Prevalence of and Clinical Characteristics Associated with Microalbuminuria in Hypertension

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Abstract

Cardiovascular disease is the leading cause of mortality. As blood pressure is one of the most important risk factors for cardiovascular disease, effective management of hypertension is critical in reducing this risk. In addition to high blood pressure, however, several factors have been identified as predictors of future cardiovascular events. These include high cholesterol, cigarette smoking, obesity and diabetes. Taken together, these traditional risk factors do not entirely explain the risk. Thus, many novel risk factors have been proposed for risk prediction of cardiovascular disease. Microalbuminuria is one such factor.

Microalbuminuria is defined as excretion of albumin in the urine above the normal level but less than gross proteinuria. As excretion of albumin exhibits high variability due to many confounders (such as urinary tract infection and strenuous exercise), diagnosis of microalbuminuria should be ideally based on screening of multiple samples using either 24-hour urine collection or first-morning voids. Much evidence suggests that microalbuminuria is a reflection of generalised endothelial dysfunction. This is supported by the observation that microalbuminuria is strongly associated with cardiovascular disease.

My main aim was to study microalbuminuria in people with hypertension attending specialist clinics. Microalbuminuria has been investigated extensively in diabetes and in patients with renal disease. However, the available information on the association of microalbuminuria with hypertension has many limitations since many studies had small sample size, restricted population or were confounded by potential misdiagnosis of microalbuminuria by the use of single samples. This has led to uncertainty about the prevalence of microalbuminuria in hypertension, where reported prevalence ranges from 4.7% to 58%, and probable underestimation of its clinical significance.

I addressed these issues by conducting a series of studies in 1059 hypertensive subjects attending the Glasgow Blood Pressure Clinic or the Aberdeen Hypertension Clinic. Each patient was invited to provide an early morning urine specimen for the assessment of albuminuria. Urinary tract infection was tested using urine strips and, where positive, samples were discarded. If the first sample showed increased albumin excretion, two further samples were requested. Albuminuria (microalbuminuria or gross proteinuria) was diagnosed when two out of the three samples showed increased albuminuria. Two definitions of microalbuminuria were used in the analysis, a conventional definition with the threshold used by most therapeutic guidelines and a new definition that accounts for low excretion of albumin. All patient information was obtained from case-records.

In the first study, I showed that microalbuminuria by the conventional definition was present in 9.5% of non-diabetic hypertensive subjects without renal impairment. Another 10% of this cohort had microalbuminuria by the new definition. Compared with people with normal urinary albumin, individuals with microalbuminuria by both definitions (n= 786, after excluding those with diabetes or severe renal impairment) had significantly higher blood pressure, higher pulse pressure, increased levels of inflammatory markers, poorer renal function, higher triglycerides levels and used more cardiovascular drugs.

In a second study, the association of microalbuminuria with clinical characteristics was investigated. Subjects with microalbuminuria had increased prevalence of risk factors / co-morbidities such as left ventricular hypertrophy (19.2% in normoalbuminuria versus 29.7% and 34.8% for microalbuminuria by the new and the conventional definitions, respectively), ECG abnormalities and cardiovascular disease. In addition, people with microalbuminuria had higher risk scores for subsequent cardiovascular events using two risk calculators, the Framingham and the Joint British Societies equations. In a subcohort with controlled blood pressure and without co-morbidities or risk, microalbuminuria (by

combining the two definitions) was found in 14%. Compared with those with normoalbuminuria, subjects with microalbuminuria had higher blood pressure, poorer renal function, higher blood glucose and higher levels of inflammatory markers although the limited sample size precluded statistical significance.

In a further study, the independent association of microalbuminuria with different risk factors was evaluated using multivariate testing. Systolic blood pressure, serum creatinine, left ventricular hypertrophy and fasting triglycerides were among factors linked with microalbuminuria. The risk of microalbuminuria increased in people with poorly controlled blood pressure. I also found that microalbuminuria was associated strongly with left ventricular hypertrophy [odds ratio 1.87 (95% CI, 1.12 - 3.12) for a composite of both definitions- the combined definition] and cardiovascular abnormalities [odds ratio 1.72 (95% CI, 1.05 - 2.80) for the combined definition].

In a fourth study, the reproducibility of microalbuminuria screening was investigated. I discovered that a large proportion of people who had increased urinary albumin excretion on first sample was categorised as normoalbuminuria based on the result of multiple samples (48% at the Glasgow Clinic and 41% at the Aberdeen Clinic). This indicates that even after controlling microalbuminuria confounders, multiple testing can be recommended for more accurate diagnosis.

In the final study, I demonstrated that subjects with microalbuminuria by both definitions attending the Glasgow Blood Pressure Clinic had relatively high blood pressure and pulse pressure at first visit and subsequently. This finding indicates that subjects with microalbuminuria require particularly rigorous blood pressure management to achieve blood target blood pressure. Furthermore, individuals with microalbuminuria may be at risk for cardiovascular disease greater than that in those with normoalbuminuria since the

eventual blood pressure remained higher in these subjects. Together with the observations that microalbuminuria is associated with clustering of cardiovascular risk factors, my finding support the importance of even small increase in urine albumin excretion as an indicator of eventual cardiovascular disease.

In conclusion, microalbuminuria is found in one-fifth of subjects with essential hypertension. Although my investigation was observational, the large sample size, the use of multiple samples and allowance for the effects of potential confounders enhances the precision of the results. Moreover, subjects involved in this study represent a real hypertension population with few restrictions. My findings support the value of microalbuminuria as a tool to identify subjects at high risk for cardiovascular disease. Before routine screening can be recommended, these observations require confirmation in clinical trials and prospective studies with long-term follow up. Linkage of the records of the patients who participated in this series of studies with national morbidity and mortality statistics offers one approach with the potential to test the clinical relevance of my findings.

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Declaration

I declare that this thesis represents my own work, unless otherwise acknowledge. Raw data from the Aberdeen Hypertension Clinic was provided by Professor John Webster and I was responsible for the rearrangement, 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.

Adel A Alharf

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Presentations

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List of abbreviations

AASK	African American Study of Kidney Disease and Hypertension
ABCD	Appropriate Blood Pressure Control in Diabetes
ACCOMPLISH	Avoiding Cardiovascular Events through Combination Therapy in Patients Living with Systolic Hypertension
ACEI	Angiotensin converting enzyme inhibitor
ACR	Albumin-to-creatinine ratio
ADVANCE	The BP arm of the Action in Diabetes and Vascular disease: preterAx and diamicroN-MR Controlled Evaluation
ALOFT	ALiskiren Observation of heart Failure Treatment
ANOVA	One-way analysis of variance
ARB	Angiotensin receptor blocker
BENEDICT	Bergamo Nephrologic Diabetes Complications Trial
BHS	British Hypertension Society
BMI	Body mass index
BP	Blood pressure
CBB	Calcium channel blocker
CHARM	Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity
CHD	Coronary heart disease
CKD	Chronic kidney disease
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DCCT/EDIC	Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications
ECG	Electrocardiography
eGFR	Estimated glomerular filtration rate
EPIC-Norfolk	European Prospective Investigation into Cancer in Norfolk
ESR	Erythrocyte sedimentation rate
ESRD	End-stage renal disease
FDA	Food and Drug Administration
GBPC	Glasgow Blood Pressure Clinic
GDF-15	Growth differentiation factor 15

GEMINI	Glycemic Effects in Diabetes Mellitus Carvedilol-Metoprolol Comparison in Hypertensives
GFR	Glomerular filtration rate
HARVEST	Hypertension and Ambulatory Recording Venetia Study
HDL	High-density lipoprotein
HOPE	Heart Outcomes Prevention Evaluation
HPLC	High performance liquid chromatography
HyperGEN	Hypertension Genetic Epidemiology Network
I-DEMAND	Italy Developing Education and awareness on MicroAlbuminuria in patients with hyperteNsive Disease
IDNT	Irbesartan Diabetic Nephropathy Trial
JBS	Joint British Societies
KEAPS	Kidney Evaluation and Awareness Program in Sheffield
LDL	Low-density lipoprotein
LIFE	Losartan Intervention For Endpoint reduction
Lp-PLA2	Lipoprotein-associated phospholipase A2
LVH	Left ventricular hypertrophy
MAGIC	Microalbuminuria: A Genoa Investigation on Complications
MARVAL	MicroAlbuminuria Reduction With VALsartan
MAU	Microalbuminuria
MDRD	Modification of Diet in Renal Disease
MESA	Multi-Ethnic Study of Atherosclerosis
MI	Myocardial infarction.
MMSE	Mini–Mental State Examination
MONICA	Multinational Monitoring of Trends and Determinants in Cardiovascular Disease Project
MSSU	Mid-stream specimen of urine
NHANES	National Health and Nutrition Examination Surveys
NKF KDOQI	National Kidney Foundation Kidney Disease Outcomes Quality Initiative
Normoalb.	Normoalbuminuria
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
ONTARGET	Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial
PREVEND	Prevention of Renal and Vascular End-stage Disease

PWV	Pulse wave velocity
RAAS	Renin-angiotensin aldosterone system
RAS	Renin aldosterone system
RBC	Red blood cell
RENAAL	Reduction of Endpoints in Non insulin Dependent Diabetes Mellitus with Angiotensin II Antagonist Losartan
ROADMAP	Randomized Olmesartan and Diabetes Microalbuminuria Prevention
SBP	Systolic blood pressure
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
SVD	Small vessels disease
TIA	Transient ischemic attack
TRANSCEND	Telmisartan Randomized AssessmeNt Study in ACE iNtolerant subjects with cardiovascular Disease
UAC	Urinary albumin concentration
UAE	Urinary albumin excretion
UK	United Kingdom
UKPDS	United Kingdom Prospective Diabetes Study
US	United States.
UTI	Urinary tract infection
VALERIA	Lisinopril in Hypertensive Patients With Microalbuminuria
VVANNTT	Verapamil Versus Amlodipine in Nondiabetic Nephropathies Treated with Trandolapril
WHO	World Health Organisation
WHR	Waist-hip ratio

1. Chapter one: Introduction

1.1 Cardiovascular disease

1.1.1 Epidemiology

Cardiovascular disease is the leading cause of mortality in the world (Lloyd-Jones et al., 2010). Nearly 16.7 million deaths per year are attributable to this condition (WHO, 2003). In Europe, almost a half of all deaths are due to cardiovascular disease (Allender et al., 2008). It has been estimated that 38% of deaths in 2003 in Scotland was attributable to cardiovascular system disease (Lowther et al., 2006). Even a decade ago, the United Kingdom (UK) expenditure on cardiovascular disease-related hospitalisation and disabilities was about £29.1 billion (Luengo-Fernández et al., 2006).

Cardiovascular disease has also become a major health concern in developing countries (Kim and Johnston, 2011). Cardiovascular mortality in the middle-age population of such countries is much higher than that in developed countries (Raymond et al., 2006). These changes are expected to result in important economic consequences due to loss of productivity of working individuals.

1.1.2 Risk factors

There are several risk factors for cardiovascular disease. Most, such as hypertension, hyperlipidaemia, obesity, diabetes and cigarette smoking, are reversible and have been used extensively in risk stratification of cardiovascular disease. However, these classic risk factors do not entirely explain future cardiovascular disease (Kuulasmaa et al., 2000). As a result, many novel risk factors such as microalbuminuria, estimated glomerular filtration rate (eGFR), C-reactive protein (CRP) and homocysteine have been evaluated as potential predictive factors (Danesh et al., 2004, Bello et al., 2011).

1.1.3 Hypertension as a risk factor for cardiovascular disease

Hypertension is one of the most challenging health problems in the world. It has been estimated that, globally, almost one billion individuals have hypertension (WHO, 2002). By 2025, that number is estimated to increase by 60%, which means that 1.56 billion people could be hypertensive (Kearney et al., 2005). Latest WHO statistics showed that hypertension is the leading cause of mortality worldwide (responsible for 13% of global deaths) (WHO, 2012). Hypertension is one of the most prevalent chronic disorders in the UK, affecting about 25% of the adult population (MacDonald and Morant, 2008). It has been estimated that the cost of hypertension treatment in the UK is approximately £1 billion (National Institute for Health and Clinical Excellence, 2011). In Scotland, two antihypertensive agents (were among the ten most commonly prescribed drugs (National Health Service, 2011).

According to the National Institute for Health and Clinical Excellence (NICE) guideline, a sustained systolic blood pressure (SBP) / diastolic blood pressure (DBP) of 140/90 mm Hg and subsequent ambulatory or home blood pressure of 135/85 mm Hg or higher is considered to be the threshold for diagnosis of hypertension (National Institute for Health and Clinical Excellence, 2011). The BHS has classified hypertension into three categories; mild, moderate and severe. The society also suggests that a sustained SBP of 160 mm Hg or more, or a sustained DBP of 100 mm Hg or more requires initiation of pharmacological intervention. The threshold of treatment is reduced in high-risk patients such as those with diabetes or established cardiovascular diseases.

Unmanaged hypertension predisposes the individual to serious cardiovascular and cerebrovascular complications, such as heart failure, stroke, myocardial infarction (MI), left ventricular hypertrophy (LVH), atrial fibrillation and peripheral vascular diseases as well as premature mortality. Several epidemiological studies have demonstrated a linear

relationship between blood pressure and cardiovascular risk. The Framingham Heart Study is of landmark importance in this field (Kannel et al., 1969). The study clearly demonstrated that high blood pressure is one of the most important cardiovascular disease risk factors.

1.1.3.1 Hypertension as a risk factor for stroke and coronary heart diseases

Hypertension is an independent risk factor for stroke (Rosamond et al., 2007). A meta-analysis of 61 observational studies involving nearly one million individuals revealed that mortality from stroke as well as ischemic heart diseases increases in a log-linear manner with an increase in SBP / DBP levels above 115/ 75 mm Hg (Lewington et al., 2002). This meta-analysis demonstrated that each 20 mm Hg increase in SBP or 10 mm Hg increase in DPB is associated with a twofold increase in mortality. The Framingham Heart Study also reported a continuous relationship between blood pressure and stroke risk (Seshadri et al., 2006). Moreover, in a case-control study with participants from 22 countries, hypertension was the most important risk factor for all types of stroke (O'Donnell et al., 2010). These studies clearly demonstrate the importance of hypertension as a potentially modifiable risk factor for stroke; strict control of high blood pressure reduces such risk.

High blood pressure is also an important modifiable risk factor for coronary heart diseases (CHD). Evidence from several epidemiological studies suggests that the risk of developing CHD increases with the increase of blood pressure (Eberly et al., 2004). For instance, the Multiple Risk Factor Intervention Trial, which involved 361,662 men, revealed a strong association between hypertension and CHD (Eberly et al., 2004). Among subjects with CHD, the prevalence of hypertension has been estimated to be 32% (Khot et al., 2003). In

the United States, reduction in SBP was the second most important factor (after total cholesterol) attributed to reduction in CHD-related mortality (Ford et al., 2007).

The use of antihypertensive agents reduces the risk of cardiovascular disease including CHD and stroke. Law et al. (Law et al., 2009) analyzed 147 randomised clinical trials to investigate the effect of antihypertensive treatment on prevention of cardiovascular risk. Reductions of 10 mm Hg in SBP or 5 mm Hg in DBP are associated with 20% and 32% lower CHD and stroke risks, respectively; regardless of which class or agent is used as first-line therapy.

1.1.3.2 Hypertension and risk of LVH and heart failure

Chronic hypertension may lead to the development of LVH, possibly due to the increased workload of the heart caused by prolonged high blood pressure (Frohlich et al., 2011). LVH is a common finding in hypertensive subjects. A recent systematic review of 30 studies involving treated and untreated hypertensive subjects showed that the prevalence of LVH using echocardiography ranged from 36% to 41%, depending on the diagnosis criteria (Cuspidi et al., 2012).

High blood pressure is also an important aetiological factor for heart failure. A meta-analysis of 23 hypertension trials conducted between 1997 and 2007 and involving 193,424 subjects reported that the risk of developing heart failure is high in hypertensive subjects even under pharmacological management (Tocci et al., 2008). The authors reported that the rate of heart failure was 8.5 events per thousand patients per year. In a case-control study in the general population, hypertension was the most important risk factor for heart failure (Dunlay et al., 2009). Another population-based cohort study in elderly subjects not receiving antihypertensive agents confirmed a linear-relationship between SBP and heart failure (Butler et al., 2011).

1.1.3.3 Hypertension and kidney function

Hypertension is closely related to kidney diseases (Bidani and Griffin, 2004). Blood pressure regulation through sodium and water excretion is one the important physiological functions of the kidneys. High blood pressure is a predisposing factor for renal abnormalities (Tozawa et al., 2003). Nearly 80% of patients with chronic kidney disease (CKD) have hypertension (Buckalew et al., 1996). Although essential hypertension is considered to be strongly associated with end-stage renal disease (ESRD) in the African-American (Klag et al., 1997), whether such finding can be extended to other population (e.g. Caucasian cohort) is still unclear. Using longitudinal data from the Glasgow Blood Pressure Clinic (GBPC), it has been found that progression to ESRD in subjects attending this tertiary/secondary clinic (predominantly Caucasian) is uncommon (only 1% of the population) (Mackinnon et al., 2008). Therefore, although high blood pressure may aggravate renal impairment in people with established kidney disease, progression to overt nephropathy and ESRD in uncomplicated hypertension is rare in the Scotland.

1.1.4 Other traditional risk factors for cardiovascular disease

Prolonged elevation of serum lipids may lead to atherosclerosis, which is the underlying pathological factor of several cardiovascular diseases such as CHD and stroke (Zhang et al., 2012, Woodward et al., 2007). A meta-analysis of 61 studies involving more than 900,000 patients from Europe and Northern America showed that the total cholesterol/high-density lipoprotein (HDL) ratio was a powerful predictor for cardiovascular mortality (Prospective Studies Collaboration., 2007). The study also revealed that 1 mmol/L difference in total cholesterol in those between 40-49 years was associated with a 50% difference in CHD mortalities. Other lipid indices such as low-density lipoprotein (LDL)-cholesterol , non–HDL-cholesterol, and apolipoprotein-B also

predict several cardiovascular events (such as CHD, myocardial infarction and angina) even in those under treatment with statins (Boekholdt et al., 2012).

Evidence from epidemiological studies show that diabetes is a major risk factor for cardiovascular disease (Grundy et al., 1999, D'Agostino et al., 2008). A direct effect of hyperglycemia on blood vessels has been suggested (Piga et al., 2007) whereby glucose induces reactive oxygen species and accelerates atherosclerosis (Renard et al., 2004). The absolute risk for cardiovascular disease in patients with diabetes mellitus is almost double that in non-diabetic individuals (Fox et al., 2004). The Nurses' Health Study reported that women with diabetes mellitus are at threefold increased risk for CHD mortality (Hu et al., 2001). One of the major concerns with diabetes is that the trends for cardiovascular diseases attributable to diabetes have increased in the last decades (Fox et al., 2007). This observation reinforces the need of more extensive measurements for reduction of cardiovascular risk in subjects with diabetes mellitus.

1.1.5 Evidence for cardiovascular protection using antihypertensive drugs

Evidence from several trials clearly demonstrated that lowering blood pressure by antihypertensive drugs reduces the risk of cardiovascular morbidity (Neal et al., 2000, Czernichow et al., 2011). These trials showed that the cardiovascular protection of antihypertensive agents is evident in people with different risk profiles and in all age groups. The reduction of cardiovascular risk seems to be dependent mainly on the magnitude of blood pressure reduction regardless which antihypertensive class was used to achieve such reduction (Law et al., 2009).

Management of blood pressure using a single agent is usually inadequate and may not reduce cardiovascular risk, especially for those with SBP/DBP above treatment goal by 20/10 mm Hg (Weber et al., 2004, Gradman et al., 2011). The benefit from early

management with combination therapy over monotherapy has been suggested by the ACCELERATE trial (Brown et al., 2011). This trial was designed to investigate whether initiating combination therapy is superior to monotherapy in terms of blood pressure outcomes. At baseline, a group of participants were assigned to monotherapy with either amlodipine or aliskiren (plus placebo) while another group received a combination of both drugs. After 6 months, all participants who received a combination of active treatment. The study revealed that those received combination therapy had better early blood pressure control. Furthermore, participants who started with monotherapy never reached the blood pressure levels achieved by those initiated with combination therapy. Long term outcomes are needed to confirm the beneficial effect of initiating a combination therapy for management of hypertension (not quite right). A more recent observational analysis confirmed that combination approach is better than monotherapy for initial hypertension management (Egan et al., 2012). It also showed that those started with monotherapy did not catch up with the blood pressure reduction achieved in those taking combination therapy during the first year of treatment. However, the retrospective nature of study and the lack of randomisation limit the interpretation of the study.

Evidence from the ACCOMPLISH (Avoiding Cardiovascular Events through Combination Therapy in Patients Living with Systolic Hypertension) trial showed that a combination of a calcium channel blocker (CCB) plus an angiotensin converting enzyme inhibitor (ACEI) was better than an ACEI plus a diuretic in reducing primary endpoint (composite of cardiovascular mortality, myocardial infarction, CHD, stroke, angina and resuscitation after sudden cardiac arrest) in high-risk individuals (Jamerson et al., 2008). However, slightly greater blood pressure reduction (1/1 mm Hg) in the CCB combination and on-treatment blood pressure difference could explain the better outcome.

In diabetes mellitus, a meta-analysis of 31 trials involving 73,913 individuals indicated that the greater the reduction in blood pressure the greater the reduction in stroke risk (Reboldi et al., 2011). Overall risk of myocardial infarction was reduced by 11%. However, when those with rigorous blood pressure control were compared with less-tight control, the risk of myocardial infarction was not reduced significantly.

Evidence from a meta-analysis of 27 trials involving 158,709 individuals demonstrated equivalent cardiovascular protection of antihypertensive treatment in those with and without diabetes mellitus (Blood Pressure Lowering Treatment Trialists' Collaboration, 2005). The analysis denied any difference between the classes of antihypertensive agents in reducing cardiovascular risk.

For high-risk people, good control of blood pressure has been shown to protect against cardiovascular events (Weber et al., 2004). The Heart Outcomes Prevention Evaluation (HOPE) study is a landmark study in which 9297 high risk subjects (with diabetes or vascular disease plus a cardiovascular risk factor) were assigned to receive an ACEI or placebo and followed for five years (Heart Outcomes Prevention Evaluation Study Investigators, 2000a). Treatment with an ACEI compared with placebo was associated with significant reduction in cardiovascular complications (such as cardiac arrest and heart failure) and cardiovascular mortality. Many recent studies have attempted to investigate any differential effects of antihypertensive drug classes in high-risk populations (Kasanuki et al., 2009, Ogihara et al., 2008). However, interpretation is difficult due to insufficiency of sample size and low number of events. Also, most of the added benefit can be ascribed to difference in blood pressure reduction between comparators.

Cardiovascular protection mediated by antihypertensive treatment is also evident in the very elderly (Beckett et al., 2012). In a cohort of 3845 subjects aged 80 years or older,

active treatment with diuretic (with/without an ACEI) for a median of 1.8 years with a target blood pressure of less than 150/90 mm Hg significantly reduced the rate of stroke (fatal and non-fatal), all-cause and cause-specific mortality (Beckett et al., 2008). A sub-analysis reported a reduction in total and cardiovascular mortality following short term management of blood pressure in the elderly population (Beckett et al., 2012). The results of these trials need careful interpretation since participants consisted of people with SBP greater than 160 mm Hg while those with cognitive impairment and frail subjects were excluded.

1.1.6 Novel risk factor for cardiovascular disease

In addition to conventional risk predictors, the usefulness of several novel biomarkers has been suggested for the prediction of future cardiovascular disease (Helfand et al., 2009). One of the most frequently reported novel risk factor is CRP (Pai et al., 2004). CRP is an acute phase protein formed by the liver following inflammatory reaction (Ridker et al., 2000). This acute phase protein has a constant half-life and therefore, the levels of CRP are controlled by its rate of synthesis, which reflects the severity of the underlying cause (Pepys and Hirschfield, 2003). A more sensitive test for CPR (high sensitivity (hs-CRP)) is increasingly used as a marker for cardiovascular diseases (Nambi et al., 2009).

Data from the Copenhagen City Heart Study demonstrated a continuous relationship between levels of CRP, measured once at baseline, and all-cause mortality (Zacho et al., 2010). A meta-analysis of 54 epidemiological studies demonstrated that elevation of CRP concentration was associated with greater risk of CHD, stroke and cardiovascular mortality (Tipping et al., 2010).

The erythrocyte sedimentation rate (ESR) has been widely used as a screening tool in clinical practice to help in the detection of systemic inflammation and autoimmune

diseases. The ESR measures the rate of red blood cells (RBCs) precipitation out of plasma after 60 minutes (mm/hour). During the inflammatory process, the level of some acute-phase proteins, including fibrinogen and globulins, increase in the plasma and as a result, the RBCs tend to stick leading to the formation of rouleaux (Lewi, 1954). Therefore, the rate of erythrocyte sedimentation increases.

In addition to chronic inflammatory diseases, elevated ESR levels have been reported in coronary artery disease, heart failure and stroke (Danesh et al., 1998, Natali et al., 2003, Ingelsson et al., 2005, Maradit-Kremers et al., 2007). In a study involving 16,673 subjects, ESR was a strong and independent risk factor for CHD in both males and females (Andresdottir et al., 2003). ESR has been reported to be significantly higher in hypertensive subjects with impaired renal function (reduced eGFR and increased urinary albumin excretion) (Catena et al., 2000).

Eosinophils are that play a pivotal role in the immune system (Sampson, 2000). These cells produce several inflammatory mediators and reactive oxygen species during infection to attack pathogens. Eosinphils are also involved in the pathogenesis of allergic conditions such as asthma (Kariyawasam and Robinson, 2007). Therefore, eosinophil count in the blood is frequently used in clinical practice when inflammatory disease is suspected.

Several studies showed positive association between increased eosinophil count (eosinophilia) and cardiovascular mortality and morbidity. For instance, eosinophilia was positively correlated with cardiovascular mortality in a study involving more than 5000 patients who had eosinophil count at baseline and followed for 30 years (Hospers et al., 1999). Eosinophilia is also a risk predictor for CHD (Sweetnam et al., 1997) and acute vasospastic angina pectoris (Umemoto et al., 2000).

The clinical utility of the measurement of novel biomarkers of cardiovascular disease is controversial. For instance, while some studies suggested that CPR provides additional information to that provided by classic risk factors used in risk stratification equations (such as the Framingham equation) (Koenig et al., 2004) others showed weaker prognostic power (Wilson et al., 2005). In order to answer the question of whether CRP, or any novel risk factor, provides information additional to traditional risk factors, patients with low cardiovascular risk profile or even apparently healthy individuals might need to be followed for a prolonged period. This is because novel biomarkers may provide very early prognostic information, before any clinically detectable changes.

1.2 Microalbuminuria as a marker for cardiovascular disease in subjects with hypertension

1.2.1 Introduction: the renal system

The kidney is the major excretory organ in the body. The main function is to filter the blood to remove any potential toxic molecules, metabolic products (such as creatinine and urea) and any extra fluid in order to maintain normal blood volume (Alpern et al., 2008). The kidney also plays an important role in reabsorbing water and some important molecules such as electrolytes (e.g. sodium and potassium) and proteins (Christensen and Gburek, 2004).

Blood filtration occurs in the nephron, the functional unit of the kidney (Alpern et al., 2008). Normally, each kidney has more than one million nephrons. Each nephron consists of tubules and glomerulus where blood filtration occurs. The glomerulus contains a filtration membrane which consists of three layers: the endothelium, the epithelial podocyte and the basement membrane (Sherwood, 2008). The glomerular filtration membrane permits some blood molecules to pass through in order to reach the tubular system which has four parts: the proximal convoluted tubule, loop of Henle, distal

convoluted tubule and collecting duct (Alpern et al., 2008). In the renal tubular system, most of reabsorption process occurs. Nutritional molecules (such as glucose) and plasma proteins are usually reabsorbed in the proximal tubule (Gekle, 1998). In the loop of Henle, concentration of urine through water reabsorption occurs. The regulation of sodium and potassium ions is among the roles of distal tubules. Final ion reabsorption occurs in the collecting duct.

Blood flows into the kidney through the renal artery which branches into segmental arteries and then to different lobular arteries (Boylu et al., 2012). These arteries further branch into interlobular arteries that divide to form afferent and efferent arterioles. The afferent arterioles are responsible for blood supply to the glomerulus while efferent arterioles remove blood from of the glomerulus (Boylu et al., 2012). Both afferent and efferent arterioles play important roles in controlling the glomerular capillary pressure by vasoconstriction or vasodilation.

1.2.1.1 Protein reabsorption

Serum proteins play major roles in the body including transporting essential molecules such as hormones, vitamins, lipids, minerals and exogenous substance such as drugs (Kratz and Elsadek, 2012). Proteins also maintain the oncotic pressure between the plasma and interstitial space, and are involved in the synthesis of enzymes and other substances. Proteins in the blood can be divided into, for example, carrier proteins such as albumin, immune system proteins such as immunoglobulin and acute phase proteins such as CRP (McClatchey, 2002).

The most abundant serum protein is albumin, accounting for 60% of serum protein and with a concentration of 3.4 - 5.4 g/dL (Kadono et al., 2010). Albumin is a highly soluble single polypeptide which consists of 585 amino acid sequence (Carter et al., 1989). Each

day, 9 - 12 g of albumin is produced by the liver (Evans, 2002). More than 70% of oncotic colloid pressure is controlled by albumin (Kadono et al., 2010). Levels of albumin are affected by factors including endogenous molecules such as insulin and cortisol (Kimball et al., 1995).

As mentioned earlier, kidney reabsorption of albumin and other proteins that pass the glomerular filtration membrane occurs in the proximal convoluted tubule. This reabsorption process is achieved by receptor-mediated endocytosis (Lazzara and Deen, 2007). A receptor complex called megalin-cubilin is involved in the endocytosis process (Birn et al., 2000). The reabsorption process can be summarised as follow: albumin binds to the megalin-cubilin receptor complex in the apical plasma membrane (Amsellem et al., 2010). After that, an adaptor molecule binds to the tail of the receptor complex to help the internalisation of the ligand-receptor complex (Cui et al., 1996). Once the internalisation process occurs, an endocytic vesicle transports the formed complex to the endosomal compartment where the protein complex dissociates by vesicle acidification (Birn and Christensen, 2006). Albumin then undergoes degradation in the lysosome to its original amino acids which return to the blood stream.

1.2.2 Definition of microalbuminuria

Normally, the kidneys reabsorb almost all the filtered albumin. A very low amount of albumin is present in the urine. However, certain illnesses such as diabetes, CKD and hypertension may mediate physiological changes that lead to excretion of larger amounts of albumin into the urine, albuminuria.

Levels of albumin in the urine can be expressed as a concentration or as a ratio of albumin-to-creatinine (table 1-1). Albumin in the urine at levels exceeding 300 mg/day (> 200 mg/L in a spot specimen or albumin-to-creatinine ratio (ACR) > 25 mg/mmol) is regarded as

macroalbuminuria (or gross proteinuria). This state of increased excretion of proteins in the urine usually reflects deterioration of kidney function. The measurement of albuminuria by ACR takes into consideration the differences in muscle mass between females and males, as males have higher muscle mass and hence higher creatinine excretion (Mattix et al., 2002). Protein excretion in the urine can be measured in terms of albumin or total protein (Atkins et al., 2003). Proteinuria can be easily detected by a standard dipstick (Guy et al., 2009). If the dipstick shows a positive result for proteinuria, quantitative measurement of proteins is usually advised.

Low amounts of albumin in the urine that cannot be detected by conventional dipsticks (microalbuminuria) was firstly reported in 1969 by Keen et al. (Keen et al., 1969) who studied the increased level of albuminuria in type 2 diabetes. A few years after that, Parving and colleagues (Parving et al., 1974) reported the presence of microalbuminuria in subjects with uncontrolled hypertension. Since then, many studies have linked microalbuminuria with several abnormalities. However, until recently, the vast majority of studies focused on the association of microalbuminuria with cardiovascular disease in subjects with diabetes mellitus and in patients with CKD.

Microalbuminuria is defined as levels of albumin between 30 - 300 mg per day (equivalent to 20 to 200 µg/minute in a timed overnight urine collection, 20-200 mg/L on spot urine specimen or ACR 2.5 to 25 mg/mmol in males or 3.5 to 25 mg/mmol in females), table 1-1.

Timed urine collection			Spot morning urine specimen			
Albuminuria Level	**24-hour albumin excretion (mg/day)**	**Overnight albumin excretion (µg/min)**	**UAC (mg/L)**	**Albumin-to-creatinine ratio**		
				Gender	**mg/mmol**	**mg/g**
Normal	< 30	< 20	< 20	Male	< 2.5	< 20
				Female	< 3.5	< 30
Microalbuminuria	30 - 300	20 - 200	20 - 200	Male	2.5 - 25	20 - 200
				Female	3.5 - 25	30 - 200
Gross proteinuria	>300	>200	>200	Male	>25	>200
				Female	>25	>200

Table 1-1: Definitions of albuminuria (Yuyun et al., 2005). UAC: Urinary albumin concentration.

1.2.3 Detection of microalbuminuria

A number of dipsticks (such as Clinitek Microalbumin and Chemstrip Micral-Test) are available to detect microalbuminuria (Sarafidis et al., 2007). These strips are inexpensive and easy to use in clinical setting but may not provide precise quantitative measurement of albumin level.

Several laboratory techniques are available for the detection and quantitative measurement of microalbuminuria (Sviridov et al., 2006). These techniques are generally characterised by high specificity for detection of albumin. The most frequently utilised techniques for detection of microalbuminuria are immunoassays. Non-immunological techniques for quantifying microalbuminuria have also been used. These tests are mainly chromatographic techniques such as size-exclusion high performance liquid chromatography (HPLC) (Sviridov et al., 2006).

1.2.4 Confounders of microalbuminuria screening

Very many confounding factors may affect microalbuminuria screening and provide false-positive results (table 1-2). Strenuous exercise may increase albumin level in the urine of healthy individuals, even above the threshold of microalbuminuria (Heathcote et al., 2009). The mechanism of exercise-induced temporary microalbuminuria is not completely established. One possibility is that this effect is caused by the increase in glomerular permeability due to the increase in glomerular filtration pressure mediated by strenuous exercise (Heathcote et al., 2009).

Urinary tract infection (UTI) is also a possible confounder. The majority of therapeutic guidelines recommend the exclusion of samples that show positive evidence of UTI and recommend that a mid-stream specimen of urine (MSSU) should be submitted for bacteriology (National Kidney Foundation, 2007). Other confounder of microalbuminuria screening include fever, upright position for a long time, pregnancy and menstruation (Metcalf et al., 1993). The excretion of albumin in the urine may exhibit high variability in these conditions. Therefore, requesting additional samples after a suitable time gap is recommended.

Confounders	
Upright posture	Pregnancy
Exercise	Menstruation
Fever	Haematuria
Symptomatic UTI	Renal impairment
Heavy protein diet	Hyperglycemia
Inflammation	Hypertension
Infections (e.g. hepatitis)	Heart failure

Table 1-2: Examples of factors associated with increased urinary albumin excretion (Czekalski, 1996, Airoldi and Weinstein, 2007).

1.2.5 Screening methods and number of samples

There is a general consensus that 24-hour urine collection is the gold standard of microalbuminuria screening (Witte et al., 2009). However, complete compliance with this method is difficult for the individual and as a result, this approach is rarely utilised. Overnight albumin excretion rate is a simpler and more easily accomplished way of timed microalbuminuria measurement (Dyer et al., 2004). One advantage of overnight urine collection is that minimum movement during bedtime leads to more stable albumin excretion (Smulders et al., 1998).

More commonly used methods include measuring urinary albumin concentration (UAC) or ACR on random samples (American Diabetes Association, 2004) or first morning voids (Lambers Heerspink et al., 2008). The random spot urine collection is the easiest and most convenient way for the patients and the practitioners. In the first morning urine sample, the patient is instructed to empty the bladder on retiring and to collect the first void after awaking up from sleep. These methods should ideally reflect the real magnitude of albumin excretion in the urine and are minimally affected by biological variations.

Several studies have recently tried to identify the best method for screening of microalbuminuria. In a subset analysis of the PREVEND (Prevention of Renal and Vascular End-stage Disease) study, the researchers compared ACR and UAC, both from first morning void and random sample; the outcome of interest was which test better replicated 24-hour urine collection (Witte et al., 2009). First morning ACR sample correlated with 24-hour urine collection with similar variability and prevalence. Another subset analysis of the PREVEND study concluded that ACR from first morning samples was equivalent to 24-hour urinary albumin excretion (UAE) in predicting all-cause mortality and cardiovascular morbidity after 7.5 years follow-up (Lambers Heerspink et al., 2008). Seven hundred patients with type II diabetes participating in the RENAAL

(Reduction of Endpoints in Non insulin Dependent Diabetes Mellitus with Angiotensin II Antagonist Losartan) trial were followed for 3.4 years (Lambers Heerspink et al., 2010). The study compared the ability of two timed collection methods (urinary protein excretion and UAE) and two first morning urine collection methods (UAC and ACR) in predicting renal outcome (doubling of creatinine or need for dialysis). The study revealed that ACR was the best predictor for renal outcomes. Taken together, these studies clearly demonstrated the reliability of ACR in detecting clinically relevant microalbuminuria.

First morning samples measured by ACR exhibit low variability and hence better reliability in microalbuminuria screening. While UAC tends to be reduced when there is high urine volume, the ACR corrects for variability in urine excretion as the amount of creatinine filtered over time is relatively constant (Newman et al., 2000). Urine from first morning void is usually concentrated and may reflect the real magnitude of urinary albumin excretion without the influence of confounding factors such as exercise.

Several international guidelines recommend repeated samples for diagnosing microalbuminuria (two out of three samples should be positive) (National Collaborating Centre for Chronic Conditions, 2008). The repeated samples are suggested in order to overcome the effect of any possible confounders of microalbuminuria screening. However, this can be difficult to achieve in clinical setting. As a result, the diagnosis of microalbuminuria in the majority of studies (especially in hypertension) has been based on single samples (see later).

1.2.6 Epidemiology of microalbuminuria

The prevalence of microalbuminuria in different populations with the same clinical condition varies significantly. This variability might be due to several factors such as the

threshold used, measurement methods, instruments or extent of co-morbidities in the study population (e.g. in hypertension; mild, moderate or severe) (table 1-2).

1.2.6.1 Prevalence in general population

In the UK, data from 20,911 participants in the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) showed that the prevalence of microalbuminuria in this British population is about 11.8% (Yuyun et al., 2004c). Earlier studies suggested that the prevalence of microalbuminuria in the UK ranges from 4.7% to 10.2% (Yudkin et al., 1988, Winocour et al., 1992, Beatty et al., 1993). The prevalence of microalbuminuria in the US population was reported by the National Health and Nutrition Examination Surveys (NHANES) in two time periods; from 1988-1994 and from 1999-2004 (Coresh et al., 2007). Microalbuminuria prevalence increased significantly between the two periods from 7.1% to 8.2% (P =0 .01). This may be attributed to the increase in the prevalence of co-morbidities such as obesity and metabolic disorders.

Several studies have examined the prevalence of microalbuminuria in non-diabetic, non-hypertensive individuals. The PREVEND study is one of the largest studies to report the prevalence of microalbuminuria in a Caucasian cohort (Hillege et al., 2001). Questionnaires and urine containers were sent to all inhabitants of the city of Groningen in the Netherlands. Nearly one half responded (40,856 participants). Microalbuminuria was present in 6.6% of non-diabetic, non-hypertensive individuals. A similar prevalence (6.2%) was reported recently by the Kidney Evaluation and Awareness Program in Sheffield (KEAPS) (Bello et al., 2010). In a subset analysis of this study, the investigators found that the prevalence increased to 9.5% in those who have a family history of CKD (Bello et al., 2008).

The prevalence of microalbuminuria varies according to ethnicity. Microalbuminuria is more common in black and Asian populations compared with whites (Summerson et al., 1995). A comparison between South Asian and white European populations living in the UK revealed that microalbuminuria is significantly more common in Asians (31% versus 20%) (Dixon et al., 2006). In this study, there were no differences between the two populations in terms of age, sex or blood pressure levels. Another study compared UAE in African Caribbeans, South Asians and white Europeans (Tillin et al., 2005). The prevalence of increased UAE was higher in African Caribbeans than in South Asians and Europeans. In a cohort of 6801 subjects from different Asian countries (China, Korea, Indonesia, Philippines, Singapore, Malaysia) the prevalence of microalbuminuria was 39.8% (Weir, 2004). However, in that study, urinary albumin detection was performed by semi-quantitative dipsticks and more than 30% of the involved subjects had strong family history of cardiovascular or metabolic disease. This may partially explain the high prevalence of microalbuminuria. In a large epidemiological study based on diabetic subjects attending primary care clinics, hypertensive Hispanic subjects and non-hypertensive Asians showed prevalence of microalbuminuria higher than that in Whites subjects (Young et al., 2005).

The relation between microalbuminuria prevalence and gender is not clear. While some studies have shown that males have higher microalbuminuria prevalence (Pontremoli et al., 1997), others reported no differences (Calviño et al., 1999). In subjects with type I diabetes, the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) showed that UAE was associated with male gender and this association was explained by central obesity (measured as waist-hip ratio- WHR) (Sibley et al., 2006). When the prevalence was adjusted for WHR, no association between UAE and gender was observed. In the EPIC-Norfolk study, the

prevalence of microalbuminuria was significantly higher in females (14.1% versus 8.4%, P<0.001) (Yuyun et al., 2004d).

1.2.6.2 Prevalence in subjects with diabetes mellitus

Microalbuminuria screening is recommended by the majority of clinical guidelines for the management of diabetes (American Diabetes Association, 2011). The prevalence of microalbuminuria in diabetic individuals has been addressed by many studies. The Oxford Regional Prospective Study recruited children aged under 16 years with type I diabetes in order to examine the development of microalbuminuria (Amin et al., 2008). The study reported that the prevalence of microalbuminuria after 10 years and 20 years was 25.7% and 50.7%, respectively. Nearly 30% of adults with type I diabetes develop microalbuminuria in the 18 years following diagnosis (Hovind et al., 2004). In a large, clinic-based study involving 24,151 patients with type II diabetes from 33 countries, ACR was measured randomly on single occasion and the prevalence of microalbuminuria and proteinuria were estimated to be 39% and 10%, respectively (Parving et al., 2006).

The United Kingdom Prospective Diabetes Study (UKPDS 64) recruited 5097 type II diabetes patients (67% newly diagnosed) from different ethnic origins and followed them for 20 years in order to observe the progression of diabetic subjects to nephropathy (Adler et al., 2003). At baseline, nearly 7% of the study population had microalbuminuria and after 5, 10 and 15 years; the prevalence was 17%, 25% and 28%, respectively.

Recently, Vupputuri et al. (Vupputuri et al., 2011) reported that the prevalence of normoalbuminuria, microalbuminuria and gross proteinuria at baseline in a population-based analysis of people with type II diabetes in the United States (US) were 57%, 31% and 12%. After mean follow-up of 5.4 years, 40% of patients with normoalbuminuria

developed microalbuminuria and nearly 6% of microalbuminurics progressed to gross proteinuria.

1.2.6.3 Prevalence of microalbuminuria in subjects with hypertension

Although several studies have attempted to define the prevalence of microalbuminuria in essential hypertension, the exact figure is still unclear. The published prevalence of microalbuminuria in hypertensive subjects ranges from 4.7% to 58.4% (Jensen et al., 1997, Böhm et al., 2007).

A longitudinal study involving 1,041 young (18-45 years) participants with mild hypertension (stage 1) demonstrated that microalbuminuria, detected using the 24-hour method, was present in 6% of the study population, and 9% had high normoalbuminuria (16–29.9 mg/24 h) (Palatini et al., 2005). The low prevalence of microalbuminuria in this particular study may be explained by the age of the participants, as microalbuminuria tends to be more common in older subjects (Agrawal et al., 1996) and possibly the early stage of hypertension.

In a multi-centre observational study conducted in Italy, the prevalence of microalbuminuria in subjects with hypertension (n=3534) was 27% (Leoncini et al., 2010). More than one-third of the population had diabetes and microalbuminuria was measured in a single sample, which may affect the precision of the estimation of microalbuminuria prevalence. A more recent study of hypertensive subjects without established cardiovascular disease or renal impairment suggested that microalbuminuria, measured on a single occasion by 24-hour collection, is present in 23% of the studied population while less than 1% of the subjects had gross proteinuria (Cerasola et al., 2010).

Using urine dipsticks in the detection of microalbuminuria seems to overestimate the prevalence. For instance, in a large cohort of non-diabetic hypertensive subjects in Germany, the prevalence of microalbuminuria was 32% (Agrawal et al., 1996). In this study, the diagnosis of microalbuminuria was based on two positive urine dipsticks (Micral-Test) out of three. A highest prevalence of microalbuminuria was observed in the i-SEARCH global study, where 58.4% of the study population had microalbuminuria (Böhm et al., 2007). Almost 21,000 subjects from 26 countries participated in this clinic-based study, making it one of the largest epidemiological studies that examine the prevalence of microalbuminuria in hypertensive subjects. The overall prevalence of microalbuminuria among participating countries was high, with rates of 53 to 71% in some developing countries. However, one of the limitations of this study was that the diagnosis of microalbuminuria was based on a single urine specimen measured using urine strips. Also, the majority of the study population was over 60 years of age and diabetic, which may contribute to the high prevalence of microalbuminuria.

In summary, the reported prevalence of microalbuminuria has been limited by measurement method, under-precision caused by single screening, small sample size or too many restrictions on population tested. There is limited reliable information on microalbuminuria in hypertension and thus, the prevalence remains to be elucidated.

1.2.7 Pathogenesis of microalbuminuria

The pathophysiological mechanisms underlying the presence of urinary protein in hypertensive individuals are not completely understood. Several mechanisms have been proposed. These mechanisms involve alteration of renal haemodynamics and endothelial changes (Mattei et al., 1997).

As mentioned earlier, the afferent arteriole plays an important role in maintaining glomerular pressure. In hypertension, the afferent arteriole tends to constrict in order to protect the kidney from the high hydrostatic pressure caused by high blood pressure (Ito et al., 1992). This process is also regulated by feedback mechanism mediated by specific cells in the distal convoluted tubule when the volume of filtrate is high resulting in constriction of afferent arteriole to maintain GFR (Mountokalakis, 1997). Prolonged high blood pressure is thought to induce impairment of this autoregulatory process leading to hyperfiltration and passage of albumin into urine (Mountokalakis, 1997). This hypothesis is supported by the observation that in an experimental animal model, partial nephrectomy led to hyperfiltration in the remaining nephrons in order to maintain GFR, which in turn led to increase in the intra-glomerular pressure and passage of protein into the urine (Metcalfe, 2007).

It has been postulated that impairment of glomerular permeability also contributes to the pathophysiology of microalbuminuria (Dalla Vestra et al., 2003). Alteration of glomerular permeability occurs as a result of changes in the structure of the glomerular filtration barrier. This barrier consist of three layers; endothelium covered by anionic glycoprotein (glycocalyx), podocyte foot processes and glomerular basement membrane, figure 1-1 (Satchell and Braet, 2009). These layers are responsible for preventing some molecules from escaping to the lumen of Bowman capsule. The negative charge of the glycocalyx helps in repelling albumin as it is also negatively charged. Therefore, any structural changes could translate into passage of larger amount of albumin in the urine. Impairment of the podocytes (White et al., 2004, Toyoda et al., 2007) and glycocalyx (Nieuwdorp et al., 2006) have been shown in diabetic patients with microalbuminuria and subjects with hypertensive nephrosclerosis (Wang et al., 2009).

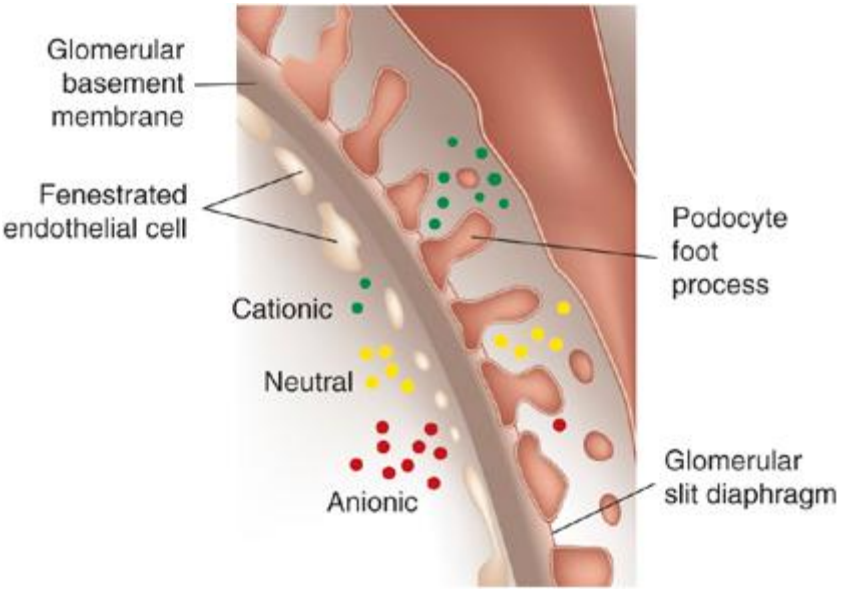


Figure 1-1: The glomerular filtration barrier. Consists of three layers; the endothelium, the glomerular basement membrane and podocyte foot processes. Each layer plays important role in preventing some molecules from being excreted as an ultrafiltrate. Adapted with permission from (Miner, 2008).

Impaired function of the renin-angiotensin aldosterone system (RAAS) has also been implicated. Angiotensin II has been found to contribute in the sclerosis of the podocytes leading to excretion of large molecules including albumin (Matsusaka et al., 2010). Activation of angiotensin II type 1 receptors leads to an increase in the production of different inflammatory mediators and reactive oxygen species (ROS) which mediate endothelial injury locally in the kidney and systemically in the blood vessels, see figure 1-2 (Warnholtz et al., 1999, Basi and Lewis, 2006). This hypothesis is supported by the observation that inhibition of angiotensin receptors using pharmacological approaches and drugs with putative anti-oxidant properties (e.g carvedilol) are associated with a significant reduction in microalbuminuria and several biomarkers of inflammation and oxidation (Persson et al., 2006, Bakris et al., 2005).

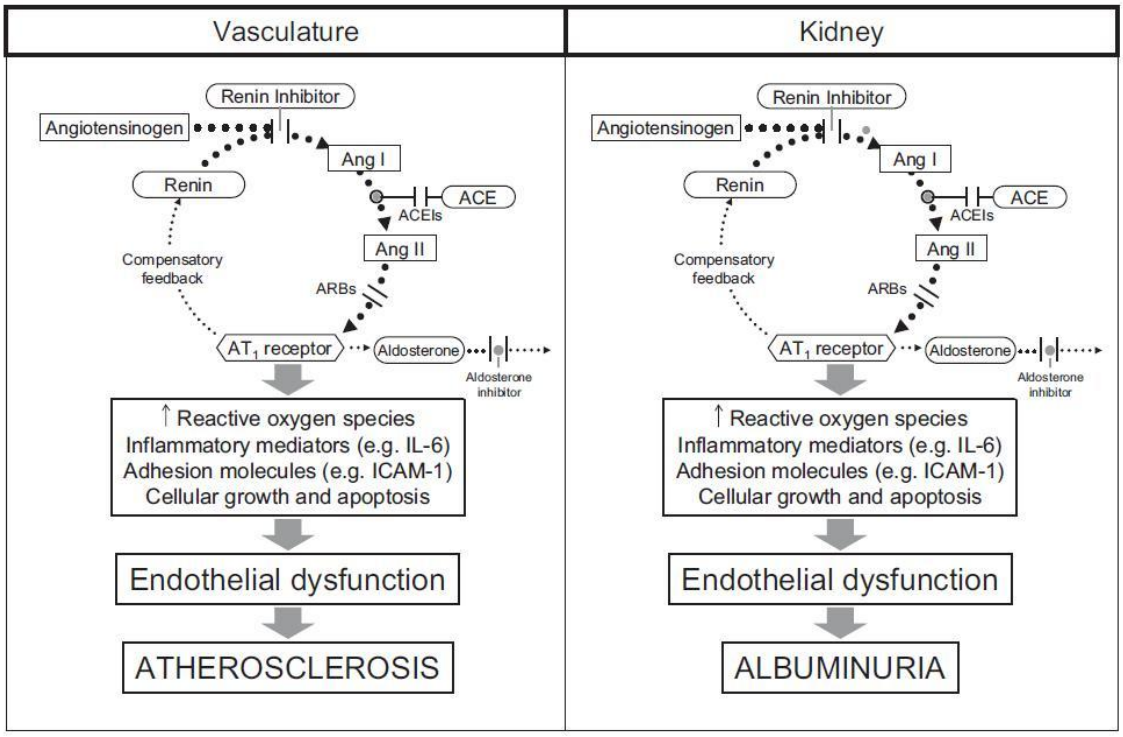


Figure 1-2: Simple illustration of mechanisms by which malfunction of RAAS could mediate albuminuria and atherosclerosis. Adapted with permission from (Basi and Lewis, 2006).

It has been suggested that albuminuria is associated with generalised endothelial dysfunction and it is widely accepted that microalbuminuria is a reflection of systemic vascular abnormalities that may explain the association of microalbuminuria with different cardiovascular diseases (Moody et al., 2012). Cottone et al. (Cottone et al., 2006) have shown that levels of several adhesion molecules that are involved in atherosclerosis of blood vessels correlated with UAE. Changes in the endothelial production of nitric oxide in diabetic mice lacking endothelial nitric oxide synthase is associated with higher levels of albuminuria compared with control mice (Mohan et al., 2008).

All previously mentioned mechanisms could mediate microalbuminuria. Renal hemodynamic abnormalities as a result of increased blood pressure may increase glomerular permeability. In addition, increased permeability may result from endothelial

dysfunction due to increased oxidative stress and high levels of inflammatory markers caused by defects in renin-angiotensin system (RAS).

1.2.8 Association of microalbuminuria with diseases and risk factors

Over the last decade, our understanding of the importance of microalbuminuria as an independent risk factor for cardiovascular and metabolic morbidities has expanded exponentially. An increasing body of evidence suggests that microalbuminuria is a useful tool for predicting several diseases (Klausen et al., 2004). This is because microalbuminuria may reflect more complex vascular changes in the body. However, the awareness of the association of microalbuminuria with various diseases among clinicians is still poor. Haller and colleagues (Haller et al., 2010) conducted a survey among 1700 clinicians (general practitioners, cardiologist and diabetologists) in five European countries to evaluate the awareness of importance of microalbuminuria in clinical practice. The vast majority (over 93%) of clinicians was aware of the association of microalbuminuria with impairment of renal function. Awareness of the association of microalbuminuria with abnormalities in other organs was extremely limited. For example, among participated clinicians in the UK, only 8%, 9%, 10%, 16% and 4% were aware of the relation between microalbuminuria and microvascular, cardiac, macrovascular, eyes and brain complications, respectively. This finding is disappointing as screening of microalbuminuria is easy and relatively inexpensive and the result may guide the clinician to identify individuals at high risk for cardiovascular complications.

1.2.8.1 Microalbuminuria and high blood pressure

The association of high blood pressure with increased UAE is plausible. High blood pressure is one of the proposed mechanisms for the presence of albumin in the urine and is one of the most frequently reported predictors of microalbuminuria (Moran et al., 2006).

In studies that aimed to characterise microalbuminuria in non-diabetic subjects with hypertension such as the MAGIC (Microalbuminuria: A Genoa Investigation on Complications) study (Pontremoli et al., 1997) and in non-diabetic subjects from general population such as the Gubbio Population studies (Cirillo et al., 1998), there were direct relationships between high blood pressure and microalbuminuria. This relation was also shown in a large cross-sectional study involving 4 subgroups; diabetic patients with severe atherosclerosis, non-diabetic with severe atherosclerosis, diabetic with mild atherosclerosis and non-diabetic with mild atherosclerosis (Hsu et al., 2009). In this study, high blood pressure, even below the threshold level for the definition of hypertension, was significantly associated with albuminuria in the four groups.

The relationship between albuminuria and increased blood pressure has also been reported in studies using 24-hours blood pressure monitoring (Clausen et al., 1998). In a small group of subjects without evidence of cardiovascular or metabolic abnormalities there were positive correlations between UAE and 24-hour SBP, DBP and pulse pressure (Clausen et al., 1998). This relationship was also evident in diabetic patients (Januszewicz et al., 2011), hypertensive subjects with end organ damage (Wiinberg et al., 2004, Moran et al., 2006), resistant hypertension (Oliveras et al., 2009) and general hypertension population (Gerber et al., 2001). These findings are of particular importance since ambulatory blood pressure is now considered the gold standard for the diagnosis of hypertension and the identification of subjects with true refractory hypertension (National Institute for Health and Clinical Excellence, 2011) and therefore, may represent the most reliable data for the association with increased UAE.

Increased UAE is found in those lacking the normal blood pressure reduction during sleeping (non-dippers) (Bianchi et al., 1994). UAE was related to non-dipping pattern in a cohort of recently diagnosed hypertension (Afsar and Elsurer, 2010), in subjects with

untreated hypertension (Syrseloudis et al., 2010) and those with resistant hypertension (Oliveras et al., 2011).

1.2.8.2 Microalbuminuria and renal function

Microalbuminuria is best recognised for the association with deterioration in renal function. Microalbuminuria may be present at an early stage of kidney disease, even when eGFR is normal (Levey et al., 2003). Recent recommendations suggest the use of albuminuria in addition to eGFR for the prediction of end stage renal disease (ESRD) (Hallan et al., 2009, Astor et al., 2011). In a study involving almost 66,000 patients followed for ten years (Hallan et al., 2009) both eGFR and albuminuria were independent predictors for ESRD. This association was also reported recently in diabetic subjects followed for 10 years (Berhane et al., 2011) and in subjects with high cardiovascular risk (Clase et al., 2011). Combining eGFR and albuminuria screening identified hypertensive subjects at high risk of cardiovascular morbidities and mortalities in a prospective cohort study (Salles et al., 2011b). In a Canadian cohort study with a large number of patients (> 900,000), increased albuminuria was a strong predictor for progression to ESRD (Hemmelgarn et al., 2010). In a sub-analysis of the MAGIC study, 917 non-diabetic hypertensive subjects with normal kidney function were followed for a median of 11.8 years (Viazzi et al., 2010). More than one-third of those who developed chronic renal insufficiency had microalbuminuria at baseline compared with 7% in the control group. The association of microalbuminuria with renal outcome was independent of eGFR or other confounders. Likewise, in a retrospective study of hypertensive subjects followed for 7 years, microalbuminuria was associated with decline in creatinine clearance and increased cardiovascular events (Bigazzi et al., 1998). Regression or significant reduction of UAE (defined as reduction exceeds 50%) in non-diabetic hypertensive subjects was associated with better renal outcomes (Pascual et al., 2006). These finding clearly suggest

that microalbuminuria provides information additional to that provided by eGFR in prediction and identification of kidney function impairment. Therefore, routine checks for those at high risk for chronic disease should include albuminuria screening in order to prevent or delay possible progression to ESRD.

1.2.8.3 Microalbuminuria and cerebrovascular abnormalities

Stroke risk

Several cross-sectional and prospective studies suggest that microalbuminuria is prevalent in those with recent acute stroke and may predict future stroke incidences (Lee et al., 2010). In a prospective case-control study involving patients with recent acute stroke (within a week) or at high risk for developing stroke (hypertension, diabetes, transient ischemic attack (TIA) or ischaemic heart disease), the prevalence of microalbuminuria in those with stroke was significantly greater than that in the high-risk group (29% versus 10%) (Beamer et al., 1999). During a mean follow-up period of 1.5 years, one-fifth of subjects with recent stroke and almost 15% of the high-risk group developed vascular events. New onset vascular events were more frequent in subjects with albuminuria even after adjusting other risk factors such as high blood pressure, diabetes and cigarette smoking.

The Losartan Intervention For Endpoint reduction (LIFE) examined the association of microalbuminuria with cardiovascular events in 8206 hypertensive subjects with LVH (Wachtell et al., 2003). After follow up for a median of 4.8 years, the study revealed that for every 10-fold increase in UAE, the risk for stroke increased by 50%. However, this result has some limitations. Microalbuminuria was detected using only one specimen and did not include any measure to exclude those with UTI. Also, the study was performed in

high-risk population and whether the same pattern may be observed in patients without LVH is not clear.

In the UK, data from the EPIC-Norfolk study, a general population study with a mean follow up of 7.2 years, revealed that stroke risk was increased by 50% in subjects with microalbuminuria (Yuyun et al., 2004a). A Cox multiple regression model showed that the hazard ratio for the association between microalbuminuria and stroke events was greater than that for some traditional risk factors such as total cholesterol, SBP and body mass index (BMI). The detection of microalbuminuria in this study was based on one random sample per patients which may overestimate albuminuria prevalence as discussed previously.

A meta-analysis involving twelve studies with more than 48,000 patients evaluated the relation of microalbuminuria with stoke (Lee et al., 2010). These studies involved different populations with or without different co-morbidities such as prior stroke, hypertension, cardiovascular disease or diabetes mellitus. Microalbuminuria was strongly associated with stroke events. In addition, subjects with microalbuminuria had a 90% greater risk for developing future stroke incidents compared with individuals with normoalbuminuria.

In the survey by Haller et al. (Haller et al., 2010), the association of microalbuminuria with brain abnormalities was the least recognised association, especially in the UK. This indicates that clinicians may miss an easy and non-invasive technique for identifying individuals at high risk of cerebrovascular diseases.

Cognitive decline and cerebrovascular changes

The largest body of evidence for the interaction between albuminuria (microalbuminuria and proteinuria) and cognitive function comes from the Ongoing Telmisartan Alone and in

Combination with Ramipril Global Endpoint Trial (ONTARGET) / the Telmisartan Randomized AssessmeNt Study in ACE iNtolerant subjects with cardiovascular Disease (TRANSCEND) (Barzilay et al., 2011). More than 28,000 patients suffering from vascular diseases or diabetes mellitus participated in these trials. Cognitive function was assessed at baseline and after 5 years using a 30-point Mini–Mental State Examination (MMSE) to evaluate the degree of dementia. Subjects with albuminuria at baseline had lower MMSE scores (less than 24) and higher odds for reduced cognitive function. Similarly, those who developed albuminuria during the study had increased odds for cognitive impairment. One of the drawbacks of the study is the population studied. The conclusions cannot be extended to those without diabetes or vascular abnormalities as these disorders may also interact with cognitive decline.

Ravera and colleagues (Ravera et al., 2002) investigated the association with subclinical cerebrovascular changes in a small number of untreated hypertensive subjects (11 with microalbuminuria and 11 with normoalbuminuria) with no clinical neurological disorders. Using magnetic resonance imaging, the researchers found that subjects with microalbuminuria had greater prevalence of cerebral lacunar infarcts. In a cohort of elderly subjects in Japan, cerebral small vessels disease (SVD) was correlated with microalbuminuria (Wada et al., 2007). In addition, ischaemic lacunar lesions and white matter hyperintensity were more prevalent in subjects with microalbuminuria. Another cross-sectional study carried out in 2316 elderly subjects showed that microalbuminuria was significantly related to dementia and mild cognitive impairment (Barzilay et al., 2008). In 285 hypertensive subjects without evidence of stroke, TIA or severely reduced renal function (Umemura et al., 2012), microalbuminuria was significantly related to deep or infratentorial cerebral microbleeds investigated by neuroimaging. Brain microbleeds were also strongly related to proteinuria in patients who had stroke or TIA events (Ovbiagele et al., 2010).

These findings are important for several reasons. First, cerebral SVD has been increasingly linked with several brain abnormalities such as cognitive decline, age-associated disorders and stroke (Leonardo, 2010). Second, white matter hyperintensities, possibly mediated by SVD-induced ischaemia, have been shown to predict stroke, cognitive decline and mortality (Stéphanie and Markus, 2010). Third, association between brain microbleeds and neurological disorders such as Alzheimer's disease (Goos et al., 2009) and mortality (Henneman et al., 2009) has been suggested.

The identification of cerebrovascular changes such as SVD, white matter lesions and cerebral microbleeds require time-consuming, expensive and are not routinely requested investigations (ultrasonography or special neuroimaging). Microalbuminuria screening could serve as an appropriate tool for identifying population at high risk for cerebrovascular complications.

1.2.8.4 Microalbuminuria and cardiovascular abnormalities

A growing body of evidence links microalbuminuria with abnormalities in the cardiovascular system. As mentioned earlier, microalbuminuria reflects generalised endothelial dysfunction which is a key factor for several cardiovascular diseases such as CHD, myocardial infarction heart failure, hyperlipidemia and atherosclerosis.

Ischaemic heart disease risk

The prognostic value of microalbuminuria for ischaemic heart diseases is suggested by evidence from a number of studies involving different population (Klausen et al., 2004). Subjects with untreated hypertension or high-normal blood pressure were followed for ten years in the Danish MONICA (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease) project (Jensen et al., 2000). Among hypertensive subjects, 9%

had future ischaemic disease. The prevalence of microalbuminuria in those who developed ischaemic heart disease was higher than that in the control group. The relative risk for developing ischaemic attack associated with microalbuminuria after adjusting for known factors such as blood pressure, lipids, gender and BMI was 3.5. These findings were confirmed in a UK population-based study where microalbuminuria and gross proteinuria predicted future episodes of CHD with hazard ratios of 1.36 and 1.59, respectively (Yuyun et al., 2004d).

The prognostic value of microalbuminuria for short and long-term outcomes following acute myocardial infarction has also been investigated. Berton et al. (Berton et al., 1997) studied albuminuria in patients hospitalised for acute myocardial infarction and compared findings with a control group (those hospitalised because of suspected myocardial infarction but who did not meet the diagnostic criteria). UAE, measured by 24-hour urine collection, was higher on the day of admission and after 2 days compared with the control group. However, participants in the control group were younger (i.e. not age-matched) and had fewer cardiovascular co-morbidities which may bias the comparison. Another study showed that more than one-third of hospitalised patients with acute myocardial infarction had increased UAE (Apostolovic et al., 2011). Increased UAE was a strong predictor for myocardial infarction complications including mortality.

A study involving 175 non-diabetic patients with acute myocardial infarction followed for 3 years aimed to identify factors associated with subsequent cardiovascular events or death (Koulouris et al., 2005). Microalbuminuria, measured by timed overnight urine collection on the third day after the event, was an independent predictor for both endpoints. Similar findings were shown in a larger non-diabetic cohort where cardiovascular complications following myocardial infarction episodes were higher than in those without microalbuminuria (Lekatsas et al., 2006). Thus, in patients with myocardial infarction,

increased UAE may identify those at high risk for complications. More rigorous treatment for these patients may lower risk for future complications.

Left ventricular hypertrophy and structural changes

The LIFE study investigated the relation between increased UAE and LVH in a population with essential hypertension (Wachtell et al., 2002). Almost 9200 hypertensive subjects with evidence of LVH on electrocardiography (ECG) but without evidence of recent stroke, low left ventricular ejection fraction (less than 40%), myocardial infarction or severe kidney impairment participated. Both microalbuminuria and proteinuria were strongly associated with LVH even after adjusting possible confounders such as high blood pressure, increased age and diabetes.

Data from the Hypertension Genetic Epidemiology Network (HyperGEN) demonstrated that albuminuria is associated with increased left ventricular size (on echocardiography) in normotensive as well as hypertensive individuals; reduced ejection fraction has seen only in microalbuminuric hypertensive subjects (Djoussé et al., 2008). Electrocardiological abnormalities such as rhythm abnormalities, intraventricular conduction defects, ventricular repolarisation alterations, and left-axis deviation were also associated with microalbuminuria (Sciarretta et al., 2009).

It has been shown recently that in hypertensive subjects with ventricular diastolic impairment, microalbuminuria is associated with increased ventricular remodelling, stiffness and increased levels of NT-proBNP, a marker for heart wall stress (Shah et al., 2011). In contrast, reduction in eGFR did not show an association with such changes in the myocardium. This means that microalbuminuria provided information additional to that offered by the classical marker of kidney disease. The importance of such findings is that

microalbuminuria could identify those with high risk for progression to heart failure and help to identify pharmacological agents with properties to prevent such progression.

Congestive heart failure (CHF)

As microalbuminuria is associated with cardiac outcomes such as CHD and myocardial infarction, an association with CHF is expected. This association was confirmed in the Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity (CHARM) trial (Jackson et al., 2009). Two thousand three hundred and ten patients with heart failure participated in the programme. Albuminuria was found in 41% of the patients (30% microalbuminuria and 11% gross proteinuria). Similar prevalences were noted after excluding diabetes and hypertension. In this patient population, albuminuria was useful predictor for mortality and further deterioration in the myocardium in the studied population.

The prevalence of microalbuminuria and gross proteinuria in a small cohort of subjects who participated in the ALiskiren Observation of heart Failure Treatment (ALOFT) study was 33% and 11%, respectively (Jackson et al., 2011). Albuminuria detected by dipstick in an Asian population was correlated with CHF with preserved left ventricular ejection fraction and predicted future death (Miura et al., 2012). In hypertensive subjects with LVH in the LIFE study, albuminuria was associated with twofold increase in risk of incident heart failure (Okin et al., 2008).

In the general population, data from the Multi-Ethnic Study of Atherosclerosis (MESA) showed that albuminuria was the strongest predictor of new-onset CHF in 6,800 individuals with different ethnic background (Bahrami et al., 2008). Although the follow-up period (4 years) was too short for accelerate estimation of such an outcome, this study is of particular interest for several reasons. First, it described the incidence of severe cardiac

outcome in apparently healthy subject without clinically detectable cardiovascular disease. Second, the study involved subjects from four different ethnicities; Caucasians, black, Hispanics and Asian. Finally, the researchers showed the association between albuminuria and heart failure even after adjustment for traditional risk factors. This observation suggests that albuminuria is important in risk stratification for severe cardiac outcomes.

Vascular diseases

Arterial stiffness measured by pulse wave velocity (PWV) has been reported in subjects with microalbuminuria (Ishikawa et al., 2008). This condition is a risk factor for future cardiovascular events (Willum Hansen et al., 2006). Loss of arterial elasticity leads to increase in the force of contractions of the heart which in turn may lead to LVH and severe complications (Willum Hansen et al., 2006, Sutton-Tyrrell et al., 2005).

In subjects without hypertension or diabetes, arterial stiffness was associated with microalbuminuria after adjusting for possible confounders (Kim et al., 2011). In small cohorts of untreated hypertensive subjects, the prevalence of microalbuminuria in those with arterial stiffness measured by carotid / femoral arteries PWV or ambulatory arterial stiffness index was significantly higher (Ratto et al., 2006, Mule et al., 2009). The association remained significant in adjusted regression models.

Microalbuminuria has been also linked with coagulation abnormalities. A sub-analysis from the PREVEND study reported an increased incidence of venous thromboembolism in subjects with microalbuminuria (Mahmoodi et al., 2009). The incidence rate was 0.40% per year for those with UAE $\geq$ 30 -300 mg per day compared with only 0.12% per year for those with UAE < 15 mg per day ($P<0.001$). A recent post-hoc analysis of the PREVEND study showed that microalbuminuria was also associated with increased risk for recurrent venous thromboembolism (van Schouwenburg et al., 2012).

A direct relationship between albuminuria and atherosclerosis has been observed in many studies. Both conditions share a common pathological mechanism, endothelial damage. In a Norwegian study, a direct correlation between ACR and de novo formation and progression of atherosclerotic plaque in the carotid artery was reported in subjects without diabetes (Jørgensen et al., 2007). Moreover, microalbuminuria predicted the formation and the progression of early marker of atherosclerotic lesion in the carotid artery (calcification) in clinically health individuals participated in the MESA study (DeFilippis et al., 2010). Increased carotid intima-media thickness, a risk factor for atherosclerosis, was also observed in hypertensive subjects with microalbuminuria (Cao et al., 2006).

1.2.8.5 Microalbuminuria and mortality

Microalbuminuria has been shown to predict those at high risk of all-cause and cardiovascular mortality, especially after myocardial infarction (Berton et al., 2001). As mentioned earlier, microalbuminuria is a common finding in subjects with very recent acute myocardial infarction (Berton et al., 1997). Therefore, studies have investigated whether such association can help in identifying subjects with increased risk of mortality. A large cohort of patients (n >1200) with acute myocardial infarction were divided into three groups according to the result of timed-urinary albumin excretion; normoalbuminuria, microalbuminuria and those with gross proteinuria (Schiele et al., 2009). The mortality rate was found to be associated with the level of albuminuria. Low survival rate was reported in patients with albuminuria two days after myocardial infarction. After one month, those with microalbuminuria had greater than twofold increased mortality risk compared with individuals with normoalbuminuria.

Data from the EPIC-Norfolk study showed a continuous relationship between albumin level in the urine and cardiovascular mortality; adjusted hazard ratio 2.03 and all-cause

mortality; 1.48 (Yuyun et al., 2004b); this relationship was evident in males and females. However, an association with non-cardiovascular death was observed only in males. The diagnosis of albuminuria was based on one random urine specimen and this is one of the limitations of this study. In a Norwegian study (the Nord-Trøndelag Health Study), 5369 treated hypertensive subjects (43% males) were followed for more than 4 years (Romundstad et al., 2003a). ACR showed a positive correlation with all-cause mortality after adjusting for common risk factors such as blood pressure, glucose, lipids, renal function and smoking. Men had higher probability for fatal end-point. These findings suggest that when using microalbuminuria for risk stratification, sex differences should be taken into account.

In a meta-analysis of data from more than 100,000 individuals in the general population, albuminuria was shown to be significantly related to all-cause and cardiovascular mortality even after adjusting for all other risk factors including eGFR (Matsushita et al., 2010). Another meta-analysis of 13 studies showed that albuminuria is an independent predictor for mortality in patients with CKD (Astor et al., 2011).

Proteinuria (>500 mg per day) has been postulated to predict cardiovascular death in diabetic patients (Cardoso and Salles, 2008). This was supported by finding from an observational study which examined the mortality rate over 10 years in type 1 diabetics and showed that 44% of subjects who died had proteinuria (Rossing et al., 1996)

The association with mortality would be of particular importance if reduction in albuminuria level is accompanied with reductions in mortality rates. In a study involving more than 23,000 high risk patients followed for two years, it has been found that cardiovascular mortality increases by 50% for every two-fold or more increase in albuminuria from baseline (Schmieder et al., 2011). The study also reported that two-fold

or more reduction in albuminuria was associated with 15% reduction in death. This finding reflects the importance of microalbuminuria not only as a biomarker for cardiovascular risk but also a useful target for pharmacological intervention.

1.2.9 Microalbuminuria and inflammatory markers

It has been suggested that individuals with microalbuminuria have increased levels of inflammatory markers (Pedrinelli et al., 2004). Such an association might contribute to the involvement of microalbuminuria in the pathology of cardiovascular disease since it is increasingly recognised that the pathogenesis of some cardiovascular disease such as atherosclerosis has an inflammatory component (Libby et al., 2009).

The vast majority of studies that have investigated subclinical inflammatory reactions in subjects with microalbuminuria have utilised CRP. Data from the National Health and Nutrition Examination Surveys showed that in the general population, levels of CRP were correlated weakly with microalbuminuria (odds ratio 1.02, 95% confidence interval 1.01 to 1.02, p = 0.0003) (Kshirsagar et al., 2008). The Oxford Regional Prospective Study reported a continuous relationship between hs-CRP levels and microalbuminuria in subjects with type I diabetes mellitus (Marcovecchio et al., 2008). The study also reported that levels of hs-CRP in microalbuminuric patients were significantly higher than in those with normal UAE.

A small cohort of subjects (n=91) with high-normal blood pressure (SBP: 120-139/ DBP: 80-89 mm Hg) were compared with those with blood pressure less than 120/80 mm Hg in terms of UAE (measured by 24-hour urine collection following two positive early morning measurements) and inflammatory markers (Navarro-Gonzalez et al., 2012). Individuals with high-normal blood pressure had high levels of UAE and hs-CPR. In non-diabetic subjects with untreated hypertension, microalbuminuria was strongly correlated with level

of hs-CRP (Pedrinelli et al., 2004, Tsioufis et al., 2010). These findings suggest that microalbuminuria is closely related to a sensitive marker of inflammation which is considered by itself as risk factor for cardiovascular disease.

In patients with type 2 diabetes mellitus, eosinophil count was significantly associated with the degree of urinary albumin excretion in men (Fukui et al., 2009). Another study showed a linear relationship between eosinophil count and the severity of diabetic nephropathy (Chung et al., 2005).

A relation between ESR and total urinary protein has been suggested (Liverman et al., 1988). In type 2 diabetic patients, ESR levels showed a linear relationship with urinary albumin excretion (Dalla Vestra et al., 2005). This was also shown in a recent study involving diabetic subjects with retinopathy where ESR levels in those with albuminuria (both microalbuminuria and gross proteinuria) were greater than in those with normal UAE (Magri et al., 2012).

1.2.10 Low-grade microalbuminuria

A mounting body of evidence suggests that low levels of urinary albumin excretion (below the conventional threshold) are associated with increased cardiovascular risk (Ärnlöv et al., 2005). The relationship between albuminuria and cardiovascular outcomes seems to exhibit a continuous relationship with no clear threshold for such association. The conventional threshold that is used currently for the definition of microalbuminuria is arbitrary and was chosen more than 25 years ago based on data of diabetic patients (Forman and Brenner, 2006).

The PREVEND study was among first studies that reported the association between albuminuria at levels greater than or equal to 3.8 mg/L and mortality in subjects without known risk factor for cardiovascular disease (Janssen et al., 2000). An increase in the UAC

from 5 to 10 mg/L was associated with almost one-third increased cardiovascular death in the that study (Hillege et al., 2002). In 1,568 individuals without diabetes or hypertension who participated in the Framingham Offspring Study, low ACR (≥ 3.9 mg/g in males and 7.5 mg/g in females) was significantly associated with three-time greater risk for cardiovascular diseases and higher death incidences (Ärnlöv et al., 2005). Low-grade microalbuminuria has also been shown to predict increase in left ventricular mass (Reffelmann et al., 2010) and heart failure incidence in the general population (Blecker et al., 2011).

Klausen et al. (Klausen et al., 2004) reported that a UAE rate of more than 4.8 µg per minute strongly predicted CHD and cardiovascular death independent of age, gender, diabetes, high BP, kidney function or lipid profile. Low-grade UAE was shown to be associated with 2-3 fold increased risk of CHD (Borch-Johnsen et al., 1999). Low albuminuria level was also associated with increased risk of target organ damage such as LVH even in healthy subjects (Lieb et al., 2006). ACR levels of 10–16 µg per mg were associated with greater cardiovascular mortality (Zamora and Cubeddu, 2008).

The Framingham Heart study reported that low-grade UAE is a strong predictor for the progression of non-diabetic subjects from normotension to hypertension (Wang et al., 2005). Low-grade microalbuminuria was also an independent predictor for progression to hypertension in females participated in the Nurses' Health Study (Forman et al., 2008). However, results based on ambulatory blood pressure from the Hypertension and Ambulatory Recording Venetia Study (HARVEST) failed to find any predictive value of microalbuminuria or low-grade microalbuminuria in the development of sustained high blood pressure (Palatini et al., 2005). Therefore, current evidence is equivocal in support of the use of low-grade microalbuminuria in identifying those at high risk for developing hypertension.

The association of low-grade microalbuminuria with worse outcomes has been also shown in subjects with essential hypertension and those with diabetes mellitus (Wachtell et al., 2003). In a cohort of untreated hypertensive subjects, overnight UAE (< 15 μg/minute) was correlated with poor renal and cardiac outcomes (Dell'Omo et al., 2002). In those with end organ damage participating in the LIFE study, there was a clear association between low-grade microalbuminuria (≥ 2.2 mg/g) and increased risk for cardiovascular and cerebrovascular outcomes (Wachtell et al., 2003). Low levels of albuminuria were also associated with poor cardiac outcome (increased thickness of left ventricle and end-systolic stiffness) in hypertensive subjects with diastolic dysfunctions (Shah et al., 2010).

Taken together, these findings emphasize the importance of examining a new definition of microalbuminuria, at least in patients with certain diseases or those more susceptible to cardiovascular complications.

1.2.11 The use of antihypertensive drugs in microalbuminuria

1.2.11.1 Role of blood pressure control

It has been reported in many studies that reduction of high blood pressure reduces urinary albumin excretion and preserves the kidney (Bakris et al., 2000). Most studies reporting the benefit of blood pressure control in attenuation of albuminuria involved patients with diabetes (either normotensive or hypertensive), mainly because of the higher prevalence of renal disease in those patients (Strippoli et al., 2005). For instance, the UKPDS study reported that "intensive" blood pressure control (<150/85 mm Hg) by either beta blockers or ACEIs was associated with 29% lower microalbuminuria risk (UK Prospective Diabetes Study Group, 1998b). In another study involving 386 patients with type I diabetes mellitus and persistent microalbuminuria, SBP lower than 115 mm Hg was among predictors for microalbuminuria regression along with lowering glycosylated haemoglobin and serum

lipids (Perkins et al., 2003). In normotensive patients with type II diabetes, blood pressure reduction with an ACEI or a CCB to levels below 128/75 mm Hg was associated with significantly fewer patients progressing to microalbuminuria or gross proteinuria compared with a group taking placebo (Schrier et al., 2002).

In a trial involving a cohort of 77 patients with type II diabetes assigned to receive either an ACEI, a dihydropyridine CCB or placebo showed that both ACEI and CCB stabilised UAE over a median follow up of 5.5 years (Jerums et al., 2004). The Modification of Diet in Renal Disease Study also demonstrated that good blood pressure control with antihypertensive drugs was associated with reduction in albuminuria (Peterson et al., 1995). The study suggested a target of < 125/75 mm Hg for patients with gross proteinuria. In the ADVANCE study (The BP arm of the Action in Diabetes and Vascular disease: preterAx and diamicroN-MR Controlled Evaluation) that involved patients with type II diabetes (normotensive and hypertensive) followed for 4.3 years , reduction in blood pressure by fixed combination of ACEI/diuretic was associated with lower incidence of renal complications (including microalbuminuria and gross proteinuria) (de Galan et al., 2009).

These findings indicate that the most crucial step in controlling albuminuria (both micro- and gross proteinuria) is rigorous blood pressure control. Although specific antihypertensive classes have been suggested to confer additional anti-proteinuric properties beyond blood pressure reduction (as I shall discuss later), the current evidence is weak. Therefore, therapeutic strategies should focus achieving blood pressure targets rather than relying on a specific pharmacological class.

1.2.11.2 Renin-angiotensin-aldosterone system blockers

RAAS blockers are the most commonly used antihypertensive drugs for the control of urinary protein excretion (Mehdi et al., 2009, Menne et al., 2010). Several mechanisms by which RAS blockers may attenuate albumin excretion have been postulated. Reduction of glomerular capillary hydrostatic pressure by RAS blockers through arteriolar vasodilation has been suggested (Iñigo et al., 2001). Normalisation of glomerular basement membrane permselectivity is another postulated mechanism (Sangalli et al., 2011).

Few studies have examined the antiproteinuric effect of RAAS blockade in patients with type I diabetes mellitus (Mauer et al., 2009). Treatment with an ACEI preserved the kidney function of patients with gross proteinuria (Lewis et al., 1993). However, whether this was independent of better blood pressure control or whether similar effects can been seen with angiotensin receptor blockers (ARBs) is unknown. In a recent trial comparing renoprotection of ACEI and ARBs (versus placebo), a lower incidence of microalbuminuria was reported with ACEI, while treatment with ARBs was associated with increased new onset microalbuminuria (Mauer et al., 2009). However, both groups did not protect the kidney over 5 years follow up. This study is limited by the exclusion of people with hypertension (>135/85 mm Hg), as high blood pressure plays a major role in nephropathy in type I diabetes (Krolewski et al., 1988) and the small number of participants (n=285). A meta-analysis of 12 studies showed that treatment with ACEIs was associated with protection against progression to gross proteinuria and regression to normoalbuminuria (The ACE Inhibitors in Diabetic Nephropathy Trialist Group, 2001).

Several major trials addressed the effect of RAAS blockade in type II diabetes (Viberti et al., 2002, Lewis et al., 2001). In the RENAAL study, diabetic subjects with nephropathy (gross proteinuria) were assigned to receive losartan or placebo plus non-RAS blocker

therapy (Brenner et al., 2001). It was reported that patients in losartan group had a reduction of albuminuria of about 35% (compared with 4% in the placebo group). In the HOPE study, ramipril lowered the risk of progression to diabetic nephropathy by 24% (Heart Outcomes Prevention Evaluation Study Investigators, 2000b). The authors suggested a possible role of bradykinin in regressing albuminuria as ACEIs are known to increase this vasodilatory substance (Gainer et al., 1998). However, other RAS blockers (e.g. ARBs) appeared to be equivalent to ACEIs in attenuating albuminuria do not induce bradykinin (McMurray, 2011, Viberti et al., 2002). One of the confounders of direct comparison between RAS blockers and other antihypertensive drugs is the possible better early blood pressure control with RAS blockers. The Irbesartan Diabetic Nephropathy Trial (IDNT) provided the best evidence for the superior effect of ARBs in retarding progression of diabetic nephropathy to ESRD in hypertensive subjects than with CCBs as the blood pressure was identical in the two treatment groups throughout the follow up period (Lewis et al., 2001).

In a randomised trial involving 599 patients with diabetic nephropathy and hypertension, the addition of aliskiren to patients concomitantly receiving losartan resulted in 20% reduction of proteinuria (Parving et al., 2008). Blood pressure reduction in the active treatment group was modestly greater (2/1 mm Hg) than in the placebo group, which may contribute to the additional proteinuria lowering effect produced by aliskiren. However, the safety of the use of aliskiren with ACEIs or ARBs has been questioned recently. The manufacturer of aliskiren has decided to stop a clinical trial investigating the safety and efficacy of aliskiren in high-risk diabetic patients (Parving et al., 2009) due to increased incidence of hyperkalemia (predictable) and stroke (unpredicted) (Novartis, 2011). The US Food and Drug Administration (FDA) announced that the use of aliskiren with RAS blockers is contra-indicated in patients with diabetes due to increased risk of kidney disease, hypotension, and hyperkalemia (Food and Drug Administration, 2012).

Spironolactone produced a 32% reduction in proteinuria compared with placebo in a small study involving 20 patients with diabetes and overt nephropathy receiving other antihypertensive agents including RAS blockers (Schjoedt et al., 2006). Addition of spironolactone produced marked reduction of the blood pressure (7/5 mm Hg) compared with placebo. Addition of spironolactone to subjects with diabetic nephropathy under chronic treatment with RAS blockers resulted in further blood pressure reduction (7/3 mm Hg) and 40% less albuminuria (van den Meiracker et al., 2006). Moreover, in a randomised trial involving 81 patients with diabetes (type I or II), hypertension and gross proteinuria who were treated with highest dose of lisinopril (80 mg), participants were assigned to receive either losartan, spironolactone or placebo (Mehdi et al., 2009). Subjects receiving spironolactone had reduction in proteinuria greater than in those treated with losartan (34% versus 17%). In a study which compared the effect of spironolactone and cilazapril on proteinuria, spironolactone alone reduced proteinuria more than cilazapril, and combination therapy produced a further reduction (Rachmani et al., 2004). However, the publication was retracted due to uncertainty about the authenticity of the results (Diabetic Medicine, 2006).

Attenuation of microalbuminuria in patients with type II diabetes has been reported in the MARVAL (MicroAlbuminuria Reduction With VALsartan) study (Viberti et al., 2002). Valsartan reduced albuminuria more than amlodipine, although both drugs exhibited similar blood pressure reduction. However, the progression of renal disease is slow which means that follow up period in the MARVAL trial (24 weeks) may limit the validity of the study conclusion. Also, many studies reported beneficial effects of dihydropyridine CCBs on albuminuria such as in the Appropriate Blood Pressure Control in Diabetes (ABCD) trial (Estacio et al., 2000).

RAAS blockers have been shown to delay and reduce new onset microalbuminuria cases in several randomized controlled trial (Ruggenenti et al., 2004). The Randomized Olmesartan and Diabetes Microalbuminuria Prevention (ROADMAP) trial revealed that the number of patients who developed new onset microalbuminuria was fewer than that in placebo group (8.2% versus 9.8%), although renal endpoint (doubling of the serum creatinine) was the same in both groups (Haller et al., 2011). The study has also shown that olmesartan prolonged time to the onset of microalbuminuria. Blood pressure was lower in the olmesartan arm and fatal cardiovascular events were statistically higher in the olmesartan group. The latter finding raised concerns about the safely of olmesartan as another study with olmesartan has reported similar finding (Imai et al., 2011).

In non-diabetic subjects with nephropathy, treatment with ramipril (versus placebo) significantly reduced blood pressure, proteinuria and slowed progression of renal deterioration (The GISEN Group, 1997). In non-diabetic subjects with hypertension, the LIFE study showed that losartan was superior to atenolol in suppressing albuminuria excretion with minimum difference between the two treatment groups in terms of blood pressure reduction (Ibsen et al., 2004).

It remains unclear whether RAAS blockers possess antiproteinuric properties beyond that provided by blood pressure reduction. For example, in the HOPE study, a blood pressure reduction of 3/2 mm Hg in the active treatment arm could explain the renoprotection mediated by ramipril (Heart Outcomes Prevention Evaluation Study Investigators, 2000b). Similarly, treatment with olmesartan was associated with greater reduction of blood pressure than in the placebo group (3.1/1.9 mm Hg) in the ROADMAP study (Haller et al., 2011). Other studies were not properly designed to show an additional effect of RAAS blockers beyond that from blood pressure reduction. For example, in the RENAAL trial (Brenner et al., 2001), the design meant that losartan resulted in earlier blood pressure

reduction and hence, more renoprotection. Spironolactone has been shown to be effective in reducing blood pressure in subjects with resistant hypertension (de Souza et al., 2010). Therefore, it is plausible that further blood pressure reduction produced by the addition of spironolactone may explain most of reduction of proteinuria in the spironolactone trials. Moreover, most spironolactone trials were underpowered, limiting the validity of the conclusions (Schjoedt et al., 2006).

The available data on whether combining drugs from different classes of RAAS blockade may provide better cardio-renal outcomes is unclear. The Valsartan in Combination With Lisinopril in Hypertensive Patients With Microalbuminuria (VALERIA) trial demonstrated the effect of lisinopril and valsartan in 133 hypertensive subjects with microalbuminuria (Menne et al., 2008). Following seven months of treatment with lisinopril, valsartan and a combination of lisinopril/valsartan, the ACR was reduced by 41%, 51% and 62%, respectively. In addition, microalbuminuria regression was achieved in 17%, 31%, and 38% of patients receiving lisinopril, valsartan and lisinopril/valsartan, respectively. It was concluded that a combination of ACEI and ARB provide better outcomes. However, the better reduction in proteinuria with the combination could be due to the better blood pressure reduction achieved by the combination. It might have been better to start with the three treatment regimens at low doses which are then titrated to the higher doses and compare the reduction in proteinuria in the three groups.

A meta-analysis of 49 studies has shown that the combination of ACEI and ARB give better reduction of proteinuria than monotherapy with drugs from these groups (Kunz et al., 2008a). However, whether such combination could be translated into better cardiovascular or renal outcome was not proven. In the ONTARGET study that involved high-risk population, combination of ramipril and telmisartan was associated with cardiovascular outcomes rates similar to that reported with each drug alone and was

associated with higher incidences of adverse drug reactions (Yusuf et al., 2008). A recent systematic review of 85 randomised controlled trials suggested that there is no evidence supporting superiority of ARB and ACEI in combination over monotherapy from either group (Maione et al., 2011). The only study that reported a clear beneficial effect of a combination of ARB/ACEI on renal outcomes was the COOPERATE trial (Nakao et al., 2003). However, serious concerns about the authenticity of the study were raised (Kunz et al., 2008b) and as a result, the paper was retracted.

The use of combination approach of any RAAS blockers needs to be based on results from long-term outcomes studies. Each group of RAAS blockers may induce hyperkalemia and a combination approach may worsen this. Patients presenting with proteinuria are usually elderly, diabetic and suffer from renal insufficiency. Such conditions are usually associated with impaired potassium excretion and therefore, drugs induce hyperkalemia could lead to serious complications. Also, drug interaction is not uncommon in such patients. This could occur with the use of non-steroidal anti-inflammatory drugs which may lead to renal deterioration and agents that lead to hyperkalemia (e.g. potassium supplements and some frequently used anti-bacterial drugs in elderly females such as trimethoprim) (Velazquez et al., 1993, Harirforoosh and Jamali, 2009).

The use of proteinuria as a surrogate marker for renal outcomes is another limitation of trials on RAAS blockade. For instance, the ONTARGET study showed that although treatment with a combination of ACEI and ARB reduced proteinuria (a surrogate marker), primary endpoint was increased (composite of death, need for dialysis or doubling of serum creatinine) (Mann et al., 2008). A meta-analysis of 70 randomised clinical trials concluded that there is insufficient evidence for the use of proteinuria as a surrogate outcome for progression of renal deterioration (Stoycheff et al., 2010). A scientific workshop sponsored by the American National Kidney Foundation and the US FDA

recommended that proteinuria can be used as a surrogate marker only in certain circumstances such as in nephrotic syndrome as the available evidence for the use of proteinuria as surrogate biomarker for CKD is limited (Levey et al., 2009). The use of microalbuminuria as a surrogate endpoint for kidney outcomes is not justified by firm evidence. Microalbuminuria is increasingly recognised as a marker of endothelial dysfunction rather than renal impairment (Moody et al., 2012).

In summary, there is some evidence of the use of ACEIs in type I diabetes mellitus for proteinuria but confounded by blood pressure differences. In type II diabetes mellitus, most of evidence is based on the use of ARBs but again most of benefit is due to blood pressure reduction. Current evidence do not support the combination approach of ACEI/ARB and therefore; the clinical practice should be based on monotherapy with either ACEI or ARB depending on the tolerability with proper doses for better blood pressure control which would be translated into better renal outcomes.

1.2.11.3 Calcium Channel Blockers

The available data on the use of different classes of CCBs in patients with increased UAE is conflicting. It has been suggested that non-dihydropyridine CCBs confer a better microalbuminuria lowering effect (Bakris et al., 2004). For instance, Smith et al. (Smith et al., 1998) compared the effect of diltiazem and nifedipine (a dihydropyridine CCB) on proteinuria in patients with type 2 diabetes mellitus. They reported that only diltiazem reduced proteinuria and improved glomerular permselectivity, while nifedipine did not produce any reduction in proteinuria. However, the magnitude of proteinuria change in each treatment group was imprecise mainly because of the small sample size (n=21).

The African American Study of Kidney Disease and Hypertension (AASK) demonstrated that amlodipine increased proteinuria by 58% from baseline (Wright et al., 2002).

Nevertheless, the lack of a control group in this study makes it difficult to interpret the influence of amlodipine. In contrast, some studies have shown renoprotective properties of dihydropyridine CCBs (Voyaki et al., 2001, Estacio et al., 2000).

In the ACCOMPLISH (Avoiding Cardiovascular Events through Combination Therapy in Patients Living with Systolic Hypertension) trial, the renoprotection properties of benazepril/amlodipine versus benazepril/hydrochlorothiazide were evaluated in high-risk hypertension population (Bakris et al., 2010). The study showed that regression of microalbuminuria and gross proteinuria was greater in those who received fixed dose of benazepril/ hydrochlorothiazide. However, renoprotection (doubling of serum creatinine) with benazepril/amlodipine was superior to that with benazepril/hydrochlorothiazide. The study was criticised as the main endpoint was doubling of serum creatinine which could be a reflection of haemodynamic changes rather than preserving renal structure (Heerspink and de Zeeuw, 2010). A proper head to head comparison for renoprotection should be long enough to detect the rate of any structural changes which is translated to end stage renal disease, as some drugs have acute haemodynamic changes (Delles et al., 2003).

The VVANNTT (Verapamil Versus Amlodipine in Nondiabetic Nephropathies Treated with Trandolapril) study showed no additive effect of combining amlodipine or verapamil with trandolapril compared with monotherapy with trandolapril (Boero et al., 2003). Data from the BENEDECT (Bergamo Nephrologic Diabetes Complications Trial) also provided no evidence of any effect of verapamil alone on lowering new onset microalbuminuria while trandolapril alone and trandolapril/ verapamil lowered the incidence of new onset microalbuminuria (Ruggenenti et al., 2004). In the VVANNTT study, the follow-up period was relatively short (8 months). Also, limited number of subjects participated in this trial (69 patients). The BENEDECT was mainly focusing on the incidence rate of new onset microalbuminuria in hypertensive diabetic subjects with normoalbuminuria. Thus,

whether verapamil helps in regressing albuminuria may not be answered in this study as participants were normoalbuminurics. Also, recent evidence suggests that new onset microalbuminuria occurred in patients under chronic treatment with RAS blockers (Cerezo et al., 2012).

1.2.11.4 Beta-blockers and diuretics

Several studies have examined the ability of beta-adrenoreceptor blocking agents in lowering albuminuria. For example, the GEMINI (Glycemic Effects in Diabetes Mellitus Carvedilol-Metoprolol Comparison in Hypertensives) trial reported that carvedilol reduced microalbuminuria and progression to microalbuminuria compared with metoprolol independently of BP reduction (Bakris et al., 2005). It has been suggested that the anti-proteinuric effect might be explained by the putative antioxidant capability of carvedilol, since microalbuminuria pathogenesis involves oxidative stress. However, there is no evidence to support such argument. It was not clear whether in-treatment blood pressure was similar in the two groups. Nebivolol was also shown to have the ability to reduce proteinuria in a study involving 2,838 diabetic patients with hypertension and this was accompanied with significant blood pressure reduction (Schmidt et al., 2007).

Early studies comparing beta blockers with ACEIs provided contradictory evidence. Several studies on patients with diabetic nephropathy and hypertension have shown that treatment with ACEIs produced reduction in albuminuria greater than with beta blockers (Nielsen et al., 1994, Nielsen et al., 1997). However, most of these studies involved small number of participants and/or short follow up. Other studies have reported comparable reduction of albuminuria using either beta blockers or ACEIs (Erley et al., 1993, Elving et al., 1994, Rudberg et al., 1999, UK Prospective Diabetes Study Group, 1998a). Again, the small sample sizes make these trials far from conclusive.

Most of the evidence of the ability of diuretics to lower proteinuria comes from studies where diuretics were used in combination with other antihypertensive drugs (Mogensen et al., 2003). Early evidence showed that treatment with metoprolol followed by addition of a thiazide diuretic significantly reduced UAE in patients with diabetic nephropathy (Christensen and Mogensen, 1987). The addition of thiazide diuretics to RAS blockers in patients with immunoglobulin nephropathy (Uzu et al., 2005) and in those with high sodium intake (Buter et al., 1998) significantly reduced blood pressure and proteinuria. Treatment with a combination of an ACEI and a diuretic was associated with reduction of new onset microalbuminuria and major cardiovascular events compared with subjects who received placebo (ADVANCE Collaborative Group, 2007). A recent trial showed that a combination of ARB and diuretic was superior to ARB plus CCB in reducing proteinuria after 12 weeks follow up (Matsui et al., 2011). The reduction in proteinuria was accompanied with reduction in nocturnal blood pressure. However, a significant reduction in eGFR was also reported with ARB/diuretic.

Such finding support the need for well-designed studies with a long follow up period to exclude any potential temporary effect and also a more emphasis should be paid to the relation between blood pressure and proteinuria reduction. It seems that most of beneficial effect on proteinuria is driven by blood pressure change with a possible of minor additional changes in some classes that need to be documented properly.

2. Chapter two: Methodology

2.1 Study population

Subjects with hypertension attending either the GBPC at the Western Infirmary or the Aberdeen Hypertension Clinic at the Aberdeen Royal Infirmary participated in this series of studies. The final sample size was 1059 subjects. Out of 1059, 884 hypertensive subjects were attending the GBPC while the remainder (n=175) were followed at the Aberdeen Hypertension Clinic. Raw data from the Aberdeen Hypertension Clinic was provided by Professor John Webster.

2.1.1 The Glasgow Blood Pressure Clinic

The clinic was established in 1968 to provide consultant-led secondary/ tertiary service for the diagnosis and treatment of hypertensive subjects in the west of Scotland (Dunn et al., 1990). The clinic maintains a registry containing the demographic and clinical information on more than 15,000 subjects. The GBPC is linked with the office of the Registrar General of Scotland which is responsible for recording all deaths, which means that the cause and date of death of the patients can be identified readily.

Patients are usually referred to the clinic by their general practitioners or hospital specialist. Each patient is seen by a specialist nurse for blood pressure recording. At the first visit, clinical details of the patient are entered on a standardised form in order to facilitate their transfer to the electronic database. These details include patient's height, weight, age, heart rate, family history of chronic diseases (hypertension, ischaemic heart disease, stroke, and diabetes) and sitting and standing systolic and diastolic blood pressures. In addition, the presence of co-morbidities or risk factors such as airways diseases, angina, cerebrovascular disease, alcohol intake, cigarette smoking, diabetes mellitus, heart failure, ischaemic heart disease, left ventricular hypertrophy, myocardial

infarction, peripheral vascular disease and renal impairment are included in the patient profile. The result of urine dipstick examination is also entered on the form.

All patients undergo standardised clinical investigations such as serum biochemistry (including random glucose and lipid profile), haematology (complete blood count and ESR), spot urine specimen for blood and protein, mid stream specimen of urine for bacteriology, ECG and full blood count. Some patients may undergo other investigations when needed such as echocardiography, 24 hours ambulatory blood pressure monitoring, chest X-ray, retinal photography, plasma renin and aldosterone, exercise tolerance testing, computerised tomography and magnetic resonance imaging.

Patients are usually followed after one month from their first visit where the results of clinical and laboratory investigations become available. The frequency of the subsequent clinic visits depends largely on the condition of each patient. New patients generally have more frequent visits during their first year until they reach the recommended blood pressure targets.

2.1.2 The Aberdeen Hypertension Clinic

The Aberdeen Hypertension Clinic was established in 1970 for hypertension-related research purposes (Webster et al., 1993). After that, the clinic started to provide a specialist service for the diagnosis and management of hypertension in patients living in the north-east of Scotland. The clinic provides a shared care system (Petrie et al., 1989). This means that hypertensive subjects are either followed by their general practitioners and the results of laboratory investigations and blood pressure records are sent to the clinic for specialist advice on how to manage their condition or they may attend the clinic at the Royal Infirmary to be seen by a specialist. Currently, more than 8,000 patients are registered in the shared care scheme.

Routinely collected data include complete blood count, lipids, glucose, height, weight, BMI, past medical history, urine electrolytes and drug intolerance history.

2.2 Detection of microalbuminuria

The detection of microalbuminuria in the two participating centres was standardised. A standard operating procedure form was distributed to the two clinics. This form included details of microalbuminuria screening and information needed from each participating subject.

The protocol of detecting microalbuminuria was the same in the two study sites (the GBPC and the Aberdeen Hypertension Clinic) except for the method of screening for microalbuminuria in the first sample. At the GBPC, the detection of microalbuminuria was carried out using laboratory quantification while a semi-quantitative dipstick method was used at the Aberdeen Hypertension Clinic. Both sites used laboratory quantification of albuminuria in the subsequent samples.

Laboratory detection of urinary albumin excretion was carried out using Architect c16000® analyser (Abbott Diagnostics, Maidenhead, UK) at the GBPC and an Advia 1200 Chemistry System (Siemens Healthcare, Camberley, UK) at the Aberdeen Hypertension Clinic. Both machines use enzymatic assay to analyse the level of creatinine and turbidimetric immunoassay to analyse the level of albumin (Brook et al., 2012, Abbott Diagnostics, 2012). Microalbuminuria was expressed as mg/mmol.

At the Aberdeen Hypertension Clinic, the initial detection of microalbuminuria was carried out using Clinitek Microalbumin 9 Reagent Strips (Siemens Medical Solutions Diagnostics, UK). These strips have a high sensitivity dye binding site for urinary albumin (Pugia et al., 1999) and another binding site for creatinine using peroxidase reaction (Pugia

et al., 2000). The colours yielded in the strips were further analysed using a Clinitek 50 Urine Chemistry Analyzer (Siemens Medical Solutions Diagnostics, UK) for semi-quantitative measurement of ACR and the results were expressed as milligrams albumin per gram or millimole creatinine (mg/mmol).

2.2.1 Protocol for detecting microalbuminuria

Each patient was invited to provide an early morning urine specimen for the assessment of albuminuria (in plain white topped container). Some of the patients were able to provide a urine sample during the clinic (9-11 A.M) while others were asked to collect urine on the morning of their next visits to the clinics. This sample was tested using Multistix 10 SG Reagent Strips (Siemens Healthcare Diagnostics, UK) for detecting leukocytes or nitrites as signs of urinary tract infection. If the dipstick showed positive result for leukocytes or nitrites, the sample was discarded and a mid-stream specimen of urine (in a red topped container) was sent for urine culture. When the result of urine culture showed no signs for urinary tract infection, the patients were requested to provide a new sample. Those with persisted UTI were excluded from my studies.

The result of first urine specimen screening fell into one of four categories:

a. Gross proteinuria: when albumin-to-creatinine ratio was greater than 25 mg/mmol in males and females.

b. Microalbuminuria according to the conventional (current) definition: when the ACR was > 2.5 mg/mmol and ≤ 25 mg/mmol in males or > 3.5 mg/mmol and ≤ 25 mg/mmol in females (as females have skeletal muscle mass lower than in males).

c. Microalbuminuria according to a new definition (a low level of albuminuria which does not include the threshold level of the conventional definition): when ACR in males was from 1.2 to 2.5 mg/mmol or 1.7 to 3.5 mg/mmol in females.

d. Normoalbuminuria: when the ACR was less than 1.2 mg/mmol in males or 1.7 mg/mmol in females.

The thresholds used in the new definition of microalbuminuria were selected as this is the current threshold used for classing patients with high normal UAE (de Jong and Curhan, 2006). Patients from categories a, b and c (without evidence of urinary tract infection) were invited to provide two further first morning void (mid-stream) urine specimens for albumin estimation. Patients were asked to collect urine for the two samples just before their next visit to the clinics.

2.3 Classifications of patients based on UAE

Patients were considered as normoalbuminurics if the first sample showed normal UAE, where no further samples were requested, or when the results of two out of three samples showed normal UAE. Positive subjects were allocated to one of the three categories of albuminuria (microalbuminuria by the new definition, microalbuminuria by the conventional definition or gross proteinuria) based on the mean of the last two urine specimens. The selection of the last two samples is because laboratory quantification of albuminuria was used in both participating centres.

2.4 Patient's information included in the analysis

Data were collected from the case-notes of the participating subjects. Collected information included patient's characteristics such as age, gender, SBP, DBP and BMI. In

addition, requests of laboratory tests such as serum total cholesterol level, serum HDL-cholesterol level, eosinophil count, serum creatinine and plasma glucose (fasting specimen in the GBPC and random specimens at the Aberdeen Hypertension Clinic) were recorded. Information on risk factors and comorbidities such as type II diabetes mellitus, LVH and established cardiovascular disease (coronary heart diseases, stroke/transient ischaemic attack and peripheral vascular disease) was also collected. A complete list of all current cardiovascular and metabolic drugs was obtained. This included all antihypertensive agents (diuretics, CCBs, ACEIs, ARBs, beta-adrenoreceptor blockers, alpha-adrenoreceptor blockers, aldosterone antagonists and imidazoline receptor agonists), antidiabetic medications, lipid lowering agents (statins and others such as fibrates and ezetimibe), antiplatelet agents (mainly aspirin), anticoagulants and vasodilators (mainly nitrates).

Some information was only available from patients attending the GBPC. This included fasting triglycerides, ESR, smoking status, evidence of ST-T changes on ECG and family history of type II diabetes mellitus, premature stroke or CHD.

2.5 Laboratory and clinical measurements

2.5.1 Blood pressure

Both centres used the same procedure for recording blood pressure. Specialist hypertension nurses were responsible for measuring blood pressure readings. Prior to blood pressure measurement, each patient was requested to rest for 5 minutes in the supine or sitting position. Well-calibrated and maintained mercury sphygmomanometers ((Accoson Dekamet MK3, UK) at the GBPC and (Trimline Mercury Sphygmomanometer, Trimline Medical Products, Branchburg, New Jersey) at the Aberdeen Hypertension Clinic)) were used for measuring blood pressure. The arm was supported and positioned at heart level and any tight cloth were removed. Appropriate cuff size was selected. The cuff was

inflated over the brachial artery until disappearance of the pulse. This was followed by deflation of the cuff until the pulse re-appeared and this was recorded as estimated SBP. The cuff was re-inflated 30 mm Hg above the estimated SBP and a stethoscope was placed. After that, cuff was deflated at the rate of 2 mm Hg per second until the appearance of a rhythmic sound (SBP). Deflation was continued until disappearance of the sound (DBP). Blood pressure was measured twice and the mean was recorded. If the second measurement was significantly lower, a third reading was obtained and the mean of the last two readings was used.

The pulse pressure was defined as the difference between the SBP and DBP (i.e. SBP minus DBP). The therapeutic target for blood pressure was defined as SBP < 140 mm Hg and DBP < 90 mm Hg.

2.5.2 Obesity

Obesity was defined as BMI equal to or more than 30 kg/m^2, according to the World Health Organisation classification of underweight, overweight and obesity in adults (WHO, 2000). A BMI between 25 and 29.99 kg/m^2 was used to define pre-obesity "over-weight" while values between 18.5 to 24.99 was considered as optimal weight. The body weight was measured using calibrated weighing machines (Seca 955 chair scale at the GBPC and a Seca Delta Model 707 at the Aberdeen Hypertension Clinic, Brash & Sons, UK). Patients were instructed to remove any heavy clothes before measuring their weight. The height was measured by a height stick in both clinics.

2.5.3 Diabetes mellitus

Patients with type II diabetes were identified based on either the diagnosis by the responsible clinician, patient report, the use of antidiabetic agents or when the fasting glucose level was ≥7.0 mmol/l or the random blood glucose ≥11.1mmol/L (WHO, 2006)

(for those who do not have fasting result). Serum glucose was measured by enzymatic colorimetric technique (hexokinase assay) using an Architect c16000 analyser (Abbott Diagnostics, Maidenhead, UK) in Glasgow and an Advia 2400 Chemistry System (Siemens Healthcare, Camberley, UK) in Aberdeen. At the GBPC, fasting serum glucose was requested. Patients were instructed not to take any food for at least 8 hours before the test. Patients were advised to attend at 9.00 for performing the test. Random serum glucose samples were obtained for subjects attending the Aberdeen Hypertension Clinic.

2.5.4 Renal function

Renal function was evaluated using estimated glomerular filtration rate (eGFR). The eGFR was calculated using the 4-variable Modification of Diet in Renal Disease (MDRD) equation (Levey et al., 1999):

eGFR= 32788 × serum creatinine (in µmol/L) $^{-1.154}$ × age $^{-0.203}$ * (1.212 if black) × (0.742 if female)

Data about race was not available but it has been reported that less than 0.5% of the Scottish population are black/African (The Scottish Government, 2004). Serum creatinine (expressed as µmol/L) was measured using the Jaffè reaction method using the Abbott Architect c16000 analyser in Glasgow and the Siemens Advia 2400 analyser in Aberdeen.

The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) classification was followed for defining different CKD stages (Levey et al., 2003). An eGFR of more than or equal to 60 mL/min/1.73 m^2 were considered as normal kidney function. CKD stage 3 was defined as an eGFR result between 30-59 mL/min/1.73 m^2 while stage 4 and 5 was defined as eGFR between 15-29 mL/min/1.73 m^2 and < 15 mL/min/1.73 m^2, respectively.

In the main analysis, subjects with CKD stage 4 or 5 were excluded. This is because advanced CKD disease is usually associated with gross proteinuria. Those with stage 3 CKD were included to allow for older participants as increased age is associated with progressive reduction in eGFR (Douville et al., 2009).

2.5.5 Hyperlipidemia

Total cholesterol and HDL were estimated using enzymatic colorimetric assay techniques from non-fasting samples by the Abbott Architect c16000 analyser in Glasgow and the Siemens Advia 2400 analyser in Aberdeen. The enzymatic colorimetric assay (by Architect c16000 analyser) was also used for measuring fasting triglycerides in Glasgow. Increased total cholesterol level was defined as values exceeded 5 mmol/L (Cooper and O'Flynn, 2008). The normal ranges for HDL-cholesterol and fasting triglycerides are > 1.0 mmol/L and <2.3 mmol/l, respectively (Manninen et al., 1992).

2.5.6 Haematological indices

ESR was measured in subjects attending the GBPC using StaRRsed III Chemistry analyser (Mechatronics, Zwaag, the Netherlands). Eosinophil count was estimated using an XE-2100 Blood Cell analyser (Sysmex, Kobe, Japan) in Glasgow and Advia 2120i Haematology analyser (Siemens Healthcare Diagnostics, UK) in those attending the Aberdeen Hypertension Clinic.

The normal range for eosinophil count is 0.04–0.44 x 10^9/L (Provan, 2005). There was no specific threshold value for ESR. However, it has been suggested that levels between 1 to 10 mm in one hour can be considered as normal (Hilder and Gunz, 1964).

2.5.7 Left ventricular hypertrophy and ECG changes

Patients with LVH or ECG changes (ST-T wave) were identified according to the diagnosis of the attending clinician and their comments on the patient's case-records. The criteria for diagnosing LVH on ECG were (Goldman, 1973):

1. Standard leads: the voltage of $R_1 + S_3 > 26$ mm.

2. Extremity leads: an R wave > 13 mm.

3. Precordial leads: the total voltage of ($SV_1 + RV_5$) or ($SV_1 + RV_6$) is > 35 mm, or an R > 27mm in V_5 or V_6.

Diagnosis of LVH on echocardiography was based on reports for echocardiography laboratory.

2.5.8 Family history of cardiovascular / metabolic diseases, smoking status and cardiovascular co-morbidities

Information regarding smoking status, co-morbidities and family history of CHD, stroke or diabetes mellitus was obtained from patient's case-records. Upon their first visit, patients are questioned by a specialist nurse and/or the attending physician about such information and a copy is kept in the case-record. In addition this information is transferred to an electronic database.

2.6 Inclusion/ exclusion criteria

All patients attending the GBPC or the Aberdeen Hypertension Clinic were invited to participate to the study. Those with secondary hypertension, persistent UTI (n=22) or age less than 18 years were excluded. No patient with type I diabetes mellitus was included in the study.

2.7 The GBPC database

Since the establishment of the GBPC, the clinical characteristics of attending patients have been transferred to an electronic database for audit and research purposes. The database nowadays stores the information of more than 15,000 patients.

At each visit, the stored information includes the date of the visit, SBP and DBP and heart rate. In addition, the database contains the demographic characteristics for each patient (such as age, gender, BMI, smoking status and alcohol intake) and drug(s) used and their doses, duration of treatment and stop date, if any, and possible side effects. The results of baseline (first visit) dipstick for proteinuria are also entered in the database. Some of the patients may have more than one result for proteinuria dipstick examination.

Other information such as type of hypertension (essential or secondary) and co-morbidities (such as airways disease, angina, cerebrovascular disease, diabetes, heart failure, ischaemic heart disease, LVH, myocardial infarction, peripheral vascular disease (PVD) and renal impairment) are frequently entered and updated in the electronic database.

2.8 Cardiovascular risk estimation

All cardiovascular risk scores were derived from the original Framingham Heart Study equation and the Joint British Societies (JBS) cardiovascular disease risk formula.

2.8.1 The Framingham risk score system

Cardiovascular disease risk calculation was based on the original Framingham formula (Wilson et al., 1998). Variables that are required for risk stratification are age, gender, smoking status, SBP, presence of diabetes mellitus, LVH, total cholesterol and HDL-cholesterol. HDL was assumed as 1 mmol/L when actual data were missing (n=11). Individuals with already established CVD were excluded from the calculation. Ten-year

cardiovascular risk was selected as the Framingham equation can predict risk within the next 4 to 12 years. Ideally, the diagnosis of LVH should be based on the electrocardiographic criteria of the Framingham study. However, the inclusion of LVH in the risk prediction was based on the diagnosis of LVH by the attending clinician, mainly based on ECG traces. The Framingham risk score was calculated using a software written by Dr. Rupert Payne, University of Edinburgh (Payne, 2010) which permits risk calculation in large number of patients. The following outcomes were calculated from the Framingham heart study equation:

1. The probability of developing any CVD over the next 10 years.
2. The probability of developing CHD over the next 10 years (including angina pectoris, myocardial infarction, death from CHD and coronary insufficiency).
3. The probability of developing stroke or transient ischaemic attack over 10 years.
4. The probability of developing myocardial infarction over 10 years.
5. The probability of dying from CHD over 10 years.
6. The probability of dying from CVD over 10 years.

Subjects were considered as high risk group when the overall CVD risk(s) was greater than 20% over 10 years. Moderate risk group was defined as having CVD of 10% to 20% over than next 10 years and those who had less than 10% CVD risk were considered as low risk group.

2.8.2 The JBS cardiovascular risk equation

The JBS risk scoring system which is based on the Framingham algorithm was used to evaluate the cardiovascular risk in those without established CVD, diabetes or LVH as subjects with these diseases are considered as high risk population (British Cardiac Society

et al., 2005). The formula uses age, gender, SBP, smoking status and total cholesterol: HDL ratio. For those with missing HDL values, HDL was assumed to be 1 mmol/L. The cardiovascular risk score was computed using the CHD and Stroke Risk Assessor, University of Manchester. The JBS recommends that subjects who stopped smoking within the last 5 years should be considered as smokers. However, as this option was not feasible as direct contact with patients was not possible, "smoker" option was selected for current smokers only.

The outcome measured by the JBS formula was the probability of developing any cardiovascular event (angina pectoris, myocardial infarction, stroke, stroke death or CHD death) over 10 years. Similar to the Framingham scoring system, high risk group were defined as having more than 20% risk while moderate and low risk groups were defined as having 10-20% and < 10% CVD risk, respectively.

It should be noted that all variables used in the Framingham or the JBS equations should be based ideally on untreated variable. However, in this analysis, most variables were based on treated values which would tend to reduce risk estimation.

2.9 Ethical approval

The protocol for the studies was reviewed by the chairs of the ethics committees in Glasgow and Aberdeen. Since the project did not require patient interventions, it was deemed that formal ethical approval was not required.

2.10 Statistical analysis

2.10.1 Statistical packages used

Statistical analysis was performed using Minitab statistical software version 16.2.1 (Minitab Inc., State College, Pennsylvania, USA) and Statistical Package for the Social

Sciences (SPSS) software for Microsoft Windows version 19.0 (IBM Corporation, Armonk, New York, US).

2.10.2 Summary statistics

Quantitative variables were summarized using mean ± standard deviation or median and interquartile range where data were not normally distributed. Some of the data that were not normally distributed were transformed using the natural logarithm (the logarithm to the base *e*) and back-transformed to the original scale and represented as geometric mean with 95% confidence interval. Categorical data were summarised as percentage of the cohort. The differences between any groups were considered to be significant when P value was less than 0.05.

2.10.3 Comparison of two means

For normally distributed continuous data, the difference between any two groups was compared using a 2 sample *t* test. When data were not normally distributed, the Mann-Whitney test was used for assessing mean difference. Categorical data were compared using Chi-square or Fisher's exact test for small sample size (for observations less than 5).

2.10.4 Comparison of more than two means

One-way analysis of variance (ANOVA) was used to compare the means of more than two groups when data showed normal distribution. The Kruskal–Wallis method was used when data were skewed. For categorical data, groups were compared using Chi-square or Fisher exact tests, as appropriate. Linear association in categorical data was examined using chi-square test for trend.

When the result of ANOVA test was significant, a post-hoc statistical analysis using Tukey's method was used to identify which group was significantly different from the

others (within-groups comparison). The mean difference for each pairwise comparison was reported (95% confidence interval of mean difference).

To allow within-groups comparisons for skewed data (e.g. ESR, eosinophil count and fasting triglycerides), natural logarithmic transformation was used to achieve normal distribution. This was followed by testing the trend using ANOVA and data were back-transformed to the original scale and represented as geometric mean with 95% confidence interval. For variables with significant result with ANOVA test, multiple group comparison using Tukey's method was carried out and expressed as geometric mean ratio with 95% confidence intervals for the ratio of the geometric mean. In categorical data, Z-test was used for pairwise comparison when the overall P value was significant.

2.10.5 Logistic regression

Binary logistic regression method was performed to investigate the association of binary outcome with possible predictors. Predictors were added either using a stepwise backward elimination method or selected based on previously known possible interaction between the outcome and the predictors. In the stepwise backward method, all covariates are placed in the first model and those with no significant contribution to the model were excluded.

3. Chapter three: prevalence of microalbuminuria in hypertensive population and its association with cardiovascular risk factors and metabolic abnormalities

3.1 Introduction

The reported prevalence of microalbuminuria varies widely (ranging from 4.7% to 58.4%) (Jensen et al., 1997, Böhm et al., 2007). This variability may be due to several factors such as the population studied, detection method and diagnostic criteria. While international therapeutic guidelines recommend that the diagnosis of microalbuminuria should be based on repeated samples due to the high variability of albumin excretion in the urine (National Collaborating Centre for Chronic Conditions, 2008), the vast majority of studies of microalbuminuria in hypertensive subjects have been based on single measurements (Leoncini et al., 2010). In addition, in studies where microalbuminuria prevalence was high, the population studied had concomitant other risk factors. For example, in two studies of untreated hypertensive subjects where the prevalence of microalbuminuria was high (≥25%), (Pedrinelli et al., 2003, Pedrinelli et al., 2004) about three-quarters of the studied subjects had LVH. Most people with high blood pressure do not have end organ damage. Therefore, the exact prevalence of microalbuminuria prevalence in hypertensive subjects is still unclear.

The current thresholds used to define microalbuminuria in spot urine samples (ACR >2.5 mg/mmol in males and > 3.5 mg/mmol in females) is arbitrary and may underestimate the clinical significance of this condition. There is evidence that levels of microalbuminuria below the conventional cut-off levels are associated with increased cardiovascular risk in general population (Hillege et al., 2002).

The purpose of this analysis was to assess the prevalence of microalbuminuria (using two definitions) in a large population of hypertensive subjects attending secondary/tertiary referral centres. The definitions were the conventional limits for microalbuminuria and a new definition with a threshold about 50% lower than that of the conventional definition. I also aimed to examine the association of microalbuminuria with cardiovascular, renal and metabolic parameters.

3.2 Methods

This analysis used data collected from 1059 hypertensive subjects attending the Glasgow Blood Pressure Clinic or the Aberdeen Hypertension Clinic. Microalbuminuria was defined as ACR >2.5-25 in males or >3.5-25 in females (conventional definition) or ACR 1.2-2.5 in males or 1.7-3.5 in females (new definition) while gross proteinuria was defined as ACR > 25 in both genders. Each patient had urinary albumin estimation. For those with positive microalbuminuria, two further samples were requested. Patients with CKD stage 4 or 5, type II diabetes mellitus or gross proteinuria were not included in the main analysis as they usually have increased UAE due to kidney disease.

Demographic and clinical characteristics were compared between subjects with microalbuminuria and those with normal UAE. Variables studied were age, gender, blood pressure, BMI, serum creatinine, eGFR, serum lipids (total cholesterol, HDL-cholesterol and fasting triglycerides), fasting and random blood glucose and inflammatory markers (ESR and eosinophil count). Normally distributed data are expressed as mean ± standard deviation while skewed data are expressed as median (interquartile range). The groups were compared using a 2 sample *t* test or Mann-Whitney test, as appropriate. One-way ANOVA or Kruskal Wallis tests were used to compare more than two groups, as appropriate. Between-groups comparisons using Tukey's procedure were used with variables that showed significant trend using ANOVA test. To allow between-groups

comparison for skewed data (fasting triglycerides, eosinophil cont and ESR) natural logarithmic transformation was used and variables were expressed as geometric mean (95% confidence interval). Gender differences were assessed using chi-square test.

3.3 Results

3.3.1 Study population:

Figure 3-1 illustrates the inclusion profile. Urine was collected from 1081 individuals. Twenty two patients were excluded since repeated urine specimens suggested urinary tract infection. Of 1059 individuals who had urinary albumin estimation, 116 subjects had positive microalbuminuria on initial screening but failed to provide repeat samples and were not included in the main analysis. Among the remainder (n= 943), 125 had type II diabetes mellitus. Thirteen patients had gross proteinuria and 6 patients had CKD stage 4 or 5. Missing data from 13 patients (mainly serum creatinine) precluded calculation of eGFR. After excluding the previously mentioned individuals, 786 patients were eligible for investigation of the prevalence of microalbuminuria in hypertensive subjects without evidence of type II diabetes or severe renal impairment (CKD stage 4 & 5 or gross proteinuria).

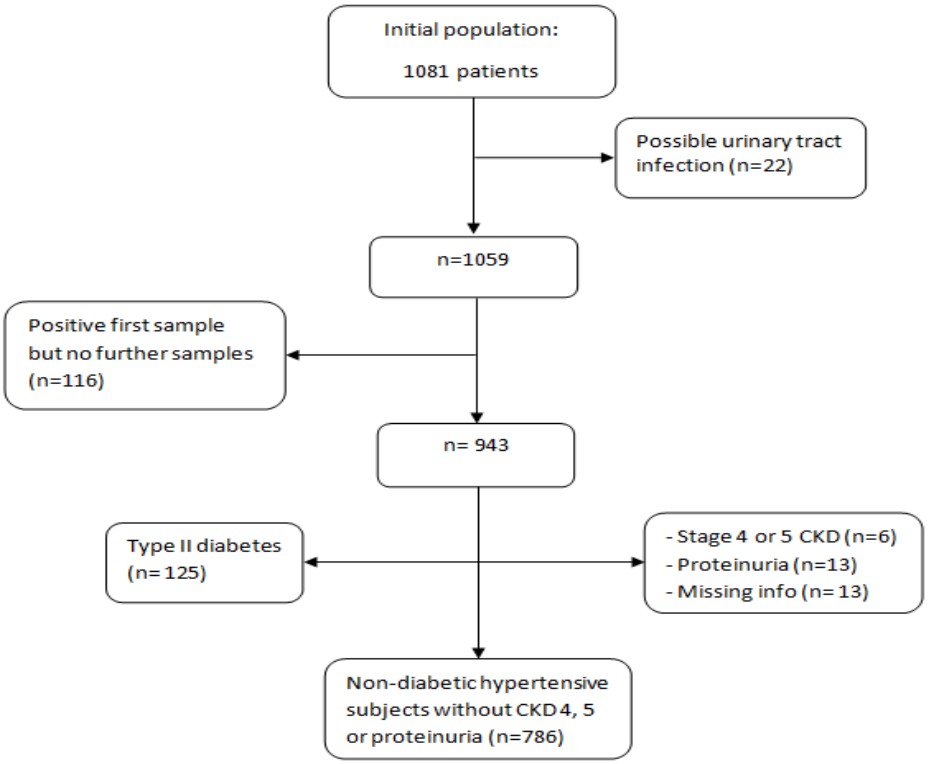


Figure 3-1: Flowchart of the study.

3.3.2 Patient characteristics

Table 3-1 summarises the demographic characteristics of the study population (n=1059). The mean age of the study population was 58 ± 14.9 years; 54% were females (detailed in chapter 4). More than 75% had a body mass index above 25 kg/m^2, with an average value of 30.0 ± 6.1 kg/m^2. Approximately 45% of total study population had a BMI of 30 kg/ m^2 or more.

The mean SBP was 140.5 ± 19.5 mm Hg. Nearly 42% of the patients had a SBP more than or equal to 140 mm Hg (above the target). The mean DBP was 86.9 ± 11.4 mm Hg. About 45% of patients had DBP of more than or equal to 90 mm Hg.

The mean serum creatinine level in the population was 85.4 ± 25.6 µmol/L. Only 4% of the patients had a serum creatinine level of more than 130 µmol/L. The mean eGFR was 78.3 ±19.5 mL/min/1.73 m^2. About 12% of the patients had stage 3 CKD. Fasting blood glucose

was estimated in 718 patients and the mean was 5.4 ± 1.4 mmol/L. For those who do not have fasting specimen, the median random glucose level was 5.4 mmol/l (interquartile range, 4.9 to 6.7).

Mean total cholesterol level was 5.1 ± 1.1 mmol/L, with a mean HDL-cholesterol level of 1.4 ± 0.47 mmol/L. More than 50% of the patients had a cholesterol measurement of ≥ 5 mmol/L. Fasting triglycerides was estimated in nearly 67% of the population. The mean value for fasting triglycerides was 1.5 ± 0.90 mmol/L (median= 1.3; interquartile range, 0.9 to 1.9).

With regards to the inflammatory marker, ESR was estimated in nearly 61% of the total population. The median ESR was 8 mm in 1 hour (interquartile range, 5 to 16). Out of 650 patients with ESR measurement, 40% had an ESR estimation of > 10 mm in 1 hour. Eosinophil count test were performed in 92% of the patients and the mean value was 0.19 ± 0.14 $\times 10^9$/L (median = 0.16, 0.1 to 0.25). The median number of cardiovascular and metabolic drugs used was 3 (interquartile range, 1 to 5).

Variable	N	Mean	SD	Median	Interquartile range	
					25%	75%
Age (years)	1058	58.0	14.9	59.0	48.0	69.0
BMI (Kg/m^2)	1029	30.0	6.1	29.1	25.8	33.1
SBP (mm Hg)	1054	140.5	19.5	136	126.0	152.0
DBP (mm Hg)	1054	86.9	11.4	88	80.0	94.0
Serum creatinine (µmol/L)	1047	85.4	25.6	81.0	70.0	94.0
eGFR (mL/min/1.73 m^2)	1046	78.3	19.5	77.9	65.4	91.5
Fasting glucose (mmol/L)	718	5.4	1.4	5.2	4.8	5.7
Random glucose (mmol/L)	265	6.2	2.4	5.4	4.9	6.7
Total cholesterol (mmol/L)	1033	5.1	1.1	5.0	4.3	5.8
HDL-cholesterol (mmol/L)	1022	1.4	0.47	1.3	1.1	1.6
Fasting triglycerides (mmol/L)	709	1.5	0.9	1.3	0.9	1.9
Eosinophil count ($\times 10^9$/L)	980	0.19	0.14	0.16	0.1	0.25
ESR (mm in 1 hour)	650	13.7	23.9	8.0	5.0	16.0
Number of drugs used*	1035	3.1	2.2	3.0	1.0	5.0

Table 3-1 : Demographic data of the study population (n= 1059). * Cardiovascular / metabolic drugs.

3.3.3 Demographic characteristics of non-diabetic hypertensive subjects without evidence of severe renal impairment

Table 3-2 summarises the clinical characteristics of those without evidence of severe renal impairment (CKD stage 4 or 5) or diabetes (n=786). The mean age was 56.5 ± 14.8 years. Approximately 40% of the subjects were obese, with an average BMI of 29.5 ± 5.8 kg/m^2. The mean SBP and DBP in this cohort were 140 ± 18.3 mm Hg and 87 ± 10.8 mm Hg, respectively. About 42% of the subjects had a SBP ≥ 140 mm Hg and 47% had a DBP record ≥ 90 mm Hg.

The mean serum creatinine level and eGFR in this cohort were 82.6 ± 18.1 µmol/L and 80.1 ±18.1 mL/min/1.73 m^2, respectively. Seventy eight patients (10%) had stage 3 CKD. While more than 90% of the cohort had an estimation of eosinophil count, ESR was measured in only 60% of the subjects. The median of eosinophil count and ESR were

0.16 $\times 10^9$/L (interquartile range, 0.10 to 0.24) and 7 mm in 1 hour (interquartile range, 4 to 15), respectively.

The mean total cholesterol and HDL-cholesterol level in this cohort were 5.2 ± 1 mmol/L and 1.5 ± 0.5 mmol/L, respectively. More than half of the subjects had an estimation of total cholesterol of more than 5 mmol/L. The median fasting triglycerides was 1.2 (interquartile range, 0.9 to 1.8). Fasting blood glucose was estimated in 561 patients with an average value of 5.2 ± 0.6 mmol/L while the mean random blood glucose in 184 subjects was 5.5 ± 1.1 mmol/L. The median number of cardiovascular or metabolic drugs used in this cohort was 3.0 (interquartile range, 1.0 to 4.0).

Variable	**N**	**Mean**	**SD**	**Median**	**Interquartile range**	
					25%	**75%**
Age (years)	786	56.5	14.8	57.0	46.0	68.0
BMI (Kg/m^2)	769	29.5	5.8	28.7	25.4	32.3
SBP (mm Hg)	784	139.8	18.3	136	126	150
DBP(mm Hg)	784	87.3	10.8	88	80	93
Serum creatinine (µmol/L)	786	82.6	18.1	79.0	70.0	93.0
eGFR (mL/min/1.73 m^2)	786	80.1	18.1	78.7	67.0	92.4
Fasting glucose (mmol/L)	561	5.2	0.6	5.1	4.8	5.5
Random glucose (mmol/L)	184	5.5	1.1	5.2	4.8	5.7
Total cholesterol (mmol/L)	771	5.2	1.0	5.1	4.4	5.9
HDL-cholesterol (mmol/L)	766	1.5	0.5	1.4	1.1	1.7
Fasting triglycerides (mmol/L)	556	1.5	0.9	1.2	0.9	1.8
Eosinophil count ($\times 10^9$/L)	733	0.18	0.13	0.16	0.10	0.24
ESR(mm in 1 hour)	480	11.9	12.7	7.0	4.0	15.0
Number of drugs used*	766	2.7	1.9	3.0	1.0	4.0

Table 3-2: Demographic characteristics of hypertensive subjects without diabetes, CKD (stage 4 or 5) or proteinuria (n=786). * Cardiovascular / metabolic drugs.

3.3.4 Demographic characteristics of non-diabetic subjects without severe renal impairment stratified by gender

Table 3-3 represents demographic characteristics by gender in non-diabetic subjects without severe renal impairment (n=786). Males had mean age (53.2 ±13.8 years), lower than in females (59.3 ± 14.9 years) (P <0001). Both males and females had mean BMI of about 30 kg/m^2. Males had mean SBP (137.8 ± 16.8 mm Hg) lower than in females (141.3 ± 19.3 mm Hg) (P<0.001). About 45% of female subjects had a SBP record of ≥ 140 mm Hg compared with 32% in male subjects. Males had higher mean DBP (88.3 ± 10.1 mm Hg in males versus 86.5 ±11.3 mm Hg in females) and the proportion of male subjects who had mean DBP of ≥ 90 mm Hg was higher than in females; 51% and 45% ,respectively.

Male subjects had mean serum creatinine higher than in females (91.1 ± 17.6 µmol/L versus 75.4 ± 15.1 µmol/L) (P<0.001). Less than 3% and 1% of male and female respectively had serum creatinine level of more than 130 µmol/L. Although males had higher creatinine level, the mean eGFR in men was higher than in females; 84.4 ±18.2 and 76.5 ± 17.2 mL/min/1.73 m^2 (P<0.001), respectively. Fasting blood glucose was higher in males than in females; mean 5.2 ± 0.6 mmol/l and 5.1 ± 0.6 mmol/l (P=0.006), respectively. However, random serum glucose was not different.

The mean total cholesterol level was significantly higher in females than in males (5.2 ±1.0 mmol/L in females versus 5.0 ± 1.0 mmol/L in males, P<0.001). Likewise, HDL-cholesterol level was higher in females than in males; 1.6 ± 0.4 mmol/L versus 1.3 ± 0.5 mmol/L (P=0.003), respectively. Females had median triglycerides level (1.3; interquartile range, 0.9 to 1.7 mmol/L lower than in males (1.7; interquartile range, 1.0 to 2.1) (P<0.001).

The median ESR was significantly lower in males (5 mm in 1 hour; interquartile range 2 to 10) than in females (10 mm in 1 hour; interquartile range, 5 to 18). Only one quarter of

male subjects had an ESR of more than 10 mm in 1 hour. In contrast, more than 45% of female subjects had an ESR of more than 10 mm in 1 hour. Eosinophil count was lower in females than in males; mean 0.17 ± 0.13×10^9/L and 0.20 ± 14×10^9/L (P=0.016), respectively. Approximately 5% in each gender had eosinophilia. The median number of cardiovascular and metabolic drugs was not statistically different between males and females.

Variable	**N**	**Male**	**N**	**Female**	**P-value**
Age (years)	359	53.2 ± 13.8	427	59.3 ± 14.9	<0.001
BMI (Kg/m^2)	353	29.7 ± 5.1	416	29.3 ± 6.3	0.307
SBP (mm Hg)	358	137.8 ± 16.8	426	141.3 ± 19.3	<0.001
DBP (mm Hg)	358	88.3 ± 10.1	426	86.5 ± 11.3	<0.001
Serum creatinine (µmol/L)	359	91.1 ± 17.6	427	75.4 ±15.1	<0.001
eGFR (mL/min/1.73 m^2)	359	84.4 ± 18.2	427	76.5 ± 17.2	<0.001
Fasting glucose (mmol/L)	255	5.3 ± 0.6	306	5.1 ± 0.6	0.006
Random glucose (mmol/L)	85	5.5 ± 1.1	99	5.5 ± 1.2	0.914
Total cholesterol (mmol/L)	352	5.0 ± 1.0	419	5.2 ± 1.0	<0.001
HDL-cholesterol (mmol/L)	351	1.3 ± 0.5	415	1.6 ± 0.4	0.003
Fasting triglycerides (mmol/L)	252	1.7 (1 to 2.1)	304	1.3 (0.9 to 1.7)	<0.001
ESR (mm in 1 hour)	210	5 (2 to 10)	270	10 (5 to 18)	<0.001
Eosinophil count (×10^9/L)	337	0.2 ± 1.4	396	0.17 ± 0.13	0.016
Number of drugs used*	414	2.0 (1 to 4)	352	3.0 (1 to 4)	0.510

Table 3-3: Demographic characteristics of non-diabetic hypertensive subjects without severe kidney impairment stratified by gender (n=786). Variables are expressed as mean ± SD or median (interquartile range), as appropriate. * Cardiovascular / metabolic drugs.

3.3.5 Demographic data in subjects with hypertension and type II diabetes mellitus:

The demographic and clinical characteristics of diabetic patients with essential hypertension are listed in table 3-4. The genders were equally distributed in this cohort. The average age was 65 ± 12.2 years which is higher than mean age of non-diabetic subjects. Diabetic subjects were characterised by increased BMI, with a mean value of 33 ± 6.7 Kg/m^2. The median SBP (134.0 mm Hg; interquartile range, 124.0 to 150.0) and mean DBP (81.4 ± 10.7 mm Hg) were slightly lower than in non-diabetic individuals.

Diabetic subjects tended to have high serum creatinine (median 88 µmol/L; interquartile range, 77.0 to 104.5) and low eGFR (mean 69.8 ± 22.0 mL/min). The median fasting glucose level in the cohort (7.3 mmol/L; interquartile range, 5.6 to 8.8) was higher than the median fasting glucose level of non-diabetic individuals. Similarly, random glucose was higher than that in non-diabetic subjects; median 8.9 mmol/l (interquartile range, 7.1 to 11.9). Compared with the non-diabetic cohort, the median of inflammatory markers in diabetic subjects were higher; 0.23×10^9 /L (interquartile range, 0.15 to 0.31) for eosinophil and 11 mm in 1 hour for ESR (interquartile range, 5 to 28).

The median total cholesterol level, HDL-cholesterol and fasting triglycerides were 4.3 mmol/L (interquartile range, 3.6 to 5.1), 1.1 mmol/L (interquartile range, 1.0 to 1.4) and 1.4 mmol/L (interquartile range, 1.1 to 2.0), respectively. While total cholesterol and HDL-cholesterol were lower than that observed in non-diabetic subjects, fasting triglycerides were higher than in non-diabetics. High number of drugs was used in this cohort (median 2.2, interquartile range, 4.0 to 7.0).

Variable	N	Mean	SD	Median	Interquartile range	
					25%	75%
Age (years)	125	64.9	12.2	66.0	56.0	75.0
BMI (Kg/m^2)	121	33.0	6.7	32.0	28.0	36.4
SBP (mm Hg)	123	139.3	20.7	134.0	124.0	150.0
DBP (mm Hg)	123	81.4	10.7	82.0	75.0	88.0
Serum creatinine (µmol/L)	125	95.5	29.3	88.0	77.0	104.5
eGFR ($mL/min/1.73\ m^2$)	125	69.8	22.0	69.0	53.8	86.0
Fasting glucose (mmol/L)	79	7.7	2.8	7.3	5.6	8.8
Random glucose (mmol/L)	38	9.4	3.7	8.9	7.1	11.9
Total cholesterol (mmol/L)	123	4.4	1.1	4.3	3.6	5.1
HDL-cholesterol (mmol/L)	123	1.2	0.36	1.1	1.0	1.4
Fasting triglycerides (mmol/L)	78	1.6	0.77	1.4	1.1	2.0
Eosinophil count ($\times 10^9/L$)	117	0.24	0.15	0.23	0.15	0.31
ESR (mm in 1 hour)	78	18.7	19.5	11.0	5	28
Number of drugs used*	125	5.4	6.0	2.2	4.0	7.0

Table 3-4: Clinical characteristics of subjects with hypertension and type II diabetes mellitus (n=125). * Cardiovascular / metabolic drugs.

3.3.6 Prevalence of microalbuminuria and gross proteinuria

3.3.6.1 Prevalence in general hypertension population (n=943):

According to the conventional definition, microalbuminuria was present in 104 patients (11%). When a lower cut-off level was used (i.e. the new definition), a further 105 patients (11.1%) were added, making the total prevalence according to both new and conventional definitions 22% (figure 3-2). Gross proteinuria, defined as ACR greater than 25, was found in only 27 patients (3%).

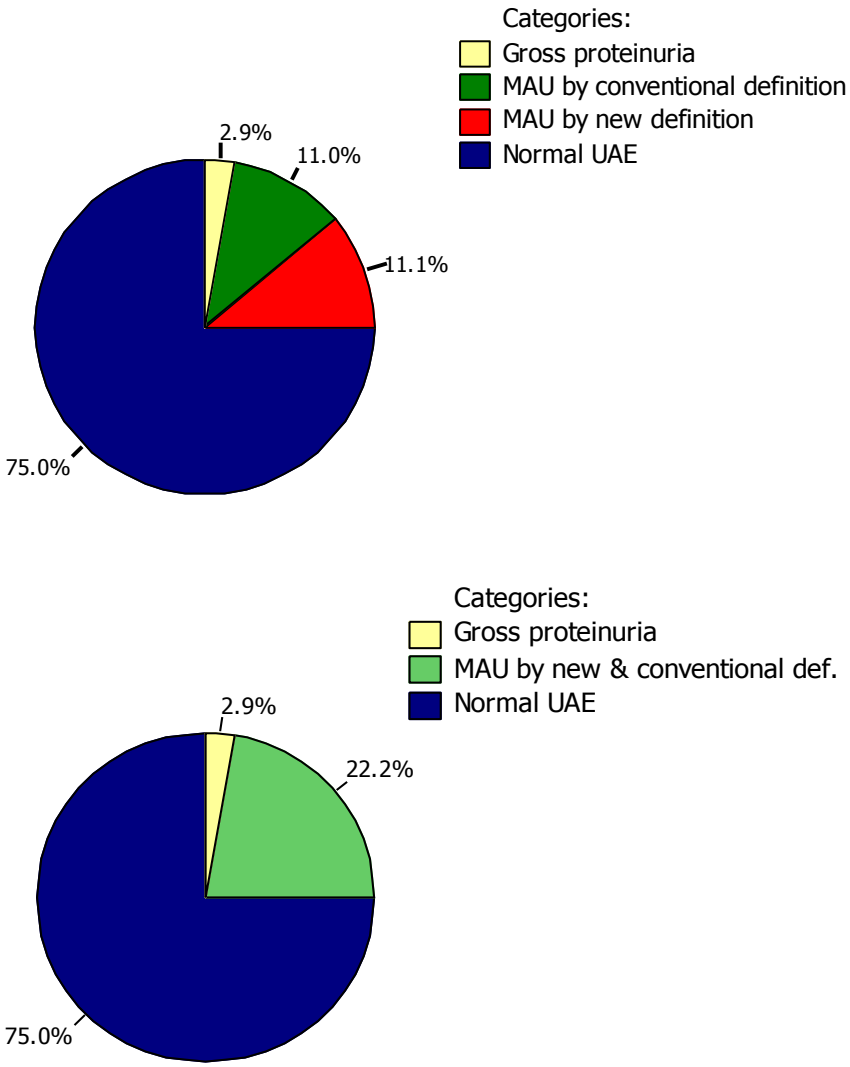


Figure 3-2: Prevalence of microalbuminuria (MAU) in the study population (n=943).

3.3.6.2 Prevalence in subjects with hypertension and type II diabetes mellitus (n=125):

Microalbuminuria, according to the conventional definition, was present in 25 patients (20%). Another 25 patients had low ACR and met the criteria of the new definition, making the total prevalence to be 40%. The prevalence of gross proteinuria in this cohort was 9.6% (figure 3-3). It should be noted that nearly half of subjects with gross proteinuria in the overall population (n=943) were diabetics.

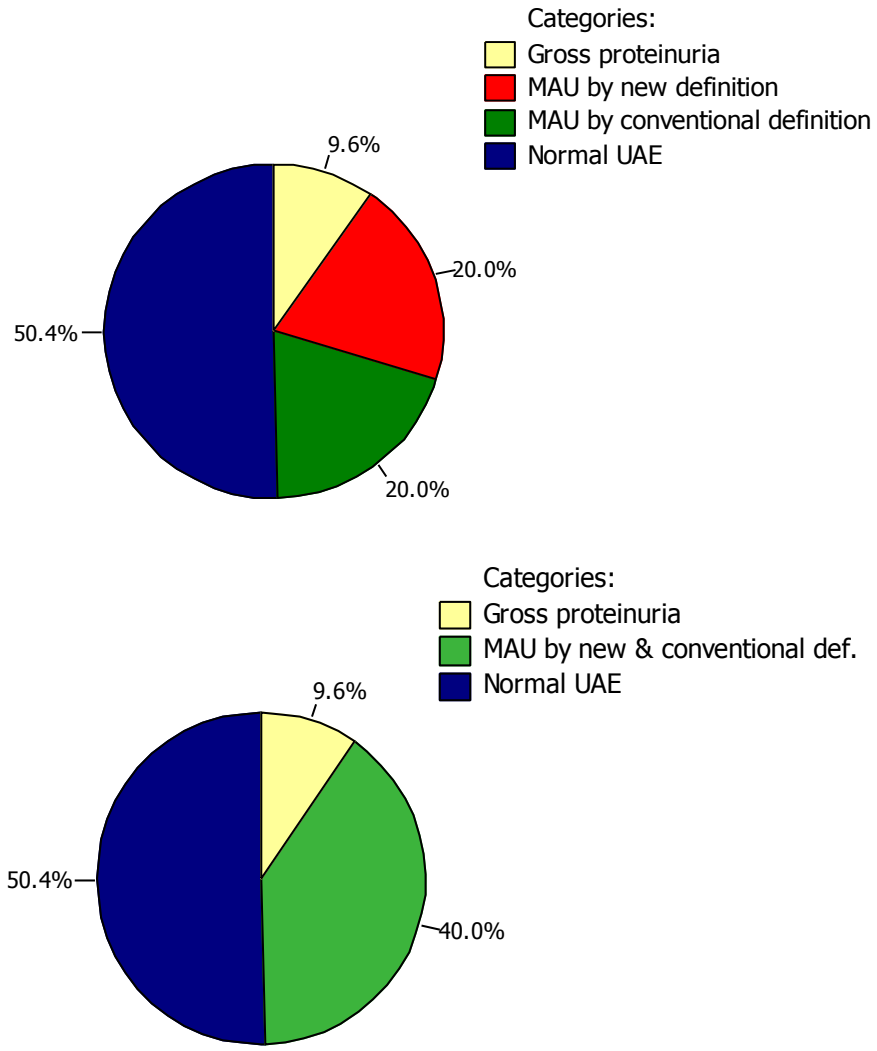
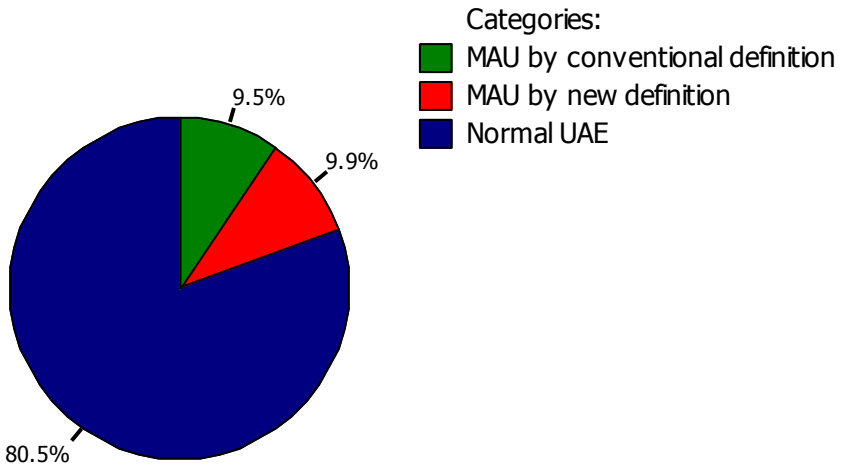


Figure 3-3: Prevalence of microalbuminuria in hypertensive patients with type II diabetes.

3.3.6.3 Prevalence in non-diabetic hypertensive subjects without evidence of severe renal impairment (n= 786):

After excluding subjects with severely reduced renal function (stage 4 CKD), those with end-stage renal failure (stage 5 CKD), subjects with gross proteinuria and those with diabetes, the prevalence of microalbuminuria using the conventional definition was 9.5% (figure 3-4). When the new definition was used, the prevalence of microalbuminuria was increased by 9.9% and combining the two definitions results in a prevalence of 19.5%.



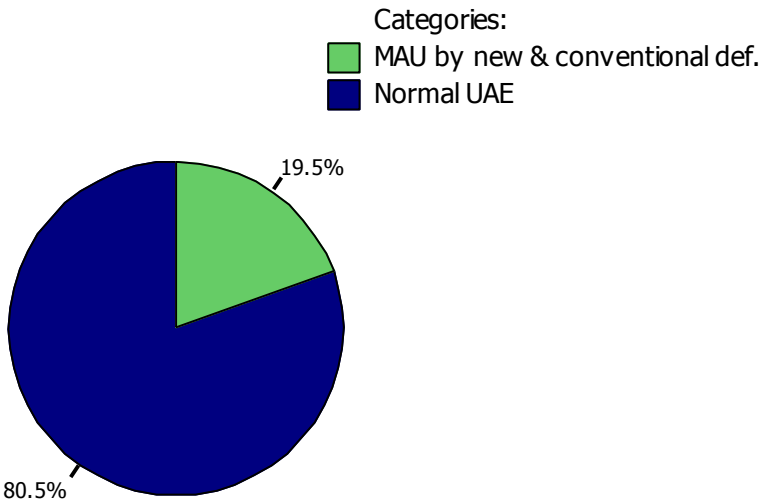


Figure 3-4: Prevalence of microalbuminuria in non diabetic hypertensive subjects without severe renal impairment.

3.3.7 Demographic characteristics of non-diabetic subjects without severe renal impairment stratified by urinary albumin excretion:

A comparison of demographic characteristics between people with normoalbuminuria and microalbuminuria (by the two definitions) is shown table 3-5. Between groups statistical comparisons are shown in table 3-6.

3.3.7.1 General characteristics:

The mean age of subjects with microalbuminuria according to the conventional definition was significantly higher than that in subjects without albuminuria, 62 ± 16.1 years versus 55 ± 14.1 years, respectively (P=0.001). Likewise, subjects with microalbuminuria according to the new definition had a higher mean age compared with individuals with normoalbuminuria, 61 ± 16.4 years versus 55 ± 14.1 years, respectively (P=0.005).

There were no differences between the groups in terms of body mass index where all groups had a mean of around 30 kg/m^2. Males were more likely than females to have microalbuminuria according to both definitions. However, the comparison between the three groups in terms of gender failed to reach statistical significance (P=0.236).

3.3.7.2 Blood pressure:

There was a clear trend of stepwise increase of blood pressure with the increase of UAE. Subjects with microalbuminuria according to the conventional definition had the highest mean SBP followed by those with the new definition of microalbuminuria and normoalbuminuric subjects; 149.7 ± 22.5 mm Hg, 140.0 ± 18.1 mm Hg and 138.5 ±17.3 mm Hg, respectively. The mean SBP of the conventional definition group was statistically higher than that in the new definition group and in normoalbuminuria, P<0.05. Although there was little evidence of a difference between the three groups in terms of mean DBP,

the pulse pressure tended to increase with the increase of UAE; 51.0 ± 15.0 mm Hg, 55.1 ± 17.2 mm Hg and 61 ± 21.1 mm Hg for subjects with normoalbuminuria, the new definition and the conventional definition of microalbuminuria, respectively (P<0.001). While the pulse pressure in subjects with microalbuminuria by the conventional definition was statistically greater than in normoalbuminuric individuals (P<0.001), the differences between subjects with the new definition and the two other groups did not achieve statistical significance.

3.3.7.3 Blood biochemistry:

Renal function

The mean serum creatinine level in subjects with microalbuminuria according to the new and the conventional definitions was higher than in individuals with normoalbuminuria. Positive subjects according to the new definition had the highest mean serum creatinine; 91.5 ± 20.9 µmol/L while the mean serum creatinine of the conventional definition group was 88.7 ± 25.7 µmol/L. Individuals with normoalbuminuria had a mean serum creatinine of 80.7 ± 16.0 µmol/L. The differences between the microalbuminuria groups and normoalbuminuric individuals were significant. Nevertheless, there were no significant differences between the two definitions groups in the mean level of serum creatinine.

Patients with microalbuminuria according to the new definition had the lowest mean of eGFR; 71.6 ± 17.9 mL/min/1.73 m^2 and this was significantly lower than that in subjects normoalbuminuria; 81.5 ± 17.1 mL/min/1.73 m^2 (P<0.001). The average eGFR in the conventional definition group was 77.4 ± 23.6 mL/min/1.73 m^2 but a post-hoc analysis failed to reveal any difference from the other groups.

Metabolic indices

Results from fasting blood glucose tests showed that patients with microalbuminuria by the new and the conventional definition had mean fasting blood glucose level higher than in the control individuals; 5.3 ± 0.5 mmol/L, 5.3 ± 0.6 mmol/L 5.1 ± 0.6 mmol/L, respectively, but the difference was not significant. People with the conventional definition of microalbuminuria had the highest random glucose level.

Lipid profile

All three groups had similar mean total cholesterol. Subjects with normoalbuminuria had mean total cholesterol of 5.2 ± 1.0 mmol/L while patients with microalbuminuria according to the two definitions had the same mean, 5.0 ± 1.0 mmol/L. Furthermore, the HDL-cholesterol levels in the three groups were not different; 1.5 ± 0.4 mmol/L for normoalbuminuric, 1.5 ± 0.5 mmol/L for the new definition and 1.4 ± 0.4 mmol/L for the conventional definition individuals. However, new definition group showed the highest geometric mean of fasting triglycerides (1.7 mmol/L, 95 % CI: 1.4, 2.1) compared with both individuals with normoalbuminuria (P=0.001) and those with microalbuminuria by the conventional definition (p=0.032), 1.3 mmol/L (95 % CI: 1.2, 1.3) and 1.3 mmol/L (95 % CI: 1.1, 1.5), respectively.

Inflammatory markers

Subjects with microalbuminuria by the conventional definition had the highest eosinophil count (P=0.012). The geometric mean in the conventional definition group was 0.18×10^{9}/L (95% CI, 0.16 - 0.22) while those with normoalbuminuria and the new definition of microalbuminuria had similar geometric means 0.14×10^{9}/L (95% CI, 0.13 - 0.15) 0.14×10^{9}/L (95% CI:, 0.11 - 0.16), respectively.

ESR was significantly higher in patients with microalbuminuria by the conventional definition (10.8 mm in 1 hour ; 95% CI, 7.9 - 15.1) than in individuals with normal UAE (7.2 mm in 1 hour; 95% CI, 6.6 - 7.9) (P =0.020). The new definition group had an intermediate ESR geometric mean: 8.4 mm in 1 hour (95% CI, 6.3 - 11.4); however, the difference failed to reach statistical significance.

Cardiovascular / metabolic drugs

The median number of drugs used in individuals with normoalbuminuria was 2 (interquartile range, 1 to 4). There was a trend of increased number of drugs used in microalbuminuric subjects; 3 (interquartile range, 2 to 4) for the conventional definition group and 4 (interquartile range, 1 to 5) for the new definition group.

Variable (unit)	Normal UAE	N	New definition	N	Conventional definition	N	P-value
Age (years)	55.4 ± 14.1	633	60.9 ± 16.4	78	62.0 ±16.1	75	<0.001
BMI (Kg/m^2)	29.4 ± 5.7	618	29.8 ± 6.0	78	29.9 ± 6.3	73	0.668
Male (%)	44.2%	280	50.0%	39	53.3%	40	0.236
SBP (mm Hg)	138.5 ± 17.3	631	140.0 ± 18.1	78	149.7 ± 22.5	75	<0.001
DBP (mm Hg)	87.5 ± 10.3	631	84.9 ± 11.1	78	88.6 ± 13.7	75	0.074
Pulse pressure (mm Hg)	51.0 ± 15.0	631	55.1 ± 17.2	78	61.0 ± 21.1	75	<0.001
Serum creatinine (µmol/L)	80.7 ± 16.0	633	91.5 ± 20.9	78	88.7 ± 25.7	75	<0.001
eGFR ($mL/min/1.73\ m^2$)	81.5 ± 17.1	633	71.6 ± 17.9	78	77.4 ± 23.6	75	<0.001
Fasting glucose (mmol/L)	5.1 ± 0.6	463	5.3 ± 0.5	44	5.3 ± 0.6	54	0.080
Random glucose (mmol/L)	5.4 ± 1.0	139	5.4 ± 0.8	29	5.9 ± 1.7	16	0.282
Total cholesterol (mmol/L)	5.2 ± 1.0	622	5.0 ± 1.0	74	5.0 ± 1.1	75	0.099
HDL-cholesterol (mmol/L)	1.5 ± 0.4	617	1.5 ± 0.5	76	1.4 ± 0.4	73	0.741
F. triglycerides* (mmol/L)	1.3 (1.2-1.3)	459	1.7 (1.4-2.1)	43	1.3 (1.1-1.5)	54	0.001
Eosinophil count ($\times 10^9/L$)	0.14 (0.13-0.15)	588	0.14(0.11-0.16)	75	0.18 (0.16- 0.22)	70	0.012
ESR (mm in 1 hr)	7.2 (6.6-7.9)	399	8.4(6.3-11.4)	38	10.8(7.9-15.1)	43	0.022
No. of drugs**	2 (1 to 4)	615	4 (1 to 5)	77	3 (2 to 4)	74	<0.001

Table 3-5: Comparison of patient characteristics in normoalbuminuric (n=633) and microalbuminuric subjects according to the new definition (n= 78) and the conventional definition (n=75) in non-diabetic hypertensive subjects without severe renal impairment. Results are summarised as mean ± standard deviation, geometric mean (95% confidence interval for the mean) median (interquartile range) or percentage of cohort. * F.: fasting. ** Cardiovascular / metabolic drugs (compared using Kruskal Wallis test).

Variable	Categories		Mean difference	P value	95% CI	
					Lower	Upper
Age (years)	Normoalb. versus:	1.New def.	-5.55	0.005	-9.66	-1.44
		2.Conv. def.	-6.62	0.001	-10.81	-2.44
	New def. versus	Conv. def.	-1.07	0.892	-6.62	4.47
SBP (mm Hg)	Normoalb. versus:	1.New def.	-1.48	0.772	-6.56	3.59
		2.Conv. def.	-11.16	<0.001	-16.33	-6.0
	New def. versus	Conv. def.	-9.68	0.003	-16.52	-2.84
Pulse pressure (mm Hg)	Normoalb. versus:	1.New def.	-4.06	0.089	-8.50	0.42
		2.Conv. def.	-9.98	<0.001	-14.5	-5.40
	New def. versus	Conv. def.	-5.92	0.056	-11.9	0.12
Serum Creatinine (µmol/L)	Normoalb. versus:	1.New def.	-10.78	<0.001	-15.77	-5.8
		2.Conv. def.	-7.97	0.001	-13.04	-2.9
	New def. versus	Conv. def.	2.81	0.586	-3.9	9.54
eGFR (mL/min/1.73 m^2)	Normoalb. versus:	1.New def.	9.89	<0.001	4.85	14.94
		2.Conv. def.	4.10	0.146	-1.03	9.24
	New def. versus	Conv. def.	-5.79	0.113	-12.59	1.01
Fasting triglycerides*† (mmol/L)	Normoalb. versus:	1.New def.	0.74	0.001	0.62	0.90
		2.Conv. def.	0.97	0.817	0.83	1.15
	New def. versus	Conv. def.	1.31	0.032	1.03	1.66
Eosinophil count*† ($\times 10^9$/L)	Normoalb. versus:	1.New def.	1.02	0.963	0.83	1.30
		2.Conv. def.	0.76	0.010	0.59	0.94
	New def. versus	Conv. def.	0.73	0.043	0.54	0.99
ESR*† (mm in 1 hr)	Normoalb. versus:	1.New def.	0.86	0.610	0.59	1.24
		2.Conv. def.	0.67	0.020	0.49	0.95
	New def. versus	Conv. def.	0.77	0.453	0.49	1.22

Table 3-6: Multiple comparisons where differences between the groups' means (95% confidence interval) were statistically significant in ANOVA test. * Geometric mean ratio instead of mean difference. † 95% confidence intervals for the ratio of the geometric mean. Normoalb.: subjects with normoalbuminuria.

3.3.8 Demographic and clinical characteristics of diabetic subjects stratified by albumin excretion

Table 3-7 compares the different clinical characteristics of diabetic cohort stratified by albuminuria level. Overall, age, blood pressure, BMI, cholesterol, fasting triglycerides, ESR, serum creatinine and fasting glucose tended to increase with the increase of albuminuria level, although comparison between groups failed to reach statistical significance, except for BMI. No differences were observed in the groups when comparing HDL-cholesterol and eosinophil count. The eGFR tended to decline with the increase of albuminuria level. The use of pharmacological agents was higher in subjects with albuminuria (P=0.029).

Variable	Normoalbuminuria	N	New definition	N	Conventional def.	N	Gross proteinuria	N	P value
Age (years)	63.1 ± 12.0	63	65.4 ± 10.0	25	68.0 ± 14	25	66.7 ± 14.1	12	0.345
BMI (kg/m^2)	32.8 ± 6.3	61	31.3 ± 5.8	24	32.7 ± 7.3	24	38.0 ± 7.7	12	**0.041**
Male (%)	44.4%	28	64.0%	16	56.0%	14	41.7%	5	0.347
SBP (mm Hg)	132 (123 to 146)	62	134 (123 to 147)	25	142 (125 to 151)	25	142 (138 to 166)	11	0.137
DBP (mm Hg)	81.8 ± 9.5	62	81.3 ± 10.0	25	78.7 ± 14.7	25	85.8 ± 6.8	11	0.312
Creatinine(µmol/L)	87 (76 to 100)	63	90 (78.5 to 103.5)	25	83 (75 to 144.5)	25	103.5 (80.2 to 131.7)	12	0.377
eGFR ($mL/min/1.73\ m^2$)	70.9 ± 18.2	63	73.2 ± 17.1	25	69.6 ± 33.4	25	57.8 ± 18.4	12	0.232
Fasting glucose (mmol/L)	6.3 (5.2 to 8.3)	39	7.8 (5.1 to 8.5)	15	8.1 (6.1 to 10.5)	19	8.1 (7.4 to 12.7)	6	0.065
Cholesterol (mmol/L)	4.4 (3.7 to 5.3)	62	3.8 (3.6 to 4.5)	25	4.2 (3.4 to 5.1)	24	4.8 (3.8 to 5.2)	12	0.297
HDL-Cholesterol (mmol/L)	1.2 (1.0 to 1.4)	63	1.1 (1.0 to 1.4)	25	1.1 (0.9 to 1.3)	23	1.2 (0.9 to 1.4)	12	0.341
F. triglycerides (mmol/L)	1.4 (1.0 to 1.9)	38	1.4 (1.1 to 2.2)	15	1.5 (0.8 to 2.0)	18	2.2 (1.3 to 2.7)	7	0.366
Eosinophil count ($\times 10^9/L$)	0.21 (0.15 to 0.29)	60	0.24 (0.14 to 0.33)	24	0.22 (0.14 to 0.32)	24	0.24 (0.17 to 0.33)	9	0.899
ESR (mm in 1 hour)	9 (5.0 to 25.5)	42	7 (5.0 to 20.0)	15	20 (8.5 to 30.7)	14	38 (7.0 to 71.0)	7	0.079
Number of drugs*	5 (3.0 to 7.0)	63	5 (4.5 to 6.0)	25	6 (5.0 to 7.0)	25	7 (5.2 to 8.0)	12	**0.029**

Table 3-7: The clinical characteristics of diabetic subjects stratified by albuminuria level (n=125). Variables are expressed as mean ± SD or median (interquartile range), as appropriate. * Cardiovascular / metabolic drugs. Def.: definition.

3.4 Summary/ discussion

The population was typical of hypertensive individuals. As well as high blood pressure, the subjects were relatively elderly, overweight and with multiple risk factors.

Microalbuminuria including a new definition incorporating lower levels of albuminuria was present in one fifth of the study population. The distribution of the new definition and the conventional definition of microalbuminuria was almost equal (11%). In diabetic subject, the prevalence of microalbuminuria and gross proteinuria was high. Almost half of diabetic patients had increased levels of albumin in the urine.

When the demographic characteristics of the non-diabetic subjects without severely reduced renal function was compared according to UAE classification, subjects with microalbuminuria had increased risk factors for poor outcomes. This was also seen with diabetic patients although the small number of patients precluded statistically significant differences.

In summary, microalbuminuria is associated with increased levels of different risk factors for cardiovascular diseases. The increased levels of such factor were also evident in those with levels of albuminuria below the current definition of microalbuminuria.

4. Chapter four: association of microalbuminuria with co-morbidities and risk factors (categorical data)

4.1 Introduction

Microalbuminuria has been linked with several comorbidities including target organ damage. For instance, in the HyperGen study, the researchers found a relationship between albuminuria (based on a single measurement) and LVH in hypertensive subjects (Djoussé et al., 2008). The purpose of the analysis presented in this chapter was to further evaluate the association of microalbuminuria with different co-morbidities and risk factors. In addition, relationships with pharmacological groups and individual cardiovascular risk score using the Framingham and the JBS equations were investigated.

4.2 Methods

Categorical data were collected from patient case-notes. Information included family history of co-morbidities, smoking status together with co-morbidities and risk factors (LVH, evidence of CVD, type II diabetes and ST-T changes on ECG). The use of antihypertensive drugs, lipid lowering agents, antidiabetics, anticoagulants and vasodilators (nitrates) were also studied. Diagnosis of LVH was based on the opinion of the attending clinician using established criteria. The Framingham equation was used to calculate an individual's cardiovascular risk. In addition, cardiovascular risk was calculated using the amended algorithm from the JBS. This method excludes diabetic subjects and those with LVH as these are considered as high risk populations. A risk score for any cardiovascular events in the next 10 years greater than 20% was regarded as high risk while a risk score between 10% - 20% or less than 10% were considered as moderate or low risk, respectively. Categorical variables were expressed as percentage of the cohort and the differences between the study's groups were assessed using Chi-square or Fisher's

exact test, as appropriate. When P value for any variable was <0.05, linear association was examined using chi-square test for trend.

4.3 Results

4.3.1 Baseline characteristics

Clinical characteristics for categorical variables are shown in table 4-1. As mentioned earlier, the majority were females (54% of the overall population). About 10% of the study population were smokers and 13% had type II diabetes mellitus. Evidence of cardiovascular complications was relatively common with LVH in about a quarter, ST-T changes on ECG evident in 15% and cardiovascular diseases, including clinical evidence of CHD, stroke/transient ischaemic attack or peripheral vascular disease, present in about one fifth of the study population. Nearly 20%, 15% and 12% of the cohort had family history of CHD, diabetes or stroke, respectively.

Table 4-1 also summarises the current therapy of the study cohort. The most commonly used antihypertensive agents were diuretics which were prescribed for nearly half of the study population. CCBs and ACEIs were also common in this cohort, each of which were used by more than 40% of the patients and nearly one third of the patients were treated with ARBs or beta blockers.

Statins were taken by 38% of the patients but other lipid lowering drugs, such as fibrates and ezetimibe, were seldom used. More than quarter of the population were prescribed antiplatelet agents and approximately 9% of the study population were treated with antidiabetic drugs.

Variable	Total available	N	Percentage
Male	1059	489	46.2%
Family history:			
- Diabetes	747	114	15.3%
- Stroke	795	94	11.8%
- CHD	795	155	19.5%
Co-morbidities/ risk:			
- Current smoker	829	76	9.2%
- Type II diabetes	1048	137	13.1%
- LVH	954	232	24.3%
- ST-T changes on ECG	761	107	14.1%
- CVD	990	174	17.6%
Pharmacotherapy:			
- Diuretics	1040	508	48.8%
- CCBs	1040	450	43.3%
- ACEIs	1040	428	41.2%
- ARBs	1040	342	32.9%
- Beta blockers	1040	321	30.9%
- Alpha blockers	1040	159	15.3%
- Aldosterone antagonists	1040	95	9.1%
- Imidazoline agonists	1040	22	2.1%
- Statins	1040	393	37.8%
- Other lipid lowering agents	1040	37	3.6%
- Anti-platelet agents	1040	272	26.2%
- Anti-diabetics	1040	88	8.5%
- Anticoagulants	1040	28	2.7%
- Vasodilators	1040	24	2.3%

Table 4-1: Clinical characteristics (categorical variables) of the study population (n=1059).

4.3.2 Cardiovascular risk assessment

Tables 4-2 and 4-3 present the 10 year cardiovascular risk assessment of the overall cohort over 10 years. Data of those with already established cardiovascular diseases including CHD, congestive heart failure, peripheral vascular disease and stroke (n=174) have been excluded. Missing information precluded the risk assessment of 29 subjects. Risk calculations were carried out in the remaining 856 subjects (table 4-2). As the age range of 137 subjects was outside the recommended age range in the Framingham study (30 – 74 years), risk calculations were also carried out to a subgroup of 719 individuals whose age met the Framingham criteria (table 4-3). It should be noted that risk score calculations were based on values usually obtained after treatment was introduced.

Overall, the population had moderate risk for CVD; median 14.1% (interquartile range, 7.5% to 23.7%). In the age-restricted group, the median CVD risk was 13.1% (interquartile range, 7.7% to 21.3%). More than one-quarter of the individuals had high risk for CVD. The mean risk score using the JBS calculation (after excluding those with LVH and diabetes) was 11.1% (SD ± 9.4) (10.5% (SD ± 8.2) in the age-restricted group).

The median risk of developing CHD was 9.1% (interquartile range, 4.4% to 16.1) in the 856 subjects and 8.9% (interquartile range, 4.8% to 15.1%) in the age-restricted group. More than a quarter of the subjects had risk score of ≥ 15% for developing CHD over the next 10 years. The median risk of stroke was 2.3% (interquartile range, 1.0% to 4.8%) (2.1% (interquartile range, 1.0% to 3.9%) in the age-restricted group. More than 18% of subjects had stroke risk of 5% or more.

The median risk score for myocardial infarction in the study cohort was 3% (interquartile range, 1.1 to 6.1) (2.9% (interquartile range, 1.3% to 5.8%) in the age-restricted group).

The risk of death from either CHD or CVD was low, with medians of 1.2% (interquartile range, 0.3% to 3.4%) for CHD death and 2.2% (interquartile range, 0.6% to 6.5%). About 2% of the population had 10% risk for fatal CHD. The risk of dying from CVD in 9.6% of the population was more than 10%. Almost similar risk scores were found in the age-restricted group.

Risk (%)	**N**	**Mean**	**SD**	**Median**	**Interquartile range**	
					25%	**75%**
CVD risk	856	16.8	12.0	14.1	7.5	23.7
CVD risk (JBS)*	607	11.1	9.4	8.8	4.7	14.6
CHD risk	856	11.4	9.4	9.1	4.4	16.1
Stroke risk	856	3.8	4.8	2.3	1.0	4.8
MI risk	856	4.4	4.7	2.9	1.1	6.1
CHD death risk	856	2.5	3.4	1.2	0.3	3.4
CVD death risk	856	5.2	7.2	2.2	0.6	6.5

Table 4-2: The probability of developing different cardiovascular diseases over 10 years based on the Framingham equation in the overall population (n=1059) after excluding those with already established cardiovascular diseases (n=174) and those with incomplete infomation (n=29). * Cardiovascular risk score based on the JBS equation after excluding additional 249 patients with LVH or diabetes mellitus.

Risk (%)	N	Mean	SD	Median	Interquartile range	
					25%	75%
CVD risk	719	15.6	10.7	13.1	7.7	21.3
CVD risk (JBS)*	515	10.5	8.2	8.7	4.9	13.7
CHD risk	719	11.1	8.6	8.9	4.8	15.1
Stroke risk	719	3.0	3.4	2.1	1.0	3.9
MI risk	719	4.2	4.2	2.9	1.3	5.8
CHD death risk	719	2.0	2.6	1.1	0.3	2.7
CVD death risk	719	3.7	4.8	1.9	0.6	4.7

Table 4-3: The probability of developing different cardiovascular diseases over 10 years based on the Framingham equation in the overall population (n=1059) after excluding those with already established cardiovascular diseases (n=174), those with incomplete infomation (n=29) and those outside the recommended age range (n=137). *Cardiovascular risk score based on the JBS equation after excluding additional 204 patients with LVH or diabetes mellitus.

4.3.3 Clinical characteristics of hypertensive subjects without diabetes or severe renal impairment

Table 4-4 lists clinical characteristics (categorical variables) of non-diabetic hypertensive subjects without severe renal impairment (stage 4, 5 CKD or gross proteinuria). Female subjects predominated, accounting for 55% of the cohort. The percentage of current smokers was 9%. Almost 22% of the cohort had evidence of LVH and over 10% had ST-T changes on ECG. Fifteen percent of the cohort had evidence of established CVD such as CHD, stroke, transient ischaemic attack or peripheral vascular disease. Family histories of CHD, diabetes or stroke were found in 20%, 15% and 12%, respectively.

The most frequently used antihypertensive drug were diuretics (44%) followed by calcium channel blockers (40%), ACEIs (39%), ARBs (30%) and beta blockers (28%). Lipid

lowering agents were used by more than 35% of the population (statins and others). About one fifth of the cohort used antiplatelet agents.

Variable	Total available	Count	Percentage
Male	786	359	45.7%
Family history:			
- Diabetes	555	72	13.0%
- Stroke	590	73	12.4%
- CHD	590	109	18.5%
Co-morbidities/ other risk factors:			
- Current smoker	617	57	9.2%
- LVH	727	158	21.7%
- ST-T changes on ECG	584	79	13.5%
- CVD	745	111	14.9%
Pharmacotherapy:			
- Diuretics	770	341	44.3%
- CCBs	770	306	39.7%
- ACEIs	770	301	39.1%
- ARBs	770	228	29.6%
- Beta blockers	770	213	27.7%
- Alpha blockers	770	98	12.7%
- Aldosterone antagonists	770	55	7.1%
- Imidazoline agonists	770	11	1.4%
- Statins	770	256	33.2%
- Other lipid lowering agents	770	21	2.7%
- Anti-platelet agents	770	165	21.4%
- Anticoagulants	770	21	2.7%
- Vasodilators	770	13	1.7%

Table 4-4: Clinical characteristics (categorical variables) of hypertensive subjects without diabetes mellitus or severe renal impairment (n=786).

4.3.4 Risk assessment for non-diabetic hypertensive subjects without severe renal impairment

Cardiovascular risk assessment calculations in non-diabetic hypertensive subjects without severe renal impairment are shown in table 4-5. The cohort had a moderate overall risk for developing CVD of 12.2% (interquartile range, 6.5% to 21%) using the Framingham equation and 10.6 (SD ± 8.7) using the JBS method. About one quarter of the cohort can be considered as a high risk group for developing CVD based on the Framingham risk score.

The median risk prediction for CHD was 8% (interquartile range, 4% to 14%). The percentage of subjects who had risk of ≥ 15% for developing CHD was more than 20%. The median risk score for stroke was 2% (interquartile: 0.8% to 4.1%) with 15% of the subjects having stroke risk estimate of 5% or more.

The median risk score for developing MI over 10 years in this cohort was 2.4% (interquartile range, 1% to 4.9%). The median risk of death from CHD was 0.9% (interquartile range, 0.2% to 2.8%). About 2% of subjects had CHD death risk of 10% or more. The median 10 years risk score for CVD was 1.7% (interquartile range, 0.5% to 5.3%) and about 8% of the cohort had CVD death risk of 10% or more.

Table 4-6 summarises the risk calculation in non-diabetic subjects without severe renal impairment after excluding those younger than 30 years and those older than 74 years. There were no differences in risk scores between this subgroup and those for the whole population.

Risk (%)	N	Mean	S.D	Median	Interquartile range	
					25%	75%
CVD risk	659	14.7	11.0	12.2	6.5	20.9
CVD risk (JBS)*	531	10.6	8.8	8.7	4.6	14.2
CHD risk	659	10.1	8.4	7.9	4.0	14.0
Stroke risk	659	3.2	3.7	2.0	0.8	4.1
MI risk	659	3.7	3.9	2.4	1.0	4.9
CHD death risk	659	2.0	2.8	0.9	0.2	2.8
CVD death risk	659	4.3	6.0	1.7	0.5	5.3

Table 4-5: The probability of developing different cardiovascular diseases over 10 years based on the Framingham equation in non-diabetic hypertensive subjects without severe renal impairment (n=786) after excluding 111 patients with established CVD and 16 patients with incomplete information. * Cardiovascular risk score based on the JBS equation after excluding additional 128 patients with LVH.

Risk (%)	N	Mean	S.D	Median	Interquartile range	
					25%	75%
CVD risk	564	14.2	9.9	11.8	6.8	19.2
CVD risk (JBS)	458	10.2	8.0	8.6	4.8	13.3
CHD risk	564	10.1	8.0	8.0	4.3	13.4
Stroke risk	564	2.8	3.0	1.9	0.8	3.6
MI risk	564	3.7	3.8	2.5	1.1	4.8
CHD death risk	564	1.8	2.4	0.8	0.2	2.4
CVD death risk	564	3.3	4.4	1.5	0.5	4.2

Table 4-6: The probability of developing different cardiovascular diseases over 10 years based on the Framingham equation in non-diabetic hypertensive subjects without severe renal impairment (n=786) after excluding those with established cardiovascular disease (n=111), incomplete information (n=16) and those younger than 30 years and those older than 74 years (n=95). * Cardiovascular risk score based on the JBS equation after excluding additional 106 patients with LVH.

4.3.5 Clinical characteristics of hypertensive subjects without diabetes or severe renal impairment stratified by gender

Table 4-7 lists the clinical characteristics of subjects with hypertension but without type II diabetes mellitus or severe renal impairment (stage 4, 5 or proteinuria) stratified by gender. There were no differences in terms of cardiovascular risk or co-morbidities except for LVH where males had double prevalence compared with females. The use of CCBs and ACEIs was more frequent in males while diuretics were more frequently used in females.

Variable	Female	N	Male	N	P value
Diabetes family history	14.1%	44	11.5%	28	0.375
Stroke family history	11.0%	36	14.1%	37	0.260
CHD family history	16.2%	53	21.3%	56	0.135
Current smoker	8.5%	29	10.1%	28	0.576
LVH	14.9%	58	30.0%	99	<0.001
ST-T changes	11.8%	38	15.7%	41	0.182
CVD	16.8%	68	12.6%	43	0.122
Pharmacotherapy:					
Diuretics	48.7%	203	39.1%	138	0.009
CCBs	34.3%	143	46.2%	163	0.001
ACEIs	35.1%	148	43.3%	153	0.027
ARBs	28.3%	118	31.2%	110	0.428
Beta blockers	30.2%	126	24.6%	87	0.090
Alpha blockers	13.2%	55	12.2%	43	0.745
Aldosterone blockers	7.0%	29	7.4%	26	0.889
Imidazoline agonists	1.4%	6	1.4%	5	1.000
Statins	31.7%	132	35.1%	124	0.319
Other lipid lowering agents	2.4%	10	3.1%	11	0.658
Anti-platelet agents	21.0%	87	22.1%	78	0.725
Anticoagulants	2.6%	11	2.8%	10	1.000
Vasodilators	2.2%	9	1.1%	4	0.401

Table 4-7: Clinical characteristics of hypertensive subjects without diabetes or severe renal impairment stratified by gender (n=786).

4.3.6 Clinical Characteristics of diabetic hypertensive subjects

Table 4-8 lists the clinical characteristics of hypertensive subjects with type II diabetes mellitus. The distribution of males and females in the cohort was equal. More than one quarter of the cohort had a family history of diabetes mellitus. Similarly, one quarter of the patients had a family history of CHD. One tenth of diabetic hypertensive subjects had a family history of stroke. Family history of diabetes, stroke and CHD in this cohort was higher than that observed with non-diabetic subjects.

Overall, this cohort was characterised by high prevalence of comorbidities and risk factors. Nearly one third of the patients had evidence of LVH which is a higher proportion than that reported with non-diabetic individuals. ST-T changes on ECG were found in 23% as compared with 13% in non-diabetic subjects. The prevalence of CVD was twice as high in diabetics as in non-diabetics (34% versus 15%).

In diabetic patients, use of cardiovascular drugs was higher than in non-diabetic individuals. As in the non-diabetic cohort, the most frequently prescribed antihypertensive drugs were diuretics. Diuretics were used by 73% of these patients compared with 44% of the non-diabetic subjects. CCBs, ACEIs, ARBs and beta blocker were also used frequently in the diabetic patients. The percentage of diabetic patients who used alpha blockers and aldosterone antagonists was two-fold higher than in non-diabetic individuals; (30% versus 13%) and (14% versus 7%), respectively. Lipid lowering agents (statins and others) were used extensively in this cohort. More than three quarters of the diabetic patients used lipid lowering agents compared with 36% in hypertensive subjects without diabetes mellitus. Use of antiplatelet agents was also common.

Variable	Total available	N	Percentage
Male	125	63	50.4%
Family history:			
- Diabetes	85	23	27.1%
- Stroke	88	9	10.2%
- CHD	87	22	25.3%
Co-morbidities/ risk:			
- Current smoker	89	4	4.5%
- LVH	103	32	31.1%
- ST-T changes on ECG	77	18	23.4%
- CVD	110	37	33.6%
Pharmacotherapy:			
- Diuretics	125	91	72.8%
- CCBs	125	76	60.8%
- ACEIs	125	71	56.8%
- ARBs	125	56	44.8%
- Beta blockers	125	56	44.8%
- Alpha blockers	125	38	30.4%
- Aldosterone antagonists	125	18	14.4%
- Imidazoline receptors agonists	125	5	4.0%
- Statins	125	84	67.2%
- Other lipid lowering agents	125	13	10.4%
- Anti-platelet agents	125	67	53.6%
- Anti-diabetics	125	76	60.8%
- Anticoagulants	125	5	4.0%
- Vasodilators	125	6	4.8%

Table 4-8: Clinical characteristics of subjects with hypertension and type II diabetes mellitus (n=125).

4.3.7 Risk assessment in diabetic hypertensive subjects

Table 4-9 shows 10 years risk assessment calculations in subjects with hypertension and type II diabetes. This cohort had higher probability of developing cardiovascular morbidities and mortalities than in non-diabetic individuals. They had high risk of developing general CVD; median 26.5% (interquartile range, 18.5% to 39.0%) which is almost double the risk score in non-diabetic subjects. More than 60% had high risk for CVD over the next 10 years while about one-third of diabetics were at medium risk.

Diabetic subjects had a median probability of developing CHD over 10 years of about 17% (interquartile range, 10.3 to 26.9%) which is also higher than that in non-diabetic individuals. While only 2% of non-diabetic subjects were at risk for developing stroke, diabetic patients had median risk of stroke of 5.7% (interquartile range, 2.7 to 9.0).

The median risk for MI in diabetic patients was about 8% (interquartile range, 4.0 to 12.8) which is higher than that in non-diabetic subjects by threefold. The median risk of dying from CHD or CVD in diabetic individuals were 3.9% (interquartile range, 1.8 to 7.5) and 7.1% (interquartile range, 2.6 to 17).

Table 4-10 summarises the risk scores in diabetic subjects after excluding those subjects outside the recommended age range. The risk scores were relatively lower than that reported with all diabetic subjects.

Risk (%)	N	Mean	SD	Median	Interquartile range	
					25%	75%
CVD	85	30.1	14.8	26.5	18.5	39.0
CHD	85	19.7	12.2	16.6	10.3	26.9
Stroke	85	7.8	7.8	5.7	2.7	9.0
MI	85	9.2	6.6	7.9	4.0	12.8
CHD death	85	5.7	5.5	3.9	1.8	7.5
CVD death	85	11.1	11.5	7.1	2.6	17.0

Table 4-9: The probability of developing different cardiovascular diseases over 10 years based on the Framingham equation in patients with hypertension and type II diabetes after excluding 37 patients with established CVD and 3 patients with incomplete information.

Risk (%)	N	Mean	SD	Median	Interquartile range	
					25%	75%
CVD	65	25.8	12.0	23.9	16.5	33.5
CHD	65	17.5	10.6	14.5	9.9	24.6
Stroke	65	5.4	4.5	4.6	2.3	6.4
MI	65	8.0	5.3	7.4	3.9	10.8
CHD death	65	3.8	3.6	2.7	1.4	5.2
CVD death	65	6.7	6.8	4.6	1.9	8.4

Table 4-10: The probability of developing different cardiovascular diseases over 10 years based on the Framingham equation in patients with type II diabetes and hypertension after exulding those with established cardiovascular disease (37), incomplete information (n=3) and those younger than 30 years and older than 74 years (20 patients).

4.3.8 A comparison of clinical characteristics according to albuminuria stages in non-diabetic hypertensive subjects without severe renal impairment

A comparison of clinical characteristics according to microalbuminuria stages in non-diabetic hypertensive subjects without CKD stage 4 or 5 or gross proteinuria is presented in table 4-11. The percentage of males tended to increase with the increase of ACR level (44% in normoalbuminuric, 50% in new definition and 53% in conventional definition subjects). Differences between the groups were not significant. Likewise, there were no significant differences in prevalence of family history, although family history of diabetes mellitus was more frequent in those with microalbuminuria according to the conventional definition.

Prevalence of LVH, ST-T changes on ECG and clinical evidence of cardiovascular disease increased in a stepwise manner as albuminuria increased. Trends were statistically significant for all three variables although there were no statistically significant differences between the two definitions of microalbuminuria.

Overall, the use of pharmacological groups tended to be high in subjects with microalbuminuria (by both definitions). For individual antihypertensive drug classes, however, differences were statistically significant only for CCBs and beta blockers. More than half of subjects with microalbuminuria (according to both definitions) used CCBs compared with 37% in subjects with normoalbuminuria. While only a quarter of subjects with normoalbuminuria were treated with beta blockers, 40% of subjects with microalbuminuria according to the new definition used these agents. A comparison between subjects with and without microalbuminuria by the new definition was significant.

The number of microalbuminuric individuals who have been treated with lipid lowering agents (including statins) was higher than in subjects with normal UAE ($P<0.05$). Statins were prescribed to half of subjects with microalbuminuria by the new definition, and the percentage of statins users was significantly higher than in subjects with normoalbuminuria and those with microalbuminuria by the conventional definition where only 31% used statins in each group. About 7% of individuals with microalbuminuria (according to the two definitions) used other lipid lowering agents compared with only 2% in individuals with normal UAE.

The use of antiplatelet agents in the three groups was not statistically different, with a percentage of around 23% in subjects with microalbuminuria (by both definitions) and 21% in individuals with normoalbuminuria. Only 1% of subjects with normal UAE used anticoagulants. On the contrary, approximately 6.5% of the new definition group and 11% of the conventional definition group used anticoagulants. The number of treated patients with anticoagulants was statistically higher in microalbuminuric subjects (by both definitions) than in normoalbuminuric individuals. While only 1% of normoalbuminuric individuals were treated with vasodilators, about 4% of subjects with microalbuminuria (by the two definitions groups) used anticoagulants ($P=0.030$).

Variable	Normoalb.	N	New def.	N	Conv. def.	N	P value
Male	44.2%	280	50.0%	39	53.3%	40	0.236
Family history:							
Diabetes	12.4%	57	11.9%	5	19.2%	10	0.374
Stroke	11.6%	57	17.8%	8	14.8%	8	0.389
CHD	17.6%	87	27.9%	12	18.5%	10	0.256
Co-morbidities/ risks:							
Current smoker	9.2%	47	10.4%	5	8.9%	5	0.924
LVH	19.2% a	112	29.7% b	22	34.8% b	24	0.003
ST-T changes	11.8% a	57	19.6% a, b	9	24.1% b	13	0.020
CVD	12.7% a	76	23.7% b	18	23.6% b	17	0.004
Pharmacotherapy							
Diuretics	43.3%	268	48.1%	37	48.6%	36	0.530
CCBs	36.7% a	227	49.4% b	38	55.4% b	41	0.001
ACEIs	37.6%	233	46.8%	36	43.2%	32	0.233
ARBs	28.1%	174	35.1%	27	36.5%	27	0.178
Beta blocker	25.5% a	158	40.3% b	31	32.4% a, b	24	0.016
Alpha blockers	11.6%	72	18.2%	14	16.2%	12	0.167
Aldosterone blockers	7.1%	44	7.8%	6	6.8%	5	1.000
Imidazoline agonists	1.5%	9	1.3%	1	1.4%	1	1.000
Statins	31.7% a	196	48.1% b	37	31.1% a	23	0.015
Other lipid lowering	1.8% a	11	6.5% b	5	6.8% b	5	0.005
Anti-platelet agents	21%	130	23.4%	18	23%	17	0.847
Anticoagulants	1.3% a	8	6.5% b	5	10.8% b	8	<0.001
Vasodilators	1.1% a	7	3.9% a, b	3	4.1% b	3	0.030

Table 4-11: Comparison of clinical characteristics of people with normoalbuminuria (n=633) and subjects with microalbuminuria by the new definition (n=78) and the conventional definition (n=75) after excluding diabetic subjects and those with severe renal impairment. All values are expressed as a percentage within each group. N represents number of cases in each relevant variable. The result of pairwise comparison (using z-test) is indicated by subscript letters (a, b) where variables that share the same letter are not statistically different from each other.

4.3.9 Cardiovascular risk assessment in subjects with normal UAE versus microalbuminuric individuals in non-diabetic subjects without severe renal impairment

Table 4-12 (A) shows a comparison of cardiovascular risk scores between subjects with normal urinary albumin excretion versus microalbuminuric subjects according to the two thresholds used. The results of multiple between-group comparisons are shown in table 4-12 (B). There was a trend of increased overall cardiovascular risk (figure 4-1) and for cause-specific outcomes with the increase of albuminuria level although the trends for CHD and MI were not statistically significant. Within group comparisons indicated that the conventional definition was associated with statistically significant influences. The trends for the new definition were similar but not significant with wide 95% confidence intervals except for CVD death where the new definition had a significant influence.

Table 4-13 represents the same comparison but after excluding those < 30 years and those > 74 years. The same trends of increased risk were observed although these were not significant for CVD (JBS), CHD, MI and CHD death possibility because of limited number of subjects involved in the analysis.

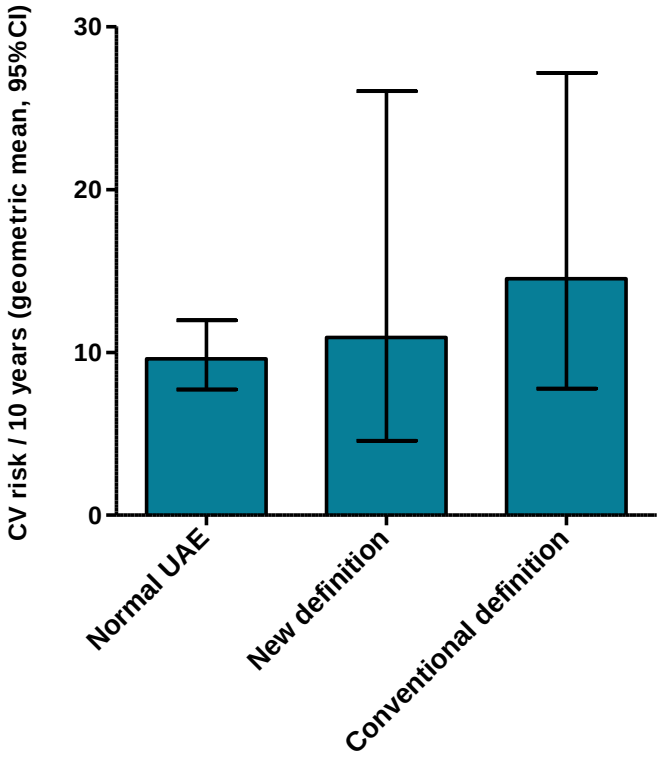


Figure 4-1: The cardiovascular risk score (using the Framingham equation) stratified by UAE in non-diabetic subjects without severe renal impairment. Data are summarised as geometric mean (95%, CI).

A. Risk score comparisons							
Risk (%)	**Normoalb.**	**N**	**New def.**	**N**	**Conv. def.**	**N**	**P value**
CVD	9.6 (8.8, 10.5)	545	10.9 (7.7, 15.5)	57	14.5 (11.3, 18.7)	57	**0.017**
CVD (JBS)	10.2 ± 8.4	447	11.7 ± 9.5	45	14.9 ± 11.3	39	**0.005**
CHD	6.1 (5.5, 6.7)	545	6.3 (4.3, 9.3)	57	8.9 (6.5, 12.1)	57	0.087
Stroke	1.8 (1.6, 1.9)	545	2.5(1.7, 3.5)	57	3.0 (2.2, 4.1)	57	**0.001**
MI	2.0 (1.8, 2.3)	545	2.2 (1.5, 3.11)	57	2.9 (2.1, 4.0)	57	0.136
CHD death	0.8 (0.75, 0.95)	545	1.2 (0.83, 1.8)	57	1.4 (0.97, 2.1)	57	**0.008**
CVD death	1.6 (1.4, 1.8)	545	2.6 (1.8, 4.0)	57	3.2 (2.2, 4.8)	57	**<0.001**

B. Tukey's post-hoc analysis						
Risk	**Categories**		**Mean ratio**	**P value**	**95% CI**	
					Lower	**Upper**
CVD	Normoalb. versus:	1.New def.	0.88	0.670	0.62	1.24
		2.Conventional def.	0.66	**0.015**	0.46	0.93
	New def. versus:	Conventional def.	0.75	0.319	0.47	1.19
CVD (JBS)*	Normoalb. versus:	1.New def.	-1.49	0.523	-4.72	1.73
		2.Conventional def.	-4.67	**0.004**	-8.12	-1.22
	New def. versus:	Conventional def.	-3.18	0.224	-7.7	1.33
Stroke	Normoalb. versus:	1.New def.	0.73	0.094	0.51	1.03
		2.Conventional def.	0.59	**0.002**	0.42	0.84
	New versus:	Conventional def.	0.81	0.565	0.50	1.30
CHD death	Normoalb. versus:	1.New def.	0.69	0.147	0.43	1.09
		2.Conventional def.	0.59	**0.022**	0.37	0.94
	New def. versus:	Conventional def.	0.85	0.829	0.46	1.59
CVD death	Normoalb. versus:	1.New def.	0.62	**0.038**	0.39	0.98
		2.Conventional def.	0.50	**0.001**	0.32	0.80
	New def. versus:	Conventional def.	0.81	0.720	0.44	1.51

Table 4-12: **A**. Comparison of cardiovascular risk assessment calculations of normoalbuminuric subjects and patient with microalbuminuria (by 2 definitions) in hypertensive subjects without severe renal impairment (n=659). Results are summarised as geometric mean (95% confidence interval) except CVD (JBS) where results were expressed as mean (±S.D). **B**. Post-hoc statistical analysis showing the multiple comparisons of the groups (95% confidence interval for the ratio of the geometric mean). * Mean difference (95% confidence interval for mean difference). Normoalb.: Normoalbuminuria

A. Risk score comparisons							
Risk (%)	**Normoalb.**	**N**	**New def.**	**N**	**Conv. def.**	**N**	**P-value**
CVD	10.4 (9.6, 11)	478	12.2 (9.4, 16.1)	42	14.0 (11.2,17.8)	44	**0.036**
CVD (JBS)	10.1 ± 7.8	395	10.0 ± 6.3	31	13.1 ± 10.7	32	0.105
CHD	6.8 (6.3, 7.5)	478	7.9 (5.8, 1.8)	42	9.0 (6.7, 12.2)	44	0.155
Stroke	1.8 (1.7, 1.97)	478	2.4 (1.8, 3.3)	42	2.7 (1.9, 3.6)	44	**0.006**
MI	2.6 (2.0, 2.5)	478	2.4 (1.7, 3.4)	42	2.9 (2.11, 4.0)	44	0.332
CHD death	0.8 (0.75, 0.59)	478	1.1(0.7, 1.62)	42	1.2 (0.8, 1.8)	44	0.142
CVD death	1.5 (1.4, 1.74)	478	2.2 (1.5, 3.3)	42	2.4(1.6, 3.6)	44	**0.028**

B. Tukey's post-hoc analysis						
Risk	**Categories**		**Mean ratio**	**P value**	**95% CI**	
					Lower	**Upper**
CVD	Normoalb. versus:	1.New def.	0.85	0.415	0.60	0.90
		2.Conventional def.	0.74	**0.050**	0.54	1.00
	New def. versus:	Conventional def.	0.87	0.723	0.60	1.34
Stroke	Normoalb. versus:	1.New def.	0.75	0.110	0.54	1.01
		2.Conventional def.	0.68	**0.021**	0.49	0.96
	New versus:	Conventional def.	0.92	0.894	0.60	1.50
CVD death	Normoalb. versus:	1.New def.	0.70	0.175	0.43	1.12
		2.Conventional def.	0.65	0.081	0.41	1.04
	New def. versus:	Conventional def.	0.94	0.969	0.49	1.77

Table 4-13: **A.** Comparison of cardiovascular risk assessment calculations of normoalbuminuric subjects and patient with microalbuminuria (by 2 definitions) in non-diabetic hypertensive subjects without CKD (stage 4 or 5) or proteinuria (n=564), after excluding those outside the recommended age range. Results are summarised as geometric mean (95% confidence interval) except CVD (JBS) where results were expressed as mean (±S.D). **B.** Post-hoc statistical analysis showing the multiple comparisons of the groups (95% confidence interval for the ratio of the geometric mean).

4.3.10 Clinical characteristics comparison in patients with type II diabetes and hypertension

A comparison of clinical characteristics of diabetic subjects with normoalbuminuria, microalbuminuria (by both definitions) and gross proteinuria is shown in table 4-14. With the exception of ARB use, there were no statistical differences between the groups for any variables. However, the number of patients in each group was small. Males were predominant in the microalbuminuric groups while female were higher in normoalbuminuric and proteinuric subjects.

Subjects with the conventional definition of microalbuminuria had the highest prevalence of family history of diabetes. Family history of stroke was more common in the new definition group than in those with normoalbuminuria or the conventional definition of microalbuminuria. Individuals with normal UAE had the highest prevalence of CHD family history.

One fifth of subjects with microalbuminuria by the new definition were smoker compared with only 2% in individuals with normoalbuminuria. Subjects with the conventional definition of microalbuminuria and those with gross proteinuria had prevalence of LVH of 40% compared with 35% in the new definition group and 25% in normoalbuminuric individuals. Patients with gross proteinuria had the highest prevalence of ST-T changes. Both patients with gross proteinuria and those with the new definition of microalbuminuria had CVD prevalence of about 42% followed by the conventional definition group (35%) and normoalbuminuric individuals (29%)

Overall, the percentage of using antihypertensive medicines increased with the increase of ACR. Similarly, subjects with increased ACR level were taking higher percentages of cardiovascular medicines, except statins.

Variable % (n)	Normal UAE	N	New def.	N	Conventional def.	N	Proteinuria	N	P value
Male	44.4%	28	64.0%	16	56.0%	14	41.7%	5	0.353
Family history:									
Diabetes	28.9%	13	13.3%	2	35.3%	6	25.0%	2	0.555
Stroke	10.6%	5	13.3%	2	11.1%	2	0	0	0.951
CHD	30.4%	14	6.7%	1	27.8%	5	25.0%	2	0.312
Co-morbidities/ risk:									
Current smoker	2.2%	1	20.0%	3	0	0	0	0	0.056
LVH	24.5%	13	34.8%	8	40.0%	8	42.9%	3	0.459
ST-T changes	22.0%	9	13.3%	2	26.7%	4	50.0%	3	0.369
CVD	28.6%	16	41.7%	10	34.8%	8	42.9%	3	0.628
Pharmacotherapy:									
Diuretics	73.0%	46	64.0%	16	76.0%	19	83.3%	10	0.672
CCBs	50.8%	32	64.0%	16	72.0%	18	83.3%	10	0.088
ACEIs	50.8%	32	56.0%	14	68.0%	17	66.7%	8	0.455
ARBs	41.3%	26	40.0%	10	40.0%	10	83.3%	10	**0.046**
Beta blocker	39.7%	25	44.0%	11	52.0%	13	58.3%	7	0.560
Alpha blockers	30.2%	19	32.0%	8	32.0%	8	25.0%	3	1.000
Aldosterone blockers	12.7%	8	12.0%	3	28.0%	7	0	0	0.135
Imidazoline agonists	3.2%	2	8.0%	2	4.0%	1	0	0	0.767
Statins	84.0%	39	72.0%	21	50.0%	18	84.0%	6	0.111
Other lipid lowering	11.1%	7	12.0%	3	0	0	25.0%	3	0.085
Anti-platelet agents	46.0%	29	48.0%	12	60.0%	15	75.0%	9	0.238
Anti-diabetics	55.6%	35	52.0%	13	72.0%	18	83.3%	10	0.150
Anticoagulants	3.2%	2	4.0%	1	8.0%	2	0	0	0.767
Vasodilators	4.8%	3	0	0	8.0%	2	8.3%	1	0.390

Table 4-14: Comparison of clinical characteristics of hypertensive patients with diabetes [normoalbuminuria (n=63), microalbuminuria by the new definition (n=25), the conventional definition (n=25) and gross proteinuria (n=12)]. All values are expressed as a percentage within each group.

4.3.11 Cardiovascular risk assessment comparison between normoalbuminuric and microalbuminuric diabetic hypertensive subjects

Table 4-15 shows a comparison of risk assessment scores in diabetic subject stratified by ACR level. The cardiovascular risk calculations were based on data of 85 diabetic patients after excluding those with established CVD. Although the Kruskal–Wallis test did not reveal any significant differences between the groups, there were trends for stepwise increase of risk with the increase of ACR level. The lack of statistical significance may be due to the small sample sizes in each group. A similar trend can be seen in the even smaller group of subjects who met the age range criteria of the Framingham study (table 4-16).

Risk factor (%)	Normal UAE (n=45)	New definition (n=15)	Conventional def. (n=17)	Proteinuria (n=8)	P value
CVD	24.2 (17.1 to 34.4)	25.9 (20.4 to 41.6)	38.4 (22.4 to 46.6)	34.9 (19.7 to 45.4)	0.123
CHD	14.7 (9.7 to 23.0)	14.5 (11.1 to 24.6)	23.6 (12.2 to 33.6)	22.6 (9.92 to 31.5)	0.263
Stroke	4.7 (2.3 to 6.9)	6.1 (3.6 to 9.2)	8.3 (3.5 to 13.0)	7.55 (5.0 to 12.5)	0.152
MI	7.1 (3.3 to 10.6)	7.9 (4.1 to 10.5)	10.8 (5.9 to 14.9)	10.8 (4.2 to 13.8)	0.148
CHD death	3.6 (1.6 to 6.2)	3.3 (1.6 to 8.8)	6.3 (2.4 to 11.2)	5.5 (2.1 to 13.2)	0.160
CVD death	4.9 (1.8 to 10.9)	5.7 (2.7 to 21.9)	16.7 (5.1 to 21.2)	7.9 (3.6 to 29.7)	0.114

Table 4-15: A comparison of the risk scores in subjects with hypertension and diabetes (n=85) stratified by ACR after excluding 37 patients with established CVD and 2 patients with incomplete information. Data are summarised as median (interquartile range). The medians of the groups were compared using the Kruskal–Wallis test. Def.: definition.

Risk factor (%)	Normal UAE (n=38)	New definition (n=11)	Conventional def. (n=10)	Proteinuria (n=6)	P value
CVD	21.9 (16.0 to 32.2)	23.3 (19.8 to 28.4)	29.0 (17.0 to 40.0)	32.7 (15.0 to 36.4)	0.612
CHD	14.6 (9.7 to 21.9)	12.9 (11.1 to 17.9)	18.4 (11.1 to 33.2)	20.2 (6.2 to 26.4)	0.680
Stroke	4.2 (1.9 to 6.2)	3.9 (3.2 to 6.5)	4.9 (1.9 to 9.65)	6.3 (4.3 to 7.9)	0.395
MI	6.5 (3.3 to 10.5)	6.6 (3.9 to 9.6)	8.0 (5.9 to 14.30)	10.8 (2.8 to 13.8)	0.435
CHD death	2.5 (1.3 to 4.9)	2.6 (1.5 to 3.4)	4.2 (1.7 to 6.5)	5.0 (1.0 to 6.3)	0.676
CVD death	4.5 (1.7 to 8.2)	3.8 (2.6 to 6.3)	8.5 (2.0 to 17.2)	6.2 (2.6 to 8.5)	0.642

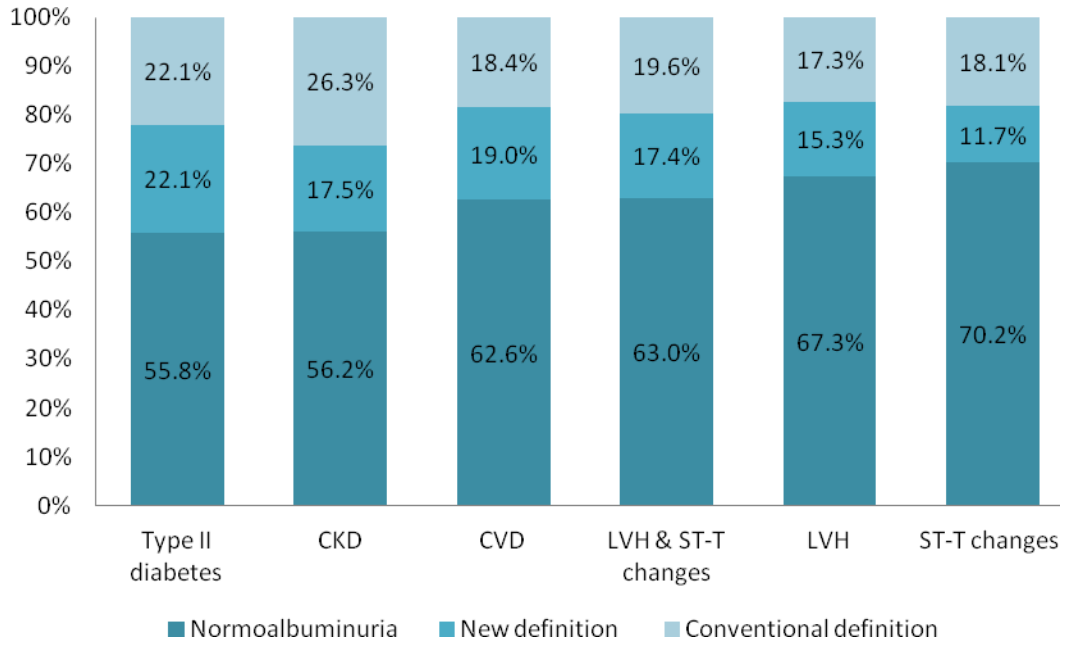
Table 4-16: A comparison of the risk scores in subjects with hypertension and diabetes (n=65) stratified by ACR. Patients <30 or >74 years were excluded. Data are expressed as median (interquartile range). The medians of the groups were compared using the Kruskal–Wallis test.

4.3.12 Prevalence in patients with and without different cardiovascular and metabolic abnormalities

Figure 4-2 A illustrates the prevalence of microalbuminuria in patients with type II diabetes (without gross proteinuria), CKD, CVD, LVH and strain (LVH and ECG changes), LVH and ST-T changes on ECG. The highest prevalence of albuminuria was found in diabetic subjects and those with CKD (stages 3, 4 or 5). About 37% of those with established CVD had microalbuminuria by the combined definition (i.e. the new definition plus the conventional definition). Similarly, those with LVH and strain had microalbuminuria prevalence of 37%. Microalbuminuria by the combined definition was found in about one-third of those with LVH or ST-T changes on ECG. In subjects with poor blood pressure control (defined as SBP ≥ 155 mm Hg and/or DBP ≥ 95 mm Hg which represented the top quartile of the cohort) but without evidence of previously mentioned diseases and risk factors, microalbuminuria was present in 18.5% of subjects (figure 4-2

B). When the data of those with poor blood pressure control were removed in addition to other co-morbidities and risk factors (no established risks), microalbuminuria was still present in 14% of the subjects.

A)



B)

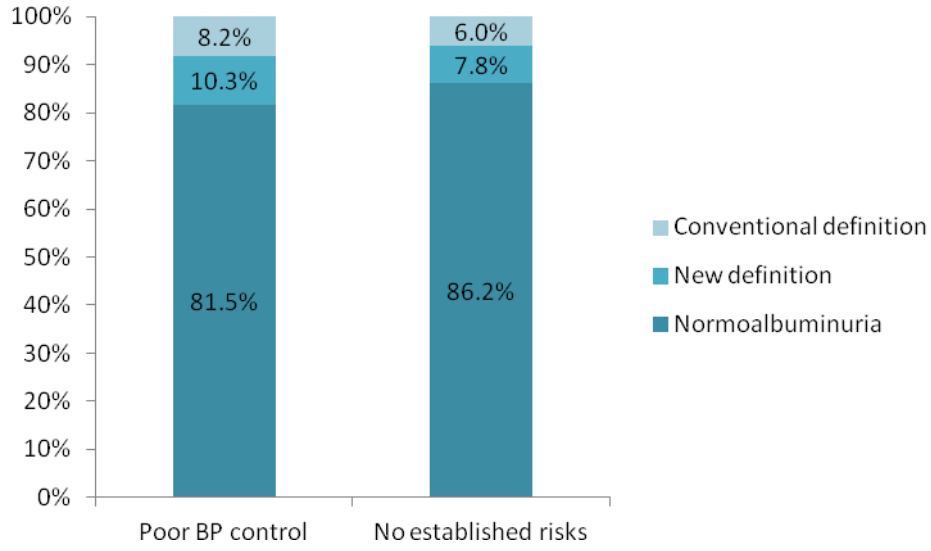


Figure 4-2: A) Prevalence of microalbuminuria in subjects without gross proteinuria but with several diseases/ risk factors. B) Prevalence of microalbuminuria in those with poor blood pressure control (defined as SBP ≥155 mm Hg and\or DBP ≥ 95 mm Hg) and those without recognised cardiovascular risk factors.

4.3.13 A comparison of demographic characteristics of those without established CVD / risk factors stratified by UAE

Table 4-17 shows comparisons of microalbuminuric (by the combined definition) and normoalbuminuric subjects in a subcohort of the hypertensive subjects with relatively well-controlled blood pressure (<155/95 mm Hg) and without evidence of established cardiovascular diseases or risk factors (CVD, LVH, ST-T changes on ECG, all stages of CKD, gross proteinuria and type II diabetes mellitus). There was a trend for increased blood pressure and some biochemical parameters although this was not supported by statistical significance, except for DBP and serum creatinine.

Variable (unit)	Normal UAE	N	Microalbuminuria	N	P-Value
Age (years)	53.2 ± 14.2	299	56.0 ± 17.4	47	0.216
BMI (Kg/m^2)	29.4 ± 5.8	293	29.6 ± 7.3	47	0.854
SBP (mm Hg)	131.1 ± 10.3	298	133.7 ± 9.5	47	0.108
DBP (mm Hg)	84.5 ± 7.5	298	86.7 ± 6.5	47	**0.054**
Serum creatinine (μmol/L)	78.0 ± 13.6	298	83.2 ± 17.3	48	**0.050**
eGFR ($mL/min/1.73\ m^2$)	84.8 ± 16.2	298	82.0 ± 17.1	47	0.264
Fasting glucose (mmol/L)	5.1 ± 0.6	220	5.1 ± 0.5	33	0.902
Random glucose (mmol/L)	5.2 (4.8 to 5.8)	59	5.3 (5.1 to 5.8)	14	0.293
Total cholesterol (mmol/L)	5.2 ± 1.0	293	5.2 ± 1.1	46	0.997
HDL-cholesterol (mmol/L)	1.5 ± 0.4	289	1.5 ± 0.6	46	0.502
Fasting triglycerides (mmol/L)	1.2 (0.9 to 1.7)	217	1.3 (0.8 to 1.9)	33	0.385
Eosinophil count ($\times 10^9/L$)	0.15 (0.1 to 0.2)	272	0.17 (0.1 to 0.3)	46	0.201
ESR (mm in 1 hr)	7.0 (4.0 to 12.5)	178	7.0 (4.0 to 16.0)	23	0.585
Number of drugs *	2.0 (1.0 to 3.0)	288	2.0 (1.0 to 3.0)	47	0.820

Table 4-17: Data of subjects without established cardiovascular diseases or risks stratified by level of albuminuria (normoalbuminuria (n=299) versus microalbuminuria by the combined definition (n=48)). Variables are expressed as mean (±SD) or median (interquartile range), as appropriate. * Cardiovascular / metabolic drugs.

4.3.14 A comparison of clinical characteristics and risk scores of those without established CVD / risk factors stratified by UAE

Tables 4-18 and 4-19 show comparisons of clinical characteristics / risk scores for those without co-morbidities, CVD or risk factors (CVD, LVH, ST-T changes on ECG, all stages of CKD, gross proteinuria and type II diabetes mellitus). Trends for increased risk scores with the increase of UAE were observed (even after excluding those outside the recommended age range, table 4-20) although this was not supported by statistical significance possibly because of the small sample size. Likewise, the use of some antihypertensive agents (such as CCBs, ARBs, beta blockers and aldosterone antagonists) tended to be higher in microalbuminuric subjects although the magnitude of differences was slight.

Variable (%)	Normoalbuminuria	N	Microalbuminuria	N	P-Value
Male	44.1%	132	52.1%	25	0.350
Diabetes family history	10.6%	24	21.4%	6	0.115
Stroke family history	12.2%	29	10%	3	1.000
CHD family history	17.2%	41	17.2%	5	1.000
Smoker	10.9%	27	5.9%	2	0.549
Diuretics	39.8%	115	29.8%	14	0.201
CCBs	34.6%	100	48.9%	23	0.072
ACEIs	34.3%	99	36.2%	17	0.869
ARBs	27.7%	80	31.9%	15	0.601
Beta blockers	20.8%	60	29.8%	14	0.185
Alpha blockers	9.3%	27	8.5%	4	1.000
Aldosterone antagonists	3.8%	11	10.6%	5	0.057
Imidazoline agonists	0.7%	2	0	0	1.000
Antiplatelet agents	18.3%	53	10.6%	5	0.296
Statins	27.3%	79	19.1%	9	0.285
lipid lowering agents	0.7%	2	0	0	1.000
Vasodilators	0.7%	2	0	0	1.000
Anticoagulants	0	0	2.1%	1	0.140

Table 4-18: Clinical characteristics of subjects without established cardiovascular diseases or risks stratified by the level of albuminuria (normoalbuminuria (n=299) versus microalbuminuria by the combined definition (n=48)). Variables are expressed as percentage of cohort. N represents total number of cases of relevant variables.

Risk (%)	Normoalbuminuria	N	Microalbuminuria	N	P-value
CVD	9.0 (4.8 to 14.8)	292	10.6 (6.4 to 17.0)	45	0.264
CVD (JBS)	7.5 (3.9 to 11.6)	292	8.5 (5.1 to 12.8)	45	0.342
CHD	5.7 (2.8 to 9.1)	292	6.4 (3.5 to 10.1)	45	0.523
Stroke	1.5 (0.7 to 2.8)	292	2.1 (0.9 to 3.3)	45	0.168
MI	1.9 (0.7 to 3.7)	292	2.0 (0.8 to 3.9)	45	0.648
CHD death	0.5 (0.2 to 1.6)	292	0.8 (0.3 to 2.2)	45	0.298
CVD death	1.0 (0.4 to 2.9)	292	1.3 (0.5 to 4.5)	45	0.163

Table 4-19: Cardiovascular risk scores for subjects without established cardiovascular diseases or risks stratified by level of albuminuria (normoalbuminuria (n=299) versus microalbuminuria by the combined definition (n=48)). Missing information precluded the calculation in 10 subjects. Variables are expressed as median (interquartile range). Groups were compared using the Mann–Whitney test.

Risk (%)	Normoalbuminuria	N	Microalbuminuria	N	P-value
CVD	9.0 (5.1 to 14)	253	9.2 (6.6 to 15)	34	0.592
CVD (JBS)	7.5 (4.3 to 11.2)	253	8.2 (5.2 to 12.6)	34	0.497
CHD	5.9 (3.4 to 8.9)	253	6.5 (3.6 to 10)	34	0.555
Stroke	1.4 (0.7 to 2.4)	253	1.4 (0.8 to 2.5)	34	0.506
MI	1.9 (0.8 to 3.5)	253	2 (1.0 to 3.7)	34	0.68
CHD death	0.4 (0.1 to 1.4)	253	0.7 (0.2 to 1.4)	34	0.687
CVD death	0.9 (0.3 to 2.3)	253	1.0 (0.4 to 2.9)	34	0.640

Table 4-20: Cardiovascular risk scores for subjects without established cardiovascular diseases or risks stratified by level of albuminuria (normoalbuminuria (n=299) versus microalbuminuria by the combined definition (n=48)). Subjects younger than 30 years or older than 74 years were excluded (n=50). Variables are expressed as median (interquartile range). Groups were compared using the Mann–Whitney test.

4.4 Summary/ discussion

The population was characterised by increased cardiovascular co-morbidities and complications. The percentages of diabetic subjects and current smokers were quite low but prescription of cardiovascular drugs such as statins and antiplatelet agents was fairly high, reflecting the complexity of the population. This was supported by the score for cardiovascular disease calculated in the population. Diabetic subjects had a prevalence of cardiovascular morbidities and risk scores higher than that in non-diabetic.

When comparing the clinical characteristics according to UAE, there were obvious trends of increased risks, co-morbidities and proportions of drugs used in subjects with microalbuminuria. Target organ damage (LVH) and CVD were strongly associated with microalbuminuria. There were trends for continuous relationships with UAE levels. Differences were not significant in diabetic patients, possibly because of number of individuals in each group. The trend was also observed in subjects without established diseases or risk factors although again the differences were not significant possibly because of the limited number of patients in the microalbuminuric group.

The use of cardiovascular risk calculators revealed that the probability of developing global cardiovascular disease and cause-specific outcomes over ten years were higher in subjects with microalbuminuria. Increased cardiovascular risk was also observed in subjects with microalbuminuria by the new definition although the difference was not significant and confidence intervals were wide. It is likely that the number of subjects in the new definition group was too small to provide clear differentiation. The increased risk in this group of patient is clinically important and may indicate that the current definition underestimate risk in subjects with low levels of albuminuria.

5. Chapter five: testing the association of microalbuminuria with clinical and laboratory characteristics by multivariate analysis

5.1 Introduction

Several factors influence microalbuminuria. For instance, microalbuminuria is highly prevalent in subjects with diabetes and an association with blood glucose level has been shown previously (Vupputuri et al., 2011). Also, deterioration of kidney function may lead to alteration of glomerular filtration and increase excretion of albumin in the urine and thus higher serum creatinine may have a strong influence (Comper et al., 2008). Therefore, the purpose of this analysis was to investigate the association of microalbuminuria with different laboratory and clinical characteristics in hypertensive subjects without severe renal impairment using regression models that adjust any potential confounders. Also, I sought to evaluate the usefulness of microalbuminuria as an independent predictor of different cardiovascular outcomes.

5.2 Method

The predictive values of laboratory and clinical characteristics of the studied subjects were analysed using binary logistic regression method. Selection of variables was based on two methods; backward elimination which starts by evaluating all variables and then eliminating any variable that has no significant effect to the model; the other method for selection was carried out by adding variables that have possible effect on the association from prior knowledge.

5.3 Results

5.3.1 Factors associated with different definitions of microalbuminuria

Table 5-1 shows the results of logistic regression models for patients with microalbuminuria grouped into three categories; the conventional definition, the new definition, and a composite of both definitions (the combined definition) versus normoalbuminuria. Covariates were included in each model using a stepwise backward elimination procedure.

Six hundred and fifteen cases out of 786 where included when the conventional definition was selected as an outcome as data for some covariates were missing. SBP, eosinophil count and LVH were associated with the conventional definition of microalbuminuria; odds ratio 1.04 (95% CI, 1.02 - 1.05), 9.63 (95% CI, 1.78 - 52.03) and 2.83 (95%, 1.59 - 5.07), respectively. Anticoagulants were highly related to the conventional definition of microalbuminuria; odds ratio 9.98 (95% CI, 3.14 - 31.74).

When the new definition group was selected as an outcome, data from 492 patients were included in the model. Microalbuminuria as categorized by the new definition was related significantly to age, serum creatinine and fasting triglycerides; odds ratios 1.03 (95% CI, 1.00 - 1.06), 1.02 (95% CI, 1.01 - 1.04) and 1.67 (95% CI, 1.27 - 2.20), respectively. CCBs and anticoagulants were associated with the new definition of microalbuminuria. The odds ratio of CCBs in this model was 2.10 (95% CI, 1.07 - 4.13). The highest odds ratio of all covariates that were associated with the new definition of microalbuminuria was again found with anticoagulants; 5.88 (95% CI, 1.45 - 23.77).

The combined definition of microalbuminuria was significantly associated with SBP, serum creatinine, LVH, fasting triglycerides, CCBs and anticoagulants. The highest odds

ratio was reported with anticoagulant use; 9.48 (95% CI, 2.98 - 30.20) followed by LVH; 1.91 (95% CI, 1.11 - 3.28), CCBs; 1.65 (95% CI, 1.01 - 2.70) and fasting triglycerides; 1.32 (95% CI, 1.05 - 1.66). Both SBP and serum creatinine had odds ratios close to one; 1.02 (95% CI, 1.01 - 1.04) and 1.02 (95% CI, 1.00 - 1.03), respectively.

Outcome	N	Predictors (unit)	O.R (95% CI)	P-value
Conventional definition	615	SBP (mm Hg)	1.04 (1.02-1.05)	<0.001
		Eosinophil count	9.63 (1.78-52.03)	0.009
		LVH	2.83 (1.59-5.07)	<0.001
		Anticoagulants	9.98 (3.14-31.74)	<0.001
New definition	492	Age (years)	1.03 (1.00-1.06)	0.026
		Serum creatinine (µmol/L)	1.02 (1.01-1.04)	0.010
		Fasting triglycerides (mmol/L)	1.67 (1.27-2.20)	<0.001
		CCBs	2.10 (1.07-4.13)	0.032
		Anticoagulants	5.88 (1.45-23.77)	0.013
Combined definition	512	SBP (mm Hg)	1.02 (1.01-1.04)	<0.001
		Serum creatinine (µmol/L)	1.02 (1.00-1.03)	0.012
		LVH	1.91 (1.11-3.28)	0.020
		Fasting triglycerides (mmol/L)	1.32 (1.05-1.66)	0.016
		CCB	1.65 (1.01-2.70)	0.045
		Anticoagulants	9.48 (2.98-30.20)	<0.001

Table 5-1: Results of multivariate logistic regression (using backward elimination method) testing the association of different variables with the three definitions of microalbuminuria versus normoalbuminuria. N represents the number of cases included in each model. O.R.: odds ratio.

5.3.2 The Association of Microalbuminuria and end-organ damage

The results of multivariate logistic regression analyses evaluating the influence of three definitions of microalbuminuria (the new, conventional and combined definitions) on LVH and ST-T changes on ECG (as outcomes) are listed on table 5-2. After adjustment for age and gender (model I), both conventional and combined definitions were significantly associated with LVH; odds ratio 1.90 (95% CI, 1.09 - 3.33) and 1.70 (95% CI, 1.11 - 2.61), respectively. In model I, ST-T changes on ECG were not associated with any of the three definitions, although the conventional and combined definitions were close to the threshold for significance with wide confidence intervals; odds ratio 1.96 (95% CI, 0.97 - 3.97) and 1.71 (0.97 - 3.01), respectively.

When logistic regression analysis was adjusted for cardiovascular risk factors such as SBP, DBP, BMI, total cholesterol, HDL-cholesterol, fasting glucose and triglycerides in addition to age and gender (model II), the combined definition remained significantly related to LVH with an odds ratio of 1.91 (95% CI, 1.08 - 3.39). The new and the conventional definitions of microalbuminuria were not individually associated with LVH (model II). ST-T changes on ECG were associated significantly with the conventional definition (odds ratio; 2.36 (95% CI, 1.05 - 5.31)), while the combined definitions just failed to achieve significance (P=0.052, odds ratio, 1.92 (95% CI, 0.99 - 3.72)).

Variables that showed significant association with microalbuminuria from model II were included in model III (age and gender). In addition, the model was adjusted for the use RAS blockers (ACEIs and ARBs). As in model I, LVH was significantly related to the conventional and combined definitions; odds ratio 1.85 (95% CI, 1.05 - 3.24) and 1.64 (95% CI, 1.07 - 2.52), respectively. Similarly, ST-T changes were associated with the conventional and combined definitions after adjustment for RAS blockers only; odds ratio 2.20 (95% CI, 1.10 - 4.39) and 1.92 (95% CI, 1.10 - 3.36), respectively.

In model IV, all available variables were included and a stepwise backward elimination logistic regression was carried out. The number of the cases included in the model was small as information in some variables included in the model was not available. Microalbuminuria by the new definition was independently associated with LVH after adjusting for age, gender , RAS blockers and ST-T changes on ECG; odds ratio of 2.12 (95% CI, 1.06 - 4.25). While the combined definition of microalbuminuria was still significantly related to LVH; odds ratio 1.87 (95% CI, 1.12 - 3.12), the conventional definition of microalbuminuria was no longer significantly associated with LVH; odds ratio 1.66 (95% C, 0.85 - 3.23). The small sample size may limit the reliability of the model as there was strong trend for all microalbuminuria measurements.

In model IV, the association between the two definitions of microalbuminuria and ST-T changes on ECG was not significant after adjusting LVH, CVD and RAS blockers. However, the odds ratio for the conventional and the combined definitions of microalbuminuria was greater than unity with relatively wide confidence interval, suggesting that the sample size might not be sufficient to detect significant differences.

	LVH				ST-T changes			
	N	**O.R**	**95% CI**	**P-value**	**N**	**O.R**	**95%-CI**	**P-value**
Model I								
New def.	658	1.55	0.89 - 2.71	0.121	530	1.50	0.68 - 3.33	0.316
Conventional	653	1.90	1.09 - 3.33	0.024	538	1.96	0.97 - 3.97	0.061
Combined	727	1.70	1.11 - 2.61	0.014	584	1.71	0.97 - 3.01	0.065
Model II								
New def.	449	1.79	0.82 - 3.91	0.145	443	1.51	0.59 - 3.86	0.389
Conventional	456	1.90	0.91 – 3.95	0.088	450	2.36	1.05 - 5.31	0.038
Combined	496	1.91	1.08 - 3.39	0.026	490	1.92	0.99 - 3.72	0.052
Model III								
New def.	650	1.49	0.85 - 2.61	0.169	522	1.66	0.75 - 3.65	0.209
Conventional	645	1.85	1.05 - 3.24	0.033	530	2.20	1.10 - 4.39	0.025
Combined	718	1.64	1.07 - 2.52	0.024	575	1.92	1.10 - 3.36	0.021
Model IV								
New def.	521	2.12	1.06 - 4.25	0.033	520	0.97	0.40 - 2.36	0.955
Conventional	529	1.66	0.85 - 3.23	0.136	519	1.59	0.75 - 3.39	0.230
Combined	574	1.87	1.12 - 3.12	0.017	572	1.30	0.70 - 2.42	0.398

Table 5-2: Mutlivariate binary logistic regression testing the association of LVH and ST-T changes on ECG (outcomes) with different definitions of microalbuminuria. Normoalbuminuria was used as a reference group. Covariates added in models I, II and III were based on the possible effect of known risk factors on the association. In model IV, backward stepwise elimination method was used. N represents number of cases used in each model. Covartiates included in each model were as follow:

Model I: adjusted for age and gender.

Model II: adjusted for age, gender and cardiovascular risk factors (SBP, DBP, BMI, total cholesterol, HDL-cholesterol, fasting blood glucose and fasting triglycerides).

Model III: adjusted for variables that showed significant association in model II in addition to RAS blockers:

1. LVH: adjusted for age, gender and RAS blockers.
2. ST-T changes on ECG: adjusted for RAS blockers.

Model IV:

1. LVH: adjusted for age, gender, RAS blockers and ST-T changes on ECG.
2. ST-T changes on ECG: adjusted for LVH, CVD and RAS blockers.

5.3.3 The Association of Microalbuminuria and Cardiovascular diseases

An evaluation of the association of different definitions of microalbuminuria with established cardiovascular diseases is presented in table 5-3. After adjustment for age and gender (model I), the combined definition was marginally significant, with an odds ratio of 1.61 (95% CI, 1.0 - 2.60). Model II was adjusted for age, gender and cardiovascular risk factors (SBP, DBP, BMI, total cholesterol, HDL-cholesterol, fasting glucose and triglycerides). No significant association was found with all three microalbuminuria definitions. However, odds ratios were above unity with wide 95% confidence intervals.

In model III, the relation between CVD risk and microalbuminuria was adjusted for RAS blockers in addition to age, HDL-cholesterol as they were the only significant variables from model II. The association between CVD risk and the three definitions of microalbuminuria was not significant, although the lower boundaries were again close to unity, especially with the combined definition; odds ratio 1.48 (95% CI, 0.91 - 2.42) P= 0.113.

Model IV was carried out using stepwise backward elimination. Only age and antiplatelet agents use were added as covariates. In this model, the combined definition was significantly related to CVD risk, with an odds ratio of 1.72 (95% CI, 1.05 - 2.80). Neither the new nor the conventional definitions of microalbuminuria alone was significantly associated with CVD; odds ratio 1.77 (95% CI, 0.94 - 3.34) and 1.76 (95% CI, 0.93 - 3.33) although again the lower 95% confidence interval was close to unity.

	CVD			
	N	**O.R**	**95% CI**	**P-value**
Model I				
New def.	673	1.66	0.90 – 3.06	0.101
Conventional	669	1.57	0.83 – 2.95	0.162
Combined	745	1.61	1.00 - 2.60	0.050
Model II				
New def.	458	1.71	0.76 – 3.87	0.194
Conventional	464	1.49	0.66 – 3.37	0.343
Combined	506	1.60	0.86 – 2.97	0.140
Model III				
New def.	651	1.53	0.82 - 2.86	0.180
Conventional	646	1.48	0.77 - 2.84	0.237
Combined	720	1.48	0.91 - 2.42	0.113
Model IV				
New def.	666	1.77	0.94 – 3.34	0.079
Conventional	662	1.76	0.93 – 3.33	0.084
Combined	737	1.72	1.05 - 2.80	0.030

Table 5-3: Mutlivariate binary logistic regression testing the association between CVD risk and different definitions of microalbuminuria.Normoalbuminuria was used as reference group. Covariates added in models I, II and III were based on the possible effect of known risk factors on the association. In model IV, backward stepwise elimination method was used. N represents number of cases used in each model. Covartiates included in each model were as follow:
Model I: adjusted for age and gender.
Model II: adjusted for age, cardiovascular risk (SBP, DBP, BMI, total cholesterol, HDL-cholesterol, fasting blood glucose and fasting triglycerides).
Model III: adjusted for variables with significant association from model II (age and HDL-cholesterol) and RAS blockers.
Model IV: adjusted for age and antiplatelet agents.

5.3.4 Association of microalbuminuria with uncontrolled blood pressure

The results presented in table 5-4 examine the association of microalbuminuria with uncontrolled hypertension. In models I and II, subjects were grouped according to their

blood pressure (obtained when microalbuminuria was measured) into two groups; those with uncontrolled hypertension (defined as SBP ≥140 or DBP ≥90 mm Hg) and those with lower blood pressure. After adjustment for age and use of antihypertensive agents (model I), uncontrolled hypertension was a significant predictor of microalbuminuria by the conventional and combined definitions; odds ratio 1.98 (95% CI, 1.14 - 3.43) and 1.56 (95% CI, 1.05 - 2.30), respectively.

In model II a stepwise backward elimination method was used. The association between uncontrolled hypertension and different definitions of microalbuminuria was examined after adjusting for any variables that showed significant association with each definition. Microalbuminuria by the conventional definition was significantly associated with the uncontrolled group; odds ratio 1.81 (95% CI, 1.01 - 3.22) after adjusting for eosinophil count, LVH and anticoagulants. Likewise, uncontrolled blood pressure was a significant predictor for the combined definition after adjustment for serum creatinine, LVH, fasting triglycerides, CCBs and anticoagulants; odds ratio 1.75 (95% CI, 1.04 - 2.95).

In order to check the pattern of the association between microalbuminuria and uncontrolled hypertension, subjects were divided into three groups according to their treated SBP and DBP records. A treated SBP to levels between 140 - 159 and/or DBP between 90 - 99 mm Hg was considered as moderately controlled hypertension. Poorly controlled hypertension was defined as SBP between 160 - 179 mm Hg and/or DBP between 100 - 109 mm Hg while very poorly controlled hypertension was defined as SBP ≥180 mm Hg and/or SBP≥110 mm Hg. When SBP or DBP fell into different classes, the higher record was used in the classification.

Model III was adjusted for age and antihypertensive drug use. The new definition of microalbuminuria was not significantly associated with any group of uncontrolled blood

pressure. On the contrary, the conventional definition was highly associated with very poorly controlled hypertension; odds ratio 7.01 (95% CI, 3.10 – 15.88). Both poorly and very poorly controlled hypertension were significant predictor of the combined definition of microalbuminuria; odds ratio 1.86 (95% CI, 1.05 - 3.29) and 2.88 (95% CI, 1.41 - 5.91), respectively (figure 5-1).

In model IV, a stepwise regression was used. After adjusting for age, serum creatinine fasting triglycerides, CCBs and anticoagulants use, there were no association with the new definition of microalbuminuria. Very poorly controlled blood pressure was significant predictor of the conventional definition of microalbuminuria and the odds ratio was 9.58 (95% CI: 3.93 - 23.33) (figure 5-2). Very poorly controlled hypertension was also associated significantly with the combined definition of microalbuminuria; odds ratio 4.91 (95% CI, 1.88 – 12.83). The relation between the combined definition of microalbuminuria and poorly controlled blood pressure control was just above the significant level; odds ratio 2.03 (95% CI, 0.97 – 4.25). Odds ratio plots (Forest plots) of the associations between different groups of uncontrolled hypertension suggest trends of stepwise increases in the risk of microalbuminuria as the blood pressure increase (figures 5-1 and 5-2).

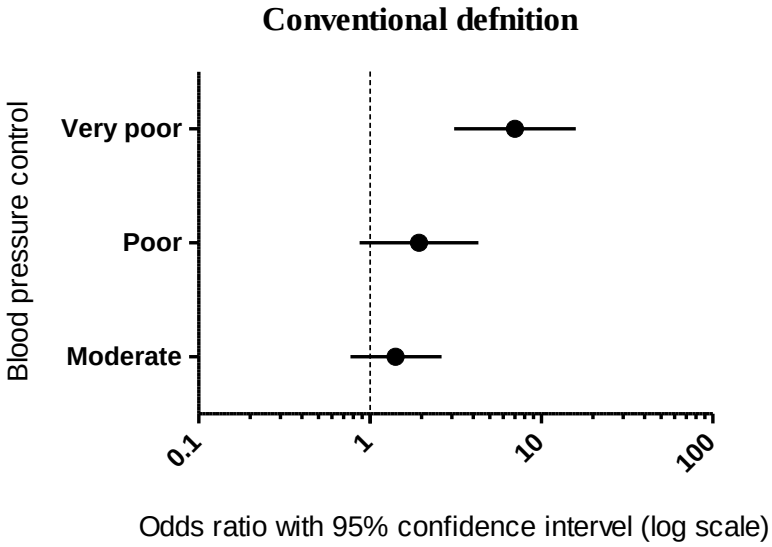
Predictors	New definition only		Conventional definition only		Combined	
Model I	**OR (95%-CI)**	**P-value**	**OR (95%-CI)**	**P-value**	**OR (95%-CI)**	**P-value**
Uncontrolled	1.23 (0.74-2.03)	0.429	1.98 (1.14-3.43)	**0.015**	1.56 (1.05-2.30)	**0.027**
Model II						
Uncontrolled	1.48 (0.73-3.01)	0.277	1.81 (1.01-3.22)	**0.045**	1.75 (1.04-2.95)	**0.036**
Model III						
Moderate	1.25 (0.72-2.17)	0.428	1.41 (0.77-2.62)	0.266	1.31 (0.85-2.02)	0.221
Poor	1.76 (0.86-3.60)	0.121	1.93 (0.87-4.29)	0.108	1.86 (1.05-3.29)	**0.033**
Very poor	0.25 (0.03-1.97)	0.188	7.01 (3.10-15.88)	**<0.001**	2.88 (1.41-5.91)	**0.004**
Model IV						
Moderate	1.44 (0.67-3.09)	0.353	1.31 (0.69-2.50)	0.403	1.42 (0.80-2.51)	0.233
Poor	1.99 (0.77-5.18)	0.158	1.55 (0.63-3.82)	0.341	2.03 (0.97-4.25)	0.060
Very poor	--	--	9.58 (3.93-23.33)	**<0.001**	4.91 (1.88-12.83)	**0.001**

Table 5-4: Model I and II represents the results of multivariate logistic regression testing the association of microalbuminuria with uncontrolled blood pressure (SBP ≥140 or DBP ≥90 mm Hg). Model III and IV tests the association of three uncontrolled blood pressure groups (defined as moderately control: SBP 140-159 or DBP 90-99, poor: SBP 160-179 or DBP 100-109 and very poor: SBP ≥180 or DBP ≥110) with microalbuminuria definitions (versus normoalbuminuria). Covariates adjusted in the models were as follow:

- Model I and III: age and antihypertensive agents. (n= 695,692 and n=769 for new, conventional and combined definitions, respectively).
- Model II and IV: (a) New definition only (n=491): age, serum creatinine, fasting triglycerides, CCBs and anticoagulants. (b) Conventional definition only (n=615): eosinophil count, LVH and anticoagulants. (c) Combined definition (n= 512): serum creatinine, LVH, fasting triglycerides, CCBs and anticoagulants

n represents number of cases used in each model (out of 786 subjects). Low number of cases were used in model II and IV because of missing information for some adjusted covariate.

A.



B.

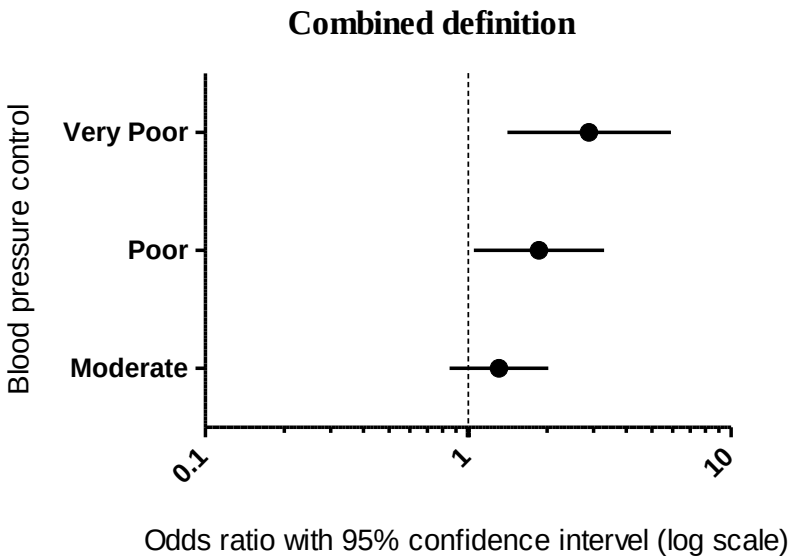
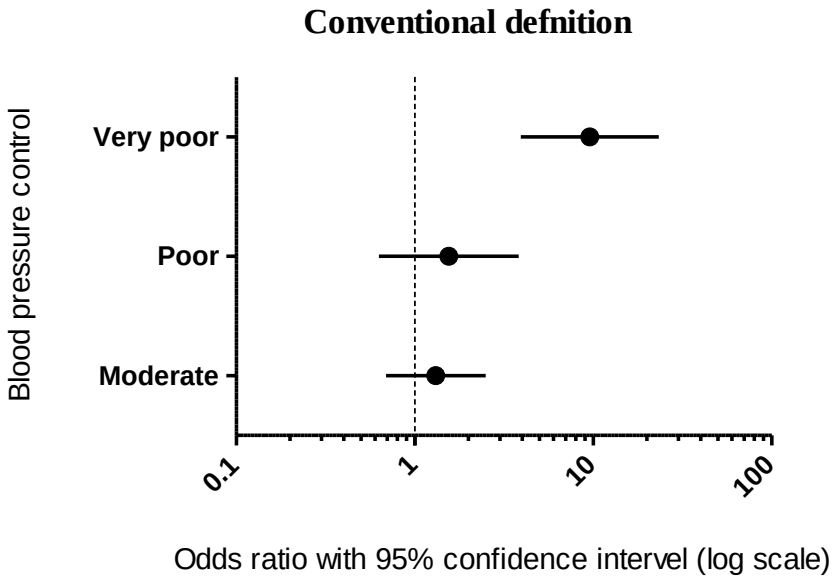


Figure 5-1: Forest plots showing the odds ratio with 95% confidence interval (on the logarithm of the base 10 scale) of three blood pressure stages as predictors of the conventional (A) and the combined (B) definitions of microalbuminuria after adjustment for age and antihypertensive agents.

A.



B.

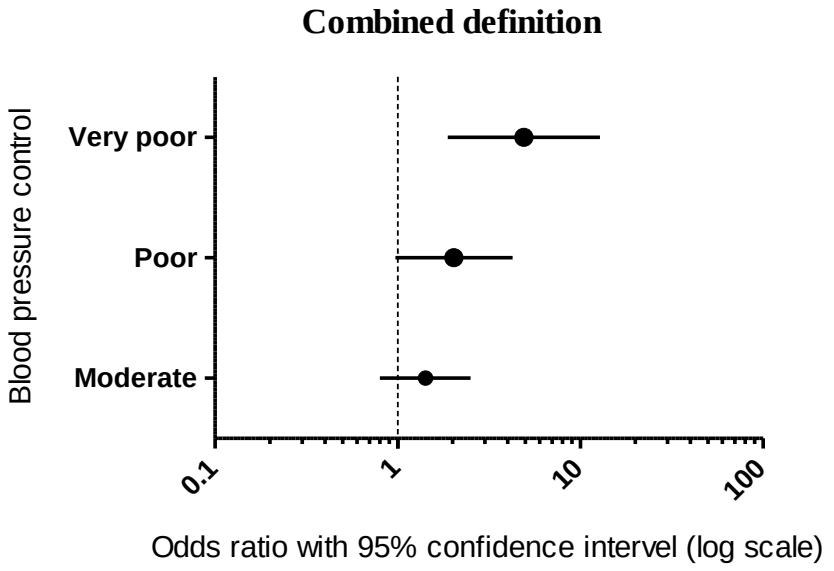


Figure 5-2: Forest plots showing the odds ratio with 95% confidence interval (on the logarithm of the base 10 scale) of three blood pressure stages as predictors of the conventional (A) and the combined (B) definitions of microalbuminuria after adjustment for:

- Age, serum creatinine and anticoagulants with the new definition group
- Age and fasting glucose with the conventional definition group.
- LVH, fasting triglycerides and CBBs with the combined definition group.

5.4 Summary/discussion

Microalbuminuria by the three definitions (new, conventional and combined) was strongly related to several laboratory and clinical characteristics. Also, microalbuminuria was associated with LVH, ST-T changes on ECG and cardiovascular disease. Blood pressure (especially SBP) was independently associated with microalbuminuria. This was also evident in models that involved a group of patients with poorly controlled blood pressure where those with very poor blood pressure were strongly associated with microalbuminuria.

Association of microalbuminuria by the new and the conventional definitions with fasting triglycerides and eosinophil count, respectively, may reflect the presence of inflammatory component of the vascular system caused by dyslipidemia. Serum creatinine was significantly related to microalbuminuria by the new and combined definitions, reflecting deterioration in kidney function in subjects with microalbuminuria. Anticoagulants were strongly associated with all three definitions of microalbuminuria. This might be explained since in subjects with hypertension, these drugs are usually utilised by those with atrial fibrillation and such patients usually suffer from complex risk factors such as LVH, increased age, higher blood pressure and vascular abnormalities. Microalbuminuria was a powerful predictor of LVH, CVD and ECG abnormalities and combining the two definitions has provided a clearer figure of the association. Although some of the associations with CVD in model IV were not significant, the confidence intervals were wide with borderline lower boundaries and odds ratio estimates were higher than one, indicating that the association might to be clinically relevant.

6. Chapter six: microalbuminuria calculated by different methods

6.1 Introduction

The gold standard method for the diagnosis of microalbuminuria is by 24-hour urine collection to account for any intra-individual variability over time (Witte et al., 2009). Several therapeutic guidelines recommend three early morning urine specimens to confirm the presence of microalbuminuria (National Collaborating Centre for Chronic Conditions, 2008). However, this method is cumbersome. Recent finding in diabetic subjects suggests that a single specimen might be sufficient to allow the diagnosis of microalbuminuria (Pugliese et al., 2011). The purpose of the analysis presented in this chapter was to test the reproducibility of microalbuminuria in cohorts of hypertensive subjects attending secondary/tertiary referral centres.

6.2 Methods

Microalbuminuria was initially detected by laboratory quantification at the GBPC and by semi-quantitative dipstick method in the Aberdeen Hypertension clinic. The prevalence of microalbuminuria was compared in the two cohorts. Reproducibility was evaluated by comparing the presence of microalbuminuria in the first sample and in subsequent measurements. Patient characteristics were explored in an exploratory analysis.

A subcohort from the GBPC population had positive result in their first sample but the collection of further samples was not possible (n=116). The characteristics of those subjects were also compared with microalbuminuric subjects defined by repeated measurement. Groups were compared using a 2-sample *t* test, Mann-Whitney test or Fisher's exact test, as appropriate.

6.3 Results

6.3.1 Demographic characteristics of subjects attending the GBPC versus the Aberdeen Hypertension Clinic

Table 6-1 compares the demographic characteristics of the GBPC and Aberdeen Hypertension Clinic cohorts. Subjects attending the GBPC were older than those attending the Aberdeen Hypertension Clinic; mean 58.4 ± 14.9 years versus 55.4 ± 14.8 years (P=0.014). There was no significant difference between the two cohorts in terms of SBP (140.1 ± 19.2 mm Hg for Glasgow cohort versus 142.8+21.2 mm Hg for Aberdeen cohort). However, subjects from the GBPC had a mean DBP of 87.4 ±11.2 mm Hg, which is statistically higher than in the Aberdeen Hypertension Clinic; 84.5 ± 12.4 mm Hg (P=0.004). The pulse pressure was higher in subjects attending the Aberdeen Hypertension Clinic; 58.3 ± 20.6 mm Hg versus 52.6 ± 16.3 mm Hg (<0.001) than in those attending the GBPC.

Subjects attending the Aberdeen Hypertension clinic had median serum creatinine level higher than in the GBPC cohort; 94 µmol/L (interquartile range 84 to 106) versus 79 µmol/L (interquartile range, 69 to 91), P<0.001. Correspondingly, the mean eGFR was lower in the Aberdeen Hypertension Clinic cohort than in the GBPC subjects, 67.3 ± 15.5 mL/min/1.73 m^2 and 80.3 ± 19.6 mL/min/1.73 m2, respectively (P<0.001).

Random blood glucose was statistically higher in the GBPC (n=88) than in the Aberdeen clinic (n=165). Both cohorts had mean BMI of about 30 kg/m2. There was no difference between the two cohorts in the total cholesterol level, with a mean value of about 5 mmol/L. Similarly, there was no significant difference between the two cohorts in the median level of HDL-cholesterol; 1.3 (interquartile range, 1.1 to 1.6) for the GBPC cohort versus 1.4 (interquartile range, 1.2 to 1.7) for the Aberdeen Hypertension Clinic cohort.

Eosinophil count was significantly higher in the Aberdeen cohort than in the GBPC cohort; 0.15 $\times 10^9$/L (0.09 to 0.24) versus 0.20 $\times 10^9$/L (0.13 to 0.28) (P <0.001), respectively.

The clinical characteristics of the two populations were similar (table 6-2). The number of cardiovascular and metabolic medicines used in both cohort were not different, with a median of 3 (interquartile range, 1 to 5). Overall, the frequency of pharmacological agents used was not statistically different between the two cohorts, except for ACEIs and ARBs. While individuals from the Aberdeen Hypertension Clinic used ACEIs more frequently than in the GBPC cohort (58% versus 38%, P<0.001), ARBs were prescribed to more than one third of the GBPC subjects compared with one-fifth of attendance at the Aberdeen Hypertension Clinic (P<0.001).

Variable (unit)	Glasgow	N	Aberdeen	N	P-value
Age (years)	58.4 ± 14.9	884	55.4 ± 14.8	174	**0.014**
SBP (mm Hg)	140.1 ± 19.2	880	142.8 ± 21.2	174	0.110
DBP (mm Hg)	87.4 ± 11.2	880	84.5 ± 12.4	174	**0.004**
Pulse pressure (mm Hg)	52.6 ± 16.3	880	58.3 ± 20.6	174	**<0.001**
BMI (kg/m^2)	30.0 ± 6.2	856	30.4 ± 5.4	173	0.393
Serum creatinine (µmol/L)	79 (69 to 91)	884	94 (84 to 106)	163	**<0.001**
eGFR ($mL/min/1.73m^2$)	80.3 ± 19.6	884	67.3 ± 15.5	162	**<0.001**
Random glucose (mmol/L)	6.6 (5.3 to 8.1)	88	5.2 (4.8 to 5.6)	165	<0.001
Total cholesterol (mmol/L)	5.1 ± 1.1	862	5.0 ± 1.1	171	0.176
HDL-cholesterol (mmol/L)	1.3 (1.1 to 1.6)	848	1.4 (1.2 to 1.7)	174	0.063
Eosinophil count ($\times 10^9/L$)	0.15 (0.09 to 0.24)	808	0.20 (0.13 to 0.28)	172	**<0.001**
Number of drugs	3 (1 to 5)	860	3 (1 to 5)	175	0.117

Table 6-1: A comparison of the demographic characteristics of subjects attending the GBPC (n=844) or the Aberdeen Hypertension Clinic (n=175). Data are summarised as mean (±S.D) or median (interquartile range), as appropriate.

Variable (total available)	Glasgow	N	Aberdeen	N	P value
Male (1059)	45.4%	401	50.3%	88	0.246
Co-morbidities/risk:					
Type II diabetes (1048)	13.1%	114	13.1%	23	1.000
LVH (954)	24.0%	187	25.9%	45	0.625
CVD (990)	16.8%	137	21.1%	37	0.189
Pharmacotherapy: (1040)					
Diuretics	48.4%	419	50.9%	89	0.563
CCBs	42.9%	371	45.1%	79	0.616
ACEIs	37.8%	327	57.7%	101	<0.001
ARBs	35.3%	305	21.1%	37	<0.001
Beta blockers	30.3%	262	33.7%	59	0.419
Alpha blockers	14.8%	128	17.7%	31	0.357
Aldosterone antagonists	9.1%	79	9.1%	16	1.000
Imidazoline receptor agonists	1.7%	15	4.0%	7	0.078
Statins	37.1%	321	41.1%	72	0.347
Other lipid lowering agents	3.7%	32	2.9%	5	0.663
Anti-platelet agents	25.4%	220	28.6%	50	0.396
Anticoagulants	2.5%	22	3.4%	6	0.607
Vasodilators	2.2%	19	2.9%	5	0.784

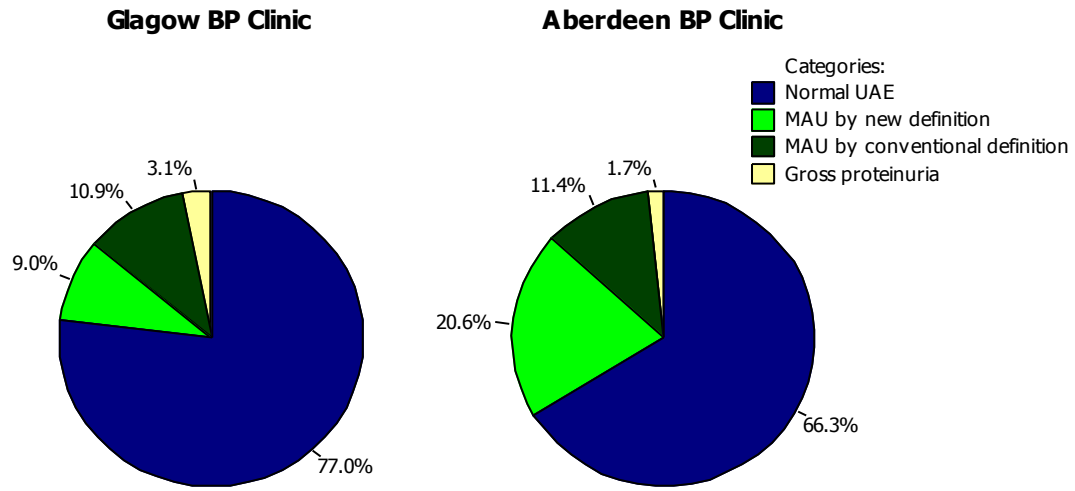
Table 6-2: The clinical characteristics of subjects attending the GBPC (n=884) and the Aberdeen Hypertension Clinic (n=175). Variables are expressed as percentage of cohort.

6.3.2 Prevalence of albuminuria stratified by the study's centres (GBPC versus Aberdeen Hypertension Clinic)

After excluding 116 patients who had only one positive sample, the prevalence of microalbuminuria in all subjects attending the GBPC (n=768) or the Aberdeen Hypertension Clinic (n=175) is shown in figure 6-1 (A). Gross proteinuria was found in 3.1% of subjects attending the GBPC compared with 1.7% in the Aberdeen Hypertension Clinic. The percentage of microalbuminuria by the conventional definition was similar in the two cohorts (11%). In contrast, the percentage of individuals with the new definition of microalbuminuria was higher in Aberdeen Hypertension Clinic than in the GBPC (21% versus 9%, P<0.001).

Figure 6-1 (B) illustrates the prevalence of microalbuminuria in non-diabetic hypertensive subjects without stage 4 or 5 CKD or gross proteinuria attending the GBPC (n = 650) or Aberdeen Hypertension Clinic (n=136). The conventional definition of microalbuminuria was found in 9.2% and 11% patients attending the GBPC or the Aberdeen Hypertension Clinic, respectively. Approximately one-fifth of the Aberdeen Hypertension Clinic cohort had microalbuminuria by the new definition compared with only 8% of the GPBC cohort (P=0.001).

A.



B.

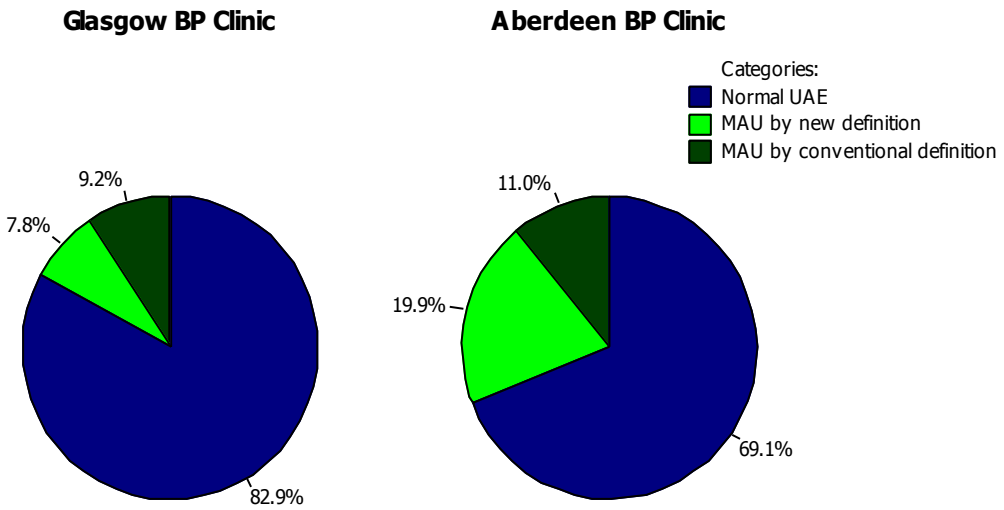


Figure 6-1: (A) The prevalence of albuminuria (microalbuminuria by the new and the conventional definitions and gross proteinuria) in all subjects attending the GBPC (n=768) versus the Aberdeen Hypertension Clinic (n=175). (B) Prevalence of microalbuminuria in non-diabetic hypertensive subjects without evidence of CKD stage 4, 5 or proteinuria (n= 650 in the GBPC and 136 in the Aberdeen Hypertension Clinic). MAU: microalbuminuria. BP: blood pressure.

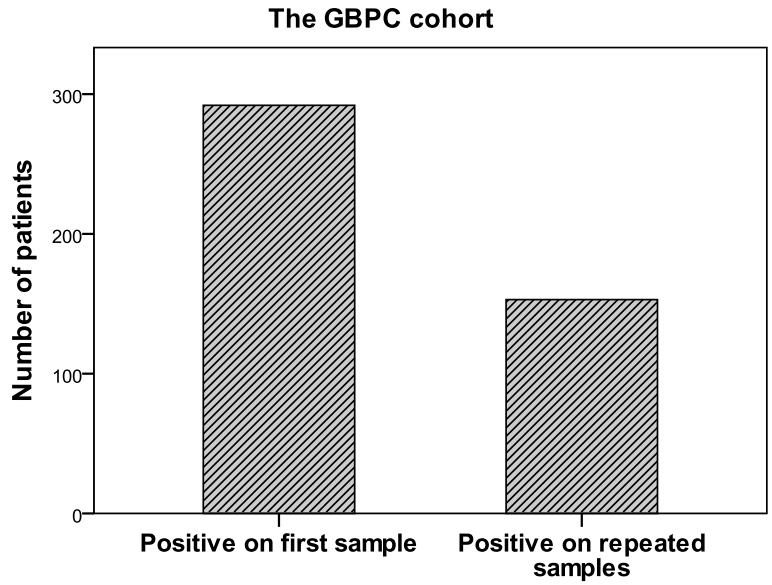
6.3.3 Reproducibility of microalbuminuria in hypertensive subjects attending the GBPC or the Aberdeen Hypertension Clinic

In the GBPC population, 292 patients out of 744 patients (39%), after excluding the data of 24 proteinuric patients, had positive microalbuminuria on first sample using laboratory quantification. When repeated samples were considered, the number of positive subjects dropped to 153 patients (52% out of 242 patients). This means that 48% of the subjects became negative when repeated samples were considered (figure 6-2 a).

As mentioned earlier, in Aberdeen cohort, the detection of microalbuminuria was carried out using dipstick method and this was followed by laboratory quantification. Ninety five subjects (55%) out of 172 patients had positive results on their first sample, after excluding the data of 3 patients with gross proteinuria. Out of those 95 subjects, 56 (59%) were positive on repeated samples using lab analysis (figure 6-2 b). This means that 41% of those positive on their first sample using dipstick method became negative when repeated samples were used in the classification.

The prevalence of microalbuminuria on first sample detected by urine dipstick in the Aberdeen Hypertension Clinic was significantly higher than in the GBPC (55% versus 39%, $P<0.001$). Also, the prevalence of microalbuminuria by repeated samples detected by laboratory method was significantly higher in the Aberdeen Hypertension Clinic (34% versus 23%, $P=0.001$). However, the difference between the two cohorts in terms of the proportion of subjects who became negative when repeated samples were considered was not significantly different ($P = 0.288$).

(a)



(b)

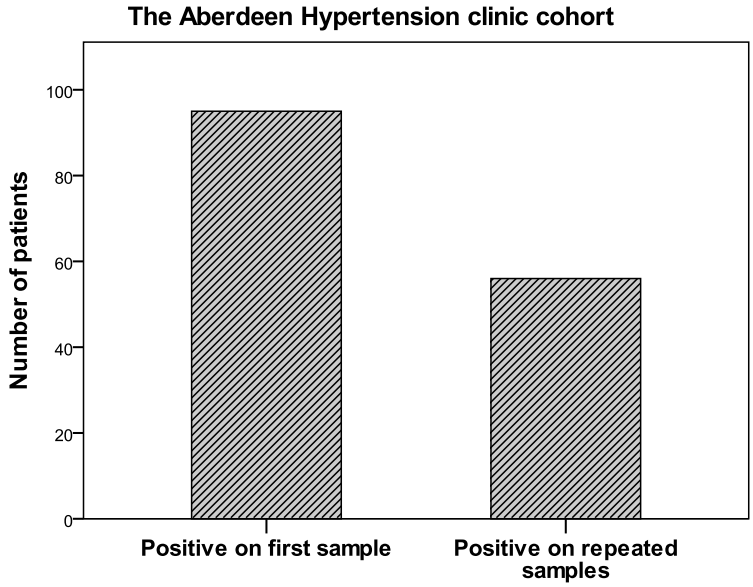


Figure 6-2: Proportion of subjects with positive UAE (by the combined definition) on their first sample versus those with positive result on repeated samples in: (a) the GBPC; (b) The Aberdeen Hypertension Clinic. In the GBPC, the first sample was estimated by laboratory quantification while semi-quantitative dipstick method was used in the Aberdeen Hypertension Clinic.

6.3.4 A comparison between those with positive microalbuminuria on repeated samples versus those who became negative on repeated samples

Table 6-3 shows a comparison between first-sample only positive individuals and those who showed persistently increased UAE on the further samples for both cohorts combined, after excluding patients with gross proteinuria and those with type II diabetes. Overall, positive subjects on repeated samples were characterised by increased age, serum creatinine, fasting glucose, fasting triglycerides and eosinophil count which were higher than in those with positive microalbuminuria on their first sample only ($P<0.05$). eGFR values were significantly lower in positive subjects on repeated samples. SBP, BMI and ESR were higher in positive subjects by repeated samples but the differences between the two groups were not significant.

While the gender distribution on subjects with positive UAE on repeated samples was almost equal, subjects with negative UAE on further samples were mainly female (60%) but the differences between the two group was not significant (table 6-4). The prevalence of LVH, ST-T changes on ECG and CVD was higher in subjects with persisted UAE than in those who showed regressed albuminuria on the further samples, although the difference in CVD prevalence failed to achieve statistical significance.

Positive subjects on repeated samples were treated with cardiovascular and metabolic medicines more often than in those with only one positive result. The use of anticoagulants and lipid lowering agents were higher in repeated samples positive subjects than those who had negative albuminuria on the further samples.

Variable (unit)	Positive group	N	Negative group	N	P-value
Age (years)	61.4 ± 16.2	153	56.8 ± 14.6	161	**0.008**
BMI (Kg/m^2)	29.8 ± 6.0	151	28.9 ± 5.3	159	0.136
SBP (mm Hg)	144.7 ± 21.0	153	141.0 ± 17.3	161	0.076
DBP (mm Hg)	86.7 ± 12.6	153	87.4 ± 10.3	161	0.613
Serum creatinine (µmol/L)	90.2 ± 23.4	153	80.9 ± 18.5	161	**<0.001**
eGFR ($mL/min/1.73\ m^2$)	74.4 ± 21.0	153	80.7 ± 17.9	161	**0.005**
Fasting glucose (mmol/L)	5.3 ± 0.6	98	5.1 ± 0.6	121	**0.048**
Total cholesterol (mmol/L)	5.0 ± 1.0	149	5.2 ± 1.0	160	**0.031**
HDL-cholesterol (mmol/L)	1.4 ± 0.5	149	1.5 ± 0.5	157	0.163
F. triglycerides (mmol/L)	1.3 (0.9 to 2.1)	97	1.2(0.9 to 1.6)	121	**0.023**
Eosinophil count ($\times 10^9/L$)	0.17 (0.11 to 0.26)	145	0.15 (0.09 to 0.23)	153	**0.034**
ESR (mm in 1 hr)	10 (5.0 to 19.0)	81	9 (4.0 to 15.7)	96	0.171
Number of drugs*	3 (2 to 5)	151	2 (1 to 4)	153	**<0.001**

Table 6-3: A comparison between subjects with positive UAE by the combined definition in repeated samples (Positive group, n=153) versus those who became negative based on multiple samples (Negative group, n=161). Subjects with diabetes mellitus and those with proteinuria were excluded. Contineous variables are expressed as mean ± SD or median (interquartile range) for non-parametric comparisons. F.: fasting. *Cariovascular/metabolic drugs.

Variable (%)	Positive group	N	Negative group	N	P value
Male	51.6%	79	40.4%	65	0.054
Co-morbidities/ risk:					
Diabetes family history	16.0%	15	8.2%	8	0.121
Stroke family history	16.2%	16	6.5%	7	**0.044**
CHD family history	22.7%	22	19.4%	21	0.609
Current smoker	9.6%	10	12.2%	14	0.666
LVH	32.2%	46	18.5%	28	**0.007**
ST-T changes	22.0%	22	11.0.%	13	**0.041**
CVD	23.6%	35	15.0%	23	0.079
Pharmacotherapy:					
Diuretics	48.3%	73	42.6%	66	0.358
CCBs	52.3%	79	40.6%	63	0.051
ACEIs	45.0%	68	38.7%	60	0.297
ARBs	35.8%	54	29.0%	45	0.223
Beta blockers	36.4%	55	27.7%	43	0.112
Alpha blockers	17.2%	26	11.0%	17	0.139
Aldosterone antagonists	7.3%	11	5.8%	9	0.649
Imidazoline agonists	1.3%	2	2.6%	4	0.685
Statins	39.7%	60	31.0%	48	0.121
Other lipid lowering	6.6%	10	1.9%	3	**0.050**
Anti-platelet agents	23.2%	35	18.7%	29	0.399
Anticoagulants	8.6%	13	1.9%	3	**0.010**
Vasodilators	4.0%	6	1.9%	3	0.331

Table 6-4: The clinical characteristics of subjects with positive UAE by both definitions in repeated samples (Positive group, n=153) versus those who became negative based on multiple samples (Negative group, n=161). Variables are expressed as percentage of cohort. N represnts number of cases of releavant variable.

6.3.5 Characteristics of those with only one-positive UAE sample versus those with positive result on single or multiple samples

Tables 6-5 and 6-6 show comparisons of the demographic characteristics of subjects who had one positive microalbuminuria result but in whom further samples were not possible versus the characteristics of subjects with microalbuminuria on repeated measurements or those who became negative in repeated samples. The combined definition was used in each group. Data of patients with diabetes and those with gross proteinuria were not included in the comparison.

With the exception of SBP, DBP and total cholesterol, microalbuminuric subjects by repeated measurement had cardiovascular risk factors higher than in those positive on one sample and those who became negative on repeated samples. Subjects with microalbuminuria on repeated samples had higher values for inflammatory markers, less good renal function, increased fasting glucose level and were treated with higher number of drugs. The proportion of those with ECG abnormalities and established CVD was also higher in microalbuminuric subjects by repeated samples. Subjects with only one positive sample had cardiovascular risk parameters higher than those who became negative such as age, BMI, SBP, DBP, total cholesterol, fasting triglycerides, ESR and LVH.

Variable (unit)	Group 1	N	Group 2	N	Group 3	N	P-value
Age (years)	56.8 ± 14.6	161	58.2 ± 15.7	97	61.4 ± 16.2	153	**0.027**
BMI (Kg/m^2)	28.9 ± 5.3	159	30.5 ± 7.0	92	29.9 ± 6.0	151	0.117
SBP (mm Hg)	141.0 ± 17.3	161	146.6 ± 20.8	97	144.7 ± 21.0	153	0.054
DBP (mm Hg)	87.4 ± 10.3	161	92.1 ± 13.0	97	86.7 ± 12.6	153	**0.001**
Serum creatinine (µmol/L)	80.9 ± 18.5	161	79.7 ± 15.7	97	90.2 ± 23.4	153	**<0.001**
eGFR (mL/min/1.73 m^2)	80.7 ± 17.9	161	82.2 ± 17.6	97	74.4 ± 21.0	153	**0.002**
Fasting glucose (mmol/L)	5.1 ± 0.6	121	5.1 ± 0.7	62	5.3 ± 0.6	98	**0.033**
Total cholesterol (mmol/L)	5.2 ± 1.0	160	5.3 ± 1.2	91	5.0 ± 1.0	149	**0.046**
HDL-cholesterol (mmol/L)	1.5 ± 0.5	157	1.4 ± 0.4	86	1.4 ± 0.5	149	0.137
Fasting triglycerides (mmol/L)	1.2 (0.9 to 1.6)	121	1.4 (0.8 to 2.1)	60	1.3 (0.9 to 2.1)	97	0.074
Eosinophil count ($\times 10^9$/L)	0.15 (0.09 to 0.23)	153	0.12 (0.07 to 0.22)	87	0.17 (0.11 to 0.26)	145	**0.004**
ESR (mm in 1 hr)	9 (4.0 to 15.7)	96	9.5 (5 to 18)	70	10 (5.0 to 19.0)	81	0.313
Number of drugs*	2 (1 to 4)	153	3 (1 to 4)	94	3 (2 to 5)	151	**0.001**

Table 6-5: A comparison of the demographic characteristics of those who became negative on repeated measurements (group 1, n=161), microalbuminuria on one sample but further samples were not available (group 2, n=97) and microalbuminuric subjects on repeated samples by the combined definition (group 3, n=153). Data are expressed as mean ± (S.D) or median (interquartile range), as appropriate. N represnts number of cases of releavant variable. * Cardiovascular/meatblic drug

Variable	Group1	N	Group2	N	Group3	N	P value
Male	40.4%	65	45.4%	44	51.6%	79	0.137
Comorbidities/risk:							
Diabetes family history	8.2%	8	17.9%	15	16%	15	0.133
Stroke family history	6.5%	7	11.1%	10	16.2%	16	0.089
CHD family history	19.4%	21	22.2%	20	22.7%	22	0.847
Current smokers	12.2%	14	11.7%	11	9.6%	10	0.833
LVH	18.5%	28	28.9%	24	32.2%	46	**0.022**
ST-T changes on ECG	11.0.%	13	9.1%	7	22.0%	22	**0.022**
CVD	15.0%	23	12.2%	11	23.6%	35	**0.046**
Pharmacotherapy:							
Diuretics	42.6%	66	46.3%	44	48.3%	73	0.603
CCBs	40.6%	63	42.1%	40	52.3%	79	0.096
ACEIs	38.7%	60	36.8%	35	45.0%	68	0.366
ARBs	29.0%	45	32.6%	31	35.8%	54	0.464
Beta blockers	27.7%	43	30.5%	29	36.4%	55	0.252
Alpha blockers	11.0%	17	13.7%	13	17.2%	26	0.291
Aldosterone blockers	5.8%	9	10.5%	10	7.3%	11	0.415
Imidazoline agonists	2.6%	4	2.1%	2	1.3%	2	0.743
Statins	31.0%	48	29.5%	28	39.7%	60	0.157
Other lipid lowering	1.9%	3	2.1%	2	6.6%	10	0.084
Anti-platelet agents	18.7%	29	24.2%	23	23.2%	35	0.502
Anticoagulants	1.9%	3	2.1%	2	8.6%	13	**0.012**
Vasodilators	1.9%	3	3.2%	3	4%	6	0.610

Table 6-6: A comparison of the categorical variables of those who became negative on repeated measurements (group 1, n=161), microalbuminurics on one sample but further samples were not available (group 2, n=97) and microalbuminuric subjects on repeated samples by the combined definition (group 3, n=153). Data are expressed as percentage of cohort. N represnts number of cases of releavant variable.

6.4 Summary/discussion

There were minor differences between the GBPC and The Aberdeen clinic populations. The use of semi-quantitative urine dipsticks resulted in a higher prevalence of microalbuminuria in the Aberdeen Hypertension Clinic. This may indicate that detecting microalbuminuria by semi-quantitative urine strips may overestimate the prevalence and give false-positive results. However, subjects attending the Aberdeen Hypertension Clinic had poorer renal function compared with the GBPC population, a finding which may explain the higher prevalence of microalbuminuria. This was supported by the observation that the prevalence of microalbuminuria on repeated sample was also higher in the Aberdeen population. Whether laboratory analysis is more accurate than urine strips in detecting microalbuminuria cannot be confirmed using my data.

There were marked reductions in the prevalence of microalbuminuria on repeated samples compared with the first sample in both cohorts. Patients with microalbuminuria only on first urine sample tended to have cardiovascular risk lower than in those with repeated positive samples but higher than in those who became negative on repeated samples. Together, these finding suggest that diagnosing microalbuminuria based on single measurement of albuminuria is not reliable and may overestimate the prevalence of clinically relevant microalbuminuria.

7. Chapter seven: blood pressure and proteinuria over time in subjects attending the GBPC

7.1 Introduction

Microalbuminuria is strongly associated with high blood pressure and is prevalent in subjects with uncontrolled hypertension (Ravera et al., 2006). However, little is known about the pattern of blood pressure changes with relation to urinary albumin excretion in treated hypertensive subjects attending specialist clinics. The main goal of this analysis was to study, longitudinally, the blood pressure changes in subjects with normal urinary albumin excretion and those with microalbuminuria. In a secondary analysis, the results of proteinuria as assessed by dipsticks at entry to the clinic (baseline) were related to the results of laboratory analysis for microalbuminuria.

7.2 Methods

Anonymised patient information was extracted from the database of the GBPC. This database stores the demographic and clinical characteristics of more than 15,000 patients, including blood pressure records from each visit. At first visit, protein excretion in the urine is routinely investigated using Multistix 10 SG Reagent Strips. Proteinuria was diagnosed when the result of the dipstick showed trace or more of protein in urine (≥15 mg/dL). The results of proteinuria examination were found in 558 subjects participated in the microalbuminuria analysis and linked to the results of laboratory analysis of albuminuria.

7.3 Results

7.3.1 Blood pressure changes

7.3.1.1 Cross-sectional analysis of blood pressure at baseline, 6 months, 1 year and 2 years

Table 7-1 shows a comparison of blood pressure changes from baseline (first-ever reading at the GBPC) to 6 months (±2 months), first year (± 3 months), second year (± 3 months) and readings obtained during the screening of microalbuminuria in all available data of subjects attending the GBPC. Patients with proteinuria (n=24) and those with only one positive sample (n=116) were excluded from this analysis making the final sample size n= 744 (the original GBPC sample size was 884). Patients were assigned to three groups, according to the result of repeated measurements of UAE (i.e. normoalbuminuria, the new definition and the conventional definition of microalbuminuria).

At baseline, subjects with increased UAE had SBP significantly higher than in normoalbuminuric individuals; 163.9 ± 23.5 mm Hg in the conventional definition group, 161.4 ± 23.1 mm Hg in the new definition group and 152.5 ± 20.0 mm Hg in normoalbuminuric individuals, $P<0.001$ using ANOVA test. The SBP in both definitions of microalbuminuria were statistically higher than that in normoalbuminuric individuals, $P<0.05$. On the other hand, DBP was not significantly different in the three groups at baseline (94.6 ± 10.4 mm Hg, 95.0 ± 12.0 mm Hg and 92.8 ± 12.0 mm Hg for normoalbuminuric subjects, the new definition and the conventional definition of microalbuminuria, respectively. Subjects with microalbuminuria had higher pulse pressure; 57.9 ± 17.6 mm Hg, 66.4 ± 22.1 mm Hg and 71.1 ± 22.5 mm Hg for normoalbuminurics, the new definition and the conventional definition of microalbuminuria, respectively.

Four hundred twenty four subjects had blood pressure records after 6 months from their first visit. Normoalbuminuric individuals achieved 8% reduction in SBP, with a mean reduction of -14.7 ± 19.3 mm Hg. The SBP of subjects with microalbuminuria by the new and the conventional definitions was reduced by approximately 11%; mean reduction -17.5 ± 22.3 mm Hg and -16.2 ± 20.3 mm Hg, respectively. Although the reduction was relatively higher in microalbuminuric subjects by the two definitions, the mean SBPs were still significantly higher than in individuals with microalbuminuria (P=010). The mean SBP in the two microalbuminuric groups was above the therapeutic target for SBP (<140 mm Hg) while normoalbuminuric individuals were just below the threshold. All the three groups achieved reductions in the DBP of about 5% after six months follow-up. The pulse pressure was again higher in individuals with microalbuminuria.

After one year, data of 492 subjects were available. There were slight reductions in both SBP and DBP in all three groups. The SBP was higher in microalbuminuric subjects by the two definitions, although the differences between the groups were just above the significance threshold (p=0.052). The mean DBPs of the three groups were not different and below the therapeutic target (< 90 mm Hg). Microalbuminuric subjects continued to have greater pulse pressure than normoalbuminuric subjects.

In their 2nd year of follow-up at the GBPC, there were slight further reductions in the SBP of microalbuminuric subjects but not in those with normal UAE. There were no differences between the three groups in terms of mean SBP. Likewise, there were no apparent differences between the levels of DBP in the 2nd year versus 1st year. However, the pulse pressure was statistically higher in microalbuminuric patients. The mean value of SBP in subjects with the conventional definition microalbuminuria was just above the therapeutic target (141.0 ± 19.4 mm Hg).

The difference between the baseline SBP and the SBP that was obtained when microalbuminuria was measured was highest in subjects with the new definition of microalbuminuria followed by the conventional definition and normoalbuminuria; - 23.2 ± 25.1 mm Hg, - 17.8 ± 27.3 mm Hg and -14.2 ± 21 mm Hg, respectively. However, a comparison between normoalbuminuric and the conventional definition subjects revealed no statistical significance (data are not shown). Also, individuals with the new definition of microalbuminuria achieved the greatest reduction of DBP but the overall difference between the groups was not significant. Subjects with microalbuminuria by the conventional definition had the highest pulse pressure, followed by the new definition group and those with normal UAE.

Variables (mm Hg)	Normal UAE	N	New definition	N	Conventional definition	N	P-value
A. Blood pressure:							
Baseline SBP	152.5 ± 20.0	593	161.4 ±23.1	69	163.9 ± 23.5	82	<0.001
6 months SBP:	139.6 ± 14.7	324	144.8 ± 18.9	44	145.1 ± 17.8	56	0.010
reduction	-14.7 ± 19.3	324	-17.5 ± 22.3	44	-16.2 ± 20.3	56	0.610
1st year SBP:	139.0 ± 17.4	375	143.4 ± 20.2	53	144.2 ± 22.5	64	0.052
reduction	-15.2 ± 22.1	375	-17.6 ± 20.3	53	- 20.1 ± 24.9	64	0.238
2nd year SBP:	138.7 ± 16.2	323	139.6 ± 18.8	50	141.0 ± 19.4	47	0.680
reduction	-16.0 ± 23.4	323	-20.7 ± 25.0	50	-25.0 ± 26.0	47	0.035
SBP (MAU)*	138.2 ± 17.9	593	138.2 ± 16.5	69	146.1 ± 21.8	82	0.001
reduction	-14.2 ± 21.0	593	- 23.2 ± 25.1	69	- 17.8 ± 27.3	82	0.003
Baseline DBP	94.6 ± 10.4	593	95.0 ± 12.0	69	92.8 ± 12.0	82	0.306
6 months DBP:	88.9 ± 9.1	324	90.8 ± 10.1	44	88.5 ± 9.5	56	0.395
reduction	-6.3 ± 10.6	324	-5.5 ± 9.4	44	-5.0 ± 8.6	56	0.657
1st year DBP:	87.6 ± 9.1	369	87.6 ± 10.7	52	87.1 ± 11.9	64	0.904
reduction	-7.7 ± 10.3	369	-7.0 ± 9.9	52	- 6.1 ± 11.5	64	0.525
2nd year DBP:	87.8 ± 9.0	290	86.0 ± 10.1	47	85.0 ± 8.7	46	0.087
reduction	-8.6 ± 11.3	290	-9.7 ± 12.0	47	-8.4 ± 9.0	46	0.810
DBP (MAU)*	87.2 ± 10.0	593	85.0 ± 11.0	69	86.3 ± 13.8	82	0.217
reduction	-7.3 ± 10.6	593	-10.0 ± 13.3	69	-6.4 ± 13.2	82	0.118
B. Pulse pressure:							
Baseline	57.9 ± 17.6	593	66.4 ± 22.1	69	71.1 ± 22.5	82	<0.001
6 months	50.6 ± 13.4	324	54.0 ± 16.2	44	56.5 ± 16.8	56	0.009
1 year	51.8 ± 16.2	355	55.8 ± 19.3	49	57.6 ± 20.8	62	0.025
2 years	51.0 ± 15.1	238	55.1 ± 16.7	46	57.7 ± 18.8	43	0.015
MAU screening	50.9 ± 15.4	593	53.2 ± 14.2	69	59.7 ± 18.9	82	<0.001

Table 7-1: Blood pressure and pulse pressure over time in normoalbuminuric (n=593) and microalbuminuric by the new definition (n=69) and the conventional definition (n=82). Blood pressure reduction was calculated by subtracting the corresponding reading from baseline record. Data are expressed as mean ± standard deviation. * SBP (MAU) and DBP (MAU) refer to the blood pressure readings obtained during microalbuminuria screening.

7.3.1.2 Longitudinal analysis at baseline, 1 year and 2 years

Table 7-2 summarises the blood pressure changes from baseline to first and second years of follow-up in those with information at all time points (n= 314). In this table, patients were divided into normoalbuminurics and microalbuminurics (by the combined definition) because of insufficient sample size in each microalbuminuria definition alone. As in the analysis that was carried out on all the available data, microalbuminuric subjects had SBP at baseline greater than in normoalbuminuric individuals while DBP did not differ in the two groups. SBP was always higher in microalbuminuric subjects at all measurement periods. There were no differences between the groups in term of DBP except after two years where DBP in individuals with normoalbuminuria was higher than in those with microalbuminuria. Microalbuminuric patients had the highest pulse pressure at all measurement periods.

Variables (mm Hg)	Normoalbuminuria	Microalbuminuria	P-value
A. Blood Pressure:			
Baseline SBP	153.8 ± 10.7	164.7 ± 14.7	0.001
1st year SBP:	139.2 ± 17.3	144.0 ± 22.0	0.089
reduction	-14.6 ± 21.9	20.7 ± 25.1	0.044
2nd year SBP:	138.4 ± 16.2	142.5 ± 20.0	0.104
reduction	-15.5 ± 23.6	-22.2 ± 25.8	0.038
SBP (MAU)*	135.4 ± 17.6	139.8 ± 19.0	0.068
reduction	-18.4 ± 23.2	-24.9 ± 26.0	0.042
Baseline DBP	96.2 ± 10.8	94.0 ± 12.4	0.140
1st year DBP:	88.5 ± 9.1	87.0 ± 12.7	0.306
reduction	-7.6 ± 11.0	-7.1 ± 10.3	0.691
2nd year DBP:	87.6 ± 9.1	85.0 ± 9.6	0.035
reduction	-8.6 ± 11.7	-8.9 ± 10.7	0.800
DBP (MAU)*	86.2 ± 9.1	84.0 ± 12.5	0.148
reduction	-10.0 ± 11.8	-10.0 ± 13.4	0.960
B. Pulse pressure:			
Baseline	57.6 ± 17.7	70.7 ± 23.8	<0.001
1 year	50.6 ± 15.2	57.1 ± 20.1	0.013
2 years	50.8 ± 15.0	57.5 ± 18.5	0.005
During MAU screening	49.1 ± 14.8	55.8 ± 17.0	0.001

Table 7-2: Blood pressure and pulse pressure in subjects with normoalbuminuria (n=240) and microalbuminuria (by the combined definition, n=74) from baseline to 12 months, 24 months and blood pressure taken during microalbuminuria study in those with records at all previously mentioned points. Data are expressed as mean ± standard deviation. * SBP (MAU) and DBP (MAU) refer to the blood pressure readings obtained during microalbuminuria screening.

7.3.2 Proteinuria changes

After excluding those with only one positive result of microalbuminuria screening, results of dipstick examination at entry (first visit) to the clinic were available in 558 patients. Four hundred thirty subjects were normoalbuminurics at microalbuminuria screening and the remainder had albuminuria (gross proteinuria or microalbuminuria by both definitions).

The results of urine dipstick analysis were evaluated with the microalbuminuria laboratory results. In those who had normal UAE, nearly 79% had negative proteinuria results on dipsticks. However, 21% of normoalbuminuric individuals had a trace of more of protein in urine when they first attended the clinic. About 72% of patients with gross proteinuria had positive baseline dipsticks but in subjects with microalbuminuria (the combined definition), 65% had negative baseline dipstick (figure 7-1).

7.3.2.1 Patient characteristics stratified by the results of baseline dipstick and laboratory analysis

Tables 7-3 and 7-4 show the demographic and clinical characteristics of the 558 patients stratified by the results of dipstick examination and laboratory assessment of albuminuria. Patients were categorised into four groups; patients with negative results in both dipstick and laboratory analysis (group 1), those with positive dipstick result but subsequent laboratory analysis showed negative result (group 2), those with negative dipstick but lab analysis showed positive result (group 3) and a group of patients with positive results in both dipstick and lab analysis (group 4).

Overall, the highest values for cardiovascular risk factors were found in patients belonging to group 4. They were older than other groups, had highest values for BMI, serum creatinine (and lowest eGFR), fasting glucose, ESR, eosinophils count, fasting triglycerides and cardiovascular/metabolic drugs. Moreover, group 4 patients had higher

proportions for co-morbidities (type II diabetes, LVH, ST-T changes on ECG) and established CVD.

The highest risk score for CVD over 10 years using the Framingham equation was found in subjects from group 3. They were also characterised by increased values for cardiovascular risk factors such as SBP, pulse pressure and frequent co-morbidities. On the other hand, subjects from either group 1 or 2 had lowest CVD risk scores and cardiovascular parameters (except DBP in group 1).

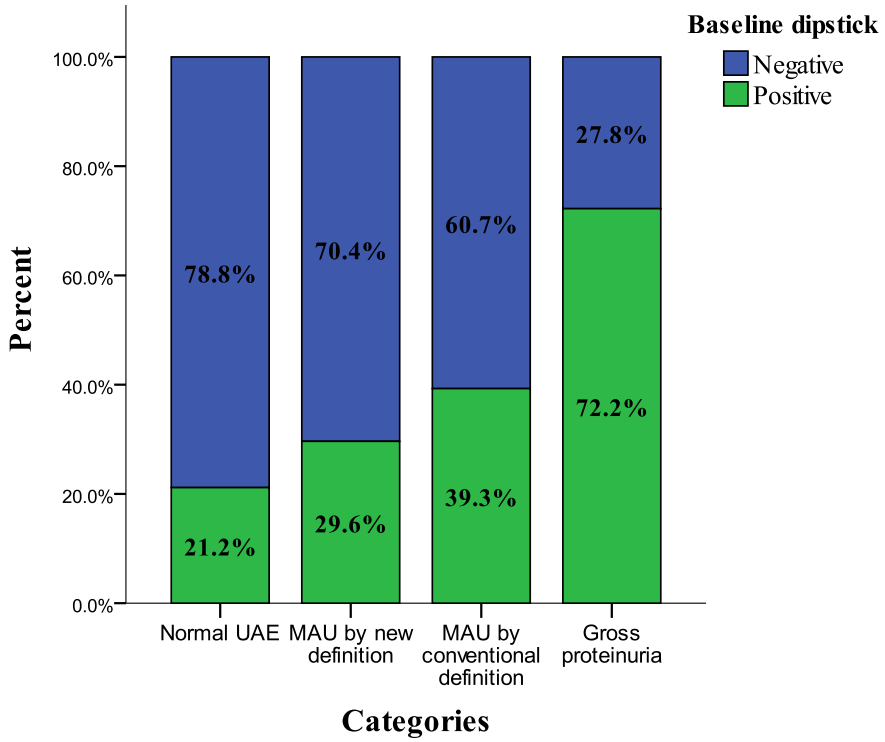


Figure 7-1: Dipstick examination in all albuminuria categories.

Variable	Group 1	N	Group 2	N	Group 3	N	Group 4	N	P-value
Age (years)	57.8 ± 12.3	339	53.9 ± 13.0	91	65.1 ± 15.1	77	65.3 ±13.8	51	<0.001
BMI (Kg/m^2)	29.3 ± 6.0	331	31.4 ± 5.8	86	29.8 ± 6.1	74	33.3 ± 6.4	51	<0.001
SBP (mm Hg)	136 (126 to 150)	337	130 (120 to 140)	91	138 (126 to 159)	76	138 (128 to 147)	51	<0.001
DBP (mm Hg)	90 (80 to 94)	337	84 (78 to 92)	91	88 (74 to 94)	76	84 (76 to 92)	51	0.166
Pulse pressure (mm Hg)	48 (42 to 58)	337	42 (38 to 52)	91	55 (44 to 66)	76	52 (44 to 66)	51	<0.001
Serum creatinine (µmol/L)	78 (70 to 89)	339	78 (66 to 93)	91	84 (71 to 110)	77	87 (77 to 131)	51	<0.001
eGFR ($mL/min/1.73\ m^2$)	80.6 ± 17.9	339	82.1 ± 15.6	91	74.9 ± 23.0	77	66.3 ± 27.0	51	<0.001
Fasting glucose (mmol/L)	5.1 (4.7 to 5.6)	290	5.1 (4.8 to 5.5)	79	5.4 (5.1 to 6.0)	68	5.4 (4.9 to 7.9)	46	<0.001
ESR (mm in 1 hour)	7 (4 to 14)	266	7 (4 to 18)	65	13 (5 to 19)	59	19 (5 to 40)	33	0.001
Eosinophil count ($\times 10^9/L$)	0.14 (0.08 to 0.23)	316	0.16 (0.12 to 0.25)	87	0.16 (0.11 to 0.27)	74	0.22 (0.15 to 0.34)	46	<0.001
Total cholesterol (mmol/L)	5.1 ± 1.0	333	5.1 ± 1.1	91	4.6 ±1.1	76	4.7 ± 1.2	51	<0.001
HDL-cholesterol (mmol/L)	1.5 ± 0.6	329	1.3 ± 0.4	91	1.3 ± 0.5	73	1.3 ± 0.5	51	0.016
Fasting triglycerides (mmol/L)	1.5 ± 0.8	287	1.4 ± 0.8	79	1.7 ± 1.2	68	1.8 ± 0.8	46	0.022
Number of drugs*	2.7 ±1.9	330	3.1 ± 2.1	90	4.2 ± 2.0	77	4.8 ± 2.3	48	<0.001
CVD risk score	11.9 (7.5 to 20)	250	12.7 (7.7 to 18.5)	66	18.7 (12.1 to 27.2)	49	16.1 (9.3 to 26.5)	36	<0.001

Table 7-3: Demographic characteristics stratified by the result of baseline dipstick and albuminuria laboratory analysis; group 1: negative baseline dipstick and lab analysis (n=339), group 2: positive baseline dipstick but negative lab analysis (n=91), group 3: negative baseline dipstick but positive lab analysis (n=77) and group 4: positive dipstick and lab analysis (n=51). Data are expressed as mean ± standard deviation or median (interquartile range), as appropriate**.** * Cardiovascular/metabolic drugs.

Variable (%)	Group 1	N	Group 2	N	Group 3	N	Group 4	N	P-value
Male	41.9%	142	44.0%	40	58.4%	45	41.2%	21	0.065
Smokers	8.4%	27	9.1%	8	12.2%	9	9.3%	4	0.811
Family history:									
Diabetes	12.8%	36	17.1%	14	12.5%	8	17.1%	7	0.704
Stroke	10.4%	32	9.4%	8	11.8%	8	9.3%	4	0.959
CHD	17.2%	53	20.2%	17	19.1%	13	27.9%	12	0.391
Co-morbidities / risks:									
Type II diabetes	7.8%	26	14.3%	13	23.4%	18	40.0%	20	**<0.001**
LVH	18.6%	57	23.3%	20	34.8%	24	42.5%	17	**<0.001**
ST-T changes	12.1%	37	9.5%	8	22.1%	15	26.3%	10	**0.014**
CVD (%)	12.8%	40	16.3%	14	28.8%	21	25.6%	11	**0.004**
Pharmacotherapy:									
Diuretics	45.3%	150	58.2%	53	58.4%	45	64.6%	31	**0.009**
CCBs	36.0%	119	46.2%	42	59.7%	46	64.6%	31	**<0.001**
ACEIs	34.7%	115	38.5%	35	49.4%	38	50.0%	24	**0.037**
ARBs	33.5%	111	37.4%	34	39.0%	30	54.2%	26	0.047
Beta blockers	26.3%	87	27.5%	25	46.8%	36	45.8%	22	**0.001**
Alpha blockers	13.3%	44	12.1%	11	20.8%	16	25.0%	12	0.068
Aldosterone blockers	7.6%	25	14.3%	13	5.2%	4	12.5%	6	0.106
Imidazoline agonists	1.2%	4	2.2%	2	1.3%	1	2.1%	1	0.739
Antidiabetic agents	4.2%	14	6.6%	6	15.6%	12	29.2%	14	**<0.001**
Antiplatelet agents	21.1%	70	28.6%	26	33.8%	26	41.7%	20	**0.005**
Statins	34.4%	114	39.6%	36	49.4%	38	58.3%	28	**0.003**
Lipid lowering	3.6%	12	3.3%	3	10.4%	8	6.3%	3	0.083
Anticoagulants	1.2%	4	1.1%	1	10.4%	8	4.2%	2	**0.001**
Vasodilators	1.2%	4	1.1%	1	6.5%	5	6.3%	3	**0.010**

Table 7-4: Clinical characteristics stratified by the result of baseline dipstick and albuminuria laboratory analysis; group 1: negative baseline dipstick and laboratory analysis (n=339), group 2: positive baseline dipstick but negative laboratory analysis (n=91), group 3: negative baseline dipstick but positive laboratory analysis (n=77) and group 4: Positive dipstick and laboratory analysis (n=51). Data are expressed as percentage of the cohort.

7.4 Summary/ discussion

Microalbuminuria is associated with higher blood pressure at first visit and subsequently. Although blood pressure fall was greater in those with microalbuminuria, blood pressure remained higher in microalbuminuric subjects. On the other hand, DBP was not different between microalbuminuric and normoalbuminuric subjects. The pulse pressure was greater in microalbuminuric subjects in all measurements indicating that these subjects might be at high risk of vascular diseases.

Subjects with persistence increase in the urinary albumin excretion from baseline were associated with complex co-morbidities and increased cardiovascular risk. Similarly those with negative baseline dipstick but later UAE laboratory analysis showed positive result were also at high risk of cardiovascular diseases compared with normoalbuminuric subjects with either negative or positive baseline dipstick. These finding reinforce the importance of screening of increased UAE as a tool of identifying subjects with increased risk for cardiovascular morbidities.

8. Chapter eight: General discussion

8.1 General overview

The current series of studies investigated the prevalence of microalbuminuria and the association with predictive factors for worse outcomes in people attending hypertension clinics. Most of previous attempts to examine the significance of microalbuminuria in hypertension population have limitations such as the selection criteria and small sample sizes. Also, many of these studies were confounded by possible misdiagnosis of microalbuminuria as the diagnosis was based on single measurement using either urine strips or laboratory analysis. This has led to uncertainty about the prevalence of microalbuminuria in hypertension and probable underestimation of its clinical significance.

Microalbuminuria by the currently accepted threshold was found in 11% of subjects attending specialist blood pressure clinics (9.5% in those without diabetes). This study also demonstrated, for the first time, that microalbuminuria by the new definition was present in 11% of the cohort (10% in non-diabetic). High prevalence of microalbuminuria was noted in people with hypertension and different co-morbidities such as diabetes, CKD and LVH. Microalbuminuria was also found in some people without apparent co-morbidities and with good blood pressure control.

This study revealed that microalbuminuria by both definitions was an independent predictor of target organ damage manifested by left ventricular hypertrophy. Microalbuminuria was also a strong predictor for cardiovascular disease and risk factors such as ECG abnormalities. The use of cardiovascular risk score equations suggested that subjects with microalbuminuria had higher chances of developing cardiovascular disease and mortality over 10 years.

Although it has been argued that single screening for microalbuminuria is enough for the diagnosis, I found that a large proportion of participants who had increased UAE on first sample was categorised as normoalbuminuric when the diagnosis was based on multiple samples. Subjects with persistent microalbuminuria had increased values for laboratory parameters associated with cardiovascular risk and higher prevalence of co-morbidities and high-risk clinical characteristics.

High blood pressure was a powerful predictor of microalbuminuria after adjusting for antihypertensive drugs and other potential confounders. The risk of microalbuminuria increased in those with poor or very poor blood pressure control. This was also evident in cross-sectional and longitudinal analysis of the blood pressure of participants from the GBPC where individuals with microalbuminuria had higher blood pressure at presentation and subsequently.

Eosinophil count was also a significant independent predictor of the conventional definition of microalbuminuria. Moreover, levels of ESR were greater in people with microalbuminuria, supporting the involvement of inflammation in the progress.

8.2 Interpretation of the findings

My results suggest that microalbuminuria is a useful tool for identifying hypertensive subjects at increased risk for future cardiovascular events. It is extremely important to determine the prevalence of microalbuminuria in hypertension in order to evaluate whether screening for this condition provides additional information that will help in planning therapeutic strategies. In diabetes mellitus, for example, it is well recognised that microalbuminuria helps in identifying patients at higher risk for developing diabetic nephropathy; periodic screening for

microalbuminuria is recommended (American Diabetes Association, 2011). This recommendation is driven by the evidence reported from many studies that investigated the prevalence and the clinical usefulness of microalbuminuria screening in diabetes.

Microalbuminuria is not uncommon in hypertension. Screening for microalbuminuria offers potential for identifying almost one-fifth of hypertensive subjects at increased cardiovascular risk. My findings showed a close association between microalbuminuria and several abnormalities. This association seems to start at levels below the current definition. By relying on the conventional definition of microalbuminuria, the clinician may miss an opportunity of identifying those with early vascular changes.

The association of microalbuminuria with increased laboratory parameters and cardiovascular risk score in people without co-morbidities or risk may reflect that those subjects are at greater risk for future cardiovascular events. Microalbuminuria offers additional information beyond that provided by conventional risk factors. Therefore, screening for microalbuminuria in apparently healthy subjects with hypertension may detect early changes that require more rigorous treatment. This needs to be confirmed on a prospective study with larger sample size.

The observation that large proportion of individuals with microalbuminuria at first screening became negative when multiple samples were considered indicates that single screening is not enough for reliable diagnosis of microalbuminuria in hypertension. This also means that any future study investigating microalbuminuria and outcomes in hypertension should be based on multiple samples for better correlation.

My findings also emphasise on the importance of blood pressure as one of the most important determinant of microalbuminuria. Thus, better control of blood pressure using

pharmacological approaches should be the main strategy for attenuating microalbuminuria in hypertension.

8.3 Study population

The individuals who participated in my studies had the typical characteristics of those attending hypertension clinics in the UK; relatively high age, a high proportion of obese and overweight subjects, sub-optimal control of blood pressure, mildly elevated serum cholesterol and relatively normal glucose level and renal function (Cuspidi et al., 1999, Cuspidi et al., 2006, Eguchi et al., 2010). The cohort was also characterised by increased co-morbidities. The prevalence of LVH was lower than that reported in several studies which have used echocardiography for the diagnosis (Mancia et al., 2002, Martinez et al., 2003, Cuspidi et al., 2012). Difference can be attributed to variability in the definitions and methods used for the identification of LVH and the population tested (high risk, untreated hypertension, etc). Also, the percentage of people with diabetes was relatively low (Lonati et al., 2008). This is because subjects with diabetes mellitus in Glasgow are usually referred to diabetologists, even if they suffer from high blood pressure.

8.4 Prevalence of microalbuminuria

The prevalence of microalbuminuria by the conventional definition was 11%. When the data of patients with diabetes and those with impaired renal function were excluded, microalbuminuria was found in 9.5% of the subjects.

This prevalence is relatively low compared with that reported in a number of studies (Böhm et al., 2007). This might be because repeated measurement were made in this study to minimise the effect of confounding factors such as requesting multiple samples and the exclusion of

UTI-positive samples. Furthermore, the prevalence of microalbuminuria was based on laboratory quantification of albumin levels in the urine.

This is the first study to report the prevalence of microalbuminuria based on multiple samples in treated hypertensive subjects attending secondary/tertiary blood pressure clinics. The MAGIC trial reported lower prevalence of microalbuminuria (6.7%) in non-diabetic subjects with hypertension (n=787) using repeated measurements. However, there were some restrictions on the population tested such as exclusion of those older than 72 years, those with liver disease, heart failure, kidney disease, severe hypertension, severe obesity and patients with hypokalemia (Pontremoli et al., 1997). The limits for defining microalbuminuria in the MAGIC study were also more restricted than that used in the current study and those of most therapeutic guidelines (ACR 2.38 – 19 mg/mmol in males and 2.96 – 20 mg/mmol in females).

The i-SEARCH global study also reported the prevalence of microalbuminuria in the setting of blood pressure clinics (Böhm et al., 2007). The overall prevalence of microalbuminuria in more than 20,000 individuals from 26 countries was 58%. This high prevalence could be due to several factors. Microalbuminuria was detected on single occasion using urine strips. In addition to the limited ability of urine strips to quantify albumin levels, strips also carry the risk of overestimation or underestimation due to concentration or dilution of the urine (Lin et al., 2011). This is because urine dipsticks detect urinary albumin concentrations which are affected by urine volume. In my analysis, I used the albumin-to-creatinine ratio that adjusts for any possible volume differences.

The authors of the i-SEARCH study did not mention whether spot or early morning samples were used. Spot urine samples are subject to confounding influences and have been shown to overestimate the prevalence of microalbuminuria (Witte et al., 2009). In a study that used 24-

hour urine collection as a reference method, the ACR from early morning urine samples was compared with the ACR from spot urine samples (Witte et al., 2009). The investigators found that the prevalence of microalbuminuria using 24-hour urine collection, early morning and spot urine samples were 10%, 7.5% and 22.4%, respectively.

The other factor that might lead to the increased prevalence of microalbuminuria reported in the i-SEARCH study is that the population studied had complex co-morbidities. For instance, the prevalence of diabetes and established cardiovascular disease were higher than that observed in the current analysis; 27.5% versus 13.1% and 36% versus 18%, respectively. Such conditions are usually associated with a higher prevalence of microalbuminuria and therefore affect the overall prevalence in the study population.

Microalbuminuria by the conventional definition was found in 20% of patients with diabetes. This prevalence was less than that reported in the literature. For example, among patients with diabetes who participated in the HOPE and Micro-HOPE studies, about one-third had microalbuminuria (ACR $\geq$ 2 mg/mmol in both genders) (Heart Outcomes Prevention Evaluation Study Investigators, 2000b). The prevalence of microalbuminuria, measured as ACR 30 to 300 mg/g, in patients with diabetes (n= 10,640) who participated in the ADVANCE study, was 27% (Ninomiya et al., 2009). In the I-DEMAND (Italy Developing Education and awareness on MicroAlbuminuria in patients with hyperteNsive Disease) study, 52% and 44% of diabetic hypertensive subjects with normal eGFR or reduced eGFR, respectively, were microalbuminurics (Leoncini et al., 2010). In the HOPE, ADVANCE and I-DEMAND studies, all prevalence estimates was based on single measurement of albumin excretion. The ADVANCE and HOPE studies, the selection criteria of patients enrolled involved the presence of cardiovascular disease in addition to diabetes mellitus. The

prevalence of microalbuminuria tends to be high in patients with concomitant cardiovascular disease (Mlačak et al., 1999).

The low percentage of patients with diabetes in the current study undoubtedly affects the microalbuminuria prevalence. In the I-DEMAND study (n=3534), 37% of the participants in the cohort had diabetes mellitus compared with 13% in my study. Also, the definition of microalbuminuria in the I-DEMAND study was ACR ≥2.5 mg/ mmol in males or ≥3.5 mg/ mmol in females, but it was not clear whether or not subjects with gross proteinuria were included. The present study allows a clearer estimate of the prevalence of microalbuminuria in hypertension, without the confounding influence of diabetes.

Albuminuria below the conventional definition has been shown to be associated with increased cardiovascular risk (Klausen et al., 2004). The current threshold of defining microalbuminuria is arbitrary. Therefore, I sought to investigate the prevalence of lower levels of microalbuminuria (the new definition) and its association with co-morbidities. The use of the new definition increased the prevalence of microalbuminuria by 11.1% in the overall population, and 10% in non-diabetic non-CKD (stage 4 or 5) subjects.

I am not aware of any study based on clinical settings of blood pressure clinic that has studied the prevalence of microalbuminuria using the new definition. In the PREVEND study, the prevalence of high-normal albuminuria defined as UAC of 10–20 mg/l was about 6% (de Jong et al., 2003). Nevertheless, the PREVEND study was population-based and measurement of microalbuminuria was carried out at single occasion without correcting any variations in urine volume. In a Japanese study, high normal albuminuria, defined as ACR >20 - <30 μg/mg, was found in 20% of untreated subjects with essential hypertension (Ohmaru et al.,

2011). However, the sample size was small (n=332) and there were many exclusions (e.g. those with CHD, arrhythmia, cardiomyopathy and cerebrovascular diseases). Microalbuminuria was estimated from single random urine specimen, a method that has been shown to overestimate microalbuminuria prevalence, as mentioned earlier.

8.5 Association of microalbuminuria with the characteristics of non-diabetic hypertensive subjects without severe kidney dysfunction

Subjects with microalbuminuria by the new and the conventional definitions were older than those with normal albumin excretion. This finding is in agreement with previous studies where advanced age was shown to be among the clinical characteristics of microalbuminuric subjects (Hillege et al., 2001, Klausen et al., 2005, Romundstad et al., 2003b). In a recent community-based study of approximately 11,000 subjects, there was a clear trend of a linear relationship between age and albumin excretion in the urine (classified into five groups; optimal, intermediate, high-normal, microalbuminuria and gross proteinuria) (Blecker et al., 2011).

Microalbuminuria based on both definitions was not associated with increased BMI. The available data of the association between microalbuminuria and obesity is conflicting. While some studies report a stepwise relationship between BMI and UAE in the general population (Kramer et al., 2005, Ferris et al., 2007), hypertension (Pontremoli et al., 1997, Martínez et al., 2001, Bello et al., 2010) and diabetes (Kramer et al., 2009), others suggest a relationship with central obesity measured by waist circumference (Bonnet et al., 2006, Böhm et al., 2007, Chandie et al., 2007, Rossi et al., 2010). However, many previous studies reported no association between BMI and microalbuminuria (Leoncini et al., 2010, Oliveras et al., 2011).

There is no specific explanation of the conflicting findings about the association between BMI and UAE. Both microalbuminuria and obesity are risk factors for cardiovascular diseases. The studied population may have an impact on the association between obesity and microalbuminuria. For instance, increased BMI and central obesity are frequent observations in advanced type II diabetes mellitus, which is also characterised by nephropathy. In this study, diabetic subjects with gross proteinuria had statistically significant higher BMI than those with lower levels of albuminuria, although the small number of diabetic patients makes it difficult to provide conclusions. In hypertension, the association is not very well established. In the study by Leoncini et al. (Leoncini et al., 2010), subjects with morbid obesity (>150% ideal weight) were excluded, which may affect the association. The finding by Martínez et al. (Martínez et al., 2001) is limited by the small number of subjects with microalbuminuria (n=16). The sample size in this study is larger, and selection was not influenced by BMI. Unfortunately, waist circumference was not measured, precluding investigation of any association with microalbuminuria.

Gender distribution was not significantly different in subjects with and without microalbuminuria. The available data on the association of microalbuminuria with specific gender is also contradictory. Among subjects who participated in the PREVEND study, microalbuminuria was more prevalent in men, almost twofold greater than in women (Verhave et al., 2003). Several studies have reported similar findings (Viazzi et al., 2010, Böhm et al., 2007, Tada et al., 2008, Jauregui et al., 2009). On the other hand, data from the third National Health and Nutrition Examination Survey revealed that microalbuminuria was more likely to be prevalent in females (Jones et al., 2002). The EPIC-Norfolk study also reported a higher prevalence of microalbuminuria in females than in males (14% versus 8%, $P<0.05$) (Yuyun et

al., 2004b). Other studies reported no gender differences between those with and without microalbuminuria (Jager et al., 1999, Calviño et al., 1999).

The inconsistency in gender prevalence of microalbuminuria might be attributed to the variability of albumin excretion. Most studies were based on a single random urine specimen. Such a collection method is more prone to bias caused by confounders such as UTI, exercise and menstruation. Therefore, gender differences in prevalence of microalbuminuria are not well supported. Also, a plausible pathophysiological basis for such an association is lacking.

Microalbuminuria was strongly associated with increased blood pressure, a finding that agrees with most reported studies on hypertensive subjects (Moran et al., 2006, Lurbe et al., 2002, Dell'omo et al., 2003). There was a stepwise relationship between SBP and UAE. Similar finding have been reported with both office and ambulatory blood pressure measurements in individuals with hypertension, and even in those with high-normal blood pressure (Moran et al., 2006). A causal relationship is supported by the observation that strict control of blood pressure prevents the development of microalbuminuria and that blood pressure control reduces albuminuria (Philipp et al., 2009, Menne et al., 2012).

Subjects with the new definition of microalbuminuria had SBP higher than that in people with normal urinary albumin excretion, although the difference was not significant. Reffelmann et al. (Reffelmann et al., 2010) demonstrated that similar association between SBP and high normal albuminuria. This study was based on general population with albumin excretion being measured only once and with inclusion based on the availability of baseline echocardiographic analysis. My finding on the association of SBP with low albumin excretion is important since most of people were under fairly extensive hypertension management in a specialist clinic setting.

DBP was not significantly different in the three albuminuria groups. However, pulse pressure was significantly higher in patients with microalbuminuria. One of the possible explanations is that subjects with microalbuminuria might suffer from stiffness of large arteries. The importance of this finding arises from the observation that high pulse pressure is associated with increased cardiovascular risk and is a frequent observation in several co-morbidities such as atherosclerosis (van Popele et al., 2001, Assmann et al., 2005, Tada et al., 2008). In my study, those with microalbuminuria by the new definition had high triglycerides and used a significantly greater proportion of lipid lowering agents, possibly reflecting a higher prevalence of hyperlipidemia and atherosclerosis. These subjects also had higher pulse pressure, with a trend towards a significant difference, than in individuals with normoalbuminuria. All these findings support the notion that subjects with microalbuminuria are at greater risk for subsequent cardiovascular events.

The association between microalbuminuria and increased pulse pressure has been previously reported (Viazzi et al., 2002, Verhave et al., 2005). In a population-based longitudinal study investigating the association of several blood pressure parameters (pulse pressure, SBP, DBP and mean blood pressure) with UAE, the study revealed that pulse pressure was the best predictor for microalbuminuria (Farasat et al., 2010). The small sample size of that study (n= 450) and the small percentage of subjects with hypertension (34%) make it difficult to extend the observation to general hypertension population. Blecker et al. (Blecker et al., 2011) also demonstrated a graded increase of pulse pressure with increasing urinary albumin levels (measured on single occasion) in general population. The ROADMAP study reported a close relationship between UAE and pulse pressure in those with type II diabetes (Januszewicz et al., 2011). But the restrictions in the population involved in the ROADMAP to diabetic patients limit the generalisability of the findings. In the current study, I have shown the

association in general hypertension population and at even lower levels of albuminuria which has not been previously reported.

Subjects with microalbuminuria by both definitions had poor renal function, manifested by high serum creatinine level and low eGFR. I have demonstrated that subjects with microalbuminuria by the new definition had the lowest grade of eGFR and the highest level of serum creatinine. The importance of this finding comes from recent evidence that showed that a combination of eGFR and albuminuria is a powerful predictor for progression to ESRD and mortality in the general population (Hallan et al., 2009) and in American Indians with diabetes (Berhane et al., 2011). Moreover, it has also been shown that microalbuminuria itself is a strong predictor of advanced renal impairment in non-diabetic hypertensive subjects (Viazzi et al., 2010). Therefore, microalbuminuria provides clinical utility for identifying subjects at high risk of worse renal outcomes. This potential needs to be confirmed in prospective studies in general hypertension population involving people with low grade albuminuria.

Fasting glucose levels in non-diabetic subjects with microalbuminuria were higher than in those with normoalbuminuria. Also, random glucose was higher in those with microalbuminuria by the conventional definition. The relationship was stronger in subjects with hypertension and diabetes mellitus. The association of albuminuria and increased blood glucose levels is firmly established in patients with diabetes mellitus (Tapp et al., 2004). On the other hand, the interpretation of such a relationship in subjects with hypertension is difficult, since the results of the majority of studies are confounded by the inclusion of patients with diabetes mellitus. One study in non-diabetic subjects with hypertension suggested no difference in glucose level between those with normal UAE and microalbuminuria (Viazzi et al., 2010).

Impaired glucose tolerance has been found in subjects with hypertension and microalbuminuria (Bianchi et al., 1995). In addition, an association of insulin resistance with microalbuminuria was reported in a non-diabetic population (Hoehner et al., 2002). A defect in insulin signalling has been suggested to play a role in the pathogenesis of microalbuminuria (Jauregui et al., 2009). Increased level of fasting glucose in non-diabetic subjects with microalbuminuria may reflect changes in the insulin-glucose pathway. Such a finding raises a question as to whether or not non-diabetic subjects with hypertension and microalbuminuria might be at higher risk of developing diabetes than those with normoalbuminuria. Longitudinal follow-up of such a group of patients may clarify possible aetiological relevance.

The threshold used for the diagnosis of diabetes mellitus is arbitrary (American Diabetes Association., 2010). The relationship between blood glucose levels and cardiovascular risk is likely to be continuous (Coutinho et al., 1999, Kay-Tee et al., 2001, Brunner et al., 2006). Therefore, the relation between blood glucose levels in non-diabetic subjects in the current study supports the suggestion that microalbuminuria is a risk predictor for cardiovascular disease.

With the exception of the triglycerides level, there were no significant differences in the lipid profile between microalbuminuric and normoalbuminuric individuals. Those with microalbuminuria by the new definition had a higher triglycerides level than in both normoalbuminuric and microalbuminuric subjects by the conventional definition. My findings are in contrast with those reported in several epidemiological studies where the prevalence of hyperlipidemia was frequent in subjects with microalbuminuria (Cirillo et al., 1998, Campese et al., 1999, Salles et al., 2011a). One possible explanation is that the use of lipid lowering agents is fairly high in the current study (more than 38% used statins and other lipid lowering

agents). The treatment of referred patients is based on overall cardiovascular risk, and hence rigorous control of serum cholesterol is of particular importance. The initiation of a lipid lowering regimen might have confounded the findings. Subjects with microalbuminuria by the new definition had a high level of fasting triglycerides. The use of statins was also highest in these subjects was also the highest.

The association of triglycerides with cardiovascular disease is ambiguous. Whether triglycerides provide additive information on cardiovascular risk beyond other lipid parameters is not well established. The Emerging Risk Factor Collaboration showed that the relationship between vascular disease and increased triglycerides levels is weak after adjusting for other potential risk factors (Di Angelantonio et al., 2009). Nevertheless, a relationship between CHD and triglycerides was reported recently, where a 16% increase in triglycerides' concentration was associated with a 10% higher risk of CHD (Sarwar et al., 2010). Two recent meta-analyses suggested a relationship between triglycerides and vascular cerebrovascular complications after adjusting for potential confounders (Labreuche et al., 2010, de Caterina et al., 2010). In view to these findings, it seems that subjects with microalbuminuria by the new definition might be at risk of vascular complication. As a result, rigours control of blood pressure and other risk factors, including serum lipids, is of particular importance.

Levels of inflammatory markers used in my study (eosinophil count and ESR) were higher in those with microalbuminuria. Although the association between microalbuminuria and inflammation has been previously reported with more sensitive markers of inflammation such as CRP (Kshirsagar et al., 2008, Festa et al., 2000), in my analysis I found that frequently

requested tests such as complete blood count (eosinophils) and ESR could serve as a useful tool for alerting the clinician about subclinical inflammation in those with microalbuminuria.

Several inflammatory markers that have been linked to cardiovascular morbidity and mortality (such as interleukin-18) were strongly correlated with microalbuminuria in patients with diabetes mellitus (Blankenberg et al., 2002, Nakamura et al., 2005). In a small cohort of non-diabetic subjects with untreated hypertension, albumin excretion was correlated with hs-CRP, interleukin-18 and soluble CD40 ligand (an atherogenic mediator) (Tsioufis et al., 2006). ACR was significantly correlated with hs-CRP, while interleukin-18 and CD40 ligand were not. However, the study is limited by the small sample size (n=118) and the selection criteria (only males, smokers and those with obesity were excluded). Another study showed a strong association between microalbuminuria and asymmetric dimethylarginine (another atherogenic mediator) in subjects with hypertension, but without diabetes and other cardiovascular diseases, limiting the generalisability of the results (Tsioufis et al., 2010).

The prevalence of a family history of cardiovascular or metabolic diseases was more often found in subjects with microalbuminuria, although the association was not significant. There was no difference between the groups in terms of the proportion of current smokers. This finding differs from earlier findings that reported a positive association between cigarette smoking and the prevalence of microalbuminuria (Ukena et al., 2010, Pinto-Sietsma et al., 2000). The low number of current smokers in my cohort might limit the ability to detect the association between UAE and smoking. In addition, it is possible that a proportion of the participating subjects had stopped smoking recently, and therefore any association would be weakened.

Target end-organ damage manifested by LVH was strongly prevalent in those with microalbuminuria. Even those with low grade albuminuria had higher prevalence of LVH than in individuals with normal UAE. A close association between albuminuria (microalbuminuria and gross proteinuria) and LVH on ECG was reported in the LIFE study (Wachtell et al., 2002). In that study, 8,029 subjects with hypertension and LVH evident on ECG were included. After 14 days of treatment with a placebo, another ECG was obtained, along with morning urine specimens for ACR measurement. Based on the second ECG, 78% of the cohort had persisting LVH. Microalbuminuria was strongly associated with LVH in those with LVH on the second ECG, more so than those who became negative. The association was evident even after controlling for possible confounders such as blood pressure, age, gender, serum creatinine and diabetes. Despite this, the LIFE study had a number of limitations. For instance, microalbuminuria was detected by a single sample without investigating for evidence of UTI in the specimen. This might overestimate the microalbuminuria prevalence. Selection bias also affected the magnitude of the association, as patients were selected based on the presence of LVH in the first ECG, and only 22% had no evidence on the second ECG. In other words, it would be better if the association had been reported in a cohort of general hypertension subjects with a normal cardiovascular disease distribution. In my analysis, the association was seen in general population with limited inclusion/exclusion criteria and after confirming the presence of microalbuminuria.

Subjects with microalbuminuria by the new definition had a high prevalence of LVH. Reffelmann et al. (Reffelmann et al., 2010) investigated the clinical utility of microalbuminuria in predicting future changes in left ventricular mass in a cohort of 1,086 subjects from the general population. They found that levels of UAE starting from 0.5 mg/mmol were a strong predictor of increased left ventricular mass over the next 5 years. The

association was stronger in sub-groups with hypertension than in those without hypertension. As mentioned earlier, single urine specimen was used for diagnosing albuminuria in the study by Reffelmann et al. (2010) which may limit their finding. Also, they investigated the predictive value of low albuminuria for subsequent ventricular changes while I reported increased LVH in those with the new definition of microalbuminuria. The finding presented in this study along with other studies emphasise the importance of early identification of people with low albumin levels in their urine, in order to implement more rigorous control of blood pressure to prevent target organ damage.

The presence of ST-T segment changes on ECG was greater in subjects with microalbuminuria than in those with normal UAE. Again, a graded increased prevalence of ST-T changes was noted. Few studies have addressed the association between microalbuminuria and ECG abnormalities (Jafar et al., 2009, Sciarretta et al., 2009) and these are confounded by selection criteria of studied population and albuminuria measurement. More importantly, the association between albuminuria and ECG abnormalities seems to predict increased risk for all-cause and cardiovascular mortality (Diercks et al., 2002). In addition, the association of ECG strain patterns with heart failure was reported in the LIFE study were combination of ECG strain patterns and albuminuria was associated with a 10% increased risk of developing heart failure, compared with 8% and 5% in those with an ECG strain alone or albuminuria, respectively (Okin et al., 2008). It should be noted that an ECG strain pattern itself is known to be a powerful risk factor for cardiovascular morbidity and mortality (Okin et al., 2004). Therefore, subjects with a combination of hypertension, microalbuminuria and ECG strain patterns should be considered as a very high risk population, and strict medical care should be followed.

In my study, minor nonspecific ST-T changes were not excluded. This is because there is evidence that this type of ECG abnormalities may predict future cardiovascular morbidities (Kumar and Lloyd-Jones, 2007, Kumar et al., 2008). For instance, a study involving 1,970 patients with hypertension who were followed for 9 years, demonstrated that minor ST-T changes on ECG were independent predictors of future CHD (Schillaci et al., 2004).

Subjects with microalbuminuria had a higher prevalence of established cardiovascular disease than those with normal UAE. The prevalence in subjects with the new definition of microalbuminuria is also high. Several epidemiological studies suggested links between microalbuminuria and cardiovascular diseases but rarely excluded those with diabetes mellitus or focused on high risk population (Wachtell et al., 2003, Cao et al., 2006, Jackson et al., 2011). Microalbuminuria has been also shown to predict future cardiovascular events (Lee et al., 2010, Ärnlöv et al., 2005). The presence of increased UAE in those with established cardiovascular disease suggests continuous vascular dysfunction. This could be translated into future cardiovascular events. Therefore, microalbuminuria might be used as a therapeutic target in those subjects.

The use of cardiovascular and metabolic drugs in subjects with microalbuminuria by both definitions was higher than in those with normoalbuminuria. This finding suggests that more drugs were required for controlling risk factors, especially blood pressure. This was supported by the observation that drugs that are usually prescribed in the management of resistant hypertension (such as alpha blockers) were more common in subjects with microalbuminuria than in those with normoalbuminuria. Microalbuminuria was common finding in high risk group who were taking multiple drugs. Many of drugs that are usually used in cardiovascular complications such as lipid lowering drugs, anticoagulants and vasodilator were taken by large

proportion of subjects with microalbuminuria even at lower levels reinforcing increased prevalence of cardiovascular disease.

8.6 Cardiovascular risk scores

I have shown that people with microalbuminuria by the two definitions are associated with complex co-morbidities and risk factors. However, whether such an association could be translated into future events can only be confirmed by following up those patients for several years, and comparing new events in microalbuminuric and normoalbuminuric individuals. As this approach is time-consuming and not feasible in the short term, I have utilised the Framingham equation and the modified equation applied by the JBS for estimating 10-year risk based on the available parameters. The Framingham equation is among the most commonly used equations in clinical practice for risk stratification, and has been shown to be valid in northern European populations (Haq et al., 1999).

The risk of overall cardiovascular and cause-specific outcomes tended to be higher in subjects with microalbuminuria. The same trend was observed using the JBS calculation of overall cardiovascular risk. However, the trend was not significant in the age-restricted group (30-74 years), probably because of the small number of patients included in the calculations. Two studies reported similar increase of risk scores with microalbuminuria but were limited by the small sample size (Asselbergs et al., 2004) and both involved the general population (Ärnlöv et al., 2005). The median risk score for overall cardiovascular disease in both definitions of microalbuminuria using the Framingham equation is intermediate. This indicates that the current conventional risk factors used in the Framingham equation might not completely

identify people at increased risk for subsequent cardiovascular events. Screening for microalbuminuria could offer additional information beyond that provided by traditional risk factors. This needs to be confirmed prospective studies with long follow up duration.

8.7 Multivariate analyses

Several classical risk factors for increased UAE were independent predictors, including increasing age, SBP, eosinophil count, serum creatinine and LVH. Two parameters were highly correlated with microalbuminuria; fasting triglycerides and anticoagulants. The probability of microalbuminuria by the new definition increases by 67% for every unit increase in fasting triglycerides (mmol/l). Such an independent association has been reported in a few studies involving patients with diabetes (Franciosi et al., 2007, Smulders et al., 1997). An Asian study reported similar finding in subjects with hypertension but without diabetes mellitus (Nishijo et al., 1999). However, participants were restricted to men and sample size was small (n=245).

There is no clear explanation of the strong association between triglycerides and microalbuminuria by the new and the combined definition. A high level of triglycerides is one of the components of the metabolic / insulin resistance syndrome (Bulhões and Araújo, 2007). This syndrome has been demonstrated to precede the onset of albuminuria in non-diabetic individuals (Fujikawa et al., 2001). Such changes could be more prevalent in those with microalbuminuria by the new definition. The other possible mechanism that may contribute to the high level of triglycerides is related to poor kidney function. The prevalence of high triglycerides levels in patients with CKD is about 50% (Miller et al., 2011). It has been

postulated that decreased renal function is associated with impaired catabolism of triglycerides, mediated by an incompletely understood mechanism (Attman and Samuelsson, 2009). In my study, those with microalbuminuria by the new definition were characterised by increased serum creatinine and low eGFR, and therefore hypertriglyceridaemia mediated by renal deterioration cannot be ruled out.

Anticoagulant (mainly warfarin) was strongly associated with microalbuminuria by the conventional and the new definitions. In a hypertension population, these agents are usually prescribed to subjects suffering from atrial fibrillation. A strong association between microalbuminuria has been reported in subjects with atrial fibrillation and hypertension (Böhm et al., 2009) and in other high atrial fibrillation risk populations (McManus et al., 2009). Thus anticoagulant use in this study is probably a marker for atrial fibrillation. The strength of the association may be overestimated since numbers were small but the lower confidence limit support a clinically significant relationship.

The use of calcium channel blockers was also significant predictor of microalbuminuria. It has been postulated that dihydropyridine CCBs may worsen albuminuria if used as monotherapy (Monster et al., 2002, Ruggenenti et al., 1998). My observations cannot confirm a causal relationship. The relationship may reflect risk which led to CCBs being used but equally could be result of CCBs use.

Results from multivariate analysis demonstrated that microalbuminuria is an independent predictor for cardiovascular diseases and risk factors (LVH and ECG abnormalities). Although some of the associations were not significant, the odds ratios were close to unity with wide confidence intervals, suggesting that the number of cases included in the models was too small. In addition to the LIFE study that reported a close relationship between

microalbuminuria and LVH (Wachtell et al., 2002), Dell'omo et al. (Dell'omo et al., 2003) also reported an independent association between microalbuminuria and LVH in untreated subjects with hypertension but without diabetes mellitus, although the sample size was small (n=330) and population selected had many restrictions (e.g. Absence of cardiovascular diseases, males, and availability of some biochemical parameters). Data from the HyperGEN study reported such an association in normotensive subjects who were selected based on the availability of echocardiographic information (Djoussé et al., 2008). I have reported such an association in subjects who were under fairly extensive control of cardiovascular risk factors. Again, classifying subjects with increased albumin excretion as a high risk population, and introducing early intervention might be translated into overall cardiovascular risk reduction.

Increased blood pressure is among the most frequently reported pathologic risk factors for microalbuminuria. In the current study, I found that uncontrolled blood pressure was associated with high probability of microalbuminuria. I also observed a clear trend of increased risk of microalbuminuria associated with the severity of blood pressure. A relationship between office and ambulatory blood pressure (especially SBP) with albuminuria has been suggested in a population-based study (Cirillo et al., 1998) and in subjects with hypertension but without cardiovascular disease (Cerasola et al., 2010).

Recently, a sub-analysis of the ROADMAP study showed that tight control of blood pressure was associated with a lower incidence of microalbuminuria in patients with hypertension and diabetes mellitus (Menne et al., 2012). A reduction of blood pressure was associated with the regression of microalbuminuria in 1,657 patients with uncontrolled hypertension (defined as blood pressure >130/85 mm Hg in those diabetes and > 140/90 mm Hg in non-diabetic subjects) (de Alvaro et al., 2005). However, 105 hypertension clinics participated in this study

and microalbuminuria screening was performed according to the practice of each clinic. This means that the detection of microalbuminuria in this particular study was prone to measurement bias. This is supported by the observation that microalbuminuria was present in 62% of the participants, indicating overestimation of the condition.

8.8 Methodology comparison

Clinical guidelines recommend multiple urine samples for the diagnosis of microalbuminuria in order to avoid overestimation caused by confounders (National Collaborating Centre for Chronic Conditions, 2008). As mentioned earlier, the diagnosis of microalbuminuria in most of the studies involving subjects with hypertension and microalbuminuria has been based on a single measurement. Diagnosis based on a single measurement of microalbuminuria may lead to an overestimation of microalbuminuria prevalence which, in turn, leads to unnecessary intervention. On the other hand, if the performance of a single screening of microalbuminuria is as good as multiple testing, the current recommendation should be changed, as the present approach might delay appropriate intervention.

Over 40% of subjects who were positive at a first screening had normal UAE on subsequent measurements. Such a reduction occurred even after minimising confounders of urinary albumin excretion, such as excluding any sample with evidence of UTI. Moreover, those with normal UAE in subsequent samples had risk factors lower than those of individuals with persistent microalbuminuria.

The Third National Health and Nutrition Examination Survey that investigated the prevalence of reduced renal function in the US general population demonstrated that about 63% of

participating subjects had persistent albuminuria at the second measurement (Coresh et al., 2003). However, urine was randomly collected in this study and was based on general population. Very few studies have addressed the reproducibility of microalbuminuria screening in hypertension. James et al. (James et al., 1994) examined the reproducibility of two consecutive urine specimens in 64 elderly subjects with untreated hypertension (mean age 74 years). The study reported a high variability of albumin excretion. The importance of this study is that the variability occurred in a cohort of elderly people where the effect of potential confounders such as rigorous exercise, high protein intake and postural changes is unlikely to relevant. The study did not mention whether urine samples were tested for evidence of UTI, although the potential effect of this on variability is unlikely as the assessment of microalbuminuria was performed on two consecutive samples. Again the small sample size was a major limitation of this study.

Pugliese et al. (Pugliese et al., 2011) recently reported a good performance of single samples in identifying persistence microalbuminuria during 3-6 months follow-up in a cohort of patients with type II diabetes mellitus. However, this observation might not be applicable to individuals with hypertension due to the higher prevalence of albuminuria in patients with diabetes. My findings suggest that the current approach of recommending multiple samples should continue to minimise false-positive microalbuminuria especially in subjects with hypertension.

8.9 Blood pressure and albuminuria over time

I investigated the blood pressure changes over time and correlated these with definitions of microalbuminuria. A strong association between SBP (and pulse pressure) with microalbuminuria was reported in cross-sectional and longitudinal analyses. Those with microalbuminuria had higher blood pressure at first visit to the clinic and subsequently.

Although those subjects had a greater blood pressure reduction, the blood pressure was always higher than that in individuals with normal UAE. These finding indicate that subjects with microalbuminuria require more aggressive treatment in order to attain recommended blood pressure targets. Furthermore, the high pulse pressure in those subjects could reflect vascular stiffness and therefore, appropriate intervention should be implemented.

Isles et al. (Isles et al., 1986) examined the mortality rate in 3783 subjects with hypertension attending the GBPC. They reported that mortality was better correlated with achieved blood pressure rather than baseline blood pressure. Subsequent evidence showed a beneficial effect of optimal control of eventual blood pressure on cardiovascular outcomes (Estacio and Schrier, 1998, Hansson et al., 1998, Berl et al., 2005). This indicates that people with microalbuminuria may be at high risk for cardiovascular disease as the eventual blood pressure was higher than that in subjects with normoalbuminuria.

Baseline dipstick examination (at first visit) was correlated with the result of laboratory analysis of microalbuminuria. The use of urine strips might not be sensitive to detect low levels of albuminuria at baseline. However, a population-based study of 2321 subjects showed that a trace of proteinuria might be a useful tool of identifying individuals with microalbuminuria (Konta et al., 2007). Therefore, I examined whether changes in albumin excretion were associated with increased or decreased cardiovascular risk factors.

There was a clear trend for increased risk depending on the result of the two measurements of microalbuminuria. For example, those with positive result in the two measurements showed the highest values for cardiovascular risk while those with negative results in both analyses had the least risk. Also, regression from positive proteinuria was associated with risk factors lower than in those who developed microalbuminuria. These findings support the notion that

reduction in urinary protein excretion are associated with reduction in cardiovascular morbidities and mortalities (Zeeuw et al., 2004). My results are consistent with those of Ibsen et al. (Ibsen et al., 2005) who investigated the effect on albuminuria changes in subjects with hypertension and LVH. During 4.8 years follow-up, the use of antihypertensive agents was associated with significant reduction in primary end point (myocardial infarction, stroke and cardiovascular mortality).

8.10 Study strengths and limitations

A major strength of the current studies is that it involved people representing a typical hypertension population being followed up in secondary/ tertiary hypertension clinics. There were minimum restrictions in terms of population examined. They represented a wide range of age, hypertension severity and were mainly non-diabetics. The majority of studies that have tested the association between microalbuminuria and hypertension were mainly part of clinical trials or involved population at high risk or with many restrictions. Another strength of my study is that several measurements were obtained to minimise the variability of urinary albumin excretion such as using repeated urine samples and minimising the effect of confounders. Also the sample size is fairly large compared with that in previous studies.

My studies have also some limitations. First, the cross-sectional design of the study precludes confirmation of causal relationship. For example, CCBs use was a strong predictor of microalbuminuria but whether these agents cause microalbuminuria cannot be answered by the current analysis. Second, screening for microalbuminuria in the Aberdeen Hypertension Clinic was carried out using semi-quantitative urine strips. However, it has been shown that the use of semi-quantitative dipsticks in general population and in diabetes for detecting microalbuminuria resulted in correct classification of albuminuria groups compared with

laboratory analysis (Graziani et al., 2009). Moreover, data from the Aberdeen Hypertension clinic represented only 17% of the total sample size. Also, the classification of participants in my study was based on the mean of last two samples which were analysed using laboratory instruments in both centres.

8.11 Future work

The current series of studies could be a basis of a more comprehensive analysis. After classifying the participant to the different albuminuric groups, prospective follow up of each group is the logical approach. For instance, a follow up of people with normal UAE to determine the rate of new onset microalbuminuria and associated factors is one of the possible approaches. Similarly, the rate of progression to gross proteinuria or regression to normoalbuminuria would be of great interest. The predictive value of each definition of microalbuminuria for future cardiovascular events is another plausible analysis. Recording mortality rates in each group is achievable since the GBPC database is linked with the Registrar General of Scotland. Linkage of the records of the patients who participated in this series of studies with national morbidity and mortality statistics offers one approach with the potential to test the clinical relevance of my findings.

A second phase of this study would include evaluation of the influence of rigorous cardiovascular risk management including different anti-hypertensive drugs on microalbuminuria, blood pressure and other risk factors. Such a study could be the basis of in-depth analysis of antihypertensive agents. As I have explained earlier, there is an ongoing debate about whether certain pharmacological groups confer additional anti-proteinuric effects beyond that provided by blood pressure lowering. This could be addressed via a randomised trial involving individuals with microalbuminuria where drugs that have putative additional

anti-proteinuric effect (as claimed for RAAS blockers) should be compared with another pharmacological group. The trial might be designed so that the group with putative anti-proteinuria might achieve less good blood pressure in order to evaluate whether they could provide better control of albuminuria. Earlier attempts have yielded unreliable results because of failure to allow for blood pressure differences.

With a larger sample size and additional information, it would be informative to investigate whether the incorporation of microalbuminuria enhances the risk the cardiovascular risk assessment compared with classical risk factors.

9. Conclusion

Microalbuminuria is present in almost one-fifth of subjects with essential hypertension attending secondary/tertiary referral centres. Microalbuminuria serves as a tool for identifying individual at risk for cardiovascular complications. Even levels of albumin excretion in the urine lower than the conventional thresholds are associated with complex co-morbidities and risk factors. Therefore, there is a need for a new definition of microalbuminuria that takes into the account early increase in the albumin level. Screening of microalbuminuria should be confirmed in multiple samples and the diagnosis should continue to rely on the results of these samples rather. Several factors are associated with microalbuminuria risk. However, it seems that increased blood pressure is the predominant risk factors associated with microalbuminuria.

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