usually lower than clinic readings. Consequently, ABPM and home BP thresholds should probably be adjusted downwards (e.g. by 10/5 mmHg) for diagnosis of hypertension and treatment target for hypertension (29).

The introduction and availability of automated BP monitoring into the clinic has shown that there are marked differences between clinic BP readings and home or ambulatory BP averages. These differences can be identified as either white coat hypertension or masked hypertension. These differences in BP has prompted consideration about whether the conventional means to measure BP in

the clinic is still the most accurate method of predicting future risk of cardiovascular disease.

With the increasing use of both self-blood pressure measurements and ambulatory blood pressure monitoring (ABPM), various subtypes of hypertension like isolated systolic hypertension, white coat hypertension (WCH) and masked hypertension are increasingly recognised and detected

Table 1-3 Advantages and disadvantages of ABPM and HBPM

HBPM	ABPM
Advantages	
Ability to detect white-coat and masked hypertension	Ability to detect white-coat and masked hypertension
Low cost and widely available	Stronger prognostic evidence
Relaxing setting since measurement are taken at home instead of clinic	Night-time readings while patient is sleep
Patient involvement in BP measurement	Measurement in real-life settings
Easily repeated and Ability to assess day-to-day BP variability changes through longer periods of use	Additional prognostic BP phenotypes
	Abundant information from a single measurement session, including short-term BP variability
Disadvantages	
Only static BP is available	Expensive
Possibility for measurement error	sometimes limited availability
No night readings while patient is sleep	Can be uncomfortable

Table is reproduced from (25)

1.1.5.8 Isolated systolic hypertension

Isolated systolic hypertension ISH occurs when the systolic blood pressure is more than 140 mmHg, while the diastolic pressure is normal (less than 90 mmHg). O'Rourke et al. (2013) suggested that young healthy individuals with ISH usually have normal, central BPs and there is no benefit to using anti-hypertensive drugs whereas in the elderly (aged >60 years), ISH occurs due to aortic stiffening (30).

1.1.5.9 White Coat hypertension

White coat hypertension (WCH) in individuals is defined as the condition in which the blood pressure is higher than normal when measured in a medical environment; however, their BP levels are normal during their daily life leading to unnecessary drug prescription. Higher prevalence of WCH is seen among females, elderly and non-smokers. The Cardiovascular prognosis of individuals

with WCH is worse or equal than normotensives, but it is better than the individuals with sustained hypertension (31, 32).

1.1.5.10 Masked Hypertension

Masked hypertension, also called “reverse white-coat hypertension” or “white-coat normotension.” “isolated home hypertension”, “isolated ambulatory hypertension” is an opposite phenomenon where a person has a normal BP in the clinical setting, but has a high BP out of the clinic (home BP or ambulatory daytime BP >135/85 mmHg) (33). The prevalence has been estimated to be around 16% in the population (34). Several factors are associated with masked hypertension including male gender, young age, smoking, alcohol, diabetes and obesity (35). Higher levels for urinary albumin, left ventricular mass index and carotid maximal intimal thickness in people with masked hypertension are similar to those with sustained hypertension but higher than controlled hypertension (36). A meta-analysis of seven studies including 11,502 persons, mean age 63 years, at 8.0-year follow-up demonstrated that people with masked hypertension had two fold increase in their initial cardiovascular events compared to normotensive (37).

1.1.5.11 Resistant hypertension

Resistant hypertension is defined as a lack of response (failure to lower SBP and DBP) to the treatment. The treatment should include lifestyle modification with 3 or more drugs at their maximum tolerated dose including a diuretic. The inadequate BP control is confirmed by ABPM or HBPM in patients with confirmed adherence to treatment. Moreover, secondary causes of hypertension and pseudo-resistant hypertension should be excluded (25). Resistant hypertension is associated with male, black African origin, old age (especially >75 years), higher initial BP at diagnosis, obesity, diabetes and Chronic kidney disease CKD (38).

1.1.5.12 Pseudo-resistant hypertension

Pseudo-resistant hypertension is a high blood pressure that appears to resist treatment; however, other factors, are causing the increase in BP. The factors include non-adherence to prescribed treatment, White-coat phenomenon, improper techniques for office BP measurements, and marked brachial artery calcification(39).

1.1.1 Blood pressure dipping

BP follows a circadian pattern in healthy individuals. BP starts dropping from late evening onwards, reaches lowest around midnight, and rises just after awakening in the morning (40). Dipping in BP have been described in three windows of sleep: BP starts declining in the vesperal window, reaches the plateau level in the basal window, rises in the preawakening window (41). The use of 24-ABPM has demonstrated this phenomenon. Behavioural factors, such as food consumption and obesity, dietary intake of sodium, drinking and smoking habits, consumption of coffee and tea, and bathing can cause alteration to the natural circadian variation of blood pressure (42).

There are 3 main groups based on the difference between day and night BP readings. A fall of >10% in systolic and diastolic BP in the night, compared to daytime readings, is normal. While patients with a nocturnal fall < 10% are defined as nondippers and those with a paradoxical rise in the night reading are defined as reverse dippers (43).

Even in the absence of provoking environmental situations during the awakening period, BP is not constant (44). The clinical measurement of BP significantly overestimate the hypertensive status when compared to 24-hour ABPM, According to a meta-analysis of 20 studies, it suggested that if ABPM was taken as the reference standard to detect hypertension, clinic measurements over 140/90 mmHg had a sensitivity of 74.6% and a specificity of 74.6% (45). Nocturnal BP represents basal BP and hence is representative of the true BP status of an individual. Office BP is affected by random error, systematic error, and a patient's alerting response in addition to numerous other physiological variables during the daytime (42).

The clinical relevance of this phenomenon lies in the fact that non-dipping has been associated with increased frequency of hypertensive target organ damage (brain, heart and kidney), as well as cerebrovascular and cardiovascular events in hypertensive patients (46).

1.1.2 **Guidelines**

The effective management and treatment of hypertension require clinicians and patients to work together to balance pharmacologic and non-pharmacologic interventions and to prevent target organ damage. Therefore, several guidelines for hypertension are provided for its effective management. The most common guidelines for hypertension are provided by NICE in the United Kingdom (47), the European Society of Cardiology (ESC)/European Society of Hypertension (ESH) in Europe (25) and the American College of Cardiology ACC/American Heart Association AHA in the United States(48). The treatment guidelines by European Society of Cardiology/European Society of Hypertension were released in 2018 (25) while the treatment guidelines by American Heart Association were published in 2017 (48). The ESC/ESH guidelines recommended that BP should be classified, and hypertension should be graded as follows (described in Table 1-4). The guidelines also recommended assessment for target organ damage, treatment target through lifestyle and pharmacotherapy.

Table 1-4 Classification of blood pressure according to ESH, AHA

ESH 2018			
Category	Systolic (mmHg)		Diastolic (mmHg)
Optimal	<120	and	<80
Normal	120–129	and/or	80–84
High normal	130–139	and/or	85–89
Grade 1 hypertension	140–159	and/or	90–99
Grade 2 hypertension	160–179	and/or	100–109
Grade 3 hypertension	>_180	and/or	>_110
Isolated systolic hypertension	>_140	and	<90
AHA			
Normal	<120 mm Hg	and	<80 mm Hg
Elevated	120-129 mm Hg	and	<80 mm Hg
Hypertension: stage 1	130-139 mm Hg	or	80-89 mm Hg
Hypertension: stage 2	≥140 mm Hg	or	≥90 mm Hg

Table is reproduced from (25, 48)

1.1.3 Cardiovascular risk, Target organ damage and comorbidities and assessment

Hypertension is a major risk factor for ischaemic and haemorrhagic stroke, myocardial infarction, heart failure, atrial fibrillation, chronic kidney disease, cognitive decline and premature death. Moreover, SBP >140 mmHg accounts for around 70% of the mortality and disability burden. The largest number of SBP-related deaths per year occur due to ischaemic heart disease (4.9 million), haemorrhagic stroke (2 million), and ischaemic stroke (1.5 million) (49). DBP tends to decline from midlife as a consequence of arterial stiffening; consequently, SBP assumes even greater importance as a risk factor from around 50 years onwards (50). Untreated hypertension is usually associated with a progressive rise in blood pressure. The relationship between BP and risk of cardiovascular events is continuous from a systolic BP (as low as 115 mmHg) and it has been shown in all ages and ethnic groups (51-53). The vascular and renal damage that hypertension may cause can culminate in a treatment-resistant state. People with treated hypertension have a clinic blood pressure target set to below 140/90 mmHg if aged under 80 years, or below 150/90 mmHg if aged 80 years and over.

1.1.3.1 Target organ damage

Hypertension mediated organ damage (HMOD) refers to structural or functional changes in arteries or end organs (heart, blood vessels, brain, eyes, and kidney) that are caused by elevated BP. Presence of any of these changes is a marker of pre-clinical or asymptomatic CVD and indicates increased CV risk especially when the damage affects multiple organs (54). Some forms of HMOD can be reversed by using antihypertensive therapy especially if used early; however, HMOD can be irreversible with long-standing hypertension regardless of BP control. Nevertheless, antihypertensive therapy is still vital because it may delay the progression of HMOD and decreases the elevated CV risk (55). Furthermore, considering both the hypertension and cardiology guidelines together, the presence of HMOD is unlikely to additionally influence BP treatment in hypertensive patients with documented CVD, diabetes, CKD, grade 3 hypertension, or familial hypercholesterolaemia as they are already at high or very high CV risk (>10% risk of a fatal event) and should already be receiving

lifestyle interventions, BP-lowering medications, statins, and antiplatelet therapy, to reduce their CV risk (56).

According to the ESH guidelines, basic screening for HMOD is recommended for all hypertensive patients and more detailed assessment is performed when the presence of HMOD might influence treatment decisions. The main advantage of detecting HMOD is that it may reclassify a patient's risk assessment from low to moderate or from moderate to high risk. In addition, specific manifestation of HMOD (e.g. LVH or CKD) might affect the choice of drug treatment for hypertension. The compelling indications for specific antihypertensive drugs are presented in Table 1-5, where some of the compelling indications are HMOD In patients more likely to have HMOD (i.e. those with high grade 1 or grade 2–3 hypertension), initial treatment with a combination of two drugs is recommended, usually an ACEI/ARB together with CCB/thiazide-type diuretic, which would be the optimal treatment for all manifestations of HMOD. More details on drugs for hypertension in different clinical scenarios are provided in the next section (Hypertension management).

1.1.3.2 The heart in hypertension

Chronically increased left ventricular (LV) workload in hypertensive patients can result in left ventricular hypertrophy (LVH), impaired LV relaxation, left atrial enlargement, increased risk of arrhythmias, especially atrial fibrillation (AF), and an increased risk of heart failure.

1.1.3.3 The kidney in hypertension

Hypertension is the second most important cause of CKD after diabetes. In addition, it can also be the presenting feature of asymptomatic primary renal disease. Changes of renal function is detected by an elevated levels of serum creatinine. However, creatinine is an insensitive marker of renal impairment because a severe deterioration in renal function is required before serum creatinine rises. Moreover, BP reduction by antihypertensive drugs often cause an acute increase in serum creatinine by as much as 20–30% especially with (RAS) blockers. The diagnosis of hypertension induced renal damage is based on the finding of reduced renal function and/or the detection of albuminuria. (57).

1.1.3.4 The brain in hypertension

Hypertension increases the risk of brain damage, transient ischaemic attack (TIA) and stroke are the most acute clinical manifestations. In the asymptomatic phase, brain damage can be detected by magnetic resonance imaging (MRI) as white matter hyperintensities, silent microinfarcts, (most of which are small and deep, i.e. lacunar infarctions), microbleeds, and brain atrophy. White matter hyperintensities and silent infarcts are associated with an increased risk of stroke and cognitive decline due to degenerative and vascular dementia(58).

1.1.3.5 Hypertensive retinopathy

The changes in hypertensive retinopathy result from damage and adaptive changes in the arterial and arteriolar circulation in response to the high blood pressure. The use of fundoscopy to detect of retinal haemorrhages, microaneurysms, hard exudates, cotton wool spots, and papilloedema indicates severe hypertensive retinopathy and highly predictive of mortality. Fundoscopy should be performed in patients with grade 2 or 3 hypertension or hypertensive patients with diabetes, in whom significant retinopathy is more likely(59).

Management for hypertensive patients with certain comorbidities will be discussed later in this chapter.

1.2 Hypertension management

The ESC/ESH guidelines recommend that the first objective of treatment is to lower BP to <140/90 mm Hg in all patients. In addition, if treatment is well tolerated, then treated BP values should be targeted to 130/80 mm Hg or lower in most patients. For patients <65 years receiving BP-lowering drugs, lowering SBP to 120-129 mm Hg is recommended while for patients ≥65 years receiving BP-lowering drugs, a target SBP range of 130-139 mm Hg is recommended. Further, lifestyle interventions and drug treatment are two well-established strategies to lower the BP. Device-based therapy is also emerging but so far, it has not been proved to be an effective treatment option.

Benefit of reducing BP

A meta analyses of RCTs which involved several 100,000 patients have concluded that a reduction of 10 mmHg in SBP or 5 mmHg in DBP is associated with 20% reduction in all major CV events and 10 to 15% in stroke, 20% in coronary events and 40% in heart failure (61 ,60).

1.2.1 Non-pharmacological treatment

According to the international guidelines, life-style modification is recommended for all patients who are suffering from hypertension or prehypertension. These modifications include salt restriction, increasing body exercise and decreasing body-weight while maintaining adequate BMI, smoking cessation and moderate alcohol consumption (5).

1.2.1.1 Salt restriction

The current recommendation is to reduce salt intake to <5g/day in hypertensive patients (39). A causal association has been shown between salt consumption and BP elevation. Excessive salt consumption can lead to resistant hypertension. In various studies, it has been demonstrated that reduction in salt intake causes BP reduction. Greater BP reduction is seen in patients who are old, black, with diabetes or metabolic syndrome. A meta-analysis of 167 studies investigated the effects of a decreased salt intake of 120 mmol less than the usual intake (150 mmol) on BP levels in normotensive and hypertensive Asian, black and Caucasian

patients. This caused SBP/DBP to decrease by -1.27/-1.68 mmHg in Asians, -4.02/-2.01 mmHg in Blacks and -1.27/-0.05 mmHg in Caucasians. The reductions of SBP/DBP were higher in hypertensive patients -10.21/-2.60, -6.44/-2.40 and -5.48/-2.75 mmHg in Asians, Blacks and Caucasians respectively. A major trial, Trials of Hypertension Prevention—Phase I (TOHP I) demonstrated that for a decrease of 100 mmol/24 h in urinary Na, the overall adjusted decrease in BP was 1.4/0.9 mm Hg (62). In addition, 10- to 15-year observational follow-up of TOHP showed that dietary salt restriction significantly reduced long term risk of cardiovascular event by 25% (63).

1.2.1.2 Diet

The Dietary Approaches to Stop Hypertension (DASH) diet showed that a diet consisted of fruits, vegetables, low-fat dairy products and low in total saturated fats decreased BP by 5.5/3.0 mmHg (64).

1.2.1.3 Weight reduction

Studies have shown that weight loss results in the lowering of BP. A meta-analysis indicated that an average of 5.1 kg decrease in body weight, resulted in decrease of SBP/DBP 4.4/3.6 mmHg respectively (65). Also, higher body mass index increases the probability of total mortality. According to a meta-analysis of 57 prospective studies involving 894,576 subjects, it was concluded that every 5 kg/m2 of BMI above the 22.5-25 kg/m2 range is associated with a 30% increase in total mortality with mortality at a minimum for this range (66). Therefore, in order to achieve weight loss and cardiovascular protection exercise should be combined with diet. A systematic review of 9 studies involving 5168 subjects demonstrated that combining physical activity with diet in weight control strategies caused an improvement in weight and reduced diabetes incidence in people with pre-diabetes.

1.2.1.4 Smoking cessation

Studies have shown that hypertensive patients that smoke have an increased risk of total, haemorrhagic and ischemic stroke. Smoking releases Nicotine which causes the arousal of the sympathetic nervous system, as a result it leads to the release of epinephrine and norepinephrine causing a hypertensive effect. Also,

the use of Cigarette causes BP to increase 4/3 mm Hg. The cardiovascular risk is related to the number of cigarettes smoked (67).

1.2.1.5 Moderate alcohol consumption

Xin et.al indicated a dose-response relationship between reducing alcohol intake and the decrease in blood pressure level. In the study, it was observed that the alcohol reduction group had a decrease in BP of 3/2 mm Hg (68, 69). The ESC/ESH guidelines recommend restriction of alcohol to <14 units/week for men and <8 units/week for women, along with avoidance of binge drinking (39).

1.2.1.6 Regular body exercise

A meta-analysis was conducted to determine the effect of aerobic exercise on blood pressure. It demonstrated an average reduction of 4 mm Hg in systolic blood pressure and 3 mm Hg in diastolic blood pressure (70). Patients, whether hypertensive or pre-hypertensive should exercise for about 30 min on most days of the week (5). The PREMIER clinical trial was one of the first trials to show that all of the recommended lifestyle changes can be combined to reduce blood pressure successfully.

1.2.2 Pharmacological treatment

There are various classes of drugs that are used for the treatment of hypertension, called antihypertensive drugs. Each class of antihypertensive drugs has its own unique mechanism of action and chemical structure. The main classes are beta-blockers (BB), diuretics, calcium channel blockers (CCB), angiotensin converting-enzyme inhibitor (ACEI), angiotensin II receptor blockers (ARB), and alpha-blockers.

1.2.3 Antihypertensive drug classes

1.2.3.1 Beta blockers

It refers to a group of medications that block the action of endogenous catecholamines epinephrine (adrenaline) and norepinephrine (noradrenaline) on beta-adrenergic receptor which is a part of the sympathetic nervous system (71). The major adrenergic receptors located in the cardiovascular system are ß1, ß2,

and α1 receptors (72). The beta blockers are classified based on their selectivity and vasodilatory properties into first, second and third generation. First generation beta-blockers are non-selective because they have identical affinity towards ß1 and ß2 receptors (e.g. propranolol). Second generation beta blockers have more affinity to ß1 than ß2 therefore they are called selective beta blockers (e.g. atenolol). The third generation of beta-blockers are known for their intrinsic vasodilatory properties (e.g. nebivolol). BB have been used as first-line therapy for hypertension in several hypertension guidelines, however they are no longer the first choice for initial treatment of most patients (73).

A meta-analysis showed that when compared with placebo, BBs significantly decreased the risk of heart failure, stroke and major CV events in patients with hypertension (74). When compared with antihypertensive classes, BBs are usually equivalent in preventing major CV events but less effective in stroke prevention. In addition, RCTs based on HMOD demonstrated that BBs are less effective than RAS blockers and CCBs in preventing or regressing LVH, small artery remodelling and aortic stiffness (75)

1.2.3.2 Diuretics

Diuretics (also known as water pills) are drugs that cause diuresis, eliminating excess salt and water. They are effective in reducing blood pressure. They are categorized into different types based on their mechanism and site of action. The most common types of diuretics are loop diuretic, thiazide, potassium sparing diuretics and osmotic diuretics.

Loop diuretics work at the ascending limb of the loop of Henle in the kidney (e.g. Furosemide) and are effective in patients with impaired kidney function (76).

Thiazide diuretics act on the distal convoluted tubules in the kidney by inhibiting the reabsorption of sodium and chloride (e.g. Hydrochlorothiazide). They are recommended as first line of therapy in the European (ESC/ESH) guidelines and US guidelines (JNC VIII). However NICE guidelines recommend that thiazide should be used as a first line of therapy only if CCBs are not suitable or if patients have high risk of developing heart failure or have oedema. Although

thiazides are cheap and effective, they are not prescribed as often as novel drugs as they are associated with increased risk of new-onset diabetes. Therefore, they are recommended for patients over 65 years old where advantage of controlling systolic blood pressure outweighs the risk of new-onset diabetes (77).

Potassium sparing diuretics work either by directly blocking sodium channels (e.g. amiloride) or by competing with aldosterone for intracellular cytoplasmic receptor site (e.g. Spironolactone) (78). They do not facilitate the secretion of potassium in urine. Additionally, osmotic diuretics cause inhibition of water reabsorption in the proximal convoluted tubule and the descending loop of Henle (e.g. Mannitol and eplerenone). Hence, diuretics are effective in preventing all types of CV morbidities and mortality and are more effective than other antihypertensive drugs at preventing heart failure(74).

1.2.3.3 Calcium channel blocker

Calcium channel blockers work by blocking the movement of calcium through calcium channels. They are classified into three groups depending on their chemical structure Dihydropyridines (e.g. amlodipine, felodipine), Benzothiazepines (e.g. diltiazem) and Phenylalkylamines (e.g. verapamil). They are recommended to be used as a first-line treatment either as monotherapy or in combination with other classes such as ACE inhibitors, thiazide-type diuretics, or angiotensin II receptor antagonists for all patients regardless of age or race (79). CCBs have a greater effect on stroke reduction than expected reduction in the BP. CCBs are reported to be more effective than BBs in slowing the progression of carotid atherosclerosis, and in decreasing LVH and proteinuria (74).

1.2.3.4 ACE inhibitors

Angiotensin is a peptide hormone which is a part of the renin–angiotensin system that regulates BP. It is responsible for vasoconstriction leading to an increase in blood pressure. In addition, it stimulates the release of aldosterone from the adrenal cortex causing sodium retention by the kidneys. ACE inhibitors prevent the conversion of Angiotensin I to Angiotensin II by inhibiting the activity of

angiotensin-converting enzyme. This causes a decrease in the arteriolar resistance and increase in venous capacity, lower the cardiac output, cardiac index, stroke work, and volume, lower resistance in blood vessels in the kidneys; and leads to increased natriuresis. In addition, bradykinin increases due to decreased inactivation by ACE (80). According to their chemical structure, they are divided into three groups that are Sulfhydryl-containing agents (e.g. Captopril), Dicarboxylate-containing agents (e.g. Ramipril) and Phosphonate-containing agents (e.g. Fosinopril) (81). The AASK trial demonstrated that ACE inhibitors are more effective than calcium channel blockers and beta blockers in reducing the decline in kidney function (82). Therefore, they should be considered as the first choice for patients with chronic kidney disease regardless of their race or diabetic status. The common side effects of ACEI are cough and angioedema which occur due to the other effects of ACE inhibition, such as degradation of bradykinins and prostaglandins (83).

1.2.3.5 ARB

The mechanism of action of Angiotensin II receptor blockers (ARBs) is competitive antagonism of the angiotensin II receptors. They displace angiotensin II from the angiotensin I receptor. As a result, it leads to their blood pressure lowering effects that occur due to their inhibiting action for angiotensin II such as vasoconstriction, aldosterone release, catecholamine release, arginine vasopressin release, water intake, and hypertrophic response (e.g. Candesartan and Losartan) (84). ARBS are significantly associated with lower treatment discontinuation rates for adverse events compared to other antihypertensive therapies (85). ACE I and ARBs should not be used together for hypertension management because there is no added benefit on outcomes but an increase in adverse renal events (86). ACE I and ARBs decrease albuminuria more than other antihypertensive classes and are effective at delaying the progression of diabetic and non-diabetic CKD (75).

The following table indicates the compelling and possible contraindications to each antihypertensive class

Table 1-5 The compelling indication and contraindications to each antihypertensive class

Drug	Compelling Indication	Contraindication
Diuretics	• Heart failure • High coronary disease risk • Diabetes • Recurrent stroke prevention	• Gout
Beta-blockers	• Heart failure • Post myocardial infarction • High coronary disease risk • Diabetes	• Asthma • Bradycardia
CCB	• High coronary disease risk • Diabetes	• Tachyarrhythmia • Pre-existing severe leg oedema
ACE inhibitors	• Heart failure • Post myocardial infarction • High coronary disease risk • Diabetes • Chronic kidney disease Recurrent stroke prevention	• Pregnancy • Previous angioneurotic oedema • Hyperkalaemia • Bilateral renal artery stenosis
ARBs	• Heart failure • Diabetes • Chronic kidney disease	• Pregnancy • Hyperkalaemia • Bilateral renal artery stenosis

1.2.3.6 Vasodilators

Vasodilators cause the relaxation of the smooth muscle of arteries by directly working on them (e.g. Hydralazine). They are only used when other medications have failed or in case of hypertensive emergencies (87, 88).

1.2.3.7 Other

There are other new classes of drugs to manage hypertension such as Alpha-2 adrenergic receptor agonists (e.g. Moxonidine and Methyldopa), Aldosterone receptor antagonists (e.g. eplerenone) and Renin Inhibitors (e.g. Aliskiren) (89).

1.2.4 **Starting antihypertensive drug**

The guidelines are focusing on stepped care approach by initiating the treatment with various monotherapies and sequentially adding other drugs until BP is controlled.

All guidelines agree that the patients with grade 2 or 3 hypertension and patients with grade 1 hypertension with high CV risk should receive antihypertensive drug treatment alongside lifestyle interventions. The ESC/ESH guidelines recommend the combination treatment for most hypertensive patients as initial therapy, preferably including a renin-angiotensin system (RAS) blocker (angiotensin-converting enzyme ACEI or ARB with CCB or diuretic; other combinations of the five major classes can be used. Combination with beta blockers is recommended with any of the other major drug classes when required by specific clinical situations require it.

It is recognised by all guidelines that <50% of patients that are treated for hypertension currently achieve a target office SBP of <140 mmHg. As a result, some differences are encountered by the European and American guidelines in their approach towards BP targets. The ESC/ESH guidelines recommend that the first objective should be the lowering of BP to <140/90 mmHg in all patients. Provided that the treatment is well tolerated, treated BP values should be targeted to 130/80 mmHg or lower in most patients, although in some groups the evidence is less compelling. In older patients (>65 years), SBP should be targeted between 130 and 140 mmHg, and DPB <80 mmHg. Treated SBP should not be targeted to <120 mmHg. In contrast, the US guidelines recommend the BP goal for all hypertensive patients, including those with diabetes, should be <130/80 mm Hg and patients over 80 years should have <150 mm Hg systolic BP (16).

The failure to achieve BP control in a majority of hypertensive patients, despite a series of treatment guidelines has resulted in the recognition that recommended treatment strategies are not working. The new guidelines are recommending more stringent BP targets in both younger and older patients which will make it even more challenging to control the BP rates. There are various reasons for the failure of current treatment strategies to achieve good BP control rates and these are listed below (39)–

- Efficacy of pharmacological therapies
- Physician or treatment inertia
- Patient adherence to treatment
- Insufficient use of combination treatment
- Complexity of current treatment strategies

These is accruing evidence that therapeutic nonadherence (not following recommended medical or health advice, including failure to “persist” with medications and recommended lifestyle modifications) is a much more important factor than previously recognised for BP control. The focus of this thesis is to study adherence in hypertensive patients.

1.2.5 **Antihypertensive classes and specific conditions**

Some of studies showed that certain antihypertensive classes are more effective than others at reducing BP with some patient’s characteristics or have the ability to prevent more risks.

1.2.5.1 Diabetes mellitus

Several RCTs in patients with diabetic nephropathy, non-diabetic nephropathy or CVD showed that RAS blockers exhibited a greater effect at lowering albuminuria compared to a placebo and other antihypertensive drugs. Moreover, RAS blockers have been shown to be effective in protection against incident microalbuminuria. For these reasons, RAS blockers have been recommended for use with diabetes mellitus, especially in patients who have proteinuria or microalbuminuria, and in hypertensive patients with nephropathy (97, 98). Combination of an ACE inhibitor with an ARB is contraindicated because it is accompanied by an excess of renal adverse events. Recent RCTs have shown that the selective inhibitors of sodium glucose cotransporter 2 in the kidney (SGLT2 inhibitors) can reduce office and ambulatory BP even when people are treated with antihypertensive drugs and can reduce the progression of CKD (90).

1.2.5.2 Metabolic syndrome

Calcium antagonists and RAS blockers are preferred to use for patients with metabolic syndrome because they have been shown to improve insulin

sensitivity, or at least not worsen it. However, beta blockers (except for the vasodilating effects) have been shown to worsen insulin sensitivity.

Hypokalaemia which may result from diuretic side effects worsens insulin sensitivity. Therefore, potassium-sparing drugs should be used in association with diuretics in metabolic syndrome to prevent hypokalaemia (99). The recent PATHWAY-3 clinical trial showed that amiloride has lower rates of dysglycemia tested by the oral glucose tolerance test compared to hydrochlorothiazide with similar BP lowering effects supporting the value of using potassium sparing diuretics in patients with metabolic syndrome (91).

1.2.5.3 Cerebrovascular disease (Stroke prevention)

Stroke prevention is the most consistent benefit of antihypertensive therapy, it has been observed in almost all large RCTs using different drug regimens, all regimens are considered acceptable for stroke prevention provided that BP is effectively reduced. Meta-analyses suggest that calcium antagonists may have a slightly greater effectiveness on stroke prevention (100). However, the incidence of stroke has also been reduced by using diuretics or a combination of diuretics and ACEIs (101). Meta-analyses found that ARBs have a greater effectiveness on cerebrovascular protection than other antihypertensive agents (102). All of these medications are acceptable for stroke protection, on the condition that BP is effectively decreased. In acute intracerebral haemorrhage, acute BP lowering to <140/90 is not recommended except when SBP is greater than 220 mmHg. In acute ischaemic stroke, for patients receiving IV thrombolysis BP should be maintained at <180/105, while in those not receiving thrombolysis, BP should be by 15%, with close monitoring, during the first 24 h after stroke onset (92). Prevention of stroke is a consistent benefit of antihypertensive

therapy and has been observed in all large RCTs using different drug regimens except for beta-blockers. The use of beta-blockers for stroke prevention is not recommended unless there is compelling indication for its use (24).

1.2.5.4 Heart disease

A. Coronary heart disease

There is strong epidemiological evidence for the beneficial effect of BP reduction in reducing CAD events. For every 10 mmHg reduction in SBP, there is a 17% reduction of CAD (93). In hypertensive patients, beta-blockers have been reported to have a greater protective effect post myocardial infarction (103). In patients with symptomatic angina, beta-blockers and calcium antagonists are the preferred antihypertensive agents. Highly beneficial effects of an ACEI have been shown with acute myocardial infarctions (104). All antihypertensive drugs have similar effects in cases of other CHDs (103). There is inconsistent evidence for a J-curve relationship between achieved BP and CAD risk and the recommendation is to achieve a target BP <130/80 but not <120/80 (94-97).

B. Heart failure

ACEIs, beta blockers and diuretics prevent heart failure better than CCB (100). In ALLHAT, diuretics demonstrated a greater effectiveness in preventing heart failure than ACEI. However, this may have resulted from a study design that depended on the initial diuretic withdrawal, which could lead to a small excess of early heart failure incidences (105). Hospitalizations for heart failure were not decreased in patients receiving ACEIs below the levels of placebo patients according to the PROFESS and TRANSCEND trials (106). In patients with heart failure or severe LVH, it is preferable to use ACEIs, ARBs, beta blockers and/or mineralocorticoid receptor antagonists to decrease hospitalization and mortality (107). In a randomized trial of patients with heart failure and reduced ejection fraction, the SGLT2 inhibitor dapagliflozin markedly reduced mortality and worsening heart failure. Remarkably, these benefits seemed to be similar in people with and without diabetes and along with the BP lowering effects of SGLT2 inhibitors there is a potential for new heart failure preventive strategies using this class of drugs in hypertension (98).

C. Left ventricular hypertrophy

BP reduction is associated with regression of LVH and consequent reduction in CV events. Randomized comparative studies showed greater effects of ARBs, ACEIs and CCB at reducing LVH compared with beta-blockers, but they have similar BP reductions (108).

D. Atherosclerosis

ACEIs and CCB delay the progression of atherosclerosis to a greater extent than beta blockers or diuretics (109). CCBs and ACEI have a greater effect on carotid intima-medial thickness regression compared to beta-blockers or diuretics.

The advantages of ACEIs was shown in more than 4,000 patients with peripheral artery disease (PAD) who had enrolled in the Heart Outcomes Prevention Evaluation (HOPE) study (110). The Appropriate Blood pressure Control in Diabetes (ABCD) demonstrated that a major benefits of ACEIs or CCB for PAD patients who had intensive BP reductions (<130/80 mm Hg) (111).

According to meta-analysis and meta-regression analysis pulse wave velocity (PWV) that is used to measure arterial stiffness, was decreased by ACEIs and ARBs (112). However, the superiority of ACEIs and ARBs to other antihypertensive drugs in reducing arterial stiffness is not clear due to the lack of properly powered and high-quality RCTs.

1.2.6 **Monotherapy and combination therapy**

Despite the availability of a various antihypertensive drugs as effective and safe treatment, poor BP control remains common worldwide (113). The ability of a single drug to reach target blood pressure levels (140/90 mmHg) are rare (114). Combination therapy of two or more medications has greater efficacy to decrease blood pressure than a single drug. A recent meta-analysis on 11,000 participants from 42 studies has concluded that a greater BP reduction was achieved by combining two drugs from two different classes of antihypertensive drugs than doubling the dose of a single drug (115). The Assessment of Lescol in Renal Transplantation (ALERT) study demonstrated the ability of combination

therapy to decrease BP better than monotherapy, low-dose combined antihypertensive therapy (ACEI and CCB) improved measures of cardiovascular structure and function compared with high-dose individual drug with either component. In addition, combination therapy results in achieving BP target more promptly. Several clinical trials have shown the importance of achieving these BP quickly, combination therapies are required for patients especially at high cardiovascular risk to achieve their treatment goals (116).

Combination therapy of different classes advantages include: first, it has different and complementary mechanisms of action. Second, favourable tolerance profile because the combination of complementary mechanisms of action reduce their individual side effects. Third, the combination therapy can decrease blood pressure greater than that of either component of the combination. Furthermore, combination therapy allows blood pressure target to be achieved earlier than monotherapy which is necessary in some cases such as patients with high risk cardiovascular disease.

1.2.7 **Fixed low dose approach**

The aim of treating hypertension is to maximize therapeutic efficacy without side effects. The accepted approach is to start therapy with a low dose of a single drug and then titrate it upward as needed to achieve a better therapeutic effect. However, higher doses of an individual drug increase the frequency and severity of side effects.

A fixed low-dose combination therapy with inexpensive BP lowering drugs has the potential to address several barriers to improve BP control. Low-dose combinations improve efficacy (117), side effects are minimized at half-standard doses (118), and the benefits are additive across blood pressure–lowering medication classes. In addition, fixed-dose combinations can improve medication adherence due to regimen simplification (119), thereby decreasing patient, physician, and health system barriers related to multiple visits and prolonged titration schedules (120). The most commonly used drug combinations are as follows: (1) diuretics with potassium-sparing agents; (2) beta blockers with diuretics (3) ACEI with diuretics (4) ARB with diuretics; and (5) ACEI with CCB.

Recent trials of triple therapy have demonstrated benefits among patients with severe hypertension not controlled by dual therapy (121).

A recent randomized clinical trial of 700 patients compared the use of low-dose triple combination antihypertensive medication vs usual care for BP Control. The combination therapy included (20 mg of telmisartan, 2.5 mg of amlodipine, and 12.5 mg of chlorthalidone). 70% of patients in the triple combination pill therapy group achieved a BP of less than 140/90 mm at 6 months compared with 55% of patients in the usual care group ($P < .001$). Use of a low-dose triple combination blood pressure–lowering pill for initiation of treatment or escalation from monotherapy increased the proportion of patients with hypertension reaching their blood pressure targets.

Despite the value of using combination therapy, there are differing recommendations in guidelines. The ESH/ESC guidelines recommend that initial therapy for the majority of patients with hypertension should be with a combination of two drugs, not a single drug (97). In contrast, the latest 2019 NICE guidelines advise a stepped care approach with monotherapy. The NICE guideline committee felt that there was not enough evidence to determine confidently the benefits or harms of starting treatment with dual therapy (24). The only trial that showed benefit of commencing dual therapy was PATHWAY-1, but the limitations of this trial were the short follow-up period of 1 year which is not suitable to assess the impact on morbidity and mortality (99).

1.3 Adherence

Patient adherence is defined as an “active, voluntary, and collaborative involvement of the patient in a mutually acceptable cause of behaviour to produce a therapeutic result” (100, 101). While according to WHO, it is defined as “the extent to which a person’s behaviour—taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider” (102). Adherence rate is described as the percentage of the prescribed drugs that is taken by patients during a specific period. Adherence may include information regarding the dose such as number of pill or information about the time (intake of medication in the correct prescribed period). Adherence rates are usually higher in patients with an acute disease in comparison to patients with a chronic disease. In addition, there is no general agreement on the proper adherence percentage, in clinical trials some researchers consider more than 80% to be acceptable while others consider more than 95% to be required especially in case of serious disease. Non-adherence can lead to deterioration in health and progression of disease state, or it can cause death. It can also increase the cost burden on the health care system (103). There is no clear data regarding the prevalence of adherence to medication, it can range from 3% and up to 65 % (104, 105).

1.3.1 Factors affecting adherence

Medication adherence for the patients on chronic treatment may change with time due to several factors related to five areas which are disease, medications, patient and close relatives, demographic and socioeconomic factors, and health care system (106, 107).

1.3.1.1 Disease (condition related factors)

Disease-related factors are related to the presence of symptoms, the severity of the disease and the rate of progression, the availability of an effective treatment, level of disability (psychological, physical social and vocational). Long term treatments for several chronic diseases often decrease significantly over time especially if the patient have fewer or no symptoms. Their effect depends on how they influence patients’ risk perception, significance of given drug regimen and priority placed on adherence.

1.3.1.2 Medication

The complexity of a drug regimen which includes total number of medications and their required daily doses, mode of administration, duration of the therapy, timing of doses and the presence of side effect and their severity, can affect patients' adherence. In addition, non-adherence may occur at different stages of the medication taking process. Non-adherence may take place at the beginning of the treatment, during treatment if the patient shows suboptimal implication of their prescribed regimen or they discontinue the treatment early (108).

1.3.1.3 Social and demographic

Several factors have an effect on adherence including low level of education, illiteracy, unemployment, poor socioeconomic status, poverty, low level of education, unemployment, absence of social support networks, unstable living conditions. The other factors related to treatment are long distance from treatment centre, expensive transportation, expensive treatment, changing environmental situations, culture and lay beliefs about illness and treatment, and family dysfunction.

1.3.1.4 Patients and close relatives

Patient factors can be either intentional or unintentional, unintentional factors can be due to most common patient forgetfulness, misunderstanding of the instructions or presence of external distraction. On the other hand, intentional causes occur when patients intentionally stop taking their prescribed drugs due to different underlying causes such as patient concern regarding side effects for example sexual dysfunction, lack of beneficial effect of a drug, a less severe disease state and feeling well, lack of agreement and trust with their treating physician, patient concern regarding the medication cost and availability. Also, physical impairments and cognitive limitations may increase the risk for nonadherence in older adults such as lack of knowledge about the disease and the reasons for which medication is needed, lack of motivation, low self-efficacy, and substance abuse.

Hawthorne effect

Hawthorne effect also known as observer effect is a behavioural reaction in which individuals change and modify an aspect of their behaviour as a response to their awareness of being observed or participating in an experiment. If patients are aware that they are being monitored for taking their medication, they modify their behaviour which can cause an increase in adherence (109).

1.3.1.5 Health care system

Clinician factors may include prescribing a complex drug regimen that is hard for the patient to follow, a poor relationship with their patient and not involving them in decision-making, improper explanation of the correct way to take a drug and how do they work, their side effects and the disease state, no concern regarding patient lifestyle or financial status, inadequate follow-up and poor empathy. The health care system can also affect patient adherence, by using a restricted formulary or using different formulary. Further, there has been a very limited development for a novel drug class that is specific for treating arterial hypertension. Instead pharmacological advances in hypertension treatment focuses on the development of multiple single pill drug combinations to simplify the drug regimen and to improve its efficacy.

Improving the relationship between the patient and physician, and the relationship between patient and health care system will lead to a good adherence. The relationship of the doctor-patient is one of the most important health care system-related factors impacting adherence. A good relationship between the patient and health care provider, which features encouragement and reinforcement from the provider, has a positive impact on adherence. Poverty or lack of communication concerning the benefits, instructions for use, and side effects of medications can also contribute to non-adherence, especially in older adults with memory problems. Long term drugs administration for many chronic illnesses and adherence to such treatment regimens often decline significantly over time. This often happens when patients have few or no symptoms and the absence of symptoms is a barrier for people to take their medication. It is important for the patient to understand the illness and the consequences if it is left untreated.

1.4 Adherence assessment

There are several reasons why adherence to medications is measured such as to identify medication taking behaviour, beliefs and barriers that affect adherence. It can provide an assessment for the medication because unrecognized non-adherence can lead to an underestimation of possible treatment effects. In addition, to determine the influence of adherence in people with a specific disease state such as hypertension or diabetes. Moreover, it assists in identifying individuals that require further education or support to improve their medication use.

Adherence is assessed using 2 different methods, either indirect or direct methods each has its own advantages and disadvantages

1.4.1 Indirect methods:

There are several indirect methods used such as self-reports, electronic measures, pharmacy refills, and prescription claims databases.

1.4.1.1 Self-reports

The self-reports include various options such as questionnaires, interviews, and diaries (where information about medication taking is provided by the patient or their family members/caregivers). They are considered as the most practical approaches to measure adherence. Also, they are commonly used in clinical and research settings because of their low cost, flexibility, low staff and respondent burden, relative unobtrusiveness and time efficiency (110, 111). They can be used in several ways that are self-administration, face-to-face interviews, telephonic interviews and computer applications. A lot of these measures differ from each other as some of these measures are developed for a specific disease or targeting a certain patient population, used in a different setting (e.g. in clinics or clinical trials). They can also differ in their question, measurement scale, and format. Garfield et.al (2011) was able to identify 58 self-report measures which consisted of 1–21 items, using Likert or visual analogue scales (112). The limitation for self-report is an overestimation which is caused by two major biases. First, Social desirability where the patient provides positive feedback answers to what the interviewer expects. Second, memory bias where

the patient is unable to recall the missed dose (113, 114). There are a large number of adherence measures which are suitable to use in clinical settings and research. Many well-validated adherence measures have been highly correlated with objective measures of adherence in a variety of patient population. An ideal scale should be easy to administer and should be able to correctly identify medication behaviours. A systematic review conducted by Nguyen et.al (115) explored and assessed different scale. They categorized self-adherence scale into 5 groups according to the information they were trying to obtain. The first group observed only the medication-taking behaviour. The second group searched for barriers to adherence and medication-taking behaviour. The third group searched for only the information regarding barriers to adherence while the fourth group looked only at the beliefs associated with adherence. The last group assessed both barriers and beliefs associated with adherence. Further, to identify patients who were not adherent, most scales classified adherence by determining the overall score and dividing the population into two groups: adherent and non-adherent. Other scales categorized non-adherence into more than 2 groups such as MMAS which divided the population into high, medium and low adherence according to the correlation with BP control. The followings are examples of self-reports: (Morisky scale will be discussed in detail in chapter 4)

Self-efficacy for Appropriate Medication Use SEAMS

A multidisciplinary team with expertise in adherence and health literacy, developed the Self-efficacy questionnaire for Appropriate Medication Use. In 436 patients with coronary heart disease and other comorbidities, the psychometric properties were evaluated. The scale was composed of 13 questions and had a good internal consistency reliability (Cronbach's alpha=0.89). SEAM is a valid and reliable scale, that is appropriate to use in patient with low literacy skills. In addition, it provided a useful assessment for medication self-efficacy in patients with chronic disease (116).

Brief Medication Questionnaire BMQ

Brief Medication Questionnaire BMQ is a self-report questionnaire to screen for adherence and barriers. It is composed of three main question headings and several sub-questions. Ben et.al assessed adherence in hypertensive and diabetic

patients using BMQ. The study concluded that it performed better than other screens in identifying low adherence in patient with uncontrolled hypertension (117).

The Hill-Bone Compliance Scale

The Hill-Bone Compliance Scale addresses barriers and self-efficacy. It focuses on the patients with hypertension and is composed of 14 items in three subscales. Each item is a four-point Likert type scale. It assesses patient's patient behaviours for three important behavioural domains for hypertension treatment. First, decrease sodium intake, second, appointment keeping and finally medication taking (118). Lambert et.al validated the scale for use in South African primary health care setting, because hypertension in Black South Africans is prevalent, under-diagnosed and poorly treated. They demonstrated criterion validity and internal consistency for a modified Hill-Bone. The results were compared favourably with those from an urban African-American setting (standardized Cronbach alpha was 0.74–0.84). The study showed that many of the behavioural aspects of the fundamental elements of hypertension care and control, such as salt intake reduction, medication taking and appointment keeping are measurable across different cultures. Moreover, the study demonstrated that in different cultural groups, vigorous psychometric methods can be used effectively. In a clinical setting both concurrent and predictive validity can be rapidly assessed (119).

Adherence to Refills and Medications Scale ARMS

The Adherence to Refills and Medications scale ARMS composed of 12-item measure. It was developed, pilot tested, and administered to 435 patients with coronary heart disease in an inner-city primary care clinic. The criterion-related validity was evaluated by comparing scores with Morisky questionnaire, medication refill adherence, and blood pressure measurements. The overall internal consistency observed was high (Cronbach's alpha=0.814). Patients with low ARMS scores (which indicated better adherence) were significantly more likely to have controlled DBP and tended to have better systolic blood pressure control. When used in a patient with chronic disease, ARMS is a valid and

reliable adherence scale with good performance characteristics even among individuals with low literacy skills (120).

Visual analogue rating scale VAS

Visual analogue rating scale VAS is a single item that involves questioning individuals to consider a specified time period. A study evaluated the validity of VAS and Morisky scale among patients with uncontrolled hypertension using electronic pillbox measurement as the gold standard. The VAS scale consists of a numbered line with intervals of 10% starting from 0% to 100% for each of the medication, patients mark an "X" on the line corresponding to their estimated adherence over the specific period. The overall VAS score is calculated by averaging scores across all antihypertensive medications. The score ranges from 0% to 100%, with 100% indicating highest adherence while a score less than 80% indicates low adherence. The study concluded that VAS was modestly useful in identifying non-adherence for antihypertensive medication (121).

1.4.1.2 Electronic measures

They are medication packages with an electronic microchip attached to it that records every date and timing of the opening of the device, an example of this method is the Medication Events Monitoring System (MEMS) cap. Further, these electronic measures allow health researchers to measure drug adherence longitudinally (real time) by giving a detailed dose-by-dose description for a patient. They provide data pertaining to the date and timing of each opening of the bottle. This data can be repeated and compared over time which allows researcher to provide feedback for patients on their behaviour for each visit such as medications holidays (where the patient stop taking the medication for a period of time from days to months) and tooth brush effect (increase in adherence immediately before and after the medical visit). In addition, it demonstrated a positive association between clinical outcomes and adherence. However, the downside to electronic measures is the cost which may not be suitable for every patient. They can also have a potential positive bias by reinforcing medication intake (Hawthorne effect) (122).

1.4.1.3 Pharmacy refills

This method utilizes the administrative pharmacy refill date to determine medication adherence. It does not involve the patient; therefore, it is an objective measurement. The data offered by this method gives information about patient's prescription and the frequency of refill. In addition, it can also give information about the number of days patient went without the prescribed medication (123).

Administrative claims or pharmacy refill databases record medication dispensation events, including patient identifier, date of event, type of medication, and quantity dispensed, and less frequently daily dosage recommended. However, between two dispensing events, there is no information on how the medication has been used by the patient. From the refill data, and assuming that the patient takes the medication as prescribed, it is easy to establish the duration that the medication would have lasted. If the time interval between the two events is longer than this number, it is likely that the patient ran out of medication before re-supplying or used less during that time. If the interval is substantially longer or there is no second event, then the patient has probably finished the supply at some point and then discontinued medication.

There are four main assumptions underlying analysis of pharmacy refill data –

1. The treatment requires the use of a fixed daily dosage of medication and not taken as required.

2. All medication supplied for that patient in that period of time is recorded and the patient does not use medication from other sources, otherwise this will lead to underestimation of adherence and/or persistence.

3. The medication supplied is used by the patient it has been supplied for (if other persons use the medication, adherence and/or persistence will be overestimated)

4. Medication is supposed to be supplied at least two times during the study period.

Estimation of adherence based on refill of repeat prescriptions is a comparatively convenient method, however, no single approach has been uniformly accepted. The main outcome that can be measured is the medication possession ratio (MPR). MPR can be defined as the total days' supply of medication prescribed divided by the number of days that the patient should have been taking the medication.

The resulting MPR usually ranges from 0 to 1. A value of 1 corresponds to 100% adherence. It is possible for the results to be greater than 1 if patients get early refills or if they have only filled the medication once. However, there are concerns that this approach could overestimates a patient's adherence. Therefore, many studies cap the ratio at 100% so it prevents the result from inflation.

Another outcome is the proportion of days covered (PDC), which is the number of days on which a drug is available to the patient divided by the total number of days in the data analysis period. PDC ranges from 0 to 1 and cannot be more than 1. A value of 1 corresponds to 100% adherence. The Pharmacy Quality Alliance (PQA) recommends that PDC be used in measuring adherence. because the results are more conservative estimate, especially in cases of frequently switched medications. These outcomes define adherence as >0.8 or 80% of days covered. Depending on the condition medications such as those for human immunodeficiency viruses (HIV) and birth control may require closer to 100% adherence for effectiveness (107, 124). The disadvantages of this method are it doesn't monitor the actual consumption of the medication, it does not consider the daily intake variation and it is not applicable if the patients get their drugs from different pharmacies (independent databases) (107). Unlike all of the previous methods discussed, the utilization of administrative refill data for medication adherence determination does not require patient participation and provides objective measurement of medication adherence in a naturalistic setting. This method also allows for an evaluation of a large number of patients over an extended period of time. However, the adherence value obtained from refill data does not produce any information on medication consumption; rather,

it solely provides assessment of acquisition and possession of medication. It is assumed that patients administer the medication between the day of dispensation and the day of the refill. Since this method is based on refill of prescriptions, it is better suited to study a chronic treatment rather than short-term treatment. Also, the use of refill data may be problematic for medications that require frequent dosage changes such as anticoagulants, anticonvulsants, and immunosuppressive medications. Finally, medications cannot be purchased outside of the closed system, where all prescription refills are documented in the same database.

There are five source of bias that may lead to an over- or under- estimation of adherence and need to be considered carefully in the interpretation of results.

1. Observation period - the period over which adherence is assessed. This may start and end at a specific fill and refill date; on arbitrary start/stop dates that are set as the index or inventory date and are independent from fills and refills; or a combination of a fixed and an arbitrary date.

2. An initial/terminal gap between dates of first/last fill and arbitrary start/end dates may be present and can be quantified as a proportion of time without supply.

3. An interim gap may exist between refills when prior supply is depleted before refill supply is available.

4. The number of days' supply dispensed at any fill/refill event may vary and requires adjustments in the calculations.

5. Overlap may occur as refill precedes depletion of the quantity from a prior dispensing and leads to stock piling of accumulated supply.

1.4.1.4 Pill count

It is defined as number of units of a prescribed medication multiplied by dosage, divided by the number of tablets that should have been consumed according to dosage and number of days within analysed period. It is provided as a percentage. It is a common method to measure adherence because it is an objective method that is simple to use. However, there are limitations such as patient can discard the mediation which can cause overestimation of adherence. There is no information of the actual intake or timing. Also, the actual starting date may not be the dispensed date (125)

1.4.2 Direct method:

Direct method is more accurate than indirect method because it gives result without relying on patient information that can be obtained by measuring drug, metabolite or biological marker in a biologic sample (urine or blood) or by direct observed therapy

1.4.2.1 Direct patient observation:

It is the most accurate method to measure adherence, where the patients ingesting their medication are monitored, and their timings are recorded. However, it is difficult to use in large studies and it is relatively costly and more labour-intensive for the health care providers. In addition, patient can pretend taking the mediation and remove it from their mouth when they are no longer monitored.(103).

1.4.2.2 measuring drug or a metabolite in a biologic sample and biological markers

The presence of a drug or its metabolite in a biologic sample (urine, serum and plasma) provides an objective method to measure patient adherence. In addition, biological markers which are non-toxic and stable, easily detected substances that are added to medication can be measured. On the other hand, they are expensive, and the results may vary among individuals due to the difference in metabolism because of genetic difference.

1.4.3 Adherence in clinical trials

It was found that the rate of BP control in clinical trials is usually much higher (around 80% or more). This is due to the strict treatment protocol such as frequent regular clinical visit. The care provider and patients are more motivated to achieve the protocol's goal. Mancia et.al conducted a post-hoc analysis of the invest trial and demonstrated that the higher number of clinical visits with normal BP was related to the decrease in a clinical outcome. This indicates that there is a gap between high success rate achieved in clinical trial as compared to real life national surveys (126, 127).

1.5 Adherence studies

The following are several studies on medication adherence using different methods (MMAS-8, pill counts, MEMS, refill data and serum drug levels). The problems with studies of medication non-adherence is the heterogeneity in assessment, interventions and outcomes. Systematic reviews are limited because of these issues that result in limiting the reviews to few studies focussing on specific adherence assessment of interventions, or because the outcome studies are blood pressure changes as an indirect measure of adherence. In view of these, I have described the major studies of hypertensive medication adherence which highlights the differences and also the range of questions that investigators tried to address.

Adherence studies

Table 1-6 Adherence studies

	Author	Study type	Year	Location	Number of patients	Adherence result High	Medium	Low	Findings
1	Jung	Urine analysis	2013	Goethe-University hospital, Germany	76	36 (47%)	(30%)	(70%)	Low adherence was the most common cause of poor blood pressure control in patients with apparent resistant hypertension
2	Tomaszewski	Urine analysis	2014	hypertension centre, Leicester, UK	208	153 (75%)	34 (14.9%)	21 (10.1%)	There was a linear relationship between BP and both the numerical difference and the ratio in detected/prescribed medications. those with all medications detected had the lowest clinic SBP, DBP and 24 h mean daytime DB. DBP showed a stronger association with nonadherence to antihypertensive medications than SBP.
3	Schmieder	Urine analysis	2016	University Hospital Erlangen, Germany,	79	Baseline 44 (56%) after 6 month 52 (66%)	22 (28%) 17 (22%)	13 (16%). 10 (13%).	Nonadherence was significantly associated higher office BP and 24-hour systolic ABPM.
4	Azizi	Urine analysis	2016	DENERHTN trial, in 15 French tertiary care,	85	Active 20 (50.0%) Placebo 21 (46.7%)	13 (32.5%) 20 (44.4%)	7 (17.5%) 4 (8.9%)	The prevalence of nonadherence to antihypertensive drugs at 6 months was high ≈50% but not different between active and placebo.
5	Lawson	Urine analysis	2016	Birmingham, UK	49	88%	8%	4%	nonadherence to BP lowering therapy is common in patients with suboptimal BP control and those referred for renal denervation
6	Pucci	Urine analysis	2017	hypertension clinic at University Hospitals Birmingham NHS Foundation Trust	131	67 (51%)	43 patients (33%)	21 (16%)	After confronting with result :30% of non-adherent patients denied the results

7	Hamdidouche	Urine analysis	2017	hypertension department of the Pompidou university hospital, Paris	174	159 (91%)	12 (7%)	3 (2%)	Non-adherent had significantly higher number of prescribed antihypertensive drugs, cardiovascular drugs and SBP and DBP were higher. There was no significant association between MMAS-4 and directly measured nonadherence
8	Cuffee	MMAS	2013	(TRUST project) Birmingham, Alabama,	780	318 (41%)	350 (45%)	112 (14%)	High adherence was associated with increased age, male gender, and greater reported trust in physicians.
9	Gabrielle	MMAS	2013	Hong Kong	1,114	65.1%		32.6%	Poor adherent patients were younger, had a shorter use duration of medication, employed and those reported a self-perceived health status as poor or very poor
10	Hou	MMAS	2015	outpatient clinic of a University hospital and communities in Suzhou, China	585	34.2% had good adherence		65.8%	poor adherence was associated with increasing age, lower level of education, lower income and no retirement pension or medical insurance. higher BP level and shorter duration of hypertension.
11	Pandey	MMAS	2015	hypertension specialty clinic at the University of Texas Southwestern Medical Centre	47	40%	34%	26%	not adherent were significantly younger, more females and had significantly higher heart rate.
12	Cumming	MMAS	2016	North Carolina	495	298 (60%)		298 (40%)	Younger age, African American race And lower perceived social standing but not sex or socioeconomic were significantly associated with lower adherence.
13	Mugwano	MMAS	2016	Kampala Uganda	112	17 %		76.8%	Non adherence: lack of knowledge for the chronicity of hypertension, the cost of medication and access to the health care system.
14	Guiradoa	Pill count	2011	Barcelona, Spain	996			12%	The educational intervention had no significant impact on patients' adherence to the medication.

15	Zeller	MEMS	2007	five general practices in Bristol, UK	239	175 (73%)		11(5%)	study concluded that inadequate control might be related to pharmacological non response or insufficient intensity of the medication rather than adherence to medication
16	Grigoryan	MEMS	2012	10 primary care clinics in Texas, USA	176			61 (34.6%)	AA ethnicity, female gender and attending a publicly funded primary care clinic were associated with lower adherence.
17	Hamilton	MEMS	2003	USA	107	58%			MEMS is effective to measure medication adherence
18	Burnier	MEMS	2001	Switzerland.	41	93%			MEMS is a useful tool in improving the management of patients with refractory hypertension
19	Santschi	MEMS	2008	Switzerland	68	96%			better BP control was achieved over one year in hypertensive patients using MEMS
20	Tang	Refill	2017	Manitoba, Canada	2199	83%			Adherence was inversely associated with death.
21	Yang	Refill	2017	USA	155,597	60.8%	30.3%	8.9%	Patient that were high adherent were slightly younger, female, non-Hispanic white, taking >1 antihypertensive drug, uses statins and had fewer comorbidities.
22	Lee	Refill	2017	South Korea	38,520	68.8%	13%	18.2%	The study concluded that Hypertensive patients with intermediate or poor adherence to antihypertensive medication had 1.13 times and 1.27 times higher risk of stroke,
23	Eakin	Refill	2013	USA	21	82%			The study showed that adherence by MPR was correlated with blood pressure control, but not with MEMS or self-report
24	Corrao	Refill	2015	Italy	622 case, 3110 control	26%	14%	54%	adherence decreased the risk of hospitalization for HF in patients that were younger, older, male gender, diuretic, ACEI and ARBS.

	Mancia	Refill	2014	Italy	493,623		57%		treatment discontinuation was more common in women and in the 40–49-year decade than in older patients
	Vrijens	Refill	2008	Belgium	4783			50%	The study concluded that the most common cause for poor adherence with once a day antihypertensive medication are: early discontinuation of treatment and suboptimal daily execution of the prescribed regimens
	Krousel-Wood	Refill	2009	USA	87	58%	33%	9%	MMAS is significantly associated with pharmacy refill adherence
	Scotti	Refill	2013	Lombardia, Italy	209,650	26%		44%	low adherence were mainly female and initially had a combination of 2 or more drugs
	Qvarnström	Refill	2013	Sweden	5225			14%	determinants of discontinuation antihypertensive medication are young age, male gender, mild to moderate rise in SBP, and birth outside of Sweden
	Wijk	Refill	2008	USA, Canada and Netherlands.	9664 patients from USA, 25 377 patients from Canada and 24 603 patients from Netherland			25%	Older age, male gender and frequent use of medications were associated with low adherence.
	Bramley	Refill	2006	USA	840	629 (74.9%)	165 (19.6%)	46 (5.5%)	association between high adherence and BP control

1.5.1 Studies on adherence using biological sample (urine)

1.5.1.1 Jung et al. 2013

Jung et.al (128) assessed patient adherence through toxicological urine screening of 76 patients with uncontrollable hypertension that were on at least 4 antihypertensive drugs. Their objective was to check for non-adherence which may be the cause for uncontrolled blood pressure rather than a true resistant hypertension. It was the first study to use urine screening with HPLC to check for adherence systematically. Patients involved in the study were referred from primary care due to uncontrolled blood pressure during period from 2004 to 2011. The median number of drugs prescribed per patient was 5, Diuretics was giving to all patients, and most patients received ACI, ARBs, BB and CCB. Out of 388 drugs prescribed for the 76 patients 368 were analyzed using LC-MS. Result showed that out of 76 patient (those that met the criteria resistant hypertension) 36 patient (47%) were adherent (all the antihypertensive drugs were detected in the urine) while 40 (53%) patient were not adherent. Non-adherent patients were divided into complete (no presence of antihypertensive drugs and their metabolite (30%) and incomplete, presence of partial amount of the prescribed drug and metabolite (70%). Non adherent patients had higher systolic and diastolic blood pressure compared to adherent patients. They concluded that low adherence was the main cause of uncontrollable hypertension. In addition, incomplete adherence was more common than non-complete adherence. Measuring a single drug as a predictor of adherence for patient on multiple drug regimen may not give accurate result because the study showed that majority of patients were partially non adherent and this could lead to false adherence status. The adherence accuracy detected was (93.4%) 71 out 76 patients. Also, because many patients were on multiple drug regimen, the result for patient assessment must include all prescribed drugs to produce a reliable result. There were several drawbacks for their study: first, urine screening test was done qualitatively no quantitatively therefore, it wasn't possible to determine the accurate time when the drug was taken especially drugs with long half-life. Second, white-coat adherence where patients improve their adherence when their appointment is near, and this could have led to overestimation of adherence rate. the study was a single center study and therefore the results cannot be generalized to different populations. The cost

for HPLC was analyzed and it was found that it is comparable to the cost of adding 1 or 2 antihypertensive drugs to patient's prescription, which is the natural clinical response for a resistant hypertension. HPLC becomes more cost effective when invasive measures are considered such as renal denervation or implantable carotid body stimulator (129). Non adherence is more common than the secondary cause for resistant hypertension and therefore, it is more efficient to use HPLC to check for adherence first.

1.5.1.2 Tomaszewski et al. 2014

Tomaszewski et.al (104) used HPLC- MS urine analysis test for detecting 40 antihypertensive drugs and their metabolites to determine patient adherence. The study involved 208 patients with hypertension (attending a specialist clinical hypertension centre, Leicester, UK) during the period between 2011 and 2013. Patients were divided into 3 groups; group A 125 patient were new referral from primary care. Group B 66 patients with poor response to their prescribed antihypertensive medication. Group C 17 patient with hypertension referred for renal denervation. HPLC-MS targeted monitoring was applied to patient urine samples. Result showed that about 25% of patient were non adherent, patients with total non-adherence (no antihypertensive drugs or metabolites were detected) were (10.1%) and those partial adherence (patient with fewer drugs than prescribed) were (14.9%). The highest prevalence of non-adherence was among patient with inadequate blood pressure control (28.8%) and those referred for consideration of renal denervation (23.5%). They reported that their study was the first study on non-adherence to antihypertensive treatment among patients referred for renal denervation. Patients that were confirmed non adherent with the urine test had the highest blood pressure level (especially diastolic blood pressure) than adherent patients. The difference in the results between this study and (Jung, et.al study) is due to the difference in inclusion criteria. Tomaszewski et.al sample size was larger and diverse which reflected a better spectrum of the hypertensive population. Another reason was the median number of medications, (Jung, et.al) had 5 drugs compared to 3 in Tomaszewski et.al. They concluded that HPLC-MS of urine has many advantages because it is non-invasive and simple, it can be done prior to appointment with physician by healthcare personnel. In addition, it can provide a clear information whether the drug is present or absent. Moreover, it has an excellent specificity and

sensitivity, and a single urine test is inexpensive (it can cost around £30) and can save a lot of resources from further investigation or treatment. On the other hand, there were limitation in their study: first, they didn't have 24 h ambulatory blood pressure for 25% of patients, this has led to lower number of observation and therefore, lower power to detect association. Second, the number of patients referred for renal denervation was small. Third, Single spot urine analysis may not be able to take into account non adherent period. That is white coat effect: when patient adhere to medication when the appointment is near. Fourth, the lack of indirect method, none were used and finally, the study was done as an audit for adherence. They acknowledged the risk from unmeasured confounders and further studies were recommended on utility/ cost HP LC-MS/MS.

1.5.1.3 Schmieder et al. 2016

The aim was to investigate adherence rates to medication before and after renal denervation and its effect on blood pressure (BP) control among patient with treatment resistant hypertension TRH. The study included 79 patients with TRH that completed 6 months follow up period after renal denervation. Patients were recruited from the clinical research centre at the Department of Nephrology and Hypertension, University Hospital Erlangen, Germany. TRH was defined as office BP ≥140/90 mm Hg regardless of being treated with at least 3 antihypertensive medications including a diuretic. This was confirmed by initial 24-hour ABP monitoring (≥130/80 mm Hg) to exclude white coat effect. Average of 3 measurements was taken for office BP while ABP was measured using automatic portable devices validated according to the ESH International Protocol. Urine samples were collected at baseline and 6-month follow-up visits. The samples were analysed for antihypertensive compounds or metabolites using high-performance liquid chromatography–mass spectrometry (LC-MS/MS). Adherence was categorized as complete adherence (all prescribed drugs were detected, partial adherence (at maximum, 1 of the prescribed drugs was missing and nonadherence (≥2 of the prescribed drugs were not found in the sample ("numeric adherence"). In addition, a second analysis was conducted where adherence was defined as detection of ≥80% of the detectable drugs and nonadherence as <80% measured in the urine analysis. The mean age was 60.4±10 years and male were 57 (72%) and Number of antihypertensive drugs

prescribed 6.0 (5.0–7.0). office systolic and diastolic BP decreased by 13±22 and 7±12 mm Hg respectively (both P<0.001) and 24-hour systolic and diastolic ABP decreased by 8±16 and 5±10 mm Hg, respectively (both P<0.001). Adherence at baseline were as follow: complete 44 (56%), partial 22 (28%) and nonadherence 13 (16%). Adherence after 6 months were: complete 52 (66%), partial 17 (22%) and nonadherence 10 (13%). There was no significant change in adherence between baseline and 6-month visits (McNemar-Bowker test, P=0.362). According to cutoff of 80% of the detectable medication, adherence was found in 59 of 79 (74.7%) patients with TRH at baseline and in 61 of 79 (77.2%) at 6-month follow-up. Nonadherence was significantly associated higher office BP and 24-hour systolic ABP (P=0.317 for office BP and P=0.049 for ABP). The main limitation of the study is the small number therefore not enough statistical power (130).

1.5.1.4 Azizi et al. 2016

The objective of the study was to investigate the effect of adherence to standardized stepped-care antihypertensive treatment for resistant hypertension at 6 months. DENERHTN trial (Renal Denervation for Hypertension) which was multicentre, prospective, randomized, open-label blinded end point evaluation-controlled trial conducted in 15 French tertiary care centres between May 22, 2012 and October 14, 2013. 106 patients were randomised to receive renal denervation plus routine treatment or control receiving only the same antihypertensive treatment. adherence to ramipril was measured using spot urine AcSDKP/creatinine ratio. a cut off of 4 nmol/mmol was used to differentiate between high and low adherence. The remaining antihypertensive drugs were measured using UPLC-MS/MS 60= urine sample and 25 plasma. 85 out of 106 patients were included in the adherence analysis. The number of antihypertensive medications was not different between the two groups however, it was lower than prescribed. the mean ratio of detected to prescribed drugs was 75.0% in both groups. Comparing the active group to control group, patients were fully adherent: 20 (50.0%) vs 21 (46.7%); partially nonadherent: 13 (32.5%) vs 20 (44.4%) and completely nonadherent: 7 (17.5%) vs 4 (8.9%) P=0.3605. Non adherence (combining partially and completely) was about 50% of the patients regardless of adherence ambulatory SBP was reduced more in the active group compared to control. both fully adherent patient (mean BP reduction of ≈7 mm Hg) and in nonadherent (mean BP reduction of ≈8 mm Hg).

Main limitation of the study because of the design it might have been at risk for Hawthorne effect (change in the behaviour of the patient and health care provider due to allocation. High rate of Nonadherence that was observed in the DENERHTN study in spite of using recommended international guidelines to improve it. this involved proving cost free medication, using long acting drugs that are more forgiving if a single dose was omitted. Patient were instructed and provided with device to monitor their BP at home (131).

1.5.1.5 Lawson et al. 2016

Lawson et.al (132) developed a novel LC–MS-MS method to detect 23 commonly prescribed antihypertensive drugs in urine (refer to appendix Table 8-1). Their goal was to identify patients who are resistant to antihypertensive drugs. 49 patients attending the hypertension clinic at Birmingham Heartlands Hospital who were on at least one antihypertensive agent were analysed. 88% of the samples were adherent to their prescribed medications while three patients 8% who were all prescribed one or more of the following: lisinopril, felodipine, furosemide, diltiazem, indapamide, doxazosin, amlodipine and hydrochlorothiazide were not detected for any drugs (however, these drugs were detected in adherent patients). The remaining 5 (4%) were not detected for Ramipril.

1.5.1.6 Pucci et al. 2017

Pucci et.al (133) conducted a retrospective observational study looking at results from the routine use of urine adherence testing in the hypertension clinic at University Hospitals Birmingham NHS Foundation Trust. Medication adherence was assessed using HPLC-MS/MS analysis on 131 urine samples form hypertensive patients. only 67 (51%) were taking fully adherent to their prescribed drugs while 43 patients (33%) were taking some of their medications and only t 21 patients (16%) were non-adherent. Adherence to Spironolactone and thiazide/thiazide like diuretics were lower compared to other classes antihypertensive medication. The study introduced a new aspect by confronting non adherent patients with their result and trying to identify causes for non-adherence. ~30% of non-adherent patients denied the results. It is unknown whether it is due to

patient refusal to admit the truth, a false-negative result or simply a misunderstanding

1.5.1.7 Hamdidouche et al. 2017

The objective of the study was to assess adherence among hypertensive patient using urine analysis. Patient were selected from hypertension department of the Pompidou university hospital in Paris, from January to April 2015. Sociodemographic and clinical information were obtained. Urine sample was analysed using ultraperformance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS). Full adherence was defined by the presence of all antihypertensive medication. Partial non-adherence was considered if at least one but not all antihypertensive medication. Full non-adherence was considered if no prescribed antihypertensive medication was detected. In addition, 4 items Morisky questionnaire was used. 174 patients were included in the study with mean age of 67±11 years, 57% were female and mean BMI was 26±4. Office SBP 133±16 mmHg and DBP 73±10 mmHg. According to urine analysis, 159 patients (91%) were full adherent while 12 patients (7%) were partially nonadherent and the three (2%) fully nonadherent. Patient that were non-adherent had significantly higher number of prescribed antihypertensive drugs and pills. A significantly higher number of cardiovascular drugs. BB was associated with non-adherence group. In addition, SBP and DBP were higher in nonadherent than adherent group. There was no significant association between MMAS-4 and directly measured nonadherence. 134 (77%) patients were adherent to both methods, whereas only two patients (1%) were considered nonadherent. A subset of 105 patient were assessed for adherence after a mean follow up of 11 month and it showed that adherence remained the same in 88% of patients. The main limitations of the study are the small number, socioeconomic, behavioural, and psychological parameters were not measured. BP was measured in supine unattended which differs from the (ESH-ESC) Guidelines. Urine analysis was assessed using qualitative yes or no response (134).

1.5.1.8 Kocianova et al. 2017 (serum drug level)

The objective of the study was to investigate the relationship between BB serum level and patient's heart rate. The study design was retrospective analysis of

patients with resistant arterial hypertension who had had beta-blocker levels measured to assess adherence. patients were referred to and followed Hypertension Unit, University Hospital Olomouc. patients were classified as adherent if the serum level was within therapeutic range. On the other hand, patients were classified as non-adherent if the serum level was undetectable or below the therapeutic range. 106 patients were involved in the study and 220 measurements of serum beta-blocker levels were performed. Patients had mean of 56.8 years and 56% were men. Mean SBP was 151.7 mm Hg and mean DBP 89.0mm Hg. Mean for Antihypertensive medication was 5.5. Mean office heart rate was 73.0 beats per minute. 55.4% of measured BB serum level was within the therapeutic range, therefore classified as adherent while the remaining 44.6% were non-adherent. Patients that were non adherent had significantly higher heart rates (80.9 vs. 66.6 bpm, p<.001), SBP (157.4 vs. 147.0mm Hg, p=.002) and DBP (91.1 vs. 87.2mm Hg, p= .041) compared to patient with high adherence. The study concluded that heart rate was a good predictor of non-adherence to BB in patients with resistant hypertension. heart rate that is more than 75.5 bpm was highly predictive of non-adherence to most beta blockers with a high specificity of 86.8%.The main limitation of the study was the small number and retrospective design (135).

1.5.2 **Studies on adherence using MMAS-8**

The following are studies on adherence using Morisky questionnaire

1.5.2.1 Cuffee et al. 2013

The aim of the study was to investigate reported racial discrimination was associated with medication nonadherence among hypertensive African Americans and whether distrust of physicians was a contributing factor. Study was cross sectional design and patient data were recruited from the TRUST project conducted in Birmingham, Alabama, from 2006 to 2008. All the patients involved in the study were African American with hypertension. Adherence was assessed using Morisky MMAS-8. Trust in physicians was measured using the Hall General Trust Scale while discrimination was measured using the Experiences of Discrimination Scale. Associations were quantified by ordinal logistic regression, adjusting for gender, age, education, and income. Result of the study involved

780 patients with mean age of 53 (±9.9) years and 553 (71%) were female. around 66% of the patient had an income of $11 999 or less, while (68%) had attended some college. racial discrimination score was 3.4 (±4.4) while trust score was 39.2 (±8.0). Regarding adherence, 112 (14%) patient had low adherence, 350 (45%) with moderate adherence, and 318 (41%) had high adherence. The study concluded that racial discrimination was associated with lower medication adherence which might be due to delay filling of their prescribed medications. On the other hand, High adherence was associated with increased age, male gender, and greater reported trust in physicians. Limitation of the study, several factors weren't included that could affect adherence such as the number of years from initial diagnoses with hypertension, racial concordance between the patient and physician and psychological aspect including stress, social support and depression. Entry criteria for the study included a single entry of hypertension in the medical record however, the diagnosis was not further verified. Another limitation is the under presentation of men, it is unclear whether men with lower adherence were less likely to participate, this could explain the adherence with men when compared to women (136).

1.5.2.2 Hou et al. 2015

The aim of the study was to investigate the adherence levels among Chinese older patients taking antihypertensive drugs and to study the association between antihypertensive medication adherence and aging perceptions. Method: the study design is cross sectional for the inward and outpatient clinic of a University hospital and communities in Suzhou, China. Patient complete a self-administered questionnaire to obtain their demographic, clinical information, perception of aging and medication adherence. Adherence was measured using the MMAS-8 scale while aging perceptions was assessed using Aging perceptions questionnaire APQ which was developed by Barker et.al. in 2007. APQ assessed "Identity'' which refers to the elders' beliefs about aging in the context of health, the "Time line chronic" which relates the extent to which awareness of ageing is chronic in nature." Time line cyclical" the extent to which one experiences variation in the awareness of aging. "Consequence" the effect of aging on one's life across a variety of domains either positive or negative consequence. Result of the study showed that 585 patients completed the study

mean age of 68.4 ± 7.48), 60.3% were male, 62.2% had hypertension duration of less than 10 years. Regarding their adherence, 65.8% had poor adherence while 34.2% had good adherence. Patient with poor adherence was associated with increasing age, lower level of education, lower income and no retirement pension or medical insurance. They also had higher BP level and shorter duration of hypertension. In addition, they had poor response to the efficacy of the antihypertensive drugs. Logistic regression analysis showed that aging perception that involved identity, timeline cyclical, control positive was significantly associated with medication adherence. Limitation for the study: first, adherence to specific prescribed drug was not evaluated. Second, the influence of number of medication and their frequency on medication adherence was not considered (137).

1.5.2.3 Cumming et al. 2016

A study conducted by Cumming et.al in 2016, the aim of the study was to find the relative importance of perceived social standing against traditional objective socioeconomic variables as correlates of lower medication adherence in a rural community-based setting in the south-eastern United States, and to characterize the relationship of lower medication adherence with BP. The study was cross sectional study that used data from longitudinal cohort study (2011-2014) at a rural county in North Carolina. The standard socioeconomic variables include level of education and annual income. On the other hand, perceived social standing is affected by past circumstances and experience, family history and resources, level of depression or distress, and future prospect and opportunities. It was assessed by showing the patient a picture of a ladder and rate themselves a score from 1 to 10 in relation to others in their community. (score = 1-10 with higher scores indicating higher perceived social standing). Result of the study, 495 patients completed the study with mean age of 57.3 ± 12.8 years, 68% female and 60% African. Non-adherence to antihypertensive medication was found to be around 40% and patients were significantly younger, from African American race with no insurance, have lower perceived social standing and had lower number of medications. 38% of patient answered to cost-related reasons as a cause for non-adherence. While 75% of non-adherent patient stop or reduced their dose without informing their doctors because they felt worse. MPR was calculated for a subsample of 32 patient and showed moderate correlation

with MMAS score (Pearson correlation coefficient = 0.54, P = .001). Medication adherence was not significantly associated with traditional socioeconomic variable (education level and annual income). However, the study showed a stronger relationship between medication adherence and patient own perceived social standing specifically among the African American. In a series wise linear regression models, older age, African race and lower adherence score were significantly associated with higher levels of systolic BP. Limitation of the study: first, a causal relationship between medication non-adherence demographic and socioeconomic characteristics and SBP couldn't be established because the study was cross sectional. Second only white and African American race were included in the study without looking at the other racial group. The conclusion was that in rural southern communities. Non-adherence to antihypertensive medication is prevalent among the African race and it is strongly associated with perceived social standing. Non-adherence contributes to elevated Bp and it not always cost related (138).

1.5.2.4 Pandey et al. 2015

The goal of the study was to investigate the specificity and sensitivity of MMAS-8 in a cohort of patients against therapeutic drug monitoring. In addition, to determine the accuracy of clinical predictors and other independent questionnaire in detecting non-adherence in patients with apparent treatment resistant hypertension a–TRH. Method: the records of all new patients that were referred to the hypertension specialty clinic at the University of Texas Southwestern Medical Centre for a–TRH between Jan 2009 and Oct 2014 were reviewed. In the clinic three BP was measured using oscillometric device (Welch Allyn, Vital Signs, Skaneateles Falls, NY). MMAS-8 was used to measure drug adherence. Since Dec 2010 the serum level of antihypertensive medication was assessed. Serum level below detection limit of at least on antihypertensive drug was defined as non-adherence. Result: 47 patients completed the study by MMAS-8 scale, 26% had poor adherence 34% had medium adherence and 40% had high adherence. Patient who were not adherent were significantly younger, more females and had significantly higher heart rate. In contrast chronic kidney disease was higher in adherent group. There were no significant different between the 2 groups for hypertension duration, history of side effect to medication frequency of drug dosing and number of medications. When

comparing MMAS against therapeutic drug monitoring the sensitivity was 26% (95% CI, 10.3%–48.4%) and specificity was 75% (95% CI, 53.3%–90.2%). Positive predictive value was 50% (95% CI, 21.1%–78.9%) and negative predictive value of 51% (95% CI, 34%–68.6%). multivariate analysis using backward selection technique was used to determine clinical predictor for non-adherence. Elevated HR was found to be an independent predictor for non-adherence. On the other hand, presence of CKD as predictor for adherence to medication (however, this could be attributed to the older age and more male in the adherent group). Age, gender, ethnicity, total daily dosing for antihypertensive drugs, BP level and MMAS-8 were not independent predictors. In conclusion, MMAS-8 had limited sensitivity and specificity to detect medication non–adherence among patients with a–TRH. Limitation of the study were first, it was done retrospectively for a referral hypertension specialty clinic, therefore the result cannot be generalised. Second, only insured patients were included and finally the sample size is small (139).

1.5.2.5 Yue et al. 2015

The aim of the study was to investigate adherence to antihypertensive drugs in relation to well-developed behavioural theory (the health belief model HBM). HBM was devised by social psychologist to explain the public participation in medical program. It involves different cognitive constructs which predict people's action to control their illness. Method: the study was cross sectional design and patient were enrolled from teaching hospital in Shanghai, China. MMAS-8 was used to measure patient's adherence while the HBM was assessed using a HBM questionnaire developed by champion. Result: 232 completed the study with mean age of 64.1 ±11.0 years, and 52.6% were females. 26.3% had low adherence, 22% had medium adherence and 51.7% had high adherence. Four risk factors were significantly associated with better medication adherence, this includes: older age ($p = 0.037$), long duration of hypertension ($p = 0.003$), long duration of medication use ($p = 0.001$) and taking combination of antiplatelet agents ($p < 0.001$). HBM explained 48.8% of the variance in adherence to antihypertensive drugs with an accuracy of 82.8%. Higher levels of perceived susceptibility, perceived barriers, cues to action, and self-efficacy were significantly associated with better antihypertensive medication adherence. Limitation of the study are HMB model didn't take into consideration the

emotional, environmental and social. Recall bias associated with the self-report and the small sample size which limit the generalization of the findings. In conclusion the HBM was reliable to predict medication adherence in Chinese hypertensive patients. The study was the first to asses medication adherence on the basis of a well-developed behaviour theory in Chinese patients and the first to provide insight on the relationship between risk factors, HBM construct and medication adherence (129) (140).

1.5.2.6 Mugwano et al. 2016

Mugwano et.al 2016 studied the level of adherence in hypertensive patients with stroke and to evaluate stroke risk factor in Kampala Uganda. The study was cross sectional design and hypertensive stroke patients were recruited from two hospitals in Kampala. adherence was assessed using Morisky questionnaire MMAS-8. Result of the study: the study included 112 patients with mean age of 63.5 years, female 66%. 70% of patients had ischemic stroke while 30 % had haemorrhagic stroke. Calcium channel blockers (amlodipine and Nifedipine) were the most common prescribed drug while Methyldopa was the least. About 14% were receiving 3 or more medication for blood pressure control. On the other hand, more than half of the patients were taking dual antihypertensive therapy. There were some patients who had been taking herbal medication that have been shown to have hypotensive effect or interfere with the bioavailability of the antihypertensive medication. MMAS score showed that around two third of patients 76.8% were non-adherent compared to 17 % who were high adherent. The main reason for non-adherence was lack of knowledge for the chronicity of hypertension, followed by the cost of medication and access to the health care system. Although medication is provided by the government, during periods where medications is not available, patients have to buy their own medication. Nevertheless, 20% of patient didn't provide a reason for their non-adherence. The main limitations of the study are the small sample size and the role of the health care provider was not explored. In conclusion the study demonstrated the lack of awareness for hypertension and the poor adherence to BP control which could contributed to the high rate of stroke in Uganda, Therefore, there is a serious need to increase the awareness of screening for hypertension and bp control(141).

1.5.2.7 Gabrielle et al. 2013

The aim of the study was to examine the adherence profiles and the factors associated with antihypertensive drug adherence among Chinese patients. The design of the study was cross sectional, adult patient that were aged 18 years or older who were taking at least one long-term antihypertensive drug were recruited form outpatient clinic located in the New Territories Region of Hong Kong from 01 February 2012 to 30 April 2012. Demographic information including age, gender, level of education, marital status, educational levels number of antihypertensive drugs taking, duration of taking antihypertensive drugs were collected. Adherence was measured using Morisky questionnaire mmas-8. 1,114 completed the study with mean age of 65.7 (±11.1), female 648 (58.3%). mean number of drugs were 1.6 (±1.0) while the duration used was 7.7 years (±6.9). The mean systolic blood pressure was 130.6(±16.9) mmHg and the mean diastolic blood pressure was 74.7(±9.3) mmHg. MMAS score was 6.7 (±1.4). A total of 65.1% of patients had a score more than 6 indicating they had good adherence while 32.6% of had a result of 6 or below indicating their adherence levels were labelled as poor. Poor adherent patients were younger, had a shorter use duration of medication, employed and those reported a self-perceived health status as poor or very poor. Limitation of the study included: the sample was taken from a single out-patient clinic; therefore, result cannot be generalized for the larger population. Also the absence of association between the MMAS and the blood pressure which might be due to not excluding patients with white-coat hypertension despite the standard methodology to measure BP (142).

1.5.2.8 Guiradoa 2011 et al. (Pill count)

The purpose of the study was to evaluate the efficacy of a healthcare education program for patients with hypertension. The study was multi-centre, prospective, cluster randomised, controlled clinical trial, using the primary healthcare centre as a randomization unit. 36 primary health care centres were involved (18 in the control group CG and 18 in the intervention group IG) located in Barcelona, Spain. Patients were included if they had hypertension, were aged between 18 and 80 years, attending the clinic for long-term follow-up and using anti-hypertensive medication, and had attended the clinic for at least a period of 6 months. Blood Pressure (BP) was measured using a regularly-calibrated a

mercury sphygmomanometer. Adherence was measured using the Haynes-Sackett and Morisky-Green tests and pill count. 432 in control group and 436 in the intervention group completed the study. The mean age was 63 years, most patents were female, and two thirds of the participants had no formal education. About half of the participants had poor control of their hypertension. Noncompliance was found to be around 4% for the Haynes-Sackett test while Morisky-Green test had noncompliance of 25% and pill count had 12%. Indirect measures of adherence did not show improvement following the intervention except or the Morisky-Green test which increased by increased by 9.6% (95% CI: 5.5–13.6) in the IG. The main limitation was inconsistency of the measure of adherence, and it is not clear which of them is the most suitable. Further studies are required in order to define adherence and to develop more specific interventions directed towards improving adherence among long term hypertensive patients in the primary health care that are applicable and feasible to the everyday practice (143).

1.5.3 Studies on adherence using MEMS

1.5.3.1 Zeller et al. 2007

The aim of this study was to investigate the between blood pressure and medication adherence using electronic pillboxes (MEMS). Patient were recruited from five general practices in Bristol, UK. Adherence was assessed using electronic box, patients were told to insert a month's supply of one antihypertensive drug into the monitor. Only one drug was selected due to cost and feasibility. The first main outcome was adherence which was defined based on timing adherence that is which is the number of doses taken at 24±6 h for a once-daily drug or 12±3 h for the twice-daily regimen, divided by the total number of days and multiplied by 100%. Blood pressure was measured using an automatic inflation blood pressure monitor. 239 patients participated in the study with mean age of 66.7±10.3 years and 125 (52.3%) were males. SBP 147.9± 19.1 mmHg while DBP 82.3± 10.1 mmHg. Diuretics were the commonest dispensed drug for pillboxes (41.4%), followed by beta blockers (20.1%), ACE inhibitors (19.3%), calcium channel blockers (12.1%), and others (7.1%). Most patients were on a once-daily regimen (96.7%). Adherence was assessed on a mean of 33± 6 days and most of patients returned administered pillboxes (90%).

Baseline systolic and diastolic blood pressure weren't different in patients who returned (n=216) or did not return (n=23) MEMS devices. Mean Timing adherence for all patient n=216 was 88% (±17%). Timing adherence was > 80% in 175 (73%), only 11 (5%) patients had timing adherence less than 50%. There was no clear relationship between adherence to antihypertensive drugs and blood pressure. Regardless of the high timing adherence blood pressure levels were not controlled, therefore the study concluded that inadequate control might be related to pharmacological non response or insufficient intensity of the medication rather than adherence to medication. The main limitation to the study is ''Hawthorne effect'' which is a change in patient behaviour due to being monitored. In addition, the short duration for the monitored period (33 days) (144).

1.5.3.2 Grigoryan et al. 2012

The aim of the study was to identify predictors of adherence to antihypertensive medication measured by (MEMS), in uncontrolled, mainly African-American (AA) hypertensives from large urban public and private primary care clinics. The study was cross sectional design that is part of a cluster-randomized trial for hypertension control. It involved 10 primary care clinics in Texas, USA. Demographic data was collected, and patients were given Aardex MEMS to record the date and time of each bottle cap opening during the monitoring period. The duration of monitoring was 30 days to evaluate up to three antihypertensive drugs. the average percentage of prescribed doses taken per day was used as measure for adherence. Patients were defined as nonadherent if they took <80% of all prescribed doses, averaged across all monitored antihypertensives. 176 patients completed the MEMS monitoring (124 patients randomly selected at baseline while 52 patients were referred for monitoring by their physicians in the intervention clinics). Mean age was 55.5±10.2, 131 (74.4%) female, the mean SBP 153.1 ± 15.0 mm Hg and mean DBP was 84.8 ± 12.1 mm Hg. The mean number of antihypertensive drugs was 2.6 ± 1.2. 61 (34.6%) had <80% of prescribed doses. Lower adherence was found in African race in comparison to Hispanic (OR 0.36 CI 0.15–0.86). In addition, female gender was associated with lower adherence (OR 0.38; 95% CI 0.15–0.91). Patients attending public clinic had lower adherence in contrast to those attending private clinic. On the other hand, patient with diabetes and those who were monitored by their physicians

had high adherence. Age and number of medications weren't associated with adherence. The limitation of the study was not assessing the effect of attitudinal factors on medication adherence (145).

1.5.3.3 Hamilton et al. 2003

The purpose of this study was to evaluate the adherence to medication taking among hypertensive patients using MEMS. The study design was Randomised clinical trial double blind where patients were assigned to receive either the medication (potassium) or placebo. Within each group patient were randomised to take part in one of three interventions related to improved adherence. Adherence was measured using first, MEMS electronic device. Patients were asked to follow protocol: to open the bottle at three times during the day spaced at least 4 hrs apart, and at each time to take two capsules. Ideal adherence would mean that the patient took the medicine 100% of the time. Second, self-report where the Medical outcomes study (MOS) General Adherence Scale was used and it includes five items that ask about adherence to health care provider medication recommendations. Third, collateral report: MOS general adherence items adapted for the physicians was used to estimate patient's perception of adherence. Fourth, capsule count: staff recorded the number of capsules handed to the patient at visit three and four and at visit 4 and five the clinical trial coordinator counted the number of capsules that were left and computed a percentage of those presumed taken. Finally, Urinary potassium excretion levels as a direct measure for adherence. 107 patients were recruited with mean age of 58 years and 55 (51.4%) were male. adherence result varied MEMS result was (around 58%) while collateral report had 59%. Pill count adherence was 83% for visit 4 and 88% for visit 5. Urinary potassium showed adherence of 62%. Self-report had the highest adherence score around 80-90%. The study concluded there is significant correlation between electronic method (MEMS), pill count and self-report measures (146).

1.5.3.4 Burnier et al. 2001

The aim of the study was to evaluate prospectively the potential benefits of measuring drug adherence using electronic monitors in the management of patients with treatment resistant hypertension. 41 patients were included in the

study and antihypertensive medication was provided in an electronically monitored device MEMS to be followed for 2 months. compliance was calculated according to the number of days (in per cent) during which the prescribed number of doses were recorded as taken referred as (taking compliance). Compliance was evaluated separately for each prescribed drug. Timing compliance wasn't considered in this study. Office blood pressure were taking 3 times and average of the last two measurements was used for the analysis. The Characteristics of the patients were mainly men 31(75%), mean age 50.5, SBP 156 ±23 (mmHg) and DBP 107 ±11 (mmHg). After 2 months of follow-up the average decreases in office SBP and DBP were -11.5 (SD 23.3) and -9.1 mmHg (SD 14.5) respectively. monitoring of compliance resulted in a normalization of office SBP in 32% of patients and of DBP in 34% of the patients. The mean taking compliance was 93% (SD 9.3%; range 30-100% for each individual treatment) and there was no difference in compliance between class of drugs. The results of the study showed that monitoring of drug compliance with electronic devices is a useful tool in the management of patients with resistant hypertension (147).

1.5.3.5 Santschi et al. 2008

The study aimed to investigate whether monitoring medication adherence with an electronic system MEMS improves long-term (BP) control in hypertensive patients followed by general practitioners. the design was one-year pragmatic cluster and open randomized controlled study conducted in networks of community-based pharmacists and general practitioners in Switzerland between 2001 and 2004. Patients with uncontrolled hypertension were assigned to either usual care (UC) where treatment were dispensed as usual, or to intervention (INT) group that used MEMS to monitor patient adherence. adherence was measured by "taking adherence" defined as the percentage of days with correct intake. 68 patients were included in the study (UC: 34; INT: 34) were enrolled. Over the duration of one year, patient in the intervention group were more likely to reach their target BP comparted to the UC group. At 4 months 38% in the INT group achieved the target BP compared to 12% in the UC group. At 12 months 21% in INT compared to 9% in UC. the likelihood of reaching the target BP was higher in the INT group compared to the UC group (pb0.05). At 4 months, 38% in the INT group reached the target BP vs. 12% in the UC group (pb0.05), and 21% vs. 9% at 12 months (p: ns). The study concluded that monitoring drug

adherence using MEMS resulted in a better BP control among hypertensive patients. However, this effect deceased with time (148).

1.5.4 **Studies on adherence using refill data**

1.5.4.1 Tang et.al 2017 MPR

The goal for this study was to compare adherence rates and associations with mortality using different methods to measure adherence. The design of the study was cohort study of patients aged ≥65 years from Manitoba, Canada that were diagnosed with incident of hypertension in 2004 and followed to 2009. Adherence to antihypertensive drugs was calculated using several methods such as interval and prescription-based medication possession ratios (MPRi & MPRp) and proportion of days covered (PDC)) while patients on polytherapy was calculated by a) MPR considering adherence to any antihypertensive b) average of the MPR's specific to each anti-hypertensive medication class. c) calculating the MPR's specific to each medication class, then taking the highest of the class-specific MPR's d) calculating the MPR's specific to each medication class, then taking the lowest of the class-specific MPR's. 2199 patient completed the study with mean age of was 75.2 ±7.0, 45.4% were male, 33.7% were new users of anti-hypertensives, and 64.7% were on monotherapy. Adherence for patient on monotherapy (n = 1422), MPRi estimates (mean 0.83, ±0.23). on the other hand, polytherapy (n = 777), adherence result varied widely, depending on whether MPRi or MPRp was used. If patient with an MPRi of ≥0.80 for each and every medication class were only considered they would be classified as adherent, only 24.1% would be adherent. On the contrary, when considering patients with an overall MPRi of ≥0.80, when all medication classes were grouped together, as adherent, over 90% of the same sample would be considered as adherent. Adherence was inversely associated with death, with the strongest association for MPRp measures. The study concluded that due to wide variation in adherence rate based on operational definition, it is recommended to use prescription based MPR's when defining medication adherence (149).

1.5.4.2 Yang et.al 2017 PDC

The purpose of the study was to assess the association between antihypertensive adherence and risk of cardiovascular disease among older hypertensive patients.

The study was a cohort of Medicare fee-for-service beneficiaries aged 66 to 79 years that were newly initiated on antihypertensives in 2008–2009. Antihypertensive medication was defined as being alpha blocker, angiotensin II receptor blocker, angiotensin-converting enzyme inhibitor, beta-blocker, calcium-channel blocker, vasodilating agents or diuretics. Adherence was measured using proportion of days covered (PDC) which is (the proportion of days during the follow-up period where the beneficiary had their prescribed medication on hand). It was calculated first by determining the beneficiaries' follow-up in months from index prescription date to either first occurrence of a CVD event (fatal or nonfatal), death from a cause other than CVD, or the end of the follow-up period. Second, within the defined follow-up period, the number of days were counted for the beneficiary where they had at least 1 antihypertensive on hand (covered days) based on the prescription fill date and days' supply. The final analytical cohort was n= 155 597 patient with mean age of 69.9 years and female were 63.7%. regarding adherence 8.9% (95% CI, 8.8–9.1%) had PDC below 40% (low adherence), 30.3% (95% CI 30.0–30.5%) had 40% to 79% PDC (intermediate adherence), and 60.8% (95% CI60.6–61.1%) had ≥80% PDC (high adherence). Patient that were high adherent were slightly younger, female, non-Hispanic white, taking >1 antihypertensive drug, uses statins and had fewer comorbidities. Patient that were considered highly adherent had a significantly lower risk of having a cardiovascular event during median follow up of 5.8 years in comparison to those with low or moderate adherence. The limitation of the study, there was no blood pressure measurements data available. Therefore, they weren't able to determine the status of blood pressure control during follow up (150).

1.5.4.3 Lee et.al 2017 MPR

The purpose of this study was to determine the effect of adherence to antihypertensive medication on stroke incidence. The study design was retrospective cohort study. It included patients with hypertension that aged over 30 years who received a check-up between 2009 and 2013 and had no history of stroke before 2009 in South Korea. Medication adherence was measured using medication possession ratio (MPR). The MPR is based on the ratio of the number of days supplied with medication to the total number of days in the year before the study year. MPR = Number of days supplied by at least one during the year.

Number of days between first fill and the last day of the year. 38 520 patients were included in the study 54.2% were male. MPR result showed that 26 512 (68.8%) were high adherent to medication while 4996 (13.0%) were intermediate and 7012 (18.2%) were poorly adherent. Poorer medication adherence was significantly associated with a higher risk of stroke. A subgroup analysis was made according to the duration of hypertension (< 2 year, 2-5 years and 5-10 years). higher MPR was associated with a lower risk of stroke in each category of the duration of hypertension. Using only MPR is a limitation to the study where the result might overestimate that amount the patent took. PDC wasn't used because there was no consideration for the type of prescription drug. There also the possibility of selection bias because check-ups in South Korea could be offered by national health insurance and private hospitals. only patients with hypertension who received a check-up from the NHI were included in this study analysis. The study concluded that Hypertensive patients with intermediate or poor adherence to antihypertensive medication had 1.13 times and 1.27 times higher risk of stroke, respectively, than those with high adherence. In addition, this was the first study in South Korea to consider the duration of hypertension and changes in adherence prior to a stroke in relation to adherence to antihypertensive medication and index stroke (151).

1.5.4.4 Eakin et.al 2013 MPR

The objective of the study was to gain preliminary estimates of adolescents' objectively measured adherence and self-reported adherence. In addition, to determine the association between adolescents' antihypertensive medication adherence, BP control and race. The study was an observational design. Children were recruited from Johns Hopkins University, Harriet Lane Kidney Centre, USA if they were adolescents aged 11–17 years who had a diagnosis of essential hypertension and were currently prescribed antihypertensive medication. Adherence was assessed using MEMS, MPR and MMAS-8. mean MEMS ratio was calculated by using the number of events the cap was opened during the 28-day monitoring period as the numerator and the number of doses prescribed for the same period as the denominator, due to cost only one prescribed antihypertensive medication was monitored. 21 patients were recruited with mean age of 14.7±2.0 years and male 57(21%). ten (48%) were African American (AA) while eleven (52%) were non-AA. 10 (48%) adolescents had systolic and/or

diastolic blood pressure readings at or above the 95th percentile for their age, sex and height. MEMS ratio (0.82±0.22) was significantly higher than MPR (0.66±0.25; p <0.04), this could be due to the short duration of the MEMS 28 days versus 12 months for MPR. African race had lower medication adherence when measured using MPR. The study showed that adherence by MPR was correlated with blood pressure control, but not with MEMS or self-report. The limitation of the study is the small size indicating that the result cannot be generalized to general population of adolescents with hypertension. In addition, only a single manual BP measurement was obtained in the clinic ignoring the possibility of variability for BP measurement (152).

1.5.4.5 Corrao et.al 2015 PDC

A study conducted by Carrao et.al to investigate the relationship between long-term adherence to the prescribed treatment regimen and risk of the first hospitalization for Heart failure (HF). The study was a case–control that was carried out in Lombardy, Italy. It involved a cohort of 76017 patient from the age of 40 to 80 year, that were newly prescribed with an antihypertensive medication during 2005. Cases were 622 patients that has been hospitalized for HF from initial prescription until 2012. For each case, up to 5 controls were randomly selected. For each case and control all prescribed antihypertensive medication during follow up period were identified. The period covered by an individual prescription was measured by dividing the total amount of the drug prescribed for the defined daily dose. In case of overlapping prescriptions, patients were assumed to have taken the drugs contained in the first prescription before starting the second. Adherence was calculated by proportion of days covered by treatment. Four groups of adherences were considered, that is, very low (≤ 25%), low (26%–50%), intermediate (51%–75%), and high (>75%) PDC values. 622 case patients were matched to 3110 controls with mean age of 67 years, about 54% of the patients were men and ACEI most common initial drugs in both cases and controls. Adherence in cases were as follow: very low 285 (46%), low 93 (15%), intermediate 82 (13%) and high 162 (26%). While for control: very low 1283 (41%), low 405 (13%), intermediate 432 (14%) and high 990 (32%). adherence to antihypertensive medication decreased the risk of hospitalization for HF markedly and progressively in patients that were younger, older, male gender, diuretic, ACEI and ARBS but wasn’t associated to women, BB and CCB.

Limitation of the study, they acknowledged that adherence was derived from drug prescriptions which is a commonly used method to estimate adherence in large populations. however, it is based on the assumption that the proportion of days covered by a prescription corresponds to the proportion of days of drug use. Moreover, allocation of antihypertensive medication was not randomized. Therefore, the results might be affected by confounding (153).

1.5.4.6 Mancia et.al 2014

The objective of the study was to investigate the factors involved in discontinuation of antihypertensive medication prescriptions in real life. The study was a cohort of 493 623 patient recruited in 2003, 2006 and 2009 that were new users of antihypertensive medication. Discontinuation was defined as lack of prescription renewal for at least 3 months. Each patient was followed at most for 1 year. Treatment was considered persistent if the time span between the end of one prescription and the beginning of the following one was 90 days or shorter. While discontinuation If the between-prescription time span was longer than 90 days. 493 623 patients were involved in the study with mean age of 59 years, 48% were men. Treatment started with monotherapy in 69% patients and combination of two more drugs in 31% patent. The most common initial monotherapies were ACEI (26%), BB (14%), ARB (11%), calcium antagonists (11%), diuretics (5%) and alpha blockers (2%). 282 117 (57%) had at least one episode of discontinuation within year. treatment discontinuation depended on the type of initial antihypertensive treatment, diuretic monotherapy associated with higher risk while less for calcium antagonists alpha-blockers, and minimal for ACEI and ARB, treatment discontinuation was lower in patient that were male and older, in patients treated for diabetes, hospitalized for cardiovascular or renal disease. In contrast it was greater in patients under receiving antidepressant drugs or hospitalization for concomitant pulmonary, rheumatic, neoplastic or neurological diseases and high-density population (in metropolitan areas). adherence to the prescribed antihypertensive treatment does not only depend on doctor's behaviour and patient characteristics, but it extends to social and environmental factors (154).

1.5.4.7 Vrijens et.al 2008

The purpose of the study was to describe characteristics of dosing history in patients prescribed a once a day antihypertensive drug. The study was longitudinal database design from clinical studies archived in database for 1989-2006. All Patients who participated in the studies whose dosing histories were available through electronic monitoring. Adherence was measured based on 2 components: persistence and execution. Persistence, the time from the first taken dose to the last taken dose. Execution is the multidimensional outcome of the comparison of two time series: the prescribed drug dosing regimen and the patient's drug dosing history while he or she is still engaged with treatment. The database had dosing histories of 4783 hypertensive patient which came from 21phase IV clinical studies, with lengths ranging from 30 to 330 days. It included 43 different antihypertensive medication: ARB (n=2088), CCB (n=937), ACEI (n=665), BB (n=195), and diuretics (n=155). Around half of the patients that were prescribed an antihypertensive drug had stopped taking it within one year and almost 95% of patients missed at least a single dose a year. failure to take a dose was more common at weekends and evening dosing were poorly executed compared to morning dose. Moreover, there was a small seasonal pattern of drug adherence. On each day of treatment dosing was omitted in around 10% of the scheduled doses: 42% were omission of a single day's dose, 15% were of one or two consecutive days and 43% were of 3 or more days. The study concluded that the most common cause for poor adherence with once a day antihypertensive medication are: early discontinuation of treatment and suboptimal daily execution of the prescribed regimens (105).

1.5.4.8 Krousel-Wood et.al 2009 MMAS and Refill data

The objective of the study was to evaluate the association and concordance of the 8-item self-report Morisky Medication Adherence Scale (MMAS) with pharmacy fill data in a managed care population of hypertensive old patient. The study was cross sectional design and participants were drawn from drawn from a large southern managed care organization which offered healthcare benefits to persons enrolled in the Medicare risk plans. Self-report adherence was measured using Morisky MMAS-8 questionnaire while the pharmacy fill data used the following to measure adherence: continuous single interval medication

availability CSA (dividing the days' supply obtained at a pharmacy fill by the number of days before the next pharmacy fill for that same drug), medication possession ratio MPR (sum of the days' supply obtained between the first pharmacy fill and the last fill divided by the total number of days in this time period), and continuous multiple-interval medication gaps CMG (dividing the total number of days without medications between the first and last pharmacy fill by the number of days in this time period). 87 patients were included in the analysis with mean age of 76 years, 31% male 48% were black and mean number of drugs was 2.2. according to MMAS-8 adherence result were 58% high, 33% medium and 9% low. The adherence based on pharmacy fill had a median CSA, MPR and CMG of 0.91, 0.91, and 0.12, respectively. MMAS is significantly associated with pharmacy refill adherence. Patients with low MMAS adherence were 6.89 (95% CI: 2.48 – 19.1) times more likely to have non-persistent pharmacy fill rates by CSA and 5.22 (95% CI:1.88 – 14.5) times more likely to have non-persistent pharmacy fill rates by MPR. Concordance between MMAS and CSA, MPR, and CMG was ≥75%. Weakness of the study were it didn't differentiate adherence based on drug class and only older age group were selected which may not be representative for the general population (155).

1.5.4.9 Scotti et.al 2013 PDC

The purpose of this study was to estimate the cost-effectiveness of improving adherence to antihypertensive medications in a large population without signs of pre-existing cardiovascular disease. Patient data were collected from health service data bases of Lombardia, Italy. Adherence was measured using PDC and were grouped as follow: very low (≤25%), low (26%–50%), intermediate (51%–75%), and high (>75%) while the cost-Effectiveness of enhancing adherence was measured by incremental cost-effectiveness ratio. 209,650 patients were included in the study with mean age of 60 ±10 years and 45% were male. Only 26% of patient had high adherence. In contrast, those who had low or very low were 44%. The characteristic of patient with low adherence compared with high were mainly female, initially had a combination of 2 or more drugs (156).

1.5.4.10 Qvarnström et.al 2013

The purpose of this study was to investigate factors associated with persistence in patients newly initiated on antihypertensive drugs. the study was Cohort design using clinical records of hypertensive patients from the Swedish Primary Care Cardiovascular Database (SPCCD) which is the largest Swedish population-based registry and comprises all patients in 48 primary health care centres with a diagnosis of hypertension during 2001–2008. Persistence was measured using dispensed drugs for two years. Patients were considered non-persistent if they had a gap of more than 30 days between end of dispensed supply and next dispensed prescription. 5225 patients were included in the study with a mean age of 61±13 years, mean SBP was 166±20 mmHg and mean DBP was 94±12 mmHg. ACEI was the most common prescribed drug in males while diuretic was in female. 736 patients (14 %) stopped treatment after being dispensed only one prescription. Among patients with a dispensed first prescription, 1356 (26 %), discontinued any antihypertensive drug treatment during the first year, and 492 (9 %) discontinued treatment during the second year of follow-up. Persistence was lower in patients that were male, younger age (30-39 years, SBP but not DBP was positively associated with persistence. In addition, Patients born in Sweden patients and other Nordic countries had lower discontinuation rates than those born outside the Nordic countries. The study concluded that important determinants of discontinuation antihypertensive medication are young age, male gender, mild to moderate rise in SBP, and birth outside of Sweden (157).

1.5.4.11 Wijk et.al 2008

The purpose of the study was to determine the rate and predictor of adherence of antihypertensive medication among elderly patient. The study was cross-national population-based study from USA(Pennsylvania), Canada (British Columbia) and the Netherlands. USA population were derived from Pharmaceutical Assistance Contract for the Elderly (PACE) programme in Pennsylvania. The Canadian population from the British Columbia Pharmacare Program and the Netherland population from PHARMO record linkage system. population included patients that were 65 years and older who started on antihypertensive drugs between 1 January 1998 and 31 December 2003. Persistence was measured using patient refill data. Patient were considered non-

persistent if they had consecutive 180-day period after the end date of a given prescription during which they filled no prescriptions for any antihypertensive drug. There were two reasons for selecting 180 days. First, it led to an adherence of at maximum 14% in the USA according to 30-day prescription and 33% in Canada and Netherland according to 90 days prescription that was sufficient to detect actual discontinuation. second, 180 days without treatment caused significant BP differences in placebo-controlled randomized controlled trials (put reference). 9664 patients from USA, 25 377 patients from Canada and 24 603 patients from Netherland were included in the study. Percentage of patient who had at least one period of 180 days without drugs were as follow for the first year: USA 23.3%, Canada 23.4, Netherland 24%. And after 6 years were: 41.1%, 36.3% and 38.2 % respectively. This indicate that during the first year almost quarter of the patient had stopped their treatment for at least 180 days. However, after 6 years USA was the highest for non-adherence. in the baseline year, older age, male gender and frequent use of prescription drugs were associated with non-persistence in the three countries while previous episode of MI and hypercholesteremia was associated with high persistence. Patient that were prescribed (ACEI and ARB) were less likely to have a 180-day medication gap compared to patient that were prescribed BB and diuretics (158).

1.5.4.12 Bramley et.al 2006

The aim of this research was to determine the relationship between medication adherence and blood pressure control among hypertensive patients who are taking antihypertensive monotherapy. The study design was retrospective, population-based that was conducted using medical and pharmacy claims from 13 health plans across the USA from 1999 to 2002. The data was used to identify patients with an International Classification of Diseases, Ninth Revision (ICD-9) code indicating the diagnosis of essential HTN. Adherence was measured using MPR and adherence was categorised into three groups: high (80%- 100%), medium (50%-79%), and low (<50%. 840 patients were selected with mean age was 59 ± 12.2 years, (50%) were women. The most common antihypertensive drugs prescribed were ACEI (27% of patients), CCB (22%), BB (20%), or diuretic (11%). 629 (74.9%) were found to have high adherence, 165 (19.6%) with medium adherence, and 46 (5.5%) with low adherence. The mean days of supply per pharmacy claims was 39.4 for the high adherence while 32.8 days for the

medium adherence and 30.2 days for the low-adherence. 270 (43%) with high adherence achieved BP control compared with 56 (34%) and 15 (33%) patients with medium and low adherence, respectively. After adjusting for age, gender, and comorbidities patients with high adherence were 45% more likely to achieve BP control compared to those with medium or low adherence (odds ratio=1.45; P =0.026). The study demonstrated the associated between high adherence and BP control. The main limitation of the study was first, using only one BP measurement. Second, not considering the potential influence of mail-service pharmacy on the measure of adherence. Third, potential confounding factors such as such as smoking, family history of cardiovascular disease, socioeconomic status were not available because of the retrospective design. Fourth, the results of the study are limited to monotherapy and cannot be generalized to patient who are on dual or multiple medications (159).

1.5.4.13 Heisleret.al 2008

The study assessed the prevalence of and relationship between patient adherence and provider treatment intensification. The study was retrospective cohort of hypertensive patients that received at least two drug refills in 2004 and had 1 or more outpatient primary care visits with an elevated BP during 2005 at Veterans' Affairs (VA)healthcare facilities in a Midwestern VA administrative. Pharmacy refill data was used over the period of 12 months investigate whether doses were increased, or BP medications were added ("intensification"). Adherence was measured by calculating continuous, multiple interval measure of gaps in therapy (CMG) which is the proportion of days the patient should have been taking medications during which the patient did not have medication available: CMG=total days on that patient did not have medications available/total days the patient should have been taking medication. Higher proportions indicate worse levels of adherence. Refill gap was categorized into: <20% (reference category), 20% to 59%, and ≥60%. 38 327 patients were included with mean age of 67.5 years, 97% were male. The mean number of elevated BP events was 1.79 per person and had a total of 68 610 elevated BP events. Mean SBP was 151.7 mm Hg and DBP was 78.3 mm Hg. intensification rates were 31% for patients with gap of <20%, 34% for patients with gap of 20% to 59% and 32% for patients with gaps of 60% or more. The study concluded that patient's adherence had little effect on providers' plan about

intensifying medications, even at very high levels of poor adherence. both patient adherence and provider intensification would most likely result in better BP control (160).

2 Methodology

I performed the following in my study:

- Patient interview, delivering the questionnaire and obtaining the consent.
- Urine sample collection transfer the sample from hospital to storage at BHF building.
- Preparing and sending the sample to the 3 different laboratories (to Birmingham lab by a special courier) and personally to Glasgow Polyomics Facility and Forensic Toxicology lab.
- Statistical analysis, analysing the result obtained from the lab and conducting statistical analysis.

In the study, I reviewed the participants which consisted of the patients attending the Glasgow Blood Pressure clinic (GBPC). The GBPC provides secondary- and tertiary-level services to hypertensive individuals from the West of Scotland. Information from patients attending the clinic is stored in a computerised database. Detailed patient history and physical examination were taken for each patient which included the basic socio-demographic data. The socio-demographic data involved Information regarding sex, age (date of birth), marital status, education, occupation, level of physical activity, smoking and drinking habits, hours of sleep, and current antihypertensive medication (number of medication and their doses). Besides that, height and weight were measured using a calibrated stadiometer and weighing scale for patients wearing light clothing without shoes. The BMI was calculated as the weight (in kilograms) divided by the square of the height (in metres).

2.1 Inclusion criteria

The patients who were 18 years old and above, male or female gender were included in the study. Additional criteria included the signed consent of patient with confirmed diagnosis of hypertension.

2.2 **BP measurement**

BP measurements were taken manually at each visit of the patient in the clinic. BP measurements were recorded three times using standardized sphygmomanometers after the patient started resting quietly for 5 min. The measurements were recorded by specialist hypertension nurses before the patients had their consultation with the physician. Three readings were taken and the mean of the last two measurements was recorded at each visit. Patients attending the clinic were advised to take their regular medications as usual.

2.2.1 **ABPM**

ABPM is performed by attaching a blood pressure monitor onto the patient arm. The device will automatically take a measurement every half an hour while the patient is practicing his normal daily activities. ABPM readings were obtained from patients record before taking the sample (first reading was around the time of sample collection and the second reading in the future). Each measurement provides a 24 detailed report which involves the following: 24 hours (systolic average/standard deviation, diastolic average/ standard deviation, mean arterial pressure average and standard deviation, pulse pressure and standard deviation, heart rate and standard deviation). The following are terms that are used in the ABPM report:

Mean arterial pressure MAP is defined as the average blood pressure in an individual during a single cardiac cycle(161) while pulse pressure PP is the difference between the systolic and diastolic BP.

24H: 24 hours ,24H SYS AVG/SD: 24 hr systolic average/standard deviation.

24H DIA AVG/SD 24 hours diastolic average/ standard deviation.

D: day, N: Night

2.3 **Lab investigation**

The routine blood investigation of patients was conducted, and their results were obtained from clinical records which included the investigations summarized in (appendix Table 8-2).

2.4 **Morisky questionnaire MMAS administration**

In the study, adherence using an eight item Morisky Medication Adherence Scale (MMAS-8). The scale was developed by professor D. Morisky and is composed of seven items with yes/no response options and one item with a 5-point Likert scale response option. These items provide information about the barriers to medication adherence, such as forgetting to take medications, not taking medications when one feels worse, and difficulties in complying with a treatment regimen (162).

2.4.1 **Scoring**

The scores on the eight items were summed to create an overall adherence (illustrated in Table 2-1), scores ranging from zero to eight, MMAS scores of 8 indicate high adherence. A score of 6 to <8 indicate medium adherence and a score <6 indicate low adherence.

Table 2-1 MMAS questionnaire

1) Do you sometimes forget to take your pills?	Yes/No
2) People sometimes miss taking their medications for reasons other than forgetting. Thinking over the past two weeks, were there any days when you did not take your medicine?	Yes/No
3) Have you ever cut back or stopped taking your medicine without telling your doctor because you felt worse when you took it?	Yes/No
4) When you travel or leave home, do you sometimes forget to bring along your medicine?	Yes/No
5) Did you take all your medicine yesterday?	Yes/No
6) When you feel like your symptoms are under control, do you sometimes stop taking your medicine?	Yes/No
7) Taking medicine every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your treatment plan?	Yes/No
8) How often do you have difficulty remembering to take all your medicine?	A. Never/rarely B. Once in a while C. Sometimes D. Usually E. All the time
Table reproduced from (162) MMAS-8	

2.5 **Sample collection**

For the study, I collected urine samples from hypertensive patients who completed the Morisky questionnaire. Prior to the collection of urine sample, patients received Patient Information Sheet about the purpose and the procedures of the study and provided written informed consent. If patients were not able to provide a urine sample, they were asked to provide it in their next visit if they were still willing to participate.

Plastic container was handed to the patient and they were asked to fill it up and return it. For female patients, a urine collection pot was provided to help collect the urine and fill the plastic container. Each container was labelled with a special ID for the corresponding patient. Samples were collected during clinic time between 9 am and 12 pm. The samples containers had a secure lid that were leak-resistant. Leak-resistant containers reduce specimen loss and healthcare worker exposure to the specimen while also protecting the specimen from contaminants.

After the samples were collected, they were transferred in an ice box filled with wet ice immediately after clinic to the lab in BHF building where the samples were centrifuged at 1000g for 10-15 min. Samples were aliquoted into 2 ml tubes using pipette. Afterwards, they were stored in the freezer under -80 °C in their corresponding racks until they were sent for urine analysis to three different laboratories. No special requirement was requested from any of the laboratories.

During the study there was one patient that wanted to withdraw from the study, her urine sample was removed.

2.6 **Drug assay analysis**

Patient's urine samples were analysed by three methods: 1) Birmingham Heartlands Hospital BIR which gave a qualitative result (drug detected or not). 2) Glasgow Polyomics POL used a novel method searching for potential drug using molecular network. 3) Glasgow Toxicology provided a qualitative and quantitative result (the concentration of drugs was provided).

2.6.1 **Analysis Birmingham Heartlands Hospital Laboratory**

Samples were analysed using HPLC-MS/MS. Prior to the HPLC-MS/MS analysis, the samples were diluted in 1:10 in distilled water. In addition, the solvent extraction of antihypertensive drugs that were weakly acidic, basic and neutral such as bisoprolol, diltiazem, amiloride was performed. In the process of solvent extraction, 5 mL of urine was mixed with an organic solvent for the duration of 10 min. It was then centrifuged at 3000 rpm for 5 min. The upper organic layer

was evaporated under nitrogen at 40°C. After that, it was reconstituted in 1 mL of 5% methanol prior to HPLC-MS/MS. For the study, the assay of HPLC-MS/MS was performed using an Agilent Technologies 1290 series High Pressure Liquid Chromatograph interfaced with an Agilent Technologies 6460 Triple Quad Mass Spectrometer that was connected to a Jetstream electrospray (ESI) source which was operable in either positive-ionization or negative-ionization mode. The nebuliser gas temperature was set at 350°C with a flow rate of 5 L/min and a pressure of 45 psi and the sheath gas temperature was set at 250°C with a flow rate of 11 L/min. The HPLC system was operated in gradient mode using 0.1% acetic acid in water for mobile phase A and 0.1% acetic acid in methanol for mobile phase B. The initial conditions of 5% B/ 95% A were maintained for 2 min and then were ramped to 60% B at 6 min and further to 100% B at 9 min. The gradient was maintained at 100% B for 1 min and then was returned to 5% B at 11 min to re-equilibrate. The total run time calculated was 12 min per sample. The HPLC separation of the sample was carried out on Agilent technologies Zorbax Eclipse Plus C18 2.1×50 mm column. Further, the mass spectrometer was operated in the targeted multiple reaction monitoring mode. The analysis of each urine sample was carried out twice. The primary analysis was carried out in the positive ion mode for drugs and metabolites listed in category 'Positive'. The secondary analysis was conducted in the negative ion mode for drugs and metabolites listed under 'Negative' category. Moreover, the total non-adherence to antihypertensive treatment was defined as complete absence of any prescribed antihypertensive medication (or their metabolites where appropriate) in a spot urine sample on screening. The patients for which the urine analysis demonstrated the presence of medications lesser than their prescribed medications were classified as partially non-adherent.

2.6.2 **Analysis Glasgow Polyomics**

1.1.1.1 Urine sample preparations

A general metabolome extraction procedure was performed (i) 5 lL urine was extracted in 200 lL chloroform/methanol/water (1:3:1) at 4 C; (ii) then vortexed for 5 min at 4 C; (iii) then centrifuged for 3 min (13,000 g) at 4 C. The resulting supernatant was stored at -80 C until analysis. A pooled aliquot of the was

prepared prior to the LC– MS runs with DDA applying higher collision dissociation (HCD).

1.1.1.2 Analytical platform

For the analysis, a Thermo Scientific Ultimate 3000 RSLCnano liquid chromatography system (Thermo Scientific, CA, USA) was utilized. This low flow liquid chromatography system was coupled to a Thermo Scientific Q-Exactive Orbitrap mass spectrometer equipped with a HESI II interface (Thermo Scientific, Hemel Hempstead,UK). Instrument control and data acquisition was acquired using Thermo Xcalibur Tune software (version 2.5).

1.1.1.3 LC settings

The HILIC separation was performed with a SeQuant ZICpHILIC column (150 9 4.6 mm, 5 lm) equipped with the corresponding pre-column (Merck KGaA, Darmstadt, Germany). A linear biphasic LC gradient was carried out from 80 % B to 20 % B over 15 min, followed by a 2 min wash with 5 % B, and 7 min re-equilibration with 80 % B, where solvent B was acetonitrile and solvent A was 20 mM ammonium carbonate in water. The flow rate was 300 lL/min, column temperature was maintained at 25 ºC, injection volume was 10 lL, and samples were maintained at 4°C in the autosampler (Creek et al. 2011).

1.1.1.4 MS and MS/MS settings

In the positive and negative ionization combined fragmentation mode, a duty cycle consisted of a full scan in positive ionization mode, followed by a TopN MS/MS (MS2) data dependent fragmentation event, subsequently followed by the same two scan events in negative ionization mode. Data acquisition was carried out in positive (+) and negative (-) switching ionization mode, using m/z 74.0964 (+) (ACN cluster), 88.07569 (-) (contaminant), and m/z 112.98563 (-) (Formic Acid cluster) as lock masses. The set up was calibrated with the help of Thermo calmix (PierceTM calibration solutions from Thermo Scientific), with additional masses at lower m/z; 74.0964 m/z (+) and 89.0244 (-), in both ionization modes prior to the analysis and a tune file targeted towards the lower m/z range was used. The full scan (MS1) data was acquired in both ionization modes in profile mode at 35,000 resolution (at m/z 200) using 1 microscan, an AGC target of 10^6 cts, a maximum injection time of 120 ms, with spray voltages +3.8 and -3.0 kV,

capillary temperature 320 ºC, sheath gas flow rate 40, auxiliary gas flow rate 15 a.u., sweep gas flow rate 1 a.u, and a full scan mass window of 70–1050 m/z.

The MS/MS (MS2) data was acquired in profile mode at 35,000 resolution using 1 microscan, an AGC target of 1×10^5 cts, a maximum injection time of 120 ms, a loop count of 10, a MSX count of 1, a TopN of 10, an isolation window of 1.0 Da, an isolation offset of 0.0 Da, a stepped normalized collision energy (NCE) (HCD) mode combining 25.2, 60.0, and 94.8 NCEs into one fragmentation scan, an underfill ratio of 20 %, an intensity threshold of 1.7×10^5 cts, and the dynamic exclusion was set to 15 s. Further, the settings such as no apex trigger, no charge exclusion were applied prior to the MS/MS analysis. Additionally, peptide match was turned off, exclude isotopes option was turned on, and the preference, if idle,the setting 'the machine does not pick up other ions' was chosen prior to the data acquisition. Positive or negative ionization separate fragmentation modes as for the combined experiments, were operated with the following modifications: full scan (MS1) resolution (at m/z 200) was set to 70,000, MS/MS (MS2) resolution (at m/z 200) was set to 17,500, MS/MS maximum injection time was set to 80 ms and the underfill ratio set to 10 %, with a resulting intensity threshold of 1.3×10^5 cts. The duty cycle consisted of one full scan (MS1) event and one Top10 MS/MS (MS2) fragmentation event. Data acquisition

In accordance with standard procedures at Glasgow Polyomics), blank runs, quality control samples extracts were run to assess the performance of the mass spectrometer in terms of chromatography and mass intensities. Additionally, in a similar manner, three standard mixes containing 150 reference compounds were run to assess the quality of the mass spectrometer to aid in metabolite annotation and identification (Creek et al. 2011). The pooled sample was run prior to and across the batch every 6th sample to monitor the stability and quality of the LC–MS run, whereas the samples were run in a randomized order. All the raw files were converted into mzXML format immediately after the acquisition, thereby centroiding the mass spectra and separating positive and negative ionization mode spectra into two different mzXML files using the command line version of MSconvert (ProteoWizard). The accurate masses of

standards were obtained well within the accuracy of 3 ppm and intensities of the quality control samples were obtained within specifications.

Data processing

The mzXML files were uploaded into the Global Natural Products Social Molecular Networking (GNPS) environment (http://gnps.ucsd.edu—a free account is needed to log in) using an FTP server (FileZilla, version 3.10.1.1). Parameter optimization for molecular network generation for the HR-MS data sets resulted in the following settings. All MS2 spectra that were obtained in the data were clustered with MS-Cluster with a so-called 'parent mass tolerance' of 0.25 Da and a MS/MS fragment ion tolerance of 0.005 Da to create consensus spectra. As a result, the consensus spectra that contained less than 2 spectra were discarded. A network was created where edges were filtered for a cosine score above 0.55 and 2 or more matched peaks. Further, the edges between two nodes were kept in the network only if each of the nodes appeared in each other's respective top 10 most similar nodes. The spectra in the network were then searched against the GNPS spectral libraries. The library spectra were filtered in the same manner as the input data. All matches kept between network spectra and library spectra were required to have a cosine score above 0.6 and at least 4 matched peaks. Analog search was then enabled against the library with a maximum mass shift of 100.0 Da. Running times were under 15 min for both combined and single mode fragmentation files. Afterwards, Cytoscape, a network visualization software (http://www.cytoscape.org/), was then used to further process and visualize the downloaded molecular network data. The recommended graphical layout style is FM3 which is available for Cytoscape versions 2.8.1 and below. Thus, the molecular network was uploaded into Cytoscape (version 2.8.1) following the documentation available on the GNPS website. After applying the FM3 layout plugin, the molecular network was saved in.cys format (Cytoscape Session File) and reopened in Cytoscape version 3.2.0, where labelling and colouring of nodes and edges was conducted. Most importantly, the nodes were labelled with precursor masses, and coloured in such a manner that the two nodes had the same colour when they were present in the same set of files (using the rainbow pallet). Accordingly, two nodes having similar colours indicates that they are present in a similar set of files, often

differing in one or two files). Subsequently, the size of the nodes was made proportional to the number of unique files from where the node spectra originated, implying that the larger the node, the more unique files its spectra originated from. The edges were labelled with the mass differences between the two nodes they connected. The resulting molecular networks for the combined and separate fragmentation modes were then inspected in the Cytoscape environment.

2.6.3 **Analysis Glasgow Toxicology**

The analysis of 10 antihypertensive drugs and their metabolites (amlodipine, atenolol, bendroflumethiazide, bisoprolol, doxazosin, furosemide, losartan, losartan carboxylic acid (losartan-COOH), nor verapamil, ramipril, ramiprilat, spironolactone ,canrenone, and verapamil) in urine samples was performed using a conventional extraction method (PPt, LLE or SPE) and HF-LPME followed by LC-MS/MS analyses.

The quantifier and the qualifier ions were chosen by their relative abundances (the first being the most abundant); the noise produced, and in addition, the specificity of the ion was also considered for this choice. The fragmentor voltage and collision energy values of the drugs and their metabolites (amlodipine, atenolol, bisoprolol, canrenone, doxazosin, losartan, losartan-COOH, norverapamil, ramipril, ramiprilat, spironolactone, verapamil, atenolol-D6, canrenone-D4, losartan-D4, and verapamil-D7) were optimised in positive ionization mode. Also the other drugs and metabolites (bendroflumethiazide, furosemide, bendroflumethiazide-D5, and furosemide-13C6) were optimized in negative ionisation mode. The positive and negative optimization modes were selected based on the literature and considered the structure of drugs. Further, the scan-to-scan polarity switching is necessary due to the combination of drugs with different properties (acidic, basic, and neutral compounds). The composition of the mobile phase additives was tested by varying their concentration (0.1% or 0.01% (v/v) of formic acid, and 1, 2, 3 and 5mM of 138 ammonium acetate) and by comparing the resultant chromatographic peak shape and ion abundance.

Preparation of Calibrator and Quality Control Solutions

A standard working solution at 10µg/mL was prepared by diluting stock standard solutions in methanol up to the mark in a 20-mL volumetric flask. A Quality Control QC working solution at 10µg/mL was prepared by diluting stock QC solutions the same way as the standard working solution.The mixed calibrators and QCs solutions were prepared by the dilution of certain volumes of these working solutions in a 5mL-volumetric flask then made up to the mark with MeOH. All calibrators and QC solutions were stored in freezer (-20oC), in amber glass flasks.

After obtaining the urine analysis result from all three laboratories, I analysed the result and applied statistical analysis to produce my results.

2.7 Statistical Analysis

2.7.1 Statistical packages used

The statistical analysis was performed using Statistical Package for the Social sciences (SPSS) software for Microsoft Windows Version 25.0 (IBM Corporation, Armonk, New York, US).

2.7.2 Summary statistics

The mean ± standard deviation (SD) was used to summarise the quantitative variables while the valid count and percentage were used to summarise the categorical variables.

The P value was calculated using chi-square for categorical variables and one-way ANOVA and T test for continuous variables. P value was considered significant when the difference between the groups was less than 0.05.

2.7.3 Comparison of two means

Two sample t test was used to compare the differences between two groups. While Chi-square or Fisher's exact tests were used to compare the categorical data.

2.7.4 Comparison of more than two means

The difference between more than two groups of continuous data were compared using one way analysis of variance (ANOVA).

2.7.5 Logistic regression

The association of a binary result with possible predictors was investigated by using the binary logistic regression.

2.8 **Ethical approval**

Ethical approval was obtained from the West of Scotland Research Ethics Committee - R&D reference: GN14CA266; REC reference: 14/LO/1887 – (Refer to appendix 8.5).

3 Systematic review of metabolomics in hypertension and adherence

3.1 Introduction

3.1.1 Metabolomics

The urinary detection of prescribed drugs is one of the methods which is recommended despite its limitations to measuring adherence. The detection of drugs in the urine and plasma is generally performed using mass spectrometry and requires specific user input in terms of specifying a list of expected drugs for screening. The enhanced sensitivity of new generation mass spectrometers including the high-resolution Orbitrap series of instruments increasingly provide more capability to understand how drugs are metabolised by the human body and may permit untargeted screening of all the drugs. Mass spectrometry fragmentation (MS/MS or MS2) is widely used to find drug-specific fragments and for the identification of an extensive range of metabolites that arise from transformations in drug metabolism such as hydroxylation, methylation, and decarboxylation may augment detection (163, 164).

3.1.2 Definition

Nicholson et al. defined metabolomic as “The quantitative measurement of the multiparametric time-related metabolic responses of a complex (multicellular) system to a pathophysiological intervention or genetic modification” (165). Metabolomics studies metabolism at a global level and involves analysing the metabolites which are small molecules that are present in a biological cell, tissues, organs and biological fluid. The specific metabolites and their concentration are the results of the relation between the genetic expressions, protein expression and the environment (diet, activity, behaviour, disease and medical treatment). It represents the last layer following genomics, transcriptomics, and proteomics. Metabolomics has many alternative names such as metabonomics, metabolic profiling or fingerprinting. The size of the metabolomics depends on the number of compounds that are included in the analytical method. It could range from a thousand and reach up to ten thousand compounds. The disease causes changes to metabolism that last long; these

changes can be identified as a metabolic signature. In addition, medications can cause an alteration in the metabolic pathways either by acting on their target or by working in a different pathway leading to side effects.

3.1.3 **Types**

Metabolomics allows us to investigate a wide range of endogenous and exogenous metabolites with the potential investigation of physiological status, diagnosis of disease, the discovery of biomarkers and identification of perturbed biochemical pathways (166). It is currently a developing field and can be generally divided into two types. Untargeted and targeted metabolomics.

3.1.3.1 Untargeted metabolomics

Untargeted metabolomics is a comprehensive general approach to investigate small metabolite molecules that are either unknown or known metabolites. It allows us to identify metabolic perturbations and discriminatory metabolites (potential disease biomarkers) that might be associated with a certain disease or condition. Also, it enables us to discover novel metabolic pathways without having a prior hypothesis. It is usually referred to as hypothesis-generating or discovery-phase experimentation (167).

3.1.3.2 targeted metabolomics

Targeted metabolomics is a quantitative analysis of a set of chemically known annotated metabolites. In targeted metabolomics pathways are already known, explored and a prior hypothesis is present; for example, metabolites from a particular metabolic pathway or a specific class of small molecule or to validate a biomarker that was discovered in an earlier untargeted experiment (168). The advantage of the targeted method is that the detection and quantification can be robustly validated in advance (especially, in case if MS is the technical platform). Moreover, post hoc metabolite annotation is not required since it is a component of the initial technical development phase.

3.1.4 **Steps**

The metabolomics study involves several steps. The primary step is sample collection (for example patient urine, serum or blood). Once the sample is obtained the compounds of interest are separated from the specimen and then analysed using a special analytical instrument such as High-pressure liquid chromatography-mass spectrometry HPLC MS. Afterwards, the dataset is collected and curated. In the next step, the dataset is managed with an advanced software. Later, this database can be generated for the same patient before and after treatment or it can be produced for cases and control depending on the type of study. The database can provide information regarding the identity of detectable metabolites and their concentration levels. In addition, it can describe the property of the metabolite such as oxidation or reduction, mass to charge ratio. The software can also provide information regarding, Disease signature such as hypertension. As an instance, Brindle et al. was able to distinguish low/normal systolic blood pressure serum samples from borderline and high blood pressure samples. Secondly, it can predict the class whether the sample is for case or control, or before or after treatment. Third, the software can identify unrecognized groups and lastly, it can identify interactions among variables and can finally link variables to the specific pathways. In this case, it is important to note that a biomarker that helps to identify a drug response or disease state not only involves a single metabolite but also involves a pattern of several metabolites. There are various types of analytical methods, and each of them has its advantages. As an example, liquid chromatography with mass spectrometry is useful to gain the largest biochemical profile, while gas chromatography with mass spectrometry is beneficial to analyse the lipid compounds. Liquid chromatography and coulometric array detection are the best methods to map neurotransmitter pathways. nuclear magnetic resonance spectroscopy is ideal for studying toxicology (169). It is important to select the ideal method and while selecting two important factors must be taken into consideration: the availability of the appropriate instrument and adequate sample. Further, in an ideal situation such as in pharmaceutical companies where multiple instruments are used to obtain the data, it combines the advantage of each method, using several techniques and combining them together to achieve a better result. However, academic institution lacks the

multiple instrument facility. Therefore, it is important to select the most appropriate method and have the qualified experts who can deal with these instruments in order to obtain the best results. However, biological samples may be limited, and sometimes multiple tests are needed to be conducted. For these reasons, it is necessary to select the most appropriate method that can yield the best results consuming the least amount of sample.

3.1.5 **Advantages**

There are various advantages of metabolomics such as the knowledge about metabolome could provide information about the diagnosis of a particular phenotype. Moreover, it could affect our understanding of various metabolic diseases and novel, unexplored pathways. The changes in metabolome may allow us to understand different biological processes which can be integrated with knowledge of genome, proteome and transcriptome to enable a system biology approach towards a particular phenotype. The current information indicates that biochemical changes within body precede the development of a clinical disease.

3.2 **Aim**

The aim of this review is to comprehensively review existing studies that used metabolomics in hypertension and to summarize the most prevalent metabolites found for different hypertension phenotypes including drug response. As metabolomics is a new method with a multitude of platforms that measure different types of metabolites, the scope of this systematic review was deliberately made broad in order to obtain a complete understanding of the evidence available even if formal synthesis of outcome may not be possible.

3.3 **Studies:**

Types of studies included:

Case-cohort studies, case-control, cohort studies, clinical trials

Inclusion criteria:

- All studies involving human

- adult subjects (>18 years of age)

- Hypertension (cardiovascular disease) and with or without controls were included.

- Analytical platform using mass spectrometry (MS) or Nuclear magnetic resonance spectroscopy,

- Sample type: Serum- Plasma– Urine

Exclusion :

- studies analysing the proteome rather than the metabolome

- animal studies

Checked in for:

Participants' number and population, metabolomic techniques, sample types, and significantly altered metabolites between disease and control groups.

3.3.1.1 Literature Search for Identification of Studies

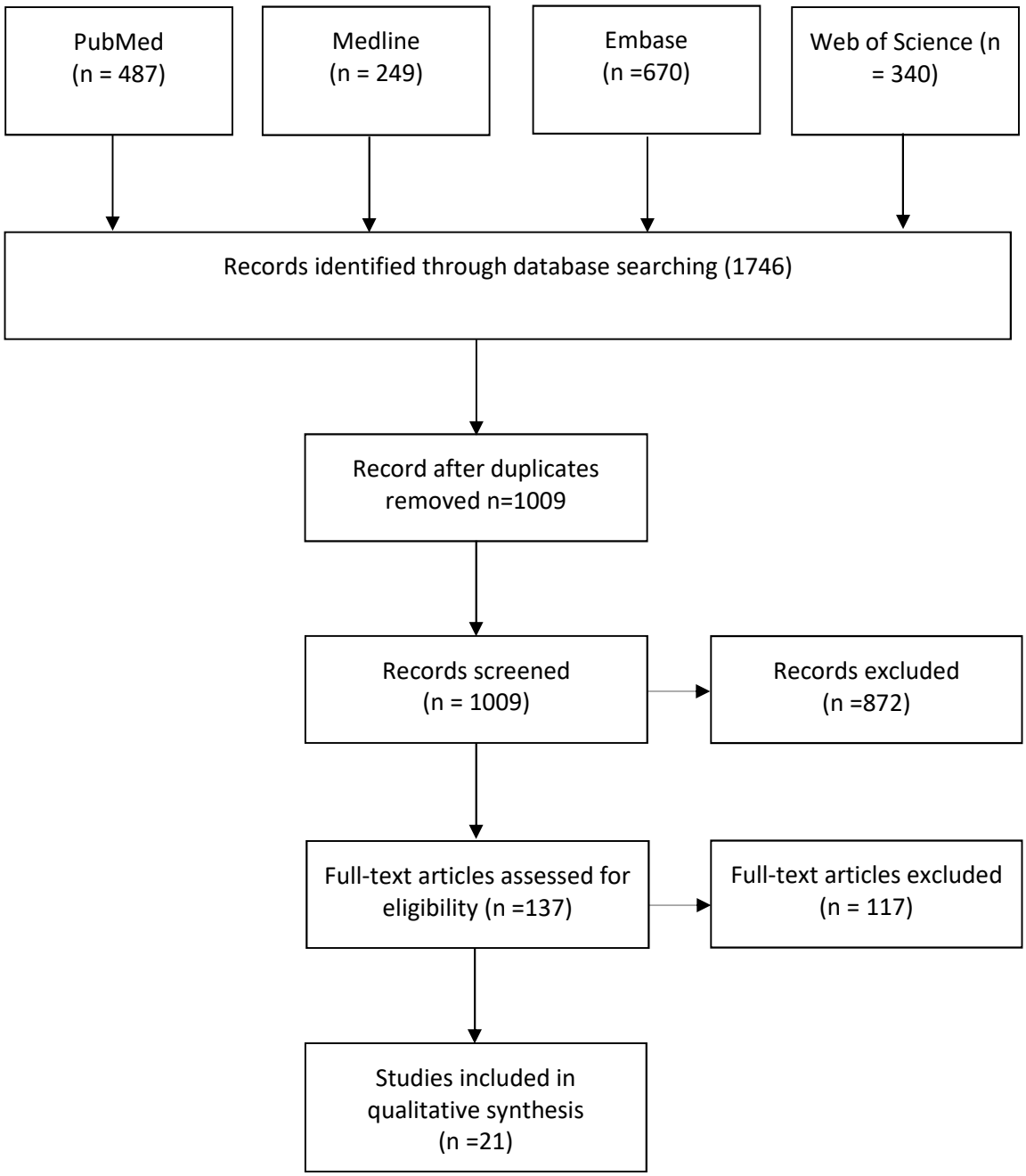
Electronic search was conducted on the following data bases: PUBMED, MEDLINE, EMBASE and Web of Science.

Search terms: metabolomics, metabonomics, metabolic profiling, Hypertension.

The review was conducted in accordance with (PRISMA) guidelines. PRISMA checklist for this systematic review is provided in appendix 8.10.

3.4 **Result**

3.4.1 **Flow chart**



3.4.2 **Tables Result**

The results of the literature review are summarised in the table below and the key features of each study are described as well.

Table 3-1 Metabolomic studies summary

	Author	Year	Specimen	N	Study population	Metabolites tested	Platform	Main finding	Conclusion
	Targeted								
1	Brindle	2003	Serum	64			NMR	Lipoprotein	One of first study to distinguish between low and high SBP
2	Graessler	2009	plasma	70	PRAEDIAS	95	LTQ MS	free cholesterol and ether lipids (Ether phosphatidylcholines and ether phosphatidylethanolamines)	Hypertensive patient had lower level
3	Liu	2011	serum	63		40	GC/TOFMS	Glucosamine, D-sorbitol, 1-stearoylglycerol and homocysteine	associated with hypertension
4	Mels	2013	serum	202	SABPA		ESI–MS/MS	L-carnitine and acylcarnitine	associated with BP
5	Kulkarni	2013	plasma	1192	SAFHS	319	LC/MS	Diacylglycerols DG 16:0/22:5 and DG 16:0/22:6 lipid species	significantly associated with SBP, DBP and MAP and risk of incident hypertension
6	Dietrich	2016	serum	1116	EPIC	127	LC/MS	Serine, glycine, and acyl alkyl-phosphatidylcholines C42:4 and C44:3 diacyl-phosphatidylcholines C38:4 and C38:3	higher predicted 10-year hypertension-free survival lower predicted 10-year hypertension-free survival
	Untargeted								
7	DeMeyer	2008	Serum	80			NMR	a-1 acid glycoprotein and choline.	Increased in hypertensive patients
8	Holmes	2008	urine	4630	INTERMAP		1H-NMR	* alanine * Formate, Hippurate	*directly associated with hypertension * inversely associated with hypertension
9	Zheng	2013	serum	896	ARIC	204	GC/MS	4-hydroxyhippurate, sex steroid: epiandrosterone sulfate, 5alpha-androstan-3beta 17beta-diol disulfate and androsterone sulfate	significantly associated with risk of incident hypertension
10	Stamler,	2013	urine	1559	NHWA		NMR	* Hippurate, succinate, trimethylamine * Dimethylamine and dimethylglycine, Creatinine and guanidinoacetate	*Higher in NHWA were hippurate, succinate, trimethylamine * High in AA :
11	Wikoff	2013	plasma	272	PEAR		GC-TOF/MS	3-hydroxybutyric acid &	Decrease in Caucasian race

								free fatty acids: saturated (palmitic), monounsaturated (oleic, palmitoleic) and polyunsaturated (arachidonic, linoleic)	
12	Menni	2015	Blood	3980	TwinsUK	280	UPLC-MS/MS & GC/MS	hexadecanedioate	Associated with BP
13	Deventer	2015	urine	25			GC/MS &LC/MS	GC:lactic, fumaric, 4–ydroxyphenyllactic, 2–hydroxyisovaleric acid LC: methyluric acid, methylguanosine, Trimethyl-L-lysine (TML) and indole carboxylic acid glucuronide.	GC showed 4 organic acids elevated in hypertensive groups LC low levels
14	Rotroff	2015		443	PEAR	489	GC TOF MS	Uric acid 5-methoxytryptamine indole-3-acetate	*increased in patient taking HCT *negatively associated with DBP in white patients on atenolol * associated with black patient on atenolol
15	Hao	2016	serum	58	CMCS	241	GC/MS	26 metabolites	
16	Shahin	2016	plasma	228	PEAR	212	GC TOF MS	Glycolic acid,Fumaric acid, Arachidonic acid, Caprylic acid, Dodecanol, Iminodiacetic acid, Trihydroxypyrazine NIST, Pyrazine 2,5 dihydroxy NIST, 2 hydroxyvaleric acid, Dihydroabietic acid, Phytol , 2 hydroxybutanoic Acid, Arabinose	associated with hydrochlorothiazide SBP and DBP responses
17	Gonzalez	2016	urine	148			LC MS	* Glutamate, glycerate, guanidinoacetate, pantothenate, oxaloacetate and 3-ureidopropionate * 3- hydroxybutyrate, malate, and pyruvate,	*Diminished levels compared to healthy individuals * Increased response in HTN with RAS inhibition
18	Hiltunen	2017		313	GENRES	600	LC MS	* long- and medium-chain acylcarnitines). * Amlodipine decrease in SBP and DBP was associated with decrease of plasma cysteinylglycine of hexadecanedioate. * Bisoprolol BP was associated with plasma fructose levels. * losartan BP associated with plasma oleamide and linoleamide .	* lower in most treated group (except for HCT * No clear relationship for HCT
19	Bujak	2017	plasma	69			LC TOF MS	19 up-regulated and 13 downregulated metabolites	Associated with hypertension
20	Zhao	2018		150			UPLC-Q-TOF/MS	10 metabolites L-methionine, Butyric acid, 5-Hyrdoxyhexanoic acid, o-Tyrosine, Cortolone, 11-hydroxyandrosterone,	Associated with hypertension

								3,4-Dihyroxyphenylglycol, 2-Aminooctanoic acid, Melatonin, %-hyrdoindoleacetic acid	
21	Ke	2018	Plasma	3464	Husermet project		GC/MS& UPLC/ MS	Hexadecenoic acid, hexadecenoic acid, and tetradecanoic acid acetylcarnitines	* associated with hypertension

The study conducted by Brindle (2003) was one of the first studies that was conducted using targeted metabolomics that was able to differentiate between low/normal systolic blood pressure (SBP 130 mm Hg), borderline SBP (131–149 mm Hg) and high SBP (150 mm Hg;) using 1H NMR spectroscopy. The resulting serum metabolic profile obtained in the study was mainly different in lipoprotein composition (170).

A study by Liu et.al. demonstrated the association of serum fatty acids with hypertension. oleic acid, nonanoic acid, ecosanoic acid, hexaenoic acid and hepatonic acid were found to be directly associated with hypertension. Serum fatty acids are proposed to increase vascular tone by increasing the sympathetic tone. In addition, they might influence cell membrane phospholipids that directly affect interaction of sodium and calcium influx and efflux causing an increase in vascular tone (171).

The INTERMAP study provided the largest evidence regarding small metabolites and pathogenesis of high blood pressure, the study included 4630 men and women aged 40–59 years from 17 population samples in the USA, UK, China, and Japan. The study has a unique collection of four interviewer-administered multi-pass 24-h dietary recalls allowing for a comprehensive assessment of dietary habits, and two 24-h urine collections from each individual. H NMR-based untargeted metabolic phenotyping of urine was performed on the stored 24-h urine collections from the 4630 participants. (alanine, hippurate, formate, and N-methylnicotinate) were associated with blood pressure. Two metabolites were inversely related to BP, formate (a byproduct of fermentation of dietary fibre by the gut microbiome), and hippurate (formed by hepatic glycine conjugation of benzoate, derived from gut microbial fermentation of plant phenolics).On the other hand, Alanine which is higher in people who eat animal rather than vegetable products, was directly associated with blood pressure (172).

A role for the gut microbiome in high BP was also reported in analysis of data from 896 normotensive black participants in the Atherosclerosis Risk in Communities (ARIC) study. Serum samples were analysed by GS-MS using the Metabolon platform; 4-hydroxyhippurate, an end product of benzoate metabolism from microbial fermentation of polyphenols, was associated with

17% higher risk of hypertension at 10 years follow-up. In addition, sex steroid metabolites (5α androstan- 3ß, 17ß diol sulfate, androsterone sulfate, and epiandrosterone sulfate)) were positively associated with elevated risk of incident hypertension (173).

In the European Prospective Investigation into Cancer and Nutrition (EPIC)-Postdam study, 127 metabolites were analysed among 135 cases (participants who developed hypertension over 10 years follow-up) and 981 non-cases, using a targeted MS platform in blood samples. Serine, glycine, acyl-alcyl-phospatidylcholines (PCs), and diacyl-PCs were associated with incident hypertension. These results suggest a possible role of inflammatory pathways in high blood pressure; both serine and glycine share anti-inflammatory and antioxidant properties, while PCs may exhibit an anti-inflammatory role and protect lipoproteins from oxidation (174)

Number of studies have supported the associations between and amino acid metabolism with blood pressure. The TWINUK study with measurement of 280 metabolites in fasting serum samples (MS-based metabolic profiling using Metabolon platform) on 3580 females with replication in two independent cohorts: Cooperative Health Research in the Augsburg Region (KORA) (n = 1494) and Hertfordshire (n = 1515) demonstrated a direct associations between hexadecanedioate (dicarboxylic acid) and both blood pressure and all-cause mortality. A causal role of this metabolite on blood pressure was supported by in vivo studies in rats highlighting the potential role of fatty acid ω-oxidation in blood pressure regulation(175).

Other smaller studies also suggested several lipids and amino acids associated with blood pressure levels and the role of inflammation and oxidative stress hypertension (176-181).

Demeyer et.al used a novel 1H –NMR – based algorithm called adaptive intelligence binning algorithm to identify bin edges in existing bins of NMR spectrum. This algorithm enables identification of low intensity metabolites. This study identified α1 – glycoprotein and choline to be associated with hypertension (182). A study of 25 black South African males enrolled in Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) cross-

sectional study found elevated levels of lactate, fumarate, 4-hydroxyphenyllactate, and 2-hydroxyvaleric acid(183).

3.4.3 Studies related to drug response

Several studies have attempted to identify potential biomarkers of drug responsiveness and to understand the molecular mechanisms that lead to drug response variation in blood pressure.

3.4.3.1 PEAR study by Rotroff

Rotroff et al. conducted an untargeted GC TOF MS on plasma samples to identify metabolites signature associated with response to antihypertensive medications: atenolol and hydrochlorothiazide (HCT) in white and black patients. Patient were selected from Pharmacogenomic Evaluation of Antihypertensive Responses study (PEAR). Metabolic profile was different in between the two drugs. The uric acid was found to be increased in both black and white patients taking HCT, which corresponded to the side effect of hyperuricemia that occur with thiazide diuretics. Further, 5-methoxytryptamine was negatively associated with DBP in white patients treated with atenolol whereas indole-3-acetate was associated with black patients on atenolol. 5-methoxytryptamine is a tryptamine derivative linked to serotonin, while indole-3-acetate is derived from tryptophan and both are involved in the gut microbiome. Additionally, O-phosphoethanolamine was significantly lower in black patients on HCT. Phosphoethanolamine is used as a component for phospholipid. In addition, patients on HCT had impaired glucose metabolism (184).

3.4.3.2 Wikoff

The study demonstrated the effect of atenolol intake on metabolites which caused a decrease in the level of free fatty acids including saturated (palmitic), monounsaturated (oleic, palmitoleic) and polyunsaturated (arachidonic, linoleic) in Caucasian race which was highly significant in Caucasian while absent in African American. It was found that atenolol increased plasma triglyceride levels and lowered HDL levels while not affecting LDL levels and affecting the plasma lipoprotein metabolism and lipolysis causing a decrease in fatty acids. Another possible mechanism is through the effect of phospholipase activity. It has been

shown that atenolol inhibits lysosomal phospholipase A1 (add reference). Also, 3-hydroxybutyric acid was also lowered in Caucasian which could possibly be the result of mitochondrial free fatty acid b-oxidation mainly in liver, increased utilization of 3- hydroxybutyric acid, which is produced from acetyl-CoA as a result of ketogenesis, and decreased production (185).

3.4.3.3 Shahin

The study by Shahin et al. demonstrated the used metabolomic and genomic profiles of hydrochlorothiazide-treated patients to discover the novel markers associated with hydrochlorothiazide BP response. The study included 228 patients from PEAR study using GC TOF MS. It was found that 13 metabolites which were Glycolic acid, Fumaric acid, Arachidonic acid, Caprylic acid, Dodecanol, Iminodiacetic acid, Trihydroxypyrazine NIST, Pyrazine 2,5 dihydroxy NIST, 2 hydroxyvaleric acid, Dihydroabietic acid, Phytol, 2 hydroxybutanoic Acid, Arabinose were significantly associated with hydrochlorothiazide SBP and DBP responses(186).

3.4.3.4 GENRES study by Hiltunen et al.

The aim of the study was to characterize the effects of several classes of antihypertensive medication on circulating metabolic profile. 313 Finnish men with high blood pressure (aged 35 to 60 years) were selected from the GENRES (Genetics of Drug Responsiveness in Essential Hypertension study). The GENRES trial is a cross-over design that studied the effects of four different classes of antihypertensive medication which are losartan angiotensin receptor antagonist, bisoprolol beta blocker, amlodipine calcium channel blocker and hydrochlorothiazide diuretic. The main finding of the study was long- and medium-chain acylcarnitines were lower in most treated groups relative to placebo periods (except for HCT) and amlodipine decrease in SBP and DBP was associated with the decrease of plasma cysteinyl glycine of hexadecanedioate. Also, the bisoprolol BP was associated with plasma fructose levels. In addition, for losartan BP associated with plasma oleamide and linoleamide there was no clear relationship established for HCT(187).

3.5 Discussion

The metabolome is the aggregate of all metabolites in a biological system and reflects the complex interactions between gene expression, protein expression, and the environment. Thus, metabolome is a reflection on organism's current physio-pathologic status and metabolomic association with a trait is potentially more informative of the involved causal pathways than associations from other omic studies such as proteomics or genomics. In this comprehensive systematic review, I identified 21 articles that evaluated the association between circulating metabolites and blood pressure or hypertension. The 21 studies were characterised by small sample sizes, predominantly cross-sectional (only 2 studies included prospective outcome data), heterogeneity in metabolomic assays(targeted or untargeted), platform used(NMR, LC/MS, LCTOF/MS, GC/MS GCTOF/MS) and phenotypes studied (blood pressure, hypertension and one study on antihypertensive response). The studies were predominantly in Caucasian subjects. The same set of metabolites was rarely found in different populations, making it difficult to draw consistent conclusions from these studies. Only one study tried to establish a causal relationship between the metabolite and blood pressure through integrated genomic and animal studies implicating pathways in the fatty acid omega oxidation pathway playing a causal role in blood pressure regulation(175, 188). Three studies showed early exploratory evidence for metabolomic characterisation of antihypertensive BP response.

Table 3-1 summarises the metabolomic studies and their significant findings. Various methods and platforms were employed in these studies that resulted in many metabolites associated with hypertension and antihypertensive drugs. there is a strong indication towards studying various metabolites in the context of hypertension. It is important to understand the types of metabolites deranged in hypertensive patients because this could help in developing potential therapeutic strategies to prevent hypertension. The studies related to hypertension had various result. This is due to the fact there is currently no standard platform or method to measure metabolites. Each study uses a different preparation and method. The result they produce are vastly different. Several pathways have been suggested in relation to blood pressure including the possible role of inflammatory, oxidative stress, lipid pathways and the gut

microflora(172-174, 182). Certain metabolites were found to be either upregulated or downregulated and were associated with hypertension.

3.5.1 **Pharmacometabolomics**

Pharmacometabolomics applies a metabolomic approach to the study of drug effects on individuals to predict patient responses to therapy, drug metabolism, efficacy and side effects. It offers the chance to tailor treatment more effectively based on the metabolic profile for each patient and avoid unwanted side effects. This approach has possible potential to develop personalized treatment. Wikoff et al.(185), Rotroff et al.(184), Shahin et al.(186) and Hiltunen et al.(187) have identified sets of blood metabolites that are associated with different antihypertensive drugs (atenolol, HCT, amlodipine, Losartan and Bisoprolol). The major limitations of these studies include small sample sizes, lack of replication and lack of functional studies. The biggest limitation for pharmacometabolomic studies has been the variety of platforms and methods to detect metabolites. Currently, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are the methods that are used most frequently for biofluid metabolic profiling. Some of the methods only detect lipid metabolites, while the untargeted methods detect a range of metabolites with the added challenge of identification of the metabolite signals. NMR metabolomics is a quantitative and highly reproducible method, which is important to study pharmacokinetic and pharmacodynamic aspects of the drug. The primary disadvantage of NMR spectroscopy compared to other analytical platforms is its low sensitivity. Nuclear magnetic resonance spectroscopy can reliably detect and quantify only metabolites present at relatively high concentrations.

Further, untargeted metabolomics using mass spectrometry is a highly sensitive technique that is capable of detecting a diversity of metabolites with high precision. The two main limitations are; the long time required for analysis and the diversity of the separation equipment components (chromatographs, columns) and separation protocols (eluents, gradients). Applying different components and protocols in chromatography limits the method’s reproducibility and leads to the collection of false data. Therefore, method standardization is one of the most important requirements in the application of MS-based methods in clinical practice.

A “metabolite profile” that can be obtained from metabolomic studies represents all interactions among various aspects of the genome, microbiome and environment. This makes the metabolite profile very informative not only to determine the effective dose of a given drug but also to modify pharmacotherapy on the basis of patients’ characteristics. The value of pharmacometabolomics over pharmacogenomics and pharmacoproteomics is the observation that the general metabolome remains invariant for each individual (189). The ability of the metabolome to provide complete information about the current physiological status of a patient makes metabolic profiling most suitable for the personalization of treatment.

Therapeutic drug monitoring TDM which is a method for the quantitative determination of drug concentration in various biofluids is a well-established technique for the assessment of drug effectiveness and toxicity. Therapeutic drug monitoring is akin to targeted metabolomics – as it is based on the existence of a direct connection between the administered dose of a drug (drug concentration) and the drug’s therapeutic effects. Thus, there is a value in the personalisation of drug treatment (190). As TDM measures the concentration of the drug that has reached its target, it is appropriate only for drugs that have a direct relationship between biofluid concentration and response and does not integrate the influence of pharmacodynamic variabilities. Thus, TDM is useful for establishing initial dosing regimens and monitoring certain medications. Metabolomics can extend the traditional TDM by generating a comprehensive analysis of all measurable metabolites in the sample including unknown chemicals and this may be crucial in the personalisation of treatment.

The critical factor in producing a metabolite profile is the identification of metabolites. This is done by the variety of methods and as noted below novel methods are being developed that would allow complete identification of metabolites reliably. In untargeted metabolomics, structural information is usually obtained from spectral libraries or previously characterised compounds. Currently, mass spectral libraries contain only a small fraction of the metabolites whose existence is known; for example, mzCloud (www.mzCloud.org) and MassBank (www.massbank.jp) contain fragmentation spectra of thousands of compounds, whereas PubChem

(http://www.ncbi.nlm.nih.gov/pccompound) contains tens of millions of chemical structures and many other compounds have yet to be catalogued in any database (191, 192). Several computational tools that predict metabolite structures and fragmentation patterns in-silico are in development phase but most are currently not capable of global analyses or comparison of large numbers of fragmentation spectra(193, 194).

Metabolomic studies provides a prospect of discovering novel biomarkers. Metabolomic changes can help us understand various biological processes and this information can be integrated with complements of genome, proteome and transcriptome to provide a system biology approach towards a particular phenotype. The current understanding of clinical disease development is that biochemical changes in the body precede the development of subclinical disease and therefore clinical disease. Information gained by complement of metabolites may prove to provide mission information regarding our understanding of hypertension. In addition, due to the dynamic nature of metabolites which increases the variance in development of predictive models. Statisticians with experience in metabolomics will be required to analyse information from the data. After that a comprehensive understanding of metabolic pathways which requires the involvement of a biochemist in interpretation of findings. Therefore, this field provides the next step in the advancement of science that requires collaboration of scientists from different fields at multiple levels.

3.5.1.1 Limitations

Despite the importance of this field, there are several issues. First, the reproducibility of metabolomic experiment can be challenging due to the dynamic nature of metabolome, with many sources of variation such as intra- and inter-sample variation, experimental conditions, methods of sample preparation and separation. Second, although different methods of chromatography that have been used to improve metabolite detection with high accuracy. There is no single mode of chromatography that can comprehensively separate the whole metabolome in a single run. Third, metabolomic differ from genomics and proteomics, genomics and proteomics commonly utilize a methodology to amplify the signal by certain polymerase chain reaction (PCR) related amplification while metabolites cannot be amplified by any method.

Moreover, the structure of proteins and genes is usually a polymer of already known simplified subunits (amino acids, and DNA). In contrast, metabolites differ generally in their shape and structure. Finally, once metabolites have been identified, many of these metabolites are still unknown and their biological role is not well understood (195-197).

4 Analysis of antihypertensive drug adherence using the Morisky questionnaire in the Glasgow BP Clinic

4.1 Introduction

Morisky et al. developed the Morisky Medication Adherence Scale (MMAS) in 1986, it was first validated in antihypertensive medication in outpatient settings (198). Morisky scale was originally composed of 4 items with dichotomous response categories with a yes or no. The rationale for selecting four items was the drug errors of omission could occur in any or all of several ways that are forgetting, carelessness, stopping the drug when feeling better or starting the drug when feeling worse. The scale was useful for identifying causes of medication underuse or omission; however, it didn't display good psychometric properties. The scale had a sensitivity of 81% while the specificity was 44%. Cronbach's alpha reliability was 0.61 which is below the acceptable values of 0.7. Despite that the lack of psychometric properties it has been used in many studies.

In 2008, Morisky et al. developed a modified eight-item Morisky Medication Adherence Scale (MMAS-8) from the original four-items scale and was published. The first seven questions are dichotomous answer categories with a yes or no while the last item was a five-point Likert response. The addition of the four items is done to identify and address the issues that are related to adherence behaviours compared to the four-item scale. The structure and wording of the questions are rearranged to avoid answers that might follow a certain behavioural pattern. The MMAS-8 is an important resource that addresses adherence concerns such as stopping to take medications without physician's advice, forgetting to take medications. In addition, it had a better psychometric property, the sensitivity was 93% and the specificity was 53%. Cronbach's alpha was 0.83 which is above the acceptance threshold. Cronbach's alpha is defined as a measure of reliability or internal consistency of a set of scale or test that is, how closely related a set of items are as a group. If a measure produces similar results under consistent conditions, it is said to have a high reliability.

Since it is the ratio of two variances, the value of Cronbach's alpha varies from 0 to 1. In psychometric tests, most fall within the range of 0.75 to 0.83 (199).

The MMAS-8 became popular and was commonly used in various patient populations. The scale has been verified by many studies on a worldwide scale with more than 110 versions and over 80 translations (162, 200).This was not the first time that the scale was validated in the hypertensive patients. Researchers had been developing and testing the questionnaire before its widespread use.

4.1.1.1 Advantages and Disadvantages of the Morisky Medication Adherence Scale

Despite the extensive use of the Morisky scale in a clinical setting, there are advantages and disadvantages for its use. The main advantage of MMAS-8 is the ease of use and cost-effectiveness. Patients need minimal effort to complete it. It can provide a convenient method to gain information regarding adherence in real-time because it is not invasive compared to direct monitoring of drug levels. Physicians experience no burden when delivering the questionnaire and after receiving the completed assessment, they can provide the direct feedback and support to determine any barriers to adherence.

Like the other self-report methods, the Morisky questionnaire can be subjected to several confounding factors such as recall bias (which might lead to overestimation of adherence). Besides, patients might feel pressured to provide acceptable answers in order to gain a positive reaction from their health care provider. These factors can potentially interfere with the consistency and can skew the rates of non-adherence.

4.2 **Aim**

The aim was to deliver MMAS-8 to patients attending the Blood pressure clinic at Queen Elizabeth university hospital to measure their level of adherence.

4.3 **Scoring**

The scale is composed of seven items with yes/no response options and one item with a 5-point Likert scale response option. These items provide information

about the barriers to medication adherence, such as forgetting to take medications, not taking medications when one feels worse, and difficulties in complying with a treatment regimen (refer to section 2.4.1).

4.4 Results

4.4.1 General characteristics for all the patients participated in the study (348 patient)

The total number of patients that completed the MMAS-8 questionnaire was 348 patients. According to Table 4-3 the demographic characteristics of the patients included in the study. The mean age of patients was 63.5 years (±14.6) with a minimum age of 21 and a maximum age of 75 years and half of the patient were males 185 (53.2%). In addition, majority of the patients were of European (white) ethnicity 322 (92.5%), two-third of them were married 227 (65.2%) and about half of them 179 (51.4%) had at least high school education, 140 (40.2%) were currently working with most of them working at daytime, only 35 (10.1%) were active smokers, 84 (24.1%) didn't drink alcohol and the mean hours of sleep were 6.93±1.53 hrs.

The perception described as the patient's feeling towards receiving antihypertensive medication had three responses: improving, when the patient felt better taking their drugs, no change, when the patient didn't notice any difference after taking their prescribed drugs and worsening, patients who felt worse on their antihypertensive drugs. The total number of patients that felt improving were 141(40.5%) while half of the patients 186 (53.4%) felt no change and only 21 (6%) patients felt worsening on antihypertensive drugs.

The mean SBP was 148 (±21) mmHg and the mean DBP was 83 (±13) mmHg and only one-third of patients 125 (35.9%) were controlled for their SBP while 259 patients (74.6%) were controlled for DBP. Also, two-third of patients responded with a score of 8 indicating high adherence, while 26.7% of patients scored (6 to <8) medium adherence and only 11.2 had low adherence (score <6).

4.4.2 **Antihypertensive Medication**

The mean number of antihypertensive medications for each patient was 2.56 (±1.4). Antihypertensive monotherapy was prescribed to 27% patients while 26.4% were receiving two antihypertensive medications and 46.6% were on 3 or more antihypertensive drugs

According to (Table 4-1), CCB was the most common class prescribed in 211 patients (60.6%), followed by diuretics in 166 (47.7%), ACEI in 149 (42.8%), ARB in 130 (37.4%) and finally BB in 116 (33,3%). In Table 4-2 The most common prescribed drugs were Amlodipine (to 41.1% of patients), Bendroflumethiazide (34%), Ramipril (25.9%), Losartan (16.4%), Candesartan (15%), Doxazosin (13.5%), Atenolol (13%), Bisoprolol (12.6%), Lercanidipine (11.8%), Perindopril (8.6%), Furosemide (7.8%), Spironolactone (6.9%).

Table 4-1 The prevalence of antihypertensive drugs according to class.

Drug class	No.	%
CCB	211	60.6
Diuretics	166	47.7
ACEI	149	42.8
ARB	130	37.4
BB	116	33.3

Table 4-2 Most common antihypertensive drugs prescribed in patient completing MMAS

Drug name	number	percent	Class
Amlodipine	143	41.1	CCB
Bendroflumethiazide	118	34.0	Diuretic
Ramipril	90	25.9	ACEI
Losartan	57	16.4	ARB
Candesartan	52	15.0	ARB
Doxazosin	47	13.5	Alpha Blocker
Atenolol	45	13.0	BB
Bisoprolol	44	12.6	BB
Lercanidipine	41	11.8	CCB
Perindopril	30	8.6	ACEI
Furosemide	27	7.8	Diuretic
Spironolactone	24	6.9	Diuretic
Indapamide	18	5.2	Diuretic
Lisinopril	18	5.2	ACEI
Irbesartan	11	3.2	ARB
Nebivolol	11	3.2	BB
Nifedipine	10	2.9	CCB
Felodipine	9	2.6	ACEI
Enalapril	7	2.0	ACEI
Eplerenone	7	2.0	mineralocorticoid receptor antagonist
Valsartan	7	2.0	ARB
Moxonidine	6	1.7	lpha-2/imidazoline receptor agonist
Metoprolol	5	1.4	BB
Propranolol	5	1.4	BB
Diltiazem	4	1.2	CCB
Hydralazine	4	1.1	Vasodilator
Labetalol	4	1.1	BB
Methyldopa	4	1.1	centrally acting antiadrenergic
Amiloride	3	0.9	Diuretic
Verapamil	3	0.9	CCB
Trandolapril	2	0.6	ACEI
Captopril	1	0.3	ACEI

4.4.3 **Morisky questionnaire result**

4.4.3.1 Demographic (categorical) variables

Table 4-3 compares the categorical demographic variables vs the Morisky adherence groups (Low, mid and High). Chi-square analysis was done to obtain the P-value. The statistically significant factors were gender, education and occupation and perception ($P<0.05$). On the other hand, ethnicity, marital status, smoking, alcohol, SBP and DBP control, number of antihypertensive drugs and eGFR were not significant.

Table 4-3 Demographic characteristics for Morisky result (categorical)

		Low		Medium		High		
		N=39	%	N=93	%	NN=216	%	P
Gender	Male	28	71.8%	47	50.5%	110	50.9%	0.047
Ethnicity	Non-white	5	12.8%	7	7.5%	14	6.5%	0.383
	White	34	87.2%	86	92.5%	202	93.5%	
Marital	Single	17	43.6%	33	35.5%	71	32.9%	0.427
	Married	22	56.4%	60	64.5%	145	67.1%	
Smoking	Non-smoker	33	84.6%	80	86.0%	200	92.6%	0.106
	Smoker	6	15.4%	13	14.0%	16	7.4%	
Alcohol	No	7	17.9%	25	26.9%	52	24.1%	0.549
	Yes	32	82.1%	68	73.1%	164	75.9%	
Education	Bachelor	26	66.7%	46	49.5%	98	45.4%	0.049
	High school	13	33.3%	47	50.5%	118	54.6%	
Occupation	Employed	27	69.2%	40	43.0%	73	33.8%	<0.0005
	Retired	6	15.4%	45	48.4%	120	55.6%	
	Unemployed	6	15.4%	8	8.6%	23	10.6%	
AntiHTN	1	15	38.5%	23	24.7%	56	25.9%	0.389
	2	11	28.2%	23	24.7%	58	26.9%	
	3 or more	13	33.3%	47	50.5%	102	47.2%	
Perception	Improving	10	25.6%	37	39.8%	94	43.5%	0.043
	No change	23	59.0%	50	53.8%	113	52.3%	
	Worsening	6	15.4%	6	6.5%	9	4.2%	
	SBP controlled	14	35.9%	35	37.6%	76	35.2%	0.919
	SBP uncontrolled	25	64.1%	58	62.4%	140	64.8%	
	DBP controlled	25	64.1%	67	72.0%	167	77.3%	0.182
	DBP uncontrolled	14	35.9%	26	28.0%	49	22.7%	
eGFR	>60	34	87.2%	76	82.6%	172	81.1%	0.886
	30 – 60	5	12.8%	15	16.3%	37	17.5%	
	<30	0	0.0%	1	1,1%	3	1.4%	

4.4.3.2 Gender

Table 4-4 shows that in the lower adherence group, the number of males 28 (71.8%) was twice the number of females of 11(28.2%). In the middle group, both males and females were around 50% and in the higher adherence group, males were 110 (50.9%) similar to the females 106 (49.1%). It was found to be statistically significant (P=0.047) indicating that males are more likely to have lower adherence. Figure 4-1 shows the relationship between gender and age, females were older compared to younger males.

Table 4-4 Gender compared with Morisky

Gender		low	medium	high	Total
Male	N	28	47	110	185
	%	71.8%	50.5%	50.9%	53.2%
Female	N	11	46	106	163
	%	28.2%	49.5%	49.1%	46.8%
Total	N	39	93	216	348
	%	100.0%	100.0%	100.0%	100.00%

Chi-square= 0.047

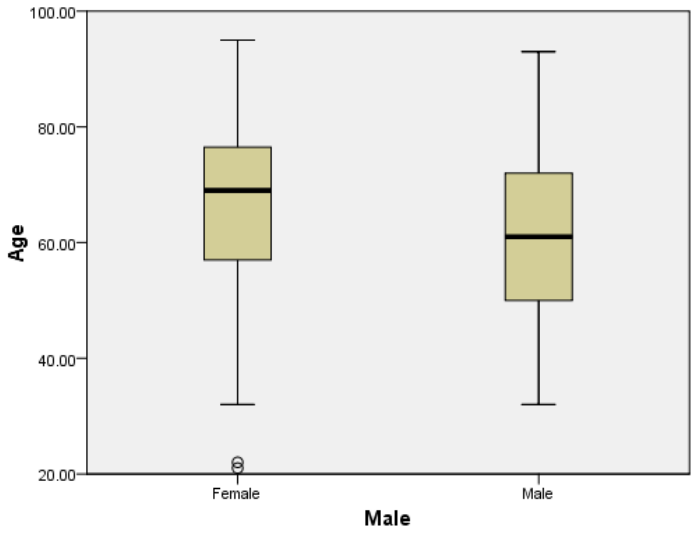


Figure 4-1 Boxplot for gender and age. The plot shows the association between age and gender.

4.4.3.3 Occupation

The results (summarised in Table 4-5) found that 171 patients were retired, 140 patients were employed and only 37 patients were unemployed. In the lower adherence group, employed patients were the most common with a number of 27 (69.2%) as compared to both retired and unemployed patients that both with a number of 6 (16.2%). In the middle adherence group, the number of retired patients was highest which was around 45 (48.4 %) while the number of employed patients were 40 (43%) and unemployed were 8 (8.6%). In the Higher adherence group, retired patients were the most common with 120 (55.6%), followed by employed with 73 (33.8%) and finally unemployed with 23 (10.6%). It was found to be statistically significant (P< 0005) implying that employed patients are more likely to be non-adherent to their medication. There is a strong association between age and occupation shown in (Figure 4-2), older patients were mostly retired.

Table 4-5 Occupation compared with Morisky

Occupation		Low	Medium	High	Total
Employed	N	27	40	73	140
	%	69.2%	43.0%	33.8%	40.2%
Retired	N	6	45	120	171
	%	15.4%	48.4%	55.6%	49.1%
Unemployed	N	6	8	23	37
	%	15.4%	8.6%	10.6%	10.6%
Total	N	39	93	216	348
	%	100.0%	100.0%	100.0%	100.00%

Chi-square P<0.0005

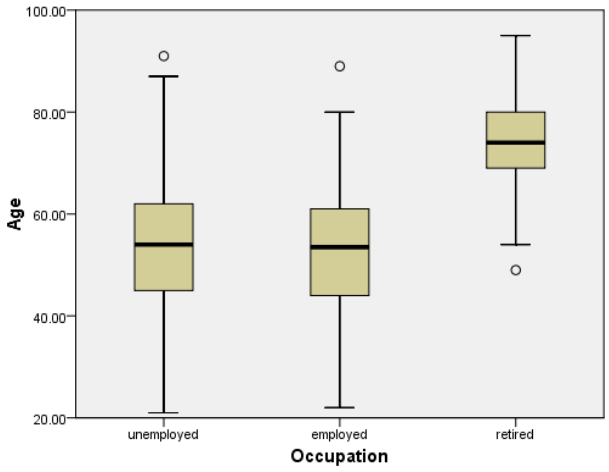


Figure 4-2 Box plot for occupation and age. The plot shows the association between age and occupation.

4.4.3.4 Education

In the area of education, it was found that (Table 4-6) 170 patients had bachelor's degree and higher education as compared to 178 patients that had only high school education. Patients with bachelor's degree and higher education were about 26 (66.7%) in the lower adherence group which was double the number of patients with high school education 13 (33.3%). While in the medium group, they had similar number 46 (50.5%) for patients with bachelor's degree education and 47 (26.4) for high school education. In the higher adherence group, high school educated patients were 118 (54.6%) more than patient with bachelor 98 (45.4%). It was found to be statistically significant (P=0.049) concluding that patients with bachelor's or higher education are more likely to have lower adherence. Younger patients had higher education compared to older patients as shown in (Figure 4-3)

Table 4-6 Education compared with Morisky

Education		Low	Medium	High	Total
Bachelor and higher	N	26	46	98	170
	%	66.7%	49.5%	45.4%	48.9%
High school	N	13	47	118	178
	%	33.3%	50.5%	54.6%	51.1%
Total	N	39	93	216	348
	%	100.0%	100.0%	100.0%	100.00%

Chi-square P= 0.049

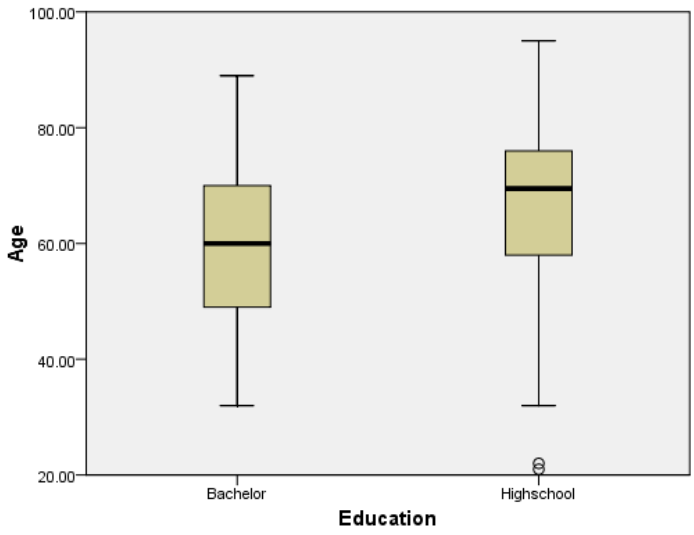


Figure 4-3 Boxplot for education and age. The plot shows the association between age and education.

4.4.3.5 Perception

Perception is defined as the patient response to how they feel about receiving the antihypertensive drugs, the responses are categorized as improving, no change and worsening. In the lower adherence group 6 patients (15.4%) felt worsening on receiving antihypertensive drugs, 23 (59%) patients responded with no change and 10 (25.6%) felt improving, in the medium adherence group half of the patients felt no change followed by about 40% who felt improving and only 6.5% who felt worsening. In the high adherence group, the highest group were those who responded (felt no change) 113 (52.3%) followed by those who felt improving on their medication 94 (43.5%), and finally 9 (4.2%) felt worse on their medications. This was statistically significant (P=0.043) patient in the lower adherence group felt worsening on their prescribed drugs in contrast to patient that were adherent who felt they were improving.

Table 4-7 Perception compared with Morisky

Perception		Low	Medium	High	Total
Improving	N	10	37	94	141
	%	25.6%	39.8%	43.5%	40.5%
No change	N	23	50	113	186
	%	59.0%	53.8%	52.3%	53.4%
Worsening	N	6	6	9	21
	%	15.4%	6.5%	4.2%	6%
Total	N	39	93	216	348
	%	100.0%	100.0%	100.0%	100.00%

Chi-square P=0.043

4.4.3.6 Continuous variables

4.4.3.7 Demographic (continuous) and Morisky groups

Table 4-8 Demonstrates the demographic chrematistics for the continuous variables across Morisky groups. Patient in the low adherence group were younger, had lower SBP but higher DBP and weight compared to patient in the middle and high group. Amount of sleep was similar across the groups. The statistically significant variables were age and DBP.

4.4.3.8 Age and Morisky adherence

Adherence was statistically significantly ($p<0.0005$) associated with age, in the lower adherence group the mean age was 53.31±13.88 years which is lower than both the middle and higher groups. The mean age for the middle group was 62.92±15.73 years and for the higher group the mean age was 65.68±13.48 years. This indicates that younger patients are more likely to be non-adherent to their medication compared to the older patients.

4.4.3.9 DBP and Morisky adherence

The mean DBP for the lower adherence group was 87±15 mmHg which was higher than DBP mean for higher adherence group 82±12 mmHg. DBP was statistically significant P=0.049. Therefore, patients with high DBP are more likely to be non-adherent to their prescribed regimen.

Table 4-8 Demographic (continuous) and Morisky groups

	Low n=39			Medium n=93			High n=216			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	P
Age	39	53.3	13.88	93	62.9	15.73	216	65.68	13.48	<0.0005
SBP	39	145.0	16	93	148.0	22	216	149	22	0.385
DBP	39	87.0	15	93	83.0	13	216	82	12	0.049
Weight	38	90.7	17.88	92	85.9	17.47	212	86.16	19.94	0.362
BMI	37	29.9	4.93	84	30.1	5.76	197	30.7	6.96	0.689
SleepHour	39	7.0	1	93	7.0	1	216	7	2	0.947

P-value was calculated using one-way ANOVA statistical test.

4.4.3.10 ABPM and Morisky groups

Table 4-9 summarises the ABPM for patients across Morisky groups. The statistically significant AMBP component were: 24H (SYS SD, DIA AVG, T MAP AVG, T PP AVG), Day (DIA AVG, MAP AVG, PP AVG), Night (DIA AVG, PP AVG). Second ABPM components: (24H DIA SD2 and Day HR AVG).

In lower adherence patient, day SBP dropped to from 143 to 130 mmHg at night (≈9%) while DBP 89 to 76.9 (≈13%) compared to higher adherence group day SBP 143 to 131 (≈8%) and DBP 81 to 71.5 (≈12%) Patients in the higher adherence group had lower DBP compared to patients in the lower adherence group .

Table 4-9 ABPM and Morisky groups

	Low n=39			Medium n=93			High n=216			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	P
First ABPM										
24H										
SYS AVG	32	140	15	73	144	19	160	140	14	0.124
SYS SD	28	12.89	2.46	69	14.91	3.75	146	14.84	3.94	0.034
DIA AVG	32	86	12	73	83	14	160	79	11	0.003
DIA SD	28	11.21	2.36	69	10.73	2.67	146	10.67	2.59	0.593
MAP AVG	32	104	12	73	104	14	160	100	10	0.022
MAP SD	28	11.45	2.28	69	12.17	2.88	146	12.05	2.71	0.48
PP AVG	28	53	7	69	61	14	146	60	12	0.012
PP SD	28	8.87	2.18	69	10.28	2.82	146	10.08	2.9	0.072
HR AVG	28	75	13	69	73	14	146	70	12	0.099
HR SD	28	9.95	3.46	69	9.2	3.73	146	9.67	3.88	0.598
Day										
SYS AVG	32	143	15	73	147	19	160	143	15	0.124
SYS SD	32	12.43	7.63	73	13.57	4.19	160	13.43	3.93	0.471
DIA AVG	32	89	13	73	86	15	160	81	11	0.003
DIA SD	32	9.38	3.43	73	9.2	2.52	160	9.31	2.66	0.942
MAP AVG	32	107	13	73	107	15	160	103	11	0.018
MAP SD	28	9.45	2.51	69	10.66	3.13	146	10.68	2.69	0.096
PP AVG	28	53	8	69	61	14	146	61	13	0.011
PP SD	28	8.98	2.23	69	10.42	3.3	146	9.95	2.99	0.103
HR AVG	28	77	13	69	75	15	146	72	12	0.095
HR SD	28	9.64	3.5	69	8.63	3.55	146	9.38	4.14	0.341
Night										
SYS AVG	32	130	16	73	136	20	159	131	17	0.12
SYS SD	32	12.72	4.7	73	12.19	5.29	158	12.81	4.79	0.67
DIA AVG	32	76.9	13.4	73	75.6	13.2	159	71.5	11.3	0.012
DIA SD	32	10.84	5.14	73	9.31	4.31	158	9.46	3.32	0.142
MAP AVG	32	95	13	73	97	14	159	93	11	0.077
MAP SD	28	11.38	4.36	69	9.82	3.93	144	10.59	3.5	0.144

PP AVG	28	52	7	69	60	15	145	58	12	0.017
SD	28	7.59	4.01	69	7.99	3.14	144	8.42	3.41	0.415
HR AVG	28	69	13	69	67	12	145	65	11	0.128
HR SD	28	6.83	3.88	69	6.24	3.14	144	5.96	2.97	0.386
Second ABPM										
24H										
SYS AVG2	10	140	12	31	144	17	75	140	16	0.463
SYS SD2	8	14.64	4.15	30	15.4	3.48	66	15.15	3.7	0.868
DIA AVG2	10	83	9	31	80	12	75	79	10	0.466
DIA SD2	8	12.14	1.89	30	11.88	2.71	66	10.36	2.64	0.015
MAP AVG2	10	103	9	31	103	11	75	100	10	0.41
MAP SD2	8	12.62	1.52	30	12.24	3.41	66	11.83	2.85	0.683
PP AVG2	8	56	12	30	64	16	66	60	12	0.229
PP SD2	8	9.24	3.75	30	9.89	2.26	66	10.19	2.46	0.569
HR AVG2	8	76	16	30	73	13	66	69	12	0.169
HR SD2	8	9.13	3.17	30	9.15	4.27	66	9.05	3.38	0.993
Day										
SYS AVG2	10	143	14	31	147	17	75	142	15	0.336
SYS SD2	10	13.28	4.02	31	13.75	3.62	75	14.46	4.45	0.577
DIA AVG2	10	85	10	31	83	13	75	81	10	0.374
DIA SD2	10	9.67	2.7	31	10.21	1.6	75	9.25	2.46	0.147
MAP AVG2	10	105	10	31	106	12	75	103	10	0.294
MAP SD2	8	10.98	2.7	30	11	2.81	66	10.67	2.83	0.844
PP AVG2	8	57	13	30	64	16	66	60	12	0.227
PP SD2	8	9.4	3.34	30	9.78	2.37	66	10.3	2.61	0.496
HR AVG2	9	85	26	30	75	14	66	71	12	0.019
HR SD2	9	9.44	3.37	30	8.79	3.72	66	8.77	3.66	0.873
Night										
SYS AVG2	10	124	19	31	134	21	74	132	19	0.382
SYS SD2	10	11.89	3.88	31	13.06	3.97	74	12.18	4.49	0.591
DIA AVG2	10	77	13	31	70	10	74	71	10	0.172
DIA SD2	9	11.17	3.91	31	10.09	3.46	74	8.84	4.16	0.128
MAP AVG2	9	95	8	31	94	13	73	94	12	0.972
MAP SD2	8	11.13	3.4	30	10.14	3.31	66	9.98	3.7	0.694
PP AVG2	8	54	9	30	63	19	66	59	14	0.231
PPSD2	8	7	3.4	30	7.58	3.43	66	8.15	2.86	0.487
HR AVG2	8	70	16	30	68	12	66	63	11	0.111
HR SD2	8	7.16	3.36	30	5.94	3.05	66	5.72	2.72	0.41

SYS: systolic DIA: diastolic, MAP: mean arterial pressure, PP pulse pressure. D: day, N: night, AVG: average, SD: standard deviation. P value was calculated using one-way ANOVA test.

4.4.3.11 Lab investigations and Morisky groups

Table 4-10summarises the lab investigation across Morisky groups. The significant investigations were: HB, TB, Albumin, HDL, CHOL/HDL ratio, Urine creatinine and aldosterone. The low adherence group had highest values for Hb, Albumin and Aldosterone.

Table 4-10 Lab investigations and Morisky groups

	Low n=39			Medium n=93			High n=216			
CBC	N	Mean	SD	N	Mean	SD	N	Mean	SD	P
WBC	38	7.374	2.693	93	7.631	3.538	214	7.257	2.01	0.507
HB	38	149	14	93	142	16	214	141	14	0.014
Platelet	38	251	59	93	252	64	214	265	76	0.248
U&E										
Na	39	140	2	93	139	3	216	139	3	0.434
K	39	4.22	0.38	93	4.67	3.63	215	4.29	0.47	0.244
CL	39	104	3	93	103	4	216	103	4	0.326
Urea	39	5.36	2.04	93	6.01	1.92	216	6.15	2.91	0.218
Creatinine	39	85	23	93	84	26	216	82	30	0.763
LFT										
TB	39	10	4	92	12	6	216	10	4	0.036
ALT	39	32	14	92	29	31	215	26	16	0.14
AST	39	26	10	93	25	20	216	24	11	0.582
ALP	39	83	19	93	88	32	216	87	33	0.687
Albumin	39	41	3	93	39	4	216	39	3	0.01
Lipid Profile										
Cholesterol	38	5.33	1.36	93	5.03	1.15	216	5.06	1.1	0.35
TGL	38	2.02	1.47	93	1.76	1.06	216	1.7	1.03	0.235
HDL	38	1.32	0.34	89	1.31	0.3	213	1.43	0.43	0.026
LDL	37	3.16	1.26	84	2.97	0.97	204	2.89	0.99	0.314
Chol/HDL ratio	38	4.24	1.29	89	4.02	1.13	213	3.75	1.11	0.019
Glucose	39	6.44	3.21	87	6.27	4.23	207	6.24	2.69	0.937
HbA1c	27	43	15	54	41	13	129	44	14	0.447
Urate	2	203.63	287.62	10	393.44	179.97	25	359.38	96.96	0.197
Renin	11	72	120	14	89	103	34	36	52	0.099
Aldosterone	10	432	489	14	603	290	31	334	158	0.015
LogTB	39	.98	0.15	92	1.03	0..19	216	0.99	0.17	0.064

WBC: White Blood Cell, HB: Haemoglobin, Platelet, U&E: Urea and Electrolyte, Na: Sodium, K: Potassium, CL: Chloride, LFT: Liver Function Test, TB: Total Bilirubin, ALT: Alanine aminotransferase, AST: Aspartate Transaminase, ALP: Alkaline Phosphatase, TGL: Triglycerides, HDL: High-density Lipoprotein, LDL: Low-density Lipoprotein, VLDL: Very low-density Lipoprotein, Chol/HDL ratio: Cholesterol: HDL ratio. P-value was calculated using one-way ANOVA test.

4.4.3.12 Binary Logistic regression

Due to the small number of patients in the lower and middle group and in order to increase power, they were combined into one group and were classified as non-adherent while the patients in high adherence group were classified as adherent because two groups were considered. Binary logistic regression was used instead of ordinal logistic regression. All significant variables were included: gender, age, education, occupation and perception.

Table 4-11 Binary logistic regression. All significant variable included.

	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
							Lower	Upper
Male	-0.114	0.235	0.236	1	0.627	0.892	0.563	1.413
Highschool	0.128	0.241	0.282	1	0.596	1.137	0.708	1.823
Unemployed			1.853	2	0.396			
Employed	-0.441	0.4	1.218	1	0.27	0.643	0.294	1.409
Retired	-0.089	0.456	0.038	1	0.845	0.915	0.375	2.235
worsening			5.721	2	0.057			
No change	-0.401	0.243	2.731	1	0.098	0.67	0.416	1.077
Improving	-1.046	0.489	4.576	1	0.032	0.351	0.135	0.916
Age	0.019	0.011	2.874	1	0.09	1.02	0.997	1.043
Constant	-0.224	0.729	0.094	1	0.759	0.799		

None of the variables were statistically significant, this may be because the variables are confounding. Due to the possibility of confounding I removed occupation from the regression analysis.

Table 4-12 Binary logistic regression. Excluding occupation from the model.

	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
							Lower	Upper
Male	-0.137	0.232	0.347	1	0.556	0.872	0.553	1.375
High school	0.191	0.235	0.659	1	0.417	1.21	0.763	1.919
Worsening			5.292	2	0.071			
No change	-0.372	0.241	2.391	1	0.122	0.689	0.43	1.105
Improving	-1.01	0.486	4.315	1	0.038	0.364	0.14	0.945
Age	0.025	0.008	9.351	1	0.002	1.026	1.009	1.042
Constant	-0.851	0.553	2.367	1	0.124	0.427		

Age was significant ($P<0.05$). a year increase in age increases the odds of high adherence in the sample by 2.6%, with the odds in the population increased by between 0.9% and 4.2%. Thus, older patients are more likely to have high adherence than younger patients.

Table 4-13 Binary logistic regression. Excluding age from the model.

	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
							Lower	Upper
Male	-0.167	0.232	0.517	1	0.472	0.847	0.538	1.333
High school	0.187	0.238	0.616	1	0.433	1.205	0.756	1.921
worsening			5.526	2	0.063			
No change	-0.376	0.241	2.436	1	0.119	0.686	0.428	1.101
Improving	-1.04	0.486	4.582	1	0.032	0.353	0.136	0.916
Unemployed			8.426	2	0.015			
Employed	-0.415	0.397	1.096	1	0.295	0.66	0.303	1.436
Retired	0.318	0.384	0.686	1	0.407	1.374	0.648	2.915
Constant	0.784	0.428	3.357	1	0.067	2.19		

Occupation (unemployed) was significant ($P<0.05$). This indicates that age and occupation are potentially confounding.

4.4.4 **Discussion**

In this chapter I have summarised the characteristics of the patients that completed Morisky questionnaire in order to assess patient adherence using an indirect method (self-report) and to determine predictors for adherence. The results indicate that out of 348 patient that completed the questionnaire nearly two third of patients were found to be adherent to their prescribed antihypertensive drugs.

Previous studies results

The results are similar to the study conducted by Lee et al. in Hong Kong where out of 1114 patients, 725 (65.1%) patients showed high adherence. However, other studies conducted in USA, China and Brazil showed lower adherence. In US, a study by Hyre et al. demonstrated that out of 295 hypertensive patients, only 35.6% had high adherence which was similar to a study conducted by Hou et al. in China on 585 older patients who showed 34.2% high adherence. While a study by Olivera et al. in Brazil was carried out on 223 hypertensive patients which found that high adherence was present in only 19.7% (137, 139, 201, 202). The rate of adherence may differ by many factors, such as study design, types of population, method of measurement, cultural factors (These include cultural health perception of hypertension, health perceptions of Western medications, self-care behaviour and social support) which could explain different levels of adherence among different populations (142) .Several studies have evaluated the factors associated with antihypertensive medication adherence. Age, gender, the number of antihypertensive drugs and socioeconomic status were found to be associated with drug adherence (203, 204).

There are several explanations for the high adherence seen in the Glasgow BP Clinic participants in contrast to other studies. One reason may be that all the Glasgow patients were attending a specialised hypertension clinic and they were referred to the clinic because general practitioners had a difficulty getting their BP under control. Secondly, MMAS may not be reliable in assessing adherence in the selected cohort of patients. The questionnaires were administered when the patients attended the clinic for clinic review which may have impacted how they completed their questionnaires. More generally, all prescriptions are freely

provided by the NHS in Scotland which may have influenced the patients to respond more positively about questions on adherence. So, further analysis of the questionnaire results in relation to actual BP control was performed to elucidate the true levels of adherence.

4.4.4.1 Adherence and SBP

The main consequence of not adhering to the prescribed regimen is failure to control BP. In our study, despite the high adherence detected, the level of BP control was low. Only 35% of patient who reported that they were adherent had controlled SBP. Several studies have demonstrated direct associations between adherence and BP control. In the study conducted in Brazil 65.1% of patients with high adherence had their BP under control while in the original study by Morisky it was 56.7% (210).

There are several reasons for the lack of BP control in my study, first it is possible that the patients are not taking their prescribed drugs and they are providing a false positive response which is a downside associated with self-reports. (In later chapters when looking at the metabolomics results, I will assess if there is evidence for this). Second, patients could be adherent to their medications, but the drugs are not effective in controlling their BP, patients may be resistant to treatment.

ABPM

ABPM measurements instead of clinic readings may be more informative and helps in defining true and apparent uncontrolled hypertension. Patients in the higher adherence group had lower DBP compared to patients in the lower adherence group. The difference between day and night SBP and DBP were around 10% in both low and high adherent group which represent the normal dipping. However, Adeoye eet.al demonstrated that low adherent patient had poor full time, daytime and night-time SBP and DBP. Four in five patients lacked nocturnal drop in the BP which was worse in women (205). Hypertensive patients who are non-dipper and those with nocturnal hypertension have 3–5 fold risk of developing major cardiovascular events such as stroke, heart attack and kidney failure (206).

Resistant Hypertension

In clinical practice, patient who are adherent to three antihypertensive drugs including at least one diuretic are considered to have resistant hypertension(207). Pseudo resistant hypertension is the lack of BP control in patients receiving the appropriate treatment who are exposed to other factors that cause increase in BP measurement including: inappropriate measurement technique, white-coat effect(208).

Based on this definition of resistant hypertension, around 50% of patients in this study have resistant hypertension. Persell et.al estimated the prevalence of resistant hypertension in the USA to be about 8.9 % of hypertensive patient (209). Therefore, it is important to identify factors that prevent BP control, such as high sodium intake, alcohol intake, obesity, use of drugs with potential to raise blood pressure, obstructive sleep apnoea syndrome and secondary forms of hypertension, and correct them. The high prevalence of resistant hypertension in my study patients may explain the discrepancy between the adherence measurements and BP control.

4.4.4.2 Drug costs

Medication cost is a widely studied predictive factor of nonadherence (103). The cost of medication is a factor that may decrease patient adherence where patients must buy their own medications. A study conducted by Maciejewski et. al compared drug adherence at four Veterans Affairs medical centres in US between veterans who were exempt from copayments and veterans who were not exempted. The hypertension sample included 3545 exempted veterans and 3545 non-exempted veterans, and the study showed the importance of medication cost to patient adherence. They concluded that a $5 copayment increase (from $2 to $7) adversely affected adherence for veterans taking antihypertensive and oral hypoglycaemic agents (210). In UK, the NHS provides medication for the patients free of charge. Thus, this may have an impact of patients answering questionnaires about adherence, also this may have a major impact as medication costs are removed as a barrier to adherence.

4.4.4.3 Common antihypertensive prescriptions

CCB was the most common prescribed class (60% of patients) which corresponds with the current guidelines that recommend the use of CCB for patient that are aged over 55 years old or of an African or Caribbean family origin of any age (25). The mean age for the patient was 63.5 years in our study. (47). The mean age for the patient was 63.5 years in my study.

4.4.4.4 Adherence and associated factors

Several factors were found significantly associated with adherence in my study- gender, age, occupation, education, perception and DBP.

Some predictors have been consistently associated with poor adherence including complexity of the regimen, medication side effects, the presence of psychological problems like depression; however, these weren't studied in this project. The number of prescribed medications is the most relevant aspect of therapeutic regimen complexity (211). The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) study is a randomized clinical trial involving a large number of patients in order to evaluate the control of hypertension and the effects of antihypertensive medications on clinical outcomes. The main predictors of lack of BP control in the study included age, black ethnicity and higher basal BP. The additional causes included female gender, diagnosis of diabetes, obesity, previous antihypertensive therapy and left ventricular hypertrophy (212).

Adherence wasn't found to be significantly associated with the number of antihypertensive drugs. However, studies have shown that patient that were taking two or more drugs were less likely to be adherent to their drugs compared to patients who were on monotherapy. Schroeder et al. demonstrated in a systematic review of randomized controlled trials that decreasing the number of daily doses of antihypertensive drugs can lead to better adherence (213). Moreover, using single tablet per day is quite simple for the patient to follow minimizing the chances to forget their dose. Therefore, combining two antihypertensive drugs as a single tablet might improve patient adherence.

4.4.4.5 Gender

In my study, males were found to be more non-adherent compared to female. The findings of my study were opposite to the findings of Holt et al., who demonstrated that women had lower adherence scores compared to men (15% vs 13.1%), the study also concluded that factors associated with low antihypertensive drug adherence scores differed based on sex and that interventions should consider for the sex of the target population (214). However, there were no differences in adherence between males and females in CoSMO study (215). Further, more studies are needed to understand the underlying factors associated with differences in non-adherence between gender.

4.4.4.6 Age

In my study, younger patients were more likely to be non-adherent to their medication compared to older patients. Bandi et al. in cross-sectional study involving 1,043 Hispanic adults with hypertension concluded that the younger patient had significantly lower prevalence of high adherence compared to older patients (216). The possible explanation could be that younger patient are more forgetful about taking their medication due to their busy life. In addition, because of the reason that hypertension is a silent disease (asymptomatic) and they are diagnosed with it at an earlier age, it causes them to less likely to take care of their health. On the other hand, older patients might have multiple comorbidities and therefore, perceive themselves as sicker causing them to be more adherent to their antihypertensive medication. In contrast, some researchers reported the opposite finding where poor adherence was associated with the increasing age. They argued that older patients are associated with less physical mobility, cognitive level and self-care which are factors that could influence the relationship between age and adherence (142, 217).

4.4.4.7 Occupation

It was observed that employed patients were less adherent to their prescribed drugs while retired patients were more adherent. It is possible that both age and occupation could be confounding. Since employed patients were younger while retired patients were older. In my study, after applying multiple regression the

age was collinear with occupation and it is difficult to determine whether age or occupation had an independent effect on adherence

4.4.4.8 Education

In my study patients with higher education were less adherent than patients with lower education. This may again reflect age as nowadays younger patients have more education compared to the older patients who had less opportunities in the past. Thus, the relationship with age, education and employment are complex and dissection of their independent effect is beyond the scope of this study.

Comorbidities and adherence

The presence of multiple comorbidities has been considered as a barrier to medication adherence and prompts nonadherence. There are two aspect to this, one is related to the nature of the disease itself, a study by Briesacher et.al reported that adherence (measured by MPR) was 80% in 72.3% of individuals with only hypertension compared to hypothyroidism comorbidity (68.4%) or gout comorbidity (36.8%) (218). On the other hand, Natarajan et.al found good adherence to antihypertensives drugs in patients with diabetes (219). The other aspect relates to the medication for the disease, the concomitant use of lipid-lowering therapy in hypertensive patients was associated with poor adherence and about 45% were shown to be adherent; this reduced to 36% at 1-year follow-up (220). The results of previous studies about the role of comorbidity are heterogeneous which might be related to the fact that adherence is a complex phenomenon.

4.4.4.9 Limitations

This study included patients only from a single centre that is a tertiary care which might limit generalization of the finding to a wider population. In addition, some predictors including psychological aspect were not studied such as depression. The timing of the medication or the method is not included in the questionnaire which may explain the lack of association between the use of three or more antihypertensive drugs with an inadequate adherence but the existence of an association between the number of these drugs and lack of BP

control. In addition, assessing adherence using patient self-report may involve recall bias and may also be subjected to social desirability bias which can lead to misclassification regarding the true prevalence of adherence. Also, the sample size was small to identify a clear relationship between adherence and various factors. Moreover, there is a possibility of overestimation the BP reading because BP reading were taking in the clinic and this could be affected by white coat effect. Thomas et.al studied the relationship between office BP, ABPM and factors that influence white coat effect in a large British cohort (n=2056) from 2 hypertension clinics. They found that 51% had white coat effect and differences between clinic BP and ABPM measurements increased with the stage of hypertension. In addition, there was a positive correlation between clinic systolic and diastolic BP and white coat effect(221).

5 Study of antihypertensive adherence using urinary drug assays by multiple methods

5.1 Introduction

Adherence to antihypertensive drugs is essential for BP control. Poor adherence to therapy is associated with Higher CV risk for the patient and increase the health care burden. The prevalence of nonadherence differs widely due to the inconsistency of study designs and the lack of objective measures to determine nonadherence to therapy. Recently, urine analysis of the drugs or their metabolites has gained increasing interest. Several studies have assessed nonadherence in hypertensive patients by measuring antihypertensive drugs and their metabolites in urine or serum using various platforms (liquid/gas chromatography, mass spectrometry). Jung et al. was one of the first studies to use urine screening with HPLC to check for adherence. Out of 76 patient, 36 patients (47%) were adherent (all the antihypertensive drugs were detected in the urine) while 40 (53%) patient were not adherent. Non-adherent patients were divided into complete (no presence of antihypertensive drugs and their metabolite (30%) and incomplete, presence of partial amount of the prescribed drug and metabolite (70%). Studies using urine analysis assay had various adherence rates showed that 25% to 65% hypertensive patients do not take their antihypertensive drugs as prescribed (104, 128, 130, 134, 222). These results are extremely robust because they come from studies that used a direct and objective measure for adherence assessment. These analyses represent spot assessments of adherence but are subject to the “white coat adherence” effect. The term white coat adherence is defined as improving in patient’s medication taking behaviour prior to their clinical appointments(103, 147).

5.1.1 Aim

In this chapter, the aim of this study was to assess patient adherence using a direct objective measure by measuring adherence in urinary sample from hypertensive patients attending GBPC using 3 different methods.

5.2 Method

Analysis on patient's urine samples were done by three methods: 1) Birmingham Heartlands Hospital BIR which gave a qualitative result (drug detected or not). 2) Glasgow Polyomics POL used a novel method searching for potential drug using molecular network. 3) Glasgow Toxicology provided a qualitative and quantitative result (the concentration of drugs was provided).

5.2.1 Number of patients for each method

Table 5-1 demonstrate he number of patients for each method. 79 patients were included in Birmingham analysis, 100 by Glasgow Polyomics and 173 for Glasgow toxicology. Figure 5-1 shows the number of shared patients. 57 patients were shared between the 3 methods. All the samples in Birmingham and Polyomics were included in Glasgow toxicology.

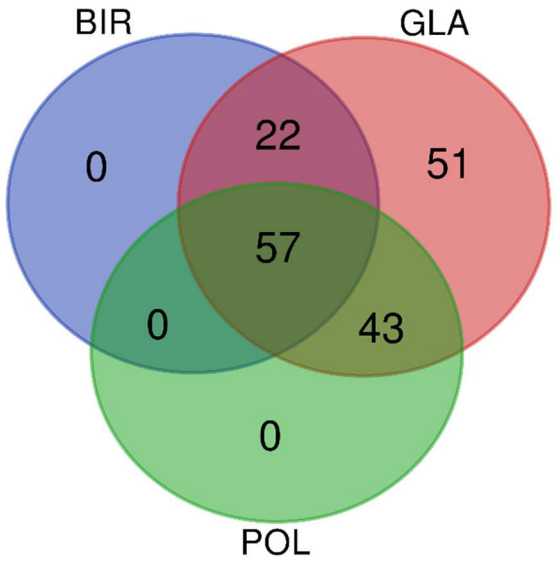


Figure 5-1 Ven diagram for the number of patients in each method.

Table 5-1 Number of patients in each method

Method	Number of patients
BIR	79
POL	100
GLA	173

5.2.2 **Number of antihypertensive drugs detected for each method**

Table 5-2 demonstrate the antihypertensive drugs for each method. Figure 5-2 illustrates the number of antihypertensive drugs for each method. 4 drugs (Amlodipine, Atenolol, Ramipril and Losartan) were shared between the 3 methods.

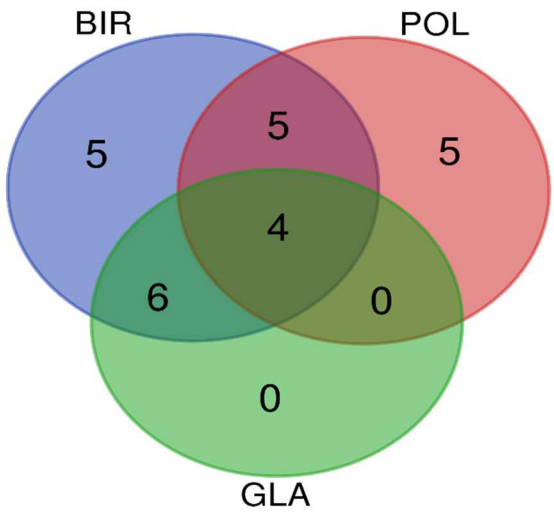


Figure 5-2 Ven diagram for the number of drugs in each method. 4 drugs were shared across the 3 methods.

5.2.3 **Shared drugs**

Table 5-2 Antihypertensive shared between each method

Names	N	Antihypertensive drugs
BIR GLA POL	4	Amlodipine Atenolol Ramipril Losartan
BIR POL	5	Irbesartan Enalapril Candesartan Diltiazem Perindopril
BIR GLA	6	Bisoprolol Verapamil Bendroflumethiazide Spironolactone Doxazosin Furosemide
BIR	5	Labetalol Indapamide Felodipine Lisinopril Metoprolol
POL	5	Sotalol Methyldopa Labetalol Trandolapril Valsartan

5.3 Result of Birmingham Laboratory drug assay

79 urine sample were collected from patients and analyzed by Birmingham laboratory

Table 5-3 summarise the prescribed antihypertensive drugs in the sample. 18 (23%) of the patient were on single antihypertensive drug while 27(34%) were on dual therapy. The remaining were on 3 or more antihypertensive drugs.

Table 5-3 Summaries the number of antihypertensive drugs prescribed in the study sample n=79

Prescribed	N	%
1	18	22.8
2	27	34.2
3	13	16.5
4	14	17.7
5	3	3.8
6	3	3.8
8	1	1.3
Total	79	100

5.3.1 Detection rate

As shown in table (Table 5-4) the analysis conducted by Birmingham heartland hospital laboratory was able to detect complete presence of antihypertensive medication in 49 (62%) of the urine samples. Only 6 samples showed complete absent of any medication and the remaining (30.4%) were detected with at least one of the prescribed antihypertensive medications (partial). No drugs were detected in patients who weren't prescribed them.

Table 5-4 summary of detection rate n=79

Detection rate	N	%
0	6	7.6
Partial	24	30.4
complete	49	62.0
Total	79	100

5.3.2 **Most common detected antihypertensive drugs**

(Table 5-5) demonstrates the detected antihypertensive drugs, Bendroflumethiazide is the most common antihypertensive drug prescribed in 33 (%) of the samples, followed by Amlodipine in 32 (%), Candesartan in 18 (%) and Losartan in 15 (19%), Ramipril in 15 (%), Doxazosin and Bisoprolol both in 11 (%) of patients.

Most of the antihypertensive drugs had detection rate of 100%. Other than Candesartan and Losartan, most of these were not commonly taken drugs (each prescribed to <10% of analysed patients). 8 drugs had incomplete detection (Bendroflumethiazide, Doxazosin, Bisoprolol, Amlodipine, Ramipril, Furosemide, Amlodipine and Atenolol). 2 drugs were not detected in any patients prescribed them: Lercanidipine (CCB) and Nebivolol (BB). None of the drugs were detected in any patients who were not prescribed them.

Table 5-5 List of antihypertensive drugs prescribed in patient sample analysed by Birmingham n=79

Drug	Prescribed	Detected
Bendroflumethiazide	33 (41.8%)	31 (93.9%)
Amlodipine	32 (40.5%)	22 (68.8%)
Candesartan	18 (22.8%)	18 (100%)
Losartan	15 (19%)	15 (100%)
Ramipril	15 (19%)	13 (86.7%)
Doxazosin	11 (13.9%)	10 (90.9%)
Bisoprolol	11 (13.9%)	10 (90.9%)
Lercanidipine	9 (11.4%)	
Atenolol	8 (10.1%)	5 (62.5%)
Furosemide	7 (8.9%)	5 (71.4%)
Indapamide	6 (7.6%)	6 (100%)
Lisinopril	6 (7.6%)	6 (100%)
Perindopril	6 (7.6%)	6 (100%)
Nebivolol	6 (7.6%)	
Spironolactone	5 (6.3%)	3 (100%)
Enalapril	3 (3.8%)	3 (100%)
Diltiazem	3 (3.8%)	3 (100%)
Labetalol	1 (1.3%)	1 (100%)
Metoprolol	1 (1.3%)	1 (100%)
Irbesartan	1 (1.3%)	1 (100%)
Verapamil	1 (1.3%)	1 (100%)

5.4 Result for Glasgow Polyomics drug assay

100 urine samples were collected and sent to Glasgow Polyomics for drug assay analysis.

Table 5-7 summarise the prescribed antihypertensive drugs in the sample. 31 (31%) of the patient were on single antihypertensive drug while 28(28%) were on dual therapy. The remaining were on 3 or more antihypertensive drugs.

Table 5-6 Summaries the number of antihypertensive drugs prescribed in the study sample n=100

Prescribed	N	%
1	31	31
2	28	28
3	13	13
4	22	22
5	4	4
6	2	2
Total	100	100

5.4.1 Detection rate

Table 5-7 demonstrates the analysis conducted by Glasgow Polyomics laboratory. It was able to detect complete presence of antihypertensive medication in 34 (34%) of the urine samples. 12 (12%) were completely absent of any medication and the remaining (54%) detected at least one of the prescribed antihypertensive medications (partial).

Table 5-7 summary of detection rate n=100

Detection rate	N	%
0	12	12
Partial	54	54
complete	34	34
Total	100	100

In the study, it was found that one patient had a drug detected in that they were not prescribed. This was for atenolol. It should be noted that that patient was prescribed metoprolol instead; this is the same class of drug as atenolol (beta blockers).

Table 5-8 Cross tabulation for atenolol between prescribed and POL

		Atenolol Prescribed		
Atenolol		not prescribed	Prescribed	Total
Not detected	N	88	1	89
POL	%	88.0%	1.0%	89.0%
Detected	N	1	10	11
	%	1.0%	10.0%	11.0%
Total	N	89	11	100
	%	89.0%	11.0%	100.0%

5.4.2 **Most common detected antihypertensive drugs**

Table 5-9 summarises the antihypertensive medication and their count, detection rate in the samples analysed by Glasgow Polyomics.

According to the results, Amlodipine, Ramipril, Candesartan, Losartan and Atenolol were the most prescribed antihypertensive drugs in the sample analysed by Glasgow Polyomics. The prescription profile was very similar in these 100 patients as in the 79 samples sent to Birmingham, with the top 5 drugs found in common by both centres being found in the same order. This is partly as 57 patients had their results analysed by both centres.

Amlodipine was the most common drug detected in 29 patients followed by Candesartan in 20 patients, subsequently Losartan in 17 patients and Ramipril 16 patients and atenolol 11patients. Most of the drugs had a complete detection rate of 100 %. Amlodipine, Candesartan and Ramipril had partial detection of 93.5%, 95.2% and 72.7% respectively. However, POL didn't test for common drugs such as Bendroflumethiazide or bisoprolol.

Table 5-9 List of antihypertensive drugs prescribed in patient analysed by Glasgow Polyomics n=100

	Drug	Prescribed in 100	Detected in 100	Detection rate %
1	Amlodipine	31	29	93.5
2	Candesartan	21	20	95.2
3	Losartan	17	17	100
4	Ramipril	22	16	72.7
5	Atenolol	11	11	100
6	Perindopril	4	4	100
7	Diltiazem	3	3	100
8	Irbesartan	3	3	100
9	Labetalol	2	2	100
10	Valsartan	2	2	100
11	Enalapril	2	2	100
12	Methyldopa	2	2	100
13	Sotalol	1	1	100
14	Trandolapril	1	1	100

5.5 Glasgow Toxicology result

The total number of patients that were included in the analysis was 173 patients.

The following tables Table 5-10 summarises the prescribed antihypertensive drugs in the sample. According to the results, 44 (25%) of the patient were on single antihypertensive drug while 54 (31%) were on dual therapy and 43% were on 3 or more antihypertensive drugs.

Table 5-10 Summaries the number of antihypertensive drugs prescribed in the study sample n=173

Prescribed	N	%
1	44	25.4
2	54	31.2
3	32	18.5
4	29	16.8
5	9	5.2
6	3	1.7
7	1	0.6
8	1	0.6
Total	173	100

5.5.1.1 Detection rate

Only 6 (3.9%) patients weren't detected for any medication in the sample (non-adherent), while 137 (89.5%) were detected with all the medication they were tested for (adherent) and 9 (5.9%) had some of their prescribed drugs detected (partial adherence). In addition, there was one patient that was detected for a drug they weren't prescribed for. The patient was prescribed Losartan however, he was prescribed Valsartan, so it is possible that the assay registered valsartan metabolites as losartan since they are under the same antihypertensive class (ARB).

For each patient, the total number of prescribed antihypertensive drugs followed by the total number of drugs tested by Glasgow toxicology. After that, the number of drugs detected and the detection rate.

Table 5-11 summary of detection rate. N=153

Detection rate	N	%
0	6	3.9
Partial	9	5.9
complete	137	89.5
False positive	1	0.7
Total	153	100.0

5.5.1.2 Most common drug prescribed

According to (Table 5-12) most common prescribed antihypertensive drugs were Amlodipine, Bendroflumethiazide, Ramipril, losartan, Bisoprolol and Doxazosin. Like the previous 2 methods, the prescription profile for the 173 patients analysed by Glasgow toxicology. 4 drugs were found common by all 3 centres. most of the drugs had detection rates of around 90%. Atenolol and Losartan had complete detection (as did Verapamil, though it was only prescribed to 1 patient). Note that Losartan had a false positive detection mentioned earlier

5.5.1.3 Concentration (Drug quantification)

This analysis included quantification of the drugs (Parent drug and its metabolites). This method used 2 terms: (refer to Appendix 8.5.3).

limit of detection LOD to detect for drugs qualitatively (present or not). LOD is defined as is the lowest concentration of a substance that can be distinguished from the absence of that substance (223) and limit of quantitation LOQ to measure the concentration of the drugs.

A drug was considered detected if its concertation was at least equal to LOD and a concentration was provided in it was at least equal to lower LOQ. Assay LLOQ=10ng/mL was adopted for all of the drugs except furosemide which has a LLOQ=50ng/mL and the upper limit was 2000 ng/ml.

The parent drugs were positive for the corresponding metabolite; however, some samples presented positive results for the metabolite but not for the parent drug, which shows the importance of choosing metabolites during this type of analysis. The following drugs had corresponding metabolites (Ramipril- losartan – verapamil).

Table 5-12 List of antihypertensive drugs prescribed in patient analysed by Glasgow toxicology n=173

	Drug	Prescribed	Detected	Detection rate %
1	Amlodipine	74	67	90.5
2	Bendroflumethiazide	60	54	90.0
3	Ramiprilat/ Ramipril	42	38	90.5
4	Losartan / Losartan Carboxylic Acid	25	26	104.0
5	Bisoprolol	29	26	89.7
6	Doxazosin	23	20	87.0
7	Furosemide	21	19	90.5
8	Atenolol	18	18	100.0
9	Spironolactone	11	8	72.7
10	Verapamil	1	1	100.0

5.5.1.4 Demographic characteristics for the patients (n=152)

5.5.1.5 Categorical variables

In this study, out of 173 patients, there were 20 patients that were prescribed antihypertensive drugs that couldn't be detected by Glasgow toxicology. 1 patient was falsely detected for Losartan but was prescribed Valsartan. The patients were divided into 3 groups based on detection rate into: complete detection: complete detection of the prescribed drugs, non-detected: no detection of any drug and partial: detection of at least one of the prescribed drugs. Table 5-13 summarise the categorical variables between the 3 groups. Most of the demographic variables were not significantly associated with detection. However, this may be partly due to the small sample sizes in the non- and partially detection groups giving low power. The one exception was for perception where none of those in the non-detection group felt their medication was improving their health, compared to 22% of those partially adherent and 45% of those detection.

Table 5-13 Demographic characteristics for patient samples assessed by Glasgow toxicology (categorical).

	GLA	Non detected n=6		Partial n=9		Complete detection n=137		
		N	%	N	%	N	%	P
Gender	male	3	50.0%	4	44.4%	60	43.8%	0.956
Education	bachelor	4	66.7%	5	55.6%	70	51.1%	0.738
	high school	2	33.3%	4	44.4%	67	48.9%	
Ethnicity	White	5	83.3%	7	77.8%	128	93.4%	0.173
Occupation	Unemployed	2	33.3%	2	22.2%	9	6.6%	0.98
	employed	1	16.7%	3	33.3%	51	37.2%	
	retired	3	50.0%	4	44.4%	77	56.2%	
Smoking	non smoker	6	100.0%	7	77.8%	121	88.3%	0.419
	smoker	0	0.0%	2	22.2%	16	11.7%	
Alcohol	No	3	50.0%	4	44.4%	33	24.1%	0.164
	Yes	3	50.0%	5	55.6%	104	75.9%	
Perception	improving	0	0.0%	2	22.2%	61	44.5%	0.032
	no change	4	66.7%	6	66.7%	68	49.6%	
	worsening	2	33.3%	1	11.1%	8	5.8%	
AntiHTN	1	1	16.7%	1	11.1%	31	22.6%	0.636
	2	3	50.0%	2	22.2%	45	32.8%	
	3 or more	2	33.3%	6	66.7%	61	44.5%	
eGFR	>60	5	83.3%	9	100.0%	109	80.1%	0.683
	30 – 60	1	16.7%	0	0.0%	25	18.4%	
	<30	0	0.0%	0	0.0%	2	1.5%	

P value calculated using Chi-square statistical test.

5.5.1.6 Continuous variables

Only for the total number of medications (TotalP) was there found to be any differences across detection groups. Notably the non-detection (mean=2.8) and in particular the partially detection group (4.7) had a higher average number of medications prescribed compared to the detection group (2.3). SBP had a p-value just greater than 5%, with those partially detected having the highest mean SBP. There is a correlation between the detection rate and Morisky questionnaire, Non detected patients had lower Morisky score while complete detected had higher Morisky score.

Table 5-14 Demographic characteristics for patient samples assessed by Glasgow toxicology (continuous)

GLA	Non detected n=6			Partial n=9			Complete detection n=137			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	P
Age	6	67.8	10.3	9	59.0	14.8	137	65.1	13.9	0.382
SBP	6	155.3	12.8	9	167.0	25.3	137	149.1	22.3	0.059
DBP	6	91.8	21.6	9	91.9	16.3	137	81.6	12.6	0.180
wt	5	94.9	33.4	9	89.5	19.8	134	85.1	21.0	0.518
BMI	5	32.9	12.5	9	32.3	8.4	123	30.6	7.1	0.644
sleep	6	5.5	2.4	9	7.0	1.7	137	7.1	1.5	0.590
Morisky	6	6.9	1.4	9	7.1	1.2	137	7.5	0.8	0.104
TotalP	6	2.8	1.3	9	4.7	2.3	137	2.5	1.2	<0.0005

P value calculated using one-way ANOVA statistical test.

5.5.1.7 ABPM

Details about ABPM and terminology are described previously in chapter 2 (refer to section 2.2.1)

Table 8-5 (see appendix) summarise the ABPM for the patients. The significant variables are: 24H (SYS AVG, DIA AVG, MAP AVG). Day (SYS AVG, MAP AVG). Night (SYS AVG, DIA AVG, DIA SD, MAP AVG, PP SD, HR AVG). The main finding is that SBP (24 hr, day and night) readings were generally lower in the completely detected group compared to non-detected. In addition, DBP (24 hr and night) reading were also lower in the completely detected group compared to non-detected

5.5.1.8 laboratory investigations

Table 8-6 summarise the lab investigations for the patients analysed by Glasgow toxicology (see appendix). Na sodium was only the significant test (p=0.03) which was lower in the non-detected (mean=136 mmol/L) group compared to the groups (mean = 139 mmol/L).

5.5.1.9 Combining partial and non-adherent into one group

Due to the small numbers of patients in the partial and non-detected groups. We combined them into one group as non-adherent (n= 15). Univariate analysis was performed instead of multi variate.

Table 5-15 Comparing adherent to non adherent

	Non adherent n=15			Adherent n=137		
		N	%	N	%	P
Gender	male	7	46.7%	60	43.8%	0.832
Highschool	bachelor	9	60.0%	70	51.1%	0.512
	high school	6	40.0%	67	48.9%	
Ethnicity	White	12	80.0%	128	93.4%	0.067
	Unemployed	4	26.7%	9	6.6%	
occupation	employed	4	26.7%	51	37.2%	0.03
	retired	7	46.7%	77	56.2%	
Smoker	non smoker	13	86.7%	121	88.3%	0.851
	smoker	2	13.3%	16	11.7%	
Alcohol	No	7	46.7%	33	24.1%	0.059
	Yes	8	53.3%	104	75.9%	
Perception	improving	2	13.3%	61	44.5%	0.021
	no change	10	66.7%	68	49.6%	
	worsening	3	20.0%	8	5.8%	
AntiHTN	1	2	13.3%	31	22.6%	0.68
	2	5	33.3%	45	32.8%	
	3 or more	8	53.3%	61	44.5%	
eGFR	>60	14	93.3%	109	80.1%	0.453
	30-60	1	6.7%	25	18.4%	
	<30	0	0.0%	2	1.5%	
Antilipid	0	11	73.3%	73	53.3%	0.138
	1	4	26.7%	64	46.7%	
Diabetic	0	12	80.0%	122	89.1%	0.303
	1	3	20.0%	15	10.9%	
Antiplatelet	0	11	73.3%	111	81.0%	0.478
	1	4	26.7%	26	19.0%	
Anticoagulant	0	15	100.0%	123	89.8%	0.194

Table 5-16 Comparing adherent to non adherent continuous

	Non adherent			Adherent			
	N	Mean	SD	N	Mean	SD	P
Age	15	62.5	13.5	137	65.1	13.9	0.496
SBP	15	162.3	21.4	137	149.1	22.3	0.03
DBP	15	91.9	17.8	137	81.6	12.6	0.005
wt	14	91.4	24.3	134	85.1	21.0	0.292
BMI	14	32.5	9.6	123	30.6	7.1	0.352
sleep	15	6.4	2.1	137	7.1	1.5	0.127
Morisky	15	7.0	1.2	137	7.5	0.8	0.037
TotalP	15	3.9	2.1	137	2.5	1.2	<0.0005

Binary logistic regression was applied for each variable individually

Table 5-17 Result of Binary logistic regression

Variable	P
Age	0.494
SBP	0.035
DBP	0.007
Wt	0.299
BMI	0.352
Sleep	0.125
Morisky	0.047
Total prescribed	0.001
Male	0.832
Highschool	0.514
White	0.083
occupation	0.052
Smoker	0.851
Alcohol	0.067
perceptin	0.045

Table 5-18 Binary logistic regression including only significant variables

	Sig.	Exp(B)	95% C.I.for EXP(B)	
			Lower	Upper
SBP	0.788	1.005	0.972	1.038
DBP	0.096	0.958	0.911	1.008
TotalP	0.004	0.487	0.299	0.794
Morisky	0.75	1.114	0.573	2.169
Perception	0.144			
Perception1	0.742	1.399	0.19	10.306
Perception2	0.114	7.036	0.624	79.331
Constant	0.138	355.072		

Only Total prescribed drug Is significant. Note however, that as there are only 15 in the not fully adherent group, this multiple binary logistic regression should be treated with great caution as it will be overfitted.

5.5.2 **Repeated subset samples: 30 patients**

A subset of 30 patients that we collected a repeated sample at a second appointment (at least 6 months after the initial sample was taken) to check for their adherence.

Table 5-19 Repeated samples

Drug	Prescribed	First detected	Not detected	Repated detected	Not detected
Amlodipine	15	13	1	14	1
Atenolol	3	2	1	2	1
Bendroflumethiazide	10	9	1	9	1
Bisoprolol	1	1		1	
Doxazosin	2	2		2	
Losartan	3	3		3	
Ramipril	6	5	1	5	1
Furosemide	4	4		4	

One patient was not adherent to Amlodipine, however in the repeated sample amlodipine was discovered (ngs50) while one patient was detected at first but was absent in the repeated sample. Both patients didn't have any change in their prescribed meds.

Shows 1 patient (NGS 64) who wasn't originally on Amlodipine, however he was later prescribed amlodipine added to his prescription medications.

For atenolol the repeated sample were similar to the originals. Out of 3 patients prescribed atenolol, 2 were detected in the original and repeated sample, while one patient was never detected for atenolol (ngs37). This patient was on poly therapy and none of his other medications was identified.

Out of 10 patients prescribed Bendroflumethiazide, 9 patients were detected both in the original and repeated samples. Only one patient wasn't detected for Bendroflumethiazide (NGS37) who were also and atenolol. Bisoprolol

For Bisoprolol, only one patient was prescribed, and he was detected in both original and repeated sample.

Two patients who were prescribed Doxazosin were detected both in the original and repeated samples. However, one patient wasn't prescribed doxazosin in the original sample. He was prescribed doxazosin later and his repeated sample showed that doxazosin was present shown in Losartan

All three patients that were prescribed Losartan were detected in both original and repeated samples.

6 patients were prescribed Ramipril, 5 patients were detected both in the original and repeated samples. Only one patient wasn't detected for Ramipril, this patient (NGS36) was prescribed Amlodipine and Ramipril but only Amlodipine was detected.

The repeated sample were similar to the originals. All three patients that were prescribed Furosemide were detected in both original and repeated samples.

5.6 Discussion

In this chapter I have summarised the analysis of three platforms to measure antihypertensive drug adherence in urine samples.

5.6.1 The Birmingham (BIR) analysis

Birmingham hospital laboratory was the initial analysis that tested adherence among 79 patients from the Glasgow Blood pressure clinic. The most commonly antihypertensive drugs prescribed in the sample that Birmingham investigated were Bendroflumethiazide, Amlodipine, Candesartan, Losartan and Ramipril.

The assay was able to detect all the ARB drugs (Losartan and Candesartan) at 100% detection while amlodipine at 69% and atenolol at 63%. In addition, there were 2 drugs (Nebivolol and Lercanidipine) that weren't detected at all although they have been prescribed in many patients. One possible explanation is that the assay wasn't able to measure these drugs. Another possibility could be related to the pharmacokinetics, where the drugs are metabolised before reaching the urine.

The half-life of Lercanidipine in the plasma is 8 to 10 hours. However, it has high membrane partition coefficient causing a long-lasting effect at receptor and membrane levels which enables it for once per day dose administration. It is metabolized in the liver by cytochrome P450 (CYP) 3A4. After that, the metabolites are excreted in urine and feces (224, 225). Nebivolol is BB and its metabolism is dependent on the phenotype of the metabolizer. The plasma half-life can range from 10.3 hrs in extensive metabolizers and up to 31.9 hrs in poor metabolizers. These drugs may be heavily metabolised making them unavailable to be picked up by the assay. Moreover, it might be an issue with the assay at detecting these classes CCB and BB because of their low rate with amlodipine and atenolol. A further possibility is that patients were not taking the drugs in cases where it was not found indicating non adherence, patients could be confronted with the result and educated about the importance of adhering to therapy.

The fact that none of the drugs investigated by BIR were found in patients who were not prescribed them, is an encouraging finding and helps to give confidence in the BIR results; however just because there were no false positives does not mean that there could not be false negative results.

In Birmingham study the adherence detection rates were 62% for complete detection,30% for partial and only 7.6 % for complete non-detection. What is not yet clear is whether the cases of non-detection are due to non-adherence or false negative results.

5.6.2 **The Glasgow Polyomics (POL) analysis**

To help understand better which factors might be leading to non-detected results, 100 samples were sent to Glasgow Polyomics laboratory (of which 57 patients were shared with Birmingham). This method identified and visualized antihypertensive drug metabolites using untargeted metabolomics experiments based on the spectral similarity of their fragmentation spectra. Amlodipine, Candesartan, Losartan and ramipril were the most common prescribed drugs.

The POL detection was higher than the other two assays as polyomics used an untargeted metabolomic profiling method. They measured over a 1000 metabolites and were able to map fragments of drug metabolites using molecular networking and drug related clusters were identified based on subsets of connected nodes in the molecular network that all relate to one parent drug (or endogenous compound class in case of non-drug compounds). This is in contrast to Birmingham and Glasgow Toxicology drug assays which used targeted screening of drugs looking for specific drug metabolites (226).

According to the result, POL had a high detection rate for the antihypertensive drugs they looked into though the vast majority of these drugs were prescribed to less than 10% of participants. Three of the 5 drugs prescribed to more than 10% of patients did not have complete detection: Amlodipine and Candesartan were around 90% and Ramipril was only detected at 72%. This is higher than BIR analysis, which could suggest that the Birmingham samples were at least in some cases failing to detect samples rather than identifying non-adherence. Moreover, it could be related to the assay method of identification of drugs. The cost-

effectiveness of the Glasgow polyomic approach is yet to be established. The cost of the assay by the Polyomic method is 10 times that of the standard targeted screening. More controlled studies are required to determine if there is added value to using this expensive assay for adherence compared to targeted screening. This is investigated further in Chapter 6.

5.6.3 **The Glasgow toxicology (GLA) analysis**

Finally, the complete set of samples of 173 patients were sent to Glasgow toxicology (GLA) for analysis. The method looked into 10 antihypertensive drugs (Amlodipine, Atenolol, Bendroflumethiazide, Bisoprolol, Doxazosin, Furosemide, Losartan, Ramipril, Spironolactone, and Verapamil). 3 drugs had complete detection (losartan, atenolol and verapamil), the remaining drugs had high detection rate at around 90% with only spironolactone lower 73%. There was one patient that had a false positive detection rate, that patient was prescribed Valsartan (which was not investigated by GLA) however the assay marked them as positive for Losartan. Valsartan and Losartan are both under the same class (ARBS) which might explain that the assay measured the metabolites of valsartan and registered it as Losartan due to similar structure. This is an issue that will need to be taken into account when assessing metabolomic drug detection methods.

137 patients (89.5%) had complete detection of their prescribed drugs while 9 (5.9%) were partially detected and only 9 (3.9%) were non adherent. This was similar to a study conducted by Hamdidouche et.al where they measure drug detection in urine sample of 174 patients attending the hypertension clinic at the hypertension department of the Pompidou university hospital in Paris. They found that 159 (91%) were completely detected, 12 patients (7%) who were partially detected and only three patients (2%) had none of their drugs detected.

High rates of medication adherence in patients attending the outpatient clinic of a university hospital is expected because most of these patients show up regularly for their appointment and had controlled BP, either under office or home conditions. Given the very low rate of false positive detection (1 case for losartan only), this gives us some confidence that the GLA analysis has high sensitivity (227).

Due to the low number of patients in the partially and non-detected groups, 6 and 9 respectively, they were combined into one group of 15 as non-detected and were compared to completely detected group. The main significant findings of their demographic characteristics were that number of total prescribed antihypertensive drugs, SBP, Morisky questionnaire and perception were associated with detection. Complete detection patients had higher Morisky score compared to not fully detected, which helps to suggest that detection might be used to measure of adherence.

The total number of drugs taken was lowest in the fully detected group (mean 2.5), and highest in the partially detected group (mean 4.7). This could be due to a greater chance of getting at least one false negative result but could also be due to patients on multiple drugs being more likely to fail to take (intentionally or accidentally) all their drugs.

Patient who were not fully detected had high higher SBP/DBP compared to fully detected patients. The mean SBP for detected patients was 149 mmHg, but higher in the other groups (155 (not detected) and 167 (detected) mmHg). Similar results were found for DBP. The fact that it is higher in partially detected groups may be related to the partially detected groups taking more drugs and therefore having higher rates of resistant hypertension.

Patients whose drugs were fully detected had a more positive perception of the effects of the drugs, as might be expected. However, it should be borne in mind that the sample sizes were small, even when the non-detected (n=6) and partially detected groups (n=9) were combined. Since there was no multiple testing adjustment, some of the differences found to be significant could be false positive results. There is a correlation between the detection rate and Morisky questionnaire, Non detected patients had lower Morisky score while complete detected had higher Morisky score. implying drugs are not being detected due to poor adherence rather than limitations of the assay

5.6.3.1 Repeated samples

We had a subset of 30 patient who took a repeated sample from future visits). Most of the repeated sample had the same detection to their original samples.

One patient had an added doxazosin to their prescribed drugs and the assay was able to detect it in the future sample. Also with Amlodipine, 1 patient was had amlodipine added to his prescription and was detected in the future sample. This showed that GLA was able to detect the addition of antihypertensive drugs in urine samples.

5.6.3.2 Limitations

There are several limitations in our study. First, characteristics of the study population might not represent the general population. This population differs from other populations that is usually included in clinical research where patients have resistant or uncontrolled BP. Our patients had been followed in a tertiary clinic and had benefited from a therapeutic education regarding the need for a long-lasting, well observed antihypertensive treatment and warning about the side effects. This could explain the high rate of adherence (90%). Second, due to the small number of nonadherent patients, we cannot exclude a chance finding regarding the differences between the adherent and groups. Our results need replication and confirmation in larger study sample including patients treated in general practice. Third, psychological, socioeconomic and behavioural parameters haven't been studied. Our focus was on measuring drug adherence using urine assay. Fourth, although urine analysis can act as an objective and direct method to measure adherence, we are aware that not all analysis method can detect all the antihypertensive medications taken by the patients. Some of these limitations can be partially assessed by comparing the results from the 3 difference analyses: BIR, POL and GLA. These comparisons are considered in the Chapter 6.

According to the result shown earlier regarding the number of antihypertensive drugs prescribed for each patients, most patients where on combination therapy, this could have be a factor that affected the adherence result which was not further looked into (I only looked at the total prescribed number of antihypertensive drugs) . According to the literature, there were several studies that demonstrated that increase in number of antihypertensive drugs caused a decrease in adherence (134).

6 Comparison of drug screening for antihypertensive medications across three methods

6.1 Aim

In this chapter I will compare between the 3 methods by Birmingham, Glasgow toxicology and Glasgow Polyomics for 4 shared drugs in a shared sample of (57 patients). After that, I will compare between Birmingham and Glasgow toxicology because they had the largest shared sample of 79 patients. The demographic characteristics will be described for patients prescribed with certain antihypertensive medications with patient not prescribed the medication. In addition, test groups will be compared with the concentration of the antihypertensive drug detected by Glasgow toxicology method, Morisky questionnaire, SBP and DBP.

6.2 Test groups

Test groups	
BIR1-GLA1	Detected in both BIR and GLA
BIR1-GLA0	Detected in BIR but not detected in GLA
BIR0-GLA1	Not detected in BIR but detected in GLA
BIR0-GLA0	Not detected in both BIR and GLA

6.3 Number of patients for each method

Described previously in chapter 5 (refer to section 5.2.1.)

6.4 Comparing between 3 methods

4 drugs were shared between the 3 methods (57 patients): Amlodipine, Atenolol, Losartan and Ramipril. Sensitivity and specificity are summarised in Table 6-1, Losartan was detected for all patient. Birmingham was the lowest method to detect amlodipine and atenolol. However, for Ramipril Birmingham identified more than the others. Glasgow Polyomics and Glasgow toxicology had high sensitivity for drugs around (80%). Glasgow Polyomics misidentified 1 patient that was prescribed metoprolol for atenolol.

Table 6-1 Sensitivity and Specificity summary for all methods

	BIR		POL		GLA	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Amlodipine	0.56	1	1	1	0.94	1
Atenolol	0.57	1	0.85	0.98	0.85	1
Losartan	1	1	1	1	1	1
Ramipril	0.9	1	0.8	1	0.8	1

6.5 Comparing BIR vs GLA

10 antihypertensive drugs were shared between Birmingham and Glasgow toxicology in 79 patients. I compared the detection rate for each drug and the result are summarized in Table 6-2. Losartan and Verapamil were detected for all prescribed patients by both methods. Both methods (Birmingham and Glasgow toxicology) detected the same number of patients for (Bendroflumethiazide, Bisoprolol, Doxazosin and Furosemide. Ramipril was prescribed for 15 patients; 13 patients were identified by both methods, but each method detected 1 patient that couldn't be detected by the other. Glasgow toxicology detected more patients than Birmingham for Amlodipine 8 patients and Atenolol 2 patients. On the other hand, Birmingham detected 1 more patient for Spironolactone. Most of the patients that weren't detected by both methods had lower SBP and DBP to those detected. There was no clear relationship between Morisky score and Urinary adherence, this could be due to the small number of sample (especially non adherent group).

Table 6-2 Summary for shared antihypertensive drugs between GLA and BIR

Drug	Prescribed	Both detect	GLA detect	BIR detect	Missed	Morisky
Bendroflumethiazide	33 (42%)	30 (91%)			2 (6%)	7.2
Amlodipine	32 (41%)	22 (69%)	8 (25%)		2 (6%)	7.4
Ramipril	15 (19%)	12 (80%)	1 (7%)	1 (7%)	1 (7%)	7.1
Losartan	15 (19%)	15 (100%)				7.8
Doxazosin	11 (14%)	10 (91%)			1 (9%)	7.4
Bisoprolol	11 (14%)	10 (91%)			1 (9%)	7.9
Atenolol	8 (10%)	5 (63%)	2 (25%)		1 (13%)	7.3
Furosemide	7 (9%)	5 (71%)			2 (29%)	7.3
Spironolactone	5 (6%)	2 (40%)		1 (20%)	2 (40%)	6.7
Verapamil	1(1%)	1 (100%)				6

Prescribed % (is out of total number of shared sample n=79), detected and missed % (out of prescribed number). Morisky score for prescribed patient

6.6 Comparing BIR vs POL

5 antihypertensive drugs were shared between Birmingham and Glasgow Polyomics. The following antihypertensive drugs (Perindopril, enalapril, Diltiazem) were only prescribed in 2 patients while Irbesartan was prescribed in only 1 patient. Due to the small number of patients, no clear relationship was identified. Candesartan was prescribed in 14 patients; Birmingham detected all the patients while Glasgow Polyomics missed 1 patient. The patient had a complete adherence based on Morisky score and high BP readings. He was prescribed 2 antihypertensive (Candesartan and Diltiazem), Diltiazem was detected in both Birmingham and Polyomics which might indicate that the drug assay failed to detect that patient.

6.7 Comparing POL vs GLA

There were no drugs shared only between POL and GLA.

6.8 Comparing Glasgow toxicology with Birmingham

Birmingham was compared with Glasgow toxicology because they had the largest shared sample (n=79 patients, refer to 5.2.1).

The following drugs were detected by both Glasgow toxicology and Birmingham: Bendroflumethiazide, Amlodipine, Ramipril, Losartan, Doxazosin, Bisoprolol, Atenolol, Furosemide, Spironolactone and Verapamil.

6.8.1 Bendroflumethiazide

6.8.1.1 Comparing BIR vs GLA

Table 6-3 Shows the total number of patients that were prescribed Bendroflumethiazide was 33 patients. 31 (93.9%) patients were identified by both methods while 2 prescribed patients were not detected by either method.

Table 6-3 BIR vs GLA (only sample prescribed with Bendroflumethiazide) Cross-tabulation of the metabolomics results of the 33 patients analysed by both Birmingham and Glasgow toxicology laboratories

Bendroflumethiazide			GLA		Total
			Not detected	Detected	
BIR	Not detected	N	2	0	2
		%	6.1%	0.0%	6.1%
	Detected	N	0	31	31
		%	0.0%	93.9%	93.9%
Total		N	2	31	33
		%	6.1%	93.9%	100.0%
Mcnemar P=1					

6.8.1.2 Morisky score across the test groups

Most patients that were detected by both methods responded with score of 8. The 2 patients that weren't detected by either method had score lower than 8.

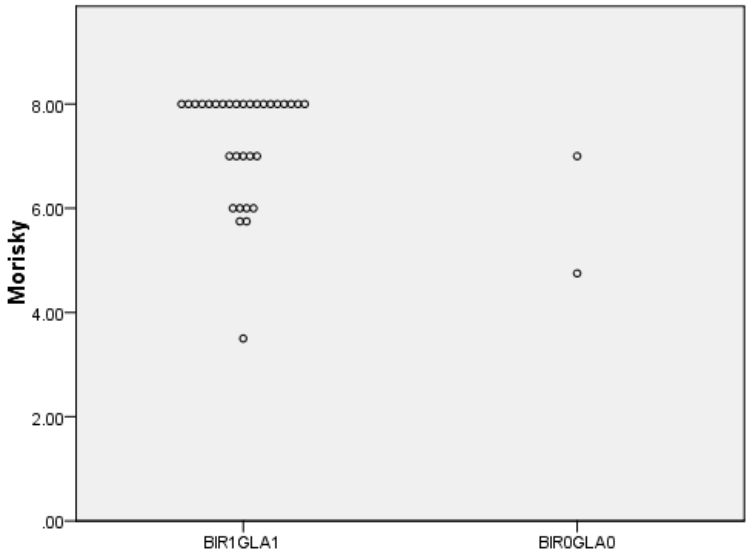


Figure 6-1 The Morisky score for 33 patients prescribed with Bendroflumethiazide using MMAS-8. The plot compares the score of the patients were detected by both Birmingham and Glasgow toxicology (n=31) with the patients that neither method could detect (n=2).

6.8.1.3 Discussion

Bendroflumethiazide is diuretic and was the most common antihypertensive drug detected in shared sample between Birmingham and Glasgow toxicology,

It was highly detected by both (Glasgow toxicology and Birmingham in 31 patients) while not detected in only 2 patients. The 2 patients had normal BP compared to those that were detected and their Morisky score was less than 8 indicating lower adherence. It is possible that the patients were not taking their prescribed drug because they felt it was overtreatment (since they both were on multiple drugs), or possible due to side effect that might be associated with diuretics such as frequent urination and dizziness.

6.8.2 **Amlodipine**

Table 6-4 shows the comparison between Birmingham and Glasgow toxicology for detection of Amlodipine. Total number of patients that were prescribed amlodipine is 32 patients. 22 (68.8%) patients identified by both methods showed presence of amlodipine while 2 prescribed patients were absent for amlodipine. However, Glasgow toxicology was able to detect 8 (25%) patients that couldn't be detected by Birmingham. This was statistically significant.

6.8.2.1 Comparing BIR vs GLA

Table 6-4 BIR vs GLA Cross-tabulation of the metabolomics results of the 32 patients analysed by both Birmingham and Glasgow toxicology laboratories for patients prescribed Amlodipine.

Amlodipine			GLA		Total
			Not detected	Detected	
BIR	Not detected	N	2	8	10
		%	6.3%	25.0%	31.3%
	Detected	N	0	22	22
		%	0.0%	68.8%	68.8%
Total		N	2	30	32
		%	6.3%	93.8%	100.0%
Chi-square P=0.030					

6.8.2.2 GLA level for

Figure 6-2 shows that majority of patient in the (BIR1GLA1) group had Amlodipine level ranging between 200 and 500 ng/ml whereas patients in (BIR0GLA1) group had patients around 1000 ng/ml. The 2 patients that couldn't be detected by either method had concentration of zero.

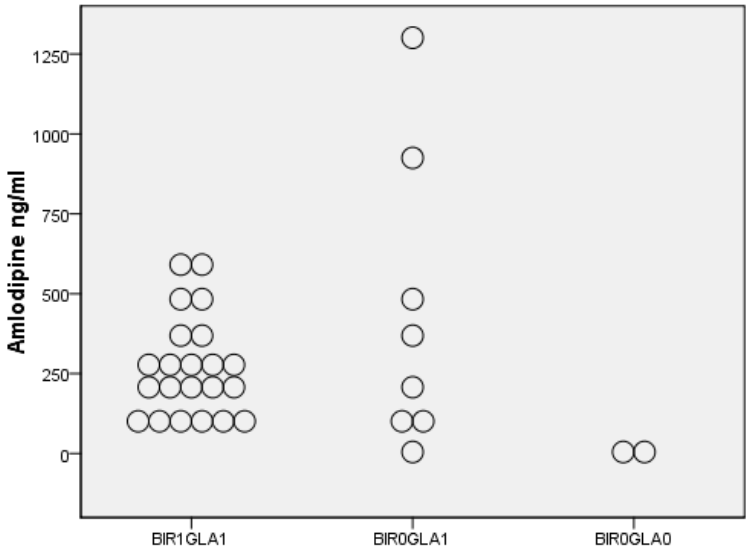


Figure 6-2 The amlodipine levels of the 32 patients prescribed with amlodipine detected by Glasgow Toxicology laboratory. The plot compares the levels of the patients also detected by Birmingham (n=22) with the patients that only Glasgow Toxicology detected.

6.8.2.3 Morisky score across the test groups

Figure 6-3 Shows that patient detected by both methods had majority of their patient with score of 8. There is no clear difference between it and those only detected by Glasgow toxicology. Only 2 patients that couldn't be detected by either method responded with a score of 8.

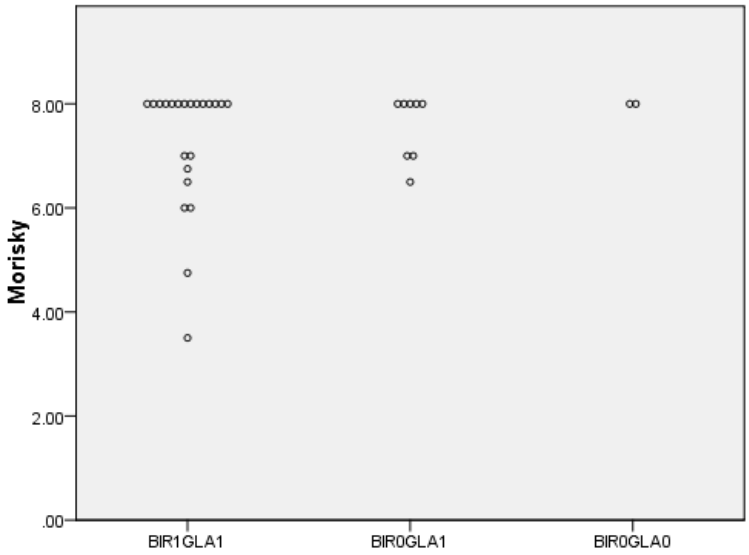


Figure 6-3 The Morisky score for 32 patients prescribed with amlodipine using MMAS-8. The plot compares the score of the patients were detected by both Birmingham and Glasgow toxicology (n=22) with the patients that only Glasgow Toxicology detected (n=8) and patients not detected by either method (n=2)

6.8.2.4 Medication dosage

Figure 6-4 demonstrates patients that were detected by both methods had higher dosages compared to patient that were only detected by Glasgow toxicology. This could explain why Birmingham was not able to identify these patients because the low dose may be related to bioavailability of the drug in urine causing the assay to fail at detecting it.

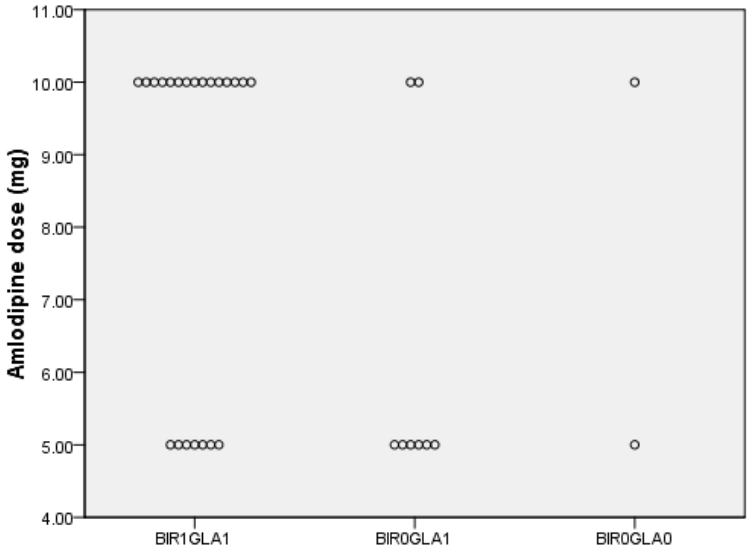


Figure 6-4 The dosage for 32 patients prescribed with amlodipine. The plot compares the Amlodipine dosage for the patients were detected by both Birmingham and Glasgow toxicology (n=22) with the patients that only Glasgow Toxicology detected (n=8) and patients not detected by either method (n=2)

6.8.2.5 Morisky vs GLA conc.

No clear relationship could be identified.

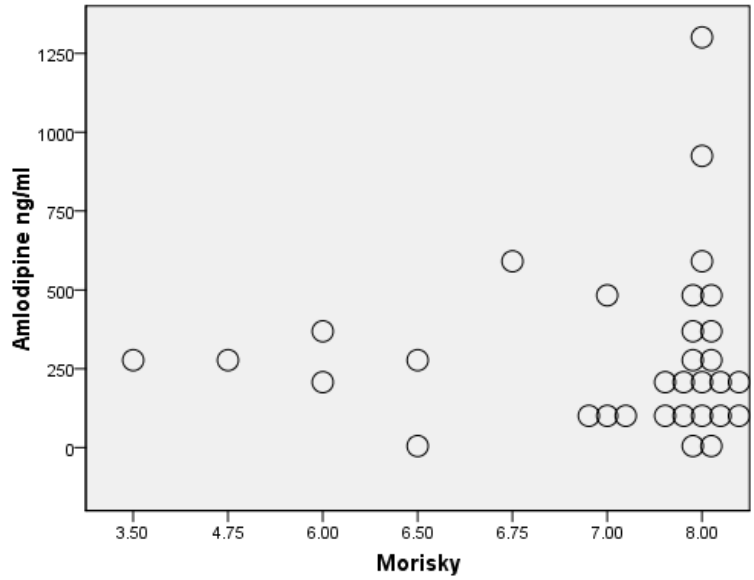


Figure 6-5 The levels for 32 patients with amlodipine detected by Glasgow toxicology with Morisky score. The plot compares the level of the patients with Morisky score adherence.

6.8.2.6 Discussion

Amlodipine is a calcium channel blocker and is considered one of the first line antihypertensive to be used in patient over 55 years old. It was the second most commonly prescribed drug in our sample (32 patient).

Both Birmingham and Glasgow toxicology method detected it in 22 patients (69% of 32 prescribed). However, Glasgow toxicology detected in 8 more patients (25% of 32). It is noted that it's hard to make any conclusions about patient who were not detected by either method, as there were only 2 of them.

Looking into the 8 patients that were detected only by Glasgow toxicology, they were almost all female (p=0.011) and had somewhat higher BP (p=0.086). It's possible that their somewhat worse perception made them less likely to be taking their drugs, although it should be noted that it was not significant (p=0.079). In addition, their dosage was on average lower which might also explain why Birmingham wasn't able to detect them.

6.8.3 **Ramipril**

6.8.3.1 Comparing BIR vs GLA

Table 6-5 shows the comparison between Birmingham and Glasgow toxicology for detection of Ramipril. Total number of patients that were prescribed Ramipril was 15 patients. 12 patients were identified by both methods while 1 patient was not detected by either method. Each method detected 1 patient that couldn't be detected by the other.

Table 6-5 BIR vs GLA (only sample prescribed with Ramipril) Cross-tabulation of the metabolomics results of the 15 patients analysed by both Birmingham and Glasgow toxicology laboratories who were prescribed Ramipril.

Ramipril			GLA		Total
			Not detected	Detected	
BIR	Not detected	N	1	1	2
		%	6.7%	6.7%	13.3%
	Detected	N	1	12	13
		%	6.7%	80.0%	86.7%
Total		N	2	13	15
		%	13.3%	86.7%	100.0%
Mcnemar P=1					

6.8.3.2 GLA level for Ramipril

The only patient detected by Glasgow toxicology and not Birmingham had relatively low detection level for Glasgow, but this was only slightly lower levels compared to the average level of those detected by both methods. Also, due to small number it is difficult to identify any relationship.

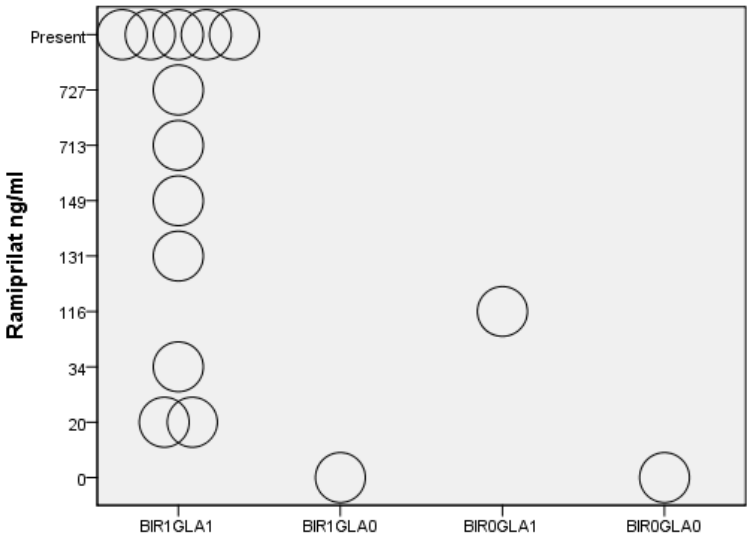


Figure 6-6 The levels Ramiprilat (metabolite of Ramipril) of the 15 patients prescribed with Ramipril detected by Glasgow Toxicology laboratory. The plot compares the levels of the patients detected by both Birmingham and Glasgow toxicology (n=12) with the patients that only Glasgow Toxicology detected (n=1).

6.8.3.3 Morisky score across the test groups

Most patients that were detected by both methods responded with score of 8 (complete adherence). The 2 patients that were detected by only one method had score of 8 however, the single patient that wasn't detected by both had score less than 6 (low adherence) Figure 6-7.

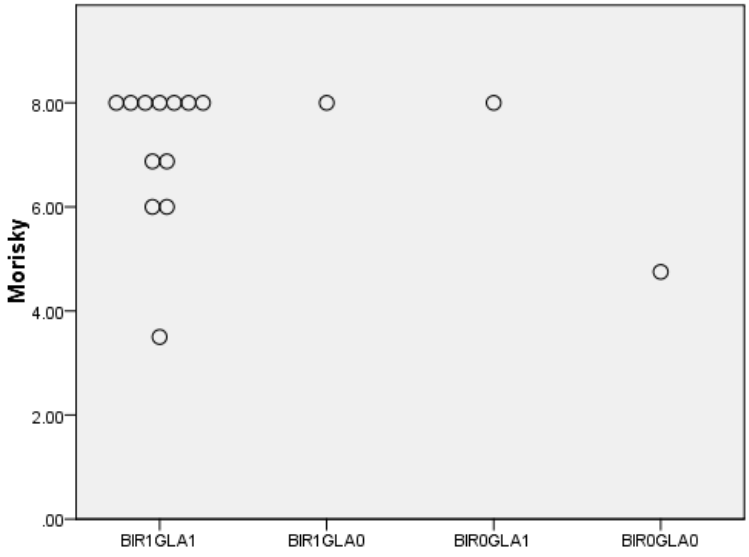


Figure 6-7 The Morisky score for 15 patients prescribed with Ramipril using MMAS-8. The plot compares the score of the patients were detected by both Birmingham and Glasgow toxicology (n=12) with patient detected only by Birmingham (n=1) and patients only detected by Glasgow toxicology (n=1) and patients that neither method could detect (n=1).

6.8.3.4 Discussion

Ramipril is and ACEI that is used as a first line agent. It was prescribed in 15 patients. The main finding in the characteristic for the prescribed patients is that they were younger (mean age of 54 years) with lower SBP compared to non-prescribed.

Ramipril was only detected by both methods in 12 patients, each method detected one patient that couldn't be detected by the other. The patient that wasn't detected by either method had lower Morisky score and a normal SBP. The reason for that could be ACEI is well known to cause dry cough as a side effect that is common with ACEI or the possibility of not taking it since the patient had a lower Morisky score.

6.8.4 **Losartan**

Table 6-6 shows the comparison between Birmingham and Glasgow toxicology for detection of Losartan. Total number of patients that were prescribed Losartan was 15. All prescribed patients were detected by both methods

6.8.4.1 Comparing BIR vs GLA

Table 6-6 BIR vs GLA Cross-tabulation of the metabolomics results of the 15 patients analysed by both Birmingham and Glasgow toxicology laboratories who were prescribe Losartan.

Losartan			GlA	Total
			Present	
BIR	Present	N	15	15
		%	100.0%	100.0%
Total		N	15	15
		%	100.0%	100.0%

6.8.4.2 Morisky score across the test groups

Most patients that were detected by both methods responded with score of 8 (complete adherence) as shown in Figure 6-8.

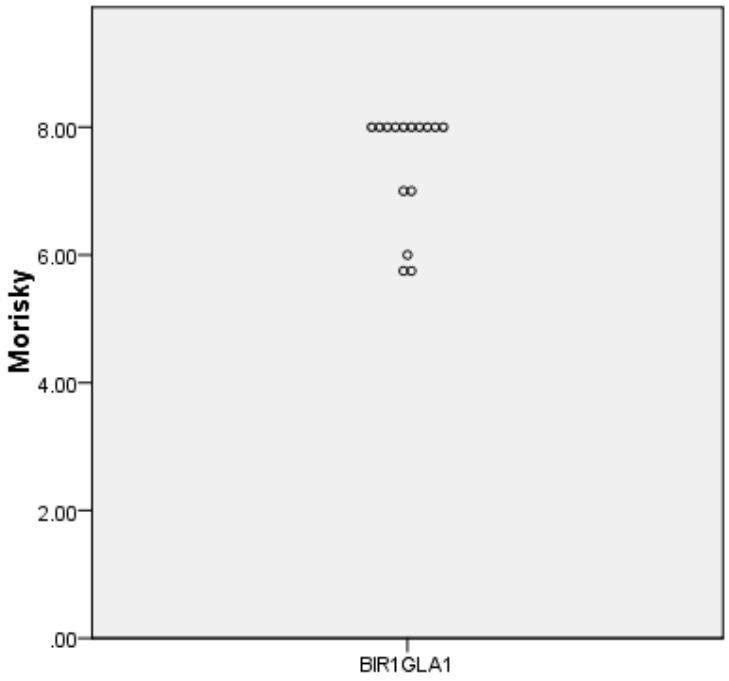


Figure 6-8 The Morisky score for 15 patients prescribed with Losartan using MMAS-8. The plot demonstrates the score of the patients were detected by both Birmingham and Glasgow toxicology (n=15)

6.8.4.3 Discussion:

Losartan is ARB that is protective to kidneys and is prescribed to patient with hypertension associated with kidney disease or diabetes. It was prescribed in 15 patients for our sample. The main finding for demographic was that prescribed patients were mainly on at least 2 antihypertensive drugs or more and had lower education compared to patients not prescribed Losartan. The detection rate for Losartan was 100% by both methods, they detected all prescribed patients which indicates a good profile to measure for adherence.

6.8.5 **Doxazosin**

6.8.5.1 Comparing BIR vs GLA

Table 6-7 shows the comparison between Birmingham and Glasgow toxicology for detection of Doxazosin. Total number of patients that were prescribed Doxazosin was 11 patients.10 (90%) patients were identified by both methods while 1 (10%) patient was not detected by either method.

Table 6-7 BIR vs GLA Cross-tabulation of the metabolomics results of the 11 patients analysed by both Birmingham and Glasgow toxicology laboratories who were prescribed Doxazosin.

Doxazosin			GLA		Total
			Not detected	Detected	
BIR	Not detected	N	1	0	1
		%	9.10%	0.00%	9.10%
	Detected	N	0	10	10
		%	0.00%	90.90%	90.90%
Total		N	1	10	11
		%	9.10%	90.90%	100.00%
Mcnemar P=1					

6.8.5.2 Morisky score across the test groups

Most patients that were detected by both methods responded with score of 8. The patient that wasn't detected by either method had score of 8 (Figure 6-9).

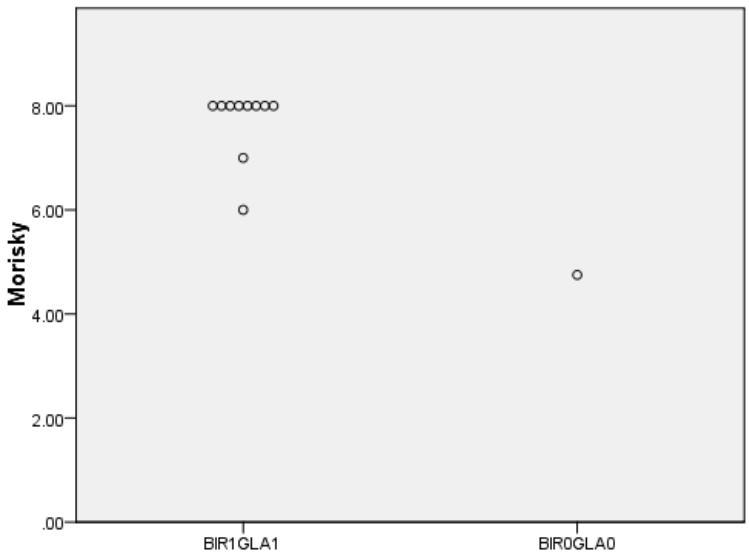


Figure 6-9 The Morisky score for 11 patients prescribed with Doxazosin using MMAS-8. The plot compares the score of the patients were detected by both Birmingham and Glasgow toxicology (n=10) with the patients that neither method could detect (n=1).

6.8.5.3 Discussion

Doxazosin is an alpha blocker for management of hypertension or symptomatic benign prostatic hyperplasia (BPH). Patient that were prescribed Doxazosin were older, retired, non-smoker and on 3 or more antihypertensive drugs. Both Glasgow toxicology and Birmingham were able to detect 10 patients. The only patient that wasn't detected by either method had low adherence Morisky score and normal BP, he was on 6 antihypertensive and only 2 were discovered (amlodipine and Furosemide). This might be related to the pharmacokinetics of the drugs and its elimination. It is heavily metabolized in the liver through hydroxylation or demethylation and about 9% is excreted in urine.

6.8.6 **Bisoprolol**

6.8.6.1 Comparing BIR vs GLA

Table 6-8 shows the comparison between Birmingham and Glasgow toxicology for detection of Bisoprolol. Total number of patients that were prescribed Bisoprolol was 11 patients. 10 (90%) patients were identified by both methods while 1 (10%) patient was not detected by either method.

Table 6-8 BIR vs GLA (only sample prescribed with Bisoprolol) Cross-tabulation of the metabolomics results of the 11 patients analysed by both Birmingham and Glasgow toxicology laboratories who were prescribed Bisoprolol.

Bisoprolol			GLA		Total
			Not detected	Detected	
BIR	Not detected	N	1	0	1
		%	9.1%	0.0%	9.1%
	Detected	N	0	10	10
		%	0.0%	90.9%	90.9%
Total		N	1	10	11
		%	9.1%	90.9%	100.0%
Mcnemar test= 1					

6.8.6.2 Morisky score across the test groups

Most patients that were detected by both methods responded with score of 8. The patient that wasn't detected by either method had score of 8 (Figure 6-10).

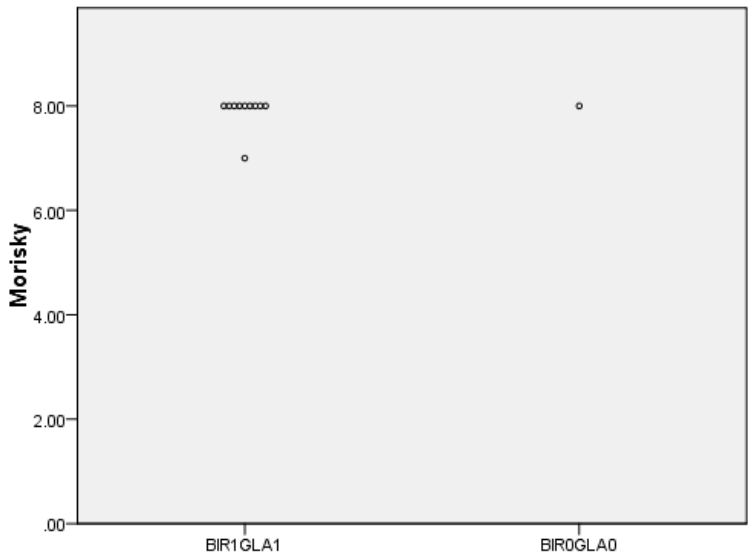


Figure 6-10 The Morisky score for 11 patients prescribed with Bisoprolol using MMAS-8. The plot compares the score of the patients were detected by both Birmingham and Glasgow toxicology (n=10) with the patients that neither method could detect (n=1).

6.8.6.3 HR across test groups

Patient that wasn't detected by either method had low HR. all the patient that were detected by both methods had HR below 100 (normal HR 60-100 beat/min). due to the small number it is difficult to obtain a clear relationship.

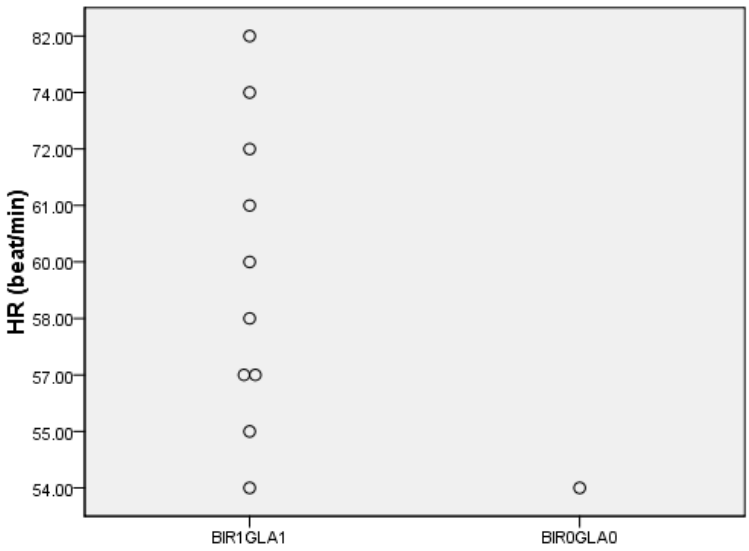


Figure 6-11 HR for 11 patients prescribed with Bisoprolol.

6.8.6.4 Discussion

Bisoprolol is BB which is not a first line of therapy but mainly used to treat patient with ischemic heart disease. It was prescribed for 11 patients that were mainly older (mean age 73) retired, non-smoker, felt improving and had higher SBP compared to non-prescribed.

Both Birmingham and Glasgow toxicology detected 10 patients. The patient that wasn't detected was only prescribed bisoprolol and had high Morisky adherence, low HR but high BP, this might indicate the patient is resistant to the medication.

6.8.7 **Atenolol**

6.8.7.1 Comparing BIR vs GLA

Table 6-9 shows the comparison between Birmingham and Glasgow toxicology for detection of Atenolol. Total number of patients that were prescribed Atenolol was 8 patients. 5 (62.5%) patients were identified by both methods while 1 patient (12.5%) was not detected by either method. Glasgow toxicology was able to detect 2 (25%) more patients that couldn't be detected by Birmingham.

Table 6-9 BIR vs GLA Cross-tabulation of the metabolomics results of the 8 patients analysed by both Birmingham and Glasgow toxicology laboratories.

Atenolol			GLA		Total
			Not detected	Detected	
BIR	Not detected	N	1	2	3
		%	12.5%	25.0%	37.5%
	Detected	N	0	5	5
		%	0.0%	62.5%	62.5%
Total		N	1	7	8
		%	12.5%	87.5%	100.0%
Mcnemar P=0.500					

6.8.7.2 GLA level for

The 2 patients that were only detected by Glasgow toxicology had high levels (2000 ng/ml) for atenolol similar to patients detected by both methods (Figure 6-12).

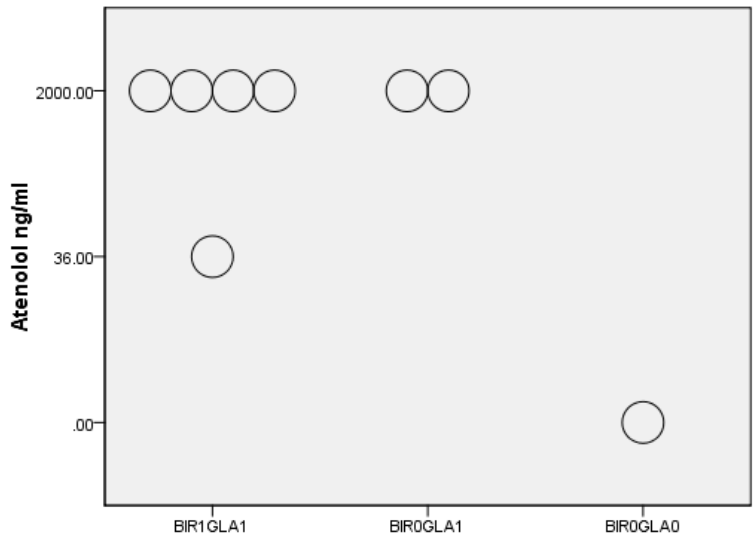


Figure 6-12 The levels of the 8 patients prescribed with Atenolol detected by Glasgow Toxicology laboratory. The plot compares the levels of the patients detected by both Birmingham and Glasgow toxicology (n=5) with the patients only detected by Glasgow toxicology.

6.8.7.3 Morisky score across the test groups

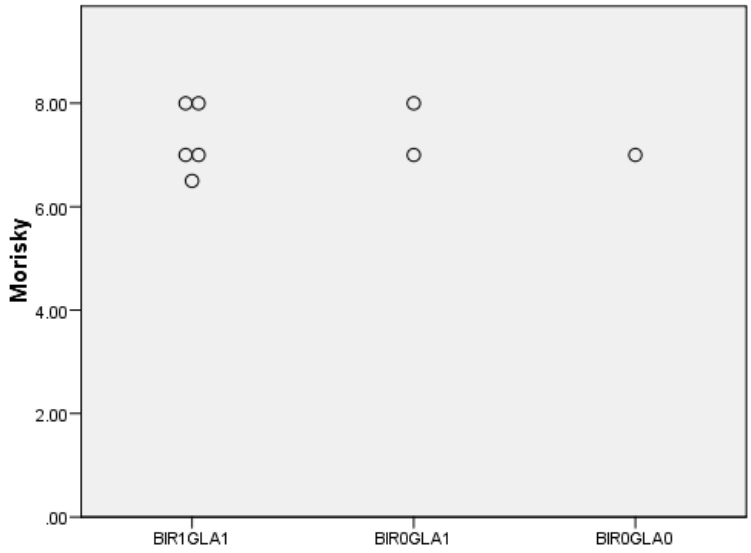


Figure 6-13 The Morisky score for 8 patients prescribed with Atenolol using MMAS-8. The plot compares the score of the patients were detected by both Birmingham and Glasgow toxicology (n=5) with the patients that only Glasgow Toxicology detected (n=2). 1 patient wasn't detected by either method.

Most patients that were detected by both methods responded with score of 8. The 2 patients that were detected by only by Glasgow toxicology had score of 8 while the only patient that wasn't detected by both methods had score lower than 8.

6.8.7.4 Heart rate across the test groups

The patient that wasn't detected by both methods had higher heart rate compared to the other groups. However due to the small number it is difficult to find a clear relationship.

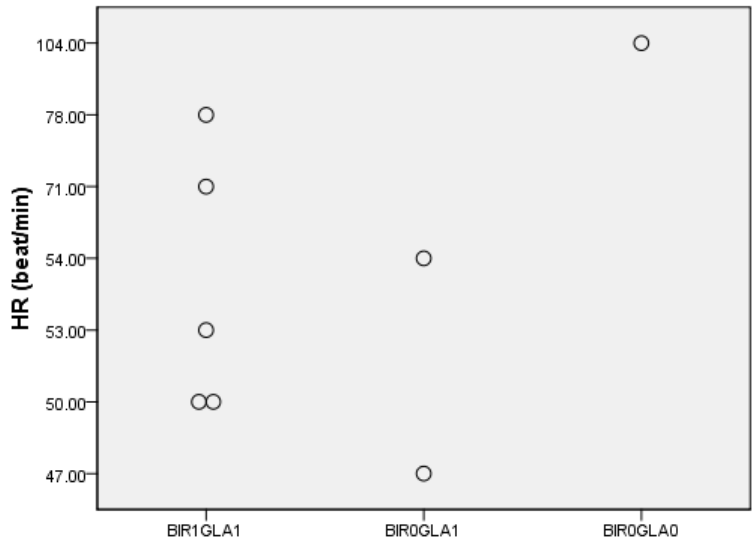


Figure 6-14 HR for 8 patients prescribed with Atenolol. The plot compares the HR of the patients were detected by both Birmingham and Glasgow toxicology (n=5) with the patients that only Glasgow Toxicology detected (n=2). 1 patient wasn't detected.

6.8.7.5 Discussion

Atenolol is a BB that not prescribed initially for hypertension but usually patient with heart disease. It was prescribed in 8 patients that were mainly older (mean age 68 years) and had higher BP and were on at least 2 antihypertensive drugs. 5 patients were detected by both methods, but Glasgow toxicology were able to detect 2 patients that weren't detected by Birmingham. These 2 patients had similar concentration and Morisky score to the patients that were detected by both methods. However, they had lower BP and HR. The single patient that didn't take atenolol had higher HR (this was opposite to the patient in the receiving Bisoprolol) which might indicate that the patient is not taking the drug.

6.8.8 **Furosemide**

6.8.8.1 Comparing BIR vs GLA

Table 6-10 shows the comparison between Birmingham and Glasgow toxicology for detection of Furosemide. Total number of patients that were prescribed Furosemide was 7 patients. 5 patients (71%) were identified by both methods while 2 (29%) patient were not detected by either method.

Table 6-10 BIR vs GLA (only sample prescribed with Furosemide) Cross-tabulation of the metabolomics results of the 7 patients analysed by both Birmingham and Glasgow toxicology laboratories who were prescribe Furosemide.

Furosemide			GLA		Total
			Not detected	Detected	
BIR	Not detected	N	2	0	2
		%	28.6%	0.0%	28.6%
	Detected	N	0	5	5
		%	0.0%	71.4%	71.4%
Total		N	2	5	7
		%	28.6%	71.4%	100.0%
Mcnemar P=1					

6.8.8.2 Morisky score across the test groups

Most patients that were detected by both methods responded with score of 8. For the patient that weren't detected by either method one had score of 8 while the other had score of less than 8 (Figure 6-15).

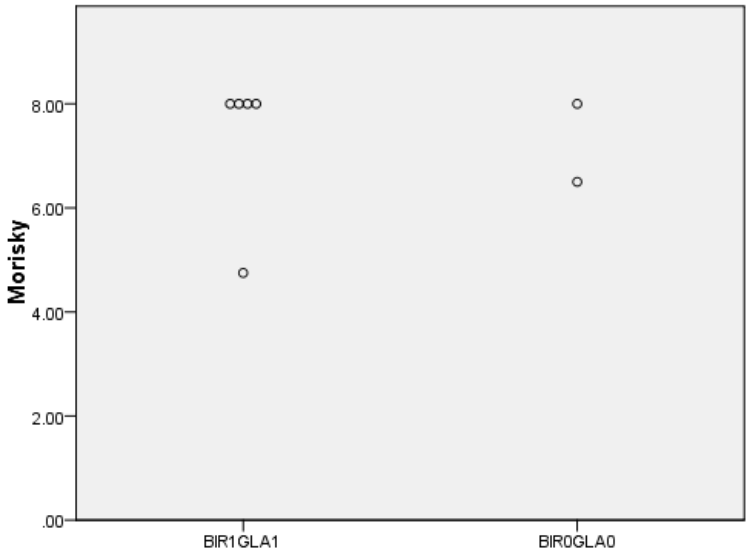


Figure 6-15 The Morisky score for 7 patients prescribed with Furosemide using MMAS-8. The plot compares the score of the patients were detected by both Birmingham and Glasgow toxicology (n=5) with the patients that neither method could detect (n=2).

6.8.8.3 Discussion

Furosemide is a loop diuretic for treating of hypertension especially if there is kidney or heart impairment. it was prescribed in only 7 patients. The main characteristics for the prescribed patient were all on 3 or more antihypertensive drugs and had on average higher weights compared to non-prescribed. Both methods detected only 5 patients. Due to the small number, no clear relationship could be identified, however the 2 patients that weren't detected were on at least 4 antihypertensive drugs and had high BP which might be caused by non-adherence.

6.8.9 **Spironolactone**

6.8.9.1 Comparing BIR vs GLA

Table 6-11 shows the comparison between Birmingham and Glasgow toxicology for detection of Spironolactone. Total number of patients that were prescribed Spironolactone was 5 patients. 2 (40%) patients were identified by both methods while 2 (40%) patients were not detected by either method. Birmingham was able to detect 1 patient that couldn't be detected by Glasgow toxicology.

Table 6-11 BIR vs GLA Cross-tabulation of the metabolomics results of the 6 patients analysed by both Birmingham and Glasgow toxicology laboratories who were prescribed Spironolactone.

Spironolactone			GLA		Total
			Not detected	Detected	
BIR	Not detected	N	2	0	2
		%	40.0%	0.0%	40.0%
	Detected	N	1	2	3
		%	20.0%	40.0%	60.0%
Total		N	3	2	5
		%	60.0%	40.0%	100.0%
Mcnemar P=1					

6.8.9.2 Morisky across the test groups

2 patients that were detected by both methods responded with score of 8 while a single patient detected only by Birmingham had score less than 8. The 2 patients who weren't detected by either method had score lower than 8 (Figure 6-16).

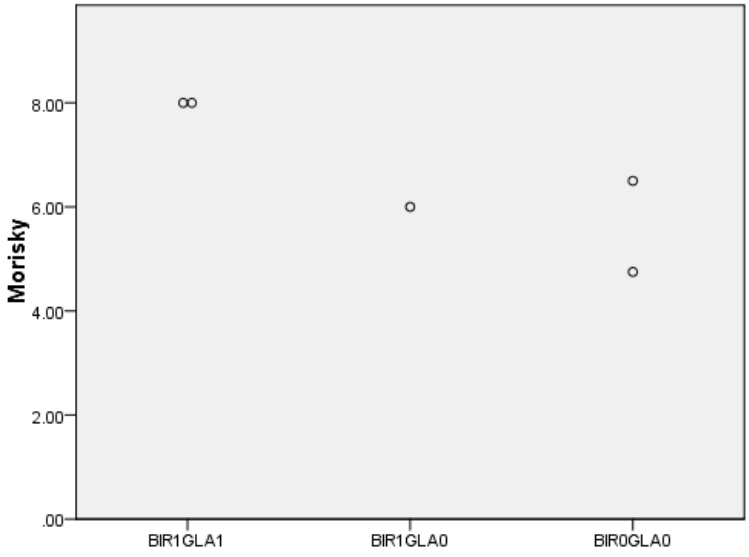


Figure 6-16 The Morisky score for 6 patients prescribed with Spironolactone using MMAS-8. The plot compares the score of the patients were detected by both Birmingham and Glasgow toxicology (n=2) with patients that were detected by Birmingham and not Glasgow toxicology (n=1) and patients that couldn't be detected by either method.

6.8.9.3 Discussion

Spironolactone is a potassium sparing diuretics that is used to treat hypertension, heart failure and kidney disease. It is usually prescribed with other antihypertensive drugs. It was only prescribed in 5 patients and was detected in 2 by both method, Birmingham detected an additional patient. Due to the small number it was difficult to identify any relationship for the patients.

6.8.10 Verapamil

6.8.10.1 Comparing BIR vs GLA

Only 1 patient was prescribed Verapamil and it was detected in both methods.

Table 6-12 Verapamil detection amongst the 79 patients analysed by both Birmingham hospital laboratory and Glasgow toxicology laboratory.

Prescribed	BIR N	BIR % Detection	GLA N	GLA % Detection
1	1	100	1	100

6.8.10.2 Discussion

Verapamil is CCB for managing hypertension (especially for patient with arrhythmia such as atrial fibrillation). In our sample it was only prescribed in 1 patient and was detected by both methods. Due to the small number it was difficult to find any relationship.

6.9 Comparing Glasgow Polyomics with Birmingham

Number of shared patients between BIR and POL is 57 patients.

6.9.1 Candesartan

Table 6-13 shows the comparison between Birmingham and Glasgow Polyomics for detection of Candesartan. Total number of patients that were prescribed Candesartan was 14 patients. Birmingham was able to detect all patient 14 while Glasgow Polyomics missed 1 patient.

6.9.1.1 Comparing BIR vs POL

Table 6-13 BIR vs POL Cross-tabulation of the metabolomics results of the 14 patients analysed by both Birmingham and Glasgow Polyomics laboratories who were prescribe Candesartan.

Candesartan			POL		Total
			Not detected	Detected	
BIR	Detected	N	1	13	14
		%	7.1%	92.9%	100.0%
Total		N	1	13	14
		%	7.1%	92.9%	100.0%

6.9.1.2 Morisky score across the test groups

Most patients that were detected by both methods responded with score of 8. the patient that was detected only by Birmingham had a score of 8 (Figure 6-17).

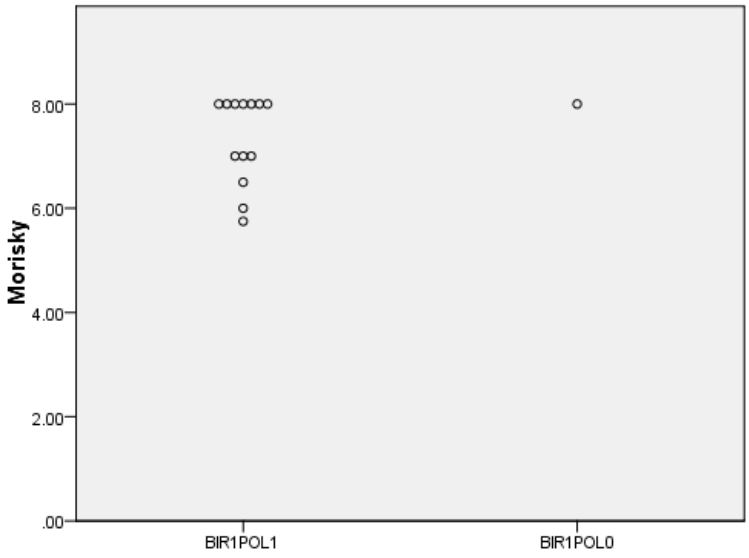


Figure 6-17 The Morisky score for 14 patients prescribed with Candesartan using MMAS-8. The plot compares the score of the patients that were detected by both Birmingham and Glasgow Polyomics (n=13) with the patient that only Birmingham detected (n=1).

6.9.1.3 Discussion

Candesartan is an ARB widely used to treat hypertension and heart failure. It is usually prescribed for patients that can't tolerate ACEI. It was prescribed in 14 patients (for the 57 shared between Birmingham and Glasgow Polyomics). Compared to non-prescribed, patient on Candesartan were mainly younger, retired, felt improving, on 3 or more antihypertensive with higher SBP.

Birmingham detected all the prescribed patient while Glasgow polyomics missed 1 patient. The patient had a complete adherence Morisky score and High BP readings. He was prescribed 2 antihypertensive (Candesartan and Diltiazem), Diltiazem was detected in both Birmingham and Polyomics which might indicate that the drug assay failed to detect that patient.

6.9.2 **Perindopril**

6.9.2.1 Detection rate

All prescribed patients were detected by both methods (Table 6-14)

Table 6-14 Numbers and percentages samples detected of the 2 patients prescribed Perindopril, amongst the 57 patients analysed by both Birmingham hospital laboratory and Glasgow Polyomics laboratory.

Prescribed	BIR N	BIR % Detection	POL N	POL % Detection
2	2	100	2	100

6.9.2.2 Morisky score across the test groups

One patient had a score of 8 while the other was less than 8 (Figure 6-18).

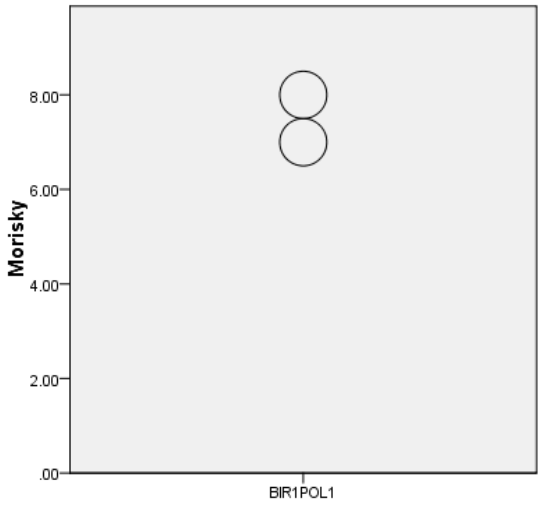


Figure 6-18 The Morisky score for 2 patients prescribed with Perindopril using MMAS-8. The plot shows the score of the patients that were detected by both Birmingham and Glasgow Polyomics (n=2)).

6.9.3 **Enalapril**

6.9.3.1 Detection rate

All prescribed patients were detected by both methods (Table 6-15).

Table 6-15 Numbers and percentages samples detected of the 2 patients prescribed Enalapril, amongst the 57 patients analysed by both Birmingham hospital laboratory and Glasgow Polyomics laboratory

Prescribed	BIR N	BIR % Detection	POL N	POL % Detection
2	2	100	2	100

6.9.3.2 Morisky score across the test groups

Both patients had score of 8 (complete adherence) Figure 6-19.

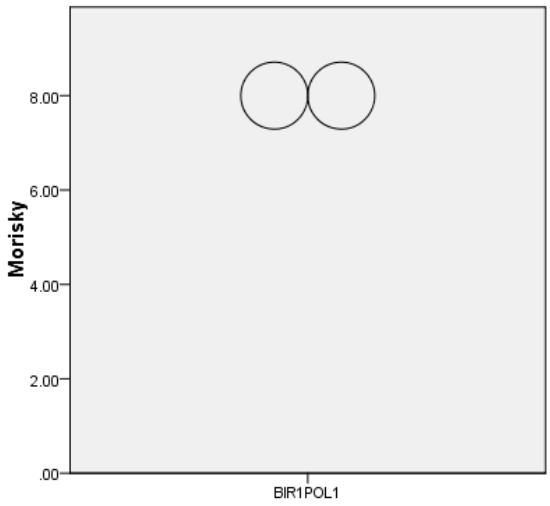


Figure 6-19 The Morisky score for 2 patients prescribed with Enalapril using MMAS-8. The plot shows the score of the patients that were detected by both Birmingham and Glasgow Polyomics (n=2)).

6.9.4 **Diltiazem**

6.9.4.1 Detection rate

All prescribed patients were detected by both methods (Table 6-16).

Table 6-16 Numbers and percentages samples detected of the 2 patients prescribed Diltiazem, amongst the 57 patients analysed by both Birmingham hospital laboratory and Glasgow Polyomics laboratory

Prescribed	BIR N	BIR % Detection	POL N	POL % detection
2	2	100	2	100

6.9.4.2 Morisky score across the test groups

Both patient prescribed Diltiazem had Morisky score of 8 (Figure 6-20)

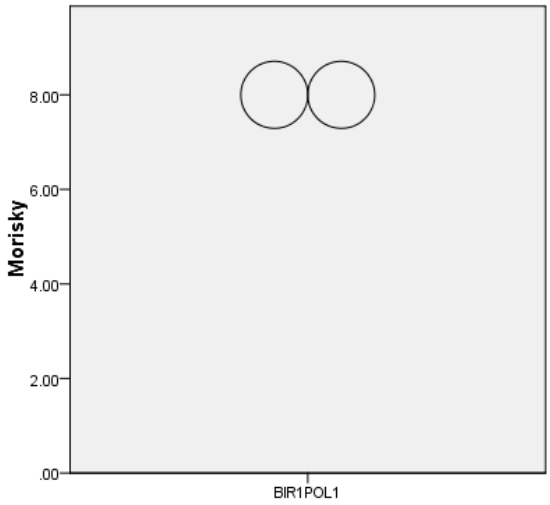


Figure 6-20 The Morisky score for 2 patients prescribed with Diltiazem using MMAS-8. The plot shows the score of the patients that were detected by both Birmingham and Glasgow Polyomics (n=2)).

6.9.5 **Irbesartan**

6.9.5.1 Detection rate

The prescribed patient was detected by both methods.

Table 6-17 Numbers and percentages samples detected of the 2 patients prescribed Irbesartan, amongst the 57 patients analysed by both Birmingham hospital laboratory and Glasgow Polyomics laboratory

Prescribed	BIR N	BIR % Detection	POL N	POL % Detection
1	1	100	1	100

6.9.5.2 Morisky score across the test groups

Patient prescribed Irbesartan had Morisky score less than 8 (Figure 6-21).

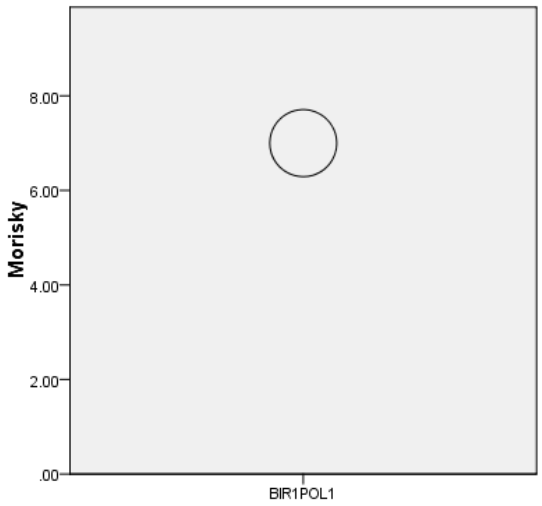


Figure 6-21 The Morisky score for patient prescribed with Irbeartan using MMAS-8. The plot shows the score of the patients# that were detected by both Birmingham and Glasgow Polyomics (n=1).

6.10 **Discussion**

In this chapter, I compared 4 antihypertensive drugs that were commonly assayed in 57 patients by 3 methods: Birmingham, Polyomics and Glasgow toxicology. The 4 drugs were amlodipine, atenolol, losartan and ramipril. Losartan was detected equally by all three methods. Amlodipine and Atenolol was detected in more patients by Polyomics and Glasgow toxicology compared to Birmingham. This could be an issue related with Birmingham method since the other 2 were able to detect the prescribed samples. Ramipril was detected in one additional patient compared to the other two methods. Polyomics detected atenolol in a patient who was prescribed metoprolol indicating untargeted methods have an advantage in detecting drugs which are not pre-specified. It is very hard to decide whether lower detection rates mean better or worse performance, but for the 4 drugs tested in all 3 centres it's possible (though not perfect) to use majority voting .

After that I compared between Birmingham and Glasgow toxicology in 79 shared samples for 10 antihypertensive drugs. were shared between Birmingham and Glasgow toxicology. I compared the detection rate for each drug and the result are summarized (Table 6-2)

Both methods agreed for most of the patients except GLA detected more for amlodipine and atenolol while Birmingham detected 1 more patient for Spironolactone. For samples where there is disagreement between BIR, GLA &/or POL it's clearly harder to say which results are likely to be true. Analyses of the concentrations found by GLA doesn't suggest those detected by GLA but not detected by BIR had lower concentrations. In the current literature there are not many studies that compares between several urine analysis methods.

Causes for non-detection

There are several possibilities for non-detection: First, patient not taking their prescribed drugs indicating true non adherence. However, urine assay doesn't identify the cause of non-adherence and patients need to be reviewed and asked for the possible causes. A study by Pucci et al analysed urine from hypertensive patients to assess for adherence. The adherence rates for 131 patients were 67

(51%) were completely detected (33%) were partially detected and only 21(16%) were non detected. They confronted the partially and non-detected patients about the result, 21 (36%) denied non adherence while 25 (43%) admitted non adherence(133). The most common reasons reported were side effect related to certain antihypertensive, forgetfulness, running out of medication, prescription cost, misunderstanding the instructions, depending on a carer to provide the drug and language barrier.

Second, failure of the assay to detect drugs. This could be from issues related to the sample such as sample collection, storage and preparation.

Unwanted sources of variations associated to sample collection and storage such as improper sample preservation (failure in the storage control of low temperature). freezer problems. Sample preparation and transport, failure of sample transport, mislabelled sample,

Lab assessment, lab not looking for certain drugs , technique related, explorative vs preset such as Insufficient coverage of MS profiling methods, limited quality of MS analyses, Incomplete metabolite identification.

Currently there is no standard method to measure urine adherence. New techniques are being developed to improve detection of medications. In my study the 3 methods each used a different technique for extraction and identifying the drugs. Third, Number of drugs screened: most of the studies that screened for antihypertensive drugs had different number of measured drugs. In a study by Tomaszewski et al. the number of antihypertensive drugs screened for were 40 (104). Another study by Lawson et al screened for 23 drugs(228). On the hand, Azizi et al, measured only 7 antihypertensive drugs (131). This could affect the rate of detection. In my study, Glasgow forensic only looked for 10 antihypertensive drugs while Glasgow Polyomics looked into 14 drugs and Birmingham searched for most drugs 20 drugs.

Fourth, the limit of detection might be different across the methods. Lawson et al. study had LOD ranging from 0.1 to 1.0 µg/L while in my study Glasgow toxicology used a LOD of .01 µg /L.

Glasgow toxicology method provided drug concentration for each antihypertensive drug analysed. This quantitative result could potentially enable us to identify reduced adherence (not always taking the correct dose every day). However, there are a lot of issues found in my result. There was no association with non-detection at BIR. In addition, issues regarding how to interpret different quantities.

Fifth, intra-individual variations due to diet and environmental factors such as diurnal variation and stress, inter-individual variations due to the genetic factors and presence/absence of disease. Failure to minimise such unwanted variation can cause a negative impact on the outcome resulting in identification of fewer drugs. In general, patients in whom prescribed drugs were not detected by two methods showed lower BP compared to patients who showed adherence confirmed by presence of prescribed drugs in the urine. Detailed analysis of the patients with discrepant detection of amlodipine by Birmingham and Glasgow methods showed that those individuals in whom amlodipine was not detected had lower blood pressure levels. As these were data on only two patients, no statistical analyses were possible. However, it raises the possibility that these patients may not be adherent with amlodipine because of their low BP levels. However, this needs to the tested formally in an independent study. This also indicates the possibility that urine drug assays for testing adherence routinely in unselected patients may identify individuals with good BP control who may be non-adherent and hence could benefit from deprescribing selected antihypertensive drugs.

My study clearly shows no correlation between the Morisky score and adherence based on urine drug assays. Results showed no significant difference and the average Morisky score was around 7. This was similar to studies that reported limited accuracy of the MMAS–8 in detecting medication non–adherence(139). A study conducted by Hamdidouche et al. used MMAS-4 and urine screening to assess for adherence. The study showed that MMAS-4 questionnaire was not significantly different between adherent and nonadherent patients based on urine drug screening. Therefore MMAS-8 scale has less accuracy than urine analysis to predict drug nonadherence (134)

Need for a gold standard

It is important to measure adherence through a reliable and validated methods. Currently each of the existing methods for measuring adherence exhibits certain strengths and limitations that should be considered before using that method and subsequently while interpreting results. Validation of a method for measuring adherence, necessitates an identification of a "gold standard," which can be concurrently compared to the measure of interest to determine its accuracy. A gold standard plays an important role in the ability to interpret results of measurements assessed in actual clinical practice and to subsequently translate and apply these results to clinical decision-making(229). Therefore, choosing a highly flawed gold standard can result in many negative consequences by introducing bias to the validation process and depending on whether the gold standard overestimates or underestimates adherence and whether it has a tendency to miscalculate in the same direction as the measure of interest, the measure may be falsely validated. Currently there is no gold standard to detect adherence in hypertensive patient, in this study we wanted to see if metabolomics could potentially be used to measure patient adherence.

Use of these techniques in terms of clinical practice

In general, direct measurements are relatively costly and are more labour-intensive for the health care provider. In addition, the complicated logistics of performing these measurements are an inherent disadvantage. Due to these disadvantages, it is unreasonable to use direct methods for measuring medication adherence in large patient populations. Indirect methods of measuring adherence are more commonly employed, due to their overall ease of use and less costly implementation. Ultimately the value of any clinical laboratory test needs to fulfil analytic validity, clinical utility and cost-effectiveness before they can be used routinely in clinical practice. Among these, clinical utility has to be clearly demonstrated showing that the use of these tests will improve clinical outcomes in practice. It is plausible that detecting non-adherence with these assays will theoretically improve adherence, however there are multiple unknowns – is a single measure of adherence sufficient? How to address white-coat adherence? Does measuring urine drug levels improve adherence and/or clinical outcomes. These require formal

blinded randomised controlled trials to establish the clinical value of adherence testing.

7 General discussion

Hypertension is a major risk factor for several cardiovascular CV diseases including stroke, coronary heart disease, myocardial infarction, congestive heart failure, stroke, chronic renal failure, peripheral vascular disease, and premature death. The estimated prevalence of HTN in Scotland in the adult population from 2014 to 2017 for all age groups in both sexes was 58.7%. All guidelines agree that patients with grade 2 or 3 hypertension and those with grade 1 hypertension with high CV risk should receive antihypertensive drug treatment alongside lifestyle interventions.

The direct and indirect costing for managing hypertension was estimated to be around $51.2 billion in 2012-2013 and total direct cost is estimated to be $200 billion by 2030 (230). Lifestyle modifications and antihypertensive medications decrease high BP and the associated morbidity and mortality. Achieving ideal BP targets and treatments optimize the balance between the complications of high BP and side effects from excessive BP lowering.

Hypertension guidelines were designed to reflect critical evaluation of the available evidence and provide recommendations for the prevention, detection, evaluation, and treatment of hypertension to provide patients with the most benefit and least amount of harm.

7.1 Changes in guidelines during study

Two hypertension guidelines were updated during my study which are AHA in 2017 and ESH in 2018. However, these are unlikely to have had an impact on my study as most of the sample collections were complete before the guidelines were published. The ESH guidelines kept the previous definition of hypertension (ie, BP >140/90 mm Hg) whereas AHA guidelines decreased the threshold to define hypertension to <130/80 mm Hg. Both sets of guidelines recommend the same therapeutic BP goal of <130/80 mm Hg.

AHA definition of normal BP remains the same <120/80 mmHg, the 2017 guideline replaces the term “prehypertension” with “elevated BP” 120-129/<80 mmHg) and “stage 1 hypertension” (average SBP of 130 to 139 mm Hg or average

DBP of 80 to 89 mm Hg). Stage 2 hypertension is defined as an average SBP of at least 140 mm Hg or an average DBP of at least 90 mm Hg instead of a BP of at least 160/100 mm Hg. The upper end of prehypertension was reclassified as stage 1 hypertension because adults with BP in this range have an approximately 2-fold increase in CVD risk compared with adults with normal BP. This change in BP classification is estimated to result in an increase of about 14% in the prevalence of hypertension in the United States but only a 1.9% increase in adults requiring antihypertensive drug therapy (231).

Table 7-1 Comparison between AHA and ESH guideline

Parameter	AHA	ESH
Definition of hypertension mm Hg	>130/80	>140/90
Normal pressure grading	Normal <120/80 Elevated 120-129/<80	Optimal <120/80 Normal 120–129/80–84 High normal 130–139/85–89
Hypertension grading	Grade 1, 130–139/80–89 Grade 2, ≥140/90	Grade 1 140–159/90–99 Grade 2, 160–179/100–109 Grade 3, ≥180/110
BP targets for treatment	≤65 y, <130/80 ≥65 y, <130/80	<65 y, <130/80 ≥65 y, <140/80

7.2 Issues associated with ABPM

Despite the benefit of ABPM for confirming the diagnosis of hypertension. There are patients who reported drawbacks associated with it including disturbance in sleep, work and daily activities and social embarrassment from the impression of having a medical problem. In a study by Beltman et.al reported side effect associated with ABPM were plan (9%), skin irritation (8%), noisy device (8%), inconvenience with work (3%), haematoma (2%) and other (4%). 61% minor disturbance while sleeping and 2% did not sleep at all. It is important to consider lifestyle, work, daily activities, family and sleep when ABPM is required. Patients who cannot tolerate ABPM need to be offered home monitoring as an alternative (232).

7.3 Adherence

Adherence to therapy is an important factor for BP control. The prevalence of adherence has varied between studies, it was reported to be high in some studies and low in others. Medication adherence is measured to Non-adherence to therapy can lead to uncontrolled blood pressure (BP), deterioration in health and progression of disease state. It can also increase the cost burden on the health care system.

Adherence is assessed using 2 different methods, either indirect or direct methods each has its own advantages and disadvantages. Measuring patient adherence accurately has historically been very challenging. However, urine analysis drug screening has recently become routinely available.

The main aim of this study was to assess adherence by indirect methods using self-report Morisky scale and direct method by untargeted and targeted drug screening in urine samples of hypertensive patients attending Glasgow Blood Pressure clinic (GBPC).

348 patients completed Morisky questionnaire and showed that 62.1% of patients had high adherence, while 26.7% of patients had medium adherence and only 11.2 had low adherence. This was generally higher than adherence rates from other studies. However, despite the high adherence detected, the level of BP

control was low. Only 35% of patient who reported that they were adherent had controlled SBP. Only DBP was significantly different (87 vs 82 mmHg in the low and higher adherence respectively. The predictor for adherence were assessed, age, DBP, gender, occupation and education were significant on univariate analysis. However, after applying binary logistic regression only age was significant which possibly could be confounding with occupation. The main advantages of Morisky scale are low cost, easy to administer, quick and short. However, the main drawbacks are the overestimation of the result which is seen in my study due to the subjective nature of self-report questionnaires and the potential recall bias. Also, Negativity in questions, suggesting blaming the patients for not fulfilling their prescribed regime can lead to bias.

Adherence was assessed using urine drug screening on urinary samples from hypertensive patients using 3 different centres: Birmingham heartland laboratory, Glasgow Polyomics and Glasgow toxicology.

Biochemical screening for adherence to antihypertensive treatment using a spot urine sample has several major advantages. It is a completely non-invasive procedure that can be conducted by a healthcare assistant prior to routine clinical appointments. Unlike many other previously used methods of screening, the HP LC-MS/MS analysis provides a clear ‘Yes/No’ answer to a question on presence/absence of antihypertensive medications based on direct measurement of urine. HP LC-MS/MS is a recognised method with good to excellent sensitivity and specificity to detect many pharmacological agents in urine.

79 urine samples were sent to Birmingham heartland laboratory and the assay was able to detect complete presence of antihypertensive medication in 49 (62%) of the urine samples. Only 6 samples were found to be completely absent of any medication and the remaining (30.4%) detected at least one of the prescribed antihypertensive medications (partial). No drugs were detected in patients who weren’t prescribed them. This method detected the largest number of antihypertensive drugs compared to the other 2 methods.

100 urine samples were sent to Glasgow Polyomics and was able to detect complete presence of antihypertensive medication in 34 (34%) of the urine samples. 12 (12%) were completely absent of any medication and the remaining

(54%) detected at least one of the prescribed antihypertensive medications (partial). The low level of complete detection in this method is possibly due to the approach they used. High-resolution untargeted mass spectrometry data-dependent fragmentation spectra and molecular networking were used to identify drugs

Out 173 urine samples sent to Glasgow toxicology 152 samples were tested for their prescribed drugs. Results showed only 6 (3.9%) patients weren't detected for any medication in the sample, while 137 (89.5%) detected all the medication they were tested for and 9 (5.9%) had some their prescribed drugs detected (partial adherence). There was one false positive result.

For the shared drugs across the 3 methods Birmingham and Glasgow toxicology agreed. In contrast Birmingham had lower detection for Amlodipine and Atenolol. This disagreement might indicate Birmingham has lower sensitivity detecting these drugs as the possibility of non-adherence is unlikely due to the other 2 methods identifying the drugs. Factors related to the sample preparation, extraction, LOD and analysis might cause the difference in detection rate.

There was no clear relationship between Morisky score and urinary adherence, this could be due to the small number of sample (especially non adherent group). This indicate that MMAS-8 has limited accuracy in detecting medication non–adherence.

My study showed the adherence assessment using MMAS-8 and 3 urine assays. In addition, I demonstrated the detection rate for each antihypertensive drug separately and compared between methods. These results could help guiding treatment in hypertensive patients. Detecting non adherence can help physicians avoid prescribing extra medications to control BP and identify patients that require further guidance and support regarding the importance of adherence to therapy

7.4 Cost implication

Nonadherence puts an enormous cost burden on the health service through medication wastage. A report of a study commissioned by the Department of Health, UK, in 2010 estimated the cost of National Health Service primary and community care prescription medicines wastage in England to be £300 million per year and that for antihypertensive medication to be at least £100 million a year(233).

HPLC-MS/MS instrumentation is expensive (cost around ≈$250,000–$300,000) and requires skilled laboratory staff. A recent predictive modelling study showed that repeated biochemical screening for non-adherence to antihypertensive therapy (therapeutic drug monitoring) is cost-effective in the management of resistant hypertension (228). The consequences of ineffective diagnostic approaches to non-adherence to antihypertensive therapy are extremely expensive reaching around $1000 to $1500 per patient in the UK. A 25% improvement in non-adherence rates in patients with hypertension can lead to decrease in adverse events by more than 2 million and lead to savings of 20 billion $ (229).

On the individual level, detecting non-adherence objectively prevents unnecessary investigations, reduce the number of hospital visits, help patients understand their illness and complying to their prescribed regimen.

Who will benefit?

There is a high incidence of non-adherence in patients presenting with ‘resistant hypertension’. Patel et.al showed that around one in three patients referred for renal denervation were non-adherent to their antihypertensive drugs (234). The recent data from DENERHTN trial suggest that non-adherence is even more common amongst patients in whom renal denervation was conducted (131). Therefore, it is worth considering screening for non-adherence in patients with resistant hypertension prior to expensive and irreversible interventions such as renal denervation. Moreover, there is a subgroup of patients who are considered to have refractory hypertension that is patients who have uncontrolled blood pressure despite being on 5 or more antihypertensive drugs (usually on two

diuretics) and under specialist care for their hypertension (235). The prevalence is estimated to be about 3-10% of patients referred with uncontrolled resistant hypertension to a specialist clinic and 0.5% of all hypertensive patients (236, 237). These patients should be excluded for non-adherence to antihypertensive therapy by an objective method prior to classifying them as truly refractory to antihypertensive medications.

Interventions Improve Non-Adherence?

Although not assessed in my study , a Cochrane review on interventions to improve non adherence which included 13 studies related antihypertensive therapy (Four used self-reported measures, five used pill counts and the remaining four used MEMS) demonstrated that adherence rates improved by 3% (pill counts) to 36% (self-report) in 11 of the 13 studies. SBP improved by 3–9.5 mmHg in seven studies while two studies only DBP improved by 3–4 mmHg (238).

7.5 Clinical implications

There is no consistency on how adherence is reported in the literature, it mainly depends on the method that is used to measure adherence. It can be reported qualitatively as patient being adherent/nonadherent or can be reported quantitatively such as percentages (calculated measure when using refill data). Traditionally, a cut-off value of 80% has been used for adherence; healthcare usage and costs are noted to be reduced in patients where medication adherence exceeds 80%. In hypertension, >80% of the prescribed medications have been shown to maintain blood pressure control (239).Using ineffective methods for detecting non-adherence will result in unnecessary treatment and additional investigations, many of which carry risks.

7.6 Limitation of the study

First, the characteristics of the study population may not reflect the general population. Patients were recruited from a tertiary care clinic that could have benefited from a therapeutic education regarding the need for a long-lasting antihypertensive therapy and acceptance for its adverse effects.

Second, small number of non-adherent/non detected patients. The differences between the nonadherent and adherent groups finding cannot exclude a chance finding.

Third, not all the antihypertensive drugs prescribed for patients were detected. In my study BIR detected 20 drugs while POL detected 14 and GLA detected only 10 drugs.

Fourth, urine drug screening is sensitive to the Hawthorne effect because the samples were collected on their clinic visit which may increase the positive result.

Fifth, hypertension detection in the study, hypertension measurement used in the study were obtained from clinic readings, this could be potentially influenced by white coat effect. (ABPM should have been ideally recorded for each patients.

Sixth, no gold standard, no way to confirm

Finally, the detection of a prescribed antihypertensive drugs in urine does not mean persistence, it only provides a snapshot of non-adherent behaviour. There is no assessment of long term adherence. The urine analysis is not immune to white-coat adherence since the samples will be collected during patient's clinic visit. This could be managed by collecting random samples from patients during different time (patient doesn't know about the collection time which is similar to drug testing in sport athlete. However, it is very difficult to apply in practice, labour intensive and is highly cost.

Further studies on utility and cost effectiveness of HP LC-MS/MS urine analysis should be conducted against indirect measures of adherence to inform future health policies and clinical practice.

7.7 **Future plans**

The relationship between medication adherence and BP control is difficult to demonstrate. However, it is important to obtain an unbiased estimate of this effect and it will inform clinical practice. Further studies in larger sample sizes are required for urine drug assays to be used in routine clinical practice. The limitations outlined above will restrict routine use of urine drug assays in clinical practice and it is likely to be more commonly used in clinical trial settings or in selected patients with resistant hypertension. There are other methods such as witnessed drug administration that are very useful and commonly used in clinical practice to assess drug adherence. In this context, urine drug assays are second line tests. There is still lack of clarity on how to manage patients who demonstrate non-adherence. Future studies should focus not just on adherence but also combining adherence studies with interventions that would improve BP control.

In summary, adherence is an important and complex area of future research that is essential to improve hypertension management and decrease the global burden of hypertension. My studies in the Glasgow BP Clinic has yielded useful pilot information which can inform future studies.

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8 Appendix

8.1 Lawson 23 antihypertensive drugs

Table 8-1 Antihypertensive drugs analysed by Lawson et.al.

CCB	Amlodipine, Diltiazem, Felodipine, Verapamil, Nifedipine
ACEI	Lisinopril, Perindopril, Ramipril, Enalapril
ARB	Losartan, Irbesartan, Candesartan
Diuretics	Indapamide, Furosemide, Bendroflumethiazide, Hydrochlorothiazide
BB	Atenolol, Labetalol, Bisoprolol, Metoprolol
other	Doxazosin, Spironolactone and Moxonidine.

8.2 Lab investigations

Table 8-2 Lab investigations

Test	**components**
Complete blood count CBC	White blood cell WBC, Haemoglobin Hb and Platelet.
Urea and electrolyte U&E	Sodium Na, Potassium K, Chloride Cl, Urea and creatinine
Liver function test LFR	Total bilirubin TB, Alanine aminotransferase ALT, Aspartate transaminase AST, Alkaline phosphatase ALP and Albumin.
Lipid Profile	Triglycerides TGL, high-density lipoprotein HDL, Low-density lipoprotein LDL, Cholesterol: HDL ratio Chol/HDL ratio.
Glucose and HbA1c	
Renin and Aldosterone	

8.3 ABPM for Morisky scale

Table 8-3 ABPM and Morisky groups

	Low n=39			Medium n=93			High n=216			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	P
First ABPM										
24H										
SYS AVG	32	140	15	73	144	19	160	140	14	0.124
SYS SD	28	12.89	2.46	69	14.91	3.75	146	14.84	3.94	0.034
DIA AVG	32	86	12	73	83	14	160	79	11	0.003
DIA SD	28	11.21	2.36	69	10.73	2.67	146	10.67	2.59	0.593
MAP AVG	32	104	12	73	104	14	160	100	10	0.022
MAP SD	28	11.45	2.28	69	12.17	2.88	146	12.05	2.71	0.48
PP AVG	28	53	7	69	61	14	146	60	12	0.012
PP SD	28	8.87	2.18	69	10.28	2.82	146	10.08	2.9	0.072
HR AVG	28	75	13	69	73	14	146	70	12	0.099
HR SD	28	9.95	3.46	69	9.2	3.73	146	9.67	3.88	0.598
Day										
SYS AVG	32	143	15	73	147	19	160	143	15	0.124
SYS SD	32	12.43	7.63	73	13.57	4.19	160	13.43	3.93	0.471
DIA AVG	32	89	13	73	86	15	160	81	11	0.003
DIA SD	32	9.38	3.43	73	9.2	2.52	160	9.31	2.66	0.942
MAP AVG	32	107	13	73	107	15	160	103	11	0.018
MAP SD	28	9.45	2.51	69	10.66	3.13	146	10.68	2.69	0.096
PP AVG	28	53	8	69	61	14	146	61	13	0.011
PP SD	28	8.98	2.23	69	10.42	3.3	146	9.95	2.99	0.103
HR AVG	28	77	13	69	75	15	146	72	12	0.095
HR SD	28	9.64	3.5	69	8.63	3.55	146	9.38	4.14	0.341
Night										
SYS AVG	32	130	16	73	136	20	159	131	17	0.12
SYS SD	32	12.72	4.7	73	12.19	5.29	158	12.81	4.79	0.67
DIA AVG	32	76.9	13.4	73	75.6	13.2	159	71.5	11.3	0.012
DIA SD	32	10.84	5.14	73	9.31	4.31	158	9.46	3.32	0.142
MAP AVG	32	95	13	73	97	14	159	93	11	0.077
MAP SD	28	11.38	4.36	69	9.82	3.93	144	10.59	3.5	0.144
PP AVG	28	52	7	69	60	15	145	58	12	0.017
SD	28	7.59	4.01	69	7.99	3.14	144	8.42	3.41	0.415
HR AVG	28	69	13	69	67	12	145	65	11	0.128
HR SD	28	6.83	3.88	69	6.24	3.14	144	5.96	2.97	0.386
Second ABPM										
24H										
SYS AVG2	10	140	12	31	144	17	75	140	16	0.463
SYS SD2	8	14.64	4.15	30	15.4	3.48	66	15.15	3.7	0.868
DIA AVG2	10	83	9	31	80	12	75	79	10	0.466
DIA SD2	8	12.14	1.89	30	11.88	2.71	66	10.36	2.64	0.015
MAP AVG2	10	103	9	31	103	11	75	100	10	0.41
MAP SD2	8	12.62	1.52	30	12.24	3.41	66	11.83	2.85	0.683
PP AVG2	8	56	12	30	64	16	66	60	12	0.229
PP SD2	8	9.24	3.75	30	9.89	2.26	66	10.19	2.46	0.569

HR AVG2	8	76	16	30	73	13	66	69	12	0.169
HR SD2	8	9.13	3.17	30	9.15	4.27	66	9.05	3.38	0.993
Day										
SYS AVG2	10	143	14	31	147	17	75	142	15	0.336
SYS SD2	10	13.28	4.02	31	13.75	3.62	75	14.46	4.45	0.577
DIA AVG2	10	85	10	31	83	13	75	81	10	0.374
DIA SD2	10	9.67	2.7	31	10.21	1.6	75	9.25	2.46	0.147
MAP AVG2	10	105	10	31	106	12	75	103	10	0.294
MAP SD2	8	10.98	2.7	30	11	2.81	66	10.67	2.83	0.844
PP AVG2	8	57	13	30	64	16	66	60	12	0.227
PP SD2	8	9.4	3.34	30	9.78	2.37	66	10.3	2.61	0.496
HR AVG2	9	85	26	30	75	14	66	71	12	0.019
HR SD2	9	9.44	3.37	30	8.79	3.72	66	8.77	3.66	0.873
Night										
SYS AVG2	10	124	19	31	134	21	74	132	19	0.382
SYS SD2	10	11.89	3.88	31	13.06	3.97	74	12.18	4.49	0.591
DIA AVG2	10	77	13	31	70	10	74	71	10	0.172
DIA SD2	9	11.17	3.91	31	10.09	3.46	74	8.84	4.16	0.128
MAP AVG2	9	95	8	31	94	13	73	94	12	0.972
MAP SD2	8	11.13	3.4	30	10.14	3.31	66	9.98	3.7	0.694
PP AVG2	8	54	9	30	63	19	66	59	14	0.231
PPSD2	8	7	3.4	30	7.58	3.43	66	8.15	2.86	0.487
HR AVG2	8	70	16	30	68	12	66	63	11	0.111
HR SD2	8	7.16	3.36	30	5.94	3.05	66	5.72	2.72	0.41

SYS: systolic DIA: diastolic, MAP: mean arterial pressure, PP pulse pressure. D: day, N: night, AVG: average, SD: standard deviation. P value was calculated using one-way ANOVA test.

8.4 LAB investigations for Morisky

Table 8-4 Lab investigations and Morisky groups

	Low n=39			Medium n=93			High n=216			
CBC	N	Mean	SD	N	Mean	SD	N	Mean	SD	P
WBC	38	7.374	2.693	93	7.631	3.538	214	7.257	2.01	0.507
HB	38	149	14	93	142	16	214	141	14	0.014
Platelet	38	251	59	93	252	64	214	265	76	0.248
U&E										
Na	39	140	2	93	139	3	216	139	3	0.434
K	39	4.22	0.38	93	4.67	3.63	215	4.29	0.47	0.244
CL	39	104	3	93	103	4	216	103	4	0.326
Urea	39	5.36	2.04	93	6.01	1.92	216	6.15	2.91	0.218
Creatinine	39	85	23	93	84	26	216	82	30	0.763
LFT										
TB	39	10	4	92	12	6	216	10	4	0.036
ALT	39	32	14	92	29	31	215	26	16	0.14
AST	39	26	10	93	25	20	216	24	11	0.582
ALP	39	83	19	93	88	32	216	87	33	0.687
Albumin	39	41	3	93	39	4	216	39	3	0.01
Lipid Profile										
Cholesterol	38	5.33	1.36	93	5.03	1.15	216	5.06	1.1	0.35
TGL	38	2.02	1.47	93	1.76	1.06	216	1.7	1.03	0.235
HDL	38	1.32	0.34	89	1.31	0.3	213	1.43	0.43	0.026
LDL	37	3.16	1.26	84	2.97	0.97	204	2.89	0.99	0.314
Chol/HDL ratio	38	4.24	1.29	89	4.02	1.13	213	3.75	1.11	0.019
Glucose	39	6.44	3.21	87	6.27	4.23	207	6.24	2.69	0.937
HbA1c	27	43	15	54	41	13	129	44	14	0.447
Urate	2	203.63	287.62	10	393.44	179.97	25	359.38	96.96	0.197
Renin	11	72	120	14	89	103	34	36	52	0.099
Aldosterone	10	432	489	14	603	290	31	334	158	0.015
LogTB	39	.98	0.15	92	1.03	0..19	216	0.99	0.17	0.064

WBC: White Blood Cell, HB: Haemoglobin, Platelet, U&E: Urea and Electrolyte, Na: Sodium, K: Potassium, CL: Chloride, LFT: Liver Function Test, TB: Total Bilirubin, ALT: Alanine aminotransferase, AST: Aspartate Transaminase, ALP: Alkaline Phosphatase, TGL: Triglycerides, HDL: High-density Lipoprotein, LDL: Low-density Lipoprotein, VLDL: Very low-density Lipoprotein, Chol/HDL ratio: Cholesterol: HDL ratio. P-value was calculated using one-way ANOVA test.

8.5 Ethical Approval

Coordinator: Dr Maureen Travers
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Dr S Padmanabhan

NHS GG&C Board Approval

Dear Dr S Padmanabhan

Study Title:	Next generation Sequencing and Metabolomic Approaches in Stratification of Resistant Hypertension
Principal Investigator:	Dr Sandosh Padmanabhan
GG&C HB site	Western Infirmary General
Sponsor	NHS Greater Glasgow & Clyde
R&D reference:	GN14CA266
REC reference:	14/LO/1887
Protocol no: (including version and date)	Version: 1.3 Date: 04.11.2014

I am pleased to confirm that Greater Glasgow & Clyde Health Board is now able to grant **Approval** for the above study.

Conditions of Approval

1. **For Clinical Trials** as defined by the Medicines for Human Use Clinical Trial Regulations, 2004
   a. During the life span of the study GGHB requires the following information relating to this site
      i. Notification of any potential serious breaches.
      ii. Notification of any regulatory inspections.

It is your responsibility to ensure that all staff involved in the study at this site have the appropriate GCP training according to the GGHB GCP policy (www.nhsggc.org.uk/content/default.asp?page=s1411), evidence of such training to be filed in the site file.

2. **For all studies** the following information is required during their lifespan.
   a. Recruitment Numbers on a quarterly basis
   b. Any change of staff named on the original SSI form
   c. Any amendments – Substantial or Non Substantial
   d. Notification of Trial/study end including final recruitment figures

Figure 8-1 Ethical approval from the NHS Greater Glasgow & Clyde Health Board

8.5.1 **ABPM for GLA toxicology**

Table 8-5 ABPM and adherence by urinary drug assay by Glasgow toxicology

GLA	Non detected n=6			Partial n=9			Complete detection n=137			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	P
ABPM1										
24H										
SYS AVG	4	168.5	17.5	9	154.9	25.0	102	141.2	14.9	<0.0005
SYS SD	3	18.4	4.7	7	15.0	3.2	90	14.9	4.3	0.393
DIA AVG	4	93.5	18.1	9	88.6	15.4	102	80.1	11.4	0.016
DIA SD	3	12.4	3.5	7	12.0	2.3	90	10.7	2.4	0.225
MAP AVG	4	119.5	15.3	9	109.9	19.0	102	101.3	10.6	0.002
MAP SD	3	14.0	3.1	7	13.3	2.3	90	12.0	2.7	0.268
PP AVG	3	70.7	14.8	7	64.4	18.0	90	60.5	12.1	0.305
PP SD	3	14.7	3.6	7	10.5	3.9	90	10.2	3.0	0.046
HR AVG	3	77.0	19.0	7	71.9	13.5	90	69.1	12.5	0.5
HR SD	3	11.1	6.7	7	7.6	3.2	90	9.4	3.7	0.32
Day										
SYS AVG	4	172.0	17.7	9	157.0	26.5	102	144.4	15.3	0.001
SYS SD	4	17.7	4.0	9	14.0	4.1	102	13.9	5.9	0.433
DIA AVG	4	94.0	17.1	9	91.1	16.5	102	82.8	12.3	0.052
DIA SD	4	10.0	3.4	9	10.3	2.6	102	9.4	2.8	0.619
MAP AVG	4	121.3	15.3	9	112.0	20.7	102	104.2	11.5	0.009
MAP SD	3	11.8	4.8	7	12.2	3.1	90	10.5	2.8	0.277
PP AVG	3	73.0	15.1	7	65.3	18.0	90	61.1	12.4	0.226
PP SD	3	14.0	3.2	7	10.6	3.9	90	10.2	3.3	0.151
HR AVG	3	77.3	18.6	7	73.3	14.0	90	70.7	13.3	0.631
HR SD	3	11.6	8.1	7	7.1	2.9	90	9.3	3.8	0.209
Night										
SYS AVG	4	157.8	21.7	9	146.3	24.4	102	131.8	16.9	0.002
SYS SD	4	14.8	2.3	9	10.3	3.0	101	12.7	4.5	0.174
DIA AVG	4	91.5	22.3	9	80.8	12.2	102	72.1	11.1	0.001
DIA SD	4	19.8	11.0	9	8.9	2.7	101	9.5	3.6	0
MAP AVG	4	113.5	17.3	9	101.7	16.3	102	93.1	11.3	0.001
MAP SD	3	15.1	6.0	7	10.0	3.7	89	10.5	3.6	0.099
PP AVG	3	61.7	15.9	7	62.4	19.2	90	58.9	12.8	0.761
PP SD	3	14.3	8.9	7	8.2	4.4	89	8.3	2.9	0.009
HR AVG	3	74.0	18.3	7	81.6	41.3	90	64.6	11.6	0.015
HR SD	3	7.8	7.8	7	5.3	1.7	89	6.3	3.3	0.553
ABPM2										
24H										
SYS AVG	3	153.3	1.5	9	143.8	15.1	41	143.6	18.0	0.638
SYS SD	3	18.4	6.5	5	15.8	5.5	38	15.5	3.4	0.467
DIA AVG	3	93.3	4.5	9	83.1	9.8	41	79.6	11.4	0.098
DIA SD	3	14.4	1.7	5	13.4	4.5	38	11.2	2.8	0.085
MAP AVG	3	115.3	3.5	9	104.8	10.0	41	102.9	11.3	0.168
MAP SD	3	16.2	3.6	5	10.9	6.6	38	12.5	3.0	0.125
PP AVG	3	60.0	3.0	5	60.0	12.7	38	61.5	13.8	0.962
PP SD	3	11.0	1.0	5	10.2	2.6	38	10.1	2.4	0.803

HR AVG	3	81.7	12.5	5	79.2	13.7	38	70.7	12.6	0.165
HR SD	3	10.4	0.9	5	7.1	2.3	38	9.9	3.6	0.218
Day										
SYS AVG	3	157.7	4.2	9	146.8	17.5	41	146.6	17.6	0.562
SYS SD	3	15.9	5.5	9	15.0	5.6	41	13.8	4.0	0.596
DIA AVG	3	97.0	5.2	9	86.0	11.9	41	82.2	11.8	0.092
DIA SD	3	12.2	1.6	9	10.3	3.3	41	9.5	2.4	0.182
MAP AVG	3	119.3	6.0	9	107.8	11.7	41	105.7	11.4	0.137
MAP SD	3	14.3	3.1	5	13.1	3.2	38	10.8	2.9	0.051
PP AVG	3	61.0	3.5	5	59.6	12.7	38	61.7	13.2	0.944
PP SD	3	10.8	0.5	5	10.3	3.3	38	10.0	2.3	0.813
HR AVG	3	84.3	12.1	5	80.4	13.2	38	72.1	13.4	0.168
HR SD	3	9.4	1.9	5	6.9	2.2	38	9.8	3.9	0.266
Night										
SYS AVG	3	137.7	13.3	8	139.0	15.7	41	134.1	21.3	0.798
SYS SD	3	15.1	2.3	8	11.7	2.8	41	13.0	4.5	0.492
DIA AVG	3	81.0	7.6	8	75.4	9.7	41	71.2	11.3	0.245
DIA SD	3	13.5	3.5	8	9.7	4.1	41	9.3	4.5	0.283
MAP AVG	3	102.0	6.1	8	97.9	10.5	41	94.2	12.9	0.469
MAP SD	3	13.4	3.1	5	10.8	2.2	38	10.6	4.1	0.505
PP AVG	3	56.7	7.5	5	60.8	15.8	38	60.5	16.8	0.925
PP SD	3	8.7	4.5	5	8.0	1.6	38	8.0	2.9	0.916
HR AVG	3	72.7	15.0	5	76.0	15.3	38	66.3	11.9	0.219
HR SD	3	7.6	1.9	5	5.5	1.9	38	6.5	3.3	0.653

8.5.2 **LAB investigations for GLA toxicology**

8.5.2.1 Laboratory tests and antihypertensive adherence

Table 8-6 Lab investigations for patients

	Non detected n=6			Partial n=9			Complete detection n=137			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	P
CBC										
WBC	6	10.2	6.3	9	7.6	2.4	136	7.4	2.7	0.078
HB	6	142.7	7.3	9	138.0	22.5	136	141.9	12.7	0.687
PLATELET	6	279.7	56.7	9	288.2	76.6	136	255.7	63.1	0.239
U&E										
Na	6	136.0	5.1	9	139.0	2.8	137	139.1	2.6	0.03
K	6	4.7	0.9	9	4.1	0.7	137	4.2	0.5	0.051
CL	6	102.7	4.5	9	103.6	3.8	137	103.1	3.3	0.881
Urea	6	5.3	2.2	9	5.3	1.3	137	6.3	2.6	0.335
Creatinine	6	76.2	19.4	9	77.1	13.2	137	81.7	25.5	0.762
LFT										
TB	6	8.7	2.0	9	9.1	3.1	137	10.9	4.9	0.298
ALT	6	20.2	10.9	8	26.5	17.4	136	26.8	15.4	0.59
AST	6	21.2	6.4	9	22.9	10.8	137	24.3	9.6	0.677
ALP	6	101.8	27.1	9	93.9	16.6	137	86.4	32.7	0.423
Albumin	6	36.8	1.3	9	37.6	1.8	137	38.9	3.3	0.148
Lipid profile										
Cholesterol	6	5.6	1.4	9	4.6	1.2	137	5.2	1.2	0.233
TGL	6	1.8	0.7	9	1.9	1.2	137	1.9	1.2	0.992
HDL	6	1.3	0.3	8	1.3	0.4	134	1.4	0.4	0.826
LDL	6	3.4	1.1	7	2.5	0.9	130	3.0	1.0	0.281
Chol/HDL ratio	6	4.3	1.2	8	4.0	1.5	134	4.0	1.2	0.841
Glucose	6	6.4	1.2	7	8.0	5.4	131	6.2	2.2	0.123
HbA1c	5	43.6	16.0	7	45.7	18.1	85	43.0	12.9	0.876
Renin Conc	3	29.2	23.5	4	49.1	38.6	18	71.7	99.5	0.707
Aldosterone	4	305.0	91.6	3	441.7	161.8	18	398.5	267.6	0.727
LogTB	6	0.9	0.1	9	0.9	0.2	137	1.0	0.2	0.365
LogALT	6	1.3	0.2	8	1.4	0.3	136	1.4	0.2	0.41
LogAST	6	1.3	0.1	9	1.3	0.2	137	1.4	0.2	0.561
LogALP	6	2.0	0.1	9	2.0	0.1	137	1.9	0.1	0.201
LogALBumin	6	1.6	0.0	9	1.6	0.0	137	1.6	0.0	0.206
LogTGL	6	0.2	0.2	9	0.2	0.3	137	0.2	0.2	0.953
LogHDL	6	0.1	0.1	8	0.1	0.1	134	0.1	0.1	0.828
LogCHOL	6	0.7	0.1	9	0.7	0.1	137	0.7	0.1	0.201
Logglucose	6	0.8	0.1	7	0.9	0.2	131	0.8	0.1	0.208
LogHba1c	5	1.6	0.1	7	1.6	0.1	85	1.6	0.1	0.904
LogRenin	3	1.3	0.4	4	1.6	0.3	18	1.6	0.5	0.715
LogAldosterone	4	2.5	0.1	3	2.6	0.2	18	2.5	0.2	0.643

8.5.3 **Concentration for each drug detected by Glasgow toxicology**

NGS	Amlodipine	Atenolol	Bendro	Bisoprolol	Canrenone	Spironolactone	Doxazosin	Furosemide	Losartan	Losartan Carboxylic Acid	Ramipril	Ramiprilat	Verapamil	Norverapamil
NGS002	NEG	NEG	935	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS003	NEG	NEG	NEG	425	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS004	NEG	NEG	847	NEG	NEG	NEG	NEG	NEG	Present	Present	NEG	NEG	NEG	NEG
NGS005	126	NEG	731	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS007	178	NEG	1720	NEG	NEG	NEG	354	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS008	<10	36	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS009	NEG	NEG	890	NEG	NEG	NEG	NEG	NEG	NEG	NEG	59	1315	NEG	NEG
NGS010	NEG	NEG	768	NEG	NEG	NEG	NEG	NEG	>2000	>2000	NEG	NEG	NEG	NEG
NGS011	121	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS012	NEG	NEG	615	NEG	NEG	NEG	NEG	NEG	NEG	NEG	<20	34	NEG	NEG
NGS013	299	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS014	NEG	NEG	1734	NEG	236	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS015	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	<20	713	NEG	NEG
NGS016	186	NEG	NEG	715	NEG	NEG	39	222	NEG	NEG	NEG	NEG	NEG	NEG
NGS017	NEG	NEG	154	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS018	73	NEG	NEG	NEG	NEG	NEG	NEG	NEG	>2000	>2000	NEG	NEG	NEG	NEG
NGS019	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS020	101	NEG	287	>2000	NEG	NEG	NEG	NEG	157	169	NEG	NEG	NEG	NEG
NGS021	237	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS022	NEG	NEG	251	NEG	NEG	NEG	NEG	NEG	1689	>2000	NEG	NEG	NEG	NEG
NGS023	NEG	NEG	NEG	898	NEG	NEG	<10	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS024	90	NEG	NEG	1452	NEG	NEG	19	NEG	NEG	NEG	NEG	NEG	NEG	NEG

NGS025	NEG	NEG	158	NEG	NEG	NEG	55	NEG	69	>2000	NEG	NEG	NEG	NEG
NGS026	NEG	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS027	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS028	256	NEG	993	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS029	NEG	NEG	319	NEG	NEG	NEG	NEG	NEG	>2000	1953	NEG	NEG	NEG	NEG
NGS030	391	NEG	NEG	1034	NEG	NEG	NEG	NEG	>2000	>2000	NEG	NEG	NEG	NEG
NGS031	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS032	NEG	NEG	1046	>2000	NEG	NEG	NEG	NEG	>2000	>2000	NEG	NEG	NEG	NEG
NGS033	NEG	NEG	1352	NEG	NEG	NEG	NEG	NEG	>2000	848	NEG	NEG	NEG	NEG
NGS034	471	NEG	NEG	NEG	NEG	NEG	NEG	NEG	>2000	>2000	NEG	NEG	NEG	NEG
NGS035	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	727	NEG	NEG
NGS036	211	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS037	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS038	200	NEG	1197	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS039	295	NEG	NEG	NEG	NEG	NEG	25	>2000	1501	1761	NEG	NEG	NEG	NEG
NGS040	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	1093	NEG	NEG
NGS043	572	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	1490	NEG	NEG
NGS045	NEG	NEG	>2000	NEG	NEG	NEG	392	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS046	NEG	NEG	NEG	NEG	NEG	NEG	18	>2000	1478	291	NEG	NEG	NEG	NEG
NGS047	610	NEG	1533	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS048	399	NEG	888	NEG	NEG	NEG	NEG	NEG	NEG	NEG	84	>2000	NEG	NEG
NGS049	NEG	>2000	773	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS050	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS051	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG

NGS052	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS053	NEG	NEG	1609	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS054	282	NEG	1333	NEG	NEG	NEG	NEG	NEG	NEG	NEG	<20	Present	NEG	NEG
NGS055	NEG	NEG	NEG	580	56	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS056	NEG	NEG	NEG	421	NEG	NEG	NEG	NEG	NEG	NEG	148	>2000	NEG	NEG
NGS057	194	NEG	47	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	116	NEG	NEG
NGS058	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS059	NEG	NEG	171	666	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS060	70	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS061	NEG	NEG	NEG	1416	258	NEG	NEG	NEG	168	1642	NEG	NEG	NEG	NEG
NGS062	NEG	>2000	NEG	NEG	NEG	NEG	70	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS063	82	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS064	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS065	NEG	>2000	NEG	NEG	NEG	NEG	25	>2000	NEG	NEG	NEG	NEG	NEG	NEG
NGS066	132	NEG	NEG	NEG	NEG	NEG	NEG	NEG	23	102	NEG	NEG	NEG	NEG
NGS067	476	>2000	706	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS068	925	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS069	339	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	318	Present	NEG	NEG
NGS070	NEG	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS071	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	46	Present	NEG	NEG
NGS072	1301	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS073	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS074	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS075	NEG	NEG	629	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG

NGS076	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	76	Present	NEG	NEG
NGS077	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS078	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS079	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS080	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	>2000	>2000
NGS081	495	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS082	NEG	NEG	998	NEG	NEG	NEG	NEG	NEG	>2000	1293	NEG	NEG	NEG	NEG
NGS083	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	158	Present	NEG	NEG
NGS084	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS085	284	NEG	NEG	NEG	NEG	NEG	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG
NGS086	NEG	NEG	38	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS087	268	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	483	Present	NEG	NEG
NGS088	409	NEG	179	1587	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS089	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	27	Present	NEG	NEG
NGS090	103	NEG	NEG	785	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS091	1093	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS092	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Inconclusive	NEG	NEG	NEG	NEG
NGS093	NEG	>2000	243	NEG	NEG	NEG	46	NEG	132	691	NEG	NEG	NEG	NEG
NGS094	140	>2000	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS095	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS096	213	NEG	872	NEG	NEG	NEG	NEG	NEG	>2000	>2000	NEG	NEG	NEG	NEG
NGS097	NEG	NEG	>2000	NEG	NEG	NEG	65	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS098	396	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS099	NEG	NEG	NEG	NEG	NEG	NEG	NEG	1116	NEG	NEG	Present	Present	NEG	NEG

NGS100	522	NEG	848	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	Present	NEG	NEG
NGS101	NEG	NEG	NEG	NEG	NEG	NEG	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG
NGS102	NEG	NEG	484	NEG	NEG	NEG	NEG	NEG	518	Present	NEG	NEG	NEG	NEG
NGS103	NEG	NEG	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	NEG	NEG
NGS104	NEG	NEG	415	NEG	NEG	NEG	NEG	NEG	984	Present	NEG	NEG	NEG	NEG
NGS105	NEG	NEG	689	NEG	49	NEG	44	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS106	NEG	NEG	NEG	NEG	NEG	NEG	108	>2000	NEG	NEG	NEG	NEG	NEG	NEG
NGS107	NEG	NEG	NEG	NEG	NEG	NEG	<10	NEG	NEG	Present	NEG	NEG	NEG	NEG
NGS108	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS109	129	NEG	40	NEG	NEG	NEG	25	NEG	NEG	NEG	NEG	Present	NEG	NEG
NGS110	NEG	NEG	NEG	1127	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS111	NEG	NEG	NEG	1380	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS112	NEG	NEG	464	NEG	95	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS113	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS114	NEG	NEG	NEG	NEG	188	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS115	NEG	>2000	NEG	NEG	NEG	NEG	NEG	>2000	NEG	NEG	NEG	Present	NEG	NEG
NGS116	547	>2000	472	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS117	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	NEG	NEG
NGS118	222	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS119	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS120	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS121	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	NEG	NEG
NGS122	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	NEG	NEG
NGS123	NEG	NEG	1056	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	NEG	NEG

NGS124	62	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	NEG	NEG
NGS126	NEG	NEG	NEG	>2000	NEG	NEG	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG
NGS127	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS128	NEG	NEG	NEG	NEG	NEG	NEG	68	>2000	NEG	NEG	NEG	NEG	NEG	NEG
NGS129	327	NEG	945	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	NEG	NEG
NGS130	353	>2000	1039	NEG	NEG	NEG	NEG	NEG	>2000	Present	NEG	NEG	NEG	NEG
NGS131	425	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	NEG	NEG
NGS132	526	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS133	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	NEG	NEG
NGS134	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS135	NEG	NEG	NEG	NEG	NEG	NEG	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG
NGS136	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	1846	NEG	NEG
NGS137	NEG	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS138	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	765	180	NEG	NEG	NEG	NEG
NGS139	254	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS140	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS141	NEG	NEG	NEG	NEG	37	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS142	NEG	NEG	NEG	1753	NEG	NEG	NEG	>2000	NEG	NEG	NEG	1179	NEG	NEG
NGS143	1048	NEG	308	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	NEG	NEG
NGS144	824	>2000	984	NEG	NEG	NEG	NEG	NEG	202	>2000	NEG	NEG	NEG	NEG
NGS145	39	NEG	NEG	386	NEG	NEG	NEG	NEG	NEG	NEG	NEG	968	NEG	NEG
NGS146	NEG	NEG	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS147	NEG	NEG	1593	NEG	NEG	NEG	NEG	NEG	674	355	NEG	NEG	NEG	NEG
NGS148	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	NEG	NEG

NGS149	NEG	NEG	NEG	NEG	NEG	NEG	11	>2000	NEG	NEG	NEG	NEG	NEG	NEG
NGS150	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	NEG	NEG
NGS151	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	369	613	NEG	NEG	NEG	NEG
NGS152	599	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	273	NEG	NEG
NGS153	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS154	141	>2000	NEG	NEG	NEG	NEG	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG
NGS155	263	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS156	489	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS157	NEG	NEG	NEG	>2000	NEG	NEG	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG
NGS158	67	NEG	NEG	NEG	212	NEG	NEG	NEG	NEG	NEG	NEG	Present	NEG	NEG
NGS159	59	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS160	260	NEG	259	1623	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS161	1030	NEG	NEG	NEG	NEG	NEG	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG
NGS162	NEG	NEG	200	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS163	328	>2000	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS164	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS165	NEG	NEG	1757	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS166	NEG	NEG	NEG	179	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Present	NEG	NEG
NGS167	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS168	655	NEG	NEG	NEG	NEG	NEG	32	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS169	1140	NEG	>2000	>2000	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS170	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS171	391	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS172	168	NEG	NEG	1252	NEG	NEG	NEG	>2000	NEG	NEG	NEG	NEG	NEG	NEG

NGS175	859	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS176	NEG	NEG	NEG	1214	NEG	NEG	NEG	961	NEG	NEG	NEG	NEG	NEG	NEG
NGS177	292	>2000	893	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS178	NEG	NEG	NEG	NEG	NEG	NEG	<10	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS179	390	>2000	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
NGS180	113	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Drugas	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG

8.5.4 **LOD and LOQ for each drug**

Extraction type	Drug	LLE parameters (n=6)							HF-LPME parameters (n=6)						
		LOD			LLOQ			Assay LLOQ (ng/mL)	LOD			LLOQ			Assay LLOQ (ng/mL)
		ng/mL	%CV	S/N	ng/mL	%CV	S/N		ng/mL	%CV	S/N	ng/mL	%CV	S/N	
Acidic	BEN	10	17%	27	10	17%	27	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	FUR	50	10%	17	50	10%	17	50	50	7%	15	50	7%	15	50
	LOS	10	19%	22	10	14%	22	10	1	10%	12	1	10%	12	10
	LOSm	10	17%	53	10	17%	53	10	1	15%	10	1	15%	10	10
	RAM	1	7%	12	10	4%	128	10	1	6%	12	1	6%	12	10
	RAMm	10	19%	16	10	19%	16	10	10	15%	20	10	15%	20	10
Basic	AML	1	19%	7	10	14%	43	10	10	5%	35	10	5%	35	10
	ATE	10	6%	13	10	6%	13	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	BIS	0.1	19%	8	1	5%	65	10	1	14%	111	1	14%	111	10
	CAN	10	2%	66	10	2%	66	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	DOX	0.1	12%	7	1	7%	50	10	0.01	11%	14	0.01	11%	14	10
	NOR	0.1	11%	12	1	7%	112	10	0.01	5%	16	0.01	5%	16	10
	SPI	10	6%	27	10	6%	27	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	VER	0.1	17%	8	1	8%	71	10	0.01	16%	14	0.1	8%	14	10

AML=amlodipine; ATE=atenolol; BEN=bendroflumethiazide; BIS=bisoprolol; CAN=canrenone; DOX=doxazosin; FUR=furosemide; LOS=losartan; LOSm=losartan-COOH; NOR=norverapamil; RAM=ramipril; RAMm=ramiprilat; SPI=spironolactone; VER=verapamil; LLE=liquid-liquid extraction; HF-LPME=hollow-fibre liquid-phase microextraction; LOD=limit of detection; LLOQ=lower limit of quantification; S/N=signal-to-noise ratio; n.a.=not assessed

Figure 8-2 LOD and LOQ - antihypertensive drugs and their metabolites in urine. Reproduced from Glasgow toxicology.

8.6 Comparing across 3 methods

8.6.1 Amlodipine across 3 methods

Amlodipine was completely detected by Polyomics sensitivity (100%) and highly detected by Glasgow toxicology (94%) while Birmingham was far lower around (56%) (Table 8-7 and Figure 8-3).

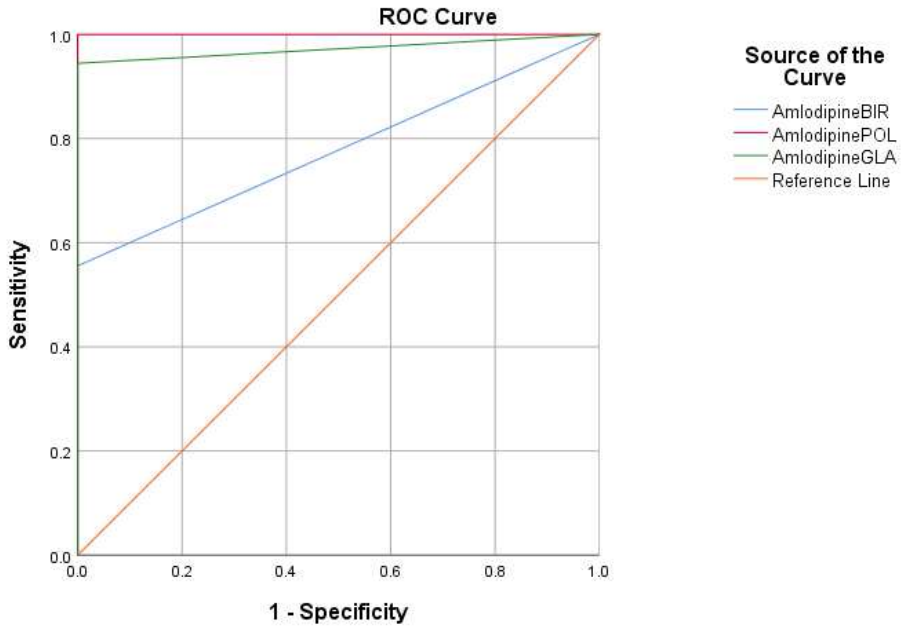

Figure 8-3 Amlodipine ROC curve for the 3 analysis. Glasgow Polyomic detected all patient prescribed amlodipine. Glasgow toxicology detected most of the patient. Birmingham was the lowest at detecting Amlodipine

Table 8-7 Amlodipine summary (prescription rate), sensitivity, specificity and area under curve AUC

Amlodipine	N=57	%	
Prescribed	18	31.6	
Not prescribed	39	68.4	
	Sensitivity	1 - Specificity	AUC
BIR	0.556	0	0.778
POL	1	0	1
GLA	0.944	0	0.972

8.6.2 **Atenolol across 3 methods**

Amongst the 7 people prescribed atenolol, Birmingham again had the lowest sensitivity (57%) compared with only 1 patient in whom it was not detected for the other two analyses. Glasgow Polyomics identified 1 patient that wasn't prescribed atenolol. When we further checked that patient, he was prescribed metoprolol Table 8-8 and Figure 8-4)

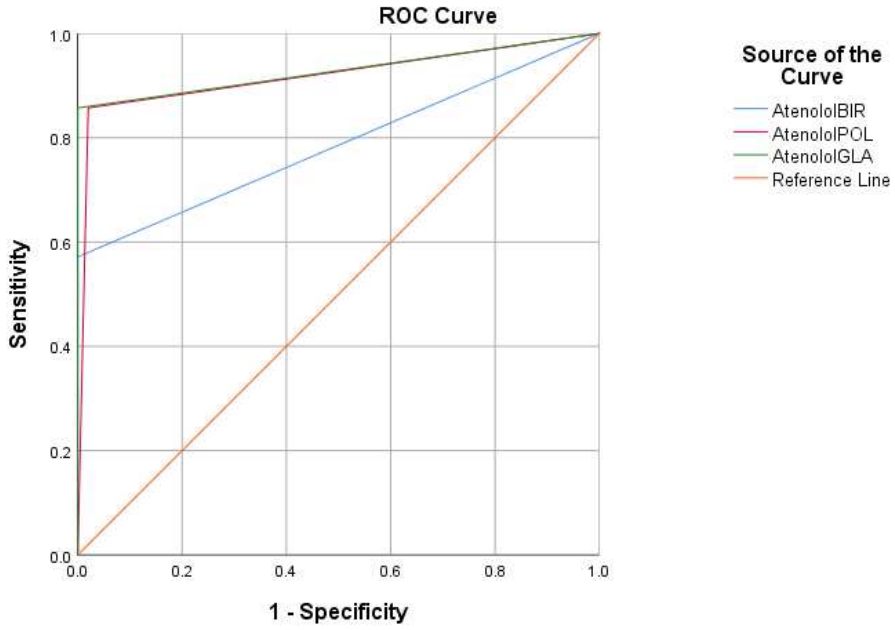

Figure 8-4 Atenolol ROC curve for the 3 analysis. Glasgow toxicology was the highest followed by Polyomics. Birmingham had the lowest detection

Table 8-8 Atenolol summary (prescription rate), sensitivity, specificity and AUC

Atenolol	N=57	%	
Prescribed	7	12.3	
Not prescribed	50	87.7	
	Sensitivity	1 - Specificity	AUC
BIR	0.571	0.00	0.786
POL	0.857	0.02	0.919
GLA	0.857	0.00	0.929

8.6.3 **Losartan across 3 methods**

All the prescribed patients were detected in all methods (Table 8-9 and Figure 8-5).

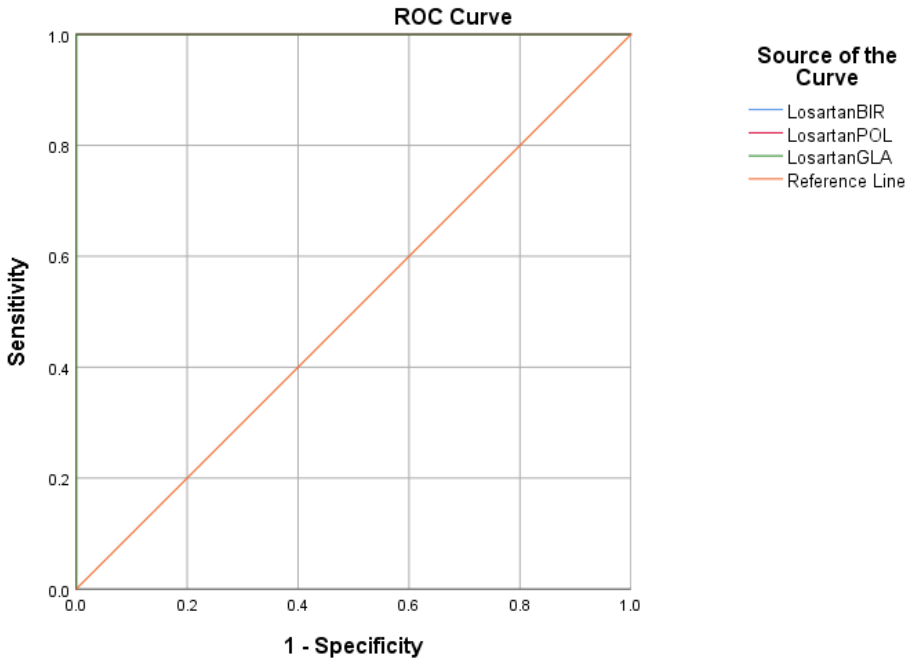

Figure 8-5 Losartan ROC curve for the 3 analysis. All methods detected all patient prescribed with losartan.

Table 8-9 Losartan summary (prescription rate), sensitivity, specificity and AUC

Losartan	N=57	%	
Prescribed	11	19.3	
Not prescribed	46	80.7	
	Sensitivity	1 - Specificity	AUC
BIR	1.0	0.0	1.0
POL	1.0	0.0	1.0
GLA	1.0	0.0	1.0

8.6.4 **Ramipril across 3 methods**

Birmingham was the highest to detect Ramipril (90%), while Glasgow Polyomics and Glasgow forensic were same at 80% (Table 8-10 and Figure 8-6).

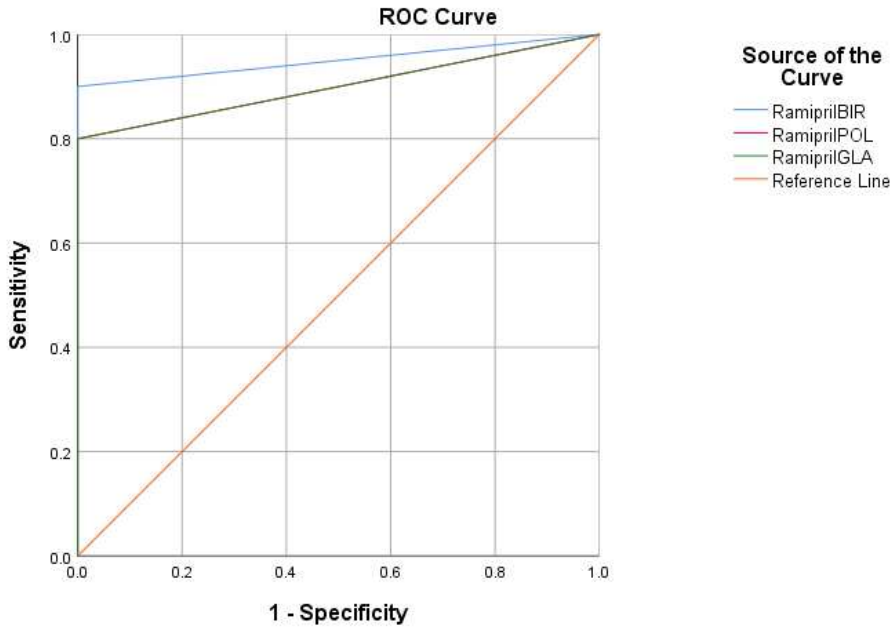

Figure 8-6 Ramipril ROC curve for the 3 analysis. Ramipril was the highest at detecting patient prescribed with Ramipril. Polyomics and Glasgow toxicology detected the same number.

Table 8-10 Ramipril summary (prescription rate), sensitivity, specificity and AUC

Ramipril	N=57	%	
Prescribed	10	17.5	
Not prescribed	47	82.5	
	Sensitivity	1 - Specificity	AUC
BIR	0.9	0	0.950
POL	0.8	0.0	0.900
GLA	0.8	0.0	0.900

8.7 BIR vs GLA

8.7.1 Bendroflumethiazide

8.7.1.1 SBP and DBP score across the test groups

Figure 8-7 and Figure 8-8 illustrate the SBP and DBP between the 2 groups. The 2 patients that couldn't be detected by either method had normal BP.

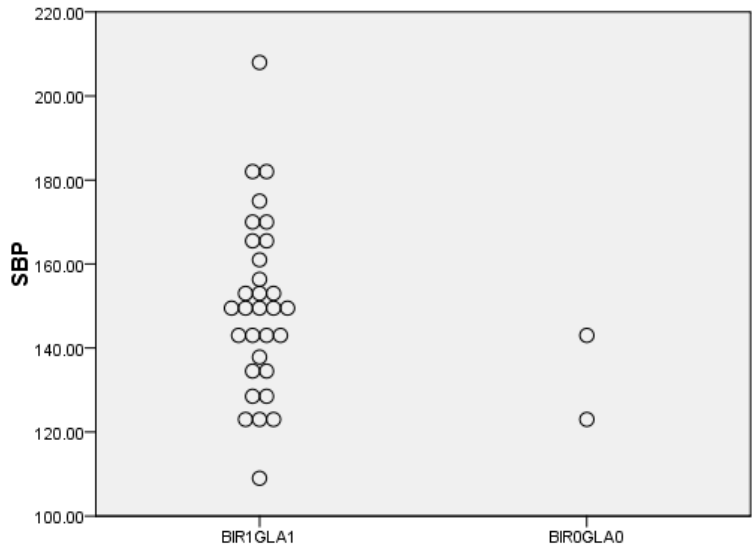

Figure 8-7 The SBP for 33 patients prescribed with Bendroflumethiazide. The plot compares the SBP of the patients were detected by both Birmingham and Glasgow toxicology (n=31) with the patients that neither method could detect (n=2).

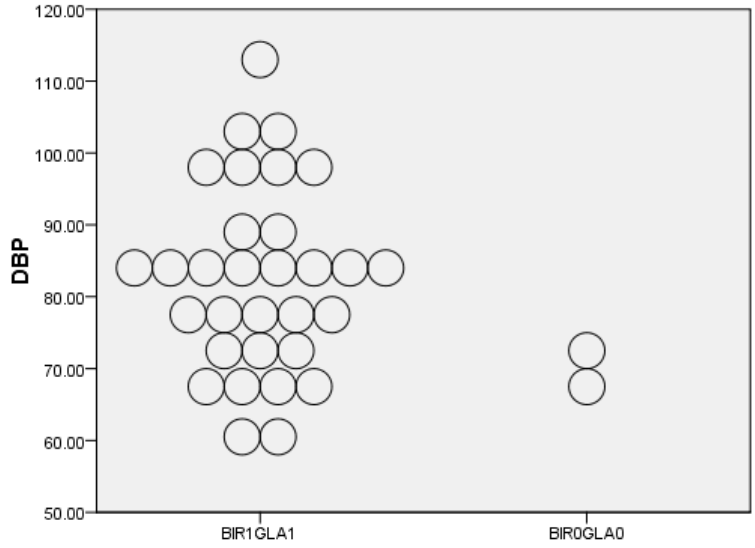

Figure 8-8 The DBP for 33 patients prescribed with Bendroflumethiazide. The plot compares the DBP of the patients were detected by both Birmingham and Glasgow toxicology (n=31) with the patients that neither method could detect (n=2).

8.7.1.2 SBP and DBP score across the test groups

Figure 8-9 and Figure 8-10 illustrate the SBP and DBP between the 2 groups. The 2 patients that couldn't be detected by either method had normal BP.

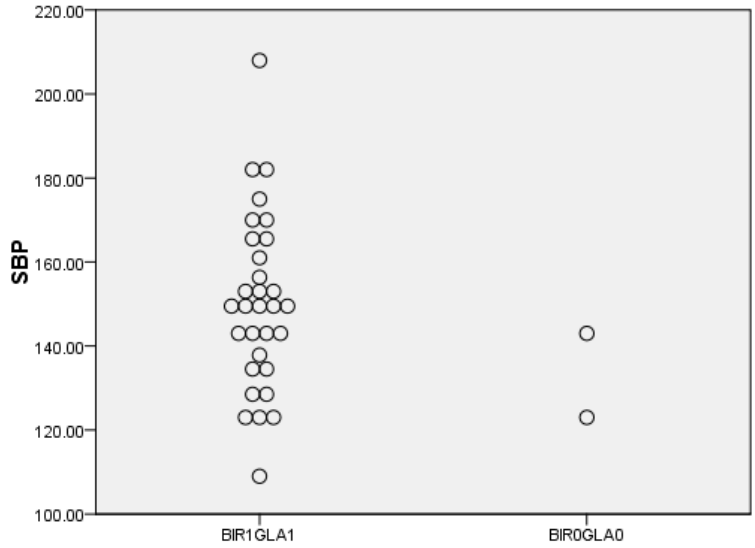

Figure 8-9 The SBP for 33 patients prescribed with Bendroflumethiazide. The plot compares the SBP of the patients were detected by both Birmingham and Glasgow toxicology (n=31) with the patients that neither method could detect (n=2).

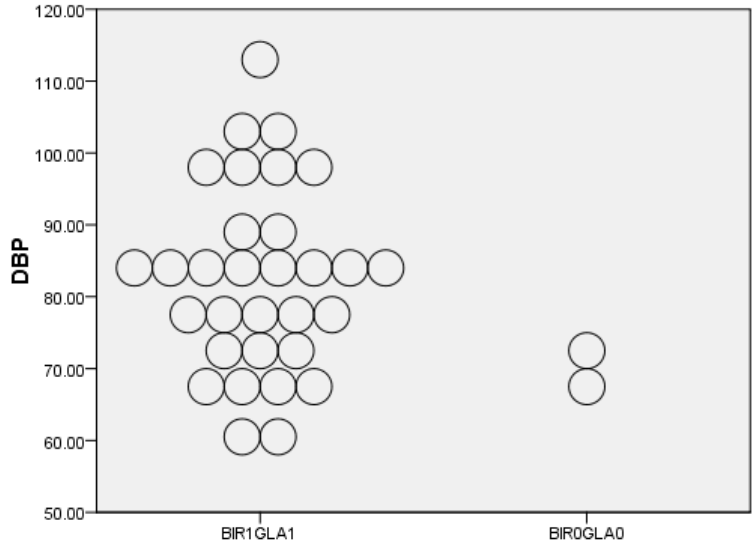

Figure 8-10 The DBP for 33 patients prescribed with Bendroflumethiazide. The plot compares the DBP of the patients were detected by both Birmingham and Glasgow toxicology (n=31) with the patients that neither method could detect (n=2).

8.7.2 **Amlodipine**

8.7.2.1 SBP and DBP score across the test groups

In Figure 8-11 the group that were detected by both methods mainly between 140 to 160 mmHg. On the other hand, patients only detected by Glasgow toxicology had SBP around 140 mmHg with few patients more than 160 mmHg. The 2 patients that couldn't be detected by either method had normal BP.

Figure 8-12 demonstrate patients that were detected by both methods had DBP around 80 mmHg. While those detected only by Glasgow toxicology had DBP ranging from 60 to 120 mmHg. Patients not detected by either method had normal DBP.

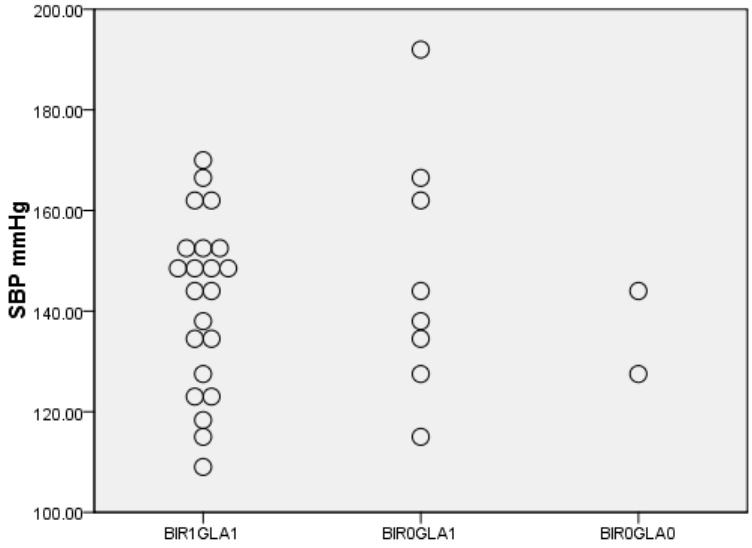

Figure 8-11 The SBP for 32 patients prescribed with amlodipine. The plot compares the SBP of the patients were detected by both Birmingham and Glasgow toxicology (n=22) with the patients that only Glasgow Toxicology detected (n=8) and patients not detected by either method (n=2).

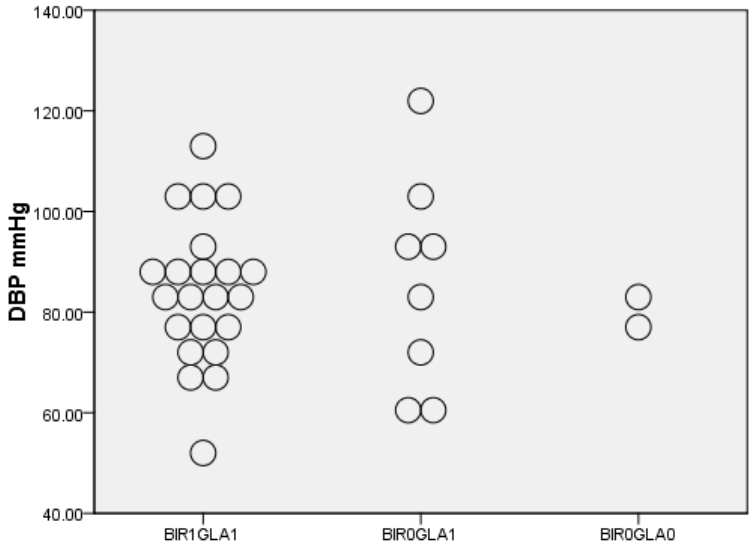

Figure 8-12 The DBP for 32 patients prescribed with amlodipine. The plot compares the DBP of the patients were detected by both Birmingham and Glasgow toxicology (n=22) with the patients that only Glasgow Toxicology detected (n=8) and patients not detected by either method (n=2).

8.7.3 **Ramipril**

8.7.3.1 SBP and DBP score across the test groups

Patients that were not detected by both methods had lower SBP and DBP compared to the patients detected (Figure 8-13 and Figure 8-14).

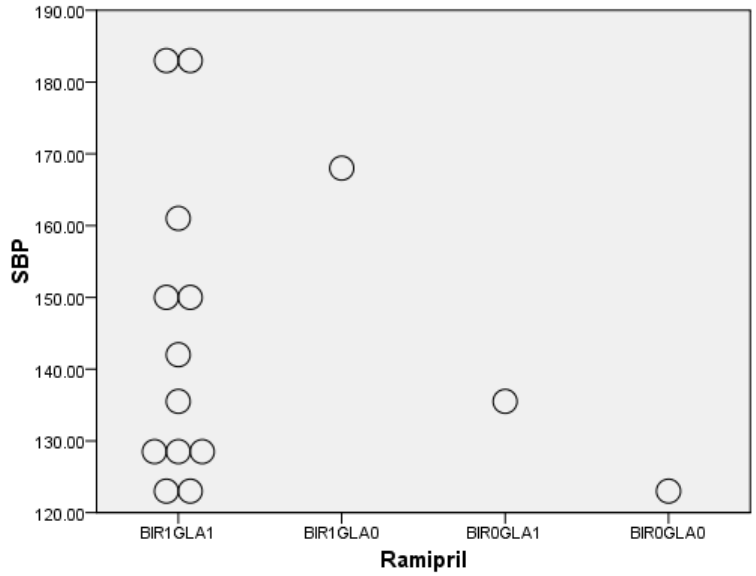

Figure 8-13 The SBP for 15 patients prescribed with Ramipril. The plot compares the SBP levels for patients detected by both Birmingham and Glasgow toxicology (n=12) with patient detected only by Birmingham (n=1) and patients only detected by Glasgow toxicology (n=1) and patients that neither method could detect (n=1).

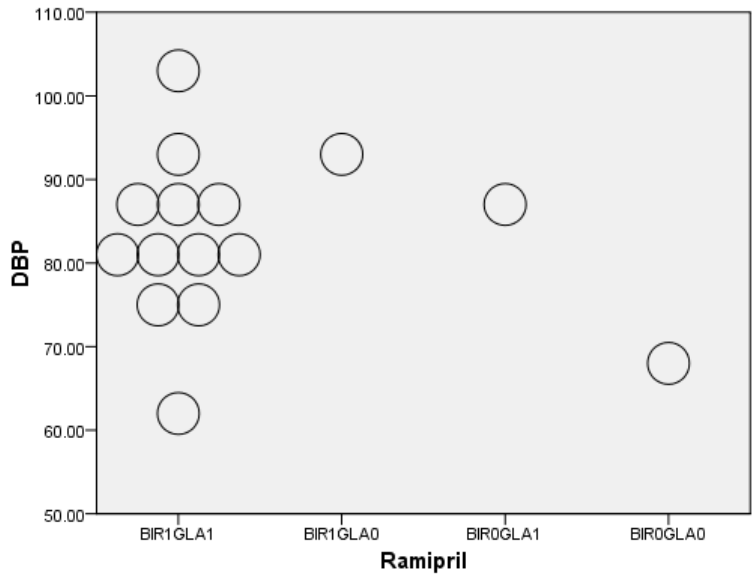

Figure 8-14 The DBP for 15 patients prescribed with Ramipril. The plot compares the DBP levels for patients detected by both Birmingham and Glasgow toxicology (n=12) with patient detected only by Birmingham (n=1) and patients only detected by Glasgow toxicology (n=1)

8.7.4 **Losartan**

8.7.4.1 SBP and DBP score across the test groups

Patients that were detected by both methods had SBP ranging from 120 to 160 mmHg and DBP ranging from 70 to 100 mmHg (Figure 8-15 and Figure 8-16).

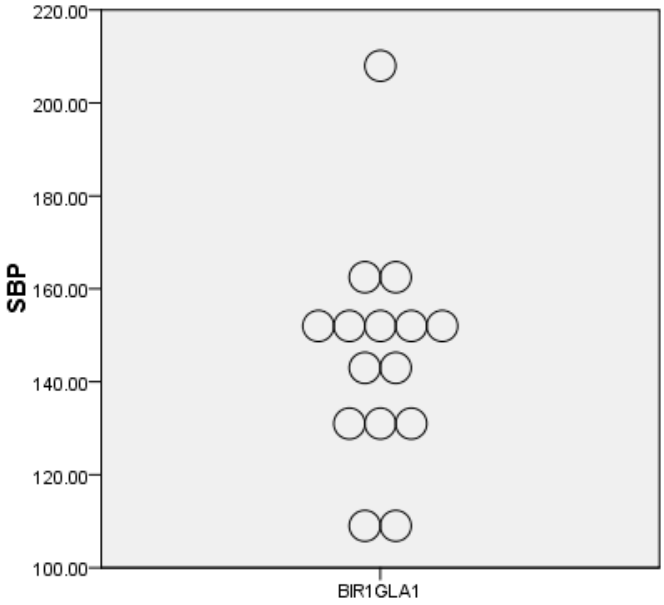

Figure 8-15 The SBP for 15 patients prescribed with Losartan. The plot demonstrates the score of the patients were detected by both Birmingham and Glasgow toxicology (n=15)

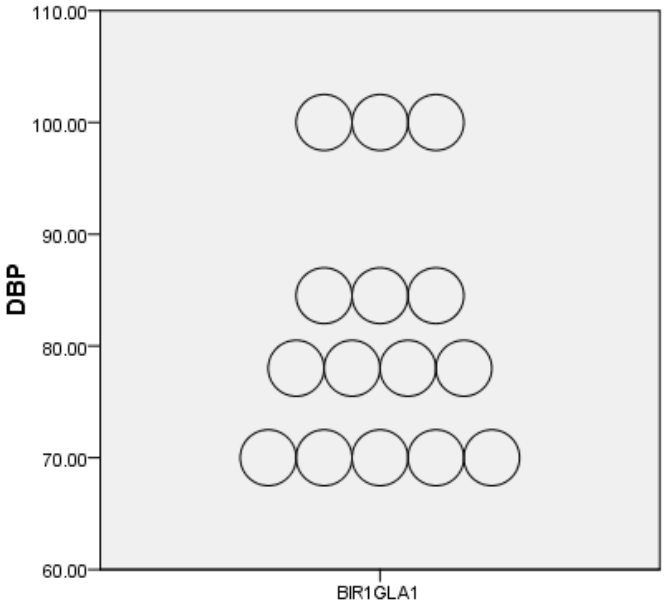

Figure 8-16 The DBP for 15 patients prescribed with Losartan. The plot demonstrates the score of the patients were detected by both Birmingham and Glasgow toxicology (n=15)

8.7.5 **Doxazosin**

8.7.5.1 SBP and DBP score across the test groups

The patient that both methods couldn't detect had normal SBP and DBP compared to those detected by both methods (Figure 8-17and Figure 8-18).

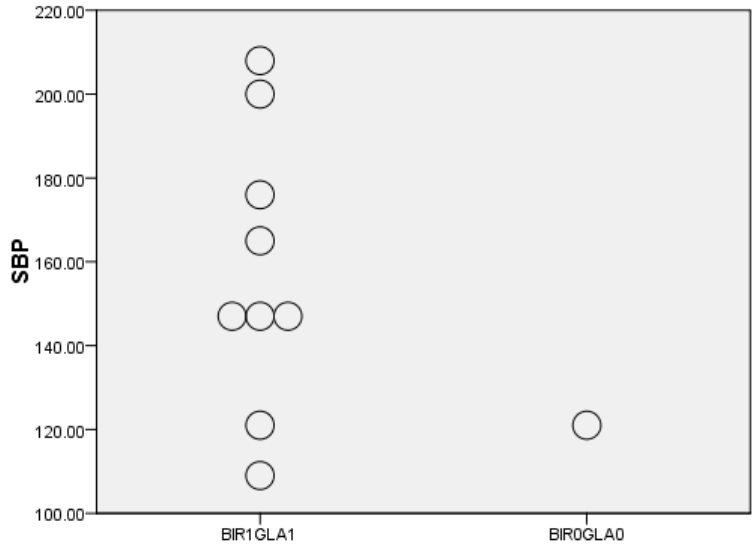

Figure 8-17 The SBP for 11 patients prescribed with Doxazosin. The plot compares the SBP of the patients were detected by both Birmingham and Glasgow toxicology (n=10) with the patient that neither method could detect (n=1).

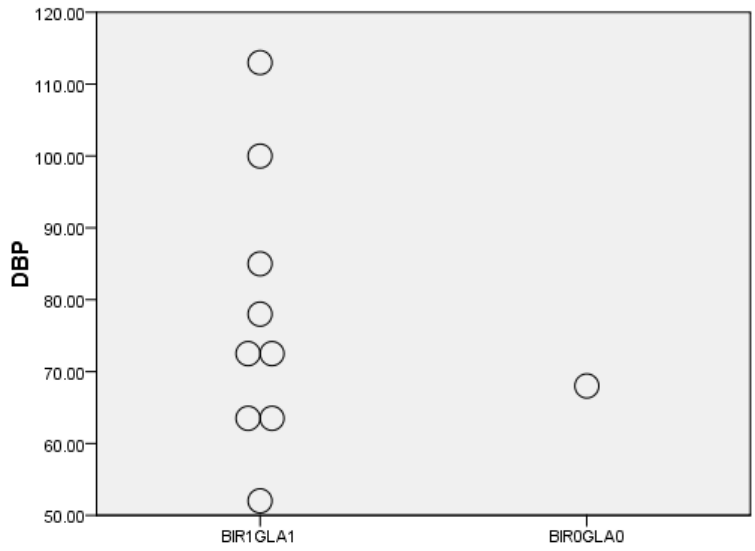

Figure 8-18 The DBP for 11 patients prescribed with Doxazosin. The plot compares the DBP of the patients were detected by both Birmingham and Glasgow toxicology (n=10) with the patient that neither method could detect (n=1).

8.7.6 **Bisoprolol**

8.7.6.1 SBP and DBP score across the test groups

Figure 8-19 and Figure 8-20 shows that the patient that couldn't be detected by both methods had a high SBP and DBP compared to the rest of patients.

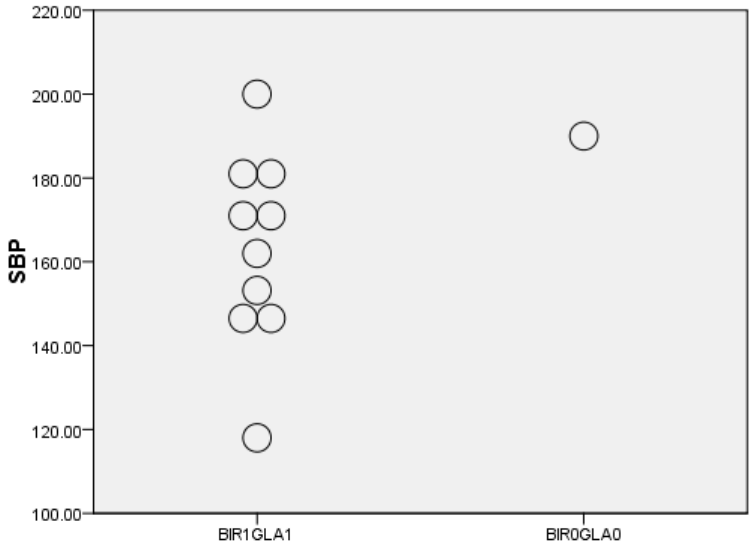

Figure 8-19 The SBP for 11 patients prescribed with Bisoprolol. The plot compares the SBP of the patients were detected by both Birmingham and Glasgow toxicology (n=10) with the patients that neither method could detect (n=1).

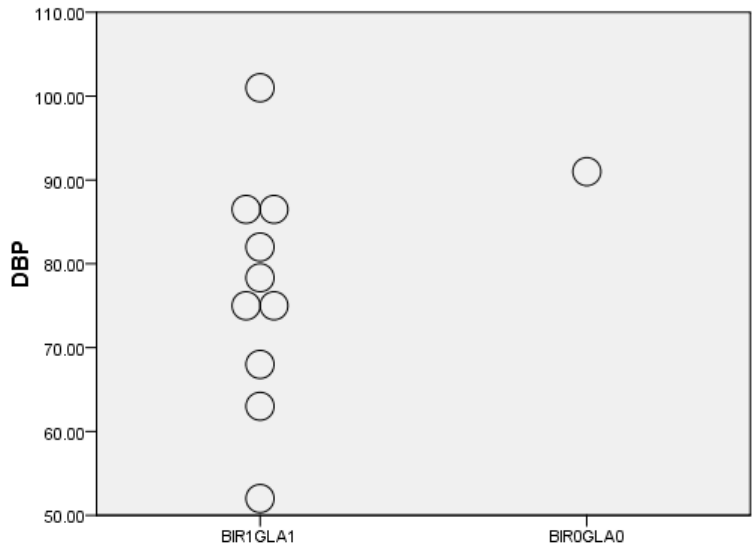

Figure 8-20 The DBP for 11 patients prescribed with Bisoprolol. The plot compares the DBP of the patients were detected by both Birmingham and Glasgow toxicology (n=10) with the patients that neither method could detect (n=1).

8.7.7 **Atenolol**

8.7.7.1 SBP and DBP score across the test groups

The SBP and DBP of patients that weren't detected by at least one method was lower than those detected by both methods (Figure 8-21and Figure 8-22).

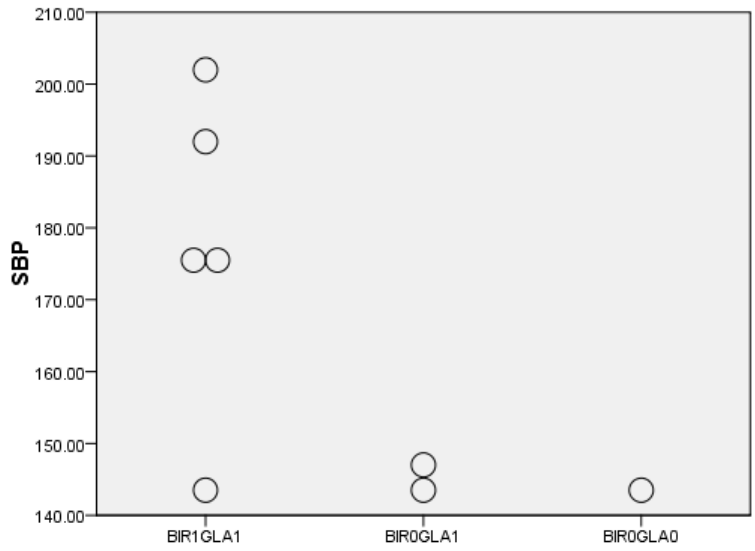

Figure 8-21 The SBP score for 8 patients prescribed with Atenolol. The plot compares the SBP of the patients were detected by both Birmingham and Glasgow toxicology (n=5) with the patients that only Glasgow Toxicology detected (n=2). 1 patient wasn't detected by either method.

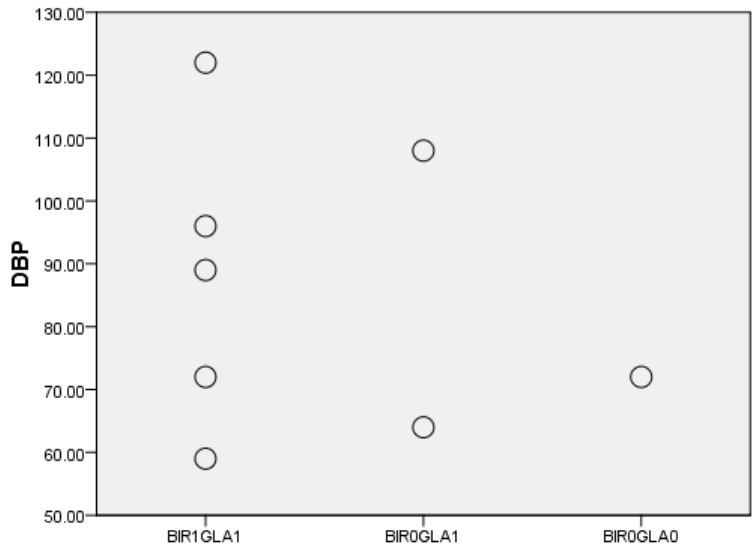

Figure 8-22 The DBP score for 8 patients prescribed with Atenolol. The plot compares the DBP of the patients were detected by both Birmingham and Glasgow toxicology (n=5) with the patients that only Glasgow Toxicology detected (n=2). 1 patient wasn't detected

8.7.8 **Furosemide**

8.7.8.1 SBP and DBP score across the test groups

The 2 patients that couldn't be detected by either method had higher BP (SBP more than 180 mmHg) compared to non-prescribed (Figure 8-23and Figure 8-24)

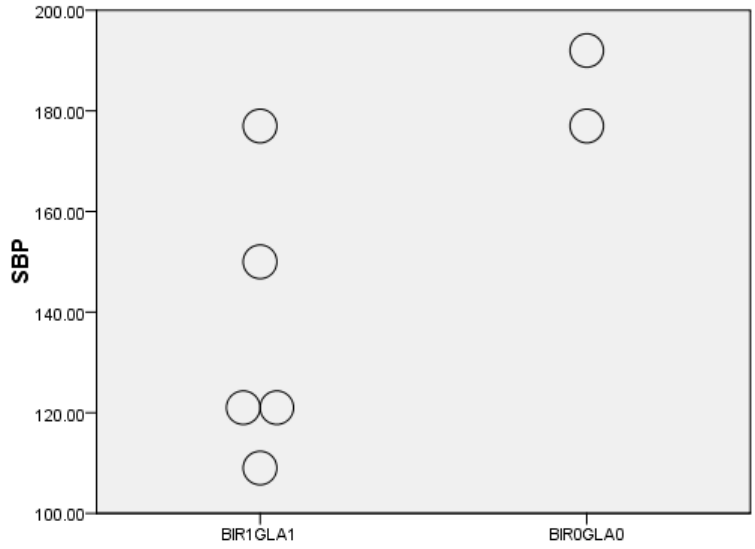

Figure 8-23 The SBP for 7 patients prescribed with Furosemide. The plot compares the SBP for the patients detected by both Birmingham and Glasgow toxicology (n=5) with the patients that neither method could detect (n=2).

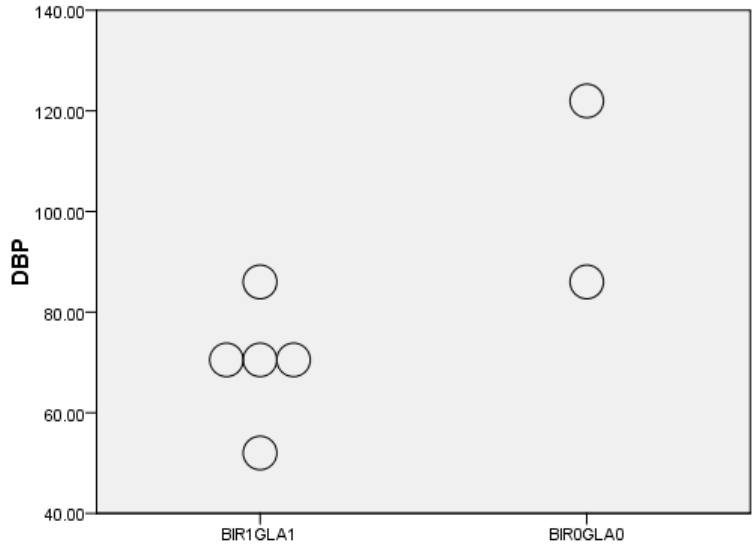

Figure 8-24 The DBP for 7 patients prescribed with Furosemide. The plot compares the DBP for the patients detected by both Birmingham and Glasgow toxicology (n=5) with the patients that neither method could detect (n=2).

8.7.9 **Spironolactone**

8.7.9.1 SBP and DBP across the test groups

Figure 8-25 and Figure 8-26 due to small number of patients no clear relationship could be identified.

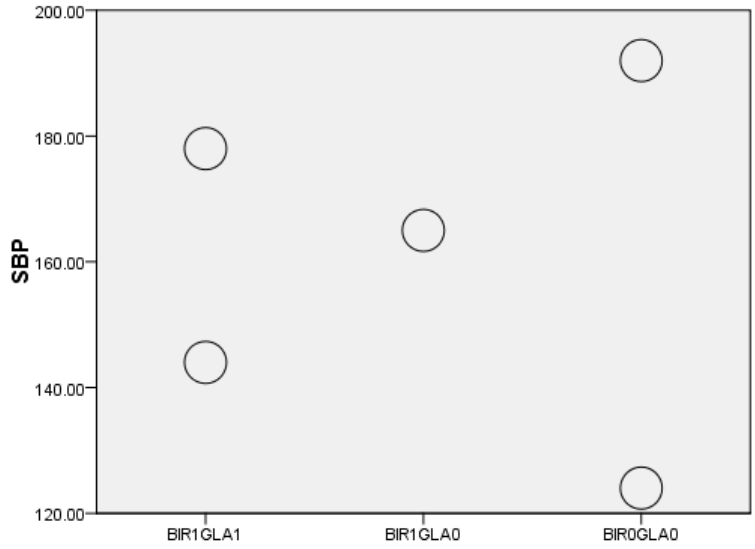

Figure 8-25 The SBP 6 patients prescribed with Spironolactone. The plot compares the SBP of the patients were detected by both Birmingham and Glasgow toxicology (n=2) with patients that were detected by Birmingham and not Glasgow toxicology (n=1) and patients that couldn't be detected by either method.

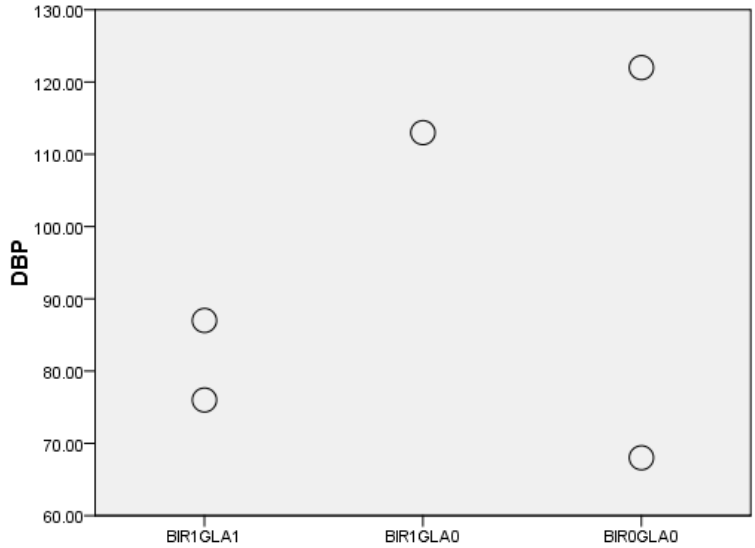

Figure 8-26 The DBP 6 patients prescribed with Spironolactone. The plot compares the DBP of the patients were detected by both Birmingham and Glasgow toxicology (n=2) with patients that were detected by Birmingham and not Glasgow toxicology (n=1) and patients that couldn't be detected by either method.

8.8 BIR vs POL

8.8.1 Candesartan

8.8.1.1 SBP and DBP score across the test groups

No clear difference for SBP and DBP due to small number of patients (Figure 8-27 and Figure 8-28).

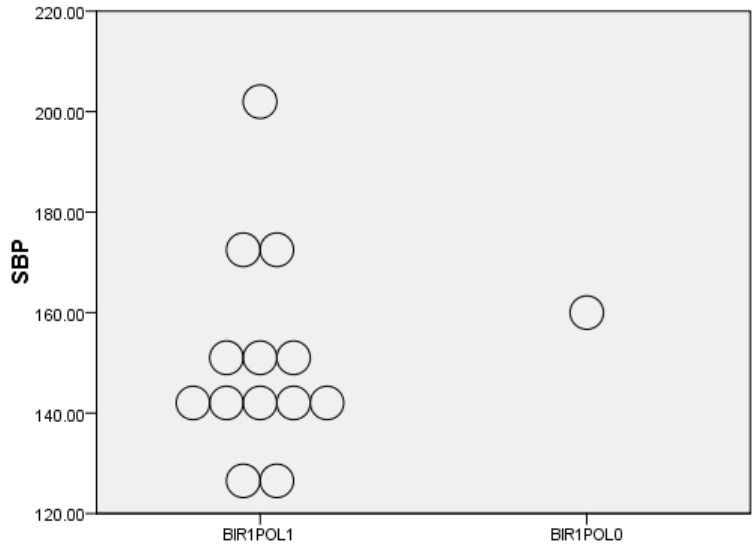

Figure 8-27 The SBP for 14 patients prescribed with Candesartan. The plot compares the SBP of the patients that were detected by both Birmingham and Glasgow Polyomics (n=13) with the patient that only Birmingham detected (n=1).

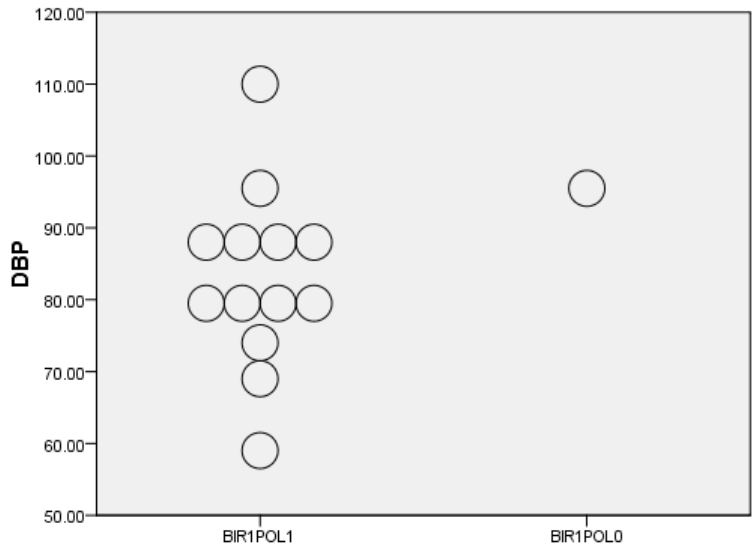

Figure 8-28 The DBP for 14 patients prescribed with Candesartan. The plot compares the DBP of the patients that were detected by both Birmingham and Glasgow Polyomics (n=13) with the patient that only Birmingham detected (n=1).

8.8.2 **Perindopril**

8.8.2.1 SBP and DBP score across the test groups

One patient had normal BP while the other had High BP. Due to small number no clear relation could be identified (Figure 8-29 and Figure 8-30).

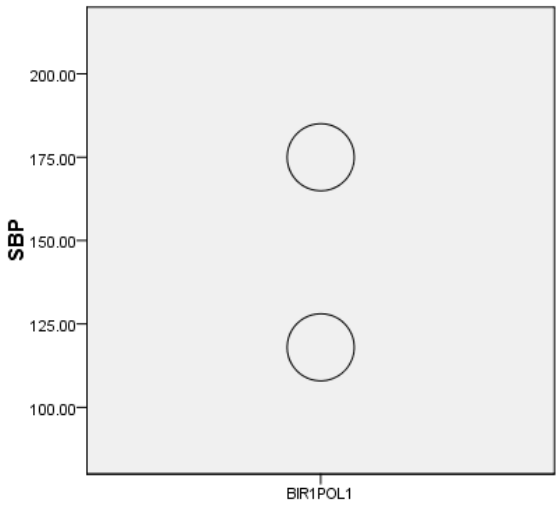

Figure 8-29 The SBP for 2 patients prescribed with Perindopril. The plot shows SBP for the patients that were detected by both Birmingham and Glasgow Polyomics (n=2).

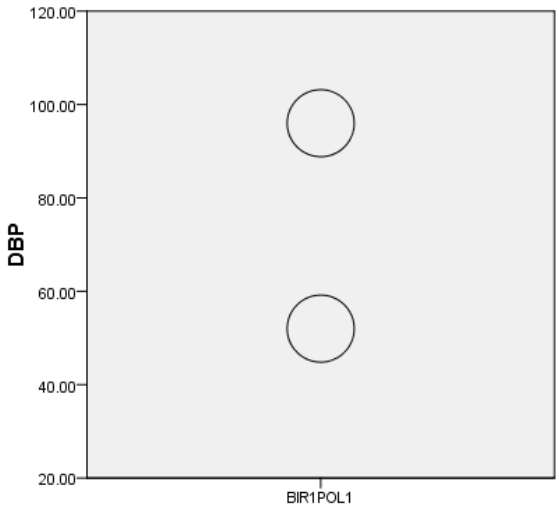

Figure 8-30 The DBP for 2 patients prescribed with Perindopril. The plot shows DBP for the patients that were detected by both Birmingham and Glasgow Polyomics (n=2)).

8.8.3 **Enalapril**

8.8.3.1 SBP and DBP score across the test groups

Due to small number, no clear relation could be identified for SBP and DBP.

Figure 8-31 The SBP for 2 patients prescribed with Enalapril. The plot shows the SBP of the patients that were detected by both Birmingham and Glasgow Polyomics (n=2).

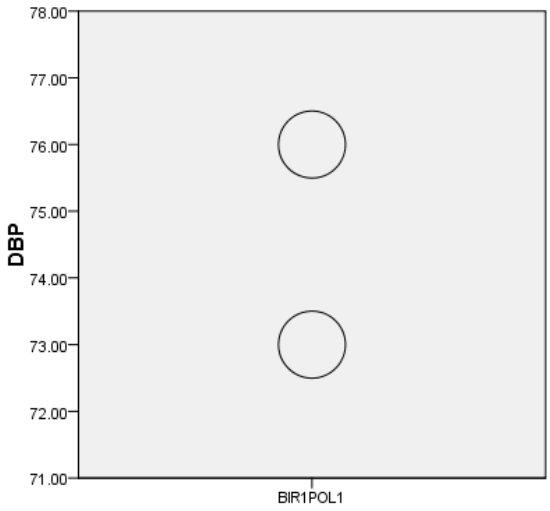

Figure 8-32 The DBP for 2 patients prescribed with Enalapril. The plot shows the DBP of the patients that were detected by both Birmingham and Glasgow Polyomics (n=2).

8.8.4 **Diltiazem**

8.8.4.1 SBP and DBP score across the test groups

SBP and DBP for patient prescribed Diltiazem, no clear relationship (Figure 8-33 and Figure 8-34)

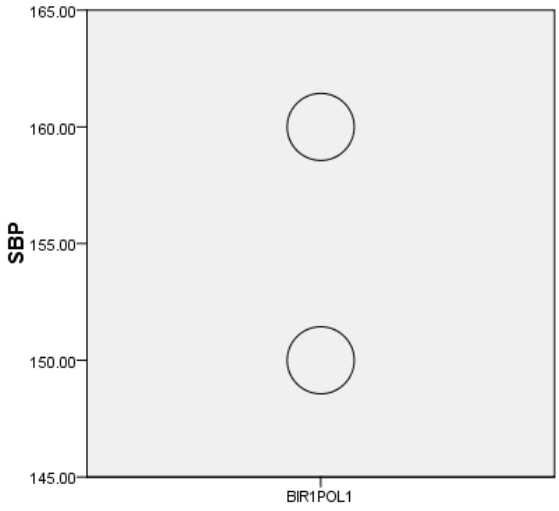

Figure 8-33 The SBP for 2 patients prescribed with Diltiazem. The plot shows the SBP of the patients that were detected by both Birmingham and Glasgow Polyomics (n=2)).

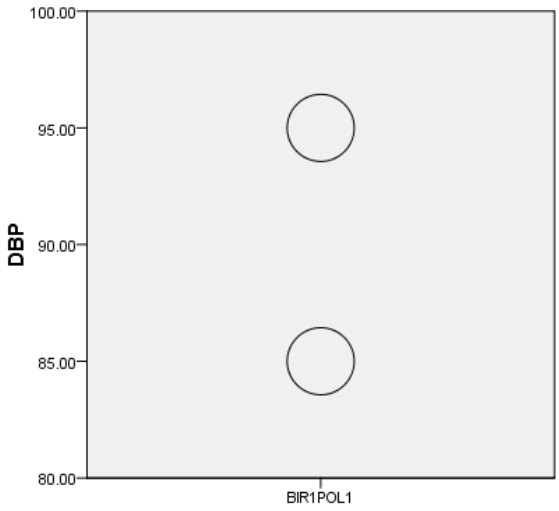

Figure 8-34 The DBP for 2 patients prescribed with Diltiazem. The plot shows the DBP of the patients that were detected by both Birmingham and Glasgow Polyomics (n=2)).

8.8.5 **Irbesartan**

8.8.5.1 SBP and DBP score across the test groups

SBP and DBP appears to be controlled for the patient with Irbesartan (Figure 8-35 and Figure 8-36)

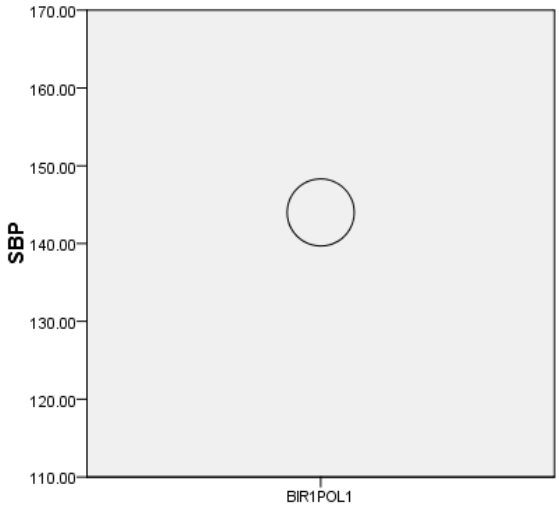

Figure 8-35 The SBP for a patient prescribed with Irbesartan. The plot shows the SBP for the patient that was detected by both Birmingham and Glasgow Polyomics (n=1).

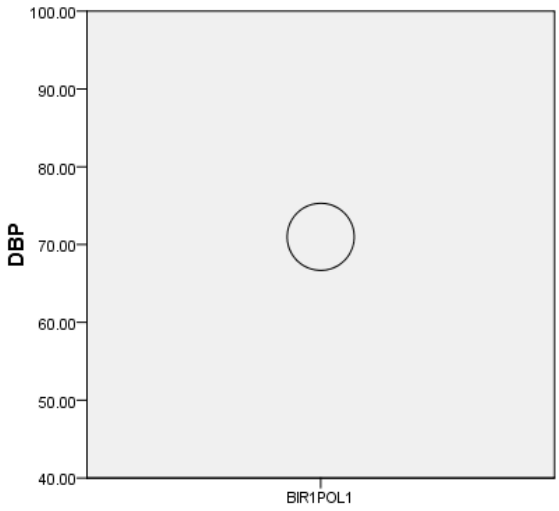

Figure 8-36 The DBP for a patient prescribed with Irbesartan. The plot shows the DBP for the patient that was detected by both Birmingham and Glasgow Polyomics (n=1).

8.8.6 **GLA concentration graph**

Bendroflumethiazide

8.8.6.1 GLA level for Bendroflumethiazide

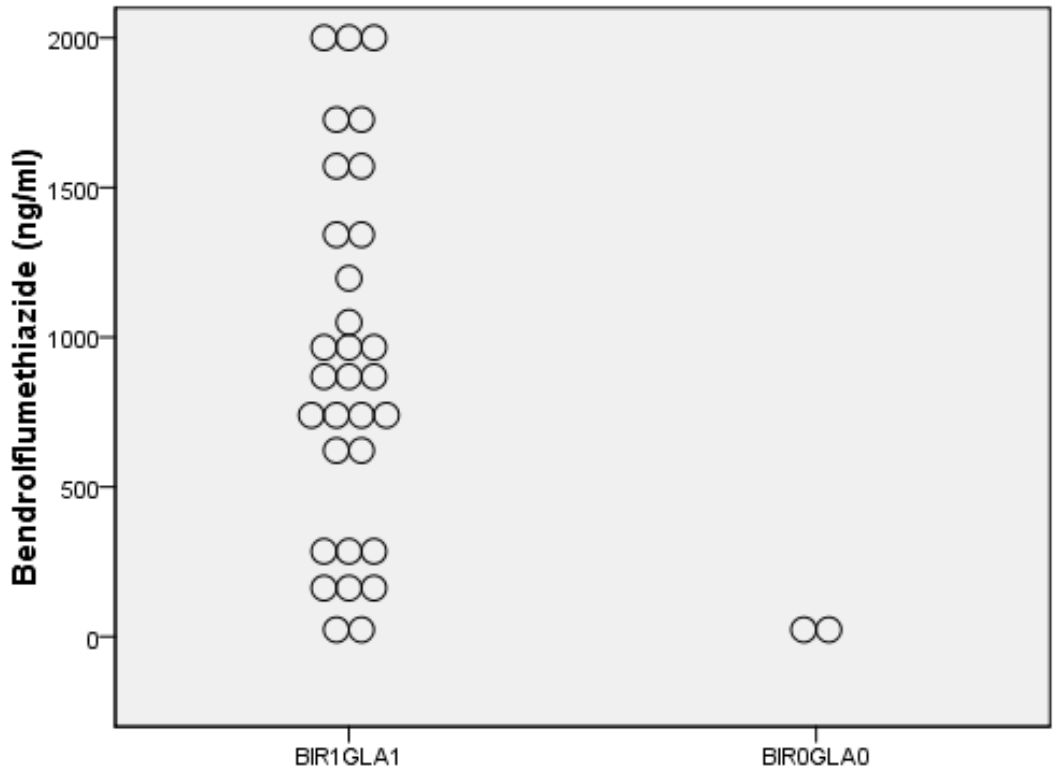

Figure 8-37 The Bendroflumethiazide levels of the 33 patients prescribed with Bendroflumethiazide detected by Glasgow Toxicology laboratory.

8.8.6.2 GLA levels for Doxazosin

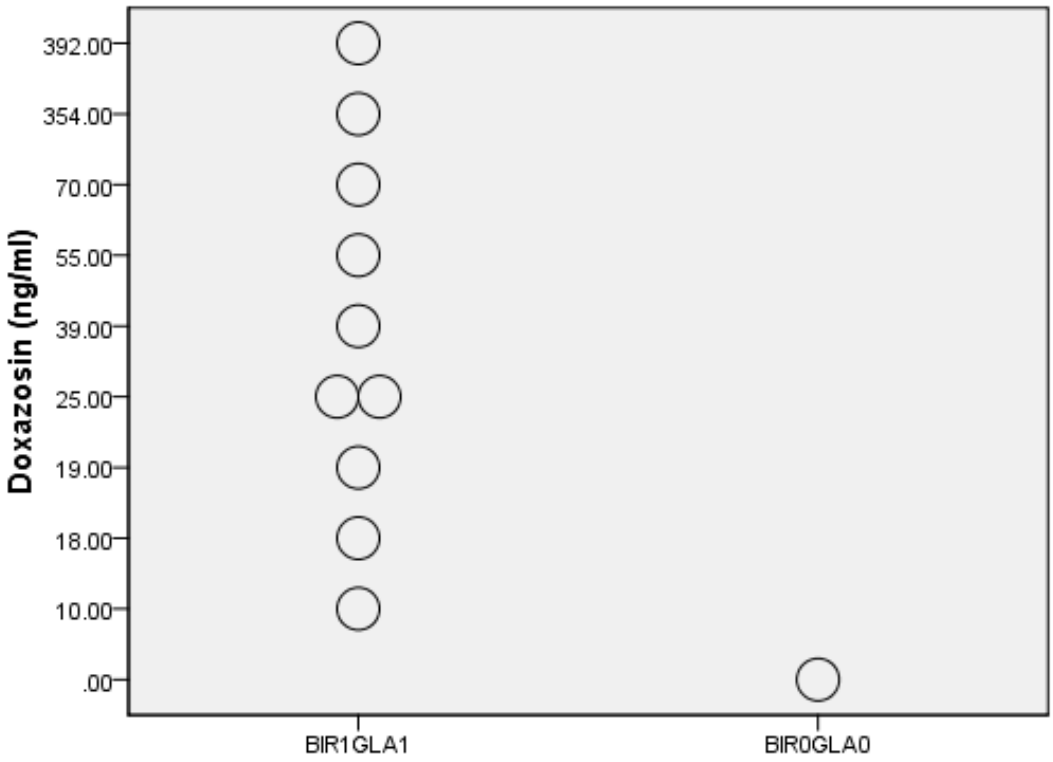

Figure 8-38 Plot demonstrate the concentration of doxazosin detected by Glasgow toxicology.

8.8.7 **Testing for normality**

The following figures demonstrate the normality plots for the significant variables in order to assess the normal distribution.

TB, Ur creatinine and aldosterone were not normally distributed. However, after applying logarithm, they appeared as normally distributed.

8.8.7.1 TB

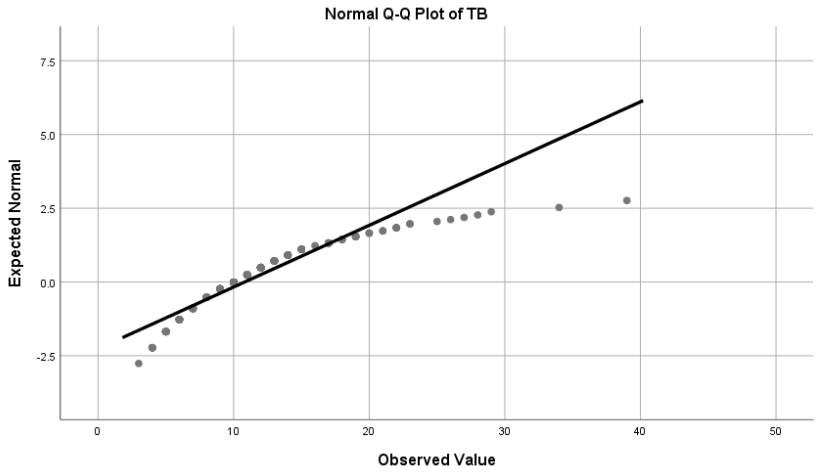

Figure 8-39 Normal probability plot for TB. Data is not normally distributed.

8.8.7.2 Log TB

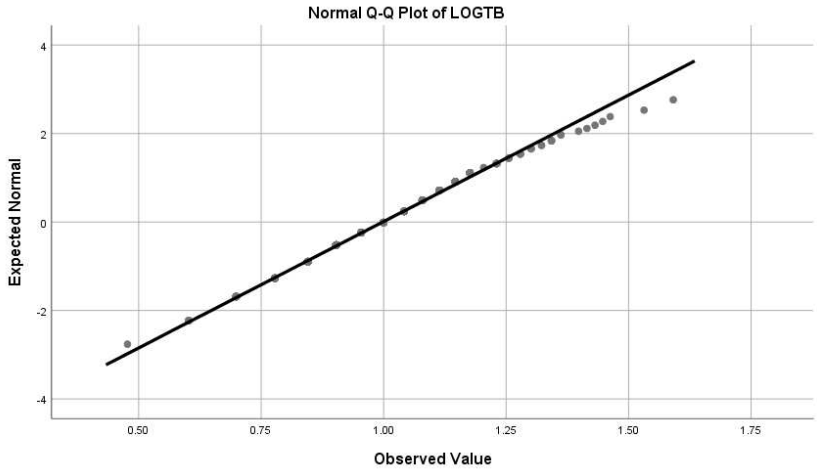

Figure 8-40 Normal probability plot for Log TB. The data is normally distributed after logging TB

8.8.7.3 Urine creatinine

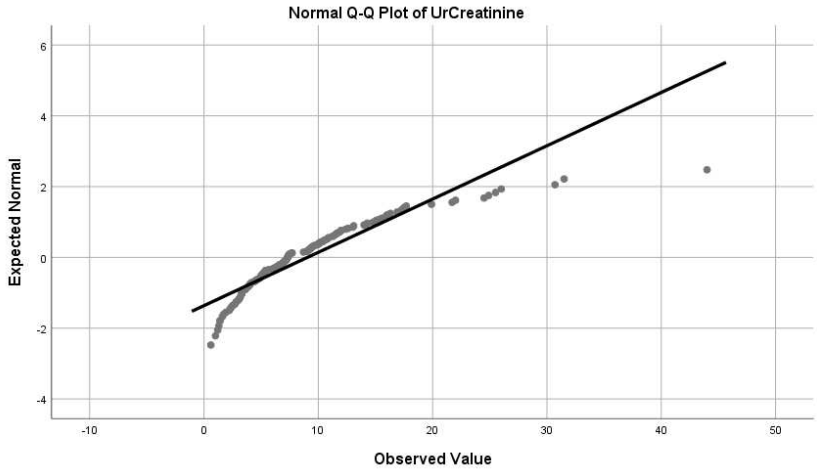

Figure 8-41 Normal probability plot for Urine creatinine. Data is not normally distributed, few outliers with high values

8.8.7.4 Log Ur Creatinine

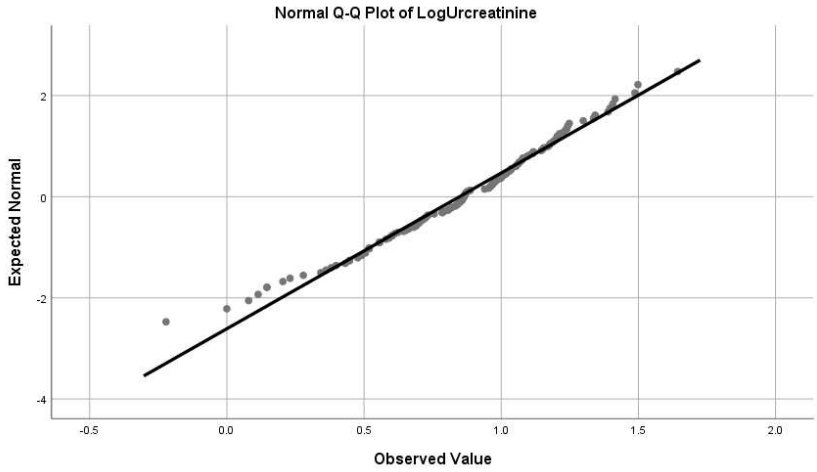

Figure 8-42 Normal probability plot for Log Urine creatinine. Data now is normally distributed

8.8.7.5 Aldosterone

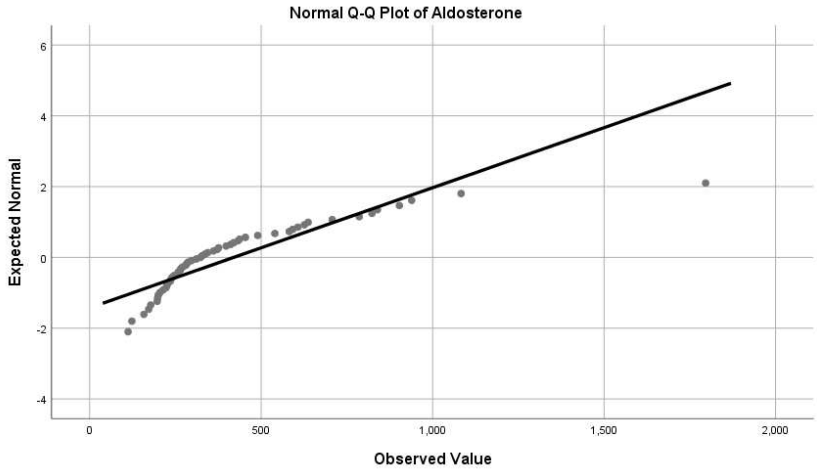

Figure 8-43 Normal probability plot for Aldosterone. Data is not normally distributed, one outlier with high reading

8.8.7.6 Log Aldosterone

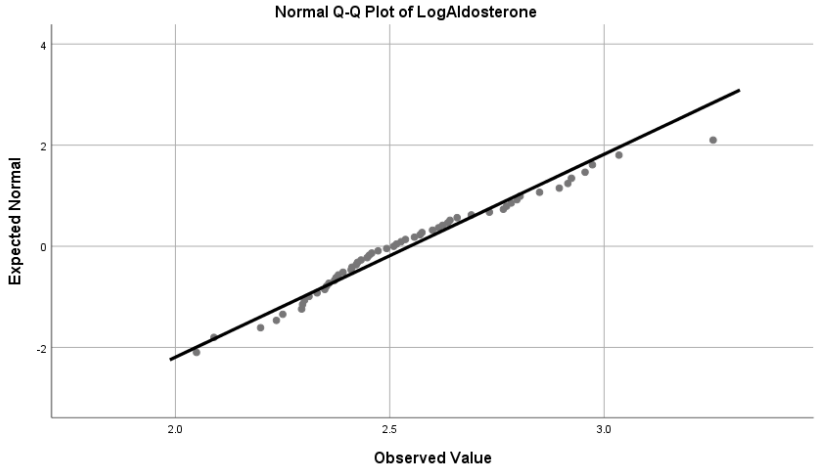

Figure 8-44 16 Normal probability plot for Log Aldosterone. Data is normally distributed

8.8.7.7 HB

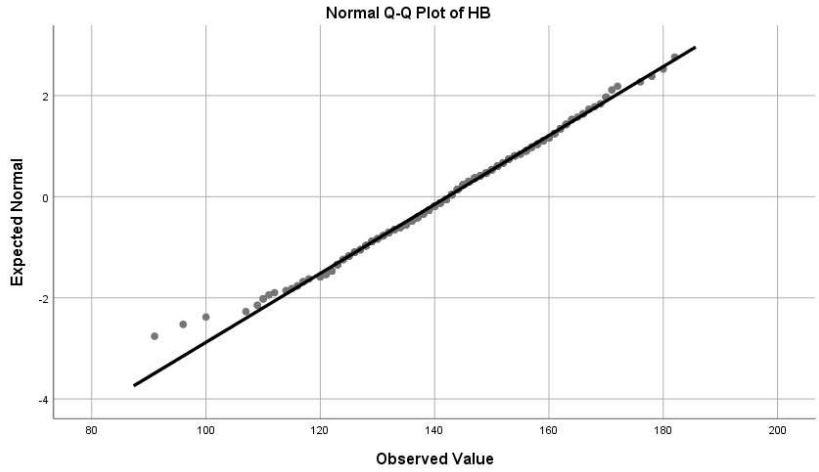

Figure 8-45 Normal probability plot for HB. Data is normally distributed,

8.8.7.8 Albumin

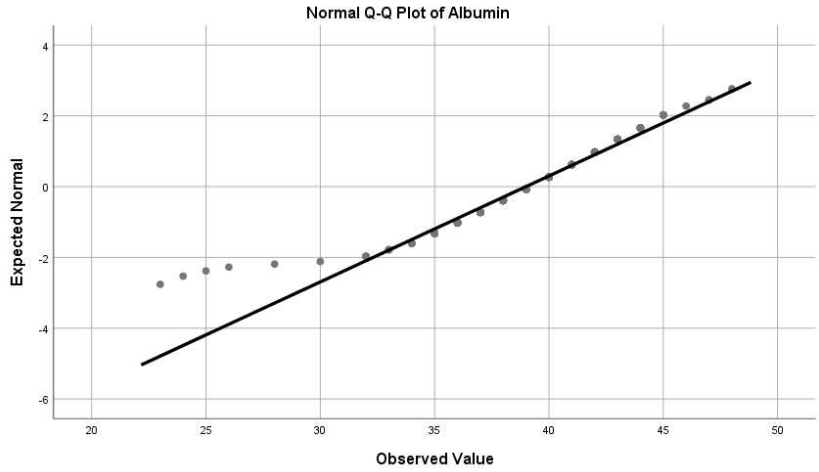

Figure 8-46 Normal probability plot for Albumin. Data is normally distributed, few patients have lower albumin levels

8.8.7.9 HDL

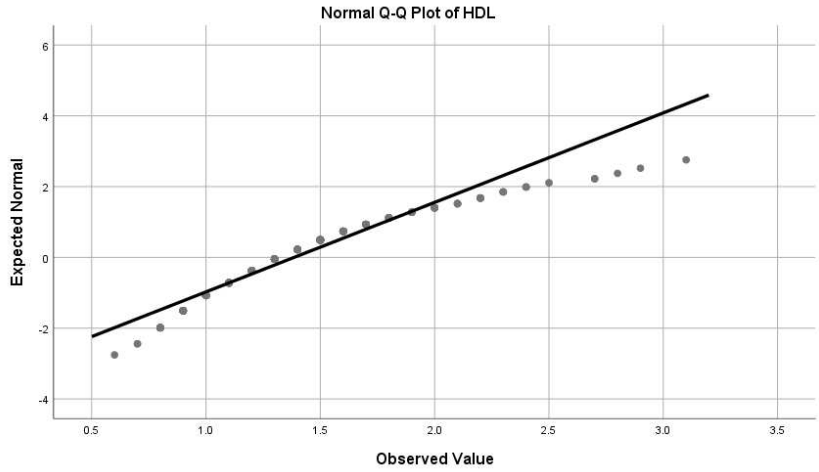

Figure 8-47 Normal probability plot for HDL. Data is normally distributed.

8.8.7.10 CHOL/HDL

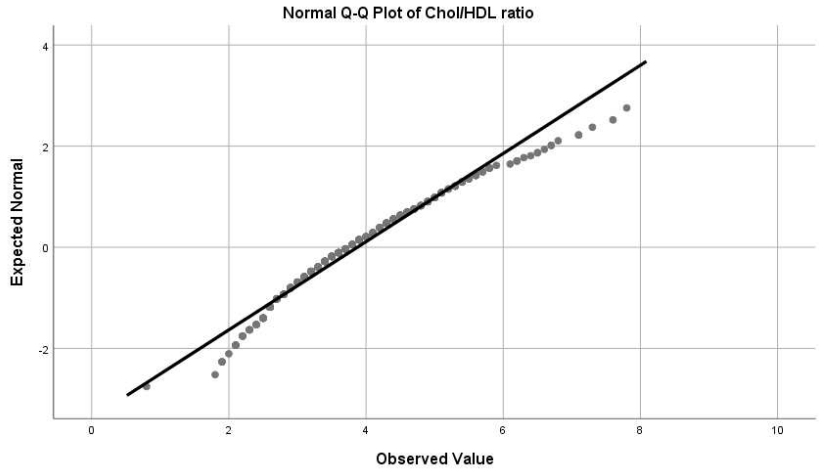

Figure 8-48 Normal probability plot for CHOL/HDL. Data is normally distributed.

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Please print, sign, and scan and email *this agreement to* dmorisky@ucla.edu

Please sign and return this contractual agreement in a PDF format, Pages 1 and 2 to Professor Morisky and he will provide you with pages 3 and 4 of the listing of the MMAS-8 items, scoring and re-coding criteria and signature authorizing full use of this copyrighted scale. I agree to use only the English version of the MMAS-8 unless I purchase a validated translation of the MMAS-8 through Professor Morisky. I understand that it is a violation of international copyright laws to either use your own translation and call it the "MMAS-8" or use an existing MMAS-8 scale that has been translated and used for another study. The validated translation is non-transferrable and is linked to a specific license agreement and cannot be reproduced, copied, distributed, placed on the internet, published, or used by another individual. If the licensee violates any copyright laws contained in this licensing agreement they will be solely responsible for a $5000.00 penalty and any associated legal costs.

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Title of Study: Metabolomics and Next Generation Sequencing Approaches in Stratification of Resistant Hypertension

Number of Anticipated Administrations of the MMAS-8: 500

Signature of Licensee:

Date: 7. July . 2015

Signature of Developer/Owner: Donald E. Morisky

Date: July 7, 2015

Figure 8-49 Morisky license

8.10 **PRISMA checklist**

Section/topic	#	Checklist item	Reported on page #
Title	1	Identify the report as a systematic review, meta-analysis, or both.	107
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	110
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	110
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	110
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	111
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	111
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	111
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	112
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	112
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	111

Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	111
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	110
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	112
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	113
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	113
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	120
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	123
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	123
FUNDING			

Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	