Blood Pressure Variability and Ambulatory Arterial Stiffness Index in Predicting Major Adverse Cardiovascular Events

Dr Aung Hein

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Background: Increased blood pressure variability (BPV) and ambulatory arterial stiffness index (AASI) are associated with major adverse cardiovascular events (MACE), including stroke, transient ischaemic attack, acute coronary syndrome and cardiovascular (CV) death. However, the prognostic value of AASI and BPV in the same population has not been previously investigated.

Aim: To assess the relationship between BPV, AASI and MACE.

Methods: This was an ambidirectional observational cohort study. BPV and AASI were measured from 24-Hr ambulatory blood pressure monitor (ABPM). Other indices included standard deviation (SD) of systolic (SBP) and diastolic (DBP) blood pressure and nocturnal dipping status. Statistical analyses included chi-square and Fisher's exact tests for categorical data. Independent sample t-tests and Mann-Whitney U tests were used for parametric and non-parametric continuous data. Univariate and multivariate logistic regression with odds ratios (OR) and 95% confidence intervals (CI) were used to assess the relationship between BPV, AASI and MACE. Multivariate Cox regression analysis with hazard ratio (HR) and (95% CI) and Kaplan-Meier analyses were conducted for time-to-event (MACE) data.

Results: A total of 829 patients (424 males, 405 females) were followed for 4.35 (±1.32) years. There were 38 MACE (4.58%) events. AASI values were significantly greater in patients with MACE compared to those without [0.54 (±0.16) vs 0.45 (±0.16); $p < 0.001$]. AASI was associated with MACE in univariate analysis (OR: 26.96, 95%CI: 3.77–195.58, $p < 0.001$), but not in multivariate analysis. SD 24-Hr SBP was a univariate and independent predictor of MACE (adjusted OR: 1.21, 95% CI: 1.07–1.37, $p = 0.002$). Multivariate Cox regression confirmed this association (HR: 1.07, 95%CI: 1.01–1.14, $p = 0.024$). Kaplan-Meier analysis showed significantly lower survival in patients with AASI >0.47 (median, $p = 0.002$).

Conclusion: SD 24-Hr SBP was an independent predictor for MACE, while AASI was a potential risk factor.

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Preface

In today's world, high blood pressure stands as a great health concern, closely related to a wide range of CV diseases. While several healthcare policies and strategies have been implemented to reduce deaths related to high blood pressure, we have limited understanding of the variability in blood pressure. It has now become evident that BPV itself poses risks for CV events. Recognising the dynamic process of blood pressure and its challenging nature, it would be very helpful to undertake research in this area to help shape further CV risk stratification. My hope is that this study will provide new information in managing BPV and thereby reduce associated CV risks.

Acknowledgements

Without unwavering support and guidance from my supervisors, I would not have been able to complete this research. I am deeply grateful to Professor Boos, Professor Khattab and Dr Felton for their patience and guidance in this journey. Through various meetings and discussions, their guidance has been instrumental in navigating the complexities of my research. I also extend my thanks to the entire Cardiology Department at University Hospitals Dorset.

Chapter 1 Introduction and background

1.1 Blood pressure variability and arterial stiffness in cardiovascular events

In 1733, Stephen Hales conducted a series of experiments that demonstrated a method for measuring blood pressure (Lewis 1994). By 1907, blood pressure measurement had become part of essential healthcare assessments for medical insurance (Fisher 1914). In the twentieth century, new methods of measurement for systolic and diastolic blood pressure were invented, and subsequently, the clinical significance of blood pressure has been increasingly recognised. By 1959, the Build and Blood Pressure study reported a correlation between increased mortality and mild increases in blood pressure (Kotchen 2011). Today, high blood pressure is one of the leading risk factors for CV diseases and death (WHO, 2023).

Blood pressure is a dynamic physiological process with fluctuations throughout life and serves as a driving force for organ perfusion (Meng 2021). These fluctuations reflect the complex interplay between extrinsic factors (environmental) and intrinsic (physical and emotional) factors. Blood pressure variation is a physiological process essential for maintaining homeostasis by meeting metabolic demands, preserving organ perfusion, and responding to environmental and emotional stimuli. The clinical significance of these fluctuations, or variability, has been debated among researchers for decades. It has gradually become evident that increased BPV is a potential CV risk factor (Grove et al. , 1997).

BPV is measured over different time frames: very short-term (beat-to-beat), short-term (within 24 hours), mid-term (day to day) and long-term (years). Oxford intra-arterial method was used to measure very short-term BPV until the Peñáz method using a finger probe sensor was introduced. For other types of BPV, office blood pressure monitors, home blood pressure monitors (HBPM), and ambulatory blood pressure monitor (ABPM) have been used. Newer devices, such as cuffless blood pressure monitors, have been introduced in recent years, and they could measure all types of BPV. However, more data are still required to validate these devices (Schutte et al. 2022).

Depending on the type of BPV, various factors influence this dynamic process. Behaviours and emotions, cardio-regulatory mechanisms, arterial stiffness, appropriateness of pharmacotherapy for high blood pressure, and medication compliance all play important roles in different types of BPV. Among these factors, arterial stiffness plays an important role in short-term, mid-term and long-term BPV (Parati et al. 2018).

In recent decades, a growing number of studies have demonstrated that increased BPV is linked to target organ disease damage (TOD), CV events, and death (Sega et al. 2002; Poortvliet et al. 2012; Hastie et al. 2013; Suchy-Dicey et al. 2013; Muntner et al. 2015). In 2002, a study was conducted in a randomly selected population enrolled for the Pressioni Arteriose Monitorate E Loro Associazioni (PAMELA) study and it showed a positive association between left ventricular mass index and BPV (Sega et al. 2002).In 2016, Stevens and colleagues conducted a landmark meta-analysis in which increased long-term BPV is associated with all-cause death and CV events, whereas mid-term and short-term BPV followed a similar pattern, but both short-term and mid-term BPV outcomes had limited data. One significant finding from that study was that increased BPV is correlated with CV events irrespective of mean arterial pressure.

Pathophysiology of the association between BPV and CV events is not fully understood yet. It has been postulated that high BPV may trigger inflammatory cascade, cause endothelial dysfunction, and make changes in vascular smooth muscles (Sheikh et al. 2023). Altered microcirculation and increased atherosclerosis associated with these sequences of events may lead to TOD (Sega et al. 2002).

As mentioned above, arterial stiffness (reduced arterial compliance), is linked to BPV. Arterial stiffness has been measured using various methods and the gold standard method is carotid-femoral pulse wave velocity (cfPWV). In 2006, a new index called AASI was introduced, and it was proposed as an index reflecting arterial function. Li and colleagues (2006) demonstrated that AASI is closely related to traditional arterial stiffness markers, and in later studies, AASI was considered as an indirect marker of arterial stiffness (Dolan et al. 2006; Kollias et al. 2012; Boos et al. 2021). Since then, AASI has been widely studied and regarded as a prognostic marker for CV events (Hansen et al. 2006; Kollias et al. 2012; Sobiczewski et al. 2019; Hoshide et al.).

AASI is calculated as one minus regression slope of diastolic blood pressure over systolic blood pressure and can readily be available in modern ABPM. ABPM can provide not just AASI but other blood indices, including day and night systolic and diastolic blood pressure (SBP and DBP), mean arterial pressure, nocturnal and morning blood pressure changes and heart rate reflecting circadian rhythm. Furthermore, ABPM can be used for short-term, mid-term and long-term BPV.

Studies on AASI and BPV have demonstrated that both indices can predict CV events (Xu et al. 2011; Suchy-Dicey et al. 2013; Muntner et al. 2015; Stevens et al. 2016; Webb et al. 2018; Cremer et al. 2021; Heshmatollah et al. 2022; Hoshide et al. 2023).

Stevens and colleagues reported that increased short-term BPV is a strong predictor for stroke but not associated with CVD or coronary heart disease events. In a meta-analysis conducted in 2012 by Kollias and colleagues, it was reported that AASI is a significant predictor for stroke but has modest predictive ability for coronary heart disease. In 2011, it was demonstrated that AASI and BPV are interlinked (Lee et al. 2011). Investigating AASI and short-term BPV as predictors for MACE, which include CV death, stroke, transient ischaemic attack (TIA), acute coronary syndromes (ACS), including myocardial infarction (MI), will contribute to our current knowledge about the potential role of these two indices in CV risk stratification.

1.2 Rationale

Hypertension is a well-recognised risk factor for CV events and TOD (Schmieder 2010; Zhou et al. 2021). We now have a growing body of evidence that not only high blood pressure but also increased BPV is linked to CV events and TOD (Sega et al. 2002; Stevens et al. 2016; Mehlum et al. 2018). Studies also demonstrated that short-term BPV can predict future CV events (Manning et al. 2015; Berry et al. 2016; Palatini et al. 2019). In 2017, a new model for CV risk assessment called QRISK-3 for general practitioners (GP) was introduced, and data were validated. The study showed that long-term BVP is the predictor for CV events in the UK (Hippisley-Cox et al. 2017).In addition, AASI has been a recognised risk factor for CV events. (Hansen et al. 2006; Muxfeldt et al. 2010; Chen et al. 2016; Koumelli et al. 2019; Raina et al. 2020; Hoshide et al. 2023).

In terms of clinical application, there are limitations for both BPV and AASI to be used as therapeutic targets. Recent data suggested that there were only modest responses demonstrated in a study conducted to assess treatment-induced changes of AASI in hypertensive patients (Kollias et al. 2015). As for BPV, it was demonstrated that calcium channel blockers could reduce BPV and associated stroke risk (Parati et al. 2023). These findings applied mainly to long-term BPV. Further studies demonstrated that indapamide, amlodipine, olmestartan and telmisartan can reduce short-term BPV but CV risk reduction was not the outcome of interest in these studies (London et al. 2006; Hermida et al. 2007, 2008; Hoshino et al. 2010). This was echoed by Stevens and colleagues (2016) that short-term blood BPV has a similar pattern of association but at that time, data were limited.

To our knowledge, these two indices have not yet been compared and investigated for their predictive abilities for MACE in the same population. In many NHS hospitals, ABPM service is widely accessible, and short-term BPV and AASI can readily be

investigated. This research will have a translational impact on future CV risk stratification and treatment strategies regarding short-term BPV and AASI.

1.3 Blood pressure and cardiovascular physiology

Blood pressure, also known as systemic arterial pressure, refers to the measurable pressure in large arteries in systemic circulation, and it corresponds to cardiac output, elasticity of arteries and resistances. SBP is the maximal pressure measured in large arteries typically measured in the brachial artery during cardiac contraction (systole), and diastolic pressure is the minimal pressure measured in large arteries during the relaxation phase of a cardiac cycle (diastole) (Brzezinski 1990). Mean arterial pressure is a critical haemodynamic factor and low mean arterial pressure can lead to reduced organ perfusion (Vedel et al. 2016). Systemic arterial blood pressure is tightly regulated primarily through complex mechanisms, including baroreceptor reflexes (high-pressure and low-pressure receptors), anti-diuretic hormone (ADH) and renin-angiotensin-aldosterone (RAA) mechanism (Chopra et al. 2011).

When arterial baroreceptors in the carotid sinus and aortic arch detect elevated blood pressure through vessel wall stretch, they transmit afferent signals via the glossopharyngeal and vagus nerves to the nucleus tractus solitarius (NTS) in the medulla oblongata. This activates central autonomic pathways that inhibit sympathetic outflow—primarily through suppression of the rostral ventrolateral medulla—and enhance parasympathetic (vagal) activity via the nucleus ambiguus. The resulting effects include reduced heart rate (negative chronotropy), decreased myocardial contractility (negative inotropy), and peripheral vasodilation, all contributing to a reduction in blood pressure. This baroreflex mechanism plays a key role in short-term blood pressure homeostasis (Persson et al. 1988; Kougias et al. 2010). During hypotension, reduced stretch of high-pressure baroreceptors in the carotid sinus and aortic arch leads to decreased afferent signaling to the nucleus tractus solitarius (NTS), which in turn increases sympathetic outflow. This result in elevated heart rate, enhanced myocardial contractility, and peripheral vasoconstriction. Simultaneously, decreased stretch of low-pressure baroreceptors in the atria and pulmonary vessels reduces inhibition of vasopressin release from the hypothalamus, promoting water retention. In parallel, renal baroreceptors sense reduced renal perfusion, activating the renin-angiotensin-aldosterone system (RAAS), which further promotes vasoconstriction and sodium/water reabsorption. Collectively, these neurohumoral mechanisms act to rapidly restore arterial pressure and circulating volume (Sved 2009) (Figure 1-1 & 1-2).

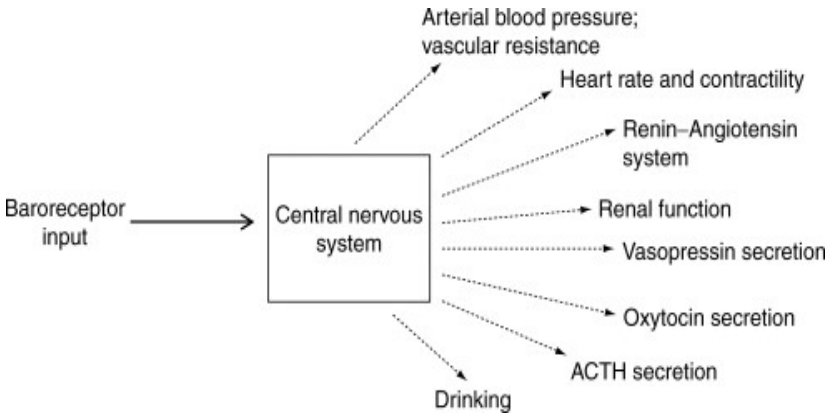


Figure 1-1: Baroreceptor reflex effector systems. This figure illustrates how changes in baroreceptor afferent activity influence CV regulation through multiple brain outputs, including autonomic and hormonal pathways. Reproduced from Sved (2009). (ACTH, Adrenocorticotrophic hormone; SNS, sympathetic nervous system; PNS, parasympathetic nervous system.)

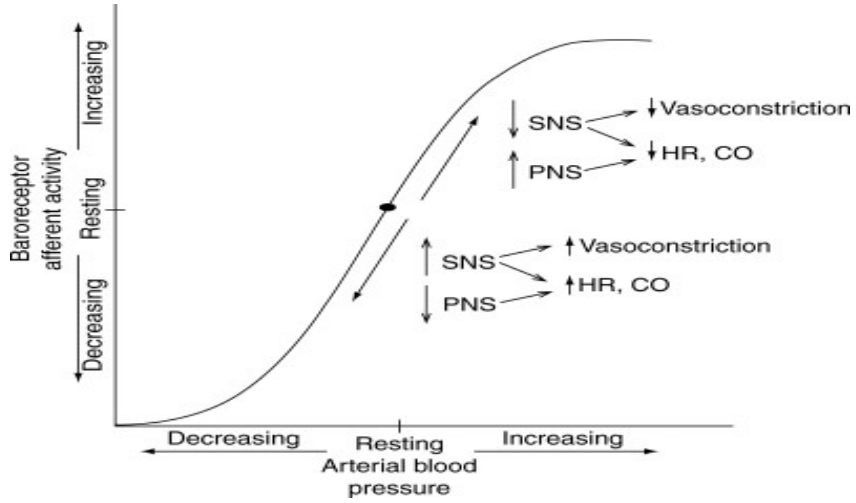


Figure 1-2: Relationship between arterial blood pressure, baroreceptor afferent activity, and CV autonomic outflow. SNS, sympathetic nervous system; PNS, peripheral nervous system; HR, heart rate; CO, cardiac output [Reproduced from (Sved 2009)].

In addition to autonomic stimulus, ADH responds to other triggers, such as increased serum osmolarity and angiotensin II (Usberti et al. 1985). Blood pressure is also regulated through RAA system which is usually initiated with release of renin from Juxta-glomerular apparatus in kidneys. Renin is released in response to low blood pressure, sympathetic stimulus and reduced sodium level in distal convoluted tubules (Morganti 2018). Renin enters systemic circulation and activates angiotensinogen into

angiotensin I. In pulmonary vessels, angiotensin converting enzyme (ACE) is released and converts angiotensin I to angiotensin II, which has multiple effects on the vessels. Angiotensin II causes systemic vasoconstriction, renal efferent arteriole vasoconstriction to maintain adequate glomerular filtration, release of ADH from posterior pituitary, and aldosterone from adrenal gland (Brewster and Perazella 2004) (Figure 1-3).

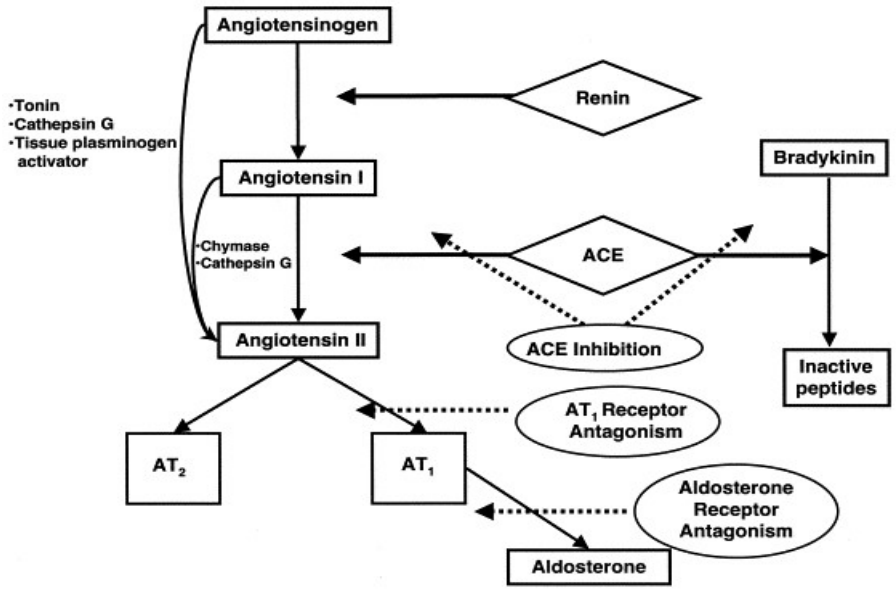


Figure 1-3: The RAA system and its inhibitors.

ACE; AT_1 =angiotensin II type 1 receptor; AT_2 =angiotensin II type 2 receptor
[Reproduced from Brewster and Perazella (2004)]

1.4 Blood pressure variability

Throughout life, blood pressure is a spontaneous oscillating process, and its variability is best categorised in different time frames, such as very short-term (beat-to-beat), short-term (minutes to hours within twenty-four hour), mid-term (day to day) and long-term (months to years) (Figure 1-4 & 1-5).

Very short-term BPV (beat-to-beat): Within seconds, the blood pressure varies, and it is influenced by respiration, increased sympathetic drive, reduced baroreceptor reflex and factors such as rheological, behavioural and emotional factors. Age, activity or sleeps also play important roles in beat-to-beat BPV (Rosei et al. 2020).

Short-term BPV (minutes to hours within 24 hour): It is also influenced by increased sympathetic drive, reduced baroreceptor reflexes, humoral, rheological, behavioural and emotional, age, and activity and sleep factors. In short-term BPV throughout a twenty-four-hour period, circadian rhythm has an influence on blood pressure pattern manifesting as a decline in blood pressure at night (nocturnal dipping) and a rise in the morning (morning surge) (Parati et al. 2013,2015, 2018). Reduced arterial compliance, or increased vascular stiffness, amplifies short-term blood pressure variability by impairing the pressure-buffering function of large arteries, diminishing baroreflex sensitivity, and enhancing wave reflections (Parati et al. 2015; Shin et al. 2019; Parati et al. 2020).

Mid-term BPV (day to day): This type of variability depends on age, arterial stiffness level and improper dosage of antihypertensive medications, poor compliance to antihypertensive therapy (Rosei et al. 2020).

Long-term BPV (visit-to-visit): It is influenced by age, increased arterial stiffness, improper dosage or titration of antihypertensive therapy, compliance to antihypertensive treatments, and seasonal changes.

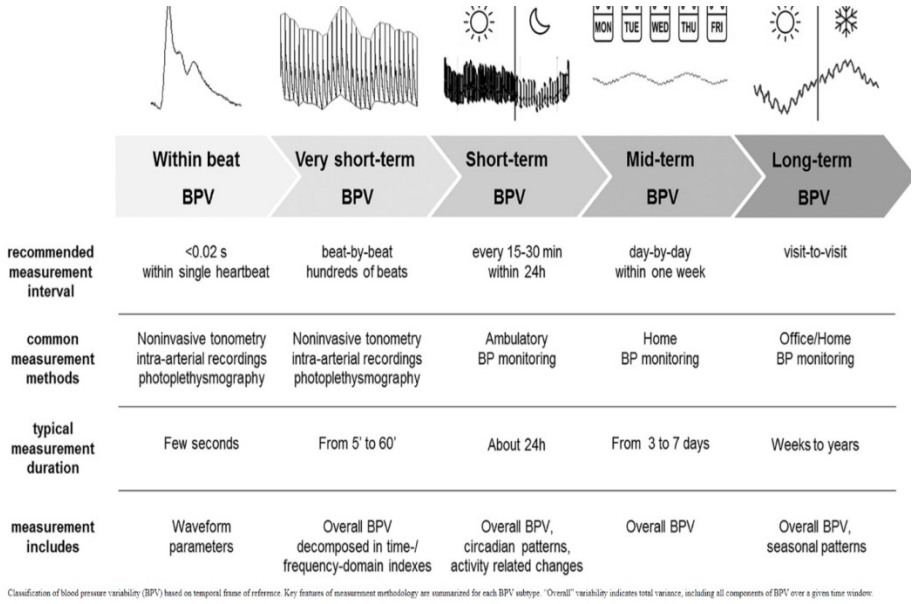


Figure 1-4: Classification of BPV based on temporal frame of reference [reproduced from Schutte et al. (2022)]

Key measurement methodology is summarised for each BPV subtype. Overall variability indicates total variance, including all components of BPV over a given time window.

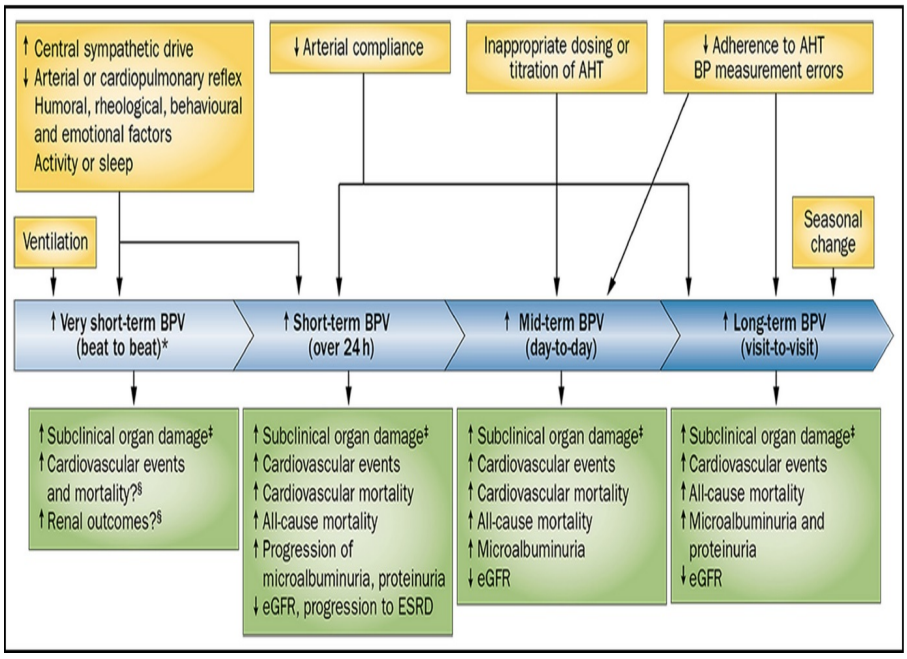


Figure 1-5: Various types of BPV, their determinants, and prognostic relevance for CV and renal outcomes.*Assessed in laboratory conditions; ‡cardiac, vascular, and renal subclinical organ damage; §BPV on a beat-to-beat basis has not been routinely measured in population studies. Abbreviations: Antihypertensive treatments (AHT); BP, blood pressure; BPV, blood pressure variability; ESRD, end-stage renal disease; eGFR, estimated glomerular filtration rate [Reproduced from Parati et al. (2018)].

1.5 Indices for measuring blood pressure variability

There are five main types of indices to measure BPV, including frequency, dispersion, sequence, instability and specific patterns of blood pressure (Parati et al. 2018) (Table 1-1)

Frequency: Very short-term and short-term BPV can be measured using the non-invasive Peñáz method. It was improved later with two finger cuffs which can assess finger arterial pressures (Staessen et al. 1995). This type of measurement provides SD of a mean BP value and estimated values from spectral analysis contributing to overall BPV (Parati et al. 2015).

Dispersion: Standard deviation (SD), coefficient of variation (CoV) and variability independent of the mean (VIM) are indices which measure the dispersion of BPV. They are useful for short-term, mid-term and long-term BPV. Short-term BPV is affected by nocturnal blood pressure decline. Therefore, a weighted 24-Hr SD, which is the average of day and night SD corrected for the respective duration of day and night is a useful index to measure short-term BPV (Parati et al. 1995).

Sequence: Interval weighted standard deviation (wSD), average real variability (ARV), and time rate of blood pressure fluctuations measure sequences of BPV. ARV is the

average absolute difference between consecutive BP readings (Parati et al. 2015; Lu et al. 2023). Time rate BP fluctuations is a measurement of the rate of SBP changes in 24-Hr and it is useful in cases with multiple changes in blood pressure trend (Schutte et al. 2022). These indices can be used for short-term, mid-term and long-term BPV.

Instability and patterns: Measuring the range of BP (maximum – minimum BP), peak size (maximum BP), trough size (mean- minimum BP) are methods focused on the instability of blood pressure (BP). Measuring specific patterns include nocturnal dipping, night/day ratio, MSI, afternoon siesta BP drop, and post-prandial BP fall (Parati et al. 2018).

Table 1-1: Different BPV measurement and indices [reproduced from Parati et al. (2018)]

Type	Indices	BPV category
Frequency	Spectral indices (HF, LF, VLF), residual variability	Short-term BPV Very short-term BPV (spectral analysis)
Dispersion	SD, CoV, VIM, weighted 24-Hr SD (wSD)	Short-term BPV Mid-term BPV Long-term BPV
Sequence	ARV, interval weighted standard deviation (wSD), time rate of BP fluctuations	Short-term BPV Mid-term BPV Long-term BPV
Instability	Range (maximum- minimum), peak size (maximum), Trough (mean-minimum)	Short-term BPV Mid-term BPV
Patterns	Nocturnal BP dipping, night/day ratio, MSI, afternoon siesta dipping, post-prandial blood pressure drop	Short-term BPV

This table summarizes the types of indices used to quantify blood pressure variability (BPV), categorized by frequency, dispersion, sequence, instability, and patterns, and their applicability to short-term, mid-term, and long-term BPV. Data [reproduced from Parati et al. (2018)].

Currently, there are multiple BPV indices and there is no perfect index of BPV. Commonly used indices include SD in long-term BPV, CoV, ARV and wSD in short-term variability. These indices provided some evidence in predicting adverse CV events (Stevens et al. 2016; Mena et al. 2017). However, nocturnal decline in blood pressure can impact both SD and CoV, therefore wSD is suggested (Schutte et al. 2022).

Since SD is related to the magnitude of value relative to the mean of all values and vulnerable to nocturnal dipping in BP, ARV has also been used. ARV calculates the absolute differences between successive BP measurements and can bypass the vulnerability of SD, but the number of readings and missing values can influence its reliability (Parati et al. 2023).

In a recent study conducted in 2022, VIM demonstrated a prognostic value in predicting stroke, MI, heart failure and death (Ebinger et al. 2022). However, the complexity of calculating VIM poses a challenge to its practicality (Schutte et al. , 2022). Different types of blood indices and their strengths, weaknesses and use in measuring four types of BPV are described. (Table 1-2 & 1-3)

Table 1-2: Type of BPV, determinants, methods, and indices

Type of BPV	Determinants	Methods	Indices
Very short – term BPV (Beat-to-beat)	Age, increased sympathetic drive, reduced cardiopulmonary reflexes, humoral, rheological, behavioural and emotional factors, ventilation	Continuous BP recordings	SD CoV ARV Spectral analysis
Short-term BPV (24-Hr)	Increased arterial stiffness, age. Increased sympathetic drive, reduced cardiopulmonary reflexes, humoral, rheological, behavioural and emotional factors,	24-Hr ABPM HBPM	SD CoV 24 Hr weighted SD ARV 24-Hr VIM Spectral analysis Nocturnal dipping, night/day ratio MSI, AASI
Mid-term BPV (Day to day)	Age, increased arterial stiffness, improper dosage of antihypertensive treatment, adherence to medications, BP measurement errors	Over 48-Hr ABPM HBPM	SD CoV ARV VIM
Long-term BPV (Visit-to-visit)	Age, increased arterial stiffness, improper dosage of antihypertensive treatment, adherence to medications, BP measurement errors. Seasonal changes	ABPM HBPM OBPM	SD CoV ARV VIM

ABPM, ambulatory blood pressure monitor; HBPM, home blood pressure monitor; OBPM, office blood pressure monitor; SD, standard deviation; CoV, coefficient of variation; ARV, average real variability; 24-Hr Weighted SD, twenty-four hour weighted standard deviation; 24-Hr VIM, twenty-four-hour variability independent of the mean; MSI, morning surge index; AASI.

Table 1-3: BPV indices with strengths and weaknesses

Indices	Strengths	Weaknesses
SD	• Easy to calculate • Less affected by extreme values	• Correlation with average BP levels • Affected by BP trends in day/night BP changes
CoV	• Easy to calculate	• Affected by BP trends in day/night BP changes
wSD Weighted 24 Hr CoV (wCoV)	• Removes nocturnal dipping	• Affected by day/night BP trend
VIM	• No correlation with the Mean	• Complexity of calculation • Coefficient may vary in different populations • Needs previous derivation of previous coefficient from a given population
ARV	• Unaffected by day/night BP changes • Shows within subject variability	• Affected by average BP levels • Vulnerable to missing values and poor data recording
Spectral indices	• Continuous BP monitoring	• Need evenly sampled series • Risk of under-sampling
Residual variability	• Continuous BP monitoring	• Depends on the statistical model
Frequency domain indices	• Continuous BP monitoring	• Discontinuous measurement • Risk of under-sampling
Complexity domain indices	• Continuous BP monitoring	• Data requirement (long-term data) • Computational complexity
Range	• Easy to calculate	• Heavily influenced by artefacts and outliers
Time rate of BP fluctuations	• Shows magnitude and speed of BP changes	• Limited in discontinuous measurements
Nocturnal dipping	• Can differentiate between dipper and non-dipper or reverse dipper	• No current consensus for SBP or DBP to be used
Morning surge index	• No obvious strength	• Correlates with nocturnal blood pressure. Alternative definition has been proposed

1.6 Clinical relevance of blood pressure variability

As discussed above, it has now been recognised that increased BPV is clearly associated with TOD, CV events and all-cause mortality. Increased BPV would be a manifestation of behavioural changes, impaired cardio-regulatory mechanisms, increased arterial stiffness, inappropriate pharmacotherapy for hypertension and poor compliance to pharmacotherapy (Parati et al. 2018).

An important feature is that increased BPV may be related to undiagnosed hypertension. It was reported that increased BPV is more pronounced in hypertensive patients than in normotensive patients (Cacciolati et al. 2013; Rosei et al. 2020). It was supported by a study that high short-term BPV can predict future development of hypertension (Özkan et al. 2022). Hence, in patients with normal BP but high BPV, clinicians should consider undiagnosed hypertension or future hypertension development.

In terms of TOD, in pre-clinical state, increased BPV is correlated with cardiac, renal, cerebral and vascular dysfunction (Sega et al. 2002; Grassi et al. 2012; Parati et al. 2023). TOD include increased left ventricular mass index, increased arterial stiffness, decline in renal function and development of proteinuria, increased carotid-intima thickness, decline in cognitive function and left ventricular dysfunction. In clinical state, there is clear evidence of association between BPV and CV events as well as all-cause mortality (Grove et al. 1997; Suchy-Dicey et al. 2013; Manning et al. 2015; Muntner et al. 2015; Stevens et al. 2016; Cuspidi et al. 2017; Wang et al. 2017; Mehlum et al. 2018; Webb et al. 2018; De Havenon et al. 2019; Liu et al. 2022).

With this body of evidence, studies were conducted to reduce BPV and CV events. Anglo-Scandinavian Cardiac Outcomes Trial: Blood Pressure-Lowering Arm (ASCOT-BPLA) demonstrated that combination of amlodipine and perindopril can reduce BPV and CV events (Östergren et al. 2008). However, more data, such as randomised controlled trials, are required to investigate the effect of different medications in reducing BPV and CV risks. Therefore, in this research, patient medications were included to assess their effect on MACE and BPV.

1.7 Arterial stiffness

Arterial stiffness plays an important role in BPV and is a biomarker for CV events, cognitive decline and all-cause mortality (Mitchell et al. 2010; Vlachopoulos et al. 2010; Hughes et al. 2018). Increased arterial stiffness is a manifestation of vascular ageing

and the progression is exacerbated by co-morbidities such as diabetes mellitus, atherosclerosis and chronic kidney diseases. Stiff arteries lose their ability to accommodate changes in blood flow, resulting in pressure fluctuations. Stiff arteries can cause an increase in SBP because they cannot expand sufficiently during the systolic phase of the heart. At the same time, DBP may not rise proportionately, or it may even decrease, leading to increased BPV.

Several key stages of structural changes are found in stiff arteries. Reduced elastin increased dysregulated collagen production, and increased smooth muscle proliferation with intimal and medial thickening are observed in stiff arteries (Xu et al. 2000; Zieman et al. 2005). Endothelial cell signalling, vascular smooth muscle tone and neuro-endocrine signalling (angiotensin II, glucose, insulin), and genetic polymorphism also play important roles in increased arterial stiffness (Figure 1-6).

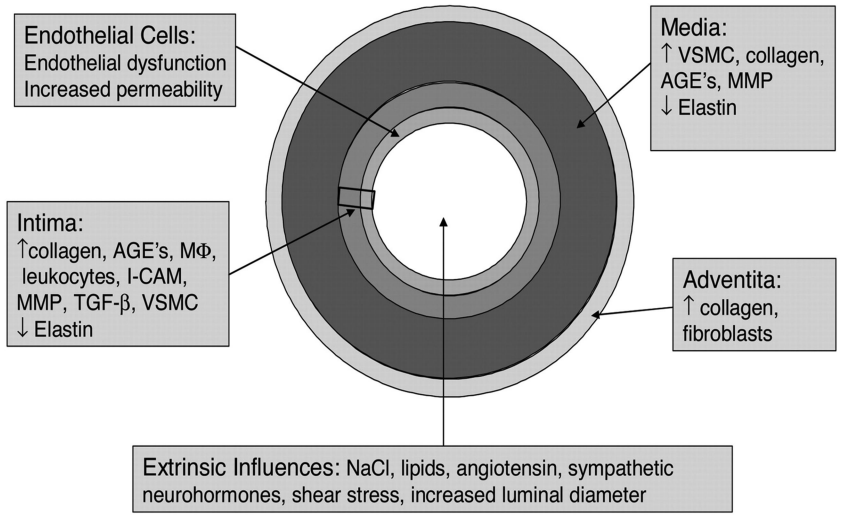


Figure 1-6: Summary of the multiple causes and locations of arterial stiffness. [Reproduced from Zieman et al. (2005)]

Stiff arteries demand greater energy expenditure by the left ventricle, resulting in left ventricular hypertrophy (Lartaud-Idjouadiene et al. 1999). Stiff arteries also lead to an increase in pulse pressure, resulting in isolated systolic hypertension (Dart and Kingwell 2001). Changes at the microcirculation and macrocirculation levels are closely related to the TOD (Figure 1-7).

1.7.1 Traditional means of measuring arterial stiffness

There are several ways of measuring arterial stiffness, and the current gold standard technique is carotid-femoral pulse wave velocity (cfPWV), which measures central aortic stiffness (Wilkinson et al. 2020). Other methods include using devices such as Complicor®, Sphygmocor®, and PulsePen® for PWV measurement and aortic PWV measurement with MRI.

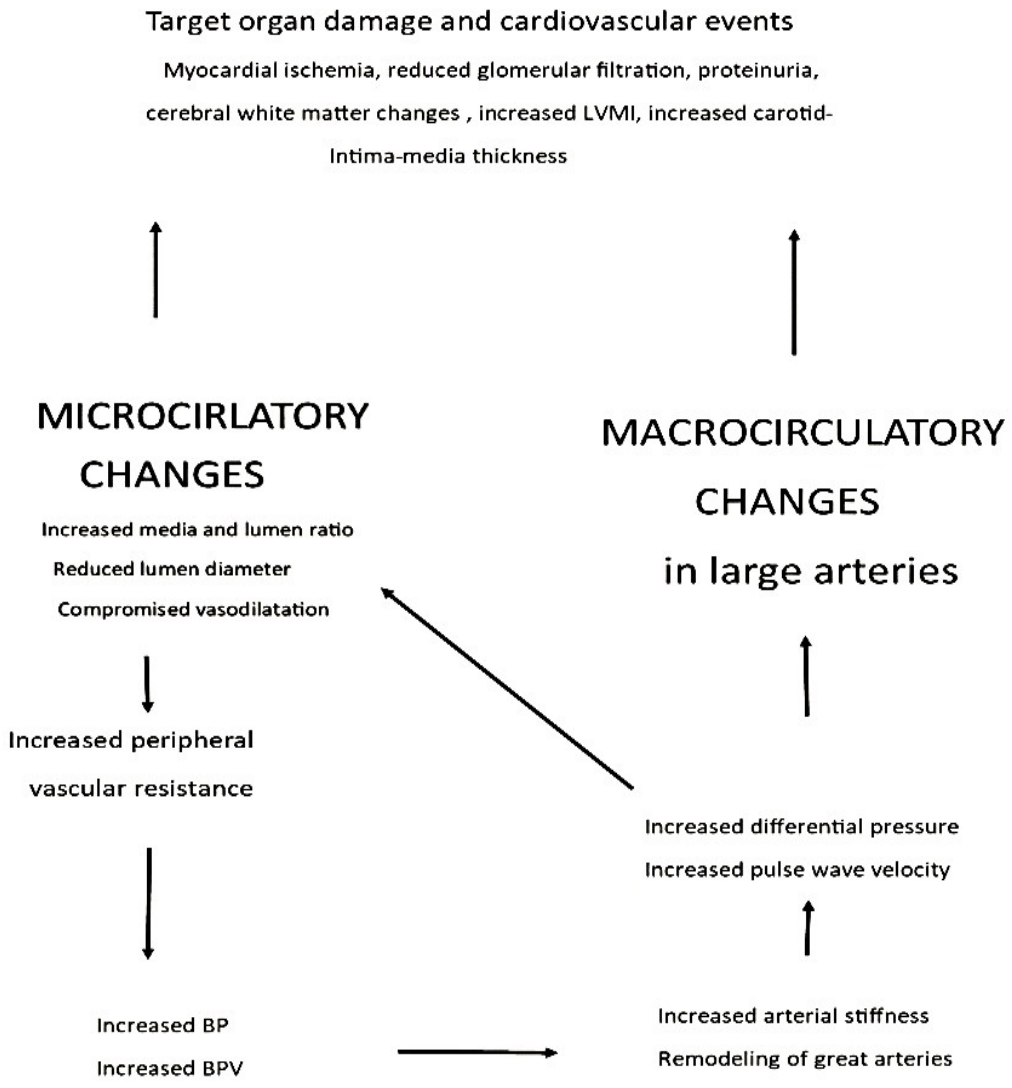


Figure 1-7: Microcirculatory changes, macrocirculatory changes, and target organ damage.

1.8 Ambulatory arterial stiffness index

The AASI is a relatively new index and is readily available on 24-Hr ABPM reports. As it is calculated from SBP, it is linked to variations in blood pressure. In a normal compliant artery, when there is an increase in systolic blood pressure (SBP), the diastolic blood pressure (DBP) also increases in a parallel fashion. In stiff arteries, an increase in SBP results in a normal or even a lower DBP. Based on this concept, it was hypothesised that 1 minus the regression slope of DPB over SBP would be a measure of arterial stiffness (Li et al. 2006). Li and colleagues demonstrated that AASI has a moderate but statistically significant correlation ($r \approx 0.3–0.5$) with established arterial stiffness measures, including aortic pulse wave velocity (aPWV), reflecting large artery stiffness, and central (CAIx) and peripheral (PAIx) augmentation indices as measures of wave reflections (Laurent et al. 2006). Central augmentation index (CAIx) and peripheral augmentation index (PAIx) are measures derived from arterial waveforms that quantify the contribution of reflected pressure waves to systolic blood pressure, with CAIx reflecting central (aortic) wave reflection and PAIx reflecting peripheral (e.g., radial) wave reflection. This correlation, validated by studies like Li et al. (2006), supports AASI's role as a practical, noninvasive tool for assessing arterial stiffness and CV risk.

Clinically, AASI has been considered a prognostic risk factor for CV events, particularly stroke (Kollias et al. 2012). In the Dublin Outcome study, it was reported that AASI could predict future CV events and its prognostic ability is significant in normotensive patients with a history of hypertension (Dolan et al. 2010).

Despite its role as a prognostic marker, targeting AASI for treatment did not show significant changes (Kollias et al. 2015). Another issue with AASI is the lack of consensus over a cut-off value for practical utility in clinical settings. Further research in this area is still required to have a consensus on the cut-off value of AASI in CV risk stratification. Therefore, in this MRes research, AASI cut-off values were investigated in relation to current and previous literature evidence.

1.9 Aims and objectives

The primary aim of this MRes clinical research project is to examine the relationship between contemporary measures of BPV and AASI and MACE, which include CV deaths, non-fatal stroke, TIA, and ACS.

This aim will be fulfilled through the following objectives.

1. To critically review the literature on BPV, AASI and MACE
2. To collect and analyse data using appropriate statistical models to establish whether there is a link between short-term BPV, AASI and MACE

1.10 Research structure

The clinical research project structure is described as follows in the table.

Chapter	Title	Description
1	Introduction	Blood pressure physiology, BPV, indices measuring BPV and AASI
2	Systematic literature review	A systematic literature review on the ambulatory arterial stiffness and major adverse cardiovascular events
3	Methodology	Research methods will be described, evaluated and justified.
4	Results and discussion	Results from data collection and statistical analysis will be discussed.
5	Conclusion	The thesis concludes with explanations of whether the aim and objectives have been met and future recommendations.

Chapter 2 Systematic literature review

In recent decades, researchers have become increasingly interested in the linear relationship between SBP and DBP. This dynamic relationship has been thought to reflect arterial wall function, and in 2006, a novel index was introduced as the AASI (Li et al. 2006).

Since it was first introduced, AASI has been examined and demonstrated to be an index which can predict MACE, particularly cerebrovascular events (Kollias et al. 2012). MACE typically includes CV deaths, stroke or TIA, and coronary events such as MI or ACS. Another common outcome in studies is all-cause mortality. There have been discussions and debates about whether or not AASI is a true arterial stiffness marker. Currently, AASI has been considered as an indirect marker of arterial stiffness and correlated with BPV (Lee et al. 2011; Boos et al. 2021).

Since 2006, two meta-analyses have been conducted to investigate the role of AASI in predicting future CV events, and both consistently demonstrated that AASI could predict CV outcomes (Aznaouridis et al. 2012; Kollias et al. 2012). A significant finding from Kollias et al. (2012) was that AASI is a significant predictor for cerebrovascular events but has limited predictive ability for coronary events. It has now been more than ten years since these meta-analyses were conducted and further studies have been conducted to investigate the role of AASI in predicting both cerebrovascular and coronary events.

There are ways of calculating AASI from SBP and DBP. Nowadays, in modern ABPM devices, AASI is an automatically reported index. Furthermore, the use of ABPMs is now a gold standard method in diagnosing and managing hypertension (Palatini 2012). An ABPM typically reports parameters such as day, night, average and mean SBP and DBP in addition to AASI. Furthermore, other indices, such as nocturnal dipping status and MSI could be reported. With the increasing availability of such equipment and the fact that it has now been more than ten years since the last two meta-analyses were carried out, there is a need for a new systematic literature review on AASI to investigate its role in predicting MACE.

2.1 Methods

2.1.1 Search strategy

A systematic review was conducted according to a pre-defined protocol in line with the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines and has been registered on PROSPERO (https://www.crd.york.ac.uk/PROSPERO; registration ID CRD42023423030). The primary outcomes were:

(i) all-cause mortality
(ii) CV death

Secondary outcomes were:

(i) stroke
(ii) MACE

Literature search was conducted using databases including MEDLINE, PubMed, CINAHL, Google Scholar, and Science Direct. The key search terms were "ambulatory arterial stiffness Index", OR "arterial stiffness" OR "ambulatory systolic-diastolic regression index" OR "AASI" AND "MACE" OR "Stroke" or "Cerebrovascular events" OR "Myocardial infarction/ Acute coronary syndrome" OR "CV deaths". Journal articles were searched with the starting point from January 2006 (matching the year) AASI was first used and the endpoint was July 2023.

2.1.2 Selection criteria and data extraction

This systematic literature review included studies that investigated the association between the AASI or the ambulatory systolic-diastolic pressure regression index (ASDPRI) and clinical outcomes including all-cause mortality, CV death, stroke/transient ischaemic attack (TIA), and myocardial infarction (MI) or acute coronary syndrome (ACS). A defined set of selection criteria was applied, and only studies meeting the inclusion criteria were included. For this review, AASI is calculated from ambulatory arterial stiffness index values obtained over a continuous 24- to 48-hour period using a single ABPM session.

Table 2-1: Inclusion criteria

Studies with participants aged ≥18 years
Studies with a cohort design
Studies with the AASI value reported
Studies with AASI calculated using ABPM only and sufficient information for the outcome (CV death, stroke, TIA, MI,

2.1.3 Data extraction, quality evaluation, synthesis and visualisation

The extraction of crucial data and the quality assessment of the study were performed independently by two investigators (AH and CJB) to ensure the accuracy and precision of data extraction. Potential disagreements were resolved through deliberation and a third investigator (AK) where necessary. The results of maximally adjusted models for the outcomes of interest were used where available. All extracted data were described in the narrative synthesis table and compared. OR and HR with a ninety-five per cent confidence interval (95% CI) were graphically displayed using Forest plots. Forest plots were used in a non-meta-analytical method and undertaken primarily to improve data visualisation. Forest plot was created using Microsoft Excel and Matplotlib with Python and R coding.

Table 2-2: Details and key characteristics of the eligible studies included

1	First author and year of publication
2	Source of journal
3	Study population
4	Exclusion criteria
5	Mean age
6	Follow-up years
7	Events
8	AASI regression model
9	Covariate adjustment factors
10	Main outcome
11	Key summary findings

2.1.4 Quality evaluation and risk of bias assessment

The Newcastle-Ottawa Scale (NOS), developed for case–control and cohort studies, was used to assess the quality and risk of bias (ROB) of all included articles. The NOS grading has three parts: (1) selection, (2) comparability, and (3) exposure and encompasses a total of eight items with a maximum of one star per criteria with two stars for comparability. The total score can range from 0-9 stars. Studies with a score of

7-9 were graded as high quality, 4-6 as medium quality and a score of < 4 as poor quality.

2.2 Results

2.2.1 Study and patient characteristics

A total of 595 articles were selected and 277 duplicates were removed. A total of 318 studies were screened. 195 studies were irrelevant and excluded. The remaining 123 studies were assessed for eligibility. 104 studies were excluded for the reasons of wrong study design, wrong outcome, wrong intervention, wrong setting, and non-English language publication and 19 studies were selected. Of the selected 19 studies, there were a total of 13 full papers, 5 abstracts and 1 research letter (Figure 2-1). Due to the limited number of studies after exclusion, all eligible studies were accepted for the systematic review. Study populations included the general population, patients who underwent ABPM, patients with hypertension or resistant hypertension, patients with diabetes, patients with MI or those referred for angiography, and patients undergoing haemodialysis (Figure 2-1, Table 2-3 & 2-4).

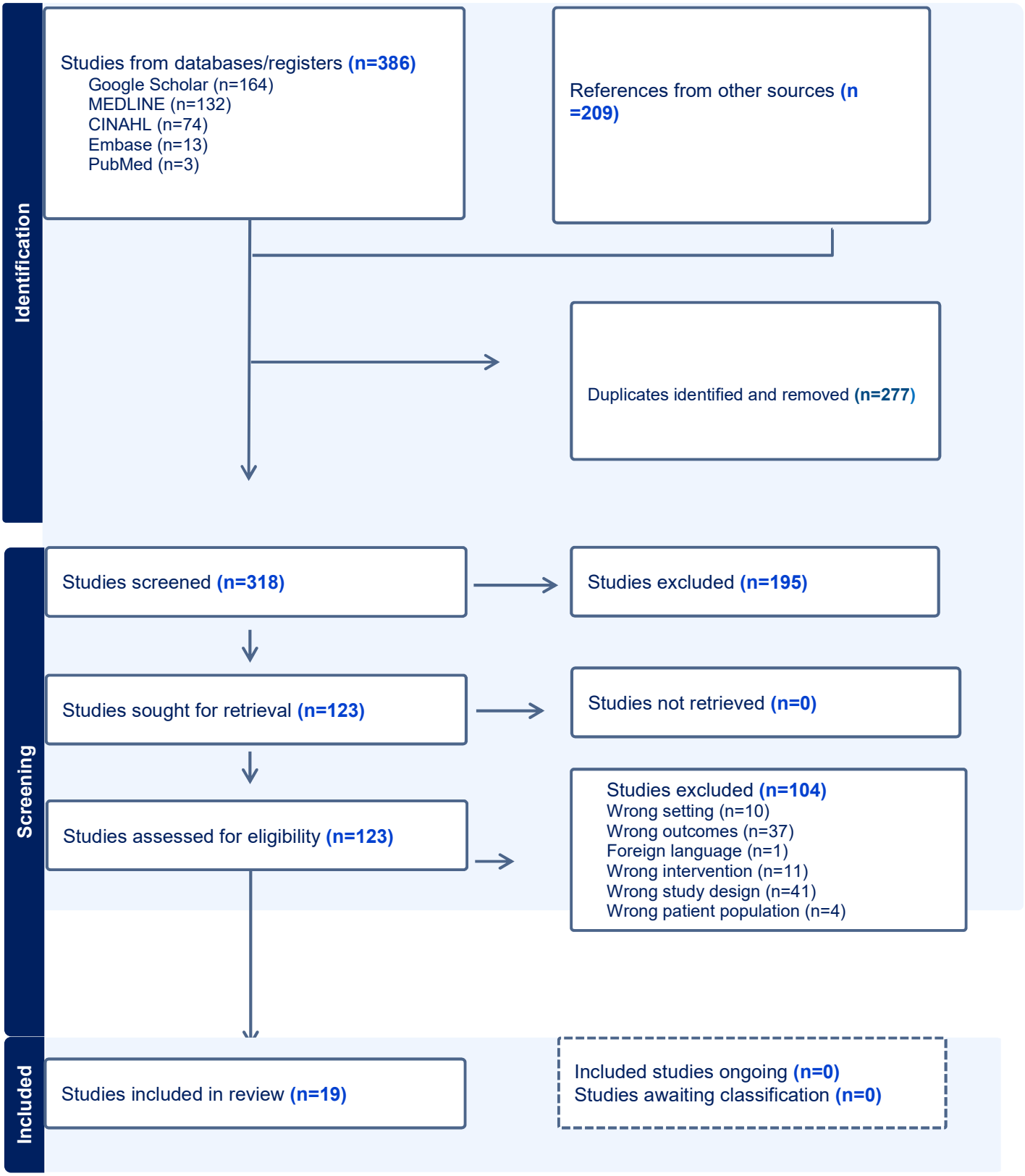


Figure 2-1: PRISMA flow diagram

2.3 Outcomes

This systematic review reported that AASI is a robust predictor for MACE. Out of nineteen studies (n = 37063), eleven studies (n=27484) supported the finding that AASI can independently predict MACE, including fatal or non-fatal stroke, CV deaths, and coronary diseases (Table 2-5 to 2-7) (Figure 2-2 to 2-5). In subgroup analyses, it was revealed that AASI is a predictor for stroke in eight studies (n = 26286) (Table 2-6, Figure 2-3). Additionally, four studies showed that AASI is also a predictor for coronary events (n = 3433) (Table 2-7, Figure 2-4). Furthermore, four studies (n = 4908) from this systematic review showed that AASI is also an independent predictor for all-cause mortality (Table 2-8, Figure 2-5). In several studies, the terms all-cause mortality and all-cause death were interchangeably used. Therefore, in this systematic review, all-cause mortality was primarily used, but original terms from studies were maintained in the study findings tables.

2.3.1 Major adverse cardiovascular events, subgroups and all-cause mortality

The review encompasses 13 studies from 2006–2012 and 6 from 2015–2023, covering diverse populations including normotensive individuals, hypertensive patients, diabetic patients, post MI patients, haemodialysis patients, and those undergoing coronary angiography (Table 2-3). Sample sizes ranged from 80 to 11,291, mean ages from 50.7 to 70.3 years, and follow-up durations from 2.19 to 13.3 years. The studies comprised 14 full papers and 5 abstracts, with exclusion criteria such as incomplete ABPM, recent CV events, pregnancy, or night-shift work (Table 2-3).

Of the 19 studies, 17 employed Cox proportional hazard models to analyse time-to-event outcomes (MACE, stroke, coronary events, all-cause mortality), while 2 studies (Lee et al. 2012; Sobiczewski et al. 2019) used logistic regression for binary outcomes (Table 2-4). Cox regression models reported HRs with 95% CIs and p-values, adjusted for CV risk factors such as age, sex, body mass index (BMI), BP, diabetes, smoking, cholesterol, and study-specific factors (e.g., dialysis status, lifestyle factors). Logistic regression models reported ORs with 95% CIs and p-values, adjusted for similar covariates (Table 2-3 & 2-4).

AASI values range from 0 to 1, but no consistent cut-off was established for predicting adverse CV events, with reported cut-offs varying from 0.30 to 0.71 depending on population (e.g., ethnicity in Dolan et al. 2006; age in Hansen et al. 2006) and outcome. Studies employed different ABPM durations and AASI categorizations, such as

quartiles (Kikuya et al. 2007) or tertiles (Palmas et al. 2009). Adjustments for CV risk factors varied, though most studies included age, sex, BMI, BP, diabetes, smoking, and study-specific covariates (e.g., dialysis factors, lifestyle). This methodological heterogeneity precluded meta-analysis, and results were synthesized narratively.

Narrative synthesis of the 17 Cox regression studies (HRs 0.65–37.65, mostly 1.3 – 2.5) and 2 logistic regression studies (ORs 1.8–3.0) confirmed AASI's consistent prediction of MACE, stroke, coronary events, and all-cause mortality, with the strongest association observed for stroke (8 studies), followed by MACE (11 studies), all-cause mortality (4 studies), and coronary events (4 studies). The wide range of HRs, particularly high values in small studies (e.g. Viazzi et al. 2020: HR 37.65, n = 80), reflects imprecision due to limited sample sizes or high-risk populations. Figure 2-2 presents a Forest plot of study-specific and combined HRs and ORs, visualizing AASI's prognostic strength. AASI's ability to identify high-risk patients supports its potential for CV risk stratification, but the lack of a standardized cut-off limits clinical applicability.

Table 2-3: Study characteristics

Study ID	Author & Year	Journal	Type	Sample Size	Population	Exclusion Criteria	Mean Age (Years)	Follow-Up (Years)
1	Dolan et al. 2006	Hypertension	Full paper	11,291	Patients who underwent ABPM, not on antihypertensive	Not reported	54.6 (± 14.6)	5.3 (median), range 2 days - 21.4
2	Hansen et al. 2006	Journal of Hypertension	Full paper	1,829	Randomly selected Danes aged 40-70	Fewer than 14 day-time or 7 night-time BP readings, night shifts	55.5	3.1 - 10.1
3	Gosse et al. 2007	American Journal of Hypertension	Full paper	469	Office BP <140/90 mm Hg, with CV complications	Various CV complications, left-bundle branch block, thyroid pathology	54 (±14)	5.8 (±3.25)
4	Kikuya et al. 2007	Stroke	Full paper	1,542	Residents of Ohasama, Japan	Incomplete ABPM, hospitalization, incapacitation	61.7	13.3
5	Hansen et al. 2008	Journal of Human Hypertension	Research Letter	1,678	Random Danish population	No aPWV, insufficient BP readings, night work, history of MI or stroke	54.8	9.4 (median)
6	Ben-Dov et al. 2008	Journal of Human Hypertension	Full paper	2,918	Patients aged 16+, no pregnant women, valid ABPM recordings	Poor quality ABPM recordings (less than 50 valid measurements)	56 (±16)	6.6 (±0.4)
7	Palmas et al. 2009	Hypertension	Full paper	1,178	Older patients with diabetes from the IDEATel study	Not reported	70.3 (±6.1) (alive), 73.1 (±6.9) (deceased)	6.6 (±0.4)
8	Gavish et al. 2009	Hypertension Research	Full paper	3,433	Patients who underwent ABPM	Younger than 16, pregnant women, poor quality ABPM (less than 15 valid measurements)	56 (±16)	7.6
9	Bastos et al. 2010	Sociedade Portuguesa Cardiologia	Full paper	1,200	Portuguese patients >18 with HTN, no prior CV events	Not reported	50.7 (±12.7)	Not reported
10	Muxfeldt et al. 2010	Journal of Hypertension	Full paper	547	Patients with resistant hypertension	Not reported	65.9 (±11.3)	4.8 (median)
11	Dolan et al. 2010	Journal of Hypertension	Abstract	1,905	Patients from ASCOT	Not reported	N/A	5.5 (median)
12	Lee et al. 2012	Journal of Hypertension	Abstract	885	Hypertensive patients	Not reported	N/A	N/A
13	Laugesen et al. 2012	Journal of Hypertension	Full paper	108	Patients with Type 2 DM	Incomplete ABPM, migration, missing CV outcome records	56 (±9)	9.5 (range 0.5–14.5)
14	Bastos et al. 2015	Journal of Hypertension	Abstract	217	Patients with resistant HTN, defined by 24-Hr ABPM	Not reported	56.4 (±14.6)	6.0 (±3.1)
15	Cieslik-Guerra et al. 2019	Journal of Hypertension	Abstract	90	Post-MI patients	Not reported	N/A	5.33
16	Sobiczewski et al. 2019	European Heart Journal	Abstract	891	Patients referred for diagnostic coronary angiography	Not reported	N/A	6.7
17	Viazzi et al. 2020	American Journal of Hypertension	Full paper	80	Haemodialysis (HD) patients	BMI > 40 kg/m², missed HD treatments, drug abuse, recent stroke or MI	67.4 (±14.1)	Not reported
18	Boos et al. 2021	BMC Cardiovascular Disorders	Full paper	508	UK adults investigated for HTN	Transplantation, AF, CKD stages IV/V, pregnancy, active cancer, severe stenosis, infection, recent hospitalization	58.8 (±14.0)	2.19
19	Hoshide et al. 2023	Hypertension Research	Full paper	6,294	Treated hypertensive patients	Not reported	68.6	4.5

This table summarizes key study characteristics, including author, year, publication type, sample size, population, exclusion criteria, mean age, and follow-up duration. Studies evaluated the prognostic role of AASI across various populations using ambulatory blood pressure monitoring (ABPM). Follow-up periods are reported as mean, median, or range, as provided in the original sources.

Table 2-4: Study findings

	Author & Year	Outcomes	AASI Regression Model	AASI Cut-Off	Adjustments	Results	Comments
1	Dolan et al. 2006	566 CV deaths (151 stroke, 358 cardiac disorders)	Cox regression	Normotensive: 0.55 (Chinese) 0.57 (European)	Sex, age, BMI, MAP, smoking, diabetes, CV history	↑1SD in AASI associated with ↑ in CV mortality. HR 1.59, 95% CI: 1.47-1.71, p < 0.001.Stroke; HR 1.21, 95% CI:1.01-1.45, p < 0.05)	AASI is a significant predictor for MACE and stroke in normotensive patients.
2	Hansen et al. 2006	40 strokes, 150 coronary events, 212 CV events	Cox regression	0.62 (40 yrs), 0.71 (70 yrs)	Sex, age, BMI, MAP, smoking, diabetes, cholesterol ratio, CV history	Stroke HR 1.62; 95% CI: 1.14-2.28, p = 0.007. Coronary events not significant	AASI is a strong predictor for stroke, less for other CV events.
3	Gosse et al. 2007	62 CV events	Cox regression	0.59 (±0.14), 0.55 (±0.13)	Age, pulse pressure	Not significant in multivariate, significant in univariate analysis	AASI is an indirect estimate of arterial stiffness and there is an element for CV risk evaluation.
4	Kikuya et al. 2007	126 CV deaths, 63 stroke deaths	Cox regression	0.46 (±0.10), Quartiles: Q1 <0.39, Q2: 0.39-0.45, Q3: 0.45-0.51, Q4 > 0.51	Sex, age, BMI, MAP, lifestyle factors	Q1 HR 1.40, p= 0.04; Q2: HR 0.82, p= 0.25; Q3 HR 0.64, p= 0.01	AASI is a significant predictor for CV events and stroke beyond pulse pressure.
5	Hansen et al. 2008	154 CV events, 31 strokes, 62 coronary events	Cox regression	Not reported	Age, MAP, BMI, night-to-day MAP ratio, alcohol intake	Stroke: HR 1.68, p= 0.001; aPWV for CV events: HR 1.15, p= 0.03	AASI predicts stroke and aPWV predicts composite MACE.
6	Ben-Dov et al 2008	215 all-cause mortality	Cox regression	Not reported	Age, gender, treatment for diabetes and HTN, 24-h SBP, SBP dipping	AASI non-significant; s-AASI HR 1.17 (95% CI: 1.01–1.37, p= 0.041)	Modified AASI (s-AASI) has a stronger association with mortality than traditional AASI.
7	Palmas et al. 2009	218 deaths (110 CV deaths)	Cox regression	Not reported	Age, gender, race/ethnicity, diabetes duration, various biomarkers	AASI tertiles: 3rd tertile HR 1.36; 95% CI: 1.01-1.83, p= 0.025	AASI is a significant predictor for mortality in elderly with diabetes, not for CV deaths.
8	Gavish et al. 2009	238 all-cause mortality	Cox regression	Not reported	Demographics, 24-h mean BP, PP, dipping	Short-term mortality: HR 2.21 (1.36-3.59); 1.5-7 yrs: HR 1.28 (1.06-1.54)	BPVR=AASI, is a significant predictor for short-term mortality independent of traditional BP.
9	Bastos et al. 2010	62 all-cause deaths, 79 strokes, 51 coronary events	Cox regression	0.41	Age, BMI, diabetes, antihypertensive therapy	CV events HR 1.27 (1.01-1.59), p < 0.02; Stroke HR 1.36(1.02-1.89), p < 0.02	AASI predicts MACE and stroke, not coronary events.

Table 2-4 (continued)

	Author & Year	Outcomes	AASI Regression Model	AASI Cut-Off	Adjustments	Results	Comments
10	Muxfeldt et al. , 2010	42 strokes, 21 MI, 14 revascularizations	Cox regression	0.55 (0.46-0.63)	CV risk factors, mean BPs, nocturnal BP reduction	24 Hr AASI for MACE: HR 1.46 (1.12-1.92); Night AASI CV mortality: HR 1.73 (1.13-2.65)	AASI is a significant predictor for CV morbidity and mortality in resistant HTN patients.
11	Dolan et al. , 2010	173 CV events	Cox regression	Not reported	Age, sex, smoking, diabetes, BMI, SBP	Univariate HR 1.31 (1.17-1.46), p <0.001; Multivariate HR 1.24 (1.10-1.41), p < 0.005	AASI is a significant predictor for MACE (both stroke and coronary events).
12	Lee et al. , 2012	185 strokes	Logistic regression	0.48 (±0.01)	Adjusted, not detailed in paper	AASI for stroke OR 0.86 (0.29-2.5, p= 0.777); higher in stroke patients	BP variability influences AASI, but not a significant predictor for stroke.
13	Laugesen et al. , 2012	45 CV events (35 non-fatal, 10 fatal)	Cox regression	0.43 (±0.15) (with events)	Established CV risk factors	AASI in events: 0.43 (±0.15), non-events: 0.38 (±0.14)	Higher AASI in CV events, but not an independent predictor
14	Bastos et al. , 2015	53 CV events, 24 deaths	Cox regression	0.41	Sex, age, BMI, SBP	CV events HR 8.34; 95% CI: 1.76-39.57, p = 0.008	AASI predicts MACE and all-cause mortality.
15	Cieslik-Guerra et al. 2019	5 CV deaths, 2 recurrent MI, 15 unstable angina	Cox regression	0.42	N/A	CV events HR 7.899; 95% CI: 1.835-33.994, p= 0.006	AASI is a predictor for CV events post-MI.
16	Sobiczewski et al. , 2019	135 ACS, 5 deaths, 55 strokes	Logistic regression	0.35 (±0.01) (ACS), 0.30 (±0.1)	Age, sex, diabetes, MI history, treatments	OR 4.0; 95% CI: 1.3-12.0; AASI in ACS: 0.35 (±0.1), p < 0.01	AASI is an independent predictor for ACS.
17	Viazzi et al. 2020	31 deaths	Cox regression	0.54	Age, sex, dialysis duration	All-cause mortality HR 37.657; 95% CI: 2.259-627.638, p = 0.012	AASI is a predictor for all-cause mortality.
18	Boos et al. 2021	39 MACE, 7 CV deaths	Cox regression	0.56 (reverse dippers), 0.48 (non-dippers), 0.39 (normal dippers)	Age, CAD, HF, PVD, stroke/TIA, diabetes, HTN, NLR	Univariate OR: 1.03 (1.01-2.05), p = 0.006; multivariate not significant	AASI is associated with BP dipping and inflammation, not independent predictor for MACE.
19	Hoshide et al. 2023	213 CV events (119 stroke, 98 coronary)	Cox regression	≥ 0.57	Age, sex, BMI, smoking, diabetes, CV history, antihypertensives	Stroke HR 2.55 (1.32-4.95) for AASI ≥0.578 + 24-Hr SBP > 130 mmHg	Day-time SBP is a risk for stroke in patients with high AASI.

This table presents key outcomes, regression models, AASI cut-offs, covariate adjustments, and main findings from included studies assessing the prognostic value of AASI. Outcomes include MACE, stroke, coronary events, and all-cause mortality. Results are reported using hazard ratios (HRs) or odds ratios (ORs) with corresponding 95% confidence intervals and p-values.

Table 2-5: Studies which demonstrated that AASI is an independent predictor for MACE

Studies	Number of patients	HR	lower 95%CI	upper 95% CI
Dolan et al. 2006 (CV death, including stroke and cardiac disorders)	11291	1.59	1.47	1.71
Hansen et al. 2006 (MACE, including stroke and coronary events)	1829	1.62	1.14	2.28
Kikuya et al. 2007 AASI <0.39 (CV mortality)	1542	1.41	1.01	1.96
Kikuya et al. 2007 AASI 0.45-0.51 (CV mortality)		0.65	0.46	0.91
Hansen et al. 2008 (MACE, including stroke and coronary events)	1678	1.68	1.11	2.59
Bastos et al. 2010 (CV events)	1200	1.27	1.01	1.59
Bastos et al. 2010 (stroke)		1.36	1.02	1.89
Muxfeldt et al. 2010 (MACE, including stroke and coronary events)	547	1.46	1.12	1.92
Dolan et al. 2010 (MACE, including stroke and coronary diseases)	1905	1.24	1.10	1.41
Bastos et al. 2015 (CV events)	217	8.34	1.79	39.57
Cieslik-Guerra et al. 2019 (MACE, including CV death and coronary events)	90	7.89	1.83	33.99
Sobiczewski et al. 2019 (ACS)	891	OR:4	1.30	12
Hoshide et al. 2023 (AASI >0.57 +DT SBP>179.4 mmHg) (CV events)	6294	1.89	1.13	3.15
All studies	27484			

Studies evaluating the association between AASI and MACE across 19 studies (total N=27,484, 2006–2023). The table reports hazard ratios (HR) or odds ratios (OR) with 95% confidence intervals (CI) for MACE, including CV deaths, stroke, coronary events, and acute ACS, as specified. Data are sourced from individual studies listed, with sample sizes ranging from 80 to 11,291. Relevant data are presented in Tables 2-3, 2-4, and Figure 2-2.

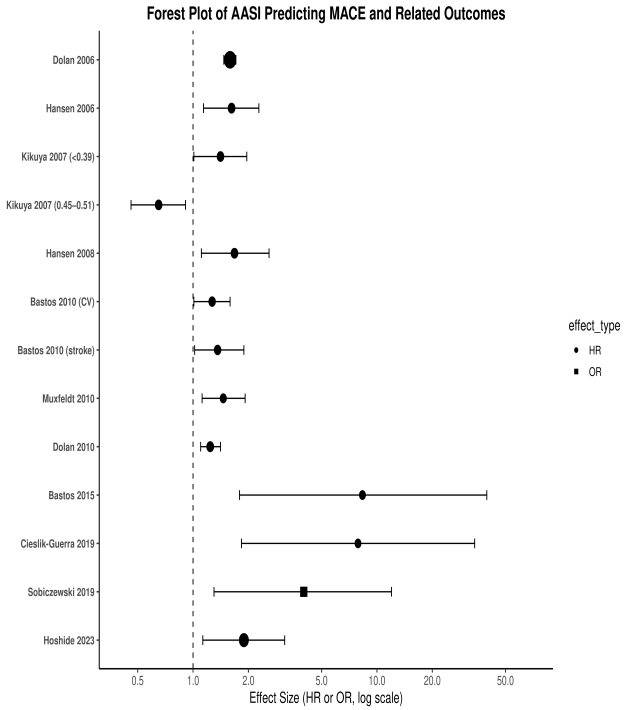


Figure 2-2; Forest plot of studies demonstrating that AASI is an independent predictor for MACE

Table 2-6: Studies which demonstrated that AASI is an independent predictor for stroke

Studies	Number of patients	HR	lower 95%CI	upper 95% CI
Dolan et al. 2006 (stroke)	11291	1.23	1.04	1.45
Hansen et al. 2006 (stroke)	1829	1.62	1.14	2.28
Kikuya et al. 2007 AASI <0.39 (stroke)	1542	1.41	1.01	1.96
Kikuya et al. 2007 AASI 0.45-0.51 (stroke)		0.52	0.30	0.89
Hansen et al. 2008 (stroke)	1678	1.68	1.11	2.59
Bastos et al. 2010 (stroke)	1200	1.36	1.02	1.89
Muxfeldt et al. 2010 (MACE, including stroke and coronary events)	547	1.46	1.12	1.92
Dolan et al. 2010 (MACE, including stroke and coronary disease)	1905	1.24	1.1	1.41
Hoshide et al. 2023 (AASI >0.57 +DT SBP>179.4 mmHg) (stroke)	6294	1.89	1.13	3.15

This table summarizes studies evaluating the prognostic role of AASI in predicting stroke events. HR and 95% CI are reported. Some studies included stroke as part of MACE outcome.

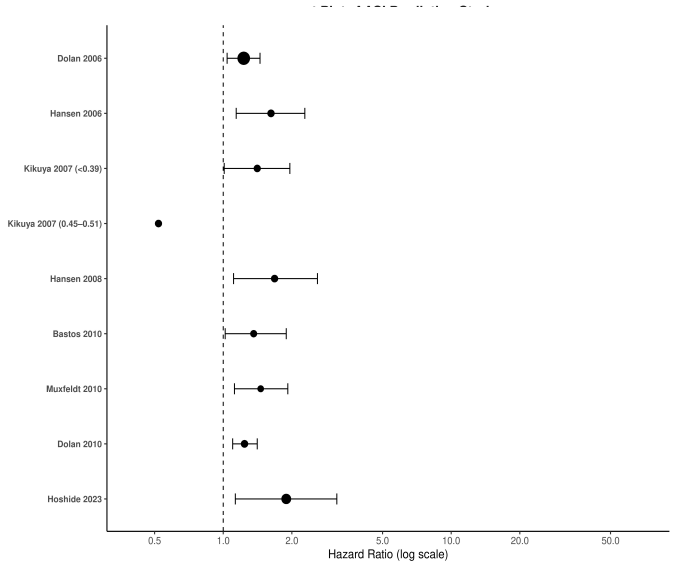


Figure 2-3: Forest plot of studies demonstrating that AASI is independent predictor for Stroke

This forest plot displays HRs and corresponding 95% CIs from studies evaluating the association between AASI and the risk of stroke or MACE. Each point represents the HR reported in an individual study, with horizontal lines indicating the 95% CI. The vertical dashed line marks the line of no effect (HR = 1.0). Some studies focused on stroke alone, while others included stroke as part of a composite MACE outcome. The results demonstrate a consistent association between higher AASI and increased cardiovascular risk

Table 2-7: Studies which demonstrated that AASI is an independent predictor for coronary events

Studies	Number of patients	HR	Lower 95%CI	Upper 95% CI
Muxfeldt et al. 2010 (MACE, including coronary disease)	547	1.46	1.12	1.92
Dolan et al. 2010 (MACE, including stroke and coronary disease)	1905	1.24	1.10	1.41
Cieslik-Guerra et al. 2019 (MACE, including coronary disease)	90	7.90	1.84	33.99
Sobiczewski et al. 2019 (ACS)	891	OR: 4	1.30	12

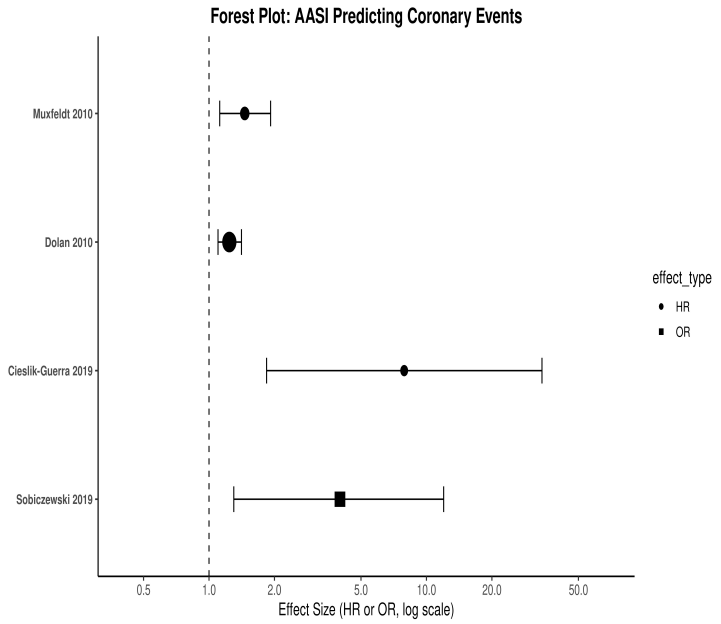


Figure 2-4: Forest plot of studies demonstrating AASI as independent predictor for coronary events with effect sizes and 95% CIs (circle: HR, square: OR). Logarithmic scale was used for visual presentation due to wide CIs.

Table 2-8: Studies which demonstrated that AASI is an independent predictor for all-cause mortality

Studies	Number of patients	HR	Lower 95%CI	Upper 95% CI
Palmas et al. 2009 AASI second tertile (all-cause mortality)	1178	0.95	0.69	1.30
Palmas et tal.2009 AASI third tertile (all-cause mortality)	1178	1.36	1.01	1.83
Gavish et al. 2009 (all-cause mortality in FU 1.5 year)	3433	2.21	1.36	3.59
Gavish et al. 2009 (all-cause mortality in FU 1.5-7 years)	3433	1.28	1.06	1.54
Gavis et al. 2009 (all-cause mortality in FU >7 years)	3433	0.98	0.68	1.20
Bastos et al. 2015 (CV events and all-cause mortality)	217	8.34	1.79	39.57
Viazzi et al. 2020 (all-cause mortality)	80	37.65	2.26	627.64

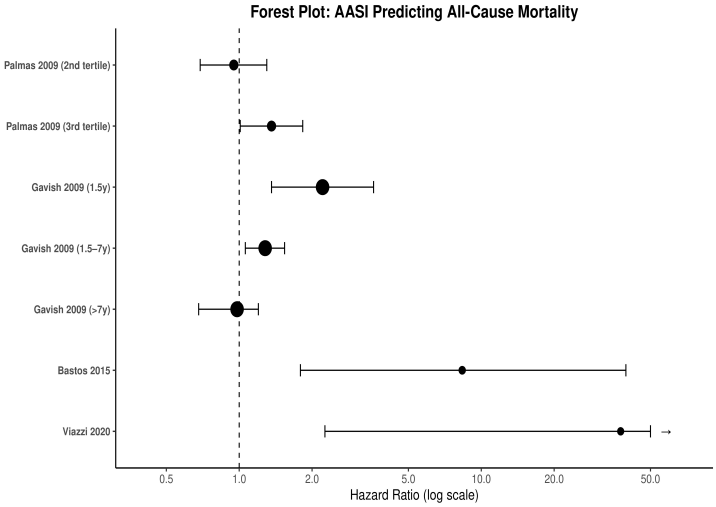


Figure 2-5: Forest plot of studies which demonstrated that AASI could predict all-cause mortality.

HR with 95% CI are shown on a logarithmic scale. Each circle represents an HR estimate from a study or subgroup. The rightward arrow indicates a truncated CI exceeding the scale. The plot highlights consistent associations between higher AASI and increased all-cause mortality risk across varying follow-up durations and populations.

2.4 Discussion

In 2012, in the first meta-analysis, ASDPRI, an equivalence index of AASI, was reported as an independent predictor for CV events, stroke and all-cause mortality with a relative risk of 1.51 (95% CI: 1.18-1.39, p = 0.001), 2.01 (95% CI:1.60-2.52, p < 0.001), and 1.25 (95% CI: 1.10-1.41, p = 0.001) respectively. A one-standard-deviation increase in ASDPRI was associated with 15% to 30% in total CV events and stroke (Aznaouridis et al. 2012).

In the same year, another systematic review with meta-analysis reported that AASI was an independent predictor for stroke and associated with indices of arterial function, though it was not a significant predictor for coronary events. The pooled HR for stroke, with a one-SD increase in AASI, was 1.26 (95% CI: 1.08-1.45) (Kollias et al. 2012). Both reviews demonstrated that AASI is a significant predictor for total CV and cerebrovascular events.

2.4.1 Ambulatory arterial stiffness index and major adverse cardiovascular events

This systematic review included studies from the two meta-analyses prior to 2012. From 2012 onward, three additional studies reported that AASI is an independent predictor for MACE. In 2015, Bastos et al. reported (n = 217) that an AASI above 0.41 (median) was associated with a worse prognosis compared to those below it. In that study, two models were constructed for AASI according to covariates, and both models demonstrated that AASI was an independent predictor for MACE, including total CV events, coronary events, and stroke and all-cause mortality.

As outlined in the results, a total of 11 studies (n = 27484) supported AASI as a predictor for MACE (Table 2-5, Figure 2-2). Studies from 2006 to 2012 consistently demonstrated that AASI was a significant predictor for CV deaths, stroke, and coronary events (Dolan et al. 2006; Hansen et al. 2006, 2008; Kikuya et al. 2007; Gavish et al. 2009; Palmas et al. 2009; Bastos et al. 2010 Muxfeldt et al. 2010). AASI cut-off values ranged from 0.30 to 0.71. The majority of studies investigating AASI as a predictor for MACE adjusted for established CV risk factors such as hypertension, diabetes, dyslipidaemia and history of MI. These studies demonstrated that AASI is a robust predictor for MACE after controlling for these confounders (Table 2-5, Figure 2-2).

2.4.2 Ambulatory arterial stiffness index and coronary heart disease

Before 2012, AASI was not widely considered a significant predictor for coronary heart disease (Kollias et al. 2012). However, studies from 2010 onwards showed that AASI could be a predictor of coronary (ischaemic) heart disease. Muxfeldt et al. (2010) demonstrated that AASI is a good predictor for coronary events (HR: 1.46, 95%CI: 1.12-1.92). Similarly, Dolan et al. (2010), in a study of 1905 patients, revealed that AASI is a predictor for both stroke and coronary artery disease in multivariate analysis (HR: 1.24, 95% CI: 1.1-1.41, $p < 0.005$).

In 2019, Cieslik-Guerra et al. (2019) conducted a study on 90 patients with MI showing that AASI values above 0.42 (median) were associated with a worse prognosis compared to those below 0.42 in Cox regression. However, that study did not describe the adjustment for CV risk factors. Sobiczewski et al. (2019) conducted a study (n = 891) on patients referred for coronary angiography with a focus on coronary events during follow-up. It was reported that AASI was a significant predictor for ACS (OR: 4.0, 95% CI: 1.83-33.99, $p = 0.006$) after adjustments for sex, age, diabetes mellitus, history of MI, antihypertensive treatments and lipid-lowering treatments. These two studies added to evidence of AASI in predicting coronary events (Table 2-7, Figure 2-4).

2.4.3 Ambulatory arterial stiffness index and all-cause mortality

Regarding all-cause mortality, four studies demonstrated that AASI was a predictor for all-cause mortality (Table 2-8, Figure 2-5). Viazzi et al. (2020) reported that AASI above 0.54 in haemodialysis patients was associated with increased mortality risk. A one-unit-increase in 44-hour AASI was associated with all-cause mortality (HR: 37.65, 95% CI: 2.25-627.63, $p = 0.011$) after adjusting for age, sex and duration of haemodialysis years (Viazzi et al. 2020). In 2009, Gavish et al. (n = 3433) reported that the BPVR, equivalent to AASI, could predict short-term all-cause mortality (HR: 1.21, 95% CI: 1.05-1.40, $p = 0.007$), after adjusting for demographics, 24-Hr mean blood pressure, 24-Hr PP and dipping (Gavish et al. 2009). Both studies had limitations, as they did not adjust for established CV risk factors, which are potential confounders. In 2009, Palmas et al. reported that AASI cannot predict CV death, but it predicted all-cause mortality when AASI was grouped into tertiles and adjusted for multiple risk factors

(Palmas et al. 2009). In 2015, Bastos et al. reported that AASI with cut-off value of 0.41 can predict both MACE and all-cause mortality.

2.5 Limitations

There are limitations which need to be acknowledged. This systematic review included a wide range of populations with different mean ages and co-morbidities such as resistant hypertension, chronic kidney disease, previous MI, and diabetes mellitus. Four studies were less than five hundred patients, and five studies were abstract only without full data available despite requests for original data sets as these are conference abstracts. Several studies included in this systematic review were retrospective studies and there will be a risk of reporting bias.

2.5.1 Ambulatory arterial stiffness index and stroke

From 2006 onwards, eight studies (n = 26286) supported AASI as a robust predictor for stroke (Table 2-6, Figure 2-3). In 2012, Kollias et al. reported that AASI is a predictor for stroke (Kollias et al. 2012). Despite two meta-analyses supporting the strength of AASI as a predictor for stroke, Lee and colleagues (2012) argued that AASI was significantly influenced by BPV and not a predictor for stroke (Lee et al. 2012). The study was conducted in 855 hypertensive patients and there was a total of 185 strokes (OR: 0.86, 95% CI: 0.29-2.5, p = 0.77).

Although the majority of studies supported AASI as a prognostic indicator, two studies concluded that AASI was not a predictor for CV events (Laugesen et al. 2012; Boos et al. 2021). Since 2012, only a limited number of studies have focused on AASI with a single endpoint, such as coronary heart disease. Most studies have a composite outcome of CV deaths, coronary events, stroke, or all-cause mortality. Moreover, as a value calculated from 24-Hr ABPM, several studies were performed to compare AASI and other blood pressure indices. AASI has been recognised as an indirect marker of arterial stiffness and is closely related to other indices reflecting arterial function.

Furthermore, AASI has been influenced by BPV and nocturnal blood pressure dipping (Schillaci et al. 2007; Lee et al. 2011). Given the correlation between increased BPV and MACE, further research is required to assess the role of AASI as a prognostic indicator (Stevens et al. 2016; Cuspidi et al. 2017; Heshmatollah et al. 2022).

2.6 Conclusion

This systematic review highlights that AASI is an index which has a prognostic value in predicting MACE. Its role in all-cause mortality is questionable. AASI has been a significant predictor for cerebrovascular events (stroke) and new studies have added its potential value in predicting coronary events (ischaemic heart disease). Future research with a focus on coronary events will contribute to our current understanding of AASI.

Chapter 3 Methods

This Chapter outlines the methods used to conduct this research project. Methods selected were to be in line with aims and objectives described in Chapter One. This research project focused on BPV indices, AASI and their association with MACE. In this Chapter, methods for clinical research project will be described including methods for data collection, literature review, technical tools and ethical considerations. Methods for systematic literature review were described in detail in Chapter 2.

This research project will be divided into two parts

(1) Systematic literature review for the relationship between AASI and MACE (Chapter 2)
(2) Clinical research project examining the association between BPV, AASI and MACE (Chapter 4)

3.1 Method for clinical research project

This clinical research project was an ambidirectional observational cohort study of 829 NHS patients who underwent ABPM for the diagnosis or management of hypertension since 2015. ABPM data were recorded in both Poole Hospital and Bournemouth Hospital databases.

The aim of the clinical research project was to examine the relationship between BPV indices, AASI and MACE The objective of this study is to evaluate the predictive ability of AASI and BPV indices for the occurrence of MACE. The definition of MACE was non-fatal stroke, TIA, ACS including MI, and CV death. MACE is the primary outcome of the research project and all-cause mortality is the secondary outcome.

3.2 Justification of methods for the study

ABPM is regarded as a gold standard in diagnosing and managing hypertension. ABPM can provide reliable and reproducible blood pressure data including circadian rhythm patterns. Furthermore, it offers indices such as AASI and MSI.

AASI and BPV have been demonstrated to be useful predictors for MACE in studies. These data can be readily accessed from hospital database. Meanwhile, electronic patient record (EPR) system is an effective clinical tool for patient care, and clinically important data can easily be retrieved from electronic system.

This study employed an ambidirectional observational cohort design, incorporating both retrospective and prospective components (Figure 3-1). Baseline clinical characteristics and ABPM data were collected retrospectively, while outcomes**,** including MACE and all-cause mortality, were tracked prospectively through electronic health records and death registries. This design enabled the evaluation of long-term CV outcomes in patients with prior ABPM, thereby enhancing the temporal relevance of AASI and BPV indices as predictive markers.

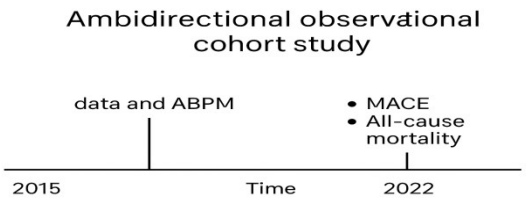


Figure 3-1: Timeline illustrating the ambidirectional observational cohort study design. Baseline clinical data and ABPM data were collected retrospectively from 2015, with prospective follow-up until 2022 to assess outcomes, including MACE and all-cause mortality.

Microsoft Excel software is a user-friendly software and available at university and hospital with data protection functions. SPSS is an excellent tool to conduct advanced statistical analysis in a time-efficient manner.

During data collection, we included demographic variables (age, ethnicity), anthropometric measures (height, weight, and BMI), established CV risk factors, relevant blood test results, and medication history. These variables were selected because they are known to influence the primary outcome, MACE. For instance, type II diabetes is a well-established CV risk factor. It was essential to include it in the analysis as a potential confounding variable. A confounder is a factor that is associated with both the exposure (e.g., AASI or BPV indices) and the outcome (e.g., MACE), which can distort the observed relationship between them. By accounting for these confounders, the analysis aims to isolate the true effect of AASI and BPV on CV outcomes. We included blood results reflecting glycaemic status, renal function, and chronic inflammation, as these might have a potential impact on the outcome. All blood pressure data from reported ABPM were collected since they will be examined appropriately.

Primary outcomes were (i) combined overall MACE, (ii) MI or ACS, (iii) non-fatal stroke or TIA, (iv) CV death, and (v) all-cause death for the purpose of subgroup analysis.

3.3 Data collection

Between 2015 and 2022, patients who underwent ambulatory blood pressure monitoring (ABPM) at Royal Bournemouth Hospital and Poole General Hospital were considered eligible for inclusion. ABPM was performed as part of routine clinical care, primarily to diagnose or manage hypertension, assess treatment response.

The sampling frame consisted of all adult patients with available ABPM reports recorded in the hospital databases during the study period. Patients were initially identified from these records and their NHS numbers were submitted to the hospital's clinical governance team to check their status in the NHS national data opt-out programme. Those who had formally opted out of research participation were excluded before screening, in accordance with national data protection and ethical guidelines.

Following this step, inclusion and exclusion criteria were applied. Duplicate ABPM entries were removed, and patients with incomplete or ineligible records were excluded. For each eligible patient, baseline data (e.g., demographic characteristics, clinical history, medications, and blood results) were collected retrospectively. Prospective outcome follow-up was conducted using electronic health records and death registries to identify events such as MACE and all-cause mortality.

Although the study cohort was limited to patients who were referred for ABPM—for the diagnosis or management of hypertension—this population reflects real-world clinical practice. These individuals may have a higher CV risk profile, making the findings directly applicable to routine hypertension management and CV risk assessment in similar healthcare settings

3.4 Inclusion criteria

1	People who underwent ABPM
2	Age >18 years-old

3.5 Exclusion criteria

1	Patients below 18 years old
2	Patients investigated for syncope or pre-syncopal symptoms
3	Pregnant patients
4	Patients with severe aortic stenosis or aortic coarctation
5	Patients with chronic kidney disease stage 4 or 5
6	Patients with recent hospital admission prior to ABPM
7	Patients diagnosed with cancer
8	Patients with valid ABPM measurements below 15

Microsoft Excel software was used for data collection. The data collection proforma was designed to include age, ethnicity, blood pressure indices, co-morbidities, medications, blood results, and outcomes. The ABPM database was retrieved from Philips software and the databases in both hospitals. The blood pressure records were reported in PDF format, and Adobe Pro software, licensed for hospital use, was used to transfer the data to the data collection form.

Co-morbidities recorded included previous MI, stroke, TIA, and diagnoses such as hypertension, diabetes mellitus, peripheral vascular disease, aortic disease, and gout. Events that occurred prior to the ABPM date (defined as time zero) were recorded as established CV risk factors. Since this was an ambidirectional observational study using patient records, the date of ABPM was used as the point of cohort entry or 'recruitment'.

Follow-up for outcomes such as MACE and all-cause mortality was conducted using hospital electronic health records and linked death registry data. The follow-up period varied among patients, depending on the time of their ABPM and their survival status. For example, a patient who underwent ABPM in 2015 and remained alive until the study end date in December 2022 would have a follow-up duration of approximately seven years. In contrast, a patient who had ABPM in 2021 and died in early 2022 would have a follow-up of less than one year. The duration of follow-up was therefore variable and dependent on both the ABPM date and patient outcome status. The range and distribution of follow-up periods are reported in Section 4.1.1.

For age, ethnicity, co-morbidities, blood results, and outcomes, the hospital electronic patient record (EPR) was searched using each patient's hospital or NHS number. Each patient file was thoroughly searched in EPR and (uploaded) scanned records. Each patient's age was taken as the age when they had ABPM. Blood results, medications,

weight, and BMI data measured closest to the ABPM data were retrieved. For those with single blood results or single body height data, we accepted available data.

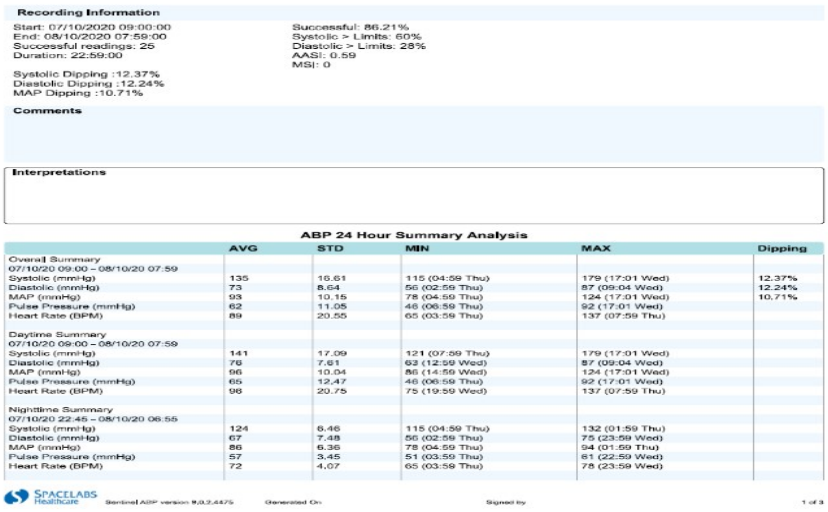
As for blood tests and biomarkers, we included blood results reflecting underlying glycaemic status, renal function, and vascular inflammation. They included lipid profile, glucose, haemoglobin A1C, urea, creatinine, estimated glomerular filtration rate (eGFR), full blood count with a focus on haemoglobin, neutrophil, lymphocyte, and platelets, and left ventricular ejection fraction as left ventricular function. As a strong CV risk factor, smoking status at the time of ABPM was also collected.

To ascertain outcomes, medical records including death certificates were reviewed. All outcomes must be documented by a clinician with the appropriate level of experience. For example, stroke diagnosis must be documented by a stroke physician or an experienced geriatrician looking after stroke patients.

The ABPM device used in both hospitals is Spacelab 90207, Spacelab Healthcare, Hertford, UK (Figure 3-2). An automated oscillometric cuff was put on the non-dominant arm, and BP measurement frequencies were set every fifteen to thirty minutes throughout a 24-Hr period. The night-time period was defined as the hours of 22:01 to 06:00 hour, and the day-time period as 06:01 to 22:00.

Figure 3-2 Spacelab 90207 ABPM device

For the blood pressure indices, ABPM automatic software (Sentinel ABP 9.0.2.4475) was used. The software reported average, day and night systolic and diastolic pressure indices. The software also reported mean and SD of average, day and night SBP, PP, heart rate, AASI, MSI and nocturnal dipping percentage (Figure 3-3).



Recording Information

Start: 07/10/2020 09:00:00
End: 08/10/2020 07:59:00
Successful readings: 26
Duration: 22:59:00

Systolic Dipping :12.37%
Diastolic Dipping :12.24%
MAP Dipping :10.71%

Successful: 86.21%
Systolic > Limits: 60%
Diastolic > Limits: 28%
AASI: 0.59
MSI: 0

Comments

Interpretations

ABP 24 Hour Summary Analysis

	AVG	STD	MIN	MAX	Dipping
Overall Summary					
07/10/20 09:00 – 08/10/20 07:59					
Systolic (mmHg)	135	16.61	115 (04:59 Thu)	179 (17:01 Wed)	12.37%
Diastolic (mmHg)	73	8.64	56 (02:59 Thu)	87 (09:04 Wed)	12.24%
MAP (mmHg)	93	10.15	78 (04:59 Thu)	124 (17:01 Wed)	10.71%
Pulse Pressure (mmHg)	62	11.05	46 (06:59 Thu)	92 (17:01 Wed)	
Heart Rate (BPM)	89	20.55	65 (03:59 Thu)	137 (07:59 Thu)	
Daytime Summary					
07/10/20 09:00 – 08/10/20 07:59					
Systolic (mmHg)	141	17.09	121 (07:59 Thu)	179 (17:01 Wed)	
Diastolic (mmHg)	78	7.61	63 (12:59 Wed)	87 (09:04 Wed)	
MAP (mmHg)	96	10.04	86 (14:59 Wed)	124 (17:01 Wed)	
Pulse Pressure (mmHg)	65	12.47	46 (06:59 Thu)	92 (17:01 Wed)	
Heart Rate (BPM)	98	20.75	75 (19:59 Wed)	137 (07:59 Thu)	
Nighttime Summary					
07/10/20 22:45 – 08/10/20 06:55					
Systolic (mmHg)	124	6.46	115 (04:59 Thu)	132 (01:59 Thu)	
Diastolic (mmHg)	67	7.48	56 (02:59 Thu)	75 (23:59 Wed)	
MAP (mmHg)	86	6.36	78 (04:59 Thu)	94 (01:59 Thu)	
Pulse Pressure (mmHg)	57	3.45	51 (03:59 Thu)	61 (22:59 Wed)	
Heart Rate (BPM)	72	4.07	65 (03:59 Thu)	78 (23:59 Wed)	

SPACELABS Healthcare Sentinel ABP version 9.0.2.4475 Generated On: Signed by: 1 of 3

Figure 3-3: Sample report of 24-Hr ABPM

3.6 Data analysis

The data were manually checked three times by AH for a total of 829 patients to ensure quality assurance and independently reviewed by another clinician. Any uncertainties were cross-checked with supervisors and amended. Patients with unclear or incomplete data were removed. Once the data review was finalised, the data was anonymised and analysed.

IBM SPSS Statistics for Windows [SPSS 28.0.1] (SPSS, Chiago, IL, USA) was used for statistical analysis. Data from Microsoft Excel spread sheet were exported to SPSS software for further processing. Data inspection and normality tests were conducted using parametric and non-parametric tests. Although some data do not follow a normal distribution, the sample size (n = 829) is large enough for the sampling distribution of the mean to be approximately normal, in accordance with the Central Limit Theorem. Therefore, parametric methods that rely on the normality of the sampling distribution may still be appropriate. Continuous variables were described as mean (±SD). For the group comparison, independent T-test and Mann-Whitney U tests were performed for continuous data. For categorical data Pearson chi-square test or Fisher's exact tests were used.

To assess collinearity, Pearson's correlation test was used. Variables with a correlation coefficient of 0.7 or higher were removed due to the risk of multicollinearity. AASI, standard deviation of 24-Hr systolic blood pressure (SD 24-Hr SBP), SD of 24-Hr diastolic blood pressure (SD 24-Hr DBP), MAP, PP, dipping percentage of MAP, coefficient of variation (CoV), day-time SBP (DTSBP), day-time DBP (DTDBP), night-time SBP (NTSBP) and night-time DBP (NDTBP) were selected as indices for the

research project. Heteroscedasticity was not assessed given its binary outcome data. The primary outcome variable was combined into one single variable as a composite of MACE, which included CV death, ACS (including acute MI), non-fatal stroke and TIA.

Statistical models were constructed to assess correlations, confounding effects, and the actual effect of predictors on the outcome. Since MACE was a dichotomous outcome, binary logistic regression models were used with MACE as the dependent variable. Selected blood pressure indices (AASI, SD-24Hr SBP, SD 24-Hr DBP, MAP MAP dipping %, PP, DTSBP, DT DBP, NT SBP and NT DBP) were tested. To control for confounders, well established CV risk factors (co-morbidities) were added to the model and examined. In the multivariate logistic regression analysis, adjustments were made for age, stroke or TIA and heart failure. A total of 13 statistical models were constructed for 13 indices to examine their individual effects while controlling the confounders. Cox and Snell R Square, -2 Log likelihood and Nagelkerke R square and the Hosmer and Lemeshow test across models were examined. The results were presented as the OR and 95% CI. The first occurrence of MACE after ABPM (time zero) was used as the dependent variable.

To obtain more robust results, Cox proportional hazards models were developed using a time-to-event framework, with the first occurrence of MACE after ABPM (time zero) as the dependent variable. Each BP-related predictor of interest—including SD 24-Hr SBP and DBP, AASI, 24-Hr SBP and DBP, PP, and MAP dipping—was analysed in a separate multivariate Cox model. Each model was adjusted for the same set of potential confounders: age, sex, IHD, stroke or TIA, HF, HTN, and smoking. This approach was adopted to isolate the independent effect of each BP variable while avoiding multicollinearity among related haemodynamic parameters. Results were presented as HR with 95% CI.

Based on the literature review on AASI, the median value of AASI (0.47) was used as a cut-off point. Kaplan-Meier survival analyses were performed to investigate higher versus lower AASI (based on the median value of 0.47) on MACE. Further exploratory analyses were conducted by constructing a linear regression model by keeping SD 24-Hr SBP as the dependent variable, whereas medications and blood results as the predictor variables. Subgroup analyses were also performed, which included: (1) ACS (including MI) as the dependent variable, (2) MACE as the dependent variable in patients aged above 69 years, which is the approximate mean age in MACE group (3) MACE as the dependent variable in normotensive patients (SBP<140 mmHg and DBP<90 mmHg), with AASI and SD 24-Hr SBP as predictor variables. As part of the

exploratory analysis, an ROC analysis was conducted using Youden's index to obtain the optimal cut-off for SD 24-Hr SBP. Based on this cut-off, SD 24-Hr SBP was dichotomised and examined for its association with MACE and ACS. A Kaplan-Meier analysis was conducted using the dichotomised SD -24-Hr SBP to assess the association with MACE.

3.7 Ethical considerations

This database included ABPM data collected from patients who had an NHS indicated investigations from the period of January 2015 to December 2022. As discussed, all patients were screened and those who opted out from NHS research were excluded from the study. This study and its experimental protocol were approved by the Poole Hospital (University Hospitals Dorset) Clinical Research and Innovation Department and the West of Scotland Research Ethics Committee (REC reference: 20/WS/0097). The study was conducted in accordance with the recommendations for physicians involved in research on human subject adopted by the 18th world medical assembly, Helsinki 1964, and later revision and in compliance with the UK Policy framework for Health and Social Care Research (2017).

Chapter 4 Results

4.1 Baseline population characteristics (at the point of inclusion)

A total of 829 patients were included, and 405 (49%) patients were females. The mean age of all patients was 58.79 (±15.21) years. 776 (97%) of patients were Caucasian. 128 (15%) patients had diagnosis of diabetes prior to enrolment in the study, 64 (7.7%) had stroke or TIA, 38 (4.6%) had heart failure, 55 (6.6%) had peripheral artery disease or aortic disease, 35 (4.2%) had gout and 529 (63%) had been diagnosed with hypertension (Table 4-1). In addition to Entresto (Sacubitril/Valsartan) and Tolvaptan, seven types of blood pressure medications were listed, and two anti-lipid therapies (statin or ezetimibe) were listed (Table 4-1). Mean value with range and SD of blood pressure indices and blood results were described as descriptive statistics (Table 4-2).

Table 4-1: Baseline characteristics of the study population- categorical variables

Variable	number	Percentage
Total	829	100%
Male	424	51%
Female	405	49%
Caucasian	776	97%
Diabetes mellitus	128	15%
Stroke/TIA	64	7.7%
Heart failure	38	4.6%
PVD/aortic disease	55	6.6%
Gout	35	4.2%
Hypertension	529	63.8%
No-smoker	428	52.6%
Current smoker	148	18.2%
Ex-smoker	237	28.6%
Entresto/tolvaptan	13	1.9%
ACE-I/ARB	444	53.6%
Calcium channel blockers	296	35.7%
Beta blockers	268	32.3%
Diuretics	150	18.1%
Alpha blockers	98	11.8%
Statin/ ezetimibe	313	37.8%
Aldosterone antagonist	37	4.5%
Hydralazine	3	0.4%

Values are presented as number (percentage).TIA, transient ischaemic attack; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; PVD, peripheral vascular disease

Table 4-2: Baseline characteristics of the study population- continuous variables

	Mean	Std. Deviation
Age (years)	58.79	15.31
AASI	0.45	0.16
MSI	10.30	12.56
Systolic dip %	9.83	8.77
diastolic dip %	13.01	9.49
map dip %	10.93	8.80
24-Hr SBP, mmHg	132.85	15.42
24-Hr DBP, mmHg	76.54	10.11
MAP, mmHg	95.89	10.26
PP, mmHg	56.36	12.94
DTSBP, mmHg	136.94	15.67
DTDBP, mmHg	79.69	10.67
DTMAP, mmHg	99.17	10.65
DTPP, mmHg	57.24	13.16
NTSBP, mmHg	123.13	18.65
NTDBP, mmHg	69.15	10.38
NTMAP, mmHg	88.17	11.56
NTPP, mmHg	54.28	13.36
SD 24-Hr SBP	14.89	4.42
CoV, mmHg	1.29	0.22
SD 24-Hr DBP, mmHg	10.36	2.64
SD MAP, mmHg	11.55	2.99
SD PP, mmHg	9.73	2.93
SD DTSBP, mmHg	12.87	4.54
SD DTDBP, mmHg	8.70	2.57
SD DTMAP, mmHg	10.26	6.85
SD DTPP, mmHg	9.67	3.20
SD NTSBP, mmHg	11.61	5.15
SD NTDBP, mmHg	8.32	3.44
SD NTMAP, mmHg	9.03	3.68
SD NTPP, mmHg	7.73	3.53
Lymphocyte, x 10*9/L	1.90	0.84
Neutrophils, x 10*9/L	4.61	1.97
NLR	2.84	2.00
Haemoglobin, g/L	139.40	14.89
Platelets, x 10*9/L	257.17	71.01
White Cell Counts, x 10*9/L	7.298	2.13
Creatinine, µmol/L	88.05	25.89
eGFR, mL/min/1.73m*2	70.30	16.50
total cholesterol, mmol/L	4.78	1.19
High-density lipoprotein, mmol/L	1.48	0.50
Low-density lipoprotein mmol/L	3.01	1.15
Triglycerides, mmol/L	1.65	1.01
Chol/HDL ratio	3.48	1.26
Glucose, mmol/L	5.91	2.23
HbA1C, mmol/mol	42.62	13.26

Baseline characteristics and blood pressure indices for 829 atients in this study (2015–2022).Values are-mean (±SD) for patients who underwent ABPM for hypertension diagnosis or monitoring. The table summarizes continuous variables, including demographic data (age), BP indices, and laboratory markers relevant to CV risk. Age is included as a continuous demographic variable alongside BP indices and laboratory values..SBP, systolic blood pressure; DBP, diastolic blood pressure; AASI, ambulatory arterial stiffness index; MSI, morning surge index; systolic dip %, systolic dipping percentage; diastolic dip %, diastolic dipping percentage; 24-Hr SBP, 24-Hr systolic blood pressure; 24-Hr DBP, 24-Hr diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; DTSBP, day-time systolic blood pressure; DTDBP, day-time diastolic blood pressure; DTMAP, day-time mean arterial pressure; DTPP, day-time pulse pressure, NTSBP, night-time systolic blood pressure; NTDBP, night-time diastolic blood pressure; NTMAP, night-time mean arterial pressure; NTPP, night-time pulse pressure, SD 24-Hr SBP, standard deviation of 24-Hr systolic blood pressure; CoV, coefficient of variation; SD 24-Hr DBP, standard deviation of diastolic blood pressure, SDMAP, standard deviation of mean arterial pressure; SD DTSBP, standard deviation of day-time systolic blood pressure; SD DTDBP, standard deviation of day-time diastolic blood pressure, SD DTMAP, standard deviation of day-time mean arterial pressure, SD DTPP, standard deviation of day-time pulse pressure; SDNTSBP, standard deviation of night-time systolic blood pressure; SD NTDBP, standard deviation of night-time diastolic blood pressure; SDNTMAP, standard deviation of night-time mean arterial pressure; SD NTPP, standard deviation of night-time pulse pressure. Note: Blood pressure values are in mmHg. Laboratory values are in standard SI units.

4.1.1 First follow-up results after 4.35 (± 1.32) years

A total of 829 patients who had ABPM for the diagnosis or monitoring of hypertension were recruited from 2015 to 2022 and were followed up for a mean of 4.35 (±1.32) years (range: 0.5–7 years) in this single-centre ambidirectional observational study. Events including stroke and MI prior to recruitment (time zero) were collected as established CV risk factors in addition to other CV risk factors such as diabetes mellitus, smoking, peripheral arterial disease or aortic disease, heart failure, hypertension, and gout. During 4.35 (±1.32) yearlong follow-ups, there were a total of 38 (4.58%) MACE events occurred, and 791 (95.42%) patients did not develop MACE. Due to the relatively limited number of outcomes for pre-defined CV events, all MACE events were combined and examined as a single variable.

4.2 Group comparison results between patients with and without major adverse cardiovascular events

4.2.1 Established cardiovascular risk factors and medications

Group comparisons were performed between MACE and non-MACE groups using Fisher's exact test and Pearson chi-square test for categorical data and the independent T-test for continuous data.

The results of this study showed that diabetes mellitus ($p = 0.009$), stroke or TIA ($p < 0.001$), heart failure ($p = 0.01$), and hypertension ($p = 0.008$) were significant variables for MACE as shown in (Table 4-3) and (Figure 4-2). Previous diagnoses of peripheral artery disease or aortic disease showed marginal significance ($p = 0.05$).

Table 4-3: Group comparison between MACE and no-MACE

Variables	MACE	No-MACE	p-value
Female	16 (1.9%)	408 (49.2%)	0.31
Male	22 (2.7%)	383 (46.2%)	0.30
Age	69.58 (± 9.84)	58 (±15.34)	< 0.001
BMI	27.85 (±7.02)	28.4 (±5.71)	0.23
Caucasian	37 (4.5%)	739 (89.1%)	0.45
Diabetes	11 (1.3%)	117 (14.1%)	0.009
Hypertension	32 (3.9%)	497 (60.0%)	0.008
Ischaemic heart disease	12 (1.4%)	152 (18.3%)	0.27
Gout	0 (0.0%)	35 (4.2%)	0.12
Peripheral artery	5 (0.6%)	50 (6.0%)	0.050
Stroke/TIA	9 (1.1%)	55 (6.6%)	< 0.001
Heart failure	5 (0.6%)	33 (6.6%)	0.01
Non-smoker	21 (2.5%)	407 (49.1%)	0.727
Current smoker	8 (1.0%)	140 (16.9%)	0.727
Ex-smoker	9 (1.1%)	228 (27.5%)	0.727
Sacubitril/Valsartan	1 (0.1%)	12 (1.4%)	0.582
ACE-I/ARB	23 (2.8%)	421 (50.8%)	0.238
Calcium channel	12 (1.4%)	284 (34.3%)	0.361
Beta blocker	16 (1.9%)	252 (30.4%)	0.128
Diuretics	9 (1.1%)	141 (17.0%)	0.235
Aldosterone antagonists	5 (0.6%)	32 (3.9%)	0.008
Statin/Ezetimibe	25 (0.0%)	288 (34.7%)	<0.001

Hydralazine	0 (0.0%)	3 (0.4%)	0.844
Alpha blocker	5 (0.6%)	92 (11.1%)	0.287

Categorical variables are shown as n (%), where percentages represent the proportion of the total cohort (N=829). Continuous variables are shown as mean (±SD). P-values compare MACE (n=38) versus No-MACE (n=791) groups using chi-square or Fisher's exact tests for categorical variables and t-tests or Mann-Whitney U tests for continuous variables.BMI, body mass index; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Figure 4-1: Comparison of mean age between patients with and without MACE in the study.Bars represent the mean age (years) for the MACE group (n=38) and No-MACE group (n=791), with error bars indicating SD. The MACE group was significantly older than the No-MACE group (69.58 ± 9.84 vs. 58 ± 15.34 years; p < 0.001, independent t-test).

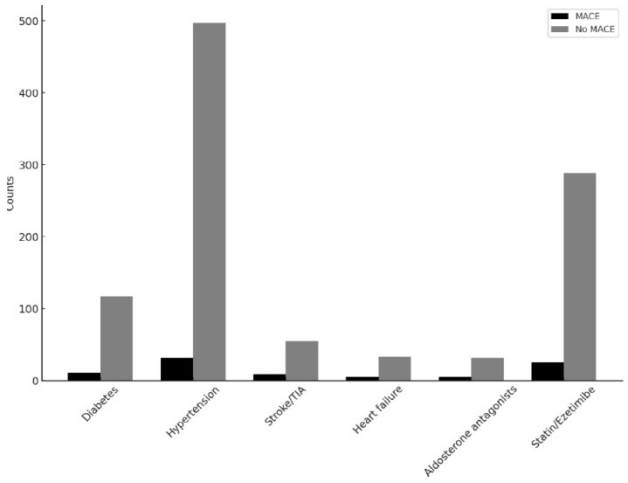


Figure 4-2: Prevalence of categorical variables in MACE and non-MACE Groups. This bar chart compares the prevalence of statistically significant categorical variables (e.g., comorbidities, smoking status) between patients MACE (MACE; black bars) and those without (non-MACE; grey bars).

4.2.2 Blood pressure and blood pressure variability indices between those with major adverse cardiovascular events and those without

The results of this study also showed that AASI, PP, 24-Hr SBP, SD 24-Hr SBP, systolic dipping percentage, diastolic dipping percentage, day-time SBP, day-time PP, night-time SBP, night-time DBP and night-time MAP were significantly higher in MACE group.

Mean values and the mean difference (MD) were used to describe the differences between MACE and no-MACE groups, as shown in (Table 4-4). AASI (MD = 0.09, 95% CI 0.03- 0.14, p < 0.001), SD 24-Hr SBP (MD = 2.25 mmHg, 95% CI 0.80-3.68, p =

0.002), CoV (MD = 0.13, 95%CI 0.04-0.21, p < 0.001), MAP (MD = 3.67, 95% CI 0.33-7.01, p = 0.016), day-time SBP (MD = 7.7 95% CI 2.61-12.78, p = 0.002), day-time PP (MD = 6.8, 95% CI 2.54-11.07, p < 0.001), NTSBP (MD = 12.11, 95% CI 6.0-18.13, p = 0.050), NTDBP (MD = 4.5, 95% CI 1.13-7.8, p = 0.009), NTMAP (MD = 6.6, 95% CI 2.86-10.35, p < 0.001) and NTPP (MD = 7.5, 95% CI 3.20-11.86, p < 0.001) were statistically significant in MACE group (Figure 4-2 & 4-3). A Forest Plot was created for data visualisation (Appendix Figure 3)

Table 4-4: Group comparison of BP indices between MACE and no-MACE

BP Indices	MACE	No-MACE Events	p-value
AASI	0.54 (±0.16)	0.45 (±0.16)	< 0.001
MSI	10.94 (±14.31)	10.27 (±12.48)	0.74
PP, mmHg	63.11 (±14.04)	56.03 (±12.80)	< 0.001
MAP, mmHg	99.39 (±10.90)	95.72 (±10.20)	0.016
24-Hr SBP, mmHg	141.39(±17.31)	132.44 (±15.21)	< 0.001
24-Hr DBP, mmHg	78.32 (±11.23)	76.46 (±10.05)	0.260
SD 24-Hr SBP, mmHg	17.03 (±6.03)	14.78 (±4.32)	0.002
SD 24-Hr DBP, mmHg	9.77 (±2.66)	10.39 (±2.65)	0.15
CoV	1.42 (±0.25)	1.29 (±0.22)	< 0.001
Systolic dipping in %	6.38 (±13.33)	9.99 (±8.47)	0.007
Diastolic dipping %	9.01 (±9.88)	13.20 (±9.43)	0.004
MAP dipping %	7.08 (±8.86)	11.11 (±10.44)	0.006
DTSBP, mmHg	144.29(±17.45)	136.59 (±15.50)	0.002
DTDBP, mmHg	80.66 (±11.07)	79.65 (±10.65)	0.585
DTMAP, mmHg	101.84(±10.85)	99.05 (±10.63)	0.064
DTPP, mmHg	63.74 (±14.75)	56.03 (±12.80)	< 0.001
NTSBP, mmHg	134.68(±23.46)	122.57 (±18.22)	0.050
NTDBP, mmHg	73.44 (±13.05)	68.94 (±10.20)	0.009
NTMAP, mmHg	94.47 (±14.37)	87.86 (±11.33)	<0.001
NTPP, mmHg	61.47 (±16.20)	53.94 (±13.13)	<0.001

Continuous variables are presented as mean (±SD). Group comparisons were conducted using independent t-tests for normally distributed variables and Mann–Whitney U tests for non-normally distributed variables. The table summarizes differences in BP indices, including AASI, MSI, MAP, PP, BP variability measures (SD, CoV), dipping percentages, and day-time/nighttime BP profiles, between patients with and without MACE. A p-value < 0.05 was considered statistically significant.

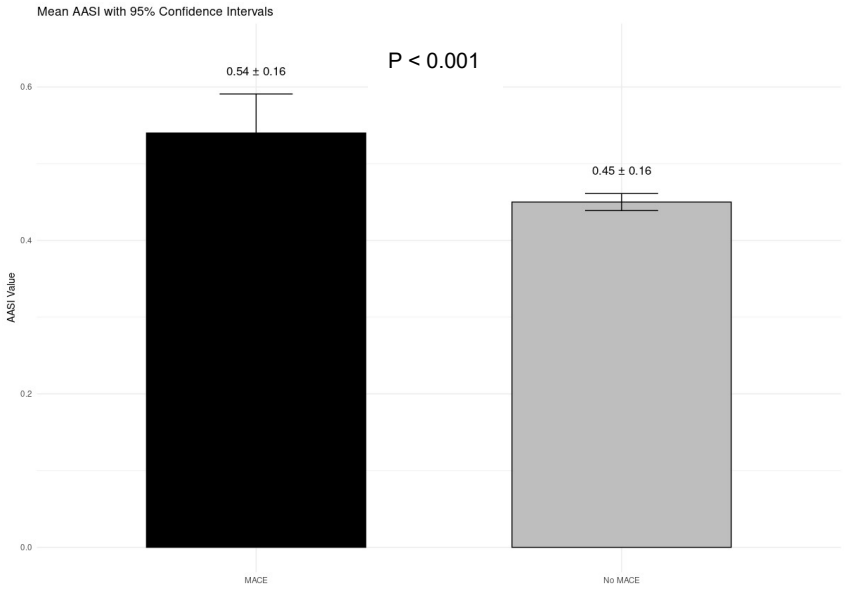


Figure 4-3: Comparison of mean AASI between patients with and without. Bars represent the mean AASI for the MACE group (n=38) and no-MACE group (n=791), with error bars indicating the SD. Patients who experienced MACE had a significantly higher mean AASI value (0.54 ± 0.16) compared to those without MACE (0.45 ± 0.16). Despite overlapping SD ranges (MACE: 0.38–0.70; No-MACE: 0.29–0.61), the difference is statistically significant ($p < 0.001$, independent t-test), indicating a reliable difference between groups.

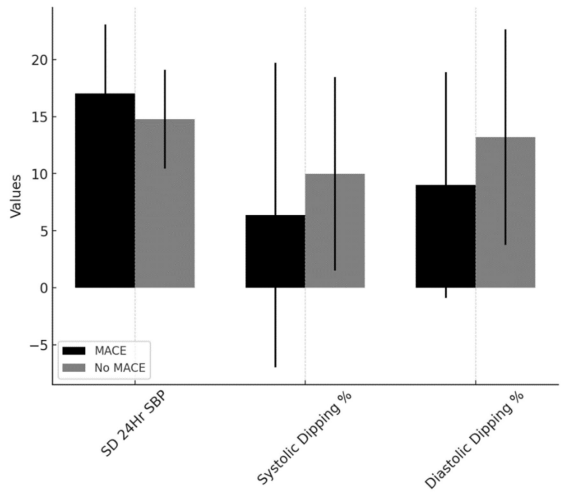


Figure 4-4: Comparison of SD 24-Hr SBP and nocturnal BP dipping percentages between patients with and without MACE. Mean SD 24-Hr SBP was higher in patients with MACE compared to those without MACE. Similarly, both systolic and diastolic dipping percentages were lower in the MACE group. Error bars represent standard deviations. Black bars indicate MACE; grey bars indicate no MACE.

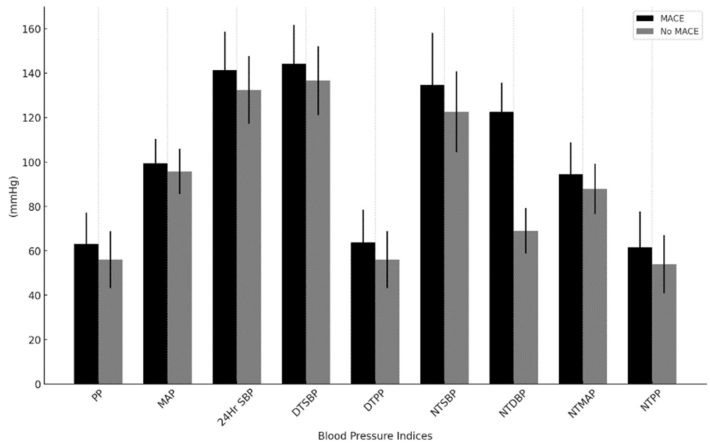


Figure 4-5: Comparison of blood pressure indices between patients with and without MACE. Patients with MACE exhibited higher values across multiple blood pressure indices, including PP, MAP, 24-Hr SBP, DTSBP, DTPP, NTSBP, and NTMAP. NTDBP and NTPP were also elevated in the MACE group. Error bars represent standard deviations. Black bars indicate MACE; grey bars indicate no MACE.

4.3 Univariate and multivariate logistic regression analyses

In univariate analysis, several indices, including AASI, were statistically significant predictors for MACE, as shown in (Table 4-5). However, the 95% CI of AASI was very wide, and it implies uncertainty despite its potential association with MACE (Table 4-5, Figure 4-6). AASI was standardised and re-examined which showed an OR of 1.74 (95% CI: 1.25-2.43, $p < 0.001$). Traditional BP indices (SBP and PP) and BPV indices (SD 24-Hr SBP and dipping %) were also statistically significant.

Table 4-5: Univariate analysis of BP indices (logistic regression)

Variables	OR	95% CI	P-value
AASI	26.96	3.77-195.58	< 0.001
zAASI	1.74	1.25-2.43	< 0.001
Systolic dipping %	0.95	0.92-0.99	0.014
Diastolic dipping %	0.95	0.92-0.98	0.008
MAP dipping %	0.95	0.92-0.98	0.006
24-Hr SBP	1.03	1.01-1.05	< 0.001
24-Hr DBP	1.01	0.98-1.05	0.26
MAP	1.03	1.0-1.06	0.32
PP	1.03	1.01-1.06	0.001
SD 24-Hr SBP	1.09	1.03-1.16	0.003
SD 24-Hr DBP	0.91	0.80-1.03	0.15

This table presents the results of univariate logistic regression analyses evaluating the association between individual blood pressure (BP) indices and the risk of MACE. ORs with corresponding 95% CIs and p-values are reported. Continuous variables were assessed without transformation, except for zAASI, which represents the standardized AASI. A p-value < 0.05 was considered statistically significant.

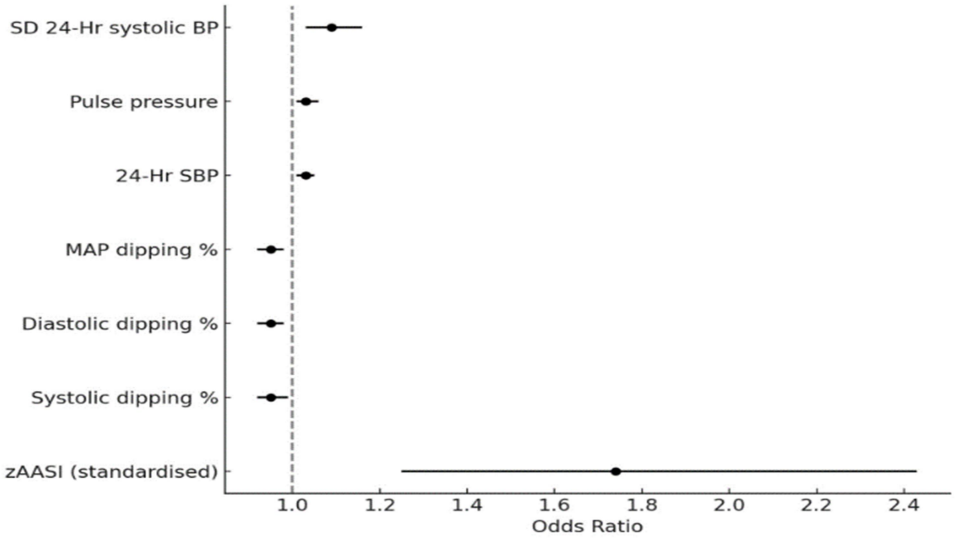


Figure 4-6: Forest plot of OR with 95% CIs for BPV indices, which are statistically significant in univariate analysis. This plot shows the ORs and 95% CIs for selected BPV and dipping indices, including zAASI, systolic and diastolic dipping percentages, MAP dipping, 24-Hr SBP, pulse pressure, and SD of 24-Hr systolic BP. All variables showed reached statistical significance ($p < 0.05$) in univariate logistic regression.

4.4 Multivariate logistic regression analysis

To reduce the risk of multicollinearity, 13 statistical models were analysed, each corresponding to one of the 13 total BP indices. Each BP index was adjusted for established CV risk factors, including age, previous history of stroke or TIA and heart failure. A total of 13 models were constructed and the models fit were compared. Cox and Snell R Square, -2 Log likelihood and Nagelkerke R square, and the Hosmer and Lemeshow test across all models showed acceptable values, indicating good model fit and similar performance (Appendix Table 3). The BP indices examined included SD 24-Hr SBP, SD 24-Hr DBP, AASI, 24-Hr SBP, 24-Hr DBP, MAP, dipping percentage of MAP, PP, CoV, day-time SBP, day-time DBP, night-time SBP and night-time DBP.

In the classification table, the model demonstrated a very strong specificity for the negative events (99.9%), but its sensitivity for detecting positive events (MACE) was only about 10.5% (Appendix Table 2). The overall correct percentage was 95.7%, indicating that it is better at capturing negative events, i.e. no-MACE events, than MACE events. There were a total of 38 events (MACE) out of 829 patients, and this limited number of outcomes likely impacted the model's predictive accuracy.

In multivariate analyses, it was demonstrated that SD 24-Hr SBP, 24-Hr SBP and DBP, day and night SBP and DBP were all independent predictors of MACE. Among BPV

indices, SD 24-Hr SBP emerged as the strongest predictor with the best model fit (-2 Log likelihood 246.157). For each unit increase in beta, there was a 21.5% increased risk of having a MACE (Table 4-6) (Figure 4-7). Additionally, a previous history of stroke or heart failure was also independent predictors for future MACE.

Table 4-6: Multivariate logistic regression analysis of BPV indices associated with MACE

Variables	OR	p-value	Lower 95% CI	Upper 95%	% change
SD 24-Hr SBP	1.215	0.002	1.077	1.372	21.5%
SD 24-Hr DBP	1.007	0.915	0.883	1.149	0.7%
AASI	3.329	0.322	0.308	35.932	232.9%
24-Hr SBP	1.029	0.009	1.007	1.051	2.9%
24-Hr DBP	1.058	0.002	1.010	1.079	5.8%
MAP	1.044	0.011	1.010	1.079	4.4%
PP	1.014	0.300	0.987	1.042	1.4%
MAP dipping %	0.982	0.326	0.946	1.019	-1.8%
CoV	3.978	0.072	0.884	17.888	297.8%
DT SBP	1.025	0.022	1.004	1.046	2.5%
DT DBP	1.051	0.006	1.014	1.088	5.1%
NTSBP	1.026	0.006	1.007	1.046	2.6%
NTDBP	1.056	0.001	1.021	1.091	5.6%
Age	1.063	<0.001	1.026	1.102	6.3%
Stroke/TIA	2.667	0.027	1.120	6.350	166.7%
Heart failure	3.261	0.030	1.118	9.512	226.1%

This table presents results from multivariate logistic regression examining the independent association of BP indices and clinical variables with MACE. OR, 95% CI, and p-values are shown for each variable. The percentage change in OR reflects the relative increase or decrease in odds per unit change in the predictor. Significant predictors include SD 24-Hr SBP, 24-Hr SBP, 24-Hr DBP, MAP, DT SBP, DT DBP, NTSBP, NTDBP, age, prior stroke/TIA, and heart failure ($p < 0.05$).

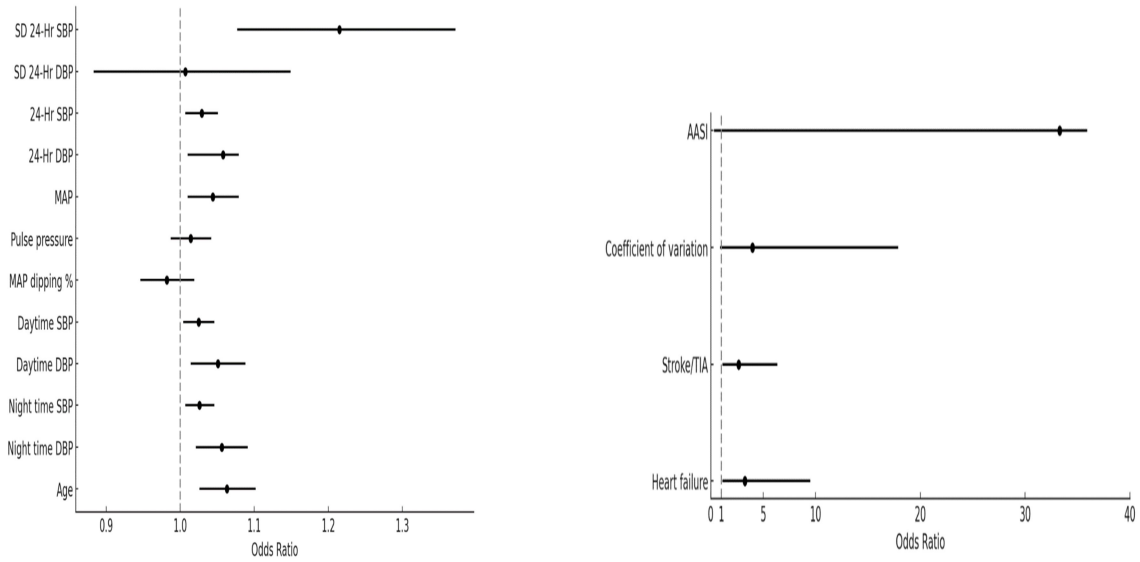


Figure 4-7: Forest plot demonstrating the OR with 95% CI for predictor variables. The line at OR = 1 represents no effect. Points to the right of this line indicate increased odds, while points to the left indicate decreased odds. Due to the wider CI for certain variables (AASI, CoV, stroke or TIA, and heart failure), two plots were created for better visualisation.

4.5 Survival analysis

Univariate Cox proportional hazards analyses identified significant associations between SD 24-Hr SBP (HR = 1.08, 95% CI: 1.010–1.149, $p < 0.001$) and AASI (HR = 24.04, 95% CI: 2.57–224.98, p = 0.005) with MACE. In multivariable analyses—comprising seven separate models, each adjusted for age, sex, ischaemic heart disease (IHD), stroke or transient ischaemic attack (TIA), heart failure (HF), hypertension (HTN), and smoking status—SD 24-Hr SBP remained independently associated with MACE (HR = 1.075, 95% CI: 1.010–1.146, p = 0.024), indicating a 7.5% increase in MACE risk per one-unit increase in SD 24-Hr SBP. AASI was no longer statistically significant after adjustment (HR = 2.74, 95% CI: 0.282–26.532, p = 0.386), which may reflect confounding by age or limited power given the wide confidence interval. Other significant BP indices included 24-Hr SBP (HR = 1.024, 95% CI: 1.005–1.044, p = 0.016) and 24-Hr DBP (HR = 1.05, 95% CI: 1.019–1.089, p = 0.002).

Each BP variable was analysed in a separate multivariable model to avoid multicollinearity, using an identical set of covariates. This strategy allowed HR to be interpreted as the independent effect of that BP parameter on MACE, consistently adjusted for key CV risk factors.

Among the adjustment covariates, age (HR = 1.061, 95% CI: 1.026–1.098, $p < 0.001$), stroke/TIA (HR = 2.51, 95% CI: 1.145–5.512, p = 0.022), and HF (HR = 2.72, 95% CI: 1.033–7.161, p = 0.043) were independently associated with MACE across models. HRs for these covariates were consistent across all seven models and are re*p*orted as representative values in (Table 4-7), which consolidates the results for clarity. Non-significant predictors included SD 24-Hr DBP (HR = 1.004, 95% CI: 0.885–1.139, p = 0.954), PP (HR = 1.01, 95% CI: 0.985–1.036, p = 0.435), MAP dipping percentage (HR = 0.98, 95% CI: 0.946–1.016, p = 0.277), sex (HR = 1.83, 95% CI: 0.934–3.598, p = 0.078), IHD (HR = 1.005, 95% CI: 0.489–2.068, p = 0.989), HTN (HR = 2.18, 95% CI: 0.892–5.313, p = 0.87), and smoking (HR = 1.29, 95% CI: 0.561–2.951, p = 0.551).

Model fit was assessed using the likelihood ratio chi-square statistic, with the representative model for SD 24-Hr SBP yielding a χ^2 value of 48.99 ($p < 0.001$, df = 8), indicating that the model significantly distinguished between patients with and without MACE. Forest plots were generated to visualise the HRs for BP indices (Figure 4-8) and clinical covariates (Figure 4-9). The Kaplan–Meier survival curve (Figure 4-10) demonstrated a stepwise decline in survival over time, while the cumulative hazard

function (Figure 4-11) showed a progressive increase in MACE risk during the four-year follow-up, both evaluated at the mean values of covariates.

The study included 829 patients, with 38 MACE events recorded, providing sufficient power for exploratory survival modelling and identification of independent risk predictors.

Table 4-7: Multivariate Cox regression analysis

No	Variable	HR	95% lower CI	95% upper CI	p-value
1	SD 24-Hr SBP	1.075	1.010	1.146	0.024
2	SD 24-Hr DBP	1.004	0.885	1.139	0.954
3	AASI	2.74	0.282	26.532	0.386
4	24-Hr SBP	1.024	1.005	1.044	0.016
5	24-Hr DBP	1.05	1.019	1.089	0.002
6	PP	1.01	0.985	1.036	0.435
7	MAP dipping %	0.98	0.946	1.016	0.277
8	Age	1.06	1.026	1.098	< 0.001
9	Male sex	1.83	0.934	3.598	0.078
10	Ischaemic heart disease	1.005	0.489	2.068	0.989
11	Stroke/ TIA	2.51	1.145	5.512	0.022
12	Heart failure	2.72	1.033	7.161	0.043
13	Hypertension	2.18	0.892	5.313	0.87
14	Smoking	1.29	0.561	2.951	0.551

This table presents HR, 95% CIs, and p-values from multivariate Cox proportional hazards models evaluating the association BP and BPV parameters and MACE. Each model included one parameter (e.g., SD of 24-Hr systolic BP, AASI) along with the same set of clinical covariates: age, sex, ischaemic heart disease, prior stroke/transient ischaemic attack (TIA), heart failure, hypertension, and smoking. Rows 1–7 report HRs for BP and BPV parameters from their respective models, while rows 8–14 list HRs for covariates, which remained consistent across all models. Statistically significant predictors ($p < 0.05$) reflect independent associations with increased MACE risk.

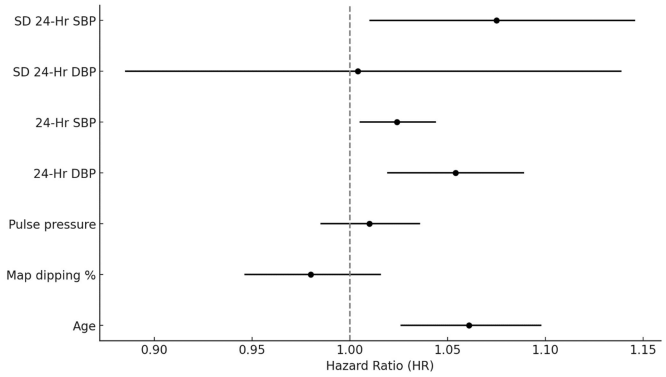


Figure 4-8: Forest plot of BPV, BP indices, and age in multivariate Cox regression analysis

This forest plot displays HR with 95% CIs for selected BPV measures, BP indices, and age, derived from multivariate Cox regression models assessing risk of MACE. Each model included one BP parameter (e.g., SD 24-Hr SBP, MAP dipping %) adjusted for the same set of clinical covariates (age, sex, ischaemic heart disease, stroke/TIA, heart failure, hypertension, and smoking). The vertical dashed line at HR = 1.0 indicates no association. Markers to the right of this line suggest increased hazard, while those to the left suggest a decreased hazard.

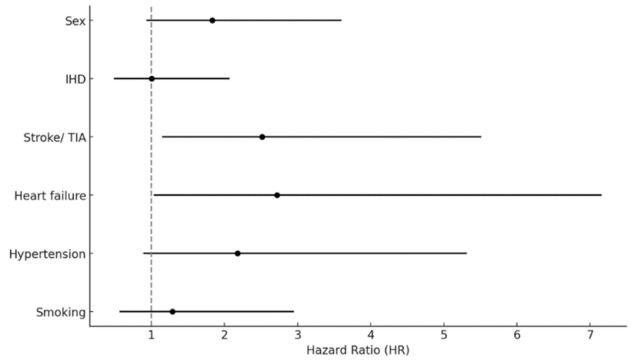


Figure 4-9: Forest plot of clinical covariates in multivariate Cox regression analysis.

This forest plot illustrates HR with 95% CIs for clinical variables included in multivariate Cox regression models assessing the risk of MACE. Variables shown include sex, IHD, prior stroke or TIA, heart failure, hypertension, and smoking. The vertical dashed line at HR = 1.0 indicates no effect. Markers to the right of the line suggest increased hazard. These covariates were included in all models as adjustment variables for BP and BPV indices.

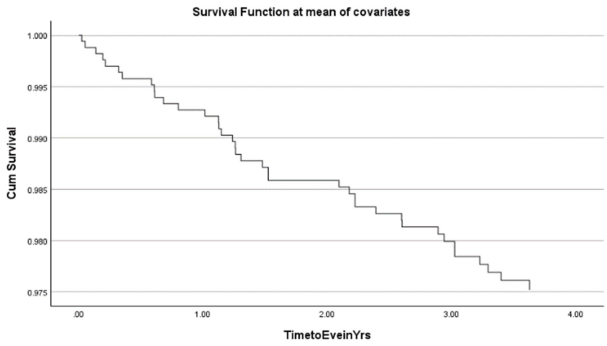


Figure 4-10: The survival function illustrates cumulative survival over time (in years) at the mean of covariates. The stepwise decline indicates the proportion of patients experiencing MACE throughout the study period.

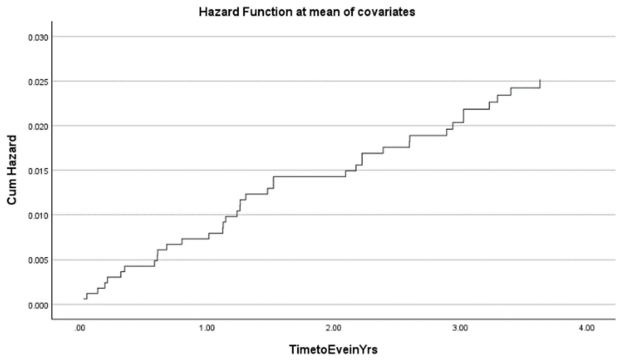


Figure 4-11: Cumulative hazard plot showing the cumulative hazard function over a four-year period, calculated at the mean covariate values. The plot demonstrates an increasing hazard rate, indicating a growing risk of MACE as time progresses.

4.6 Ambulatory arterial stiffness and survival function

A survival analysis using the Kaplan–Meier method was conducted to evaluate the association between AASI and time to MACE. Based on findings from the systematic review and the distribution of the dataset, the AASI was dichotomised at the median value of 0.47. Kaplan–Meier survival curves were generated to estimate event-free survival over time for two groups: patients with AASI ≥ 0.47 and those with AASI < 0.47. A log-rank (Mantel–Cox) test was used to compare survival distributions between the two groups. Survival time was defined as the period from baseline ABPM to the occurrence of MACE. Patients who were lost to follow-up or did not experience a MACE by the end of the study period were censored.

The estimated mean survival time was 6.3 years (SE = 0.032) for patients with AASI < 0.47 and 6.19 years (SE = 0.050) for those with AASI ≥ 0.47. The overall mean survival time across the cohort was 6.3 years (SE = 0.031). The log-rank test indicated a statistically significant difference in survival distributions between the two AASI groups ($\chi^2(1) = 10.016$, $p = 0.002$), suggesting that AASI level was significantly associated with time to MACE. The Kaplan–Meier survival curve demonstrated a separation between the groups, with patients in the higher AASI group showing a lower probability of remaining MACE-free over time. This finding was further supported by the cumulative hazard plot, which showed a greater hazard of MACE in patients with AASI ≥ 0.47 (Figure 4-12 and Figure 4-13).

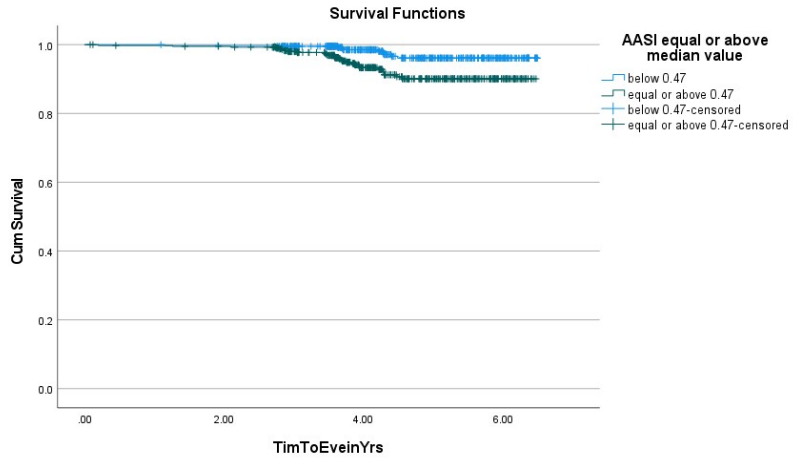


Figure 4-12: Kaplan-Meier survival curves by AASI category, describing event-free survival over time for two groups: patients with AASI < 0.47 and those with AASI ≥ 0.47. Patients with higher AASI showed decreased survival over time, indicating an increased risk of MACE.

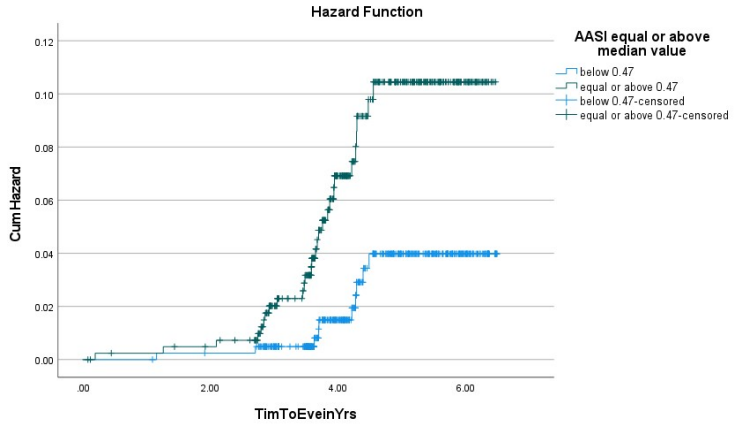


Figure 4-13: Cumulative hazard functions by AASI category, illustrating the cumulative hazard for MACE over time by AASI categories. A higher cumulative hazard for MACE is observed in patients with AASI ≥ 0.47 compared to patients with AASI < 0.47.

4.7 Effects of medications and other blood pressure indices on blood pressure variability

A linear regression model was constructed to examine the effect of medications and other blood pressure indices on SD 24-Hr SBP. Age was a significant predictor of SD 24-Hr SBP, with a standardised coefficient β= 0.04 (95% CI: 0.02-0.06, p < 0.001) (Appendix Table 6). Meanwhile, PP significantly predicted SD 24-Hr SBP, β= 0.162, (95% CI: 0.028-0.089, p < 0.001). AASI did not predict SD 24-Hr SBP. Medications did not show any statistical significance on SD 24-Hr SBP (Appendix Table 7). None of the CV co-morbidities can predict SD 24-Hr SBP (Appendix Table 8). Haemoglobin showed a borderline significance. Other blood tests were examined but excluded as none of the blood results showed any significance (Appendix Table 9).

4.8 Neutrophil-lymphocyte ratio in predicting major cardiovascular events and all-cause death

NLR has been regarded as a novel marker of inflammation in various studies. It has been reported that NLR is associated with increased arterial stiffness and is likely related to vascular inflammation (Wang et al. 2015, 2017). Verma et al. found that NLR is an independent predictor for MACE, CV events, and all-cause death. In this study, it was demonstrated that NLR is a statistically significant predictor for AASI (p = 0.001) (Appendix Table 17). In multivariate Cox regression, NLR is an independent predictor for MACE (HR: 1.12, 95% CI: 1.01-1.12, p = 0.031) and all-cause death (HR: 1.20, 95% CI: 1.07-1.34, p = 0.001) (Appendix Table 15 & 16).

4.9 Further exploratory subgroup analyses

Exploratory analyses were conducted for AASI and SD 24-Hr SBP as predictor variables in subgroups. The first subgroup (n= 829) analysis included ACS as a single outcome. The second subgroup analysis (n = 286) was for patients above 69 years with a combined MACE as the primary outcome, and the third subgroup analysis was for normotensive patients (n = 560) (SBP < 140 mmHg and DBP < 90 mmHg) and MACE (Appendix Table 10 -15). Neither AASI nor SD 24-Hr SBP were predictors for ACS and MACE in all analyses. In the ACS outcome subgroup (n=829), AASI did not significantly predict ACS (OR: 1.719, 95% CI: 0.066–44.616, p = 0.745), and SD 24-Hr SBP also did not demonstrate significance (OR: 1.052, 95% CI: 0.961–1.152, p = 0.275). For patients aged 69 years and above, AASI was not a significant predictor of

MACE (OR: 0.029, 95% CI: 0.000–10.940, p = 0.243), and SD 24-Hr SBP similarly showed no statistical significance for MACE (OR: 0.959, 95% CI: 0.824–1.116, p = 0.588). In the normotensive subgroup (SBP < 140 mmHg and DBP < 90 mmHg), AASI was not an independent predictor of MACE (OR: 0.016, 95% CI: 0.000–1.241, p = 0.062), while SD 24-Hr SBP remained non-significant (OR: 0.975, 95% CI: 0.863–1.101, p = 0.678).

Table 4-8: AASI in predicting ACS and MACE in subgroup analyses

Subgroup	Variable	OR	95% CI	p-value
ACS (outcome) (n = 829)	Age	1.075	1.019 - 1.135	0.009
	AASI	1.719	0.066 - 44.616	0.745
	Smoking (current)	0.507	0.130 - 1.971	0.327
	Smoking (ex)	0.290	0.097 - 0.861	0.026
	Ischaemic Heart Disease	5.628	2.207 - 14.353	< 0.001
	Diabetes Mellitus	2.415	0.895 - 6.517	0.082
	Hypertension	3.821	0.849 - 17.202	0.081
Age ≥ 69 (Outcome,MACE) (n = 286)	AASI	0.029	0.000 - 10.940	0.243
	Age	1.226	1.020 - 1.475	0.030
	Ischaemic Heart Disease	1.263	0.254 - 6.282	0.775
	Diabetes Mellitus	0.000	-	0.998
	Stroke/TIA	2.244	0.448 - 11.240	0.326
	Hypertension	0.836	0.189 - 3.706	0.814
Normotensive (Outcome, MACE) (n = 560)	AASI	0.016	0.000 - 1.241	0.062
	Age	1.111	1.043 - 1.184	0.001
	Diabetes Mellitus	0.469	0.057 - 3.835	0.480
	Hypertension	1.177	0.377 - 3.678	0.779
	Smoking (current)	1.318	0.255 - 6.820	0.742
	Smoking (ex)	1.368	0.412 - 4.542	0.609

Logistic regression showing ORs with 95% CIs and p-values for AASI and clinical covariates in three subgroups: total cohort (ACS outcome), age ≥ 69 (MACE outcome), and normotensive patients (MACE outcome). Variables include age, AASI, IHD, DM, HTN, stroke/TIA, and smoking status.

Table 4-9: SD 24-Hr SBP in predicting ACS and MACE in subgroup analyses

Subgroup	Variable	OR	95% CI for OR	p-value
ACS (outcome) (n = 829)	SD 24-Hr SBP	1.052	0.961 - 1.152	0.275
	Age	1.073	1.018 - 1.130	0.009
	Smoking (current)	0.499	0.128 - 1.937	0.315
	Smoking (ex)	0.281	0.094 - 0.842	0.023
	Ischaemic Heart Disease	5.953	2.293 - 15.458	< 0.001
	Diabetes Mellitus	2.395	0.912 - 6.286	0.076
	Hypertension	3.677	0.817 - 16.545	0.090
Age ≥ 69 (outcome, MACE) (n = 286)	SD 24-Hr SBP	0.959	0.824 - 1.116	0.588
	Age	1.212	1.013 - 1.449	0.035
	Ischaemic Heart Disease	1.272	0.263 - 6.143	0.765
	Diabetes Mellitus	0.000	-	0.998
	Stroke/TIA	2.329	0.475 - 11.429	0.297
	Hypertension	0.835	0.196 - 3.564	0.807
Normotensive (Outcome, MACE) (n = 560)	SD 24-Hr SBP	0.975	0.863 - 1.101	0.678
	Age	1.087	1.026 - 1.152	0.004
	Diabetes Mellitus	0.384	0.047 - 3.112	0.370
	Hypertension	1.159	0.372 - 3.613	0.799
	Smoking (current)	1.103	0.218 - 5.570	0.905
	Smoking (ex)	1.307	0.396 - 4.314	0.661

Logistic regression results showing ORs with 95% CIs and p-values for SD 24-Hr SBP and clinical covariates in three subgroups: total cohort (ACS outcome), age ≥ 69 (MACE outcome), and normotensive patients (MACE outcome). Variables include age, SD 24-Hr SBP, IHD, DM, HTN, stroke/TIA, and smoking status.

A receiver operative curve analysis was conducted to obtain a cut-off value for SD 24-Hr SBP (Appendix Figure 5). Area under the curve of SD 24-Hr SBP in discriminating MACE and no-MACE event was 0.604 and optimal cut-off value of SD 24-Hr SBP obtained from Youden's index was 17.21 mmHg. Those with value above 17.21 mmHg of SD 24-Hr SBP were dichotomised into 0 (below <17.21 mmHg) and 1(≥ 17.21 mmHg) and then further analysed using multivariate Cox regression, with MACE and ACS as dependent variables. It was demonstrated that dichotomised SD 24-Hr SBP was an independent predictor for MACE (HR: 2.25, 95% CI: 1.17-4.31, p = 0.014) and ACS (HR: 2.54, 95%CI 1.06-6.0, p = 0.035) (Appendix Figure 5, Table 18 & 19). A Kaplan-Meier analysis was conducted for MACE and no-MACE events using dichotomous SD 24-Hr SBP, and there was a significant difference between the survival curve of the groups (Appendix Figure 6, Table 20).

Chapter 5 Discussion

This research project investigated the predictive ability of BPV indices and AASI derived from ABPM for MACE. Statistical models were constructed to examine different BPV indices. In addition to SD 24-HrSBP, SD 24-Hr DBP and AASI, other traditional BP indices including PP, MAP and nocturnal dipping percentage, were examined. For multivariate logistic regression analysis, established CV risk factors, including age, stroke or TIA and heart failure, were adjusted as potential confounders. For Cox regression analysis, age, gender, ischaemic heart disease, history of stroke or TIA, hypertension, and smoking were adjusted.

5.1 Blood pressure variability and major adverse cardiovascular events

In univariate analyses, BPV indices (SD 24-Hr SBP, SD 24-Hr DBP and nocturnal dipping) and multiple BP indices (24-Hr, day and night SBP and DBP) can predict MACE (Table 4-5). Nocturnal blood pressure dipping was negatively associated with the outcome of MACE. Reverse nocturnal dipping is a recognised CV risk factor for MACE (Gavriilaki et al. 2020). In this research, it was demonstrated that MACE group had reduced nocturnal dipping including, systolic, diastolic and mean dipping, compared to no-MACE group. This finding is consistent with current knowledge and evidence (Gavriilaki et al. 2020; Palatini et al. 2022). However, after adjusting for CV risk factors (age, ischaemic heart disease, diabetes, stroke or TIA, heart failure, peripheral vascular disease, or aortic disease, the mean nocturnal dipping was no longer significant in multivariate analysis. This implies that failure to drop blood pressure nocturnally would be a potential risk factor for MACE but not an independent predictor.

5.1.1 Short-term systolic blood pressure variability and major adverse cardiovascular events

In multivariate logistic regression and Cox regression analyses after adjusting for CV risk factors, SD 24-Hr SBP was as an independent predictor for MACE. As discussed in Chapter 1, increased BPV is associated with increased arterial stiffness and CV events (Mehlum et al. 2018; Parati et al. 2018). Increased arterial stiffness is related to TOD and may mediate relations with CVD (Vasan et al. 2019).

BPV is a haemodynamic component, and high BPV may cause shear stress and circumferential stretch on the arterial vessel walls, which may affect endothelial cell function via epigenetic or mechanically sensitive cation channels, potentially impacting

atherosclerosis (Liu et al. 2022). Increased BPV may also trigger inflammation by upregulating inflammatory cascade (Abramson et al. 2006). In animal studies, increased BPV has been demonstrated to promote vascular smooth muscle proliferation and migration via angiotensin II pathway (Aoki et al. 2014). Furthermore, BPV is correlated with endothelial dysfunction (Diaz et al. 2013). These all are linked to increased atherosclerosis and potential TOD.

In this research study, patients in MACE group demonstrated that they have higher NLR, which is a marker of systemic inflammation. These factors may reflect that there is some element of increased vascular inflammation in patients with MACE. Additional discussion regarding NLR is described in subgroup analysis section. In 2019, a novel concept called Systemic Haemodynamic Athero-thrombotic Syndrome (SHATS) was proposed and BPV was included as a BP biomarker (Kario 2019). This research finding may support this novel concept, linking higher BPV and vascular inflammation to MACE.

Current evidence demonstrates that high BPV is associated with TOD (Parati et al. 1987; Cho et al. 2018; Chowdhury et al. 2018; Hisamatsu and Ohkubo 2022). High BPV is linked to an increased risk of coronary heart disease, the progression of chronic kidney disease and cerebrovascular disease (De Havenon et al. 2019; Wang et al. 2020; Harefa et al. 2021). In this research, high short-term BPV index (SD 24-Hr SBP) predicted MACE in logistic regression and Cox regression analyses. However, when subgroup analyses were conducted for specific single outcomes, for instance, coronary heart disease, it is no longer significant. Neither SD 24-Hr SBP nor any other the BPV indices predicted these primary outcomes. This may be due to the relative rarity of outcome events and the models' limitations in subgroup analyses given the sample size of population examined.

In multivariate logistic regression analysis, a one-unit increase in SD of 24-Hr SBP corresponds to a 21.5% higher risk of MACE. SD 24-Hr SBP remained a significant predictor in both univariate and multivariate Cox regression models. After adjusting for CV risk factors, a one-unit increase in SD 24-Hr SBP was associated with a 7.5% increased risk of MACE.

Although it was described that diastolic BPV may be a key risk factor for cerebrovascular function decline (Peters et al. 2022), in this research, diastolic BPV was not a significant predictor. However, it was demonstrated that patients with MACE have higher AASI, an indirect marker of arterial stiffness, which may have a better correlation with systolic BPV. Arterial stiffness may play a role in different predictive

ability between systolic BPV and diastolic BPV. Predictive ability of systolic BPV is supported by a study conducted in 2015 that higher systolic BPV is associated with poor outcomes in patients with ischaemic or haemorrhagic strokes (Manning et al. 2015). As previously discussed, in 2017, QRISK-3 CV risk calculator accepted SD systolic BP (long-term BPV) as a CV risk factor (Hippisley-Cox et al. 2017). In 2016, Stevens et al. reported that short-term BPV follow similar pattern. This research study's findings demonstrate that short-term systolic BPV is a potentially significant CV risk factor.

5.1.2 Ambulatory arterial stiffness index and major adverse cardiovascular events

Arterial stiffness is considered a risk marker for vascular ageing and a new biomarker for CVD (Franklin 2008). There have been discussions about whether AASI is a true arterial stiffness marker (Gavish et al. 2007; Schillaci et al. 2007; Westerhof et al. 2007; Kips et al. 2012). However, it has been accepted as a CV risk factor and is an indirect marker of arterial stiffness (Li et al. 2006; Mahmud et al. 2007; Laugesen et al. 2011; Palmiero et al. 2011; Kollias et al. 2012; Parati and Schillaci 2012; Schillaci and Pucci 2015).

AASI has been demonstrated in several studies to be a robust predictor for TOD (Leoncini et al. 2006; Ratto et al. 2006; Natale et al. 2010; Gómez-Marcos et al. 2012; Eriksen et al. 2017). However, the practical challenge for AASI is that there is no consensus over its cut-off value for clinical use and in Chapter 2, it was discussed that AASI value varies from 0.3 to 0.72. In this research study, the mean value of AASI in patients with MACE was 0.54 (±0.16), and without MACE was 0.45 (±0.16). It implies that AASI would be a CV risk factor, with a higher mean value observed in the MACE group.

In this study, a researcher proposed AASI cut-off value was 0.47 (median). A Kaplan-Meier analysis was conducted, and it was demonstrated that patients with AASI value above 0.47 showed lower probability of survival and it is significantly associated with time to MACE.

In univariate analyses of both logistic regression and Cox regression, AASI was a significant predictor. However, when age was adjusted, it lost its significance in predicting MACE. Age itself is a recognised cause of vascular ageing with increased arterial stiffness (Mikael et al. 2017). Therefore, age may have a significant impact on AASI in this research study and in group comparison; the MD of age between MACE and non-MACE is 11.58 years ($p < 0.001$). It was cross-checked by conducting a linear

regression analysis by keeping AASI as dependent variable and age as predictor variable. In that linear regression, age was a predictor for AASI. In multivariate logistic regression and Cox regression analyses, when age was adjusted, AASI was no longer an independent predictor of MACE.

5.1.3 Subgroup analyses of ambulatory arterial stiffness index and SD 24-Hr systolic blood pressure in predicting myocardial infarction, major adverse cardiovascular events in patients above sixty-nine, and normotensive patients

Further exploratory subgroup analyses were conducted with AASI and SD 24-Hr SBP as predictor variables, with ACS as the dependent variable. Neither AASI nor SD 24-Hr SBP showed significance in this analysis, which is likely related to the limited number of events. Given that the average age in the MACE group was approximately 69 years, an additional subgroup analysis was performed, where both AASI and SD 24-Hr SBP were examined as predictors of MACE. In this age-specified analysis, neither variable demonstrated statistical significance. A further analysis was conducted in patients with SBP < 140 mmHg and DBP < 90 mmHg, but again, both AASI and SD 24-Hr SBP failed to show any significant associations. In all analyses, adjustments were made for co-morbidities such as age, smoking, ischaemic heart disease, diabetes mellitus, and hypertension. The rarity of events likely influenced the outcomes of these subgroup analyses, contributing to the lack of significant findings.

5.1.4 Further exploratory analysis

A ROC curve analysis was conducted for SD 24-Hr SBP, and the area under the curve showed 0.604, which has a moderate discriminating capacity between MACE and no-MACE. Using optimal cut-off (17.21 mmHg), SD 24-Hr SBP was dichotomised and survival analyses were conducted using both multivariate Cox regression and Kaplan-Meier analyses. It was demonstrated that dichotomised SD 24-Hr SBP can predict MACE in Cox regression (HR: 2.25, 95% CI: 1.17-4.31, p = 0.014). The Kaplan-Meier analysis showed a significant difference in survival between MACE and no-MACE group. Dichotomised data may have revealed a non-linear relationship in high-risk groups, specifically patients with high BPV. Additionally, dichotomised SD 24-Hr SBP was found to independently predict ACS in multivariate Cox regression analysis.

5.1.5 Subgroup analyses of neutrophil-lymphocyte ratio in predicting all-cause mortality, major adverse cardiovascular events and ambulatory arterial stiffness index

It has been demonstrated that high NLR is an independent predictor for CV events and increased arterial stiffness (Wang et al. 2015, 2017; Ning et al. 2022; Verma et al. 2023). Furthermore, it is also noted that higher NLR is found in hypertensive patients than in normotensive patients (Sarejloo et al. 2023). In 2020, Chung et al. reported that high NLR is associated with large cerebral artery atherosclerosis (Chung et al. 2020). These suggest the role of systematic inflammation, including vascular components and TOD. In this study, it was demonstrated that NLR showed modest predictive value for AASI, which is an indirect marker of arterial stiffness. More importantly, it has emerged as an independent predictor for MACE and all-cause deaths which are in keeping with recent literature evidence (Angkananard et al. 2018; Verma et al. 2023). The role of vascular inflammation due to high BP load and reduced arterial compliance may play a role in increased BPV.

5.2 Future recommendations

ABPM is the gold standard tool for the diagnosis and management of hypertension. It can provide reliable blood pressure data, including the measurement of BPV and circadian rhythm data. In this study, it was noticeable that a larger sample size should be considered, and an age cut-off should be applied to minimise data imbalances. Replication studies with some additional exclusion criteria should be considered to re-investigate AASI and SD 24-Hr SBP. In this study, the outcome events were not as high, likely related to factors such as age. Therefore, retrospective case-control studies could be considered. In current research project, SD was used as the primary BPV index and in future studies, other indices such as ARV should be considered. BPV is associated with TOD and in future studies, further secondary outcomes could be added, for instance, incidence of new atrial fibrillation, progression of chronic kidney disease or heart failure.

5.3 Limitations

It needs to be acknowledged that there are some limitations in the current research project. Firstly, our study is an observational ambidirectional study, and there is a selection bias risk. Secondly, the outcome event is relatively rare in relation to the total number of patients, with only 38 out of 829 patients (4.58%) experiencing MACE, which limited the statistical power to detect weaker associations and increased the risk of model bias toward no-events, potentially affecting the reliability of predictive models; data re-sampling would provide more balanced data to the current data set, but due to time and technological constraints, it was not achieved. Thirdly, the majority of patients were Caucasian, with a paucity of data on other ethnic groups. Fourthly, medication compliance plays a role in blood pressure variability, and this study was unable to assess medication compliance. Finally, this study was exploratory in nature, aiming to identify potential associations between blood pressure variability indices and MACE, which require further validation in larger, prospective studies.

5.4 Conclusion

This research project compared the prognostic role of AASI and short-term BPV index (SD 24-Hr SBP). It was demonstrated that short-term BPV is an independent predictor for MACE in different statistical models. Short-term BPV as a prognostic risk factor is a consistent finding with current evidence.

Short-term BPV is under the influence of environmental, behavioural CV regulatory mechanisms and arterial stiffness. Higher BPV may indicate that there would be underlying impaired CV regulatory mechanisms or increased arterial stiffness. High BPV, increased arterial stiffness, increased vascular inflammation and TOD are interconnected and clinicians should raise suspicion of these risk factors when a patient presents with higher BPV.

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Abbreviations

1. AASI: Ambulatory arterial stiffness index
2. ABPM: Ambulatory blood pressure monitoring
3. ACS: Acute coronary syndrome
4. ACTH: Adrenocorticotropic hormone
5. AHT: Antihypertensive therapy
6. APBM: Ambulatory blood pressure monitor
7. ARB: Angiotensin II receptor blocker
8. ARV: Average real variability
9. BMI: Body mass index
10. BP: Blood pressure
11. BPV: Blood pressure variability
12. BPVR: Blood pressure variability ratio
13. CI: Confidence interval
14. CoV: Coefficient of variation
15. DBP: Diastolic blood pressure
16. DTSBP: Day-time systolic blood pressure
17. DTDBP: Day-time diastolic blood pressure
18. DTMAP: Day-time mean arterial pressure
19. DTPP: Day-time pulse pressure
20. EPR: Electronic patient record
21. ESRD: End-stage renal disease
22. HD: Hemodialysis
23. HDL: High-density lipoprotein
24. HR: Hazard ratio
25. HTN: Hypertension
26. MAP: Mean arterial pressure
27. MD: Mean difference
28. MI: Myocardial infarction
29. MSI: Morning surge index
30. MACE: Major cardiovascular events
31. NLR: Neutrophil-lymphocyte ratio
32. NTDBP: Night-time diastolic blood pressure
33. NTMAP: Night-time mean arterial pressure

34. NTPP: Night-time pulse pressure
35. NTS: Nucleus Tractus Solitarius
36. NTSBP: Night-time systolic blood pressure
37. NOS: Newcastle-Ottawa Scale
38. OBPM: Office blood pressure monitor
39. OR: Odds ratio
40. PNS: Parasympathetic nervous system
41. PP: Pulse pressure
42. PRISMA: Preferred Reporting Items for Systematic Review and Meta-Analyses
43. PVD: Peripheral vascular disease
44. PWV: Pulse wave velocity
45. RAA: Renin-angiotensin-aldosterone
46. REC: Research Ethics Committee
47. ROB: Risk of bias
48. ROC: Receiver operating characteristic
49. SBP: Systolic blood pressure
50. SD: Standard deviation
51. SD 24-Hr SBP: Standard deviation of 24-hour systolic blood pressure
52. SD 24-Hr DBP: Standard deviation of 24-hour diastolic blood pressure
53. SDMAP: Standard deviation of mean arterial pressure
54. SD DTSBP: Standard deviation of day-time systolic blood pressure
55. SD DTDBP: Standard deviation of day-time diastolic blood pressure
56. SD DTMAP: Standard deviation of day-time mean arterial pressure
57. SD DTPP: Standard deviation of day-time pulse pressure
58. SDNTSBP: Standard deviation of night-time systolic blood pressure
59. SD NTDBP: Standard deviation of night-time diastolic blood pressure
60. SDNTMAP: Standard deviation of night-time mean arterial pressure
61. SD NTPP: Standard deviation of night-time pulse pressure
62. SHATS: Systemic haemodynamic athero-thrombotic syndrome
63. SNS: Sympathetic nervous system
64. TIA: Transient ischaemic attack
65. TOD: Target organ damage
66. VIM: Variability independent of the mean
67. wSD: Weighted standard deviation

Appendix

Table 1: ROB assessment using Newcastle-Ottawa Scale

	Author year	Selection				Comparability	Outcome			
		Representativeness of exposed cohort	Selection of non-exposed	Ascertainment of exposure	Demonstration that outcome of Interest Was	Comparability of Cohorts on the basis of the design or	Assessment of outcome	Follow-up long enough for outcomes	Adequacy of Follow-up of cohorts	Total score
1	Dolan et al. 2006	*		*	*	**	*	*	*	8
2	Hansen et al. 2006	*		*	-	**	*	*	*	7
3	Gosse et al. 2007	*		-	-	*	-	*	-	3
4	Kikuya et al. 2007	*		*	*	**	*	*	*	8
5	Hansen et al. 2008	*		*	*	**	-	*	-	6
6	Ben-Dov et al. 2008	*		*	*	*	*	*	*	7
7	Palmas et al. 2009	*		*	*	**	*	*	*	8
8	Gavish et al. 2009	*		*	*	*	*	*	-	6
9	Bastos et al. ,2010	*		*	*	*	*	*	-	6
10	Muxfeldt et al. 2010	*		*	*	*	-	*	*	6
11	Laugesen et al. , 2012	*		*	*	**	*	*	-	7
12	Viazzi et al. 2020	*		*	*	**	*	*	-	7
13	Boos et al. 2021	*		*	*	**	*	*	-	7
14	Hoshide et al. 2023	*		*	*	**	*	*	-	7

Table 2: Classification table of model constructed in predicting MACE

Classification Table					
	Observed		Predicted		
			MACE		Percentage Correct
			No-MACE event	MACE	
Step 1	MACE	No-MACE event	773	1	99.9
		MACE	34	4	10.5
	Overall Percentage				95.7
a. The cut value is .500					

Table 3: Model fit data comparison of total 13 model, chi-square (χ2)

Model Number with predictor	-2 Log likelihood	Cox and Snell R square	Nagelkerke R square	Hosmer and Lemeshow test
Model 1: SD-24-Hr SBP	246.157	0.072	0.229	χ2=7.789, df=8,p = 0.454
Model 2: SD-24 Hr DBP	267.591	0.047	0.150	χ2=5.497, df=8, p = 0.703
Model 3: AASI	266.652	0.048	0.154	χ2=5.216, df=8, p = 0.734
Model 4: 24-Hr SBP	260.778	0.055	0.175	χ2=7.367, df=8, p = 0.498
Model 5: 24-Hr DBP	258.314	0.058	0.185	χ2=3.579, df=8, p = 0.893
Model 6: MAP dipping %	266.681	0.048	0.154	χ2=6.446, df=8, p = 0.597
Model 7: pulse pressure	266.571	0.048	0.154	χ2=8.089, df=8, p = 0.425
Model 8: MAP	261.227	0.055	0.174	χ2=9.237, df=8, p = 0.323
Model 9: Coefficient of variation	264.364	0.051	0.162	χ2=6.475, df=8, p = 0.594
Model 10: Day-time SBP	262.350	0.053	0.170	χ2=3.278, df=8, p = 0.916
Model 11: Day-time DBP	259.968	0.056	0.178	χ2=4.648, df=8, p = 0.794
Model 12: Night-time SBP	260.100	0.056	0.178	χ2=13.841, df=8, p = 0.086
Model 13: Night-time DBP	257.202	0.059	0.188	χ2=3.823, df=8, p = 0.872

Table 4: Omnibus test of multivariate Cox regression analysis

	Omnibus Tests of Model Coefficients									
	-2 Log Likelihood	Overall (score)			Change From Previous Step			Change From Previous Block		
		Chi-square	df	Sig.	Chi-square	df	Sig.	Chi-square	df	Sig.
Model 1	495.144	10.539	1	.001	9.051	1	.003	9.051	1	.003
Model 2	473.040	27.199	3	<0.001	22.104	2	<0.001	22.104	2	<0.01
Model 3	456.594	48.985	10	<0.001	16.446	7	0.021	16.446	7	0.021

Table 5: Group comparison of blood results, left ventricular ejection fraction between MACE and no-MACE

Blood results	MACE	Non-MACE Events	P-value
Haemoglobin (g/dl)	135.21 (±3.05)	139.61 (±14.95)	0.038
Lymphocytes	1.616 (±0.65)	1.92 (±0.85)	0.015
Neutrophils	5.113 (±1.98)	4.58 (±1.97)	NS
Platelets	245 (±60.88)	257.14 (±71.46)	NS
White Cell Counts	7.629 (±2.12)	7.28 (±2.13)	NS
Neutrophil/ lymphocyte ratio	3.96 (±3.42)	2.78 (±1.93)	<0.001
Creatinine	100.11 (±33.79)	87.84 (±27.73)	0.005
eGFR	61.76 (±18.96)	70.72 (±16.27)	<0.001
Total cholesterol	4.49 (±1.47)	4.80 (±1.18)	NS
High-density lipoprotein	1.45(±0.46	1.48 (±0.50)	NS
Low-density lipoprotein	2.53 (±1.29)	3.041.138	0.004
Triglycerides	1.65 (±0.8))	1.65 (±1.01)	NS
Cholesterol/HDL ratio	3.10 (±1.05)	3.50 (±1.26)	NS
Glucose	6.72 (±2.33)	5.87 (±2.21)	0.013
HbA1C	48.6(±18.72	42.32 (±12.86)	0.004
LVEF	55.93 (±9.49)	59.07 (±7.41)	0.013

eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HbA1C, haemoglobin A1C; LVEF, left ventricular ejection fraction

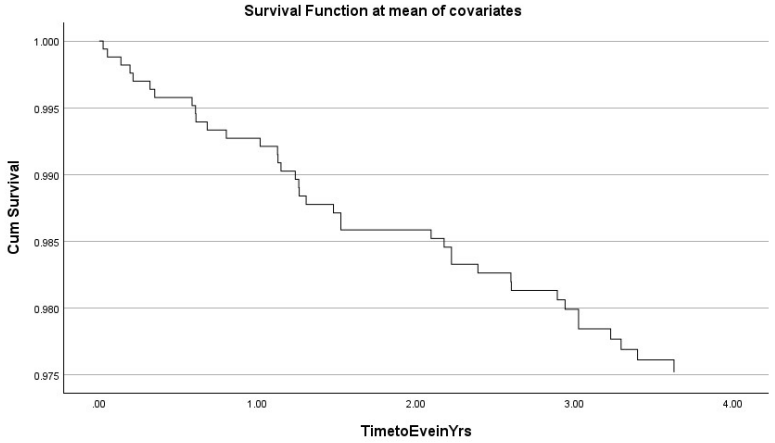

Figure 1: Survival function illustrating cumulative survival against time to events (in years) at the mean of covariates. The stepwise decline indicates that proportion of patients experiencing MACE over the study period.

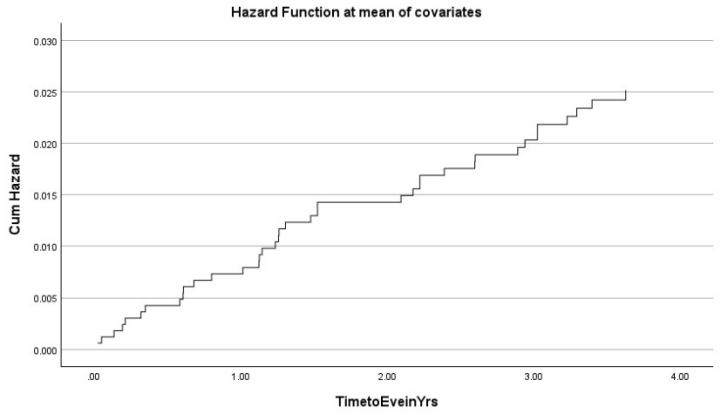

Figure 2:

Table 6: Regression analysis of age, AASI and PP (predictors) on SD 24-Hr SBP

Coefficients[a]								
Model		Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95.0% CI for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	9.544	0.743		12.841	<.001	8.085	11.003
	Age	0.044	0.012	0.152	3.808	<.001	0.021	0.066
	AASI	-.785	1.105	-0.030	-0.710	.478	-2.954	1.385
	PP	.056	0.014	0.162	3.992	<.001	0.028	0.083
a. Dependent Variable: SD 24-Hr SBP								

Table 7: Regression analysis of effects of medications on SD 24-Hr SBP

Coefficients[a]								
Model		Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95.0% CI for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	15.031	0.299		50.343	<0.001	14.445	15.618
	ENTRESTO / TOLVAPTAN	-0.931	1.287	-0.028	-0.723	0.470	-3.458	1.597
	ace-i/arb	0.060	0.370	0.007	0.162	0.871	-.0666	0.786
	Calcium channel blockers	0.084	0.378	0.009	0.222	0.824	-.0658	0.826
	Beta blockers	-0.121	0.377	-0.012	-0.319	0.750	-0.862	0.621
	Diuretics	0.730	0.476	0.063	1.533	0.126	-0.205	1.664
	alpha blocker	-0.086	0.552	-0.006	-0.156	0.876	-1.170	0.998
	ALD ANTAG	0.317	0.831	0.015	0.382	0.703	-1.314	1.948
	HYDRALAZINE OTHR	-2.779	2.677	-0.041	-1.038	0.300	-8.036	2.478

a. Dependent Variable: SD 24-Hr SBP

Table 8: Regression analysis of co-morbidities on SD 24-Hr SBP

Coefficients								
Model		Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95.0% CI for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	14.285	0.265		53.818	<0.001	13.764	14.806
	Ischaemic heart disease	0.100	0.404	0.009	0.248	0.804	-0.692	0.893
	Diabetes mellitus	0.209	0.444	0.017	0.471	0.638	-0.662	1.080
	stroke/TIA2	-0.257	0.581	-0.015	-0.442	0.658	-10.397	0.883
	Heart failure	-0.600	0.747	-0.028	-0.804	0.422	-20.066	0.866
	pvd / AORTI1 DISEASE	0.419	0.623	0.024	0.673	0.501	-0.804	1.642
	Gout	0.390	0.776	0.018	0.503	0.615	-1.133	1.914
	Hypertension	0.866	0.326	0.094	2.652	0.008	0.225	1.507
a. Dependent Variable: SD 24-Hr SBP								

Table 9: Regression analysis of blood results on SD 24-Hr SBP

Coefficients								
Model		Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95.0% CI for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	19.511	2.621		7.445	<.001	14.366	24.656
	lymphocyte	-0.286	0.224	-0.054	-1.277	0.202	-0.725	0.154
	Neutrophils	0.059	0.162	0.026	0.361	0.718	-0.260	0.377
	Haemoglobin	-0.031	0.012	-0.107	-2.670	0.008	-0.055	-0.008
	Platelets	-0.004	0.003	-0.060	-1.500	0.134	-0.009	0.001
	White Cell Counts	0.125	0.160	0.060	0.779	0.437	-0.190	0.439
	Creatinine	-0.014	0.012	-0.085	-1.195	0.233	-0.038	0.009
	eGFR	0.005	0.019	0.017	0.239	0.811	-0.033	0.042
	total cholesterol	0.214	0.140	0.058	1.535	0.125	-0.060	0.488
a. Dependent Variable: SD 24-Hr SBP								

Table 10: Regression analysis of SD 24-Hr SBP, AASI and other variables on coronary events (ACS)

Variables in the Equation									
		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age	.072	.028	6.904	1	.009	1.075	1.019	1.135
	AASI	.541	1.662	.106	1	.745	1.719	.066	44.616
	smoking CURRENT =1 NEVER = 0 EX =2			5.189	2	.075			
	smoking CURRENT =1 NEVER = 0 EX =2(1)	-.679	.693	.961	1	.327	.507	.130	1.971
	smoking CURRENT =1 NEVER = 0 EX =2(2)	-1.239	.556	4.968	1	.026	.290	.097	.861
	Ischaemic heart disease(1)	1.728	.478	13.088	1	<.001	5.628	2.207	14.353
	Diabetes mellitus(1)	.882	.506	3.033	1	.082	2.415	.895	6.517
	stroke/TIA2(1)	-18.618	4492.402	.000	1	.997	.000	.000	.
	Hypertension(1)	1.341	.768	3.050	1	.081	3.821	.849	17.202
	Constant	-10.019	1.970	25.863	1	<.001	.000		
Dependent variable: ACS									
Variable(s) entered on step 1: smoking CURRENT =1 NEVER = 0 EX =2, Ischaemic heart disease, Diabetes mellitus, stroke/TIA2, Hypertension									

Table 10 (continued)

Variables in the Equation									
		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age	0.070	0.027	6.907	1	0.009	1.073	1.018	1.130
	SD 24-Hr SBP	0.051	0.046	1.192	1	0.275	1.052	0.961	1.152
	smoking CURRENT =1 NEVER = 0 EX =2			5.360	2	0.069			
	smoking CURRENT =1 NEVER = 0 EX =2(1)	-0.696	0.692	1.010	1	0.315	0.499	0.128	1.937
	smoking CURRENT =1 NEVER = 0 EX =2(2)	-1.270	0.560	5.138	1	0.023	0.281	0.094	0.842
	Ischaemic heart disease(1)	1.784	0.487	13.427	1	<.001	5.953	2.293	15.458
	Diabetes mellitus(1)	0.873	0.492	3.144	1	0.076	2.395	0.912	6.286
	stroke/TIA2(1)	-18.695	4405.189	.000	1	0.997	0.000	0.000	.
	Hypertension(1)	1.302	0.767	2.880	1	0.090	3.677	0.817	16.545
	Constant	-10.364	1.985	27.268	1	<.001	0.000		
Dependent variable: ACS									
a. Variable(s) entered on step 1: smoking CURRENT =1 NEVER = 0 EX =2, Ischaemic heart disease, Diabetes mellitus, stroke/TIA2, Hypertension									

Table 11: Regression analysis of AASI and SD-24Hr SBP with other variables on MACE for patients age equal or above 69 (n=286 patients)

Variables in the Equation[a]									
		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[b]	AASI	-3.529	3.021	1.365	1	0.243	0.029	.000	10.940
	Age	0.204	0.094	4.694	1	0.030	1.226	1.020	1.475
	Ischaemic heart disease(1)	0.234	0.818	.081	1	0.775	1.263	.254	6.282
	Diabetes mellitus(1)	-18.735	7022.890	.000	1	0.998	0.000	.000	.
	stroke/TIA2(1)	0.808	0.822	.966	1	0.326	2.244	.448	11.240
	Hypertension(1)	-0.179	0.760	.055	1	0.814	.836	.189	3.706
	smoking CURRENT =1 NEVER = 0 EX =2			1.727	2	0.422			
	smoking CURRENT =1 NEVER = 0 EX =2(1)	-18.122	7994.152	.000	1	0.998	0.000	0.000	.
	smoking CURRENT =1 NEVER = 0 EX =2(2)	.991	0.754	1.727	1	0.189	2.695	0.614	11.822
	Constant	-16.598	6.941	5.718	1	0.017	0.000		

a.Dependent variable: MACE

b. Variable(s) entered on step 1: Ischaemic heart disease, Diabetes mellitus, stroke/TIA2, Hypertension, smoking CURRENT =1 NEVER = 0 EX =2

Table 11 (continued)

Variables in the Equation[a]									
		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[b]	Age	0.192	0.091	4.435	1	0.035	1.212	1.013	1.449
	SD 24-Hr SBP	-0.042	0.077	0.294	1	0.588	0.959	0.824	1.116
	Ischaemic heart disease(1)	0.240	0.804	0.089	1	0.765	1.272	0.263	6.143
	Diabetes mellitus(1)	-18.940	7110.013	0.000	1	0.998	0.000	0.000	.
	stroke/TIA2(1)	0.846	0.812	1.086	1	0.297	2.329	0.475	11.429
	Hypertension(1)	-0.180	0.741	.059	1	0.807	0.835	0.196	3.564
	smoking CURRENT =1 NEVER = 0 EX =2			1.408	2	0.495			
	smoking CURRENT =1 NEVER = 0 EX =2(1)	-18.393	8001.264	0.000	1	0.998	0.000	0.000	.
	smoking CURRENT =1 NEVER = 0 EX =2(2)	0.866	0.730	1.408	1	0.235	2.378	0.569	9.941
	Constant	-16.729	6.755	6.134	1	0.013	0.000		

a. Dependent variable: MACE

b. Variable(s) entered on step 1: Ischaemic heart disease, Diabetes mellitus, stroke/TIA2, Hypertension, smoking CURRENT =1 NEVER = 0 EX =2

Table 12: Regression analysis of AASI and other variables on MACE in normotensive patients (n=560)

Variables in the Equation[a]									
		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[b]	AASI	-4.153	2.229	3.472	1	0.062	0.016	0.000	1.241
	Age	0.106	0.032	10.654	1	0.001	1.111	1.043	1.184
	Diabetes mellitus(1)	-0.757	1.072	0.498	1	0.480	0.469	0.057	3.835
	Hypertension(1)	0.163	.581	0.079	1	0.779	1.177	0.377	3.678
	smoking CURRENT =1 NEVER = 0 EX =2			0.293	2	0.864			
	smoking CURRENT =1 NEVER = 0 EX =2(1)	0.276	.839	0.108	1	0.742	1.318	0.255	6.820
	smoking CURRENT =1 NEVER = 0 EX =2(2)	0.313	.612	0.262	1	0.609	1.368	0.412	4.542
	Constant	-8.830	2.101	17.667	1	<.001	0.000		

a. Normotensives = 1.00

b. Variable(s) entered on step 1: Diabetes mellitus, Hypertension, smoking CURRENT =1 NEVER = 0 EX =2.

Table 13: Regression analysis of SD 24-Hr SBP and other variables on MACE in normotensive patients (n=560)

Variables in the Equation[a]									
		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[b]	Age	0.084	0.029	8.089	1	0.004	1.087	1.026	1.152
	SD 24-Hr SBP	-0.026	0.062	0.172	1	0.678	0.975	0.863	1.101
	Diabetes mellitus(1)	-0.957	1.067	0.804	1	0.370	0.384	0.047	3.112
	Hypertension(1)	0.147	0.580	0.065	1	0.799	1.159	0.372	3.613
	smoking CURRENT =1 NEVER = 0 EX =2			0.193	2	0.908			
	smoking CURRENT =1 NEVER = 0 EX =2(1)	0.098	0.826	0.014	1	0.905	1.103	0.218	5.570
	smoking CURRENT =1 NEVER = 0 EX =2(2)	0.268	0.609	0.193	1	0.661	1.307	0.396	4.314
	Constant	-8.776	2.185	16.135	1	<.001	0.000		
a. Dependent variable: MACE									
b. Variable(s) entered on step 1: Diabetes mellitus, Hypertension, smoking CURRENT =1 NEVER = 0 EX =2									

Table 14: Univariate analysis of age on AASI

Dependent Variable: AASI						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	7.404[a]	67	0.111	5.167	<0.001	0.313
Intercept	76.695	1	76.695	3586.507	<0.001	0.825
age	7.404	67	0.111	5.167	<0.001	0.313
Error	16.273	761	0.021			
Total	198.524	829				
Corrected Total	23.677	828				
a. R Squared= .313 (Adjusted R Squared= .252)						

Table 15: Cox regression for NLR for MACE

Variables in the Equation								
	B	SE	Wald	df	Sig	Exp(B)	95.0% CI for Exp(B)	
							Lower	Upper
NLR	0.112	0.052	4.668	1	0.031	1.119	1.010	1.239
Age	0.058	0.017	11.691	1	<0.001	1.060	1.025	1.096
Ischaemic heart disease	0.077	0.372	0.042	1	0.837	1.080	0.521	2.237
Diabetes mellitus	0.489	0.380	1.654	1	0.198	1.631	0.774	3.437
stroke/TIA2	0.672	0.405	2.756	1	0.097	1.958	0.886	4.326
Hypertension	0.871	0.453	3.700	1	0.054	2.388	0.984	5.798
Heart failure	0.835	0.490	2.901	1	0.089	2.306	0.882	6.030
smoking CURRENT =1 NEVER = 0 EX =2			3.244	2	0.198			
smoking CURRENT =1 NEVER = 0 EX =2(1)	0.374	0.426	.770	1	0.380	1.454	0.630	3.351
smoking CURRENT =1 NEVER = 0 EX =2(2)	-.518	0.403	1.653	1	0.199	0.596	0.271	1.312

Table 16: Cox Regression for NLR for all-cause death

Variables in the Equation								
	B	SE	Wald	df	Sig	Exp(B)	95.0% CI for Exp(B)	
							Lower	Upper
NLR	0.186	0.057	10.568	1	0.001	1.205	1.077	1.348
Age	0.068	0.021	10.814	1	0.001	1.070	1.028	1.114
Ischaemic heart disease	0.168	0.397	.180	1	0.672	1.183	0.543	2.577
Diabetes mellitus	1.204	0.394	9.359	1	0.002	3.334	1.541	7.210
stroke/TIA2	0.050	0.569	.008	1	0.930	1.051	0.345	3.208
Hypertension	-0.204	0.428	.228	1	0.633	.815	0.352	1.887
Heart failure	0.914	0.549	2.767	1	0.096	2.493	0.850	7.317
smoking CURRENT =1 NEVER = 0 EX =2			7.144	2	0.028			
smoking CURRENT =1 NEVER = 0 EX =2(1)	1.271	0.576	4.860	1	0.027	3.563	1.151	11.025
smoking CURRENT =1 NEVER = 0 EX =2(2)	0.964	0.414	5.431	1	0.020	2.622	1.166	5.898

Table17: Linear regression of NLR for AASI

Coefficients[a]						
Model		Unstandardised Coefficients		Standardised Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	0.431	0.010		42.615	<0.001
	NLR	0.010	0.003	0.116	3.369	<0.001
a. Dependent Variable: AASI						

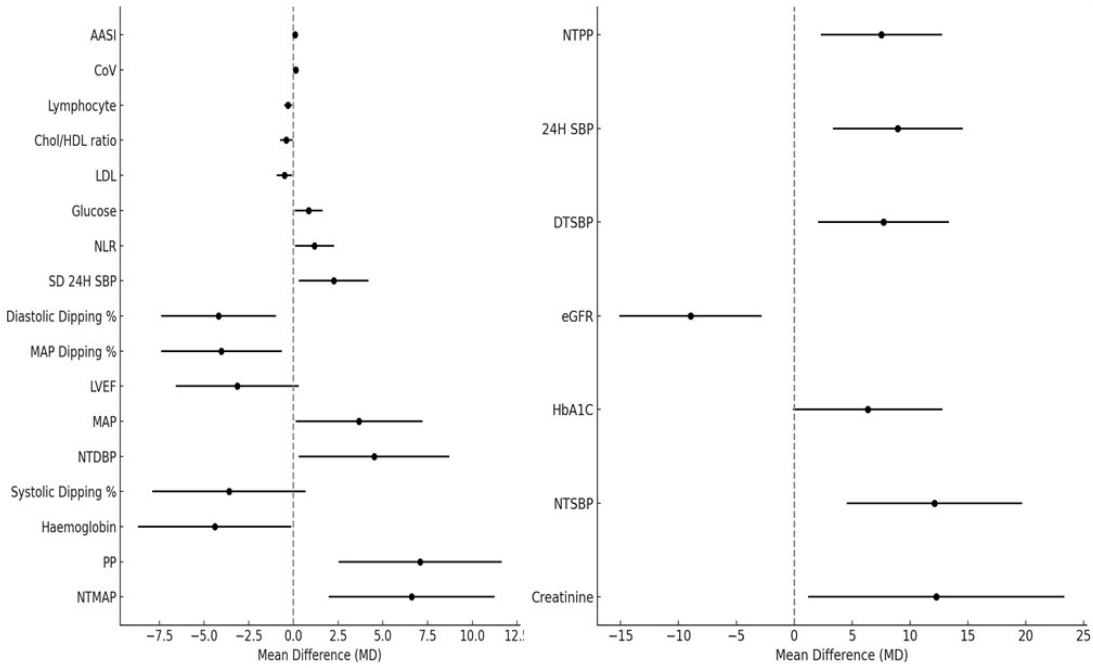

Figure 3: Forest Plot of MDs. This plot showed the MD and 95% CI for variables including blood pressure indices and blood results. Each point represents the MD for a variable MACE and no-MACE group, with the line spanning the 95% CIs

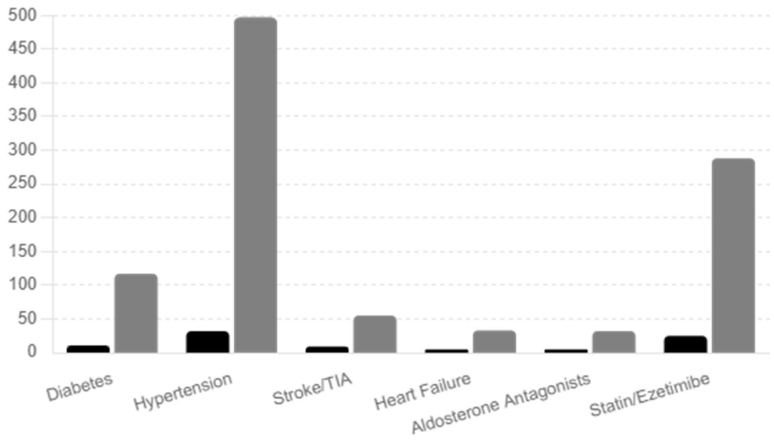

Figure 4: Bar chart of statistically significant categorical variables between MACE and non-MACE group. (Grey colour=non-MACE, black=MACE)

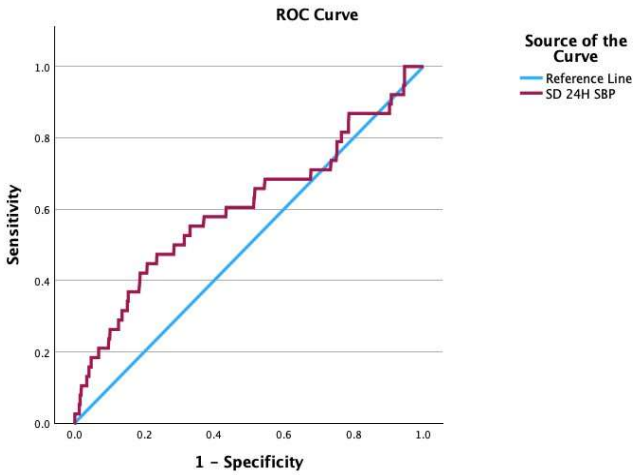

Figure 5: ROC curve analysis of SD 24-Hr SBP for MACE

Table 18: Multivariate Cox regression analysis of dichotomised SD 24-Hr SBP in predicting MACE

Variable	B	SE	Wald	df	Sig	Exp(B)	95% CI for Exp(B) (Lower)	95% CI for Exp(B) (Upper)
Dichotomised SD 24-Hr SBP	0.813	0.332	6.007	1	0.014	2.254	1.177	4.316
Age	0.057	0.017	11.100	1	<0.001	1.059	1.024	1.095
Ischaemic heart disease	0.101	0.369	0.074	1	0.785	1.106	0.536	2.280
Diabetes mellitus	0.361	0.383	0.887	1	0.346	1.434	0.677	3.039
Stroke/TIA	0.812	0.399	4.142	1	0.042	2.252	1.031	4.923
Heart failure	0.971	0.491	3.904	1	0.048	2.639	1.008	6.911
Hypertension	0.837	0.453	3.419	1	0.064	2.309	0.951	5.606
Smoking CURRENT = 1 NEVER = 0 EX = 2			2.737	2	0.255			
Smoking CURRENT = 1 NEVER = 0 EX = 2(1)	0.248	0.421	0.349	1	0.555	1.282	0.562	2.924
Smoking CURRENT = 1 NEVER = 0 EX = 2(2)	-0.528	0.401	1.734	1	0.188	0.590	0.269	1.294

Table 19: Multivariate Cox regression analysis of dichotomised SD 24-Hr SBP in predicting ACS

Variable	B	SE	Wald	df	Sig	Exp(B)	95% CI for Exp(B) (Lower)	95% CI for Exp(B) (Upper)
Dichotomised SD 24-Hr SBP	0.935	0.443	4.452	1	0.035	2.547	1.069	6.070
Age	0.076	0.027	7.895	1	0.005	1.079	1.023	1.138
Ischaemic heart disease	1.700	0.470	13.096	1	<0.001	5.473	2.180	13.743
Diabetes mellitus	0.734	0.466	2.485	1	0.115	2.084	0.836	5.190
Stroke/TIA	-14.321	463.370	0.001	1	0.975	0.000	0.000	(undefined)
Heart failure	-1.475	1.060	1.934	1	0.164	0.229	0.029	1.829
Hypertension	1.085	0.758	2.052	1	0.152	2.960	0.670	13.072
Smoking CURRENT = 1 NEVER = 0 EX = 2			5.561	2	0.062			
Smoking CURRENT = 1 NEVER = 0 EX = 2(1)	-0.207	0.663	0.097	1	0.755	0.813	0.222	2.983
Smoking CURRENT = 1 NEVER = 0 EX = 2(2)	-1.255	0.535	5.506	1	0.019	0.285	0.100	0.813

Figure 6: Kaplan-Meier analysis of dichotomised SD 24-Hr SBP for MACE

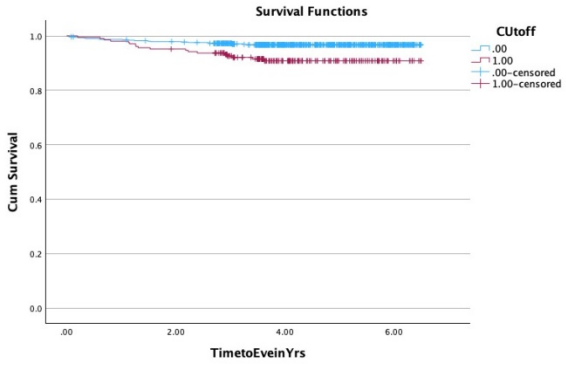

Table 20: Kaplan-Meier analysis

	Chi-Square	Degrees of Freedom (df)	p-value (Sig)
Long Rank	10.764	1	0.001

Table 21: Means for survival time

Dichotomised SD 24-Hr SBP	Mean Estimate	Std. Error	95% CI (Lower Bound)	95% CI (Upper Bound)
0	6.325	0.037	6.251	6.398
1	6.072	0.096	5.883	6.261
Overall	6.264	0.037	6.191	6.337