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PULSE PRESSURE AS A PREDICTIVE MARKER FOR
CARDIOVASCULAR EVENTS. RELATION TO BIOMARKERS
AND ANTIHYPERTENSIVE TREATMENT

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PULSE PRESSURE AS A PREDICTIVE MARKER FOR
CARDIOVASCULAR EVENTS. RELATION TO BIOMARKERS AND
ANTIHYPERTENSIVE TREATMENT

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Blood pressure (BP), in particularly ambulatory blood pressure (ABP), is a strong predictor for cardiovascular (CV) disease (CVD). Pulse pressure (PP) is related to vascular disease and ambulatory PP (APP) may have a specific value in certain populations. It is unknown whether ABP is a better predictor for CV events compared to office BP in patients with peripheral arterial disease (PAD). NT-proBNP, hs-CRP and cystatin C are biomarkers that are increasingly used for risk prediction but prospective studies on the predictive value of these biomarkers adjusted for ABP are scarce. Although PP may have a clinical value, the relation to outcomes in interventional antihypertensive studies has not been sufficiently studied. The overall aim of this thesis was to study the predictive value of ABP with special reference to PP in relation to the biomarkers NT-proBNP, hs-CRP, and cystatin C and to evaluate whether ABP and these biomarkers improved risk prediction when added to traditional risk factor models. We further aimed to study whether the antihypertensive treatment effect on CV events was dependent on baseline PP.

Material and methods. This thesis was based on studies in patients with PAD, elderly men and high-risk hypertensives. We investigated the relations of ABP with special reference to APP and the biomarkers NT-proBNP, hs-CRP, and cystatin C to CV events during long-term follow-up. We used Cox regression models and C-statistics, net reclassification improvement and integrated discrimination improvement. We studied whether the difference in CV events between two different antihypertensive treatments was dependent on baseline PP.

Results. APP was a better predictor of CV events compared to office BP in PAD patients and a combination of APP, NT-proBNP, and hs-CRP improved discrimination and net reclassification. In elderly male subjects, the substitution of office BP with ABP in a model with traditional risk factors improved discrimination and reclassification. The addition of NT-proBNP to the ABP model improved reclassification but not discrimination. However, the addition of ABP to a traditional model that included any of the biomarkers did not improve discrimination or reclassification. In high-risk hypertensive patients, we observed a positive relationship between baseline PP and incident CVD. However, the superior treatment effect of amlodipine as compared to hydrochlorothiazide when combined with benazepril was independent of baseline PP. The absolute treatment effect was higher in the higher tertiles of PP.

Conclusion. Pulse pressure is a predictor for CV events and seems to be most useful in patients with established CVD. NT-proBNP has additive value for risk prediction in patients with CVD as well as in the elderly. Combinations of pulse pressure and NT-proBNP may help to tailor treatment in subjects to prevent incident CVD. The difference in reduction of

CV events between two different antihypertensive treatments was not dependent on baseline pulse pressure. That is, there is presently no evidence to support that a subject's pulse pressure per se should direct the choice of antihypertensive drugs for treatment.

LIST OF SCIENTIFIC PAPERS

I. Ambulatory pulse pressure predicts cardiovascular events in patients with peripheral arterial disease

Per H. Skoglund, Jan Östergren, Per Svensson

Blood Pressure, 2012; 21: 227–232

II. Amino-terminal pro-B-type natriuretic peptide and high-sensitivity C reactive protein but not cystatin C predict cardiovascular events in male patients with peripheral artery disease independently of ambulatory pulse pressure

Per H. Skoglund, Johannes Arpegård, Jan Östergren, Per Svensson

American Journal of Hypertension, 2014;27(3):363-371

III. NT-proBNP but not cystatin C or CRP improve risk prediction for cardiovascular disease beyond ambulatory blood pressure and traditional risk factors in elderly men

Per H. Skoglund, Jonas Höijer, Johan Ärnlöv, Björn Zethelius, Per Svensson

in submission

IV. Amlodipine+benazepril is superior to hydrochlorothiazide+benazepril irrespective of baseline pulse pressure: Subanalysis of the ACCOMPLISH trial

Per H. Skoglund, Per Svensson, Joline Asp, Björn Dahlöf, Sverre E. Kjeldsen, Kenneth A. Jamerson, Michael A. Weber, Yan Jia, Dion H. Zappe, Jan Östergren, the ACCOMPLISH investigators

Journal of Clinical Hypertension, 2015 Feb;17(2):141-6.

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LIST OF ABBREVIATIONS

ABI	Ankle-to-Brachial Systolic Pressure Index
ABP	Ambulatory Blood Pressure
ACC	American College of Cardiology
AHA	American Heart Association
APP	Ambulatory Pulse Pressure
ASCVD	Atherosclerotic Cardiovascular Disease
AUC	Area under the Receiver-Operating Characteristic Curve
BP	Blood Pressure
CABG	Coronary Artery Bypass Graft
CI	Confidence Interval
CV	Cardiovascular
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
ESC	European Society of Cardiology
ESH	European Society of Hypertension
HR	Hazard Ratio
hs-CRP	High Sensitivity C-reactive Protein
IDI	Integrated Discrimination Improvement
MI	Myocardial Infarction
NRI	Net Reclassification Improvement
NT-proBNP	Amino-terminal pro-B-Type Natriuretic Peptide
PAD	Peripheral Arterial Disease
PCI	Percutaneous Coronary Intervention
PP	Pulse Pressure
SBP	Systolic Blood Pressure
WHO	World Health Organization

1 INTRODUCTION

Ever since the 1950s with the introduction of tolerable antihypertensive treatment, much effort has been made to reduce the development of cardiovascular disease (CVD) and cardiovascular (CV) morbidity and mortality. Risk factors for CVD such as hypertension have been identified from observational and longitudinal studies and much progress has been made in identifying subjects at risk. However, hypertension is still a major health problem, responsible for approximately 45% of deaths caused by heart disease and 54% of deaths caused by stroke (1-3). Further, CVD is the leading cause of death globally and account for almost one third of all deaths (4) with 7.4 million coronary heart disease deaths and 6.7 million deaths from stroke annually (2). The World Health Organization (WHO) has identified hypertension as one of the most important global risk factors for premature death.

Advances within medicine over the decades have increased survival from CVD, thus resulting in a growing elderly population worldwide. One of the strongest risk factors for CVD is age. Risk factors that are commonly combined in estimating a person's risk of future CVD include age, gender, systolic blood pressure, total cholesterol, high-density lipoprotein, smoking and whether or not the subject is undergoing treatment for hypertension. However, these risk factors seem to lose their predictive value with age (5) and new variables that can be used to improve risk prediction are warranted.

Pulse pressure is an indicator of arterial stiffness and vascular disease and is related to an increased risk for CV events (6-13). Pulse pressure may be a better predictive marker in the elderly as well as in subjects with hypertension compared to systolic blood pressure (6, 14-17). Biomarkers such as hs-CRP, NT-proBNP and cystatin C are also associated with worse outcome in subjects with CVD (18-22). Ambulatory blood pressure is superior to blood pressure measured in the office both in providing a subjects blood pressure during daily activities and sleep and also in improving risk stratification in hypertensive patients with (23) or without a history of CVD (24, 25). However, the value of the biomarkers hs-CRP, NT-proBNP and cystatin C for risk stratification has not been evaluated in relation to ambulatory blood pressure.

The overall aim of this thesis was to study the predictive value of ambulatory blood pressure in relation to biomarkers in a population with advanced vascular disease and in a population of healthy elderly men. A further aim was to investigate whether ambulatory blood pressure and these biomarkers could improve risk prediction and discrimination compared to a basic CVD risk factor model. In addition we aimed to study whether the superior effect on incident

CVD of a specific antihypertensive treatment in a large, randomized clinical, ACCOMPLISH trial, was dependent on baseline pulse pressure.

2 BACKGROUND

2.1 Blood Pressure

Stephen Hales made the first known measurement of blood pressure in 1733 (26). A cannula with a fitted glass tube was inserted into an artery of a horse and Hales could see the blood rising to a certain level in the tube and then varying with the pulse (pulse pressure). However, it was not until 1828 that the study of blood pressure began with the introduction of the mercury manometer made by Poiseuillein (27). During the 1800s methods developed and in 1896 Riva-Rocci presented a non-invasive sphygmomanometer on which our present measurement technique is based. Riva-Rocci's device could however only measure systolic blood pressure as it was based on pulse palpation. Further, the device design was not optimal giving inaccurate measurements and Von Recklinghausen refined the design in 1901 (27). In 1905, a Russian surgeon named Korotkoff presented the technique that is still in use today – the auscultatory technique in combination with Riva-Rocci's cuff sphygmomanometer, which determines the systolic blood pressure, the diastolic blood pressure and the pulse pressure.

Blood pressure is dynamic during normal life, responding to internal factors such as hormones and signaling substances but also to external stimuli such as physical activity and stressful environment (28). This may cause problems in the diagnosis of hypertension since diagnosis is usually derived from a couple of measurements made by a nurse or a physician in an office setting. Subjects with high blood pressures in the office setting may suffer from “White-coat syndrome”, a stress reaction due to the environment of the doctor's office, causing the blood pressure to rise (29). Conversely, some patients may suffer from “masked hypertension” with normal blood pressure in the office, but elevated levels over a 24-hour period or daytime period even in patients treated for hypertension (30). This uncertainty can be ruled out by the use of ambulatory blood pressure monitoring (discussed below) and provide a better decision basis for the diagnosis of hypertension.

Blood pressure is the result of the pumping action of the heart, and the resistance in the vessels. Systolic blood pressure peaks at the opening of the aortic valve and then rapidly declines. The systolic pressure, built up from the heart, is partly absorbed by the arterial wall and when the vessels return from their distended state, the absorbed energy transcends back into the blood and results in the diastolic pressure. In addition, as the pulse wave travels through the arterial vessel tree, every junction echoes a backward traveling pulse-wave that augments the diastolic pressure (figure 1). With age, the arteries become stiffer and thicker with reduced compliance due to vascular aging (reduction of elastin and increased collagen in

the arterial wall) and atherosclerosis (31). The reduced ability of the arterial wall to absorb the kinetic energy of the blood results in an increased pulse wave velocity, causing the backward traveling pulse-wave to return faster thus leading to augmentation of the late systolic pressure instead. The result is an elevated systolic blood pressure value and decreased diastolic blood pressure (elevated pulse pressure) (32). The augmentation of the pulse wave reflection in these two conditions is illustrated in figure 1 (33).

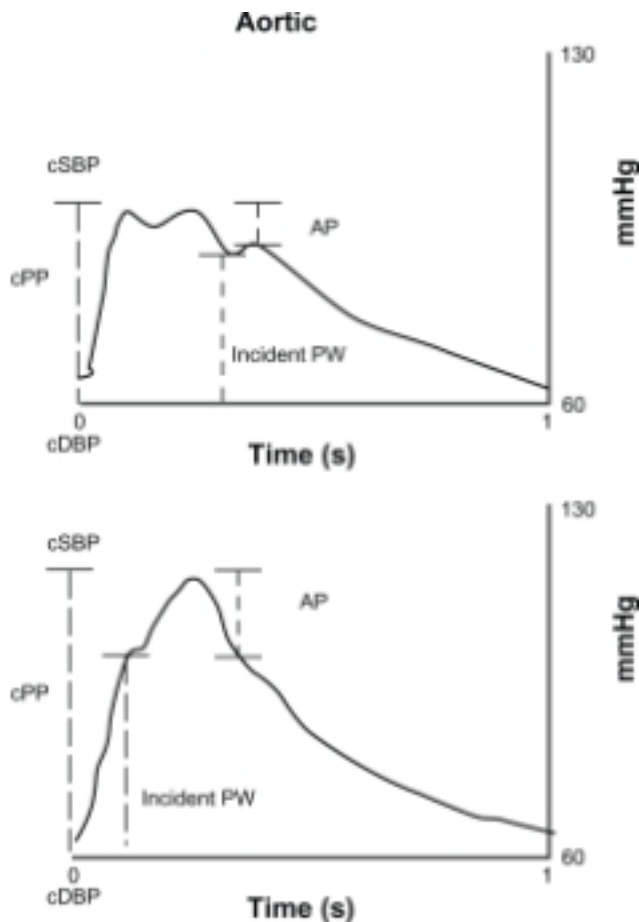


Figure 1.
Normal pulse wave reflection when augmenting pressure (AP) occurs in post systolic descending pressure curve (upper figure).

Early wave reflection when augmentation pressure (AP) increases systolic blood pressure (lower figure).

c, central; DBP, diastolic blood pressure; SBP, systolic blood pressure; PP, pulse pressure; PW, pulse wave
Adopted from Palatini, 2011.

2.2 Cardiovascular Risk Factors

The first major attempt in cardiovascular epidemiology started post World War II. It began with prospective studies in Minnesota where coronary heart disease was observed among professional men (34). The methods used led to the first major study in cardiovascular epidemiology, the Seven Countries Study (35). A lot of our knowledge today about the

associations between lifestyle and diet to coronary heart disease and stroke descends from the Seven Countries Study. The major finding was the direct correlation between cholesterol and the incidence of heart attacks and stroke. During the same time period, the Framingham Heart Study (36) started. The first report revealed that elevated blood pressure, overweight and cholesterol predicted coronary heart disease (37). The Framingham Heart Study has since then produced numerous papers, identifying risk factors for coronary heart disease such as cholesterol (38), smoking (39), hypertension (40), high-density lipoprotein (41), diabetes (42) and the role of blood pressure in stroke (43), all of which are the foundation for CVD risk assessment today. The Framingham researchers also noted that several risk factors were often present at the same time and that this phenomenon was directly related to coronary heart disease rates (44). This led to the introduction of multivariable risk assessment (45), finalized in the Framingham Risk Score (45) (for coronary heart disease) and its modified version (taking stroke into account) that are currently used.

Risk factor assessment and treatment decisions in individuals are based on scoring systems for cardiovascular disease such as the Framingham Risk Score and SCORE (46) (discussed below) among others. These scoring systems include among other risk factors, systolic blood pressure as a variable but do not account for diastolic blood pressure or pulse pressure. In the Framingham Heart Study, it was clear that the different blood pressure variables associate contrarily with coronary heart disease in different age groups. The best predictive variable shifts from diastolic blood pressure to systolic blood pressure at around 50 years of age (47). Further, the Framingham Heart Study showed that for any given systolic blood pressure, coronary heart disease rates increased with lower diastolic blood pressure values (14), or put another way, increasing pulse pressure. In the latest guidelines (48), having a pulse pressure >60 mm Hg was introduced as a marker of end organ damage, implying that such patients should be regarded in the same risk category as other high risk groups like patients with diabetes or left ventricle hypertrophy when deciding on antihypertensive treatment. Apart from the introduction of pulse pressure as a binary variable in the guidelines there are no treatment goals and pulse pressure is not considered as a continuous variable like systolic or diastolic pressure. However, in other guidelines, little is mentioned how to address pulse pressure in risk assessment (49) and hypertension treatment (50-52).

2.3 Hypertension

During the early 1900s, little was known about the danger of hypertension, except for the outcome of subjects with very high blood pressure (malignant hypertension) and even up until the 1950s, subjects with "mild benign hypertension" (<210/100 mm Hg) were not considered for treatment (53). Since the 1950s, it has been recognized that elevated blood pressure is associated with cardiovascular disease development and death much thanks to longitudinal studies such as the Framingham Heart Study (40).

For many years, the diastolic blood pressure was used in decisions on indication for antihypertensive treatment and for diagnosis of hypertension. However, in the early 1990s, systolic blood pressure was proposed as the best blood pressure variable to use, since several studies reported that systolic blood pressure had a superior association to CVD compared to diastolic blood pressure (54-56). As a result, WHO treatment recommendations on hypertension in 1993 (57) added systolic blood pressure as one of the criteria for hypertension. Since then, systolic blood pressure has emerged as the main blood pressure variable used in cardiovascular disease risk assessment, clinical decision-making and target for antihypertensive treatment (48, 51).

Classification of hypertension is displayed in table 1. Normal blood pressure is defined differently in Europe and the United States whereas hypertension is defined as systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg by both. The hypertension definition is based on evidence of better outcome with blood pressure reduction following treatment in patients with blood pressure above this definition. However, the risk of CVD starts at lower values and initiation of treatment and treatment goals must be considered together with other risk factors for CVD. This explains why the classification differs between Europe and the United States in values below 140 mm Hg and 90 mm Hg.

Table 1
Classification of Hypertension

Category (ESH)	Systolic		Diastolic	Category (AHA)
Optimal	<120	and	<80	Normal
Normal	120–129	and/or	80–84	Prehypertension
High normal	130–139	and/or	85–89	
Grade 1 hypertension	140–159	and/or	90–99	Grade 1 hypertension
Grade 2 hypertension	160–179	and/or	100–109	Grade 2 hypertension
Grade 3 hypertension	≥180	and/or	≥110	Hypertensive Crisis
Isolated systolic hypertension	≥140	and	<90	

Adopted from European Society of Hypertension (ESH)/ European Society of Cardiology (ESC) and American Heart Association (AHA). Numbers presented in mm Hg.

2.4 Risk Scoring

There are many risk scores (risk score calculators) to consider in evaluating a person's future risk of incident CVD. They are developed from different populations, on different continents, with different methods and outcomes, and therefore consist of different variables. The most well-known is the Framingham Risk Score that was published in 1998 by Wilson et al (45). The 1998 version included age, gender, systolic blood pressure, total cholesterol, high-density lipoprotein, smoking and diabetes as variables predicting coronary heart disease morbidity and mortality. It was revised 2002 into Framingham/ATPIII (58) excluding diabetes but instead including blood pressure treatment. To extend the Framingham Risk Score to a wider endpoint including stroke, intermittent claudication and heart failure, the Framingham General Cardiovascular Risk Score (59) was presented in 2008, again including diabetes. Reynolds risk score for women (2007) (60) and men (2008) (61) are two similar scoring systems. They are similar to other risk scores at base, but unique as they include hs-CRP. In Europe SCORE was presented in 2003 (46). SCORE was based on the same risk factors as the Framingham Risk Score excluding blood pressure treatment and diabetes but taking into account high or low-risk region of Europe as a factor. QRISK and QRISK2 (2007, 2008) (62, 63) were based on subjects from England and Wales, excluding diabetes but incorporating a regional score, family history of CVD and BMI. The most recent risk calculators are ACC/AHA pooled cohort hard CVD risk calculator (US 2013) (48) and the JBS3 risk score calculator (Britain 2014) (64).

The risk scoring systems usually provide a subject's 10-30 year CVD risk (some for mortality, some for morbidity and mortality) with risk cut-off values to aid the subjects and physicians in making treatment decisions. Some calculators are also designed to estimate lifetime risk. Risk below 5-10% (depending on what risk score is used) is regarded as low risk implying that the harm of treatment may be greater than the benefit. Above 20% is defined as high risk where everything possible should be done in risk factor management in order to prevent future CV events. In the latest ACC/AHA guidelines on cardiovascular risk management (48) the low risk threshold is 7.5% but the guidelines lack a definition of high risk. They also introduced the outcome atherosclerotic cardiovascular disease (ASCVD), including coronary heart disease death, non-fatal myocardial infarction (MI), fatal- and non-fatal stroke.

There is unfortunately not one risk score estimator that can be applicable for all individuals. They all have advantages and limitations and the physician should use the most appropriate score system considering the patients risk factor profile, ethnicity, comorbidity and social status. In addition, they are all designed for risk assessment in clinically healthy individuals. Subjects with established CVD or diabetes (type 2) are already at high risk and require intensive risk factor attention. However, due to the increasing population of high-risk individuals, better predictive markers and improved stratification of CVD risk within high risk populations are warranted. Below, two major scoring systems are discussed, the Framingham Risk Score, the first scoring system developed and SCORE, which is recommended in Sweden today.

Framingham Risk Score

The Framingham Risk Score is based on data from the Framingham Heart Study. As mentioned above, the scoring system was created after researchers noticed that risk factors were often clustered together and that this strongly correlated with coronary heart disease. The scoring system was created in 1998 to predict the 10-year risk of developing coronary heart disease (45). It has been revised on several occasions and the current version is from 2002. One limitation of the Framingham risk score is that it only predicts coronary heart disease risk. Therefore, the Framingham General Cardiovascular Risk Score was developed in 2008 to predict CVD risk including coronary artery disease, cerebrovascular disease, peripheral arterial disease and heart failure (59). Another limitation is that it has only been validated in the United States (in European Americans and African Americans but not

Hispanic Americans and Native Americans), raising questions of usage outside the US and even within the US in certain groups (65). However, it has been validated to some extent in non-US populations like Australian women (66) and in an Asian population (67). It has been suggested that pulse pressure should be a variable in the Framingham Risk Score (68) since pulse pressure is a strong predictor of cardiovascular events, especially in the elderly.

SCORE (Systematic Coronary Risk Evaluation)

ESH/ESC recommended SCORE in their 2007 European Society of Cardiology guidelines on cardiovascular disease prevention (69) since the Framingham Risk Score overestimated the risk in European populations. SCORE is based on data from 12 different prospective studies including over 200,000 men and women (46). The result, however, was not applicable on Europe as a whole but it was successfully adapted to regions of low risk and high risk. SCORE estimates the 10-year risk of any first fatal atherosclerotic event (including stroke or ruptured abdominal aneurysm), which is different from the Framingham Risk Score. Note that SCORE only estimates CV mortality rather than both morbidity and mortality. An Internet based, interactive version of SCORE is available with scoring systems customized for a specific country, e.g. Sweden.

2.5 Guidelines

ACC/AHA in the United States and ESH/ESC in Europe continuously write and update guidelines (70-73) for different aspects of heart disease, hypertension treatment, cardiovascular risk assessment, management of peripheral arterial disease and diabetes to name a few areas covered. These guidelines provide evidence-based knowledge and are the basis for care and treatment options for physicians and health institutions. Guidelines for cardiovascular disease risk assessment promote a multi-variable approach, considering a subject's total CVD risk. Although this approach identifies subjects at risk who need treatment for all aspects of CVD risk, it may lead to inattention to single risk factors that do not calculate an overall risk above the suggested thresholds for treatment. Inattention to single risk factors may cause irreversible organ damage over time. Further, in CVD risk assessment there is a need for better predictive markers to further distinguish subjects within high-risk groups such as patients with peripheral arterial disease and patients with hypertension. In the latest guidelines (48), having a pulse pressure >60 mm Hg was introduced as a marker of end organ damage, implying that such patients should be regarded in the same risk category as

other high risk groups like patients with diabetes or left ventricle hypertrophy when deciding on antihypertensive treatment. Apart from the introduction of pulse pressure as a binary variable in the guidelines there are no treatment goals and pulse pressure is not considered as a continuous variable like systolic or diastolic pressure. However, in other guidelines, little is mentioned how to address pulse pressure in risk assessment (49) and hypertension treatment (50-52).

2.6 Peripheral Arterial Disease

Peripheral arterial disease is a clinical manifestation of a general atherosclerotic vascular disease and with a high risk for cardiovascular events, mainly due to concomitant coronary artery disease (74-76). In 2010, approximately 202 million people in the world had PAD, of which about 70% in low- or middle-income countries (77). PAD patients are considered to have at least the same risk for future CV events as subjects with prior coronary events (78) and it has been estimated that the five-year CV mortality in PAD patients (symptomatic and asymptomatic) is around 7-8% (79). It is of great importance to identify and treat CV risk factors such as hypertension in PAD patients (80-82). Although this is known, PAD patients are often undertreated regarding hypertension (83) and when treated, few PAD patients are aggressively treated to reach blood pressure goals (84). PAD predominantly consists of intermittent claudication, although all atherosclerotic artery disease, except coronary artery disease, is included in the definition. Intermittent claudication is manifested by pain in the lower extremities during physical activity. This is due to insufficient oxygen delivery to the muscles secondary to the reduced blood flow caused by atherosclerotic arteries. The Ankle-brachial index (ABI) is commonly used to diagnose PAD (intermittent claudication) with a ratio <0.9 indicating PAD. In this thesis, our PAD population consists of patients with symptomatic intermittent claudication although CV risk is high even in PAD patients with ABI <0.9 but without symptoms (79).

2.7 Hypertensive Subjects

Hypertensive subjects constitute a group that is at high risk for future CV complications. Hypertension is associated with many aspects of CVD (85) and CVD mortality. The risk of coronary heart disease and stroke increases with higher blood pressure, even below values that define hypertension (86, 87). Evidence of the importance of blood pressure is supported by results from interventional studies that show a reduction of CVD outcome with blood

pressure lowering pharmacotherapy compared to placebo (88-90) as well as comparison between different treatment goals. Hypertensive subjects are often asymptomatic for a long time, and may thus have developed end organ damage (91) before detected in a physician's office, which may put them at greater risk even before treatment starts. Considering the increasing prevalence of hypertension with age (the strongest risk factor for CVD), elderly (even asymptomatic) populations are at very high risk for future CV events.

2.8 Ambulatory Blood Pressure

Ambulatory blood pressure measurement was introduced 1962 by Maurice Sokolow (92). He noted that some hypertensive patients with high blood pressure values measured in the office, lived normal lives and had the same life expectancy as normotensive subjects. He developed an ambulatory blood pressure monitoring device together with his colleague Hinman and concluded after a series of research, that there was a poor correlation between office blood pressure and a subject's actual blood pressure over time. They also showed that ambulatory blood pressure had better correlation to hypertensive complications than office blood pressure (93) and that ambulatory blood pressure predicted CVD risk (94). This has also been shown subsequently in various populations such as untreated hypertensive patients (95), in population-based cohorts (96, 97), in high-risk diabetic (type 2) patients (98), elderly men (99), hypertensives (100) and hemodialysis patients (101). Ambulatory blood pressure monitoring is commonly used for patients with large variability in blood pressure readings in the office and when there is a suspicion of white-coat hypertension or masked hypertension. It should, however, be considered in more blood pressure evaluations or even routinely used due to the additive information it provides. The superiority of ambulatory blood pressure, compared to office blood pressure, in individuals is likely due to several reasons, one of which is the number of measurements carried out by ambulatory blood pressure monitoring versus office blood pressure measurements. Obviously, the mean value of the ambulatory blood pressure monitoring readings is a better estimate of a subject's average blood pressure than office blood pressure, revealing both masked hypertension which is associated with high risk for incident CVD (102) or the presence of an abnormally large white coat effect (indicating white coat hypertension) which is associated with a relatively lower risk for incident CVD (102). However, subjects with white coat hypertension may be at risk for actual hypertension later in life (103). Ambulatory blood pressure also represents a patient's blood pressure during normal life circumstances and not least importantly at night. Blood pressure is normally reduced during the nighttime period but subjects without blood pressure

reduction during the night, referred to as non-dippers, are at higher risk for CV events (104). Night blood pressure has also been proven to be a stronger predictor compared to day blood pressure in hypertensive patients (105, 106). This may partly explain why ambulatory blood pressure monitoring improves risk stratification beyond that of office blood pressure measurements in hypertensive patients with (23) or without a history of cardiovascular disease (24, 25). Apart from ambulatory blood pressure variables mentioned above, the most common variable used from ambulatory blood pressure monitoring are 24-hour or daytime systolic and diastolic blood pressure and relatively few studies have evaluated the predictive value of ambulatory pulse pressure.

Despite the important information that ambulatory blood pressure can provide, ambulatory blood pressure monitoring is not routinely used in the assessment of hypertension and CVD risk assessment. Perhaps expense and lack of knowledge of the additive value may contribute to this matter. The normal values of ambulatory blood pressure monitoring are different from office-measured values. The adopted normal values of ambulatory blood pressure are shown in table 2 (51).

Table 2
Normal values for ambulatory blood pressure

24-hour	<130/80 mm Hg
Daytime	<135/85 mm Hg
Nighttime	<120/70 mm Hg

Adopted from the 2013 ESH/ESC Guidelines for the management of arterial hypertension

2.9 Pulse Pressure

Pulse pressure is calculated by subtracting the diastolic blood pressure from the systolic blood pressure. Systolic blood pressure increases with age and the diastolic blood pressure decreases gradually after approximately 55 years of age (47), resulting in a gradual increase in pulse pressure. Pulse pressure is related to more advanced vascular disease with increased vascular stiffness (107, 108). The strong predictive power of systolic blood pressure for incident CVD is evident in all age groups in contrast to the diastolic blood pressure that loses its predictive value in the elderly (6). Pulse pressure is more closely correlated with systolic blood pressure than diastolic blood pressure and in addition, it takes both the increase in

systolic and the decline of the diastolic blood pressure into account. This may partly explain the predictive value of pulse pressure in older subjects. Pulse pressure has also been shown to have superior predictive value compared to systolic blood pressure in some studies (6, 14-17). A rise in pulse pressure affects the arterial wall in a negative way, promoting degeneration of the elasticity and endothelial damage (31). Increased pulse pressure is also associated with elevated stress on the heart that may cause left ventricular hypertrophy and heart failure. The predictive power of pulse pressure has been shown in several cohort studies such as the Framingham heart study (14) but also several others (6, 15-17). Ambulatory pulse pressure as a predictor for CV events has also been reported (109-111) but studies on the predictive value of ambulatory pulse pressure are relatively few.

2.10 Biomarkers

Over the recent decades new biomarkers for cardiovascular disease have evolved. In search for new predictive markers, several studies have reported biomarker associations with CVD (19-21, 112-118). New biomarkers include high sensitivity C-reactive protein (hs-CRP), interleukin 6 (IL-6), uric acid, aldosterone, oxidized LDL antibodies, amino-terminal pro-B-type natriuretic peptide (NT-proBNP), cystatin C, coagulation factors and serum phosphate to name a few (21). However, the only biomarker recommended in current guidelines is hs-CRP, but it is still debated as to whether or not hs-CRP improves risk assessment in addition to traditional risk factors (119). One reason for the increasing interest in using biomarkers may be that the traditional risk factors for CVD tend to lose their predictive value with age (5) and there is a need for better tools in risk assessment in the elderly and in already diseased populations. Also, biomarkers are easily obtainable from the patients by means of a simple blood test and may detect subclinical organ dysfunctions or damage at an early stage. Research has been conducted to identify biomarker associations with cardiovascular disease both in short-term prognosis (21) and in long-term follow-up (120, 121). The biomarkers NT-proBNP, hs-CRP, and cystatin C have all been significant predictors for CV events in various groups of patients and subjects (19, 20, 112-118).

NT-proBNP

NT-proBNP is mainly used in clinical settings for diagnosis and control in patients with congestive heart failure (122). NT-proBNP is a byproduct of proBNP conversion into BNP, the 32-amino acid active form, and NT-proBNP, the amino terminal byproduct, is mainly

produced in and secreted by cardiac myocytes on the stimulus of myocardial stretch. Production is strongly upregulated in congestive heart failure due to increased production of proBNP1-108, as evidenced by increased circulating levels of this prohormone in patients with congestive heart failure (123). Elevated levels of NT-proBNP are also seen in older subjects, women (compared to men) and in several other situations (124). NT-proBNP has been associated with risk of developing heart failure and for CV and CV death (112, 113) including patients with established CVD, hypertensive patients as well as apparently healthy subjects.

hs-CRP

CRP is a plasma protein synthesized mainly by the liver in response to inflammation (125). Its function is thought to be activation of the complement system. The clinical use of CRP is mainly for detecting and monitoring infectious diseases although elevated levels of CRP are seen in all conditions of systemic inflammation. However, high sensitivity (hs-) assays have been developed that can detect variations of CRP at low levels (126). Hs-CRP has been associated with CVD in various populations both in short-term prognosis (114) and in long-term outcome (115). The relation of hs-CRP in the inflammatory process of atherosclerosis has been debated but it is clear that the role of hs-CRP is not causal (127). Hs-CRP have been suggested as a predictor of adverse events in PAD (19). It has also been suggested that hs-CRP correlates with ambulatory blood pressure (128). Hs-CRP is one of the basic prediction variables in the Reynold Risk Score for both men and women (60, 61) from 2003 but not in any of the other presented risk scores. It is suggested as a risk assessment marker in the current American guidelines for assessment of cardiovascular risk (48) but only if treatment decision is unclear after traditional CVD risk assessment. However, as stated above, there is still debate as to whether or not hs-CRP has a role in cardiovascular risk assessment models (119).

Cystatin C

Cystatin C is routinely used in estimating glomerular filtration rate (GFR). GFR is associated with CV events in various populations (117). However, it has been suggested that cystatin C is not simply a marker of GFR since it predicts future CV events independently of estimated GFR (116). This might be due to the limitations of creatinine-based estimations of GFR, the most common calculation estimate of renal function in most studies. Associations between

the level of cystatin C and incident CVD have been reported (117) in various populations including in the elderly, in CKD patients and in the general population. An earlier longitudinal study suggested that cystatin C was a predictor for mortality, independent of renal function, in PAD patients (118). In a previous study, it was shown that cystatin C concentration, corrected for differences in estimated GFR, was higher in PAD patients compared to controls, suggesting that cystatin C may be an independent marker of atherosclerotic disease in this group (129).

Biomarkers may be useful (in addition to traditional risk factors) in calculating risk for CVD (20), but few studies have adjusted for ambulatory blood pressure. Considering the correlation of NT-proBNP, hs-CRP and cystatin C to ambulatory blood pressure, and these biomarkers' associations with incident CVD, it is still unclear whether these biomarkers are completely independent predictors or not when adjusted optimally for blood pressure with ambulatory blood pressure. Only Paget et al (130) showed that NT-proBNP was predictive of mortality in hypertensive patients in adjusted analysis with ambulatory blood pressure, but there are no such studies for hs-CRP and cystatin C.

2.11 Risk Prediction Models And Statistics

There are several statistical methods used to evaluate the predictive improvement of adding a new marker to an existing predictive model. C-statistics is commonly used. However, the increase in the area under the receiver operating characteristic curve (AUC) is small when the existing model already includes powerful predictors. Therefore, net reclassification improvement (NRI) and integrated discrimination improvement (IDI) (131) were introduced as new methods to study the value of adding a new marker to an existing model. NRI and IDI have become very popular measurements of model performance. However, to evaluate a new marker properly, several methods should be considered and the differences should be taken into account before interpretations of the results.

2.12 Antihypertensive Treatment

Before the start of pharmacological treatment for hypertension in the 1950s, there were three treatment options for high blood pressure available; strict restrictive sodium diet, sympathectomy and pyrogenic therapy in which bacteria were injected in order to infect the

patient and thus cause blood pressure drop (53). Pharmacological treatment with several agents was introduced during the 1950s but all with considerable side effects and compliance problems (53). The first tolerable, effective pharmacological treatment came with the introduction of chlorothiazide in 1958 (53). Today, there are many tolerable antihypertensive agents to choose from both as single pills or in combinations with different agents. What agents to use is dependent on co-existing risk factors and comorbidity (see guidelines above), although recently, results from a meta-analysis propose that all of the classes of antihypertensive agents are equally preventive of CVD for a given reduction in blood pressure, regardless of established CVD or not and regardless of pre-treatment hypertension (132).

Up until the mid-1990s, treatment was focused on absolute blood pressure values, regardless of other CV risk factors involved but since then, a general CVD risk approach is recommended considering the total CVD risk of a subject (48, 49). Thus, a subject with high total risk but only moderate elevation of blood pressure may be aggressively treated in all aspects of CV risk factors including blood pressure reduction. Further, it has been suggested that different antihypertensive agents affect central pulse pressure differently and that there are differences in blood pressure-lowering effect between brachial and aortic pressures (133-136). This raises the question of whether outcomes in interventional antihypertensive studies are dependent on baseline pulse pressure, that is, if those with higher pulse pressures at baseline can benefit more from specific classes of antihypertensive treatment.

2.13 Background Summary

Pulse pressure and systolic blood pressure are strong predictors of incident CVD in the elderly (14) and pulse pressure has been shown to have superior predictive value compared to systolic blood pressure in some studies (6, 14-17). Further, it is suggested that office blood pressure underestimates ambulatory blood pressure in PAD patients (137) but it is not known if ambulatory blood pressure is a better predictor of CV events compared to office blood pressure in PAD patients.

Ambulatory blood pressure, NT-proBNP, hs-CRP, and cystatin C associate with CV and CVD mortality in various populations. Ambulatory blood pressure and these biomarkers have also been shown to correlate with each other. However, it is currently unknown if ambulatory blood pressure and the biomarkers NT-proBNP, hs-CRP, and cystatin C predict CV events independently of each other and further, if ambulatory blood pressure in combination with

these biomarkers improves risk prediction compared to basic risk factor models in apparently healthy and in CVD populations. Paget et al (130) showed that NT-proBNP was predictive of mortality in hypertensive patients in adjusted analysis with ambulatory blood pressure, but other prospective studies on the predictive value of NT-proBNP, hs-CRP, and cystatin C adjusted for ambulatory blood pressure are very few or non-existent. Finally, different antihypertensive agents may affect central pulse pressure differently and differences in blood pressure-lowering effect between brachial and aortic pressures have been suggested. Hence, outcomes in interventional antihypertensive studies may be dependent on baseline pulse pressure, and those with higher pulse pressures at baseline may benefit more from specific classes of antihypertensive treatment. This thesis aimed to investigate these gaps in knowledge further.

3 AIMS OF THE THESIS

The overall aim of this thesis was to study the predictive value of ambulatory blood pressure with a special reference to pulse pressure, and the biomarkers NT-proBNP, hs-CRP, and cystatin C and to evaluate whether ambulatory blood pressure and these biomarkers could improve risk prediction and discrimination compared to basic CVD risk factor models. In addition we aimed to study whether the outcome of antihypertensive treatment was dependent on baseline pulse pressure.

Specific aims:

3.1 Study I

-To study the value of ambulatory blood pressure as a predictive marker for cardiovascular events in PAD patients.

3.2 Study II

-To study the predictive values of NT-proBNP, hs-CRP, and cystatin C in relation to ambulatory pulse pressure for cardiovascular events in patients with PAD. Secondary aims were to study whether predictive models including these biomarkers and ambulatory pulse pressure resulted in better discrimination and reclassification of patients in comparison with a model containing other significant risk factors previously identified in this cohort.

3.3 Study III

-To study if the association between NT-proBNP, hs-CRP and cystatin C for incident CVD was independent of traditional cardiovascular disease risk factors including ambulatory blood pressure in a population-based cohort of elderly men and if the addition of biomarkers improved risk discrimination and reclassification in this setting.

3.4 Study IV

-To study whether the superiority of the combination treatment benazepril+amlodipine compared to benazepril+hydrochlorothiazide on cardiovascular events in the ACCOMPLISH trial was dependent on baseline pulse pressure.

4 SUBJECTS AND METHODS

4.1 Subjects

4.1.1 Study I-II

Male patients were consecutively recruited from patients referred for symptoms of intermittent claudication to the vascular clinics of Karolinska and St Görans hospitals, Stockholm, Sweden, between 1998 and 2001. Inclusion criteria were: male sex, aged >45 years, a history of intermittent claudication and an ABI <0.9 by Doppler ultrasonography at rest based on initial study examination. Twenty-seven patients had a history of previous peripheral vascular surgery. In these patients, a higher baseline ABI than <0.9 at the time of the study investigation was accepted. Patients with rest pain, previous amputation, or reasons for a reduced walking performance other than intermittent claudication, diabetes mellitus type 1, and atrial fibrillation were excluded. A history of ischemic heart disease was not an exclusion criterion, and patients were included irrespective of presence of ischemic heart disease. Referred patients that met the inclusion criteria were asked for informed consent to participate in the study.

A total of 99 patients with intermittent claudication were finally included. In one patient, ambulatory blood pressure monitoring was not performed since office systolic blood pressure was repeatedly >210 mm Hg, leaving 98 patients for the final analysis. No alteration of medication was done before the investigations. Potential control subjects matched for sex and age were identified from the population registry of Stockholm County. Eligible subjects were invited to the clinic for a screening visit, at which time a medical history, blood pressure, ABI, and a 12-lead electrocardiogram were obtained. Subjects who did not have a history of ischemic heart disease, stroke, or PAD and with an ABI >0.9 were selected as control subjects. A total of 92 control subjects were sampled, 90 of whom performed ambulatory blood pressure monitoring.

4.1.2 Study III

Uppsala Longitudinal Study of Adult Men (ULSAM) was initiated in 1970 when all 50-year-old men born in 1920-1924 living in Uppsala, Sweden, were invited to participate in a study that aimed to identify risk factors for cardiovascular disease. The study is described in detail at www.pubcare.uu.se/ULSAM. Study III was based on data from follow-up in 1991-1995

when subjects reached approximately 70 years of age. From the original patient inclusion, 1681 were still alive living in Uppsala and 1221 of these subjects participated in the 1991-1995 follow-up. All patients with missing values in ambulatory blood pressure, or any of the investigated variables were excluded, leaving 1024 subjects. Prior to the baseline examination, 114 subjects had been hospitalized due to coronary heart disease or cerebrovascular disease (International Classification of Diseases, ICD-9: 410, 411.8, 431, 433, 434 and ICD-8: 410, 411, 431, 432, 433, 434) or surgical codes (Swedish classification of interventions and procedures) for percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG) used as proxy for coronary heart disease (KVA: FNA, FNB, FNC, FND, FNE, FNF, FNG and before 1996; 3066, 3067, 3080, 3105, 3127, 3158) and were defined as having cardiovascular disease at baseline and were excluded, resulting in 910 subjects for final analyses.

4.1.3 Study IV

The complete design of the ACCOMPLISH study has been published previously (138). In brief, the ACCOMPLISH trial was a randomized, double-blinded, multicenter trial (a total of 548 centers in the United States, Sweden, Norway, Denmark, and Finland) that compared the effect of benazepril+amlodipine with benazepril+hydrochlorothiazide in preventing a composite of fatal and nonfatal cardiovascular outcomes. Participants included in the trial were 55 years or older with either systolic blood pressure ≥ 160 mm Hg or currently receiving antihypertensive therapy. Included patients had evidence of CVD and/or renal disease or other target organ damage or diabetes. 13,782 patients were screened and 11,499 underwent randomization (5741 to benazepril+amlodipine and 5758 to benazepril+hydrochlorothiazide) in ACCOMPLISH all of which were included in this subanalysis. There was no formal washout period for patients with ongoing antihypertensive treatment. Patients already receiving treatment for hypertension were to discontinue ongoing treatment after the first visit, resulting in a two-week period of no antihypertensive treatment until switching to the blinded study drugs after randomization.

4.2 Methods

4.2.1 Ambulatory blood pressure

4.2.1.1 Study I-II

Ambulatory blood pressure values were obtained using a noninvasive oscillometric system (Spacelabs 90207; Spacelabs, Redmond, WA) (139). An experienced nurse fit the device to the patient. Patients were instructed not to restrict their daily activities during the monitoring periods. Before start of the monitoring period, the automatic readings were crosschecked against manually measured blood pressure by auscultation. Blood pressure and heart rate were recorded automatically every 15 minutes for a 24-hour period. The blood pressure data was auto-edited by the Spacelabs program, which excludes presumably erroneous data. No manual editing of data was carried out so as not to induce bias. Means were calculated for the whole 24-hour period, as well as for day (7:00 am–9:00 pm), and night (midnight–6:00 am) periods.

4.2.1.2 Study III

Ambulatory blood pressure was measured with Accutracker II (Suntech Medical Instruments, Raleigh, NC) (140). The device was fitted to the patients' non-dominant arm by a skilled lab technician. Systolic blood pressure and diastolic blood pressure were measured every 30 min during daytime (0600-2300) and every hour during nighttime over 24 hours. From November 1993, blood pressure was measured every 20 minutes during the whole 24-hour period. Limited editing was done excluding all readings of zero, diastolic blood pressure >170 mm Hg, systolic blood pressure >270 mm Hg or < 80 mm Hg and all readings with pulse pressure less than 10 mm Hg.

4.2.2 Office blood pressure

4.2.2.1 Study I-II

Office blood pressure was recorded in both arms by an experienced nurse using a mercury sphygmomanometer with the subject in the supine position after 5 minutes of rest. The mean of two consecutive readings was calculated. If there was a difference in systolic or diastolic blood pressure between the arms of >10 mm Hg, the arm with the highest reading was used when defining office blood pressure; otherwise the non-dominant arm was used. The same arm was used for office and ambulatory blood pressure measurements.

4.2.2.2 *Study III*

Office blood pressure was measured in the right arm with a sphygmomanometer using the appropriate cuff size. Recordings were taken with the subject in the supine position after resting for 10 minutes. The values were recorded twice and to the nearest even figure and presented as means of the two values.

4.2.2.3 *Study IV*

Blood pressure was measured according to the 1988 American Heart Association committee report on blood pressure determination (141) using a calibrated standard sphygmomanometer or a calibrated digital device and an appropriately sized cuff. Blood pressure was measured three times at each study visit at 1- to 2-minute intervals after the patient had remained in a seated position for 5 minutes and was recorded as the average of the three measurements.

4.2.3 **Laboratory examination**

Plasma NT-proBNP concentration was measured using a commercial test kit and instrument (ELECSYS 2010; Roche Diagnostics, Basel, Switzerland) (**study II-III**). Cystatin C and hs-CRP measurements were performed according to the instructions of the manufacturer using particle-enhanced immunonephelometric assays with kits and instrument (BN II analyzer; Dade Behring, Marburg, Germany) (126) (**study II**) and by latex enhanced reagent (Dade Behring, Deerfield, IL, USA) using a Behring BN ProSpec analyzer (Dade Behring) (**study III**). Glomerular filtration rate (GFR) was calculated according to modification of diet in renal disease (142) from creatinine, age, race, and sex (**study II**). Plasma glucose in samples from the oral glucose tolerance test was measured by the glucose dehydrogenase method (Gluc-DH, Merck, Darmstadt, Germany) (**study III**). Cholesterol and triglyceride concentrations were analyzed in serum and in the isolated lipoprotein fractions by enzymatic techniques using IL Test Cholesterol Trinders's Method and IL Test Enzymatic-colorimetric Method for use in a Monarch apparatus (Instrumentation Laboratories, Lexington, USA) (**study III**). High-density lipoproteins were separated by precipitation with magnesium chloride/phosphotungstate (**study III**). LDL cholesterol was calculated using Friedewald's formula: $LDL = \text{serum cholesterol} - \text{high-density lipoprotein} - (0.45 \cdot \text{serum triglycerides})$ (mmol/L) (**study III**). Diabetes was diagnosed according to the 1985 WHO criteria (143).

The body mass index (BMI) was calculated as the weight (in kg with one decimal) divided by the height (in meters with two decimals) squared (kg/m^2) (**study III**).

Treatment for hypertension was defined as treatment with antihypertensive drugs (**study I-III**). Men treated with these drugs for other reasons, i.e. congestive heart failure, were not included in this definition in study III.

4.2.4 Survival, Hospitalization Data

4.2.4.1 The Swedish National Patient Register

The Swedish National Board of Health and Welfare started the National Patient Register in 1964. The National Patient Register has provided data and hospital discharge diagnosis according to the International Classification of Disorders (ICD-codes) on in-patient care in the whole country since 1987 (parts of the country since 1964) and outpatient public and private care since 2001. Primary care is not yet covered in the National Patient Register. The in-patient care records form a part of the National Patient Register called the Swedish National Inpatient Register. External validation of the Swedish National Inpatient Register (also called the Hospital Discharge Register), show reliable overall positive predictive values between 85-95% for diagnose specific validity and low drop-out rates (144). Positive predictive values for MI (145) and stroke (144) are as high as 98%.

4.2.4.2 The Cause of Death Register

This is held by the Swedish National Board of Health and Welfare and provides information on all deceased Swedish citizens since 1952 and is considered to have almost complete coverage from 1961. The data contains date and location of death and ICD codes on main and contributory causes of death. Missing records of main diagnosis of death comprise about 1-2% and are coded without known cause of death. An external validation of the Cause of Death Register, published in 2009, showed correct diagnosis in 77% of the cases in general but 87% for ischemic heart disease (146).

4.2.5 Endpoints

4.2.5.1 Study I-II

The primary outcome variable was time to first cardiovascular event, defined as either cardiovascular mortality or any hospitalization for acute MI, stroke or coronary revascularization (PCI or CABG). Data were obtained from the Swedish Hospital Discharge

and Cause of Death Registries. Data included ICD codes for all-cause mortality and all-cause hospitalization based on main discharge diagnosis. Cardiovascular mortality was defined as ICD codes I 10–25 and I 30–79. Hospital records for each hospitalization were obtained to verify the event and diagnosis.

4.2.5.2 Study III

Patients were followed for 10 years after the baseline visit (1991-1995). The end point was a new event of atherosclerotic cardiovascular disease event (ASCVD) defined as fatal or non-fatal MI or fatal or non-fatal stroke in accordance with the recently updated AHA prevention guidelines (48). Event data were obtained from the Swedish Hospital Discharge and Cause of Death Registries according to ICD-10: I21, I22, I61, I63, I64 and ICD-9: 410, 411.8, 431, 433 and 434. Only main discharge and main death diagnosis were used to identify events.

4.2.5.3 Study IV

The primary endpoint in the subanalysis of the ACCOMPLISH trial was a combined endpoint of cardiovascular morbidity (nonfatal MI or nonfatal stroke) and/or mortality (death caused by sudden cardiac death, fatal MI, fatal stroke, death caused by coronary intervention, or death caused by congestive heart failure or other cardiovascular causes). This was the same as the overall primary endpoint in ACCOMPLISH (time to first event for cardiovascular death or cardiovascular event) except for the removal of the following (in cardiovascular events): hospitalization for unstable angina, coronary revascularization, or resuscitation after sudden cardiac arrest. Secondary endpoints in the subanalysis consisted of nonfatal and fatal MI and nonfatal and fatal stroke. An endpoint committee adjudicated all endpoints according to standard criteria. The members of the endpoint committee were unaware of the study group assignments and were not active investigators or staff of the sponsor, Novartis Pharmaceuticals.

4.2.6 Statistics

4.2.6.1 Study I

The crude predictive values of ambulatory and office blood pressure variables, age and relevant clinical variables were assessed by Cox regression analysis. Hazard ratios (HR, with 95% confidence intervals (CI)) were calculated for a 10-unit increase in blood pressure variables and age. Backward stepwise variable selection was performed to determine independent predictors in an adjusted Cox regression analysis in PAD patients. The following

variables were entered in the analysis; 24-hour pulse pressure, history of hypertension, history of diabetes, history of previous MI, treatment with blood pressure-lowering drugs, history of stroke, current smoker and age. Night pulse pressure was omitted, as it covariates with 24-hour pulse pressure and we considered 24-hour pulse pressure to be more clinically relevant. Statistical analysis and database management were performed with StatSoft, Inc. (2010), Statistica data analysis software system, version 9.1 (www.statsoft.com). Student's t-tests for dependent or independent samples or a χ^2 test were used when appropriate. Pearson r correlation coefficient was calculated for linear correlation. A p-value < 0.01 was considered significant.

4.2.6.2 *Study II*

The incidence of events was compared between high vs low tertiles of biomarkers and ambulatory pulse pressure. Since the distribution of the biomarkers NT-proBNP, hs-CRP, and cystatin C were skewed, logarithmic values were used in the final analyses. In addition, since a U-shaped relation between cystatin C levels and CV events was seen, a quadratic and centered term for cystatin C was analyzed. The crude predictive values were assessed by Cox regression analysis, and hazard ratios for a 1 SD increase (with 95% CI) were calculated for 24-hour pulse pressure and logarithmic values of biomarkers. Ambulatory blood pressure variables, office blood pressure variables, log(hs-CRP), log(NT-proBNP), and log(cystatin C) were separately adjusted for basic cardiovascular risk factors (age, treatment with blood pressure-lowering drugs, and previous MI) in adjusted analysis. Further, the biomarkers were separately adjusted for basic CV risk factors and 24-hour pulse pressure, day pulse pressure, night pulse pressure, and night systolic blood pressure, respectively, since these ambulatory blood pressure variables were significant predictors in crude analyses reported previously (study I). Finally, backward variable selection from all of the above (P to enter <0.05; P to remove >0.10) was performed to determine independent predictors in adjusted Cox regression analysis. No interactions were found among included variables in interactional analysis. A discriminant analysis was performed calculating area under the receiver-operating characteristic curve (AUC) of adjusted models predicting risk for CV events. AUC was compared adding biomarkers, significant ambulatory blood pressure variables, and office pulse pressure (as a clinically relevant comparator) separately and together to a model containing basic cardiovascular risk factors, using methodology described by DeLong et al (147). AUC calculations were done using MedCalc version 12.7.5 (www.medcalc.org). Finally, net reclassification improvement (NRI) was calculated when biomarkers and blood pressure variables were added (separately and together) to a model with basic cardiovascular

risk factors. Risk cutoffs for categorical NRI estimation were based on tertiles of risk as predicted by the different models. NRI was calculated according to Pencina et al (131). Statistical analysis and database management were performed with StatSoft 2010 STATISTICA data analysis software system version 9.1 (www.statsoft.com). Student t-tests, Mann–Whitney U tests, or χ^2 tests were used for dependent or independent variables when appropriate. $P < 0.05$ was considered significant.

4.2.6.3 Study III

A basic ASCVD risk model was used that included the traditional CVD risk factors: age, smoking, diabetes, treatment for hypertension, lipid-lowering medication, BMI, cholesterol, high-density lipoprotein and office systolic blood pressure, all of which have previously been used as risk factors in the ULSAM-cohort. The basic ASCVD risk model was altered by adding ambulatory blood pressure variables one at a time, and by exchanging office systolic blood pressure for ambulatory blood pressure variables one at a time. Cox regression analysis was used to estimate the association between the variables in the models and incident ASCVD. For each of these models, a measure of predictive power, Harrell's C, was calculated (148). The method described by Newson (149) was used for comparison between two models in terms of Harrell's C. Two ambulatory blood pressure models (24-hour systolic blood pressure and 24-hour pulse pressure) were chosen for further analyses. The biomarkers were added, alone and in combinations, to the basic ASCVD risk model and to the chosen ambulatory blood pressure models and new models were created. Finally the new models were compared to the same model that also included 24-hour systolic blood pressure and 24-hour pulse pressure respectively. The variables NT-proBNP, hs-CRP and cystatin C were logarithmically transformed due to skewness. All continuous variables presented in the models were standardized, consequently the interpretation of the regression coefficients was in terms of one change in standard deviation. Observations with missing values on at least one covariate were excluded from the analysis. Schoenfeld residuals were used to test the assumptions of proportional hazards.

NRI and IDI were calculated according to Pencina et al (131). In order to define the risk categories for NRI, a cut off point of 7.5% for low risk of ASCVD during a 10-year follow-up was used as recommended by the 2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk (48). Since the current guidelines do not define a cut off for high risk, previous high risk cut off (20%) from the 2008 Framingham General Cardiovascular Risk Score (59) was extrapolated to 25% similarly to the increase of low risk from 6 to 7.5%. NRI and IDI were in this study based on logistic regression and having an ASCVD event before

10 years after inclusion in the study. Censored observations were treated as non-events. Statistical analyses and database management were performed with the statistical software Stata (version 13). P-values <0.05 were considered statistically significant.

4.2.6.4 Study IV

Patients were divided into pulse pressure tertiles (high, medium, and low) based on their baseline pulse pressure. Normally distributed data were presented as mean standard deviation in the three tertiles. First, HRs with 95% CIs for the primary and secondary endpoints for each of the tertiles (high vs low, high vs medium, and medium vs low) were calculated pooling the two treatment groups using a Cox regression model that included age, coronary artery disease (yes/no), and diabetes mellitus (yes/no) as covariates. Secondly, HRs with 95% CIs for the primary and secondary endpoints for treatment effect (benazepril+amlodipine over benazepril+hydrochlorothiazide) were calculated in all pulse pressure tertiles using the same Cox regression model. Finally, HRs for treatment effects were compared among all pulse pressure tertiles. SAS version 9.3 (SAS Institute Inc, Cary, NC) was used for statistical analysis. P-values <0.05 was considered statistically significant.

5 RESULTS

5.1 Study I-II

Baseline patient characteristics are shown in table 3. Office and ambulatory pulse pressure were significantly higher in PAD-patients compared to control subjects ($p < 0.001$).

Table 3
Main characteristics of the PAD population (n=98) and control subjects (n=90)

	<i>PAD-patients</i>	<i>Control subjects</i>	<i>P-value</i>
Age (years)	68 ± 7 (45-79)	68 ± 8	
Smokers (current/former/never)	23/69/6 (23/70/6)	15/40/35	
ABI (Ankle brachial index)	0.66 ± 0.19	1.11 ± 0.11	
Duration of symptomatic IC (years)	2 (1, 10)	-	
History of hypertension (yes)	59 (60)	14 (16)	
Treatment with BP lowering drugs (yes)	69 (70)	19 (21)	
Diabetes mellitus type II (yes)	16 (16)	7 (8)	
Clinical ischemic heart disease	40 (41)	0	
Previous MI	23 (23)	0	
Previous stroke	17 (17)	0	
Estimated GFR (ml/min/1.73m ²)	75.5 ± 21.7	80.2 ± 13.8	
cystatin C (mg/L)	0.96 (0.86, 1.23)	0.92 (0.82, 1.01)	
hs-CRP (mg/L)	2.58 (1.32, 4.87)	1.46 (0.74, 2.56)	
NT-proBNP (ng/L)	166 (76, 418)	59 (36, 123)	
Office SBP	151 ± 22	139 ± 20	<0.001
Office DBP	79 ± 10	79 ± 9	
Office Pulse Pressure	71 ± 19	60 ± 15	<0.001
24h SBP (mm Hg)	142 ± 14	133 ± 14	<0.001
24h DBP (mm Hg)	78 ± 8	79 ± 8	
24h Pulse Pressure (mm Hg)	64 ± 12	54 ± 10	<0.001

Values are expressed as mean ± SD, median (interquartiles) or numbers (percentage). P-values denote comparisons between preceding column groups using Students t-test, Mann-Whitney U-test or chi-square where appropriate. ACE, angiotensin converting enzyme; MI, myocardial infarction; ARB, angiotensin 2 receptor blockers; BP, blood pressure; DBP, diastolic blood pressure; CRP, high sensitivity C-reactive protein; GFR, glomerular filtration rate; IC, intermittent claudication; NT-proBNP, amino-terminal pro B-type natriuretic peptide; SBP, systolic blood pressure; 24h, 24 hour

Median observation time was 71 months (range 50–88). A total of 55 events occurred in 36 of 98 patients including 14 MIs, 7 PCIs, 9 CABGs, 10 strokes and 15 cardiovascular deaths. A total of 8 events occurred in 7 of 90 control subjects (1 MI, 2 PCIs, 1 CABG, 3 strokes and 1 cardiovascular death).

Study I

24-hour pulse pressure and night pulse pressure predicted time to first cardiovascular event in PAD patients with an increased risk of 48 percent and 44 percent for a 10 mm Hg increase respectively in crude analyses. Office blood pressure did not predict CV events in PAD patients. In the control subjects, office systolic blood pressure and pulse pressure were significant predictors ($p < 0.01$), however ambulatory blood pressure did not improve prognostic information above office blood pressure in this group. After backward variable selection (including; 24-hour pulse pressure, history of hypertension, history of diabetes, history of previous MI, treatment with blood pressure-lowering drugs, history of stroke, current smoker and age) 24-hour pulse pressure ($p < 0.01$) still predicted cardiovascular events in PAD patients, along with history of previous MI, treatment with blood pressure-lowering drugs (figure 2).

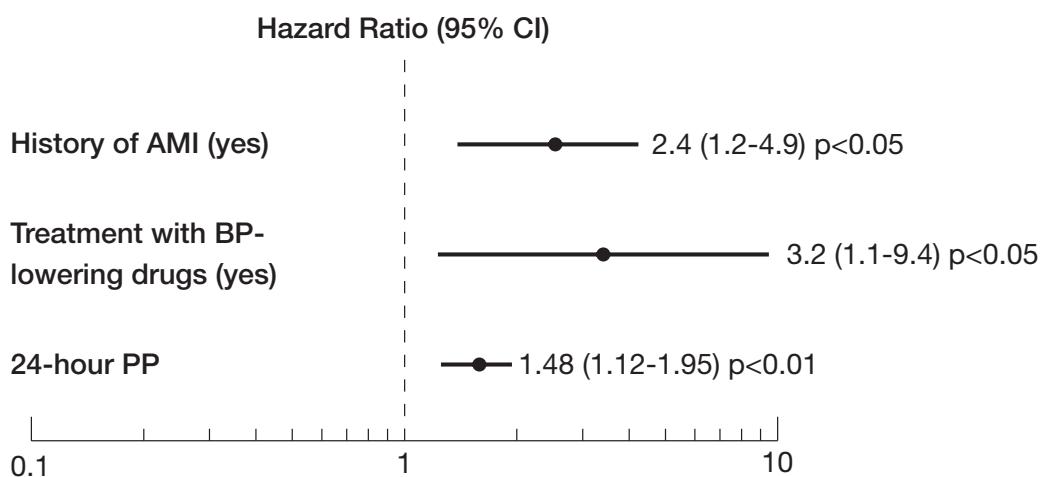


Figure 2. Predictors of cardiovascular events in peripheral arterial disease patients in adjusted analysis (n= 98) MI, myocardial infarction; BP, blood pressure; CI, confidence interval; PP, pulse pressure Hazard ratios apply for a 10-mmHg increase in 24-hour PP.

Event free survival in controls and in relation to tertiles of 24-hour ambulatory pulse pressure in PAD patients is presented in figure 3. The hazard ratio for CV events in PAD patients in comparison to control subjects was 5.4 (2.4-12.1, $p < 0.001$).

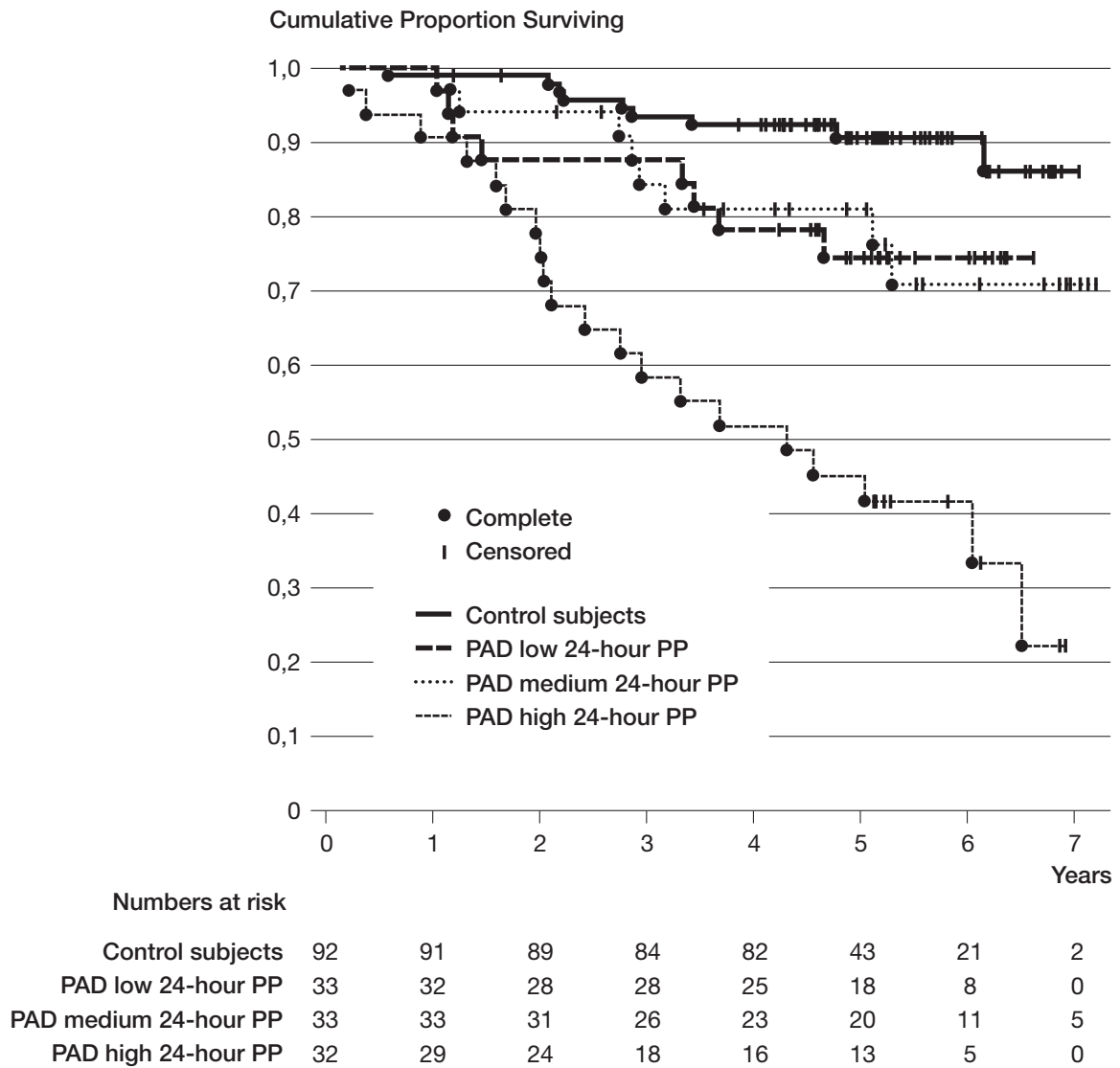


Figure 3. Event-free survival and numbers at risk in relation to tertiles of 24-hour pulse pressure (PP) in peripheral arterial disease (PAD) patients (n=97) and in control subjects (n=90). Low tertile corresponds to 24-hour PP below 58.7 mmHg, medium tertile to 24-hour PP between 58.7 and 68.7 mmHg, and high tertile to 24-hour PP greater than 68.7 mmHg.

Study II

In study II, higher values of NT-proBNP and hs-CRP were associated with higher incidence of cardiovascular events in PAD patients, whereas this was not the case for cystatin C. For

NT-proBNP, the incidence of cardiovascular events was 64% versus 15% ($p < 0.001$) in the high compared to the low tertile. For hs-CRP, the incidence was 55% versus 21% ($p < 0.01$) and for cystatin C it was 51% versus 42% respectively (figure 4).

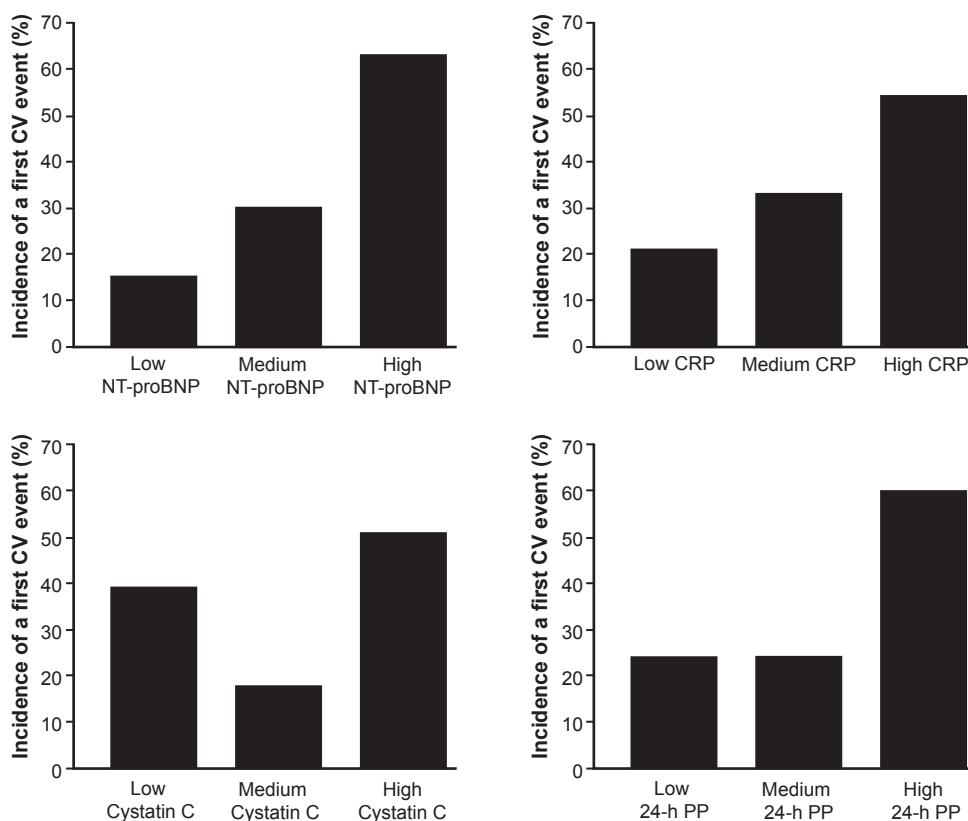


Figure 4. Incidence (%) of cardiovascular events (CV) in relation to tertiles of amino-terminal pro-B-type natriuretic peptide (NT-proBNP; low tertile: < 97 ng/L; medium tertile: 97–319 ng/L; high tertile: > 320 ng/L), high-sensitivity C-reactive protein (CRP; low tertile: < 1.7 mg/L; medium tertile: 1.7–4 mg/L; high tertile: > 4 mg/L), cystatin C (low tertile: < 0.89 mg/L; medium tertile: 0.89–1.06 mg/L; high tertile: > 1.06 mg/L), and 24-hour pulse pressure (24-h PP; low tertile: < 58.7 mm Hg; medium tertile: 59–69 mm Hg; high tertile: > 69 mm Hg). Comparison with χ^2 for NT-proBNP high vs. low tertile: $P < 0.001$. Comparison for CRP high vs. low tertile: $P < 0.01$. Comparison for cystatin C high vs. low tertile: $P = 0.30$. Comparison for 24-h PP high vs. low tertile: $P < 0.01$.

The patients were divided into 4 categories of 24-hour pulse pressure and NT-proBNP; above or below median 24-hour pulse pressure (62 mm Hg) and above or below median NT-proBNP in the respective category (114 and 318 ng/L respectively). Patients with the combination of a high 24-hour pulse pressure and high NT-proBNP had the worst outcome (figure 5), whereas other combinations did not differ significantly in event-free survival. The combination of a below median 24-hour pulse pressure and below median NT-proBNP, had the same outcome as matched control subjects free of cardiovascular disease (figure 5). A corresponding analysis for 24-hour pulse pressure and hs-CRP (2,2 and 2,9 mg/L respectively) showed similar results (figure 5). No corresponding analysis of cystatin C was made due to the lack of association between cystatin C levels and cardiovascular events.

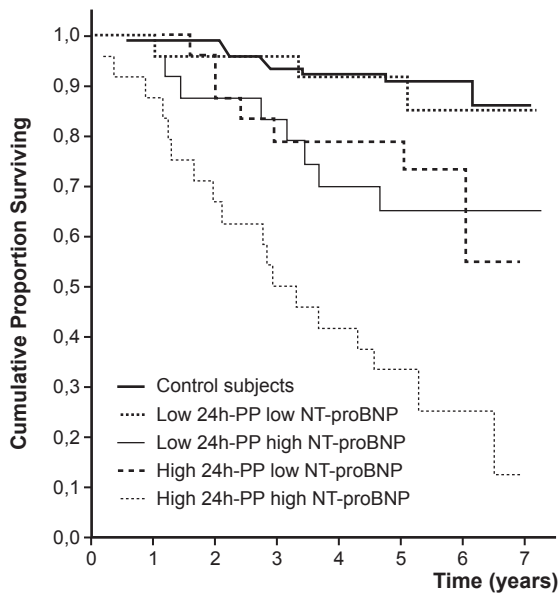
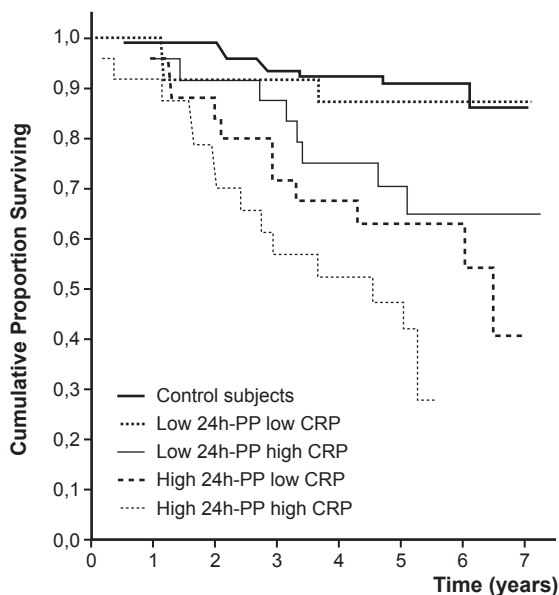


Figure 5.

Event-free survival in relation to 4 categories of ambulatory pulse pressure and amino-terminal pro-B-type natriuretic peptide (NT-proBNP): greater or less than median 24-hour pulse pressure (24-h PP; 62 mm Hg) and greater or less than median NT-proBNP in respective category (114 and 318 ng/L, respectively).



Event-free survival in relation to 4 categories of ambulatory pulse pressure and high-sensitivity C-reactive protein (hs-CRP): greater or less than median 24-h PP (62 mm Hg) and greater or less than median hs-CRP in respective category (2.2 and 2.9 mg/L, respectively)

When used as continuous variables, 24-hour pulse pressure, $\log(\text{NT-proBNP})$ and $\log(\text{hs-CRP})$ all predicted cardiovascular events in crude analysis ($p < 0.01$), whereas $\log(\text{cystatin C})$ did not ($p = 0.16$) (table 4). In multivariate analysis both $\log(\text{NT-proBNP})$ and $\log(\text{hs-CRP})$ still predicted cardiovascular events when adjusted for age, previous MI and treatment with blood pressure lowering drugs and remained significant when further adjusted for day pulse pressure, night pulse pressure, night systolic blood pressure, or 24-hour pulse pressure respectively (table 4).

Day pulse pressure, $\log(\text{NT-proBNP})$, $\log(\text{hs-CRP})$, and previous MI were all independent predictors of cardiovascular events (table 4) in adjusted analysis with backward stepwise variable selection, including all of the biomarkers, day pulse pressure, night pulse pressure,

night systolic blood pressure, and 24-hour pulse pressure, age, previous MI and treatment with blood pressure lowering drugs.

Table 4
HR and 95% CI for CV events and independent predictive markers for CV events in PAD patients, multivariate (n=98)

	HR	95% CI	P value
<i>Adjusted for basic CV risk factors*</i>			
Log(NT-proBNP)/ SD	1.68	1.09-2.60	<0.05
Log(hs-CRP)/ SD	1.53	1.13-2.08	<0.01
Quadratic and centered term of cystatin C	1.27	0.74-2.16	0.39
<i>Adjusted for basic CV risk factors and ambulatory BP</i>			
Log(NT-proBNP)/ SD	1.62 ^a	1.05-2.51	<0.05
	1.65 ^b	1.06-2.56	<0.05
	1.59 ^c	1.02-2.45	<0.05
	1.62 ^d	1.05-2.50	<0.05
Log(hs-CRP)/ SD	1.63 ^a	1.19-2.24	<0.01
	1.65 ^b	1.20-2.26	<0.01
	1.58 ^c	1.15-2.14	<0.01
	1.59 ^d	1.16-2.17	<0.01
Quadratic and centered term of cystatin C	1.32	0.76-2.27	0.32
<i>Independent predictors for cardiovascular events**</i>			
Day PP/ SD	1.64	1.12-2.40	<0.05
Log(NT-proBNP)/ SD	1.55	1.05-2.27	<0.05
Log(hs-CRP)/ SD	1.54	1.10-2.15	<0.05
Previous MI (yes)	2.87	1.38-5.97	<0.01

MI, myocardial infarction; BP, blood pressure; CI, confidence interval; CV, cardiovascular; DBP, diastolic blood pressure; HR, hazard ratio; CRP, high sensitivity C-reactive protein; NT-proBNP, amino-terminal pro B-type natriuretic peptide; PAD, peripheral arterial disease; PP, pulse pressure; SBP, systolic blood pressure; 24h, 24-hour

*Basic CV risk factors; (age, treatment with BP lowering drugs, previous MI)

**Backward stepwise variable selection including variables log(hs-CRP), log(NT-proBNP), 24 hour PP, day PP, night PP, night SBP and basic CV risk factors

Adjusted for ^a24h PP, ^bday PP, ^cnight PP, ^dnight SBP

Finally, the ability of the biomarkers and ambulatory blood pressure to improve risk discrimination when added to a basic cardiovascular risk factor model including age, previous MI and treatment with blood pressure lowering drugs were analyzed (table 5). When added separately to the basic cardiovascular risk factor model none of the ambulatory blood pressure variables, office pulse pressure, log(NT-proBNP) or log(hs-CRP) improved discrimination. However, several combinations of these variables significantly improved risk discrimination with the combination of day pulse pressure, log(NT-proBNP) and log(hs-CRP) resulting in the highest AUC value (p=0.02 for the difference in AUC compared to the

basic CV risk factor model). The percentage of correctly reclassified patients, adding day pulse pressure, log(NT-proBNP) and log(hs-CRP) to the basic cardiovascular risk factor model was 36.7% (95% CI 11-62.5%). However, the addition of any of the variables, separate as well as in combinations, significantly improved NRI with a range between 17.6-43.9% compared to the basic cardiovascular risk factor model.

Table 5
AUC of multivariable models and NRI using multivariable models predicting risk for CV events in PAD patients (n=98)

Model	AUC			NRI		
	AUC	95% CI	P value**	%	95% CI	P value
Basic CV risk factor model *	0.736	0.637 to 0.820				
+ 24h PP and NT-proBNP and CRP	0.825	0.735 to 0.895	0.030	32.3	7.0 to 57.7	0.0125
+ Day PP and NT-proBNP and CRP	0.833	0.744 to 0.901	0.020	36.7	11.0 to 62.5	0.0052
+ Night PP and NT-proBNP and CRP	0.810	0.717 to 0.882	0.056	32.6	8.2 to 56.9	0.0087
+ Night SBP and NT-proBNP and CRP	0.813	0.721 to 0.885	0.047	37.0	12.3 to 61.7	0.0034
+ Office PP and NT-proBNP and CRP	0.816	0.725 to 0.887	0.052	32.3	6.6 to 58.1	0.0139

AUC, area under the receiver-operating characteristic curve; CI, confidence interval; CRP, high sensitivity C-reactive protein; CV, cardiovascular; NRI, Net reclassification improvement; NT-proBNP, amino-terminal pro B-type natriuretic peptide; PAD, peripheral arterial disease; PP, pulse pressure; SBP, systolic blood pressure; 24h PP, 24-hour pulse pressure

*Basic CV risk factor model includes age, treatment with blood pressure lowering drugs and previous acute MI.

NRI is based on tertiles of risk; NT-proBNP and CRP are logarithmic (log) values.
AUC from logistic regression analysis of different models used to predict the outcome.

**Probability values for the comparison of AUCs with the traditional risk factor model using the DeLong test.

5.2 Study III

Baseline characteristics are displayed in table 6. Mean follow-up time was 8.5 years. All variables in table 6 were predictive of outcome in crude analysis except for age (p=0.30), lipid-lowering medication (p=0.81) and BMI (p=0.26). Ambulatory blood pressure variables were analysed one at a time with all covariates in a basic CVD risk factor model (age, smoking, diabetes, treatment for hypertension, lipid-lowering medication, body mass index (BMI), cholesterol, high-density lipoprotein and office systolic blood pressure). All ambulatory blood pressure variables were significant predictors in adjusted analyses with the basic CVD risk factor model (range; 24-hour systolic blood pressure (HR 1.35, p<0.001) to night pulse pressure (HR 1.21, p=0.003)). NT-proBNP (HR 1.46, p<0.001) and hs-CRP (HR

1.19, $p=0.048$) but not cystatin C (HR 1.15, $p=0.096$) were predictive in adjusted analyses. Two models were chosen for further analyses with biomarkers; the 24-hour systolic blood pressure model (ASBPm) and the 24-hour pulse pressure model (APPm) (ambulatory pulse pressure HR 1.25, $p=0.002$). AUC for the ASBPm was higher ($p=0.034$) compared to the basic CVD risk factor model but NRI was not improved. However, all models that included

Table 6
Baseline characteristics at 71 years of age for the ULSAM cohort

Variable	N/ Mean	Range
Age (years)	71.0 (0.61)	69.4-73.6
Smoker (yes)	186 (20.4)	
Hypertension (yes)	324 (32)	
Diabetes (yes)	133 (14.6)	
Treatment for Hypertension (yes)	290 (31.9)	
Lipidmedication (yes)	76 (8.4)	
BMI (kg/m ²)	26.1 (3.4)	16.7-46.3
Cholesterol (mmol/L)	5.80 (0.99)	2.43-8.97
HDL cholesterol (mmol/L)	1.30 (0.35)	0.51-3.1
NT-proBNP (ng/L)	199 (340)	5-3992
hs-CRP (mg/L)	3.25 (4.58)	0.16-47.8
cystatin C (mg/L)	1.23 (0.27)	0.75-4.87
SBP office (mm Hg)	147.1 (18.5)	100-222
24h SBP (mm Hg)	135.7 (16.5)	101-208
24h DBP (mm Hg)	76.6 (7.93)	55-109
24h PP (mm Hg)	59.0 (12.3)	34-146
24h SBP night (mm Hg)	121.3 (19.2)	86-218
24h DBP night (mm Hg)	67.8 (8.8)	45-98
24h PP night (mm Hg)	53.5 (14.5)	30-165

24h, 24 hour; BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; hs-CRP, high sensitivity C-reactive protein; NT-proBNP, amino-terminal pro-B-Type natriuretic peptide; PP, pulse pressure; SBP, systolic blood pressure; SD, standard deviation
Values expressed as N (%) for categorical variables and as means (SD) for continuous variables

a combination of NT-proBNP and 24-h systolic blood pressure increased the AUC ($p<0.02$), as well as improved NRI compared to basic CVD risk factor model. The comparisons between the ambulatory blood pressure biomarker models and the ambulatory blood pressure models are shown in table 7. No significant improvement in C-statistics was observed when

we compared combined ambulatory blood pressure biomarker models to the ASBPm or APPm respectively (table 7). However, the addition of NT-proBNP, but none of the other biomarkers, significantly improved NRI as well as IDI compared to both to the ASBPm and APPm (table 7).

To determine the additive effect of ambulatory blood pressure on models including biomarkers, we compared the ASBPm and the APPm biomarker models to the basic CVD risk model with the corresponding biomarker combination. None of the combined ambulatory blood pressure biomarker models improved AUC compared to the corresponding basic CVD-biomarker risk models (table 8). The addition of 24-hour systolic blood pressure to the basic CVD risk factor model with all biomarkers improved NRI (+8.1%, $p=0.019$) but not when added to the NT-proBNP model (+6.4%, $p=0.071$) or any other combinations of biomarkers (table 8). IDI did not improve when we added 24-hour systolic blood pressure or 24-hour pulse pressure to the basic CVD risk factor model combined with biomarkers (table 8).

Table 7
Comparisons of models with combinations of ambulatory blood pressure variables and biomarkers in relation to ambulatory blood pressure models.

Model	HR			AUC			NRI			IDI		
	HR	95% CI	P value	AUC diff	95% CI	P value	%	P value	coefficient	95% CI		
Basic CVD risk factor model* (office SBP)	1.24	1.05-1.48	0.014	0.664 (ref)	0.621-0.708							
24h SBP model	1.35	1.17-1.56	<0.001	0.023	0.002-0.044	0.034	3.0	0.323	0.010	-0.005-0.031		
24h PP model	1.25	1.09-1.43	0.002	0.013	-0.005-0.031	0.169	-0.9	0.779	0.003	-0.012-0.021		
24h SBP model				0.687 (ref)	0.644-0.731							
+ log(NT-proBNP)	1.49	1.25-1.77	<0.001	0.012	-0.013-0.036	0.354	14.3	<0.001	0.015	0.002-0.039		
+ log(hs-CRP)	1.17	0.98-1.40	0.080	0.0003	-0.010-0.011	0.960	0.7	0.746	0.001	-0.000-0.008		
+ log(Cystatin C)	1.17	0.99-1.38	0.073	0.001	-0.010-0.012	0.885	2.6	0.279	0.002	-0.000-0.013		
+ log(NT-proBNP) and log(hs-CRP)				0.013	-0.011-0.038	0.289	16.1	<0.0001	0.015	0.002-0.037		
+ log(NT-proBNP) and log(Cystatin C)				0.013	-0.011-0.037	0.292	14.2	<0.001	0.015	0.002-0.038		
+ log(Cystatin C) and log(hs-CRP)				0.002	-0.013-0.016	0.824	2.1	0.426	0.002	-0.000-0.011		
+ log(NT-proBNP), log(Cystatin C) and log(hs-CRP)				0.014	-0.010-0.039	0.260	16.0	<0.0001	0.015	0.002-0.034		
24h PP model				0.677 (ref)	0.634-0.720							
+ log(NT-proBNP)	1.50	1.26-1.78	<0.001	0.016	-0.010-0.043	0.216	13.9	<0.001	0.015	0.002-0.040		
+ log(hs-CRP)	1.18	0.99-1.41	0.065	0.001	-0.011-0.013	0.899	0.5	0.838	0.001	-0.000-0.009		
+ log(Cystatin C)	1.16	0.98-1.37	0.080	0.002	-0.009-0.014	0.712	1.7	0.183	0.002	-0.000-0.012		
+ log(NT-proBNP) and log(hs-CRP)				0.019	-0.007-0.046	0.152	14.5	<0.001	0.015	0.002-0.039		
+ log(NT-proBNP) and log(Cystatin C)				0.018	-0.008-0.044	0.170	14.0	<0.001	0.015	0.002-0.039		
+ log(Cystatin C) and log(hs-CRP)				0.003	-0.012-0.018	0.705	-0.6	0.776	0.002	-0.000-0.010		
+ log(NT-proBNP), log(Cystatin C) and log(hs-CRP)				0.021	-0.006-0.047	0.126	14.5	<0.001	0.015	0.002-0.035		

24h, 24 hour; AUC, area under the receiver-operating characteristic curve; CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio (apply for a 1 SD increase in the variable added to the model); hs-CRP, high sensitivity C-reactive protein; IDI, integrated discrimination improvement; NRI, net reclassification improvement; NT-proBNP, amino-terminal pro-B-Type natriuretic peptide; PP, pulse pressure; SBP, systolic blood pressure
 *basic CVD risk factor model consists of SBP, office, serum cholesterol, HDL, BMI, age, treatment for hypertension (yes/no), diabetes (yes/no), lipid lowering treatment (yes/no) and smoking status (yes/no)
 AUC is based on Harrell's C, NRI is based on risk categories <7.5%, 7.5-25% and >25%. IDI calculated with bias corrected bootstrap confidence interval¹⁹.

Table 8
Comparisons of models with combinations of ambulatory blood pressure and basic cardiovascular disease risk biomarker models in relation to basic cardiovascular disease risk biomarker models

Model	AUC			NRI			IDI	
	AUC diff	95% CI	P value	%	P value	coefficient	95% CI	
Basic CVD risk factor model* + log(NT-proBNP)								
+24h SBP	0.013	-0.004-0.030	0.133	6.4	0.071	0.011	-0.002-0.033	
+24h PP	0.007	-0.007-0.022	0.299	2.1	0.545	0.005	-0.007-0.021	
Basic CVD risk factor model + log(hsCRP)								
+24h SBP	0.018	-0.001-0.038	0.066	2.9	0.407	0.010	-0.006-0.030	
+24h PP	0.009	-0.009-0.026	0.331	1.4	0.658	0.003	-0.012-0.020	
Basic CVD risk factor model + log(Cystatin C)								
+24h SBP	0.019	-0.001-0.039	0.065	4.7	0.180	0.010	-0.005-0.031	
+24h PP	0.010	-0.007-0.027	0.256	0.3	0.915	0.004	-0.012-0.020	
Basic CVD risk factor model + log(NT-proBNP), log(hsCRP) and log(Cystatin C)								
+24h SBP	0.012	-0.004-0.028	0.130	8.1	0.019	0.011	-0.002-0.032	
+24h PP	0.008	-0.005-0.022	0.227	2.7	0.404	0.005	-0.008-0.022	

24h, 24 hour; AUC, area under the receiver-operating characteristic curve; CI, confidence interval; CVD, cardiovascular disease; hs-CRP, high sensitivity C-reactive protein; IDI, integrated discrimination improvement; NRI, net reclassification improvement; NT-proBNP, amino-terminal pro-B-type natriuretic peptide; PP, pulse pressure; SBP, systolic blood pressure
 *basic CVD risk factor model consists of SBP office, serum cholesterol, HDL, BMI, age, treatment for hypertension (yes/no), diabetes (yes/no), lipid lowering treatment (yes/no) and smoking status (yes/no)
 Office blood pressure is exchanged for either 24h SBP or 24h PP in each basic CVD risk factor model with biomarkers.
 AUC is based on Harrell's C; NRI is based on risk categories <7.5%, 7.5-25% and >25%. IDI calculated with bias corrected bootstrap confidence interval¹⁵.

5.3 Study IV

The ACCOMPLISH trial was terminated early when the limit of the pre-specified stopping criterion was reached after a mean study duration of 35.7 months. There was a highly significant treatment effect in favor of the benazepril+amlodipine combination (150).

Baseline characteristics of the study patients are shown in table 9 in relation to tertiles of pulse pressure and treatment. Of randomized patients, most (97.2%) were on antihypertensive treatment before the trial, although only 37.3% had a normal blood pressure at baseline.

Table 9
Baseline characteristics of the study patients in ACCOMPLISH according to tertiles of pulse pressure and treatment.

	Low tertile (mean PP=50,3 mm Hg) (<58 mm Hg)		Medium tertile (mean PP=63.9 mm Hg) (58-70.7 mm Hg)		High tertile (mean PP=82.2 mm Hg) (≥70.7 mm Hg)	
	B+A (n=1888)	B+H (n=1881)	B+A (n=1924)	B+H (n=1887)	B+A (n=1929)	B+H (n=1990)
Sex (male/female)	1213/675	1247/634	1175/749	1157/730	1059/870	1109/881
Mean age (years)	66.9 (6.49)	66.4 (6.36)	68.4 (6.70)	68.4 (6.74)	70.0 (7.02)	70.0 (6.97)
Antihypertensive treatment at start (yes)	1886	1874	1896	1857	1834	1914
SBP (mm Hg)	129.7 (11.9)	129.7 (11.4)	144.0 (11.2)	144.1 (11.2)	161.9 (15.1)	161.4 (14.7)
DBP (mm Hg)	80.3 (10.2)	80.6 (9.8)	80.1 (10.6)	80.3 (10.6)	79.9 (11.6)	79.0 (14.7)
History of CV disease:						
MI (yes)	452 (23.9)	487 (25.9)	459 (23.9)	450 (23.8)	426 (22.1)	435 (21.9)
Unstable angina (yes)	241 (12.8)	235 (12.5)	201 (10.4)	221 (11.7)	210 (10.9)	215 (10.8)
CABG (yes)	394 (20.9)	374 (19.9)	441 (22.9)	393 (20.8)	412 (21.4)	430 (21.6)
PCI (yes)	424 (22.5)	436 (23.2)	333 (17.3)	340 (18.0)	296 (15.3)	346 (17.4)
History of stroke (yes)	254 (13.5)	257 (13.7)	249 (12.9)	243 (12.9)	258 (13.6)	236 (11.9)
Diabetes mellitus (yes)	1099 (58.2)	1083 (57.6)	1198 (62.3)	1121 (59.4)	1180 (61.2)	1262 (63.4)
Other risk factors:						
Current smoking (yes)	216 (11.4)	230 (12.2)	228 (11.9)	226 (12.0)	197 (10.2)	202 (10.2)
Total cholesterol (mg/dL)	182.9 (41.2)	180.3 (37.4)	184.4 (39.7)	183.9 (39.2)	187.5 (40.5)	187.9 (40.8)
HDL-cholesterol (mg/dL)	48.9 (14.0)	48.1 (13.3)	49.5 (13.8)	49.4 (14.2)	50.4 (14.5)	51.0 (14.6)

Data are number of patients, (%) or mean (SD) were appropriate if nothing else is stated. Missing data in each subgroup varied from 0-4.

B+A, benazepril+amlodipine; B+H, benazepril+hydrochlorothiazide; CABG, coronary angioplastic bypass surgery; CV, Cardiovascular; DBP, Diastolic blood pressure; HDL, high density lipoprotein; MI, Myocardial infarction; PCI, percutaneous coronary intervention; SBP, Systolic blood pressure; Unstable angina, Hospitalization for unstable angina
eGFR (estimated glomerular filtration rate) was calculated according to MDRD (Modification of Diet in Renal Disease)

Comparisons between tertiles of pulse pressure, pooling the two treatment groups, showed an increased incidence of the primary end-point (cardiovascular mortality/non-fatal MI/non-fatal stroke) in the high tertile compared to the low tertile and in the high compared to the medium

tertile of pulse pressure ($P<0.01$) (Table 10). For the secondary end-point (all MI), a similar association was observed. No significant association was observed between pulse pressure and the incidence of stroke.

Table 10
Number of events according to tertiles of pulse pressure and between-tertile hazard ratios

	High vs. Low	Medium vs. Low	High vs. Medium
CV mortality/ non-fatal MI/ non-fatal stroke	284 (7.2) vs. 164 (4.4) 1.48 (1.22-1.80)*	204 (5.4) vs. 164 (4.4) 1.16 (0.94-1.42)	284 (7.2) vs. 204 (5.4) 1.28 (1.07-1.54)*
All MI	136 (3.5) vs. 64 (1.7) 2.01 (1.48-2.73)*	84 (2.2) vs. 64 (1.7) 1.29 (0.93-1.79)	136 (3.5) vs. 84 (2.2) 1.56 (1.19-2.05)*
All Stroke	104 (2.7) vs. 66 (1.8) 1.22 (0.89-1.78)	75 (2.0) vs. 66 (1.8) 1.00 (0.72-1.40)	104 (2.7) vs. 75 (2.0) 1.22 (0.91-1.65)

Data are number of patients with events (%) and Hazard Ratio (95% CI). * $p<0.01$.
CV=cardiovascular; MI=myocardial infarction

Secondly, HRs for benazepril+amlodipine over benazepril+hydrochlorothiazide were calculated in the three tertiles of pulse pressure in a Cox regression model adjusted for age, diabetes mellitus and previous MI. The HRs for the primary endpoint for benazepril+amlodipine over benazepril+hydrochlorothiazide were significant in the high and medium tertiles of pulse pressure but not in the low tertiles (Table 11). There were, however, no significant differences between tertiles of pulse pressure when comparing HRs (Table 11).

Table 11
Between-treatment hazard ratios across pulse pressure tertiles for the primary endpoint cardiovascular mortality/non-fatal myocardial infarction/non-fatal stroke

Baseline PP tertiles	CV events/ N (B+A)	CV events/ N (B+H)	HR	95% CI	P value
High	120/ 1929 (6.2)	164/ 1990 (8.2)	0.75	(0.60-0.95)	0.018
Medium	89/ 1929 (4.6)	115/ 1887 (6.1)	0.74	(0.56-0.98)	0.034
Low	79/ 1888 (4.2)	85/ 1881 (4.5)	0.91	(0.67-1.23)	0.54

Comparing treatment HRs between tertiles (High vs. Low $p=0.34$, Medium vs. Low $p=0.33$, High vs. Medium $p=0.93$, Overall among tertiles $p=0.56$)
Values are expressed as numbers and (%) in each tertile and treatment group respectively.
B+A, benazepril+amlodipine; B+H, benazepril+hydrochlorothiazide; CI, Confidence interval; CV, cardiovascular; HR, Hazard Ratio; PP, pulse pressure

The difference in event rates of the secondary end-points all MI and all stroke between the two treatment groups across the pulse pressure tertiles is shown in figure 6. The HRs favored benazepril+amlodipine in both end-points and tertiles except for all MI in the low pulse pressure tertile. However, none of these HRs were significant. Differences in HRs between tertiles were not significant.

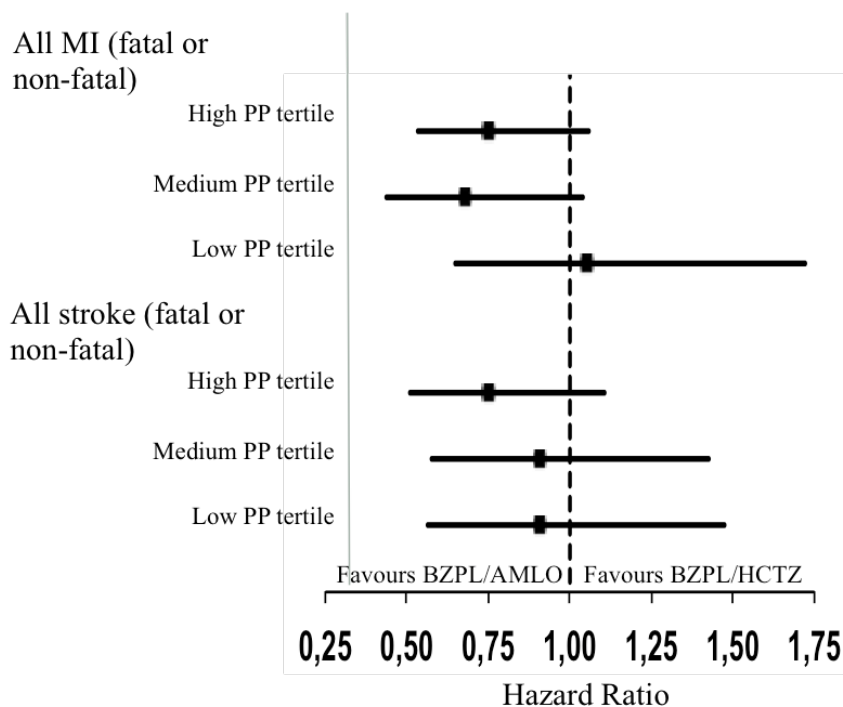


Figure 6. Between-treatment hazard ratios across pulse pressure tertiles by baseline pulse pressure for the indicated endpoint.

AMLO, amlodipine; BZPL, benazepril; CAD, coronary artery disease; HCTZ, hydrochlorothiazide; MI, myocardial infarction; PP, pulse pressure
 Bars express the 95% confidence interval. Hazard ratio for BZPL/AMLO over BZPL/HCTZ is based on a Cox regression model with treatment, baseline PP tertile, and treatment-by-PP tertile interaction as factors and baseline age, CAD (yes/no), and Diabetes Mellitus (yes/no) as covariates. Hazard ratios comparing treatments between tertiles were not significant at $P < 0.05$ (High vs Low, Medium vs Low, High vs Medium, and overall among tertiles $P = 0.39$ for all MI and $P = 0.76$ for all stroke) by baseline PP.

6 DISCUSSION

In this thesis, we studied the predictive value of ambulatory blood pressure with special reference to ambulatory pulse pressure and its relation to NT-proBNP, hs-CRP and cystatin C in PAD patients. We further analyzed if combinations of ambulatory blood pressure and these biomarkers could improve risk prediction models compared to traditional risk prediction models in PAD patients and in healthy, elderly men. Finally we studied if antihypertensive treatment effects were dependent on baseline pulse pressure.

We observed that ambulatory pulse pressure predicted CV events in PAD patients and also that NT-proBNP and hs-CRP were independent predictors of CV events in PAD patients. A combination of ambulatory pulse pressure and these biomarkers improved risk discrimination and reclassification in PAD patients. Further, we found that ambulatory blood pressure (both systolic and pulse pressure) predicted CV events in healthy, elderly men and that NT-proBNP, but not hs-CRP, was an independent predictor when adjusted for ambulatory blood pressure. Cystatin C had no predictive value in any of the studies. The combination of ambulatory systolic blood pressure and NT-proBNP improved risk discrimination and net reclassification for incident CVD compared to office systolic blood pressure in healthy elderly men. Finally, in a subanalysis of a large randomized controlled trial in hypertensive patients (the ACCOMPLISH trial), we observed that pulse pressure at baseline was a predictor for CV events. However, the superiority of amlodipine over hydrochlorothiazide when combined with benazepril in reducing CV events was not dependent on baseline pulse pressure.

6.1 Ambulatory Blood Pressure

We demonstrated that ambulatory blood pressure was an independent predictor, superior to office blood pressure, for CV events in patients with PAD (study I-II) and in elderly men (study III). This confirms earlier reported results in other populations such as, patients with untreated (95) and treated (24, 151) hypertension, isolated systolic hypertension (100) and type 2 diabetes mellitus (152). The predictive superiority of ambulatory blood pressure over office blood pressure could be due to the detection of suboptimal antihypertensive treatment in subjects with hypertension and/or masked hypertension. In a previous study of PAD patients, Svensson et al found that office blood pressure underestimated 24-hour blood pressure as compared to the corresponding situation in healthy controls (137). This indicates

that there may be important differences in results when assessing blood pressure with ambulatory and office measurements respectively in different groups of subjects. This is particularly important in high-risk groups such as patients with PAD, since correct monitoring and proactive treatment of hypertension in this group is effective to prevent future CV events (153). Further, in patients with intensive antihypertensive treatment (e.g. several drugs taken in the morning), daytime blood pressure may be more affected than nighttime levels (137). The use of ambulatory blood pressure to detect differences in blood pressure over the whole 24-hour period in treated hypertensive subjects may thus be of value for deciding on timing of intake of blood pressure lowering drugs.

However, ambulatory blood pressure just modestly improved discrimination in C-statistics (study II and III) and did not at all improve reclassification analyses (NRI and IDI) in study III. Further, ambulatory blood pressure did not add predictive value beyond the addition of NT-proBNP to the basic CVD risk factor models (study III). This was surprising, given the strong associations between ambulatory blood pressure and CV events in the adjusted survival analyses, although the result might be due to methodological limitations (discussed below). However, little additive effect of ambulatory blood pressure in discrimination and reclassification analyses was also shown in a study by Bell et al, in which they used methodology different from ours in a Framingham Risk Score based model in 780 men free of CVD from ULSAM (154). As discussed above, one gain of ambulatory blood pressure monitoring is detecting elevated nighttime blood pressure levels, despite normal office blood pressure values in treated hypertensive patients. Thus, one possible explanation for the results in our study (III), as well as in Bell's study, is the low rate of subjects on antihypertensive treatment (approximately 30 % in both studies) limiting the predictive value of ambulatory blood pressure in the analyses. Although strong predictive capabilities in adjusted survival analyses, more studies are needed to evaluate whether ambulatory blood pressure improves discrimination or not. Nevertheless, in clinical practice, ambulatory blood pressure monitoring contributes with information beyond office blood pressure in patient assessment. It is important to reveal insufficient antihypertensive treatment and masked hypertension, both with elevated risk of incident CVD if undetected. In addition, other blood pressure variables such as nighttime pressure and non-dipping and blood pressure load, all of which have been suggested as predictors of CVD (95, 155, 156), can assist in tailoring treatment for high-risk individuals. In our studies (I-III), among ambulatory blood pressure variables, ambulatory pulse pressure proved to be a strong predictor compared to office blood pressure and in study III, ambulatory pulse pressure was predictive even when adjusted for office systolic blood pressure.

6.2 Pulse Pressure

We demonstrated that ambulatory pulse pressure was the strongest predictor among ambulatory blood pressure variables in PAD patients (study I) and a strong predictor in healthy, elderly men (study III) confirming results from previous studies (99, 109-111, 157). Pulse pressure is related to more advanced vascular disease with increased vascular stiffness (107, 108). Benetos et al demonstrated the predictive value of pulse pressure in a large French study in 1997 including more than 19 000 men, 40 to 69 years old and concluded that pulse pressure was a significant independent predictor of all-cause, cardiovascular, and, especially, coronary mortality (9). Pulse pressure has a close correlation to systolic blood pressure and an inverse relationship to diastolic blood pressure. Hence, pulse pressure increases with age as the diastolic blood pressure level descends from the age of 55 (47). More than fifteen years ago Franklin et al (14) reported the relation between systolic and diastolic blood pressures and the risk of coronary heart disease using Framingham data. The study included 1924 men and women, 50-79 years of age, free of coronary heart disease and not on antihypertensive treatment. In conclusion, Franklin et al showed that for a fixed systolic blood pressure, risk increased with every increase in pulse pressure i.e. a decrease in diastolic blood pressure. This was true for a normal systolic blood pressure of 130 mm Hg when pulse pressure exceeded 50 mm Hg (14). Further, Franklin et al studied the predictive value of different blood pressure variables in a wide age-range population of healthy men and women. The results demonstrated that the predictive blood pressure variable shifts from diastolic in the young (<50 years) to pulse pressure in the elderly (>60 years) (6). However, other studies have showed contrasting results. In a follow-up study of the MRFIT study including more than 340,000 men, 35-57 years of age, Domanski et al compared the association of blood pressure variables and CV mortality in two age-groups (35-44 and 45-57) (15). The subjects were further divided into groups according to the classification of blood pressure. The overall conclusion was for a joint use of both diastolic and systolic blood pressure (15). However, as pulse pressure closely correlates to systolic blood pressure but is also a function of the diastolic pressure, the results are not surprising in the young where systolic and diastolic pressure is usually concordant. In fact, the results showed a significant association between pulse pressure and CVD mortality in the older age group, in the high normal blood pressure group as well as in all groups with higher blood pressure values. There was a 22% elevated risk for CV mortality between the highest and the lowest quartile of pulse pressure within the optimal blood pressure (<120 and <80 mmHg) group (15). The relatively weak association for pulse pressure overall in the MRFIT study can be due to the relatively young patients, in whom diastolic blood pressure has not started to descend (14, 47). Other studies have come to

different conclusions. A follow-up study of the NHANES including 7830 white and African-American men and women free of CVD (30-74 years) showed that pulse pressure was associated with increased risk, decreased risk, or no change in risk depending on age and systolic and diastolic blood pressure. The conclusion was that pulse pressure should not be recommended for prognostic or therapeutic decisions (87). However, single measurements of office pulse pressure may not be adequate. Ambulatory blood pressure measurement provides more information on pulse pressure over a 24-hour period.

Ambulatory pulse pressure as a predictor for CV events has been reported previously in other patient groups (109-111). Verdecchia et al tested the hypothesis that ambulatory pulse pressure would be superior to office pulse pressure as a predictive marker for CV morbidity and CV mortality in 2000 men and women (mean age, 51.7) with essential uncomplicated hypertension (109). There was a significant increase in event rate (CV morbidity and mortality) from the first to the third tertile of office pulse pressure as well as in ambulatory pulse pressure tertiles. This increase in event rates was also evident for every tertile of ambulatory pulse pressure within every office pulse pressure tertile. Verdecchia concluded that ambulatory pulse pressure was a potent marker in hypertension and superior to office pulse pressure in predicting CV morbidity (but not mortality) (109). More recently, Kao et al investigated 412 hypertensive patients in Taiwan and found that ambulatory pulse pressure was a good predictor for long-term outcomes (including CV morbidity and mortality, coronary heart disease and stroke) in hypertensive patients and suggested that ambulatory, rather than office blood pressure, could be applied for risk stratification either before or during antihypertensive treatment (157). Further, in a small study of patients with on-going hemodialysis (subjects with a very high risk for CV events) conducted by Amar et al, ambulatory pulse pressure was the strongest predictor for CV mortality (101).

Although the uncertainty in some populations (86, 158, 159), data suggests that pulse pressure, measured in the office as well as ambulatory monitored, is a predictor for CV morbidity and mortality in the elderly (>60 years of age) with or without hypertension and possibly in other high-risk groups with established CVD. Our results extend this knowledge to include patients with PAD. In the latest guidelines (48), having a pulse pressure >60 mm Hg was introduced as a marker of end organ damage, implying that such patients should be regarded in the same risk category as other high risk groups like patients with diabetes or left ventricle hypertrophy when deciding on antihypertensive treatment. Apart from the introduction of pulse pressure as a binary variable in the guidelines there are no treatment goals and pulse pressure is not considered as a continuous variable like systolic or diastolic

pressure. However, in other guidelines, little is mentioned how to address pulse pressure in risk assessment (49) and hypertension treatment (50-52).

6.3 Biomarkers

NT-proBNP

The results from study II and III identifies NT-proBNP to be a powerful, independent predictor of CV events in adjusted analyses. NT-proBNP also improved risk discrimination and reclassification in combination with ambulatory pulse pressure and hs-CRP in PAD patients (study II) and by itself in elderly men (study III). The predictive power of NT-proBNP has been reported in several previous studies (112, 113, 160, 161). NT-proBNP is mainly produced in, and secreted by cardiac myocytes upon the stimulus of myocardial stretch and production is strongly upregulated in congestive heart failure (123).

Atherosclerotic heart disease with subclinical congestive heart failure could therefore be one explanation for poor outcomes in subjects with elevated NT-proBNP. Elevated levels of NT-proBNP are also seen in older subjects (compared to younger), women (compared to men) diabetes and in several other situations (124) that must be taken into consideration in statistical analyses.

In studies by Olsen et al (162, 163), NT-proBNP predicted CV events in 945 hypertensive patients (age 55-80 years) (162) and also in a population based study of 2656 individuals (age 41–71 years) (163). However, NT-proBNP did only improve risk prediction compared to a basic CV risk factor model in the hypertensive population (C-statistics) (162). In comparison, the average level of NT-proBNP at baseline in their population-based study was much lower than in our cohort of elderly men (ULSAM), indicating that subclinical heart disease might be more prevalent among our subjects and therefore explain the different results of the two studies. The mean age in the ULSAM cohort was 71 years (range 69.4-73.6) but in the study of Olsen et al, only 45% were 61 and 71 years old (55% were 41 and 51 years old), thus differences in age may also explain the different results. In a prospective study by Wannamethee et al, including more than 3600 men (60-79 years) with and without pre-existing CVD (164), NT-proBNP predicted CV events in both groups with similar HRs. Further, in risk prediction, Wannamethee et al showed improvement in discrimination with C-statistics and in reclassification with NRI in both groups when NT-proBNP was added to a Framingham based risk factor model (164). Although our models were based on ambulatory blood pressure, the results are similar, suggesting that NT-proBNP has additive value to

traditional risk factor models in both healthy elderly men and subjects with established CVD. Further, NT-proBNP is associated with ambulatory pulse pressure (165, 166) but very few studies have adjusted for ambulatory blood pressure when evaluating the predictive value of NT-proBNP. Paget et al (130) showed that NT-proBNP predicted mortality in hypertensive patients in adjusted analysis with ambulatory blood pressure although neither discrimination nor reclassification analyses were done in that study.

hs-CRP

Hs-CRP was an independent predictor of CV events in PAD patients (study II). In the elderly population (study III), hs-CRP was predictive in adjusted analysis with basic CV risk factors but not in ambulatory blood pressure models. Hs-CRP improved discrimination and reclassification alone and in combination with ambulatory blood pressure and NT-proBNP in PAD patients (study II) but did not have additive value in discrimination or reclassification in the elderly population (study III).

The predictive value of hs-CRP has been reported in several previous studies (160, 161). However, our results imply that hs-CRP only has a predictive value in high-risk populations such as PAD patients. This is supported by a study conducted by Urbonaviciene et al, in which they showed that risk prediction was improved by using hs-CRP in addition to traditional risk factors in PAD patients (167). Contrarily, Olsen et al reported that hs-CRP did not predict CV events in 945 hypertensive patients, of whom approximately 25% of the subjects had established CVD at baseline (162) and neither in a population based study including 2656 individuals (age 41–71 years) (163). In another prospective study, including more than 3600 older men (age 60–79 years) with and without pre-existing CVD (164), hs-CRP did only predict events in the group without pre-existing CVD but hs-CRP did not improve C-statistics and NRI when added to a Framingham based risk factor model. However, none of the studies have adjusted for ambulatory blood pressure, which hampers comparisons to our studies (II-III). The inconsistent findings regarding the predictive value of hs-CRP for CV events and CV mortality is further reported in several large studies. Both positive (168, 169) and negative (170, 171) findings have been reported. The latter suggest that elevated hs-CRP are related to traditional risk factors and only reflect the presence of such (170). The results from our studies contribute to the uncertainty regarding the value of hs-CRP in risk prediction in CVD. Still, hs-CRP is recommended in the 2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk, but first after initial assessment with

traditional risk factors and when the treatment decision is unclear (48). The European Guidelines on Cardiovascular Disease Prevention in Clinical Practice, has a similar approach recommending that hs-CRP may be used in subjects at moderate risk but should not be used in asymptomatic low-risk individuals and in high-risk patients (49).

Cystatin C

Kidney function, measured by estimated GFR is associated with CV events in various populations (117). The association between cystatin C and CVD is thus probably due to its close relation to kidney function. Cystatin C is more likely to detect subclinical reductions in kidney function than estimated GFR (based on creatinine) and this could explain why cystatin C is predictive of CV events independent of renal function estimated by creatinine based formulas (such as MDRD). Cystatin C has been shown to be a predictive marker for incident CVD (117, 172, 173) and to be predictive of mortality in PAD patients independent of renal function (118). Surprisingly, cystatin C was not predictive of CV events in our studies (II and III), and cystatin C did not improve discrimination or reclassification when added alone or together with other biomarkers and/or ambulatory blood pressure variables. The predictive value of cystatin C has been reported from the Multi-Ethnic Study of Atherosclerosis (6600 subjects free of CVD, mean age approximately 63 years), in which cystatin C was added to a Framingham based risk factor model. In that study, cystatin C was predictive of CV events in adjusted analysis with traditional risk factors whereas creatinine was not. However, cystatin C did not improve discrimination and reclassification compared to the Framingham based model alone (174). Similarly, in a Swedish cohort study involving more than 5000 men and women (mean age 58 years) with no evidence of CVD, cystatin C was predictive of CV events (175). Further, cystatin C modestly (but significantly) improved discrimination with C-statistics but did not improve reclassification when added alone to traditional risk factors. However, in that study, cystatin C was removed in backward variable selection together with other biomarkers (hs-CRP and NT-proBNP among others) and was not tested in a multi-biomarker approach (175). None of the studies, however, have adjusted for ambulatory blood pressure. Cystatin C has been shown to correlate to ambulatory pulse pressure in a small cohort including 87 men and women (176). Studies on cystatin C for CV endpoints adjusted for ambulatory blood pressure are unfortunately non-existing, which limits comparisons to our results.

6.4 Antihypertensive Treatment

In study IV, the main outcome of the ACCOMPLISH trial in favor of amlodipine in combination with benazepril was not dependent on baseline pulse pressure according to the statistical method used. However, the treatment effect was larger in the highest tertile in absolute numbers. Studies on the relation of pulse pressure to treatment effect are scarce. The Losartan Intervention For Endpoint reduction in hypertension (LIFE) study demonstrated the superiority of treatment with losartan (an angiotensin II receptor antagonist) over atenolol (a beta-blocker) in reducing cardiovascular events in high risk hypertensive patients (177). In a post hoc analysis of that study, a higher pulse pressure was significantly related to an increased number of cardiovascular events in the atenolol-treated group whereas the same pattern although not significant, was observed in the group treated with losartan (177, 178). Although the relation of the reported numbers of events in categories of pulse pressure and treatment show similar patterns compared to study IV, the differences in baseline characteristics and statistical methodology hamper comparisons between the two studies.

In absolute numbers, a larger treatment effect was observed in the two higher tertiles compared to the lowest tertile. The lack of significant differences between pulse pressure tertiles for the relative treatment effect in study IV could be due to a type 2 error. Further, a categorization of pulse pressure based on standard blood pressure measurements may be inferior in comparison to ambulatory blood pressure (179) and central blood pressure measurements (180, 181). As ambulatory blood pressure monitoring is a more precise method for determining a subject's blood pressure, it is a better marker for cardiovascular risk and outcomes (182). As discussed above, office blood pressure has been suggested to underestimate ambulatory blood pressure in patients with established cardiovascular disease (137). Ambulatory blood pressure measurement was only performed in a subset of the ACCOMPLISH patients and analysis showed that achieved ambulatory blood pressure after 2 years did not differ between the two treatment arms (183). However, the reduction of ambulatory blood pressure from baseline blood pressure in relation to the two treatment arms in ACCOMPLISH have not (yet) been reported. Differences in achieved ambulatory pulse pressure have been reported between different antihypertensive treatment-regimes in a small study of diabetic patients (184). In that study, similar ambulatory pulse pressures at baseline and non-significant differences in treatment effect (systolic and diastolic pressures) between the two treatment arms was reported, but there was a significant reduction in pulse pressure in favor of lisinopril+candesartan versus high dose lisinopril. Another trial that studied the effect of a calcium channel blocker (as in ACCOMPLISH) in patients with high ambulatory pulse

pressure is the Syst-Eur (Systolic Hypertension in Europe) trial (185). In that study, the 24-hour average pulse pressure, before the initiation of drug therapy, was the most important factor predicting CV disease risk (186). Further, it was shown that the reduction of CV events correlated with the pulse pressure reduction from calcium channel blocker treatment, thus implying that calcium channel blocker treatment may be beneficial in subjects with high pulse pressure. Although a clear association between the number of events and pulse pressure tertiles was observed in our study (IV), the power to observe a difference in treatment effect in relation to pulse pressure tertiles was diminished since the compared groups were further divided within each treatment arm. The power to significantly detect clinically relevant differences in HR between these tertiles may thus have been insufficient in study IV.

In the ACCOMPLISH trial, the difference in systolic blood pressure between the two treatment groups was less than 1 mm Hg. This indicates that there are other mechanisms, than the reduction of brachial blood pressure, that are responsible for the superiority of benazepril+amlodipine over benazepril+hydrochlorothiazide in preventing CV disease events. It was concluded in a meta-analysis by Law et al (132), that all classes of antihypertensive agents are equally effective in reducing incident CVD for a given reduction in blood pressure, arguing against the presence of a pleiotropic effect of antihypertensive agents as a mechanism. However, it has been suggested that different antihypertensive agents affect central pulse pressure differently and that the blood pressure-lowering effect differs between brachial and aortic pressures (133-136). In the CAFÉ trial (187), a substudy of the ASCOT trial, Williams and colleagues suggested that atenolol reduced central blood pressures less (hence, less improvement in pulse pressure) compared to amlodipine+perindopril despite similar brachial blood pressures (133). Further, a recent meta-analysis concluded that different antihypertensive treatments affect central aortic blood pressure differently which results in a varied reduction in central aortic blood pressure despite similar achievements in brachial target pressure. It was further concluded that this could explain different outcomes in randomized clinical trials when beta-blocker- and/or diuretic-based antihypertensive therapy are compared to other antihypertensive regimes (188). In our study we did not have central aortic pressure recordings, which makes assumptions that the outcome in ACCOMPLISH was based on different changes in central pressures purely speculative.

Rapsomaniki et al recently reported results of different blood pressure variables and their relation to specific CVDs in 1.25 million patients (85). The results indicate that different variables of blood pressure (systolic, diastolic and pulse pressure) are important for different

manifestations of CVDs. It was further concluded that new strategies for lowering blood pressure are needed. Hence, new studies are needed to evaluate if antihypertensive treatment, directed towards specific variables of blood pressure, will affect outcome.

6.5 Methods Discussion

Cox regression analysis is a semi-parametrical statistical model used for survival analyses (189). By the use of time to event, this model does not only estimate the risk for an event but also the time it takes for the event to occur. Further, the model takes censoring into account, that is, subjects that part from the study before the end of the planned observation time without having the event (for example death from another cause than the study outcome). Cox regression can be used with dichotomous variables as well as continuous and time-dependent variables. Results are expressed as Hazard ratios (HR) reflecting a subject's risk of an event anytime during the time period studied. This has to be verified, testing if the assumption of proportional hazards is fulfilled. Cox regression analysis was used in study I-IV.

Logistic regression is used to describe the relationship between several independent continuous or dichotomous variables (exposures) and a dependent dichotomous variable (outcome) (189). Results are expressed as Odds Ratios (OR). NRI (study II and III) and IDI (study III) were based on logistic regression.

C-statistics

C-statistics is commonly used as a measure of performance of predictive models. The receiver operating characteristic curve (ROC) displays the true positive rate versus the false positive rate for all possible values of prediction from a model. The area under the receiver operating characteristic curve (AUC) is commonly used as a measure of model performance. However, C-statistics can be misleading in terms of deciding if a new marker improves risk prediction that could presumably lead to management change (190). This is because the change in AUC might be small even though the marker is significantly associated with the outcome. The increase in AUC is small when the existing model already includes powerful predictors. In addition, various C-statistic methods perform differently with the same data. This can result in predictions that may lead to different conclusions (131) depending on which C-statistic model being used. Also, in comparisons between different models in C-

statistic performance, the use of the DeLong test in nested models might show inaccurate results (191). In conclusion, evaluating new markers by only using C-statistics should be done with caution.

NRI and IDI

The test of differences in Harrell's C has low statistical power when the basic model already has reasonably good predictability (131). Therefore, Pencina et al. developed two additional measures of predictive power, net reclassification improvement (NRI) and integrated discrimination improvement (IDI) (131). NRI is based on having predefined risk categories and results heavily depend on relevant cut offs. In NRI, a model is considered better in terms of predictability if individuals without events move to a lower risk category, and individuals with events move to a higher risk category mathematically expressed as the sum of differences in proportions of patients moving up minus the proportion moving down for patients with events and the proportion of patients moving down minus the proportion moving up for patients without events. In our cohort of elderly men (study III), most subjects were already in intermediate or high risk due to age and other risk factors, which may have affected our results of NRI.

IDI is based on continuous predictive probabilities of events. A new model is considered better if the estimated probability of events is higher for individuals with events, and lower for individuals without events. This method does not depend on risk cut offs and may be a better discrimination method for high-risk subjects.

Although NRI and IDI have become very popular in addition to C-statistics, they have also been criticized for various reasons (192-194). NRI does not account for time-to-event data, which is a limitation in evaluating new markers in survival analyses (192). Further, NRI is highly dependent on cut-off values for categories of risk, which hampers comparisons between studies with different cut-off values, and can also alter the results within the same study if the cut-off values are changed. Pencina et al have dealt with these two issues and presented the category free NRI (cNRI). cNRI does not rely on categories of risk at all and also take time-to-event data into account (195). Further, Hilden et al proposed that NRI and IDI overestimated model performance and should not be trusted (194). There has also been criticism against IDI since the sampling distribution of IDI is unknown (193) and therefore we used a bias corrected bootstrap confidence interval for IDI in study III as suggested by Kerr et al (193). In conclusion, to properly evaluate the predictive value of new markers in

relation to an existing model, several methods should be considered and the differences should be taken into account before interpretations of the results.

6.6 Limitations

Study I-II. Our study population was quite small, which limits the power of this study. The study population consisted of men only. Morbidity and mortality data were acquired from The Swedish National Board of Health and Welfare and these data are based on hospital records from treating physicians, which might be associated with ascertainment biases. To limit the risk of misdiagnosis, we have reviewed the hospital records to validate the diagnosis of hospitalization and causes of mortality. Further, the interpretation of NRI should be carried out with caution as it can cause overconfident risk predictions to appear advantageous (194) and the use of the DeLong test may have affected the results (191).

Study III. We tested several different models in different combinations that increase the possibility of type 1 errors, which may limit the interpretation of the results. Other limitations are the lack of variety in age, (in general one of the strongest predictors for incident CVD) and that the cohort only consisted of men. Although this limits the findings of our study, it may enable the identification of new associations, as the effects of age and gender are limited or non-existent. Our results need to be confirmed in populations with both genders and with a wider age distribution. In study III, we excluded patients with CV events prior to baseline, but congestive heart failure was not an exclusion criteria. Because congestive heart failure is highly correlated with NT-proBNP, these patients (if any) could affect the results. As this manuscript has not been published yet, we intend to exclude patients with established congestive heart failure and recalculate our results. In addition, NRI was based on risk categories and the upper category limit (25%) is extrapolated from earlier guidelines and may not be appropriate. Further, NRI was based on logistic regression, not considering time-to-event data and this could also have affected the results. We intend to recalculate and use cNRI instead to resolve these issues.

Study IV. The subjects in ACCOMPLISH had a high prevalence of comorbidity. Established CVD was common with approximately 25% having a previous MI, 13% having prior stroke, 50% had BMI >30, and 60% of the patients had diabetes. Hence, most patients had advanced vascular disease with a high or very high risk for CVD events. Analyses were based on office pulse pressure that may not be the best blood pressure measurement in this high-risk group. Unfortunately, ambulatory blood pressure was only measured in a subset of patients in

ACCOMPLISH. They were performed two years after randomization on 573 subjects and these measurements are not applicable to our study as these measurements were on achieved blood pressure. There were also 218 baseline ambulatory blood pressure measurements but these data were not available to us for analysis. In addition, the size of such a study population would be underpowered in order to draw any conclusions regarding relations between pulse pressure and treatment effects. Consequently only office blood pressures were used in our study, which might probably have limited the ability to show a treatment effect in relation to pulse pressure.

6.7 Clinical Implications

NT-proBNP and ambulatory blood pressure is widely available and easy to use methods. The application of these methods in tailoring treatment of patients with high blood pressure could be of benefit and may further reduce the risk for incident CVD, especially in patients with PAD and possibly in other patients with established CVD. Hs-CRP is already to some extent recommended in risk assessment in current guidelines but may have additional value in specific groups of subjects, both alone and in combinations with ambulatory blood pressure and NT-proBNP. However, our results need to be confirmed in further studies in PAD patients and in other populations before they can be recommended as a clinical routine.

6.8 Future Perspectives

Ambulatory blood pressure is superior to office blood pressure as a predictive marker for CV events in male PAD patients and in healthy elderly men. Although we believe our findings may be of clinical importance, the underlying mechanisms leading to adverse cardiovascular events are not fully known. Within our research group, we are studying whether achieved ambulatory blood pressure will differ when ambulatory blood pressure and office blood pressure respectively are used as a base for treatment decisions. It would further be important to study if antihypertensive treatment based on ambulatory blood pressures and office blood pressures respectively affect CV outcome.

NT-proBNP is a strong predictive marker and may be used to stratify CVD risk alone and in combination with ambulatory blood pressure in PAD patients and elderly men. However, studies on the predictive value of biomarkers such as hs-CRP and NT-proBNP for CV

outcome in relation to ambulatory blood pressure are scarce and new prospective studies in other populations are needed.

Individuals with high pulse pressure at baseline benefit more from antihypertensive treatment because of a higher absolute risk and more studies are needed to investigate if the effects of treatments to prevent CV events are dependent on pulse pressure levels or not. Although suggested by some studies it is so far not proven whether patients with stiff arteries, higher central blood pressure and higher pulse pressure benefit more from a calcium-channel blocker-based treatment compared to antihypertensive treatments and such studies should be performed.

Future studies on cost-effectiveness on the use of biomarkers and ambulatory blood pressure that investigate the number needed to screen (the number of subjects that need to be screened for a given duration to prevent one event) would be of interest. One hypothesis to be tested is that the use of ambulatory blood pressure and biomarkers for risk prediction will be more cost-effective in subjects with established CVD and higher absolute risk and perhaps also in hypertensive patients undergoing antihypertensive treatment.

7 CONCLUSIONS

Study I. Ambulatory pulse pressure predicts CV events in patients with PAD and was a better predictor for CV events compared to office blood pressure.

Study II. A combination of ambulatory pulse pressure, NT-proBNP, and hs-CRP can be used to stratify risk in PAD patients.

Study III. Our results indicate that NT-proBNP and ambulatory blood pressure can be used in order to improve risk prediction in elderly male patients.

Study IV. High pulse pressure is related to higher incidence of CV death, nonfatal MI, and stroke in high-risk hypertensive patients. The superiority of the combination treatment benazepril+amlodipine over benazepril+hydrochlorothiazide in hypertensive patients existed irrespective of baseline pulse pressure, but the absolute treatment effect was higher in the higher tertiles of pulse pressure.

Overall conclusion. Pulse pressure is a predictor for CV events and seems to be most useful in patients with established CVD. NT-proBNP has additive value for risk prediction in patients with CVD as well as in the elderly. Combinations of pulse pressure and NT-proBNP may help to tailor treatment in subjects to prevent incident CVD. The difference in reduction of CV events between two different antihypertensive treatments was not dependent on baseline pulse pressure. That is, there is presently no evidence to support that a subject's pulse pressure per se should direct the choice of antihypertensive drugs for treatment.

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