Panniyammakal, Jeemon (2013) *Studies of mortality risk predictors in hypertensive patients.* PhD thesis.

http://theses.gla.ac.uk/4031/

Studies of mortality risk predictors in hypertensive patients

by

Jeemon Panniyammakal

Institute of Cardiovascular and Medical Sciences
University of Glasgow

A thesis submitted for the degree of Doctor of
Philosophy in the School of Medicine, College of Medical,
Veterinary and Life Sciences of the
University of Glasgow

February 2013

Summary

Hypertension is a leading cause of death and disability worldwide. Despite effective treatment regimens the mortality among hypertensive subjects are much higher than that of normal individuals. Several studies have been carried out to identify prognostic factors that have predictive value for mortality in the general population. New biomarkers that are readily available and cost-effective are important in risk stratification and management of hypertension. While important prognostic information can be learned from routine blood tests that are often conducted in hypertension clinics, the usefulness of these markers in predicting survival are not studied in detail. The thesis aims to explain the relationship between such inexpensive and commonly available markers and survival in a hypertensive population.

The thesis is divided into five main results chapters (chapters 3 to 7) based on studies conducted to assess the independent role of blood pressure variability (BPV), haematocrit, serum phosphate, serum electrolytes and indices of liver dysfunction or injury in predicting mortality in hypertensive patients. The study settings (Glasgow Blood Pressure Clinic) provided an opportunity to examine these relationships in a treated hypertensive cohort of more than 15,000, predominantly white population, from the West of Scotland. The hypertension clinic database was linked with the electronic records of General Register Office for Scotland. This electronic linking allowed extraction of primary cause of death data (if patients died during the course of follow-up) according to the International Classification of Diseases, 10th Revision, Version for 2007 (ICD-10), codes. The type of mortality was ascertained (namely; ischaemic heart disease, stroke, cardiovascular, non-cardiovascular and all-cause) from the ICD-10 codes. The independent relationships between predictor variables of interests and mortality were estimated after employing appropriate survival models. The main study findings are summarised below.

Blood pressure variability and mortality: Long term average BPV is an independent predictor of mortality. Longitudinal changes in BPV also predict mortality independent of underlying mean BP. While sustained high variability increases mortality, sustained low variability decreases mortality in this hypertensive cohort. The findings indicate that BPV is likely a fundamental

physiologic trait and it is a marker of early mortality. Visit-to-visit BPV is an important prognostic indicator of long-term mortality, and physicians should be made aware that long term clinic BPV should not be disregarded as random fluctuation between visits.

Haematocrit and mortality: Haematocrit (Hct) is the proportion of blood volume occupied by red blood cells. It is associated with follow-up BP and is an independent predictor of mortality in the hypertensive population. There are distinct differences both in terms of the strength and magnitude of the association of Hct and mortality between men and women that have not previously been known. While Hct is associated with CV mortality in men ('U' shaped, non-linear), it is more closely associated with non-CV mortality in women ('U' shaped, non-linear). In the assessment and management of newly diagnosed hypertensive patients, Hct levels should be taken into consideration as an important risk predictor.

Serum phosphate and mortality: Inorganic phosphate is an important mineral that is directly linked to energy metabolism, bone mineralisation, signal transduction, storage and translation of genetic information and maintenance of lipid membrane structure. A positive linear association between serum phosphate and mortality is reported in the present study. Deprivation status, serum calcium and serum alkaline phosphatase levels do not attenuate the mortality risk associated with serum phosphate in men and women. While serum phosphate is associated with CV mortality in men, it is more closely associated with non-CV mortality in women.

Serum electrolytes and mortality: Electrolytes, especially sodium, chloride, potassium and bicarbonates, play a vital role in maintaining homeostasis within the human body. While the relationship with all-cause mortality is non-linear across the entire range of serum chloride, there is a linear increase in mortality with decrease in serum chloride level below 100 mEq/L. The relationship between serum chloride and mortality is independent of serum sodium and bicarbonate levels. While serum potassium shows a non-linear "U" shaped relationship with mortality, serum bicarbonate shows a positive linear association.

Indices of liver dysfunction or liver injury and mortality: Serum albumin, bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT) and alakaline phosphatase (ALP) are widely used markers of liver function or injury to liver cells. These markers of liver function or injury to liver cells independently predict mortality outcomes in the hypertensive population. While there is a linear association of both GGT and ALP with mortality outcomes, it is a more complex, non-linear and 'U' shaped association for AST. Both ALT and bilirubin show inverse linear association with mortality. Age and body mass index significantly influence the relationship between ALT and mortality.

Strengths and limitations: The strengths of the studies conducted as part of this thesis include; a large cohort of nearly 15,000 hypertensive adults, a real life clinical setting, 35 years of follow-up with median survival time of 32 years, the ability to link predictor variables with differing causes of mortality outcomes, and adjustment for several potential confounding factors. Exclusion of individuals without predictor variables assessed at baseline and the bias introduced by the missing covariates in the adjusted Cox-proportional hazard models are the major weaknesses.

Future recommendations: Although the above mentioned inexpensive markers predict mortality in hypertensive population, the mechanism involved in their association with mortality is not clear. Future studies are required to explain the missing links. Usefulness of inclusion of these markers in predicting mortality should be tested in an independent population.

Acknowledgement

I have received immense support from my family, friends, teachers, mentors and my PhD supervisors throughout the PhD period. I wish to express my sincere gratitude to all those who have supported me throughout my PhD journey.

It is almost impossible to name everyone and weigh their level of support. However, it would be unfair if I do not acknowledge the important contributions.

I improved my skills in epidemiology as I followed the PhD path. Dr Sandosh Padmanabhan and Prof Anna Dominiczak helped me to become a better epidemiologist during this journey. I am grateful to their support.

The support that I received from Prof Dorairaj Prabhakaran as guide, teacher and mentor is commendable. I am indebted to the guidance and support from Prof Nikhil Tandon, and Prof K Srinath Reddy. I am grateful to Dr Claire Haistie, Dr Manesh Stephen, Dr Manju Stephen, Dr Dimple Kondal and Mr Stephen Mathunny for their valuable inputs on my writing and analytical skills.

I am thankful to my colleagues at the Centre for Chronic Disease Control, New Delhi for providing valuable comments and their encouragement. I am grateful to the Wellcome Trust secretariat at the Public Health Foundation of India for their administrative and financial support.

I am indebted to Mr Jaison Mathew and his family in Glasgow and Jiji Joseph and his family in London, UK for extending their hospitality, warmth, and friendship during my stay in UK. I am fortunate to have several good friends and well-wishers. Their support during the PhD period was admirable.

I dedicate this work to my wife, our parents and our daughter Meryl Jee George. They supported me at each step, sacrificed many things and stood with me throughout this journey.

Above all, I received showers of blessings from the Lord, Almighty and he protected me at every stage.

List of contents

List of Tables

List of Figures

Publications during the PhD period

Prabhakaran D, **Jeemon P**. Should your family history of coronary heart disease scare you? Mt Sinai J Med. 2012 Nov-Dec;79(6):721-32.

Jeemon P, Prabhakaran D. Does uric acid qualify as an independent risk factor for cardiovascular mortality? Clinical Science. 2012 Oct 9. [Epub ahead of print].

Paul L, **Jeemon P (Joint first author)**, Hewitt J, McCallum L, Higgins P, Walters M, McClure J, Dawson J, Meredith P, Jones GC, Muir S, Dominiczak AF, Lowe G, McInnes GT, Padmanabhan S. Hematocrit predicts long-term mortality in a nonlinear and sex-specific manner in hypertensive adults. Hypertension. 2012 Sep;60(3):631-8.

Jeemon P, Prabhakaran D, Goenka S, Ramakrishnan L, Padmanabhan S, Huffman M, Joshi P, Sivasankaran S, Mohan BV, Ahmed F, Ramanathan M, Ahuja R, Sinha N, Thankappan KR, Reddy KS; Sentinel Surveillance in Industrial Populations Study Group. Impact of comprehensive cardiovascular risk reduction programme on risk factor clustering associated with elevated blood pressure in an Indian industrial population. Indian J Med Res. 2012 Apr;135(4):485-93.

Dhillon PK, **Jeemon P (Joint first author)**, Arora NK, Mathur P, Maskey M, Sukirna RD, Prabhakaran D. Status of epidemiology in the WHO South-East Asia region: burden of disease, determinants of health and epidemiological research, workforce and training capacity. Int J Epidemiol. 2012 Jun;41(3):847-860.

Manimunda SP, Benegal V, Sugunan AP, **Jeemon P**, Balakrishna N, Thennarasu K, Pandian D, Pesala KS. Tobacco use and nicotine dependency in a cross-sectional representative sample of 18,018 individuals in Andaman and Nicobar Islands, India. BMC Public Health. 2012 Jul 10;12(1):515.

Jeemon P, Prabhakaran D, Huffman MD, Ramakrishnan L, Goenka S, Thankappan KR, Mohan V, Joshi PP, Mohan BV, Ahmed F, Ramanathan M, Ahuja R, Chaturvedi V, Lloyd-Jones DM, Reddy KS; Sentinel Surveillance in Industrial Populations Study Group. Distribution of 10-year and lifetime predicted risk for

cardiovascular disease in the Indian Sentinel Surveillance Study population (cross-sectional survey results). BMJ Open. 2011 Apr 29;1(1):e000068.

Jeemon P, Pettigrew K, Sainsbury C, Prabhakaran D, Padmanabhan S. Implications of discoveries from genome-wide association studies in current cardiovascular practice. World J Cardiol. 2011 Jul 26;3(7):230-47.

Jeemon P. Socio-economic status and cardiovascular risk among Indians. Prev Med. 2011 Jun;52(6):471-2.

Jeemon P, Prabhakaran D, Ramakrishnan L, Gupta R, Ahmed F, Thankappan K, Kartha C, Chaturvedi V, Reddy K; Sentinel Surveillance in Industrial Populations Study Group. Association of high sensitive C-reactive protein (hsCRP) with established cardiovascular risk factors in the Indian population. Nutr Metab (Lond). 2011 Mar 28;8(1):19.

List of Abbreviations, Acronyms & Symbols

Δ	Change or Difference
≤	Less than or Equal to
≥	Greater than or Equal to
β	Beta (Regression coefficient)
$\bar{X}$	Mean
μ	Micro
μmol/L	Micromols Per Litre
AASK	African American Study of Kidney Diseases and Hypertension
ABPM	Ambulatory Blood Pressure Monitoring
ACCOMPLISH	Avoiding Cardiovascular Events through Combination-Therapy in Patients Living with Systolic Hypertension
ACEI	Angiotensin Converting Enzyme Inhibitor
ACTH	Adrenocorticotropic Hormone
ADMA	Assymmetric Dimethyl Argenine
ALDH2	Aldehyde Dehydrogenase 2 family
ALLHAT	Antihypertensive and Lipid-Lowering Treatment to-Prevent Heart Attack Trial
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ANBP2	Australian Comparative Outcome Trial of Angiotensin-converting enzyme inhibitor-and diuretic-based treatment of hypertension in the elderly
ANOVA	Analysis of Variance
ARB	Angiotensin II Receptor Blocker
ARIC	Atherosclerosis Risk in Communities
ARV	Average Real Variability
ASCOT-BPLA	Anglo-Scandinavian Cardiac Outcomes Trial Blood-Pressure Lowering Arm
AST	Aspartate Transaminase
AT1	Angiotensin II receptor, type 1
AT2	Angiotensin II receptor, type 2
ATP	Adenosine Tri-Phosphate
AUC	Area Under the Curve
Bili	Bilirubin
BIP	Bezafibrate Infection Prevention Study
BIRNH	Belgian Interuniversity Research on Nutrition and Health
BLL	Blood Lead Level
BMI	Body Mass Index
BNP	Brain Natriuretic Peptide
BP	Blood Pressure
BPV	Blood Pressure Variability
Ca	Calcium
CA	Calcium Antagonists
CAD	Coronary Artery Disease
CAPP	CAptopril Prevention Project
CARE	Cholesterol and Recurrent Events Study
CASTEL	CArdiovascular *STudy* in the ELderly

CHARM	Candesartan in Heart Failure: Assessment of-Reduction in Mortality and Morbidity
CHD	Coronary Heart Disease
CHF	Congestive Heart Failure
CI	Confidence Interval
CKD	Chronic Kidney Disease
Cl	Chloride
cm	Centimeters
CO2	Carbon Dioxide
CoeffV	Coefficient of Variation
CONVINCE	Controlled ONset Verapamil INvestigation of Cardiovascular Endpoints Trial
COPD	Chronic Obstructive Pulmonary Diseases
CV	Cardiovascular
CVD	Cardiovascular Disease
D_1	Dopamine Receptor
DALY	Disability-adjusted life year
DBP	Diastolic Blood Pressure
DBPtw	Time Weighted Diastolic Blood Pressure
DOPPS	Dialyses Outcome and Practice Pattern Study
ECF	Extracellular Fluid
eGFR	Estimated Glomerular Filtration Rate
eNaC	Epithelial Sodium Channel
eNOS	Endothelial Nitric Oxide Synthase
EOs	Endogenous Ouabain
EPIC-Norfolk	Norfolk Cohort of the European Prospective-Investigation into Cancer
ESRD	End Stage Renal Disease
ETA	Endothelin Alpha Receptor
ETB	Endothelin Beta Receptor
FGH	Fibroblast Growth Factor
FHS	Framingham Heart Study
g	Grams
GBD	Global Burden of Diseases
GBPC	Glasgow Blood Pressure Clinic
GEE	Generalized Estimating Equations
GFR	Glomerular Filtration Rate
GGT	Gamma Glutamyl Transpeptidase
GOF	Goodness-of-fit
H^+	Hydrogen
Hb	Haemoglobin
HCO3	Bicarbonate
Hct	Haematocrit
HCTZ	Hydrochlorothiazide
HR	Hazard ratio
HTN	Hypertension
HYVET	HYpertension in the Very Elderly Pilot Trial
ICD	International Classification of Diseases
IDH	Isolated Diastolic Hypertension
IHD	Ischaemic Heart Disease
IMT	Intima Media Thickness
INSIGHT	International Nifedipine GITS Study- Intervention as a Goal for Hypertension Treatment

INTERSALT	International study of Electrolyte Excretion and Blood-Pressure
INVEST	International Verapamil-Trandolapril Study
IQR	Inter Quartile Range
ISH	Isolated Systolic Hypertension
IU	International Units
JACD	Japanese Coronary Artery Disease Study
JNC	Joint National Council
K	Potassium
kg	Kilograms
KLoSHA	Korean Longitudinal Study of Health and Aging
K-M	Kaplan-Meir
KORA	The Cooperative Health Research in the Region of-Augsburg (*KORA*) *study*
LDL	Low Density Lipoprotein
LIFE	Losartan Intervention for Endpoint Reduction
log	Natural Logarithm
$\log_{10}$	Logarithm with Base 10
LVF	Left Ventricular Failure
LVH	Left Ventricular Hypertrophy
M	Median
MDRD	Modification of Diet in Renal Disease Study Group
mEq/L	Milliequivalents per Litre
mg	Miligrams
MI	Myocardial Infarction
MIDAS	Multicenter Isradipine Diuretic Atherosclerosis Study
mmHg	Milimeter of Mercury
mmol	Millimoles
mmol/L	Millimoles Per Litre
MORE	Multiple Outcome of Raloxifene Evaluation Trial
MRFIT	Multiple Risk Factor Intervention Trial
MSNA	Muscle Sympathetic Nerve Activity
Na	Sodium
NaCl	Sodium Chloride
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NAFLD	Non-alcoholic Fatty Liver Disease
NCX	Sodium Calcium Exchanger
NE	Norepinephrine
NHANES	National Health and Nutrition Examination Surveys
NHS	National Health Service
NICE	National Institute for Health and Clinical Excellence
NORDIL	NORdic DILtiazem study
OR	Odds Ratio
PAF	Population Attributable Fraction
PAMELA	Pressioni Arteriose Monitorate E Loro Associazioni
PGE2	Prostaglandin E2
PH	Proportional Hazard
PO4	Phosphate
PPARG	Peroxisome Proliferator Activated Receptor Gamma
PTH	Parathyroid Hormone
p-y	Person Years
QALY	Quality-adjusted Life Year
r	Spearman Correlation Coefficient

RAAS	Renin Angiotensin Aldosterone System
RAS	Renin Angiotensin System
RBC	Red Blood Cells
RCT	Randomised Controlled Trial
ROC	Receiver Operator Characteristics Curve
RR	Relative risk
SBP	Systolic Blood Pressure
SBPtw	Time Weighted Systolic Blood Pressure
SCOPE	Study on COgnition and Prognosis in the Elderly
SD	Standard Deviation
SIMD	Scottish Index of Multiple Deprivation
SNS	Sympathetic Nervous System
STATA	Data Analysis and Statistical Software
STOP2	The Second Swedish Trial in Old Patients with Hypertension
sVCAM	Soluble Vascular Cell Adhesion Molecule
TC	Total Cholesterol
TGF-α	Transforming Growth Factor Alpha
THIN	The Health Improvement Network
TIA	Transient Ischaemic Attack
TNF-α	Tumour Necrosis Factor Alpha
UGT1A1	Uridine Diphosphate-glucuronosyltranferase 1
UK	United Kingdom
UKPDS	The UK Prospective Diabetes Study
ULN	Upper Limit of Normal
UN	United Nations
USA	United States of America
VALUE	The Valsartan Antihypertensive Long-term Use-Evaluation
Vit.D	Vitamin D
VSM	Vascular Smooth Muscle
WHO	World Health Organization
WoRES	West of Scotland Research Ethics Services
WRI	Wave Reflection Index

Author's Declaration

I declare that the work presented in this thesis is, to the best of my knowledge and belief, original and my own work, unless specified otherwise in the text. The Glasgow Blood Pressure Clinic data are extracted with help from Dr Sandosh Padmanabhan. Use of the anonymised database for analyses is approved by the West of Scotland research ethics service (WoSRES) of the National Health Service (11/WS/0083). This work has not been submitted previously for a higher degree and was carried out under the supervision of Dr Sandosh Padmanabhan, Prof Dorairaj Prabhakaran and Prof Anna Dominiczak.

Jeemon Panniyammakal
February 2013

1   Introduction

1.1 Elevated blood pressure as a risk factor: Historical perspectives

Reverend Stephen Hales, an English clergy, is considered to be the first person to measure arterial blood pressure (BP) in horses in the first half of 18th century [1]. Sphygmomanometers were introduced in the late 19th century by Riva-Rocci and Korotkov [2]. Diastolic blood pressure (DBP) measurements were made possible after the vital description of sounds associated with the appearance of the pulse wave by Korotkov [3]. The Hawksley random zero sphygmomanometer was introduced later as an alternative to reduce the potential for digit preference associated with using the standard devices [4]. Later in the 20th century aneroid (mechanical) and electronic devises replaced the mercury manometer mainly because of the health hazards associated with mercury use and disposal [5]. Recently, ambulatory blood-pressure monitoring (ABPM) has been recognised as a superior option for the cost-effective diagnosis of hypertension in adults [6].

Richard Bright (1827) and Ludwig Traube (1856) described the association between "increased arterial pulse tension" with cardiac and renal conditions such as "dropsy" and "nephritis" [2,3]. High tension in the arterial system was recognised as a condition prior to the commencement of any kidney damage or appearance of albumin in urine in the late 19th century by Fred Mahomed [2,3]. Clifford Allbutt (1986) and Henri Huchard subsequently demonstrated that hypertension might occur without overt renal disease and might precede arteriosclerosis [5,7]. In the early 20th century Otto Frank named the condition "essential hypertension" which implied that the elevation of BP was a compensatory reaction to overcome ischaemia of the tissue caused by constricted arterioles [3,8]. In the tenth edition of Osler's medical text book in 1925, the normal systolic blood pressure (SBP) was described as 120-130 mmHg and 130-150 mmHg if over age 50 [9].

Insurance companies in the United States of America (USA) started measuring BP using sphygmomanometer in the early 20th century [5]. The first evidence of an

age related increase in BP, and the relationship between elevated BP and mortality were published by the Actuarial Society of USA in 1925 [10]. Subsequently, a series of reports by the Actuarial Society of USA documented a positive association between elevated BP and mortality [5 11 12]. A sharp increase in mortality associated with an incremental increase in BP was also documented in 1959 by the Build and Blood Pressure Studies [13]. Sir George Pickering in 1960 highlighted hypertension as a "quantitative trait" with a continuous positive relationship between BP over the whole range and mortality [14].

The main limitation of the Actuarial Society of USA data was that it represented only those individuals who applied for and who were issued a life insurance policy. Later epidemiological studies in the general population such as the large Multiple Risk Factor Intervention Trial (MRFIT) and data from Framingham Heart Study (FHS) corroborated and extended the basic conclusions of the Actuarial Society reports. The MRFIT cohort (1993), reported a graded and continuous influence of both systolic and diastolic BP on coronary heart disease (CHD) mortality and end-stage renal disease [15]. Later in the year 2001, the FHS investigators confirmed the incremental increase in mortality associated with increase in BP even within the non-hypertension range [16]. A large meta-analysis in 2002, involving data for more than one million adults further strengthened the concept of a positive, graded relationship between BP and mortality [17]. This meta-analysis reported that there was no evidence of an abnormal BP threshold and the mortality increased progressively throughout the range of BP. Since BP is normally distributed in the population with the majority of individuals in the middle of the distribution and the risk operates across the continuum of BP, a large number of events arise from the 'moderate' middle of the distribution than from the 'high-risk' tail.

1.2 Evolution of hypertension definitions

Defining hypertension is extremely difficult due to the 'quantitative' relationship between BP and morbidity or mortality. The operational definition of hypertension is the level at which the benefits of action exceed those of inaction [18]. As discussed in the previous section, a significant proportion of the adverse cardiac outcomes and mortality occur in individuals in the middle of the BP distribution. Having said that, a threshold BP level along with the assessment

of the global cardiovascular risk may be useful in clinical practice for screening high risk patients and initiating beneficial therapy. For this very reason clinical guidelines for detection, evaluation and treatment of hypertension have been modified several times in the last few decades.

The first report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-1) in 1976 agreed DBP as the basis for detection and treatment of high BP. It was consistent with the prevailing belief that DBP contributes to greater cardiovascular (CV) risk than SBP and rising SBP is an inconsequential part of the aging process [19]. Although SBP was incorporated into BP staging as early as in 1984 (JNC III), it was used in the proper definition of hypertension for consideration of therapy only in the 5th JNC guidelines in 1993 [20-24]. A Clinical Advisory Statement issued in the year 2000 [25], after evaluating the accumulating evidence on the importance of SBP [26-29], recommended SBP as the principal measure for the detection, evaluation and the treatment of hypertension in both middle-aged and older adults. JNC-6 published in the year 1999 [30], and the subsequent guidelines from European [31][32] (guidelines Committee, 2003), World Health Organization-International Society of Hypertension [33] (WHO/ISH Writing Group, 2003) and the British Hypertension Societies [34][35] classified BP above 140 mmHg systolic and 90 mmHg diastolic as hypertension.   In 2003, JNC 7 [36-38] defined normal BP as SBP below 120 mmHg and DBP below 80 mmHg. While individuals with SBP 140-159 mmHg and/or DBP 90-99 mmHg were classified as stage 1 hypertension, those at or above SBP 160 mmHg and/or DBP 100 mmHg were classified as stage 2 hypertension. Thus, hypertension was defined as SBP 140 mmHg or greater and/or DBP 90 mmHg or greater. Furthermore, a new category named "pre-hypertension" was defined as SBP between 120 and 139 mmHg and DBP between 80 and 90 mmHg. Various JNC classifications since 1976 are shown in figure 1-1 and figure 1-2. The United Kingdom's National Institute for Health and Clinical Excellence (NICE) 2011 hypertension guideline document also recommends the same criteria for hypertension classification [39]. However, NICE recommends using ABPM or home blood pressure monitoring (HBPM) in patients with clinic BP $\geq$ 140/90 mmHg with no evidence of target organ damage to confirm the diagnosis of hypertension [39] and an ABPM/HBPM of <135/85 is classified as normal BP.
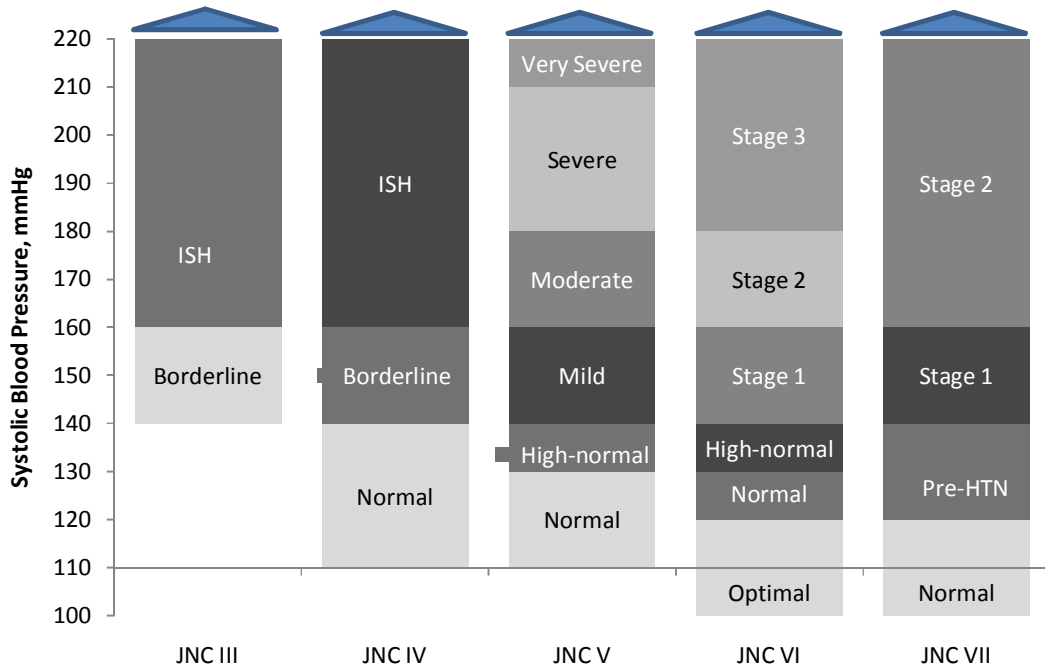
Figure 1-1: Joint National Committee (JNC) blood pressure classifications for systolic blood pressure.

Adapted from Giles 2003 [40]. ISH=Isolated Systolic Hypertension. Systolic blood pressure is not part of JNC I and JNC II staging.
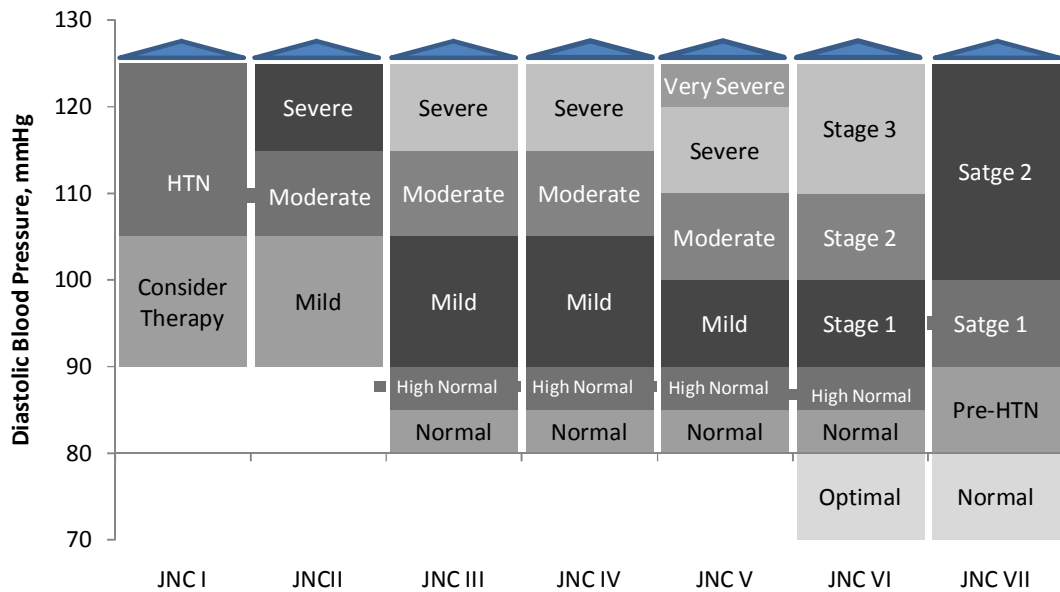
Figure 1-2: Joint National Committee (JNC) blood pressure classifications for diastolic blood pressure.

Adapted from Giles 2003 [40]. HTN=Hypertension.

Subtypes of hypertension include isolated systolic hypertension (ISH), isolated diastolic hypertension (IDH), white coat hypertension (elevated office BP but normal home BP) and masked hypertension (normal office BP but elevated mean arterial BP). ISH is the dominant form of hypertension in the elderly especially after the sixth decade of life [28] [41] [42]. White coat hypertension is defined as BP that, if measured by a physician or a nurse, is persistently > 140 mmHg systolic, > 90 mmHg diastolic, or both, whereas out-of-office BP is within the normal range (i.e., <125-130 mmHg systolic and <80 mmHg diastolic for 24-h mean BP, or <135/85 mmHg for home BP) [43]. The European Society of Hypertension and European Society of Cardiology guidelines often use the term "isolated clinic hypertension" for white-coat-hypertension. Masked hypertension was first described by Pickering [44] and it was defined as a clinic BP of <140/90 mmHg, and a 24-h or home BP value above normal values.

1.3 Historical perspectives in the pathogenesis of primary hypertension

In 1844 Richard Bright attributed hypertension to intrinsic renal disease [2]. Subsequently several others demonstrated that hypertension may occur without overt renal disease. Sir William Osler in 1912 misinterpreted hypertension as a purely mechanical affair, a necessity and discouraged early attempts to develop drugs to lower BP [45]. Later, Pickering [46] suggested that hypertension might simply reflect the right end of a normal Gaussian curve for BP in the population, and thus might not be a true 'disease'.

Goldblatt postulated that essential hypertension is primarily a disease of the pre-glomerular renal arterioles, leading to ischaemic renal injury and the release of some factors that would raise systemic vascular resistance and BP [47]. This concept was also supported by the observations of Perera who showed that renal arteriolar changes progressively became more severe depending on the magnitude of BP and duration [48]. Although elevated SBP is considered as an essential component of aging, Jim Henry in 1969 described several societies where age related change was negligible [49]. Henry correlated the annual rate of increase in BP with "social tension score" in different societies. Later, hypertension was considered as a disease of adaptation to stress [50] or exposure to Western civilization [51]. Page's "mosaic theory" [52] explains the aetiology of

hypertension where essential hypertension was designated as a disease of control or regulation that was influenced by many known and unknown factors.

Guyton postulated a theory of "hierarchy of pressure control systems" that provides both short-term damping and long-term control of arterial pressure and involves cardiovascular reflexes, capillary fluid shifts, vascular compliance and hormones [53]. Based on the relationship of plasma renin activity to 24-hour urine sodium excretion, Bunner et al [54] identified two forms of vasoconstriction in essential hypertension namely renin-angiotensin-mediated vasoconstriction (high renin) and a volume-mediated vasoconstriction (low renin). Neural mechanisms especially the evidence of an activated sympathetic nervous system (SNS) leading to increased heart rate, BP and plasma catecholamnes in response to stimuli that includes mental stress, exercise, and a cold pressor test are well documented [55-58].

Borst and Borst-De Geus [59] proposed that an acute rise in BP was accompanied by a reflex increase in urinary sodium excretion namely 'pressure natriuresis'. Guyton et al [60] further developed this concept using mathematical and computer modelling and suggested that most systems that would raise BP, such as activation of SNS, would result only in transient elevations and that permanent increase in BP would require a resetting of the pressure natriuresis curve.

Ecological associations of salt intake with BP led to the conclusion that salt intake explains the age-related increase in BP [61][62]. Insignificant increases of BP with age in certain societies as demonstrated in the INTERSALT study further strengthened the notion of a causal association of salt intake with BP [63]. However, in a study conducted in nuns living in Italy, there was a significantly lower increase in BP with age and relatively fewer cardiovascular events than their counterparts living in the surrounding villages despite the same 24-hour sodium excretion in both the groups [64][65]. In a study conducted in the Kuna Indians, initial residents of the San Blas Islands off the coast of Panama, the sodium excretion levels were similar in migrants and non-migrants at different levels of BP [66]. However, this study was grossly under powered to detect such differences. In general, migrant studies or ecological studies consistently show that salt intake is associated with increase in BP levels.

1.4 Mechanisms of primary hypertension

Blood pressure is the product of cardiac output and peripheral resistance. Changes in any of these two conditions may result in high BP. Hypertension can sustain persistently only in response to an increase in cardiac output or a rise in peripheral resistance. Thus defects may be present in one or more of the multiple factors that affect these two forces and lead to hypertension. Despite decades of clinical research, a specific cause for most cases of hypertension is not identified except in a minority of individuals who have secondary hypertension. Therefore the condition is often referred to as 'primary' in preference to 'essential' hypertension. The increase in BP is usually slow and gradual and therefore some of the initiating factors may no longer be apparent at the time of diagnosis of hypertension due to various compensatory mechanisms that reverse or normalise these factors. The peripheral artery resistance often seen in sustained hypertension is generally considered as a compensatory mechanism to adjust the raised BP caused by a raised cardiac output. Regardless of how hypertension begins, increased peripheral resistance ultimately becomes the haemodynamic pattern seen in sustained hypertension. Available evidence suggests a complex interplay of neural, renal, hormonal and vascular mechanisms (Table 1-1, Page 31) and they are discussed in detail in the following sections.

1.4.1 Neural mechanisms

In the early 1990's Julius and his team suggested that a 'hyperdynamic' circulation resulting from an adrenergically driven increase in cardiac output often resulted in elevated BP [67]. This elevated BP is sustained by subsequent vasoconstriction, vascular remodelling and autoregulation (autoregulation further increases vasoconstriction with an appropriately normal cardiac output). In animal models, overactivity of the sympathetic nervous system (SNS) often leads to elevated BP and it is believed to play a key role in the pathogenesis of hypertension [68]. However, the inhibitory reflexes arising from baro-receptors of the carotid sinus, carotid arch, heart and great veins evoke a reflex increase in efferent parasympathetic and decrease in efferent sympathetic activity. These reflexes lead to bradycardia and peripheral vasodilatation and often act as a

compensatory mechanism to control the increase in BP [68].   Excitatory neural reflexes from the activation of renal afferents by ischaemic metabolites and activation of sensory afferents of the muscles during exercise evoke reflex increases in BP and cardiac output [69]. Activation of the sympathetic nerves to the kidneys increases tubular sodium absorption, renin release and renal vascular resistance.

Catecholamines (epinephrine and nor-epinephrine) induce their effects via both β-adrenergic and α-adrenergic receptors. While the β-adrenergic stimulation of the heart increases ventricular contractility and heart rate, the α-adrenergic stimulation of the peripheral vasculature causes vasoconstriction, vascular remodelling and hypertrophy [69,70]. The renal nerve stimulates vasoconstriction via α_1 adrenergic receptors, renin release via β_1 adrenergic receptors, and enhances renal sodium and water re-absorption via α_1 adrenergic receptors [71].

The early stage of primary hypertension is associated with increased heart rate, cardiac output, plasma and urinary nor-epinephrine, regional nor-epinephrine spillover, decreased nor-epinephrine uptake, peripheral postganglionic sympathetic nerve firing and α-adrenergic receptor mediated vasoconstrictor tone in the peripheral circulation all of which indicate sympathetic overactivity [68,69,72,73]. However, the sympathetic overactivity is difficult to quantify in clinical settings and plasma norepinephrine levels are an insensitive measure of sympathetic overactivity. Invasive radiotracer measurements of regional norepineprine spillover suggest that early stages of primary hypertension are characterised by sympathetic activation targeted to the kidney, heart, and skeletal muscle vasculature [73]. The microneurographic measurements (a real-time measure of sympathetic nerve activity) of muscle sympathetic nerve activity (MSNA) show higher levels of MSNA in hypertensive subjects than normotensive individuals [68,74]. Increased MSNA alone does not cause hypertension as it is often accompanied by compensatory decreases in cardiac output and α-adrenergic receptor sensitivity to norepinephrine [75]. However, increased MSNA accompanied by impaired norepinephrine reuptake by sympathetic nerve terminals can lead to elevated BP and sustained hypertension [76].

Table 1-1: Main mechanisms involved in primary hypertension

Mechanisms	Mediators and specific actions
Neural	Adrenergically driven hyperdynamic circulation α and β adrenergic stimulation Vascular re-modelling and autoregulation Overactivity of sympathetic nervous system Inhibitory reflexes from baroreceptors of large vessels
Renal	Renoprival (Glomerular filtration rate dependent) Transport mechanism (re-absorption of sodium from renal tubules; action on eNaC) Renal ischaemia (Vaso-constriction, oxidative stress, inflammation) Low nephron numbers
Hormonal	Renin angiotensin system (Sodium handling, vascular remodelling, inflammation, oxidative stress, Sodium-Calcium exchange and increase in cystosolic calcium, pressure-natriuresis) Mineralocortocoid receptors (Sodium re-absorption, salt appetite, vascular contraction, endothelial dysfucntion, inflammation, vascular remodelling) Renal dopaminergic system (Pressure-natriuresis) Insulin Vasopressin Carditonic steroids
Additional vascular mechanisms	Increase in cytosolic calcium, vascular contractility, endothelial dysfunction, oxidative stress, eutrophic remodelling, hypertrophic remodelling Microvascular rarefaction
Other mechanisms	Obesity related hypertension Uric acid Androgens Haematocrit, whole blood viscosity and elevated plasma fibrinogen Nicotine, Caffeine, alcohol, high altitude, cold weather and air pollution. Vitamin D levels Blood lead levels

eNaC=epithelial sodium channel

1.4.2 Renal mechanisms

In general kidneys are considered to be both culprits and victims in hypertension. Johnson et al [77] propose three primary renal mechanisms in the aetiology of hypertension and they are; (a) glomerular filtration rate dependent (renoprival mechanism), (b) transport mechanisms (stimulation of sodium re-absorption in collecting duct), and (c) renal ischaemia (vasoconstriction, oxidative stress and inflammation).

There is a strong association between renal function and hypertension [47]. Hypertension develops rapidly in animals whose kidneys are removed (renoprival) or injured [78]. However, hypertension can occur even in the absence of clinically evident renal diseases. In humans, hypertension increases with worsening glomerular filtration rate and is often seen in individuals with mild renal dysfunction [79]. The reduction in GFR can lead to sodium retention and volume expansion. Since the tubular renal handling is impaired in renal dysfunction the excess sodium load due to reduced GFR cannot be compensated by reduced tubular re-absorption.

Renal vasoconstriction mainly in the afferent arteriole limits sodium excretion, alters the sodium balance and results in elevated BP [80,81]. The vasoconstriction is mediated by different mechanisms and involves hyperactive SNS, activation of the renin-angiotensin system, endothelial dysfunction with impaired release of nitric oxide, hypokalemia, oxidative stress, thromboxane, and by drugs such as cyclosporine [82-89]. In the long run the multiple episodes of renal vasoconstriction, particularly if coupled with local angiotensin II stimulation, can lead to renal arteriolar disease.

The inflammatory cells consisting of the interstitial T cells and macrophages infiltrate the renal vessels and produce oxidants and angiotensin II [89,90]. Intrarenal injury can also activate SNS [91], which further activates renal efferent nerves and augments renal vasoconstriction and facilitates sodium retention. Continued stimulation of sodium re-absorptive mechanisms leads to impaired pressure natriuresis and persistent hypertension [77].

Low nephron numbers commonly seen in low birth weight infants and those suffering from intra-uterine growth retardation leads to hypertension [92-94]. In

maternal malnutrition models in which rats pups are born with low nephron numbers, they develop preglomerular arteriolar disease and tubulointerstitial inflammation [95]. This suggests that low nephron numbers probably lead to hypertension because of its propensity to cause renal microvascular disease and inflammation. Young Caucasian individuals with essential hypertension have only half the number of nephrons compared with age-matched controls [96].

1.4.3 Role of sodium and potassium in hypertension

While a surplus of sodium (the main extracellular cation) increases arterial BP [97-100], mounting evidence indicates the role of potassium (the main intracellular cation) deficit in hypertension [99 101] and its cardiovascular complications. While exchangeable sodium (measured by the isotope dilution technique) is increased in hypertensive subjects and correlates positively with arterial BP [102], exchangeable potassium correlates negatively with arterial pressure [103 104]. Since human physiology is evolved in a low-sodium and high-potassium environment, we are currently under-equipped to manage the exposure of high sodium and low potassium diet [99]. Prospective data from the third NHANES suggest that a higher sodium potassium ratio is associated with significantly increased risk of CV and all-cause mortality [105 106].

In a landmark intervention study on chimpanzees, a high sodium diet increased BP rapidly and it was reversed after withdrawal of the sodium chloride supplement [107]. Interestingly BP rose in only seven out of ten chimpanzees potentially due to varying degrees of salt sensitivity. In dietary intervention studies in human beings, a low salt diet caused a moderate to significant decrease in BP both among normotensive and hypertensive subjects [108-111]. A high-salt diet, beyond raising BP, is an independent risk factor for target organ damage leading to fatal and non-fatal cardiovascular events that include stroke, aortic stiffness, cardiac hypertrophy, diastolic dysfunction and renal failure [112-114]. In a recent meta-analysis of the long term effects of interventions aimed at reducing dietary salt on mortality and cardiovascular morbidity, clinically significant impact on mortality or cardiovascular morbidity was not evident in both normotensive or hypertensive populations [115 116]. However, in a subsequent meta-analysis conducted after combining the event rates in both normotensive and hypertensive subjects, salt reduction was associated with significantly lower cardiovascular events [117]. Although it is probably not appropriate to combine the

studies in normotensive and hypertensive subjects (hypertensive subjects are highly selected group) as in the latter meta-analyses, inadequate sample size in the intervention studies may be the reason for the absence of clinically significant impact in the former meta-analyses. Based on BP response to salt reduction at the population level in the USA, the projected effect of dietary salt reduction on future deaths were estimated at the population level. Reducing the dietary salt by 3 g per day is projected to reduce mortality by 2-12% in all age groups, men and women and in blacks and non-blacks (Figure 1-3) [118].
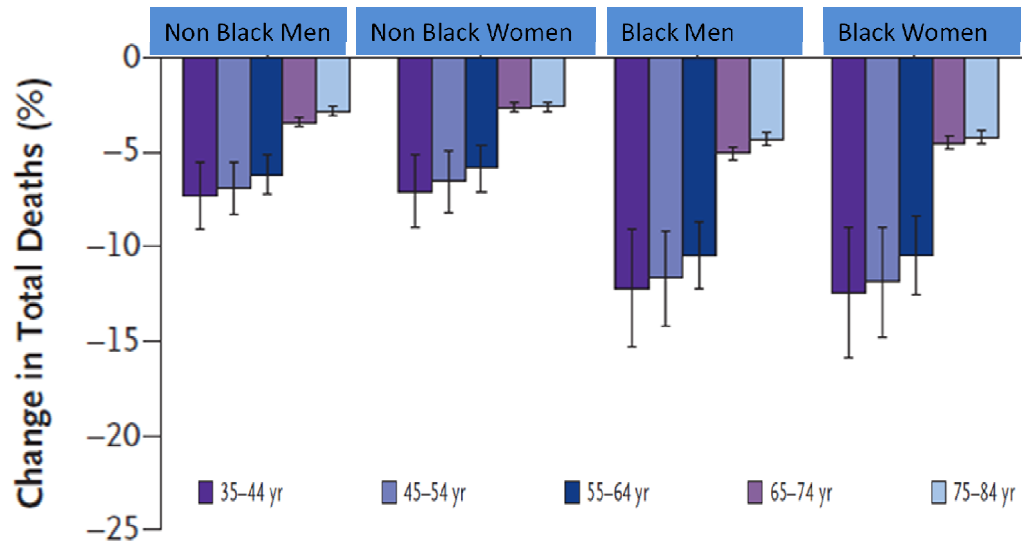
Figure 1-3: Projected annual reduction in mortality (best case scenario) given a dietary salt reduction of 3 g per day in the US population.

Adapted from Bibbins-Domingo et al 2010 [118].

A diet low in potassium (10 to 16 mmol/day) with the usual sodium intake (120-200 mmol/day) causes sodium retention and elevation of BP [119] [120]. The long-term effects of potassium depletion are to stimulate the activity and expression of the renal sodium pump that leads to sodium retention [121] [122]. In animal studies a high potassium diet even within the high sodium environment lowered BP and reduced the risk of stroke and stroke-related death [123]. A high potassium diet prevents cardiac hypertrophy, mesenteric vascular damage and renal injury [124]. A meta-analysis of intervention studies evaluating the effects of an increased potassium intake on BP in human beings concluded that potassium supplementation lowered BP both in hypertensive and normotensive subjects [125]. Potassium supplementation in hypertensive subjects can reduce the need for anti-hypertensive medication [126] and it exerts a powerful and dose-dependent inhibitory effect on sodium sensitivity [127].

How does sodium increases BP? There is no clear explanation on the mechanisms by which altered sodium levels influence BP. The potential mechanisms include expansion of plasma volume, increase in cardiac output, vasoconstriction, and vascular remodelling. Although increased cardiac output may initiate rises in BP, small vessel vasoconstriction and large-vessel stiffness are essential to sustain the elevated BP.

Rodriguez-Iturbe and his colleagues elaborate both volume-dependent and volume-independent mechanisms in salt-dependent hypertension [128]. Autoregulation and endogenous-ouabian-like compounds have been suggested to be involved in the volume-dependent pathway. Autoregulation is the process by which the small arteries constrict in response to an increased cardiac output. The resultant increase in cardiac after load returns cardiac output to normal but increases systemic vascular resistance and BP [59 67 129 130]. Endogenous ouabain-like inhibitors (EOs), an endogenous "digitalis-like factor" identical to ouabain or a stereoisomer of ouabian, are released from adrenal glomerulosa cells in response to salt retention and inhibit Na/K-ATPase in vascular smooth muscles and cardiac muscles [131-133]. The resultant increase in sodium flux drives the Na-Ca-exchanger (NCX) to increase cytosolic Ca^{2+} and enhances vasoconstriction, cardiac contractility and Ca^{2+} dependent cardiac and vascular hypertrophy [134]. Volume independent mechanisms include angiotensin-mediated CNS effects, increase in SNS activity, hypertrophy in cardiac myoblasts and contractility of vascular smooth muscles, increase in production of nuclear factor-κB (pro-inflammatory cytokines), increase in expression of angiotensin II type 1 receptor (AT_1) in renal tissue and increase in transforming growth factor ß production [128].

Re-absorption of the filtered sodium is enhanced in primary hypertension mainly because of the stimulation of several sodium transporters and the sodium pump. The activity of the sodium-hydrogen exchanger type 3 is enhanced in the proximal tubule and in the thick ascending limb of the loop of Henle in animal models with hypertension [135]. Potassium depletion further enhances sodium-hydrogen exchanger type 3 by inducing intracellular acidosis and by stimulating the SNS and the RAAS [136].

Pressure-natriuresis is the mechanism by which renal excretion of sodium and water increases when BP rises. This leads to shrinking fluid volume and return to normal BP in normotensive people [130 137]. While salt-resistant hypertension is

characterised by a shift in pressure-natriuresis curve towards the right, the salt sensitive hypertension is accompanied by an exaggerated increase in BP with increase in salt intake (changing the slope of the curve) [138] [139]. The renal function curve depicting the effect on mean arterial BP is shown in figure 1-4. The resetting of the pressure-natriuresis is mediated by changes in tubular sodium transport with unchanged glomerular filtration rate [137]. Renal medulla is the key site in which pressure-natriuresis occurs. An imbalance between an overactive RAAS and a defective nitric oxide (NO) pathway is involved in resetting of the pressure-natriuresis [140]. AT_1 receptors in the kidney stimulate renal medullary vasoconstriction and increase sodium re-absorption [140]. Studies have also demonstrated that angiotensin II alters pressure-natriuresis [139]. Angiotensin II triggers calcium signal in the pericytes of the descending vasa recta and promotes vasoconstriction. It also acts on the tubular epithelial cells of the thick ascending limb and releases NO. This offsets the angiotensin II dependent vasoconstriction. Any imbalance between the vasoconstrictor and the dilator factors (tubule-vascular crosswalk) can cause medullary ischaemia, impaired pressure-natriuresis and salt induced hypertension [140]. While AT_1 receptors promote sodium retention, AT_2 receptors promote natriuresis which is mediated partly by release of NO [141].
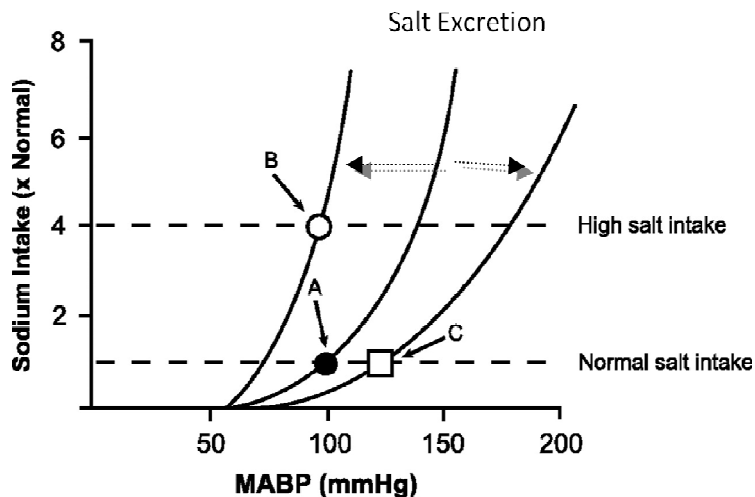
Figure 1-4: Renal function curve showing the effect of mean arterial blood pressure (MABP) on renal sodium excretion.

A: the equilibrium pressure that is maintained through adjustment in sodium balance. *B*: on sustained increases in salt intake, function curve shifts to the left to give a higher level of excretion at any given pressure. *C*: if these adjustments fail, curve shifts to the right so that a higher equilibrium pressure is required to match sodium output to input (**Adapted from Mullins et al 2006** [142])

Stimulation of dopamine receptors (D_1) enhances natriuresis and in animal studies the dopaminergic system explains part of the sodium excretion seen with salt loading [143]. Both the D_1 receptor-g-protein coupling defect and the decreased generation of dopamine could lead to renal sodium retention and hypertension [144]. Dopamine simultaneously inhibits Na/K-ATPase activity [145] [146] and the Na^+/H^+ exchanger [147] [148] within the renal tubule system. This results in decrease in sodium transport and re-absorption. A cytoskeletal protein named α-adducin modulates Na/K-ATPase activity in renal epithelial cells and the polymorphisms within the adducin gene are associated with high BP [149]. Furthermore in adducin dependent hypertension, the renal dopamine system is unable to inhibit Na/K-ATPase activity and thereby decrease renal sodium re-absorption [150].

Endothelin, a potent endothelium derived vasoconstrictor, causes natriuresis [151]. Endothelin is plentiful in the renal medulla and protects against salt induced hypertension. While the vasodilatory effect of endothelin is mediated by endothelin B receptor (ETB), the vasoconstrictor effect of endothelin is mediated by endothelin A receptor (ETA). A high salt diet influences endothelin expression in the renal medulla and changes the renal medullary blood flow via PGE2 and NO [152].

Renal inflammation is very often associated with renal medullary ischaemia and it is believed to be involved in the initiation and progression of experimental salt-sensitive hypertension [128]. However, whether it is a cause or effect of salt-sensitive hypertension or the renal medullary ischaemia is still not clear. Nocturia is very often a clinical sign of abnormal pressure-natriuresis and hypertensives have more nocturia that probably reflect the resetting of natriuresis [153]. It is suggested that the fluid retained peripherally during daytime can lead to central volume expansion at night. The resulting elevated nocturnal BP compels the pressure-natriuresis [154].

The extrarenal mechanisms involved in resetting the natriuresis and implicated in causing salt-sensitive hypertension involve activation of renal sympathetic nerves [71], α-melanocyte stimulating hormone which exacerbates salt-sensitive hypertension via stimulation of the central melanocortin system and activation of SNA [155], and dysfunction of the natriuretic peptides [156]. Interstitial tissue macrophages regulate lymphatic fluid drainage and salt-dependent volume by

affecting the vascular-endothelial growth-factor-C-dependent buffering mechanisms [157].

A homeostasis of sodium and potassium plays an important role in control of BP. In the following section the mechanisms by which potassium controls BP are explained. Potassium deficiency inhibits the sodium pump of arterial and arteriolar vascular smooth muscle cells and thereby increases the intracellular sodium [158]. Furthermore, hypokalemia inhibits potassium channels in the cell membrane and depolarises the membrane. This in turn activates voltage-dependent calcium channels in the membrane and in the sarcoplasmic reticulum and promotes the sodium calcium exchange [159] [160]. The excess cytosolic calcium increases the contraction of the vascular smooth muscles. By contrast, an increase in serum potassium hyperpolarises the endothelial cell, decreases the cytosolic calcium and promotes vasodilation [161] [162]. Furthermore, a potassium rich diet decreases cardiovascular risk by inhibiting arterial thrombosis, atherosclerosis, and medial hypertrophy of the arterial wall [163-165].

Potassium depletion depresses the baro-receptor sensitivity and thereby affects this compensatory mechanism in the control of increase in BP [166]. Furthermore, the decrease in baro-receptor sensitivity is restored when adequate potassium is supplemented. While potassium depletion inhibits insulin secretion, potassium infusion increases insulin secretion by changing the membrane potential of pancreatic beta cells [167] [168]. The increase in insulin promotes endothelium-dependent vasodilation in skeletal muscles by influencing the release of NO [169] [170].

1.4.4 Hormonal mechanisms

Multiple hormonal mechanisms including the renin-angiotensin system (RAS), mineralocorticoid and mineralocorticoid receptors, the renal dopaminergic system, insulin, vasopressin and carditonic steroids have been demonstrated to play a role in primary hypertension. The renin-angiotensin-aldosterone system (RAAS) activation is probably the most important hormonal mechanism contributing to increases in BP and hypertension [171]. The following section briefly describes these hormonal mechanisms in control of BP. Some of these hormonal

mechanisms are also involved in salt induced hypertension and they are described in the previous section (Section 1.4.3).

Renin produced by the renal juxtaglomerular cells cleaves angiotensinogen to angiotensin I and it is then converted to angiotensin II by angiotensin-converting enzyme (ACE). Chymase in the heart and systemic arteries also provides an alternative pathway for conversion of angiontensin I to angiotensin II. The interaction of angiontensin II with G protein coupled AT_1 receptors activates several cellular processes that contribute to elevated BP and hypertension [172].

The earliest pathophysiologic change seen in primary hypertension is probably the process of vascular remodelling. Vascular remodelling in primary hypertension is characterised by increases in the media to lumen ratio resulting from vascular smooth muscle (VSM) cell growth, apoptosis, elongation of cells, and altered composition of extracellular matrix [173]. Angiotensin II initiates vascular remodelling by stimulating both VSM cell hyperplasia and hypertrophy [174]. Furthermore, Angiotensin II is considered to be an inflammatory mediator in the vasculature and induces its effects by oxidative stress [175] [176]. Angiotensin II stimulates the formation of intracellular reactive oxygen species, including superoxide anion, hydrogen peroxide, hydroxyl free radical and peroxynitrite [177] [178]. Angiotensin II evokes enhanced vasoconstriction and in patients with primary hypertension, AT_1 receptor blockade reduces both BP and arterial resistance [179].

Harrison et al proposed a new mechanism that involves T-cell stimulation by angiotensin II and CNS activation in the pathogenesis of hypertension [180]. The activated T cells release tumour necrosis factor (TNFα) and other diverse inflammatory stimuli, further activates vascular and renal nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and leads to local production of reactive oxygen species, vasoconstriction and vascular remodelling [180]. The cellular level changes induced by Angiotensin II are also associated with many signal pathways, including tyrosine kinase, mitogen-activated protein kinase, RhoA/Rho kinase and increased generation of reactive oxygen species [181-188]. Interactions between these pathways are highly complex and dysregulation at any level could manifest as pathophysiologic vascular changes in hypertension. However, it is still not clear whether Angiotensin II plays a primary initiating role in hypertension or whether it simply mediates the vascular damage.

Kidney cross-transplantation experiments suggest that AT_1 receptors exclusively in the kidney regulate BP [189]. The proximal tubule AT_1 receptors are critical for the sodium retaining action of Angiotensin II and its influence on BP. While the inappropriate activation of intrarenal RAS prevents the kidney from maintaining normal sodium balance [190] [191], the inhibition of intrarenal RAS markedly reduces glomerular filtration rate, and sodium excretion [192] [193]. Furthermore, an inappropriately active intrarenal RAS causes renal vascular, glomerular and tubule-interstitial injury and fibrosis [194].

Aldosterone is produced in the adrenal zona glomerulosa in response to Angiotensin II, potassium and adrenocorticotropin (ACTH). Aldosterone promotes unidirectional sodium flux, and an increase in extracellular fluid volume that leads to elevated BP [195]. Interaction of aldosterone with mineralocorticoid receptors in the renal collecting duct cells recruits sodium channels from the cytosol to the surface of the renal epithelium. The epithelial sodium channels increase sodium absorption leading to expansion of plasma volume and increase in BP. Suppression of serum aldosterone increases endocytosis, de-phosporilate the epithelial membrane, diminish the epithelial sodium channels and sodium re-absorption [196]. In large epidemiological studies such as the Framingham study a 17% increase in risk of hypertension was observed for each quartile increase in plasma aldosterone [197]. While aldosterone influences the vascular contractility, it acts on the CNS and increases salt appetite [198]. Increased concentration of aldosterone in combination with high sodium intake induces myocardial interstitial fibrosis [199] [200]. Aldosterone induced oxidative stress, endothelial dysfunction, inflammation, vascular remodelling and fibrosis in the vasculature, heart and kidneys are mediated via the mineralocorticoid receptor and sodium [201-203]. Aldosterone also induces progressive renal and cerebrovascular damage [204] [205]. However, the adverse effects of aldosterone are only seen in the presence of a high-sodium diet [206].

1.4.5 Additional vascular mechanisms

Application of Poiseuille's law [207] suggests that BP is directly related to the first power of cardiac output and inversely proportional to the fourth power of blood vessel radius. In addition to changes in cardiac output, alterations in small changes in the diameter of both the small and large arteries therefore play an

important role in the pathogenesis of elevated BP and hypertension. The neural and hormonal mechanisms involved in the sodium calcium exchange (NCX) and the role of sodium and potassium intake in controlling the cytosolic calcium are described in detail in the previous sections. An increase in cytosolic calcium is the final common pathway that mediates contraction of vascular smooth muscles.

Endothelial cell dysfunction characterised by the impaired release of endothelial derived relaxing factors such as NO and enhanced release of endothelial derived constricting, proinflammatory, prothrombotic and growth factors (endothelin, thromboxane, and TGF-α) are involved in the pathogenesis of primary hypertension [208] [209]. Overproduction of superoxide anion and other reactive oxygen species (oxidative stress) activate signalling molecules that lead to cell growth, fibrosis inflammation and eventually vascular remodelling [208] [210]. While the RAAS mediated inward eutrophic remodelling initiated by vasoconstriction is the dominant mechanism in small arteries, hypertrophic remodelling mediated by SNS and angiotensin II with increase in size of vascular smooth muscle cells, accumulation of extracellular matrix proteins such as collagen and fibronectin due to activation of TGF-α is the dominant mechanism of remodelling in larger arteries [171].

Elevated BP and hypertension are commonly accompanied by microvascular rarefaction i.e., reduced number or combined length of small vessels in a given volume of tissue. Both functional rarefaction (decreased capillary recruitment during metabolic demand) mediated by reactive oxygen species and anatomic rarefaction (vascular smooth muscle cell death and vessel dropout) mediated by apoptosis are believed to be involved in the pathogensis of hypertension [211].

1.4.6 Other factors or mechanisms related to hypertension

Primary hypertension is multi-factorial and several distinct and related mechanisms are involved in its pathogenesis. Overweight and obesity in children appears to be the lead contributor to essential hypertension prevalence in children and adolescents [212] [213]. The mechanisms of obesity-related hypertension are discussed briefly in the following section.

Several mechanisms are involved in the characteristic haemodynamic pattern of volume expansion, increased cardiac output, and systemic vascular resistance seen in obesity related hypertension. Sympathetic overactivity [214-216], selective leptin release [217] [218], adipokines including leptin, free fatty acids and angiotensin II [219], RAAS overactivity, reactive oxygen species and NO deficiency [219], T cell activation [220] [221], and the overactivation of endocannabinoid pathway [222] are some of the mechanisms associated with obesity related hypertension. Obesity and associated increase in adipose tissue results in decrease in adiponectin levels and increase in leptin levels. The imbalance between the prohypertensive and antihypertensive adipokines mediates cell proliferation, endothelial dysfunction, inflammatory reaction, oxidative stress and thrombosis in the vascular structure [219]. Furthermore, the reduced insulin sensitivity often seen in obesity is directly associated with elevated BP and hypertension [219].

Large epidemiological studies suggest that increased uric acid levels predict the development of hypertension [223-225]. A one month treatment with allopurinol, the xanthine oxidase inhibitor, reduced BP in a randomised, double blind, and placebo controlled trial [226]. However, conclusive evidence supporting the use of allopurinol in reducing the risk of developing hypertension is lacking [227].

While androgens increase BP and risk of primary hypertension [228] [229], the impact of oestrogen on BP is not very clear [229]. Up-regulation of thromboxane A2 expression, NE, and angiontensin II are involved in the androgen mediated vasoconstriction and hypertension [228].

Various haemorheological factors are associated with elevated BP [230] and include increased haematocrit [231] and whole blood viscosity [232], elevated plasma fibrinogen [233] and plasminogen activator inhibitor, increased tissue plasminogen activator antigen levels [234], and T cell activation [180].

Nicotine in tobacco especially in smoking form acutely raises BP by stimulating the release of NE from sympathetic nerve terminals [235]. Nicotine in cigarette smoke also impairs NO dependent vasodilatation both by increasing oxidative stress and increasing the plasma levels of an endogenous NOS inhibitor asymmetric dimethyl arginine (ADMA) [236]. However, the effect of each cigarette is transient and is over within 30 minutes. Although a vasoconstriction mediated by nicotine causes acute but transient increase in systolic BP, it is followed by a

decrease in BP as a consequence of depressant effects played chronically by nicotine itself. Nicotine along with carbon monoxide from the tobacco smoke may act directly on the arterial wall and in the long run causes structurally irreversible alterations and sustained hypertension [237].

Caffeine in coffee or other beverages acutely raises BP by blocking the vasodilatory adenosine receptors and also by increasing plasma NE [238]. Although the risk of hypertension did not vary with coffee consumption in the Nurses' Health Study, the risk increased steeply when caffeine was consumed in soft drinks [239]. While regular caffeine intake increases BP, when ingested through coffee, the BP effect of caffeine was small [240]. In a meta-analysis in hypertensive individuals, caffeine intake produced an acute increase in BP for ≥3 hours with no longer-term association between coffee consumption and increased BP [241]. Cytochrome P-450 metabolises caffeine in the liver in the human body and it is known that people carrying a polymorphism of the P-450 gene (CYP12A) are at excess risk for a future myocardial infarction if they are heavy coffee drinkers [242].

The relationship between alcohol consumption and BP levels is not very clear. While alcohol can raise BP due to increases in SNA, it can reduce BP due to vasodilatation [243]. In binge drinkers the risk of hypertension is relatively high due to sympathetic activation with each intervening period of alcohol withdrawal [244]. Interestingly, in large epidemiological studies a J-shaped relationship is observed between alcohol intake and risk of hypertension [245] [246]. However, the risk of hypertension was higher even in light drinkers among Japanese men [247]. While a J-shaped association was observed between alcohol intake and risk of hypertension in women in the Women's Health Study, a positive linear association was observed in men in the Physicians' Health Study [248]. A linear gene-dose effect with the gene encoding alcohol dehydrogenase (ALDH2) gene with no evidence of an initial J-climb is reported in Japanese men [243].

Sympathetic activation in cold weather [249] and high altitude [250] may raise BP. Cumulative exposure to decreasing ambient and apparent temperature may increase BP [251]. Decrease in outdoor temperature increases BP in both men and women [252]. Seasonal variation in BP correlates with outdoor temperature, with each 10 degree Celsius drop in outdoor temperature is associated with a 5.7mmHg increase in SBP in Chinese adults aged 30-79 years [253]. In a study of

ABPM measurements in more than six thousand patients, normal room temperature was associated with lower day-time BP and higher night-time BP [249].

Vitamin D deficiency is linked to hypertension and in many prospective studies 25-hydroxy-vitamin D_2 has been independently associated with an increased risk of hypertension [254-256]. However in a large randomised trial of over 36000 postmenopausal women, calcium and vitamin D supplements had no effect on BP or on the risk of developing hypertension [257].

While occupational lead exposure is associated with renal damage and hypertension [258], other epidemiological studies indicate a positive but modest association between blood lead levels with BP and incident hypertension [259]. In the NHANES 1999-2006, blood lead levels (BLL) were significantly correlated with higher SBP among black men and women, but not white or Mexican-American participants [260]. Cumulative environmental lead exposure in mothers is a predisposing factor to higher BP levels in female offspring [261].

Short-term exposure to air pollution rapidly increased DBP under experimental settings in normotensive subjects [262]. Long-term exposure to particulate matter increased arterial BP in a population-based study of individuals in the age group of 45-75 years [263]. The particulate matter activates the excitatory neural reflexes in the lungs and increases SNA. Furthermore, the smallest particles can also enter the systemic circulation, cause oxidative stress and vascular inflammation [264 265].

1.5 Natural history of primary hypertension

1.5.1 Tracking of blood pressure from childhood to adulthood

High BP in children is a long-term health problem and a systematic review in diverse populations showed strong evidence for BP tracking from childhood into adulthood [266]. In the Fels Longitudinal study, childhood BP levels predicted hypertension in adulthood [267]. Data on association of childhood BP with cardiovascular events in later adulthood are lacking. However, intermediate markers of target organ damage, such as left ventricular hypertrophy (LVH) [268-

[271], increased carotid intima-media thickness (cIMT) [272-274], and retinal vascular changes [275] are more common in children and adolescents with high BP.

1.5.2 Pre-hypertension

In the Framingham cohort, hypertension developed in 5%, 18% and 37% of individuals with baseline BP less than 120/80 mmHg, BP less than 130/85 mmHg and BP between 130-139 and/OR 85-89 mmHg, respectively over a four year interval [16]. Pre-hypertension was associated with increased left ventricular mass (LVM) and carotid intima-media thickness (cIMT) in adolescents and young adults [276]. Target organ damages in pre-hypertension in adults include left ventricular hypertrophy [277-279], coronary calcification [280], reduced coronary flow reserve [281], progression of coronary atherosclerosis [282], increases in IHD and stroke [283 284], poor cognitive function [285], retinal vascular changes [286], proteinuria [287 288], and renal arteriosclerosis [289].

1.5.3 Established hypertension

Long-term observations of hypertensive patients reveal premature deaths in this group of patients with an average of 15-20 years less than normal life expectancy [48]. Adolescent BP and BP tracking into young adulthood are associated with subclinical atherosclerosis [290]. Atherosclerosis leads to premature morbidity and mortality in hypertensive patients more often than in the general population. While hypertension doubles the risk for CHD, it triples the risk for congestive heart failure (CHF) [291]. Brain micro-bleeds are reported more often in individuals with hypertension [292]. Individuals with hypertension are at 3-4 times greater risk of stroke [293]. Left ventricular hypertrophy seen very often in hypertensive patients is strongly related to subsequent cardiovascular mortality [294 295].

1.5.4 Resistant hypertension

Resistant hypertension is defined as failure to achieve BP goal in patients who are adhering to full tolerated doses of an appropriate three-drug regimen that includes a diuretic [296 297]. In a retrospective cohort analysis conducted among over 0.2 million patients with incident hypertension, 1.9% developed resistant

hypertension during a median follow-up period of 1.7 years with the incidence rate of 0.7 cases per 100 person-years of follow-up [298]. While non-compliance with treatment is considered as the foremost reason for resistant hypertension, there are other reasons such as secondary hypertension, fluid retention resulting from kidney failure that influences hypertension control. Choice of anti-hypertensive therapy, baseline SBP and associated co-morbidities also influence development of resistant hypertension [299].

While comparing the clinical outcomes in hypertensive population, a retrospective electronic clinical records review found greater risk for CHD and congestive heart failure (CHF) in individuals with resistant hypertension in comparison to non-resistant hypertension [300]. There was no difference in total cardiovascular events between the two groups in this study. However in another retrospective cohort analysis, there was a 47% increase in risk (HR=1.47, 95% CI: 1.33-1.62) of cardiovascular events in patients with resistant hypertension [298].

1.5.5 Natural history of sub-types of hypertension

Subtypes of hypertension such as isolated systolic hypertension (ISH) and isolated diastolic hypertension (IDH) have different prognostic implications [41 301 302]. For example, individuals with IDH were 23 times more likely to develop systolic hypertension in comparison to individuals with optimum BP (<120/80 mmHg) during a ten year follow-up [302]. Furthermore, subjects with IDH at baseline have high likelihood of developing a cluster of features of increased cardiovascular risk such as insulin resistance and other metabolic abnormalities. Rise in SBP and widening of pulse pressure are typical changes associated with aging and associated with mortality outcomes in elderly patients with ISH [303]. ISH is associated with a 2.7 times greater risk of strokes than seen in individuals with normal blood pressure [304]. However, data on long-term natural history and the clinical importance of ISH and IDH in young individuals are scarce.

1.6 Global burden of hypertension

Hypertension is a significant global public health problem and remains as the most common risk factor for cardiovascular morbidity and mortality. Worldwide hypertension affects approximately 1 in 4 adult individuals which translates to

nearly one billion in absolute numbers in the year 2000 and this is expected to grow to >1.5 billion by 2025 [305]. While in higher income regions hypertension was predicted to grow by 70 million people from 2000 to 2025, it was >500 million in lower-income regions during the same period. The lifetime risk of hypertension is estimated to be approximately 90% among individuals who are non-hypertensive at 55-65 years and survived to age 80-85 years [306]. Demographic changes in populations, progress in economic development, globalisation, increase in food availability and reduction in physical activity are considered to be the major determinants of this rapid rise in hypertension [307].

Burden of hypertension using the most recent global, regional and country-wise data are summarised in the following section. The global burden of disease (GBD) estimates for year 2004 and 2008 are the principle data sources [308]. The disease and injury outcomes caused by hypertension are quantified in terms of deaths and disability-adjusted life year (DALYs) for 2004, as described in a recently released WHO report [309]. DALY is a time-based measure that combines years of life lost due to premature mortality and years of life lost due to time lived in states of less than full health [310]. For population estimates, the GBD 2004 uses the 2006 revision of the 2004 population estimates for WHO Member States prepared by the UN Population Division [311].

1.6.1 Prevalence of hypertension

Of the 189 WHO member countries with available data[312], age standardised prevalence of hypertension in men aged 25 years or more was lowest and highest in United States of America (17.0%) and Niger (50.3%), respectively.   Consistent with the mean SBP levels, the prevalence of hypertension in women aged 25 years or more was lowest in the Republic of Korea (13.1%) and highest in Sao Tome and Principe (42.4%). The age standardised hypertension prevalences for the year 2008 in all WHO member countries are summarised in figure 1-5.

There are distinct regional variations in prevalence of hypertension. Although the average BP is decreasing worldwide since 1980 at the rate of 1mmHg SBP per decade, the change in BP is different across regions and countries in a global analysis of data covering 5.4 million participants [313]. However, the number of people with uncontrolled hypertension increased from 605 million (537-680

million) in 1980, to 978 million (921-1040 million) in 2008 [313]. The increase in hypertension prevalence is mainly attributable to increase in average SBP in the African, and South East Asian regions. SBP is currently highest in the low-income and middle-income countries [313].

1.6.2 Mortality and morbidity attributable to high blood pressure

Overall high BP is responsible for 7.6 million premature deaths (13.5% of all deaths) and 92 million disability adjusted life years (DALYs) lost (6% of all) worldwide, annually [293]. Furthermore, 54% of all stroke and nearly half (47%) of all ischaemic heart disease (IHD) were attributable to non-optimal BP [293]. Of the 7.6 million deaths attributable to high BP worldwide, 4 million were in women. While the high BP associated population attributable fraction (PAF) of total mortality worldwide was nearly 12.8% (highest among all other risk factors) [314], they were 11.4% and 14.3% in men and women respectively as per the WHO 2004 estimates. The revised estimates of mortality attributable to leading risk factors are summarised in figure 1-6.

High BP is associated with significant mortality in all regions of the world with the PAF ranging from 4.6% in the African region to 14.46% in the Western Pacific region. Although the mortality rates are falling in both hypertensive men and women in industrialised countries [315], hypertension still remains as a major contributor to overall mortality. Among the WHO regions, European region tops the chart with 2.5 million deaths attributable to high BP followed by Western Pacific (1.8 million deaths) and South East Asian Region (1.4 million). Low Income and Lower Middle Income countries contribute nearly 4.9 million deaths attributable to high BP. The region wise mortality statistics attributable to high BP are summarised in figure 1-7.

In terms of disability adjusted life years lost (DALYs), the overall PAF associated with high BP was 3.7% worldwide (3.9% and 3.6% in men and women, respectively). The DALYs lost due to high BP was highest in the European region followed by South East Asian region and Western Pacific Region (Figure 1-7). Nearly 70% of the total DALYs burden attributable to high BP was from Low Income and Lower Middle Income countries.
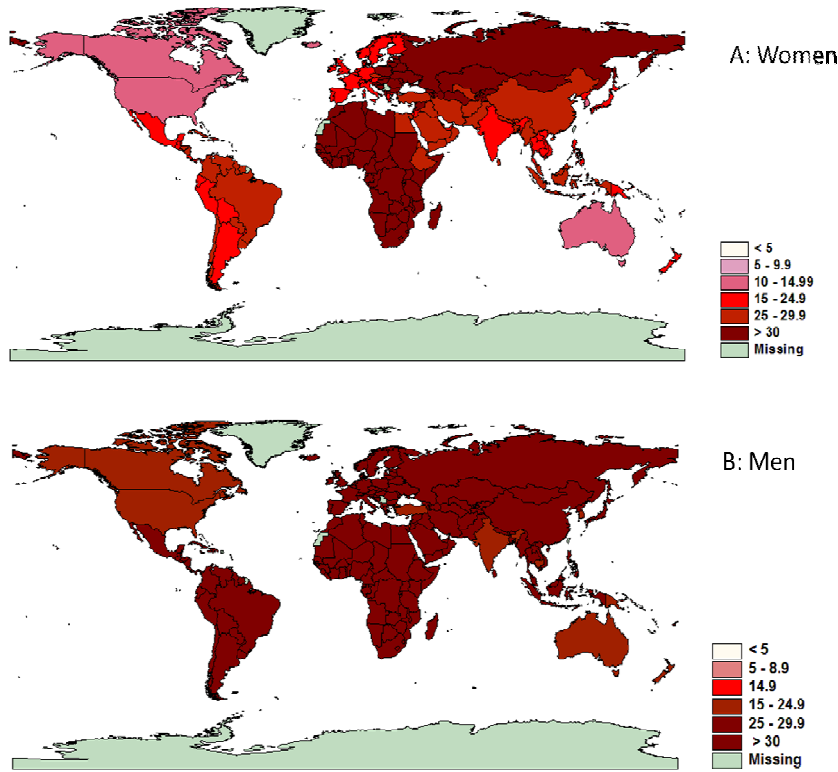
Figure 1-5: World map showing age standardised prevalence range of hypertension in women (Panel A) and men (Panel B).

Data source: WHO Global Burden of Disease database [312].
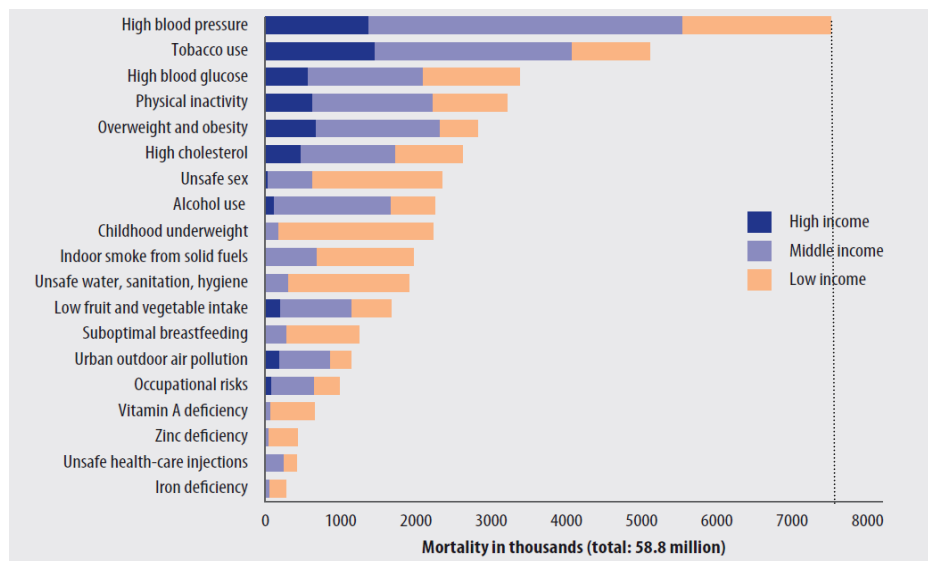
Figure 1-6: Mortality attributable to 19 leading risk factors, 2004 estimates.

Adapted from WHO publication [312]. Countries grouped into income regions by gross national income per capita; low income (USD 825 or less), middle income (USD 826-10065) and high income (USD 10066 or more).

Death attributable to high blood pressure (Numbers in 1000s)

DALYs attributable to high blood pressure (Numbers in 1000s)
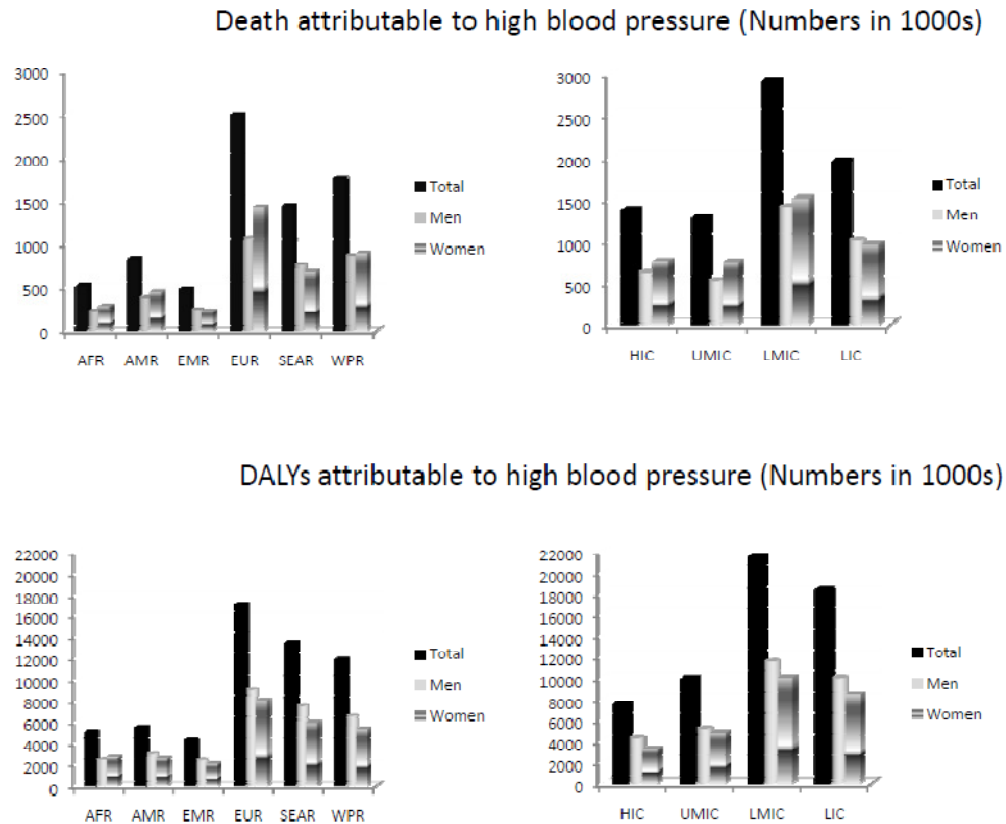
Figure 1-7: The region wise mortality and morbidity statistics attributable to high blood pressure (Numbers are in thousands).
AFR=African Region, AMR=American Region, EMR=Eastern Mediterranean Region, EUR=European Region, SEAR=South East Asian Region, and WPR=Western Pacific Region. HIC=High Income Countries, LIC=Low Income Countries, LMIC=Lower Middle Income Countries, and UMIC=Upper Middle Income Countries. Data source: WHO Global Burden of Disease database [312].

1.7 Predictors of adverse outcomes in hypertension

1.7.1 Blood pressure and mortality outcomes

Elevated BP as a leading factor associated with cardiovascular mortality and morbidity is well established [17]. In hypertensive individuals treated in clinical practice, SBP is a good predictor of CVD and coronary heart disease (CHD) [316 317]. A sustained SBP reduction of 12 mmHg over ten years in individuals with stage 1 hypertension and one additional cardiovascular risk factors is adequate to prevent 1 death for every 11 patients treated [318]. The latest United Kingdom National Institute for Health and Clinical Excellence (NICE)-2011 hypertension guideline recommends anti-hypertensive drug therapy to people under the age of 80 years with stage I hypertension (BP≥140/90 mmHg and <160/100 mmHg)

who have any of the following conditions; (a) target organ damage, (b) established CVD, (c) diabetes, (d) renal diseases and (e) a 10 year CHD risk equivalent of 20% or greater [319]. It also recommends pharmacological treatment for people at any age with stage II hypertension irrespective of accompanying risk conditions.

Randomised controlled trials of anti-hypertensive agents have unequivocally proved the causal role of elevated BP on CVD events and mortality outcomes [320]. However, sub-optimal adherence to anti-hypertensive therapy and poor BP control are observed in a substantial proportion of patients with hypertension [321] [322]. As per the latest available estimates in the Unites States of America (USA), approximately 30% of adults are unaware of their hypertension, >40% of individuals with hypertension are not on treatment and two-thirds of treated hypertensive patients are not being controlled to the desired BP levels of <140/90 mmHg [36]. The corresponding rates in England are 34%, 46% and 48%, respectively [323]. The awareness, treatment and control rates of hypertension are even lower in middle income and lower middle income countries [324].

In general, lower BP values are associated with better clinical outcomes in treated hypertensive patients and all major anti-hypertensive classes can effectively lower BP. Effects of antihypertensive treatment on total mortality in trials comparing new (CCBs, ACEIs, ARBs and alpha blockers) with old (diuretics and β blockers) antihypertensive drugs show no differences in outcomes between the drug groups (Figure 1-8) [325]. Similarly, the Blood Pressure Lowering Treatment Trialists' collaborative group's assessment on the comparative effects of different BP-lowering regimens suggests no major difference between drug classes in terms of cardiovascular prevention [326] [327]. Tolerability of anti-hypertensive therapy influences discontinuation of treatment during follow-up and hence treatment outcomes. While ARBs have the lowest discontinuation rate, it is highest in diuretics and beta blockers. Alpha blockers and calcium channel blockers also report significantly higher rate of discontinuation in comparison to ACEI [328]. In general, 50% of patients with hypertension do not take all of their prescribed medications due to various reasons [329]. Poor adherence to BP medications can lead to inadequate BP control [330] and adverse clinical outcomes.
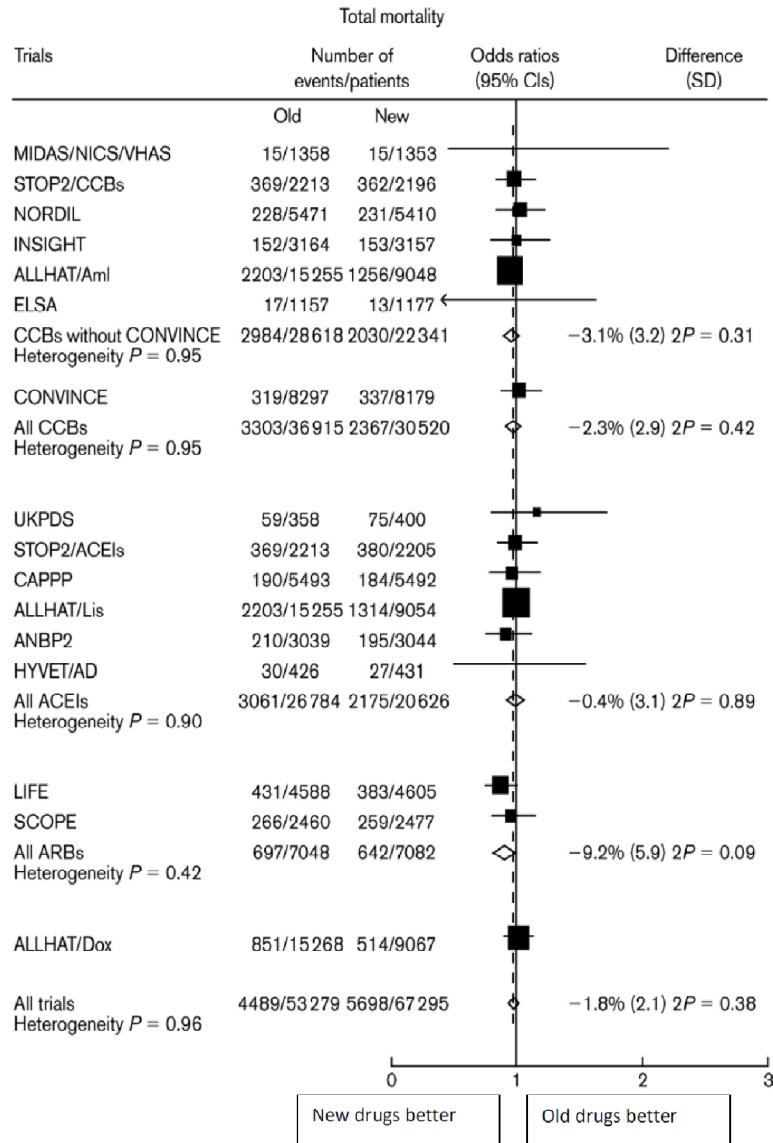
Figure 1-8: Effects of antihypertensive treatment on all-cause mortality in trials comparing new with old antihypertensive drugs.

Solid squares represent the odds ratios in trials and have a size proportional to the number of events. The 95% confidence intervals for individual trials are denoted by lines and those for the pooled odds ratios by diamonds. Adapted from Staessen et al 2003 [325]. **ALLHAT/Aml** (Antihypertensive and Lipid-Lowering treatment to prevent Heart Attack Trial – amlodipine versus chlorthalidone), **ALLHAT/Dox** (Antihypertensive and Lipid-Lowering treatment to prevent Heart Attack Trial – doxazosin versus chlorthalidone), **ALLHAT/Lis** (Antihypertensive and Lipid-Lowering treatment to prevent Heart Attack Trial – lisinopril versus chlorthalidone), **LIFE** (Losartan Intervention For Endpoint reduction in hypertension study), **MIDAS** (Multicenter Isradipine Diuretic Atherosclerosis Study), **STOP2/ACEIs** (angiotensin-converting-enzyme inhibitor arm of STOP2), **STOP2/CCBs** (calcium-channel blocker arm of STOP2), **NORDIL** (NOrdic DILtiazem study), **INSIGHT** (International Nifedipine GITS Study – Intervention as a Goal for Hypertension Treatment), **ELSA** (European Lacidipine Study on Atherosclerosis), **CONVINCE** (Controlled ONset Verapamil INvestigation of Cardiovascular Endpoints Trial), **STOP2/ACEIs** (angiotensin-converting-enzyme inhibitor arm of STOP2), **CAPPP** (CAptopril Prevention Project), **ANBP2** (Australian comparative outcome trial of angiotensin-converting enzyme inhibitor- and diuretic-based treatment of hypertension in the elderly), **HYVET/AD** (HYpertension in the Very Elderly pilot trial – ACE inhibition versus diuretic treatment), **SCOPE** (Study on COgnition and Prognosis in the Elderly).

Recent pooled data suggest that various classes of anti-hypertensive agents are not equivalent in their ability to protect against target organ damages and adverse mortality and morbidity outcomes [331]. Although all antihypertensive drug classes reduce cardiovascular disease morbidity and mortality, clinical trials in the last two decades demonstrate a class effect especially in specific baseline demographic and clinical conditions such as ethnicity, diabetes, left ventricular hypertrophy, heart failure, atrial fibrillation, previous myocardial infarction, stroke, peripheral artery diseases, proteinuria and renal dysfunction [331][332]. While renin angiontensin system (RAS) blockers are the preferred choice of drugs in hypertensive subjects with heart failure, microalbuminuria, renal dysfunction, end stage renal disease (ESRD), recurrent atrial fibrillation, metabolic syndrome and diabetes mellitus, calcium antagonists (CA) are the preferred choice of anti-hypertensive agents in asymptomatic atherosclerosis, peripheral artery disease, and pregnancy. Similarly, diuretics are the preferred choice of first line anti-hypertensive treatment in heart failure, isolated systolic hypertension in the elderly and among hypertensive blacks [331][332]. Heart failure is also a compelling indication for use of aldosterone antagonists especially among patients with severe left ventricular dysfunction [36][333]. Although, effective BP control can be achieved in almost all of hypertensive patients, majority of them will require two or more anti-hypertensive agents [36].

Despite similar BP control in the treatment arms of the losartan intervention for endpoint reduction (LIFE) study among patients with essential hypertension and LVH, losartan (angiotensin receptor blocker, ARB) was clearly superior to atenolol (beta blocker, BB) in reducing cardiovascular morbidity and mortality [334]. In the Anglo-Scandinavian cardiac outcomes (ASCOT-BPLA) trial the amlodipine-based regimen reduced the relative risk of cardiovascular events more effectively than the atenolol-based regimen in both older and younger patients [335]. However, the achieved BP difference alone could not explain the greater risk reduction in the amlodipine (CA) based regimen with added perindropil (ACEI) in comparison to the atenolol (BB) based regimen [336]. Better BP control was partially responsible for the lower incidence of fatal and non-fatal myocardial infarction in the amlodipine (CA) based regimen in comparison to the valsartan (ARB) based regimen in the valsartan antihypertensive long-term use evaluation (VALUE) trial [337]. More recent data suggest significant differences in outcomes even in combination therapies with different anti-hypertensive

agents. For example, the avoiding cardiovascular events through combination therapy in patients living with systolic hypertension (ACCOMPLISH) trial stopped prematurely due to favourable outcomes in the benazepril-amlodipine fixed dose regimen group in comparison to the benazepril-thiazide fixed dose regimen group [338]. Although the BP reduction was better in the ACEI-CA (ACE-inhibitor, Calcium antagonists) group in the ACCOMPLISH trial, the clinical benefit observed in this group was beyond the clinical benefits explained by BP reduction achieved. Even though there are different explanations for the disparity observed between anti-hypertensive drug classes in their ability to protect against target organ damage, none of them fully explain the clinical benefits observed in some anti-hypertensive classes in comparison to other agents.

The effect of anti-hypertensive agents on central aortic BP has been suggested as one of the mechanisms that influences outcomes. For example, in the conduit artery functional endpoint (CAFE) study central aortic BP was significantly lower in the amlodipine based arm than in the atenolol-thiazide arm [339]. The difference in central aortic BP between the treatments arms in the CAFÉ study partially explain the preference of amlodipine based regimen in reducing the composite outcome of total cardiovascular events, CV procedures and the development of renal impairment. In the ASCOT-BPLA trial sub-study, differences in magnitude of wave reflection index (WRI) account for differential effects of amlodipine and atenolol based regimens (lower carotid systolic BP in people randomized to amlodipine based regimen) on central BP [340]. Furthermore, higher WRI predicts future cardiovascular events independent of brachial BP and other conventional risk factors in the ASCOT-BPLA sub-study [341].

1.7.2 Blood pressure variability and mortality outcomes

Contrary to the normal BP hypothesis that extensively relies on effect of anti-hypertensive agents in reducing the mean office BP during follow-up, several other mechanisms influence the CV outcomes. In the Pressioni Arterios Monitorate e Loro Associazioni (PAMELA) study among the general adult population of Italy, the steepest increase in mortality rate was observed with 24-hour SBP measurement in comparison to risk associated with home and office SBP [342]. Increases in SBP during night-time and the mean difference between day-time and night-time DBP were associated with CVD mortality [343] [344]. Even a

morning surge in BP alone independently predicts stroke [345-347]. For example in the International Database on Ambulatory BP in relation to Cardiovascular Outcome (IDACO) study, a morning surge exceeding the 90th percentile of the population, was an independent risk factor for mortality and CV events [348]. The average of several readings from ABPM predicts risk of vascular events better than one-office based BP reading [349] [350]. Masked hypertension (normal office BP but raised BP at home or on ambulatory BP monitoring) is associated with increased risk of vascular events [351] [352]. The accumulating data suggest that out-of-office measures such as self-measured BP at home, ambulatory BP and central pressure are more closely associated with risk of target organ damage and adverse CVD outcomes than office-based BP [43] [353-356]. In the ASCOT-BPLA trial nocturnal ambulatory BP readings provide complimentary and incremental utility over clinic BP in the prediction of cardiovascular risk [357].

Visit-to-visit variability in BP is significantly associated with increase in risk of all-cause mortality [358]. Independent of mean SBP, visit-to-visit office SBP and maximum SBP predict future stroke events [359]. Anti-hypertensive drug classes like calcium antagonists (CA) and beta blockers (BB) have differential effects on BP variability which partially accounts for the disparity in observed effect on risk of stroke and the expected effect based on mean BP [360]. Treatments that affect the greatest reductions in BP variability (for example; CA) are associated with the greatest reductions in risk of stroke and coronary heart disease in the secondary analysis of data from major randomised controlled trials of antihypertensive treatment and of secondary stroke prevention [361]. The comparison of estimated relationship between usual BP and stroke risk with relations based on actual measured mean SBP across seven clinic visits in the pooled analysis of the UK and Dutch transient ischaemic attacks (TIA) trials suggests that the usual clinic BP substantially overestimates the risk of stroke among individuals with variable BP [362]. Effects of anti-hypertensive drugs on SBP variability are dose-dependent and even persist when used in combinations. High dose of a CA alone or in combination with other agents is therefore effective in prevention of stroke [363]. The International Verapamil SR-Trandolapril (INVEST) trial results suggest that consistent BP control (BP control in >75% follow-up visits) reduces the composite primary outcome of deaths, non-fatal MI and non-fatal stroke [342]. Although patients with consistently normal SBP have very few vascular events, those with high variability and normal mean SBP are at

increased risk. However, the results of post-hoc analyses can be considered as only hypothesis generating and prospective studies in real life settings are required to test these hypotheses. Therefore, hypertension treatment guidelines often ignore residual variability in clinic BP in treated hypertensive patients [362].

1.7.3 Magnitude of blood pressure reduction and cardiovascular risk

The magnitude of BP reduction achieved is an important determinant of favourable cardiovascular outcomes in hypertensive populations [326]. However, a recent systematic review concluded that treating to lower than standard DBP goals does not always reduce mortality or morbidity [364]. Moreover in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial, there was no significant reduction in CV events in the intensive treatment group targeting a SBP<120 mmHg compared to targeting <140mmHg [365]. Furthermore, serious adverse events were more common in the intensive treatment group. In patients with CHD lowering of DBP to <55 or 60 mmHg was associated with an increase in CV events, including myocardial infarction [366]. Consistent with the available evidence, the UK NICE 2011 hypertension guideline document does not recommend such intensive pharmacological treatment of hypertension [319].

1.7.4 Race, ethnicity and CVD outcomes in hypertension

In general, patients of African origin are less responsive to β-blockers, ACEIs, and ARBs as monotherapy and more responsive to CCBs and diuretics [367-371]. However, a systematic review of racial differences in the efficacy of anti-hypertensive therapy for the prevention of CV outcomes in hypertensive subjects suggests no major differences between the ethnic groups [372]. While Anti-hypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) reports a greater magnitude of benefit for 'blacks' on diuretic therapy compared with 'non-blacks' [373], post hoc analyses of The Losartan Intervention for Endpoint Reduction (LIFE) trial on usefulness of angiotensin-receptor blockers (ARBs) as first-line antihypertensive agents suggests poor outcomes in 'Blacks, in comparison to non-blacks [374]. However, no large outcome studies have been carried out with ARBs in sufficiently large number of African Americans, South Asians and Chinese to make any valid conclusions.

In the BP lowering arm of the Anglo-Scandanavian Cardiac Outcomes Trial (ASCOT-BPLA), black patients were significantly less responsive to atenolol monotherapy. Although the BP response to CA monotherapy did not differ by ethnic groups, black patients had lesser BP response on addition of perindropil as second line agent to CA in comparison to whites [375]. In the African American Study of Kidney Disease and Hypertension, African American patients treated with the ACEI ramipril had a significantly lower incidence of the primary composite end point (glomerular filtration rate reduction, end-stage renal disease, or death) than African Americans treated with the calcium channel blocker amlodipine [376]. Conversely, African Americans and Asians have 3-4 fold higher risk of angioedema [377] attributable to ACEIs than Caucasians.

1.7.5 Clustering of CVD risk factors with hypertension

Clustering of cardiovascular risk factors with hypertension is common and increases the risk of adverse cardiovascular outcome [378] [379]. Clustering of risk factors with hypertension significantly inflates health care costs and the cost rises incrementally with additional number of risk factors as a consequence of relatively higher incidence of cardiovascular events and mortality outcomes [380].

1.7.6 Hypertension and Chronic Kidney Disease

Chronic Kidney Disease (CKD) is a major determinant of cardiovascular mortality and morbidity outcomes in patients with hypertension [381]. It is important to note that while kidney dysfunction leads to hypertension (cause), hypertension is a leading factor in promotion of progressive loss of renal function (consequence) [382]. Hypertension is considered as a major risk factor for both diabetic and non-diabetic CKD [383]. More than one quarter of the population with hypertension in the United States have concomitant CKD [384]. Often, hypertensive individuals with CKD report significantly poorer BP control [385] and it leads to adverse CVD outcomes. Furthermore, lowering of SBP to targets less than 130 mmHg reduces both cardiovascular risk and the progression of CKD in proteinuric cases [386].

1.8 Other potential biomarkers/predictors of mortality in hypertension

Haematocrit, heamoglobin and basic metabolic profile are commonly ordered, relatively inexpensive laboratory tests that contain potentially important health information and are readily accessible in almost all practice settings. Furthermore, the elements of the tests are quantitative and easily electronically encoded, and have international standards for measurement. The following section describes the relationship between haematocrit and basic metabolic profile with CVD and mortality outcomes. Studies in the general population and in special population groups are reviewed in detail with the objective to study some of the important findings in a hypertensive population.

1.8.1 Association between RBC indices and mortality

Haemoglobin is the iron-containing oxygen transport metallo-protien in the red blood cells (RBC) of human beings and in most of the other vertebrates. The prognostic value of haemoglobin within the normal reference range was examined in a 20 year follow-up study (Tromso Study) of 6541 men aged between 20-49 years [387]. A U-shaped relationship between quintiles of haemoglobin and all-cause mortality (excess risk in quintile 1 and quintile 5) was reported and the relationship was more pronounced in smokers in comparison to non-smokers.

Haematocrit (Hct), the proportion of blood volume occupied by red blood cells (RBC), is a major determinant of blood viscosity, BP, venous return, cardiac output and platelet adhesiveness [388-392]. Several prospective studies have established associations between Hct and risk of cardiovascular disease, including coronary heart disease (CHD) and stroke [393-397]. A 'J' or 'U' shaped relationship between Hct and all-cause mortality (with significantly higher mortality rates among those with Hct in the lowest and highest quartile) has been reported among women, while an inverse relationship between Hct and all-cause mortality was seen in men in the Framingham Heart Study [395]. In contrast, a population based 17 years follow-up study reported a U-shaped relationship between Hct and CHD mortality (with the lowest CHD mortality rates being seen among those with Hct in the middle tertile and higher rates among those with

Hct in the upper tertile) in males, and a positive linear relationship in females (with linear increase in CHD mortality from those with Hct in the lowest tertile to highest tertile) [393]. In a 28 year follow-up study among men aged 55 years, borderline polycythemia (Hct>0.50), was associated with a 1.8 fold increase in CHD death even after adjustment for established coronary risk factors [396]. In the Northwick Park heart Study, after 30 years of follow-up haematocrit was found to be associated with CHD mortality [398]. Major study findings are summarized in table 1-2.

Table 1-2: Haematocrit and risk of coronary heart disease

Studies	Details	Comparison	Effect size
Danesh, 2000 [394]	Meta-analyses of prospective cohort studies (N=8020 in general population and N=1162 with pre-existing CVD).	Hct (Tertile 1 Vs tertile 3) and CHD	General population: RR=1.16, 95% CI: 1.05-1.29. Pre-existing CVD: RR=1.81, 95% CI: 1.19-2.76.
Brown, 2001 [393]	NHANES II Mortality study, 16.8 years of follow-up, 8896 adults.	Hct (Tertile 1 Vs tertile 3) and CHD mortality.	Men: No association. Women: HR=1.3, 95% CI: 0.9-1.90. Women younger than 65 years: HR=2.2, 95% CI: 1.0-4.6.
Kunnas, 2009 [396]	TAMRISK study, 28 years follow-up in 650 men aged over 55 years.	Hct<50% Vs Hct≥50% and CHD mortality.	HR=1.8, 95% CI: 1.1-2.7.
Pizzi, 2010 [398]	NPHS-I, 30 year follow-up in 3108 individuals over 30 years.	RR per 1 SD increase in PCV and CHD mortality.	RR=1.17, 95% CI: 1.00-1.37.

CHD=Coronary Heart Disease, CI=Confidence Interval, CVD=Cardiovascular Disease, Hct=Haematocrit, HR=Hazard Ratio, NHANES=National Health and Nutrition Examination Survey, NPHS= Northwick Park Heart Study, PCV=Packed Cell Volume, RR=Relative Risk, SD=standard deviation, TAMRISK= The Tampere Adult Population Cardiovascular Risk Study.

1.8.2 Renal function indices and mortality

Estimated glomerular filtration rate (eGFR) is a biomarker of chronic kidney disease (CKD) and the 2002 Kidney Disease Outcomes Quality Research Initiative guidelines [399] define CKD as a persistent kidney damage marked by albuminuria or reduced GFR and assign disease stages on the basis of GFR. Several studies in the general population report the association between low eGFR and adverse clinical outcomes including all-cause mortality [400-404]. While individuals with eGFR<60 mL/min have an approximate 16% increase in CV mortality, it is more than 30% in individuals with eGFR<30 mL/min [405]. Similarly, the presence of microalbuminuria and macroalbuminuria increase the risk of CV mortality by 50%

and 350%, respectively [406]. In a meta-analysis involving more than 100,000 participants from general population cohorts, eGFR<60 mL/min and albumin creatinine ratio of >1.1 mg/mmol are independent predictors of mortality [407]. In a recent collaborative meta-analysis, the hazard ratio (HR) for cardio-vascular disease mortality and all-cause mortality were significantly elevated among individuals with eGFR<60 mL/min/1.73m^2) in comparison to individuals with eGFR>95 mL/min/1.73m^2 [408].

1.8.3 Serum uric acid and mortality

A positive association of elevated serum uric acid with all-cause mortality was observed in the World Health Organization (WHO) Monitoring Trends and Determinants in Cardiovascular Diseases (MONICA) Augsburg cohort of men in the age group of 45-64 years [409]. In the MONICA/KORA- (Cooperative Health Research in the Region Augsburg) cohort study, elevated serum uric acid was independently associated with both CV and all-cause mortality [410]. Elevated serum uric acid levels independently predicted the risk of CV mortality in the NHANES follow-up study of 5926 subjects [411]. In 10,615 Japanese individuals from a cohort of atomic bomb survivors, elevated serum uric acid was independently associated with CV mortality in women and all-cause mortality in both men and women [412]. In a large prospective cohort of 83683 Austrian men followed-up for a median of 14 years, serum uric acid was independently related to mortality from congestive heart failure (CHF) and stroke. However, after adjustment for potential confounding factors serum uric acid was not significantly associated with deaths from CHD [413]. Similarly in a large cohort of 28613 elderly Austrian post-menopausal women followed-up for a median of 15 years, serum uric acid independently predicted all major forms of death from CVD [414]. The HR for all-cause mortality [1.22, 95% CI; 1.09-1.37] and CHD mortality (1.29, 95% CI; 1.05-1.58) were increased in the upper serum uric acid quintile in comparison to the first quintile in a cohort of middle-aged workers (9125 men) free of CHD at entry [415]. However, fatal stroke showed a U-shaped relationship as both the upper and bottom quintiles were associated with excess risk [415]. Hyperuricemia was an independent risk factor of mortality from all causes, total CVD, and ischaemic stroke after adjustment for multiple CV risk factors in a prospective cohort study of 41,879 Taiwanese men and 48,514 women aged ≥35 years [416]. In a meta-analysis involving 402,997 adults, hyperuricemia was associated with an

increased risk of CHD incidence and mortality even after adjustment for potential confounding factors. However, the relationship was significant only among women [417]. Similarly, in a meta-analysis involving 238,449 adults, hyperuricemia was associated with modest increase in risk of both stroke incidence and mortality [418].

Serum uric acid levels show a 'J-shaped' association (elevated mortality risk in the lowest and highest quintiles) with all-cause mortality in haemodialysis patients [419]. In a follow-up study of 1,017 patients with angiographically proven coronary artery disease, serum uric acid was an independent predictor of mortality [420]. Similarly, in another follow-up study of 647 consecutive patients with angiographically proven significant coronary artery disease, serum uric acid was an independent predictor of all-cause mortality with no significant association with CV mortality [421] [422]. In the sub-analysis of Japanese Coronary Artery Disease (JCAD) study involving 8832 patients with severe coronary artery stenosis, elevated uric acid was an independent predictor of CV events and all-cause mortality [423]. In the Bezafibrate Infarction Prevention [BIP] study, elevated uric acid was significantly associated with the primary end-point of fatal or nonfatal myocardial infarction or sudden cardiac death [424]. Hyperuricemia after acute myocardial infarction is associated with the development of heart failure and independently predicts mortality [425]. Furthermore, serum uric acid independently predicts deaths in patients at high risk of CVD [426].

In a follow-up study of 560 consecutive patients with an acute heart failure event admitted in a single university centre, serum uric acid level independently predicted all-cause mortality [427]. The relative risk (RR) of all-cause mortality was 2.13 (95% CI, 1.78-2.55) for serum uric acid>6.5 mg/dL compared with serum uric acid<6.5 mg/dL in a meta-regression analysis involving 1456 heart failure patients with a median ejection fraction of 32% [428].

In hypertensive patients, serum uric acid is independently associated with left ventricular mass and the combination of hyperuricemia with left ventricular hypertrophy is an independent and powerful predictor of CVD [429]. In the Fremantle Diabetes Study participants (1,268 participants with Type 2 Diabetes and Southern European Ancestry), after a mean follow-up of four years serum uric acid did not independently predict CVD or all-cause mortality [430]. In contrast, independent of several potential confounding factors higher serum uric

acid levels increased the risk of cardiovascular mortality in Type 2 Diabetic patients. However, the relationship was not statistically significant for all-cause mortality [431]. In a recent study in Type 2 Diabetes patients [432], higher levels of serum UA above 7.70 mg/dl was associated with increased mortality.

1.8.4 Serum phosphate and mortality

Hyperphosphatemia (plasma inorganic phosphate concentration >5mg/dl or 1.6mmol/l) is associated with vascular calcification, CVD morbidity and mortality in populations with CKD [433-440]. In a meta-analysis on quality of evidence for the association between levels of serum phosphorus and CV mortality in individuals with CKD, the risk of death increased 18% for every 1-mg/dL increase in serum phosphorus [441]. Summary of main cohort studies on serum phosphate and mortality outcomes are shown in table 1-3 on page 63.

Mechanistic studies suggest that higher phosphate levels could lead to vascular calcification and CVD [442] [443]. In post-myocardial infarction statin trials [444], BP lowering trials [445] among diabetic patients and in medical out-patients [446] serum phosphate was significantly associated with cardiovascular events and mortality. Furthermore, in a population based cohort of 13,340 subjects free of overt cardiovascular and renal diseases in the age group of 45-64 years, serum phosphorus was positively associated with carotid intima-media thickness independent of traditional risk factors in men [447]. In the Bogalusa Heart Study, among asymptomatic young adults, serum phosphate within the normal range was found to be significantly associated with carotid intima-media thickness with smoking potentiating this adverse association [448].

Recent studies in the general population also indicate significant association between elevated phosphate and CVD events or mortality [449-451]. The elevated risk of cardiovascular events linked to hyperphosphatemia in individuals with CKD are actually similar to those linked to higher-normal phosphate in non-uremic populations [452]. However, any prospective interventional data showing improved CVD and mortality outcomes are lacking and additional research is required to confirm a causal association between phosphorous and CVD [453].

Table 1-3: Serum phosphate and mortality outcomes

Studies	Details	Comparison	Effect size
Tonelli, 2005 [444]	Post-hoc analyses of CARE study (4127 participants with prior MI, 5 years follow-up).	1 mg/dl increase in serum phosphate and risk of mortality.	RR=1.27, 95% CI: 1.02-1.58.
Foley, 2008 [450]	15762 ARIC study participants with 12.6 years of follow-up.	Phosphate per 0.5 mg/dl increase and mortality.	HR=1.12, 95% CI: 1.07-1.17.
Chonchol, 2009 [445]	Post-hoc analyses of Appropriate Blood Pressure Control in Diabetes Study (950 participants with diabetes, 4.8 years of follow-up)	Serum phosphorus >3.9 mg/dl Vs low serum phosphorus and cardiovascular deaths (time dependent model)	HR=4.25, 95% CI: 1.15-16.55.
Abramowitz, 2010 [446]	Retrospective cohort study of 10743 outpatient attendees with GFR≥60 ml/min (6.8 years of follow-up).	Highest Vs lowest quartile of phosphate and mortality.	HR=1.29, 95% CI: 1.07-1.55.
Larsson, 2010 [451]	Community based cohort of 2176 men (30 years follow-up).	Phosphate per 1 SD increase and mortality.	HR=1.06, 95% CI: 1.01-1.12.
Slinin, 2011 [454]	Post-hoc analyses of 7259 postmenopausal women with osteoporosis in the MORE study with 4 years of follow-up.	Phosphate per 1 SD increase and mortality.	HR=1.00, 95% CI: 0.80-1.25.
Palmer, 2011 [441]	Meta-analyses in individuals with chronic kidney diseases. 47 cohort studies included with 327644 individuals.	1 mg/dl increase in serum phosphate and mortality.	RR=1.18, 95% CI: 1.12-1.25.

SD=Standard Deviation, MORE=Multiple Outcomes of Raloxifene Evaluation, ARIC=Atherosclerosis Risk in Communities, HR=Hazard Ratio, RR=Relative Risk, CI=Confidence Interval.

1.8.5 Serum calcium and mortality

In a meta-analysis on quality of evidence for the association between levels of serum calcium and CV mortality in individuals with CKD, there was no independent association between serum levels of calcium and the risk of death or CV events [441]. In the United States Renal Data System Waves 1, 3, and 4 Study of 14,829 patients who were on haemodialysis, higher calcium levels were associated with fatal or nonfatal cardiovascular events and the association was independent of other risk factors [438]. Serum calcium and albumin-corrected calcium were associated with all-cause mortality in the Dialysis Outcomes and Practice Patterns Study (DOPPS), an international study of haemodialysis practices and associated outcomes [455]. In the CORES Study of 16,173

haemodialysis patients, elevated and reduced serum levels of albumin-corrected calcium were associated with increase in all-cause mortality [456]. In haemodialysis patients cumulatively (time-averaged calcium values to reflect the 'cumulative' effect of calcium burden over time) high (>10.0 mg/dl) or low calcium (<9.0 mg/dl) levels were associated with higher death risk [457]. In a post hoc data analysis from the Multiple Outcomes of Raloxifene Evaluation (MORE) trial of raloxifene treatment in 7259 postmenopausal women with osteoporosis, higher baseline serum calcium levels were independently associated with cardiovascular events [458]. Serum calcium was associated with all-cause mortality and non-CV mortality in a community-based cohort of 2176 men who were followed-up for 30 years [459].

1.8.6 Serum electrolytes and mortality

In the CArdiovascular STudy in the ELderly (CASTEL), a population-based study performed in Northeast Italy, elevated serum potassium > or = 5 mEq/l was associated with incident stroke [460]. Higher serum potassium was independently associated with increased cardiovascular mortality in the NHANES I Epidemiological Follow-up Study [461]. Elevated potassium levels > or = 5.2 mmol/litre were associated with a significant increase in mortality, particularly non-CV mortality, even after adjustment for potential confounding factors in a prospective study of middle-aged British men who were not on anti-hypertensive treatment and followed-up for 11.5 years [462]. However, the increased risk of mortality associated with elevated potassium was seen only among current smokers. Serum electrolytes and mortality outcomes data are summarised in table 1-4 on page 65.

A statistically significant inverse association between serum sodium concentration and all-cause mortality was reported in a prospective study of 1069 consecutive patients who were scheduled to undergo coronary angiography for suspected or known coronary atherosclerosis [463]. Lower serum sodium levels were associated with progressive heart failure deaths [464]. In prospective evaluation of 3,282 elderly subjects with CHF, lower serum sodium (serum sodium level < or = 139 mmol/L) was associated with increased risk of deaths [465]. In the United Kingdom heart failure evaluation and assessment of risk trial (UK-heart), increase in serum sodium was associated with all-cause mortality [466].

However, there were only 54 deaths during the follow-up period in this study and it was underpowered to detect an association between serum sodium and mortality.

Table 1-4: Serum electrolytes and mortality outcomes

Studies	Details	Comparison	Effect size
Wannamethee, 1997 [462]	Prospective study of 7262 middle age British men, 11.5 years of follow-up.	SK≥5.2 mmol/L Vs SK=3.7-5.1 mmol/L and mortality.	HR=1.7, 95% CI: 1.2-2.6.
Nolan, 1998 [466]	433 CHF patients in the UK-heart trial, 16 months follow-up.	1 SD increase in SK and mortality.	HR=1.64, 95% CI: 1.03-2.63.
Fang, 2000 [461]	NHANES-I, 2836 subjects, 16 years of follow-up.	SK≥4.5 mEq/L Vs SK=3.8-4.4 mEq/L and mortality.	HR=1.51, 95% CI: 1.15-1.97.
Nolan, 1998 [466]	433 CHF patients in the UK-heart trial, 16 months follow-up.	1 SD increase in SNa and mortality.	HR=1.59, 95% CI: 1.13-2.22.
Mazza, 2005 [465]	3282 CHF patients aged over 65 years, 12 years follow-up.	SNa≤139 mmol/L Vs >139 mmol/L and mortality.	RR=1.95, 95% CI: 1.44-2.63.
Mehta, 2008 [464]	396 incident heart failure patients in two UK centres, 10 months follow-up.	High Vs Low SNa and mortality.	HR=0.91, 95% CI: 0.86-0.97
Jia, 2009 [463]	1069 consecutive patients who underwent coronary angiography in a Chinese hospital, 3 years follow-up.	SNa Quartile 4 Vs Quartile 1 and mortality.	HR=0.46, 95% CI: 0.26-0.81.
De Bacquer, 1998 [467]	9106 individuals in the BIRNH study, 10 years follow-up.	SCl≤100 Vs SCl>100 mmol/L and mortality.	RR=1.65, 95% CI: 1.06-2.57 in men and RR=2.16, 95% CI: 1.11-4.22 in women.
Kovesdy, 2009 [468]	1240 male patients with moderate CKD.	SHCO3 and mortality.	U shaped relationship with mortality.
Menon, 2010 [469]	1881 individuals with CKD in the MDRD study.	SHCO3 Quartile 1 Vs Quartile 4 and mortality.	HR=1.39, 95% CI: 1.07-1.18.
Navaneethan, 2011 [470]	41749 CKD patients.	SHCO3 and mortality.	A 'J' shaped relationship with mortality.
Raphael, 2011 [471]	1094 patients in the AASK trial, 6 years follow-up.	One unit increase in SHCO3 and mortality.	HR=0.96, 95% CI: 0.92-0.88

AASK=African American Study of Kidney Disease and Hypertension, BINRH=Belgian Interuniversity Research on Nutrition and Health, MDRD=Modification of Diet in Renal Disease, NHANES=National Health and Nutrition Examination Survey, SK=Serum Potassium, SNa=Serum Sodium, SCl=Serum Chloride, SHCO3=Serum Bicarbonate, HR=Hazard Ratio, RR=Relative Risk, CI=Confidence Interval, SD=Standard Deviation, CHF=Congestive Heart Failure, CKD=Chronic Kidney Disease.

Serum chloride level is routinely assessed in clinical laboratories in the management of patients with hypertension. However, serum chloride level is often ignored in clinical decision making in routine medical practice. In a ten year follow-up study of >9100 participants aged 25-74 years, free of symptomatic coronary heart disease at baseline, the estimated adjusted hazard ratio for CV death in subjects with a serum chloride level ≤100 mmol/l in comparison to those with levels above that limit was 1.65 (95% CI; 1.06-2.57) and 2.16 (95% CI; 1.11-4.22) in men and women, respectively [467].

A J-shaped relationship was noted between serum bicarbonate and mortality in CKD patients [470]. Both lower and higher serum bicarbonates were associated with increased all-cause mortality (U-shaped relationship) in patients with non-dialysis-dependent CKD [468]. In the Modification of Diet in Renal Disease (MDRD) Study, although low serum bicarbonate level was associated with all-cause mortality the relationship was not independent of baseline glomerular filtration rate [469]. In the African American Study of Kidney Disease and Hypertension (AASK) trial, higher serum bicarbonate level within the normal range was associated with better survival and renal outcomes [471].

1.8.7 Indices of liver dysfunction/injury and mortality

In a ten year follow-up study of 3704 adults, low serum albumin was associated with high mortality in men and women [472]. In dialysis patients the associations of serum phosphorus and albumin concentrations with mortality are modified by each other over time and the lowest risk was found with concurrent low phosphorus and high albumin values [473]. In a multivariate analysis, significantly higher risk for total mortality was reported in haemodialysis patients with serum albumin <3.8 g/dL (odds ratio 5.04, 95% CI; 1.30-19.60] when compared with those with albumin >4.1 g/dL. However, serum albumin did not independently predict mortality outcomes in a 4 year follow-up study of chronic haemodialysis patients [474]. In a systematic review of the relationship between laboratory based measures and mortality outcomes in haemodialysis patients, serum albumin was significantly associated with all-cause mortality [475].

The independent associations of serum alkaline phosphatase (ALP) and mortality are well established. For example, after adjustment for serum phosphate,

hepatic enzymes, and other potential confounders, post- myocardial infarction participants with ALP in the third tertile reported a HR of 1.43 (95% CI: 1.08-1.89) compared with those in the lowest tertile. This association was also validated in the US general population [476] and it was independent of the baseline prevalence of metabolic syndrome [477]. In outpatients with normal kidney function ALP is associated with all-cause mortality [478]. In the Korean Longitudinal study of Health and Aging (KLoSHA), the group with low ALP had better survival rates in comparison to the group with high ALP [479].

Elevated aspartate transaminase (AST) is associated with mortality in ST elevation myocardial infarction patients [480]. In an elderly cohort of people aged 75 years and above abnormal AST level was associated with increased risk (adjusted HR=1.27, 95% CI: 1.09-1.47) of all-cause mortality [481]. The association of AST with mortality outcomes in the general population has not been studied in detail.

Positive linear association between serum ALT on a logarithmic scale and mortality is reported in both men and women with prior history of stable CVD [482]. However, in the US general population elevated ALT was associated only with liver disease mortality [483]. Studies evaluating the association between non-alcoholic fatty liver diseases (using liver enzyme levels as surrogate markers) and mortality in samples from the general population also provide conflicting results [484-486]. An inverse relationship between ALT in the normal range with all-cause mortality was reported in three independent populations of middle-to-older aged subjects without evidence of clinically significant liver damage and independent of traditional risk factors [487].

Serum GGT is independently associated with mortality. In the US adult population, all-cause mortality increased with elevated GGT (HR, 1.5; 95% CI, 1.2-1.8), as did mortality from liver disease (HR, 13.0; 95% CI, 2.4-71.5), neoplasms (HR, 1.5; 95% CI, 1.01-2.2), and diabetes (HR, 3.3; 95% CI, 1.4-7.6) [483]. In the European Prospective Investigation into Cancer and Nutrition study, higher GGT plasma-activity was associated with increased all-cause mortality in individuals with diabetes [488]. In a prospective study of 6997 men aged 40-59 with no history of CVD (CHD or stroke) or diabetes drawn from general practices in Britain, elevated GGT was associated with significantly increased risk of stroke, fatal CHD events and CV mortality independent of established CV risk factors [489].

Several other studies also report increased CV mortality with elevated GGT levels [490-492].

Serum bilirubin is inversely associated with both cardiovascular diseases [493-495] and cancer [496] in cross-sectional epidemiological studies. In the Korean Longitudinal study of Health and Aging (KLoSHA), the group with high bilirubin had better survival rates in comparison to the group with low bilirubin [479]. In a large cohort study among over a half million adults from a UK primary care research database, 0.1 mg/dl increase in bilirubin decreased the mortality in men by 3% (95% CI: 2%-3%) [497].

1.9 Summary of literature review and rationale for the present study

Hypertension is a leading cause of death and disability worldwide. The pathophysiology of hypertension is very complex and involves the interplay of multiple neural, hormonal, renal and environmental factors. Despite effective treatment regimens the mortality among hypertensive subjects is much higher than that of normal individuals. Although reduction in clinic BP is an important and a major determinant in mortality reduction, several other factors influence survival in hypertensive patients.

Several studies have been carried out to identify prognostic factors that have predictive value for mortality in hypertensive populations. However, the search for a new biomarker that is readily available and cost-effective is of special interests. While important prognostic information can be learned from BP measurements and routine blood tests that are often conducted in hypertension clinics, the usefulness of these markers in predicting survival have not been studied in detail. This thesis aims to study such factors and the specific aim and objectives are listed in the following section.

1.10 Aim and objectives of the thesis

1.10.1   Aim

To assess the independent predictors of long term mortality in hypertensive population.

1.10.2   Objectives

1. To assess the relationship between long term blood pressure variability and mortality in a hypertensive population.

2. To explain the relationship of haematocrit and mortality in a hypertensive population.

3. To evaluate the relationship between serum phosphate and mortality in a hypertensive population.

4. To assess the relationship between serum electrolytes and mortality in a hypertensive population.

5. To establish the relationship of liver enzymes (serum aspartate transaminase-AST, alanine transaminase-ALT, alkaline phosphatase-ALP, gamma glutamyl transpeptidase-GGT) and bilirubin with mortality in a hypertensive population.

2 Materials and Methods

2.1 Study setting and study population

The Glasgow blood pressure clinic (GBPC) provides secondary and tertiary level service to individuals with hypertension from the West of Scotland. It was set-up in 1968 to record standardised information on patients attending the special hypertension clinics at the Western Infirmary, the Royal Infirmary, Stobhill Hospital and the Southern General Hospital [498]. All the clinics were held in out-patient departments. It was initially supervised by an executive committee with GB Shaw as its first chairman. A small sub-committee looked after the medical records maintenance and computer infrastructure. Patients were referred to the specialist hypertension clinic mostly by their general practitioners or by other doctors in these hospitals. The primary objective of the GBPC was to store accessible data from a large number of hypertensive patients and to use this information for management and research [498]. Data from patients attending these clinics are stored in a computerised database, which contains information on individuals attending the clinic from 1968 to 2011.   Use of the anonymised database for research studies is approved by the West of Scotland research ethics service (WoSRES) of the National Health Service (11/WS/0083).

2.2 Collection of data and follow up

All patients were treated at GBPC until they achieve target BP and are maintained at that level for at least three months. In the early days, a document management system developed by Kennedy and his colleagues was used to record the data collected [499] [500]. While an initial structured acceptance document and a detailed history and examination document were completed at the patient's first visit, a shorter structured document was used to record follow-up data. The results of investigations were entered separately as an additional document. The frequency of visits to GBPC mainly depends on individual patients BP levels and presence of other co-morbidities. After completion, the acceptance and follow-up documents reach the data processing unit situated at the department of Medical Computing, the Western Infirmary. Demographic and clinical data on these documents together with the results of the clinical investigations were transferred to a main computer file.

Magnetic tapes were used to store the data in the initial days and later they were reorganised and stored by using the NHS information technology system in dedicated computers.

2.3 Measurements

Blood pressure measurements were taken manually 3 times, using standardised sphygmomanometers at each visit by specialist hypertension nurses; the mean of the last 2 measurements was recorded at each visit. All BP measurements used in this study were taken in a seated position. The Korotkoff sounds (phase V) were taken as DBP. Patients attending the clinic were advised to take their regular medications as usual. Height and weight of all patients were measured using standardised equipment during each visit. Patients were classified as being smokers if they had ever smoked cigarettes. Frequency of consumption of alcohol was also recorded. Alcohol consumption was categorised into two groups; ≤ 5 and ≥ 6 standard units per week.

2.4 Laboratory measurements

Venous blood samples were collected at baseline and at regular intervals for estimation of routine haematological and biochemical indices. Haematocrit (Hct) was analysed in the hospital laboratory auto-analyser, which calculates Hct as the product of red blood cell count and the erythrocyte mean cell volume. Serum electrolytes, creatinine, phosphate, liver enzymes and bilirubin were measured routinely in the hypertension clinic using the auto-analysers. Estimated glomerular filtration rate (eGFR) was calculated from the baseline serum creatinine values using the Modification of Diet in Renal Disease Study Group (MDRD) equation [501]. The laboratory assays were performed by the central laboratory services of the Western Infirmary. All data were electronically captured and maintained as a large single database. The baseline, repeat measurements and outcome variables are summarised in table 2-1 on page 72.

Table 2-1: Study variables in the Glasgow Blood Pressure Clinic Database.

Baseline variables	Number of individuals at baseline	Repeat measurements*	Outcome variables
Age at first visit (Yrs)	16011		Date of
Sex (Men or Women)	16011		death (DD
Date of first visit (DD MM YR)	16011	Y	MM YR)
Height (Meters)	15405	Y	
Weight (Kg)	15739	Y	Primary
Systolic BP (mmHg)	15727	Y	Cause of
Diastolic BP (mmHg)	15727	Y	death (ICD
Current tobacco use	14665		10 codes).
Current alcohol use (Frequency of use)	14188		
Co-morbidities (CVD, and Diabetes)	15002		
Heamatocrit (%)	10951	Y	
Serum Creatinine (mg/dl)	11033	Y	
Serum Phosphate (mg/dl)	9820	Y	
Serum AST (U/L)	11258	Y	
Serum ALT (U/L)	10125	Y	
Serum ALP (U/L)	11427	Y	
Serum GGT (U/L)	10555	Y	
Serum Bilirubin (mg/dl)	11037	Y	
Serum Sodium (mEq/L)	13830	Y	
Serum Potassium (mEq/L)	14012	Y	
Serum Chloride (mEq/L)	13430	Y	
Serum Bi-carbonate (mEq/L)	10550	Y	

*3 months and up to 10 years (3-6 months interval). Y=Yes, CVD=cardiovascular disease, BP=blood pressure, AST=aspartate transaminase, ALT=alkaline transaminase, ALP =alkaline phosphatase, GGT=gamma glutamyl transpeptidase, ICD=international classification of disease.

2.5 Outcome assessment

Record linkage with the office of the Register General for Scotland allowed identification of all deaths and causes of death in clinic attendees. Records kept by the General Register Office for Scotland ensured notification of a subject's death (provided that it occurred in the United Kingdom) together with the primary cause of death according to the International Classification of Diseases, 10th Revision, Version for 2007 (ICD-10), codes[502][503]. Initial validation checks in a sample of 300 patients did not find any instance of failure to notify a death [504]. The International Classification of Diseases (ICD), endorsed by the World Health Assembly, is the standard diagnostic tool for epidemiology, health management and clinical purposes especially to monitor the incidence and prevalence of diseases and other health problems [505]. ICD classifies diseases and other health problems recorded on many types of health and vital records including death certificates and health records. It helps in international comparisons and is used

as a standard tool for comparison of national mortality and morbidity statistics of the member countries of World Health Organization (WHO) in the global burden of disease analyses [506]. It is available in the six official languages of WHO (Arabic, Chinese, English, French, Russian and Spanish) as well as in 36 other languages. The disease classification system is updated regularly, and is available as annual list of changes [502]. The updates are approved annually at the meeting of the Heads of WHO collaborating centres for the Family of International Classifications.

As per ICD-10 convention, diseases of the circulatory system are identified with the codes I00-I99 [503]. Of the diseases of the circulatory system, ischaemic heart diseases (IHD) and cerebrovascular diseases are coded as I20-I25 and I60-69, respectively. However, transient cerebral ischaemic attacks and related syndromes are excluded and classified separately as G45. The following mortality events were considered in the analyses: Cardiovascular deaths (CV mortality; ICD-10 codes I00-I99), ischaemic heart disease deaths (IHD mortality; ICD-10 codes 120-I25), and stroke deaths (stroke mortality; ICD-10 codes I60-I69). Deaths other than due to cardiovascular causes were classified as non-cardiovascular deaths (non-CV mortality). All deaths irrespective of their corresponding ICD codes were included in the all-cause mortality category. Mortality data were collected up to April 2011.

2.6 Survival analysis

Survival analysis was the central statistical method employed for the analysis and interpretation of the data. In the context of this thesis, survival analysis refers to analysing the time to occurrence of death. The survival time was estimated as number of years from the beginning of the follow-up of an individual until death occurs. As in most of the survival models the vector of survival time was not completely observed [507] and the survival time was right censored at 35 years of follow-up allowing a maximum of 35 years for participants who had been under follow up for the longest time. Survival characteristics are often summarised by quoting survival probabilities or cumulative survival at a given time point in the follow-up. Survival probabilities are assumed to be the same for subjects recruited early and late into the study. The survivor function S(t) provides the probability that a person survives longer

than some specific time 't' during the follow-up [508] [509]. The hazard function h(t) gives the instantaneous potential per unit time (one year) for the event to occur, given that the individual has survived up to time 't' [509]. The most popular method used in comparing the survival between different groups is the log rank test [510]. It takes the whole follow-up period into account in the analyses and test the hypothesis that there is no difference between populations being studied in the survival probability at any given time point in follow-up. Median survival time is another option to summarise survival data. The point in time from the time of inclusion in the study where the cumulative survival is 50% is the median survival time. However, median survival time derived from raw data is often not correct especially when there is censoring. Although it is reliable to interpret the median survival time from the survival curves, it cannot be estimated unless the survival curve drops below 0.5.

The relative survival in two groups can be estimated by comparing the observed number of events with the expected number of events. The relative risk ratio is a measure of how many events have occurred in a study and expressed as proportion of events occurring in one group in comparison to the reference group. It is often calculated over the average or median duration of the study. A hazard ratio (HR) is different from relative risk estimates. In simple terms, the HR is the relative probability of an individual reaching a certain point in time without experiencing an event in one group compared to the reference group.

2.6.1 Kaplan-Meier survival analysis

Initially the Kaplan-Meier (KM) method was employed to summarise the survival data separately for all categories of mortality events [508]. A plot of the KM survival probabilities (KM curve) corresponding to each ordered failure time (i.e., one year) are normally plotted as a step function that starts with a horizontal line at a survival probability of 1 and then steps down to other survival probabilities separately for all categories of the baseline predictor variables [511] [512]. It is recommended to present the survival plots as cumulative incidence (mortality) data displaying the proportion of patients with events increasing over time [513]. This approach was followed for presentation of KM curves. To estimate the survival probability at any given time the risk set available at that time was used [514]. A subject might not be available in the risk

set at given time 't' if the subject died prior to time 't' or if the subject did not enter into the study at the given time 't'. The general KM formula for survival probability at event time $t_{(j)}$ is as follows;

$$\hat{S}(t_{(j)}) = \hat{S}(t_{(j-1)}) \times \hat{P}r(T > t_{(j)} | T \geq t_{(j)}) \qquad (1)$$

In other words, the general KM formula gives the probability of survival past the previous failure time $t_{(j-1)}$, multiplied by the conditional probability of surviving past time $t_{(j)}$, given survival to at least time $t_{(j)}$. The letter 'T' in the equation 1 denotes a random variable for a person's survival time in the study where 'T' is always equal to or greater than zero and equal to or less than 35 years. To determine whether the KM curves for the different categories of baseline predictor variable are statistically equivalent, the log-rank test for multiple groups was used [510]. In general, the log-rank test is a large-sample chi-square test where the test statistic provides an overall comparison of the KM curves being compared. The test statistic is derived from the observed versus expected cell counts over categories of mortality outcomes. The categories for the log-rank statistic are defined by each of the ordered failure times for the entire set of data being analysed. For example, if there are 4 groups based on the baseline predictor variable, the log-rank test statistic estimation involved estimation of variance and covariance of observed and expected cell count in all four groups. Furthermore, the log-rank test statistic would have approximately a large chi-square distribution with 3 degrees of freedom under the null hypothesis that all four groups have common survival time. The estimated power of two-sample comparison of survivor functions in log-rank test at different sample sizes and hazard ratios are given in table 2-2.

Table 2-2: Estimated power of two-sample comparison of survivor functions.

Power	N	N1	N2	HR	Alpha*
0.85	80	40	40	2.00	0.05
0.82	100	50	50	1.80	0.05
0.81	200	100	100	1.50	0.05
0.83	500	250	250	1.30	0.05
0.82	1000	500	500	1.20	0.05
0.88	1200	600	600	1.20	0.05
0.93	1400	700	700	1.20	0.05
0.81	3600	1800	1800	1.10	0.05

N; total number of events, N1 and N2; Number of events in each group, HR=effect size, Alpha=two sided significance level. They were generated using 'Stata version 12' which implements the Freedman method[515] for sample size calculation under the null hypothesis that H0=S1(t)=S2(t).

There are some weaknesses in the KM method. Since the log rank test is just a significance test, an estimate of the effect size (size of the difference between groups compared) cannot be generated using this test. The KM method and the log rank test can study the effect of one factor at a time and therefore they are not often used in multivariate analyses. Additionally, it cannot model a continuous predictor variable.

2.6.2 Cox proportional hazards models

Since the KM method and the log-rank test can only study the effect of one factor at a time, Cox proportional hazards (Cox PH) models [516] were set up to analyse the influence of baseline predictor variables on all-cause, CVD, IHD, stroke and non-CVD mortality after adjustment for potential confounding variables. The Cox PH model assesses the hazard of an event to occur at time 't' for an individual with a given specification of a set of variables, provided that the individual has not experienced that event up to time 't' [517].

The Cox PH formula can be written as;

$$H(t, X) = h_0(t)e^{\sum_{i=1}^{p} \beta_i X_i} \qquad (2)$$

where X=(predictor variable of interests and other confounding variables).

The Cox PH model in equation 2 gives the hazard at time 't' as a product of baseline hazard involving time 't' (an unspecified arbitrary non-negative function of the time) and an exponential expression to the linear sum of $\beta_i X_i$, where the sum is over all the explanatory variables and β is an unknown parameter reflecting the effect of X on survival. In other words, it models the effect of covariates on the hazard rate and leaves the baseline hazard rate unspecified. The baseline hazard function is interpreted as the hazard when all covariates are zero. This model is a semi-parametric model as it contains finite dimensional relative risk parameters (β's) and an infinite dimensional parameter $h_0(t)$. The exponential part ensures that the fitted model will always give estimated hazards that are non-negative. Cox model does not assume knowledge of absolute risk and only estimates relative risk. An additional advantage of Cox model over the K-M method is that it can accommodate both discrete and continuous measures of event times.

A stepwise backward selection of variables was used in the selection of models [518]. Exposure variables with p values less than 0.10 were retained in the model and others were excluded. When the linearity of the effect of exposure variable on outcome was in doubt, they were divided into groups. In order to ensure an equal proportion of individuals at risk at baseline in all groups, often the quartiles or quintiles of the exposure variable was taken. Variables with skewed distribution were transformed into logarithmic scale to avoid undue influence of extreme values on the choice of the model. Finally, if there were strong correlations between variables in the model (correlation coefficient of more than 0.60), one of them was removed from the model.

The covariates included were baseline age, gender, body mass index (BMI), smoking status, systolic and diastolic blood pressure (SBP and DBP), alcohol use, tobacco use, eGFR and cardiovascular co-morbidity. A variable on year of first visit strata (epochs) was used to adjust the secular trend in mortality and was divided into five categories (first visit 1977 or before, between years 1978-1985, 1986-1993, 1994-2001, 2002 and after). This variable was included in all models to adjust for any secular trend in treatment practices and mortality.

The Cox's method is a 'semi-parametric' approach and no particular type of distribution is assumed for survival. However, there are some strong basic assumptions made on the effect of exposure variable on survival. The main assumptions in the basic Cox-PH model are [516] [519]; (a) the hazard rate of an individual at time 't' is proportional to the hazard rate at any other given time point in the follow-up period and (b) the exposure variable of interests and other covariates contribute linearly to the natural log of the hazard ratio.

Although the Cox-PH model makes no assumption about the form of baseline hazard function (non-parametric part of the model), it assumes a parametric form for the effect of predictors on the hazard. Therefore, parameters estimates like the maximum likelihood estimates of the Cox model were derived by maximising the partial likelihood function (L) [519]. The likelihood function is a mathematical expression which describes the joint probability of obtaining the data actually observed on the subjects in the study as a function of the unknown parameters (the ß's) in the model being considered. To maximise the likelihood function the partial derivatives of log of 'L' with respect to each parameter in the model were carried out using an iteration procedure. In other words, a

solution is obtained in a stepwise manner, which starts with guessed value for the solution, and then successfully modifies the guessed value until a solution is finally obtained. While the HRs were computed by taking the exponential of the coefficient of the predictor variable, the statistical inferences of the HRs were derived in terms of the maximum likelihood estimates by computing a Wald test (a Z statistic known as the Wald statistic) and a likelihood ratio (LR) test. Variance is the inverse of the observed information evaluated at the maximum partial likelihood estimation. The standard error of ß's derived from the resulting variance-covariance matrix is used for estimation of 95% confidence limits of the HR.

Cox-PH model assumes that time to event is a continuous variable. The partial likelihood in the Cox-PH model is only valid when there is no ties in the dataset i.e., no two subjects have the same event time. In instances where ties are present, the Breslow approximation to the partial log likelihood was used [520]. There are different approaches to manage tied events in the Cox-PH models. The most common method used is the exact method. It assumes that ties result from imprecise measurement of time and there is a true unknown order of events in time. Mathematically the exact method calculates the event probability of all possible ordering of events. It is largely a complex computation when there are multiple ties and often requires approximation. Breslow[520] and Efron [521] are approximation to the exact method. Breslow does not perform well when the number of ties at a particular time point is a large proportion of the number of case at risk. In such situations, Efron is preferred over Breslow.

The estimated sample size requirements for Cox-PH regression to obtain 80% power at different hazard ratios are given in table 2-3. They were generated using 'Stata version 12' which implements the Hsieh and Lavori (2000) method[522] for sample size calculation. The 'Stata' command provides options to account for possible correlation between a covariate of interest and other predictors and for withdrawal of subjects from the study. The required sample size was estimated under two assumptions; (a) the predictor variables are independent of each other and (b) there is correlation between predictor variables and the multiple correlation coefficients is 0.3.

Table 2-3: Estimated sample size for Cox regression.

Power	N	N1	HR	Alpha*
0.80	3457	4938	1.1	0.05
0.80	945	1350	1.2	0.05
0.80	457	652	1.3	0.05
0.80	278	397	1.4	0.05
0.80	191	273	1.5	0.05
0.80	143	204	1.6	0.05
0.80	112	160	1.7	0.05
0.80	91	130	1.8	0.05
0.80	77	109	1.9	0.05
0.80	66	94	2.0	0.05

N; number of events, N1; number of events required assuming the covariate of interest is correlated with other covariates (r = 0.3), HR; hazard ratio, Alpha=two sided p value.

2.6.3 Testing proportional hazard assumption

The proportional hazard (PH) assumption requires that the hazard ratio (HR) is constant over time and that the hazard of one individual is proportional to the hazard for any other individual, where the proportionality constant is independent of time. The PH assumption is tested by comparing the estimated – ln(-ln) survivor curves of the baseline predictor variable as in the research question and also by the goodness-of-fit (GOF) tests [523] [524]. In general, the log-log survival curve is a transformation of an estimated survival curve that results from taking the natural log of an estimated survival probability twice. Since the log of the survival probability is always a negative number, a second log is taken to negate the first log. The PH assumption is violated when the log-log curves are not parallel to each other. In the GOF test, Z or large sample chi-square statistics were computed for each variable in the model, after adjustment for other variables in the model and p values were derived from comparing it with a standard normal statistic obtained for each variable in the model.

2.6.4 Cox proportional hazard model with restricted spline
functions

In the Cox PH model described earlier (2.1.3), the modelled response is the hazard rate of death with a log hazard ratio linear in the predictor variables. However, this assumption is often violated especially when the effect of predictor variables are best represented as non-linear functions. In order to model efficiently the non-linear relationship, multivariable Cox-regression models with restricted spline functions were used [525]. This model can be used as a flexible alternative model in case of violation of PH assumption. The Cox PH model incorporating an arbitrary covariate effect is of the form;

$$H(t, X) = h_0(t)e^{g(X)} \qquad (3)$$

where the log hazard ratio function g is an unspecified smooth function of X.

A spline is a polynomial of degree d or order d+1, on any interval $(\varepsilon_{i-1}, \varepsilon_i)$. A knot is often described as a free parameter for a piecewise linear spline that represents a break point in the log hazard function which may be interpreted as a threshold value. The 'mvrs' algorithm in 'Stata' was used to fit the regression spline (RS) Cox PH models with all predictor variables [525]. Unlike in the previous Cox PH model, the RS Cox PH model provides more flexibility in modelling a continuous predictor variable.

For all the predictor variables, the classical maximising gradient based method was employed in the multivariate model with equally spaced knots. In order to avoid the problem of local optima, different sets of initial knots values were placed on a grid constructed within the range of the variable. Although ten knots were placed, the knot localised on the bounds of the interval would not have any influence on the estimation. Therefore, in total $\binom{9}{K} = \frac{9!}{K!(9-K)!}$ different vectors of knots were used for initialising the algorithm.

Given the fact that the classical linear model is nested in each spline model, the models were compared by using likelihood ratio test. A best fit was arrived based on the knot positions by bootstrapping method [525]. Since we used all predictor variables, the algorithm cycled over each predictors by changing the model according to the type-1 error rate of individual variable in the model

(excluded when α>0.05). The process stopped after several cycles when there was no further change in predictor variables included in the model and their spline functions. Finally, the log relative hazard for each predictor variable in the multivariable RS model and their 95% point wise confidence interval estimates were plotted against actual values of the predictor variable. The exponent of the log HR provides the actual HR and its interpretation is same as that in the Cox-PH model.

2.6.5 Stratified Cox proportional hazard model

The stratified Cox PH model was used to analyse data when one or more of the predictor variables violated the PH assumption. The stratified Cox PH model is a modification of the Cox-PH model and often used in survival analyses when one or more of predictor variables violate the PH assumption [526]. While the predictors that satisfy the PH assumption are included in the model, predictors that do not satisfy the PH assumption are not included in the model and used for stratification [524].

The general stratified Cox PH model can be written as;

$$h_g(t, X) = H_{0g}(t)e^{(\beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_p X_p)} \qquad (4)$$

where g=1, 2,..............k*, strata defined from Z predictor variable that do not satisfy the PH assumption. This model is designated as a "no-interaction" model because of the β coefficients in the model are the same for each subscript g [524].

However, this model assumes that the β coefficients do not vary over strata. In other words, the variables being stratified are assumed not to interact with the predictor variables in the model. The estimated survival curves for each stratum in the fitted stratified Cox-PH model will be different due to differences in the baseline hazard function for each stratum. Conversely, the β coefficients of the X's are the same for each stratum. Hence the baseline hazard ratios are the same for each stratum. However if we allow for interaction, then we would expect to obtain different β coefficients for each of the stratified categories [524]. This model allows the underlying hazard function to vary across levels of stratification variables. A likelihood ratio test can be performed to compare the log likelihood statistics for the interaction model and the non-interaction model

under the null hypothesis that the ß coefficients of each of these product terms are all zero.

2.7 Analysis of repeat measurement data

2.7.1 *Generalised estimating equation regression*

Generalised estimating equations (GEE) were used to analyse longitudinal correlated response data [527] [528] such as repeat measurements of BP variables and eGFR. The normal linear model assumes that the measurements are independent while estimating the variance component. Conversely, the GEE model accounts for the correlation between observations in generalised linear regression models by use of empirical variance estimator [528].

The GEE model characterises the marginal expectation (average response of observations sharing the same covariates; population averaged effects) as a function of covariates. There are no distributional assumptions in GEE models. It avoids the need for multivariate distribution of the longitudinal response variable by assuming a functional form for the marginal distribution at each time point. The functional form should have a mean zero if the true parameters are entered into that estimating function. The GEE model generalise and extend the usual likelihood equations for a generalised linear model with a univariate response by incorporating the covariance matrix of the vector of response variables. The estimating equations approach of population average model provides a more efficient approximation to the truth than mixed effect models [529]. The covariance structure is often treated as a nuisance in the model. While the "independence estimating equations", a GEE model with an independent working correlation matrix, incorrectly assumes that the observations within subject are independent, the sandwich estimator [528] provides a good estimate of the covariance in large samples. The main focus in the GEE analysis are to estimate the population averaged mean effect and the estimation of inferences of the regression coefficients in the model. It allows robust inferences even if the underlying correlation structure is incorrectly specified [529].

In GEE models, the estimated regression coefficient represent the effect of the explanatory variables on the population average effect of the response variable. In other words it describes the change in population mean given the changes in

covariates after accounting for correlation between observations. For quantitative traits, the GEE regression coefficient is interpreted as change in mean outcome for a unit change in the exposure in the entire population.

The major limitations of the GEE are described below. There is no likelihood function in GEE as it does not specify completely the joint distribution. Instead it uses a quasi-likelihood estimate of the regression coefficient by maximising the normality-based likelihood without assuming that the response is normally distributed. In quasi-likelihood estimations, the mean response is expressed as a parametric function of covariates and the variance is assumed to be a function of the mean [530]. Therefore, testing the model fit and comparing the models are not possible in GEE. GEE requires specifying the covariance structure of the correlated response for the appropriate standard error estimations. Empirical based standard errors often underestimate the true ones. However, the effect can be minimised by increasing the sample size. Although GEE makes less computational restrictions on data, it treats missing data as completely missing at random. However, this assumption may not hold true in longitudinal repeat measurement data especially when the follow-up schedule is not decided in advance. In the GBPC, the patient follow-up is partially influenced by the presence of other co-morbidities and status of BP control. Patients with other co-morbidities and uncontrolled BP may visit the clinic more frequently than others. To minimise the bias introduced by irregular follow-up in the analyses, the GEE model was restricted to individuals with a minimum number of follow-up visits (at least 4 annual visits in the first five years).

2.7.2 Extended Cox proportional hazard model with time dependent variables

In the general Cox PH model, the baseline hazard is a function of time 't' but does not involve the predictor variables (X's). However, the exponential function involves the X's but does not involve 't'. Hence the X's in this model are generally referred as "time independent" X's [524]. Under the PH assumption, the hazard for one individual is proportional to the hazard for any other individual and the proportionality constant is independent of time. However, some of the baseline predictor variables are "time-dependent" and change their value with time. In such situations, the Cox PH model will be extended to involve interaction terms with the predictor variable and some function of time. The basic assumption is

that the effect of a time-dependent variable $X_j(t)$ on survival probability at time 't' depends on the value of this variable at the same time 't', and not on the value at an earlier or later time [524]. This model is generally referred as "time-dependent extended Cox PH model". Regular follow-up schedule is essential for time dependent Cox PH models [526]. As the clinic visits are dependent largely on clinical progress and given the fact that repeat measurement data are available only in a sub-group of patients, this method was not used in the GBPC data analyses.

2.7.3 Four groups analyses

The 'four groups' models for contrasting combinations of change in variables ranges during follow-up are also considered in the survival analyses [531]. Median values of initial assessment and final assessment of the same variable were taken as the cut-off points to define the 'four groups'. The four groups comprised of high-high, high-low, low-high and low-low groups based on initial and final values of the variable. In specific cases clinically relevant cut-off were used to classify individuals into four groups. The classification details are provided in the respective results chapters. They were considered in both the Kaplan-Meier and the Cox-PH models to analyse the impact of change in predictor variables during the course of follow-up on mortality.

2.8 Measuring correlation between variables

Different measures of correlations are described in the literature to describe the association between two variables. A perfect correlation occurs when a scatter plot of one variable against another one results in a straight line. The methods used in the analyses of GBPC data are described in the following section.

2.8.1 Pearson correlation coefficient

Pearson's correlation coefficient was used to assess the relationship between two quantitative variables. Pearson correlation coefficient (r) is a measure of linear association between two variables. The correlation coefficient can assume a value anywhere between -1 to +1. The interpretation of correlation coefficient is as follows; the larger the correlation coefficient the stronger the linear association between two variables [532]. The sign of the correlation coefficient

indicates the direction of the association. The strength of the association between two variables can vary depending on the range of values observed on the variables.

2.8.2 Spearman's rank correlation coefficient

Spearman's rank correlation (r_s) was used to assess the relationship between quartiles or quintiles of two variables. It uses ranks of the two variables to calculate correlation. While Spearman's correlation assesses monotonic relationship, Pearson's correlation benchmarks linear relationship [532]. The Spearman's correlation coefficient (r_s) also takes values from +1 to -1. The closer r_s is to zero, the weaker the association between the ranks.

Correlation coefficients are often used incorrectly to indicate agreement between two variables. A systematic error can results in perfect correlation with absolutely no agreement. Correlation also depends on the range of the true quantity in the sample. The correlation will be greater if the range of the quantity is wider. Although the test of significance of the correlation coefficient may indicate that the two methods are related (it will be invariably significant in large samples with relatively small correlation coefficient), it needs to be interpreted with the absolute value of correlation coefficient. Evidently, simple correlation is a poor tool with which to assess reproducibility [533].

2.8.3 Intra-class correlation coefficient

Intra-class correlation coefficient (ICC) was used to assess the reliability of measurements at repeated time points. ICC is a general measurement of agreement, where the measurements used are assumed to be parametric (i.e. continuous and has a normal distribution). The coefficient represents agreement between two or more evaluation methods on the same set of subjects, after corrections for agreement expected by chance [534]. Two-way mixed model analysis of variance (ANOVA) with measures of consistency was used to generate ICC between different measurements. The advantage of ICC over correlation coefficient is that it is adjusted for the effects of the scale of measurements [535]. However, it is dependent on the range of measurement as in the correlation coefficients.

The basic assumption in all ICC models as in any other parametric models is that the study subjects are a random sample from a larger population. However, this is often not the case and it makes the interpretation more difficult. The ICC estimates are based on mean squares obtained by applying analysis of variance (ANOVA) models to the data. The ICC in general is the proportion of relevant variance associated with differences among individuals in the study group [534] and it is calculated as the ratio of between-subjects variance to the total variance. In other words, the larger the heterogeneity among the subjects, with lower or equal random error the easier it is to differentiate between subjects. The values of the ICC can theoretically range from 0 to 1. It can be also used to measure consistency between different measurements. However, it is difficult to say whether one measurement can be used in place of another measurement using the ICC [534].

2.8.4 *Cramer's V statistic*

To measure the degree of congruence between different measurements Cramer's V statistics was generated. Cramer's V is a post-hoc test to give additional information on the significance of findings over and above Chi-square test [536]. Cramer's V statistic assumes that the marginal differences in rows are similar to those for columns and that the change from one category to another is equally likely. Cramer's V varies between 0 and 1 and a value close to zero shows little association between variables.

2.9 Statistical software for analyses

Stata, Version 12.1, StataCorp, Texas, USA was used for data analyses.

3 Long-term blood pressure variability and mortality

3.1 Introduction

Blood pressure is inherently variable and variability of BP in an individual could be as important as the magnitude of the BP [537]. Even within controlled environment, BP is subject to biological variation from beat to beat. Factors such as temperature, weather [249], and physical activity [538] influence blood pressure variability (BPV). Studies also reveal that SBP varies to a greater extent than DBP [539]. There are mainly two forms of variability—short-term BPV, which is the variability of BP over minutes or hours, such as is seen on 24-hour ABPM, and long-term BPV, such as is seen with repeated recordings over weeks or months (visit-to-visit variability).

BPV increases with increase in mean BP [538] and it is often ignored as a prognostic marker in epidemiological studies. One of the earliest studies that examined the prognostic benefit of BPV was in a group of 286 hypertensive patients who had been followed-up for more than three years. In this study the carotid artery intima media thickness was higher in individuals with higher SBP variability (BPV_{SBP}) compared to individuals with similar mean BP but lower variability [540]. In another study conducted among 1433 Japanese men living in Hawaii, there was a 100% increase in the risk of incident CHD events in individuals with high SBP variability as compared to individuals with low SBP variability [541].

Recent evidence from post-hoc analysis of large clinical trials and population cohorts indicates that long-term visit-to-visit BPV may be an independent cardiovascular risk predictor [342 359 542]. The association of short-term variability of BP (24 hour period) with end-organ damage and cardiovascular events is well established [346 543]. There is evidence to suggest that visit-to-visit variability in SBP has prognostic value, independent of average BP [359 544], and that the variability is reproducible and not a random phenomenon [545 546]. Long-term intra-individual BP variability, which reflect BP fluctuations between measurements separated by months, has been shown to have poor correlation with concomitant 24 hour BP variability (correlation coefficient <0.4). Thus the prognostic impact associated with these two measures relates to different underlying mechanisms

[547]. This would imply that long-term BPV is a marker of risk that is currently not captured by the office and ambulatory BP measurements routinely used in clinical practice and may be a fundamental physiologic trait. If BPV is a true independent prognostic marker then visit-to-visit BPV during different time-frames over a long follow-up period should show similar patterns of risk. The present study assessed the relationship between BPV measurements at different time-points of follow-up and mortality in a treated hypertensive cohort.

3.2 Methods

3.2.1 Study Setting and Population

The study population comprised of all patients attending the Glasgow Blood Pressure Clinic (GBPC). Detailed description of study settings and measurements are provided in section 2.1-2.4 of chapter 2. Data from 16,011 attendees are stored within a computerised database. Detailed study flow-chart is presented in figure 3-1 on page 92.

3.2.2 Blood Pressure Measurements

The GBPC employs specialist hypertension nurses, who are experienced and highly trained in BP measurement. The procedure requires subjects to rest for 5 minutes in a seated position before BP is manually measured using standardised sphygmomanometers. The procedure is performed 3 times and the mean of the second and third measurements is recorded. Heart rate is also recorded. The approximate timing of BP measurement was between 9.00am and 11.00am during each clinic visit. Consumption of food and drink, and level of physical exertion before each clinic appointment could not be controlled. Drug substitution, addition and dose adjustment occurred during follow-up and in accordance with clinical guidelines. Prescribed medications are cross checked with patients at each clinic visit, and they are advised to comply with their treatment at all times. However formal concordance testing was unavailable.

3.2.3 Blood pressure trait definitions

In total four time frames for analysis were defined as periods of follow-up from the first visit to clinic - year 1 (Y1), years 2-5 (Y2-5), years 5-10 (Y5-10), and years 10+ (Y10+). To be included in each time frame for analysis, subjects were required to have a minimum of 3 BP readings at least 30 days apart. Other exclusion criteria were extremes of age (<20 and >80 years) and BMI (<15 and >50Kg/m2). For each time frame the coefficient of variation (CoeffV) and average real variability (ARV) as metrics of BP variability (ARVSBP, ARVDBP, CoeffVSBP, CoeffVDBP) were calculated. CoeffV provides a normalized measure of variability and ARV takes the order of the BP measurements into account and quantifies variability between adjacent readings. The mean BP was calculated for each time frame using a time-weighted average of all BP measurements (SBPtw, DBPtw) during the interval, to remove any upward bias of the mean BP caused by more frequent appointments when BP was uncontrolled. The formulae used for calculating all these metrics are presented in box 1 on page 90. All CoeffV values were then multiplied by 100 to produce CoeffV percent. Finally, quartiles of ARV and CoeffV percent were determined for each time period.

In addition to the above analyses, the study population was divided into 4 groups depending on the longitudinal pattern of change in BPV over time. Using a threshold ARVSBP of 17 and comparing Y1 and Y2-5, study subjects were classified into those who had a persistently high (high-high) or low (low-low) BPV, and subjects whose BPV increased (low-high), or decreased (high-low) over time. A cut-off of ARVSBP=17 was used as it facilitated the division of the cohort into 4 groups with approximately equal sample sizes. The same categorisation procedure was undertaken for DBP using a threshold of ARVDBP=9, again comparing Y1 and Y2-5. Similar analyses were performed excluding Y1, i.e. comparing Y2-5 with Y5-10, because Y1 can be considered a period where patients would be exposed to intense drug alterations and dose titration of antihypertensive agents in order to reach target BP.

3.2.4 Outcome assessment

Outcome assessment is explained in detail in section 2.5 of chapter 2.

Box 1: Calculation of BP metrics

Coefficient of Variation

$$\text{CoeffV} = \frac{SD}{Mean}$$

Average Real Variability

$$\text{ARV}_{BP} = \frac{1}{n-1}\sum_{i=1}^{n-1}|BP_{i+1} - BP_i|$$

Time-weighted average BP

$$\text{Mean BP}_{tw} = \sum_{t=0}^{n-1}\left[\frac{BP_t + BP_{t+1}}{2}\right] * [T_{t+1} - T]$$

BP= Blood Pressure; T= Time; SD= Standard deviation; BP = SBP or DBP

3.2.5 Statistical Analysis

Summary statistics of baseline participant characteristics were calculated by time periods (Y1, Y2-5, Y5-10, and Y10+), stratified by sex. Categorical variables are shown as counts and percentages and continuous variables shown as means (SD). ARVSBP and ARVDBP were summarised as mean (SD), stratified by follow-up period and by categories of baseline covariates. Appropriate statistical comparisons were performed using independent 't' tests or analysis of variance (ANOVA).

To measure degree of congruence of BPV classification between time frames, Cramer's V statistics and intra-class correlation coefficients were calculated for both SBP and DBP. Cramer's V statistic assumes that the marginal differences in rows are similar to those for columns and that the change from one category to another is equally likely. The intraclass correlation coefficient provides a scalar measure of agreement or concordance between the BPV categories in different time-frames. More details are provided in the sections 2.8.3 to 2.8.4. In order to assess the relationship between BPV and average blood pressure, Spearman's correlation coefficients (section 2.8.2) were calculated between ARVBP and mean BP, and ARVBP and mean BPtw, for each time frame.

Survival analyses were performed as explained in section 2.6. The survival data were right censored at 35 years of follow-up. Cox proportional hazards (Cox-PH) models (section 2.6.2) were used to determine the risk of all-cause mortality, IHD, stroke, cardiovascular or non-cardiovascular mortality for each quartile of ARVBP, with the lowest quartile serving as the referent. Separate analyses were performed for SBP and DBP, and for each follow-up time frame: Y1, Y2-5, Y5-10, and Y10+. Furthermore, all analyses were repeated using CoeffV percent as an alternative measure of BP variation. The models were adjusted for mean SBPtw and DBPtw, and baseline cardiovascular risk factors including: age, sex, smoking, alcohol consumption, BMI, total cholesterol, prevalent cardiovascular disease (CVD), and eGFR. Furthermore, in analyses of Y1 and Y2-5 a variable on year of first visit strata (epochs) was included to adjust the secular trend in mortality and was divided into five categories (first visit 1976 or before, between years 1977-1987, 1988-1997, 1998-2004, 2005 and after). Log-minus-log plots (section 2.6.3) were analysed for any suggestion of deviation from the proportional hazards assumption [548].

A sub-group analysis was also performed after stratifying the Y1 and Y2-5 average real blood pressure variability measurements by mean baseline SBP categories (<140 mmHg, 140-160 mmHg and >160 mmHg). The HRs for both all-cause and cardiovascular mortality were generated using cox-proportional hazard models as explained in the previous paragraph.

The four groups of change in BPV at different time frames were compared visually using Kaplan Meier survival plots (as explained in section 2.7.1), and statistically using Log Rank tests. Additionally, Cox proportional hazards models were used to determine the risk conferred by membership in one of the four groups after adjustment for the covariates described above.
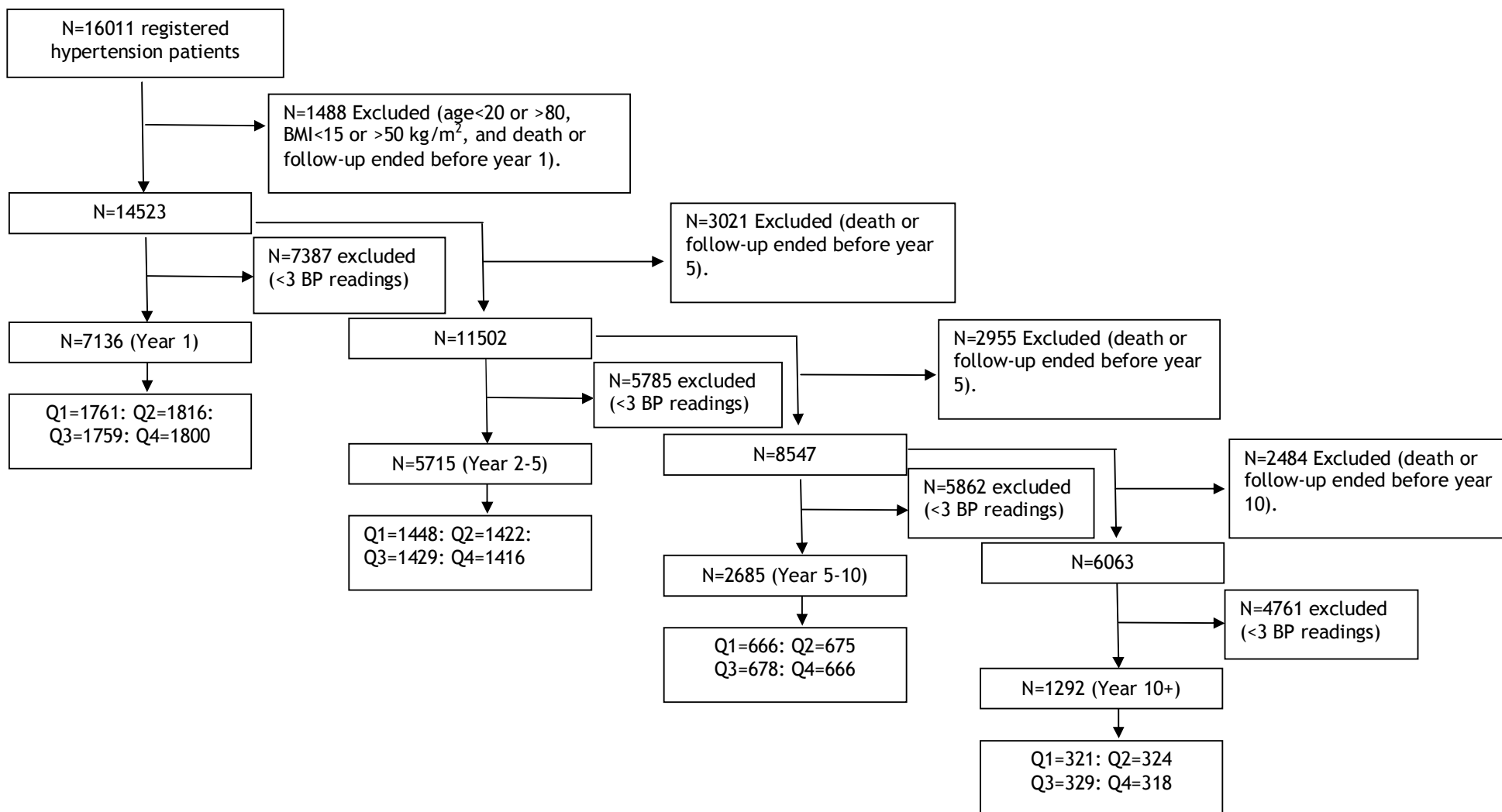
Figure 3-1: Study flow chart.

3.3 Results

3.3.1 Sample baseline demographic characteristics

At baseline, after exclusions based on age and BMI, 14,523 participants had basic demographic information recorded. Of these, 6952 (47.9%) were males and 6094 (42.5%) smokers.   The median survival time was 29.32 years. The mean (SD) SBP at baseline was 165mmHg (29.19) and the mean (SD) DBP was 98mmHg (20.02). The baseline characteristics for each time period, stratified by sex, are presented in table 3-1 on page 95. BPV as measured by ARV, CoeffV or SD were all higher in Y1 compared to Y2-5 and later. The summary statistics of baseline BPV data are presented in table 3-2 on page 95.

3.3.2 Blood pressure variability and baseline characteristics

Table 3-3 presents mean (SD) ARVSBP, categorised by follow-up time-frames and by baseline characteristics. ARVSBP was consistently higher during all follow-up time-frames in women ($p < 0.01$), older age ($p < 0.001$), participants with baseline CKD ($p < 0.01$), and baseline prevalent CVD ($p < 0.01$). High baseline SBP and high baseline mean SBPtw, were both associated with greater ARVSBP ($p < 0.001$). Table 3-4 (page 97) presents mean (SD) ARVDBP, categorised by follow-up time-frames and by baseline characteristics. Albeit with a few exceptions and poor consistency across the follow-up period, the ARVDBP results were similar to ARVSBP. In contrast with ARVSBP, ARVDBP was not associated with sex, and it decreased with age beyond 50 years in Y1 and Y2-5 time periods ($p<0.001$). Furthermore, among users of alcohol ARVDBP was higher up to five years follow-up ($p<0.001$).

3.3.3 Agreement between BP variability measurements

In general there was poor agreement between ARVBP quartiles and different time frames though the association was significant. The correlation was higher between adjacent time frames but the magnitude of correlation was small (ICC<0.3, Cramer's V <0.3) (Tables 3-5 and 3-6 on page 98). This was similar for both SBP and DBP measures.

3.3.4 Blood pressure variability and mean blood pressure

ARVSBP and ARVDBP were moderately correlated with both mean BP and mean BPtw, at each follow-up time period (p<0.01; Table 3-7 on page 99). The highest Spearman correlations were observed during Y2-5 (mean SBP (mean SBPtw) and ARVSBP = 0.47, mean DBP and ARVDBP = 0.28, mean DBPtw and ARVDBP = 0.27).

3.3.5 Blood pressure variability and mortality

Data from previous BPV studies suggest that when compared with SD of BP, ARV seems to be a more appropriate index for BPV and a better predictor of risk. I therefore highlighted the relationship between ARV and mortality in this chapter. However, the association between SD and CoeffV of BP with mortality are also presented for better clarity. Unadjusted event rates per 1000 person years of follow-up, stratified by quartiles of ARV, are presented in tables 3-8 on page 100 (SBP) and 3-9 on page 101 (DBP). For all mortality outcomes the number of events increased with increasing BP variability. The PH assumption was tested graphically using log-log survival curves (Figures 3-2 to 3-3 on page 102). There were no indications of violation of PH assumption. Cox-PH models of ARVSBP (Figures 3-4 to 3-5 on pages 103-104) and ARVDBP (Figures 3-6 to 3-7 on pages 105-106) are presented as Forest plots. The results of the Cox-PH analysis of ARV and CoeffV percent BP for each time period are also presented in tables 3-10 to 3-14 on pages 107-109. Increasing quartiles of ARVSBP in Y1 were associated with increased risk of all-cause (p for trend <0.001), cardiovascular (p for trend <0.001), and IHD mortality (p for trend <0.001) (Figures 3-4 to 3-5 on pages 103-104). The results were consistent during other follow-up time points as well. Similarly, increasing quartiles of ARVDBP in Y1 were associated with increased risk of all-cause (p for trend <0.01), and cardiovascular mortality (p for trend <0.05) (Figure 3-6 on page 105). However, the results were not consistent in the other follow-up time points analysed. Non-CV mortality was consistently elevated in higher ARVSBP quartiles in all follow-up time points up to year 10 (Figure 3-5 on page 104). CoeffV of SBP quartiles showed a significant association with all-cause mortality in all time frames except Y1 (Table 3-10 on page 107).

Table 3-1: Baseline population characteristics by follow-up period, stratified by sex.

| Variables | Year 1 (n=7136) | | Years 2-5 (n=5715) | | Years 5-10 (n=2685) | | Year 10+ (n=1292) | |
| | Men | Women | Men | Women | Men | Women | Men | Women |
	n=3518	n=3618	n=2787	n=2928	n=1279	n=1406	n=579	n=713
Age at first visit, years(SD)	51.22 (11.77)	52.90 (13.59)	51.43 (11.12)	52.88 (13.03)	51.03 (10.51)	52.47 (12.18)	49.20 (9.62)	49.60 (11.43)
Smoker, n (%)	1673 (49.42)	1467 (42.00)	1352 (48.90)	1171 (40.23)	590 (46.17)	553 (39.33)	256 (44.21)	242 (33.94)
IHD, n (%)	527 (14.98)	449 (12.41)	488 (17.51)	447 (15.27)	301 (23.53)	292 (20.77)	180 (31.09)	159 (22.30)
CVD, n (%)	876 (25.69)	701 (19.88)	819 (29.45)	692 (23.65)	464 (36.28)	431 (30.65)	248 (42.83)	249 (34.92)
Alcohol >6 units, n (%)	2544 (77.04)	1641 (48.05)	2043 (75.75)	1351 (47.54)	961 (76.27)	627 (45.53)	435 (75.39)	310 (43.79)
Cholesterol (mmol/l), mean (SD)	5.92 (1.35)	6.18 (1.36)	5.87 (1.17)	6.18 (1.33)	5.97 (1.17)	6.23 (1.25)	6.10 (1.17)	6.37 (1.27)
eGFR<60 ml/min/1.73m2, n (%)	657 (20.25)	999 (29.92)	554 (20.28)	846 (29.46)	240 (18.78)	381 (27.10)	87 (15.05)	160 (22.47)
BMI (kg/m2), mean(SD)	27.47 (4.45)	27.50 (5.63)	27.55 (4.39)	27.55 (5.54)	27.45 (4.14)	27.31 (5.34)	26.87 (3.69)	26.82 (4.99)
Baseline SBP mmHg, mean (SD)	169.04 (26.66)	173.59 (30.25)	167.25 (27.40)	172.11 (30.21)	167.61 (26.74)	171.41 (30.22)	170.04 (25.80)	172.49 (28.97)
Baseline DBP mmHg, mean (SD)	102.13 (14.53)	100.14 (15.03)	101.55 (15.01)	99.53 (21.49)	101.85 (14.92)	99.46 (14.58)	102.89 (14.82)	100.28 (14.68)

SD = standard deviation, IHD = ischaemic heart disease, CVD = cardiovascular disease, eGFR = estimated glomerular filtration rate, BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure.

Table 3-2: Baseline blood pressure variability summary statistics by follow-up period, stratified by sex.

| Variables | Year 1 (n=7136) | | Years 2-5 (n=5715) | | Years 5-10 (n=2685) | | Year 10+ (n=1292) | |
| | Men | Women | Men | Women | Men | Women | Men | Women |
	n=3518	n=3618	n=2787	n=2928	n=1279	n=1406	n=579	n=713
ARV_{SBP}, mean (SD)	20.55 (11.29)	21.91 (12.53)	17.21 (9.87)	18.08 (9.94)	16.86 (9.71)	17.85 (9.51)	15.91 (8.04)	17.52 (8.77)
ARV_{DBP}, mean (SD)	11.57 (6.79)	11.28 (6.74)	9.69 (5.61)	9.54 (5.49)	9.27 (5.11)	9.18 (5.03)	8.67 (4.27)	8.94 (4.53)
SD_{SBP}, mean (SD)	18.67 (6.53)	19.68 (6.88)	17.48 (6.79)	18.32 (6.70)	17.59 (6.71)	17.96 (6.63)	17.25 (6.52)	18.65 (6.77)
SD_{DBP}, mean (SD)	10.29 (3.69)	10.23 (3.72)	9.61 (3.75)	9.66 (3.74)	9.44 (3.56)	9.49 (3.60)	9.64 (3.56)	9.97 (3.74)
$CoeffV_{SBP}$, mean (SD)	0.121 (0.039)	0.124 (0.040)	0.116 (0.042)	0.119 (0.040)	0.119 (0.044)	0.119 (0.041)	0.117 (0.043)	0.123 (0.043)
$CoeffV_{DBP}$, mean (SD)	0.110 (0.038)	0.111 (0.040)	0.105 (0.040)	0.107 (0.040)	0.107 (0.040)	0.108 (0.041)	0.111 (0.042)	0.115 (0.045)
Mean SBP, mean (SD)	154.38 (17.30)	157.87 (19.08)	149.80 (16.78)	153.04 (17.80)	147.52 (14.90)	150.64 (16.70)	148.05 (14.22)	151.53 (16.06)
Mean DBP, mean (SD)	93.87 (9.00)	92.78 (9.56)	91.34 (8.90)	90.13 (9.10)	88.54 (7.65)	88.00 (8.23)	87.05 (7.02)	87.06 (7.68)
Mean SBP_{tw}, mean (SD)	154.70 (19.76)	159.04 (22.13)	148.00 (18.04)	152.00 (19.63)	146.29 (16.74)	149.81 (18.35)	146.07 (14.16)	149.50 (17.17)
Mean DBP_{tw}, mean (SD)	94.92 (10.40)	93.61 (10.90)	91.37 (9.56)	90.14 (9.99)	88.71 (8.45)	88.22 (8.86)	85.14 (7.29)	85.36 (8.39)

ARV=average real variability, SD = standard deviation, SBP = systolic blood pressure, DBP = diastolic blood pressure, CoeffV=coefficient of variation, $_{tw}$=time weighted estimates.

Table 3-3: Baseline characteristics and average real systolic blood pressure variability.

Variables	Year 1 ARV$_{SBP}$ (n=7136)	Years 2-5 ARV$_{SBP}$ (n=5715)	Years 5-10 ARV$_{SBP}$ (n=2685)	Year 10+ ARV$_{SBP}$ (n=1292)
Gender				
Men, Mean (SD)	20.55 (11.29)	17.21 (9.87)	16.86 (9.71)	15.91 (8.04)
Women, Mean (SD)	21.91 (12.53)	18.08 (9.94)	17.85 (9.51)	17.52 (8.77)
P Value (ttest)	<0.001	<0.001	0.008	<0.001
Age Groups				
20-29, Mean (SD)	16.33 (9.15)	14.90 (8.48)	13.49 (9.04)	13.38 (5.54)
30-39, Mean (SD)	18.43 (11.19)	16.11 (9.34)	14.79 (8.48)	15.74 (7.84)
40-49, Mean (SD)	20.50 (11.47)	16.92 (9.60)	17.20 (9.20)	15.99 (7.84)
50-59, Mean (SD)	22.09 (12.37)	17.96 (9.53)	17.64 (9.48)	17.40 (8.58)
60-69, Mean (SD)	22.84 (12.12)	19.22 (11.33)	18.50 (10.24)	18.55 (9.88)
70 above, Mean (SD)	23.32 (12.24)	18.34 (9.13)	20.21 (10.85)	20.16 (10.58)
P Value (ANOVA)	<0.001	<0.001	<0.001	<0.001
Smoking				
Smokers, Mean (SD)	21.73 (12.10)	18.00 (9.91)	17.26 (10.18)	16.99 (8.29)
Non-Smokers, Mean (SD)	20.89 (11.83)	17.40 (9.89)	17.46 (9.19)	16.68 (8.61)
P Value (t test)	0.004	0.02	0.59	0.53
Alcohol Use				
Users, Mean (SD)	21.30 (11.77)	17.82 (10.04)	17.16 (9.57)	16.37 (7.98)
Non-Users, Mean (SD)	21.40 (12.22)	17.55 (9.72)	17.70 (9.70)	17.42 (9.11)
P Value (t test)	0.74	0.33	0.16	0.03
Obesity				
BMI (<19), Mean (SD)	23.52 (12.70)	21.35 (12.07)	13.49 (9.04)	15.48 (4.06)
BMI (19-24.99), Mean (SD)	21.68 (11.92)	18.13 (9.55)	14.79 (8.48)	16.79 (7.96)
BMI (25-29.99), Mean (SD)	21.04 (11.65)	17.83 (10.27)	17.20 (9.20)	16.50 (9.08)
BMI>30, Mean (SD)	21.01 (12.35)	16.85 (9.58)	20.21 (10.85)	17.41 (8.15)
P Value (ANOVA)	0.05	<0.001	0.21	0.45
CKD Status				
eGFR<60, Mean (SD)	23.42 (12.54)	19.28 (10.24)	18.89 (10.03)	18.27 (9.86)
eGFR>60, Mean (SD)	20.36 (11.51)	16.96 (9.55)	16.93 (9.45)	16.43 (8.05)
P Value (t test)	<0.001	<0.001	<0.001	0.002
CVD Status				
CVD Present	22.75 (12.44)	18.35 (9.44)	17.98 (8.76)	17.64 (8.39)
CVD Absent	20.86 (11.79)	17.41 (10.07)	17.07 (10.01)	16.28 (8.51)
P Value (t test)	<0.001	0.002	0.02	0.005
Baseline SBP				
<160	16.92 (10.44)	14.99 (8.62)	15.60 (8.85)	15.47 (7.74)
>160	23.75 (12.07)	19.44 (10.34)	18.52 (9.93)	17.54 (8.80)
P Value (t test)	<0.001	<0.001	<0.001	<0.001
Mean SBP$_{tw}$				
Low	17.94 (10.12)	14.10 (7.82)	14.41 (8.11)	14.33 (7.40)
High	24.54 (12.72)	21.21 (10.49)	20.34 (19.80)	19.27 (8.79)
P Value (t test)	<0.001	<0.001	<0.001	<0.001

SBP=Systolic Blood Pressure, ARV=Average Real Variability, CKD=Chronic Kidney Disease, SD=Standard Deviation, BMI=Body Mass Index, CVD=Baseline Cardiovascular Diseases.

Table 3-4: Baseline characteristics and average real diastolic blood pressure variability.

Variables	Year 1 ARV$_{DBP}$ (n=7135)	Years 2-5 ARV$_{DBP}$ (n=5714)	Years 5-10 ARV$_{DBP}$ (n=2684)	Year 10+ ARV$_{DBP}$ (n=1292)
Gender				
Men, Mean (SD)	11.57 (6.79)	9.69 (5.61)	9.26 (5.11)	8.67 (4.27)
Women, Mean (SD)	11.28 (6.74)	9.54 (5.49)	9.18 (5.03)	8.94 (4.53)
P Value (t test)	0.07	0.33	0.65	0.28
Age Groups				
20-29, Mean (SD)	11.31 (6.34)	9.57 (5.14)	9.53 (4.79)	8.60 (3.62)
30-39, Mean (SD)	11.70 (6.88)	10.14 (5.79)	9.23 (5.18)	8.61 (4.38)
40-49, Mean (SD)	11.71 (6.92)	9.89 (5.57)	9.55 (5.03)	8.84 (4.53)
50-59, Mean (SD)	11.67 (6.78)	9.72 (5.33)	9.05 (4.93)	8.88 (4.29)
60-69, Mean (SD)	11.23 (6.78)	9.42 (6.04)	9.17 (5.48)	8.77 (4.55)
70 above, Mean (SD)	9.83 (6.09)	7.89 (4.42)	8.71 (4.44)	9.65 (5.92)
P Value (ANOVA)	<0.001	<0.001	0.32	0.89
Smoking				
Smokers, Mean (SD)	11.58 (6.66)	9.78 (5.65)	9.03 (5.15)	8.92 (4.53)
Non-Smokers, Mean (SD)	11.30 (6.77)	9.46 (5.46)	9.36 (5.01)	8.76 (4.35)
P Value (t test)	0.08	0.03	0.09	0.52
Alcohol Use				
Users, Mean (SD)	11.89 (6.83)	9.92 (5.62)	9.35 (4.98)	8.65 (4.01)
Non-Users, Mean (SD)	10.82 (6.50)	9.16 (5.36)	9.04 (5.24)	9.05 (4.93)
P Value (t test)	<0.001	<0.001	0.11	0.10
Obesity				
BMI (<19), Mean (SD)	13.13 (6.82)	10.84 (5.66)	9.23 (4.15)	10.21 (5.03)
BMI (19-24.99), Mean (SD)	11.74 (6.69)	9.85 (5.12)	9.53 (4.81)	9.09 (4.25)
BMI (25-29.99), Mean (SD)	11.33 (6.72)	9.73 (5.78)	9.22 (5.11)	8.74 (4.65)
BMI>30, Mean (SD)	11.19 (6.90)	9.20 (5.56)	8.92 (5.27)	8.57 (4.16)
P Value (ANOVA)	0.003	0.001	0.13	0.89
CKD Status				
eGFR<60, Mean (SD)	11.68 (6.95)	9.91 (5.61)	9.54 (5.19)	8.99 (4.27)
eGFR>60, Mean (SD)	11.22 (6.55)	9.43 (5.43)	9.12 (5.03)	8.76 (4.41)
P Value (t test)	0.015	0.004	0.068	0.45
CVD Status				
CVD Present	11.70 (6.76)	9.88 (5.37)	9.33 (4.82)	8.77 (3.89)
CVD Absent	11.37 (6.72)	9.52 (5.61)	9.17 (5.19)	8.85 (4.72)
P Value (t test)	0.092	0.031	0.43	0.74
Baseline SBP				
<160	9.99 (6.38)	8.70 (5.27)	8.73 (4.92)	8.57 (4.42)
>160	12.25 (6.85)	10.22 (5.65)	9.52 (5.08)	8.96 (4.43)
P Value (t test)	<0.001	<0.001	<0.001	0.14
AV Area DBP				
Low	10.29 (6.16)	8.52 (4.94)	8.64 (4.81)	8.61 (4.31)
High	12.56 (7.15)	10.71 (5.90)	9.80 (5.25)	9.02 (4.52)
P Value (t test)	<0.001	<0.001	<0.001	0.10

DBP=Diastolic Blood Pressure, ARV=Average Real Variability, CKD=Chronic Kidney Disease, SD=Standard Deviation, BMI=Body Mass Index, CVD=Baseline Cardiovascular Diseases.

Table 3-5: Dependency between average real blood pressure variability measurements in quartiles (Cramer's V statistics).

Variables	Year 1 ARV$_{SBP}$ (n=7136)	Years 2-5 ARV$_{SBP}$ (n=5715)	Years 5-10 ARV$_{SBP}$ (n=2685)	Year 10+ ARV$_{SBP}$ (n=1292)
Year 1 ARV$_{SBP}$ (n=7136)	1.00	0.161***	0.118***	0.113***
Years 2-5 ARV$_{SBP}$ (n=5715)	0.161***	1.00	0.169***	0.103***
Years 5-10 ARV$_{SBP}$ (n=2685)	0.118***	0.169***	1.00	0.141***
Year 10+ ARV$_{SBP}$ (n=1099)	0.113***	0.103***	0.141***	1.00
	Year 1 ARV$_{DBP}$ (n=7136)	Years 2-5 ARV$_{DBP}$ (n=5715)	Years 5-10 ARV$_{DBP}$ (n=2685)	Year 10+ ARV$_{DBP}$ (n=1292)
Year 1 ARV$_{DBP}$ (n=7136)	1.00	0.184***	0.127***	0.121***
Years 2-5 ARV$_{DBP}$ (n=5715)	0.184***	1.00	0.193***	0.114***
Years 5-10 ARV$_{DBP}$ (n=2685)	0.127***	0.193***	1.00	0.140***
Year 10+ ARV$_{DBP}$ (n=1099)	0.121***	0.114***	0.140***	1.00

***$p<0.001$. SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure, ARV=Average Real Variability.**

Table 3-6: Intra-class correlation coefficients (ICC) between average real blood pressure variability in quartiles.

Variables	Year 1 ARV$_{SBP}$ (n=7136)	Years 2-5 ARV$_{SBP}$ (n=5715)	Years 5-10 ARV$_{SBP}$ (n=2685)	Year 10+ ARV$_{SBP}$ (n=1292)
Year 1 ARV$_{SBP}$ (n=7136)	1.00	0.27 (0.25-0.30)***	0.19 (0.15-0.23)***	0.19 (0.13-0.25)***
Years 2-5 ARV$_{SBP}$ (n=5715)	0.27 (0.25-0.30)***	1.00	0.28 (0.24-0.31)***	0.17 (0.11-0.23)***
Years 5-10 ARV$_{SBP}$ (n=2685)	0.19 (0.15-0.23)***	0.28 (0.24-0.31)***	1.00	0.22 (0.16-0.28)***
Year 10+ ARV$_{SBP}$ (n=1099)	0.19 (0.13-0.25)***	0.17 (0.11-0.23)***	0.22 (0.16-0.28)***	1.00
	Year 1 ARV$_{DBP}$ (n=7136)	Years 2-5 ARV$_{DBP}$ (n=5715)	Years 5-10 ARV$_{DBP}$ (n=2685)	Year 10+ ARV$_{DBP}$ (n=1292)
Year 1 ARV$_{DBP}$ (n=7136)	1.00	0.31 (0.28-0.30)***	0.20 (0.16-0.25)***	0.14 (0.08-0.20)***
Years 2-5 ARV$_{DBP}$ (n=5715)	0.31 (0.28-0.33)***	1.00	0.31 (0.27-0.34)***	0.13 (0.07-0.19)***
Years 5-10 ARV$_{DBP}$ (n=2685)	0.20 (0.16-0.25)***	0.31 (0.27-0.34)***	1.00	0.22 (0.16-0.28)***
Year 10+ ARV$_{DBP}$ (n=1099)	0.14 (0.08-0.20)***	0.13 (0.07-0.19)***	0.22 (0.16-0.28)***	1.00

Numbers in parenthesis indicate 95% CI of intra-class correlation coefficient. ***$p<0.001$. SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure, ARV=Average Real Variability.

Table 3-7: Spearman's correlation coefficient (r) between mean blood pressure variables and average real blood pressure variability.

Variables	Year 1 ARV$_{SBP}$ (n=7136)	Years 2-5 ARV$_{SBP}$ (n=5715)	Years 5-10 ARV$_{SBP}$ (n=2685)	Year 10+ ARV$_{SBP}$ (n=1292)
Year 1 Mean SBP	0.35***			
Year 1 Mean SBP$_{tw}$	0.34***			
Years 2-5 Mean SBP		0.47***		
Years 2-5 Mean SBP$_{tw}$		0.47***		
Years 5-10 Mean SBP			0.41***	
Years 5-10 Mean SBP$_{tw}$			0.42***	
Year 10+ Mean SBP				0.41***
Year 10+ Mean SBP$_{tw}$				0.40***

	Year 1 ARV$_{DBP}$ (n=7136)	Years 2-5 ARV$_{DBP}$ (n=5715)	Years 5-10 ARV$_{DBP}$ (n=2685)	Year 10+ ARV$_{DBP}$ (n=1292)
Year 1 Mean DBP	0.24***			
Year 1 Mean DBP$_{tw}$	0.19***			
Years 2-5 Mean DBP		0.28***		
Years 2-5 Mean DBP$_{tw}$		0.27***		
Years 5-10 Mean DBP			0.19***	
Years 5-10 Mean DBP$_{tw}$			0.17***	
Year 10+ Mean DBP				0.10***
Year 10+ Mean DBP$_{tw}$				0.07**

SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure, $_{tw}$=time weighted, ARV=Average Real Variability, AUC=area under the curve, r=Spearman's correlation coefficient, **p<0.01, ***_p_<0.001

Table 3-8: Event rates per 1000 person years of follow-up (p-y) and their 95% confidence interval (95% CI) stratified by quartiles of average real systolic blood pressure variability.

Variables	ACD	CV	IHD	Stroke	non-CV
Year 1 ARV$_{SBP}$ Quartile1	14.61 (13.15-16.23)	8.13 (7.06-9.36)	4.52 (3.50-5.17)	2.19 (1.67-2.87)	6.48 (5.54-7.59)
Year 1 ARV$_{SBP}$ Quartile2	20.67 (19.03-22.46)	12.00 (10.76-13.38)	7.26 (6.31-8.35)	2.45 (1.92-3.11)	8.67 (7.63-9.85)
Year 1 ARV$_{SBP}$ Quartile3	24.74 (22.91-26.71)	15.61 (14.17-17.19)	8.41 (7.37-9.59)	4.39 (3.66-5.27)	9.13 (8.05-10.36)
Year 1 ARV$_{SBP}$ Quartile4	34.37 (32.13-36.76)	21.20 (19.46-23.10)	11.83 (10.55-13.27)	5.37 (4.53-6.37)	13.17 (11.81-14.68)
Year 2-5 ARV$_{SBP}$ Quartile1	13.01 (11.24-15.06)	6.47 (5.25-7.96)	3.27 (2.44-4.38)	1.53 (1.00-2.34)	6.54 (5.32-8.04)
Year 2-5 ARV$_{SBP}$ Quartile2	21.21 (19.12-23.52)	12.23 (10.67-14.01)	7.38 (6.20-8.80)	2.66 (1.98-3.56)	8.98 (7.67-10.53)
Year 2-5 ARV$_{SBP}$ Quartile3	28.58 (26.14-31.25)	16.22 (14.41-18.27)	9.21 (7.88-10.78)	3.45 (2.66-4.46)	12.36 (10.79-14.16)
Year 2-5 ARV$_{SBP}$ Quartile4	38.29 (35.37-41.44)	23.77 (21.50-26.29)	13.39 (11.71-15.31)	6.07 (4.97-7.40)	14.51 (12.76-16.51)
Year 5-10 ARV$_{SBP}$ Quartile1	17.35 (14.11-21.33)	8.48 (6.31-11.40)	4.63 (3.10-6.90)	2.51 (1.46-4.32)	8.87 (6.64-11.84)
Year 5-10 ARV$_{SBP}$ Quartile2	27.89 (24.07-32.31)	16.70 (13.81-20.20)	10.56 (8.31-13.41)	2.36 (1.42-3.92)	11.19 (8.86-14.12)
Year 5-10 ARV$_{SBP}$ Quartile3	33.96 (29.84-38.64)	17.86 (14.95-21.35)	10.48 (8.31-13.23)	3.69 (2.49-5.46)	16.09 (14.34-19.42)
Year 5-10 ARV$_{SBP}$ Quartile4	39.18 (34.36-44.67)	21.96 (18.43-26.17)	12.82 (10.20-16.13)	5.09 (3.54-7.33)	17.22 (14.12-20.99)
Year 10+ ARV$_{SBP}$ Quartile1	20.94 (15.78-27.79)	9.16 (5.97-14.05)	3.49 (1.75-6.98)	3.49 (1.75-6.98)	11.78 (8.08-17.18)
Year 10+ ARV$_{SBP}$ Quartile2	22.29 (17.67-28.13)	11.62 (8.42-16.03)	6.91 (4.55-10.49)	2.51 (1.26-5.02)	10.67 (7.63-14.94)
Year 10+ ARV$_{SBP}$ Quartile3	31.59 (26.02-38.36)	16.41 (12.54-21.49)	10.22 (7.27-14.38)	2.17 (1.03-4.55)	15.18 (11.47-20.08)
Year 10+ ARV$_{SBP}$ Quartile4	39.88 (32.47-48.99)	22.35 (16.98-29.41)	14.02 (9.92-19.83)	4.82 (2.67-8.71)	17.53 (12.86-23.90)

ACD=all-cause mortality, CV=cardiovascular mortality, IHD=ischemic heart disease mortality, Stroke=stroke mortality, non-CV=non-cardiovascular mortality, ARV=average real variability, SBP=systolic blood pressure.

Table 3-9: Event rates per 1000 person years of follow-up (p-y) and their 95% confidence interval (95% CI) stratified by quartiles of average real diastolic blood pressure variability.

Variables	ACD	CV	IHD	Stroke	non-CV
Year 1 ARV$_{DBP}$ Quartile1	17.24 (15.53-19.14)	9.40 (8.16-10.83)	5.34 (4.43-6.44)	1.96 (1.44-2.67)	7.84 (6.71-9.15)
Year 1 ARV$_{DBP}$ Quartile2	20.94 (19.31-22.72)	12.86 (11.59-14.26)	7.08 (6.15-8.14)	3.29 (2.68-4.04)	8.09 (7.10-9.22)
Year 1 ARV$_{DBP}$ Quartile3	25.39 (23.51-27.41)	15.50 (14.06-17.10)	8.89 (7.80-10.10)	3.80 (3.12-4.63)	9.88 (8.74-11.17)
Year 1 ARV$_{DBP}$ Quartile4	29.38 (27.43-31.44)	18.08 (16.57-19.72)	9.93 (8.83-11.17)	4.93 (4.17-5.83)	11.29 (10.11-12.61)
Year 2-5 ARV$_{DBP}$ Quartile1	16.07 (13.91-18.57)	8.30 (6.79-10.14)	4.63 (3.54-6.06)	1.75 (1.13-2.71)	7.77 (6.31-9.57)
Year 2-5 ARV$_{DBP}$ Quartile2	23.76 (21.50-26.24)	13.18 (11.52-15.08)	7.55 (6.32-9.02)	3.16 (2.40-4.15)	10.58 (9.11-12.29)
Year 2-5 ARV$_{DBP}$ Quartile3	25.84 (23.60-28.30)	14.89 (13.21-16.78)	8.19 (6.97-9.62)	3.65 (2.87-4.65)	10.96 (9.53-12.59)
Year 2-5 ARV$_{DBP}$ Quartile4	33.44 (30.86-36.24)	20.87 (18.86-23.10)	12.12 (10.61-13.85)	4.71 (3.81-5.84)	12.57 (11.03-14.33)
Year 5-10 ARV$_{DBP}$ Quartile1	22.98 (18.91-27.92)	11.15 (8.42-14.75)	7.51 (5.34-10.56)	1.59 (0.76-3.34)	11.83 (9.01-15.52)
Year 5-10 ARV$_{DBP}$ Quartile2	28.79 (24.82-33.38)	17.77 (14.71-21.45)	9.54 (7.38-12.34)	4.28 (2.91-6.28)	11.02 (8.67-14.00)
Year 5-10 ARV$_{DBP}$ Quartile3	29.49 (25.73-33.81)	15.75 (13.06-18.99)	9.59 (7.55-12.19)	3.29 (2.19-4.96)	13.74 (11.25-16.79)
Year 5-10 ARV$_{DBP}$ Quartile4	36.38 (32.04-41.31)	19.72 (16.59-23.43)	11.77 (9.41-14.72)	3.97 (2.71-5.84)	16.66 (13.81-20.10)
Year 10+ ARV$_{DBP}$ Quartile1	20.55 (15.44-27.35)	11.80 (8.09-17.21)	7.43 (4.62-11.96)	1.75 (0.66-4.66)	8.74 (5.64-13.55)
Year 10+ ARV$_{DBP}$ Quartile2	26.00 (20.83-32.46)	14.00 (10.35-18.94)	8.00 (5.36-11.94)	3.00 (1.56-5.77)	12.00 (8.66-16.64)
Year 10+ ARV$_{DBP}$ Quartile3	28.30 (22.99-34.83)	13.67 (10.14-18.43)	7.95 (5.37-11.76)	3.50 (1.94-6.32)	14.63 (10.95-19.53)
Year 10+ ARV$_{DBP}$ Quartile4	38.35 (31.46-46.75)	19.57 (14.83-25.82)	11.35 (7.89-16.33)	3.91 (2.11-7.27)	18.79 (14.16-24.93)

ACD=all-cause mortality, CV=cardiovascular mortality, IHD=ischemic heart disease mortality, Stroke=stroke mortality, non-CV=non-cardiovascular mortality, ARV=average real variability, DBP=diastolic blood pressure.
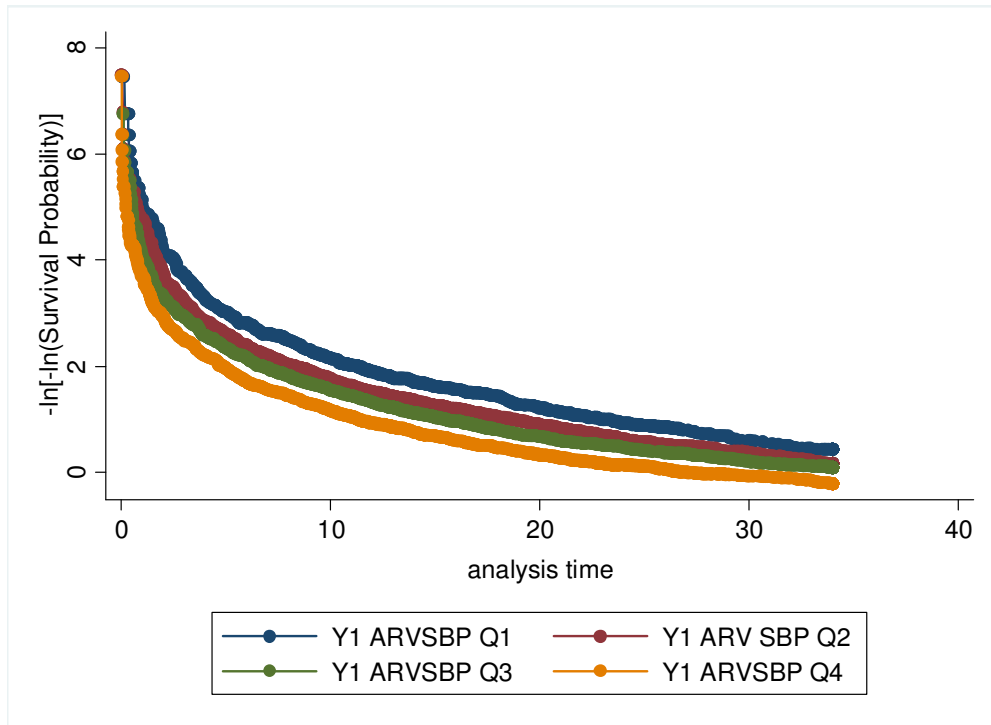
Figure 3-2: Log-log survival curves of average real systolic blood pressure (ARVSBP) variability in quartiles.
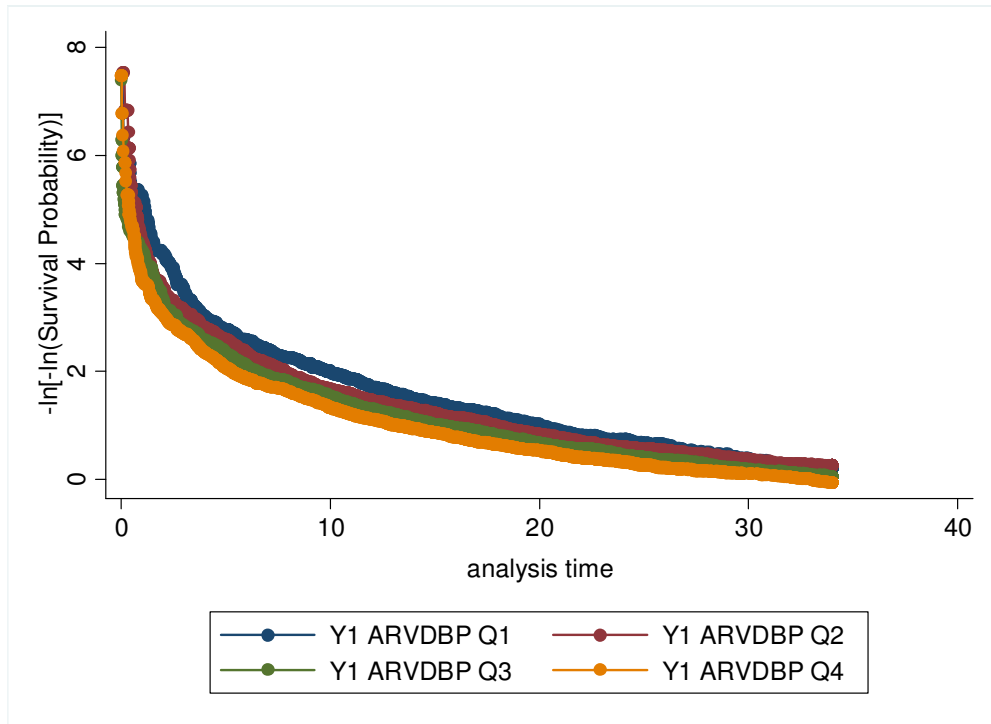
Figure 3-3: Log-log survival curves of average real diastolic blood pressure (ARVDBP) variability in quartiles.

Deaths BPV	Group	Hazard Ratio (95% CI)
All-cause (n=2170)		
	Q1	1.00 (1.00, 1.00)
ARV Y1	Q2	1.18 (1.02, 1.36)
	Q3	1.13 (0.98, 1.30)
	Q4	1.37 (1.20, 1.57)
All-cause (n=1559)		
	Q1	1.00 (1.00, 1.00)
ARV Y2-5	Q2	1.12 (0.93, 1.35)
	Q3	1.35 (1.12, 1.61)
	Q4	1.39 (1.16, 1.67)
All-cause (n=707)		
	Q1	1.00 (1.00, 1.00)
ARV Y5-10	Q2	1.24 (0.95, 1.60)
	Q3	1.48 (1.14, 1.91)
	Q4	1.55 (1.19, 2.01)
All-cause (n=309)		
	Q1	1.00 (1.00, 1.00)
ARV Y10+	Q2	0.83 (0.57, 1.21)
	Q3	1.06 (0.74, 1.51)
	Q4	1.40 (0.96, 2.03)
CV (n=1287)		
	Q1	1.00 (1.00, 1.00)
ARV Y1	Q2	1.19 (0.99, 1.44)
	Q3	1.19 (0.99, 1.43)
	Q4	1.44 (1.20, 1.72)
CV (n=902)		
	Q1	1.00 (1.00, 1.00)
ARV Y2-5	Q2	1.21 (0.94, 1.56)
	Q3	1.38 (1.08, 1.77)
	Q4	1.50 (1.16, 1.92)
CV (n=388)		
	Q1	1.00 (1.00, 1.00)
ARV Y5-10	Q2	1.45 (1.00, 2.08)
	Q3	1.47 (1.02, 2.10)
	Q4	1.64 (1.14, 2.36)
CV (n=161)		
	Q1	1.00 (1.00, 1.00)
ARV Y10+	Q2	1.05 (0.61, 1.80)
	Q3	1.30 (0.77, 2.19)
	Q4	1.72 (1.00, 2.96)
Non-CVD (n=883)		
	Q1	1.00 (1.00, 1.00)
ARV Y1	Q2	1.16 (0.94, 1.43)
	Q3	1.05 (0.85, 1.30)
	Q4	1.28 (1.04, 1.58)
Non-CVD (n=657)		
	Q1	1.00 (1.00, 1.00)
ARV Y2-5	Q2	1.03 (0.79, 1.35)
	Q3	1.33 (1.02, 1.72)
	Q4	1.28 (0.98, 1.67)
Non-CVD (n=319)		
	Q1	1.00 (1.00, 1.00)
ARV Y5-10	Q2	1.03 (0.70, 1.50)
	Q3	1.51 (1.05, 2.16)
	Q4	1.46 (1.00, 2.11)
Non-CVD (n=148)		
	Q1	1.00 (1.00, 1.00)
ARV Y10+	Q2	0.67 (0.40, 1.12)
	Q3	0.87 (0.54, 1.42)
	Q4	1.14 (0.68, 1.93)

Hazard Ratio

Figure 3-4: Forest plots showing the multivariate adjusted hazard ratios of quartiles of ARVSBP and all-cause, cardiovascular, and non-cardiovascular mortality.

SBP=systolic blood pressure, ARV=average real variability, CV=cardiovascular, Non-CVD=non-cardiovascular, CI=confidence interval.

Deaths BPV	Group	Hazard Ratio (95% CI)
IHD (n=721)		
ARV Y1	Q1	1.00 (1.00, 1.00)
	Q2	1.43 (1.11, 1.85)
	Q3	1.24 (0.96, 1.59)
	Q4	1.63 (1.27, 2.09)
IHD (n=513)		
ARV Y2-5	Q1	1.00 (1.00, 1.00)
	Q2	1.55 (1.09, 2.21)
	Q3	1.69 (1.19, 2.40)
	Q4	1.85 (1.30, 2.63)
IHD (n=231)		
ARV Y5-10	Q1	1.00 (1.00, 1.00)
	Q2	1.66 (1.03, 2.69)
	Q3	1.60 (0.99, 2.60)
	Q4	1.83 (1.12, 2.98)
IHD (n=95)		
ARV Y10+	Q1	1.00 (1.00, 1.00)
	Q2	1.64 (0.73, 3.69)
	Q3	2.05 (0.93, 4.53)
	Q4	2.66 (1.18, 5.99)
Stroke (n=318)		
ARV Y1	Q1	1.00 (1.00, 1.00)
	Q2	0.87 (0.59, 1.29)
	Q3	1.25 (0.88, 1.79)
	Q4	1.18 (0.83, 1.69)
Stroke (n=210)		
ARV Y2-5	Q1	1.00 (1.00, 1.00)
	Q2	1.01 (0.60, 1.72)
	Q3	1.05 (0.63, 1.77)
	Q4	1.34 (0.80, 2.23)
Stroke (n=80)		
ARV Y5-10	Q1	1.00 (1.00, 1.00)
	Q2	0.68 (0.31, 1.47)
	Q3	1.01 (0.50, 2.05)
	Q4	1.26 (0.63, 2.55)
Stroke (n=33)		
ARV Y10+	Q1	1.00 (1.00, 1.00)
	Q2	0.56 (0.20, 1.56)
	Q3	0.42 (0.14, 1.22)
	Q4	0.93 (0.33, 2.63)

Hazard Ratio (x-axis: 0, 1, 2, 3)

Figure 3-5: Forest plots showing the multivariate adjusted hazard ratios of quartiles of ARVSBP and ischemic heart disease and stroke mortality.

SBP=systolic blood pressure, ARV=average real variability, IHD=ischemic heart disease, CI=confidence interval.

Deaths BPV	Group		Hazard Ratio (95% CI)

All-cause (n=2170)

	Q1		1.00 (1.00, 1.00)
ARV Y1	Q2		1.02 (0.89, 1.18)
	Q3		1.12 (0.97, 1.29)
	Q4		1.25 (1.09, 1.44)

All-cause (n=1559)

	Q1		1.00 (1.00, 1.00)
ARV Y2-5	Q2		1.01 (0.84, 1.22)
	Q3		1.06 (0.88, 1.27)
	Q4		1.15 (0.95, 1.38)

All-cause (n=707)

	Q1		1.00 (1.00, 1.00)
ARV Y5-10	Q2		1.20 (0.94, 1.54)
	Q3		1.17 (0.91, 1.49)
	Q4		1.48 (1.17, 1.89)

All-cause (n=309)

	Q1		1.00 (1.00, 1.00)
ARV Y10+	Q2		1.19 (0.83, 1.72)
	Q3		1.25 (0.87, 1.79)
	Q4		1.79 (1.25, 2.57)

CV (n=1287)

	Q1		1.00 (1.00, 1.00)
ARV Y1	Q2		1.14 (0.94, 1.38)
	Q3		1.21 (1.00, 1.46)
	Q4		1.30 (1.08, 1.57)

CV (n=902)

	Q1		1.00 (1.00, 1.00)
ARV Y2-5	Q2		0.99 (0.77, 1.28)
	Q3		1.04 (0.81, 1.34)
	Q4		1.19 (0.93, 1.53)

CV (n=388)

	Q1		1.00 (1.00, 1.00)
ARV Y5-10	Q2		1.52 (1.08, 2.15)
	Q3		1.25 (0.88, 1.76)
	Q4		1.57 (1.11, 2.20)

CV (n=161)

	Q1		1.00 (1.00, 1.00)
ARV Y10+	Q2		1.10 (0.67, 1.79)
	Q3		1.04 (0.64, 1.71)
	Q4		1.46 (0.89, 2.37)

Non-CVD (n=883)

	Q1		1.00 (1.00, 1.00)
ARV Y1	Q2		0.88 (0.72, 1.09)
	Q3		1.01 (0.82, 1.25)
	Q4		1.20 (0.97, 1.47)

Non-CVD (n=657)

	Q1		1.00 (1.00, 1.00)
ARV Y2-5	Q2		1.04 (0.79, 1.36)
	Q3		1.09 (0.83, 1.42)
	Q4		1.09 (0.83, 1.43)

Non-CVD (n=319)

	Q1		1.00 (1.00, 1.00)
ARV Y5-10	Q2		0.90 (0.63, 1.31)
	Q3		1.10 (0.77, 1.56)
	Q4		1.40 (1.00, 1.98)

Non-CVD (n=148)

	Q1		1.00 (1.00, 1.00)
ARV Y10+	Q2		1.31 (0.75, 2.27)
	Q3		1.54 (0.90, 2.64)
	Q4		2.24 (1.31, 3.81)

Hazad Ratio

Figure 3-6: Forest plots showing the multivariate adjusted hazard ratios of quartiles of ARVDBP and all-cause, cardiovascular, and non-cardiovascular mortality.

DBP=diastolic blood pressure, ARV=average real variability, CV=cardiovascular, Non-CVD=non-cardiovascular, CI=confidence interval.
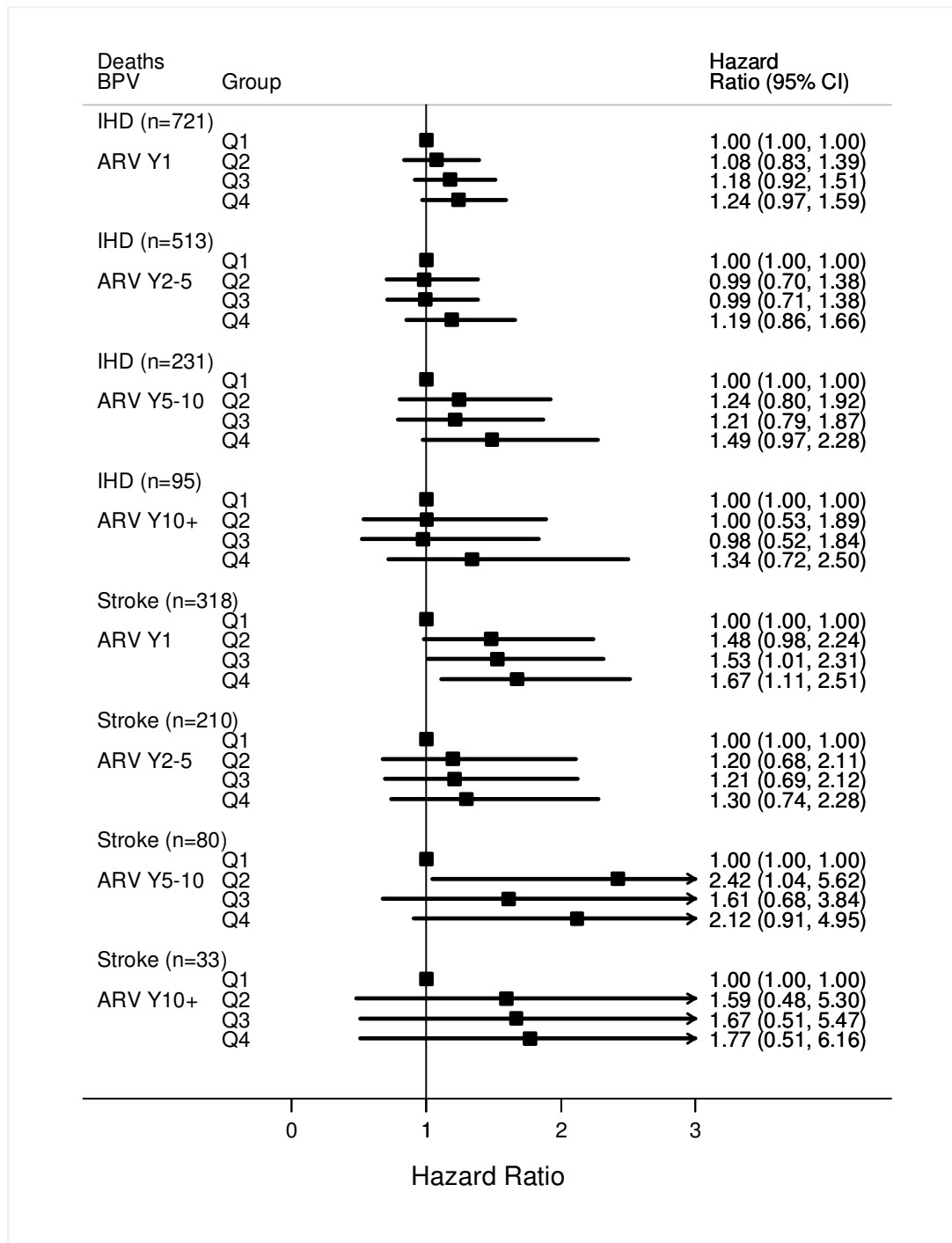
Figure 3-7: Forest plots showing the multivariate adjusted hazard ratios of quartiles of ARVDBP and IHD and stroke mortality.

DBP=diastolic blood pressure, ARV=average real variability, IHD=ischemic heart disease, CI=confidence interval.

Table 3-10: Cox proportional hazards model data for all-cause mortality, by quartiles of blood pressure variability during each period of follow-up.

All cause mortality		Year 1 2170 events	Years 2-5 1559 events	Years 5-10 707 events	Year 10+ 309 events
ARV$_{SBP}$	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	1.18 (1.02-1.36)*	1.12 (0.93-1.35)	1.24 (0.95-1.60)	0.83 (0.57-1.21)
	Q3	1.13 (0.98-1.30)	1.35 (1.12-1.61)**	1.48 (1.15-1.91)**	1.06 (0.74-1.51)
	Q4	1.37 (1.20-1.57)***	1.39 (1.16-1.67)**	1.55 (1.19-2.01)**	1.40 (0.96-2.03)
ARV$_{DBP}$	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	1.02 (0.89-1.18)	1.01 (0.84-1.22)	1.20 (0.94-1.54)	1.19 (0.83-1.72)
	Q3	1.12 (0.97-1.29)	1.06 (0.88-1.27)	1.17 (0.91-1.49)	1.25 (0.87-1.79)
	Q4	1.25 (1.09-.44)**	1.15 (0.96-1.38)	1.483(1.17-1.89)**	1.79 (1.25-2.57)**
CoeffV$_{SBP}$ %	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	1.10 (0.96-1.26)	1.12 (0.95-1.32)	1.10 (0.87-1.38)	1.13 (0.80-1.60)
	Q3	1.08 (0.95-1.24)	1.13 (0.96-1.32)	1.28 (1.02-1.61)*	1.73 (1.23-2.44)**
	Q4	1.07 (0.94-1.23)	1.27 (1.08-1.50)**	1.3 (1.09-1.73)**	1.49 (1.04-2.13)*
CoeffV$_{DBP}$ %	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	1.05 (0.91-1.22)	0.95 (0.79-1.13)	0.87 (0.68-1.12)	1.06 (0.74-1.51)
	Q3	0.92 (0.80-1.06)	0.98 (0.83-1.16)	1.12 (0.89-1.41)	1.27 (0.90-1.78)
	Q4	0.99 (0.8-1.14)	1.04 (0.89-1.24)	1.17 (0.92-1.49)	160 (1.11-2.30)*

Numbers in parenthesis indicate 95% CI of hazard ratio. *p<0.05, **p<0.01, ***p<0.001, ARV=average real variability, SBP=systolic blood pressure, DBP = diastolic blood pressure, CoeffV = coefficient of variation.

Table 3-11: Cox proportional hazards model data for cardiovascular mortality, by quartiles of blood pressure variability during each period of follow-up.

Cardiovascular mortality		Year 1 1287 events	Years 2-5 902 events	Years 5-10 388 events	Year 10+ 161 events
ARV$_{SBP}$	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	1.19 (0.99-1.44)	1.21 (0.94-1.56)	1.45 (1.01-2.08)*	1.05 (0.61-1.80)
	Q3	1.19 (0.99-1.43)	1.38 (1.08-1.77)*	1.47 (1.02-2.10)*	1.30 (0.77-2.19)
	Q4	1.44 (1.20-1.72)***	1.50 (1.16-1.92)**	1.64 (1.14-2.36)**	1.72 (1.00-2.96)*
ARV$_{DBP}$	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	1.14 (0.95-1.3)	0.99 (0.77-1.28)	1.52 (1.08-2.15)*	1.10 (0.67-1.80)
	Q3	1.21 (1.00-1.46)	1.04 (0.81-1.34)	1.25 (0.88-1.77)	1.04 (0.64-1.71)
	Q4	1.30 (1.09-1.57)**	1.19 (0.93-1.53)	1.57 (1.11-2.20)*	1.46 (0.89-2.37)
CoeffV$_{SBP}$ %	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	1.13 (0.94-1.35)	1.07 (0.86-1.33)	1.04 (0.77-1.40)	1.27 (0.78-2.07)
	Q3	1.08 (0.91-1.29)	1.11 (0.89-1.36)	1.12 (0.83-1.52)	1.79 (1.11-2.90)*
	Q4	1.04 (0.87-1.25)	1.23 (1.00-1.53)	1.19 (0.87-1.63)	1.69 (1.02-2.77)*
CoeffV$_{DBP}$ %	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	0.95 (0.79-1.15)	0.79 (0.63-0.99)*	0.78 (0.57-1.08)	1.03 (0.64-1.67)
	Q3	0.91 (0.75-1.09)	0.86 (0.69-1.07)	1.051 (0.78-1.42)	1.33 (0.84-2.09)
	Q4	0.86 (0.71-1.04)	0.91 (0.73-1.13)	0.91 (0.66-1.26)	1.46 (0.88-2.42)

Numbers in parenthesis indicate 95% CI of hazard ratio. *p<0.05, **p<0.01, ***p<0.001, ARV=average real variability, SBP=systolic blood pressure, DBP = diastolic blood pressure, CoeffV = coefficient of variation.

Table 3-12: Cox proportional hazards model data for non-cardiovascular mortality, by quartiles of blood pressure variability during each period of follow-up.

Non-CV mortality		Year 1 883 events	Years 2-5 657 events	Years 5-10 319 events	Year 10+ 148 events
ARV$_{SBP}$	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	1.16 (0.94-1.43)	1.03 (0.79-1.35)	1.03 (0.70-1.51)	0.67 (0.40-1.12)
	Q3	1.05 (0.85-1.30)	1.33 (1.02-1.72)*	1.51 (1.05-2.160)*	0.87 (0.54-1.42)
	Q4	1.28 (1.04-1.56)*	1.28 (0.98-1.67)	1.46 (1.00-2.11)*	1.14 (0.68-1.93)
ARV$_{DBP}$	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	0.88 (0.72-1.09)	1.04 (0.79-1.36)	0.91(0.63-1.31)	1.31 (0.75-2.27)
	Q3	1.01 (0.82-1.25)	1.09 (0.83-1.42)	1.10 (0.78-1.56)	1.54 (0.90-2.64)
	Q4	120 (0.97-1.47)	1.09 (0.83-1.44)	1.41 (1.00-1.98)	2.24 (1.31-3.81)**
CoeffV$_{SBP}$%	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	1.05 (0.85-1.30)	1.19 (0.93-1.53)	1.18 (0.83-1.69)	1.00 (0.61-1.65)
	Q3	1.07 (0.87-1.32)	1.15 (0.90-1.47)	1.50 (1.06-2.12)*	1.67 (1.04-2.68)*
	Q4	1.11 (0.90-1.37)	1.32 (1.03-1.67)*	1.61 (1.13-2.29)**	1.27 (0.76-2.13)
CoeffV$_{DBP}$%	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	1.21 (0.96-1.52)	1.20 (0.91-1.57)	1.01 (0.69-1.47)	1.08 (0.64-1.83)
	Q3	0.93 (0.74-1.17)	1.15 (0.88-1.51)	1.20 (0.83-1.73)	1.18 (0.71-1.98)
	Q4	1.18 (0.94-1.49)	1.24 (0.95-1.62)	1.54 (1.07-2.21)*	1.68 (0.99-2.86)

Numbers in parenthesis indicate 95% CI of hazard ratio.   *p<0.05, **p<0.01, ***p<0.001, ARV=average real variability, SBP=systolic blood pressure, DBP = diastolic blood pressure, CoeffV = coefficient of variation.

Table 3-13: Cox proportional hazards model data for IHD mortality, by quartiles of blood pressure variability during each period of follow-up.

IHD mortality		Year 1 721 events	Years 2-5 513 events	Years 5-10 231 events	Year 10 95 events
ARV$_{SBP}$	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	1.43 (1.11-1.85)**	1.55 (1.09-2.21)*	1.66 (1.03-2.69)*	1.64 (0.73-3.69)
	Q3	1.24 (0.96-1.594)	1.69 (1.19-2.41)**	1.60 (0.99-2.60)	2.05 (0.93-4.53)
	Q4	1.63 (1.28-2.09)***	1.85 (1.30-2.63)**	1.83 (1.12-2.98)*	2.66 (1.18-5.99)*
ARV$_{DBP}$	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	1.08 (0.84-1.39)	0.99 (0.70-1.38)	1.24 (0.80-1.93)	1.00 (0.53-1.89)
	Q3	1.18 (0.92-1.51)	0.99 (0.71-1.38)	1.21 (0.79-1.87)	0.98 (0.52-1.84)
	Q4	1.24 (0.97-1.59)	1.19 (0.86-1.66)	1.49 (0.97-2.28)	1.34 (0.72-2.50)
CoeffV$_{SBP}$%	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	1.23 (0.97-1.57)	1.25 (0.93-1.68)	1.10 (0.73-1.64)	1.06 (0.56-2.02)
	Q3	1.18 (0.93-1.50)	1.28 (0.96-1.71)	1.25 (0.84-1.86)	1.73 (0.93-3.21)
	Q4	1.09 (0.85-1.40)	1.35 (1.01-1.82)*	1.23 (0.81-1.86)	1.58 (0.83-3.02)
CoeffV$_{DBP}$%	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	0.97 (0.75-1.25)	0.81 (0.60-1.10)	0.84 (0.54-1.31)	0.70 (0.37-1.32)
	Q3	0.87 (0.68-1.11)	0.88 (0.66-1.18)	1.38 (0.92-2.07)	1.12 (0.64-1.95)
	Q4	0.84 (0.65-1.08)	0.89 (0.66-1.20)	1.06 (0.68-1.64)	1.17 (0.62-2.21)

Numbers in parenthesis indicate 95% CI of hazard ratio.   *p<0.05, **p<0.01, ***p<0.001, ARV=average real variability, SBP=systolic blood pressure, DBP = diastolic blood pressure, CoeffV = coefficient of variation, IHD=ischemic heart disease.

Table 3-14: Cox proportional hazards model data for stroke mortality, by quartiles of blood pressure variability during each period of follow-up.

Stroke mortality		Year 1 318 events	Years 2-5 210 events	Years 5-10 80 events	Year 10+ 33 events
ARV_SBP	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	0.870 (0.589-1.286)	1.014 (0.597-1.724)	0.679 (0.315-1.467)	0.556 (0.198-1.559)
	Q3	1.254 (0.878-1.791)	1.053 (0.627-1.767)	1.007 (0.495-2.048)	0.419 (0.143-1.224)
	Q4	1.180 (0.826-1.687)	1.338 (0.803-2.227)	1.264 (0.628-2.545)	0.931 (0.330-2.627)
ARV_DBP	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	1.482 (0.980-2.241)	1.195 (0.677-2.108)	2.421 (1.043-5.619)**	1.592 (0.479-5.295)
	Q3	1.530 (1.011-2.313)*	1.211 (0.692-2.122)	1.610 (0.676-3.836)	1.668 (0.508-5.474)
	Q4	1.672 (1.114-2.511)*	1.298 (0.739-2.279)	2.120 (0.908-4.951)	1.771 (0.509-6.159)
CoeffV_SBP%	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	0.941 (0.655-1.353)	0.928 (0.577-1.491)	0.960 (0.483-1.910)	1.087 (0.339-3.483)
	Q3	0.989 (0.698-1.399)	1.115 (0.712-1.747)	1.214 (0.624-2.360)	1.649 (0.534-5.094)
	Q4	1.003 (0.706-1.423)	1.485 (0.952-2.314)	1.305 (0.664-2.562)	2.289 (0.780-6.717)
CoeffV_DBP%	Q1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Q2	0.837 (0.578-1.210)	0.698 (0.443-1.100)	0.540 (0.289-1.012)	1.022 (0.351-2.979)
	Q3	0.806 (0.565-1.150)	0.747 (0.486-1.148)	0.514 (0.277-0.954)*	1.405 (0.515-3.836)
	Q4	0.771 (0.536-1.109)	0.787 (0.509-1.217)	0.521 (0.273-0.996)*	1.510 (0.493-4.625)

Numbers in parenthesis indicate 95% CI of hazard ratio.   *$p<0.05$ **$p<0.01$ ***$p<0.001$

ARV=average real variability, SBP=systolic blood pressure, DBP = diastolic blood pressure, CoeffV = coefficient of variation.

3.3.6 Sub-group analysis

The sub-group analyses (stratified by baseline BP) for all-cause mortality and CV mortality are presented as forests plots in figures 3-8 and 3-9 on pages 111-112. All BP sub-groups showed consistently elevated all-cause mortality in ARVSBP quintiles 3 and 4 as compared to quintile 1. Similar results were observed for CV mortality.

3.3.7 Four Groups Analysis

When Y1 was compared with Y2-5, significant associations were observed between all-cause mortality and ARVSBP four groups (Log rank p < 0.001). The lowest mortality risk was in the low-low variability group, and the highest risk in the high-high variability group. When Y2-5 was compared with Y5-10 the pattern of results was the same. Figures 3-10 to 3-15 presents (pages 113-115) the Kaplan Meier curves for the four groups' analysis of ARVSBP for all-cause, CV, and non-CV mortality outcomes.

For ARVDBP, membership in one of the four groups was associated with all-cause mortality when Y1 was compared with Y2-5 (Log rank p < 0.05). However, when Y2-5 was compared with Y5-10 the picture was less clear. Membership in one of the four groups was associated with all-cause, CV, and IHD mortality (Log rank p < 0.05), but neither non-CV nor stroke mortality. Figures 3-16 to 3-21 (pages 116-118) presents the Kaplan Meier curves for the four groups analysis of ARVSBP for all-cause, CV, and non-CV death.

The Cox-PH model data by four groups of ARVSBP and ARVDBP BPV comparing follow-up periods Y1 and Y2-5, for all mortality outcomes, are presented in table 3-14 on page 109.   For all-cause and CV mortality the highest risk was in the high-high group (HR = 1.44, 95% CI = 1.21-1.71; and HR = 1.46, 95% CI 1.16-1.84, respectively), whereas non-CV mortality risk was highest in the low-high group (HR = 1.57, 95% CI = 1.17-2.12). No association between group membership and mortality was observed for diastolic BP variability.

ARVSBP &
All-cause
Deaths

Hazard
Ratio (95% CI)

Y1 (<140 mmHg), n=183
Q1   1.00 (1.00, 1.00)
Q2   1.24 (0.83, 1.85)
Q3   1.32 (0.86, 2.02)
Q4   1.42 (0.92, 2.17)

Y2-5 (<140 mmHg), n=219
Q1   1.00 (1.00, 1.00)
Q2   1.65 (1.16, 2.36)
Q3   1.64 (1.11, 2.42)
Q4   1.90 (1.23, 2.94)

Y1 (140-160 mmHg), n=678
Q1   1.00 (1.00, 1.00)
Q2   1.27 (1.02, 1.59)
Q3   1.15 (0.91, 1.45)
Q4   1.60 (1.27, 2.01)

Y2-5 (140-160 mmHg), n=600
Q1   1.00 (1.00, 1.00)
Q2   1.10 (0.84, 1.44)
Q3   1.40 (1.08, 1.82)
Q4   1.41 (1.08, 1.84)

Y1 (>160 mmHg), n=1309
Q1   1.00 (1.00, 1.00)
Q2   1.13 (0.92, 1.39)
Q3   1.19 (0.98, 1.45)
Q4   1.47 (1.22, 1.77)

Y2-5 (>160 mmHg), n=740
Q1   1.00 (1.00, 1.00)
Q2   1.01 (0.70, 1.46)
Q3   1.36 (0.96, 1.93)
Q4   1.53 (1.08, 2.15)

.5   1   1.5   2   2.5   3

Hazard Ratio

Figure 3-8: Forest plot for quartiles of average real systolic blood pressure variability and all-cause mortality stratified by baseline blood pressure categories.
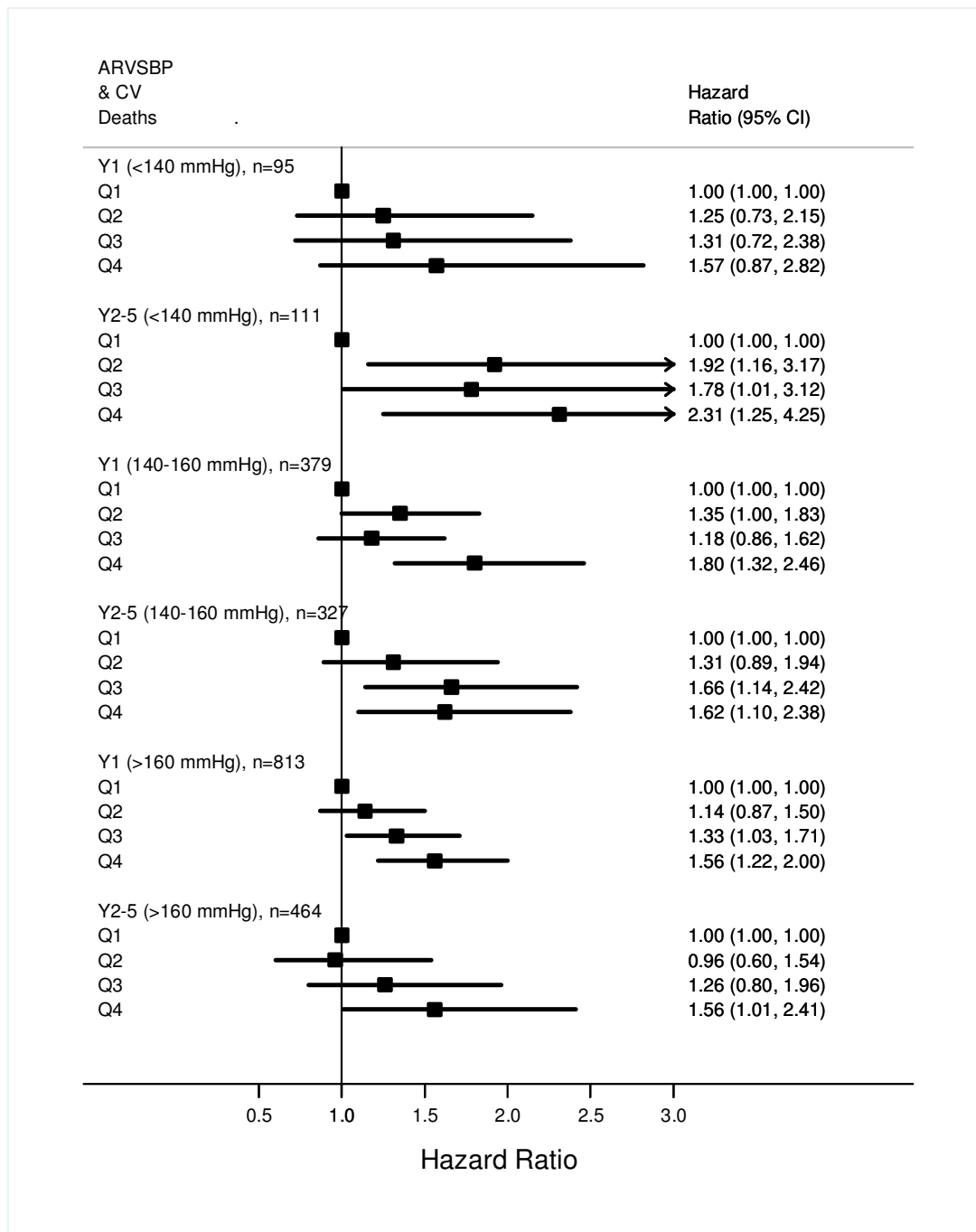
Figure 3-9: Forest plot for quartiles of average real systolic blood pressure variability and cardiovascular mortality stratified by baseline blood pressure categories.
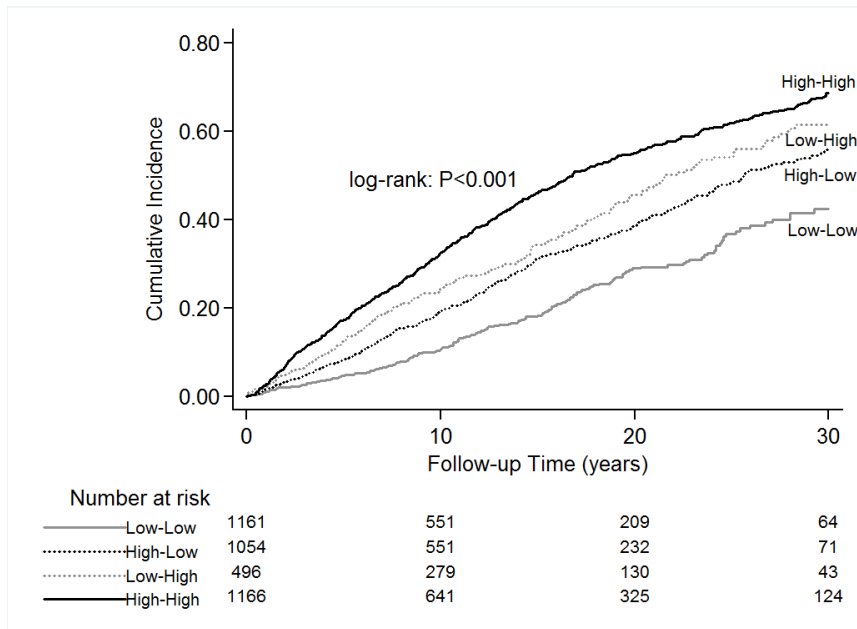
Figure 3-10: Kaplan-Meir survival curves of ARV$_{SBP}$ changes between Y1 and Y2-5 for all-cause mortality.
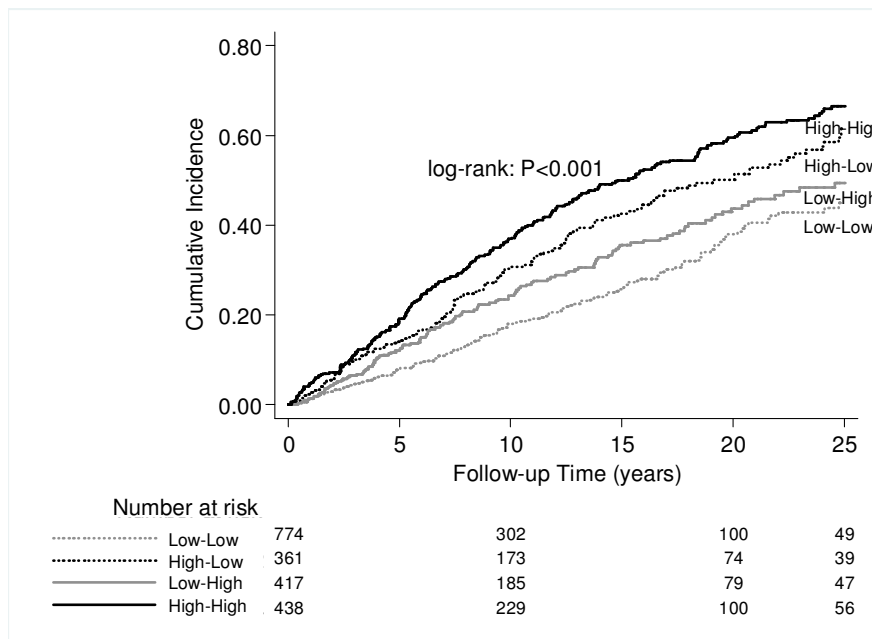
Figure 3-11: Kaplan-Meir survival curves of ARV$_{SBP}$ changes between Y2-5 and Y5-10 for all-cause mortality.

Comparison of ARVSBP between Y2-5 and Y5-10. Four groups of BPV: High-High (high ARVSBP at both time points of comparison), High-Low (high ARVSBP at initial assessment and low ARVSBP at later assessment), Low-High (low ARVSBP at initial assessment and high ARVSBP at later assessment), Low-Low (low ARVSBP at both time points). SBP=Systolic Blood Pressure. ARV=Average Real Variability.
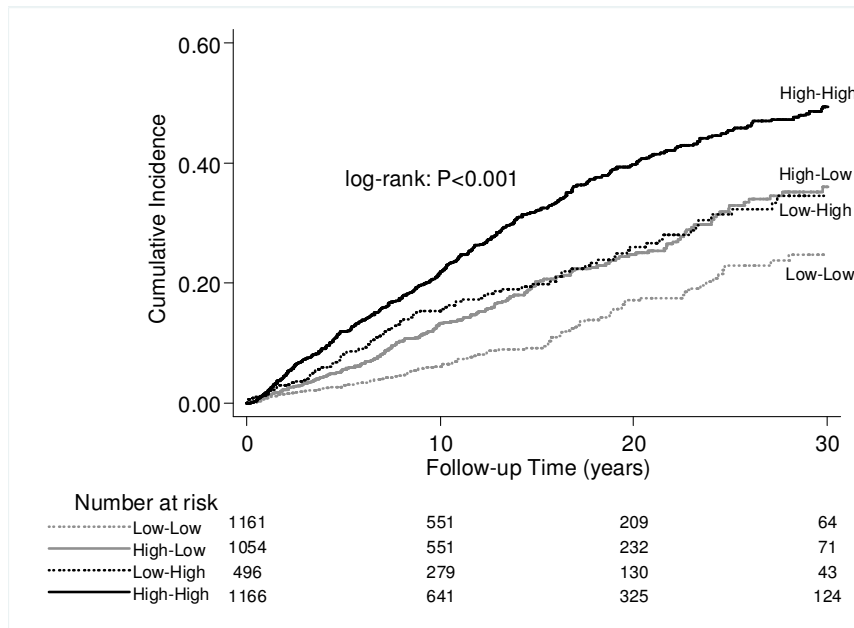
Figure 3-12: Kaplan-Meir survival curves of ARV$_{SBP}$ changes between Y1 and Y2-5 for cardiovascular mortality.

Comparison of ARVSBP between Y1 and Y2-5.

Figure 3-13: Kaplan-Meir survival curves of ARV$_{SBP}$ changes between Y2-5 and Y5-10 for cardiovascular mortality.

Comparison of ARVSBP between Y2-5 and Y5-10. Four groups of BPV: High-High (high ARVSBP at both time points of comparison), High-Low (high ARVSBP at initial assessment and low ARVSBP at later assessment), Low-High (low ARVSBP at initial assessment and high ARVSBP at later assessment), Low-Low (low ARVSBP at both time points). SBP=Systolic Blood Pressure. ARV=Average Real Variability.

Number at risk				
Low-Low	1161	551	209	64
High-Low	1054	551	232	71
Low-High	496	279	130	43
High-High	1166	641	325	124

Figure 3-14: Kaplan-Meir survival curves of ARV$_{SBP}$ changes between Y1 and Y2-5 for non-cardiovascular mortality.

Comparison of ARVSBP between Y1 and Y2-5.

Number at risk				
Low-Low	774	302	100	49
High-Low	361	173	74	39
Low-High	417	185	79	47
High-High	438	229	100	56

Figure 3-15: Kaplan-Meir survival curves of ARV$_{SBP}$ changes between Y1 and Y2-5 for non-cardiovascular mortality.

Comparison of ARVSBP between Y2-5 and Y5-10. Four groups of BPV: High-High (high ARVSBP at both time points of comparison), High-Low (high ARVSBP at initial assessment and low ARVSBP at later assessment), Low-High (low ARVSBP at initial assessment and high ARVSBP at later assessment), Low-Low (low ARVSBP at both time points). SBP=Systolic Blood Pressure. ARV=Average Real Variability.

Figure 3-16: Kaplan-Meir survival curves of ARV$_{DBP}$ changes between Y1 and Y2-5 for all-cause mortality.

Comparison of ARVDBP between Y1 and Y2-5.
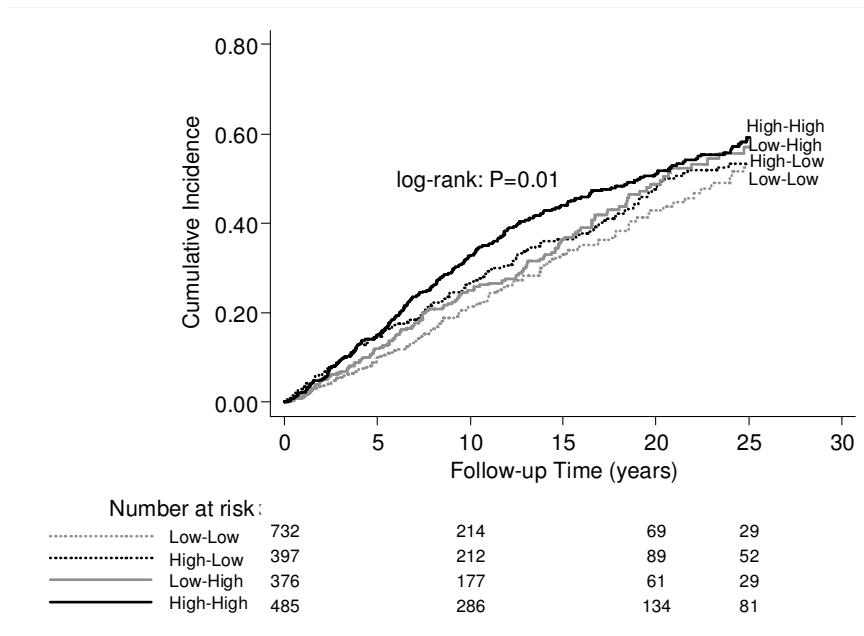
Figure 3-17: Kaplan-Meir survival curves of ARV$_{DBP}$ changes between Y2-5 and Y5-10 for all-cause mortality.

Comparison of ARVDBP between Y2-5 and Y5-10. Four groups of BPV: High-High (high ARVDBP at both time points of comparison), High-Low (high ARVDBP at initial assessment and low ARVDBP at later assessment), Low-High (low ARVDBP at initial assessment and high ARVDBP at later assessment), Low-Low (low ARVDBP at both time points). DBP=Systolic Blood Pressure. ARV=Average Real Variability.
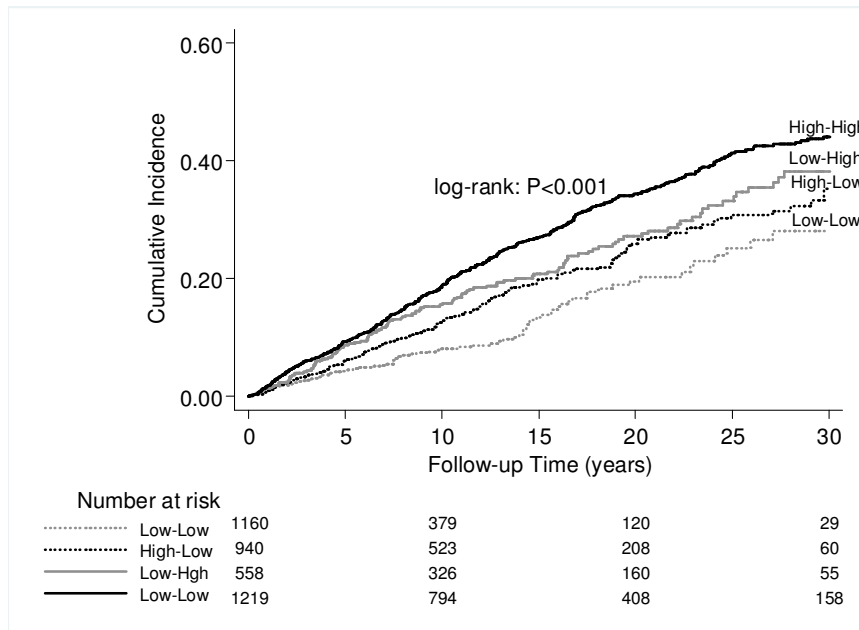
Figure 3-18: Kaplan-Meir survival curves of ARV$_{DBP}$ changes between Y1 and Y2-5 for cardiovascular mortality.

Comparison of ARVDBP between Y1 and Y2-5.
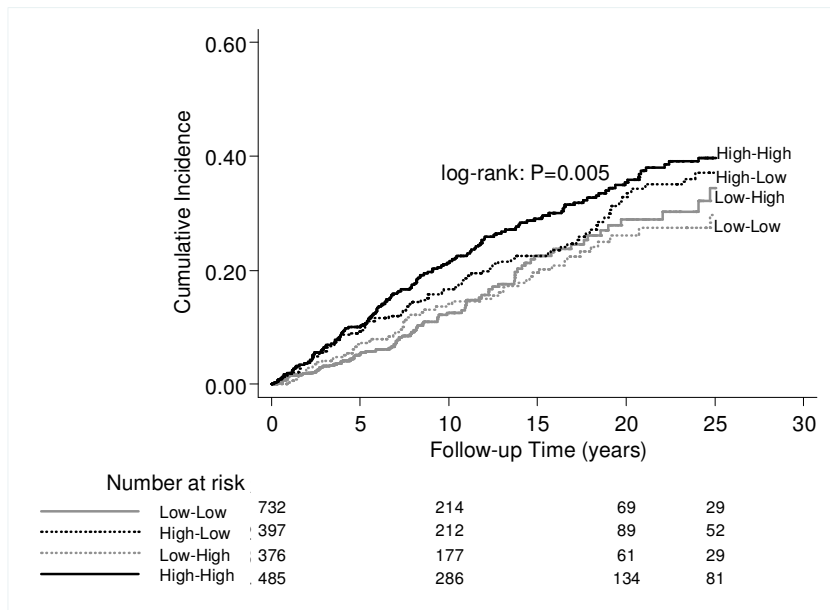
Figure 3-19: Kaplan-Meir survival curves of ARV$_{DBP}$ changes between Y2-5 and Y5-10 for cardiovascular mortality.

Comparison of ARVDBP between Y2-5 and Y5-10. Four groups of BPV: High-High (high ARVDBP at both time points of comparison), High-Low (high ARVDBP at initial assessment and low ARVDBP at later assessment), Low-High (low ARVDBP at initial assessment and high ARVDBP at later assessment), Low-Low (low ARVDBP at both time points). DBP=Systolic Blood Pressure. ARV=Average Real Variability.
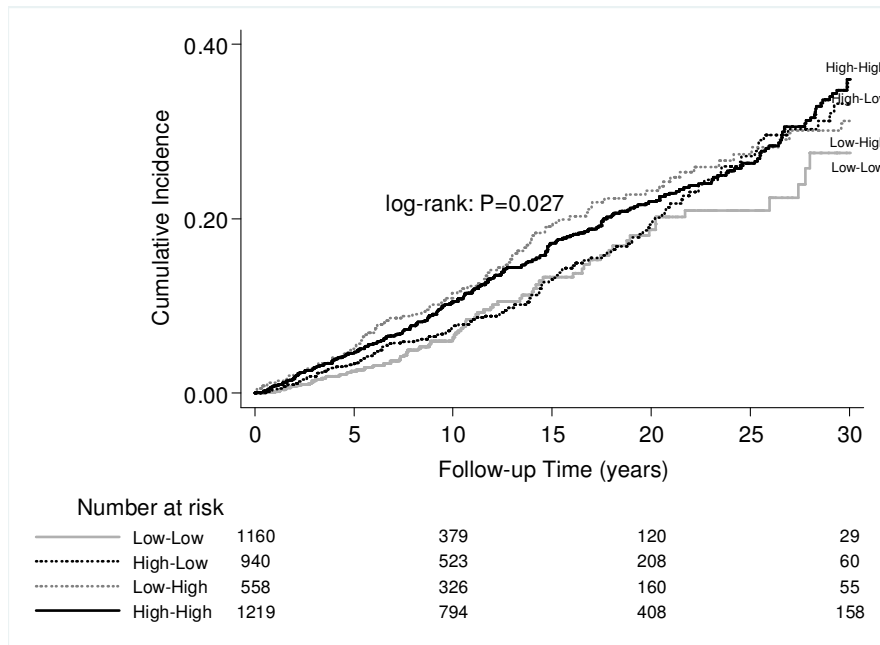
Figure 3-20: Kaplan-Meir survival curves of ARV_DBP changes between Y1 and Y2-5 for non-cardiovascular mortality.

Comparison of ARVDBP between Y1 and Y2-5.
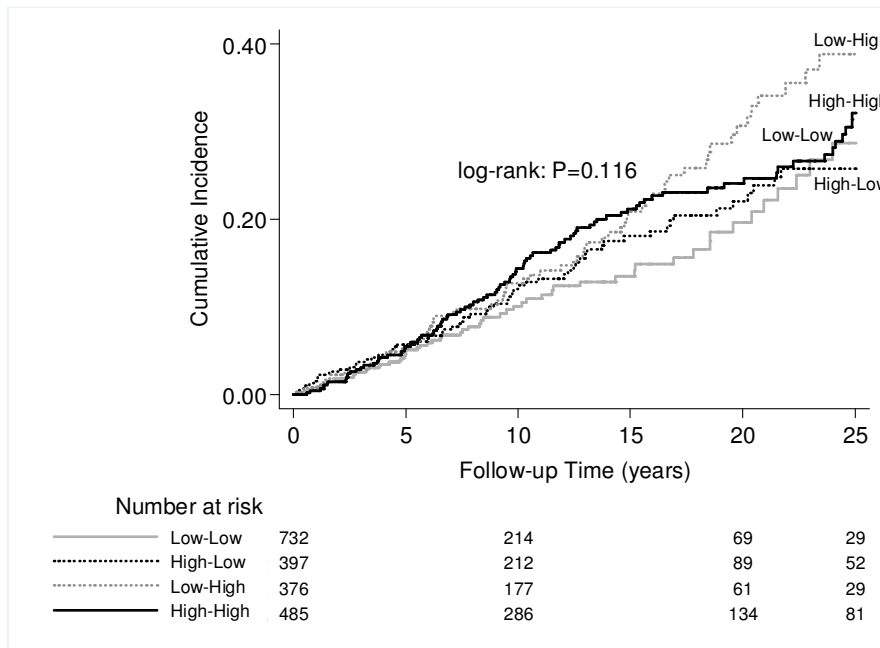
Figure 3-21: Kaplan-Meir survival curves of ARV_DBP changes between Y2-5 and Y5-10 for non-cardiovascular mortality.

Comparison of ARVDBP between Y2-5 and Y5-10. Four groups of BPV: High-High (high ARVDBP at both time points of comparison), High-Low (high ARVDBP at initial assessment and low ARVDBP at later assessment), Low-High (low ARVDBP at initial assessment and high ARVDBP at later assessment), Low-Low (low ARVDBP at both time points). DBP=Systolic Blood Pressure. ARV=Average Real Variability.

Table 3-15: Cox proportional hazards model data for all cause mortality, cardiovascular mortality, non-cardiovascular mortality, IHD mortality, and stroke mortality, by four groups of blood pressure variability comparing follow-up periods Year 1 and Years 2-5.

Mortality		Y1 vs Y2-5 ARV_{SBP}	Y1 vs Y2-5 ARV_{DBP}
All cause mortality 1267 events	low-low	1.00 (Ref)	1.00 (Ref)
	high-low	1.224 (1.021-1.468)*	1.079 (0.891-1.305)
	low-high	1.336 (1.089-1.640)**	1.218 (0.993-1.495)
	high-high	1.437 (1.208-1.710)***	1.218 (1.017-1.458)*
CV mortality 739 events	low-low	1.00 (Ref)	1.00 (Ref)
	high-low	1.290 (1.012-1.644)*	1.104 (0.853-1.428)
	low-high	1.168 (0.881-1.548)	1.176 (0.892-1.550)
	high-high	1.461 (1.159-1.842)**	1.230 (0.966-1.565)
NonCV mortality 528 events	low-low	1.00 (Ref)	1.00 (Ref)
	high-low	1.137 (0.864-1.496)	1.054 (0.793-1.400)
	low-high	1.573 (1.167-2.121)**	1.288 (0.950-1.748)
	high-high	1.398 (1.074-1.819)*	1.200 (0.914-1.576)
IHD mortality 425 events	low-low	1.00 (Ref)	1.00 (Ref)
	high-low	1.291 (0.944-1.764)	0.910 (0.651-1.273)
	low-high	1.128 (0.781-1.630)	1.008 (0.705-1.441)
	high-high	1.381 (1.023-1.864)*	1.105 (0.813-1.504)
Stroke mortality 170 events	low-low	1.00 (Ref)	1.00 (Ref)
	high-low	1.497 (0.863-2.595)	1.535 (0.873-2.699)
	low-high	1.533 (0.833-2.822)	1.237 (0.657-2.329)
	high-high	1.747 (1.034-2.951)*	1.522 (0.886-2.615)

ARV=average real variability, SBP=systolic blood pressure, DBP = diastolic blood pressure, CV = cardiovascular, IHD = ischaemic heart disease. *$p<0.05$ **$p<0.01$ ***$p<0.001$.

3.4 Discussion

In this study the relationship between BPV during different follow-up time points and mortality in a large cohort of hypertensive patients with treated hypertension was investigated. Blood pressure variability calculated over different sequential time-frames (of 1 to 5 years duration) are strong predictors of mortality independent of long-term average BP. The study also shows that even with well controlled mean SBP<140 mmHg there is a linear increase in mortality with increasing BPV.

The linear relationship between BPV and mortality is maintained in all time-frames including Y_1 despite higher mean BPV in Y_1. While the higher BPV during the first year of follow-up reflects variability primarily induced by a greater degree of physician related dose titration to get BP to target, BPV during successive time frames, Y2-5, Y5-10, Y10+ more likely reflect basal variability on an established treatment regime. Since these individuals were attending the BP clinic regularly, it is unlikely that the effect of BPV during later follow-up time

points on mortality after adjustment for mean BP is influenced by drug adherence.

The relationship between BPV and mortality independent of mean BP suggests that the BPV captures a risk trait that is a marker of haemodynamic response rather than BP. The present study data contrasts with BPV from RCTs where drugs were titrated based on a fixed protocol thus minimising within-subject and between-subject variability. This can reflect the greater likelihood of compliance of patients who enrol in clinical trials. In a real-life hypertension clinic practice, the spectrum of patients is not selected and the treatment titration/escalation varies with each patient, though they tend to follow current guidelines.

Despite low overall reproducibility of BPV over sequential time-frames, 60% of individuals show sustained high or low BPV over even longer time-frames of 6 to 9 years and this sustained BPV status is also a strong predictor of mortality independent of long-term average BP. The results also suggest that the poor reproducibility of BPV is not a reflection of a trait that is completely random, but instead there is an underlying structure within subgroups of individuals who show consistent BPV in different time frames or do not. The consistent BPV sustained over long time-periods occurs irrespective of the length of time over which BPV was calculated - the study compared BPV calculated over 1 year and 4 year periods and, in another analysis, over 4 year and 5 year periods. The low reproducibility of BPV in different time-frames may indicate a context-dependency on BPV status; however despite this participants who maintained a low BPV over time had the lowest mortality and those who showed consistently high variability over time showed the highest mortality. All these indicate that BPV captures a risk trait that is unrelated to BP and could reflect specifically a response to external challenge. The present study findings also indicate that ARV_{SBP} =17, calculated over any follow-up time frame greater than 1 year and using a minimum of 3 readings more that 30 days apart, can be used as a threshold to define treated hypertensive patients as low or high risk. A threshold of 17 was taken as it was the median of ARV_{SBP} in the study population.

The present study findings are consistent with the recent publications that have suggested a prognostic role for visit-to-visit BPV, independent of mean blood

pressure [362] [544]. One of the original studies in this area, carried out in 1997, identified an association with visit-to-visit BPV and an increased risk of coronary events [541]. Post hoc analyses of large randomised control trials (RCTs) have found associations between high visit-to-visit BPV and increased stroke incidence in a population of subjects at increased risk of vascular events [359]. Moreover, additional analyses of treated hypertensive populations in the Anglo-Scandinavian Cardiac Outcomes Trial- Blood Pressure Lowering Arm (ASCOT) found that increased systolic BPV between clinic visits was associated with stroke and coronary events [361]. However, the present study did not find any association with stroke mortality which is not surprising as stroke incidence is a more specific outcome and different from stroke mortality. Additionally, there was no separate analysis for types of stroke events (ischemic or hemorrhagic) in the previous studies. More serious stroke events (hemorrhagic) may have contributed to a larger proportion of stroke mortality in the present study. This would have partially explained the differences observed in the association between BPV and stroke mortality. The only study which evaluated mortality was the NHANES study, which demonstrated a 50% increased risk in subjects in the higher tertiles of BP standard deviation compared to the lowest tertile [542]. However, this study was relatively small (n=958 and 240 deaths) and BP measurements were recorded during in-home visits and at mobile examination centres by different staff. In addition, variability was calculated over just 3 clinic visits separated by days, suggesting intermediate variability may be the most appropriate classification of BP phenotype investigated here. In the present study, a large treated hypertensive population not randomised to intervention was examined, and found significant independent associations with increasing BPV and all cause mortality, and additionally discovered significant independent associations with CV and non-CV mortality. This study mimics real life practice unlike the previous studies.

The BPV was calculated and analysed in different time frames rather than the entire follow-up period for the following reasons - the determination of BPV depends on multiple BP measurements over a period of time, and in real-life practice it is impossible to maintain the same frequency of BP measurements over long time periods, in addition other factors that may affect BPV over time such as age, drug treatment, renal function etc vary over time, and hence BPV is more informative and accurate when calculated over shorter periods. The

observed poor reproducibility is due to the subgroup of individuals who swing between higher and lower BPV categories during follow-up. This can be explained parsimoniously if the visit-to-visit variation in BP is considered as a waveform. In individuals with BPV sustained over long time periods this would be a simple waveform where the frequency domain remains constant during any window of observation, and the risk is attributed to the amplitude of the waveform (magnitude of excursion of BP). In individuals who change BPV categories between time-frames, the frequency domain of the BP waveform would be more complex, and reflects BPV occurring over a longer time period than the time-frame over which it is calculated. Thus individuals whose BPV changes between time frames will behave as if their BPV is intermediate to those with sustained high BPV and sustained low BPV due to a mixture of waveforms of differing amplitude and frequency underlying their BPV. Interestingly, Mancia et al showed that Fourier analysis–derived residual component of 24 hour BP waveform was a strong predictor of cardiovascular events and all-cause death [344]. The present study observations indicate that long-term variability may also be amenable to spectral analyses, which may make it possible to extract prognostic information of variability from a shorter window of observation.

It may be argued that differences in risk seen in the sustained low BPV and high BPV groups may simply reflect the differences in the burden of co-morbidities, despite statistically adjusting for them in the models. While this is a plausible explanation, there are certain indicators that the relationship is real. For example, the proportion of women is significantly lower in the low-low group compared to the high-high group (women report longer survival time than men). While both BP and BPV increase with age, we show that BPV remains an independent predictor of risk at all strata of mean BP. More importantly, the survival analyses were conditional on the individual surviving for 5 years in the Y_1-Y_{2-5} group and 9 years in the Y_{2-5}-Y_{10} group, thus minimising reverse causation confounding the results in the four-groups analyses.

Interestingly the present study found the relationship to mortality more consistent for ARV rather than coefficient of variation. However, both these measures reflect different methods of measuring variability and may represent different phenotypes. ARV measures variability by taking into account the order

of BP readings while coefficient of variation is an absolute measure of variation. However, the exact mechanisms at play which create this "variability" remain unconfirmed [545] [549] [550].

Studies so far have not identified a prognostic role for diastolic BPV. In Rothwell's analyses of ASCOT, an association with stroke risk was present only in the highest deciles of variability [359]. The present study offers new insight into the prognostic role of DBP variability, presenting significant associations with all-cause, CV and non-CV mortality outcomes independent of mean DBP. Better understanding of the mechanisms of BPV induced survival risk may help enhance risk stratification and prediction strategies in hypertension management.

3.5 Limitations

The present study is however not without limitations; the observational nature lends itself to bias, and all subject data are derived from a predominantly White hypertensive cohort. The role of antihypertensive agents in determining BP variability was not studied due to incomplete prescribing data available for this analysis. However, previous publication on heart rate and mortality in these patients demonstrate that >90% of those who were prescribed beta-blockers had heart rate <70/min [551]. This would suggest that non-adherence to treatment may not be a major factor among the patients who attend clinic regularly and these are patients included in the current analyses. Additionally, compliance was not a major issue in the ASCOT study. The role of specific antihypertensive agents was not studied - however, heart rate was looked at as a surrogate for beta blockers (presumed to increase BPV [360] [547]) during follow-up, to see if patients whose variability changed from low to high produced a similar change in heart rate, either due to commencing beta blocker therapy or discontinuing calcium channel blocker therapy (presumed to decrease BPV [360]). There was no change in heart rate over Y_{2-5} and Y_{5-10} of follow-up, which supports the impression that BPV during later years of follow-up is largely dissociated from treatment induced BPV. There were no data on the physical activity levels in the GBPC cohort to facilitate its inclusion into the PH models. Data on patient compliance would have been insightful to include in the analyses. The nature of the present study analyses lead to exclusion of deaths which occurred during the time-frame over which BPV was calculated. Furthermore, individuals who did not have the

requisite number of BP measurements within the time-frame were also excluded. This would have introduced some bias in our analyses. The present study mimics real life practice and given the high prevalence of hypertension, the results of our study can be considered generalisable.

3.6 Summary

The linear association of increasing BPV with higher mortality, independent of average BP and irrespective of the duration or context in which BPV was measured, indicates that BPV is likely a fundamental physiologic trait that is a marker of early mortality. Visit-to-visit BPV is an important prognostic indicator of long-term mortality, and physicians should be made aware that variability is measureable in clinical practice and should not be disregarded as random fluctuation between visits. The clinical implications of BPV depend on whether there are interventions that can change BPV and whether these interventions will result in improvement in outcomes. New diagnostic strategies should incorporate the known effect of BPV on mortality risk and antihypertensive drugs should be chosen to minimise variability as much as possible whilst also lowering usual office BP.

4 Haematocrit and mortality

4.1 Introduction

Haematocrit (Hct) is the proportion of blood volume occupied by red blood cells. It is a major determinant of blood viscosity, BP, venous return, cardiac output, and platelet adhesiveness [388-390 392]. Although, plasma is mostly water it is about 1.8 times more viscous than water at 37 degree Celsius. An increase in red cell count leads to increase in relative viscosity, resistance to blood flow, increase in work load of heart and impairment of organ perfusion [552]. Blood viscosity is also dependent on the flow velocity, and a low-flow state permits increased molecular interaction between red cells and plasma proteins. This leads to red cells to stick together and form chains of several cells and further increases the blood viscosity and impairs blood flow [552]. Experiments in healthy individuals suggest that an eleven percent increase in Hct is associated with 20% increase in relative viscosity, 17% reduction in flow rate and 20% increase in BP [553]. It is also estimated that the prevalence of hypertension is at least two times greater with 10 units increase in Hct [554].

Associations between Hct and risk of cardiovascular disease, including CHD and stroke have been demonstrated in several prospective studies [393-395]. A sex specific effect of Hct on carotid atherosclerosis is reported in men but not in women [555-557]. Multiple studies report independent association of plasma viscosity and carotid artery thickening in terms of intima-media thickness (IMT) [558 559]. Furthermore, higher Hct is independently associated with reduced reperfusion and greater infarct size in survivors of ischemic stroke [560]. Diabetes risk is also elevated in individuals with high Hct levels. Compared to individuals in the lowest quartile of Hct, adults in the higher quartile were 63% more likely to develop diabetes in the Atherosclerosis Risk in Communities (ARIC) study [561].

While the association between Hct and mortality has been described as "J" or "U" shaped in women, it is inverse-linear in men [393-395]. In contrast, a population-based 17-year follow-up study reported a U-shaped relationship between Hct and CHD mortality (with the lowest CHD mortality rates being seen among those with Hct in the middle tertile) in men and a positive linear relationship in women (with linear increase in CHD mortality from the lowest to

highest tertile of Hct) [393]. Coronary heart disease risk was 80% higher in individuals with borderline polycythemia (Hct >0.50) even after adjustment for established coronary risk factors in a 28-year follow-up study among men aged 55 years [396]. Elevated Hct is an independent predictor of mortality within the first 28 days of ischemic stroke in women [562].

The observed association between Hct and BP [392 563] and the sex differences in blood viscosity and Hct levels [564] may be important factors to consider in the management of hypertensive patients. The independent association between Hct and long-term mortality was examined to dissect the sex-specific differences in risk associated with Hct in a large hypertensive cohort after the initiation of antihypertensive treatment.

4.2 Methods

4.2.1 Study setting and study population

General details of the study setting and study population are described in section 2.1. Briefly, the study population comprised of attendees of the Glasgow Blood Pressure Clinic (GBPC). The GBPC provides secondary and tertiary level service to individuals with hypertension from the west of Scotland.

4.2.2 Clinical Measurements

Blood samples collected at the first visit and during follow-up at the BP clinic were analysed by the Western Infirmary central laboratory services using an auto-analyser, which calculates Hct as the product of red blood cell count and the erythrocyte mean cell volume. BP measurements at the clinic were taken manually 3 times using standardized sphygmomanometers at each visit by specialist hypertension nurses; the mean of the last 2 measurements was recorded at each visit. Estimated glomerular filtration rate was calculated from the baseline serum creatinine values using the MMRD Study Group equation [501]. More details are provided in section 2.2-2.4.

4.2.3 Outcome Assessment

The details of outcome assessment are described in detail in the section 2.5.

4.2.4 Subject Classification

The study population was divided into 4 different groups based on Hct quartiles. Men and women have different reference ranges of Hct, hence 2 groups of quartiles separately for men and women were derived. These were <=0.420, 0.421 to 0.440, 0.441 to 0.460, and >0.461 for men and <=0.380, 0.381 to 0.400, 0.401 to 0.420, and >0.421 for women. The response to treatment for a subgroup of study population was examined to explore potential causal associations among BP, Hct, and mortality. Participants who had been followed for a minimum of 5 years were selected and categorised into two groups, responders and non-responders. Responders were people in whom BP was lowered to <=140/90 mmHg measured >30 days after the first visit and remained below that level for at least a year, and non-responders were subjects who did not meet these criteria.

4.2.5 Statistical Analysis

The baseline characteristics of study subjects in different Hct quartiles were compared using ANOVA for continuous variables and Chi square test for categorical variables.

The details of survival analysis are explained in section 2.6. Kaplan-Meir survival model was used to generate cumulative incidence of mortality over the follow-up period (as explained in section 2.6.1) stratified by Hct quartiles in men and women. The PH assumption was tested by visually comparing the estimated log (-log) survival curves of Hct quartiles (as explained in section 2.6.3) in men and women. Cox proportional hazards models (section 2.6.2) were set up to analyse the influence of baseline Hct on all-cause, CV, ischemic heart disease (IHD), stroke, and non-CV mortality. The covariates included were baseline age, sex, body mass index, smoking status, systolic BP (SBP), diastolic BP, alcohol use, tobacco use, eGFR, and CVD co-morbidity. A variable on year of first visit strata (epochs) was used to adjust the secular trend in mortality and was divided into 5 categories (first visit 1977 or before, between years 1978 and 1985, 1986 and 1993, 1994 and 2001, or 2002 and after). Initially Hct was assessed as a continuous variable in the overall population (model 1). Because Hct range was different in men and women and since a non-linear trend in mortality was

observed, further analyses were performed using Hct as a categorical variable based on sex-specific quartiles (model 2 in men and model 3 in women). The final models included all of the covariates that were significant (P<0.10) in the multivariate analysis. A similar analysis protocol was followed for responder and non-responder subgroups. To model efficiently the nonlinear relationship observed for Hct and mortality, multivariable Cox regression models with restricted spline functions (section 2.6.4) were used [565].

Generalized estimating equation (GEE) regression models were used to study the change in SBP and diastolic BP over the initial 5 years of follow-up across Hct quartiles. Details of GEE analysis are presented in section 2.7.1. The GEE model was adjusted for age, sex, smoking, alcohol use, body mass index, and estimated glomerular filtration rate.

The association between Hct quartiles, the time to reach target BP, and the number of antihypertensive drugs prescribed were assessed using log-rank test in a bivariate model (as in section 2.6.1) and Cox proportional hazards regression in a multivariate model (as in section 2.6.2) after adjustment for all of the covariates that were significant (P<0.1). Data were censored after 5 years of follow-up and analyses performed in men and women separately. All of the analyses were performed using Stata, version 12 (Stata Corp).

4.3 Results

4.3.1 Baseline Characteristics

A total of 10951 patients with baseline Hct values were included in the current analysis. The full demographic and clinical characteristics for men and women are given in table 4-1 on page 130. More than half of the study patients were women (51.3%; n=5722). Men were younger than women (49.6 versus 51.3 years), and both were similarly overweight (body mass index, 27.5). Compared with women, men had significantly lower values for SBP (mean SBP: 164 Vs 169) and higher estimated glomerular filtration rate (mean eGFR: 76 Vs 72), and a significantly higher proportion of men reported alcohol (86% Vs 69%) and tobacco use (50% Vs 42%). A total of 192 and 503 people had missing data for tobacco use and alcohol use, respectively.

Hct was higher in men (median, 0.44; interquartile range, 0.42– 0.47) than in women (median, 0.41; interquartile range, 0.38–0.43). The distributions of Hct in men and women are shown as probability density plots in figure 4-1 on page 130. The baseline characteristics of the study population stratified by sex and sex-specific Hct quartiles are shown in table 4-2 on page 131. The lowest Hct quartile in men was <=0.420 (range, 0.280–0.420), whereas for women it was <=0.380 (range, 0.220–0.380). The highest quartile was also different for men and women (>0.461 [range, 0.461 0.670] and 0.421 [range, 0.421– 0.550], respectively).

4.3.2 Follow-Up Period and Event Rates

Complete follow-up event data with information on all of the baseline risk variables were available for 10007 participants. During the 173246 person-years of follow-up, there were 3484 (34.8%) all-cause deaths; 52.6% of these deaths were in men. There were 1920 cardiovascular deaths (19.2%), 56.1% in men; 1077 IHD deaths (10.8%), 60.4% in men; 458 stroke deaths (4.6%), 50.7% in men; and 1379 non-CVD deaths (13.8%), 48.1% in men. Unadjusted mortality rates in deaths per 1000 person-years are presented in table 4-3 on page 134. A J-shaped pattern in men for all-cause and CVD mortality and a U-shaped pattern in women for all-cause and non-CVD mortality were observed (Table 4-3 on page 134 and figure 4-2 on page 132). The lowest mortality rate in both men and women for all-cause mortality was in quartile 2, corresponding with a Hct range of 0.421 to 0.440 and 0.381 to 0.400, respectively. The median survival rates for each quartile 1 to 4, in men and women, were 24.10/29.94/26.56/24.93 and 34.08/34.76/32.79/28.38 years, respectively (log-rank P value for both men and women <0.001).

Table 4-1: Baseline characteristics of the study population.

Variables	Men (N=5229)	Women (N=5722)	P value
Age at first visit (years), mean (SD)	49.58 (12.86)	51.28 (15.10)	<0.001
BMI (Kg/m^2), mean (SD)	27.47 (4.83)	27.47 (7.02)	0.96
SBP (mmHg), mean (SD)	164.35 (27.08)	168.49 (30.25)	<0.001
DBP (mmHg), mean (SD)	99.67 (14.91)	97.50 (15.04)	<0.001
eGFR (mL/min per 1.73 m^2), mean (SD)	76.28 (27.12)	71.50 (33.26)	<0.001
Alcohol Use, n (%)	4297 (86)	3764 (69.0)	<0.001
Tobacco Use, n (%)	2555 (49.8)	2348 (41.7)	<0.001
Baseline CVD, n (%)	1145 (21.9)	975 (17.0)	<0.001
Hct (%), mean (SD)	0.443 (0.04)	0.406 (0.04)	<0.001

BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, eGFR=estimated glomerular filtration rate based on MDRD equation, Baseline CVD=prevalent cardiovascular disease at baseline, Hct=heamatocrit, SD=standard deviation
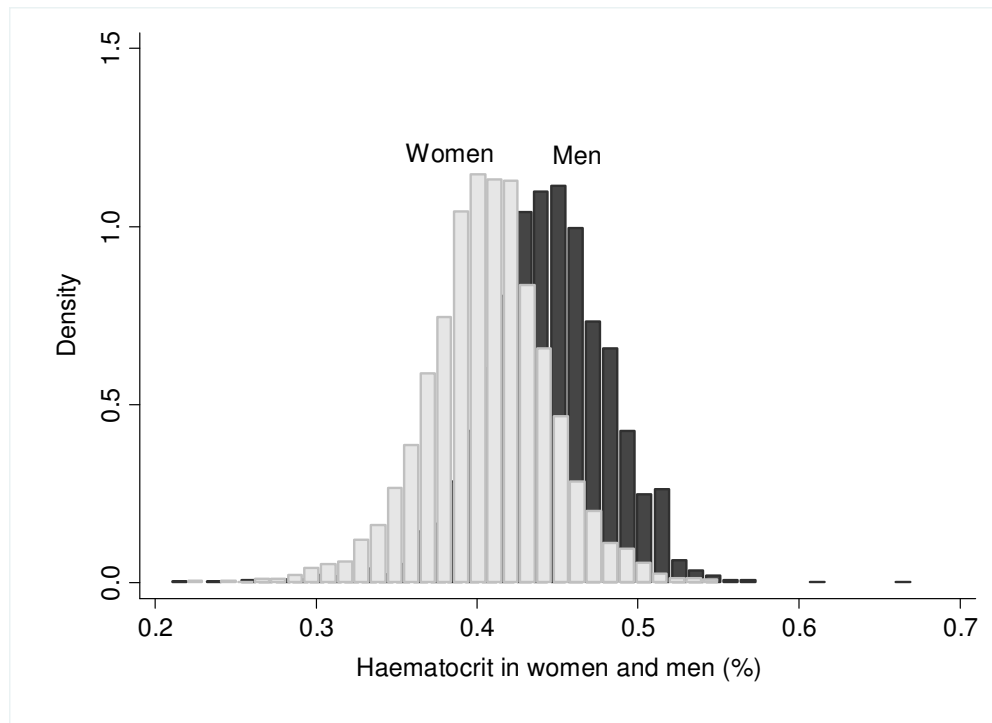
Figure 4-1: Haematocrit distribution in men and women.

Table 4-2: Baseline characteristics stratified by haematocrit quartiles.

Characteristics (Men)	<=0.420 (N=1454)	0.421-0.440 (N=1179)	0.441-0.460 (N=1183)	>=0.461 (N=1413)	P Value
Age at first visit (years), mean (SD)	52.95 (13.31)	48.84 (12.51)	48.26 (12.56)	47.85 (12.27)	<0.001
BMI (Kg/m2), mean (SD)	26.99 (4.55)	27.56 (4.54)	27.55 (4.91)	27.82 (5.23)	<0.001
SBP (mmHg), mean (SD)	165.78 (28.25)	162.14 (26.41)	163.41 (26.36)	165.51 (26.87)	<0.001
DBP (mmHg), mean (SD)	97.81 (15.93)	98.71 (14.52)	100.74 (14.35)	101.48 (14.33)	0.036
eGFR (mL/min per 1.73 m2), mean (SD)	71.51 (31.86)	78.06 (26.40)	77.84 (19.95)	78.41 (27.05)	<0.001
Alcohol Use, n (%)	1173 (84.82)	968 (86.66)	987 (86.58)	1169 (86.34)	0.483
Tobacco Use, n (%)	663 (46.72)	532 (45.86)	572 (48.85)	788 (56.90)	<0.001
CVD, n (%)	326 (22.42)	246 (20.87)	258 (21.81)	315 (22.29)	0.777
First visit <= Year 1977, n (%)	217 (14.92)	226 (19.17)	248 (20.96)	435 (30.79)	
First visit between years 1978 - 1985, n (%)	275 (18.91)	256 (21.71)	243 (20.54)	258 (18.26)	
First visit between years 1986 - 1993, n (%)	382 (26.27)	252 (21.37)	226 (19.10)	170 (12.03)	
First visit between years 1994 - 2001, n (%)	299 (20.56)	216 (18.32)	198 (16.74)	217 (15.36)	
First visit >= Year 2002, n (%)	281 (19.33)	229 (19.42)	268 (22.65)	333 (23.57)	<0.001
Characteristics (Women)	<=0.380 (N=1422)	0.381-0.400 (N=1283)	0.401-0.420 (N=1341)	>=0.421 (N=1676)	P Value
Age at first visit (years), mean (SD)	52.33 (17.01)	50.42 (15.77)	50.88 (14.25)	51.36 (13.37)	<0.001
BMI (Kg/m2), mean (SD)	27.23 (6.21)	26.92 (6.26)	27.81 (6.02)	27.80 (8.72)	<0.001
SBP (mmHg), mean (SD)	167.33 (30.05)	165.49 (29.15)	167.68 (29.50)	172.42 (31.78)	0.059
DBP (mmHg), mean (SD)	94.87 (15.22)	96.32 (14.86)	97.83 (14.25)	100.34 (15.16)	0.004
eGFR (mL/min per 1.73 m2), mean (SD)	69.23 (30.92)	73.40 (51.28)	72.07 (23.26)	71.53 (22.66)	<0.001
Alcohol Use, n (%)	883 (65.31)	857 (70.30)	889 (69.89)	1135 (70.45)	0.009
Tobacco Use, n (%)	432 (31.01)	414 (32.91)	581 (44.15)	921 (55.58)	<0.001
CVD, n (%)	234 (16.46)	220 (17.15)	325 (19.39)	1145 (21.90)	0.006
First visit <= Year 1977, n (%)	212 (14.91)	204 (15.90)	234 (17.45)	480 (28.64)	
First visit between years 1978 - 1985, n (%)	294 (20.68)	282 (21.98)	269 (20.06)	325 (19.39)	
First visit between years 1986 - 1993, n (%)	359 (25.25)	287 (22.37)	324 (24.16)	262 (15.63)	
First visit between years 1994 - 2001, n (%)	286 (20.11)	244 (19.02)	237 (17.67)	250 (14.92)	
First visit >= Year 2002, n (%)	271 (19.06)	266 (20.73)	277 (20.66)	359 (21.42)	<0.001

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; CVD, prevalent cardiovascular disease.
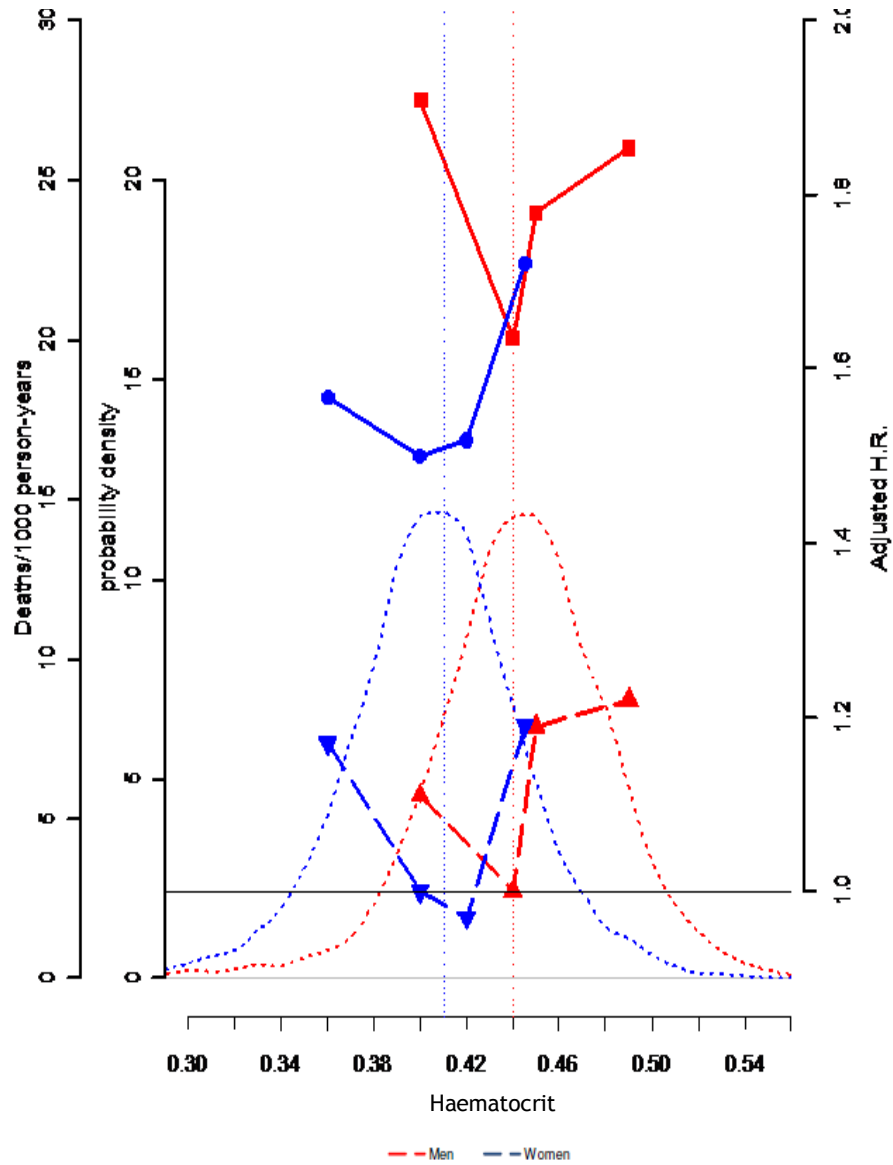
Figure 4-2: Haematocrit in quintiles and all-cause mortality in men and women.

The top curves are deaths per 1000 person years and the bottom curves are adjusted hazard ratios. A J-shaped pattern is seen in men and a U-shaped pattern is seen in women both in unadjusted and multivariate adjusted measures of risk. HR=Hazard Ratio.

4.3.3 Kaplan-Meir survival models

The PH assumption was tested across Hct categories by plotting log-log survival curves (Figure 4-3 on page 136). The log-log survival curves for haematocrit categories were parallel to each other in both men and women. All-cause mortality rates for men and women by Hct quartiles are presented as Kaplan-Meier plots (Figures 4-4 to 4-5 on page 137). While the survival time was shortest in quintile 4 of haematocrit in men, it was shortest in quintile 1 in women.

4.3.4 Cox-proportional hazard models

Using Hct as a continuous variable, a 1% increase in Hct augmented the overall adjusted event rates for all-cause mortality (hazard ratio [HR], 1.15 [95% CI, 1.05–1.26]), CV mortality (HR, 1.21 [95% CI, 1.08 –1.37]), IHD (HR, 1.3 [95% CI, 1.10 –1.53]), and borderline for stroke mortality (HR, 1.28 [95% CI, 1.00 –1.65]) but not non-CV mortality (HR, 1.05 [95% CI, 0.91–1.21]). Considering Hct as a categorical variable, the relationship between Hct quartiles and all-cause mortality showed a U-shaped pattern in women and a J-shaped pattern in men after adjusting for all baseline risk factors (P<0.02 and 0.001 in men and women, respectively; Table 4-4 and Figure 4-6 on pages 135 and 138). In men, compared with quartile 2, the lowest quartile was associated with a 25% (HR, 1.25 [95% CI, 1.04 –1.50]) increased risk of CV mortality, and higher quartiles showed a linear increase in risk of CV mortality of 21% (HR, 1.21 [95% CI, 1.01–1.46]) and 28% (HR, 1.28 [95% CI, 1.08 –1.53]) higher risk for quartiles 3 and 4, respectively. This reflected the nonlinear pattern for all-cause mortality (J-shaped) in men but not for non-CV mortality (Figures 4-6 to 4-10 on pages 138-140). In women, quartiles 2 and 3 showed no difference in risk for any of the causes of mortality (U-shaped). However, the highest and the lowest quartiles showed, respectively, 17% (HR, 1.17 [95% CI, 1.01–1.36]) and 19% (HR, 1.19 [95% CI, 1.04 –1.37]) increased risk of all-cause mortality (P<0.001) and 47% (HR, 1.47 [95% CI, 1.18 –1.84]) and 25% (HR, 1.25 [95% CI, 1.00 –1.55]) higher risk of non-CV mortality (P<0.001). The hazard ratios in men and women are plotted as forests plots for all-cause (Figure 4-6 on page 138), CV (Figure 4-7 on page 138), IHD (Figure 4-8 on page 139), stroke (Figure 4-9 on page 139) and non-cardiovascular (Figure 4-10 on page 140) mortality outcomes.

Table 4-3: Person years of follow-up and mortality event rates in men and women.

Men	Data				
Haematocrit quartile (N)	<=0.420 (N=1454)	0.421-0.440 (N=1179)	0.441-0.460 (N=1183)	>=0.461 (N=1413)	Total (N=5229)
Person years of follow-up (p-y)	19350.34	18361.72	18220.12	23083.96	79016.14
IHD mortality, n/1000 p-y (95% CI)	8.94 (7.70-10.38)	6.97 (5.86-8.29)	8.89 (7.62-10.37)	9.62 (8.43-10.97)	8.67 (7.98-9.34)
Stroke mortality, n/1000 p-y (95% CI)	3.98 (3.18-4.98)	2.12 (1.55-2.91)	3.07 (2.37-3.99)	3.21 (2.55-4.03)	3.11 (2.89-3.44)
CVD mortality, n/1000 p-y (95% CI)	16.54 (14.82-18.45)	11.16 (9.74-12.80)	13.89 (12.28-15.71)	15.60 (14.06-17.29)	14.40 (13.94-14.97)
Non-CVD mortality, n/1000 p-y (95% CI)	9.25 (7.99-10.71)	7.84 (6.66-9.23)	9.17 (7.88-10.67)	9.10 (7.95-10.41)	8.86 (8.21-9.50)
All-cause mortality, n/1000 p-y (95% CI)	27.49 (25.25-29.93)	20.04 (18.10-22.20)	23.98 (21.84-26.34)	25.99 (23.99-28.16)	24.51 (23.42-25.63)
Women	**Data**				
Haematocrit quartile (N)	<=0.380 (N=1422)	0.381-0.400 (N=1283)	0.401-0.420 (N=1341)	>=0.421 (N=1676)	Total (N=5722)
Person years of follow-up (p-y)	22293.95	21324.47	22058.07	28553.19	94229.67
IHD mortality, n/1000 p-y (95% CI)	3.72 (3.00-4.62)	4.46 (3.64-5.45)	5.44 (4.55-6.51)	5.85 (5.03-6.81)	4.93 (4.32-5.41)
Stroke mortality, n/1000 p-y (95% CI)	2.29 (1.74-3.01)	2.39 (1.82-3.15)	2.40 (1.84-3.15)	3.29 (2.69-4.03)	2.64 (1.44-2.83)
CVD mortality, n/1000 p-y (95% CI)	7.89 (6.81-9.15)	8.82 (7.64-10.17)	9.38 (8.19-10.75)	12.08 (10.87-13.43)	9.72 (9.19-10.25)
Non-CVD mortality, n/1000 p-y (95% CI)	9.29 (8.10-10.64)	6.71 (5.69-7.90)	6.48 (5.50-7.64)	9.14 (8.10-10.32)	8.01 (7.48-8.61)
All-cause mortality, n/1000 p-y (95% CI)	18.17 (16.48-20.03)	16.37 (14.74-18.18)	16.87 (15.24-18.67)	22.38 (20.71-24.18)	18.73 (17.54-19.89)

CVD=Cardiovascular disease and IHD=ischemic heart disease

Table 4-4: Cox regression analysis for the association between haematocrit and mortality.

	All-cause mortality		CV mortality		IHD mortality		Stroke Mortality		Non-CV mortality	
Cox-PH Models	N=3484/10007		N=1920/10007		N=1077/10007		N=458/10007		N=1379/10007	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Baseline Haematocrit (Model 1), (All, 162398.62 p-y)	1.15*	1.05-1.26	1.21*	1.08-1.37	1.30*	1.10-1.53	1.28	1.00-1.65	1.05	0.91-1.21
Baseline Haematocrit (Model 2), (Men)	N=1875/4931		N=1105/4931		N=665/4931		N=239/4931		N=675/4931	
<=0.420	1.11	0.97-1.28	1.25*	1.04-1.50	1.09	0.87-1.38	1.46	0.98-2.18	0.89	0.70-1.12
0.421-0.440	1		1		1		1		1	
0.441-0.460	1.19*	1.04-1.37	1.21*	1.01-1.46	1.21	0.96-1.54	1.47	0.97-2.24	1.20	0.96-1.50
>=0.461	1.22*	1.06-1.39	1.28*	1.08-1.53	1.22	0.98-1.53	1.50*	1.01-2.24	1.12	0.90-1.40
Baseline Haematocrit (Model 3), (Women)	N=1650/5226		N=842/5226		N=426/5226		N=226/5226		N=716/5226	
<=0.380	1.17*	1.01-1.36	0.95	0.76-1.18	0.91	0.66-1.24	0.94	0.63-1.41	1.47*	1.18-1.84
0.381-0.400	1		1		1		1		1	
0.401-0.420	0.97	0.83-1.13	0.97	0.79-1.20	1.16	0.87-1.54	0.84	0.56-1.26	0.95	0.75-1.21
>=0.421	1.19*	1.04-1.37	1.14	0.94-1.38	1.12	0.86-1.48	1.10	0.77-1.58	1.25*	1.00-1.55

*p value <0.05, Model 1 is adjusted for age at first visit, gender, BMI=body mass index, baseline cardiovascular disease, CKD=Chronic kidney disease, tobacco smoking, alcohol use, year of first visit (epochs), SBP=systolic blood pressure, and DBP=diastolic blood pressure. Model 2 is adjusted for age at first visit, BMI=body mass index, baseline cardiovascular disease, CKD=Chronic kidney disease, tobacco smoking, year of first visit (epochs), SBP=systolic blood pressure, and DBP=diastolic blood pressure. Model 3 is adjusted for age at first visit, BMI=body mass index, baseline cardiovascular disease, CKD=Chronic kidney disease, tobacco smoking, alcohol use, year of first visit (epochs), SBP=systolic blood pressure, and DBP=diastolic blood pressure. CV=Cardiovascular disease and IHD=ischemic heart disease, HR=hazard Ratio, CI=confidence interval, p-y=person years.
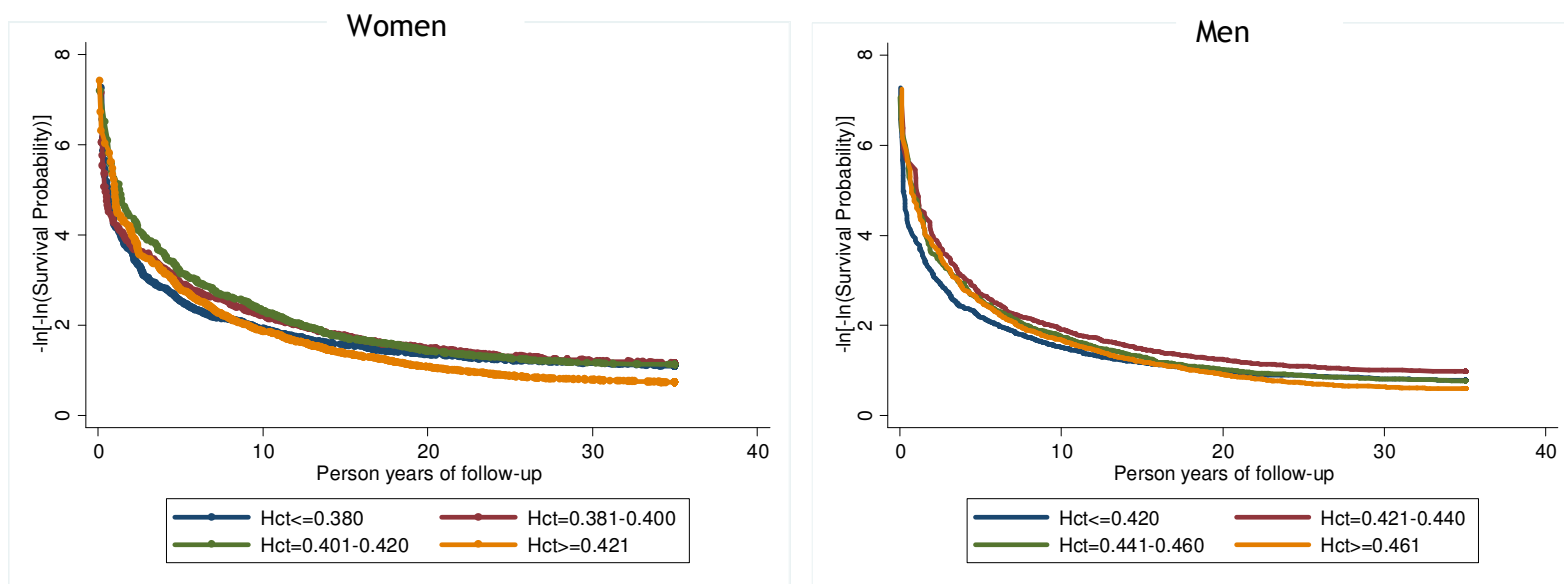
Figure 4-3: Log-log survival curves of haematocrit in quartiles

Proportionality hazard assumption is met when the curves are parallel to each other.
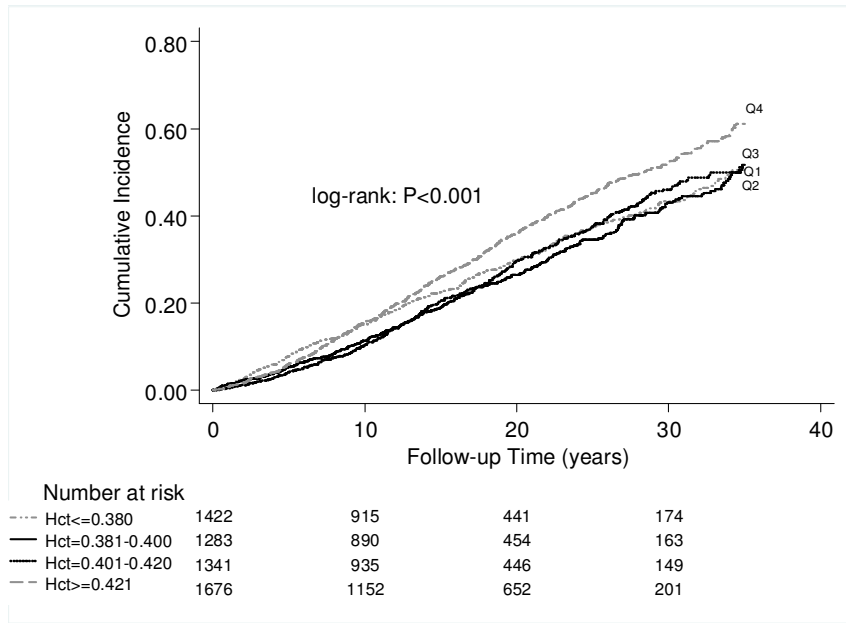
Number at risk				
---- Hct<=0.380	1422	915	441	174
—— Hct=0.381-0.400	1283	890	454	163
•••• Hct=0.401-0.420	1341	935	446	149
— — Hct>=0.421	1676	1152	652	201

Figure 4-4: Kaplan-Meir survival curves for Hct quartiles and all-cause mortality (Men).

Number at risk				
—— Hct<=0.420	1454	829	285	97
••••• Hct=0.421-0.440	1179	775	362	105
---- Hct=0.441-0.460	1183	761	360	107
—— Hct>=0.461	1413	938	522	123
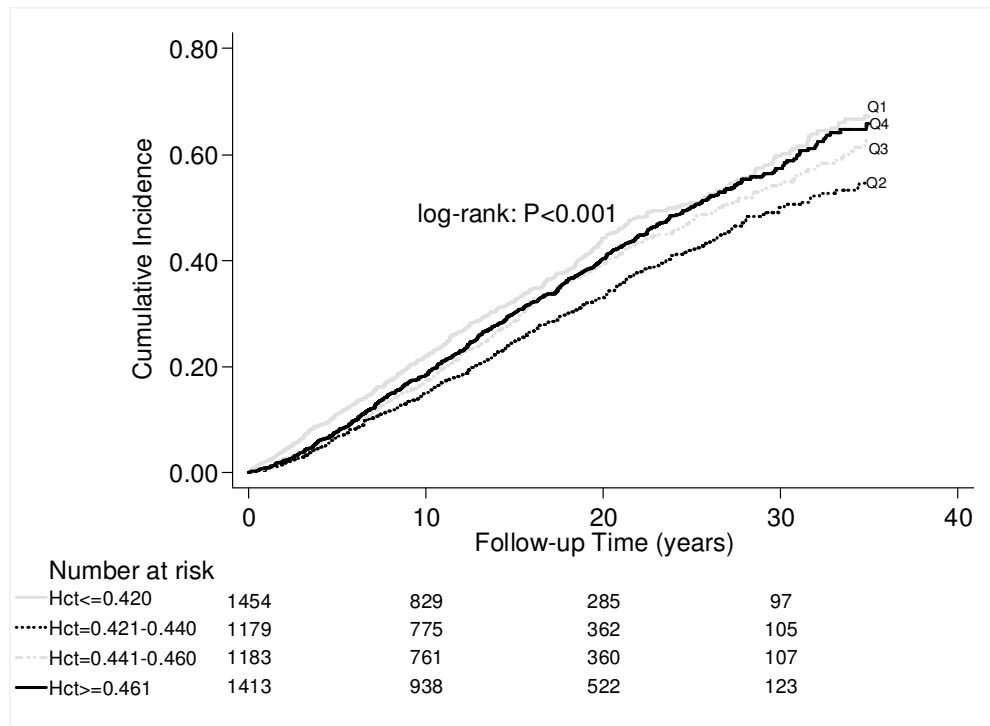
Figure 4-5: Kaplan-Meir survival curves for Hct quartiles and all-cause mortality (Women).
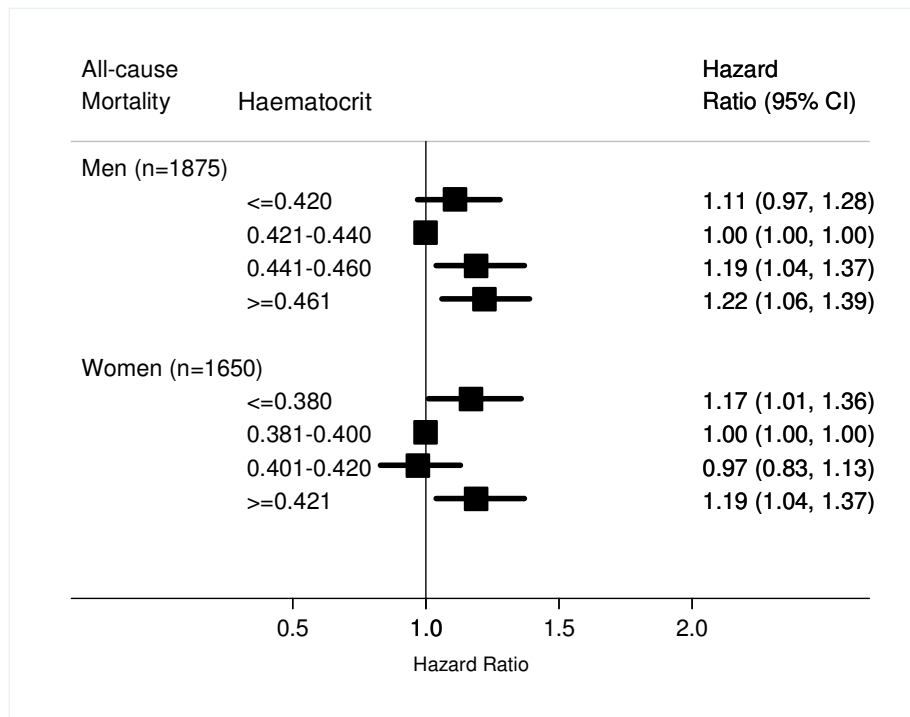
Figure 4-6: Cox-proportional hazard model for haematocrit and all-cause mortality in men and women.
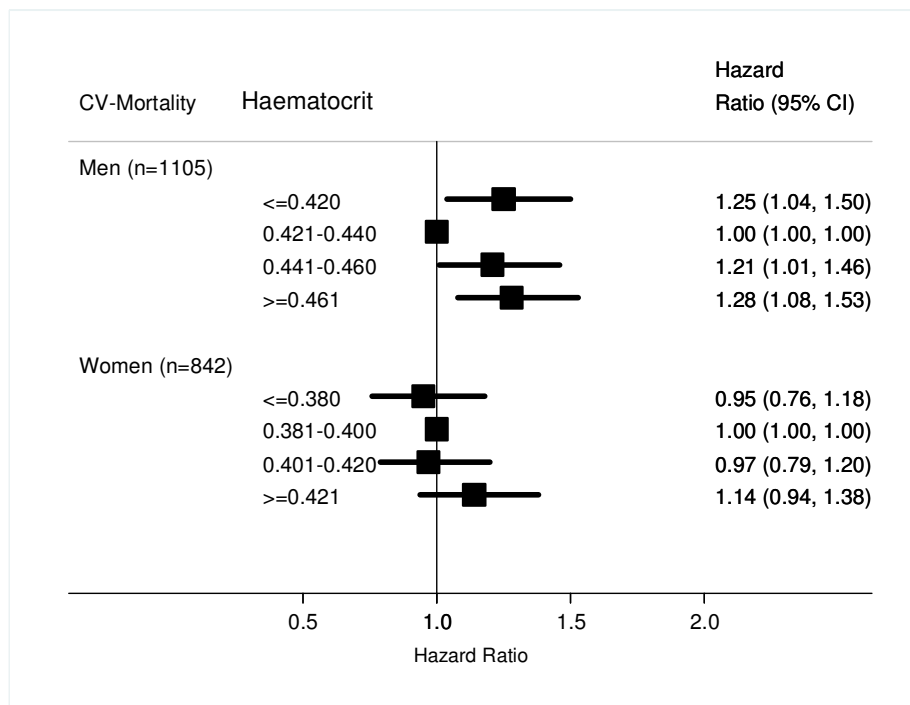
Figure 4-7: Cox-proportional hazard model for haematocrit and cardiovascular mortality in men and women.
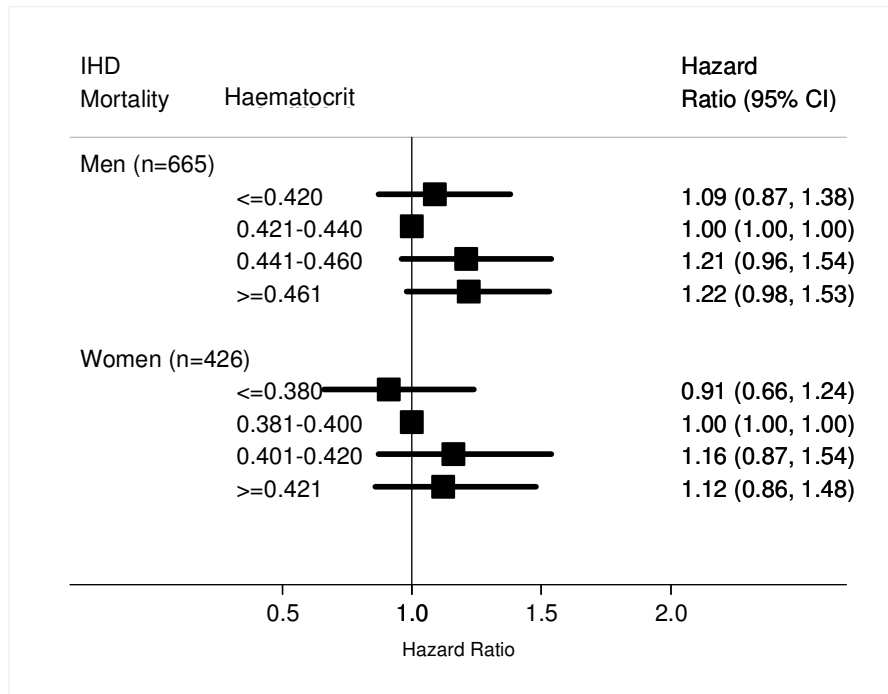
Figure 4-8: Cox-proportional hazard model for haematocrit and ischemic heart disease mortality in men and women.
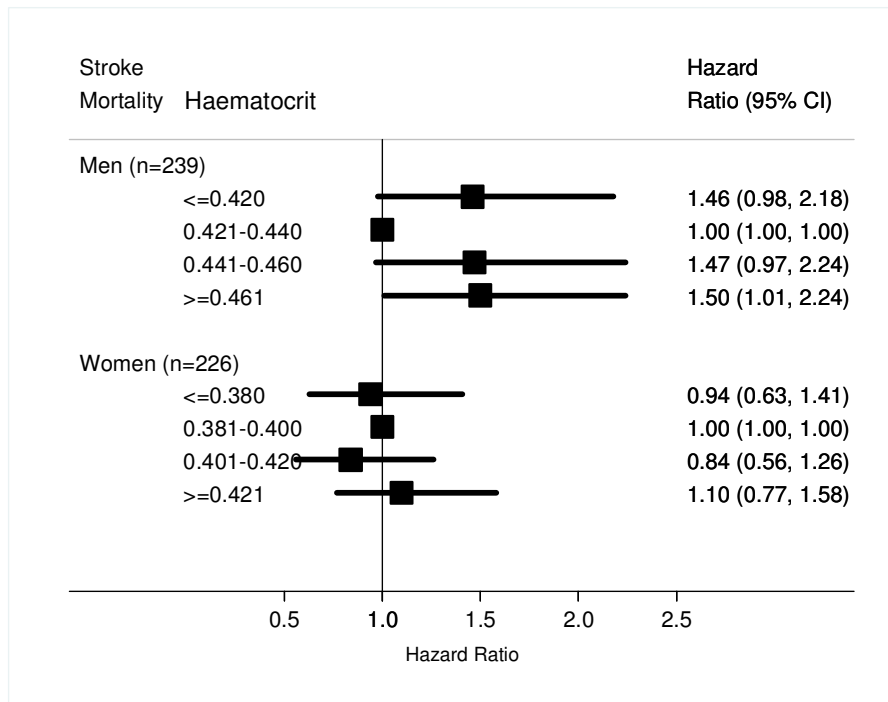
Figure 4-9: Cox-proportional hazard model for haematocrit and stroke mortality in men and women.
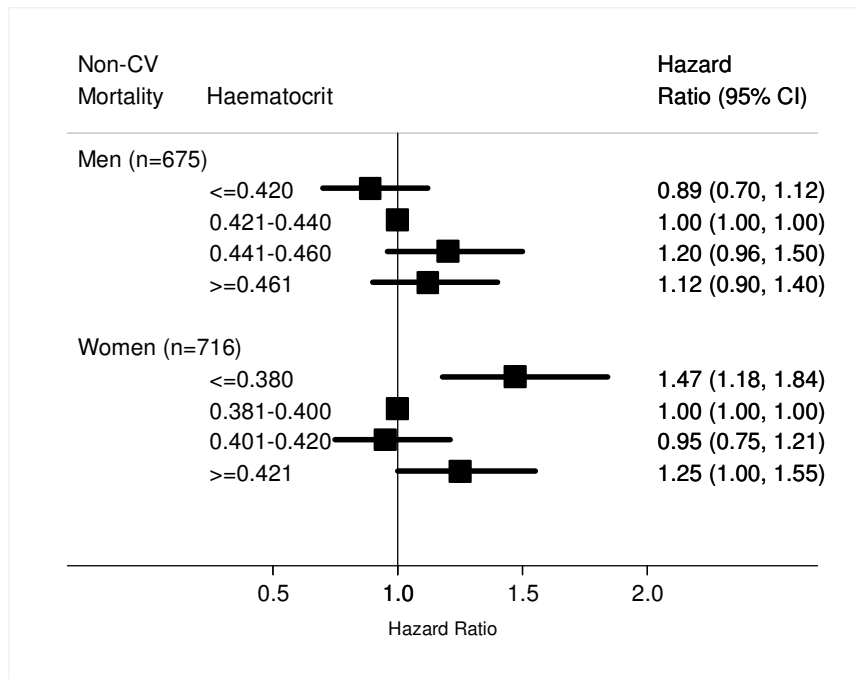
Figure 4-10: Cox-proportional hazard model for haematocrit and non-cardiovascular (Non-CV) mortality in men and women.

4.3.5 Regression spline Cox-proportional hazard model

To fully examine the nonlinear relationship between Hct and mortality, the association of Hct was examined, represented as a cubic spline, with CV and non-CV mortality in men and women and the results were consistent with the primary analyses (Figures 4-11 to 4-14 on pages 141-142).

4.3.6 Cox-proportional hazard models in sub-groups

To address reverse causality and to also demonstrate that the baseline Hct of our study population was stable, the analysis was repeated in a subset of the population who had >=3 Hct readings in the first 5 years of follow-up (n=4477). The coefficient of variation of Hct in 94% (n=4122) of this subgroup was <=0.1. The same pattern of results was seen in the subgroup overall and in those with coefficient of variation <=0.1 (Tables 4-5 and 4-6 on page 143).
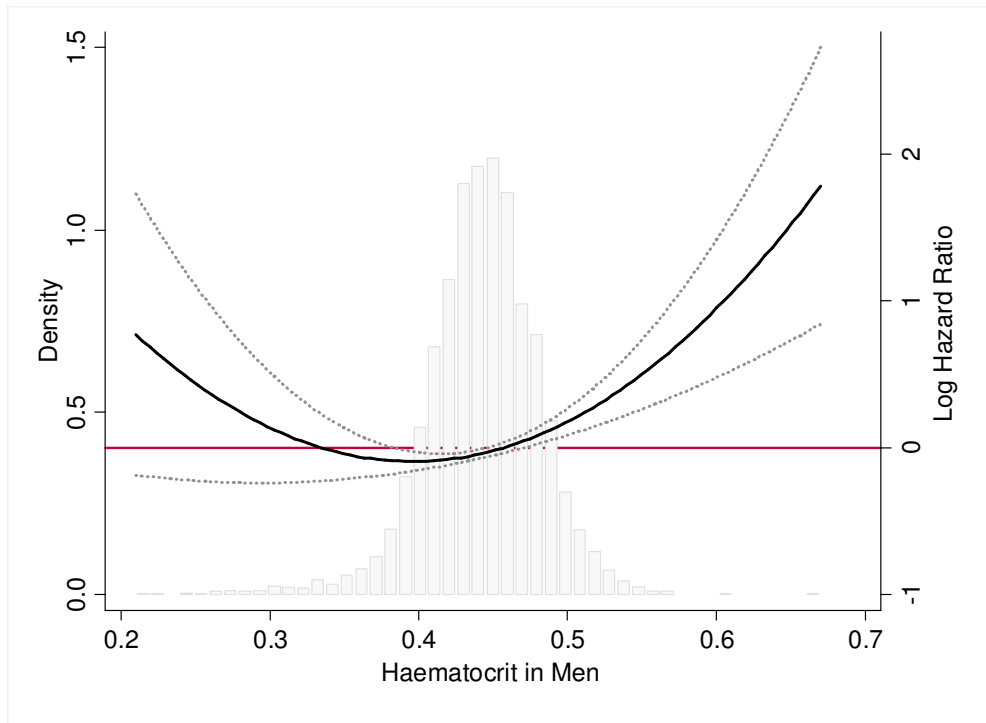
Figure 4-11: Haematocrit in men and cardiovascular disease mortality.
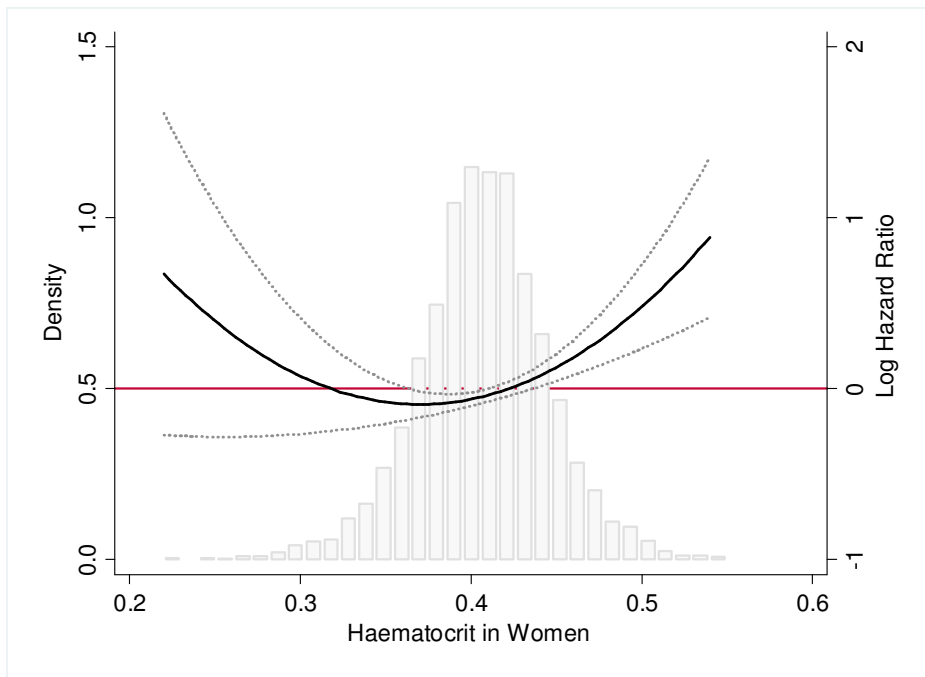
Figure 4-12: Haematocrit in women and cardiovascular disease mortality.
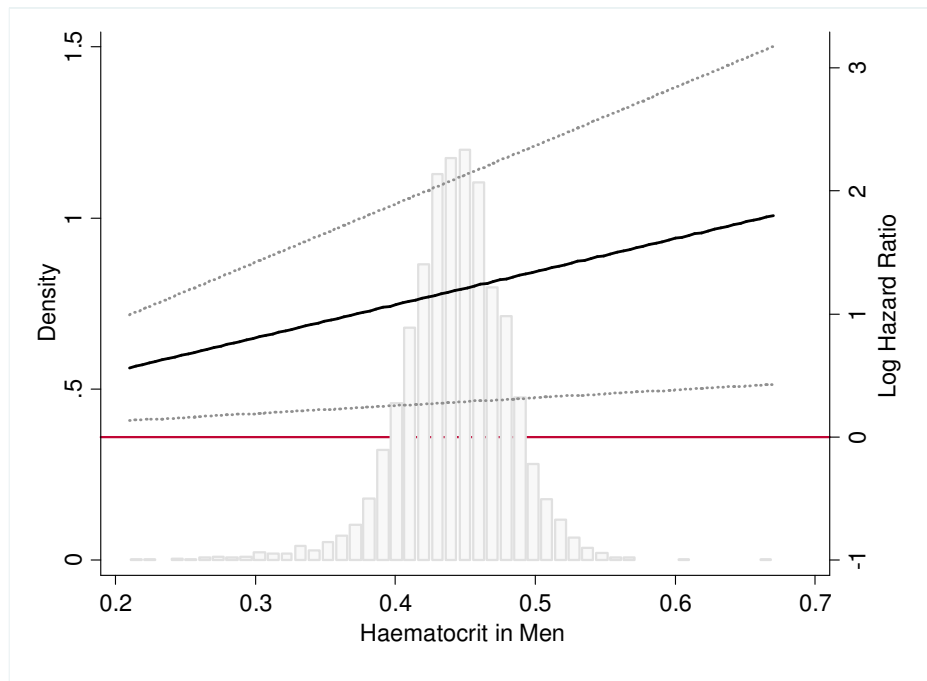
Figure 4-13: Haematocrit in men and non-cardiovascular disease mortality.
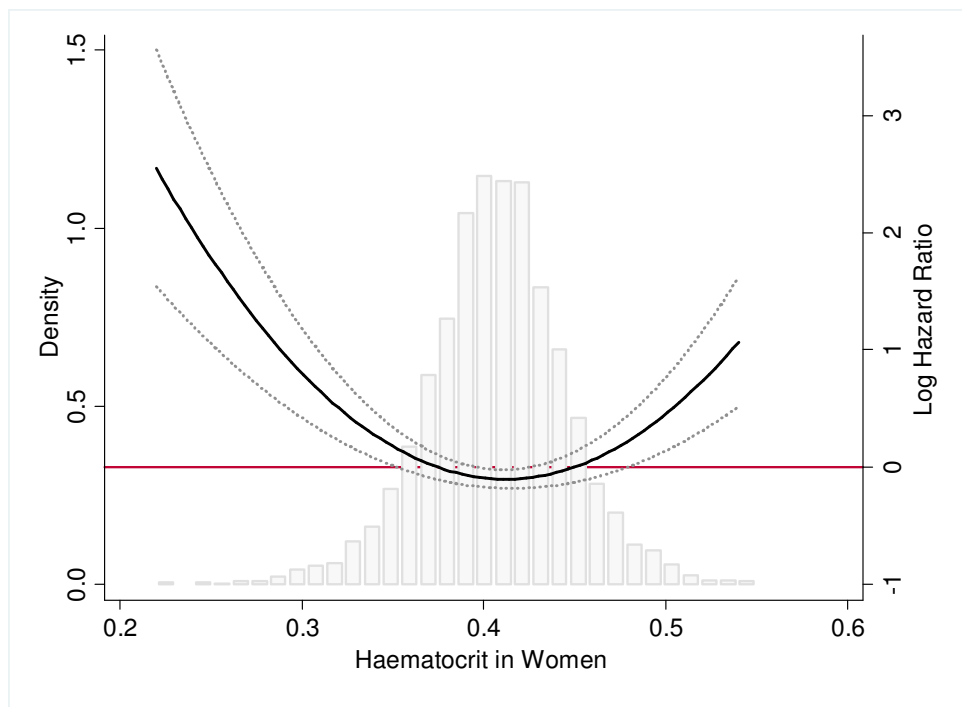
Figure 4-14: Haematocrit in women and non-cardiovascular disease mortality.

Table 4-5: Haematocrit and all-cause mortality in a subset of population who had repeat haematocrit measurements and with a coefficient of variation of <=0.1.

Hematocrit	All-cause mortality (Men: N=755/1978)	
	HR	95% CI
<=0.420	1.16	0.92-1.43
0.421-0.440	1.00 (Ref)	
0.441-0.460	1.13	0.90-1.42
>=0.461	1.21*	1.02-1.51
	All-cause mortality (Women: N=692/2093)	
	HR	95% CI
<=0.380	1.26*	1.03-1.62
0.381-0.400	1.00 (Ref)	
0.401-0.420	1.12	0.87-1.39
>=0.421	1.36*	1.01-1.50

HR=Hazard Ratio, CI=Confidence Interval, *p<0.05. 4122/4477 individuals with >=3 repeat haematocrit measurements in the first five years of follow-up is included in this analysis. HRs are adjusted for age at first visit, gender, BMI=body mass index, baseline cardiovascular disease, CKD=Chronic kidney disease, tobacco smoking, alcohol use, year of first visit (epochs), SBP=systolic blood pressure, and DBP=diastolic blood pressure. 406 individuals did not have one of the above mentioned variables.

Table 4-6: Haematocrit and all-cause mortality in all individuals who had repeat haematocrit measurements available.

Hematocrit	All-cause mortality (Men: N=766/2010)	
	HR	95% CI
<=0.420	1.11	0.89-1.38
0.421-0.440	1.00 (Ref)	
0.441-0.460	1.14	0.91-1.43
>=0.461	1.24*	1.01-1.54
	All-cause mortality (Women: N=704/2130)	
	HR	95% CI
<=0.380	1.28*	1.02-1.61
0.381-0.400	1.00 (Ref)	
0.401-0.420	1.09	0.86-1.39
>=0.421	1.36*	1.10-1.70

HR=Hazard Ratio, CI=Confidence Interval, *p<0.05. 4477 individuals with >=3 repeat haematocrit measurements in the first five years of follow-up is included in this analysis. HRs are adjusted for age at first visit, gender, BMI=body mass index, baseline cardiovascular disease, CKD=Chronic kidney disease, tobacco smoking, alcohol use, year of first visit (epochs), SBP=systolic blood pressure, and DBP=diastolic blood pressure. 334 individuals did not have one of the above mentioned variables.

4.3.7 Longitudinal change in blood pressure

The population averaged effect of change in BP across Hct quartiles in men and women over the first 5-year follow-up period are presented in Figures 4-15 to 4-16 on pages 145-146. Compared with Hct quartile 2, the mean SBP during the five year follow-up in men was relatively higher in Hct quartiles 3 and 4 by 1.29 mmHg (95% CI, 0.35–2.23) and 3.22 mmHg (95% CI, 2.30–4.14), respectively. In women, the mean SBP was relatively higher by 3.79 mmHg (95% CI, 2.85– 4.74) in the Hct quartile 4 in comparison with quartile 2.

4.3.8 Responders and Non-responders

In the study population, 2297 and 2218 subjects were classified as non-responders and responders, respectively. Among male responders and non-responders, quartile 2 was associated with the lowest mortality and quartile 4 associated with the highest mortality risk. In women, the relationship was more linear, with the quartiles 1 and 4 associated with the lowest and the highest risks, respectively. The Kaplan-Meier plots illustrating this for all-cause mortality are presented in Figures 4-17 to 4-20 (on pages 147-148), and the multivariate-adjusted survival analyses are presented in table 4-7 on page 149. The time to reach target BP among responders was similar across Hct quartiles on bivariate analysis (log rank test P=0.072 and P=0.654 for men and women, respectively) and multivariate analysis adjusting for covariates significant at P<0.1 (P=0.204 and P=0.280 for men and women, respectively).
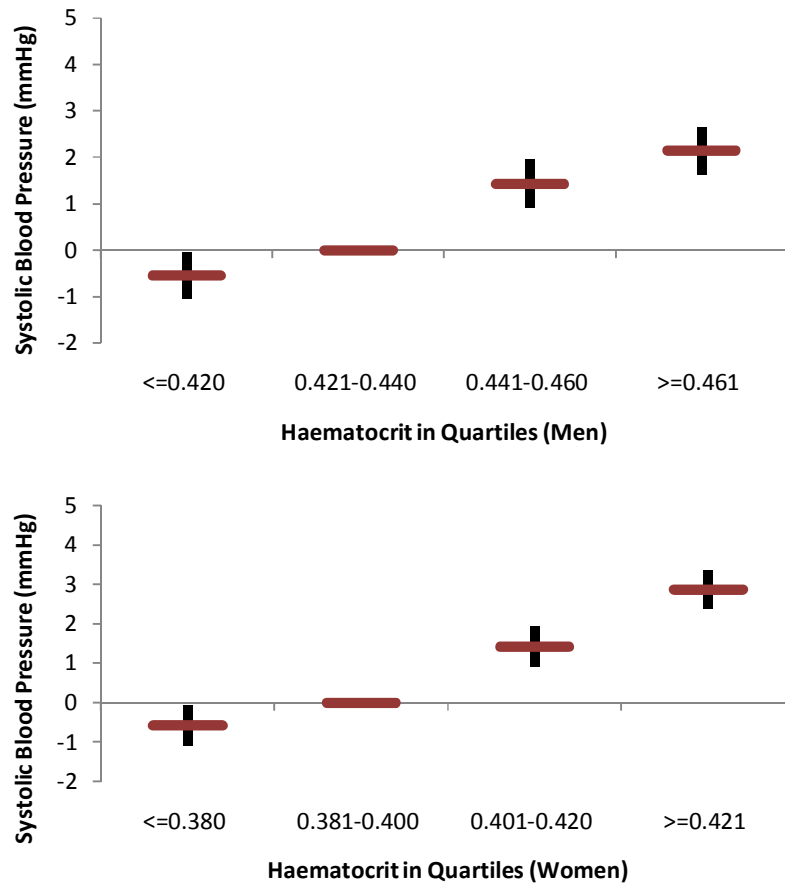
Figure 4-15: Longitudinal change in systolic blood pressure by haematocrit (Hct) quartile during the first 5 years of follow-up.
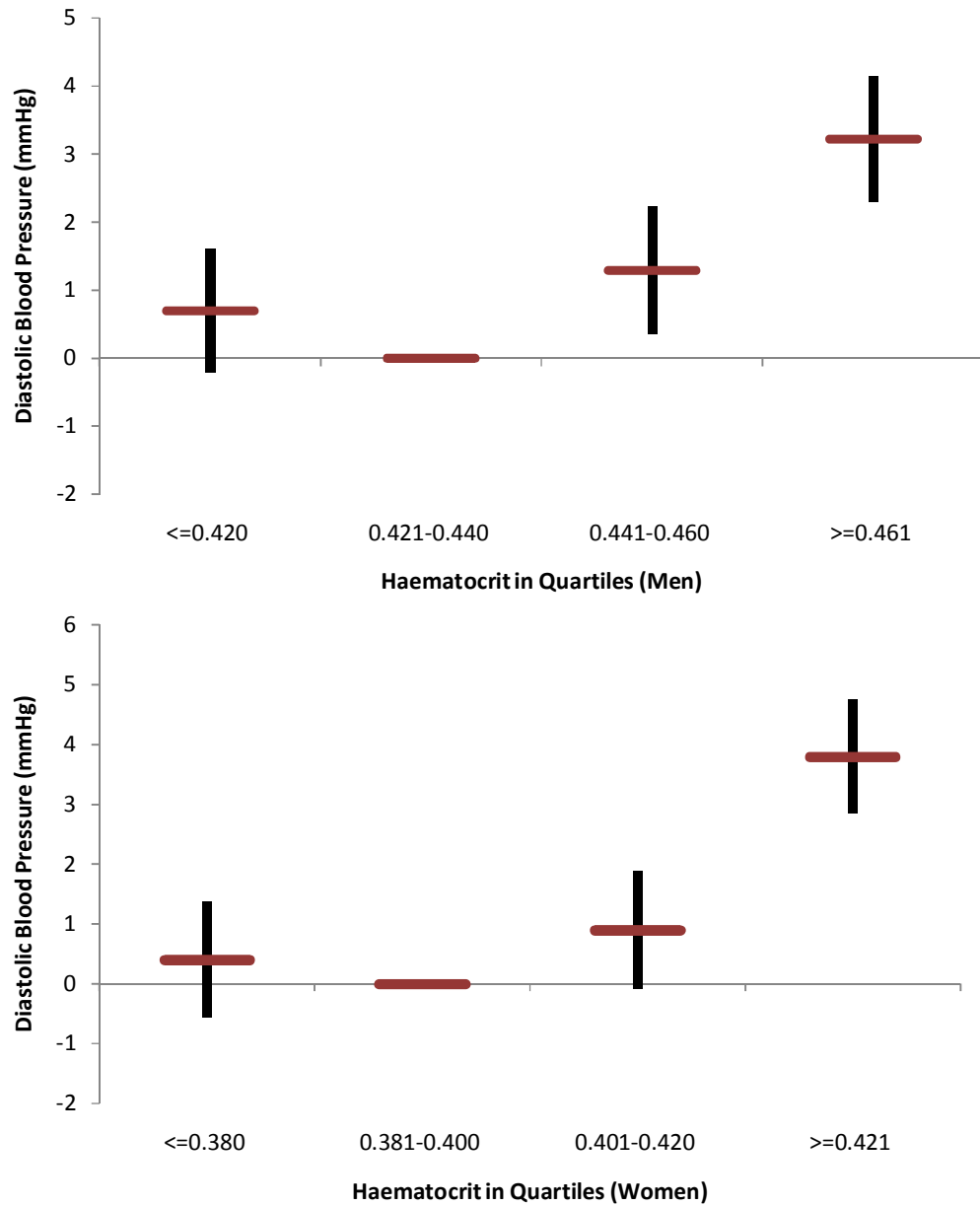
Figure 4-16: Longitudinal change in diastolic blood pressure (DBP) by haematocrit (Hct) quartile during the first 5 years of follow-up.
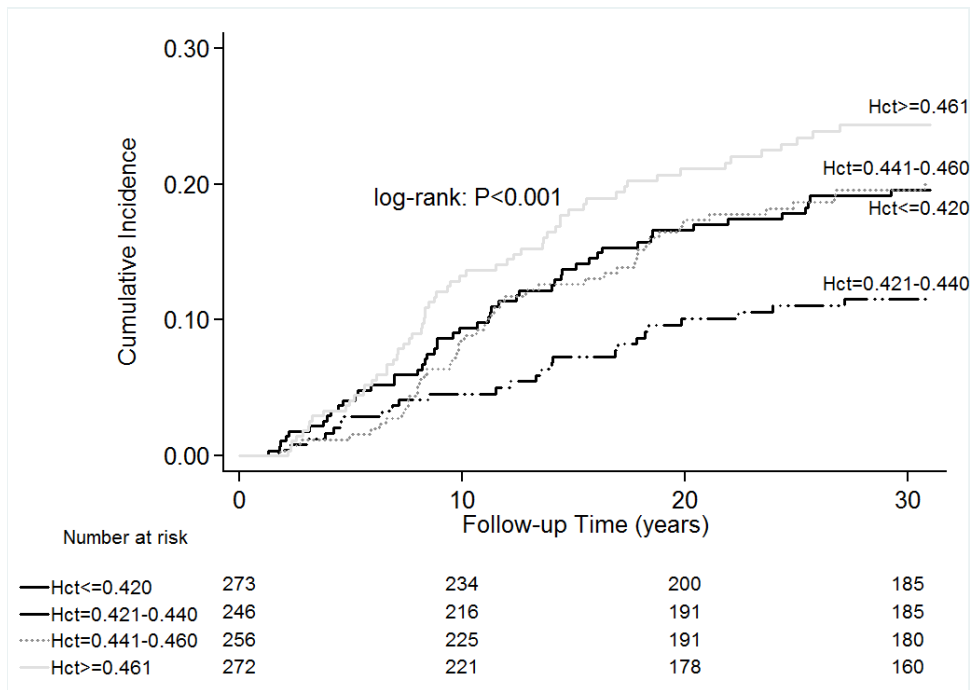
Figure 4-17: Kaplan-Meier all-cause mortality curves of haematocrit (Hct) categories in quartiles among responders (Men).
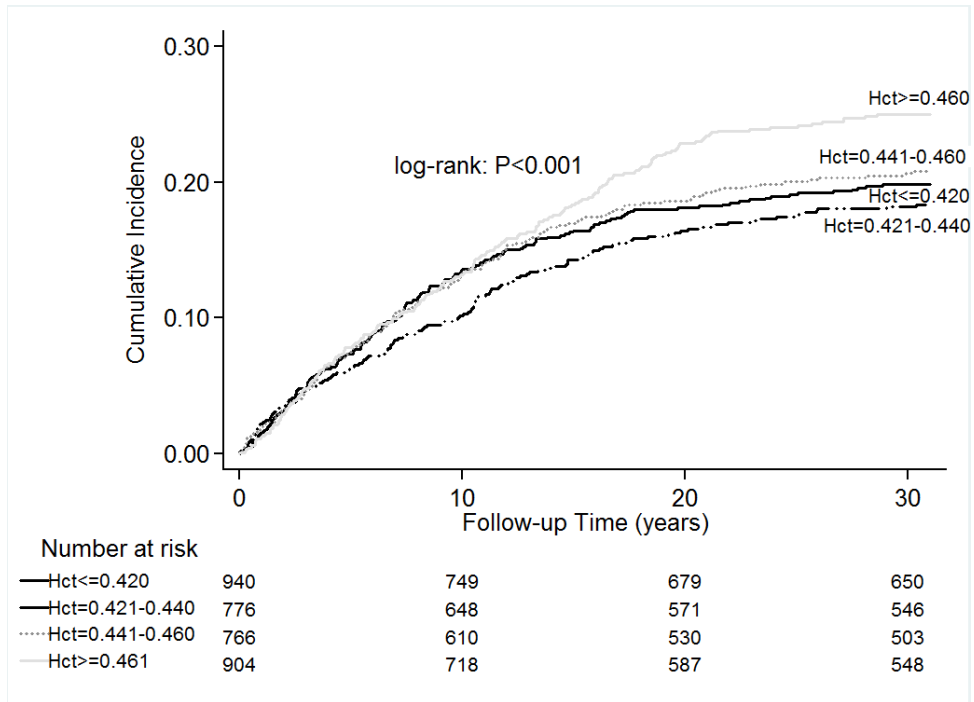
Figure 4-18: Kaplan-Meier all-cause mortality curves of haematocrit (Hct) categories in quartiles among non-responders (Men).
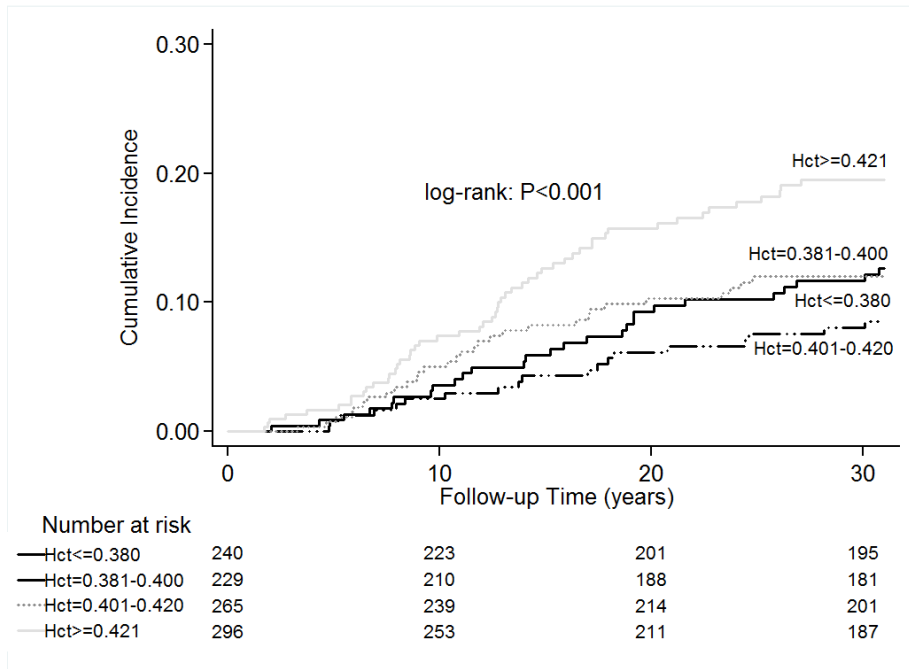
Figure 4-19: Kaplan-Meier all-cause mortality curves of haematocrit (Hct) categories in quartiles among responders (Women).
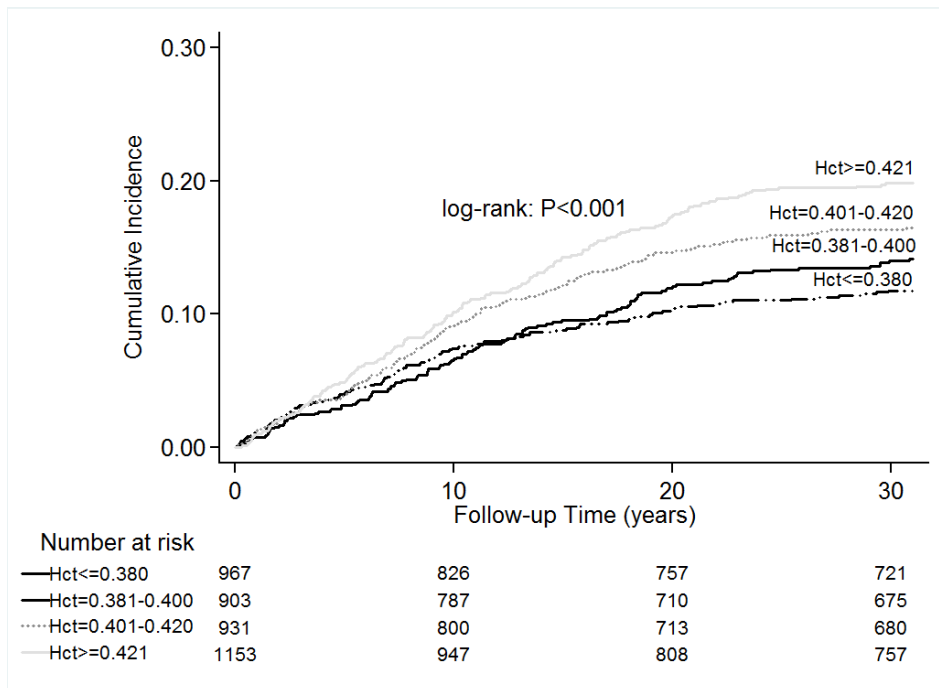
Figure 4-20: Kaplan-Meier all-cause mortality curves of haematocrit (Hct) categories in quartiles among non-responders (Women).

Table 4-7: Haematocrit and all-cause mortality in hypertensive individuals classified by achievement of target blood pressure at final clinic visit.

Cox-PH Models	All-cause mortality				CV Mortality			
	Non-responders		Responders		Non-responders		Responders	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Baseline Haematocrit, (Men)	N=1103/3449		N=332/1025		N=635/3449		N=182/1025	
<=0.420	0.87	0.73-1.04	1.17	0.83-1.69	1.06	0.84-1.34	1.53	0.94-2.51
0.421-0.440	1.00		1.00		1.00		1.00	
0.441-0.460	1.22*	1.02-1.46	1.10	0.78-1.57	1.20	0.95-1.53	1.50	0.91-2.48
>=0.461	1.31*	1.10-1.55	1.28	0.91-1.78	1.24	0.99-1.56	1.53	0.94-2.51
Baseline Haematocrit, (Women)	N=1080/3909		N=332/1004		N=549/3909		N=125/1004	
<=0.380	1.15	0.95-1.38	1.17	0.83-1.64	0.96	0.73-1.26	0.69	0.38-1.27
0.381-0.400	1.00		1.00		1.00		1.00	
0.401-0.420	1.04	0.87-1.25	1.10	0.78-1.57	1.12	0.87-1.43	0.99	0.57-1.71
>=0.421	1.20*	1.01-1.42	1.28	0.91-1.78	1.13	0.89-1.43	1.33	0.81-2.21

Adjusted for age, epochs, smoking, alcohol use, baseline CVD, BMI, achieved SBP, and eGFR. CVD=Cardiovascular Disease, BMI=Body Mass Index, SBP=Systolic Blood Pressure, eGFR=estimated Glomerular Filtration Rate.

4.4 Discussion

Haematocrit is an independent predictor of all-cause, CV, IHD, and non-CV mortality in hypertensive patients. However, the strength and magnitude of relationship vary widely according to the Hct levels and outcome of interests. The Hct ranges with the lowest mortality risk in men and women are 0.421 to 0.44 (quartile 2; J-shaped) and 0.381 to 0.420 (quartiles 2,3; U-shaped), respectively. While in men risk of death increases at Hct<0.42, it increases in women only at a lower threshold of Hct <0.38. The association between Hct and mortality observed in the present study is independent of the effect of Hct on BP.

The present study shows a non-linear relationship between Hct and mortality in a hypertensive cohort, and this is consistent with several other reports of non-linear relationships between Hct and mortality outcomes in more general populations [393] [395]. Haematocrit, a major determinant of whole blood viscosity, is associated with an increased risk of IHD, stroke, and all-cause mortality in subjects with and without pre-existing CVD [394] [566-569]. However, in some studies, after adjustment for multiple risk factors, Hct failed to significantly predict all-cause or CVD mortality events [566] [568]. The non-linear association between Hct and mortality may explain the lack of association of Hct and mortality observed in some studies. The general Cox-PH models assume that the association between a predictor variable and survival is linear. However, the present study modelled the non-linear relationship efficiently by using cubic-regression spline models.

Gender differences in blood viscosity and normal Hct are known to exist [564]. The Hct distribution was shifted to the right side of the distribution curve in men with higher mean Hct levels than in women in the present study. Tobacco use was less common in women than in men in the present study. Tobacco use increases Hct [390] [570] and it is independently associated with mortality. In the present study, the proportion of tobacco users in the last quartile of Hct was significantly higher than that in the first quartile in both men and women. Haematocrit is also associated with eGFR and the filtration fraction decreases with fall in Hct [571]. Women had significantly lower eGFR and Hct in the present

study than men. These clinical characteristics may explain the differences in association between heamatocrit and mortality in men and women.

The strength and magnitude of association of Hct and mortality appears to differ depending upon Hct levels and sex. Men and women also show differences in causes of Hct-related mortality in the present study. While quartiles of Hct predict CV mortality in men, it was associated with non-CV mortality in women. The reason for this apparent dichotomy is unclear. However, the differences in clinical characteristics in men and women as explained in the previous paragraph may partially explain this dichotomy. The evidence linking Hct with cardiovascular events are plenty in the literature [390] [394] [396] [568] [569]. The relatively high effect sizes in the association between CV mortality and Hct observed in the present study are also consistent with previous reports [390] [396]. The relatively lower risk for IHD observed in women may be due to low Hct and reduced plasma viscosity. The hormone estrogen has been linked with platelet aggregation [572] and smooth muscle cell proliferation [573] [574] and in combination with a high Hct may predispose to increased morbidity in women. A dose dependent relationship between oestrogen containing oral contraceptives and platelet activation in women is also reported [575]. Hct is therefore a more potent risk factor in younger women [393] [395]. A lower fibrinogen and plasma viscosity were observed in older women (aged 52–65 years) taking hormone replacement therapy, which would suggest that estrogen is only partly responsible for mediating the effects of Hct [576]. The present study shows that the different reference ranges of Hct in men and women influence mortality in a complex manner and can explain the variable results in previous studies. Further studies in larger cohorts are required to explain the biological reasons for the differences in association between Hct and mortality outcomes in men and women.

The lack of significant association between stroke death and Hct in women may be attributable to a relatively low number of events in this group. There were only 226 stroke deaths in the Cox model for women. Stroke mortality risk was however 50% higher in the last quartile of Hct in comparison to the first quartile in men. Inconsistent findings have been reported previously. High mean Hct predicted lacunar stroke in an analysis of 2077 people who had experienced a stroke event [577]. A non-linear U-shaped relationship between Hct quartiles and stroke mortality was reported in the Framingham Heart Study women

participants in the age group of 65 to 94 years [393]. In another prospective study of 7735 men aged 40 to 59 years at baseline, an Hct of >0.51 was found to be associated with fatal and nonfatal stroke [397]. However, these studies failed to include diastolic BP, a strong independent predictor of stroke, as a covariate in their analysis models in comparison to the present study.

A positive linear association between Hct and BP in both normotensive and hypertensive subjects have been demonstrated in several epidemiological studies [554] [563] [578]. Hct was positively correlated with both SBP (r=0.085, P<0.01 and r=0.264, P<0.001 for men and women, respectively) and DBP (=0.214, P<0.001 and r=0.266, P<0.001) in a mixed hypertensive and normotensive cohort [554]. Among British men free of IHD, Hct was associated with both DBP and SBP [397]. Since Hct increases blood viscosity and vascular resistance and thus affect total peripheral resistance to blood flow, which is abnormally high in essential hypertension, these results are not unexpected [563] [579-581]. The present study shows that higher baseline Hct is associated with higher on-treatment BP during follow-up, and this may partly explain the higher risk associated with higher Hct. Despite this relationship, baseline Hct did not affect the time to reach target SBP or DBP in this cohort. The increased risk of death because of higher Hct was seen in men and women irrespective of their achievement of target BP, indicating that the risk is independent of the effect of Hct on BP. However, the present study did not have the data to determine whether more aggressive management of BP in the higher Hct groups with earlier time-to-target BP would have reduced the risk.

4.5 Strengths and limitations

The main strengths of this study are the prospective design, long-term follow-up, high event rates, almost equal numbers of men and women, the outcomes based on fatal end points, and the effect sizes adjusted for multiple confounding factors, including baseline pre-existing disease conditions. In the final Cox models, there were 1875 and 1650 mortality events in men and women, respectively. This is probably highest among similar epidemiological studies. For example in the Framingham Heart study with 34 years of follow-up, the number of mortality events were 1234 and 1097 in men and women, respectively. The participants in the present study were however taken from a hypertensive,

overwhelmingly white population, and, therefore, the findings cannot be generalised to other populations. The changes in management of hypertension during the course of follow-up would have influenced the outcomes. Although there was an attempt to adjust for changes in management of hypertensive individuals by including a variable on year of attendance in the models, bias may still be present. We could not adjust for treatment adherence due to unavailability of validated adherence data. Inference about mortality relating to Hct measures taken many years before mortality occurred may be subject to bias. However, the variation in Hct observed in a subgroup of the study population with repeat Hct measurements was relatively small. Additionally, the Cox proportional hazard model results were consistent even in a subgroup of individuals with coefficients of variation of Hct <=0.1. Residual confounding due to unmeasured risk exposures cannot be ruled out completely.

4.6 Summary

Haematocrit is associated with follow-up BP and is an independent predictor of mortality in the hypertensive population. There are distinct differences both in terms of the strength and magnitude of the association of Hct and mortality between men and women that have not previously been shown. While Hct is associated with CV mortality in men, it is more closely associated with non-CV mortality in women. Nevertheless in the assessment and management of newly diagnosed hypertensive patients, Hct levels should be taken into consideration as an important risk predictor, and further research is needed to elucidate the mechanism of this risk and to define management strategies. Haematocrit is an inexpensive marker that is routinely measured in clinical practice and patients with Hct levels outside of the sex-specific low-risk reference ranges as identified in this study should be targeted for more aggressive BP and CV risk reduction treatment.

5   Serum phosphate and mortality

5.1 Introduction

Inorganic phosphate is an important mineral that is directly linked to energy metabolism, bone mineralisation, signal transduction, storage and translation of genetic information and maintenance of lipid membrane structure. It is regulated within a narrow range in human body by a complex interplay of multiple mechanisms that involve factors associated with intestinal absorption, exchange between intracellular and bone storage pools, renal filtration and renal tubular re-absorption of phosphate [582].

Serum phosphate has a putative adverse role in arterial stiffness and atherosclerosis. Higher levels of phosphate have been shown in vitro and in vivo to be associated with vascular calcification, arterial stiffness and atherosclerosis [583-585]. In animal studies, a high phosphate diet accelerates atherosclerosis independent of cholesterol levels [583].

Several studies report association between elevated serum phosphate and mortality in CKD patients. In a systematic review and meta-analyses of the association between serum phosphate and mortality in individuals with CKD, each 1 mg/dl increase in serum phosphorous increased the risk of all-cause mortality by 18% (RR=1.18, 95% CI: 1.12-1.25) [441]. Overall, one mg/dl increase in serum phosphorous increased the risk of CV mortality by 10% [441]. However there were serious limitations in the quality across studies included in the meta-analyses and many of them were not fully adjusted for all potential confounding factors. Serum phosphate is also associated with decline in renal function [586]. In non-diabetic CKD patients, higher serum phosphate is independently associated with left ventricular mass [587].

The association between serum phosphate and mortality in individuals with normal renal function has not been investigated in detail in longitudinal epidemiological studies. In a middle aged general population cohort of more than 15000 individuals, all cause-mortality increased linearly with phosphate levels [450]. However, the results were not adjusted for many important variables including BP. In the post hoc analyses of the Cholesterol and Recurrent Events (CARE) study, higher serum phosphate was independently associated with

adverse cardiovascular outcomes in patients with coronary artery disease and after myocardial infarction but normal renal function [444]. In the Framingham Offspring study cohort, in individuals with no prior CV events and normal renal function, serum phosphate within the normal range linearly increased the incidence of heart failure [588]. Similarly, higher phosphate was associated with incident CVD in the same cohort [449].

The association of serum phosphate with vascular calcification and arterial stiffness indicates that serum phosphate may positively influence BP levels. By contrast multiple small studies report an inverse association between serum phosphate and BP [589-591]. Higher serum phosphate is associated with a non-dipping pattern of nocturnal BP in hypertensive patients [592], while low serum phosphate is associated with a non-dipping pattern of BP in individuals with white coat hypertension [593]. It is however unclear whether serum phosphate influences follow-up clinic BP and of outcomes in hypertensive patients.

Factors that determine serum phosphate level remain unclear. In the NHANES III, a week relationship was observed between dietary phosphorous intake and serum phosphorous concentration [594]. However, inorganic phosphate in food addictives is quickly absorbed from the intestine unlike the natural phosphate in food items and can increase the serum phosphate levels [595]. In general, there is a socio-economic gradient in eating patterns with individuals in the lower socio-economic status consuming more processed food. Consequent to that, a low socio-economic status or greater social deprivation is often associated with higher serum phosphate levels [596][597]. A low deprivation score (indicates greater social deprivation) is independently associated with increased mortality [598][599]. Therefore, the deprivation index as an indicator of socio-economic status may confound the association between serum phosphate and mortality.

The aim of the present study was to elucidate the association between serum phosphate with BP control and long-term mortality in a large treated hypertensive cohort independent of other known risk factors and deprivation status.

5.2 Materials and methods

5.2.1 Study Setting and Study Population

Subjects used in this analysis were identified from the Glasgow Blood Pressure Clinic (GBPC). The study setting is described in detail in section 2.1. Use of the anonymised database for analyses is approved by the West of Scotland research ethics service (WoSRES) of the National Health Service (11/WS/0083).

5.2.2 Clinical Measurements

The Glasgow Blood Pressure Clinic (GBPC) uses specialist hypertension nurses for initial assessment.   Details of study measurements are described in detail in section 2.2-2.4.

5.2.3 Laboratory measurements

As part of their clinical care, patients underwent venepuncture during initial and regular follow-up visits to the GBPC for biochemical and haematological assessment.   All laboratory assays were performed at the central laboratory services of the Western Infirmary. More details of laboratory measurements are explained in section 2.4.

5.2.4 Outcome assessment

Details of outcomes assessment are described in detail in section 2.5

5.2.5 Study definitions

Systolic and diastolic BP control were defined as at least 3 consecutive clinic measurements of BP (usually 3 months apart) less than 140 mmHg or 90 mmHg respectively. Scottish Index of Multiple Deprivation (SIMD) data based on the postcode was used to stratify individuals into five different groups (Quintile 1-Quintile 5 of SIMD). Quintile 1 represents the most deprived group whereas quintile 5 represents the least deprived group.

5.2.6 Statistical Analysis

Summary statistics of baseline participant characteristics were calculated in men and women separately. Continuous variables were summarised as means (±SDs).

They were compared between groups using independent 't' tests. Categorical variables are shown as counts and percentages and were compared between groups using Chi square tests for association. The summary statistics estimation process repeated after stratification by deprivation status. Histograms were plotted for phosphate levels in men and women to check the distribution.

The Cox proportional hazards (Cox-PH) model as explained in section 2.6.2 was used to determine the risk of all-cause death for each increment of 0.1 mmol/L increase in baseline phosphate. The model was adjusted for major CV risk factors: age, gender, BMI, smoking, alcohol use, SBP, DBP, total cholesterol, eGFR, calcium, and alkaline phosphatase. A variable on year of first visit strata (epochs) was included to adjust the secular trend in mortality and was divided into five categories (first visit 1976 or before, between years 1977-1987, 1988-1997, 1998-2004, 2005 and after). The final model included all of the covariates that were significant (p<0.10) in the multivariate analysis. The same model is repeated in gender groups and for all other mortality outcomes. Another model after adjustment for the deprivation index in quintiles was also generated in men and women separately. Separate models for all-cause mortality were generated in diuretic users at baseline and non-diuretic users. Furthermore, a sub-group analysis was also conducted for all-cause, CV and non-CV mortality outcomes stratified by the baseline characteristics. The PH assumption was tested in the multivariate models (details are explained in section 2.6.3) by the goodness-of-fit tests. The association of serum phosphate on a continuous scale with all-cause mortality was tested using multivariate Cox-PH models with restricted spline functions (details are explained in section 2.6.4) in men and women separately. The regression spline models were repeated after adjustment for deprivation status. Finally, serum phosphate in quartiles based on sex specific cut-off points was used to generate multivariate Cox-PH models in men and women separately.

Generalized estimating equations (GEE) regression models as explained in section 2.7.1 were employed to study the change in SBP and DBP over the initial five years of follow-up with each increment increase in 0.1 mmol/L of baseline phosphate in men and women separately. Individuals with at least four annual BP assessments in the first five years of follow-up and survival up to a minimum of five years period were included in the GEE analyses. The GEE model was

adjusted for age, sex, smoking, alcohol use, BMI and eGFR. The analysis was repeated after adjustment for deprivation index. All of the analyses were carried out using Stata version 12 (Stata Corp, LP).

5.3 Results

5.3.1 Baseline characteristics

A total of 9260 patients with baseline phosphate were included in the current analysis. Of these, 47% (n=4318) were men. The population was middle aged with mean age of 51.7±14.6 years. The baseline characteristics of the study population in men and women are described in table 5-1 on page 159.The baseline mean BMI was in the overweight region and different in men (28.4±5.6) and women (28.0 ±6.4). While the mean SBP was 161.6±26.2 in men, it was 165.0±29.3 in women. Smokers and alcohol users were more common in men. Compared with men, women had significantly higher levels of serum calcium.

The distributions of phosphate in men and women are presented as probability density plots in figure 5-1 on page 159. The mean phosphate was higher in women (1.10±0.20) than in men (1.02±0.21). The baseline characteristics of the study population in men and women stratified by phosphate quartiles are presented in table 5-2 and 5-3 on pages 167-168. Age and SBP show an inverse linear relationship with phosphate in men whereas they show positive linear relationships in women. Chronic kidney disease status and tobacco use gradually increased with phosphate quartiles in both men and women. Serum ALP level was significantly elevated in quartile 1 of phosphate in comparison to other quartiles in men and women. The prevalence of CVD and mean total cholesterol level at baseline increased with phosphate quartiles in women but not in men.

Table 5-1: Baseline characteristics of the study population stratified by gender

Variables	Men (n=4318)	(n=4942)	P value
Age at first visit (years), mean (SD)	50.36 (13.33)	52.84 (15.57)	<0.001
BMI (Kg/m^2), mean (SD)	28.39 (5.63)	27.98 (6.42)	0.001
SBP (mmHg), mean (SD)	161.59 (26.20)	164.99 (29.33)	<0.001
DBP (mmHg), mean (SD)	98.01 (14.29)	95.40 (14.13)	<0.001
Total Cholesterol (mmol/l), mean (SD)	5.79 (1.18)	6.00 (1.35)	<0.001
Calcium (mg/dl), mean (SD)	2.33 (0.12)	2.35 (0.12)	<0.001
Alkaline Phosphatase (IU/L), median (IQR)	124 (78-178)	124 (78-185)	0.342
Phosphate (mmol/L), mean (SD)	1.02 (0.21)	1.10 (0.20)	<0.001
eGFR<60 mL/min, n (%)	803 (18.60)	1447 (29.28)	<0.001
Alcohol >6 units, n (%)	2985 (73.09)	2044 (43.73)	<0.001
Tobacco smoking, n (%)	2042 (48.24)	1953 (40.22)	<0.001
CVD, n (%)	955 (22.12)	880 (17.81)	<0.001
SIMD Quintiles, n(%)	-	-	0.003
Quintile 1	1016 (25.63)	1313 (28.82)	
Quintile 2	719 (18.14)	869 (19.07)	
Quintile 3	604 (15.24)	659 (14.46)	
Quintile 4	639 (16.12)	689 (15.12)	
Quintile 5	986 (24.87)	1026 (22.52)	

BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, eGFR=estimated glomerular filtration rate based on MDRD equation, Baseline CVD=prevalent cardiovascular disease at baseline, SD=standard deviation, SIMD=Scottish Index of Multiple Deprivation.
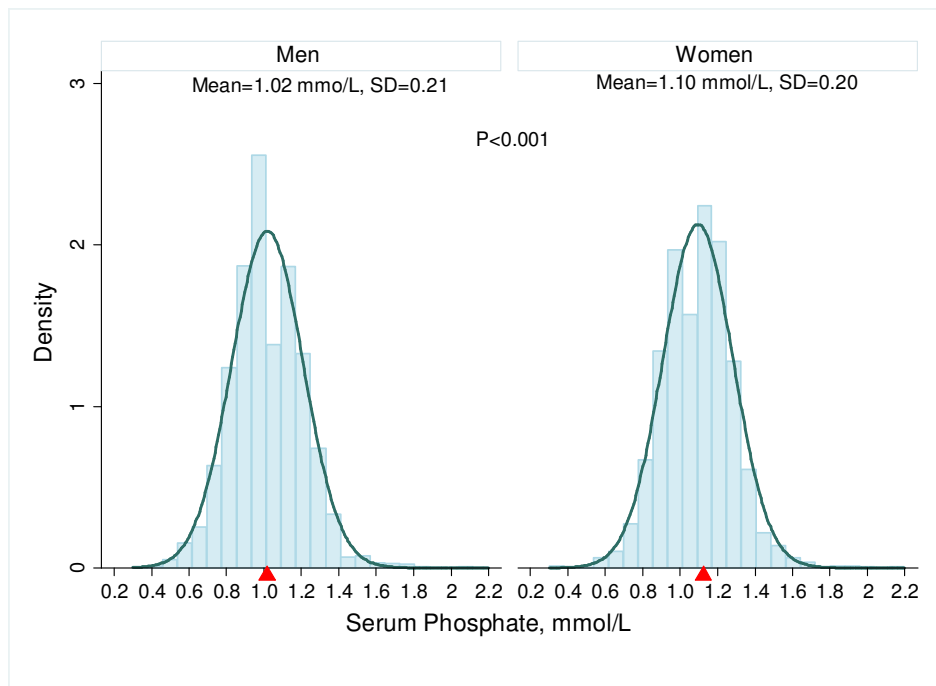
Figure 5-1: Distribution of serum phosphate in men and women

Table 5-2: Characteristics of study population stratified by quintiles of phosphate in men.

Variables	PO4≤0.9 (N=1272)	PO4=0.91-1.0 (N=960)	PO4=1.01-1.14 (N=1050)	PO4>1.14 (1036)	P value
Age (years), mean (SD)	51.12 (12.56)	50.86 (12.95)	49.63 (13.40)	49.69 (14.43)	<0.001
BMI (Kg/m^2), mean (SD)	28.61 (5.91)	28.61 (5.80)	28.44 (5.61)	27.85 (5.06)	0.358
SBP (mmHg), mean (SD)	163.33 (26.66)	162.07 (25.47)	159.60 (25.57)	161.01 (26.78)	0.01
DBP (mmHg), mean (SD)	98.73 (14.20)	98.28 (13.56)	98.47 (14.18)	97.43 (15.14)	0.233
TC (mmol/l), mean (SD)	5.76 (1.16)	5.80 (1.11)	5.80 (1.22)	5.81 (1.22)	0.385
eGFR≤60, n (%)	209 (16.43)	164 (17.08)	186 (17.71)	244 (23.55)	<0.001
Ca (mg/dl), mean (SD)	2.34 (0.12)	2.33 (0.11)	2.32 (0.11)	2.34 (0.12)	0.105
ALP, Median (IQR)	136 (85-185)	119 (78-176)	130 (78-181)	106 (72-164)	<0.001
Alcohol >6 units, n (%)	1033 (86.59)	794 (87.93)	870 (87.09)	848 (85.74)	0.554
Tobacco smoking, n (%)	539 (43.05)	460 (48.88)	488 (47.56)	555 (54.73)	<0.001
CVD, n (%)	272 (21.28)	222 (23.13)	229 (21.81)	232 (22.39)	0.785

Table 5-3: Characteristics of study population stratified by quintiles of phosphate in women.

Variables	PO4≤0.98 (N=1256)	PO4=0.99-1.1 (N=1469)	PO4=1.11-1.2 (N=1010)	PO4>1.2 (1207)	P value
Age (years), mean (SD)	49.49 (14.21)	52.16 (15.57)	54.42 (15.77)	55.83 (16.02)	<0.001
BMI (Kg/m^2), mean (SD)	28.31 (4.74)	28.31 (4.81)	28.24 (4.94)	27.77 (4.84)	0.002
SBP (mmHg), mean (SD)	160.94 (27.85)	165.76 (29.16)	165.81 (29.06)	167.60 (30.83)	0.016
DBP (mmHg), mean (SD)	95.82 (13.18)	95.95 (14.14)	94.88 (14.35)	94.73 (14.85)	0.004
TC (mmol/l), mean (SD)	5.80 (1.20)	6.06 (1.35)	6.04 (1.38)	6.13 (1.43)	0.027
eGFR≤60, n (%)	282 (22.45)	390 (26.55)	308 (30.50)	467 (38.69)	<0.001
Ca (mg/dl), mean (SD)	2.33 (0.13)	2.35 (0.13)	2.35 (0.12)	2.35 (0.13)	<0.001
ALP, Median (IQR)	139 (85-193)	111 (73-176)	118 (78-185)	124 (79-187)	<0.001
Alcohol >6 units, n (%)	855 (71.61)	1006 (71.96)	665 (69.56)	777 (69.01)	0.296
Tobacco smoking, n (%)	420 (33.95)	576 (39.81)	417 (41.99)	540 (45.80)	<0.001
CVD, n (%)	176 (14.01)	271 (18.45)	183 (18.12)	250 (20.71)	<0.001

PO4=Serum Phosphate in mmol/L, BMI=Body Mass Index, SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure, TC=Total Cholesterol, eGFR=estimated Glomerular Filtration Rate, Ca=Serum Calcium, ALP=Serum Alkaline Phosphatase, CVD=Prevalent Cardiovascular Diseases, SD=Standard Deviation, IQR=Inter Quartile Range, , Kg=Kilogram, mmHg=millimeter of mercury, mg/dl=milligram per decilitre

5.3.2 Deprivation status and baseline variables

Despite lower mean age, men from the most deprived regions had significantly higher mean levels of BMI, SBP, DBP and serum alkaline phosphatase in comparison to men from least deprived regions (Table 5-4 on page 163). Similar trends were seen in women (Table 5-5 on page 164). Serum calcium was elevated in men from the most deprived regions in comparison to their counterparts from the least deprived regions. Smoking rates were significantly higher in individuals from the most deprived regions in both men and women. However, alcohol use was less frequently observed in women from the most deprived regions in comparison to women from least deprived regions. Baseline mean serum phosphate was higher in men from the most deprived regions in comparison to men from least deprived regions. However, phosphate levels were similar in women from all groups based on SIMD quintiles (Table 5-5 on page 164).

5.3.3 Follow-up period and event rates

The total duration of follow-up was 129272.64 person years (p-y) with median survival time of 33.3 years. The time at risk in men and women were 59286.13 p-y and 69986.51 p-y, respectively. The overall event rates per 1000 p-y of follow-up were 16.50 (95% CI: 15.82-17.22), 8.95 (8.44-9.48), 4.70 (4.34-5.09), 2.31 (2.06-2.59), and 7.56 (7.10-8.05) for all-cause, cardiovascular, IHD, Stroke and non-CVD mortality, respectively. The event rates per 1000 p-y of follow-up stratified by quartiles of phosphate in men and women are presented in table 5-6 on page 165. All-cause mortality increased linearly with phosphate quartiles.

5.3.4 Survival analysis

In the Kaplan-Meir survival curves of serum phosphate in quartiles and all-cause mortality (Figure 5-2 on page 162), the shortest survival time was observed in the quartile 4. Using phosphate as a continuous variable, each increment of 0.1 mmol/L phosphate augmented the all-cause mortality by 6% (HR=1.06, 95% CI: 1.04-1.08) after adjustment for all other relevant variables. In men the increase in mortality was 7% (HR=1.07, 95% CI: 1.03-1.10), while in women it was 6% (HR=1.06, 95% CI: 1.02-1.10). Adjustment for deprivation did not change the risk estimates in the Cox-PH models (Table 5-7 on page 166). The cubic spline

regression analyses of phosphate and all-cause mortality showed a linear increase in mortality with serum phosphate >1 mmol/L in men, while in women mortality increased linearly with serum phosphate >0.4 mmol/L (Fig 5-3 on page 168). Despite social deprivation influencing serum phosphate levels in men, it did not have an influence on the association between phosphate and all-cause mortality in both men and women in the regression spline Cox models.

Cardiovascular mortality increased with each increment of 0.1 mmol/L phosphate by 8% (HR=1.08, 95% CI: 1.03-1.12) in men. The association did not change significantly after adjustment for deprivation in men. However, the moderate effect seen in women disappeared after adjustment for deprivation (Table 5-7 on page 167) and indicates residual confounding in the unadjusted analyses (Table 5-6 on page 165). Similarly, the IHD mortality was also increased with unit increase in phosphate in men. Phosphate had no effect on stroke mortality in men and women.

Although 0.1 mmol/L increase in phosphate was associated with 5% (HR=1.05, 95% CI: 1.00-1.11) increase in nonCV mortality in men with borderline significance level, the association almost disappeared after adjustment for deprivation. However, in women 0.1 mmol/L increase in phosphate increased the non-CV mortality by 8% (HR=1.08, 95% CI: 1.03-1.14) and 9% (HR=1.09, 95% CI: 1.02-1.15) respectively before and after adjustment for deprivation (Table 5-7 on page 167).
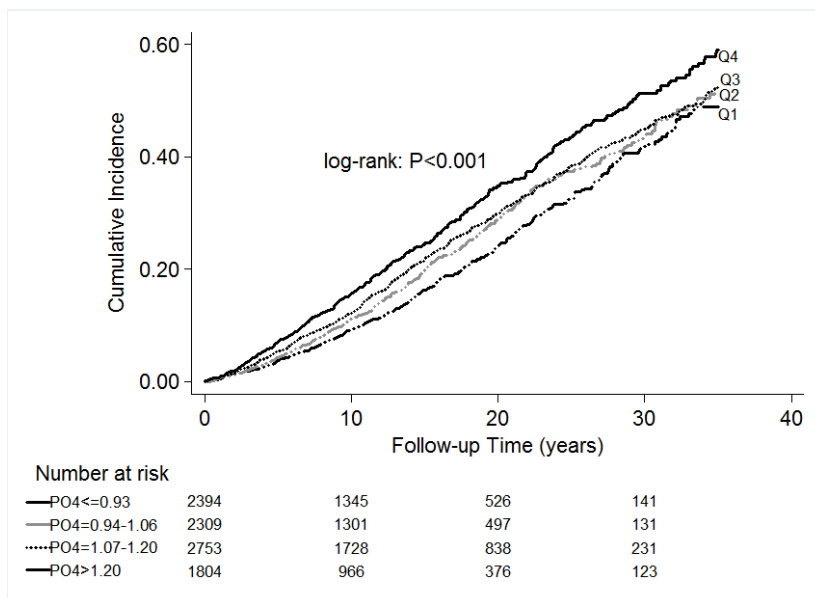
Figure 5-2: Kaplan-Meir survival curves of serum phosphate in quartiles

Serum phosphate in quartiles and mortality are presented in men (Figure 5-4 on page 169) and women (Figure 5-5 on page 169) separately after adjustment for all variables. While CV mortality was associated with higher serum phosphate in men, the relationship was stronger in women for non-CV mortality.

In the sub-group analysis, 0.1 mmol/L increase in phosphate increased all-cause mortality consistently in all sub-groups (Figure 5-6 on page 170). Stratification by baseline SBP, follow-up BP control status, serum calcium and alkaline phosphatase levels and smoking status did not have a significant influence on the association between phosphate and mortality. However, the association was very strong in individuals with poor kidney function in comparison to individuals with better kidney function. However, this could be a chance finding as I had tested the differences in more than 20 subgroups. All-cause mortality hazard ratios were similar in users (HR=1.07, 95% CI: 1.00-1.14) and non-users (HR=1.07, 95% CI: 1.03-1.120) of diuretics in men. Consistent results were reported in women in non-users of diuretics (HR=1.07, 95% CI: 1.02-1.12) and users of diuretics (HR=1.04, 95% CI: 0.98-1.10).

Table 5-4: Baseline characteristics of the study population stratified by deprivation status in men

Variables	Men SIMD Quintile 1 (N=1016)	SIMD Quintile 2 (N=719)	SIMD Quintile 3 (N=604)	SIMD Quintile 4 (N=639)	SIMD Quintile 5 (N=986)	P value
Age at first visit (years), mean (SD)	49.67 (13.57)	50.21 (13.87)	49.95 (13.63)	50.10 (13.36)	51.97 (12.94)	0.003
BMI (Kg/m^2), mean (SD)	29.19 (6.54)	28.51 (5.68)	28.55 (5.52)	28.40 (5.73)	27.91 (4.86)	<0.001
SBP (mmHg), mean (SD)	163.78 (28.08)	162.30 (26.86)	160.83 (25.37)	159.67 (24.80)	158.59 (24.17)	<0.001
DBP (mmHg), mean (SD)	99.03 (15.27)	97.91 (14.17)	98.09 (14.49)	96.48 (13.51)	96.48 (13.01)	<0.001
Total Cholesterol (mmol/l), mean (SD)	5.81 (1.20)	5.80 (1.19)	5.66 (1.16)	5.79 (1.17)	5.71 (1.12)	0.061
Calcium (mg/dl), mean (SD)	2.34 (0.12)	2.33 (0.12)	2.32 (0.11)	2.32 (0.12)	2.32 (0.11)	<0.001
Alkaline Phosphatase (IU/L), median (IQR)	129 (82-181)	132 (83-187)	131 (78-187)	120 (73-170)	86 (63-140)	0.004
Phosphate (mmol/L), mean (SD)	1.03 (0.20)	1.03 (0.19)	1.04 (0.24)	1.01 (0.20)	1.00 (0.19)	0.003
eGFR<60 mL/min, n (%)	188 (18.50)	127 (17.66)	103 (17.05)	117 (18.31)	188 (19.07)	0.873
Alcohol >6 units, n (%)	690 (71.73)	485 (70.09)	414 (73.14)	435 (72.50)	677 (73.27)	0.655
Tobacco smoking, n (%)	539 (53.95)	369 (52.12)	288 (48.81)	257 (41.19)	422 (43.91)	<0.001
CVD, n (%)	245 (24.11)	174 (24.20)	130 (21.52)	132 (20.66)	191 (19.37)	0.052

ALP=Serum alkaline phosphatase, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, eGFR=estimated glomerular filtration rate based on MDRD equation, Baseline CVD=prevalent cardiovascular disease at baseline, SD=standard deviation, SIMD=Scottish Index of Multiple Deprivation

Table 5-5: Baseline characteristics of the study population stratified by deprivation status in women

Variables	Women					P value
	SIMD Quintile 1 (N=1313)	SIMD Quintile 2 (N=869)	SIMD Quintile 3 (N=659)	SIMD Quintile 4 (N=689)	SIMD Quintile 5 (N=1026)	
Age at first visit (years), mean (SD)	52.26 (15.46)	54.47 (16.33)	52.12 (14.45)	51.83 (15.63)	55.03 (15.46)	<0.001
BMI (Kg/m^2), mean (SD)	28.72 (6.71)	28.61 (6.79)	28.15 (6.43)	27.83 (6.41)	27.03 (5.73)	<0.001
SBP (mmHg), mean (SD)	166.62 (30.92)	165.50 (29.80)	163.69 (28.77)	161.30 (27.53)	162.31 (27.48)	<0.001
DBP (mmHg), mean (SD)	96.12 (14.84)	94.87 (14.27)	95.30 (13.49)	94.20 (13.42)	93.70 (12.91)	<0.001
Total Cholesterol (mmol/l), mean (SD)	6.01 (1.35)	5.91 (1.37)	5.97 (1.31)	5.93 (1.32)	5.99 (1.29)	0.503
Calcium (mg/dl), mean (SD)	2.35 (0.13)	2.34 (0.12)	2.34 (0.12)	2.35 (0.13)	2.34 (0.12)	0.172
Alkaline Phosphatase (IU/L), median (IQR)	128 (80-192)	129 (83-195)	127 (77-185)	118 (77-174)	90 (63-147)	0.084
Phosphate (mmol/L), mean (SD)	1.10 (0.20)	1.11 (0.20)	1.08 (0.19)	1.09 (0.20)	1.10 (0.20)	0.199
eGFR<60 mL/min, n (%)	394 (30.01)	305 (35.10)	178 (27.01)	179 (25.98)	305 (29.73)	0.001
Alcohol >6 units, n (%)	519 (41.85)	317 (38.75)	240 (38.46)	293 (45.15)	439 (45.59)	0.006
Tobacco smoking, n (%)	643 (49.73)	356 (41.78)	223 (34.63)	227 (33.24)	339 (33.90)	<0.001
CVD, n (%)	284 (21.63)	171 (19.68)	105 (15.93)	102 (14.80)	147 (14.33)	<0.001

ALP=Serum alkaline phosphatase, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, eGFR=estimated glomerular filtration rate based on MDRD equation, Baseline CVD=prevalent cardiovascular disease at baseline, SD=standard deviation, SIMD=Scottish Index of Multiple Deprivation

Table 5-6: Person years of follow-up and event rates stratified by groups based on quintiles of phosphate in men and women.

	Men			
Follow-up and event rates	**PO4≤0.9 mmol/L (N=1272)**	**PO4=0.91-1.0 mmol/L (N=960)**	**PO4=1.01-1.14 mmol/L (N=1050)**	**PO4>1.14 mmol/L (1036)**
Person years of follow-up (p-y)	17283.24	13301.58	14327.82	14373.49
IHD mortality, n/1000 p-y (95% CI)	5.09 (4.13-6.27)	5.71 (4.56-7.15)	5.10 (4.05-6.41)	7.51 (6.22-9.07)
Stroke mortality, n/1000 p-y (95% CI)	2.03 (1.45-2.82)	3.01 (2.21-4.10)	1.88 (1.29-2.48)	2.85 (2.10-3.87)
CV mortality, n/1000 p-y (95% CI)	8.98 (7.66-10.48)	11.13 (9.47-13.07)	9.77 (8.28-11.53)	12.52 (10.82-14.49)
Non-CV mortality, n/1000 p-y (95% CI)	7.58 (6.39-9.00)	7.44 (6.11-9.06)	7.05 (5.80-8.57)	8.00 (6.66-9.61)
All-cause mortality, n/1000 p-y (95% CI)	16.55 (14.74-18.58)	18.57 (16.39-21.04)	16.82 (14.83-19.08)	20.52 (18.31-23.01)
	Women			
Follow-up and event rates	**PO4≤0.98 mmol/L (N=1256)**	**PO4=0.99-1.1 mmol/L (N=1469)**	**PO4=1.11-1.2 mmol/L (N=1010)**	**PO4>1.2 mmol/L (1207)**
Person years of follow-up (p-y)	16685.93	22954.54	14072.01	16274.02
IHD mortality, n/1000 p-y (95% CI)	2.16 (1.56-2.99)	3.88 (3.15-4.77)	4.05 (3.12-5.25)	4.92 (3.95-6.12)
Stroke mortality, n/1000 p-y (95% CI)	1.74 (1.21-2.50)	2.13 (1.61-2.82)	2.56 (1.85-3.55)	2.64 (1.96-3.56)
CV mortality, n/1000 p-y (95% CI)	4.91 (3.96-6.10)	7.67 (6.61-8.89)	7.96 (6.61-9.58)	10.14 (8.70-11.81)
Non-CV mortality, n/1000 p-y (95% CI)	4.91 (3.96-6.10)	6.80 (5.81-7.95)	9.38 (7.91-11.13)	9.83 (8.42-11.48)
All-cause mortality, n/1000 p-y (95% CI)	9.83 (8.43-11.45)	14.46 (12.99-16.11)	17.34 (15.29-19.666)	19.97 (17.91-22.26)

p-y=Person Years of Follow-up, PO4=Serum Phosphate, mmol/L=millimol/Litre, IHD=Ischeamic Heart Disease, CV=Cardiovascular, Non-CV=Non Cardiovascular, CI=Confidence Interval.

Table 5-7: Cox-Proportional Hazard models of phosphate and mortality outcomes.

Cox-Proportional Hazard Models	All-cause mortality		CVD mortality		IHD mortality		Stroke Mortality		Non-CVD mortality	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
	N=1026/4042		N=591/4042		N=327/4042		N=136/4042		N=435/4042	
Baseline Phosphate, Men (Model 1)	1.07*	1.03-1.10	1.08*	1.03-1.12	1.08*	1.02-1.14	1.04	0.95-1.14	1.05	1.00-1.11
	N=847/3704		N=482/3704		N=260/3704		N=114/3704		N=365/3704	
Baseline Phosphate, Men (Model 2)	1.05*	1.01-1.09	1.07*	1.02-1.13	1.09*	1.01-1.16	1.03	0.93-1.14	1.03	0.97-1.09
	N=1010/4606		N=507/4606		N=247/4606		N=146/4606		N=503/4606	
Baseline Phosphate, Women (Model 1)	1.06*	1.02-1.10	1.04	0.99-1.09	1.03	0.96-1.11	1.01	0.92-1.11	1.08*	1.03-1.14
	N=858/4230		N=427/4230		N=208/4230		N=126/4230		N=431/4230	
Baseline Phosphate, Women (Model 2)	1.05*	1.01-1.10	1.02	0.96-1.08	1.02	0.94-1.11	0.98	0.88-1.09	1.09*	1.02-1.15

Model 1: adjusted for age, epochs, smoking, alcohol use, baseline systolic blood pressure, diastolic blood pressure, serum calcium, and serum alkaline phosphatase. Model 2: adjusted for all variables as in model 1 and deprivation index in quintiles. The hazard ratios are for each increment of 0.1 mmol/L of phosphate.
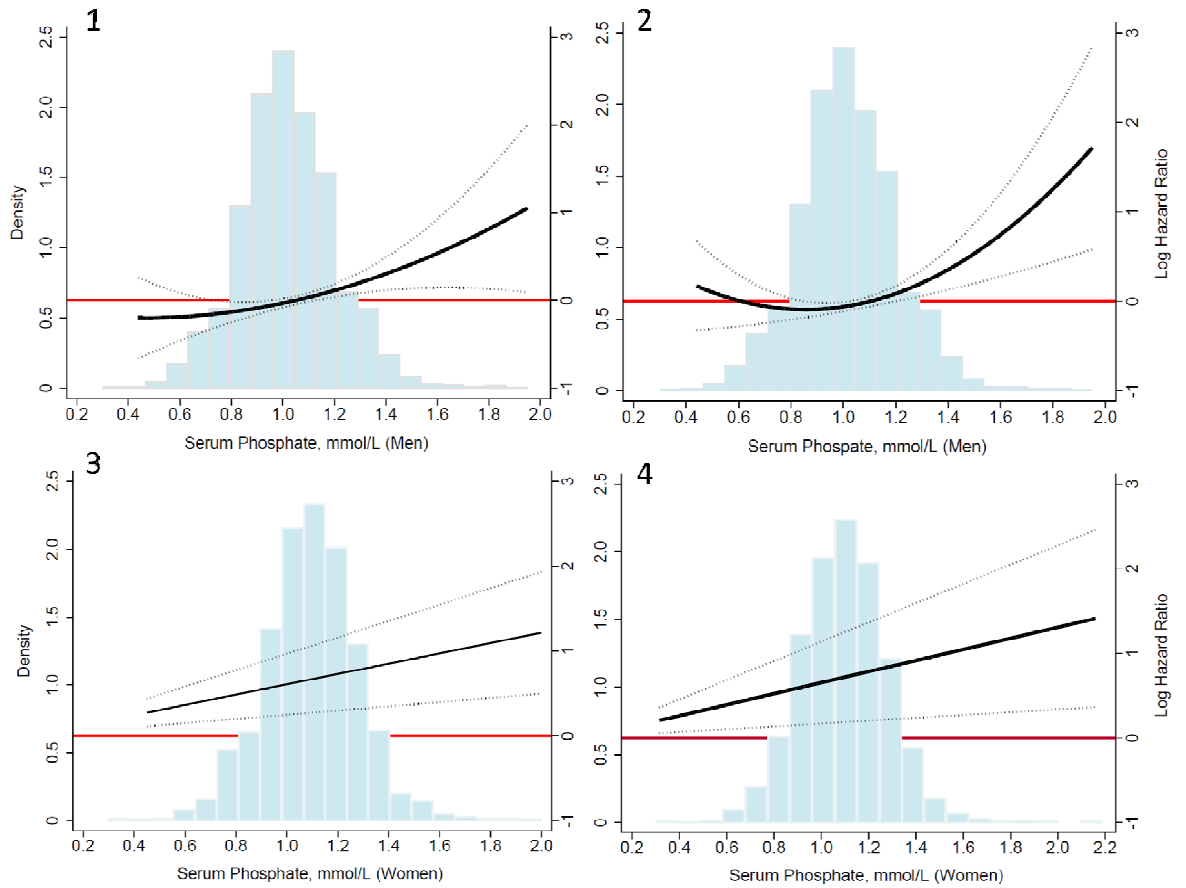
Figure 5-3: Regression spline Cox hazard model for serum phosphate and all-cause mortality.

Panel 1: Before adjustment for deprivation in men. Panel 2: Adjusted for deprivation in men. Panel 3: Before adjustment for deprivation in women. Panel 4: Adjusted for deprivation in women. All models also include age, epochs, smoking, alcohol use, baseline systolic blood pressure, diastolic blood pressure, serum calcium, and serum alkaline phosphatase.
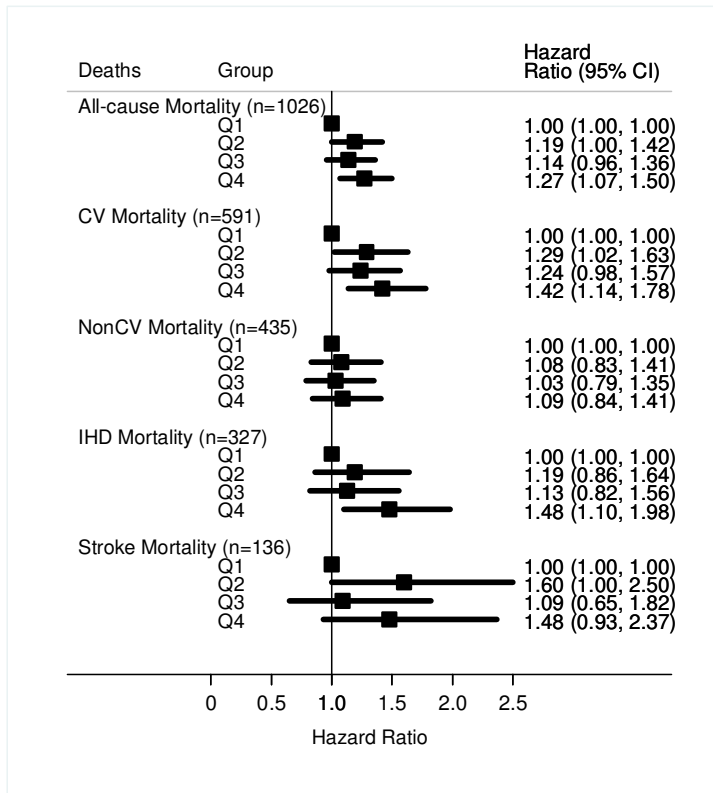
Figure 5-4: Serum phosphate in quartiles and mortality in men.
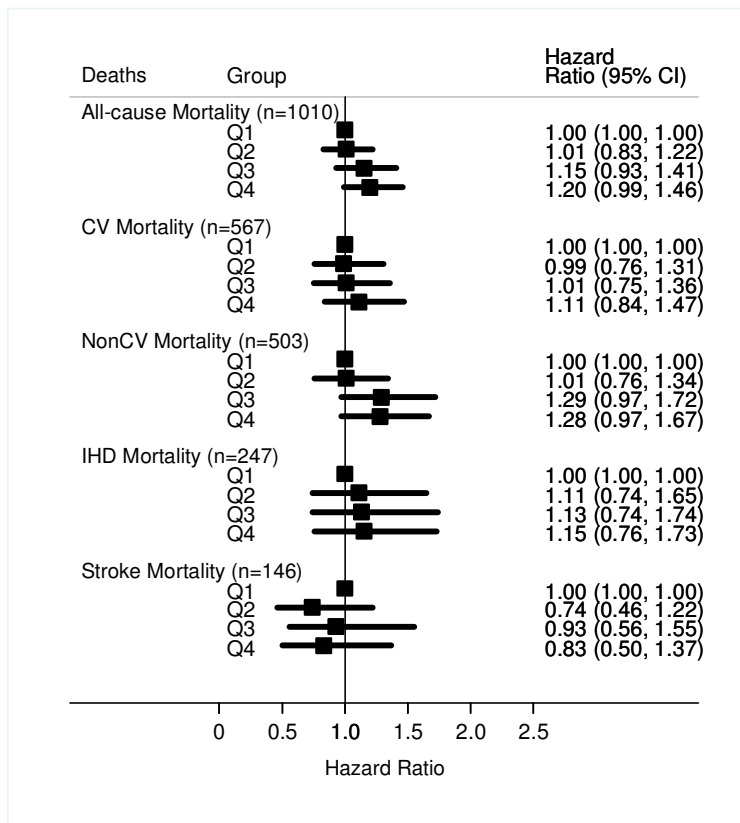
Figure 5-5: Serum phosphate in quartiles and mortality in women.

Group	Deaths	Hazard Ratio (95% CI)
All-cause Mortality		
Overall	2036	1.06 (1.04, 1.08)
Men	1026	1.07 (1.03, 1.10)
Women	1010	1.06 (1.02, 1.10)
Deprived	653	1.06 (1.01, 1.11)
Non-Deprived	464	1.08 (1.02, 1.13)
Smokers	1101	1.08 (1.05, 1.12)
Non-Smokers	935	1.04 (1.01, 1.08)
SBP>=160	1519	1.06 (1.03, 1.09)
SBP<160	517	1.07 (1.02, 1.12)
CVD	588	1.03 (0.99, 1.08)
No CVD	1448	1.08 (1.05, 1.11)
eGFR<60	728	1.11 (1.07, 1.15)
eGFR>=60	1308	1.03 (1.00, 1.06)
High-ALP	680	1.11 (1.07, 1.16)
Low-ALP	1356	1.03 (1.00, 1.07)
High Calcium	1022	1.06 (1.03, 1.10)
Low-Calcium	1014	1.07 (1.03, 1.11)
Responders	338	1.05 (0.99, 1.12)
Non-Responders	1570	1.07 (1.04, 1.10)

0.90 0.95 1.00 1.05 1.10 1.15 1.20 1.25

Hazard Ratio

Figure 5-6: Sub-group analyses of serum phosphate and all-cause mortality.
The hazard ratios are for each increment of 0.1 mmol/L of phosphate.

5.3.5 Association of phosphate with follow-up blood pressure

The annual reduction in SBP and DBP were 3.64(95% CI: 3.48-3.86) and 1.92 (95% CI: 1.80-2.10) mmHg, respectively. During the initial five years of follow-up, longitudinal change in mean SBP was not influenced by serum phosphate in men and women after adjustment for age, BMI, smoking status, alcohol use, serum calcium, epochs and eGFR (Table 5-8 on page 171). While mean DBP did not change with serum phosphate in men, it decreased moderately in women by 0.13 mmHg (95% CI: 0.25 to 0.04) with each increment of 0.1 mmol/L increase in serum phosphate.

Table 5-8: Longitudinal increase in blood pressure across baseline phosphate levels in men and women

| | SBP mmHg | | DBP mmHg | |
| | Men | Women | Men | Women |
GEE Models	GEE β [95% CI]	GEE β [95% CI]	GEE β [95% CI]	GEE β [95% CI]
Phosphate (Model 1)	-0.09 [-0.33; 0.16]	0.16 [-0.09; 0.41]	-0.05 [-0.18; 0.09]	0.13 [-0.25; 0.04]

Model 1: Adjusted for age, body mass index, smoking, alcohol use, serum calcium, epochs, and eGFR CKD status. The regression coefficients (βs) are for each increment of 0.1 mmol/L of phosphate.

5.4 Discussion

The present study demonstrates that in a large cohort of hypertensive individuals followed-up for over 30 years, elevated initial serum phosphate is an independent predictor of all-cause mortality. While serum phosphate is associated with CV mortality in men, it is more closely associated with non-CV mortality in women.

Although there are consistent reports on the association between serum phosphate and mortality in patients with CKD and end stage renal disease [441], the relationship is not clear in individuals with normal kidney function. Post-hoc analyses of the post myocardial infarction CARE cohort have demonstrated a significant association between elevated serum phosphate and adverse CV outcomes in individuals with normal renal function [444]. Even though a large study in the general population reported a positive association of high phosphate with mortality, the results were partially adjusted for confounding factors [450]. By contrast in a recent 8 year follow-up study of 1206 patients with acute cardiovascular events, serum phosphate was not independently associated with all-cause mortality or CV events [600]. However, the total number of events reported was relatively small in this cohort and all-cause mortality showed a non-significant increase with increase in serum phosphate. The present study in a large hypertensive cohort supports the evidence that serum phosphate is independently associated with mortality. The findings are important as they are adjusted for all the conventional risk factors, serum calcium and alkaline phosphatase.

Vascular calcification, vascular stiffness and associated peripheral arterial resistance are hypothesised as potential mechanisms mediating the association

of serum phosphate with incident CVD and mortality. Higher serum phosphorous is associated with coronary artery calcification and obstruction in individuals with suspected coronary artery disease [601]. Higher phosphate levels even within the normal range is associated with coronary artery calcium deposits as estimated by computed tomography measurements in young adults in the CARDIA study [584]. Similar findings are reported in Koreans with normal renal function [602]. In vitro experiments suggest that exogenous serum phosphorous induces phenotypic transformation of aortic vascular smooth muscle cells to osteoblast like cells, production of calcium binding proteins and secretion of matrix vesicles [603] [604]. These changes invariably facilitate calcium deposition on smooth muscle cell walls of the vasculature. Interestingly, the sodium-phosphate co-transporter, PT1-I is present in vascular smooth muscles which is essential for calcification [585] [605]. A low phosphate diet in animal models decreased hyperphosphatemia and ameliorated endothelial dysfunction associated with increased serum phosphate [606].

Mechanisms that link hyperphosphataemia and CVD also allude to dysregulation of vitamin D (Vit. D) synthesis and elevated parathyroid hormone (PTH) levels. However, animal studies provide evidence on independent association of serum phosphate with vascular calcification and mortality. Fibroblast growth factor FGF-23 regulates phosphate re-absorption from the renal tubules and therefore the phosphate levels in serum [607]. Despite higher serum phosphate levels in FGF-23 gene knocked-out mice, a phosphate deficient diet decreased serum phosphate and prevented vascular calcification and mortality independent of Vit.D and calcium levels [608]. Over expression of FGF-23 and Klotho (the gene regulating the synthesis of FGF-23) is associated with hypophosphatemia and increased longevity [609].

Type of mortality associated with phosphate differs in men and women. While phosphate is associated with CV mortality in men, the association is stronger in women for non-CV mortality. Consistent findings for CV mortality are reported in the literature [610]. Despite women having significantly higher serum phosphate in comparison to men [611], the association of phosphate with CV mortality is weaker in women in comparison to men. The reasons for these apparent differences are not clear.   The hormonal status has a role in phosphate metabolism and oestrogens in general have a phosphaturic effect [612]. Menopausal status and

hormone replacement therapy therefore may have an independent effect on phosphate levels [611]. However, in the post hoc analyses of MORE study participants (7259 postmenopausal women with osteoporosis), serum phosphate was not associated with all-cause mortality [454]. There are no clear explanations on the influence of hormonal status on association between serum phosphate and mortality. It is impossible to tease out the potential causes of this difference in the present study, but may merit further investigation in future studies.

The present study also demonstrates a gender specific effect of phosphate and degree of deprivation. It is unclear why phosphate level remained significantly higher only in men from more deprived background compared to their less deprived counterparts. The lack of association of phosphate with deprivation status in women could possibly be explained by the phosphaturic effect of oestrogens which may act as an additional homeostatic mechanism of maintaining tight control of serum phosphate in pre-menopausal women [613].

Inappropriate adjustment for confounding factors is often cited as a major criticism in the association between phosphate and mortality in previous studies [440] [441]. In the present study we adjust for all conventional risk factors, serum calcium, alkaline phosphatase and deprivation status. Serum phosphate is associated with intake of processed food items which contain significant amount of inorganic phosphate. There is evidence to suggest that poor socio-economic status and greater deprivation are associated with intake of more processed food and high serum phosphate levels [596]. The models adjusting for Scottish deprivation index provide reasonable evidence to suggest that the association between phosphate and mortality observed in the present study is not confounded by deprivation. Thiazides like diuretics are associated with phosphaturia[614] and may have a confounding effect on the association between serum phosphate and mortality. However, the results were consistent in users and non-users of diuretics at baseline. The longitudinal change in SBP in this hypertensive cohort is not influenced by baseline serum phosphate levels. Therefore, the association of phosphate and mortality observed in this study is not subjective to the effect of phosphate on BP.

The factors associated with higher serum phosphate remain unclear. Environmental factors include increased dietary intake of protein rich and

processed foods and liquids [594]. However there are no dietary intervention studies in the literature that evaluates the role of a low phosphate diet on serum phosphate, BP and other cardiovascular outcomes. Such studies are required to establish the causal role of phosphate on BP and mortality outcomes.

5.5 Strengths and limitations

The strengths of the current study include; a large cohort of nearly 10,000 hypertensive adults, 35 years of follow-up with median survival time of 33 years, the ability to link serum phosphate with differing causes of mortality outcomes, inclusion of the entire distribution of serum phosphate in the analyses, and adjustment for all potential confounding factors including the deprivation status. Since serum phosphate distribution was different in men and women, all analyses were conducted in men and women separately. The exclusion of individuals without serum phosphate assessed at baseline from our analysis and the bias introduced by the missing covariates in the adjusted Cox-PH models especially the deprivation status are the major limitations of this study. However, the results were consistent across multiple models with and without such variables.

5.6 Summary

Serum phosphate is an independent predictor of mortality in a treated hypertensive population. While CV mortality is more closely associated with higher serum phosphate in men, women show prominent association with non-CV mortality. Deprivation status, serum calcium and serum alkaline phosphatase levels do not attenuate the mortality risk associated with serum phosphate in men and women. Further study is required to address the effect of dietary intervention to reduce intake of phosphate rich foods on long term achieved BP, CV and all-cause mortality outcomes. Clinical trials of alternative strategies for lowering serum phophate (e.g. phosphate binding drugs) may represent a further strategy for improving outcomes in hypertensive subjects.

6 Serum electrolytes and mortality

6.1 Introduction

Electrolytes, especially sodium, chloride, potassium and bicarbonates, play a vital role in maintaining homeostasis within the human body. Serum sodium is an essential electrolyte that regulates blood volume, BP, osmotic equilibrium and the acid-base balance [615] [616]. The volume of extracellular fluid (ECF) is directly proportional to the content of sodium in plasma. The normal plasma concentration of sodium is 135-145 mEq/L. An increase in dietary sodium intake from very low to moderately high level results in an 18% increase in the ECF volume [617]. Moreover, an increase in daily sodium excretion of 200 mmol (equivalent to the effect of a 5g increase in sodium intake), increases mean arterial BP by 1 mmHg [618]. The association between dietary sodium intake and mortality shows a non-linear "j" shaped pattern with significantly higher mortality at intakes above and below the range of 2.5-6.0 g/day [619].

Chloride is the major extracellular anion and is principally responsible for maintaining proper hydration, osmotic pressure, and normal cation-anion balance in the vascular and intestinal fluid compartments [620]. Furthermore, chloride is the main determinant of metabolic acid-base state in critical illnesses [621]. The normal plasma concentration of chloride is 95-105 mEq/L. Most of the chloride in the body comes from table salt (sodium chloride) or other salt contained in the diet. While chloride is almost completely absorbed from the intestinal tract, it is removed from the blood by glomerular filtration and then reabsorbed by the kidney tubules [620]. Contrasting findings are reported in the literature on the association between dietary chloride intake and BP in animal models. While earlier studies did not find any association of dietary chloride with BP independent of dietary sodium content [622] [623], some of the new animal studies suggest an independent positive association [624-626]. Data on association of serum chloride level and mortality are scarce in the general population. In a general population cohort study conducted in Belgium, serum chloride level below the range of 100 mEq/L was independently associated with mortality [467]. Consistent findings are reported in the post hoc analyses of North American CHARM (Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity) study cohort [627].

Similar to serum chloride, bicarbonates are also important electrolytes that determine the acid base balance in the body and are routinely measured in hypertensive patients as part of renal function evaluation tests. Low serum bicarbonate levels (acidosis) precede the development of high BP in animal models [628]. Those who were in the highest quintile of serum bicarbonate had SBP 2.73 mmHg lower (95% CI: 1.26 to 4.20 mmHg) than participants in the lowest quintile in the 1999-2000 and 2001-2002 NHANES [629]. A non-linear relationship between serum bicarbonate and progression of kidney disease (with significant elevation of mortality at the lower end of distribution and a moderate elevation at the higher end of the distribution) was evident in a retrospective cohort study of 5422 participants. Similarly, a 'j' shaped relationship between serum bicarbonate and mortality is reported in patients with stage 3 and 4 CKD [470].

Potassium is the most abundant cation in the body. Normal serum potassium is in the range of 3.5 to 5.5 mEq/L, and long-term potassium homeostasis is maintained by the kidneys. Although potassium is freely filtered, 90% is reabsorbed in the tubular segments proximal to the distal convoluted tubule. Minor departure of serum potassium from its tightly controlled range is associated with significant mortality and morbidity.

Hypertension is a significant global public health problem that affects approximately one in four adults [305]. It is an important risk factor for CVD [293] and has a bi-directional relationship with renal function [382]. While the associations between dietary salt (sodium chloride) and BP have been assumed to be related primarily to the sodium content of salt [630] [631], the hypertensive effect of sodium chloride is also dependent on the concomitant presence of both sodium and chloride ions [632] [633]. Furthermore, increased production of organic acids, increase in unmeasured anions, and metabolic acidosis are linked to the development and progression of hypertension [634-636]. Despite the involvement of serum electrolytes in a variety of physiological functions in the human body such as regulation of blood volume, BP, osmotic equilibrium and the acid-base balance, the epidemiological significance of serum electrolytes especially serum chloride in relation to long term mortality outcomes have not been studied in detail. In this study associations of serum chloride and other electrolytes with mortality outcomes are explored by employing survival analysis in a high risk hypertensive cohort followed-up for more than 35 years.

6.2 Materials and Methods

6.2.1 Study setting and study population

Detailed description of study settings and population are described in section 2.1

6.2.2 Clinical measurements

Clinical, anthropometric and demographic data are collected as described in section 2.2.-2.4. Blood samples were collected at baseline and at regular intervals for estimation of routine haematological and biochemical indices. The serum electrolytes, especially, sodium, potassium, chloride and bicarbonate were part of the routine blood assay and available in almost all clinic attendees at baseline and at specific intervals. All biochemical assays were performed at the central laboratory services of the Western Infirmary.

6.2.3 Outcome assessment

The details are explained in section 2.5.

6.2.4 Statistical Methods

All analyses were restricted to the hypertensive individuals in the database with serum chloride assessed at the registration visit (N=12,968). In the presence of free water disturbances, which are common in treated hypertensive patients, serum sodium, chloride, and bicarbonate concentrations is expected to move concordantly, except when a competing acid-base disorder is present. Adjusted serum chloride (adjusted for serum sodium levels) and bicarbonate (adjusted for serum sodium and anion-gap) were estimated based on the following conversion equations.

Adjusted serum chloride ($Cl_{adjusted}$) = measured chloride + 0.76*(140-measured sodium). Adjusted serum bicarbonate ($HCO3_{adjusted}$) = measured bicarbonate + ΔAnion Gap + 0.19*(140-measured sodium)[637]where 'ΔAnion Gap' is the difference between the patient's anion gap and the normal anion gap.

The study population was divided into five groups based on quintiles of serum chloride. The characteristics of the study population across these five groups

were compared using one way ANOVA for continuous variables and Chi square test for categorical variables. Spearman correlation coefficients were generated to study the overall association between serum electrolytes in the whole cohort, and among individuals who were on treatment with diuretics. Participants were also categorized into four groups based on serum chloride and bicarbonate measured at baseline, to examine the effects of both variables on mortality. The groups were; chloride ≤100 mEq/L and bicarbonate ≤25 mEq/L, chloride ≤100 mEq/L and bicarbonate >25 mEq/L, chloride >100 mEq/L and bicarbonate ≤25 mEq/L, and chloride >100 and bicarbonate >25 mEq/L. Finally, they were categorized into four groups based on serum chloride and sodium measured at baseline. The groups were; chloride ≤100 mEq/L and sodium ≤135 mEq/L, chloride ≤100 mEq/L and sodium >135 mEq/L, chloride >100 mEq/L and sodium ≤135 mEq/L, and chloride >100 and sodium >135 mEq/L.

A generalized estimating equation (GEE) model as explained in section 2.7.1 was used to study the association between baseline serum chloride in quintiles and change in mean BP during the follow-up period after adjusting for age, sex, year of first visit, BMI, and eGFR.

The survival probabilities of serum chloride groups in quintiles and groups stratified based on serum sodium and bicarbonate levels were compared using a Kaplan-Meier plot and log-rank test (details are explained in section 2.6.2). Cox proportional hazards (Cox-PH) models as explained in section 2.6.3 were set up to analyze the influence of baseline serum chloride on all-cause, CV, IHD, stroke and non-CV mortality. The covariates included were baseline age, gender, BMI, smoking status (never versus ever), SBP, DBP, alcohol use, tobacco use, eGFR, and last SBP. A variable on year of first visit strata (epochs) was used to adjust for the secular trend in mortality and was divided into three categories (first visit 1987 or before, between years 1988-1997, 1998 and after). Hazard ratios were then generated. The PH assumption was evaluated by plotting the –ln(-ln(survival)) curves for each category of the ordinal covariate versus ln(analysis time) [548]. Initially, serum chloride was assessed as a quantitative trait (Model 1). A second model was generated after adding anion-gap as a predictor variable to Model 1 (Model 2). The anion-gap variable in model 2 was replaced with individual serum electrolytes values (Model 3). Model 4 contained the same covariates as 3, with the exception of chloride which was coded as quintiles.

Finally, model 4 was extended by stratification by concomitant diuretic use (Model 5). The KM and Cox-PH models were repeated by replacing the measured serum chloride in quintiles with adjusted serum chloride and serum bicarbonate in quintiles. Given the potential for a non-linear relationship of serum chloride with time to mortality, regression spline Cox-PH models (details are provided in section 2.6.5) were also set up to smoothen the hazard functions of each of the predictor variables.

6.3 Results

6.3.1 Demographic and clinical characteristics of the study population

Baseline characteristics of the sample are presented in table 6-1 (page 181). The study population was middle aged (50.55+14.17) and predominantly women (52.2%). It was also overweight (mean BMI=27.49+5.66), and hypertensive (SBP 166.23+29.23 and DBP 98.38+16.97). While 45% were smokers, >6 units of alcohol use per week was reported in 61.3% of the population. Less than one fifth of the population (18.2%) reported co-existing CVD morbidity at the time of registration. More than one fifth (22.0%) of the population were on diuretics. The achieved SBP and DBP were significantly lower than the baseline BP. The distribution of serum sodium, chloride, potassium and bi-carbonate are presented as density plots in figure 6-1 (page 180). Serum sodium, chloride, potassium and bicarbonate were normally distributed in the study population with mean and standard deviation of 140.06±2.76 mEq/L, 102.74±3.51 mEq/L, 4.10±0.45 mEq/L and 26.01±3.06 mEq/L, respectively.

The highest positive Spearman correlation coefficient was observed for association between serum chloride and sodium (0.39, $p<0.001$), while the lowest negative correlation coefficient was observed for association between serum chloride and bicarbonate (-0.37, $p<0.001$). Consistent results were observed in users of diuretics (Table 6-2 on page 181). However, adjusting serum chloride and bicarbonate for free water changes resulted in a near perfect correlation (-0.99, p<0.001) between them.

While serum sodium and potassium showed a positive linear association with increase in serum chloride quintiles (P<0.001), mean serum bicarbonate

confirmed an inverse linear association across groups based on serum chloride (P<0.001) (Table 6-3 on page 182). Consequently, mean anion gap was higher in the first quintile of serum chloride in comparison to other quintiles (P<0.001). The proportion of men in the first quintile (51.4%) of serum chloride was significantly higher than other groups (P<0.001) (Table 6-3 on page 182). Age, BMI, SBP, DBP, TC, eGFR, alcohol use, and prevalence of CVD at baseline were significantly different across groups based on quintiles of serum chloride (P<0.001). The proportions of individuals with eGFR<60 mL/min, and alcohol users, were lowest in the fifth quintile of serum chloride (Table 6-3 on page 182). Participants with lower serum chloride (≤100 mEq/L) were older and had higher BP, cholesterol, and CVD prevalence, regardless of bicarbonate and sodium levels (Table 6-4 and Table 6-5 on pages 183-184). The achieved SBP and DBP were also higher at lower levels of serum chloride (p<0.001).
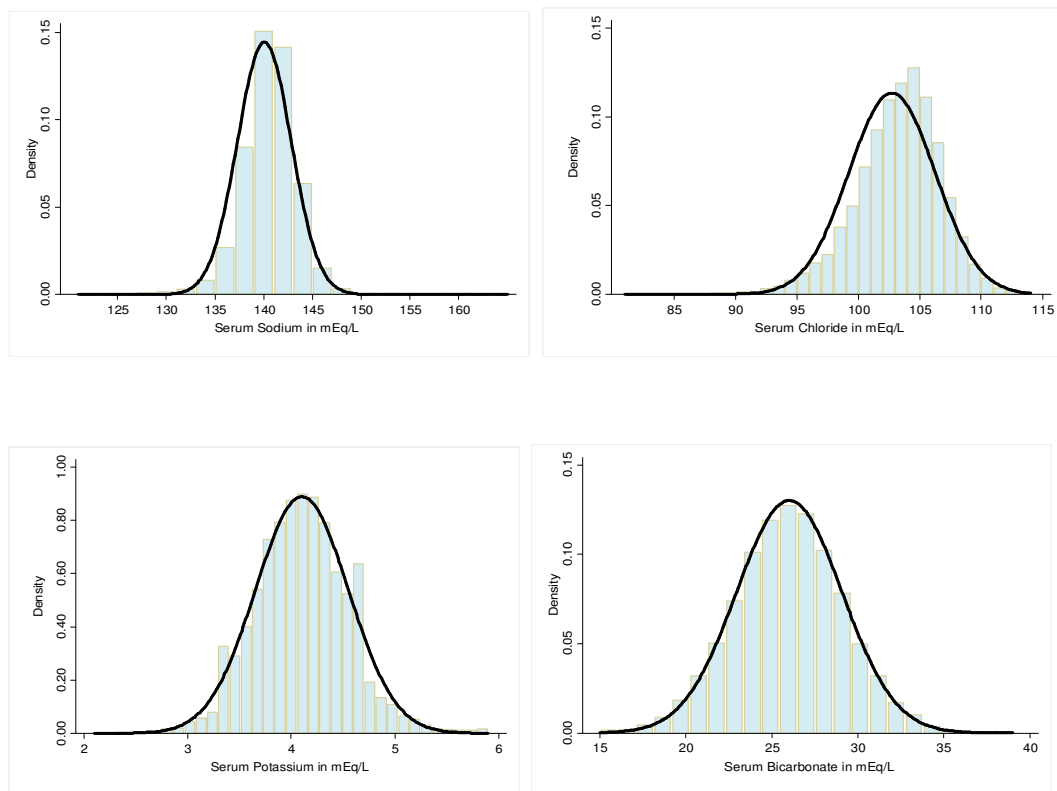
Figure 6-1: Distribution of serum sodium, chloride, potassium and bicarbonate in the study population.

Table 6-1: Correlation matrix of serum electrolytes (Spearman correlation coefficient)

Serum electrolytes	Sodium	Potassium	Bicarbonate	Chloride	Anion Gap
Sodium	1.00				
Potassium	0.02 (0.041) -0.01 (0.479)*	1.00			
Bicarbonate	0.10 (<0.001) 0.07 (<0.001)*	-0.12 (<0.001) -0.15 (<0.001)*	1.00		
Chloride	0.39 (<0.001) 0.39 (<0.001)*	0.22 (<0.001) 0.20 (<0.001)*	-0.37 (<0.001) -0.39 (<0.001)*	1.00	
Anion Gap	0.25 (<0.001) 0.28 (<0.001)*	0.03 (0.008) 0.04 (<0.333)*	-0.46 (<0..001) -0.46 (<0.001)*	-0.30 (<0.001) -0.27 (<0.001)*	1.00

*Pearson Correlation coefficient and corresponding p values in diuretic users.

Table 6-2: Characteristics of the study population

	Total N=12968	Men (N=6193)	Women (N=6775)
Age at first visit (years), mean (SD)	50.55 (14.16)	49.68 (12.94)	51.34 (13.90)
BMI (Kg/m2), mean (SD)	27.48 (5.66)	27.49 (5.06)	27.48 (6.16)
SBP (mmHg), mean (SD)	166.22 (29.25)	164.07 (27.41)	168.18 (30.71)
DBP (mmHg), mean (SD)	98.38 (16.97)	99.35 (15.19)	97.50 (18.41)
Total cholesterol (mmol/l), mean (SD)	5.95 (1.46)	5.84 (1.58)	6.05 (1.35)
eGFR <60 mL/min per 1.73 m2, n(%)	2922 (23.70)	1099 (18.70)	1823 (28.26)
Alcohol use, n (%)	7549 (61.31)	4450 (75.73)	3099 (48.15)
Tobacco use, n (%)	5691 (44.80)	2972 (49.08)	2719 (40.91)
CVD, n (%)	2354 (18.15)	1266 (20.45)	1088 (16.06)
Serum sodium, mean (SD)	140.04 (3.47)	140.25 (3.16)	139.84 (3.72)
Serum potassium, mean (SD)	4.11 (0.73)	4.12 (0.62)	4.10 (0.82)
Serum bicarbonate, mean (SD)	25.92 (3.28)	26.31 (3.21)	25.56 (3.30)
Anion gap, mean (SD)	15.81 (3.28)	15.91 (3.20)	15.72 (3.35)
Diuretic use, n (%)	2857 (22.03)	1306 (21.09)	1551 (22.89)
Achieved SBP (mmHg), mean (SD)	151.20 (24.41)	149.68 (22.25)	152.59 (25.34)
Achieved DBP (mmHg), mean (SD)	86.87 (13.03)	90.60 (12.98)	89.20 (13.04)

SD=standard deviation, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, eGFR=estimated glomerular filtration rate, CVD=prevalent cardiovascular disease at baseline.

Table 6-3: Characteristics of study population stratified by serum chloride in quintiles.

	Cl<=100 (N=3085)	Cl=101-102 (N=2670)	Cl=103-104 (N=3228)	Cl=105-106 (N=2508)	Cl>=107 (N=1477)	P Value
Age at first visit (years), mean (SD)	52.58 (13.04)	50.35 (13.90)	49.44 (14.44)	50.93 (15.19)	50.01 (16.40)	<0.001
Men,n(%)	1587 (51.44)	1364 (51.09)	1511 (46.81)	1118 (44.58)	613 (41.50)	<0.001
BMI (Kg/m2), mean (SD)	26.78 (5.05)	27.45 (5.61)	27.80 (6.00)	27.77 (6.01)	28.30 (6.28)	<0.001
SBP (mmHg), mean (SD)	172.14 (30.14)	167.79 (28.71)	164.92 (28.76)	161.46 (29.42)	161.46 (29.42)	<0.001
DBP (mmHg), mean (SD)	100.88 (16.32)	99.63 (22.19)	97.51 (14.76)	96.86 (14.24)	95.39 (15.22)	<0.001
Total cholesterol (mmol/l), mean (SD)	6.24 (1.35)	6.08 (1.46)	5.93 (1.21)	5.84 (1.22)	5.62 (1.19)	<0.001
eGFR <60 mL/min per 1.73 m2, n(%)	539 (18.82)	481 (19.13)	532 (17.43)	403 (16.81)	194 (13.72)	<0.001
Alcohol use, n (%)	1952 (65.61)	1642 (64.22)	1876 (60.99)	1362 (58.16)	717 (52.64)	<0.001
Tobacco use, n (%)	1419 (46.88)	1165 (44.23)	1351 (42.63)	1114 (45.60)	642 (44.93)	0.015
CVD, n (%)	656 (21.26)	476 (17.83)	527 (16.33)	438 (16.47)	257 (17.40)	<0.001
Serum sodium, mean (SD)	138.47 (3.20)	139.78 (2.49)	140.38 (2.28)	140.92 (2.23)	141.78 (2.44)	<0.001
Serum potassium, mean (SD)	3.95 (0.51)	4.08 (0.42)	4.14 (0.43)	4.19 (0.42)	4.24 (0.44)	<0.001
Serum bicarbonate, mean (SD)	27.48 (3.08)	26.37 (2.75)	25.52 (2.78)	24.91 (2.76)	23.91 (3.01)	<0.001
Anion gap, mean (SD)	17.15 (3.38)	15.98 (3.14)	15.48 (2.95)	14.83 (2.40)	14.25 (3.08)	<0.001
Diuretic use, n(%)	811 (25.95)	647 (23.79)	657 (19.82)	475 (18.01)	267 (16.50)	<0.001
Achieved SBP (mmHg), mean (SD)	156.22 (25.63)	151.69 (23.81)	150.30 (24.07)	147.53 (22.56)	147.99 (24.80)	<0.001
Achieved DBP (mmHg), mean (SD)	91.62 (13.77)	89.92 (12.74)	89.71 (12.99)	88.14 (13.19)	88.23 (12.84)	<0.001

Cl=serum chloride, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, eGFR=estimated glomerular filtration rate based on MDRD equation, Baseline CVD=prevalent cardiovascular disease at baseline, SD=standard deviation

Table 6-4: Characteristics of the study population stratified by serum chloride and bicarbonate levels.

Variables	Cl<=100 & HCO3<=25 (N=692)	Cl>100 & HCO3<=25 (N=3693)	Cl<=100 & HCO3>25 (N=2003)	Cl>100 & HCO3>25 (N=3681)	P Value
Age at first visit (years), mean (SD)	51.01 (13.26)	48.31 (14.19)	51.82 (12.18)	49.70 (13.64)	<0.001
Men, n (%)	329 (47.54)	1534 (41.54)	1091 (54.47)	1894 (51.45)	<0.001
BMI (Kg/m2), mean (SD)	26.49 (4.71)	27.35 (5.55)	26.64 (4.78)	27.35 (5.26)	<0.001
SBP (mmHg), mean (SD)	175.32 (31.26)	168.06 (29.30)	172.76 (29.79)	167.31 (28.30)	<0.001
DBP (mmHg), mean (SD)	102.57 (16.83)	99.37 (15.25)	101.99 (15.85)	99.19 (14.57)	<0.001
Total cholesterol (mmol/l), mean (SD)	6.40 (1.41)	6.03 (1.28)	6.33 (1.29)	5.99 (1.36)	<0.001
eGFR <60 mL/min per 1.73 m2, n(%)	174 (27.62)	845 (24.27)	471 (24.45)	762 (21.36)	<0.001
Alcohol use, n (%)	464 (68.64)	2720 (63.04)	1316 (67.42)	2248 (62.93)	<0.001
Tobacco use, n (%)	334 (48.76)	1736 (47.21)	932 (47.17)	1618 (44.24)	0.02
CVD, n (%)	152 (21.97)	628 (17.01)	443 (22.12)	695 (18.88)	<0.001
Serum sodium, mean (SD)	137.73 (3.13)	140.18 (2.32)	138.90 (2.85)	140.92 (2.40)	<0.001
Serum potassium, mean (SD)	4.02 (0.49)	4.14 (0.41)	3.90 (0.49)	4.11 (0.42)	0.655
Anion gap, mean (SD)	19.82 (3.05)	16.74 (2.79)	16.23 (2.96)	13.91 (2.71)	<0.001
Diuretic use, n (%)	153 (22.01)	801 (21.58)	597 (29.78)	921 (24.95)	<0.001
Achieved SBP (mmHg), mean (SD)	157.57 (26.09)	152.42 (24.65)	157.01 (24.45)	150.73 (23.12)	<0.001
Achieved DBP (mmHg), mean (SD)	92.36 (13.09)	90.83 (13.07)	92.58 (13.69)	89.95 (12.21)	<0.001

Cl=serum chloride, HCO3=bicarbonate, SD=standard deviation, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, eGFR=estimated glomerular filtration rate based on MDRD equation, CVD=prevalent cardiovascular disease at baseline.

Table 6-5: Characteristics of the study population stratified by serum chloride and sodium levels.

Variables	Cl<=100 & Na<=135 (N=430)	Cl>100 & Na<=135 (N=170)	Cl<=100 & Na>135 (N=2653)	Cl>100 & Na>135 (N=9703)	P Value
Age at first visit (years), mean (SD)	58.49 (14.60)	52.91 (16.93)	51.61 (12.51)	49.86 (14.39)	<0.001
Men, n (%)	260 (60.47)	116 (68.24)	1236 (46.59)	5154 (53.12)	<0.001
BMI (Kg/m2), mean (SD)	26.51 (5.11)	27.45 (5.08)	26.83 (5.04)	27.72 (5.84)	0.02
SBP (mmHg), mean (SD)	172.26 (27.75)	163.73 (30.45)	172.12 (30.52)	164.38 (2868)	<0.001
DBP (mmHg), mean (SD)	97.07 (16.33)	93.79 (15.07)	101.51 (15.24)	97.67 (17.13)	<0.001
Total cholesterol (mmol/l), mean (SD)	6.18 (1.45)	5.77 (1.50)	6.25 (1.33)	5.87 (1.49)	<0.001
eGFR <60 mL/min per 1.73 m2, n(%)	116 (28.02)	48 (29.63)	628 (25.35)	2128 (22.97)	0.003
Alcohol use, n (%)	221 (54.70)	81 (50.94)	1731 (67.35)	5512 (60.12)	<0.001
Tobacco use, n (%)	209 (50.12)	71 (44.38)	1210 (46.40)	4194 (44.12)	0.026
CVD, n (%)	87 (20.23)	33 (19.41)	569 (21.45)	1664 (17.15)	<0.001
Serum bicarbonate, mean (SD)	26.35 (3.11)	24.38 (3.27)	27.64 (3.04)	25.44 (2.89)	<0.001
Serum potassium, mean (SD)	4.04 (0.57)	4.24 (0.54)	3.94 (0.50)	4.15 (0.43)	<0.001
Anion gap, mean (SD)	14.75 (3.61)	11.47 (3.61)	17.49 (3.21)	15.39 (3.01)	<0.001
Diuretic use, n (%)	102 (23.08)	27 (15.00)	709 (26.46)	2019 (19.99)	<0.001
Achieved SBP (mmHg), mean (SD)	155.62 (26.49)	152.24 (24.69)	156.34 (25.49)	149.59 (23.78)	<0.001
Achieved DBP (mmHg), mean (SD)	88.54 (14.13)	87.44 (13.64)	92.13 (13.65)	89.35 (12.73)	0.018

Cl=serum chloride, Na=sodium, SD=standard deviation, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, eGFR=estimated glomerular filtration rate based on MDRD equation, CVD=prevalent cardiovascular disease at baseline.

6.3.2 Association of serum chloride and blood pressure

The population averaged effects of unit increase in adjusted serum chloride and other electrolytes, on both longitudinal SBP and DBP, are described in table 6-6 on page 186. Serum chloride showed no effect on SBP change over time. Among the electrolytes, only serum bicarbonate showed a significant independent effect on longitudinal SBP, with each unit rise in serum bicarbonate associated with a 0.34 mmHg reduction in SBP over 5 years. For DBP, serum chloride showed no effect while serum sodium, potassium and bicarbonate showed small effects on DBP over time.

6.3.3 Survival characteristics

The total time at risk was 197102 person years (p-y) with median survival time of 30.32 years. While the median survival time was lowest in the first quintile (22.46 years), it was highest in the fourth quintile of serum chloride (33.89 years). The incidence rates were 20.84 (95% CI: 20.21-21.49), 12.48 (95% CI: 12.00-12.98), 6.82 (95% CI: 6.47-7.20), 3.18 (2.94-3.44), and 8.36 (7.97-8.77) per 1000 p-y of follow-up for all-cause, CV, IHD, stroke and non-CV mortality outcomes, respectively (Table 6-7 on page 186).

6.3.4 Association of serum chloride and mortality outcomes

The shortest survival time was observed in the first quintile of serum chloride in the unadjusted K-M analysis (log rank P<0.001) (Figure 6-2 on page 188). Consistent relationship was seen in K-M analysis with adjusted serum chloride quintiles (Figure 6-3 on page 188). Mortality risk was greater in the groups with chloride ≤100 mEq/L, regardless of sodium or bicarbonate levels (log-rank *p*<0.001; Figures 6-4 and 6-5 on page 189). The log-log survival curves of serum chloride categories were parallel to each other and confirmed the agreement with the PH assumption (Figure 6-6 on page 190).

In the Cox-PH models, there was an inverse linear association between serum chloride and all-cause mortality. For example, each 1 mEq/L increase in serum chloride was associated with decrease in all-cause mortality by 3% (HR=0.97, 95% CI: 0.96-0.98) after adjustment for baseline confounding variables, achieved BP and other serum electrolytes levels as in model 3. In the Cox-PH models with serum chloride in quintiles, the HRs were significantly lower in all other quintiles

in comparison to quintile 1 (model 4). In the final model (model 5), even after accounting for the influence of diuretic use, the HRs for all-cause mortality were 0.82 (95% CI: 0.74-0.90), 0.79 (95% CI: 0.71-0.87), 0.79 (95% CI: 0.70-0.89) and 0.80 (0.68-0.93) in quintiles 2, 3 , 4 and 5 respectively in comparison to quintile 1 of serum chloride. Similar results were observed for CV mortality as well as non-CV mortality (Table 6-8). In the RS Cox-PH model, the risk for all-cause mortality increased linearly below serum chloride level 100 mEq/L (Figure 6-7 on page 190).

Table 6-6: Longitudinal association between serum electrolytes and blood pressure.

Serum electrolytes	GEE β (SBP)*	95% CI β	P value
Adjusted serum chloride	0.01	-0.19; 0.21	0.893
Serum sodium	0.16	-0.03; 0.35	0.105
Serum potassium	-0.65	-1.82; 0.53	0.281
Serum bicarbonate	-0.34	-0.53; -0.15	0.001
	GEE β (DBP)*	95% CI β	P value
Adjusted serum chloride	-0.01	-0.11; 0.10	0.876
Serum sodium	0.15	0.05; 0.25	0.003
Serum potassium	-1.23	-1.85; -0.61	<0.001
Serum bicarbonate	-0.19	-0.29; -0.09	<0.001

*per one unit (1 mEq/L) increase in serum electrolytes. Number of groups in the analyses=3599 and average number of blood pressure observations in each groups=5.2.

Table 6-7: Person years of follow-up and mortality event rates stratified by chloride categories in quintiles.

Follow-up and event rates	Cl<=100 (N=3085)	Cl=101-102 (N=2670)	Cl=103-104 (N=3228)	Cl=105-106 (N=2508)	Cl>=107 (N=1477)	Total (N=12968)
Person years of follow-up (p-y)	50700.86	43858.01	48665.01	35664.03	18170.12	197101.81
IHD mortality, (95% CI)	9.96 (9.13-10.87)	6.34 (5.64-7.13)	5.96 (5.31-6.69)	4.88 (4.21-5.66)	5.34 (4.38-6.51)	6.82 (6.47-7.20)
Stroke mortality, (95% CI)	4.46 (3.91-5.08)	2.90 (2.43-3.45)	2.65 (2.23-3.15)	2.66 (2.18-3.26)	2.75 (2.09-3.63)	3.18 (2.94-3.44)
CV mortality, (95% CI)	18.19 (17.05-19.40)	11.86 (10.88-12.92)	10.46 (9.59-11.41)	9.11 (8.17-10.16)	10.07 (8.71-11.64)	12.48 (12.00-12.98)
Non-CV mortality, (95% CI)	11.16 (10.28-12.12)	7.80 (7.01-8.67)	7.38 (6.65-8.18)	6.65 (5.85-7.55)	7.93 (6.73-9.33)	8.36 (7.97-8.77)
All-cause mortality, (95% CI)	29.35 (27.90-30.88)	19.65 (18.39-21.01)	17.84 (16.69-19.06)	15.76 (14.51-17.12)	17.80 (16.15-20.06)	20.84 (20.21-21.49)

Mortality rates are given as deaths/1000 person years (p-y). Cl=Serum chloride, CVD=Cardiovascular disease and IHD=ischemic heart disease

Table 6-8: Cox regression analysis for the association between serum chloride and mortality.

	All-cause mortality N=3434/11043		CV mortality N=2019/11043		IHD mortality N=1132/11043		Stroke Mortality N=485/11043		Non-CV mortality N=1415/11043	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Baseline Serum Chloride (Model 1)	0.97*	0.96-98	0.97*	0.96-0.99	0.97*	0.96-0.99	0.99	0.97-1.02	0.97*	0.96-0.99
	N=3373/9035		N=1993/9035		N=1119/9035		N=477/9035		N=1380/9035	
Baseline Serum Chloride (Model 2)	0.98*	0.97-0.99	0.98*	0.97-0.99	0.97*	0.96-0.99	0.99	0.97-1.02	0.98*	0.96-0.99
	N=3373/9035		N=1993/9035		N=1119/9035		N=477/9035		N=1380/9035	
Baseline Serum Chloride (Model 3)	0.97*	0.96-0.98	0.97*	0.96-0.99	0.97*	0.95-0.99	0.99	0.97-1.03	0.97*	0.95-0.99
Baseline Serum Chloride (Model 4)	N=3373/9035		N=1993/9035		N=1119/9035		N=477/9035		N=1380/9035	
Serum Chloride <=100	1		1		1		1		1	
Serum Chloride =101-102	0.82*	0.73-0.90	0.83*	0.73-0.94	0.81*	0.68-0.96	0.84	0.64-1.09	0.81*	0.69-0.94
Serum Chloride =103-104	0.79*	0.71-0.87	0.79*	0.69-0.90	0.88	0.74-1.05	0.80	0.61-1.06	0.78*	0.67-0.92
Serum Chloride =105-106	0.79*	0.70-0.89	0.79*	0.67-0.92	0.76*	0.62-0.95	1.03	0.75-1.40	0.80*	0.66-0.96
Serum Chloride >=107	0.80*	0.68-0.93	0.79*	0.64-0.97	0.77	0.58-1.01	1.07	0.73-1.58	0.81	0.64-1.03
Baseline Serum Chloride (Model 5)	N=3373/9035		N=1993/9035		N=1119/9035		N=477/9035		N=1380/9035	
Serum Chloride <=100	1		1		1		1		1	
Serum Chloride =101-102	0.82*	0.74-0.90	0.83*	0.73-0.94	0.81*	0.68-0.96	0.84	0.64-1.09	0.81*	0.69-0.94
Serum Chloride =103-104	0.79*	0.71-0.87	0.79*	0.69-0.91	0.88	0.74-1.05	0.80	0.61-1.06	0.78*	0.66-0.91
Serum Chloride =105-106	0.79*	0.70-0.89	0.79*	0.67-0.93	0.77*	0.62-0.95	1.03	0.76-1.41	0.79*	0.65-0.95
Serum Chloride >=107	0.80*	0.68-0.93	0.79*	0.65-0.97	0.77	0.58-1.02	1.08	0.73-1.59	0.81	0.64-1.03

*p value <0.05, Model 1 is adjusted for age at first visit, gender, BMI=body mass index, baseline cardiovascular disease, CKD=Chronic kidney disease, tobacco smoking, alcohol use, year of first visit (epochs), SBP=systolic blood pressure, DBP=diastolic blood pressure, and achieved SBP. Model 2 is adjusted for all variables in model 1 and anion gap. Model 3 is adjusted for all variables in model 1 and serum sodium, serum potassium and serum bicarbonate. Model 4 is same as model 3 but the serum chloride was considered as a categorical variable in quintiles. Model 5 is adjusted for all variables in model 5 and consistent diuretic use in the first five years of follow-up (Cox model stratified for diuretics use). CVD=Cardiovascular disease and IHD=ischemic heart disease, HR=hazard Ratio, CI=confidence interval.
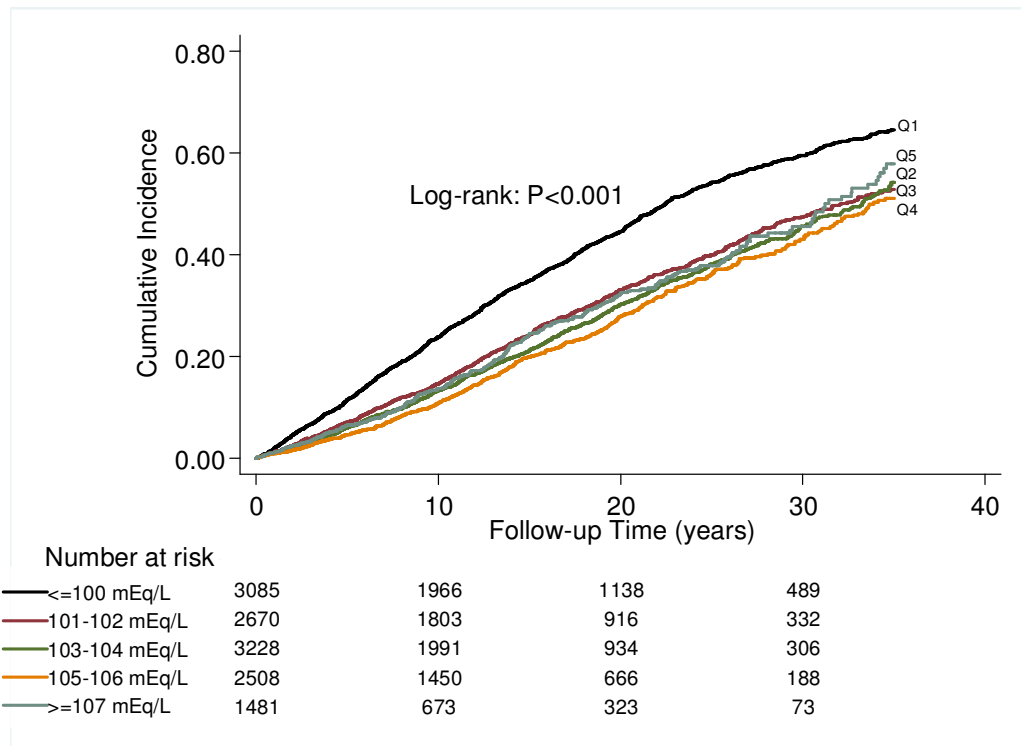
Figure 6-2: Kaplan Meir survival curves of serum chloride in quintiles.
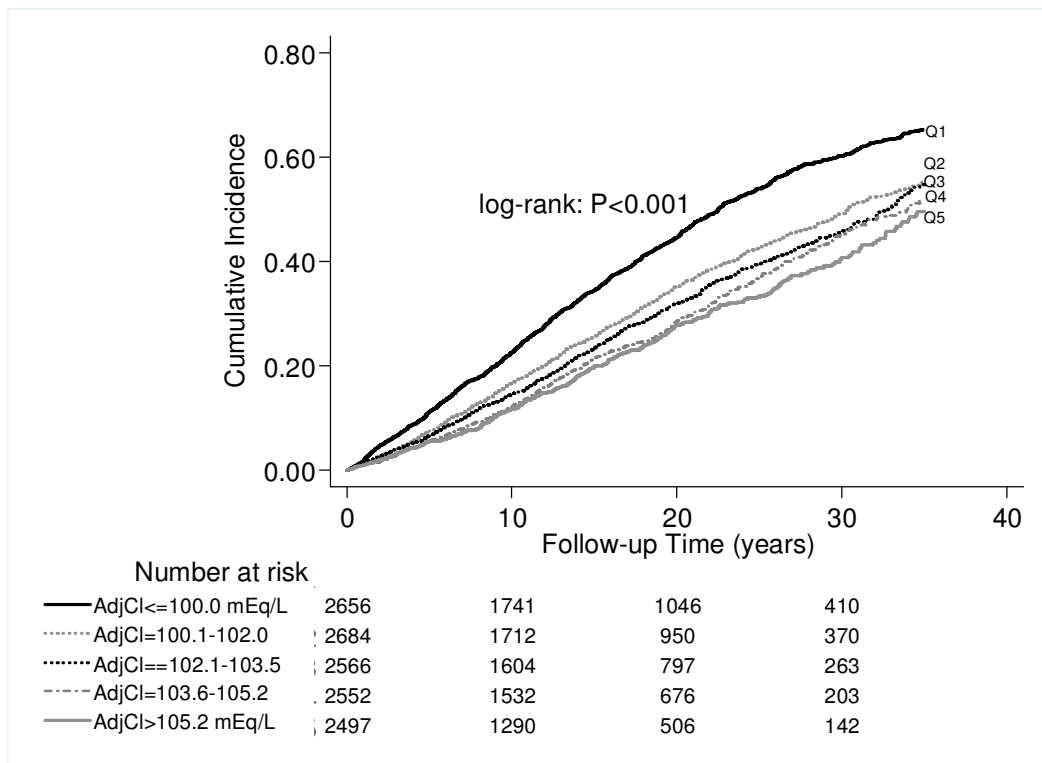
Figure 6-3: Kaplan Meir survival curves of adjusted serum chloride in quintiles.
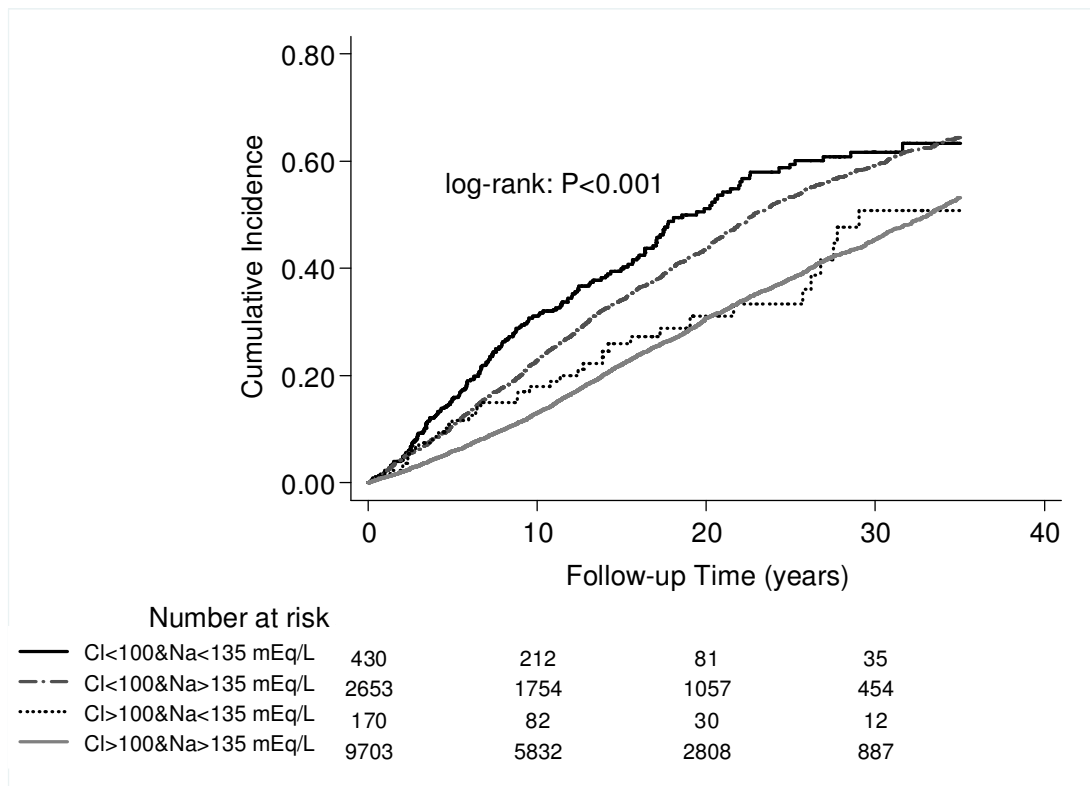
Figure 6-4: Serum chloride stratified by sodium and cumulative mortality.
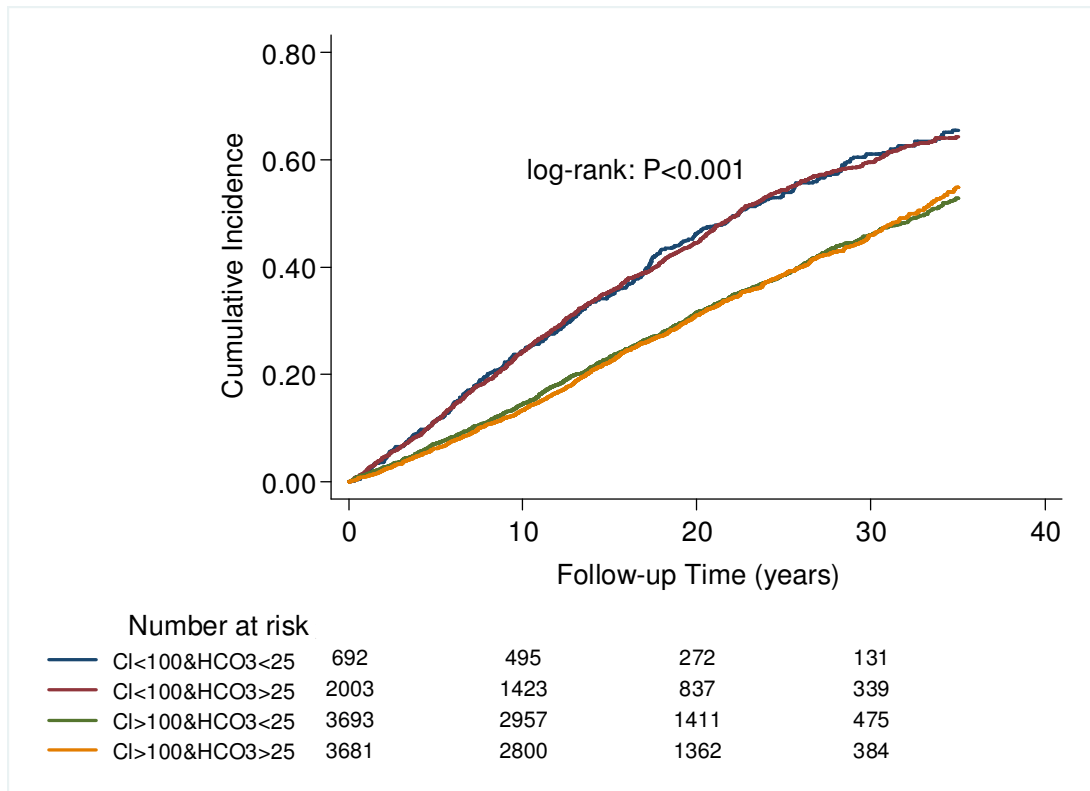
Figure 6-5: Serum chloride stratified by bicarbonate levels and cumulative mortality.
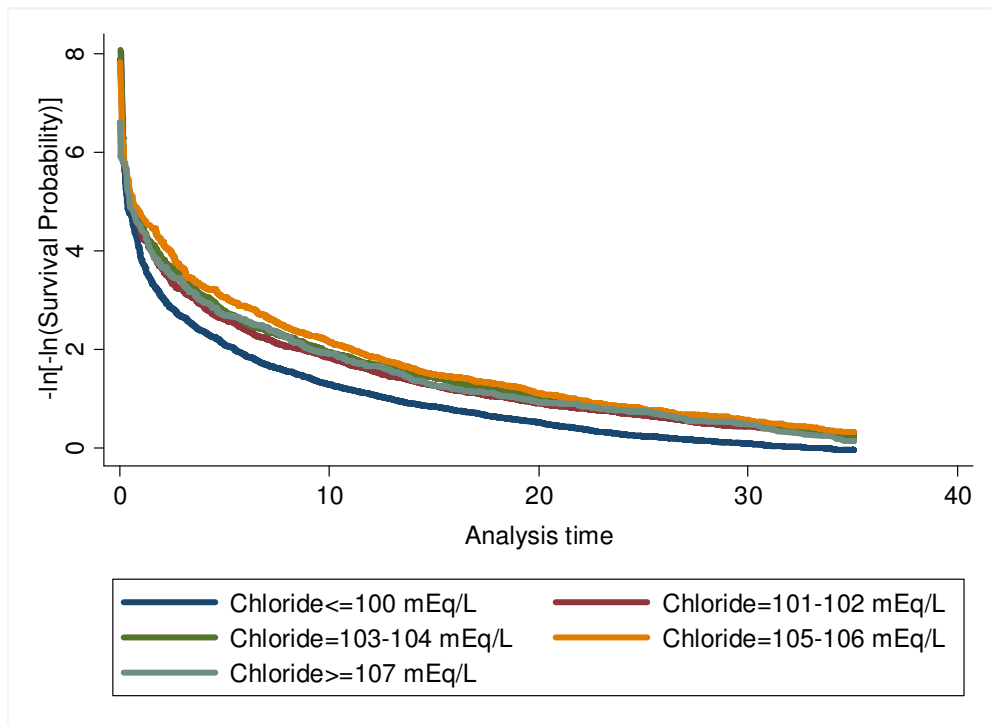
Figure 6-6: Log-Log survival curves of serum chloride in quintiles
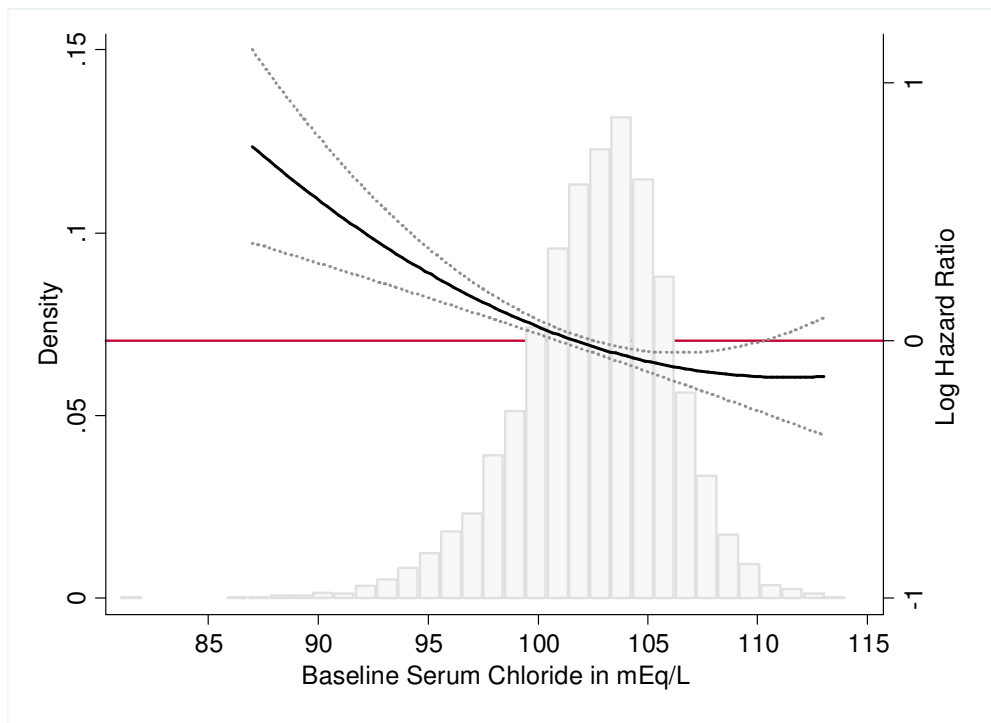
Figure 6-7: Regression spline Cox-PH model for all-cause mortality and serum chloride.

6.3.5 Serum sodium and mortality outcomes

The shortest survival time was observed in the fifth quintile of serum chloride in the unadjusted Kaplan-Meir analysis (log rank P<0.001) (Figure 6-8 on page 191). There was no deviation from the proportional hazard assumption (Figure 6-9 on page 192). However, there was no significant relationship with serum sodium and all-cause mortality in the multivariate adjusted regression spline Cox-PH model (Figure 6-10 on page 192).

Number at risk				
<=138 mEq/L	3385	2026	829	342
139-140 mEq/L	4050	2578	1258	440
141-141 mEq/L	2117	1323	725	228
142-142 mEq/L	1650	961	548	186
>=143 mEq/L	2178	1235	663	196
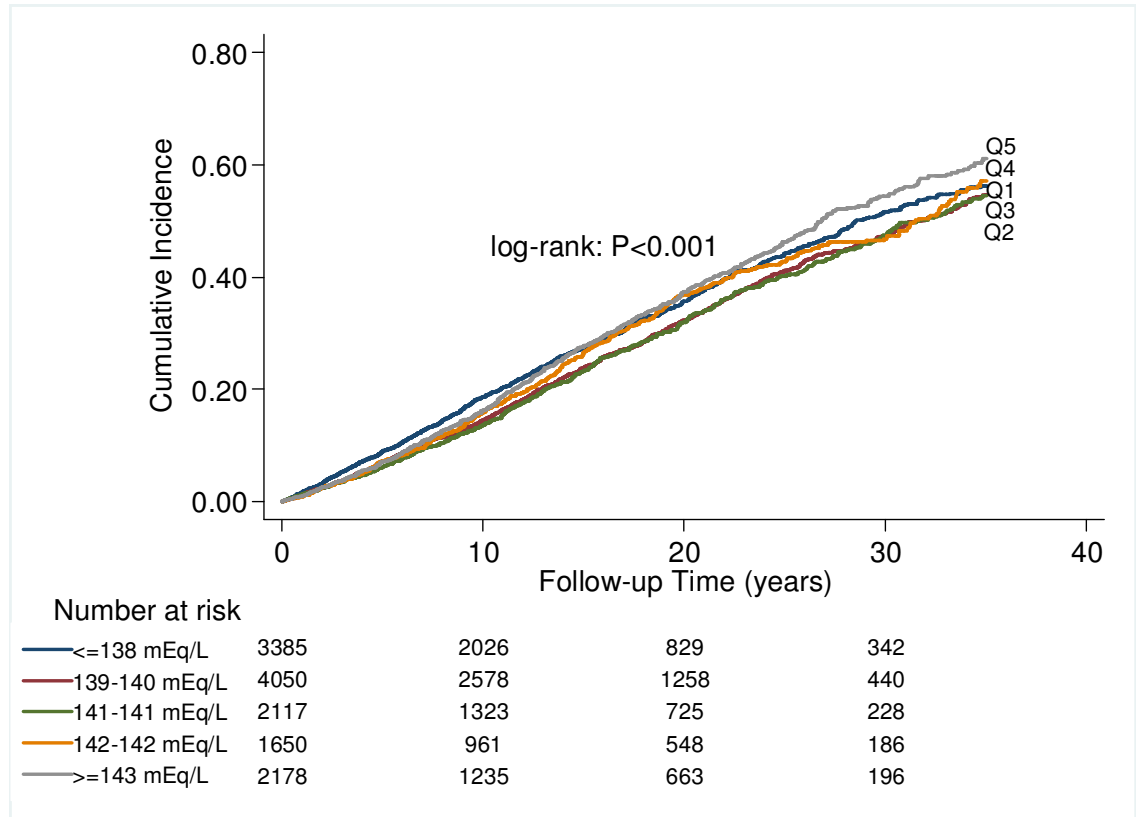
Figure 6-8: Serum sodium in quintiles and cumulative mortality
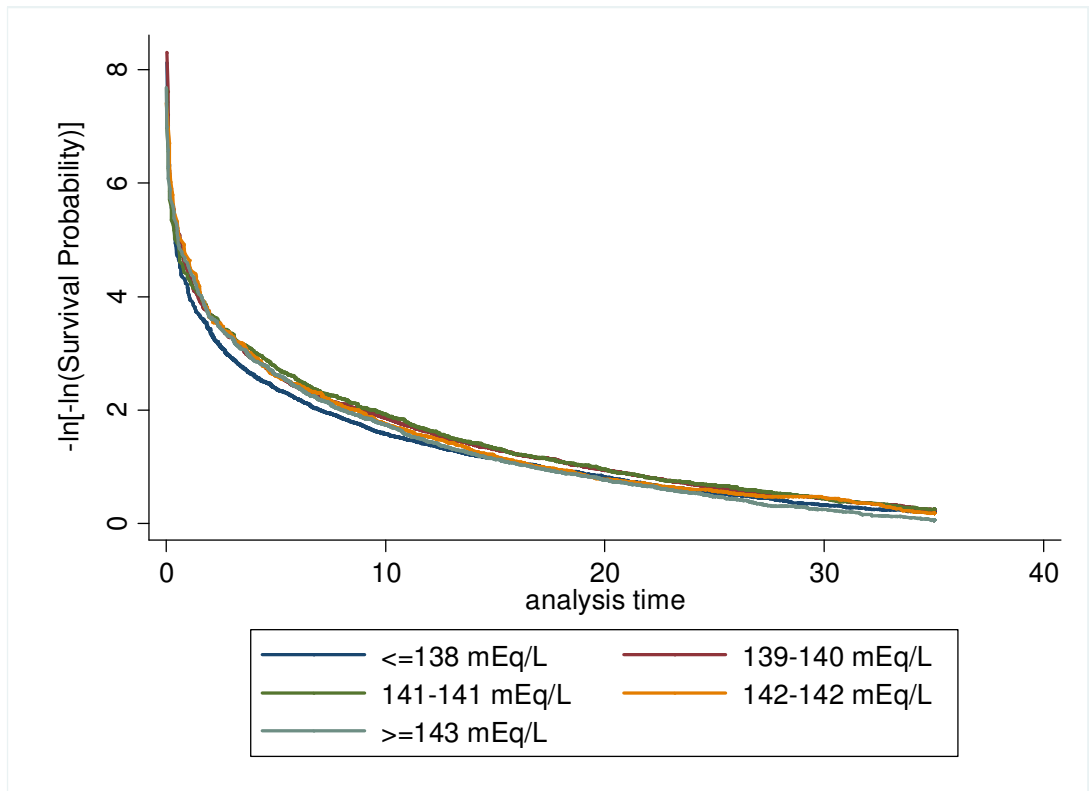
Figure 6-9: Log-log survival curves of serum sodium in quintiles
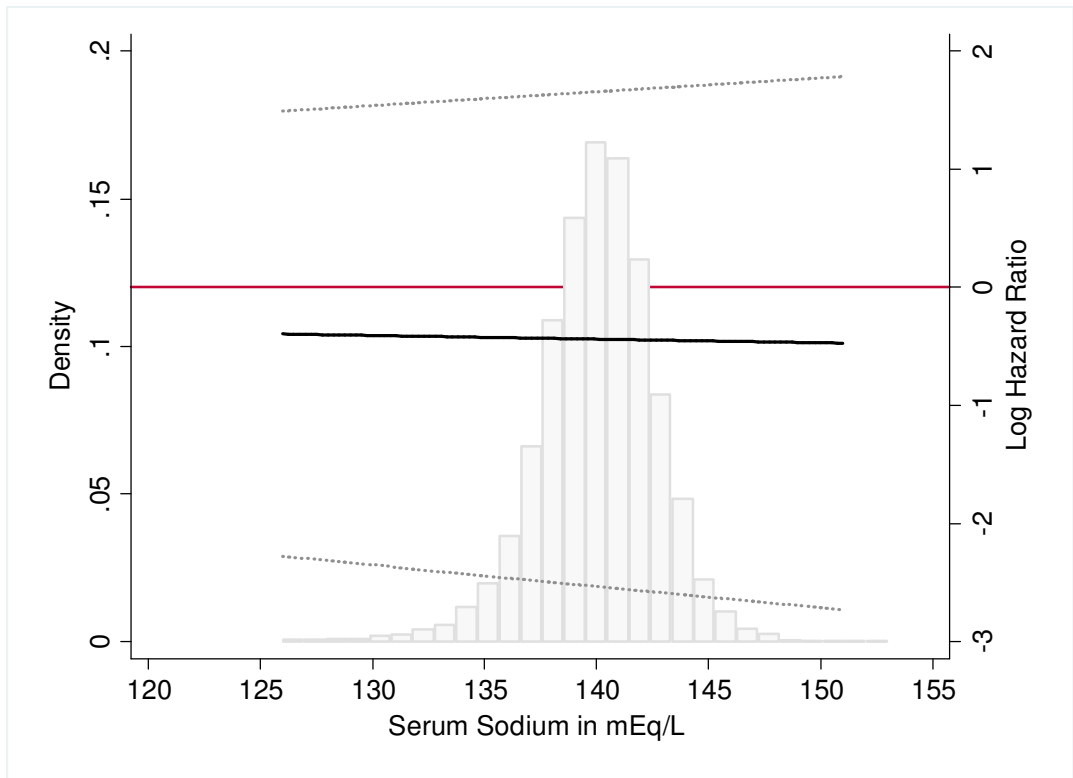
Figure 6-10: Regression spline Cox-PH model for serum sodium and all-cause mortality.

6.3.6 Serum potassium and mortality outcomes

The shortest survival time was observed in the fifth quintile of serum potassium in the unadjusted Kaplan-Meir analysis (log rank P<0.001) (Figure 6-11 on page 193). There was no deviation from the proportional hazard assumption (Figure 6-12 on page 194). In the multivariate adjusted regression spline Cox-PH model, there was a non-linear 'U' shaped relationship between serum potassium and mortality (Figure 6-13 on page 194).
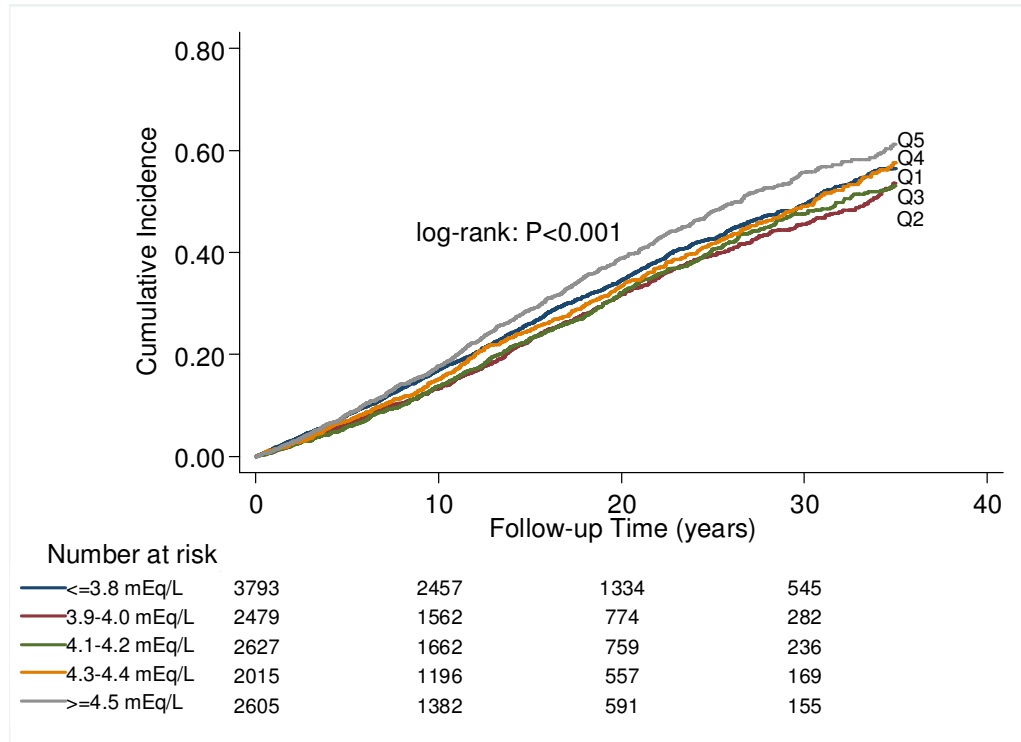
Figure 6-11: Serum potassium in quintiles and cumulative mortality.
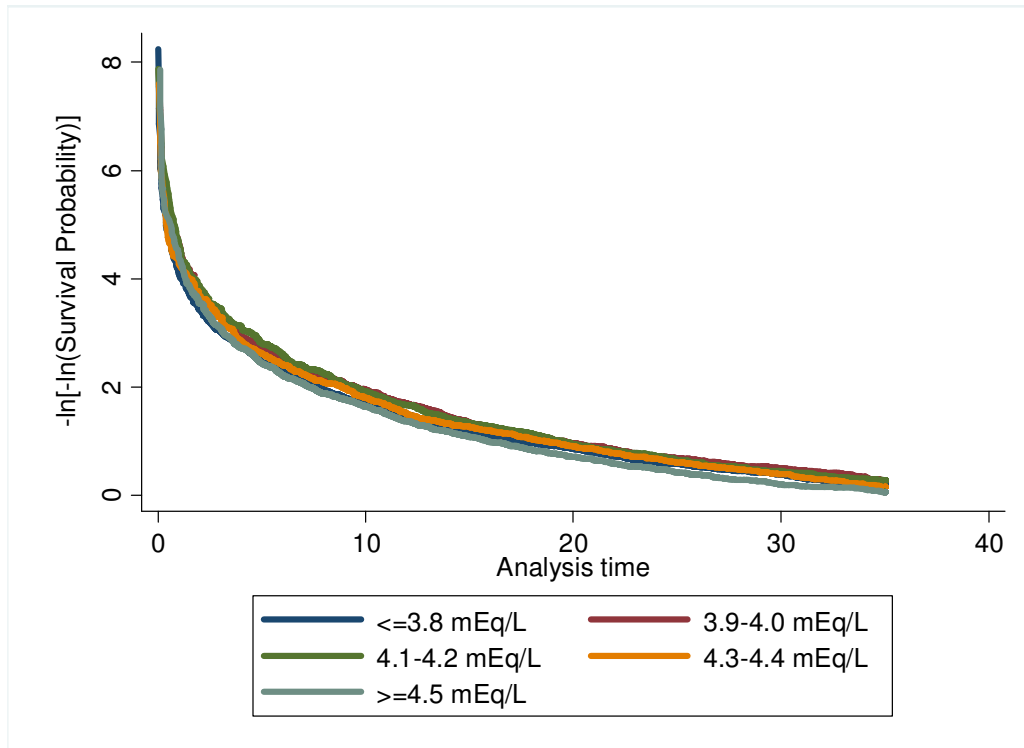
Figure 6-12: Log--log survival curves of serum potassium in quintiles.
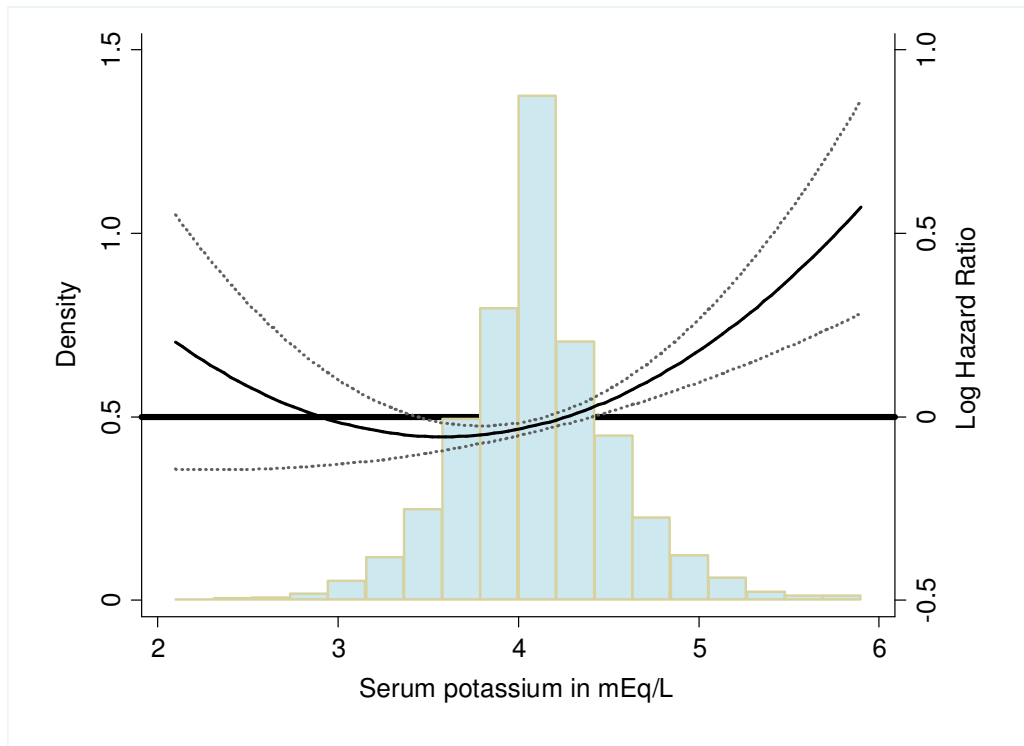
Figure 6-13: Regression spline Cox-PH model for serum potassium and all-cause mortality.

6.3.7 Serum bicarbonate and mortality outcomes

The shortest survival time was observed in the fifth quintile of serum bicarbonate in the unadjusted Kaplan-Meir analysis (log rank P<0.001) (Figure 6-14 on page 195). A positive linear relationship was evident in the KM survival analysis of adjusted serum bicarbonate in quintiles (Figure 6-15 on page 196). There was no deviation from the PH assumption (Figure 6-16 on page 196). In the multivariate adjusted regression spline Cox-PH model, there was positive linear increase in mortality with adjusted serum bicarbonate (Figure 6-17 on page 197).

Number at risk				
<=24 mEq/l	3184	2494	1186	419
25-25 mEq/L	1307	1029	520	188
26-27 mEq/L	2732	2076	1030	336
28-29 mEq/L	1974	1449	758	250
>=30 mEq/L	1277	867	436	140
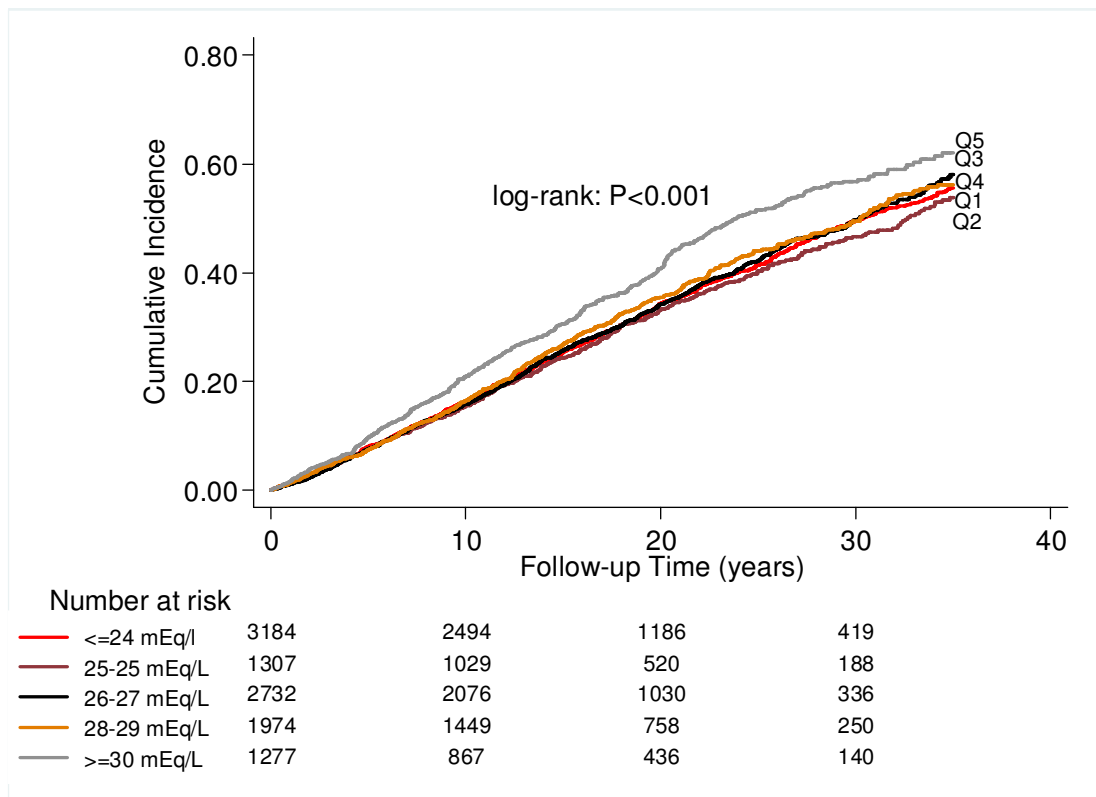
Figure 6-14: Serum bicarbonate in quintiles and cumulative mortality.

Number at risk				
AdjHCO3<=23.4 mEq/L	2005	1573	637	183
AdjHCO3=23.5-25.1	2023	1596	712	221
AdjHCO3=25.2-26.6	1996	1519	777	266
AdjHCO3=26.7-28.4	2014	1522	858	318
AdjHCO3>28.4	2012	1456	893	339
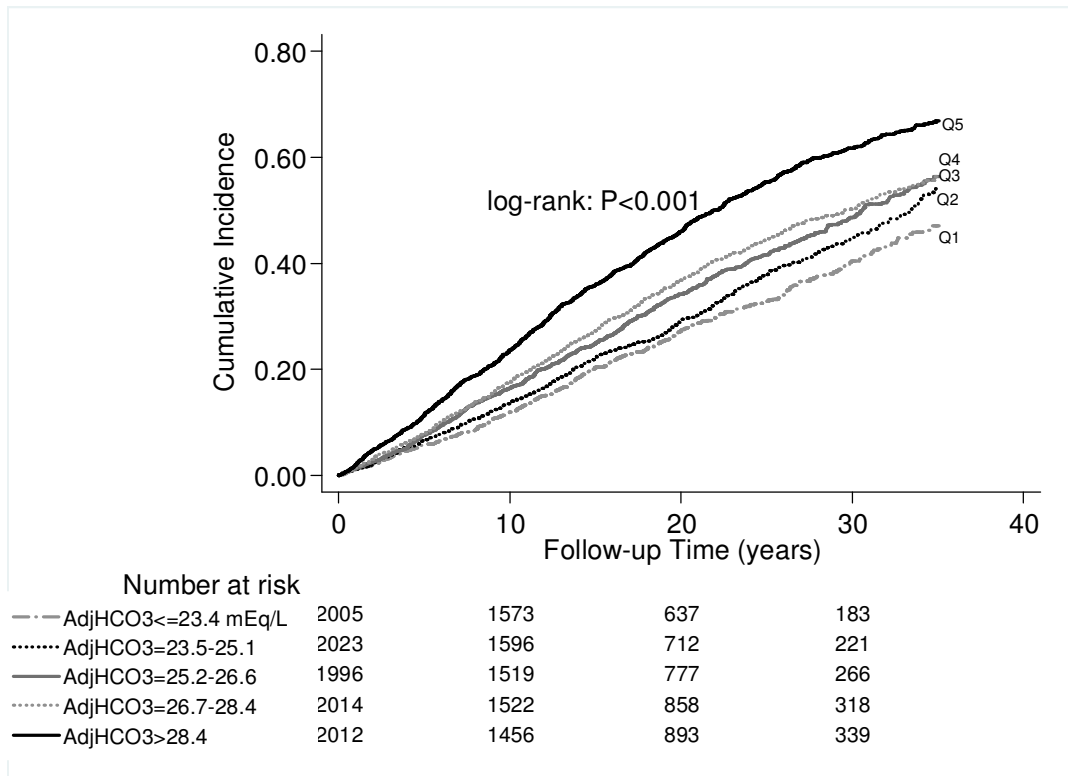
Figure 6-15: Adjusted serum bicarbonate in quintiles and mortality.
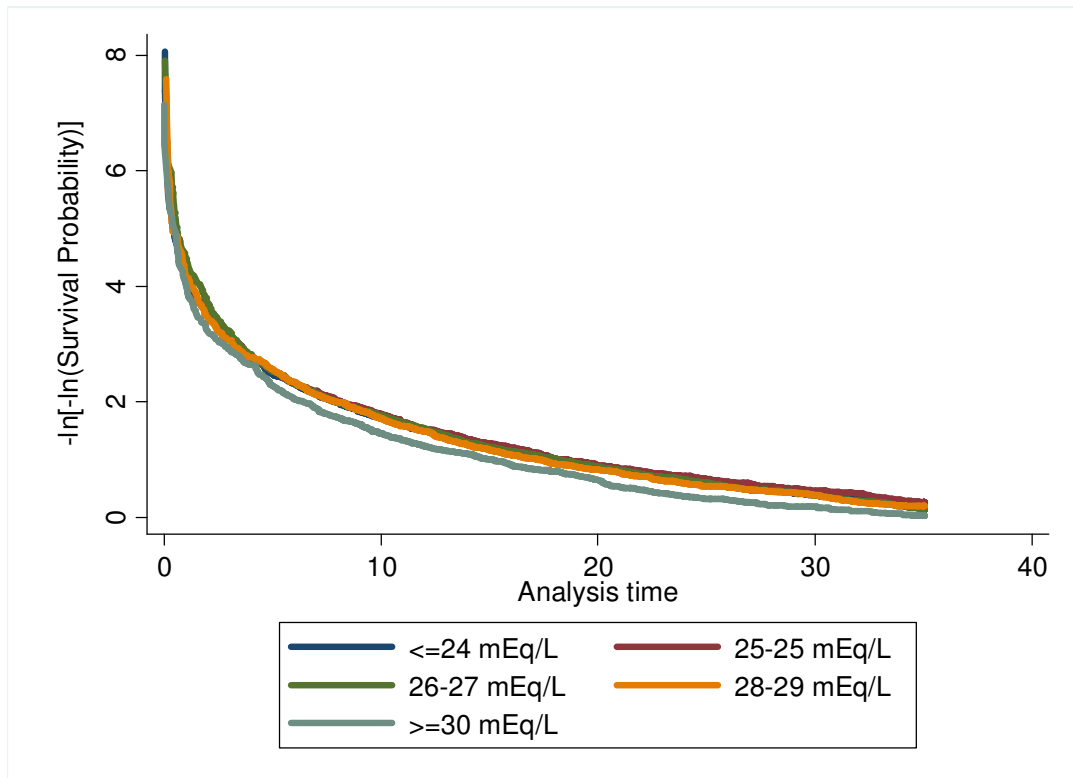
Figure 6-16: Log-log survival curves for serum bicarbonate in quintiles.
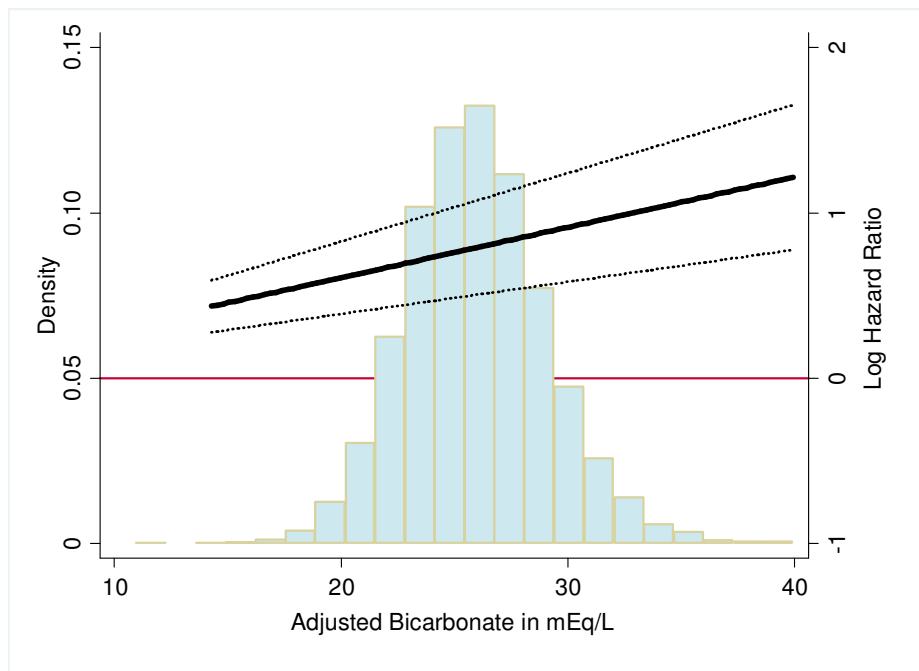
Figure 6-17: Regression spline Cox-PH model for adjusted serum bicarbonate and all-cause mortality.

6.4 Discussion

In an large cohort of >13,000 high risk hypertensive patients followed-up for 35 years, serum chloride, potassium and bicarbonate are independently associated with mortality. While the relationship with all-cause mortality is non-linear across the entire range of serum chloride, there is a linear increase in mortality with decrease in serum chloride level below 100 mEq/L. The relationship between serum chloride and mortality is independent of serum sodium and bicarbonate levels. While serum potassium shows a non-linear "U" shaped relationship with mortality, serum bicarbonate shows a positive linear association.

Although serum chloride is part of the standard screening biochemistry panel in outpatient clinics and is routinely measured in hypertensive patients, it does not feature in routine risk stratification. The current study findings suggest that serum chloride is a risk marker for mortality. Similar findings were reported in a general population cohort of the Belgian Interuniversity Research on Nutrition and Health (BIRNH) study who were followed-up for ten years [467]. The excess risk for all-cause mortality in individuals with serum chloride below 100 mEq/L was 1.48 (95% CI: 1.01-2.19) after adjustment for age, BMI and serum sodium levels

in the BIRNH study. Furthermore, even in multivariate models after incorporating all confounding variables serum chloride remained a significant predictor of all-cause, CV and non-CV mortality outcomes. Consistent findings are reported in individuals with heart failure [627]. The present study, involving a larger sample with longer follow-up (35 year of follow-up and median survival time of 30 years), confirms this relationship in a hypertensive cohort independent of other risk factors.

In order to maintain electro-neutrality, chloride deficiency often leads to increased absorption of bicarbonate [621] [638]. Sodium and potassium levels also influence chloride absorption so as to maintain the cation-anion balance. Therefore, other electrolytes can act as confounders in the relationship between serum chloride and mortality outcomes. Furthermore, most acid-base disorders cause opposite and equal changes in serum chloride and bicarbonate concentrations, and this inverse relationship is influenced by changes in the anion gap and/or water balance [637]. The findings of the present study are consistent in different models even after adjustment for other electrolytes and anion gap. They are also supported by the recent report that higher anion gap (which can be associated with low chloride) in early CKD is a marker of early mortality [639]. Moreover, the risk posed by low chloride in the present study is independent of concomitant sodium or bicarbonate levels, suggesting that this is a chloride specific finding and does not reflect risks associated with hyponatremia or acid-base disturbances.

Diuretics such as frusemide and thiazides interfere with re-absorption of chloride in the renal tubules [640]. During diuretic use, urinary loss of chloride exceeds that of bicarbonate. As our study population were treated hypertensive patients, confounding due to diuretic use is a likely possibility. However, only 22% of patients in the present study were on diuretics at the first visit when serum chloride was measured, and the relationship between low chloride and mortality persisted even when the analyses were stratified for diuretic use. Moreover, we attempted to adjust the chloride levels for free water changes that are commonly induced by diuretic use and found that the relationship persisted with adjusted chloride. The observed association of low serum chloride with mortality is therefore independent of all known potential confounders including diuretic use.

All-cause, CV, IHD and non-CV mortality outcomes were significantly higher among individuals with low serum chloride in comparison with individuals with higher serum chloride in our study. However, the mechanisms by which chloride influences mortality outcomes are not clear. High anion-gap and the corresponding low levels of serum chloride are associated with inflammatory markers such as higher leukocyte count and higher C-reactive protein level in healthy individuals [641]. Higher levels of inflammatory biomarkers are associated with increased mortality and the development of CVD [642] [643]. However further studies are required to understand the role of inflammatory markers in mediating the relationship between low serum chloride and mortality.

Contrasting findings are reported in the literature on the association between dietary chloride intake and BP in animal models. While earlier studies did not find any association of dietary chloride with BP independent of dietary sodium content [622], some of the new animal studies suggest an independent positive association [624-626]. It is also relevant to note that normal BP is a characteristic feature of Bartter syndrome despite its association with selective chloride deprivation and salt wasting. It is however not clear whether dietary chloride increases BP by some mechanisms other than by influencing the renal tubular re-absorption of chloride. By contrast, high anion gap and the corresponding low serum chloride levels are associated with higher BP [629]. In the present study serum chloride showed no independent effect on BP change over time. Hence we believe the findings on association of chloride and mortality are not confounded by BP control.

There is a near perfect negative correlation between serum chloride (adjusted) and bicarbonate (adjusted) after corrections for serum sodium and anion gap. Serum bicarbonate also shows a positive linear increase in mortality with increasing bicarbonate levels in the present study. Consistent to these observations, high serum bicarbonate level are associated with mortality in both stage 3 and stage 4 chronic kidney disease patients [470]. By contrast, each unit increase in serum bicarbonate within the normal range was associated with reduced risk of death and progression to CKD among African Americans [644]. However, in both of the above mentioned studies the serum bicarbonate level was not corrected for serum sodium and anion gap.

Serum potassium independently predicts mortality and the relationship is non-linear in the present study with elevated mortality risk at both ends of the distribution. Higher levels of potassium increase mortality despite achieving greater BP reduction during follow-up. Similar findings on BP are reported in other studies in hypertensive population [645]. Consistent results on mortality are seen in individuals with myocardial infarction [646], heart failure [647] [648], abnormal renal function [649] and individuals selected from the general population [461] [462].

6.5 Strengths and limitations

A large cohort of nearly 13,000 hypertensive adults, 35 years of follow-up with median survival time of 30 years, ability to link serum electrolytes with differing causes of mortality outcomes and most importantly the competence to model the potential non-linear association of serum chloride on mortality outcomes are the strengths of our study.

The exclusion of individuals without serum chloride assessed at baseline from the analyses, and the bias introduced by the missing covariates in the adjusted Cox-PH models are the major limitations. The present study was conducted in a treated hypertensive cohort and hence the results may not be generalisable even though the prevalence of hypertension in the adult population is high. Long term estimates of mortality based on a single measurement of serum electrolytes at baseline would have introduced some bias. A time-dependent analysis on serum electrolytes on mortality is required to establish the association of serum electrolytes and mortality outcomes.

6.6 Summary

In summary, serum chloride is an independent predictor of mortality outcomes in hypertensive population. Although the relationship with mortality outcomes are non-linear across the entire range of serum chloride, an inverse linear relationship of serum chloride levels below 100 mEq/L with mortality outcomes is evident. Similar studies in other population are required to confirm this independent association. Further studies are also necessary to elucidate the underlying mechanisms involved in the association of low serum chloride levels with mortality outcomes.

7 Indices of liver dysfunction or injury and mortality

7.1 Introduction

Serum albumin, alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP) and bilirubin are widely used markers of liver function or injury to liver cells [650]. While serum albumin levels indicate liver function, the enzymes transaminases are associated with the integrity of liver cells. Serum GGT and alkaline phosphatase (ALP) are markers of biliary tract diseases. Serum bilirubin is a marker of hepatic processing of haemoglobin breakdown products. All of them are components of the panel of laboratory assays performed to assess liver function, but except for albumin and bilirubin these are not specific markers of liver function. Clearly an elevation of any of these liver tests notionally above twice the upper limit of normal (ULN) is an indicator of liver disease that may have an adverse impact on survival. Infection, non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease are the commonest causes of deranged liver tests that are consequently associated with early mortality.

Abnormal liver enzymes not only indicate liver injury or dysfunction, but also significant predictors of mortality outcomes in the general population. The enzyme ALT is a more specific marker of hepatocyte damage and is involved in catalyzing the transfer of amino groups to generate products in gluconeogenesis and amino acid metabolism. Limited evidence suggests that ALT is the liver enzyme most closely associated with liver fat content and NAFLD [651]. High ALT levels have been associated with increased risk of diabetes [652], metabolic syndrome [653] and mortality in the general population [654]. In the NHANES-III cohort, the HR for all-cause mortality among participants with unexplained elevation of ALT (attributed to NAFLD) was 1.37 [95% confidence interval (CI) 0.98, 1.91] compared with other participants [485]. ALT is also found to be associated with mortality among participants with a BMI below the median (22.7 kg/m2) in a Japanese cohort, but not among participants with a BMI at or greater than the median [655]. By contrast, Ford et al recently demonstrated an inverse relationship with ALT within the normal range and total mortality, in middle-to-older aged subjects, independent of traditional cardiovascular risk

factors in three independent populations [656]. Furthermore, a non-linear relationship with ALT and mortality is emerging [654 656-659] and further studies are warranted to model this potential complex relationship.

While abnormal AST levels well above the upper limit of normal range are associated with increased mortality, AST in the normal range was associated with lower than expected mortality [654]. This association has not been studied in detail across the entire range of AST using appropriate survival analysis methods.

Although GGT has long been considered a marker of excessive alcohol intake, recently it has been suggested to reflect oxidative stress [660]. It is linked to NAFLD [659 661], metabolic syndrome [659 662] and coronary heart disease [659 663]. The independent associations of GGT with mortality [489-491 664 665] is well established.

Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary duct of the liver [650]. Although, ALP is part of the routine liver function test assay, it is elevated in both obstructive liver conditions and bone disorders. Serum ALP is associated with coronary artery calcification [666] and cardiovascular mortality [478]. Multiple studies confirm the independent association of serum ALP with mortality [476 667].

Serum bilirubin is also routinely measured as it is traditionally part of the panel of markers in the regular liver function test [650]. Serum bilirubin levels are determined by enzymes involved in bilirubin production, namely heme oxygenase and uridine diphosphate–glucuronosyltransferase 1 (UGT1A1). The former is involved in production of bilirubin from haemoglobin, and the latter convert insoluble bilirubin to a form suitable for renal and biliary excretion [668] [669]. Bilirubin has been shown to be an effective antioxidant both in vitro [670] and in vivo [671]. As an antioxidant, it has been shown to suppress the oxidation of lipids and lipoproteins, especially LDL cholesterol [672], and to be directly related to the total serum antioxidant capacity in humans [673]. Serum bilirubin is inversely associated with both cardiovascular diseases [493-495 674 675] and cancer [496] in cross-sectional epidemiological studies. In the Korean Longitudinal study of Health and Aging (KLoSHA), the group with high serum bilirubin and low ALP had better survival rates in comparison to the group with low bilirubin and high ALP [676]. It is not clear whether this relationship is independent of potential confounding factors associated with mortality in this population. Consistent

findings of association between serum bilirubin levels and mortality have been reported in other studies [497][677].

Hypertension is a global public health problem that affects one in four individuals worldwide and contributes to 7.6 million deaths annually [293]. Liver function tests are frequently conducted in outpatient settings in the management of hypertension. However, the usefulness of these markers in predicting long-term mortality outcomes in hypertensive population has not been studied in detail. The aim of this study was to describe the long-term mortality outcomes and their association with liver enzymes and serum bilirubin in a large treated hypertensive cohort.

7.2 Methods

7.2.1 Study setting and study population

The Glasgow blood pressure clinic (GBPC) database has information on more than 15,000 hypertensive patients. More details of the study settings, population and study measurements are described in section 2.1-2.3.

7.2.2 Laboratory measurements

Venous blood samples were collected at baseline and at regular intervals for estimation of routine hematological and biochemical indices, including liver function and renal function tests. Liver function tests were part of the initial assessment of patients and were measured centrally by the Western Infirmary central clinical laboratory services. All biochemical analyses were performed in auto-analysers using standardised estimation methods. More details are provided in section 2.4.

7.2.3 Outcome assessment

Outcome assessment is described in section 2.5.

7.2.4 Statistical analysis

All analyses were restricted to individuals in the database with at least one of the liver enzymes assessed at the registration visit (N=12,000). Analyses were

performed in the overall population and in the subset after excluding individuals with high liver test values - defined as AST > 100mg/dl (n=665, 5.7%) and/or ALT > 100mg/dl (n=115, 1.2%) and /or GGT > 100mg/dl (n=665, 6.4%) and/or ALP > 240mg/dl (n=977, 8.6%) and/or bilirubin>30mg/dl (n=145, 1.3%). The distributions of liver enzymes were observed in the remaining dataset (AST, N=11059; ALT, N=9832; ALP, N=11362; GGT, N=10454; bilirubin, N=10890) using histograms.   All except serum albumin showed non-normal distributions and were logarithmically transformed for analysis. The detailed study flow chart is presented in figure 7-1 on page 207.

The characteristics of the study population in men and women were compared using independent 't' tests and analysis of variance, where appropriate, for continuous variables and Chi-Square tests for categorical variables. The study population was divided into groups based on quartiles of serum liver enzymes. Kaplan-Meier (KM) time to event curves were generated for each liver enzyme variable in quartiles as explained in section 2.6.1. Cox proportional hazard (Cox-PH) models were used to analyse the influence of baseline liver enzymes on all-cause, CV, IHD, stroke and non-CV mortality (details are provided in section 2.6.2). The covariates included were baseline age, sex, BMI, smoking status (never versus ever), SBP, DBP, alcohol use, eGFR and final achieved SBP. A variable on year of first visit strata (epochs) was used to adjust the secular trend in mortality and was divided into five categories (first visit 1977 or before, between years 1978-1985, 1986-1993, 1994-2001, 2002 and thereafter). The PH assumption was assessed (as explained in section 2.6.3) through examination of log-minus-log plots (23). Initially, the liver enzymes were assessed as categorical variables in quartiles (Models 1-5) adjusted for the described covariates. Next the analysis was repeated with inclusion of all other liver function variables as covariates (Models 6 and 7).

Given the non-linear relationship of the liver function variables and time to mortality, a regression spline model was also employed to further smoothen the hazard functions. The relative log hazard functions with associated 95% confidence intervals were plotted in a graph. Details of regression spline models are provided in section 2.6.4. In order to elucidate the independent effects of ALT, bilirubin and GGT on mortality, the regression spline models were performed in sub-groups stratified by age, smoking, BMI and alcohol status.

Regression modelling with generalised estimating equations (GEE) was used to study the association of markers of liver dysfunction or injury with follow-up BP. Initially, individuals with at least four annual BP assessments in the first five years of follow-up and survival up to a minimum of five years period were selected. Particulars of the GEE model are explained in detail in section 2.7.1. Separate GEE models were used to study the relationship between liver biochemistry variables and follow-up SBP and DBP. The association was adjusted for baseline age, sex, epochs, alcohol and tobacco use, and eGFR based renal function in all models. All non-missing pairs of data were used in estimating the working correlation parameters. The models were repeated after stratifying the population based on different baseline variables such as age, body mass index, alcohol use status and smoking status. Stata Version 12.0 (Statacorp) was used for all statistical analysis.

7.3 Results

7.3.1 Baseline characteristics of the study population

The study population was middle aged (mean age=50.8 years), overweight (mean BMI=27.6 kg/m^2), and hypertensive (mean SBP=164 and DBP=97 mmHg). It comprised a relatively lower proportion of men (47%) than women (53%). While 44% were smokers, nearly two thirds (60%) drank more than 6 units of alcohol per week. The achieved BP after five years of follow-up was significantly lower than the baseline BP (achieved SBP=148 and DBP=88 mmHg). The full demographic and clinical characteristics are given in table 7-1 on page 206.

Tables 7-2 to 7-6 (pages 208-211) provide demographic and clinical characteristics stratified by quartiles of liver function/injury markers. ALT levels decreased with age in contrast to GGT and ALP which increased. Bilirubin showed no age relationship and AST showed a non-linear association. ALT, GGT and ALP showed a linear increase with BMI while bilirubin and AST did not. All the liver tests were higher in men compared to women. A greater proportion of individuals who consume alcohol >6units/week were present in the higher quartiles of ALT, AST, GGT and Bilirubin but not ALP which showed a reverse association with alcohol use. ALT, ALP and Bilirubin showed an inverse linear association with SBP but a direct association with DBP. GGT showed no association with SBP, but showed a direct association with DBP. AST showed no

association with BP. CKD (eGFR<60) prevalence was significantly lower in the higher quartiles of all liver tests. GGT and ALP levels were higher and bilirubin lower among smokers. There was no association between transaminases and smoking.

7.3.2 Survival characteristics

The total time at risk was 173805.67 person years (p-y) with median survival time of 32.19 years. The incidence rates were 17.59 (95% CI: 16.98-18.22), 10.08 (95% CI: 9.62-10.56), 5.40 (95% CI: 5.07-5.76), 2.50 (2.27-2.74), and 7.51 (7.11-7.93) per 1000 p-y of follow-up for all-cause, CV, IHD, stroke and non-CV mortality outcomes, respectively.

Table 7-1: Baseline characteristics of the study population

Variables	Total (N=12,000)	Men (N=5680)	Women (N=6320)	P Value
Age yrs, $\bar{X}$ (SD)	50.88 (14.51)	49.87 (13.28)	51.79 (15.48)	<0.001
BMI kg/m^2, $\bar{X}$ (SD)	27.79 (5.82)	27.86 (5.26)	27.73 (6.27)	0.23
Smoking, n (%)	5254 (44.63)	2719 (48.89)	2535 (40.81)	<0.001
Alcohol >6, n (%)	6676 (58.62)	3967 (73.56)	2709 (45.19)	<0.001
SBP mmHg, $\bar{X}$ (SD)	163.53 (28.45)	161.78 (26.69)	165.10 (29.86)	<0.001
DBP mmHg, $\bar{X}$ (SD)	96.91 (17.89)	98.00 (14.62)	95.93 (20.33)	<0.001
TC mmol/l, $\bar{X}$ (SD)	5.94 (1.47)	5.83 (1.58)	6.03 (1.35)	<0.001
eGFR, M (IQR)	72.59 (60.92-84.60)	75.54 (64.07-87.42)	69.68 (58.33-82.05)	<0.001
ALP IU/L, M (IQR)	125.0 (78.1-183.0)	125.0 (78.1-181.0)	125 (78.1-185.0)	0.91
ALT IU/L, M (IQR)	24.0 (17.0-34.0)	29.0 (21.0-41.0)	20.0 (15.0-28.0)	<0.001
AST IU/L, M (IQR)	23.0 (18.0-28.0)	25.0 (20.0-31.0)	21.0 (17.0-26.0)	<0.001
GGT IU/L, M (IQR)	27.0 (17.0-45.0)	34.0 (22.0-57.0)	21.0 (15.0-34.0)	<0.001
Bilirubin µmol/l, M (IQR)	10.0 (7.0-13.0)	11.0 (8.0-14.0)	9.0 (7.0-12.0)	<0.001
Albumin g/L, $\bar{X}$ (SD)	43.82 (3.79)	44.35 (3.87)	43.34 (3.65)	<0.001
Last SBP mmHg, $\bar{X}$ (SD)	147.97 (22.46)	146.67 (21.30)	149.14 (23.39)	<0.001
Last DBP mmHg, $\bar{X}$ (SD)	87.93 (11.85)	88.78 (11.77)	87.17 (11.88)	<0.001

$\bar{X}$=mean, M=median, n=number of individuals, SD=standard deviation, SBP=systolic blood pressure, DBP=diastolic blood pressure, TC=total cholesterol, IQR=interquartile range, ALP=alkaline phosphatase, ALT=alanine transaminase, AST=aspartate transaminase, GGT=gamma gultamyl transferase.

Figure 7-1: Study flow chart

*Numbers included in the cubic regression spline models.

Table 7-2: Baseline characteristics of the study population, stratified by ALT quartiles.

	ALT<=16 U/L (n=2168)	ALT 17-24 U/L (n=3034)	ALT 25-34 U/L (n=2184)	ALT>=35 U/L (n=2331)	*p* value
Age yrs, mean (SD)	53.24 (16.38)	52.80 (14.45)	50.83 (12.50)	47.89 (12.11)	<0.001
Men, n(%)	578 (26.7)	1212 (40.0)	1232 (56.4)	1618 (69.4)	<0.001
BMI kg/m2, mean (SD)	26.21 (5.44)	27.41 (6.09)	28.25 (5.48)	29.22 (5.57)	<0.001
Smoking, n(%)	950 (45.1)	1316 (44.0)	962 (44.7)	1022 (44.9)	0.881
Alcohol >6 units, n(%)	1064 (51.9)	1582 (54.6)	1325 (63.7)	1591 (71.8)	<0.001
SBP mmHg, mean (SD)	167.45 (29.72)	166.60 (28.68)	164.20 (27.77)	161.08 (25.93)	<0.001
DBP mmHGg, mean (SD)	96.07 (15.29)	96.73 (14.92)	98.19 (23.23)	98.36 (13.94)	<0.001
TC mmol/l, mean (SD)	5.84 (1.33)	5.92 (1.27)	6.01 (1.92)	6.08 (1.51)	0.011
eGFR<60, n(%)	667 (31.0)	834 (27.7)	444 (20.5)	355 (15.4)	<0.001
ALP IU/L, M (IQR)	113 (71-174)	123 (78-182)	122 (78-180)	121 (78-184)	<0.001
AST IU/L, M (IQR)	18 (16-22)	21 (18-25)	25 (21-29)	31 (26-40)	<0.001
GGT IU/L, M (IQR)	18 (13-26)	23 (16-35)	31 (21-48)	46 (28-80)	<0.001
Bilirubin μmol/l, M (IQR)	9 (7-11)	9 (7-12)	10 (8-13)	11 (8-14)	<0.001
Albumin g/L, mean (SD)	42.81 (3.66)	43.40 (3.95)	44.10 (3.51)	44.61 (3.64)	<0.001

SD=standard deviation, M=Median, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, TC=total cholesterol, eGFR=estimated glomerular filtration rate, IQR=interquartile range, ALP=alkaline phosphatase, ALT=alanine transaminase, AST=aspartate transaminase, GGT=gamma gultamyl transpeptidase.

Table 7-3: Baseline characteristics of the study population, stratified by AST quartiles.

	AST<=18 U/L (n=2794)	AST 19-22 U/L (n=2582)	ALT 23-28 U/L (n=2945)	AST>=29 U/L (n=2738)	p value
Age yrs, mean (SD)	49.99 (14.98)	52.41 (15.07)	52.11 (14.16)	49.69 (13.25)	<0.001
Men, n(%)	916 (32.8)	1084 (42.0)	1535 (52.1)	1715 (62.3)	<0.001
BMI kg/m2, mean (SD)	27.39 (6.07)	27.38 (5.54)	27.87 (5.54)	28.49 (5.79)	<0.001
Smoking, n(%)	1277 (46.5)	1152 (45.4)	1250 (43.5)	1188 (44.3)	0.105
Alcohol >6 units, n(%)	1458 (54.8)	1351 (55.1)	1609 (58.0)	1769 (68.5)	<0.001
SBP mmHg, mean (SD)	165.28 (29.68)	164.29 (28.18)	164.03 (27.47)	163.07 (27.60)	0.406
DBP mmHGg, mean (SD)	96.97 (15.37)	96.03 (14.15)	96.86 (14.39)	98.57 (21.62)	0.112
TC mmol/l, mean (SD)	5.87 (1.31)	5.90 (1.26)	5.91 (1.28)	6.06 (1.80)	0.325
eGFR<60, n(%)	704 (25.5)	670 (26.2)	723 (24.8)	515 (19.0)	<0.001
ALP IU/L, M(IQR)	114 (71-173)	126 (79-180)	131 (82-187)	135 (83-193)	<0.001
ALT IU/L, M(IQR)	16 (13-21)	20 (16-25)	26 (20-34)	40 (28-55)	<0.001
GGT IU/L, M(IQR)	20 (14-30)	23 (16-35)	27 (18-42)	45 (26-79)	<0.001
Bilirubin µmol/l, M (IQR)	9 (6-11)	10 (7-12)	10 (8-13)	11 (8-14)	<0.001
Albumin g/L, mean (SD)	43.13 (3.84)	43.60 (3.40)	44.09 (3.67)	44.59 (3.65)	<0.001

SD=standard deviation, M=median, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, TC=total cholesterol, eGFR=estimated glomerular filtration rate, IQR=interquartile range, ALP=alkaline phosphatase, ALT=alanine transaminase, AST=aspartate transaminase, GGT=gamma gultamyl transpeptidase.

Table 7-4: Baseline characteristics of the study population, stratified by GGT quartiles.

	GGT<=18 U/L (n=2385)	GGT 19-22 U/L (n=3040)	ALT 23-28 U/L (n=2384)	GGT>=29 U/L (n=1980)	*p* value
Age yrs, mean (SD)	48.75 (16.68)	52.51 (15.03)	52.42 (13.80)	51.07 (12.78)	<0.001
Men, n(%)	562 (23.6)	1266 (41.6)	1366 (57.3)	1273 (64.3)	<0.001
BMI kg/m2, mean (SD)	26.25 (5.32)	27.82 (5.84)	28.50 (5.93)	29.44 (6.09)	<0.001
Smoking, n(%)	830 (35.2)	1249 (41.8)	1138 (48.5)	997 (51.6)	<0.001
Alcohol >6 units, n(%)	1064 (46.8)	1468 (51.0)	1313 (58.3)	1258 (67.8)	<0.001
SBP mmHg, mean (SD)	159.94 (28.31)	162.31 (27.82)	162.72 (27.18)	161.94 (26.53)	0.164
DBP mmHGg, mean (SD)	94.59 (14.37)	95.12 (19.23)	96.05 (13.58)	97.79 (23.69)	<0.001
TC mmol/l, mean (SD)	5.68 (1.25)	5.83 (1.26)	5.90 (1.50)	6.14 (1.28)	<0.001
eGFR<60, n(%)	565 (24.1)	777 (26.0)	545 (23.2)	382 (19.6)	<0.001
ALP IU/L, M (IQR)	108 (70-159)	133 (84-184)	141 (87-194)	146 (92-200)	<0.001
ALT IU/L, M (IQR)	18 (14-23)	21 (16-28)	26 (19-35)	32 (23-46)	<0.001
AST IU/L, M (IQR)	20 (17-24)	21 (18-26)	23 (19-28)	27 (21-34)	<0.001
Bilirubin µmol/l, M (IQR)	9 (7-12)	10 (7-13)	10 (8-13)	10 (8-13)	<0.001
Albumin g/L, mean (SD)	43.36 (3.86)	43.65 (3.65)	44.17 (3.48)	44.32 (3.62)	<0.001

SD=standard deviation, M=median, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, TC=total cholesterol, eGFR=estimated glomerular filtration rate, IQR=interquartile range, ALP=alkaline phosphatase, ALT=alanine transaminase, AST=aspartate transaminase, GGT=gamma gultamyl transpeptidase.
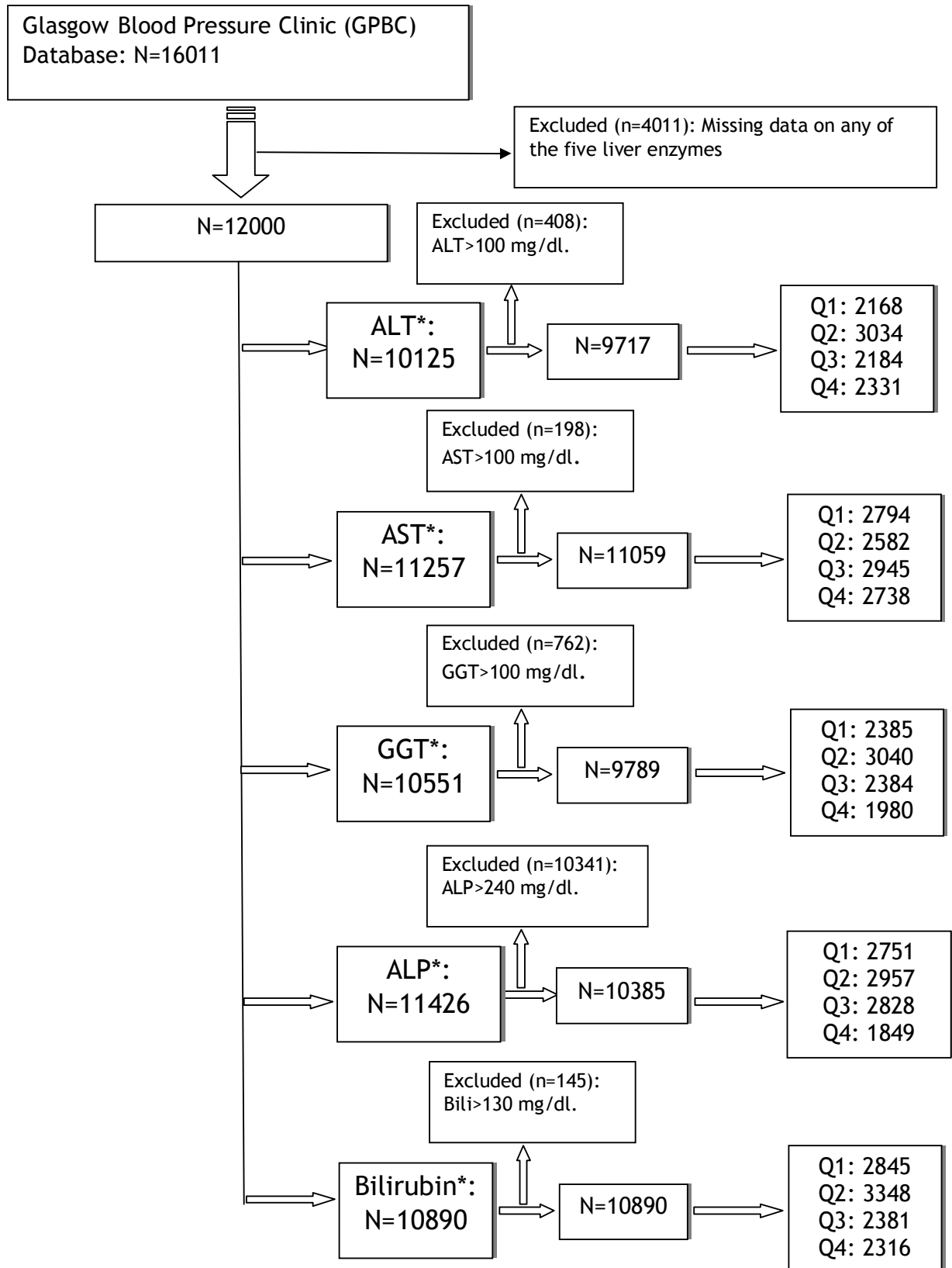
Table 7-5: Baseline characteristics of the study population, stratified by ALP quartiles.

	ALP<=78 U/L (n=2751)	ALP 79-125 U/L (n=2957)	ALP 126-183 U/L (n=2828)	ALP>=184 U/L (n=1849)	p value
Age yrs, mean (SD)	48.31 (13.88)	50.67 (13.86)	51.14 (14.52)	52.94 (14.87)	<0.001
Men, n(%)	1302 (47.3)	1409 (47.7)	1378 (48.7)	925 (50.0)	0.266
BMI kg/m2, mean (SD)	26.85 (5.20)	27.43 (5.59)	28.18 (5.68)	28.64 (6.36)	<0.001
Smoking, n(%)	1075 (40.3)	1310 (45.3)	1194 (42.8)	880 (48.1)	<0.001
Alcohol >6 units, n(%)	1801 (69.1)	1788 (63.3)	1449 (54.7)	935 (53.5)	<0.001
SBP mmHg, mean (SD)	164.66 (28.23)	165.93 (29.17)	160.33 (26.96)	163.70 (27.65)	<0.001
DBP mmHGg, mean (SD)	98.04 (15.40)	98.01 (14.72)	96.8 (20.88)	96.31 (14.11)	<0.001
TC mmol/l, mean (SD)	6.00 (1.50)	6.03 (1.31)	5.80 (1.21)	5.91 (1.99)	0.002
eGFR<60, n(%)	565 (24.1)	777 (26.0)	545 (23.2)	382 (19.6)	<0.001
ALT IU/L, M (IQR)	22 (16-34)	24 (17-35)	23 (17-34)	24 (18-35)	<0.001
AST IU/L, M (IQR)	22 (17-28)	23 (18-28)	23 (19-29)	24 (20-30)	<0.001
GGT IU/L, M (IQR)	22 (14-36)	26 (17-43)	27 (18-46)	30 (20-49)	<0.001
Bilirubin μmol/l, M (IQR)	9 (7-12)	9 (7-12)	11 (8-14)	10 (8-13)	<0.001
Albumin g/L, mean (SD)	43.46 (4.05)	43.56 (3.76)	44.28 (3.39)	44.29 (3.59)	<0.001

SD=standard deviation, M=median, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, TC=total cholesterol, eGFR=estimated glomerular filtration rate, IQR=interquartile range, ALP=alkaline phosphatase, ALT=alanine transaminase, AST=aspartate transaminase, GGT=gamma gultamyl transpeptidase.

Table 7-6: Baseline characteristics of the study population, stratified by bilirubin quartiles.

	Bili<=7 U/L (n=2845)	Bili 8-10 U/L (n=3348)	Bili 10.1-13 U/L (n=2381)	Bili>=13.1 U/L (n=2316)	*p* value
Age yrs, mean (SD)	50.88 (14.60)	51.70 (14.56)	51.37 (14.23)	50.10 (14.50)	0.587
Men, n(%)	890 (31.3)	1448 (43.3)	1307 (54.9)	1502 (64.9)	<0.001
BMI kg/m2, mean (SD)	27.60 (6.21)	28.00 (5.78)	27.80 (5.42)	27.80 (5.67)	<0.001
Smoking, n(%)	1371 (49.3)	1497 (45.6)	970 (41.5)	906 (40.0)	<0.001
Alcohol >6 units, n(%)	1467 (54.5)	1773 (56.1)	1391 (62.1)	1425 (64.7)	<0.001
SBP mmHg, mean (SD)	166.23 (29.39)	163.81 (28.34)	163.36 (27.28)	162.17 (27.47)	0.002
DBP mmHGg, mean (SD)	96.13 (15.42)	96.44 (14.38)	98.03 (22.46)	97.96 (13.87)	<0.001
TC mmol/l, mean (SD)	6.16 (1.88)	5.90 (1.26)	5.83 (1.16)	5.78 (1.45)	<0.001
eGFR<60, n(%)	737 (26.2)	796 (23.9)	524 (22.3)	459 (20.0)	<0.001
ALP IU/L, M (IQR)	100 (71-165)	133 (81-190)	142 (86-193)	141 (86-189)	<0.001
ALT IU/L, M (IQR)	21 (15-30)	23 (17-33)	25 (18-36)	27 (19-40)	<0.001
AST IU/L, M (IQR)	21 (17-26)	22 (18-28)	24 (20-30)	25 (20-32)	<0.001
GGT IU/L, M (IQR)	24 (15-41)	26 (17-42)	28 (18-47)	31 (19-53)	<0.001
Albumin g/L, mean (SD)	42.98 (3.76)	43.78 (3.60)	44.22 (3.66)	44.53 (3.71)	<0.001

SD=standard deviation, M=median, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, TC=total cholesterol, eGFR=estimated glomerular filtration rate, IQR=interquartile range, ALP=alkaline phosphatase, ALT=alanine transaminase, AST=aspartate transaminase, GGT=gamma gultamyl transpeptidase.

7.3.3 Association of ALT and mortality

Unadjusted KM time to death curves of ALT in quartiles are given in Figure 7-2 on page 214. Time to all-cause mortality was significantly lower in quartile 1 of ALT compared with other quartiles (log rank p<0.001). In the Cox-PH model, ALT quartiles 2, 3 and 4 had significantly lower all-cause mortality in comparison to ALT quartile 1 after adjustment for multiple baseline confounding variables and final achieved SBP (Table 7-7; Model 1 on page 216).　Both CV and non-CV mortality show the same trend as all-cause mortality. Adding baseline total cholesterol (which was not available in 734 individuals with baseline ALT) to this model did not change the HR estimates significantly. The hazard estimates for IHD and stroke are presented in table 7-8 on page 217. Adjustment for other liver enzymes did not materially affect the results (Table 7-9 on page 218). There were no indications of violation of PH assumption in the log-log curves.

Number at risk				
ALT<=16 IU/L	2168	1269	651	179
ALT=17-24 IU/L	3034	1856	923	233
ALT=25-34 IU/L	2184	1344	636	163
ALT=35+ IU/L	2331	1434	724	187
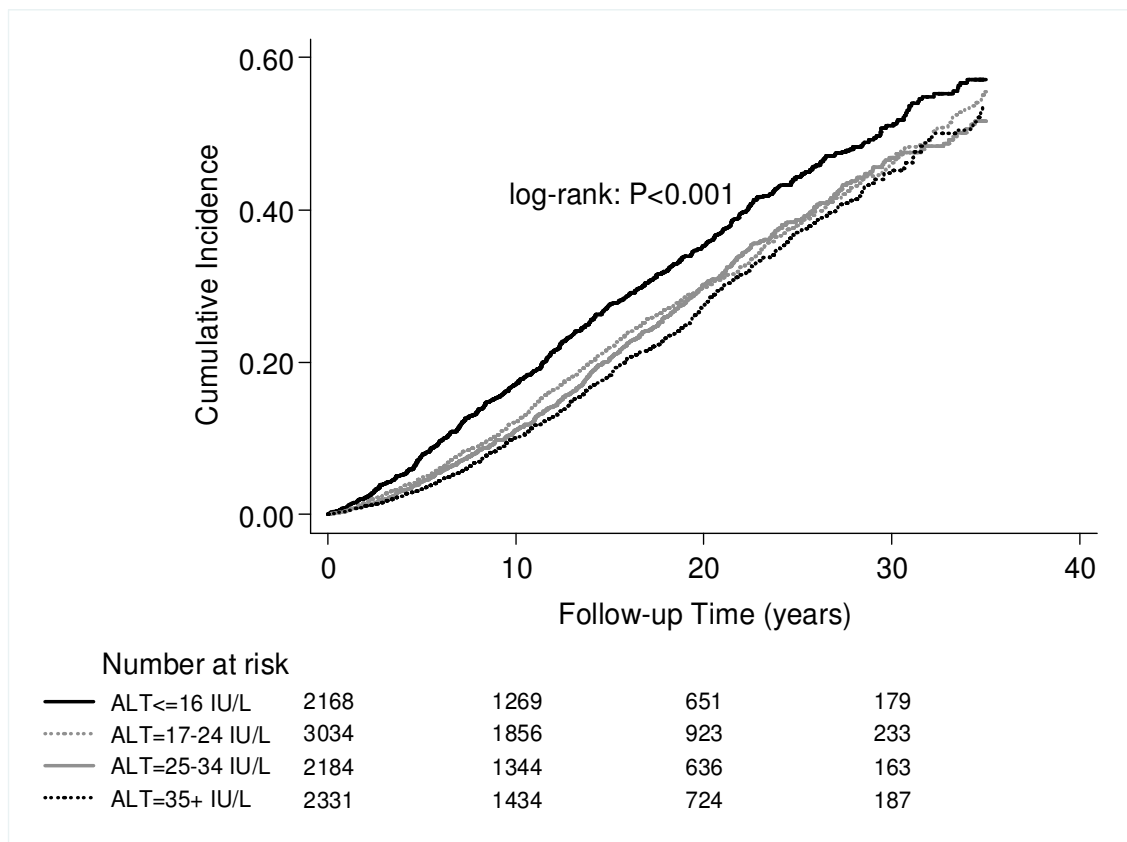
Figure 7-2: Kaplan-Meier survival curves for ALT in quartiles

In the cubic spline regression Cox-PH model after incorporating all confounding variables, an inverse linear relationship between logALT in full range and all-cause mortality was observed (Figure 7-3 on page 214). In individuals younger than 55 years of age, ALT showed no relationship to mortality, while in older individuals >55 years of age (Figure 7-4 on page 215, panels A and B), ALT showed an inverse relationship with lower ALT associated with higher mortality and risk decreasing up to around 60 IU/L. In individuals BMI≤25, ALT showed inverse relationship to mortality, while in individuals BMI>25 (Figure 7-4 on page 215, panels C and D), the association did not reach statistical significance.
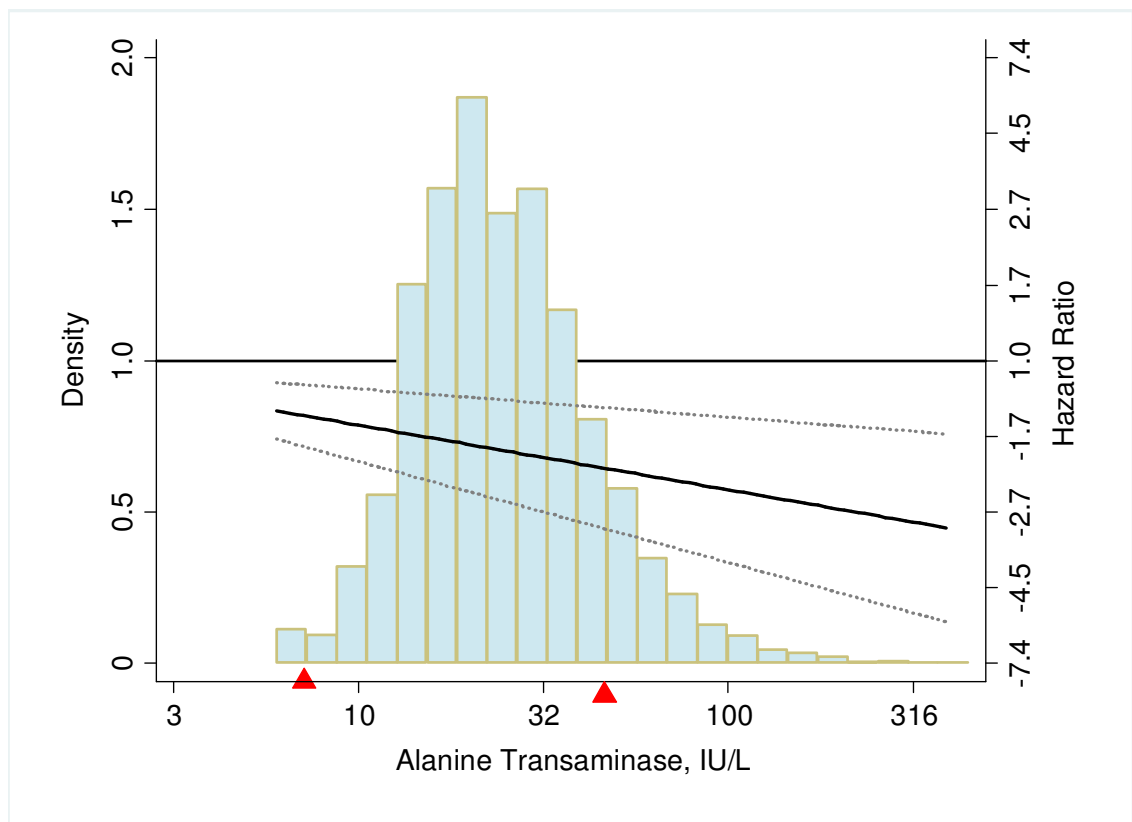
Figure 7-3: Regression spline Cox proportional hazard model for ALT and all-cause mortality.

The ALT distribution is in log scale. Red triangles on the horizontal axis indicate lower and upper limits of normal range.
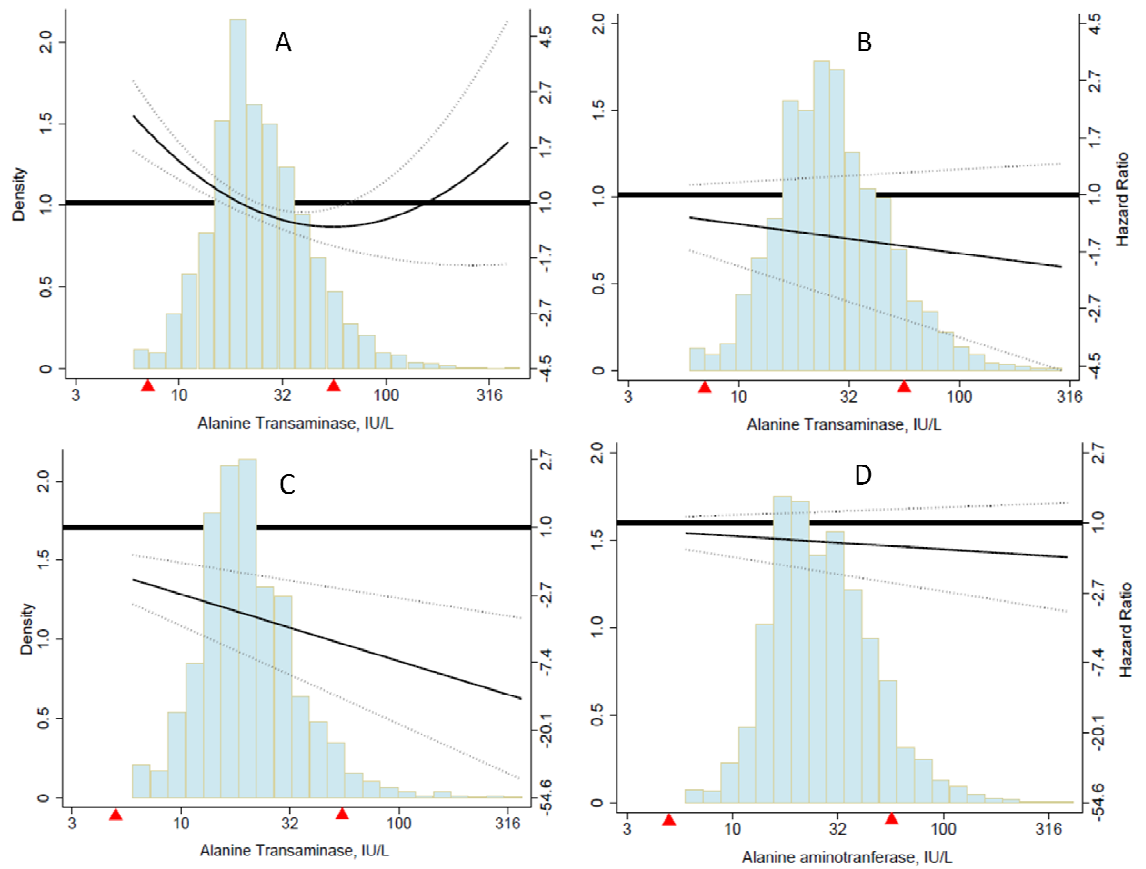
Figure 7-4: Regression spline Cox proportional hazard model for ALT and all-cause mortality (Sub-group analyses).

The ALT distribution is in log scale. Red triangles on the horizontal axis indicate lower and upper limits of normal range. A; age≤55 years, B; age>55 years, C; BMI≤25 kg/m^2, D; BMI>25 kg/m^2.

Table 7-7: Cox proportional hazard models of liver tests and mortality.

	All-cause mortality		CV Mortality		Non-CV Mortality	
	HR	95% CI	HR	95% CI	HR	95% CI
ALT (Model 1)	**N=2436/8677**		**N=1393/8677**		**N=1043/8677**	
ALT Quartile 1	1		1		1	
ALT Quartile 2	0.79*	0.71-0.87	0.80*	0.69-0.92	0.77*	0.66-0.91
ALT Quartile 3	0.80*	0.71-0.90	0.74*	0.63-0.87	0.88	0.74-1.06
ALT Quartile 4	0.81*	0.72-0.92	0.81*	0.69-0.95	0.83*	0.68-1.00
AST (Model 2)	**N=2653/9825**		**N=1503/9825**		**N=1150/9825**	
AST Quartile 1	1		1		1	
AST Quartile 2	0.83*	0.74-0.93	0.86*	0.75-0.99	0.79*	0.67-0.93
AST Quartile 3	0.83*	0.75-0.92	0.84*	0.73-0.97	0.82*	0.70-0.96
AST Quartile 4	1.00	0.90-1.11	1.00	0.87-1.15	0.99	0.85-1.17
GGT (Model 3)	**N=1887/8572**		**N=1030/8572**		**N=857/8572**	
GGT Quartile 1	1		1		1	
GGT Quartile 2	1.01	0.90-1.15	0.97	0.82-1.15	1.06	0.89-1.28
GGT Quartile 3	1.12	0.98-1.28	1.11	0.93-1.33	1.13	0.92-1.38
GGT Quartile 4	1.27*	1.10-1.46	1.24*	1.02-1.50	1.31*	1.06-1.62
ALP (Model 4)	**N=2429/9215**		**N=1383/9215**		**N=1046/9215**	
ALP Quartile 1	1		1		1	
ALP Quartile 2	1.03	0.93-1.13	1	0.88-1.14	1.07	0.92-1.24
ALP Quartile 3	1.14*	1.00-1.29	1.07	0.91-1.27	1.22*	1.01-1.48
ALP Quartile 4	1.28*	1.11-1.46	1.31*	1.10-1.57	1.25*	1.01-1.54
Bili (Model 5)	**N=2510/9641**		**N=1394/9641**		**N=1116/9641**	
Bili Quartile 1	1		1		1	
Bili Quartile 2	0.96	0.87-1.06	0.94	0.83-1.08	0.98	0.84-1.13
Bili Quartile 3	0.90	0.80-1.00	0.87	0.75-1.02	0.93	0.78-1.11
Bili Quartile 4	0.93	0.83-1.05	0.92	0.78-1.08	0.95	0.79-1.13

$p<0.05$, ALT=alanine aminotransferase, AST=aspartate transaminase, ALP=alkaline phosphatase, GGT=gamma-glutamyltransferase (GGT), Bili=bilirubin, CVD=Cardiovascular disease, Non-CVD=non-cardiovascular disease, HR=hazard Ratio, CI=confidence interval. All models are adjusted for age, gender, BMI=body mass index, tobacco smoking, alcohol use, year of first visit (epoch), baseline SBP and DBP, eGFR, and final achieved SBP.

Table 7-8: Cox proportional hazard models of liver tests and IHD and Stroke mortality.

	IHD Mortality		Stroke Mortality	
	HR	95% CI	HR	95% CI
ALT (Model 1)	**N=763/8677**		**N=341/8677**	
ALT Quartile 1	1		1	
ALT Quartile 2	0.89	0.73-1.07	0.73*	0.55-0.96
ALT Quartile 3	0.75*	0.60-0.94	0.76	0.55-1.04
ALT Quartile 4	0.77*	0.61-0.96	0.84	0.61-1.17
AST (Model 2)	**N=804/9825**		**N=368/9825**	
AST Quartile 1	1		1	
AST Quartile 2	0.98	0.81-1.19	0.79	0.58-1.08
AST Quartile 3	0.77*	0.63-0.94	0.99	0.75-1.31
AST Quartile 4	0.94	0.77-1.14	1.15	0.87-1.53
GGT (Model 3)	**N=576/8572**		**N=231/8572**	
GGT Quartile 1	1		1	
GGT Quartile 2	1.01	0.80-1.26	0.99	0.69-1.42
GGT Quartile 3	1.06	0.83-1.36	1.41	0.96-2.05
GGT Quartile 4	1.20	0.93-1.56	1.58*	1.05-2.38
ALP (Model 4)	**N=732/9215**		**N=343/9215**	
ALP Quartile 1	1		1	
ALP Quartile 2	0.96	0.81-1.15	1.09	0.85-1.41
ALP Quartile 3	1.16	0.92-1.46	0.98	0.68-1.39
ALP Quartile 4	1.38*	1.08-1.75	1.29	0.89-1.86
Bilirubin (Model 5)	**N=743/9641**		**N=346/9641**	
Bili Quartile 1	1		1	
Bili Quartile 2	0.95	0.79-1.14	1.04	0.79-1.36
Bili Quartile 3	0.91	0.74-1.14	0.92	0.66-1.27
Bili Quartile 4	0.91	0.73-1.13	1.13	0.82-1.54

*p<0.05, ALP=alkaline phosphatase, ALT=alanine transaminase, AST=aspartate transaminase, GGT=gamma gultamyl transferase, Bili=bilirubin, IHD=ischemic heart disease, HR=hazard Ratio, CI=confidence interval.

Table 7-9: Cox proportional hazard model after accounting for other markers of liver dysfunction.

	All-cause Mortality		CV Mortality		IHD Mortality		Stroke Mortality		Non-CV mortality	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
ALT (Model 6)	N=2203/8223		N=1251/8223		N=683/8223		N=307/8277		N=952/8233	
ALT Quartile 1	1		1		1		1		1	
ALT Quartile 2	0.78*	0.70-0.87	0.79*	0.68-0.91	0.84	0.68-1.03	0.75	0.56-1.00	0.77*	0.65-0.91
ALT Quartile 3	0.80*	0.71-0.90	0.74*	0.62-0.88	0.72*	0.57-0.91	0.75	0.53-1.05	0.89	0.74-1.08
ALT Quartile 4	0.84*	0.74-0.97	0.83*	0.70-0.98	0.77*	0.61-0.97	0.81	0.57-1.15	0.86	0.70-1.05
AST (Model 7)	N=1737/8124		N=929/8124		N=508/8124		N=209/8124		N=808/8124	
AST Quartile 1	1		1		1		1		1	
AST Quartile 2	0.87*	0.76-0.99	0.89	0.74-1.07	1.05	0.83-1.34	0.75	0.50-1.11	0.84	0.70-1.02
AST Quartile 3	0.77*	0.67-0.87	0.83*	0.69-0.99	0.78	0.61-1.01	0.83	0.57-1.22	0.70*	0.57-0.85
AST Quartile 4	0.94	0.82-1.08	0.99	0.82-1.20	1	0.77-1.30	1.03	0.69-1.53	0.89	0.72-1.09
GGT (Model 7)	N=1737/8124		N=929/8124		N=508/8124		N=209/8124		N=808/8124	
GGT Quartile 1	1		1		1		1		1	
GGT Quartile 2	0.97	0.85-1.11	0.92	0.76-1.10	0.87	0.68-1.12	0.96	0.66-1.44	1.04	0.86-1.27
GGT Quartile 3	1.02	0.88-1.18	0.97	0.80-1.19	0.85	0.64-1.11	1.22	0.81-1.86	1.08	0.87-1.34
GGT Quartile 4	1.18*	1.02-1.38	1.10	0.90-1.36	0.98	0.74-1.29	1.34	0.87-2.07	1.29*	1.03-1.62
ALP (Model 7)	N=1737/8124		N=929/8124		N=508/8124		N=209/8124		N=808/8124	
ALP Quartile 1	1		1		1		1		1	
ALP Quartile 2	1.05	0.92-1.20	1.06	0.88-1.26	1.06	0.83-1.36	0.91	0.63-1.31	1.04	0.85-1.27
ALP Quartile 3	1.25*	1.07-1.46	1.20	0.97-1.49	1.38*	1.04-1.84	0.78	0.49-1.26	1.30*	1.04-1.63
ALP Quartile 4	1.61*	1.39-1.87	1.68*	1.37-2.05	1.90*	1.45-2.49	1.35	0.89-2.07	1.54*	1.23-1.92
Bilirubin (Model 7)	N=1737/8124		N=929/8124		N=508/8124		N=209/8124		N=808/8124	
Bili Quartile 1	1		1		1		1		1	
Bili Quartile 2	0.89	0.79-1.00	0.90	0.77-1.06	0.92	0.74-1.15	1.11	0.79-1.55	0.88	0.73-1.04
Bili Quartile 3	0.81*	0.70-0.94	0.77*	0.63-0.94	0.82	0.63-1.08	0.77	0.49-1.21	0.85	0.69-1.04
Bili Quartile 4	0.79*	0.68-0.92	0.80*	0.65-0.99	0.87	0.66-1.14	0.95	0.62-1.47	0.77	0.61-0.96

7.3.4 Association of AST and mortality

Shortest survival time was observed in quartile 4 of AST in comparison to other quartiles (Figure 7-5 on page 219). A clear 'U' shaped association was observed with significantly lower mortality in quartile 2 (HR=0.83, 95% CI: 0.74-0.93) and 3 (HR=0.83, 95% CI: 0.75-0.92) compared with quartile 1 (Table 7-7 on page 216) after adjustment for all confounding variables as in Model 2. The trend was similar for both CV and non-CV mortality outcomes. Incorporation of baseline total cholesterol (which was not available in 865 individuals with baseline AST) to this model did not change the HR estimates significantly. Results were similar when other liver enzymes were added to the model as predictor variables (Table 7-9 on page 218). In the cubic spline regression Cox-PH model, after incorporating all confounding variables, a 'U' shaped relationship between logAST and all-cause mortality was observed with significantly higher mortality at both ends of the distribution (Figure 7-6 on page 220). Similar association was seen even in the BMI stratified analyses (Figure 7-7 on page 220).
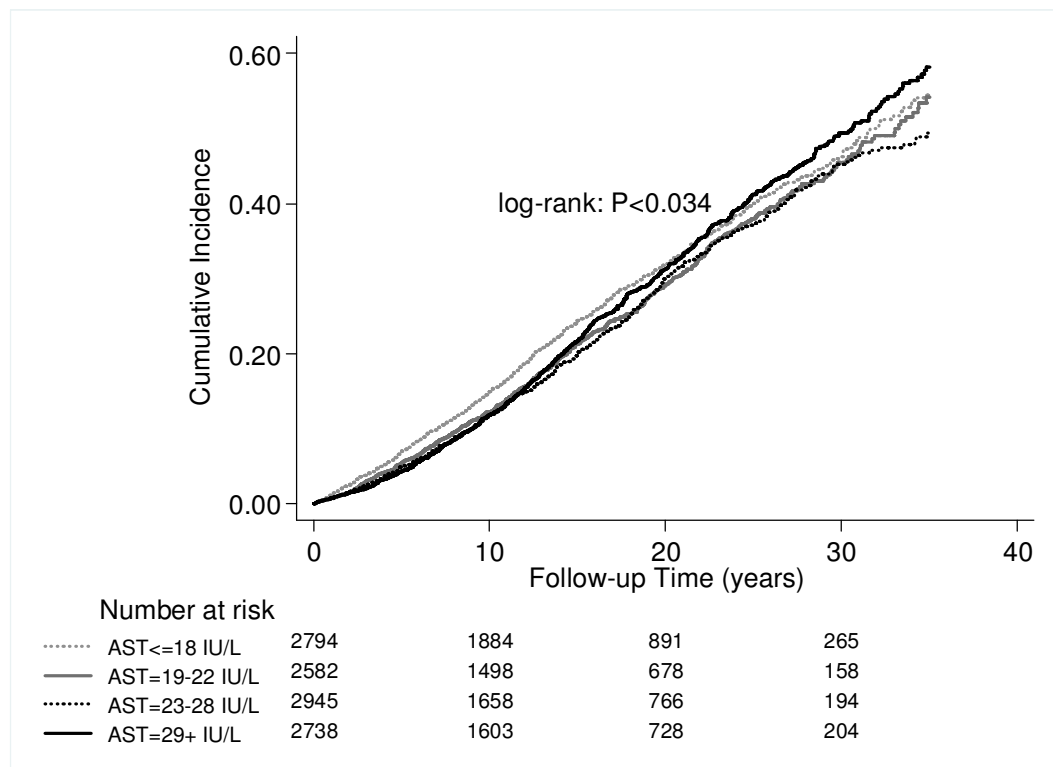
Figure 7-5: Kaplan-Meier survival curves for AST in quartiles
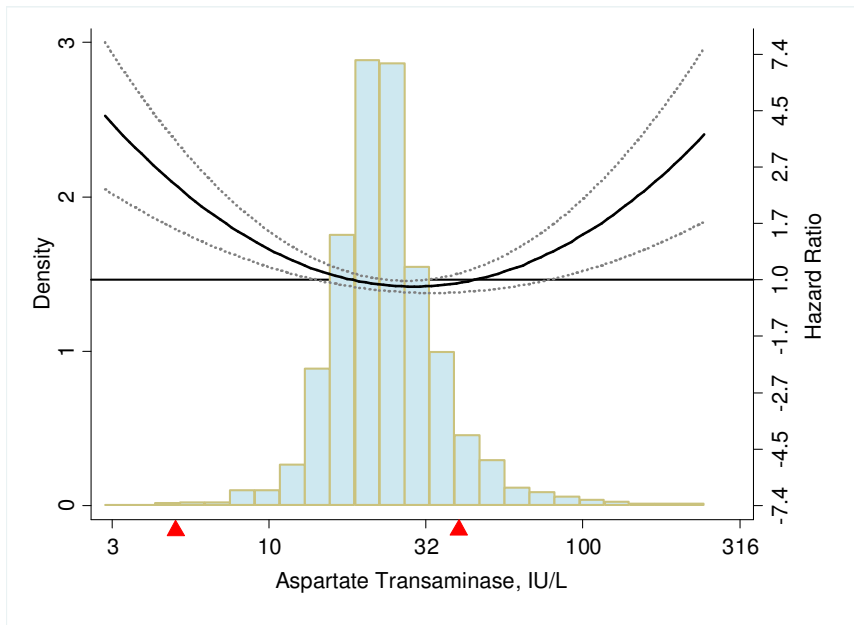
Figure 7-6: Cubic regression Cox proportional hazard model for AST and all-cause mortality.

AST distribution is in log scale. Red triangles on the horizontal axis indicate lower and upper limits of normal range.
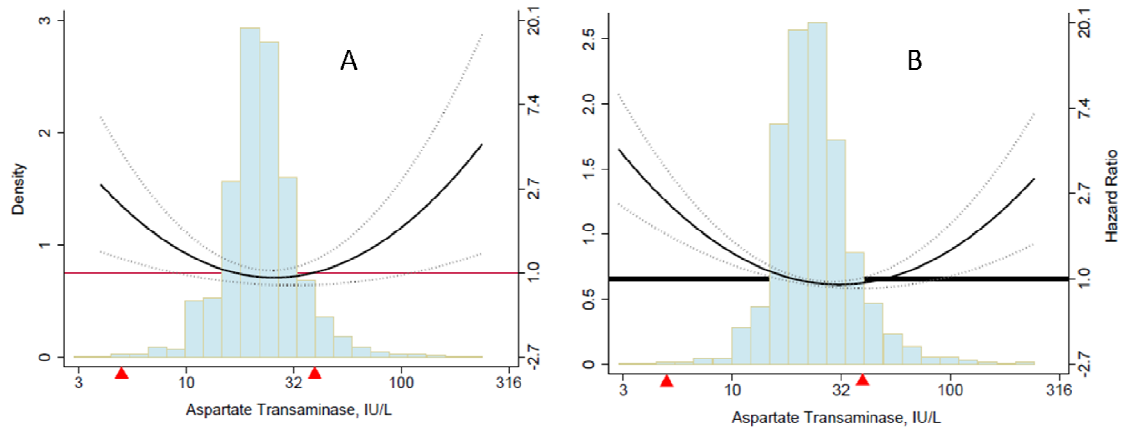
Figure 7-7: Regression spline Cox proportional hazard model for AST and all-cause mortality (Sub-group analyses).

AST distribution is in log scale. Red triangles on the horizontal axis indicate lower and upper limits of normal range. A; BMI≤25 kg/m^2, B; BMI>25 kg/m^2.

7.3.5 Association of GGT and mortality

In the KM analysis the shortest survival time was observed in the last quartile of GGT (Figure 7-8 on page 221). It had significantly higher all-cause mortality (HR=1.27, 95% CI: 1.10-1.46), CV mortality (HR=1.24. 95% CI: 1.02-1.50), stroke mortality (HR=1.58, 95% CI: 1.05-2.38) and non-CV mortality (HR=1.31, 95% CI: 1.06-1.62) compared with quartile 1 of GGT (Table 7-7 on page 216) after adjustment for confounding variables as in Model 3. Further adjustment for other liver enzymes reduced the magnitude of HRs between mortality risk in quartile 1 and quartile 4 (Table 7-8 on page 217; all-cause mortality HR=1.18, 95% CI: 1.02-1.38, CV mortality HR=1.10. 95% CI: 0.90-1.36, stroke mortality HR=1.34, 95% CI: 0.87-2.07, and non-CV mortality HR=1.29, 95% CI: 1.03-1.62). A positive linear relationship between logGGT and all-cause mortality was observed in the cubic regression spline Cox-PH model (Figure 7-9 on page 222). In individuals BMI≤25, GGT showed positive linear relationship to mortality (Figure 7-10 on page 222), while in individuals BMI>25 (Figure 7-11 on page 223), the association did not reach statistical significance. Alcohol intake (Figures 7-12 on page 223) appears to have no effect on mortality risk associated with GGT.

| Number at risk	0	10	20	30
········ GGT<=16 IU/L	2385	1487	798	133
········ GGT=17-27 IU/L	3040	1813	682	103
—— GGT=28-44 IU/L	2384	1362	479	91
—— GGT=25+ IU/L	1980	1084	394	66
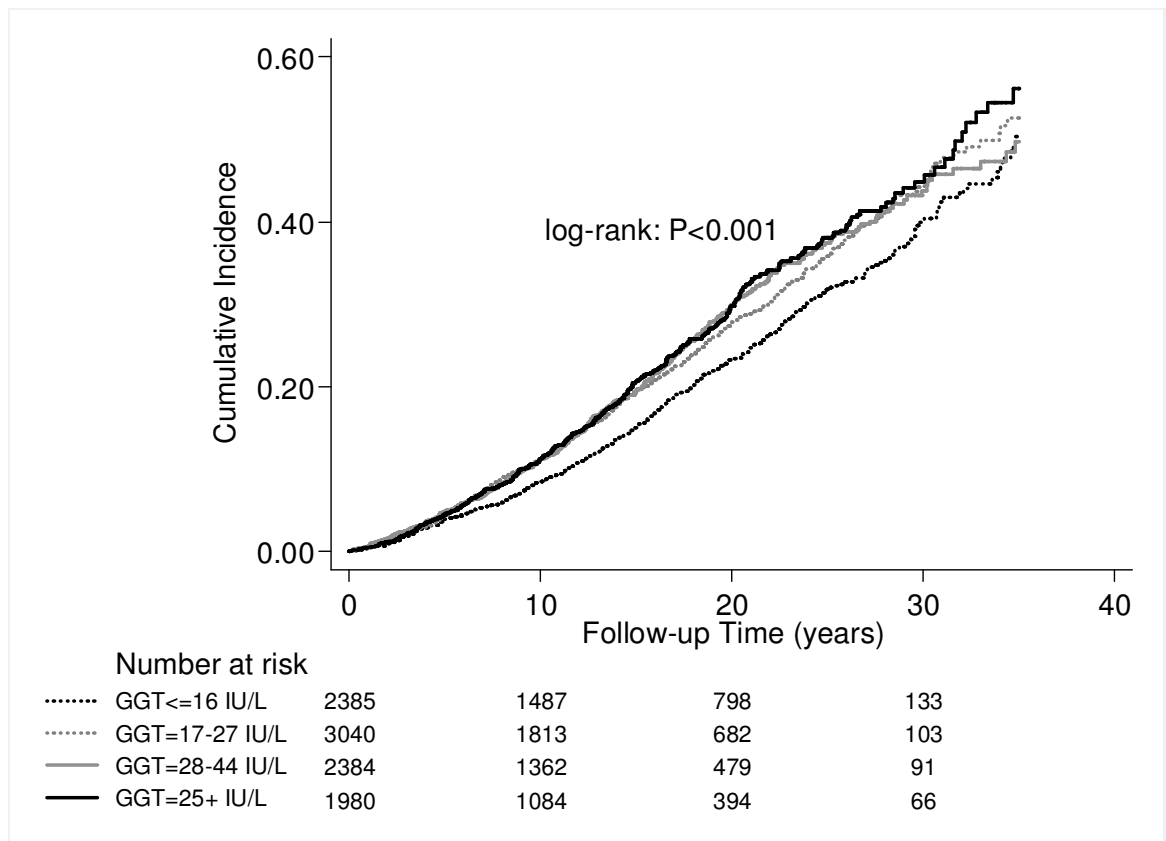
Figure 7-8: Kaplan-Meier survival curves for GGT in quartiles
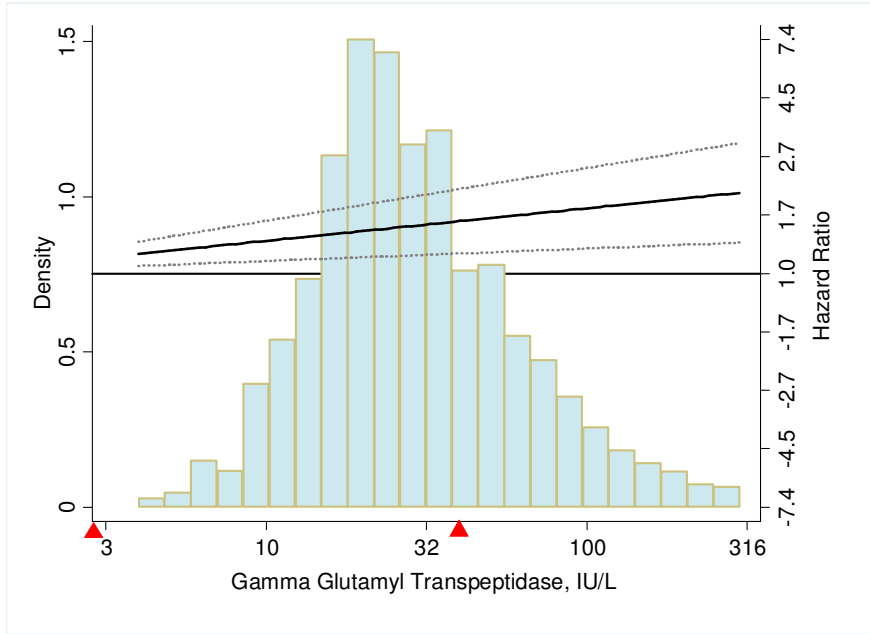
Figure 7-9: Regression spline Cox proportional hazard model for GGT and all-cause mortality.

GGT distribution is in log scale. Red triangles on the horizontal axis indicate lower and upper limits of normal range.
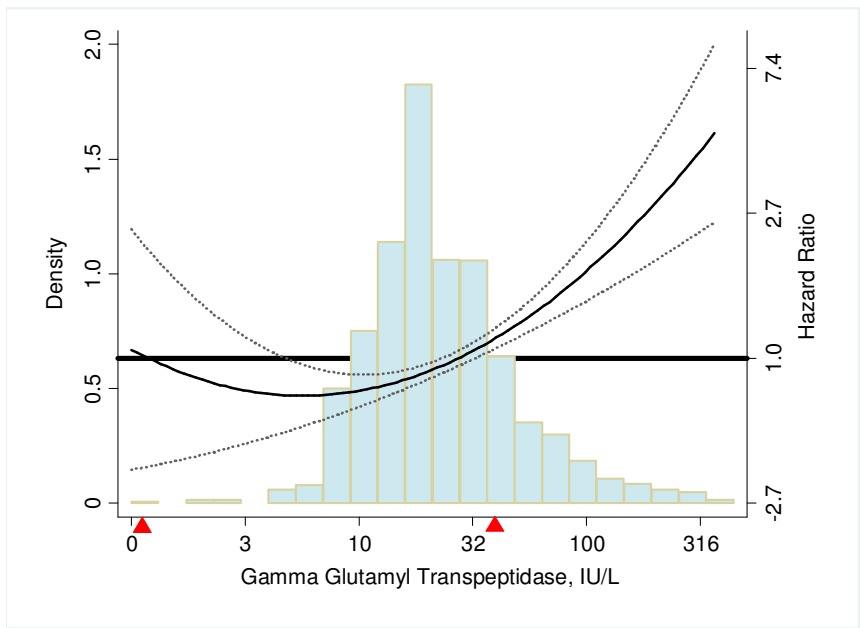
Figure 7-10: Regression spline Cox proportional hazard model for GGT and all-cause mortality in individuals with BMI≤25 kg/m^2.

GGT distribution is in log scale. Red triangles on the horizontal axis indicate lower and upper limits of normal range.
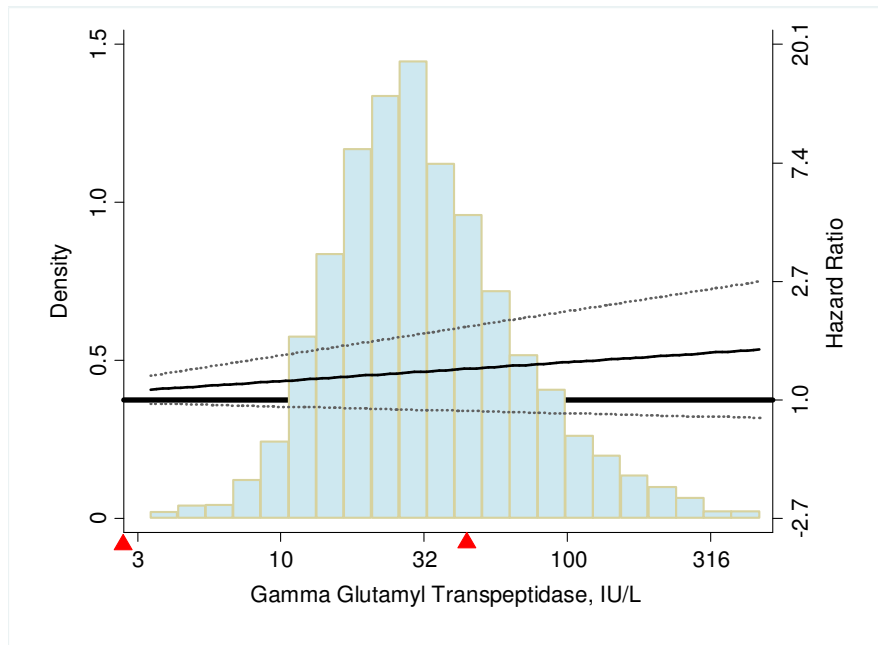
Figure 7-11: Regression spline Cox proportional hazard model for GGT and all-cause mortality in individuals with BMI>25 kg/m^2.

GGT distribution is in log scale. Red triangles on the horizontal axis indicate lower and upper limits of normal range.
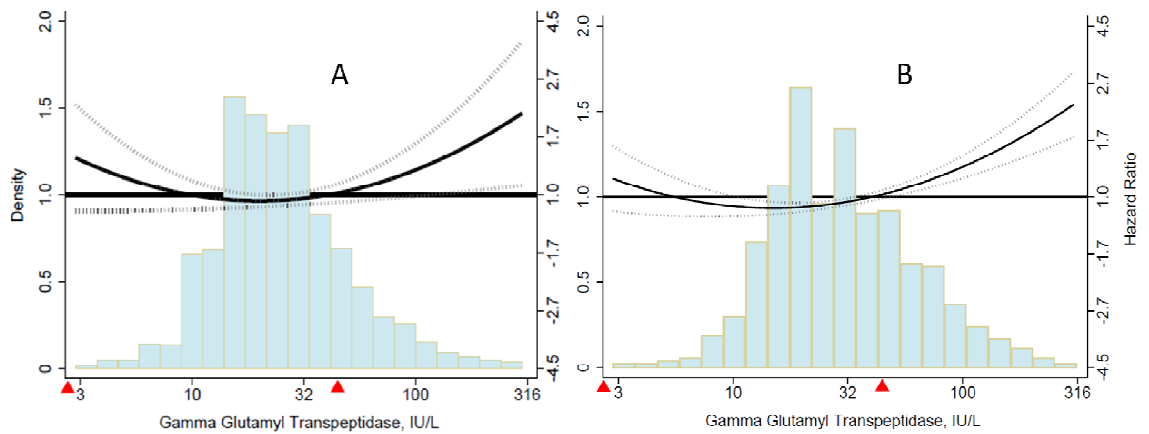
Figure 7-12: Regression spline Cox proportional hazard model for GGT and all-cause mortality in alcohol users (A) and non-users (B).

GGT distribution is in log scale. Red triangles on the horizontal axis indicate lower and upper limits of normal range.

7.3.6 Association of ALP and mortality

In the unadjusted KM model the shortest survival time was observed in quartile 4 of ALP (Figure 7-13 on page 224). While quartile 3 of ALP had 14% (HR=1.14, 95% CI: 1.00-1.29) excess mortality, it was 28% (HR=1.28, 95% CI: 1.11-1.46) in quartile 4 compared with quartile 1 after adjustment for multiple risk factors as in Model 4 (Table 7-7 on page 216). The pattern of risk was similar for CV, IHD, and non-CV mortality. Further adjustment for other liver enzymes had the effect of increasing the hazard ratios for quartile 3 and quartile 4 compared with quartile 1, for all-cause, CV, IHD, and non-CV mortality (Table 7-8 on page 217). A positive linear trend in mortality with logALP was observed in the cubic regression spline analysis (Figure 7-14 on page 225). While a similar positive linear trend was seen in individuals with BMI>25 kg/m^2 (Figure 7-15 on page 225), the association shows non-linear relationship with increase in mortality above the upper limit normal value of ALP in individuals with BMI≤25 (Figure 7-16 on page 226).

Number at risk				
— ALP<=78 IU/L	2751	1897	1296	448
⋯⋯⋯ ALP=79-125 IU/L	2957	1944	1028	226
⋯⋯ ALP=126-183 IU/L	2828	1489	444	85
— ALP=184+ IU/L	2826	1448	358	78
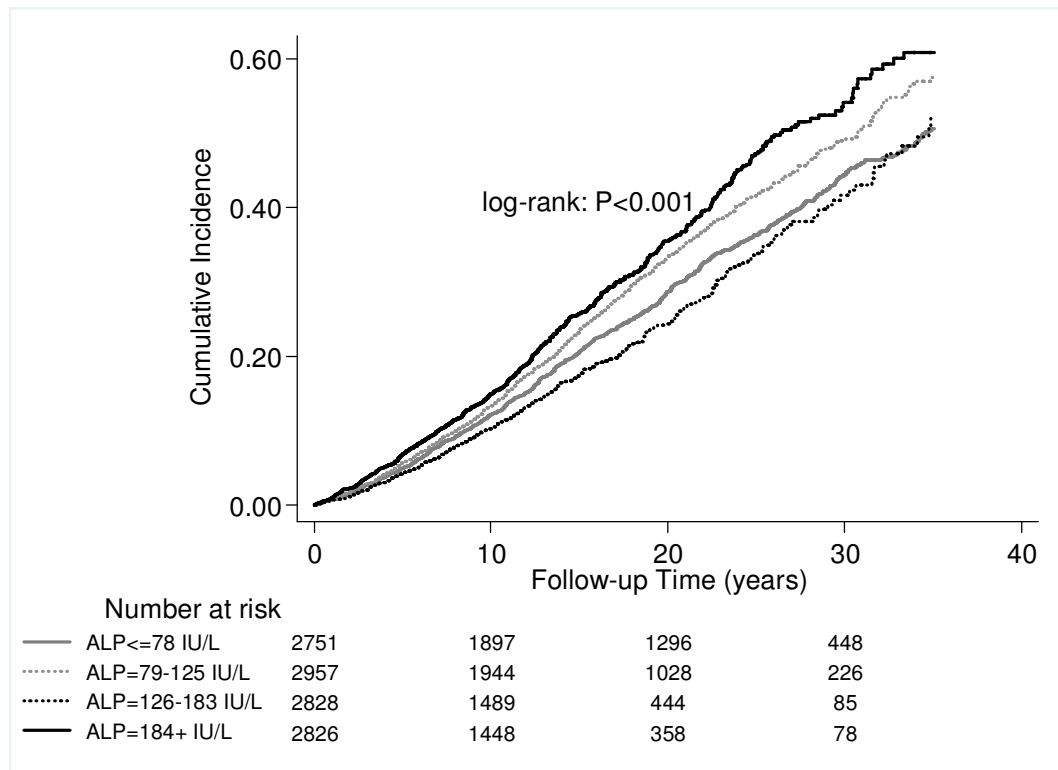
Figure 7-13: Kaplan-Meier survival curves of ALP in quartiles.
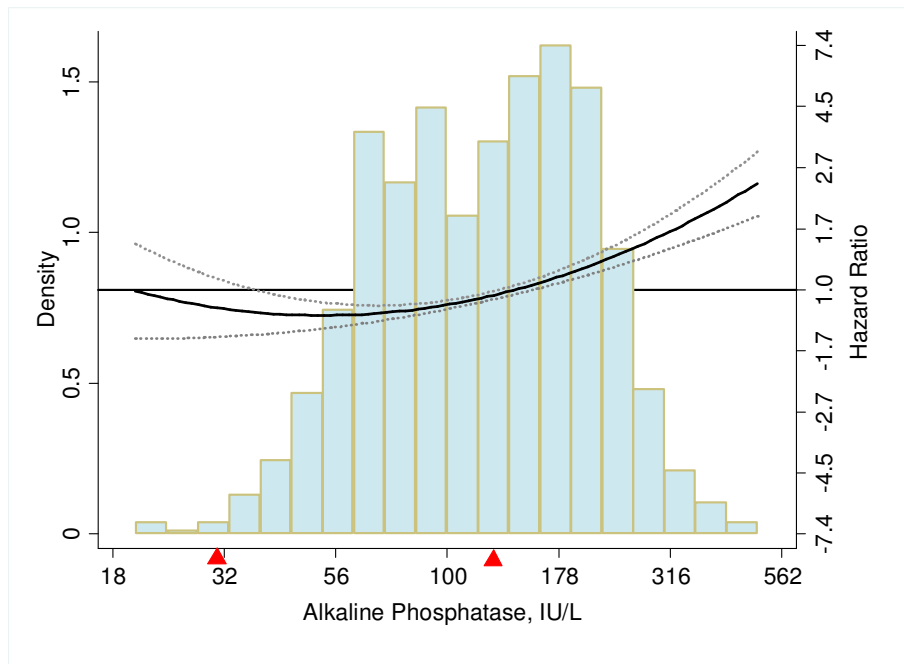
Figure 7-14: Regression spline Cox model for ALP and all-cause mortality.

ALP distribution is in log scale. Red triangles on the horizontal axis indicate lower and upper limits of normal range.
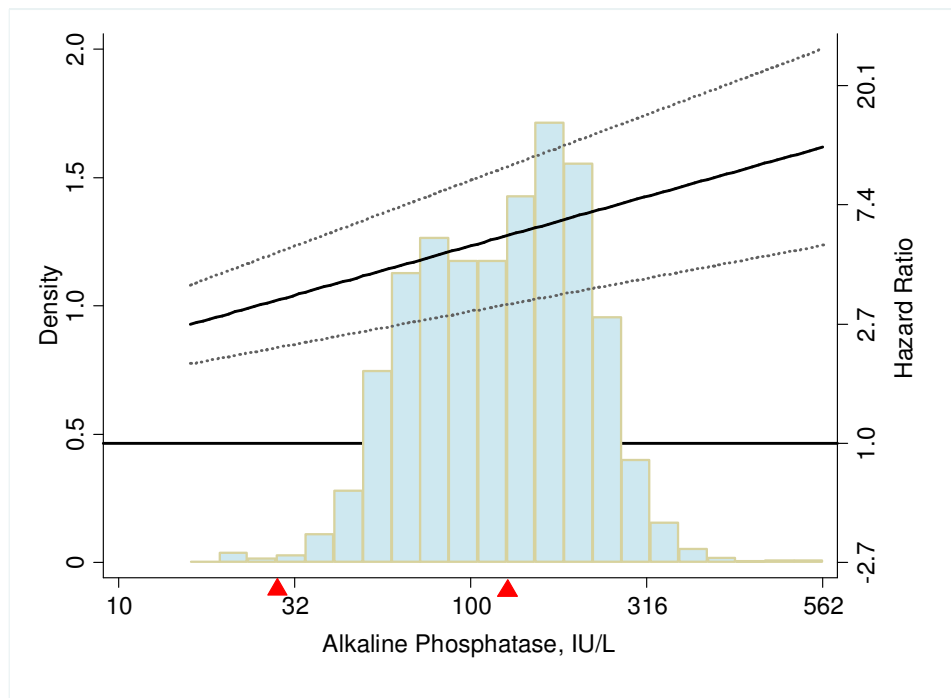
Figure 7-15: Regression spline Cox model for ALP and all-cause mortality in individuals with BMI>25 kg/m^{2}.

ALP distribution is in log scale. Red triangles on the horizontal axis indicate lower and upper limits of normal range.
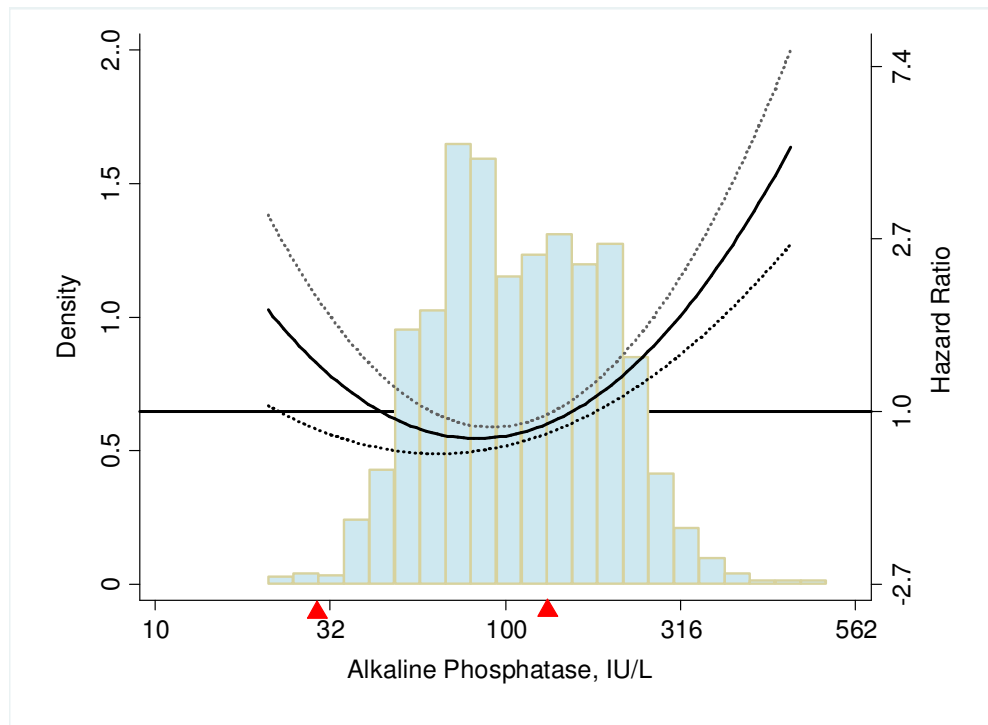
Figure 7-16: Regression spline Cox model for ALP and all-cause mortality in individuals with BMI≤25 kg/m^2.

ALP distribution is in log scale. Red triangles on the horizontal axis indicate lower and upper limits of normal range.

7.3.7 Association of Bilirubin and Mortality

In the unadjusted KM model the shortest survival time was observed in quartile 1 of bilirubin (Figure 7-17 on page 227). While the median survival time in quartile 1 of bilirubin was 30.75 years, they were 32.09, 33.54 and 33.48 in quartiles 2, 3 and 4 respectively. In Model 5, adjusted for baseline variables and final achieved SBP, bilirubin was not associated with mortality outcomes (Table 7-7 on page 216). However, once adjustment was made for other liver enzymes, bilirubin quartiles 3 and 4 had significantly lower all cause (HR=0.81, 95% CI: 0.70-0.94 and HR=0.79, 95% CI: 0.68-0.92 respectively) and cardiovascular (HR=0.77, 95% CI: 0.63-0.94 and HR=0.80, 95% CI: 0.65-0.99 respectively) mortality risk than quartile 1 (Table 7-8 on page 217).   An inverse linear relationship between log bilirubin and all-cause mortality was observed in the cubic regression spline Cox-PH model (Figure 7-18 on page 227). The inverse relationship between bilirubin and mortality persisted irrespective of smoking status (Figures 7-19 on page 228, panels A and B) and BMI levels (Figures 7-19 on page 228, panels C and D).
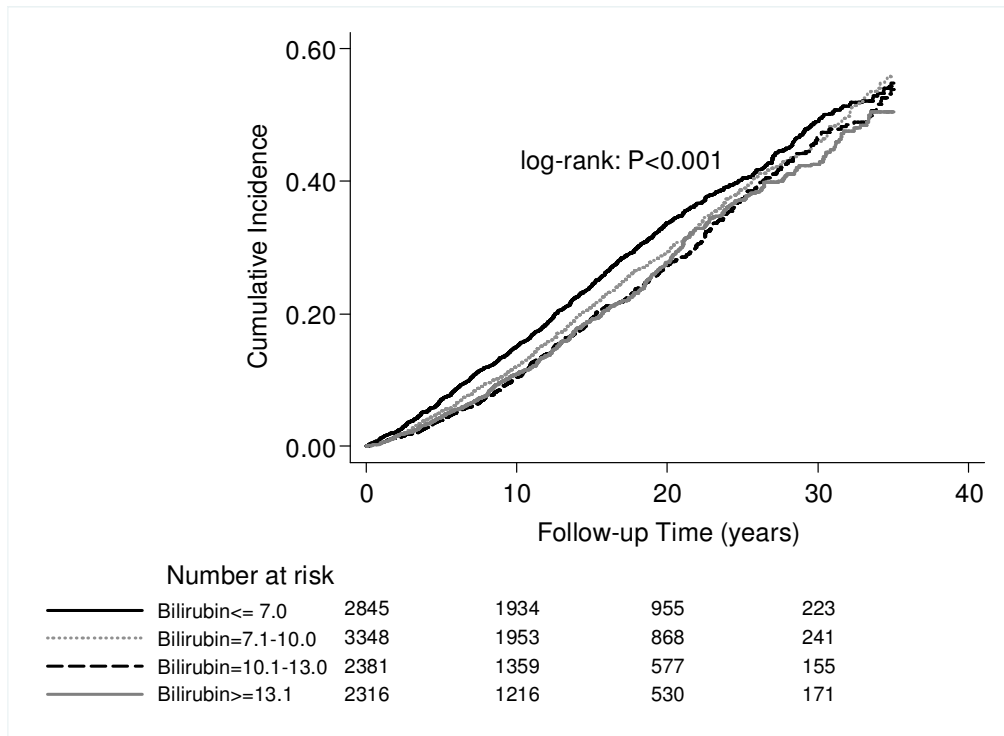
**Figure 7-17: Kaplan-Meier survival curves for serum bilirubin in quartiles**
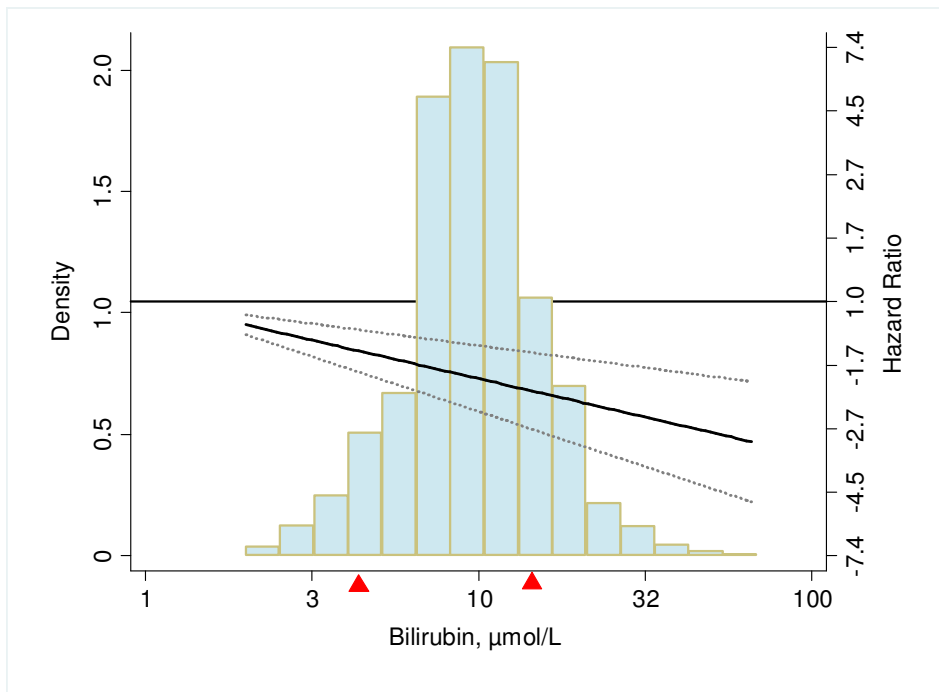
Figure 7-18: Regression spline Cox model for serum bilirubin and all-cause mortality.

Serum bilirubin distribution is in log scale. Red triangles on the horizontal axis indicate lower and upper limits of normal range.
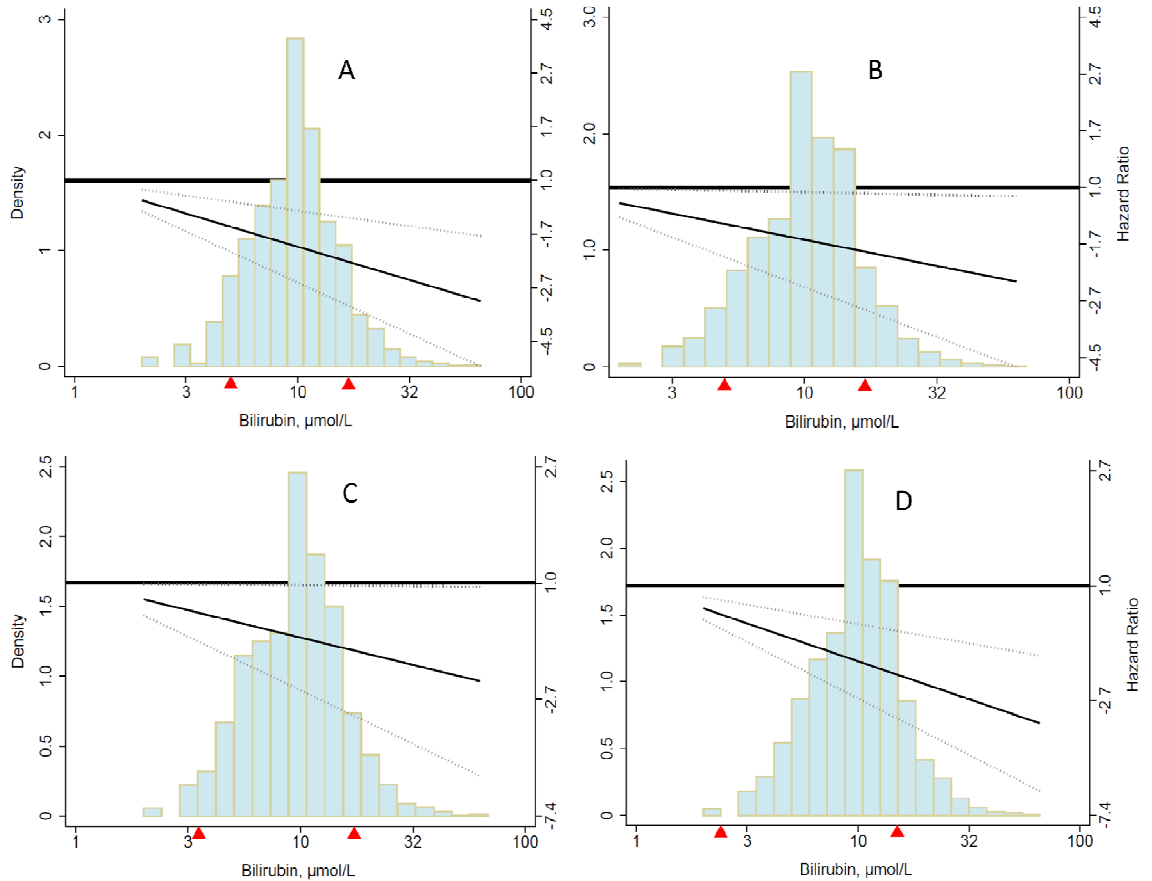
Figure 7-19: Regression spline Cox model for serum bilirubin and all-cause mortality (Sub-group analyses).

Serum bilirubin distribution is in log scale. Red triangles on the horizontal axis indicate lower and upper limits of normal range. A; smokers, B; non-smokers, C; BMI≤25 kg/m^2, D; BMI>25 kg/m^2.

7.3.8 Liver enzymes, bilirubin and follow-up blood pressure

In the GEE models, the average number of BP observations in each individual were 5.2. The annual reduction in SBP and DBP were 3.66 (95% CI: 3.49-3.83) and 1.96 (95% CI: 1.85-2.07) mmHg, respectively. The number of observations, number of individuals, and the GEE regression coefficients with their 95% CI in each model are explained in tables 7-10 to 7-12 on page 231-232. Each unit increase in ALT on a logarithmic scale decreased SBP by 1.94 (95% CI: 0.42-3.46) mmHg. Similarly each unit increase in bilirubin on a logarithmic scale decreased longitudinal SBP by 2.03 (95% CI: 0.29-3.78) mmHg. Each unit increase in both GGT and ALP on a logarithmic scale increased SBP by 2.92 (95% CI: 1.85-3.98) and 8.96 (7.48-10.45) mmHg, respectively. Blood pressure responses to AST quartiles were non-linear with maximum increase in first and last quartiles in comparison to quartile 2.

While the SBP response was more prominent in individuals over 55 years of age for ALT, AST and bilirubin, both ALP and GGT did not show any such differences. Although the SBP response to unit increase in ALT in overweight group was consistent with the overall response, SBP increased with unit increase in ALT in normal weight individuals. The SBP responses to log bilirubin and log ALT were prominent only in smokers and alcohol users in comparison to non-smokers and non-users of alcohol.

Consistent to the SBP responses to both GGT and ALP, similar changes in DBP was also observed. Unit increase in GGT and ALP on a logarithmic scale increased follow-up DBP by 2.45 (95% CI: 1.73-3.16) and 4.03 (95% CI: 3.06-5.01) mmHg, respectively. However in the stratified analyses, increase in follow-up DBP with GGT was seen only among individuals ≤55 years. Overall, ALT, AST and bilirubin did not influence follow-up DBP. In the stratified analyses the follow-up DBP decreased with bilirubin in overweight individuals, while it increased significantly with bilirubin in normal weight individuals. Similar contrasting results are observed in the stratified analyses based on alcohol use status (Table 7-11 on page 231) and smoking (Table 7-12 on page 232).

Table 7-10: Liver enzymes and bilirubin based group average effect on longitudinal changes in systolic blood pressure.

Variable	Overall	Age≤55	Age>55	BMI≤25	BMI>25	Alcohol Users	Alcohol non-users
	GEE β, 95% CI	GEE β, 95% CI	GEE β, 95% CI	GEE β, 95% CI	GEE β, 95% CI	GEE β, 95% CI	GEE β, 95% CI
ALT	N=19367, G=3733	N=11510, G=2231	N=7857, G=1502	N=5396, G=1045	N=13971, G=2688	N=7673, G=1472	N=12156, G=2351
logALT	-1.94, -3.46; -0.42*	-1.25, -3.12; 0.62	-3.27, -5.90; -0.64*	2.33, -0.88; 5.54	-3.24, -4.98; -1.51**	-2.89, -5.54; -0.24*	-1.39, -3.22; 0.45
AST	N=20053, G=3868	N=11907, G=2311	N=8146, G=1557	N=5705, G=1104	N=14348, G=2764	N=8005, G=1537	N=12527, G=2425
Q1	1.21, 0.33; 2.09*	1.12, 0.02; 2.23*	1.18, -0.26; 2.61	2.07, 0.48; 3.65*	0.82, -0.22; 1.88	-0.49, -1.87; 0.89	2.63, 1.51; 3.75**
Q2	REF	REF	REF	REF	REF	REF	REF
Q3	0.85, 0.01; 1.70*	1.65, 0.58; 2.72*	-0.07, -1.43; 1.28	1.18, -0.43; 2.80	0.83, - 0.15; 1.82	-0.04, -1.35; 1.27	1.67, 0.60; 2.85*
Q4	0.94, 0.07; 1.82*	1.25, 0.17; 2.32*	0.22, -1.25; 1.69	1.76, 0.03; 3.50*	0.56, -0.45; 1.58	0.34, -1.14; 1.82	1.48, 0.41; 2.55*
GGT	N=18083, G=3497	N=10558, G=2055	N=7525, G=1442	N=4921, G=955	N=13162, G=2542	N=7561, G=1456	N=10988, G=2132
logGGT	2.92, 1.85; 3.98**	3.16, 1.84; 4.48**	2.80, 0.99; 4.62*	2.27, .12; 4.42*	3.03, 1.80; 4.26**	4.10, 2.27; 5.92**	2.22, 0.95; 3.51**
ALP	N=20328, G=3920	N=12050, G=2338	N=8278, G=1582	N=5743, G=1111	N=14585, G=2809	N=8108, G=1557	N=12703, G=2458
logALP	8.96, 7.48; 10.45**	9.00, 7.24; 10.78**	9.00, 6.37; 11.65**	8.22, 5.59; 10.85	8.90, 7.09; 10.71	7.33, 4.82; 9.83**	9.76, 7.94; 11.57**
Bilirubin	N=19583, G=3779	N=11584, G=2249	N=7999, G=1530	N=5482, G=1063	N=14101, G=2716	N=7875, G=1512	N=12167, G=2357
logBilirubin	-2.03, -3.78; -0.29*	-0.64, -2.80; 1.52	-3.70, -6.60; -0.80*	-1.23, -4.46; 2.01	-2.61, -4.68; -0.55*	-5.79, -8.53; -3.04**	0.65, -1.57; 2.87

*p<0.05, **p<0.001**, ALT=alanine aminotransferase, AST=aspartate transaminase, ALP=alkaline phosphatase, GGT=gamma-glutamyltransferase, CI=confidence interval, REF=reference group, N=total number of observations, G=total number of individuals/groups and GEE β=generalised estimating equations β coefficient . All models are adjusted for age, gender, epochs, BMI=body mass index, tobacco smoking, alcohol use and eGFR CKD status.

Table 7-11: Liver enzymes and bilirubin based group average effect on longitudinal changes in diastolic blood pressure.

Variable	Overall	Age≤55	Age>55	BMI≤25	BMI>25	Alcohol Users	Alcohol non-users
	GEE β, 95% CI	GEE β, 95% CI	GEE β, 95% CI	GEE β, 95% CI	GEE β, 95% CI	GEE β, 95% CI	GEE β, 95% CI
ALT	N=19366, G=3733	N=11510, G=2231	N=7856, G=1502	N=5396, G=1045	N=13970, G=2688	N=7672, G=1472	N=12156, G=2351
logALT	0.74, -0.30; 1.78	0.80, -0.65; 2.26	-1.91, -3.30; -0.53*	2.42, -0.04; 4.88	0.06, -1.05; 1.18	-0.25, -1.66; 1.16	1.48, 0.08; 2.88*
AST	N=20052, G=3868	N=11907, G=2311	N=8145, G=1557	N=5705, G=1104	N=14347, G=2764	N=8004, G=1537	N=12527, G=2425
Q1	0.02, -0.50; 0.55	0.24, -0.49; 0.97	-0.38, -1.10; 0.33	-0.01, -1.19-1.16	-0.01, -0.55; 0.55	-0.91, -1.60; -0.22*	0.64, -0.09; 1.37
Q2	REF	REF	REF	REF	REF	REF	REF
Q3	0.30, -0.20; 0.80	0.42, -0.29; 1.13	0.06, -0.62; 0.73	0.28, -0.91; 1.47	0.28, -0.24; 0.80	-0.20, -0.86; 0.45	0.76, 0.06; 1.47*
Q4	0.44, -0.09; 0.96	0.99, 0.27; 1.70*	-0.85, -1.59; -0.12*	0.92, -0.36; 2.20	0.15, -0.38; 0.68	-0.60, -1.34; 0.14	1.02, 0.32; 1.71*
GGT	N=18082, G=3497	N=10558, G=2055	N=7524, G=1442	N=4921, G=955	N=13161, G=2542	N=7560, G=1456	N=10988, G=2132
logGGT	2.45, 1.73; 3.16**	3.34, 2.30; 4.38**	0.32, -0.59; 1.22	3.56, 1.91; 5.22**	1.84, 1.07; 2.62	1.57, 0.66; 2.48*	3.04, 2.05; 4.04**
ALP	N=20327, G=3920	N=12050 G=2338	N=8277, G=1582	N=5743, G=1111	N=14584, G=2809	N=8107, G=1557	N=12703, G=2458
logALP	4.03, 3.06; 5.01**	4.65, 3.30; 5.99**	2.64, 1.33; 3.96**	5.34, 3.39; 7.28**	3.17, 2.06; 4.29**	3.19, 1.93; 4.45	4.42, 3.09; 5.77**
Bilirubin	N=19582, G=3779	N=11584, G=2249	N=7998, G=1530	N=5482, G=1063	N=14100, G=2716	N=7874, G=1592	N=12167, G=2357
logBilirubin	0.15, -0.99; 1.29	1.37, -0.26; 3.00	-0.88, -2.32; 0.56	3.75, 1.33; 6.18*	-1.53, -2.80; -0.27*	-2.41, -3.80; -1.01	2.30, 0.67; 3.93*

*p<0.05, **p<0.001**, ALT=alanine aminotransferase, AST=aspartate transaminase, ALP=alkaline phosphatase, GGT=gamma-glutamyltransferase, CI=confidence interval, REF=reference group, N=total number of observations, G=total number of individuals/groups and GEE β=generalised estimating equations β coefficient . All models are adjusted for age, gender, epochs, BMI=body mass index, tobacco smoking, alcohol use and eGFR CKD status.

Table 7-12: Liver enzymes and bilirubin based group average effect on longitudinal changes in blood pressure stratified by smoking status.

Variable	SBP Smokers	Non-Smokers	DBP Smokers	Non-smokers
	GEE β, 95% CI	GEE β, 95% CI	GEE β, 95% CI	GEE β, 95% CI
ALT	N=8567, G=1656	N=10800, G=2077	N=8566, G=1656	N=10800, G=2077
logALT	-2.79, -5.09; -0.50*	-0.97, -3.00; 1.06	1.74, -0.13; 3.60	-0.06, -1.20; 1.08
AST	N=8898, G=1720	N=11155, G=2148	N=8897, G=1720	N=11155, G=2148
Q1	1.34, 0.03; 2.65*	0.88, -0.31; 2.07	0.19, -0.69; 1.07	-0.16, -0.79; 0.46
Q2	REF	REF	REF	REF
Q3	1.01, -0.24; 2.27	0.77, -0.36; 1.90	0.39, -0.46; 1.24	0.20, -0.39; 0.80
Q4	1.34, 0.02; 2.65*	0.58, -0.59; 1.75	0.75, -0.14; 1.64	0.12, -0.50; 0.73
GGT	N=7998, G=1551	N=10085, G=1946	N=7997, G=1551	N=10085, G=1946
logGGT	3.27, 1.65; 4.89**	2.62, 1.21; 4.04**	2.59, 1.25; 3.93**	2.27, 1.53; 3.01**
ALP	N=8983, G=1737	N=11345, G=2183	N=8982, G=1737	N=5743, G=1111
logALP	11.15, 8.97; 13.34**	6.61, 4.58; 8.64**	5.05, 3.32; 6.77**	3.09, 2.02; 4.15**
Bilirubin	N=8623, G=1669	N=10960, G=2110	N=8622, G=1669	N=10960, G=2110
logBilirubin	-3.56, -6.19; -0.93*	-0.90, -3.23; 1.44	2.10, 0.07; 4.14*	-1.53, -2.79; -0.29*

*p<0.05, **p<0.001**, ALT=alanine aminotransferase, AST=aspartate transaminase, ALP=alkaline phosphatase, GGT=gamma-glutamyltransferase, CI=confidence interval, REF=reference group, N=total number of observations, G=total number of individuals/groups and GEE β=generalised estimating equations β coefficient . All models are adjusted for age, gender, epochs, BMI=body mass index, tobacco smoking, alcohol use and eGFR CKD status.

7.4 Discussion

In a large hypertensive cohort followed-up for more than 35 years, indices of liver function or injury are significant predictors of mortality. I show that higher ALT and bilirubin levels (up to 4 SD from mean) are associated with lower mortality and lower follow-up BP, while increasing GGT and ALP are associated with higher mortality risk and higher follow-up BP. The mortality risk association of the liver tests mirror their effect on longitudinal BP change, though the BP effect was blunted for ALT and bilirubin in younger, non-obese or low alcohol-intake subgroups.

Although our findings on GGT and ALP are consistent with the current literature [476 489 492 678-681] they contradict some of the previous findings for both ALT and AST [654 682]. ALT is a non-specific marker of liver fat, a biomarker for NAFLD and it is associated with diabetes, metabolic syndrome and atherosclerosis [679]. There is inconsistency in the literature on the direction of association with ALT and mortality outcomes in population based studies. Although the recently published Ford et al study reports significantly higher mortality in the lowest quartile of ALT distribution, the findings were limited in the normal range of ALT and in older age group [487]. In the current study the association with mortality and ALT is inverse linear in the entire range of ALT. The inverse relationship between ALT and mortality (all-cause, CVD and non-CVD) is independent of all other concomitant liver tests and is blunted in the younger subgroup (<55 years). In the older group (>55y years), the inverse risk appears to be driven by increased risk in those with lower ALT. The reason for the increased risk seen with lower ALT is not clear. A recent study in the elderly (>70 years) showed that the increased risk associated with low ALT disappeared after adjustment for frailty [683]. This indicates that ALT is more a marker of aging and frailty. The reduced ALT levels with increasing age would indicate that ALT may be a marker of functioning hepatocyte mass or sarcopenia as ALT is primarily produced by the liver (with a small proportion from skeletal muscle). It is therefore reasonable to speculate that the greater reduction in BP in this cohort of treated hypertensive patients with higher ALT levels may indicate better antihypertensive efficacy, as the majority of commonly used antihypertensive agents are pro-drugs which require activation in the liver. The protection offered by ALT in the treated

hypertensive cohort may reflect greater BP reduction in addition to protection offered by greater functioning hepatocyte mass.

While AST is another enzyme associated with liver parenchymal cells, like ALT, it is also present in several other organ systems including cardiac muscle, skeletal muscle, kidneys, and brain tissue [684]. Despite strong correlation between AST and ALT[658] the pattern of risk is different with AST showing a U-shaped association with all-cause, CV and non-CV mortality in contrast to ALT. Interestingly, both AST and ALT show increased all-cause, CV and non-CV mortality outcomes at the lower end of their distribution and the associations remain robust even after adjustment for multiple confounding factors.

The inverse linear association of serum bilirubin and all-cause mortality observed in our study is consistent with the previous study reports. In a study conducted over half a million adults from the United Kingdom primary care research database (The Health Improvement Network, THIN), a 3% decrease in mortality was observed with 0.1 mg/dl incremental increase in bilirubin in both men and women [497]. The anti-oxidant [672], and anti-inflammatory [685-687] properties of bilirubin may partially explain the biological mechanisms associated with this inverse relationship. Low bilirubin is also associated with conditions that increase CVD mortality such as hypertension [688 689], dysglycemia [690], metabolic syndrome [691], coronary artery calcification [692], CHD [493 494 675], peripheral arterial disease [675], and stroke [674 693]. Bilirubin levels are strongly determined by genetic [668]and environmental influences. For example bilirubin levels are reduced in smokers possibly due to depletion on exposure to reactive oxygen species found in cigarette smoke [694 695]. In the Framingham Offspring study, a prospective population based cohort study, the UGTA1 genotype was associated with both serum bilirubin and CHD [696]. Individuals with the homozygous for UGTA1*28 (genotype 7/7) in the Framingham study participants had significantly higher serum bilirubin concentrations and one third risk of CHD events (HR=0.30, 95% CI=0.12-0.74). The present study confirms the protective effect of raised bilirubin and shows that it is robust to smoking both by adjusting for smoking and by analysis stratified by smoking status indicating that confounding in unlikely to be a major issue. Although there may exist residual confounding due to unmeasured environmental factors, this is also likely to be minimal because of the extremely large effect of smoking on mortality. The protective effect of

bilirubin is also independent of other concomitant liver enzymes. Higher bilirubin is associated with a lower baseline BP and with greater longitudinal reduction in BP which suggest that the protective effect of bilirubin can be partly explained by BP effects. It is also likely that in the GBPC cohort of treated hypertensive patients, the reduction in longitudinal BP may also reflect better activation of antihypertensive pro-drugs, as bilirubin levels reflect hepatic processing capacity. One way of elucidating the mechanism would be to study total and unconjugated bilirubin; however the present study was limited by the unavailability of unconjugated bilirubin levels in this cohort.

Both ALP and GGT are associated with increased mortality risk which is significant only at the higher end of the distribution. But the striking finding is the association of both GGT and ALP with higher longitudinal BP consistently. Despite a 4 mmHg annual decline in SBP related to the antihypertensive treatment seen in this cohort, the BP increases with both ALP and GGT. The magnitude of BP increase is higher with ALP than GGT. GGT has pro-oxidant effects due to its role in the extracellular catabolism of glutathione [697], and high GGT has been associated with incident diabetes [663] metabolic syndrome and fatty liver [681]. Recent prospective studies show that high GGT is positively associated with increased mortality or incidence of cardiovascular disease [489,663,678], and the present study results reflect this. However, a clear association between GGT and CV mortality was not observed once the effects of other liver tests are accounted for in the model. There is a linear increase of bilirubin with GGT levels and the 24% excess risk of CV mortality associated with the highest quartile of GGT disappears once bilirubin is included in the model. The pro-oxidant and anti-oxidant properties respectively of GGT and bilirubin associated with opposite effects on CV risk when considered jointly indicates that GGT does not have a major independent impact on CV mortality. The mechanism by which ALP and GGT increase follow-up BP levels is unclear and clearly merits further study.

7.5 Strengths and limitations

The strengths of the current study include; a large cohort of nearly 10,000 hypertensive adults, 35 years of follow-up with median survival time of 32 years, the ability to link liver enzymes with differing causes of mortality outcomes, inclusion of the entire distribution of liver enzymes (except the level suggestive

of obstructive liver disease), and most importantly the competence to model the potential non-linear association of markers of liver dysfunction on mortality outcomes. The exclusion of individuals without liver enzymes assessed at baseline from our analysis, the bias introduced by the missing covariates in the adjusted Cox-PH models were the major limitations. However, our results were consistent across multiple models with and without such variables.

7.6 Summary

Indices of liver function or injury independently predict mortality outcomes in the hypertensive population. A positive linear association was observed for both GGT and ALP with mortality outcomes, while it was more complex, non-linear and 'U' shaped association for AST. Both ALT and bilirubin show inverse linear association with mortality. These markers independently influence follow-up BP. These findings highlight the need for more tailored assessment of liver tests, in the absence of obvious liver disease, for cardiovascular risk stratification in hypertension management. While assessment of these additional markers help the physician to identify high risk groups for targeted management, addition of these variables may increase the complexity on the current risk calculations.

8 Conclusion

Blood pressure tops the listing of common global risk factors according to their population attributable fraction of mortality burden [312]. Hypertension is common among adults, affecting at least one in four worldwide [305]. It imposes a 2-3 fold excess risk for cardiovascular mortality compared with that of normotensive individuals of the same age [291]. However, not all individuals with hypertension appear to be equally susceptible to early mortality. Current risk reduction strategies focus on a combination of environmental (smoking), biochemical (lipids), disease (diabetes) and demographic factors (age, sex) to calculate future cardiovascular risk [698]. There are many modifications to this using social deprivation, albuminuria etc to enhance the prediction model [699-701]. The absolute benefit of anti-hypertensive therapy depends not only on level of BP, but also on the presence or absence of additional risk factors [702]. The search for a new biomarker or a predictor variable that is readily available and cost-effective is therefore of special interest in hypertensive population.

Several studies have been carried out to identify additional prognostic factors but these have focussed on novel biomarkers, for example B-type natriuretic peptides (BNP) [703] and C-reactive protien [704]. While important prognostic information can be learned from routine blood tests that are often conducted in hypertension clinics, the usefulness of these markers in predicting survival has not previously been studied in detail. The aim of the current project was to explain the relationship between such inexpensive and commonly available markers and survival in hypertensive population.

Altogether, five studies were conducted to assess the independent role of BP variability (BPV), haematocrit, serum phosphate, serum electrolytes and indices of liver dysfunction/injury in predicting mortality in hypertensive patients as part of this thesis work. The main strengths of these independent studies conducted were; a large cohort of nearly 15,000 hypertensive adults, a real life clinical settings, 35 years of follow-up with median survival time of 32 years, the ability to link predictor variables with differing causes of mortality outcomes, and adjustment for all potential confounding factors.

I show that both BPV_{SBP} and BPV_{DBP} calculated over different sequential time-frames (of 1 to 5 years duration) are strong predictors of mortality independent of long-term average BP. Moreover, 60% of individuals show sustained high or low BPV over even longer time-frames of 6 to 9 years and this sustained BPV status is also a strong predictor of mortality independent of long-term average BP. From this study, ARV_{SBP} =17, calculated over any follow-up time frame greater than 1 year and using a minimum of 3 readings more that 30 days apart, can be used as a threshold to define treated hypertensive patients as low or high risk. I propose that BPV is a prognostic marker that is independent of BP and probably captures a different biologic process to BP regulation. The clinical application of BPV will be to use a specific threshold (for example ARV_{SBP}=17) to allow risk-stratification of treated hypertensive patients. Further clinical implications depend on whether there are interventions that can change BPV and whether these interventions will result in improvement in outcomes. The results indicate that BPV should be studied from a non-hypertension centric viewpoint to elucidate its pathogenic mechanisms.

Among the biochemical parameters analysed in this thesis, the most interesting and novel finding is the association of serum chloride with mortality risk independent of sodium and other electrolytes. Serum chloride levels (<100 mEq/L) identify high risk patients who should be targeted for more intensive investigation to achieve and maintain target BP. The underlying mechanism for this risk is unclear. A parsimonious explanation would be that serum chloride reflects abnormal physiology better than serum sodium, levels of which are perhaps more homeostatically regulated than chloride. If other studies validate and extend these findings, further studies are necessary to elucidate the underlying mechanisms involved in the association of low serum chloride levels with mortality outcomes. However, as chloride measurement is part of routine clinical screening, our results are potentially translatable into clinical practice to identify hypertensive patients for more intensive investigation and BP management strategies.

The increasing prevalence of alcohol use and obesity have focussed attention on abnormalities in liver function test (LFT) being early predictors of cardio-metabolic risk. In current clinical practice, the definition of abnormal LFT is usually considered to be >2 SD from the mean, which triggers further

investigations based on the pre-test probability of liver disease. I show that higher ALT and bilirubin levels (up to 4 SD from mean) are associated with lower mortality and lower follow-up BP, while increasing GGT and ALP are associated with higher mortality risk and higher follow-up BP. The mortality risk association of the liver tests mirror their effect on longitudinal BP change, though the BP effect was blunted for ALT and bilirubin in younger, non-obese or low alcohol-intake subgroups. The protective effect of ALT and bilirubin and their effect on longitudinal BP may reflect functioning liver mass and in addition the antioxidant property of bilirubin. This is the first study to show the effect of LFT on longitudinal BP control in treated hypertensive patients. These results need to be confirmed in independent studies as the usual caveats of observational studies apply.

I demonstrate that Hct influences follow-up BP and it is an independent predictor of mortality in the hypertensive population. However, there are distinct differences both in terms of the strength and magnitude of the association of Hct and mortality between men and women that have not previously been known. The association of Hct and CV mortality is prominent among men in comparison to women, while non-CV mortality is prominently associated with Hct in women. Based on these findings, Hct levels should be taken into consideration as an important risk predictor in the assessment and management of hypertensive patients. However, further research is needed to elucidate the mechanism of this risk and to define management strategies. Hypertensive patients with Hct levels outside of the sex-specific low-risk reference ranges as identified in this study should be targeted for more aggressive BP and cardiovascular risk reduction treatment.

The study of the association between phosphate and mortality demonstrates that elevated initial serum phosphate is an independent predictor of all-cause mortality in hypertensive patients. Although the association of serum phosphate with CV mortality is well known, especially in the CKD population, there are some additional novel findings in the present study. They are as follows; (a) the association between serum phosphate and mortality is independent of deprivation status, serum calcium and serum alkaline phosphatase levels and (b) while serum phosphate is associated with CV mortality in men, it is more closely associated with non-CV mortality in women. Phosphate is not currently

considered as a potentially modifiable risk factor for therapeutic interventions in hypertensive subjects. The impact of reduced intake of phosphate rich foods on serum phosphate level and mortality outcomes is not known. It is therefore reasonable to propose dietary intervention studies that target reduced intake of phosphate rich foods. The role of aggressive BP and cardiovascular risk reduction treatment in individuals with high serum phosphate also needs to be tested.

There have been significant changes in the management of hypertension in the past two decades. Several hypertension management guidelines have been updated with new emerging data mainly from clinical trials. However, in community settings less than 50% of hypertensive patients are achieving the recommended systolic BP goals [323][705]. Although there are several reasons for this underachievement, co-morbidities are often suggested as one of the main reasons for less intensive anti-hypertensive therapy and poor BP control. The studies conducted as part of this thesis in real life settings suggest that haematocrit, serum electrolytes and markers of liver dysfunction or liver injury significantly influence follow-up BP levels. These are novel findings and need to be confirmed in an independent population, and their cumulative effect on survival needs to be assessed using receiver operator characteristics curve (ROC) analysis or other reclassification methods such as integrated discrimination improvement (IDI) [706] to show if these add any additional information to improve the predictive potential of existing algorithms. However, the results with follow-up BP are applicable to clinical practice, as they can enable clinicians to target the patients for more intensive management. If individuals with a specific set of serum markers require more intensive BP therapy to achieve treatment goals, monitoring of these variables in individuals with hypertension will help the physician to titrate the drugs more efficiently to achieve the desired level of BP control. Some of these markers may also have a role in resistant hypertension that needs to be investigated further.

List of References

1. Wallis F. The Osler Library Newsletter. Montreal (Quebec), Canada The Osler Library of the History of Medicine 2010.

2. Ruskin A. *Classics in Arterial Hypertension* Springfield, III., Thomas C, 1956.

3. Rutan GH, McDonald RH, Kuller LH. A historical perspective of elevated systolic vs diastolic blood pressure from an epidemiological and clinical trial viewpoint. *J Clin Epidemiol* 1989;42(7):663-73.

4. Evans JG, Prior IA. Experience with the random-zero sphygmomanometer. *Br J Prev Soc Med* 1970;24(1):10-5.

5. Kotchen TA. Historical trends and milestones in hypertension research: a model of the process of translational research. *Hypertension* 2011;58(4):522-38.

6. Lovibond K, Jowett S, Barton P, Caulfield M, Heneghan C, Hobbs FD, et al. Cost-effectiveness of options for the diagnosis of high blood pressure in primary care: a modelling study. *Lancet* 2011;378(9798):1219-30.

7. Pickering G. *Systemic arterial pressure*. New York, NY: Oxford University Press 1964.

8. Wain H. *The Story Behind the World* Springfield,III., Thomas C, 1958.

9. Osler W, McCrae T. *The Principles and Practice of Medicine* 10 ed. New York: D. Appleton 1925.

10. Society of Actuaries. Blood Pressure: Report of the Joint Committee on Mortality of the Association of Life Insurance Medical Directors and the Actuarial Society of America. New York, NY, 1925.

11. The Actuarial Society of America and the Association of Life Insurance Medical Directors. Blood Pressure Study, 1939 New York: 1940.

12. Society of Actuaries and Association of Life Insurance Medical Directors of America. Blood Pressure Study, 1979. New York, NY: Society of Actuaries and Association of Life Insurance Medical Directors of America 1980.

13. Society of Actuaries. Build and Blood Pressure Study Chicago, IL: Society of Actuaries 1959.

14. Pickering G. Hypertension. Definitions, natural histories and consequences. *Am J Med* 1972;52(5):570-83.

15. Stamler J, Stamler R, Neaton JD. Blood pressure, systolic and diastolic, and cardiovascular risks. US population data. *Arch Intern Med* 1993;153(5):598-615.

16. Vasan RS, Larson MG, Leip EP, Evans JC, O'Donnell CJ, Kannel WB, et al. Impact of high-normal blood pressure on the risk of cardiovascular disease. *N Engl J Med* 2001;345(18):1291-7.

17. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data

for one million adults in 61 prospective studies. *Lancet* 2002;360(9349):1903-13.

18. Rose G. *Epidemiology* Kent, UK. Pitman Medical 1980.

19. Franklin SS. Ageing and hypertension: the assessment of blood pressure indices in predicting coronary heart disease. *J Hypertens Suppl* 1999;17(5):S29-36.

20. Frohlich ED. The fifth Joint National Committee report on the detection, evaluation and treatment of high blood pressure. *J Am Coll Cardiol* 1993;22(2):621-2.

21. Gifford RW, Jr. The fifth report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure: insights and highlights from the chairman. *Cleve Clin J Med* 1993;60(4):273-7.

22. JNC. Fifth Joint National Committee report on high blood pressure. *Am Fam Physician* 1993;47(2):526-8.

23. JNC. The fifth report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (JNC V). *Arch Intern Med* 1993;153(2):154-83.

24. Weber MA, Laragh JH. Hypertension: steps forward and steps backward. The Joint National Committee fifth report. *Arch Intern Med* 1993;153(2):149-52.

25. Izzo JL, Jr., Levy D, Black HR. Clinical Advisory Statement. Importance of systolic blood pressure in older Americans. *Hypertension* 2000;35(5):1021-4.

26. Kostis JB, Davis BR, Cutler J, Grimm RH, Jr., Berge KG, Cohen JD, et al. Prevention of heart failure by antihypertensive drug treatment in older persons with isolated systolic hypertension. SHEP Cooperative Research Group. *JAMA* 1997;278(3):212-6.

27. Staessen JA, Thijs L, Fagard R, O'Brien ET, Clement D, de Leeuw PW, et al. Predicting cardiovascular risk using conventional vs ambulatory blood pressure in older patients with systolic hypertension. Systolic Hypertension in Europe Trial Investigators. *JAMA* 1999;282(6):539-46.

28. Kannel WB. Elevated systolic blood pressure as a cardiovascular risk factor. *Am J Cardiol* 2000;85(2):251-5.

29. Cushman WC. The clinical significance of systolic hypertension. *Am J Hypertens* 1998;11(11 Pt 2):182S-85S.

30. Sheps SG. Overview of JNC VI: new directions in the management of hypertension and cardiovascular risk. *Am J Hypertens* 1999;12(8 Pt 2):65S-72S.

31. European Society of Hypertension. 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. *J Hypertens* 2003;21(6):1011-53.

32. O'Brien E, Asmar R, Beilin L, Imai Y, Mallion JM, Mancia G, et al. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. *J Hypertens* 2003;21(5):821-48.

33. Whitworth JA. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. *J Hypertens* 2003;21(11):1983-92.

34. Williams B, Poulter NR, Brown MJ, Davis M, McInnes GT, Potter JF, et al. British Hypertension Society guidelines for hypertension management 2004 (BHS-IV): summary. *BMJ* 2004;328(7440):634-40.

35. Williams B, Poulter NR, Brown MJ, Davis M, McInnes GT, Potter JF, et al. Guidelines for management of hypertension: report of the fourth working party of the British Hypertension Society, 2004-BHS IV. *J Hum Hypertens* 2004;18(3):139-85.

36. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 2003;42(6):1206-52.

37. Lenfant C, Chobanian AV, Jones DW, Roccella EJ. Seventh report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7): resetting the hypertension sails. *Hypertension* 2003;41(6):1178-9.

38. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 2003;289(19):2560-72.

39. Wonderling D, Sawyer L, Fenu E, Lovibond K, Laramee P. National Clinical Guideline Centre cost-effectiveness assessment for the National Institute for Health and Clinical Excellence. *Ann Intern Med* 2011;154(11):758-65.

40. Giles TD. Examining therapeutic goals: population versus individual-based approaches. *Am J Hypertens* 2003;16(11 Pt 2):26S-30S.

41. Staessen J, Amery A, Fagard R. Isolated systolic hypertension in the elderly. *J Hypertens* 1990;8(5):393-405.

42. Kannel WB, Dawber TR, McGee DL. Perspectives on systolic hypertension. The Framingham study. *Circulation* 1980;61(6):1179-82.

43. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, et al. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2007;25(6):1105-87.

44. Pickering TG. The ninth Sir George Pickering memorial lecture. Ambulatory monitoring and the definition of hypertension. *J Hypertens* 1992;10(5):401-9.

45. Osler W. An Address ON HIGH BLOOD PRESSURE: ITS ASSOCIATIONS, ADVANTAGES, AND DISADVANTAGES: Delivered at the Glasgow Southern Medical Society. *Br Med J* 1912;2(2705):1173-7.

46. Pickering GW, Keen H, Rose G, Smith A. The NATURE of essential hypertension. *Lancet* 1959;2(7110):1027-30.

47. Goldblatt H. The renal origin of hypertension. *Physiol Rev* 1947;27(1):120-65.

48. Perera GA. Hypertensive vascular disease; description and natural history. *J Chronic Dis* 1955;1(1):33-42.

49. Henry JP, Cassel JC. Psychosocial factors in essential hypertension. Recent epidemiologic and animal experimental evidence. *Am J Epidemiol* 1969;90(3):171-200.

50. Selye H. The alarm reaction and the diseases of adaptation. *Ann Intern Med* 1948;29(3):403-15.

51. Page LB, Damon A, Moellering RC, Jr. Antecedents of cardiovascular disease in six Solomon Islands societies. *Circulation* 1974;49(6):1132-46.

52. Page IH. *The mosaic theory* New York, NY Grune & Stratton 1987.

53. Guyton AC, editor. *The body's approach to arterial pressure regulation* Philadelphia, PA WB Saunders Co, 1980.

54. Brunner HR, Laragh JH, Baer L, Newton MA, Goodwin FT, Krakoff LR, et al. Essential hypertension: renin and aldosterone, heart attack and stroke. *N Engl J Med* 1972;286(9):441-9.

55. Julius S, Schork MA. Predictors of hypertension. *Ann N Y Acad Sci* 1978;304:38-58.

56. Goldstein DS. Plasma catecholamines and essential hypertension. An analytical review. *Hypertension* 1983;5(1):86-99.

57. Mancia G, Grassi G, Giannattasio C, Seravalle G. Sympathetic activation in the pathogenesis of hypertension and progression of organ damage. *Hypertension* 1999;34(4 Pt 2):724-8.

58. Mancia G. Bjorn Folkow Award Lecture. The sympathetic nervous system in hypertension. *J Hypertens* 1997;15(12 Pt 2):1553-65.

59. Borst JG, Borst-De Geus A. Hypertension explained by Starling's theory of circulatory homoeostasis. *Lancet* 1963;1(7283):677-82.

60. Guyton AC, Coleman TG, Cowley AV, Jr., Scheel KW, Manning RD, Jr., Norman RA, Jr. Arterial pressure regulation. Overriding dominance of the kidneys in long-term regulation and in hypertension. *Am J Med* 1972;52(5):584-94.

61. Poulter NR, Khaw KT, Hopwood BE, Mugambi M, Peart WS, Rose G, et al. The Kenyan Luo migration study: observations on the initiation of a rise in blood pressure. *BMJ* 1990;300(6730):967-72.

62. Oliver WJ, Cohen EL, Neel JV. Blood pressure, sodium intake, and sodium related hormones in the Yanomamo Indians, a "no-salt" culture. *Circulation* 1975;52(1):146-51.

63. Carvalho JJ, Baruzzi RG, Howard PF, Poulter N, Alpers MP, Franco LJ, et al. Blood pressure in four remote populations in the INTERSALT Study. *Hypertension* 1989;14(3):238-46.

64. Timio M, Saronio P, Verdura C, Schiaroli M, Timio F, Monarca C. A link between psychosocial factors and blood pressure trend in women. *Physiol Behav* 2001;73(3):359-63.

65. Timio M, Lippi G, Venanzi S, Gentili S, Quintaliani G, Verdura C, et al. Blood pressure trend and cardiovascular events in nuns in a secluded order: a 30-year follow-up study. *Blood Press* 1997;6(2):81-7.

66. Hollenberg NK, Martinez G, McCullough M, Meinking T, Passan D, Preston M, et al. Aging, acculturation, salt intake, and hypertension in the Kuna of Panama. *Hypertension* 1997;29(1 Pt 2):171-6.

67. Julius S, Krause L, Schork NJ, Mejia AD, Jones KA, van de Ven C, et al. Hyperkinetic borderline hypertension in Tecumseh, Michigan. *J Hypertens* 1991;9(1):77-84.

68. Guyenet PG. The sympathetic control of blood pressure. *Nat Rev Neurosci* 2006;7(5):335-46.

69. Victor RG, Shafiq MM. Sympathetic neural mechanisms in human hypertension. *Curr Hypertens Rep* 2008;10(3):241-7.

70. Bleeke T, Zhang H, Madamanchi N, Patterson C, Faber JE. Catecholamine-induced vascular wall growth is dependent on generation of reactive oxygen species. *Circ Res* 2004;94(1):37-45.

71. DiBona GF. Physiology in perspective: The Wisdom of the Body. Neural control of the kidney. *Am J Physiol Regul Integr Comp Physiol* 2005;289(3):R633-41.

72. Grassi G, Quarti-Trevano F, Dell'oro R, Mancia G. Essential hypertension and the sympathetic nervous system. *Neurol Sci* 2008;29 Suppl 1:S33-6.

73. Schlaich MP, Lambert E, Kaye DM, Krozowski Z, Campbell DJ, Lambert G, et al. Sympathetic augmentation in hypertension: role of nerve firing, norepinephrine reuptake, and Angiotensin neuromodulation. *Hypertension* 2004;43(2):169-75.

74. Grassi G, Seravalle G, Trevano FQ, Dell'oro R, Bolla G, Cuspidi C, et al. Neurogenic abnormalities in masked hypertension. *Hypertension* 2007;50(3):537-42.

75. Joyner MJ, Charkoudian N, Wallin BG. A sympathetic view of the sympathetic nervous system and human blood pressure regulation. *Exp Physiol* 2008;93(6):715-24.

76. Esler MD, Eikelis N, Lambert E, Straznicky N. Neural mechanisms and management of obesity-related hypertension. *Curr Cardiol Rep* 2008;10(6):456-63.

77. Johnson RJ, Feig DI, Nakagawa T, Sanchez-Lozada LG, Rodriguez-Iturbe B. Pathogenesis of essential hypertension: historical paradigms and modern insights. *J Hypertens* 2008;26(3):381-91.

78. Ledingham JM, Pelling D. Haemodynamic and other studies in the renoprival hypertensive rat. *J Physiol* 1970;210(1):233-53.

79. Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *Am J Kidney Dis* 2003;41(1):1-12.

80. Johnson RJ, Rodriguez-Iturbe B, Nakagawa T, Kang DH, Feig DI, Herrera-Acosta J. Subtle renal injury is likely a common mechanism for salt-sensitive essential hypertension. *Hypertension* 2005;45(3):326-30.

81. Johnson RJ, Rodriguez-Iturbe B, Kang DH, Feig DI, Herrera-Acosta J. A unifying pathway for essential hypertension. *Am J Hypertens* 2005;18(3):431-40.

82. Modlinger P, Chabrashvili T, Gill PS, Mendonca M, Harrison DG, Griendling KK, et al. RNA silencing in vivo reveals role of p22phox in rat angiotensin slow pressor response. *Hypertension* 2006;47(2):238-44.

83. Rodriguez-Iturbe B, Vaziri ND, Herrera-Acosta J, Johnson RJ. Oxidative stress, renal infiltration of immune cells, and salt-sensitive hypertension: all for one and one for all. *Am J Physiol Renal Physiol* 2004;286(4):F606-16.

84. Wilcox CS. Oxidative stress and nitric oxide deficiency in the kidney: a critical link to hypertension? *Am J Physiol Regul Integr Comp Physiol* 2005;289(4):R913-35.

85. Johnson RJ, Gordon KL, Suga S, Duijvestijn AM, Griffin K, Bidani A. Renal injury and salt-sensitive hypertension after exposure to catecholamines. *Hypertension* 1999;34(1):151-9.

86. Lombardi D, Gordon KL, Polinsky P, Suga S, Schwartz SM, Johnson RJ. Salt-sensitive hypertension develops after short-term exposure to Angiotensin II. *Hypertension* 1999;33(4):1013-9.

87. Quiroz Y, Pons H, Gordon KL, Rincon J, Chavez M, Parra G, et al. Mycophenolate mofetil prevents salt-sensitive hypertension resulting from nitric oxide synthesis inhibition. *Am J Physiol Renal Physiol* 2001;281(1):F38-47.

88. Suga SI, Phillips MI, Ray PE, Raleigh JA, Vio CP, Kim YG, et al. Hypokalemia induces renal injury and alterations in vasoactive mediators that favor salt sensitivity. *Am J Physiol Renal Physiol* 2001;281(4):F620-9.

89. Rodriguez-Iturbe B, Pons H, Quiroz Y, Gordon K, Rincon J, Chavez M, et al. Mycophenolate mofetil prevents salt-sensitive hypertension resulting from angiotensin II exposure. *Kidney Int* 2001;59(6):2222-32.

90. Andoh TF, Johnson RJ, Lam T, Bennett WM. Subclinical renal injury induced by transient cyclosporine exposure is associated with salt-sensitive hypertension. *Am J Transplant* 2001;1(3):222-7.

91. Campese VM, Park J. The kidney and hypertension: over 70 years of research. *J Nephrol* 2006;19(6):691-8.

92. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D. Fetal and childhood growth and hypertension in adult life. *Hypertension* 2000;36(5):790-4.

93. Woods LL, Weeks DA, Rasch R. Programming of adult blood pressure by maternal protein restriction: role of nephrogenesis. *Kidney Int* 2004;65(4):1339-48.

94. Zandi-Nejad K, Luyckx VA, Brenner BM. Adult hypertension and kidney disease: the role of fetal programming. *Hypertension* 2006;47(3):502-8.

95. Tapia E, Franco M, Sanchez-Lozada LG, Soto V, Avila-Casado C, Santamaria J, et al. Mycophenolate mofetil prevents arteriolopathy and renal injury in subtotal ablation despite persistent hypertension. *Kidney Int* 2003;63(3):994-1002.

96. Keller G, Zimmer G, Mall G, Ritz E, Amann K. Nephron number in patients with primary hypertension. *N Engl J Med* 2003;348(2):101-8.

97. Williams GH, Hollenberg NK. Non-modulating hypertension. A subset of sodium-sensitive hypertension. *Hypertension* 1991;17(1 Suppl):I81-5.

98. O'Shaughnessy KM, Karet FE. Salt handling and hypertension. *J Clin Invest* 2004;113(8):1075-81.

99. Adrogue HJ, Madias NE. Sodium and potassium in the pathogenesis of hypertension. *N Engl J Med* 2007;356(19):1966-78.

100. Intersalt. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. Intersalt Cooperative Research Group. *BMJ* 1988;297(6644):319-28.

101. He FJ, MacGregor GA. Fortnightly review: Beneficial effects of potassium. *BMJ* 2001;323(7311):497-501.

102. Hansen J. Blood volume and exchangeable sodium in essential hypertension. *Acta Med Scand* 1968;184(6):517-23.

103. Beretta-Piccoli C, Davies DL, Boddy K, Brown JJ, Cumming AM, East WB, et al. Relation of arterial pressure with exchangeable and total body sodium and with plasma exchangeable and total body potassium in essential hypertension. *Clin Sci (Lond)* 1981;61 Suppl 7:81s-84s.

104. Lever AF, Beretta-Piccoli C, Brown JJ, Davies DL, Fraser R, Robertson JI. Sodium and potassium in essential hypertension. *Br Med J (Clin Res Ed)* 1981;283(6289):463-8.

105. Yang Q, Liu T, Kuklina EV, Flanders WD, Hong Y, Gillespie C, et al. Sodium and potassium intake and mortality among US adults: prospective data from the Third National Health and Nutrition Examination Survey. *Arch Intern Med* 2011;171(13):1183-91.

106. Silver LD, Farley TA. Sodium and potassium intake: mortality effects and policy implications: comment on "Sodium and potassium intake and mortality among US adults". *Arch Intern Med* 2011;171(13):1191-2.

107. Denton D, Weisinger R, Mundy NI, Wickings EJ, Dixson A, Moisson P, et al. The effect of increased salt intake on blood pressure of chimpanzees. *Nat Med* 1995;1(10):1009-16.

108. Vollmer WM, Sacks FM, Svetkey LP. New insights into the effects on blood pressure of diets low in salt and high in fruits and vegetables and low-fat dairy products. *Curr Control Trials Cardiovasc Med* 2001;2(2):71-74.

109. Vollmer WM, Sacks FM, Ard J, Appel LJ, Bray GA, Simons-Morton DG, et al. Effects of diet and sodium intake on blood pressure: subgroup analysis of the DASH-sodium trial. *Ann Intern Med* 2001;135(12):1019-28.

110. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, et al. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. *N Engl J Med* 2001;344(1):3-10.

111. He FJ, MacGregor GA. How far should salt intake be reduced? *Hypertension* 2003;42(6):1093-9.

112. Geleijnse JM, Witteman JC, Stijnen T, Kloos MW, Hofman A, Grobbee DE. Sodium and potassium intake and risk of cardiovascular events and all-cause mortality: the Rotterdam Study. *Eur J Epidemiol* 2007;22(11):763-70.

113. du Cailar G, Mimran A, Fesler P, Ribstein J, Blacher J, Safar ME. Dietary sodium and pulse pressure in normotensive and essential hypertensive subjects. *J Hypertens* 2004;22(4):697-703.

114. Perry IJ, Beevers DG. Salt intake and stroke: a possible direct effect. *J Hum Hypertens* 1992;6(1):23-5.

115. Taylor RS, Ashton KE, Moxham T, Hooper L, Ebrahim S. Reduced dietary salt for the prevention of cardiovascular disease. *Cochrane Database Syst Rev* 2011(7):CD009217.

116. Taylor RS, Ashton KE, Moxham T, Hooper L, Ebrahim S. Reduced dietary salt for the prevention of cardiovascular disease: a meta-analysis of randomized controlled trials (Cochrane review). *Am J Hypertens* 2011;24(8):843-53.

117. He FJ, MacGregor GA. Salt reduction lowers cardiovascular risk: meta-analysis of outcome trials. *Lancet* 2011;378(9789):380-2.

118. Bibbins-Domingo K, Chertow GM, Coxson PG, Moran A, Lightwood JM, Pletcher MJ, et al. Projected effect of dietary salt reductions on future cardiovascular disease. *N Engl J Med* 2010 362(7):590-9.

119. Krishna GG, Miller E, Kapoor S. Increased blood pressure during potassium depletion in normotensive men. *N Engl J Med* 1989;320(18):1177-82.

120. Krishna GG, Kapoor SC. Potassium depletion exacerbates essential hypertension. *Ann Intern Med* 1991;115(2):77-83.

121. Hayashi M, Katz AI. The kidney in potassium depletion. I. Na+-K+-ATPase activity and [3H]ouabain binding in MCT. *Am J Physiol* 1987;252(3 Pt 2):F437-46.

122. Imbert-Teboul M, Doucet A, Marsy S, Siaume-Perez S. Alterations of enzymatic activities along rat collecting tubule in potassium depletion. *Am J Physiol* 1987;253(3 Pt 2):F408-17.

123. Tobian L, Lange J, Ulm K, Wold L, Iwai J. Potassium reduces cerebral hemorrhage and death rate in hypertensive rats, even when blood pressure is not lowered. *Hypertension* 1985;7(3 Pt 2):I110-4.

124. Liu DT, Wang MX, Kincaid-Smith P, Whitworth JA. The effects of dietary potassium on vascular and glomerular lesions in hypertensive rats. *Clin Exp Hypertens* 1994;16(4):391-414.

125. Whelton PK, He J, Cutler JA, Brancati FL, Appel LJ, Follmann D, et al. Effects of oral potassium on blood pressure. Meta-analysis of randomized controlled clinical trials. *JAMA* 1997;277(20):1624-32.

126. Siani A, Strazzullo P, Giacco A, Pacioni D, Celentano E, Mancini M. Increasing the dietary potassium intake reduces the need for antihypertensive medication. *Ann Intern Med* 1991;115(10):753-9.

127. Morris RC, Jr., Sebastian A, Forman A, Tanaka M, Schmidlin O. Normotensive salt sensitivity: effects of race and dietary potassium. *Hypertension* 1999;33(1):18-23.

128. Rodriguez-Iturbe B, Romero F, Johnson RJ. Pathophysiological mechanisms of salt-dependent hypertension. *Am J Kidney Dis* 2007;50(4):655-72.

129. Guyton AC, Coleman TG. Quantitative analysis of the pathophysiology of hypertension. *Circ Res* 1969;24(5 Suppl):1-19.

130. Guyton AC. Kidneys and fluids in pressure regulation. Small volume but large pressure changes. *Hypertension* 1992;19(1 Suppl):I2-8.

131. Haddy FJ, Overbeck HW. The role of humoral agents in volume expanded hypertension. *Life Sci* 1976;19(7):935-47.

132. Blaustein MP. Sodium ions, calcium ions, blood pressure regulation, and hypertension: a reassessment and a hypothesis. *Am J Physiol* 1977;232(5):C165-73.

133. Ferrari P, Ferrandi M, Valentini G, Bianchi G. Rostafuroxin: an ouabain antagonist that corrects renal and vascular Na+-K+- ATPase alterations in ouabain and adducin-dependent hypertension. *Am J Physiol Regul Integr Comp Physiol* 2006;290(3):R529-35.

134. Iwamoto T. Na+/Ca2+ exchange as a drug target--insights from molecular pharmacology and genetic engineering. *Ann N Y Acad Sci* 2007;1099:516-28.

135. Meneton P, Jeunemaitre X, de Wardener HE, MacGregor GA. Links between dietary salt intake, renal salt handling, blood pressure, and cardiovascular diseases. *Physiol Rev* 2005;85(2):679-715.

136. Soleimani M, Bergman JA, Hosford MA, McKinney TD. Potassium depletion increases luminal Na+/H+ exchange and basolateral Na+:CO3=:HCO3- cotransport in rat renal cortex. *J Clin Invest* 1990;86(4):1076-83.

137. Cowley AW, Jr. Renal medullary oxidative stress, pressure-natriuresis, and hypertension. *Hypertension* 2008;52(5):777-86.

138. Hall JE, Brands MW, Shek EW. Central role of the kidney and abnormal fluid volume control in hypertension. *J Hum Hypertens* 1996;10(10):633-9.

139. Hall JE, Guyton AC, Brands MW. Pressure-volume regulation in hypertension. *Kidney Int Suppl* 1996;55:S35-41.

140. Dickhout JG, Mori T, Cowley AW, Jr. Tubulovascular nitric oxide crosstalk: buffering of angiotensin II-induced medullary vasoconstriction. *Circ Res* 2002;91(6):487-93.

141. Carey RM, Padia SH. Angiotensin AT2 receptors: control of renal sodium excretion and blood pressure. *Trends Endocrinol Metab* 2008;19(3):84-7.

142. Mullins LJ, Bailey MA, Mullins JJ. Hypertension, kidney, and transgenics: a fresh perspective. *Physiol Rev* 2006;86(2):709-46.

143. Wang X, Villar VA, Armando I, Eisner GM, Felder RA, Jose PA. Dopamine, kidney, and hypertension: studies in dopamine receptor knockout mice. *Pediatr Nephrol* 2008;23(12):2131-46.

144. Carey RM. Theodore Cooper Lecture: Renal dopamine system: paracrine regulator of sodium homeostasis and blood pressure. *Hypertension* 2001;38(3):297-302.

145. Bertorello A, Hokfelt T, Goldstein M, Aperia A. Proximal tubule Na+-K+-ATPase activity is inhibited during high-salt diet: evidence for DA-mediated effect. *Am J Physiol* 1988;254(6 Pt 2):F795-801.

146. Bertorello AM, Katz AI. Short-term regulation of renal Na-K-ATPase activity: physiological relevance and cellular mechanisms. *Am J Physiol* 1993;265(6 Pt 2):F743-55.

147. Bacic D, Kaissling B, McLeroy P, Zou L, Baum M, Moe OW. Dopamine acutely decreases apical membrane Na/H exchanger NHE3 protein in mouse renal proximal tubule. *Kidney Int* 2003;64(6):2133-41.

148. Felder CC, Albrecht FE, Campbell T, Eisner GM, Jose PA. cAMP-independent, G protein-linked inhibition of Na+/H+ exchange in renal brush border by D1 dopamine agonists. *Am J Physiol* 1993;264(6 Pt 2):F1032-7.

149. Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, Devoto M, et al. Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension. *Lancet* 1997;349(9062):1353-7.

150. Efendiev R, Krmar RT, Ogimoto G, Zwiller J, Tripodi G, Katz AI, et al. Hypertension-linked mutation in the adducin alpha-subunit leads to higher AP2-mu2 phosphorylation and impaired Na+,K+-ATPase trafficking in response to GPCR signals and intracellular sodium. *Circ Res* 2004;95(11):1100-8.

151. Kohan DE. The renal medullary endothelin system in control of sodium and water excretion and systemic blood pressure. *Curr Opin Nephrol Hypertens* 2006;15(1):34-40.

152. Schneider MP, Ge Y, Pollock DM, Pollock JS, Kohan DE. Collecting duct-derived endothelin regulates arterial pressure and Na excretion via nitric oxide. *Hypertension* 2008;51(6):1605-10.

153. Fukuda M, Goto N, Kimura G. Hypothesis on renal mechanism of non-dipper pattern of circadian blood pressure rhythm. *Med Hypotheses* 2006;67(4):802-6.

154. Bankir L, Bochud M, Maillard M, Bovet P, Gabriel A, Burnier M. Nighttime blood pressure and nocturnal dipping are associated with daytime urinary sodium excretion in African subjects. *Hypertension* 2008;51(4):891-8.

155. da Silva AA, do Carmo JM, Kanyicska B, Dubinion J, Brandon E, Hall JE. Endogenous melanocortin system activity contributes to the elevated arterial pressure in spontaneously hypertensive rats. *Hypertension* 2008;51(4):884-90.

156. Oliver PM, John SW, Purdy KE, Kim R, Maeda N, Goy MF, et al. Natriuretic peptide receptor 1 expression influences blood pressures of mice in a dose-dependent manner. *Proc Natl Acad Sci U S A* 1998;95(5):2547-51.

157. Machnik A, Neuhofer W, Jantsch J, Dahlmann A, Tammela T, Machura K, et al. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med* 2009;15(5):545-52.

158. Dolson GM, Wesson DE, Adrogue HJ. Vascular relaxation probably mediates the antihypertensive effect of a high-potassium diet: a role for enhanced vascular Na,K-ATPase activity. *J Hypertens* 1995;13(12 Pt 1):1433-9.

159. Iwamoto T, Kita S. Topics on the Na+/Ca2+ exchanger: role of vascular NCX1 in salt-dependent hypertension. *J Pharmacol Sci* 2006;102(1):32-6.

160. Iwamoto T. Vascular Na+/Ca2+ exchanger: implications for the pathogenesis and therapy of salt-dependent hypertension. *Am J Physiol Regul Integr Comp Physiol* 2006;290(3):R536-45.

161. Haddy FJ, Vanhoutte PM, Feletou M. Role of potassium in regulating blood flow and blood pressure. *Am J Physiol Regul Integr Comp Physiol* 2006;290(3):R546-52.

162. Amberg GC, Bonev AD, Rossow CF, Nelson MT, Santana LF. Modulation of the molecular composition of large conductance, Ca(2+) activated K(+) channels in vascular smooth muscle during hypertension. *J Clin Invest* 2003;112(5):717-24.

163. Tobian L, Jahner TM, Johnson MA. High K diets markedly reduce atherosclerotic cholesterol ester deposition in aortas of rats with hypercholesterolemia and hypertension. *Am J Hypertens* 1990;3(2):133-5.

164. McCabe RD, Bakarich MA, Srivastava K, Young DB. Potassium inhibits free radical formation. *Hypertension* 1994;24(1):77-82.

165. McCabe RD, Young DB. Potassium inhibits cultured vascular smooth muscle cell proliferation. *Am J Hypertens* 1994;7(4 Pt 1):346-50.

166. Biglieri EG, McIlroy MB. Abnormalities of renal function and circulatory reflexes in primary aldosteronism. *Circulation* 1966;33(1):78-86.

167. Rowe JW, Tobin JD, Rosa RM, Andres R. Effect of experimental potassium deficiency on glucose and insulin metabolism. *Metabolism* 1980;29(6):498-502.

168. Dluhy RG, Axelrod L, Williams GH. Serum immunoreactive insulin and growth hormone response to potassium infusion in normal man. *J Appl Physiol* 1972;33(1):22-6.

169. Baron AD, Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G. Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. *J Clin Invest* 1995;96(2):786-92.

170. Feldman RD, Bierbrier GS. Insulin-mediated vasodilation: impairment with increased blood pressure and body mass. *Lancet* 1993;342(8873):707-9.

171. Duprez DA. Role of the renin-angiotensin-aldosterone system in vascular remodeling and inflammation: a clinical review. *J Hypertens* 2006;24(6):983-91.

172. Kaplan NM, Victor RG. *Primary hypertension: Pathogensis* 10th ed. Philadelphia, PA: Lippincott Williams and Wilkins 2010.

173. Berk BC. Vascular smooth muscle growth: autocrine growth mechanisms. *Physiol Rev* 2001;81(3):999-1030.

174. Touyz RM. Molecular and cellular mechanisms in vascular injury in hypertension: role of angiotensin II. *Curr Opin Nephrol Hypertens* 2005;14(2):125-31.

175. Suzuki Y, Ruiz-Ortega M, Lorenzo O, Ruperez M, Esteban V, Egido J. Inflammation and angiotensin II. *Int J Biochem Cell Biol* 2003;35(6):881-900.

176. Dandona P, Kumar V, Aljada A, Ghanim H, Syed T, Hofmayer D, et al. Angiotensin II receptor blocker valsartan suppresses reactive oxygen species generation in leukocytes, nuclear factor-kappa B, in mononuclear cells of normal subjects: evidence of an antiinflammatory action. *J Clin Endocrinol Metab* 2003;88(9):4496-501.

177. Taniyama Y, Griendling KK. Reactive oxygen species in the vasculature: molecular and cellular mechanisms. *Hypertension* 2003;42(6):1075-81.

178. Wolf G. Role of reactive oxygen species in angiotensin II-mediated renal growth, differentiation, and apoptosis. *Antioxid Redox Signal* 2005;7(9-10):1337-45.

179. Hall JE, Brands MW, Henegar JR. Angiotensin II and long-term arterial pressure regulation: the overriding dominance of the kidney. *J Am Soc Nephrol* 1999;10 Suppl 12:S258-65.

180. Harrison DG, Guzik TJ, Goronzy J, Weyand C. Is hypertension an immunologic disease? *Curr Cardiol Rep* 2008;10(6):464-9.

181. Touyz RM. Activated oxygen metabolites: do they really play a role in angiotensin II-regulated vascular tone? *J Hypertens* 2003;21(12):2235-8.

182. Schiffrin EL, Touyz RM. Inflammation and vascular hypertrophy induced by angiotensin II: role of NADPH oxidase-derived reactive oxygen species independently of blood pressure elevation? *Arterioscler Thromb Vasc Biol* 2003;23(5):707-9.

183. Touyz RM, Yao G, Schiffrin EL. c-Src induces phosphorylation and translocation of p47phox: role in superoxide generation by angiotensin II in human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 2003;23(6):981-7.

184. Touyz RM. The role of angiotensin II in regulating vascular structural and functional changes in hypertension. *Curr Hypertens Rep* 2003;5(2):155-64.

185. Chitaley K, Weber D, Webb RC. RhoA/Rho-kinase, vascular changes, and hypertension. *Curr Hypertens Rep* 2001;3(2):139-44.

186. El Mabrouk M, Touyz RM, Schiffrin EL. Differential ANG II-induced growth activation pathways in mesenteric artery smooth muscle cells from SHR. *Am J Physiol Heart Circ Physiol* 2001;281(1):H30-9.

187. Touyz RM, He G, El Mabrouk M, Diep Q, Mardigyan V, Schiffrin EL. Differential activation of extracellular signal-regulated protein kinase 1/2 and p38 mitogen activated-protein kinase by AT1 receptors in vascular smooth muscle cells from Wistar-Kyoto rats and spontaneously hypertensive rats. *J Hypertens* 2001;19(3 Pt 2):553-9.

188. Berry C, Touyz R, Dominiczak AF, Webb RC, Johns DG. Angiotensin receptors: signaling, vascular pathophysiology, and interactions with ceramide. *Am J Physiol Heart Circ Physiol* 2001;281(6):H2337-65.

189. Crowley SD, Gurley SB, Oliverio MI, Pazmino AK, Griffiths R, Flannery PJ, et al. Distinct roles for the kidney and systemic tissues in blood pressure regulation by the renin-angiotensin system. *J Clin Invest* 2005;115(4):1092-9.

190. Navar LG, Harrison-Bernard LM, Nishiyama A, Kobori H. Regulation of intrarenal angiotensin II in hypertension. *Hypertension* 2002;39(2 Pt 2):316-22.

191. Navar LG, Kobori H, Prieto-Carrasquero M. Intrarenal angiotensin II and hypertension. *Curr Hypertens Rep* 2003;5(2):135-43.

192. Levens NR, Freedlender AE, Peach MJ, Carey RM. Control of renal function by intrarenal angiotensin II. *Endocrinology* 1983;112(1):43-9.

193. Hall JE. Regulation of glomerular filtration rate and sodium excretion by angiotensin II. *Fed Proc* 1986;45(5):1431-7.

194. Ruster C, Wolf G. Renin-angiotensin-aldosterone system and progression of renal disease. *J Am Soc Nephrol* 2006;17(11):2985-91.

195. Waanders F, de Vries LV, van Goor H, Hillebrands JL, Laverman GD, Bakker SJ, et al. Aldosterone, from (patho)physiology to treatment in cardiovascular and renal damage. *Curr Vasc Pharmacol* 2011;9(5):594-605.

196. Victor RG. Pathophysiology of target-organ disease: does angiotensin II remain the key? *J Clin Hypertens (Greenwich)* 2007;9(11 Suppl 4):4-10.

197. Vasan RS, Evans JC, Larson MG, Wilson PW, Meigs JB, Rifai N, et al. Serum aldosterone and the incidence of hypertension in nonhypertensive persons. *N Engl J Med* 2004;351(1):33-41.

198. Rossi G, Boscaro M, Ronconi V, Funder JW. Aldosterone as a cardiovascular risk factor. *Trends Endocrinol Metab* 2005;16(3):104-7.

199. Brilla CG, Weber KT. Reactive and reparative myocardial fibrosis in arterial hypertension in the rat. *Cardiovasc Res* 1992;26(7):671-7.

200. Weber KT, Brilla CG. Factors associated with reactive and reparative fibrosis of the myocardium. *Basic Res Cardiol* 1992;87 Suppl 1:291-301.

201. Brown NJ. Aldosterone and end-organ damage. *Curr Opin Nephrol Hypertens* 2005;14(3):235-41.

202. Joffe HV, Adler GK. Effect of aldosterone and mineralocorticoid receptor blockade on vascular inflammation. *Heart Fail Rev* 2005;10(1):31-7.

203. Marney AM, Brown NJ. Aldosterone and end-organ damage. *Clin Sci (Lond)* 2007;113(6):267-78.

204. Rocha R, Chander PN, Zuckerman A, Stier CT, Jr. Role of aldosterone in renal vascular injury in stroke-prone hypertensive rats. *Hypertension* 1999;33(1 Pt 2):232-7.

205. Rocha R, Chander PN, Khanna K, Zuckerman A, Stier CT, Jr. Mineralocorticoid blockade reduces vascular injury in stroke-prone hypertensive rats. *Hypertension* 1998;31(1 Pt 2):451-8.

206. Williams JS, Williams GH, Jeunemaitre X, Hopkins PN, Conlin PR. Influence of dietary sodium on the renin-angiotensin-aldosterone system and prevalence of left ventricular hypertrophy by EKG criteria. *J Hum Hypertens* 2005;19(2):133-8.

207. Simon AC, Flaud P, Levenson J. Non-invasive evaluation of segmental pressure drop and resistance in large arteries in humans based on a Poiseuille model of intra-arterial velocity distribution. *Cardiovasc Res* 1990;24(8):623-6.

208. Munzel T, Sinning C, Post F, Warnholtz A, Schulz E. Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. *Ann Med* 2008;40(3):180-96.

209. August P, Suthanthiran M. Transforming growth factor beta signaling, vascular remodeling, and hypertension. *N Engl J Med* 2006;354(25):2721-3.

210. Paravicini TM, Touyz RM. NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities. *Diabetes Care* 2008;31 Suppl 2:S170-80.

211. Levy BI, Schiffrin EL, Mourad JJ, Agostini D, Vicaut E, Safar ME, et al. Impaired tissue perfusion: a pathology common to hypertension, obesity, and diabetes mellitus. *Circulation* 2008;118(9):968-76.

212. Barlow SE. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. *Pediatrics* 2007;120 Suppl 4:S164-92.

213. Stabouli S, Papakatsika S, Kotsis V. The role of obesity, salt and exercise on blood pressure in children and adolescents. *Expert Rev Cardiovasc Ther* 2011;9(6):753-61.

214. Esler M, Straznicky N, Eikelis N, Masuo K, Lambert G, Lambert E. Mechanisms of sympathetic activation in obesity-related hypertension. *Hypertension* 2006;48(5):787-96.

215. Mancia G, Bousquet P, Elghozi JL, Esler M, Grassi G, Julius S, et al. The sympathetic nervous system and the metabolic syndrome. *J Hypertens* 2007;25(5):909-20.

216. Lambert E, Straznicky N, Schlaich M, Esler M, Dawood T, Hotchkin E, et al. Differing pattern of sympathoexcitation in normal-weight and obesity-related hypertension. *Hypertension* 2007;50(5):862-8.

217. Correia ML, Haynes WG. Obesity-related hypertension: is there a role for selective leptin resistance? *Curr Hypertens Rep* 2004;6(3):230-5.

218. Yang R, Barouch LA. Leptin signaling and obesity: cardiovascular consequences. *Circ Res* 2007;101(6):545-59.

219. Katagiri H, Yamada T, Oka Y. Adiposity and cardiovascular disorders: disturbance of the regulatory system consisting of humoral and neuronal signals. *Circ Res* 2007;101(1):27-39.

220. Wu H, Ghosh S, Perrard XD, Feng L, Garcia GE, Perrard JL, et al. T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. *Circulation* 2007;115(8):1029-38.

221. Guzik TJ, Hoch NE, Brown KA, McCann LA, Rahman A, Dikalov S, et al. Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. *J Exp Med* 2007;204(10):2449-60.

222. Grassi G, Quarti-Trevano F, Seravalle G, Arenare F, Brambilla G, Mancia G. Blood pressure lowering effects of rimonabant in obesity-related hypertension. *J Neuroendocrinol* 2008;20 Suppl 1:63-8.

223. Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. *N Engl J Med* 2008;359(17):1811-21.

224. Alper AB, Jr., Chen W, Yau L, Srinivasan SR, Berenson GS, Hamm LL. Childhood uric acid predicts adult blood pressure: the Bogalusa Heart Study. *Hypertension* 2005;45(1):34-8.

225. Sundstrom J, Sullivan L, D'Agostino RB, Levy D, Kannel WB, Vasan RS. Relations of serum uric acid to longitudinal blood pressure tracking and hypertension incidence. *Hypertension* 2005;45(1):28-33.

226. Feig DI, Soletsky B, Johnson RJ. Effect of allopurinol on blood pressure of adolescents with newly diagnosed essential hypertension: a randomized trial. *JAMA* 2008;300(8):924-32.

227. Trachtman H. Treatment of hyperuricemia in essential hypertension. *Hypertension* 2007;49(6):e45; author reply e46.

228. Kienitz T, Quinkler M. Testosterone and blood pressure regulation. *Kidney Blood Press Res* 2008;31(2):71-9.

229. Qiao X, McConnell KR, Khalil RA. Sex steroids and vascular responses in hypertension and aging. *Gend Med* 2008;5 Suppl A:S46-64.

230. Lee AJ. Haemorheological, platelet and endothelial factors in essential hypertension. *J Hum Hypertens* 2002;16(8):529-31.

231. Smith S, Julius S, Jamerson K, Amerena J, Schork N. Hematocrit levels and physiologic factors in relationship to cardiovascular risk in Tecumseh, Michigan. *J Hypertens* 1994;12(4):455-62.

232. Devereux RB, Case DB, Alderman MH, Pickering TG, Chien S, Laragh JH. Possible role of increased blood viscosity in the hemodynamics of systemic hypertension. *Am J Cardiol* 2000;85(10):1265-8.

233. Landin K, Tengborn L, Smith U. Elevated fibrinogen and plasminogen activator inhibitor (PAI-1) in hypertension are related to metabolic risk factors for cardiovascular disease. *J Intern Med* 1990;227(4):273-8.

234. Poli KA, Tofler GH, Larson MG, Evans JC, Sutherland PA, Lipinska I, et al. Association of blood pressure with fibrinolytic potential in the Framingham offspring population. *Circulation* 2000;101(3):264-9.

235. Grassi G, Seravalle G, Calhoun DA, Bolla GB, Giannattasio C, Marabini M, et al. Mechanisms responsible for sympathetic activation by cigarette smoking in humans. *Circulation* 1994;90(1):248-53.

236. Zhang WZ, Venardos K, Chin-Dusting J, Kaye DM. Adverse effects of cigarette smoke on NO bioavailability: role of arginine metabolism and oxidative stress. *Hypertension* 2006;48(2):278-85.

237. Leone A. Does Smoking Act as a Friend or Enemy of Blood Pressure? Let Release Pandora's Box. *Cardiol Res Pract* 2011;2011:264894.

238. Bonita JS, Mandarano M, Shuta D, Vinson J. Coffee and cardiovascular disease: in vitro, cellular, animal, and human studies. *Pharmacol Res* 2007;55(3):187-98.

239. Winkelmayer WC, Stampfer MJ, Willett WC, Curhan GC. Habitual caffeine intake and the risk of hypertension in women. *JAMA* 2005;294(18):2330-5.

240. Noordzij M, Uiterwaal CS, Arends LR, Kok FJ, Grobbee DE, Geleijnse JM. Blood pressure response to chronic intake of coffee and caffeine: a meta-analysis of randomized controlled trials. *J Hypertens* 2005;23(5):921-8.

241. Mesas AE, Leon-Munoz LM, Rodriguez-Artalejo F, Lopez-Garcia E. The effect of coffee on blood pressure and cardiovascular disease in hypertensive individuals: a systematic review and meta-analysis. *Am J Clin Nutr* 2010 94(4):1113-26.

242. Cornelis MC, El-Sohemy A, Kabagambe EK, Campos H. Coffee, CYP1A2 genotype, and risk of myocardial infarction. *JAMA* 2006;295(10):1135-41.

243. Chen L, Davey Smith G, Harbord RM, Lewis SJ. Alcohol intake and blood pressure: a systematic review implementing a Mendelian randomization approach. *PLoS Med* 2008;5(3):e52.

244. Kloner RA, Rezkalla SH. To drink or not to drink? That is the question. *Circulation* 2007;116(11):1306-17.

245. O'Keefe JH, Bybee KA, Lavie CJ. Alcohol and cardiovascular health: the razor-sharp double-edged sword. *J Am Coll Cardiol* 2007;50(11):1009-14.

246. Shaper AG, Wannamethee G, Whincup P. Alcohol and blood pressure in middle-aged British men. *J Hum Hypertens* 1988;2(2):71-8.

247. Ohmori S, Kiyohara Y, Kato I, Kubo M, Tanizaki Y, Iwamoto H, et al. Alcohol intake and future incidence of hypertension in a general Japanese population: the Hisayama study. *Alcohol Clin Exp Res* 2002;26(7):1010-6.

248. Sesso HD, Cook NR, Buring JE, Manson JE, Gaziano JM. Alcohol consumption and the risk of hypertension in women and men. *Hypertension* 2008;51(4):1080-7.

249. Modesti PA, Morabito M, Bertolozzi I, Massetti L, Panci G, Lumachi C, et al. Weather-related changes in 24-hour blood pressure profile: effects of age and implications for hypertension management. *Hypertension* 2006;47(2):155-61.

250. Wolfel EE, Selland MA, Mazzeo RS, Reeves JT. Systemic hypertension at 4,300 m is related to sympathoadrenal activity. *J Appl Physiol* 1994;76(4):1643-50.

251. Halonen JI, Zanobetti A, Sparrow D, Vokonas PS, Schwartz J. Relationship between outdoor temperature and blood pressure. *Occup Environ Med* 2011;68(4):296-301.

252. Madsen C, Nafstad P. Associations between environmental exposure and blood pressure among participants in the Oslo Health Study (HUBRO). *Eur J Epidemiol* 2006;21(7):485-91.

253. Lewington S, Li L, Sherliker P, Guo Y, Millwood I, Bian Z, et al. Seasonal variation in blood pressure and its relationship with outdoor temperature in 10 diverse regions of China: the China Kadoorie Biobank. *J Hypertens* 2012;30(7):1383-91.

254. Forman JP, Curhan GC, Taylor EN. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension among young women. *Hypertension* 2008;52(5):828-32.

255. Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, et al. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension* 2007;49(5):1063-9.

256. Wang L, Manson JE, Buring JE, Lee IM, Sesso HD. Dietary intake of dairy products, calcium, and vitamin D and the risk of hypertension in middle-aged and older women. *Hypertension* 2008;51(4):1073-9.

257. Margolis KL, Ray RM, Van Horn L, Manson JE, Allison MA, Black HR, et al. Effect of calcium and vitamin D supplementation on blood pressure: the Women's Health Initiative Randomized Trial. *Hypertension* 2008;52(5):847-55.

258. Vaziri ND. Mechanisms of lead-induced hypertension and cardiovascular disease. *Am J Physiol Heart Circ Physiol* 2008;295(2):H454-65.

259. Navas-Acien A, Guallar E, Silbergeld EK, Rothenberg SJ. Lead exposure and cardiovascular disease--a systematic review. *Environ Health Perspect* 2007;115(3):472-82.

260. Scinicariello F, Abadin HG, Edward Murray H. Association of low-level blood lead and blood pressure in NHANES 1999-2006. *Environ Res* 2011;111(8):1249-57.

261. Zhang A, Hu H, Sanchez BN, Ettinger AS, Park SK, Cantonwine D, et al. Association between Prenatal Lead Exposure and Blood Pressure in Female Offspring. *Environ Health Perspect* 2011.

262. Urch B, Silverman F, Corey P, Brook JR, Lukic KZ, Rajagopalan S, et al. Acute blood pressure responses in healthy adults during controlled air pollution exposures. *Environ Health Perspect* 2005;113(8):1052-5.

263. Fuks K, Moebus S, Hertel S, Viehmann A, Nonnemacher M, Dragano N, et al. Long-term urban particulate air pollution, traffic noise, and arterial blood pressure. *Environ Health Perspect* 2011;119(12):1706-11.

264. Bhatnagar A. Environmental cardiology: studying mechanistic links between pollution and heart disease. *Circ Res* 2006;99(7):692-705.

265. Brook RD. Cardiovascular effects of air pollution. *Clin Sci (Lond)* 2008;115(6):175-87.

266. Chen X, Wang Y. Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis. *Circulation* 2008;117(25):3171-80.

267. Sun SS, Grave GD, Siervogel RM, Pickoff AA, Arslanian SS, Daniels SR. Systolic blood pressure in childhood predicts hypertension and metabolic syndrome later in life. *Pediatrics* 2007;119(2):237-46.

268. Daniels SR, Loggie JM, Khoury P, Kimball TR. Left ventricular geometry and severe left ventricular hypertrophy in children and adolescents with essential hypertension. *Circulation* 1998;97(19):1907-11.

269. Hanevold C, Waller J, Daniels S, Portman R, Sorof J. The effects of obesity, gender, and ethnic group on left ventricular hypertrophy and geometry in hypertensive children: a collaborative study of the International Pediatric Hypertension Association. *Pediatrics* 2004;113(2):328-33.

270. McNiece KL, Gupta-Malhotra M, Samuels J, Bell C, Garcia K, Poffenbarger T, et al. Left ventricular hypertrophy in hypertensive adolescents: analysis of risk by 2004 National High Blood Pressure Education Program Working Group staging criteria. *Hypertension* 2007;50(2):392-5.

271. Brady TM, Fivush B, Flynn JT, Parekh R. Ability of blood pressure to predict left ventricular hypertrophy in children with primary hypertension. *J Pediatr* 2008;152(1):73-8, 78 e1.

272. Raitakari OT, Juonala M, Kahonen M, Taittonen L, Laitinen T, Maki-Torkko N, et al. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. *JAMA* 2003;290(17):2277-83.

273. Li S, Chen W, Srinivasan SR, Bond MG, Tang R, Urbina EM, et al. Childhood cardiovascular risk factors and carotid vascular changes in adulthood: the Bogalusa Heart Study. *JAMA* 2003;290(17):2271-6.

274. Sorof JM, Alexandrov AV, Garami Z, Turner JL, Grafe RE, Lai D, et al. Carotid ultrasonography for detection of vascular abnormalities in hypertensive children. *Pediatr Nephrol* 2003;18(10):1020-4.

275. Mitchell P, Cheung N, de Haseth K, Taylor B, Rochtchina E, Islam FM, et al. Blood pressure and retinal arteriolar narrowing in children. *Hypertension* 2007;49(5):1156-62.

276. Urbina EM, Khoury PR, McCoy C, Daniels SR, Kimball TR, Dolan LM. Cardiac and vascular consequences of pre-hypertension in youth. *J Clin Hypertens (Greenwich)* 2011;13(5):332-42.

277. Kokkinos P, Pittaras A, Narayan P, Faselis C, Singh S, Manolis A. Exercise capacity and blood pressure associations with left ventricular mass in prehypertensive individuals. *Hypertension* 2007;49(1):55-61.

278. Manios E, Tsivgoulis G, Koroboki E, Stamatelopoulos K, Papamichael C, Toumanidis S, et al. Impact of prehypertension on common carotid artery intima-media thickness and left ventricular mass. *Stroke* 2009;40(4):1515-8.

279. Markus MR, Stritzke J, Lieb W, Mayer B, Luchner A, Doring A, et al. Implications of persistent prehypertension for ageing-related changes in left ventricular geometry and function: the MONICA/KORA Augsburg study. *J Hypertens* 2008;26(10):2040-9.

280. Pletcher MJ, Bibbins-Domingo K, Lewis CE, Wei GS, Sidney S, Carr JJ, et al. Prehypertension during young adulthood and coronary calcium later in life. *Ann Intern Med* 2008;149(2):91-9.

281. Erdogan D, Yildirim I, Ciftci O, Ozer I, Caliskan M, Gullu H, et al. Effects of normal blood pressure, prehypertension, and hypertension on coronary microvascular function. *Circulation* 2007;115(5):593-9.

282. Sipahi I, Tuzcu EM, Schoenhagen P, Wolski KE, Nicholls SJ, Balog C, et al. Effects of normal, pre-hypertensive, and hypertensive blood pressure levels on progression of coronary atherosclerosis. *J Am Coll Cardiol* 2006;48(4):833-8.

283. Kshirsagar AV, Carpenter M, Bang H, Wyatt SB, Colindres RE. Blood pressure usually considered normal is associated with an elevated risk of cardiovascular disease. *Am J Med* 2006;119(2):133-41.

284. Liszka HA, Mainous AG, 3rd, King DE, Everett CJ, Egan BM. Prehypertension and cardiovascular morbidity. *Ann Fam Med* 2005;3(4):294-9.

285. Knecht S, Wersching H, Lohmann H, Bruchmann M, Duning T, Dziewas R, et al. High-normal blood pressure is associated with poor cognitive performance. *Hypertension* 2008;51(3):663-8.

286. Nguyen TT, Wang JJ, Wong TY. Retinal vascular changes in pre-diabetes and prehypertension: new findings and their research and clinical implications. *Diabetes Care* 2007;30(10):2708-15.

287. Kim BJ, Lee HJ, Sung KC, Kim BS, Kang JH, Lee MH, et al. Comparison of microalbuminuria in 2 blood pressure categories of prehypertensive subjects. *Circ J* 2007;71(8):1283-7.

288. Ogunniyi MO, Croft JB, Greenlund KJ, Giles WH, Mensah GA. Racial/ethnic differences in microalbuminuria among adults with prehypertension and hypertension: National Health and Nutrition Examination Survey (NHANES), 1999-2006. *Am J Hypertens* 2010 23(8):859-64.

289. Ninomiya T, Kubo M, Doi Y, Yonemoto K, Tanizaki Y, Tsuruya K, et al. Prehypertension increases the risk for renal arteriosclerosis in autopsies: the Hisayama Study. *J Am Soc Nephrol* 2007;18(7):2135-42.

290. Vos LE, Oren A, Uiterwaal C, Gorissen WH, Grobbee DE, Bots ML. Adolescent blood pressure and blood pressure tracking into young adulthood are related to subclinical atherosclerosis: the Atherosclerosis Risk in Young Adults (ARYA) study. *Am J Hypertens* 2003;16(7):549-55.

291. Kannel WB. Blood pressure as a cardiovascular risk factor: prevention and treatment. *JAMA* 1996;275(20):1571-6.

292. Henskens LH, van Oostenbrugge RJ, Kroon AA, de Leeuw PW, Lodder J. Brain microbleeds are associated with ambulatory blood pressure levels in a hypertensive population. *Hypertension* 2008;51(1):62-8.

293. Lawes CM, Vander Hoorn S, Rodgers A. Global burden of blood-pressure-related disease, 2001. *Lancet* 2008;371(9623):1513-8.

294. Vakili BA, Okin PM, Devereux RB. Prognostic implications of left ventricular hypertrophy. *Am Heart J* 2001;141(3):334-41.

295. Oikarinen L, Nieminen MS, Viitasalo M, Toivonen L, Jern S, Dahlof B, et al. QRS duration and QT interval predict mortality in hypertensive patients with left ventricular hypertrophy: the Losartan Intervention for Endpoint Reduction in Hypertension Study. *Hypertension* 2004;43(5):1029-34.

296. Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, et al. Resistant hypertension: diagnosis, evaluation, and treatment: a scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Circulation* 2008;117(25):e510-26.

297. Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, et al. Resistant hypertension: diagnosis, evaluation, and treatment. A scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Hypertension* 2008;51(6):1403-19.

298. Daugherty SL, Powers JD, Magid DJ, Tavel HM, Masoudi FA, Margolis KL, et al. Incidence and prognosis of resistant hypertension in hypertensive patients. *Circulation* 2012;125(13):1635-42.

299. Gupta AK, Nasothimiou EG, Chang CL, Sever PS, Dahlof B, Poulter NR. Baseline predictors of resistant hypertension in the Anglo-Scandinavian Cardiac Outcome Trial (ASCOT): a risk score to identify those at high-risk. *J Hypertens* 2011;29(10):2004-13.

300. Sim JJ, Shi J, Bhandari S, Rasgon S, Calhoun D, Kalantar-Zadeh K. Comparative outcomes in resistant hypertension (RH) vs non RH: kaiser permanente southern california cohort [abstract]. *Journal of Clinical Hypertension* 2012;14(Suppl 1):9.

301. Smulyan H, Safar ME. Systolic blood pressure revisited. *J Am Coll Cardiol* 1997;29(7):1407-13.

302. Franklin SS, Pio JR, Wong ND, Larson MG, Leip EP, Vasan RS, et al. Predictors of new-onset diastolic and systolic hypertension: the Framingham Heart Study. *Circulation* 2005;111(9):1121-7.

303. Staessen JA, Gasowski J, Wang JG, Thijs L, Den Hond E, Boissel JP, et al. Risks of untreated and treated isolated systolic hypertension in the elderly: meta-analysis of outcome trials. *Lancet* 2000;355(9207):865-72.

304. Qureshi AI, Suri MF, Mohammad Y, Guterman LR, Hopkins LN. Isolated and borderline isolated systolic hypertension relative to long-term risk and type of stroke: a 20-year follow-up of the national health and nutrition survey. *Stroke* 2002;33(12):2781-8.

305. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet* 2005;365(9455):217-23.

306. Vasan RS, Beiser A, Seshadri S, Larson MG, Kannel WB, D'Agostino RB, et al. Residual lifetime risk for developing hypertension in middle-aged women and men: The Framingham Heart Study. *JAMA* 2002;287(8):1003-10.

307. Perkovic V, Huxley R, Wu Y, Prabhakaran D, MacMahon S. The burden of blood pressure-related disease: a neglected priority for global health. *Hypertension* 2007;50(6):991-7.

308. WHO. Global health risks: mortality and burden of disease attributable to selected major risks. Geneva World Health Organization, 2009.

309. WHO. The global burden of disease: 2004 update. Geneva.: World Health Organization., 2008.

310. Lopez AD. The evolution of the Global Burden of Disease framework for disease, injury and risk factor quantification: developing the evidence base for national, regional and global public health action. *Global Health* 2005;1(1):5.

311. UN. World Population Prospects - the 2006 revision. New York: United Nations 2007.

312. World Health Organization. Global health risks: mortality and burden of disease attributable to selected major risks. Geneva World Health Organization, 2009.

313. Danaei G, Finucane MM, Lin JK, Singh GM, Paciorek CJ, Cowan MJ, et al. National, regional, and global trends in systolic blood pressure since 1980: systematic analysis of health examination surveys and epidemiological studies with 786 country-years and 5.4 million participants. *Lancet* 2011;377(9765):568-77.

314. World Health Organization. The World Health Report 2002-Reducing Risks, Promoting Healthy Life Geneva World Health Organization 2002

315. Ford ES. Trends in mortality from all causes and cardiovascular disease among hypertensive and nonhypertensive adults in the United States. *Circulation* 2011;123(16):1737-44.

316. Benetos A, Thomas F, Bean K, Gautier S, Smulyan H, Guize L. Prognostic value of systolic and diastolic blood pressure in treated hypertensive men. *Arch Intern Med* 2002;162(5):577-81.

317. Benetos A, Thomas F, Bean K, Gautier S, Smulyan H, Guize L. Prognostic value of systolic and diastolic blood pressure in treated hypertensive men: clarification. *Arch Intern Med* 2003;163(1):121.

318. Ogden LG, He J, Lydick E, Whelton PK. Long-term absolute benefit of lowering blood pressure in hypertensive patients according to the JNC VI risk stratification. *Hypertension* 2000;35(2):539-43.

319. National Clinical Guideline Centre. Hypertension: The clinical management of primary hypertension in adults London 2011.

320. Chalmers J. Comparison of various blood pressure lowering treatments on the primary prevention of cardiovascular outcomes in recent randomised clinical trials. *Clin Exp Hypertens* 2004;26(7-8):709-19.

321. Kettani FZ, Dragomir A, Cote R, Roy L, Berard A, Blais L, et al. Impact of a better adherence to antihypertensive agents on cerebrovascular disease for primary prevention. *Stroke* 2009;40(1):213-20.

322. Esposti LD, Saragoni S, Benemei S, Batacchi P, Geppetti P, Di Bari M, et al. Adherence to antihypertensive medications and health outcomes among newly treated hypertensive patients. *Clinicoecon Outcomes Res*;3:47-54.

323. Falaschetti E, Chaudhury M, Mindell J, Poulter N. Continued improvement in hypertension management in England: results from the Health Survey for England 2006. *Hypertension* 2009;53(3):480-6.

324. Pereira M, Lunet N, Azevedo A, Barros H. Differences in prevalence, awareness, treatment and control of hypertension between developing and developed countries. *J Hypertens* 2009;27(5):963-75.

325. Staessen JA, Wang JG, Thijs L. Cardiovascular prevention and blood pressure reduction: a quantitative overview updated until 1 March 2003. *J Hypertens* 2003;21(6):1055-76.

326. Turnbull F. Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. *Lancet* 2003;362(9395):1527-35.

327. Law MR, Morris JK, Wald NJ. Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. *BMJ* 2009;338:b1665.

328. Corrao G, Zambon A, Parodi A, Poluzzi E, Baldi I, Merlino L, et al. Discontinuation of and changes in drug therapy for hypertension among newly-treated patients: a population-based study in Italy. *J Hypertens* 2008;26(4):819-24.

329. Luscher TF, Vetter H, Siegenthaler W, Vetter W. Compliance in hypertension: facts and concepts. *J Hypertens Suppl* 1985;3(1):S3-9.

330. Hershey JC, Morton BG, Davis JB, Reichgott MJ. Patient compliance with antihypertensive medication. *Am J Public Health* 1980;70(10):1081-9.

331. Wright JM, Musini VM. First-line drugs for hypertension. *Cochrane Database Syst Rev* 2009(3):CD001841.

332. Forni V, Wuerzner G, Pruijm M, Burnier M. Treating high blood pressure: is reaching the target more important than the means? No, the means are important. *Eur J Intern Med* 2010;21(6):478-83.

333. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med* 1999;341(10):709-17.

334. Dahlof B, Devereux RB, Kjeldsen SE, Julius S, Beevers G, de Faire U, et al. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet* 2002;359(9311):995-1003.

335. Collier DJ, Poulter NR, Dahlof B, Sever PS, Wedel H, Buch J, et al. Impact of amlodipine-based therapy among older and younger patients in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA). *J Hypertens* 2011;29(3):583-91.

336. Poulter NR, Wedel H, Dahlof B, Sever PS, Beevers DG, Caulfield M, et al. Role of blood pressure and other variables in the differential cardiovascular event rates noted in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA). *Lancet* 2005;366(9489):907-13.

337. Julius S, Kjeldsen SE, Weber M, Brunner HR, Ekman S, Hansson L, et al. Outcomes in hypertensive patients at high cardiovascular risk treated with regimens based on valsartan or amlodipine: the VALUE randomised trial. *Lancet* 2004;363(9426):2022-31.

338. Jamerson K, Weber MA, Bakris GL, Dahlof B, Pitt B, Shi V, et al. Benazepril plus amlodipine or hydrochlorothiazide for hypertension in high-risk patients. *N Engl J Med* 2008;359(23):2417-28.

339. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, et al. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. *Circulation* 2006;113(9):1213-25.

340. Manisty CH, Zambanini A, Parker KH, Davies JE, Francis DP, Mayet J, et al. Differences in the magnitude of wave reflection account for differential effects of amlodipine- versus atenolol-based regimens on central blood

pressure: an Anglo-Scandinavian Cardiac Outcome Trial substudy. *Hypertension* 2009;54(4):724-30.

341. Manisty C, Mayet J, Tapp RJ, Parker KH, Sever P, Poulter NR, et al. Wave reflection predicts cardiovascular events in hypertensive individuals independent of blood pressure and other cardiovascular risk factors: an ASCOT (Anglo-Scandinavian Cardiac Outcome Trial) substudy. *J Am Coll Cardiol* 2010;56(1):24-30.

342. Mancia G, Messerli F, Bakris G, Zhou Q, Champion A, Pepine CJ. Blood pressure control and improved cardiovascular outcomes in the International Verapamil SR-Trandolapril Study. *Hypertension* 2007;50(2):299-305.

343. Sega R, Facchetti R, Bombelli M, Cesana G, Corrao G, Grassi G, et al. Prognostic value of ambulatory and home blood pressures compared with office blood pressure in the general population: follow-up results from the Pressioni Arteriose Monitorate e Loro Associazioni (PAMELA) study. *Circulation* 2005;111(14):1777-83.

344. Mancia G, Bombelli M, Facchetti R, Madotto F, Corrao G, Trevano FQ, et al. Long-term prognostic value of blood pressure variability in the general population: results of the Pressioni Arteriose Monitorate e Loro Associazioni Study. *Hypertension* 2007;49(6):1265-70.

345. Kario K, Shimada K, Pickering TG. Clinical implication of morning blood pressure surge in hypertension. *J Cardiovasc Pharmacol* 2003;42 Suppl 1:S87-91.

346. Kario K, Pickering TG, Umeda Y, Hoshide S, Hoshide Y, Morinari M, et al. Morning surge in blood pressure as a predictor of silent and clinical cerebrovascular disease in elderly hypertensives: a prospective study. *Circulation* 2003;107(10):1401-6.

347. Li Y, Thijs L, Hansen TW, Kikuya M, Boggia J, Richart T, et al. Prognostic value of the morning blood pressure surge in 5645 subjects from 8 populations. *Hypertension* 2010 55(4):1040-8.

348. Stolarz-Skrzypek K, Thijs L, Richart T, Li Y, Hansen TW, Boggia J, et al. Blood pressure variability in relation to outcome in the International Database of Ambulatory blood pressure in relation to Cardiovascular Outcome. *Hypertens Res* 2010 33(8):757-66.

349. Clement DL, De Buyzere ML, De Bacquer DA, de Leeuw PW, Duprez DA, Fagard RH, et al. Prognostic value of ambulatory blood-pressure recordings in patients with treated hypertension. *N Engl J Med* 2003;348(24):2407-15.

350. Salles GF, Cardoso CR, Muxfeldt ES. Prognostic influence of office and ambulatory blood pressures in resistant hypertension. *Arch Intern Med* 2008;168(21):2340-6.

351. Mancia G, Bombelli M, Facchetti R, Madotto F, Quarti-Trevano F, Polo Friz H, et al. Long-term risk of sustained hypertension in white-coat or masked hypertension. *Hypertension* 2009;54(2):226-32.

352. Pickering TG, Eguchi K, Kario K. Masked hypertension: a review. *Hypertens Res* 2007;30(6):479-88.

353. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, et al. 2007 ESH-ESC Practice Guidelines for the Management of Arterial Hypertension: ESH-ESC Task Force on the Management of Arterial Hypertension. *J Hypertens* 2007;25(9):1751-62.

354. Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Baou K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. *Eur Heart J* 2010;31(15):1865-71.

355. Thompson AM, Pickering TG. The role of ambulatory blood pressure monitoring in chronic and end-stage renal disease. *Kidney Int* 2006;70(6):1000-7.

356. Pickering TG, Shimbo D, Haas D. Ambulatory blood-pressure monitoring. *N Engl J Med* 2006;354(22):2368-74.

357. Dolan E, Stanton AV, Thom S, Caulfield M, Atkins N, McInnes G, et al. Ambulatory blood pressure monitoring predicts cardiovascular events in treated hypertensive patients--an Anglo-Scandinavian cardiac outcomes trial substudy. *J Hypertens* 2009;27(4):876-85.

358. Hsieh YT, Tu ST, Cho TJ, Chang SJ, Chen JF, Hsieh MC. Visit-to-visit variability in blood pressure strongly predicts all-cause mortality in patients with type 2 diabetes: a 5.5-year prospective analysis. *Eur J Clin Invest* 2012.

359. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlof B, et al. Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. *Lancet* 2010;375(9718):895-905.

360. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlof B, et al. Effects of beta blockers and calcium-channel blockers on within-individual variability in blood pressure and risk of stroke. *Lancet Neurol* 2010;9(5):469-80.

361. Webb AJ, Fischer U, Mehta Z, Rothwell PM. Effects of antihypertensive-drug class on interindividual variation in blood pressure and risk of stroke: a systematic review and meta-analysis. *Lancet* 2010 375(9718):906-15.

362. Rothwell PM. Limitations of the usual blood-pressure hypothesis and importance of variability, instability, and episodic hypertension. *Lancet* 2010;375(9718):938-48.

363. Webb AJ, Rothwell PM. Effect of dose and combination of antihypertensives on interindividual blood pressure variability: a systematic review. *Stroke* 2011;42(10):2860-5.

364. Arguedas JA, Perez MI, Wright JM. Treatment blood pressure targets for hypertension. *Cochrane Database Syst Rev* 2009(3):CD004349.

365. Cushman WC, Evans GW, Byington RP, Goff DC, Jr., Grimm RH, Jr., Cutler JA, et al. Effects of intensive blood-pressure control in type 2 diabetes mellitus. *N Engl J Med* 2010;362(17):1575-85.

366. Somes GW, Pahor M, Shorr RI, Cushman WC, Applegate WB. The role of diastolic blood pressure when treating isolated systolic hypertension. *Arch Intern Med* 1999;159(17):2004-9.

367. Seedat YK, Parag KB. A comparison of lisinopril and atenolol in black and Indian patients with mild-to-moderate essential hypertension. *S Afr Med J* 1987;71(3):149-53.

368. Holland OB, Gomez-Sanchez CE, Kuhnert LV, Poindexter C, Pak CY. Antihypertensive comparison of furosemide with hydrochlorothiazide for black patients. *Arch Intern Med* 1979;139(9):1015-21.

369. Cruickshank JK, Anderson NM, Wadsworth J, Young SM, Jepson E. Treating hypertension in black compared with white non-insulin dependent diabetics: a double blind trial of verapamil and metoprolol. *BMJ* 1988;297(6657):1155-9.

370. Nguyen TT, Kaufman JS, Whitsel EA, Cooper RS. Racial differences in blood pressure response to calcium channel blocker monotherapy: a meta-analysis. *Am J Hypertens* 2009;22(8):911-7.

371. Preston RA, Materson BJ, Reda DJ, Williams DW, Hamburger RJ, Cushman WC, et al. Age-race subgroup compared with renin profile as predictors of blood pressure response to antihypertensive therapy. Department of Veterans Affairs Cooperative Study Group on Antihypertensive Agents. *JAMA* 1998;280(13):1168-72.

372. Park IU, Taylor AL. Race and ethnicity in trials of antihypertensive therapy to prevent cardiovascular outcomes: a systematic review. *Ann Fam Med* 2007;5(5):444-52.

373. Wright JT, Jr., Dunn JK, Cutler JA, Davis BR, Cushman WC, Ford CE, et al. Outcomes in hypertensive black and nonblack patients treated with chlorthalidone, amlodipine, and lisinopril. *JAMA* 2005;293(13):1595-608.

374. Julius S, Alderman MH, Beevers G, Dahlof B, Devereux RB, Douglas JG, et al. Cardiovascular risk reduction in hypertensive black patients with left ventricular hypertrophy: the LIFE study. *J Am Coll Cardiol* 2004;43(6):1047-55.

375. Gupta AK, Poulter NR, Dobson J, Eldridge S, Cappuccio FP, Caulfield M, et al. Ethnic differences in blood pressure response to first and second-line antihypertensive therapies in patients randomized in the ASCOT Trial. *Am J Hypertens* 2010 23(9):1023-30.

376. Wright JT, Jr., Bakris G, Greene T, Agodoa LY, Appel LJ, Charleston J, et al. Effect of blood pressure lowering and antihypertensive drug class on progression of hypertensive kidney disease: results from the AASK trial. *JAMA* 2002;288(19):2421-31.

377. Brown NJ, Ray WA, Snowden M, Griffin MR. Black Americans have an increased rate of angiotensin converting enzyme inhibitor-associated angioedema. *Clin Pharmacol Ther* 1996;60(1):8-13.

378. Jeemon P, Prabhakaran D, Goenka S, Ramakrishnan L, Padmanabhan S, Huffman M, et al. Impact of comprehensive cardiovascular risk reduction programme on risk factor clustering associated with elevated blood pressure in an Indian industrial population. *Indian J Med Res* 2011;135(4):485-93.

379. Kannel WB. Risk stratification in hypertension: new insights from the Framingham Study. *Am J Hypertens* 2000;13(1 Pt 2):3S-10S.

380. Scholze J, Alegria E, Ferri C, Langham S, Stevens W, Jeffries D, et al. Epidemiological and economic burden of metabolic syndrome and its consequences in patients with hypertension in Germany, Spain and Italy; a prevalence-based model. *BMC Public Health* 2010 10:529.

381. Couser WG, Remuzzi G, Mendis S, Tonelli M. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. *Kidney Int* 2011.

382. Klahr S. The kidney in hypertension--villain and victim. *N Engl J Med* 1989;320(11):731-3.

383. Ritz E, Bakris G. World Kidney Day: hypertension and chronic kidney disease. *Lancet* 2009;373(9670):1157-8.

384. Collins AJ, Foley RN, Herzog C, Chavers B, Gilbertson D, Ishani A, et al. United States Renal Data System 2008 Annual Data Report. *Am J Kidney Dis* 2009;53(1 Suppl):S1-374.

385. Sarafidis PA, Li S, Chen SC, Collins AJ, Brown WW, Klag MJ, et al. Hypertension awareness, treatment, and control in chronic kidney disease. *Am J Med* 2008;121(4):332-40.

386. Khosla N, Bakris G. Lessons learned from recent hypertension trials about kidney disease. *Clin J Am Soc Nephrol* 2006;1(2):229-35.

387. Skjelbakken T, Wilsgaard T, Forde OH, Arnesen E, Lochen ML. Haemoglobin predicts total mortality in a general young and middle-aged male population. The Tromso Study. *Scand J Clin Lab Invest* 2006;66(7):567-76.

388. Guyton AC, Richardson TQ. Effect of hematocrit on venous return. *Circ Res* 1961;9:157-64.

389. Hellem AJ, Borchgrevink CF, Ames SB. The role of red cells in haemostasis: the relation between haematocrit, bleeding time and platelet adhesiveness. *Br J Haematol* 1961;7:42-50.

390. Lowe GD, Robertson J, Dunn FG, Lorimer AR, Forbes CD. Haematocrit in patients attending a hypertension clinic. *Scott Med J* 1985;30(3):168-72.

391. Lowe GD. Blood rheology in general medicine and surgery. *Baillieres Clin Haematol* 1987;1(3):827-61.

392. Vazquez BY, Martini J, Tsai AG, Johnson PC, Cabrales P, Intaglietta M. The variability of blood pressure due to small changes of hematocrit. *Am J Physiol Heart Circ Physiol* 2010 299(3):H863-7.

393. Brown DW, Giles WH, Croft JB. Hematocrit and the risk of coronary heart disease mortality. *Am Heart J* 2001;142(4):657-63.

394. Danesh J, Collins R, Peto R, Lowe GD. Haematocrit, viscosity, erythrocyte sedimentation rate: meta-analyses of prospective studies of coronary heart disease. *Eur Heart J* 2000;21(7):515-20.

395. Gagnon DR, Zhang TJ, Brand FN, Kannel WB. Hematocrit and the risk of cardiovascular disease--the Framingham study: a 34-year follow-up. *Am Heart J* 1994;127(3):674-82.

396. Kunnas T, Solakivi T, Huuskonen K, Kalela A, Renko J, Nikkari ST. Hematocrit and the risk of coronary heart disease mortality in the TAMRISK study, a 28-year follow-up. *Prev Med* 2009;49(1):45-7.

397. Wannamethee G, Perry IJ, Shaper AG. Haematocrit, hypertension and risk of stroke. *J Intern Med* 1994;235(2):163-8.

398. Pizzi C, De Stavola BL, Meade TW. Long-term association of routine blood count (Coulter) variables on fatal coronary heart disease: 30-year results from the first prospective Northwick Park Heart Study (NPHS-I). *Int J Epidemiol* 2010 39(1):256-65.

399. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002;39(2 Suppl 1):S1-266.

400. Wen CP, Cheng TY, Tsai MK, Chang YC, Chan HT, Tsai SP, et al. All-cause mortality attributable to chronic kidney disease: a prospective cohort study based on 462 293 adults in Taiwan. *Lancet* 2008;371(9631):2173-82.

401. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004;351(13):1296-305.

402. Astor BC, Hallan SI, Miller ER, 3rd, Yeung E, Coresh J. Glomerular filtration rate, albuminuria, and risk of cardiovascular and all-cause mortality in the US population. *Am J Epidemiol* 2008;167(10):1226-34.

403. Ford I, Bezlyak V, Stott DJ, Sattar N, Packard CJ, Perry I, et al. Reduced glomerular filtration rate and its association with clinical outcome in older patients at risk of vascular events: secondary analysis. *PLoS Med* 2009;6(1):e16.

404. Cheng TY, Wen SF, Astor BC, Tao XG, Samet JM, Wen CP. Mortality risks for all causes and cardiovascular diseases and reduced GFR in a middle-aged working population in Taiwan. *Am J Kidney Dis* 2008;52(6):1051-60.

405. Manjunath G, Tighiouart H, Ibrahim H, MacLeod B, Salem DN, Griffith JL, et al. Level of kidney function as a risk factor for atherosclerotic cardiovascular outcomes in the community. *J Am Coll Cardiol* 2003;41(1):47-55.

406. Hillege HL, Fidler V, Diercks GF, van Gilst WH, de Zeeuw D, van Veldhuisen DJ, et al. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation* 2002;106(14):1777-82.

407. Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet* 2010 375(9731):2073-81.

408. Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet*;375(9731):2073-81.

409. Liese AD, Hense HW, Lowel H, Doring A, Tietze M, Keil U. Association of serum uric acid with all-cause and cardiovascular disease mortality and incident myocardial infarction in the MONICA Augsburg cohort. World Health Organization Monitoring Trends and Determinants in Cardiovascular Diseases. *Epidemiology* 1999;10(4):391-7.

410. Meisinger C, Koenig W, Baumert J, Doring A. Uric acid levels are associated with all-cause and cardiovascular disease mortality independent of systemic inflammation in men from the general population: the MONICA/KORA cohort study. *Arterioscler Thromb Vasc Biol* 2008;28(6):1186-92.

411. Fang J, Alderman MH. Serum uric acid and cardiovascular mortality the NHANES I epidemiologic follow-up study, 1971-1992. National Health and Nutrition Examination Survey. *JAMA* 2000;283(18):2404-10.

412. Hakoda M, Masunari N, Yamada M, Fujiwara S, Suzuki G, Kodama K, et al. Serum uric acid concentration as a risk factor for cardiovascular mortality: a longterm cohort study of atomic bomb survivors. *J Rheumatol* 2005;32(5):906-12.

413. Strasak A, Ruttmann E, Brant L, Kelleher C, Klenk J, Concin H, et al. Serum uric acid and risk of cardiovascular mortality: a prospective long-term study of 83,683 Austrian men. *Clin Chem* 2008;54(2):273-84.

414. Strasak AM, Kelleher CC, Brant LJ, Rapp K, Ruttmann E, Concin H, et al. Serum uric acid is an independent predictor for all major forms of cardiovascular death in 28,613 elderly women: a prospective 21-year follow-up study. *Int J Cardiol* 2008;125(2):232-9.

415. Gerber Y, Tanne D, Medalie JH, Goldbourt U. Serum uric acid and long-term mortality from stroke, coronary heart disease and all causes. *Eur J Cardiovasc Prev Rehabil* 2006;13(2):193-8.

416. Chen JH, Chuang SY, Chen HJ, Yeh WT, Pan WH. Serum uric acid level as an independent risk factor for all-cause, cardiovascular, and ischemic stroke mortality: a Chinese cohort study. *Arthritis Rheum* 2009;61(2):225-32.

417. Kim SY, Guevara JP, Kim KM, Choi HK, Heitjan DF, Albert DA. Hyperuricemia and coronary heart disease: a systematic review and meta-analysis. *Arthritis Care Res (Hoboken)* 2010 62(2):170-80.

418. Kim SY, Guevara JP, Kim KM, Choi HK, Heitjan DF, Albert DA. Hyperuricemia and risk of stroke: a systematic review and meta-analysis. *Arthritis Rheum* 2009;61(7):885-92.

419. Hsu SP, Pai MF, Peng YS, Chiang CK, Ho TI, Hung KY. Serum uric acid levels show a 'J-shaped' association with all-cause mortality in haemodialysis patients. *Nephrol Dial Transplant* 2004;19(2):457-62.

420. Bickel C, Rupprecht HJ, Blankenberg S, Rippin G, Hafner G, Daunhauer A, et al. Serum uric acid as an independent predictor of mortality in patients with

angiographically proven coronary artery disease. *Am J Cardiol* 2002;89(1):12-7.

421. Tsai TH, Chen YL, Chen SM, Yang CH, Fang CY, Hsieh YK, et al. Uric Acid is not an independent predictor of cardiovascular death in patients with angiographically proven coronary artery disease. *Chang Gung Med J* 2009;32(6):605-13.

422. Tsai WC, Huang YY, Lin CC, Li WT, Lee CH, Chen JY, et al. Uric acid is an independent predictor of arterial stiffness in hypertensive patients. *Heart Vessels* 2009;24(5):371-5.

423. Okura T, Higaki J, Kurata M, Irita J, Miyoshi K, Yamazaki T, et al. Elevated serum uric acid is an independent predictor for cardiovascular events in patients with severe coronary artery stenosis: subanalysis of the Japanese Coronary Artery Disease (JCAD) Study. *Circ J* 2009;73(5):885-91.

424. Brodov Y, Behar S, Goldenberg I, Boyko V, Chouraqui P. Usefulness of combining serum uric acid and C-reactive protein for risk stratification of patients with coronary artery disease (Bezafibrate Infarction Prevention [BIP] study). *Am J Cardiol* 2009;104(2):194-8.

425. Kojima S, Sakamoto T, Ishihara M, Kimura K, Miyazaki S, Yamagishi M, et al. Prognostic usefulness of serum uric acid after acute myocardial infarction (the Japanese Acute Coronary Syndrome Study). *Am J Cardiol* 2005;96(4):489-95.

426. Ioachimescu AG, Brennan DM, Hoar BM, Hazen SL, Hoogwerf BJ. Serum uric acid is an independent predictor of all-cause mortality in patients at high risk of cardiovascular disease: a preventive cardiology information system (PreCIS) database cohort study. *Arthritis Rheum* 2008;58(2):623-30.

427. Alimonda AL, Nunez J, Nunez E, Husser O, Sanchis J, Bodi V, et al. Hyperuricemia in acute heart failure. More than a simple spectator? *Eur J Intern Med* 2009;20(1):74-9.

428. Tamariz L, Harzand A, Palacio A, Verma S, Jones J, Hare J. Uric acid as a predictor of all-cause mortality in heart failure: a meta-analysis. *Congest Heart Fail* 2011;17(1):25-30.

429. Iwashima Y, Horio T, Kamide K, Rakugi H, Ogihara T, Kawano Y. Uric acid, left ventricular mass index, and risk of cardiovascular disease in essential hypertension. *Hypertension* 2006;47(2):195-202.

430. Ong G, Davis WA, Davis TM. Serum uric acid does not predict cardiovascular or all-cause mortality in type 2 diabetes: the Fremantle Diabetes Study. *Diabetologia* 2010 53(7):1288-94.

431. Zoppini G, Targher G, Negri C, Stoico V, Perrone F, Muggeo M, et al. Elevated serum uric acid concentrations independently predict cardiovascular mortality in type 2 diabetic patients. *Diabetes Care* 2009;32(9):1716-20.

432. Ndrepepa G, Braun S, King L, Cassese S, Tada T, Fusaro M, et al. Prognostic Value of Uric Acid in Patients with Type 2 Diabetes Mellitus and Coronary Artery Disease. *Clin Sci (Lond)* 2012.

433. Sigrist MK, Taal MW, Bungay P, McIntyre CW. Progressive vascular calcification over 2 years is associated with arterial stiffening and increased mortality in patients with stages 4 and 5 chronic kidney disease. *Clin J Am Soc Nephrol* 2007;2(6):1241-8.

434. Adeney KL, Siscovick DS, Ix JH, Seliger SL, Shlipak MG, Jenny NS, et al. Association of serum phosphate with vascular and valvular calcification in moderate CKD. *J Am Soc Nephrol* 2009;20(2):381-7.

435. Block GA, Hulbert-Shearon TE, Levin NW, Port FK. Association of serum phosphorus and calcium x phosphate product with mortality risk in chronic hemodialysis patients: a national study. *Am J Kidney Dis* 1998;31(4):607-17.

436. Ganesh SK, Stack AG, Levin NW, Hulbert-Shearon T, Port FK. Association of elevated serum PO(4), Ca x PO(4) product, and parathyroid hormone with cardiac mortality risk in chronic hemodialysis patients. *J Am Soc Nephrol* 2001;12(10):2131-8.

437. Stevens LA, Djurdjev O, Cardew S, Cameron EC, Levin A. Calcium, phosphate, and parathyroid hormone levels in combination and as a function of dialysis duration predict mortality: evidence for the complexity of the association between mineral metabolism and outcomes. *J Am Soc Nephrol* 2004;15(3):770-9.

438. Slinin Y, Foley RN, Collins AJ. Calcium, phosphorus, parathyroid hormone, and cardiovascular disease in hemodialysis patients: the USRDS waves 1, 3, and 4 study. *J Am Soc Nephrol* 2005;16(6):1788-93.

439. Kestenbaum B, Sampson JN, Rudser KD, Patterson DJ, Seliger SL, Young B, et al. Serum phosphate levels and mortality risk among people with chronic kidney disease. *J Am Soc Nephrol* 2005;16(2):520-8.

440. Covic A, Kothawala P, Bernal M, Robbins S, Chalian A, Goldsmith D. Systematic review of the evidence underlying the association between mineral metabolism disturbances and risk of all-cause mortality, cardiovascular mortality and cardiovascular events in chronic kidney disease. *Nephrol Dial Transplant* 2009;24(5):1506-23.

441. Palmer SC, Hayen A, Macaskill P, Pellegrini F, Craig JC, Elder GJ, et al. Serum levels of phosphorus, parathyroid hormone, and calcium and risks of death and cardiovascular disease in individuals with chronic kidney disease: a systematic review and meta-analysis. *JAMA* 2011;305(11):1119-27.

442. Mathew S, Tustison KS, Sugatani T, Chaudhary LR, Rifas L, Hruska KA. The mechanism of phosphorus as a cardiovascular risk factor in CKD. *J Am Soc Nephrol* 2008;19(6):1092-105.

443. Kendrick J, Chonchol M. The Role of Phosphorus in the Development and Progression of Vascular Calcification. *Am J Kidney Dis* 2011.

444. Tonelli M, Sacks F, Pfeffer M, Gao Z, Curhan G. Relation between serum phosphate level and cardiovascular event rate in people with coronary disease. *Circulation* 2005;112(17):2627-33.

445. Chonchol M, Dale R, Schrier RW, Estacio R. Serum phosphorus and cardiovascular mortality in type 2 diabetes. *Am J Med* 2009;122(4):380-6.

446. Abramowitz M, Muntner P, Coco M, Southern W, Lotwin I, Hostetter TH, et al. Serum alkaline phosphatase and phosphate and risk of mortality and hospitalization. *Clin J Am Soc Nephrol* 2010;5(6):1064-71.

447. Onufrak SJ, Bellasi A, Shaw LJ, Herzog CA, Cardarelli F, Wilson PW, et al. Phosphorus levels are associated with subclinical atherosclerosis in the general population. *Atherosclerosis* 2008;199(2):424-31.

448. Ruan L, Chen W, Srinivasan SR, Xu J, Toprak A, Berenson GS. Relation of serum phosphorus levels to carotid intima-media thickness in asymptomatic young adults (from the Bogalusa Heart Study). *Am J Cardiol* 2010 106(6):793-7.

449. Dhingra R, Sullivan LM, Fox CS, Wang TJ, D'Agostino RB, Sr., Gaziano JM, et al. Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. *Arch Intern Med* 2007;167(9):879-85.

450. Foley RN, Collins AJ, Ishani A, Kalra PA. Calcium-phosphate levels and cardiovascular disease in community-dwelling adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am Heart J* 2008;156(3):556-63.

451. Larsson TE, Olauson H, Hagstrom E, Ingelsson E, Arnlov J, Lind L, et al. Conjoint effects of serum calcium and phosphate on risk of total, cardiovascular, and noncardiovascular mortality in the community. *Arterioscler Thromb Vasc Biol* 2010 30(2):333-9.

452. Ellam TJ, Chico TJ. Phosphate: The new cholesterol? The role of the phosphate axis in non-uremic vascular disease. *Atherosclerosis* 2012

453. Hruska K, Mathew S, Lund R, Fang Y, Sugatani T. Cardiovascular risk factors in chronic kidney disease: does phosphate qualify? *Kidney Int Suppl* 2011(121):S9-13.

454. Slinin Y, Blackwell T, Ishani A, Cummings SR, Ensrud KE. Serum calcium, phosphorus and cardiovascular events in post-menopausal women. *Int J Cardiol* 2011;149(3):335-40.

455. Tentori F, Blayney MJ, Albert JM, Gillespie BW, Kerr PG, Bommer J, et al. Mortality risk for dialysis patients with different levels of serum calcium, phosphorus, and PTH: the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Am J Kidney Dis* 2008;52(3):519-30.

456. Naves-Diaz M, Passlick-Deetjen J, Guinsburg A, Marelli C, Fernandez-Martin JL, Rodriguez-Puyol D, et al. Calcium, phosphorus, PTH and death rates in a large sample of dialysis patients from Latin America. The CORES Study. *Nephrol Dial Transplant* 2011;26(6):1938-47.

457. Miller JE, Kovesdy CP, Norris KC, Mehrotra R, Nissenson AR, Kopple JD, et al. Association of cumulatively low or high serum calcium levels with mortality in long-term hemodialysis patients. *Am J Nephrol* 2010 32(5):403-13.

458. Slinin Y, Blackwell T, Ishani A, Cummings SR, Ensrud KE. Serum calcium, phosphorus and cardiovascular events in post-menopausal women. *Int J Cardiol*;149(3):335-40.

459. Larsson TE, Olauson H, Hagstrom E, Ingelsson E, Arnlov J, Lind L, et al. Conjoint effects of serum calcium and phosphate on risk of total, cardiovascular, and noncardiovascular mortality in the community. *Arterioscler Thromb Vasc Biol*;30(2):333-9.

460. Mazza A, Pessina AC, Pavei A, Scarpa R, Tikhonoff V, Casiglia E. Predictors of stroke mortality in elderly people from the general population. The CArdiovascular STudy in the ELderly. *Eur J Epidemiol* 2001;17(12):1097-104.

461. Fang J, Madhavan S, Cohen H, Alderman MH. Serum potassium and cardiovascular mortality. *J Gen Intern Med* 2000;15(12):885-90.

462. Wannamethee SG, Lever AF, Shaper AG, Whincup PH. Serum potassium, cigarette smoking, and mortality in middle-aged men. *Am J Epidemiol* 1997;145(7):598-606.

463. Jia EZ, Xu ZX, Yang ZJ, Zhu TB, Wang LS, Chen B, et al. Association of serum sodium concentration with coronary atherosclerosis in China: follow-up study. *Acta Pharmacol Sin* 2009;30(4):494-500.

464. Mehta PA, Dubrey SW, McIntyre HF, Walker DM, Hardman SM, Sutton GC, et al. Mode of death in patients with newly diagnosed heart failure in the general population. *Eur J Heart Fail* 2008;10(11):1108-16.

465. Mazza A, Tikhonoff V, Casiglia E, Pessina AC. Predictors of congestive heart failure mortality in elderly people from the general population. *Int Heart J* 2005;46(3):419-31.

466. Nolan J, Batin PD, Andrews R, Lindsay SJ, Brooksby P, Mullen M, et al. Prospective study of heart rate variability and mortality in chronic heart failure: results of the United Kingdom heart failure evaluation and assessment of risk trial (UK-heart). *Circulation* 1998;98(15):1510-6.

467. De Bacquer D, De Backer G, De Buyzere M, Kornitzer M. Is low serum chloride level a risk factor for cardiovascular mortality? *J Cardiovasc Risk* 1998;5(3):177-84.

468. Kovesdy CP, Anderson JE, Kalantar-Zadeh K. Association of serum bicarbonate levels with mortality in patients with non-dialysis-dependent CKD. *Nephrol Dial Transplant* 2009;24(4):1232-7.

469. Menon V, Tighiouart H, Vaughn NS, Beck GJ, Kusek JW, Collins AJ, et al. Serum bicarbonate and long-term outcomes in CKD. *Am J Kidney Dis* 2010 56(5):907-14.

470. Navaneethan SD, Schold JD, Arrigain S, Jolly SE, Wehbe E, Raina R, et al. Serum bicarbonate and mortality in stage 3 and stage 4 chronic kidney disease. *Clin J Am Soc Nephrol* 2011;6(10):2395-402.

471. Raphael KL, Wei G, Baird BC, Greene T, Beddhu S. Higher serum bicarbonate levels within the normal range are associated with better survival and renal outcomes in African Americans. *Kidney Int* 2011;79(3):356-62.

472. Sidorenkov O, Nilssen O, Grjibovski AM. Determinants of Cardiovascular and All-Cause Mortality in Northwest Russia: A 10-Year Follow-Up Study. *Ann Epidemiol* 2012.

473. Zitt E, Lamina C, Sturm G, Knoll F, Lins F, Freistatter O, et al. Interaction of Time-Varying Albumin and Phosphorus on Mortality in Incident Dialysis Patients. *Clin J Am Soc Nephrol* 2011.

474. Amemiya N, Ogawa T, Otsuka K, Ando Y, Nitta K. Comparison of Serum Albumin, Serum C-Reactive Protein, and Pulse Wave Velocity as Predictors of the 4-Year Mortality of Chronic Hemodialysis Patients. *J Atheroscler Thromb* 2011.

475. Desai AA, Nissenson A, Chertow GM, Farid M, Singh I, Van Oijen MG, et al. The relationship between laboratory-based outcome measures and mortality in end-stage renal disease: a systematic review. *Hemodial Int* 2009;13(3):347-59.

476. Tonelli M, Curhan G, Pfeffer M, Sacks F, Thadhani R, Melamed ML, et al. Relation between alkaline phosphatase, serum phosphate, and all-cause or cardiovascular mortality. *Circulation* 2009;120(18):1784-92.

477. Krishnamurthy VR, Baird BC, Wei G, Greene T, Raphael K, Beddhu S. Associations of serum alkaline phosphatase with metabolic syndrome and mortality. *Am J Med* 2011;124(6):566 e1-7.

478. Abramowitz M, Muntner P, Coco M, Southern W, Lotwin I, Hostetter TH, et al. Serum alkaline phosphatase and phosphate and risk of mortality and hospitalization. *Clin J Am Soc Nephrol*;5(6):1064-71.

479. Han SS, Na KY, Chae DW, Kim YS, Kim S, Chin HJ. Survival benefit of high serum bilirubin and low alkaline phosphatase in older adults. *J Am Geriatr Soc* 2010 58(7):1413-5.

480. Lofthus DM, Stevens SR, Armstrong PW, Granger CB, Mahaffey KW. Pattern of liver enzyme elevations in acute ST-elevation myocardial infarction. *Coron Artery Dis* 2012 23(1):22-30.

481. Fleming KM, West J, Aithal GP, Fletcher AE. Abnormal liver tests in people aged 75 and above: prevalence and association with mortality. *Aliment Pharmacol Ther* 2011 34(3):324-34.

482. Hovinen SM, Pitkala KH, Tilvis RS, Strandberg TE. Alanine aminotransferase activity and mortality in older people. *J Am Geriatr Soc* 2010 58(7):1399-401.

483. Ruhl CE, Everhart JE. Elevated serum alanine aminotransferase and gamma-glutamyltransferase and mortality in the United States population. *Gastroenterology* 2009;136(2):477-85 e11.

484. Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. *J Hepatol* 2008;49(4):608-12.

485. Dunn W, Xu R, Wingard DL, Rogers C, Angulo P, Younossi ZM, et al. Suspected nonalcoholic fatty liver disease and mortality risk in a population-based cohort study. *Am J Gastroenterol* 2008;103(9):2263-71.

486. Ruhl CE, Everhart JE. Non-alcoholic fatty liver disease (NAFLD) and mortality. *J Hepatol* 2009;51(3):593; author reply 94-5.

487. Ford I, Mooijaart SP, Lloyd S, Murray HM, Westendorp RG, de Craen AJ, et al. The inverse relationship between alanine aminotransferase in the normal range and adverse cardiovascular and non-cardiovascular outcomes. *Int J Epidemiol* 2011;40(6):1530-8.

488. Sluik D, Beulens JW, Weikert C, van Dieren S, Spijkerman AM, van der AD, et al. Gamma-glutamyltransferase, cardiovascular disease and mortality in individuals with diabetes mellitus. *Diabetes Metab Res Rev* 2012

489. Wannamethee SG, Lennon L, Shaper AG. The value of gamma-glutamyltransferase in cardiovascular risk prediction in men without diagnosed cardiovascular disease or diabetes. *Atherosclerosis* 2008;201(1):168-75.

490. Strasak AM, Kelleher CC, Klenk J, Brant LJ, Ruttmann E, Rapp K, et al. Longitudinal change in serum gamma-glutamyltransferase and cardiovascular disease mortality: a prospective population-based study in 76,113 Austrian adults. *Arterioscler Thromb Vasc Biol* 2008;28(10):1857-65.

491. Ruttmann E, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H. Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality: an epidemiological investigation in a cohort of 163,944 Austrian adults. *Circulation* 2005;112(14):2130-7.

492. Targher G. Elevated serum gamma-glutamyltransferase activity is associated with increased risk of mortality, incident type 2 diabetes, cardiovascular events, chronic kidney disease and cancer - a narrative review. *Clin Chem Lab Med* 2010 48(2):147-57.

493. Schwertner HA, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. *Clin Chem* 1994;40(1):18-23.

494. Hopkins PN, Wu LL, Hunt SC, James BC, Vincent GM, Williams RR. Higher serum bilirubin is associated with decreased risk for early familial coronary artery disease. *Arterioscler Thromb Vasc Biol* 1996;16(2):250-5.

495. Breimer LH, Wannamethee G, Ebrahim S, Shaper AG. Serum bilirubin and risk of ischemic heart disease in middle-aged British men. *Clin Chem* 1995;41(10):1504-8.

496. Zucker SD, Horn PS, Sherman KE. Serum bilirubin levels in the U.S. population: gender effect and inverse correlation with colorectal cancer. *Hepatology* 2004;40(4):827-35.

497. Horsfall LJ, Rait G, Walters K, Swallow DM, Pereira SP, Nazareth I, et al. Serum bilirubin and risk of respiratory disease and death. *JAMA* 2011;305(7):691-7.

498. Members of Glasgow Blood Pressure Clinic. The Glasgow blood pressure clinic. *J R Coll Physicians Lond* 1972;7(1):87-97.

499. Kennedy F. SWITCH--hospital case history on computer. *Scott Med J* 1970;15(11):391-4.

500. Kennedy F, Cleary JJ, Roy AD, Kay AW. "Switch": a system producing a full hospital case-history on computer. *Lancet* 1968;2(7580):1230-3.

501. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999;130(6):461-70.

502. World Health Organization. List of Official ICD 10 Updates World Health Organization 2011.

503. World Health Organization. ICD-10 Version: World Health Organization 2010

504. Isles CG, Walker LM, Beevers GD, Brown I, Cameron HL, Clarke J, et al. Mortality in patients of the Glasgow Blood Pressure Clinic. *J Hypertens* 1986;4(2):141-56.

505. World Health Organization. International Classification of Diseases (ICD): World Health Organization 2012.

506. World Health Organization. The global burden of disease: 2004 update. Geneva: World Health Organization, 2008.

507. Prinja S, Gupta N, Verma R. Censoring in clinical trials: review of survival analysis techniques. *Indian J Community Med* 2010;35(2):217-21.

508. Bland JM, Altman DG. Survival probabilities (the Kaplan-Meier method). *BMJ* 1998;317(7172):1572.

509. Clark TG, Bradburn MJ, Love SB, Altman DG. Survival analysis part I: basic concepts and first analyses. *Br J Cancer* 2003;89(2):232-8.

510. Bland JM, Altman DG. The logrank test. *BMJ* 2004;328(7447):1073.

511. Bewick V, Cheek L, Ball J. Statistics review 12: survival analysis. *Crit Care* 2004;8(5):389-94.

512. Swinscow TDV, Campbell MJ, . *Statistics at Square One* London BMJ Books 2002.

513. Pocock SJ, Clayton TC, Altman DG. Survival plots of time-to-event outcomes in clinical trials: good practice and pitfalls. *Lancet* 2002;359(9318):1686-9.

514. Stel VS, Dekker FW, Tripepi G, Zoccali C, Jager KJ. Survival analysis I: the Kaplan-Meier method. *Nephron Clin Pract* 2011;119(1):c83-8.

515. Freedman LS. Tables of the number of patients required in clinical trials using the logrank test. *Stat Med* 1982;1(2):121-9.

516. Cox D, R. . Regression models and life-tables. . *Journal of the Royal Statistical Society* 1972;34(Series B):187-220.

517. Bender R, Augustin T, Blettner M. Generating survival times to simulate Cox proportional hazards models. *Stat Med* 2005;24(11):1713-23.

518. Bradburn MJ, Clark TG, Love SB, Altman DG. Survival analysis Part III: multivariate data analysis -- choosing a model and assessing its adequacy and fit. *Br J Cancer* 2003;89(4):605-11.

519. Bradburn MJ, Clark TG, Love SB, Altman DG. Survival analysis part II: multivariate data analysis--an introduction to concepts and methods. *Br J Cancer* 2003;89(3):431-6.

520. Breslow N. Covariance analysis of censored survival data. *Biometrics* 1974;30(1):89-99.

521. Efron B. The efficiency of Cox's likelihood function for censored data. *J Am Stat Assoc* 1997;72(359):557-65.

522. Hsieh FY, Lavori PW. Sample-size calculations for the Cox proportional hazards regression model with nonbinary covariates. *Control Clin Trials* 2000;21(6):552-60.

523. Bourdais-Mannone C, Quantin C, Abrahamowicz M, Mannone L, Brunet-Lecomte P, Aho LS, et al. Testing the proportional hazards assumption in medical survival studies--application to a population-based study of acute myeloid leukaemia. *J Epidemiol Biostat* 1999;4(2):83-92.

524. Kleinbaum DG, Klein M. *Survival Analysis: A Self-Learning Text* New York Springer 2005.

525. Royston P, Sauerbrei W. Multivariable modeling with cubic regression splines: A principled approach. *Stata Journal* 2007;7(1):45-70.

526. Clark TG, Bradburn MJ, Love SB, Altman DG. Survival analysis part IV: further concepts and methods in survival analysis. *Br J Cancer* 2003;89(5):781-6.

527. Hardin JW, Hilbe JM. *Generalized Estimating Equations*. London Chapman & Hall/CRC 2003.

528. Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. *Biometrics* 1988;44(4):1049-60.

529. Hubbard AE, Ahern J, Fleischer NL, Van der Laan M, Lippman SA, Jewell N, et al. To GEE or not to GEE: comparing population average and mixed models for estimating the associations between neighborhood risk factors and health. *Epidemiology* 2010 21(4):467-74.

530. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* 1986;42(1):121-30.

531. Noon JP, Walker BR, Webb DJ, Shore AC, Holton DW, Edwards HV, et al. Impaired microvascular dilatation and capillary rarefaction in young adults with a predisposition to high blood pressure. *J Clin Invest* 1997;99(8):1873-9.

532. Chen PY, Popovich PM. *Correlation: Parametric and nonparametric measures* Thousand Oaks, CA: Sage Publications 2002.

533. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1(8476):307-10.

534. Bland JM, Altman DG. A note on the use of the intraclass correlation coefficient in the evaluation of agreement between two methods of measurement. *Comput Biol Med* 1990;20(5):337-40.

535. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;33(1):159-74.

536. Cramer H. *Mathematical Methods of Statistics*. Princeton Princeton University Press, 1999.

537. Soueidan K, Chen S, Dajani HR, Bolic M, Groza V. Augmented blood pressure measurement through the noninvasive estimation of physiological arterial pressure variability. *Physiol Meas* 2012 33(6):881-99.

538. Watson RD, Stallard TJ, Littler WA. Factors determining the variability of arterial pressure in hypertension. *Clin Sci (Lond)* 1979;57 Suppl 5:283s-85s.

539. Musini VM, Wright JM. Factors affecting blood pressure variability: lessons learned from two systematic reviews of randomized controlled trials. *PLoS One* 2009;4(5):e5673.

540. Sander D, Kukla C, Klingelhofer J, Winbeck K, Conrad B. Relationship between circadian blood pressure patterns and progression of early carotid atherosclerosis: A 3-year follow-up study. *Circulation* 2000;102(13):1536-41.

541. Grove JS, Reed DM, Yano K, Hwang LJ. Variability in systolic blood pressure--a risk factor for coronary heart disease? *Am J Epidemiol* 1997;145(9):771-6.

542. Muntner P, Shimbo D, Tonelli M, Reynolds K, Arnett DK, Oparil S. The relationship between visit-to-visit variability in systolic blood pressure and all-cause mortality in the general population: findings from NHANES III, 1988 to 1994. *Hypertension* 2011;57(2):160-6.

543. Mancia G, Bertinieri G, Cavallazzi A, Di Rienzo M, Parati G, Pomidossi G, et al. Mechanisms of blood pressure variability in man. *Clin Exp Hypertens A* 1985;7(2-3):167-78.

544. Brickman AM, Reitz C, Luchsinger JA, Manly JJ, Schupf N, Muraskin J, et al. Long-term blood pressure fluctuation and cerebrovascular disease in an elderly cohort. *Arch Neurol* 2010 67(5):564-9.

545. Howard SC, Rothwell PM. Reproducibility of measures of visit-to-visit variability in blood pressure after transient ischaemic attack or minor stroke. *Cerebrovasc Dis* 2009;28(4):331-40.

546. Muntner P, Joyce C, Levitan EB, Holt E, Shimbo D, Webber LS, et al. Reproducibility of visit-to-visit variability of blood pressure measured as part of routine clinical care. *J Hypertens* 2011;29(12):2332-8.

547. Mancia G, Facchetti R, Parati G, Zanchetti A. Visit-to-visit blood pressure variability in the European Lacidipine Study on Atherosclerosis: methodological aspects and effects of antihypertensive treatment. *J Hypertens* 2012 30(6):1241-51.

548. Grambsch PM, Therneau TM, Fleming TR. Diagnostic plots to reveal functional form for covariates in multiplicative intensity models. *Biometrics* 1995;51(4):1469-82.

549. Dekker JM, Crow RS, Folsom AR, Hannan PJ, Liao D, Swenne CA, et al. Low heart rate variability in a 2-minute rhythm strip predicts risk of coronary

heart disease and mortality from several causes: the ARIC Study. Atherosclerosis Risk In Communities. *Circulation* 2000;102(11):1239-44.

550. Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, et al. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. *Hypertension* 2004;43(6):1239-45.

551. Paul L, Hastie CE, Li WS, Harrow C, Muir S, Connell JM, et al. Resting heart rate pattern during follow-up and mortality in hypertensive patients. *Hypertension* 2010;55(2):567-74.

552. Klabunde RE. *Cardiovascular Physiology Concepts* 2nd ed: Lippincott Williams & Wilkins, 2011.

553. Cinar Y, Demir G, Pac M, Cinar AB. Effect of hematocrit on blood pressure via hyperviscosity. *Am J Hypertens* 1999;12(7):739-43.

554. Cirillo M, Laurenzi M, Trevisan M, Stamler J. Hematocrit, blood pressure, and hypertension. The Gubbio Population Study. *Hypertension* 1992;20(3):319-26.

555. Irace C, Ciamei M, Crivaro A, Fiaschi E, Madia A, Cortese C, et al. Hematocrit is associated with carotid atherosclerosis in men but not in women. *Coron Artery Dis* 2003;14(4):279-84.

556. Carallo C, Pujia A, Irace C, De Franceschi MS, Motti C, Gnasso A. Whole blood viscosity and haematocrit are associated with internal carotid atherosclerosis in men. *Coron Artery Dis* 1998;9(2-3):113-7.

557. DeBacker TL, DeBuyzere M, Segers P, Carlier S, De Sutter J, Van de Wiele C, et al. The role of whole blood viscosity in premature coronary artery disease in women. *Atherosclerosis* 2002;165(2):367-73.

558. Levenson J, Gariepy J, Del-Pino M, Salomon J, Denarie N, Simon A. Association of plasma viscosity and carotid thickening in a French working cohort. *Am J Hypertens* 2000;13(7):753-8.

559. Lee AJ, Mowbray PI, Lowe GD, Rumley A, Fowkes FG, Allan PL. Blood viscosity and elevated carotid intima-media thickness in men and women: the Edinburgh Artery Study. *Circulation* 1998;97(15):1467-73.

560. Allport LE, Parsons MW, Butcher KS, MacGregor L, Desmond PM, Tress BM, et al. Elevated hematocrit is associated with reduced reperfusion and tissue survival in acute stroke. *Neurology* 2005;65(9):1382-7.

561. Tamariz LJ, Young JH, Pankow JS, Yeh HC, Schmidt MI, Astor B, et al. Blood viscosity and hematocrit as risk factors for type 2 diabetes mellitus: the atherosclerosis risk in communities (ARIC) study. *Am J Epidemiol* 2008;168(10):1153-60.

562. Sacco S, Marini C, Olivieri L, Pistoia F, Carolei A. Contribution of hematocrit to early mortality after ischemic stroke. *Eur Neurol* 2007;58(4):233-8.

563. Fowkes FG, Lowe GD, Rumley A, Lennie SE, Smith FB, Donnan PT. The relationship between blood viscosity and blood pressure in a random sample of the population aged 55 to 74 years. *Eur Heart J* 1993;14(5):597-601.

564. Rosenson RS, McCormick A, Uretz EF. Distribution of blood viscosity values and biochemical correlates in healthy adults. *Clin Chem* 1996;42(8 Pt 1):1189-95.

565. Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med* 1989;8(5):551-61.

566. Woodward M, Rumley A, Tunstall-Pedoe H, Lowe GD. Does sticky blood predict a sticky end? Associations of blood viscosity, haematocrit and fibrinogen with mortality in the West of Scotland. *Br J Haematol* 2003;122(4):645-50.

567. Lowe GD, Lee AJ, Rumley A, Price JF, Fowkes FG. Blood viscosity and risk of cardiovascular events: the Edinburgh Artery Study. *Br J Haematol* 1997;96(1):168-73.

568. Tzoulaki I, Murray GD, Lee AJ, Rumley A, Lowe GD, Fowkes FG. Relative value of inflammatory, hemostatic, and rheological factors for incident myocardial infarction and stroke: the Edinburgh Artery Study. *Circulation* 2007;115(16):2119-27.

569. Lowe G, Rumley A, Norrie J, Ford I, Shepherd J, Cobbe S, et al. Blood rheology, cardiovascular risk factors, and cardiovascular disease: the West of Scotland Coronary Prevention Study. *Thromb Haemost* 2000;84(4):553-8.

570. Harrison MJ, Pollock S, Thomas D, Marshall J. Haematocrit, hypertension and smoking in patients with transient ischaemic attacks and in age and sex matched controls. *J Neurol Neurosurg Psychiatry* 1982;45(6):550-1.

571. Myers BD, Deen WM, Robertson CR, Brenner BM. Dynamics of glomerular ultrafiltration in the rat. VIII. Effects of hematocrit. *Circ Res* 1975;36(3):425-35.

572. Miller VM. Gender and vascular reactivity. *Lupus* 1999;8(5):409-15.

573. Fitzpatrick LA, Ruan M, Anderson J, Moraghan T, Miller V. Gender-related differences in vascular smooth muscle cell proliferation: implications for prevention of atherosclerosis. *Lupus* 1999;8(5):397-401.

574. Moraghan T, Antoniucci DM, Grenert JP, Sieck GC, Johnson C, Miller VM, et al. Differential response in cell proliferation to beta estradiol in coronary arterial vascular smooth muscle cells obtained from mature female versus male animals. *Endocrinology* 1996;137(11):5174-7.

575. Norris LA, Bonnar J. Effect of oestrogen dose on whole blood platelet activation in women taking new low dose oral contraceptives. *Thromb Haemost* 1994;72(6):926-30.

576. Frohlich M, Schunkert H, Hense HW, Tropitzsch A, Hendricks P, Doring A, et al. Effects of hormone replacement therapies on fibrinogen and plasma viscosity in postmenopausal women. *Br J Haematol* 1998;100(3):577-81.

577. LaRue L, Alter M, Lai SM, Friday G, Sobel E, Levitt L, et al. Acute stroke, hematocrit, and blood pressure. *Stroke* 1987;18(3):565-9.

578. Smith WC, Lowe GD, Lee AJ, Tunstall-Pedoe H. Rheological determinants of blood pressure in a Scottish adult population. *J Hypertens* 1992;10(5):467-72.

579. Chien S. Blood rheology in myocardial infarction and hypertension. *Biorheology* 1986;23(6):633-53.

580. Linde T, Sandhagen B, Hagg A, Morlin C, Wikstrom B, Danielson BG. Blood viscosity and peripheral vascular resistance in patients with untreated essential hypertension. *J Hypertens* 1993;11(7):731-6.

581. Sharp DS, Curb JD, Schatz IJ, Meiselman HJ, Fisher TC, Burchfiel CM, et al. Mean red cell volume as a correlate of blood pressure. *Circulation* 1996;93(9):1677-84.

582. Takeda E, Taketani Y, Sawada N, Sato T, Yamamoto H. The regulation and function of phosphate in the human body. *Biofactors* 2004;21(1-4):345-55.

583. Ellam T, Wilkie M, Chamberlain J, Crossman D, Eastell R, Francis S, et al. Dietary phosphate modulates atherogenesis and insulin resistance in apolipoprotein E knockout mice--brief report. *Arterioscler Thromb Vasc Biol* 2011;31(9):1988-90.

584. Foley RN, Collins AJ, Herzog CA, Ishani A, Kalra PA. Serum phosphorus levels associate with coronary atherosclerosis in young adults. *J Am Soc Nephrol* 2009;20(2):397-404.

585. Giachelli CM, Jono S, Shioi A, Nishizawa Y, Mori K, Morii H. Vascular calcification and inorganic phosphate. *Am J Kidney Dis* 2001;38(4 Suppl 1):S34-7.

586. Chue CD, Edwards NC, Davis LJ, Steeds RP, Townend JN, Ferro CJ. Serum phosphate but not pulse wave velocity predicts decline in renal function in patients with early chronic kidney disease. *Nephrol Dial Transplant* 2011;26(8):2576-82.

587. Chue CD, Edwards NC, Moody WE, Steeds RP, Townend JN, Ferro CJ. Serum phosphate is associated with left ventricular mass in patients with chronic kidney disease: a cardiac magnetic resonance study. *Heart* 2011;98(3):219-24.

588. Dhingra R, Gona P, Benjamin EJ, Wang TJ, Aragam J, D'Agostino RB, Sr., et al. Relations of serum phosphorus levels to echocardiographic left ventricular mass and incidence of heart failure in the community. *Eur J Heart Fail* 2010 12(8):812-8.

589. Ljunghall S, Hedstrand H. Serum phosphate inversely related to blood pressure. *Br Med J* 1977;1(6060):553-4.

590. Daniels J, Goodman AD. Hypertension and hyperparathyroidism. Inverse relation of serum phosphate level and blood pressure. *Am J Med* 1983;75(1):17-23.

591. Gudmundsdottir H, Strand AH, Kjeldsen SE, Hoieggen A, Os I. Serum phosphate, blood pressure, and the metabolic syndrome--20-year follow-up of middle-aged men. *J Clin Hypertens (Greenwich)* 2008;10(11):814-21.

592. Kanbay M, Isik B, Akcay A, Ozkara A, Karakurt F, Turgut F, et al. Relation between serum calcium, phosphate, parathyroid hormone and 'nondipper'

circadian blood pressure variability profile in patients with normal renal function. *Am J Nephrol* 2007;27(5):516-21.

593. Vyssoulis G, Karpanou E, Tzamou V, Kyvelou SM, Michaelidis A, Stefanadis C. Serum phosphate in white-coat hypertensive patients: focus on dipping status and metabolic syndrome. *Hypertens Res* 2010 33(8):825-30.

594. de Boer IH, Rue TC, Kestenbaum B. Serum phosphorus concentrations in the third National Health and Nutrition Examination Survey (NHANES III). *Am J Kidney Dis* 2009;53(3):399-407.

595. Ritz E, Hahn K, Ketteler M, Kuhlmann MK, Mann J. Phosphate additives in food--a health risk. *Dtsch Arztebl Int* 2012 109(4):49-55.

596. Gutierrez OM, Anderson C, Isakova T, Scialla J, Negrea L, Anderson AH, et al. Low socioeconomic status associates with higher serum phosphate irrespective of race. *J Am Soc Nephrol* 2010 21(11):1953-60.

597. Gutierrez OM, Isakova T, Enfield G, Wolf M. Impact of poverty on serum phosphate concentrations in the Third National Health and Nutrition Examination Survey. *J Ren Nutr* 2010 21(2):140-8.

598. Leyland AH, Dundas R, McLoone P, Boddy FA. Cause-specific inequalities in mortality in Scotland: two decades of change. A population-based study. *BMC Public Health* 2007;7:172.

599. McLoone P, Boddy FA. Deprivation and mortality in Scotland, 1981 and 1991. *BMJ* 1994;309(6967):1465-70.

600. Grandi NC, Brenner H, Hahmann H, Wusten B, Marz W, Rothenbacher D, et al. Calcium, phosphate and the risk of cardiovascular events and all-cause mortality in a population with stable coronary heart disease. *Heart* 2012 98(12):926-33.

601. Cancela AL, Santos RD, Titan SM, Goldenstein PT, Rochitte CE, Lemos PA, et al. Phosphorus is associated with coronary artery disease in patients with preserved renal function. *PLoS One* 2012 7(5):e36883.

602. Park KS, Chang JW, Kim TY, Kim HW, Lee EK, Kim HS, et al. Lower concentrations of serum phosphorus within the normal range could be associated with less calcification of the coronary artery in Koreans with normal renal function. *Am J Clin Nutr* 2011;94(6):1465-70.

603. Moe SM, Chen NX. Mechanisms of vascular calcification in chronic kidney disease. *J Am Soc Nephrol* 2008;19(2):213-6.

604. Chen NX, O'Neill KD, Duan D, Moe SM. Phosphorus and uremic serum up-regulate osteopontin expression in vascular smooth muscle cells. *Kidney Int* 2002;62(5):1724-31.

605. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, et al. Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res* 2000;87(7):E10-7.

606. Van TV, Watari E, Taketani Y, Kitamura T, Shiota A, Tanaka T, et al. Dietary phosphate restriction ameliorates endothelial dysfunction in adenine-induced kidney disease rats. *J Clin Biochem Nutr* 2012 51(1):27-32.

607. Tenenhouse HS. Regulation of phosphorus homeostasis by the type iia na/phosphate cotransporter. *Annu Rev Nutr* 2005;25:197-214.

608. Stubbs JR, Liu S, Tang W, Zhou J, Wang Y, Yao X, et al. Role of hyperphosphatemia and 1,25-dihydroxyvitamin D in vascular calcification and mortality in fibroblastic growth factor 23 null mice. *J Am Soc Nephrol* 2007;18(7):2116-24.

609. Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, et al. Suppression of aging in mice by the hormone Klotho. *Science* 2005;309(5742):1829-33.

610. Onufrak SJ, Bellasi A, Cardarelli F, Vaccarino V, Muntner P, Shaw LJ, et al. Investigation of gender heterogeneity in the associations of serum phosphorus with incident coronary artery disease and all-cause mortality. *Am J Epidemiol* 2009;169(1):67-77.

611. Ferraro PM, Bonello M, Gambaro A, Sturniolo A, Gambaro G. Age- and sex-tailored serum phosphate thresholds do not improve cardiovascular risk estimation in CKD. *J Nephrol* 2011;24(4):446-52.

612. Uemura H, Irahara M, Yoneda N, Yasui T, Genjida K, Miyamoto KI, et al. Close correlation between estrogen treatment and renal phosphate reabsorption capacity. *J Clin Endocrinol Metab* 2000;85(3):1215-9.

613. Faroqui S, Levi M, Soleimani M, Amlal H. Estrogen downregulates the proximal tubule type IIa sodium phosphate cotransporter causing phosphate wasting and hypophosphatemia. *Kidney Int* 2008;73(10):1141-50.

614. Haas JA, Larson MV, Marchand GR, Lang FC, Greger RF, Knox FG. Phosphaturic effect of furosemide: role of PTH and carbonic anhydrase. *Am J Physiol* 1977;232(2):F105-10.

615. Rose BD. *Clinical physiology of acid-base and electrolyte disorders, 4th edition.* New York McGraw-Hill 1994.

616. Ellison DH. Disorders of sodium balance In: Berl T, Bonventre JV, editors. *Disorders of water electrolytes and acid-base* Philadelphia Blackwell Science, 1999.

617. Simpson FO. Sodium intake, body sodium, and sodium excretion. *Lancet* 1988;2(8601):25-9.

618. Guyton AC. Blood pressure control--special role of the kidneys and body fluids. *Science* 1991;252(5014):1813-6.

619. Alderman MH, Cohen HW. Dietary sodium intake and cardiovascular mortality: controversy resolved? *Am J Hypertens* 2012 25(7):727-34.

620. Morrison G. Serum Chloride In: Walker HK, Hall WD, Hurst JW, editors. *Clinical Methods: The History, Physical and Laboratory Examinations, 3rd edition* Boston Butterworths, 1990.

621. Yunos NM, Bellomo R, Story D, Kellum J. Bench-to-bedside review: Chloride in critical illness. *Crit Care* 2010 14(4):226.

622. Wyss JM, Liumsiricharoen M, Sripairojthikoon W, Brown D, Gist R, Oparil S. Exacerbation of hypertension by high chloride, moderate sodium diet in the salt-sensitive spontaneously hypertensive rat. *Hypertension* 1987;9(6 Pt 2):III171-5.

623. Boegehold MA, Kotchen TA. Importance of dietary chloride for salt sensitivity of blood pressure. *Hypertension* 1991;17(1 Suppl):I158-61.

624. Tanaka M, Schmidlin O, Yi SL, Bollen AW, Morris RC, Jr. Genetically determined chloride-sensitive hypertension and stroke. *Proc Natl Acad Sci U S A* 1997;94(26):14748-52.

625. Schmidlin O, Tanaka M, Bollen AW, Yi SL, Morris RC, Jr. Chloride-dominant salt sensitivity in the stroke-prone spontaneously hypertensive rat. *Hypertension* 2005;45(5):867-73.

626. Schmidlin O, Tanaka M, Sebastian A, Morris RC, Jr. Selective chloride loading is pressor in the stroke-prone spontaneously hypertensive rat despite hydrochlorothiazide-induced natriuresis. *J Hypertens* 2010 28(1):87-94.

627. Felker GM, Allen LA, Pocock SJ, Shaw LK, McMurray JJ, Pfeffer MA, et al. Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank. *J Am Coll Cardiol* 2007;50(1):40-7.

628. Lucas PA, Lacour B, McCarron DA, Drueke T. Disturbance of acid-base balance in the young spontaneously hypertensive rat. *Clin Sci (Lond)* 1987;73(2):211-5.

629. Taylor EN, Forman JP, Farwell WR. Serum anion gap and blood pressure in the national health and nutrition examination survey. *Hypertension* 2007;50(2):320-4.

630. Luft FC, Weinberger MH. Sodium intake and essential hypertension. *Hypertension* 1982;4(5 Pt 2):III14-9.

631. Haddy FJ, Pamnani MB. Role of dietary salt in hypertension. *J Am Coll Nutr* 1995;14(5):428-38.

632. Kaup SM, Greger JL, Marcus MS, Lewis NM. Blood pressure, fluid compartments and utilization of chloride in rats fed various chloride diets. *J Nutr* 1991;121(3):330-7.

633. Whitescarver SA, Ott CE, Jackson BA, Guthrie GP, Jr., Kotchen TA. Salt-sensitive hypertension: contribution of chloride. *Science* 1984;223(4643):1430-2.

634. Sharma AM, Distler A. Acid-base abnormalities in hypertension. *Am J Med Sci* 1994;307 Suppl 1:S112-5.

635. Sharma AM, Cetto C, Schorr U, Spies KP, Distler A. Renal acid-base excretion in normotensive salt-sensitive humans. *Hypertension* 1993;22(6):884-90.

636. Sharma AM, Kribben A, Schattenfroh S, Cetto C, Distler A. Salt sensitivity in humans is associated with abnormal acid-base regulation. *Hypertension* 1990;16(4):407-13.

637. Feldman M, Soni NJ, Dickson B. Use of sodium concentration and anion gap to improve correlation between serum chloride and bicarbonate concentrations. *J Clin Lab Anal* 2006;20(4):154-9.

638. Galla JH, Gifford JD, Luke RG, Rome L. Adaptations to chloride-depletion alkalosis. *Am J Physiol* 1991;261(4 Pt 2):R771-81.

639. Abramowitz MK, Hostetter TH, Melamed ML. The serum anion gap is altered in early kidney disease and associates with mortality. *Kidney Int* 2012.

640. Sarafidis PA, Georgianos PI, Lasaridis AN. Diuretics in clinical practice. Part II: electrolyte and acid-base disorders complicating diuretic therapy. *Expert Opin Drug Saf* 2010;9(2):259-73.

641. Farwell WR, Taylor EN. Serum anion gap, bicarbonate and biomarkers of inflammation in healthy individuals in a national survey. *CMAJ* 2010 182(2):137-41.

642. Ruggiero C, Metter EJ, Cherubini A, Maggio M, Sen R, Najjar SS, et al. White blood cell count and mortality in the Baltimore Longitudinal Study of Aging. *J Am Coll Cardiol* 2007;49(18):1841-50.

643. Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet* 2010 375(9709):132-40.

644. Raphael KL, Wei G, Baird BC, Greene T, Beddhu S. Higher serum bicarbonate levels within the normal range are associated with better survival and renal outcomes in African Americans. *Kidney Int* 2010;79(3):356-62.

645. Pikilidou MI, Lasaridis AN, Sarafidis PA, Tziolas IM, Zebekakis PE, Dombros NV, et al. Blood pressure and serum potassium levels in hypertensive patients receiving or not receiving antihypertensive treatment. *Clin Exp Hypertens* 2007;29(8):563-73.

646. Goyal A, Spertus JA, Gosch K, Venkitachalam L, Jones PG, Van den Berghe G, et al. Serum potassium levels and mortality in acute myocardial infarction. *JAMA* 2012 307(2):157-64.

647. Ahmed MI, Ekundayo OJ, Mujib M, Campbell RC, Sanders PW, Pitt B, et al. Mild hyperkalemia and outcomes in chronic heart failure: a propensity matched study. *Int J Cardiol* 2009;144(3):383-8.

648. Ahmed A, Zannad F, Love TE, Tallaj J, Gheorghiade M, Ekundayo OJ, et al. A propensity-matched study of the association of low serum potassium levels and mortality in chronic heart failure. *Eur Heart J* 2007;28(11):1334-43.

649. Korgaonkar S, Tilea A, Gillespie BW, Kiser M, Eisele G, Finkelstein F, et al. Serum potassium and outcomes in CKD: insights from the RRI-CKD cohort study. *Clin J Am Soc Nephrol* 2010 5(5):762-9.

650. McClatchey KD. *Cilinical Laboratory Medicine, 2nd Edition* Philadelphia Lippincott Williams Wilkins 2003.

651. Westerbacka J, Corner A, Tiikkainen M, Tamminen M, Vehkavaara S, Hakkinen AM, et al. Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. *Diabetologia* 2004;47(8):1360-9.

652. Schindhelm RK, Diamant M, Dekker JM, Tushuizen ME, Teerlink T, Heine RJ. Alanine aminotransferase as a marker of non-alcoholic fatty liver disease in relation to type 2 diabetes mellitus and cardiovascular disease. *Diabetes Metab Res Rev* 2006;22(6):437-43.

653. Adams LA, Waters OR, Knuiman MW, Elliott RR, Olynyk JK. NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: an eleven-year follow-up study. *Am J Gastroenterol* 2009;104(4):861-7.

654. Lee TH, Kim WR, Benson JT, Therneau TM, Melton LJ, 3rd. Serum aminotransferase activity and mortality risk in a United States community. *Hepatology* 2008;47(3):880-7.

655. Nakamura K, Okamura T, Kanda H, Hayakawa T, Okayama A, Ueshima H. The value of combining serum alanine aminotransferase levels and body mass index to predict mortality and medical costs: a 10-year follow-up study of National Health Insurance in Shiga, Japan. *J Epidemiol* 2006;16(1):15-20.

656. Ford I, Mooijaart SP, Lloyd S, Murray HM, Westendorp RG, de Craen AJ, et al. The inverse relationship between alanine aminotransferase in the normal range and adverse cardiovascular and non-cardiovascular outcomes. *Int J Epidemiol*;40(6):1530-8.

657. Elinav E, Ackerman Z, Maaravi Y, Ben-Dov IZ, Ein-Mor E, Stessman J. Low alanine aminotransferase activity in older people is associated with greater long-term mortality. *J Am Geriatr Soc* 2006;54(11):1719-24.

658. Elinav E, Ben-Dov IZ, Ackerman E, Kiderman A, Glikberg F, Shapira Y, et al. Correlation between serum alanine aminotransferase activity and age: an inverted U curve pattern. *Am J Gastroenterol* 2005;100(10):2201-4.

659. Ghouri N, Preiss D, Sattar N. Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. *Hepatology*;52(3):1156-61.

660. Lee DH, Blomhoff R, Jacobs DR, Jr. Is serum gamma glutamyltransferase a marker of oxidative stress? *Free Radic Res* 2004;38(6):535-9.

661. Radetti G, Kleon W, Stuefer J, Pittschieler K. Non-alcoholic fatty liver disease in obese children evaluated by magnetic resonance imaging. *Acta Paediatr* 2006;95(7):833-7.

662. Jo SK, Lee WY, Rhee EJ, Won JC, Jung CH, Park CY, et al. Serum gamma-glutamyl transferase activity predicts future development of metabolic syndrome defined by 2 different criteria. *Clin Chim Acta* 2009;403(1-2):234-40.

663. Fraser A, Harris R, Sattar N, Ebrahim S, Smith GD, Lawlor DA. Gamma-glutamyltransferase is associated with incident vascular events independently of alcohol intake: analysis of the British Women's Heart and Health Study and Meta-Analysis. *Arterioscler Thromb Vasc Biol* 2007;27(12):2729-35.

664. Sluik D, Beulens JW, Weikert C, van Dieren S, Spijkerman AM, van der AD, et al. Gamma-glutamyltransferase, cardiovascular disease and mortality in individuals with diabetes mellitus. *Diabetes Metab Res Rev*.

665. Targher G. Elevated serum gamma-glutamyltransferase activity is associated with increased risk of mortality, incident type 2 diabetes, cardiovascular events, chronic kidney disease and cancer - a narrative review. *Clin Chem Lab Med*;48(2):147-57.

666. Shantouf R, Kovesdy CP, Kim Y, Ahmadi N, Luna A, Luna C, et al. Association of serum alkaline phosphatase with coronary artery calcification in maintenance hemodialysis patients. *Clin J Am Soc Nephrol* 2009;4(6):1106-14.

667. Krishnamurthy VR, Baird BC, Wei G, Greene T, Raphael K, Beddhu S. Associations of serum alkaline phosphatase with metabolic syndrome and mortality. *Am J Med*;124(6):566 e1-7.

668. Lin JP, Vitek L, Schwertner HA. Serum bilirubin and genes controlling bilirubin concentrations as biomarkers for cardiovascular disease. *Clin Chem* 2010;56(10):1535-43.

669. Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de Boer A, Oostra BA, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N Engl J Med* 1995;333(18):1171-5.

670. Neuzil J, Stocker R. Free and albumin-bound bilirubin are efficient co-antioxidants for alpha-tocopherol, inhibiting plasma and low density lipoprotein lipid peroxidation. *J Biol Chem* 1994;269(24):16712-9.

671. Vitek L, Jirsa M, Brodanova M, Kalab M, Marecek Z, Danzig V, et al. Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. *Atherosclerosis* 2002;160(2):449-56.

672. Sedlak TW, Saleh M, Higginson DS, Paul BD, Juluri KR, Snyder SH. Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. *Proc Natl Acad Sci U S A* 2009;106(13):5171-6.

673. Yesilova Z, Serdar M, Ercin CN, Gunay A, Kilciler G, Hasimi A, et al. Decreased oxidation susceptibility of plasma low density lipoproteins in patients with Gilbert's syndrome. *J Gastroenterol Hepatol* 2008;23(10):1556-60.

674. Perlstein TS, Pande RL, Creager MA, Weuve J, Beckman JA. Serum total bilirubin level, prevalent stroke, and stroke outcomes: NHANES 1999-2004. *Am J Med* 2008;121(9):781-88 e1.

675. Perlstein TS, Pande RL, Beckman JA, Creager MA. Serum total bilirubin level and prevalent lower-extremity peripheral arterial disease: National Health and Nutrition Examination Survey (NHANES) 1999 to 2004. *Arterioscler Thromb Vasc Biol* 2008;28(1):166-72.

676. Han SS, Na KY, Chae DW, Kim YS, Kim S, Chin HJ. Survival benefit of high serum bilirubin and low alkaline phosphatase in older adults. *J Am Geriatr Soc*;58(7):1413-5.

677. Temme EH, Zhang J, Schouten EG, Kesteloot H. Serum bilirubin and 10-year mortality risk in a Belgian population. *Cancer Causes Control* 2001;12(10):887-94.

678. Lee DS, Evans JC, Robins SJ, Wilson PW, Albano I, Fox CS, et al. Gamma glutamyl transferase and metabolic syndrome, cardiovascular disease, and mortality risk: the Framingham Heart Study. *Arterioscler Thromb Vasc Biol* 2007;27(1):127-33.

679. Ghouri N, Preiss D, Sattar N. Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. *Hepatology* 2010 52(3):1156-61.

680. Fraser A, Thinggaard M, Christensen K, Lawlor DA. Alanine aminotransferase, gamma-glutamyltransferase (GGT) and all-cause mortality: results from a population-based Danish twins study alanine aminotransferase, GGT and mortality in elderly twins. *Liver Int* 2009;29(10):1494-9.

681. Mason JE, Starke RD, Van Kirk JE. Gamma-glutamyl transferase: a novel cardiovascular risk biomarker. *Prev Cardiol* 2010;13(1):36-41.

682. Yun KE, Shin CY, Yoon YS, Park HS. Elevated alanine aminotransferase levels predict mortality from cardiovascular disease and diabetes in Koreans. *Atherosclerosis* 2009;205(2):533-7.

683. Le Couteur DG, Blyth FM, Creasey HM, Handelsman DJ, Naganathan V, Sambrook PN, et al. The association of alanine transaminase with aging, frailty, and mortality. *J Gerontol A Biol Sci Med Sci* 2010;65(7):712-7.

684. Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, et al. *Harrison's Principles of Internal Medicine*. New York McGraw-Hill Professional, 2008.

685. Vitek L, Schwertner HA. The heme catabolic pathway and its protective effects on oxidative stress-mediated diseases. *Adv Clin Chem* 2007;43:1-57.

686. Mazzone GL, Rigato I, Ostrow JD, Bossi F, Bortoluzzi A, Sukowati CH, et al. Bilirubin inhibits the TNFalpha-related induction of three endothelial adhesion molecules. *Biochem Biophys Res Commun* 2009;386(2):338-44.

687. Tapan S, Dogru T, Tasci I, Ercin CN, Ozgurtas T, Erbil MK. Soluble CD40 ligand and soluble P-selectin levels in Gilbert's syndrome: a link to protection against atherosclerosis? *Clin Biochem* 2009;42(9):791-5.

688. Papadakis JA, Ganotakis ES, Jagroop IA, Mikhailidis DP, Winder AF. Effect of hypertension and its treatment on lipid, lipoprotein(a), fibrinogen, and bilirubin levels in patients referred for dyslipidemia. *Am J Hypertens* 1999;12(7):673-81.

689. Bhuiyan AR, Srinivasan SR, Chen W, Sultana A, Berenson GS. Association of serum bilirubin with pulsatile arterial function in asymptomatic young adults: the Bogalusa Heart Study. *Metabolism* 2008;57(5):612-6.

690. Ko GT, Chan JC, Woo J, Lau E, Yeung VT, Chow CC, et al. Serum bilirubin and cardiovascular risk factors in a Chinese population. *J Cardiovasc Risk* 1996;3(5):459-63.

691. Lin LY, Kuo HK, Hwang JJ, Lai LP, Chiang FT, Tseng CD, et al. Serum bilirubin is inversely associated with insulin resistance and metabolic syndrome among children and adolescents. *Atherosclerosis* 2009;203(2):563-8.

692. Tanaka M, Fukui M, Tomiyasu K, Akabame S, Nakano K, Hasegawa G, et al. Low serum bilirubin concentration is associated with coronary artery calcification (CAC). *Atherosclerosis* 2009;206(1):287-91.

693. Kimm H, Yun JE, Jo J, Jee SH. Low serum bilirubin level as an independent predictor of stroke incidence: a prospective study in Korean men and women. *Stroke* 2009;40(11):3422-7.

694. Van Hoydonck PG, Temme EH, Schouten EG. Serum bilirubin concentration in a Belgian population: the association with smoking status and type of cigarettes. *Int J Epidemiol* 2001;30(6):1465-72.

695. Frei B, Stocker R, Ames BN. Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc Natl Acad Sci U S A* 1988;85(24):9748-52.

696. Lin JP, O'Donnell CJ, Schwaiger JP, Cupples LA, Lingenhel A, Hunt SC, et al. Association between the UGT1A1*28 allele, bilirubin levels, and coronary heart disease in the Framingham Heart Study. *Circulation* 2006;114(14):1476-81.

697. Whitfield JB. Gamma glutamyl transferase. *Crit Rev Clin Lab Sci* 2001;38(4):263-355.

698. Grundy SM, Pasternak R, Greenland P, Smith S, Jr., Fuster V. AHA/ACC scientific statement: Assessment of cardiovascular risk by use of multiple-risk-factor assessment equations: a statement for healthcare professionals from the American Heart Association and the American College of Cardiology. *J Am Coll Cardiol* 1999;34(4):1348-59.

699. Hippisley-Cox J, Coupland C, Vinogradova Y, Robson J, Minhas R, Sheikh A, et al. Predicting cardiovascular risk in England and Wales: prospective derivation and validation of QRISK2. *BMJ* 2008;336(7659):1475-82.

700. Woodward M, Brindle P, Tunstall-Pedoe H. Adding social deprivation and family history to cardiovascular risk assessment: the ASSIGN score from the Scottish Heart Health Extended Cohort (SHHEC). *Heart* 2007;93(2):172-6.

701. Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J* 2003;24(11):987-1003.

702. Vasan RS, Kannel WB. Are guidelines effectively guiding antihypertensive therapy? *Am J Cardiol* 2007;100(1):143-4.

703. Paget V, Legedz L, Gaudebout N, Girerd N, Bricca G, Milon H, et al. N-terminal pro-brain natriuretic peptide: a powerful predictor of mortality in hypertension. *Hypertension* 2011;57(4):702-9.

704. Blake GJ, Rifai N, Buring JE, Ridker PM. Blood pressure, C-reactive protein, and risk of future cardiovascular events. *Circulation* 2003;108(24):2993-9.

705. Egan BM, Zhao Y, Axon RN. US trends in prevalence, awareness, treatment, and control of hypertension, 1988-2008. *JAMA* 2010;303(20):2043-50.

706. Pencina MJ, D'Agostino RB, Sr., D'Agostino RB, Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med* 2008;27(2):157-72; discussion 207-12.