ESTIMATING NORMAL AND REFERENCE AORTIC PULSE WAVE VELOCITY FOR THE NEW ZEALAND POPULATION: IMPROVING STROKE AND CARDIOVASCULAR RISK PREDICTION

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Abstract

Arterial stiffness is expressed as the reduced capability of physiological expansion and contraction of arteries during changes in blood pressure (BP). It is measured as carotid-femoral pulse wave velocity (cf-PWV) and is well-established as an additional, independently predictive metric for cardiovascular disease (CVD) events, particularly in the presence of diabetes, obesity and stroke. Age and hypertension are the two risk factors that are consistently and independently associated with arterial stiffness. Current New Zealand (NZ) guidelines make no recommendations for measuring arterial stiffness during CVD risk assessment for any of the major ethnic groups. This is partly due to limited information about the normal and reference values of PWV in the NZ population. The present study is a first step towards building this evidence-base to enhance the accuracy of prediction of stroke and CVD risk in the healthy adult population in NZ. It is aimed at estimating normal and reference PWV values and investigating their association with established cardiovascular risk factors.

This observational cross-sectional study included 120 participants grouped into three age groups (18-30, 31-60, and >60 years) and four BP categories (normal, elevated, stage 1 and stage 2). Participants were subjected to measurement of cf-PWV using Doppler ultrasound imaging for three to five cardiac cycles in a supine position. Descriptive and inferential analysis was made for the Normal Value Population (NVP) and Reference Value Population (RVP). Based on the positive correlation of cardiovascular risk factors to PWV, linear regression models for predicting PWV were developed.

The mean PWV for the whole study population was 5.88 ± 1.49 m/s and increased systematically with age ($p<0.001$) with a more profound rise in the RVP group ($p<0.001$, $z =-4.98$). This overall mean PWV value is lower than that found in studies conducted

overseas. However, mean PWV values among the age groups were not different between the NVP and RVP groups ($p>0.05$). Additionally, a significant difference in mean PWV in the European and non-European population ($p= 0.004$) indicated ethnicity being an important part of the assessment. The cf-PWV was found to be positively correlated with age ($r=0.71$), mean BP ($r=0.49$), BP categories ($r=0.50$), and being in the NVP or RVP group ($r=0.46$) (each having $p<0.01$). Our multiple linear regression model accounted for 57% of the variance in predicting PWV using variables- age, BMI, BP category and being in NVP or RVP group. Mean PWV in the 18-30 age group was unexpectedly lower at high BP levels when compared to all age groups in the normal BP group category. Mean PWV at 'elevated BP' levels were lower than for other BP categories.

In conclusion, this study provides an initial dataset of normative PWV values in healthy New Zealanders. A positive correlation was found between PWV and being in the NVP or RVP group besides other cardiovascular risk factors, and this was a study demographic specific finding. The overall low PWV values in the NZ cohort compared with the regression equations reported from European, American, and African studies may be due to a unique ethnic mix of the study population and warrants further investigation. This study can serve as a starting point for future studies to refine the use of PWV for CVD risk prediction, with larger sample sizes and the inclusion of other cardiovascular risk predictors.

Keywords: pulse wave velocity, arterial stiffness, normal and reference PWV values, cardiovascular risk factors, bioimpedance

Table of Contents

List of Figures

List of Tables

Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

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Chapter 1: Introduction

1.1 Overview

There is significant interest in cardiovascular risk prediction in order to better target preventative therapy in those individuals considered by current guidelines to be at low or moderate risk. This thesis describes quantitative cross-sectional research focused on the estimation of normative values of aortic pulse wave velocity (PWV) as a clinical biomarker for arterial stiffness and its association with existing cardiovascular risk factors. This chapter will outline the context of the doctoral research by outlining the background of the research, aims and objectives, rationale of the research and a brief structural snapshot of the thesis.

1.2 Background

The World Health Organisation (WHO) factsheet states that for the year 2016, coronary heart disease (ischemic heart disease) and cerebrovascular diseases (stroke) have been the leading cause of mortality for the last 15 years. Furthermore, these two diseases have been predicted to hold this position even up to 2030 (Deaton et al., 2011; WHO, 2016, 2018). Cardiovascular diseases (CVD) cross geographic, socioeconomic or gender boundaries (Deaton et al., 2011). Developed countries and lower-middle income countries have higher a prevalence of cardiovascular risk factors, incidence of CVD and stroke, and all-cause mortality (Deaton et al., 2011; Feigin et al., 2015). Additionally, the 2015-Update on Heart Disease and Stroke Statistics by the American Heart Association (AHA) highlighted that both CVD and stroke are the leading causes of health and economic burden in the US and worldwide. The reported number of CVD deaths is expected to reach >23.6 million by 2030; up from 17.3 million in 2015 (Mozaffarian et

al., 2015). Stroke burden is expected to rise to 61 million disability-adjusted life years globally in the year 2020 from ≈38 million in 1990; this is due to an ageing population (Feigin, Krishnamurthi, Barber, & Arroll, 2014). These figures will surely increase in the near future as cardiovascular risk factors continue to rise.

Cardiovascular disease risk factors such as age, sex, ethnicity, and family history are non-modifiable, and hence cannot be targeted for prevention of cardiovascular events. On the other hand, risk factors such as physical inactivity, obesity, diet, smoking, diabetes, alcohol consumption, high cholesterol and hypertension are modifiable; controlling these would lower the incidence of CVD and stroke globally (O'Donnell et al., 2016; Truthmann et al., 2015). In a quest to identify modifiable cardiovascular risk factors, various population-based scoring systems have been used in clinical practice, such as the Framingham Risk Score (Mitchell et al., 2007), CVD risk assessment and a management decision support system (called 'PREDICT') (Wells et al., 2015), and the Heart Systemic Coronary Risk Evaluation (HeartSCORE) (Pereira et al., 2014). These scores are tools for assessing cardiovascular risk which is commonly estimated based on the weighting of five conventional cardiovascular risk factors (age, gender, systolic blood pressure, cholesterol and smoking). However, the questionable predictability of the scores in low cardiovascular risk individuals may necessitate the incorporation of various new risk markers, such as aortic PWV, to improve the risk prediction (Pereira et al., 2014). Additional cardiovascular biomarkers include the level of C-reactive protein, carotid intima-media thickness, renin, fibrinogen and a variety of genetic variants. A study evaluating these additional multi-biomarker approaches in ambulatory person added little to the existing risk estimates, such as that derived from the Framingham Heart Study (T. J. Wang et al., 2006).

Loss of elasticity and distensibility in the arteries is a physiological degenerative process leading to arterial stiffness (Cecelja & Chowienczyk, 2012; Palombo & Kozakova, 2016). Arterial stiffness has been recognised and defined as a clinical predictive marker for cardiovascular events, particularly in the presence of diabetes and obesity (McLean, Williams, Mann, Miller, & Parnell, 2013; Ministry of Health, 2012). Arterial stiffness predominantly shows the association between two risk factors; age and hypertension (Cavalcante, Lima, Redheuil, & Al-Mallah, 2011; Cecelja & Chowienczyk, 2012). It is assessed by a variety of invasive and non-invasive methods among which the most frequently used, validated and established reference method utilises the carotid-femoral pulse wave velocity (cf-PWV). PWV is the rate at which a pulse wave travels between two sites along an arterial segment and is considered to be the current gold standard to measure arterial stiffness (Cavalcante et al., 2011).

There are several approaches for measuring PWV, such as assessment conducted either locally (at a specific arterial site), or regionally (along an arterial segment), that have led to methodological variations among the vast research work published over time. This has led to concerns about irregularities in recording the distance, transit time, different protocols for measuring devices, and varying inclusion/exclusion criteria for recruitment. Expert consensus documents were published after a rigorous review of the literature and present guidelines for measuring PWV (Laurent et al., 2006; Van Bortel et al., 2012). The cf-PWV methods measured regionally at the common carotid and femoral artery were chosen as the recommended standard. The measurement of the superficial arterial distance and pulse transit time was standardised. The cf-PWV has been related to the prediction of future cardiovascular risk even after accounting for other accepted cardiovascular risk factors. PWV improves the prediction of risk, and it does so equally

for cardiac and cerebrovascular events (Cavalcante et al., 2011; Laurent et al., 2006; Milan et al., 2019b; Van Bortel et al., 2012). A review paper on the current understanding of arterial stiffness and its future directions has commented that cf-PWV is a global measure of arterial stiffness and is a strong predictor of CVD, stroke, hypertension, and all-cause/cardiovascular mortality and morbidity (Cavalcante et al., 2011).

In the context of New Zealand (NZ), NZ census data reveals that the NZ population shares a large ethnic diversity and is getting older (Statistics New Zealand, 2014). In addition to this, it was also revealed that CVD risk for Māori people with diabetes is 30% higher than other ethnic groups (Kenealy et al., 2008). It was evident through the NZ census report that CVD and stroke are the leading cause of mortality and morbidity in NZ (Statistics New Zealand, 2014). According to a report published by the New Zealand Institute of Economic Research in 2018, the annual stroke cohort hospitalisation costs at least $NZ 880 million. Each stroke is also associated with a cost of $NZ 60,000 to 99,000 over a period of five years (Hogan & Siddharth, 2018). Therefore, a readily applicable means for early and more accurate identification of people at high CVD risk will reduce the medical, economic, and social burden and provide a better chance to mitigate the risk of CVD. However, in order to establish an accurate association of PWV with CVD risk, the normative values specific to the NZ population needs to be established.

1.3 Aims and objectives

The main aim of this study is to define and estimate normal and reference PWV values in the NZ population. In addition to this, the study also explores the association of PWV with CVD risk factors and ethnicity.

1.4 Context of the doctoral research

It has been shown that PWV values vary due to differences in measurement methods and populations studied (Boutouyrie & Vermeersch, 2010). In 2007, the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH) guidelines for the management of arterial hypertension in Europe included PWV assessment as a routine cardiovascular assessment practice based on the expert consensus document (Laurent et al., 2006; Mancia et al., 2007). Mancia et al. (2013) stated: "Although the relationship between aortic stiffness and events is continuous, a threshold of 12 m/s has been suggested by the 2007 ESH/ESC Guidelines as a conservative estimate of significant alterations of aortic function in middle-aged hypertensive patients." (p 2176). The European guidelines were further updated in 2012 which have recommended a new threshold value of 10 m/s quoting: "Adapted to the new standard distance (common carotid artery - common femoral artery x 0.8), it would become 9.6 m/s. We propose 10 m/s as a new standard cut-off value for cf-PWV because this is an easy figure to use in daily practice." (Van Bortel et al., 2012). A study estimated the normal and reference PWV values in a European population with respect to age and blood pressure categories (Boutouyrie & Vermeersch, 2010). Later, the AHA made recommendations for standardising research on arterial stiffness and PWV (Townsend, 2015). The updated NZ guidelines for CVD risk assessment and management for primary care published in 2018 advised assessments to be based on new five-year CVD risk prediction equations published from the NZ PREDICT study (Ministry of Health, 2018). In 2002, the University of Auckland established the PREDICT cohort and developed a web-based CVD risk assessment and management tool (Wells et al., 2015). It is an ongoing, ever-growing database in NZ that is integrated into the primary healthcare system (Pylypchuk et al.,

2018; Wells et al., 2015). The PREDICT study did overcome the limitation of the Framingham study taking into account NZ's ethnic variability and newly identified risk factors (Ministry of Health, 2018). However, NZ stroke guidelines make no recommendations for measuring arterial stiffness during CVD risk assessment, in contrast to the European or AHA guidelines (Ministry of Health, 2018; New Zealand Guidelines Group, 2012).

Given that NZ is officially a bicultural nation along with being a home to people belonging to multiple cultures, and extensive ethnic variability, the reference values from other published guidelines cannot just be applied to the NZ population. Limited information on the normative values of PWV in the NZ population has restricted the application of arterial stiffness assessment in clinical settings. Estimating the normative values of PWV with a proactive approach to CVD risk assessment would help in attaining the long-term community health goals of the NZ government. Therefore, the current doctoral research study mainly focusses on the estimation of normative PWV values for the NZ population. Secondly, the work also attempts to study the association of PWV with cardiovascular risk factors and different ethnic groups.

The present doctoral research work is a pilot study that begins with an attempt to construct PWV normal and a reference value database in the NZ population. The area covered by this study is confined within the Auckland area, for practical reasons. The study population consisted of healthy adults from all age groups and blood pressure (BP) categories, with and without existing cardiovascular risk factors. Moreover, being the first attempt, the assessments were made using the reference standard 'Doppler ultrasound' instead of any other commercially available devices. Furthermore, the mean PWV scores are analysed to establish the correlation and predictability of PWV with

cardiovascular risk factors. The findings from this research work can be used to guide future studies to assess the PWV predictability in different physiological conditions using other commercial devices, feasibility studies and eventually expanding the data pool to contribute to official CVD risk prediction and management guidelines.

1.5 Thesis structure

Chapter 1: Introduction

This chapter mainly defines the background of the study in the context of NZ CVD status. In addition to this, it also describes the aims and objectives, and the context of the doctoral research.

Chapter 2: Literature review

This chapter provides an overview of the literature relevant to this study by referring to various articles, books and journals which are related to the arterial system, arterial stiffness, pulse wave velocity, current devices to measure PWV, the clinical application of aortic PWV as an indicator, factors affecting PWV estimation, and the status quo of CVD risk assessment in NZ.

Chapter 3: Research Methods

This chapter defines various research methods which are used to collect the data in the form of research design, the study population, instruments, procedure, location and training techniques.

Chapter 4: Results

This chapter presents the collected data by using a visual presentation in the form of bar graphs, and pie charts. In addition to this, various kinds of statistical tools were used in order to analyse and determine accurate results.

Chapter 5: Discussion and Conclusion

This chapter analyses, interpret and discusses the findings observed during the study by the researcher. Additionally, it points out the novel contributions of the research, limitations and future research work needs identified by this research.

Appendix M: Preliminary trials measuring PWV with bioimpedance

This appendix reports the findings of experiments conducted as a "proof of concept" for measuring PWV with bioimpedance. The concept was included as a part of the PhD to validate the prototype of a novel bioimpedance-measuring device against Doppler ultrasound (Study I) and estimate the normal and reference PWV values in the NZ population using this device (Study II). However, the initial tests results were not promising. With time and resource restrictions to the study protocol, preliminary experiments were carried out using 'PhysioFlow' (a non-invasive haemodynamic monitor; Manatec Biomedical, Paris, France) and directed towards investigating methodological issues for bioimpedance measurement of PWV. The three experimental trials shown in Appendix M are the observations for trying different electrode placement and sitting positions at different arterial sites to measure the transit time and PWV.

Chapter 2: Literature review

2.1 Overview

This chapter will introduce the anatomy and physiology of the human blood circulatory system, arterial system and the concept of arterial pulse wave reflection. The chapter will then define arterial stiffness and ways to measure it supported by the reviewed literature. Furthermore, aligning with the scope of the research, pulse wave velocity (PWV) will be discussed in detail as a method to measure arterial stiffness. Different methodological approaches to measuring the PWV and the issues/factors affecting the estimation related to it will be mentioned. It will be followed by a structured literature review of available commercial devices for PWV measurement. The chapter will later focus on the literature highlighting the clinical application of aortic PWV as a measure for arterial stiffness and a predictor of CVD. The final section of this chapter presents a picture of cardiovascular risk assessment in NZ.

2.2 Arterial system

2.2.1 Anatomy and physiology

The blood circulatory system consists of two components: the heart and blood vessels. The blood vessels are a dynamic closed delivery system that starts and finishes at the heart. Physiologically, this network supplies all tissues and cells with oxygen, nutrients and also removes deoxygenated blood and waste products (Marieb & Hoehn, 2016). The arteries, capillaries and veins are the three major types of blood vessels. With every contraction of the left ventricle, blood rushes through the aorta to larger arteries and smaller arteries to the smallest arterioles, finally reaching the capillaries feeding the organs and tissues. Moving through the other half of the circulation, blood is then

drained into venules (smallest veins), to small and larger veins to get emptied into the heart's right atrium (Marieb & Hoehn, 2016; Nichols et al., 2008; Shirwany & Zou, 2010). Figure 1 summarises the systemic vessel channel and its relationship to the pulmonary circulation. Deoxygenated blood pumped through the right ventricle enters the pulmonary circulation to get oxygenated in the lungs, and ultimately, be a part of the systemic circulation for the next cardiac cycle.

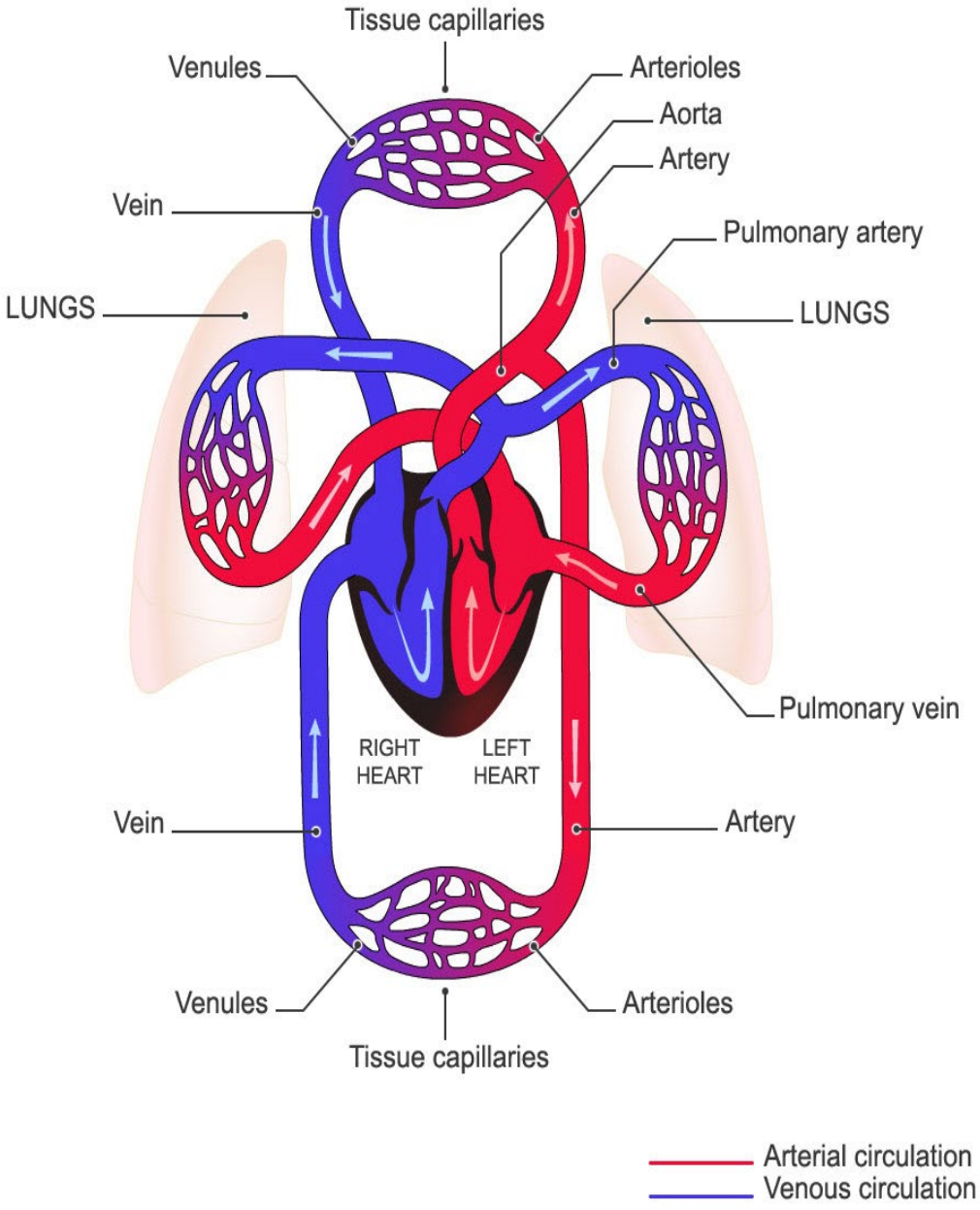

Figure 1: Human circulatory system consisting of the systemic and pulmonary circulation (Urgo Medical, 2009)

Structurally, most blood vessels walls are made up of three concentric layers. The outermost layer is made up of collagen fibres and is known as the *tunica externa* or *tunica adventitia* (coming from outside). It protects, reinforces and anchors the vessel to the surrounding structures. The middle layer, or *tunica media,* contains elastic tissues and smooth muscles. It actively regulates the sympathetic vasomotor nerve fibres causing vasoconstriction and vasodilation. Moreover, it bears the main responsibility for regulating blood pressure (BP) and blood circulation. Moving inside, the layer that covers the hollow cavity (lumen) is the *tunica intima* and comprises mainly of endothelial cells. This is the layer or lumen that the circulating blood comes into intimate contact with. Figure 2 shows a graphical representation of the three main layers of an arterial wall.

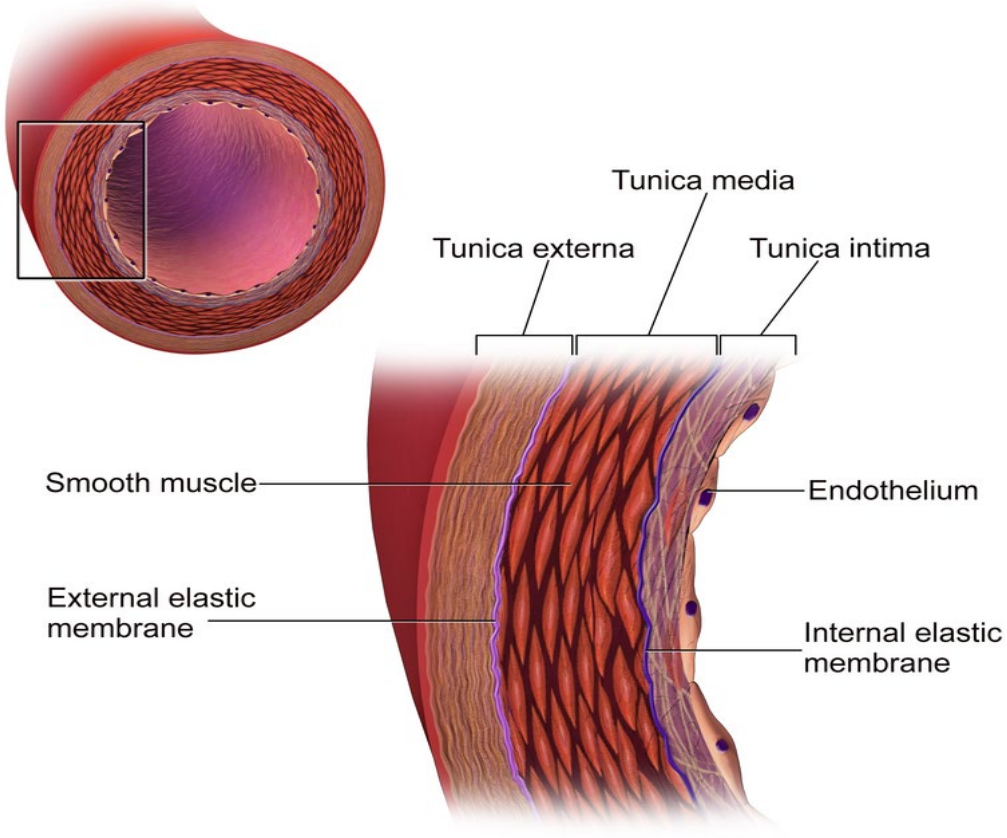

Figure 2: Structure of the three layers of an arterial wall (Blausen.comstaff, 2014)

Based on the functionality and size, the arterial system is sub-classified into three groups: elastic arteries, muscular arteries and arterioles. The structural difference between the elastic and muscular arteries is shown in Figure 3.

Elastic arteries: also known as conducting arteries, are large thick arteries (average lumen diameter: 1 to 2.5 cm) that receive the blood ejected from the heart during systole and assist in distributing it out to the peripheral system during diastole. These arteries have large lumens and contain elastin giving rise to the low-resistance vessel pathways. Because of the elastic artery's ability to contract and expand with every heartbeat, the blood flows continuously instead of switching on and off with the pulsating rhythm of the heart.

Muscular arteries: are comparatively small arteries with an average lumen diameter of 6.0 mm. These arteries (also known as the distributing arteries) are usually found in the lower limbs and can alter their tone (stiffness) to modulate the velocity of the pressure wave received by them from the large elastic arteries. They contain more smooth muscles than elastin making them less stretchable.

Arterioles: are the smallest in size (average lumen diameter: 0.3 mm to 10 µm). Large arterioles are mainly composed of smooth muscles and have low elastin content. Smaller arterioles are found near the capillary beds and have a thin layer of smooth muscle cells around the endothelial lining. Change in the lumen diameter alters peripheral resistance to blood flow and maintains mean blood pressure (MBP). Therefore, it is aptly known as a resistance vessel (Marieb & Hoehn, 2016; Nichols et al., 2008; Shirwany & Zou, 2010).

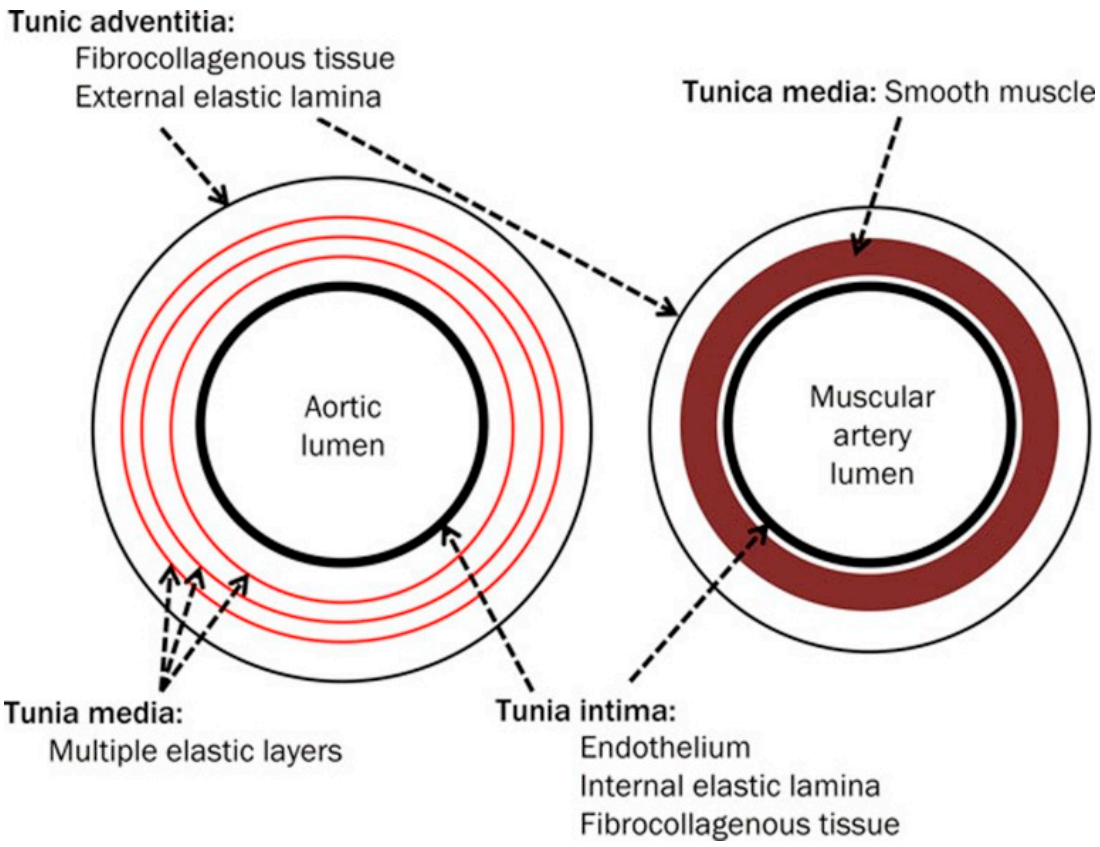

Figure 3: Structure of elastic and muscular artery (Shirwany & Zou, 2010)

2.2.2 Pulse wave reflection

During a normal cardiac cycle, when the heart contracts it sends a forward propagating pressure wave, which is distinct from the forward displacement of blood. This wave, also known as the 'incident wave,' travels along the aorta and reaches the branched arteries. At this point, it encounters a characteristic impedance mismatch due to the varying sizes of the vascular arteries. As a result, the forward wave tends to amplify further while simultaneously producing a 'reflected wave' (Figure 4). The reflected waves from the several branches aggregate and travel back toward the heart to meet the forward wave. The arrival of the reflected wave in the ascending aorta in late systole and early diastole produces an augmented pressure wave. The pressure of this pulse wave depends on a mixture of factors: stroke volume, aortic diameter, aortic compliance, pulse wave

velocity and the distance of the site of reflection from the heart (Hirata, Kawakami, & O'Rourke, 2006; Shirwany & Zou, 2010; Vasan, 2008).

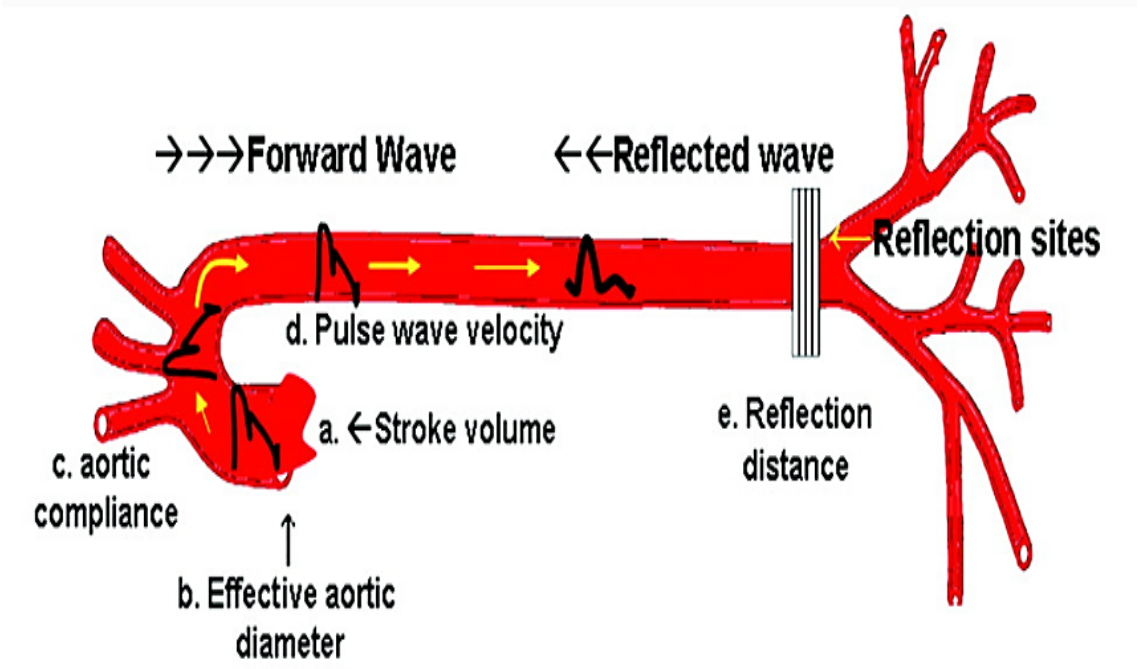

Figure 4: The incident and reflected pressure wave (Vasan, 2008)

The physiological phenomenon of pressure wave reflection can be recorded and depicted as an aortic pressure waveform. A typical pressure waveform, as shown in Figure 5, will have the following constituents; the *incident wave*, starting from the heart gives rise to two further components: a *forward pressure wave* and a *reflected pressure wave*. The forward pressure wave travels distally along the aorta, whereas, the reflected pressure wave runs back to the aortic root. The major sites of reflection are the high-resistance arterioles; however, it is often considered that the major functional reflection site is in the proximal thoracic aorta (near the termination of the abdominal aorta, corresponding to the highly perfused renal arteries). The total pressure recorded as the pressure waveform in the aorta is the sum of the forward and backward pressure components. The backward wave increases the pressure, and this is seen as *pressure*

augmentation in the pressure waveform. In a healthy person, because of a compliant vasculature, both the forward wave and backward waves travel relatively slowly. Because of this slow speed, the reflected wave arrives during diastole and contributes to the augmented *diastolic blood pressure* (DBP), whereas the systolic blood pressure (SBP) is mainly determined by the forward wave. Conversely, in a stiff aorta, the backward reflected wave moves at a fast pace and superimposes on the incident forward wave during systole leading to a rise in the pressure augmentation and pulse pressure (Hirata et al., 2006; O'Rourke, Pauca, & Jiang, 2001; Shirwany & Zou, 2010; Vasan, 2008).

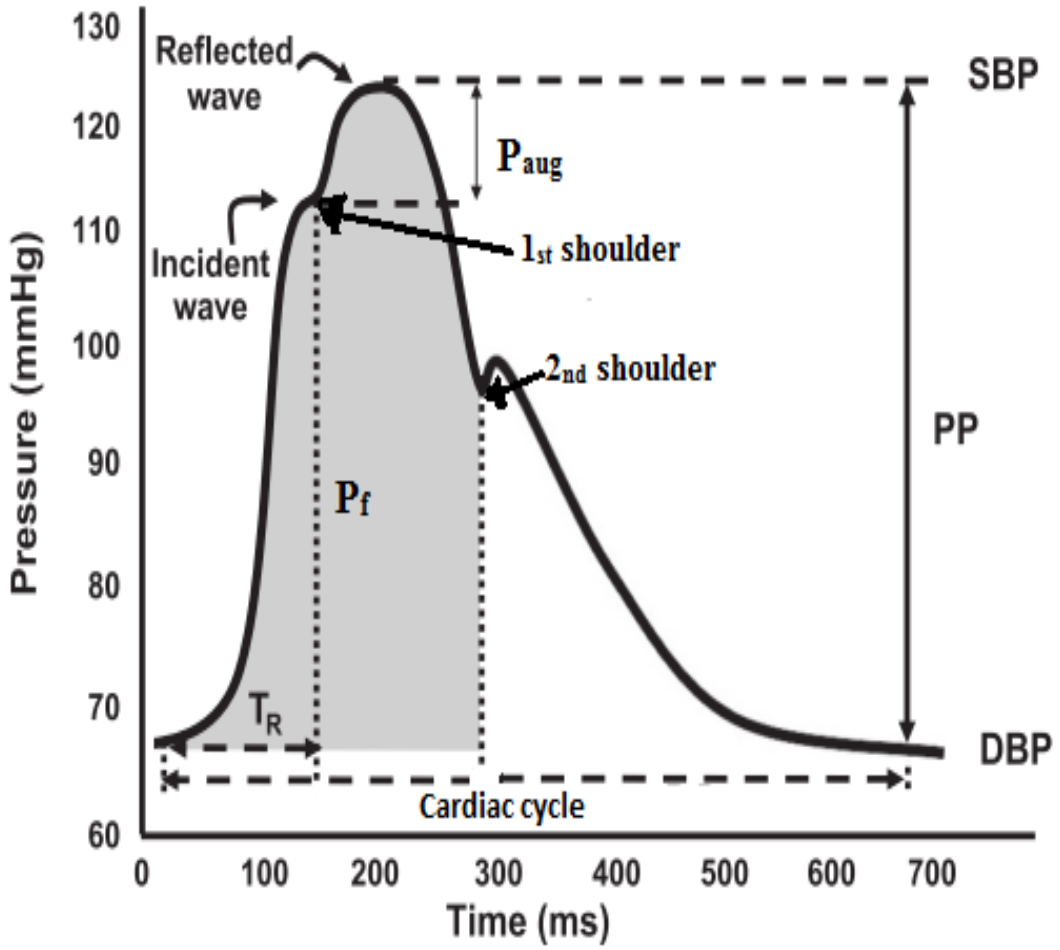

Figure 5: Aortic pressure waveform of a healthy adult (Donley et al., 2014)
P_f: Forward Pressure Wave, P_{aug}: Pressure Augmentation, T_R: Transit time; PP: Pulse Pressure, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure

The graph shown in Figure 5 represents the pressure at the aortic root and starts with the opening of the aortic valve. The first shoulder in the waveform resulting from left ventricular ejection corresponds with the arrival of the reflected wave which augments the pulse pressure to the maximum. The fall in the pressure continues until it reaches the second shoulder, which represents the closure of the aortic valve. Augmentation pressure is the pressure added by the reflected wave to the incident wave. The peak of the pressure waveform is the recorded SBP, and the trough is the DBP marking the end of the cardiac cycle. The transit time is related to the time taken by the incident wave starting from the aorta to reach the reflection site and come back to the aortic valve (Cecelja & Chowienczyk, 2012; Donley et al., 2014; Shirwany & Zou, 2010). The normal physiological process of pressure wave reflection gets modulated in an older or unhealthy person with stiffened and thickened arteries. The incident wave travels faster; hence, it gets reflected and returns earlier during the systolic phase to superimpose on the incident wave. As a result, there is an increase in the augmented pressure, and SBP, while the DBP falls sharply (Laurent et al., 2006; Shirwany & Zou, 2010).

Although the above discussion has primarily focused on the blood pressure waves propagating in the arteries, blood velocity waves exhibit similar wave propagation, speed and reflection behaviour. The term “pulse wave” can, therefore, refer to either pressure or velocity waves.

2.3 Arterial stiffness

2.3.1 Definition

Arterial stiffness or hardening of the arteries is a physiological phenomenon that occurs because of the degenerative process of the extracellular matrix of the arteries. Due to the stiffening, the arteries lose their elasticity and cushioning property. The ability to

expand and contract is reduced in response to pressure change. Two measures of arterial stiffness are 'compliance' and 'distensibility.' Compliance is the ability of a blood vessel to change in volume under pressure, and distensibility refers to the ability to distend or stretch under pressure; this relates more to arterial wall stiffness. In other words, compliance is a ratio of absolute volume change (ΔV) and pressure change (ΔP), C= ΔV/ ΔP. The inverse of compliance is the elastance or stiffness (E= ΔP/ ΔV). Another way to express compliance relative to the initial volume (V) is as a distensibility coefficient (Di), Di= ΔV/V x ΔP. Stiffened arteries tend to reduce these two parameters leading eventually to an increase in the velocity of the pressure pulse along the arterial tree (Briet, Boutouyrie, Laurent, & London, 2012; Cecelja & Chowienczyk, 2012; Palombo & Kozakova, 2016; Shirwany & Zou, 2010).

2.3.2 Mechanism of arterial stiffness

Clinical and epidemiological evidence suggests the emergence of arterial stiffness as a powerful predictor of cardiovascular risk. It is therefore of importance to elucidate the underlying mechanical and biological mechanisms that lead to the degeneration of arterial properties resulting in stiffening of the conducting vessel, a significant risk factor for cardiovascular morbidity and mortality (Avolio, 2013). As described by Chen et al. (2017), the pathophysiological mechanisms resulting in arterial stiffness can be classified into three major components: vascular structure, vascular function and blood pressure. Besides these components, there are several other factors, such as inflammation, oxidative stress, and the renin-angiotensin-aldosterone system (RAAS) that are linked to inducing arterial stiffness (Chen, Shen, Liu, & Yang, 2017; Neves, Cunha, Cunha, Gismondi, & Oigman, 2018). Moreover, a polymorphic variation of the fibrillin-1, angiotensin II type 1 receptor, and endothelin receptor genes have been reported to

influence the arterial stiffness (Avolio, 2013; Chen et al., 2017; Oliver & Webb, 2003). The mechanisms related to material properties such as collagen, elastin, and haemodynamics are considered to be 'passive mechanisms,' whereas mechanisms regulated at the cellular and molecular level, for example, inflammatory and nitric oxide pathways, aldosterone signalling and genetic factors are grouped together as 'active mechanisms' (Avolio, 2013).

Vascular structure

On analysing stiffened arteries under a microscope, it was observed that there was an increase in the number of collagen and matrix metalloproteinase. The elastin was also fragmented and diminished, and the endothelium was disorganised with infiltration of smooth muscle cells, macrophages and mononuclear cells (Chen et al., 2017). As mentioned earlier in section 2.3.1, the proteins, namely, collagen and elastin, are closely linked and important in providing arterial elasticity and strength. Larger arteries have a high elastin to collagen ratio compared to peripheral arteries (Chen et al., 2017; Oliver & Webb, 2003). Factors such as inflammation, and haemodynamic or genetic variation interfere with the balance of these proteins which leads to an increase in the stiffness of arteries. A study was conducted that aimed at investigating the effect of developing hypertension on thoracic aortic wall properties in spontaneously hypertensive rats. Functional alteration of the arterial wall properties of low compliance and distensibility was noted before developing hypertension. These changes were attributed to *tunica media* hypertrophy rather than loss of elasticity (vanGorp et al., 2000). Also, the intima-media thickness and cross-sectional area were found to be significantly higher, indicating vascular smooth muscle hypertrophy, adding to the development of arterial stiffness (Chen et al., 2017).

Vascular function

A condition where the endothelial layer of the small arteries does not function normally, is known as endothelial dysfunction. It results in a reduction of the levels of nitric oxide in the arteries and increases expression of pro-inflammatory factors (Hadi, Carr, & Al Suwaidi, 2005; Stoner, Young, & Fryer, 2012). Nitric oxide is a vasodilator and has anti-atherogenic properties inhibiting vascular smooth muscle proliferation. Blocking of the endothelium-released nitric oxide is associated with high arterial stiffness. Furthermore, vasodilators reduce smooth muscle tone causing a reduction of pulse wave reflection and an increase in arterial distensibility. On the other hand, a vasoconstrictor such as angiotensin II leads to a loss in arterial elasticity (Chen et al., 2017).

Blood pressure

The stress-strain relationship of arterial physiology plays a significant role in maintaining arterial health. The strain due to a change in the BP affects the functionality and stiffness of the arterial wall. Moreover, blood pressure plays a significant role in determining vessel wall structure, with remodelling occurring to compensate for changes in wall stress. At normal or low BP, elastin is more than capable of maintaining the elasticity, and the arterial wall is flexible/extensible. However, at high BP ranges, collagen in the artery wall uncoils and becomes the limiting elastic element. Collagen is stiffer than elastin, so the arterial wall becomes inextensible and stiff without structural changes (Chen et al., 2017; Oliver & Webb, 2003). In other words, with an increase in BP, the arterial wall compliance and distensibility decreases (Safar, Blacher, & Jankowski, 2011). The relevance of arterial stiffness as a fundamental property of the relationship between pulsatile BP and flow, and wave propagation phenomena, has been firmly established from investigations in the physical sciences. Arterial stiffness is closely linked to, but by

no means synonymous with, raised BP, and its physiopathology is still not fully understood. Aortic stiffness and arterial pulse wave reflections are key determinants of elevated central systolic pressure and are associated with adverse cardiovascular outcomes, independent of BP (Avolio, 2013).

2.3.3 Arterial stiffness and clinical relevance

This section will review the literature related to the history of examining the arterial pulse, assessing the pulse wave, structural and functional alteration due to stiffness, and association of arterial stiffness with ageing.

From ancient times, the physical examination of the arterial pulse has been an important part of the clinical examination by healers and medical professionals. One of the early records of examining the pulse comes from Egypt in 1600 BC (Breasted, 1930; Nelson et al., 2010). However, the modern era of recording the pulse started in the 19th century with the invention of the sphygmograph by Elienne Jules Marey (Snellen, 1980). The art of recording a pulse wave graphically and interpreting the shape of a pulse wave was refined over time by Mahomed (Mohamed, 1872; Nelson et al., 2010), Broadbent (Broadbent, 1890; Nelson et al., 2010) and Mackenzie (I. Mackenzie, Wilkinson, & Cockcroft, 2002; J. Mackenzie, 1902). Sphygmography was well established in the early 20th century as a means of describing cardiovascular conditions but rapidly fell out of favour with the introduction of the cuff-mercury sphygmomanometer by Riva-Rocci to measure BP in 1896 (I. Mackenzie et al., 2002; Nelson et al., 2010; O'Rourke et al., 2001).

Mohamed introduced the concept of pulse wave analysis by showing the difference in the radial pressure waveform and the carotid waveform, and its relationship with high BP and radial pressure waveform (Mohamed, 1872). Taking this further, McDonald explained the phenomenon of the different pressure wave at the central and peripheral

arterial sites through pulse wave reflection (McDonald, 1996; O'Rourke et al., 2001). In 1929, Werner Forssman used cardiac catheterisation to cement the correlation between central and peripheral pressure waveforms (Nelson et al., 2010). The original sphygmogram suffered from impracticality and was prone to artefacts. Modern tonometer devices using the piezo-electric principle were developed with better accuracy, reliability and ease of use (O'Rourke et al., 2001). These devices have reinstated the clinical importance of studying the arterial waveforms and arterial stiffness along with the regular BP assessment. What started as an understanding of fluid mechanics, haemodynamic and reflected wave properties (Nichols et al., 2008; Oliver & Webb, 2003), have now changed with advancements in technology to reflect the role of arterial stiffness in CVD and other physiological conditions at the molecular level.

Arterial stiffness has been regarded as a reliable marker of arterial structural and functional alteration after abundant experimental and clinical studies (Chen et al., 2017). It has been named as a growing epidemic concerning increased cardiovascular risk, dementia and mortality (Zieman, Melenovsky, & Kass, 2005). The physical stiffening of the large arteries is the central paradigm of vascular ageing. Indeed, stiffening in the larger central arterial system, such as the aortic tree, significantly contributes to cardiovascular diseases in older individuals and is positively associated with systolic hypertension, coronary artery disease, stroke, heart failure and atrial fibrillation, which are the leading causes of mortality in developed countries (Nilsson, Boutouyrie, & Laurent, 2009; Shirwany & Zou, 2010).

Ageing is closely related to changes in our arterial system and especially to increased stiffness of the arterial wall. The arteries progressively change with chronological ageing.

Diseases such as diabetes, hypertension, and renal failure can accelerate the ageing of the arteries. However, it is debatable whether biological ageing or chronological ageing should be used in predicting cardiovascular risk (Boutouyrie & Sønder, 2015). As the ageing of the arteries can be regarded as a risk factor of CVD, intervention on accelerated vascular ageing seems important. Nilsson et al. (2009) commented that early vascular ageing could be assessed non-invasively by parameters such as arterial stiffness, central BP, carotid intima-media thickness, and endothelial dysfunction. These parameters are considered to be "tissue biomarkers" and are better additional predictors to standard cardiovascular risk scores as opposed to a "circulating biomarker" (like c-reactive protein) (Boutouyrie, Briet, Collin, Vermeersch, & Pannier, 2009; Boutouyrie & Sønder, 2015; Hamilton, Lockhart, Quinn, & McVeigh, 2007; Nilsson et al., 2009; Steppan, Barodka, Berkowitz, & Nyhan, 2011).

The ongoing development of methods for evaluating arterial stiffness *in vivo* will facilitate the translation of some of this clinical research into practical application. Indeed, clinical studies have already established that arterial stiffness is independently associated with cardiovascular outcomes in most of the situations where it has been examined (Ben-Shlomo et al., 2014; Boutouyrie & Bruno, 2018; Chen et al., 2017). The reviewers commented that although there are a number of therapies available to reduce arterial stiffness, it is yet to be shown whether the agents that work for arterial stiffness will also reduce cardiovascular events independently of any of their other effects on recognised risk factors (Payne, Wilkinson, & Webb, 2010). Though there is evidence of arterial stiffness being a better predictor of target organ damage, for example, renin-angiotensin blockers improve arterial stiffness irrespective of BP in end-stage renal diseases (Boutouyrie & Bruno, 2018). A recent review by Boutouyrie (2018) commented

that the use of arterial stiffness as a tool for monitoring the effectiveness of treatment of CVD has yet to be proven.

2.3.4 Measurement of arterial stiffness

Over the past decade, arterial stiffness measurement has been acknowledged clinically as a predictor of cardiovascular outcome (Shirwany & Zou, 2010; Townsend, 2016). The gold standard to measure the arterial stiffness haemodynamically would be to directly approach the blood vessel in a surgical manner. However, this is not practical nor desirable. Therefore, more indirect approaches and techniques to assess arterial stiffness have evolved recently. There are several non-invasive methods to measure arterial stiffness, mainly based on recording the change of blood vessel size and mechanical properties of arteries by measuring the increase in pressure, change in arterial diameter, or wall-thickness, PWV, bioimpedance, or central pulse wave analysis. The following methods can be classified further on the location into systemic, central, local, and regional (Julio A. Chirinos, 2012; Hamilton et al., 2007; Laurent et al., 2006; Sakuragi & Abhayaratna, 2010) as shown in Figure 6. Central, local, and regional assessments are based on the propagative method, which assumes that a pulse wave travelling along a given artery has a finite velocity. By contrast, the systemic measurement of arterial stiffness is a non-propagative method (Hamilton et al., 2007).

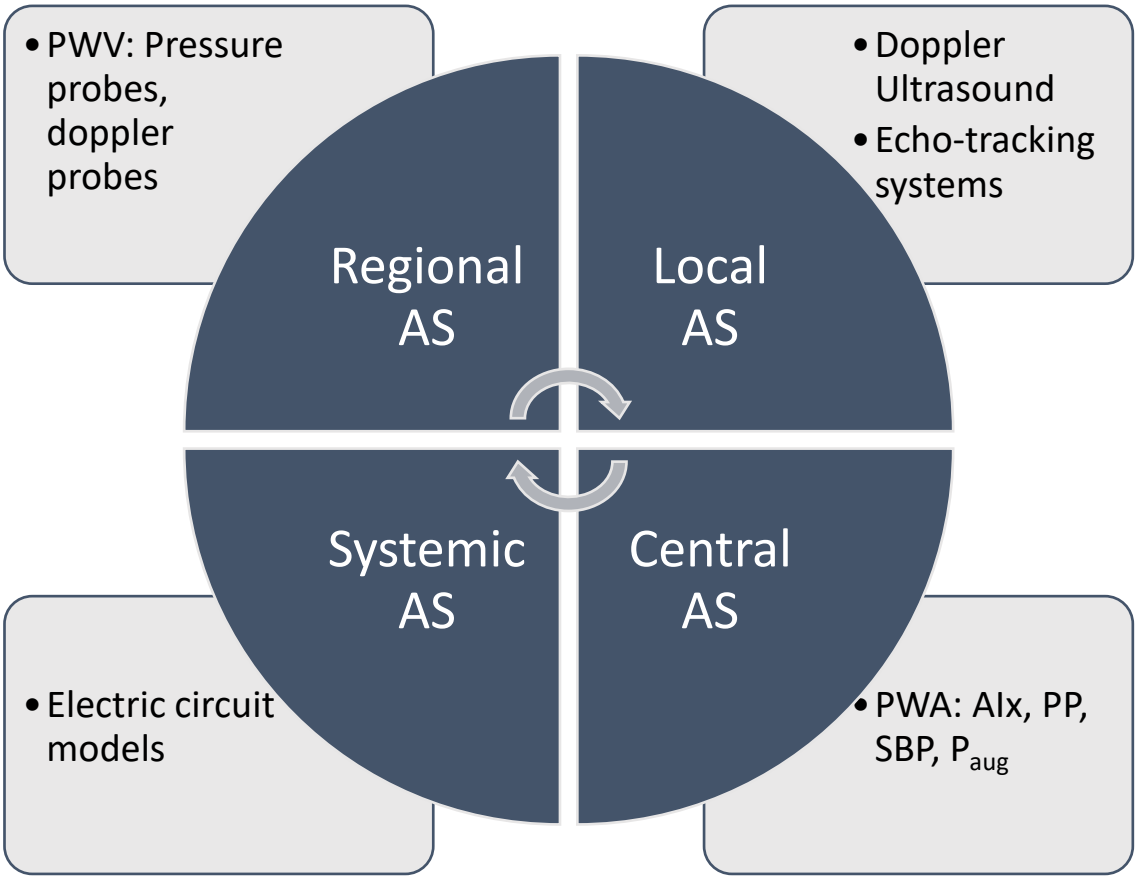

Figure 6: Different methods to measure arterial stiffness (AS) (Hamilton et al., 2007)
PWV: Pulse wave velocity, PWA: Pulse wave analysis, AIx: Augmentation index, PP: Pulse pressure, SBP: Systolic blood pressure, P_{aug}: Pressure augmentation

- **Systemic arterial stiffness:** This method is based on analogous lumped-parameter models of the circulation, particularly, a modified *Windkessel model* and *area method*. The Windkessel model was first developed by Otto Frank, who considered the heart to be the only source of pumping blood into an arterial system with a single resistance and compliance. The modified Windkessel model, or the third-element Windkessel model, is an electric circuit that can reproduce intra-beat variations in pressure waveforms by dividing the arterial tree into proximal and distal compartments (Laurent et al., 2006; Westerhof, Lankhaar, & Westerhof, 2009). A graphical representation of the third-element Windkessel model is depicted in Figure 7.

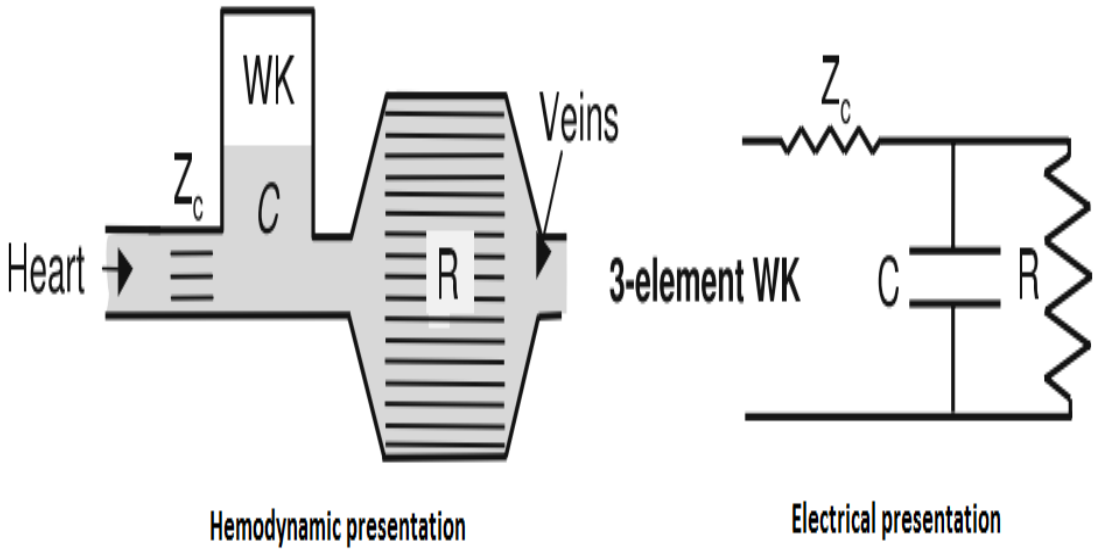

Figure 7: Third-element Windkessel model (Westerhof et al., 2009)

The 'area method' was derived by Liu et al. in 1986 as an improvement on the two-element (or as came to be known, the original) Windkessel model (Z. Liu, Brin, & Yin, 1986). On a comparative note, the 'area method' does not rely on the exponential shape of the pressure waveform; instead, it depends on the area under the blood pressure curve (Francis, 2007). This method needs a measurement of the pulse using applanation tonometry at the proximal common carotid artery to calculate the arterial compliance. It has been remarked that the applicability of this method is limited because of its dependence on diastole. Physiologically, arterial compliance and impedance are affected significantly during systole (Shim et al., 1994). Both mentioned methods, the modified Windkessel model and area method are electrical models relying on theoretical assumptions of direct measurement of the proximal and distal arterial site. Moreover, this same fact of theoretically assuming the direct distance between arterial sites limits their use in practical clinical settings (Laurent et al., 2006).

- **Central arterial stiffness:** Analysis of central pulse wave reflection can be used to study arterial stiffness, which is called pulse wave analysis (PWA). PWA is used to calculate four main parameters: the augmentation index (AIx), central pulse pressure (PP), central SBP, and the augmentation pressure (P_{aug}). It has been highlighted in the previous section that the incident and reflected waves merge to augment the pulse pressure (P_{aug}) (refer to Figure 5). The extent of this augmented pressure can be expressed as the 'augmentation index' (AIx). It is the percentage change in pressure from the first shoulder of the incident wave to the maximum pressure of the pulse wave (ΔP) divided by the pulse pressure (PP).

$$\boldsymbol{AIx = \frac{\Delta P}{PP} \times 100}$$

The augmentation index was first introduced for invasive hemodynamic study comparing the shape of aortic wave form and aortic input impedance (Murgo, Westerhof, Giolma, & Altobelli, 1980). But later, when used with applanation tonometry, it was possible to measure non-invasively in clinical studies. The index can be obtained from central blood pressure waveforms inferred from the radial pressure waveform and waveforms obtained by applanating the carotid artery (Julio A. Chirinos, 2012; Hirata et al., 2006; Laurent et al., 2006).

- **Local arterial stiffness:** Understandably, arterial stiffness is different at different arterial sites along the arterial tree. Measuring the stiffness at the local artery is performed by studying the cross-section of the artery. Superficial arteries can be studied locally using ultrasound or echo-tracking. Ultrasound probes allow determining the change in arterial diameter during diastole and systole. Ultrasound is a video-based image analysis that lacks precise measurements. Echo-tracking devices fill this gap by being more precise

using radio-frequency signals. Both methods record the distensibility of a local artery, i.e. change in the area during the heartbeat (or change in diameters: diameter diastole (Dd) and diameter systole (Ds)), as shown in Figure 8, and the local pulse pressure. Furthermore, with echo-tracking, the intima-media thickness can be measured. For deeper arteries, such as the aorta, techniques such as computerised tomography (CT) or Magnetic Resonance Imaging (MRI) could be used. Local arterial stiffness measurement does not rely on any model of circulation and is a direct method to detect carotid stiffness and media thickness. On the other hand, due to their requirements for technical expertise, local estimations of arterial stiffness are usually indicated for pathophysiological analysis or pharmacological studies, rather than in clinical practice (Cavalcante et al., 2011; Laurent et al., 2006; Van Bortel, De Backer, & Segers, 2014).

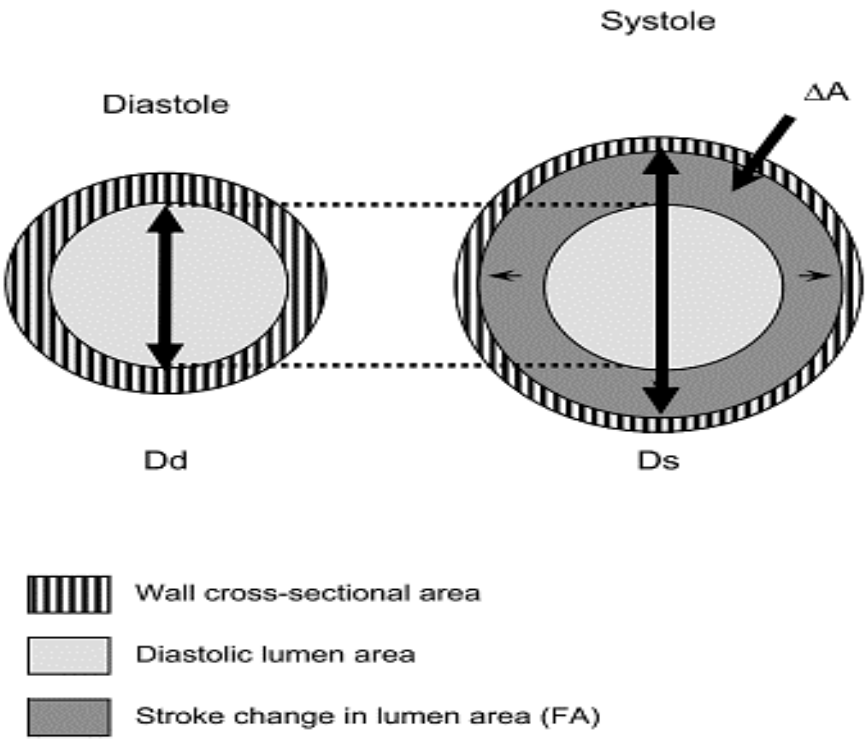

Figure 8: Distensibility of a local artery (Laurent et al., 2006)

- **Regional arterial stiffness:** In contrast to local arterial stiffness that reflects the direct mechanical stiffness of the artery at a specific point, the regional arterial stiffness

measurement focuses more on the average stiffness of a particular segment of the arterial tree. The aorta, predominantly the thoracic and abdominal aorta, plays an important role in the regional assessment of arterial stiffness. One method of measuring arterial stiffness that has been acclaimed internationally both for research and clinical practice is a measurement of PWV. Referring again to the aortic pressure waveform in Figure 5, PWV is simply the velocity of the propagated arterial pressure (or velocity) wave. In other words, PWV is the distance (D) between the two arterial sites divided by the time taken (ΔT) by the pulse wave to travel between these two sites. It is expressed in units of a meter per second (m/s) and written as:

$$\boldsymbol{PWV}\ (\frac{\boldsymbol{m}}{\boldsymbol{s}}) = \frac{\boldsymbol{D}}{\boldsymbol{\Delta T}}$$

The details of this PWV approach of measuring arterial stiffness will be discussed in the following sections. Regional PWV measurement that only provides information over a long segment of arteries with different mechanical properties would miss the initial variations in arterial properties in a small arterial segment. To overcome that, local PWV measurement of a short segment provides an indication of early stages of stiffness. Both local and regional PWV assessments are based on the external measurement of distance and travel time of the pulse waveform at two arterial sites. Furthermore, these approaches hold an advantage over the systemic or central arterial stiffness in that their parameters are measured directly and are strongly correlated to wall thickness (Laurent et al., 2006; Pereira, Correia, & Cardoso, 2015; Sahani, Shah, Radhakrishnan, Joseph, & Sivaprakasam, 2016). In summary, systemic arterial stiffness can only be measured using mathematical models of the circulation, whereas, regional and local methods measurements of arterial stiffness are directly linked to the arterial wall properties.

2.4 Pulse wave velocity

2.4.1 Definition

Among all the methods mentioned above, PWV is considered a 'gold standard' measure of arterial stiffness. In daily routine practice, assessing the carotid-femoral pulse wave velocity (cf-PWV) covering the whole central aorta has been defined as the standard practise by expert consensus (Van Bortel et al., 2012). Reasons for this consensus are that PWV is the most studied, validated in clinical settings, non-invasive, simple with minimal technical training, accurate, reproducible and strongly associated with CVD risk (Cavalcante et al., 2011; Milan et al., 2019a; Palombo & Kozakova, 2016; Shirwany & Zou, 2010; Van Bortel et al., 2012; Vlachopoulos, Alexopoulos, & Stefanadis, 2010). The Framingham Heart Study and 2007 European Society of Hypertension (ESH) and European Society of Cardiology (ESC) guidelines for the management of hypertension have suggested assessment of arterial stiffness by PWV for people at increased CVD risk (Mancia et al., 2013; Mitchell et al., 2010). Arterial Stiffness and PWV shares a direct relationship; as the stiffness of arteries increases, there is a relative increase in the PWV. A stiffer artery would make the pulse wave travel faster as compared to a compliant and distensible blood vessel (Cavalcante et al., 2011; Milan et al., 2019a).

Pulse wave velocity is defined as the velocity at which the pulse waves, generated by the systolic contraction of the heart, propagate along the arterial tree. As mentioned in the previous section, practically, PWV is calculated by measuring the distance travelled and transit time between two arterial sites. The Moens-Korteweg equation, derived in 1920 using physical modelling approach, describes the main determinants of PWV. The equation considers the relation of the velocity of the pulse wave and the distensibility of the blood vessel. According to the equation, PWV for any particular arterial length is

dependent on elastic modulus, vessel thickness and radius. The equation is expressed as

$$\boldsymbol{PWV = \sqrt{E.h/2r\rho}}$$

where,

E: elastic modulus (specifically, Young's modulus),

h: vessel wall thickness,

r: radius of the vessel, and

ρ: density of blood

Provided that the blood has a fixed density (usually around 1.050 kg/m^3), the PWV is proportional to the square root of Young's modulus, also known as elasticity of the vessel. It supports the clinical findings that stiffer arteries have higher PWV (Hamilton et al., 2007; Hirata et al., 2006; Wentland, Grist, & Wieben, 2014). However, Hamilton et al. (2007) commented that because of the square root relationship between the PWV and Young's modulus, the changes in PWV are less than changes in the mechanical properties of the artery studied.

2.4.2 Measurement of PWV

Researchers have reported using carotid-radial, femoral-tibial, carotid-femoral, or brachial-ankle arterial sites to measure PWV. However, expert consensus is that PWV between the common carotid and common femoral artery has a strong association with the risk of cardiovascular events (Laurent et al., 2006). A cf-PWV value of more than 10 m/s is an indicative index of asymptomatic organ damage (Mancia et al., 2013). The PWV values in people with CVD are higher (≈6-9 m/s) compared to those who do not (≈8-13 m/s) (Kim & Kim, 2019). Besides the ease of identification, the carotid and femoral are

superficial arteries and have the additional advantage of having a length that is similar to aortic length. Practically, PWV is measured by recording a pulse waveform at the common carotid artery and common femoral artery. The two variables to be measured are distance (m) between the arterial sites and the transit time (s) taken by the pulse wave to reach the distal artery. There are two approaches to measure the distance; *direct measurement* from the palpated carotid artery to the femoral artery, or the *indirect method* as the upper edge of the sternum notch to femoral artery distance minus carotid artery to the sternum. The timing or the transit time is made using the 'foot-to-foot' method (refer to Figure 9), as this is the part of the pulse wave least affected by the reflected wave. The transit time measurement can be done directly on the same pulse by recording simultaneously or indirectly by subtracting the time delay using ECG as a reference at the two arterial sites. Further discussion on the distance and transit time measurement appears in the following section. The readings of distance and time are used to calculate the PWV value. (Laurent & Boutouyrie, 2007; Laurent et al., 2006; Mancia et al., 2007; Milan et al., 2019b; Pereira et al., 2015; Rhee et al., 2015; Sakuragi & Abhayaratna, 2010; Shirwany & Zou, 2010; Van Bortel et al., 2012).

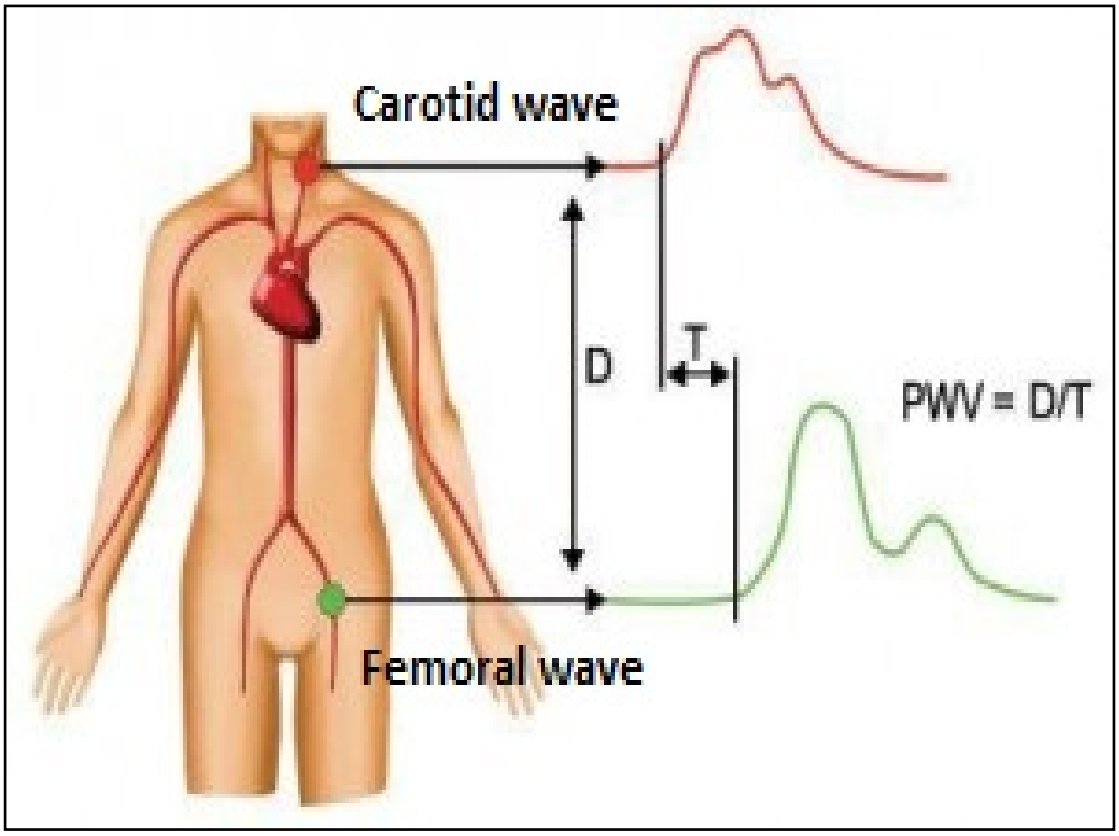

Figure 9: Determination of PWV using direct distance and foot-to-foot method (AlamMedical, 2017)

2.4.3 Methodological issues

Standardising the methodologies of measuring PWV, several factors should be taken into consideration regarding the parameters: distance and transit time. The applicability of PWV as a predictive marker is challenged by its dependence on distance measurement (Vermeersch et al., 2009). The problem is that surface distances are not the same as the length of the artery segment. There are two different approaches to tape measure the distance between the common carotid and femoral artery.

a) *Direct distance method*: as the name suggests, distance is measured directly between the measuring sites at carotid and femoral arteries.

b) *Indirect distance method*: it is the difference in distance obtained between the carotid artery-sternal notch and sternal notch-femoral artery.

It has been found that different distance measurement approaches could lead to variations in PWV values of up to 30% (Boutouyrie & Vermeersch, 2010; Wentland et al., 2014). In 2009, Vermeersch et al. validated a population-based model equation for the conversion of distance as follows (Vermeersch et al., 2009):

$$\boldsymbol{Direct\ Distance}\ (\boldsymbol{m}) = \mathbf{0.45} \times \boldsymbol{Subtracted\ Distance} + \mathbf{0.21} \times \boldsymbol{height} + \mathbf{0.08}$$

$$\boldsymbol{Subtracted\ Distance}\ (\boldsymbol{m}) = \mathbf{1.04} \times \boldsymbol{Direct\ Distance} - \mathbf{0.11} \times \boldsymbol{height} - \mathbf{0.02}$$

A study conducted in 2011, aimed at standardising and validating the carotid-femoral distance measure methods (direct and subtracted distance) using tape measures against the reference (MRI) measurement. Based on the research findings, a consensus was made to use 80% of the direct distance, i.e. multiplying the distance by 0.8 (Huybrechts et al., 2011; Van Bortel et al., 2012). The 2013 ESH-ESC Guidelines for the Management of Hypertension has given a normal cut-off PWV value as 12 m/s based on the 100% direct carotid-femoral distance measurement (Mancia et al., 2013), although, based on the 0.8 factor, a value of 10 m/s has been proposed as a new normal for cf-PWV (Van Bortel et al., 2012). Distance measurement over the body surface can be difficult and inaccurate in the case of an obese person. The ideal approach would be to use a 3-D method such as MRI or ultrasound. The over-body distance should be measured as a straight line, therefore, instead of using a tape measure, a slide calliper rule or an infantometer used upside down could be chosen (Alecu et al., 2008; Van Bortel et al., 2012).

The transit time is defined as the time taken by the propagating wave to reach from the carotid to femoral site. Past studies have recorded PWV using different types of

waveforms, such as pressure wave, flow wave, and distension wave. Although these waveforms are a part of the cardiac cycle and could be used interchangeably; out of these, the pressure wave has been shown to produce consistent results in most non-invasive methods to measure PWV (MRI and Doppler ultrasound are the exceptions as these devices record the flow waves). The 'foot-to-foot' method relies on the pressure or flow waveform and a reference point should be identified for the calculation of the time delay. The shape of the pressure wave differs at different arterial sites based on the systolic upstroke. On the other hand, the foot of the wave is usually well-defined with the minimum transition from diastole to systole, which makes assessing the foot of the wave a better marker than shape. There are three algorithms for the detection of the foot of the wave in a pressure waveform, namely, *intersecting tangents*, *maximum of the second derivative* and *10% of the pulse pressure*. These methods are explained in a graphical representation in Figure 10. Reportedly, Millasseau et al. showed that different algorithms applied to the same pressure waveform gave 5-15% variation in the PWV values. All approaches of locating the foot of the wave being consistent, it was suggested to use the intersecting tangent algorithm (Boutouyrie et al., 2009; Boutouyrie & Sønder, 2015; Millasseau, Stewart, Patel, Redwood, & Chowienczyk, 2005).

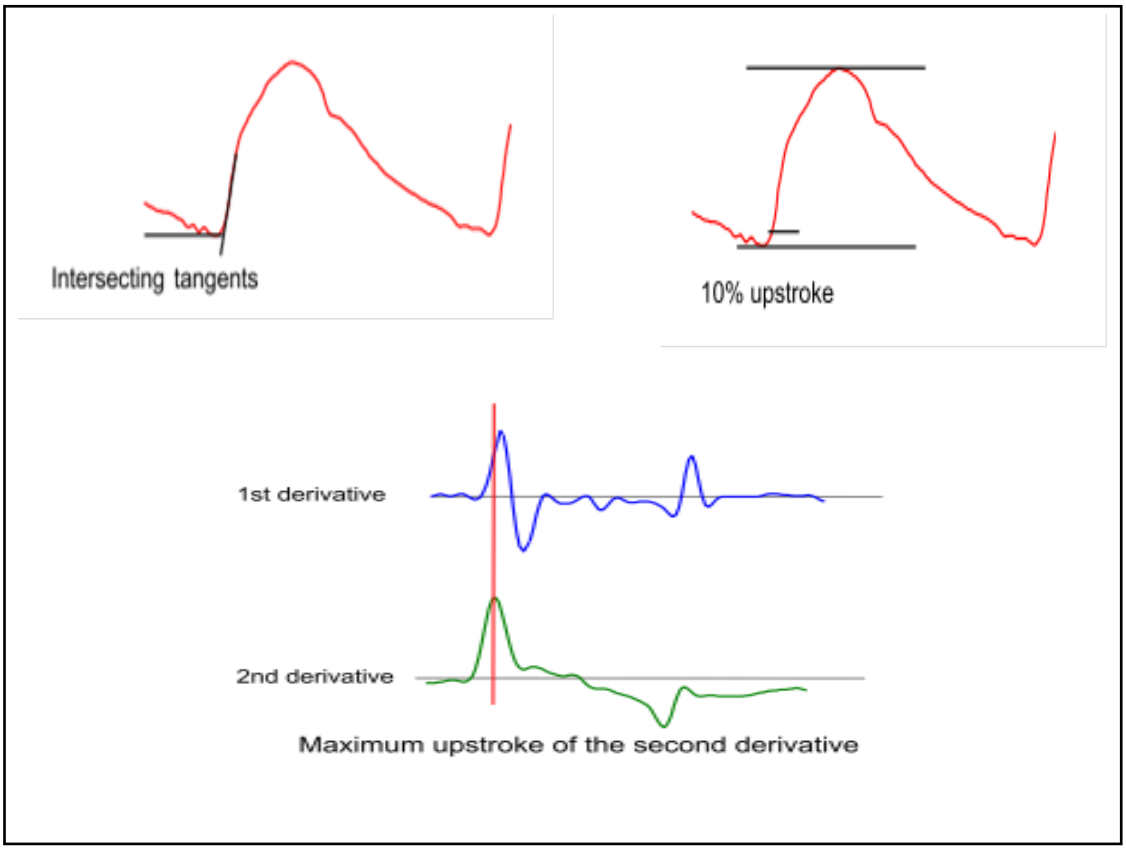

Figure 10: Detection of the foot of the wave using algorithms (Boutouyrie et al., 2009)

The expert consensus on behalf of the Artery Society, the ESH Working Group on Vascular Structure and function and the European Network for Non-invasive Investigation of Large Arteries published an advisory in 2012 on the measurement of arterial stiffness using cf-PWV (Van Bortel et al., 2012). To minimise procedural variations, a consensus was reached for the distance measurement, formula adjustment, and PWV threshold value. The recommendations made by the consensus are summarised below:

- The cf-PWV is to be considered as the gold standard for measuring PWV as a marker for arterial stiffness.
- A validated device is to be used to measure the transit time (t) taken by a pulse wave between the carotid and femoral artery.

- Use a straight tape measure for direct distance (d) from carotid and femoral arteries and take 80% of the direct distance.
- Calculate PWV (m/s) using the formula d/t x 0.8.
- Use 10 m/s as a cut-off for the cf-PWV as a predictor of cardiovascular events.
- Measurement should be made in a quiet, temperature-controlled room in a supine position after a rest of 10 min.
- The right carotid and femoral sites are preferred; data should be recorded for at least two to three cardiac cycles and a mean of at least two to three measurements should be made.
- Conditions to be excluded while estimating cf-PWV includes arrhythmia, unstable clinical conditions, high-grade stenosis of the carotid artery and cardiac sinus syndrome (abnormal heart rhythm caused by malfunction of sinus node).

2.5 Current devices to measure PWV

Several devices have been developed and validated since the invention of sphygmography and introduction of the concept of pulse wave analysis by Mohamed (I. Mackenzie et al., 2002; O'Rourke, Staessen, Vlachopoulos, & Duprez, 2002). Broadly, commercially available devices are classified as *invasive* or *non-invasive*. The invasive method is the reference point but difficult to practice in the day-to-day clinical setting. On the other hand, non-invasive methods are easier to conduct in both research and clinical settings. Most of the devices work on the principles of determining arterial stiffness, such as pressure sensors (applanation tonometry, and mechanotransducers). Figure 11 describes the different techniques and commercially available devices to measure PWV by invasive and non-invasive methods further sub-categorised as using a

local or regional approach (Pereira et al., 2015; Rajzer et al., 2008; Wentland et al., 2014).

Based on the mechanism or technique, these devices are also categorised into the following: applanation tonometry, piezo-electric mechanotransducers, cuff-based oscillometry, Doppler ultrasound, MRI and invasive methods. Each device has been validated against the invasive method of measuring PWV for their clinical viability (Milan et al., 2019a). The use of these devices commercially depends on various factors such as training required, price of the device, inter-reliability, predictability factor of PWV, validation, and ease of use. The devices mentioned in Figure 11 are explained further with their principle of action in the following sections.

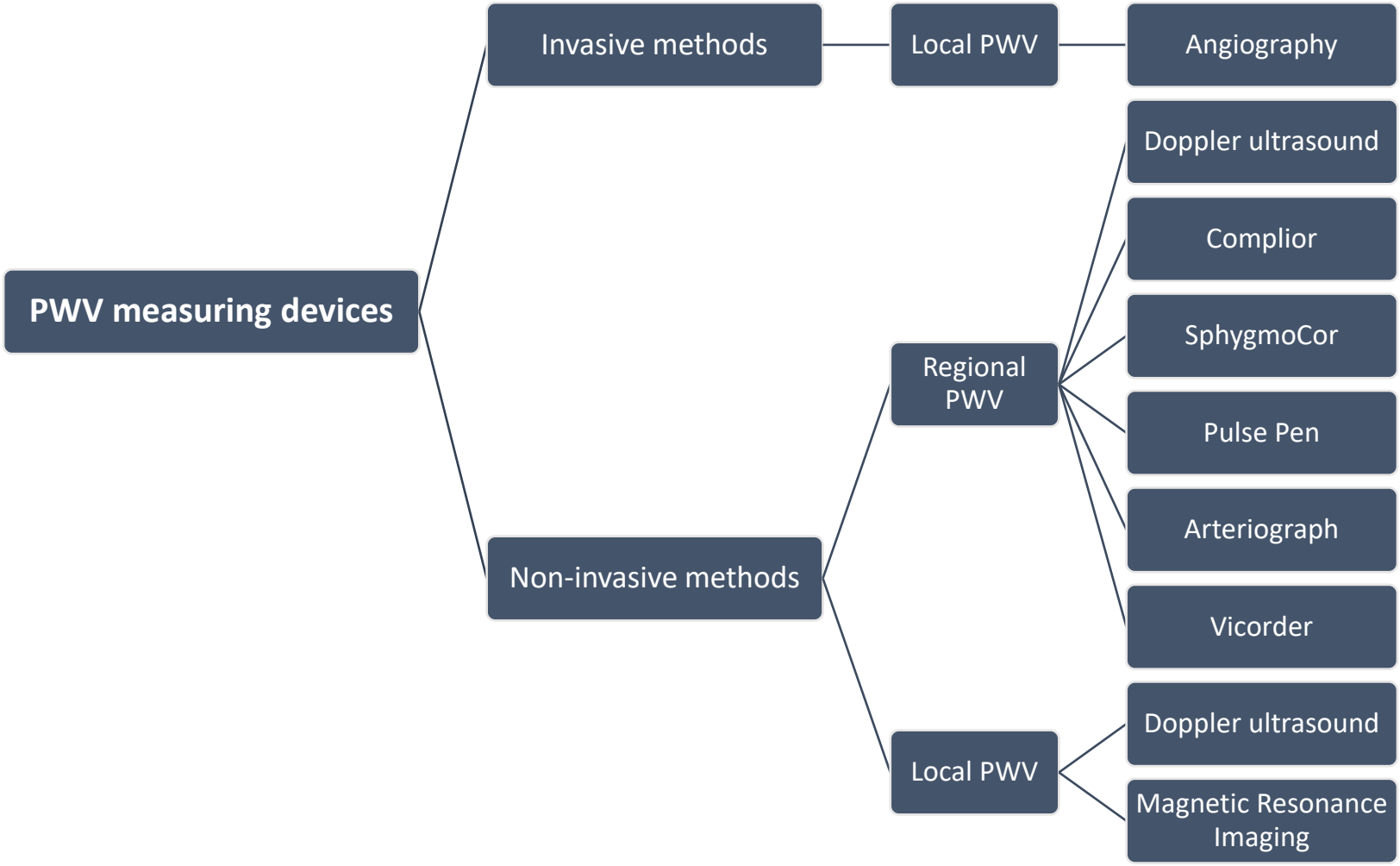

Figure 11: Classification of PWV measuring devices (Pereira et al., 2015; Rajzer et al., 2008; Wentland et al., 2014)

2.5.1 Angiography

Angiography is the only invasive method for measuring local or regional PWV and provides high accuracy in the aortic blood vessel. It is a medical imaging technique that uses X-rays for the examination of blood vessels. A catheter is introduced into the body through a peripheral artery, guided by the angiography images. The pressure wave propagation time is calculated from the electrocardiogram (ECG), and BP recording and the distance is calculated from the length of the catheter between two different sites (proximal and distal). It is mainly used in CVD diagnosis or surgical procedures. Despite being regarded as the gold standard, its clinical use is limited due to its invasiveness, cost and the technical skills needed (S. M. Park et al., 2004; Pereira et al., 2015; Wentland et al., 2014).

One example of where invasive PWV measurements may find use is in the measurement of coronary artery PWV. It was hypothesised that to predict coronary artery diseases, assessing the aortic PWV as a marker of large artery stiffness is an appropriate surrogate. However, because of the proximity of aorta and small arteries, a stiffness gradient exists, leading to a compliance mismatch. Therefore, assessing the local coronary artery instead of the aorta is more relevant for the prediction of plaque rupture. Research conducted in France presented a new approach to measure the coronary PWV invasively to assess the local compliance. Values were then compared with the invasive aortic PWV and cf-PWV in acute coronary syndrome and stable coronary artery disease. In a study population of 53 patients, it was found that coronary PWV was lowest in acute vs stable coronary artery diseases while the aortic PWV and cf-PWV values were not statistically different (Harbaoui, Courand, Cividjian, & Lantelme, 2017).

2.5.2 Complior Analyse

Complior Analyse (Alam Medical, France) works on the principle of piezo-electric technology that allows simultaneous recording of the pulse waves from two different sites. It uses two mechanotransducers to be applied on the skin at the common carotid and femoral arteries. The transducers can be placed at various sites, i.e. carotid-femoral, carotid-brachial, or femoral-ankle arteries. This device was first validated against a manual calculation with a significant correlation (automatic vs manual, $r=0.88$, $p<0.001$) (Asmar et al., 1995) and has been used in several epidemiological studies. It consequently constituted the majority of research work marking the predictability of PWV for CVD (Stea et al., 2014). As the device is able to record pulse wave simultaneously, there is no need to record an ECG along with it (Blacher, Asmar, Djane, London, & Safar, 1999; Laurent et al., 2006; Pereira et al., 2015; Rajzer et al., 2008; Wentland et al., 2014).

Another device that works on the principle of a piezo-electric transducer with simultaneous pulse wave recordings is Aortic (Exxer, Argentina). Following the Association for Research into Arterial Structure and Physiology (ARTERY) society guidelines for non-invasive haemodynamic measurement devices, Aortic was validated against the SphygmoCor in 85 subjects with a strong level of agreement (mean difference of 0.2 ± 0.84) for lower PWV ranges (Morales et al., 2015).

2.5.3 SphygmoCor

The SphygmoCor device (AtCor Medical, Australia) is a pen-like device based on applanation tonometry that measures the amount of force required to flatten the artery against a bony structure. The pressure sensors are placed at the proximal site, and a pulse wave is recorded simultaneously with an ECG. Similar recordings are made at the

distal site. Usually, these sites are the carotid and femoral arteries. The transit time is calculated sequentially by referencing the foot of the R wave in the ECG using the intersecting algorithm. With time the procedure has become quicker and less user-dependent. This device has been widely used to study the correlation of PWV with CVD risk factors, and in many cohort and intervention studies (Boutouyrie & Vermeersch, 2010; Davies, Bailey, Griffin, & Scott, 2012; Laurent et al., 2006; Milan et al., 2019a; Rajzer et al., 2008; Weber et al., 2009).

The newer version of this device is the SphygmoCor Xcel that allows estimation of PWV and PWA. A simultaneous recording of the pulse wave is possible using the pen device at the carotid artery and a thigh oscillometric cuff to read the femoral artery. The Xcel device has been validated against the well-validated non-invasive SphygmoCor device and invasive coronary arteriography with a strong correlation observed for central systolic BP (r=0.91) and central PP (r=0.89) (Butlin & Qasem, 2016; M. Hwang et al., 2014; Shoji, Nakagomi, Okada, Ohno, & Kobayashi, 2017).

2.5.4 PulsePen

PulsePen (Dia Tecne SRL, Italy) is another device designed as a pocket-sized instrument to measure PWV and works on the principles of tonometry. The device has pressure sensor/probes, an integrated ECG unit, a wireless receiver, and a USB memory stick with computer software. When the two stages of recording pulse waves are followed sequentially, the readings are synchronised with the ECG. However, two probes can be used simultaneously at two different sites without requiring the ECG; which is the option available in another variant of this device- PulsePen ETT (Pereira et al., 2015; Paolo Salvi et al., 2004). These tonometry-based devices contributed to a better understanding of the determinants that influenced large artery stiffness and showed the independent role

of PWV measurements in the cardiovascular prognosis. A comparative analysis of the two-tonometer PulsePen device was made against the one tonometer PulsePen, Complior and PulseTrace devices. The results showed a positive correlation with PulsePen (r=0.99) and Complior (r=0.83), but not with the PulseTrace (r=0.55) device (Milan et al., 2019a; Paolo. Salvi et al., 2008).

2.5.5 Arteriograph

Measuring PWV with an Arteriograph (TensioMed, Hungary) is unique and easy to operate as it replicates taking a blood pressure measurement on the upper arm. It works on the theory of oscillometry. The arteriograph consist of a brachial cuff attached to a piezo-electric sensor and placed over the brachial artery. The cuff pressure is raised by 35 mmHg above the SBP to occlude the artery completely. This device analyses the time difference between the two systolic peaks. The first will result from the blood ejecting out of the left ventricle to the systemic circulation, while the second one is interpreted to occur when the first wave gets reflected at the peripheral site that is assumedly the iliac bifurcation. The pressure variations are detected by a pressure receptor and transferred to computer software. PWV is then calculated using the distance from the sternal notch (or jugulum) to the pubic symphysis. In 2010, a group validated measuring AIx, PWV, and central BP by Arteriograph against the invasive cardiac catheterisation process. The result showed a strong correlation for all three parameters measured invasively and non-invasively; with limits of agreement being 11.4% (AIx) and 1.59 m/s (PWV) (Baulmann et al., 2008; Horvath et al., 2010; Parati & De Buyzere, 2010; Rajzer et al., 2008; Segers et al., 2009).

2.5.6 Mobil-O-Graph

Mobil-O-Graph (IEM, Germany) is another cuff-based oscillometric device that can estimate single point cf-PWV. The pressure cuff inflates first to record the systolic and diastolic BP, and then holds for 10 seconds to get the PWV and PWA. Various parameters are derived based on the mathematical ARCSolver algorithm model using the age, central PP and aortic impedance (Baumann, Wassertheurer, Suttmann, Burkhardt, & Heemann, 2014). For regional PWV/PWA assessment, these oscillometric devices hold an advantage over other invasive and non-invasive devices by using a simple pressure cuff; they are fast and, easy to use for both operator and subject (Diaz, Zócalo, Bia, & Cabrera Fischer, 2018). The Mobil-O-Graph has been validated against reference invasive intra-aortic catheter measurement and reported an acceptable linear correlation ($R= 0.81$, $p<0.0001$). The intra-observer reproducibility for PWV measurement was within limits, having the mean difference of 0.05 m/s (95% limits of agreement -0.47 to 0.57 m/s) (Hametner et al., 2013). However, a recently published work by the Salvi group has reported an attempt to see the predictive element of Mobil-O-Graph beyond the effect of age and BP. It was found that Mobil-O-Graph is well suited for subjects with an ideal age and BP, but not for subjects with early vascular ageing and high PWV scores (Marfan syndrome) (Paolo Salvi et al., 2019).

The Mobil-O-Graph 24h PWA monitor (IEM GmbH, Germany) is a variant for 24h ambulatory BP monitoring. The ARCSolver algorithm allows the measurement of aortic PWV and PWA. Many feasibility and reproducibility studies have been successfully conducted, and the device has been used to establish an association between continuous ambulatory PWV monitoring with CVD risks (Luzardo et al., 2012; Milan et al., 2019a; Papaioannou et al., 2013).

2.5.7 Vicorder

Vicorder (Skidmore Medical, Germany) is a small, portable non-invasive device that uses the oscillometric technique. It has two inflatable cuffs to be placed at two different arterial sites (carotid-femoral or brachial-ankle). For assessing cf-PWV, the first cuff is a 30 mm pad placed on the right carotid artery around the neck and the second 100 mm pad goes on the upper right thigh over the femoral artery. The cuffs are inflated to 65 mmHg simultaneously, and the pulse wave is recorded; and distance is then adjusted by subtracting that measurement at the middle of each cuff. Vicorder has an additional advantage of being able to record PWV for children or young adults and even infants, while being easy to operate. Vicorder has been validated against the tonometric device (SphymoCor) in healthy subjects ($r=0.85$, $p<0.001$), and people with peripheral arterial diseases (limit of agreement between Vicorder and SphygmCor as -1.07 to 1.09 m/s and -1.79 to 1.85 m/s) (Hickson et al., 2009; Shahin, Barakat, Barnes, & Chetter, 2013; van Leeuwen-Segarceanu et al., 2010).

2.5.8 Doppler ultrasound

Doppler ultrasound has been used widely to study, diagnose and monitor larger arteries of the circulatory system regionally and locally (Baguet et al., 2003). Estimation of local PWV can be performed using ultrasound by calculating the ratio of the change in cross-sectional area and flow at the artery locally (Julio A. Chirinos, 2012). Furthermore, ultrasound can also be used to study regional PWV by analysing two sites (e.g. carotid and femoral) simultaneously, by using two ultrasound flowmeters or sequentially with one ultrasound transducer gated with ECG (Milan et al., 2019a). Like other non-invasive techniques, ultrasound also shares the error in measuring the distance over the body surface. The accurate estimation of PWV relies on accurate identification of the foot of

the waveforms and a higher sampling frequency rate. However, 2D imaging of the artery allows a direct arterial biomechanical analysis of several arterial disease characteristics affected by age and other risk factors (Calabia et al., 2011; Jiang, Liu, McNeill, & Chowienczyk, 2008; Z. Wang et al., 2015).

Doppler ultrasound has been considered the gold standard for non-invasive PWV assessment (Milan et al., 2019a). A study conducted to test the methodology and reliability of Doppler found low variation coefficients (4.6-7.5 % in intra-observer and 4.7-8.6% in inter-observer) and concluded that this is a feasible and reproducible method (Baguet et al., 2003). Several tonometric and oscillometric devices have been validated against the ultrasound (Calabia et al., 2011; Jiang et al., 2008). This method has been used widely to establish the relationship between PWV and various health conditions, such as mortality in diabetes and glucose intolerance, end-stage renal disease and hypertension (Baguet et al., 2003; Cruickshank et al., 2002b; Jiang et al., 2008; Lehmann et al., 1998; Sutton-Tyrrell et al., 2005; Z. Wang et al., 2015).

2.5.9 Magnetic resonance imaging

Magnetic resonance imaging (MRI) is a direct non-invasive imaging method that gives an accurate measurement of path length and transit time between the thoracic and abdominal aorta. One major advantage of MRI is that it does not need geometric assumptions associated with surface distance measurements. Besides the assessment of aortic PWV, it could also be used for other vascular parameters such as aortic compliance and distensibility, Young's modulus, and AIx. There is another approach to compute PWV globally over a large vasculature (e.g. thoracic aorta) by recording a few velocity waveforms from the region. Although, this approach is not influenced by sampling bias, and areas of stiffness, the higher PWV are averaged with normal areas of

the vasculature (Wentland et al., 2014). The averaging of measures make it hard to differentiate between the individual with healthy or stiff arteries (Ibrahim, Johnson, Miller, Shaffer, & White, 2010; Joly et al., 2009; Pereira et al., 2015; van der Meer et al., 2007; Wentland et al., 2014).

Cardiac Magnetic Resonance is a technique that allows visualisation of the entire thoracic and abdominal aorta regardless of blood vessel depth and angle. Measurement of PWV based on Cardiac Magnetic Resonance has been validated against catheters both in humans and phantoms. This approach has advantages over Doppler ultrasound by being less susceptible to transducer probe placement and angle. Several methods have been used to calculate the determinants of PWV from the velocity encoded Cardiac Magnetic Resonance images such as transit time, flow area and cross-correlation. A study comparing these methods concluded that there was no inter/intra-observer variability among the different techniques, and the choice would depend on the type of vessel segment and image quality. At high field strength, transit time and cross-correlation methods were more reproducible with less user interaction than the flow area method to assess the aortic PWV (Ibrahim et al., 2010).

Another technique developed to assess PWV is phase-contrast MRI. Working with the same principle, it allows acquiring the blood flow velocity on accumulated phase shifts along any given anatomical plane through a superimposed magnetic field gradient. The measurements can be taken ‘through-plane’ or ‘in-plane’ to determine the transit time at the thoracic or abdominal aorta. The ‘in-plane’ approach of measuring PWV has been highly correlated to age and arterial stiffness in the proximal aorta. Phase-contrast MRI has been used to study cross-sectional aorta to measure flow, or at proximal and distal sites to measure PWV or long axis images of the entire aorta. This gives an opportunity

for determination of PWV at multiple locations in a single acquisition. Besides the benefits of visualising the whole aorta, MRI does need specific training, is costly and time-consuming (Aquaro et al., 2013; Joly et al., 2009; Rogers et al., 2001). Two of the transcutaneous PWV measurement devices, Complior and PulsePen, when compared with the phase-contrast MRI in obese subjects, were found to be moderately correlated (Complior, $r=0.43$, $p=0.01$ and PulsePen, $r=0.47$, $p=0.005$), whereas, the two non-invasive devices showed a strong correlation with $r=0.75$ ($p<0.0001$). It was implied that though the readings were statistically significant, the strength of correlation observed is different. This was due to the fact that MRI PWV measures thoracic stiffness with different proximal and distal PWV values, whereas, transcutaneous PWV values are recorded from the abdominal and iliac aorta (Joly et al., 2009).

Table 1: Compilation of commercially available devices measuring pulse wave velocity (modified from (Milan et al., 2019a))

Devices	**Method**	**Clinical viability***	**Clinical validation^**	**Features**
Complior	Piezo-electric mechanotransducer	+++	+++	Simultaneous recording, no need for ECG, can measure at carotid-femoral, carotid-brachial, and femoral-ankle arteries
Aortic	Piezo-electric mechanotransducer	+	-	Simultaneous recording with two piezo-electric transducers, follows same principle as the Complior
SphygmoCor	Applanation tonometry	+++	+++	Sequential recording with pressure sensors, needs ECG recording to calculate the pulse transit time, measurement at carotid-femoral arteries
SphygmoCor Xcel	Applanation tonometry	+	-	Simultaneous recording using a pen device and oscillometric cuff, same working principle as SphygmoCor
PulsePen	Applanation tonometry	+++	-	Sequential recording with pressure sensors, need ECG recording, option for extended recording for up to 24 h
PulsePen ETT	Applanation tonometry	+++	-	Simultaneous recording using two tonometer versions, no need for ECG recording, option for extended recording for up to 24 h
Arteriograph	Cuff-based oscillometry	++	-	Easy to use, uses inflatable cuff at brachial artery to read pressure variation
Mobil-O-Graph	Cuff-based oscillometry	++	++	Cuff-based device, can estimate single point cf-PWV, easy to use, fast, operator friendly

Vicorder	Cuff-based oscillometry	++	-	Simultaneous recording using proximal and distal cuffs, can read carotid-femoral and brachial-ankle sites, can be used in infants, children, and young adults
Doppler ultrasound	Doppler transducer	++	++	Measure pulse wave locally or regionally, can perform sequential and simultaneously measurements, gives accurate identification of foot of the waveform, is the gold standard for non-invasive assessments, need specific training
MRI	Resonance imaging	+	-	Direct non-invasive, accurate method, no need for surface distance measurement, need specific training, time consuming, costly

ECG: Electrocardiogram, cf-PWV: carotid-femoral pulse wave velocity, MRI: Magnetic Resonance Imaging
* Assessed considering sample size in validation studies; availability of the device; repeatability; invasiveness; invasive validation
^ Assessed considering the number of studies with clinical outcomes for each device, -: no clinical outcome; +: one study; ++: two studies; +++: more than two studies available

Table 1 compiles the reviewed PWV measuring devices with their mechanism and corresponding features. Milan et al. (2019) reviewed the validation studies for assessment of PWV against the 2015 American Heart Association Guidelines of 'Recommendations for Improving and Standardising Vascular Research on Arterial Stiffness.' Clinical viability of each device was assessed based on the data presented in the validation studies published, which included the sample size, availability of device, and invasiveness. Clinical validation was based on the number of studies available with the clinical outcome for each device (Milan et al., 2019a). Comparing the methods, features, limitations and availability of the device at the time of this study planning, it was decided to use Doppler ultrasound for measuring PWV.

2.6 Clinical application of aortic PWV as an indicator of arterial stiffness

It is very evident from the published literature in the last several decades that clinical importance is given to arterial stiffness and PWV measurement for cardiovascular risk assessment, which has been on the rise. In 2016, recommendations and standardisation for measuring arterial stiffness were published, and a graph was presented based on a search made in PubMed using the title of publications found on non-invasive measure of arterial stiffness (refer to Figure 12) (Townsend, 2016). A large amount of literature has collectively reported that increases in arterial stiffness and PWV are correlated with pathophysiological conditions. Researchers have studied the effect of arterial stiffness on ageing, genetic background with family history, cardiovascular risk factors, and CVD. Cardiovascular risk factors such as age, gender, obesity (rated as according to body mass index (BMI)), heart rate (HR), diabetes mellitus, impaired glucose tolerance, high density lipoprotein, low-density lipoprotein, total cholesterol, triglycerides, smoking, hypertension, and metabolic syndromes have all been linked with increased PWV

(Cecelja & Chowienczyk, 2009, 2012; Laurent et al., 2006; Palombo & Kozakova, 2016). In a systematic review, Cecelja et al. reviewed cross-sectional research to study the association of cf-PWV with cardiovascular risk factors other than hypertension. It was reported that the only significant independent association of PWV was found with age and hypertension (Cecelja & Chowienczyk, 2009). The summarised result of this review, including 77 studies, is shown as a graph in Figure 13. The cardiovascular risk factors included in the studies are shown as bars on the x-axis, while the proportion of these in which the risk factor was significantly independently associated with PWV is shown as a solid line. Age and BP were unanimously found to be significantly associated with cf-PWV in 90% of the studies reviewed. The review further commented that PWV has a low association with diabetes and no independent association was found with sex, total cholesterol, LDL, HDL, TG, smoking or BMI (Cecelja & Chowienczyk, 2009). The same group later presented findings through another review summarising the association of PWV with atherosclerosis risk factors (Cecelja & Chowienczyk, 2012). The review showed that the presence of aortic stiffness was linked with atherosclerosis. However, they reported no or little association of traditional cardiovascular risk factors and PWV other than age and BP. Additionally, PWV did not hold its predictive value during the early stages of atherosclerosis measured through carotid intima-media thickness and non-calcified deposition of plaque. However, it had a positive correlation with advanced stages of atherosclerosis with the calcified aorta (Cecelja & Chowienczyk, 2012).

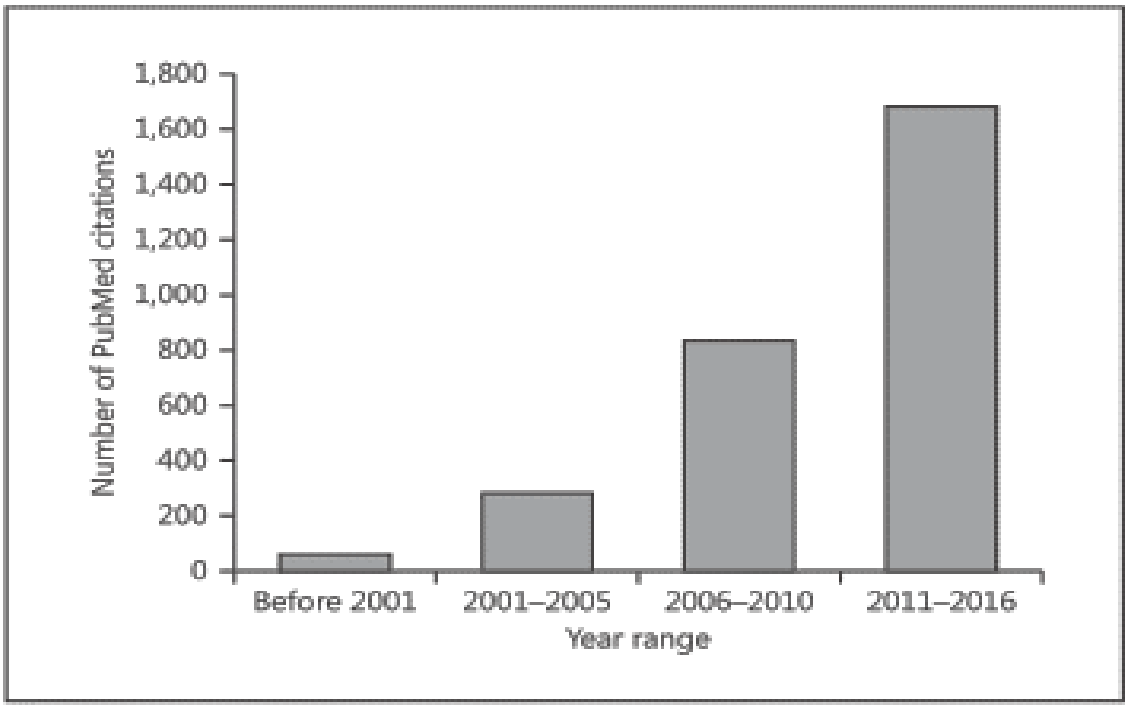

Figure 12: A graph showing the frequency of literature published on non-invasive measures of arterial stiffness (Townsend, 2016)

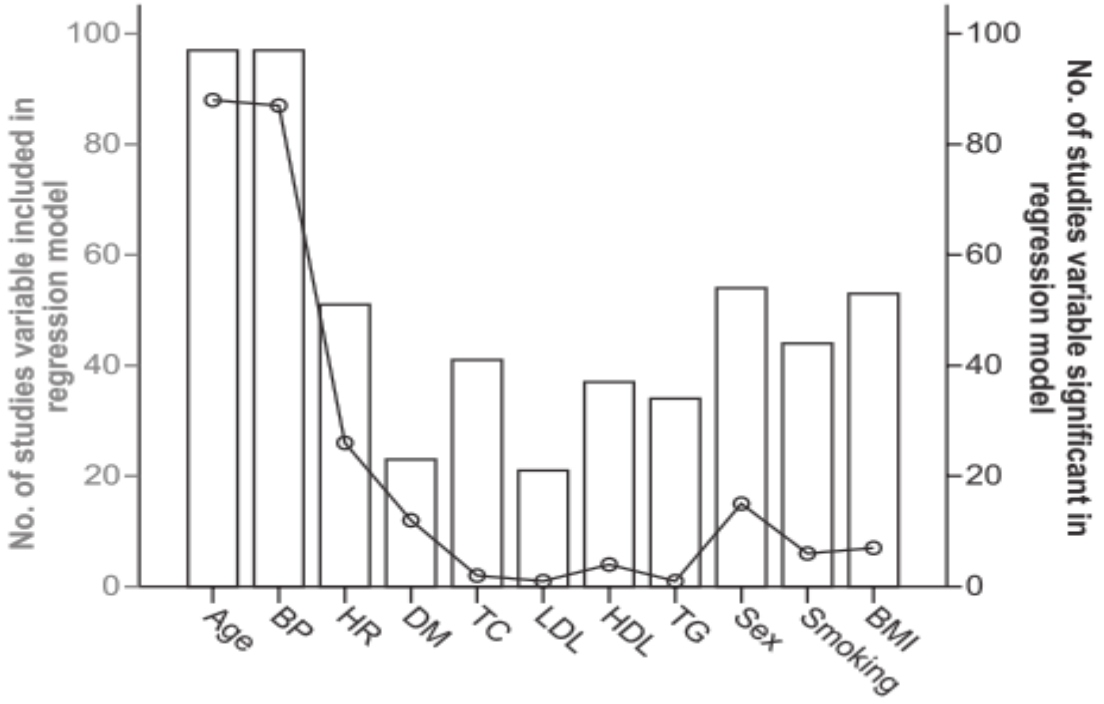

Figure 13: Findings of the systemic review showing the association of cardiovascular risk factors with PWV (Cecelja & Chowienczyk, 2009).

The cardiovascular risk factors are shown as bars and proportion of risk factors significantly correlated with PWV as a solid line. BP: blood pressure, HR: heart rate, DM: diabetes mellitus, LDL: low-density lipoprotein, HDL: high-density lipoprotein, TG: triglyceride, BMI: body mass index

In the early 20th century, although reports from cross-sectional studies had shown the correlation of arterial stiffness/PWV and cardiovascular risk factors, predictability for CVD risk was not yet proven. To validate that arterial stiffness has predictive value for cardiovascular events, several longitudinal epidemiological cohort studies were performed in which the subjects were included from the normal population, people with hypertension, end-stage renal diseases, diabetes, healthy children and the elderly. The studies summarised in Table 2 have reported the relationship of PWV with different clinical conditions. Based on the study outcomes, cf-PWV has been utilised as a global measure of arterial stiffness, and an independent predictor of coronary heart disease and stroke, changes in systolic BP, hypertension, and all-cause/cardiovascular mortality (Cavalcante et al., 2011). A meta-analysis published in 2014 systematically reviewed 16 studies with more than 17,000 subjects to determine the improved predictability of aortic PWV of CVD events beyond the conventional risk factors. Individual-level subject data was randomised and analysed through a Cox proportional hazards model to calculate the hazard ratio (HR) for studying associations of PWV with CVD adjusted for age, sex, systolic BP and other risk factors. The age and sex-adjusted HRs per 1 SD change in $\log_e$ PWV were 1.35, 1.54, and 1.45 for coronary heart disease, stroke, and CVD, respectively. Even after adjusting for the conventional risk factors, PWV maintained the predictability for all three conditions. Also, the addition of PWV to the risk prediction model (Framingham risk score) adds 13% in cardiovascular event prediction to the prediction score for 10-years CVD intermediate risk in younger participants (Ben-Shlomo et al., 2014).

Arterial stiffness is now considered as a complementary assessment to BP measurement and has added value when adjusted for the Framingham risk score (Boutouyrie & Vermeersch, 2010; Townsend, 2016). The proposed reason that arterial stiffness

contributes to risk assessment is that measurement of BP, lipid profile or glycaemia only shows the condition in that time frame (i.e. is a snapshot of that time and could fluctuate over time). Conversely, arterial stiffness assessment with cf-PWV has predictive value for up to 10-15 years and reflects the actual course of arterial wall damage (Laurent & Boutouyrie, 2007; Laurent et al., 2006; Vlachopoulos et al., 2010; Vlachopoulos, Aznaouridis, & Stefanadis, 2014).

Table 2: Independent predictive value of cf-PWV in longitudinal studies with cardiovascular events as outcomes summarised yearly

Reference	Events	Type of patients (number)	Device
(Blacher et al., 1999)	CV mortality	ESRD (241)	Doppler US
(Laurent et al., 2001)	CV mortality	Hypertension (1980)	Complior
(Meaume, Benetos, Henry, Rudnichi, & Safar, 2001)	CV mortality	Elderly >70 years of age (141)	Complior
(Shoji et al., 2001)	CV mortality	ESRD (265)	PWV meter PWV-200
(Guerin et al., 2001)	CV mortality	ESRD (150)	Doppler US
(Boutouyrie et al., 2002)	CHD events	Hypertension (1045)	Complior
(Cruickshank et al., 2002b)	CV mortality	Impaired glucose tolerance (470)	Doppler US
(Laurent et al., 2003)	Fatal strokes	Hypertension (1715)	Complior
(Sutton-Tyrrell et al., 2005)	CV mortality and events	Elderly (2488)	Doppler US
(Shokawa et al., 2005)	CV mortality	General population (492)	Complior
(Pannier, Guerin, Marchais, Safar, & London, 2005)	CV events	ESRD (305)	Complior
(Hansen et al., 2006)	CV mortality	General population (1678)	Arteriograph

(Mattace-Raso et al., 2006)	CV mortality, CHD	Elderly (2835)	Complior
(Zoungas et al., 2007)	CV events	Chronic kidney disease stage 4-5 (315)	Doppler US
(Terai et al., 2008)	Stroke, CV events	Essential hypertension (676)	Pressure transducer
(Inoue et al., 2009)	CV mortality	General population, men (3960)	PWV meter
(Anderson, Sanders, & Cruickshank, 2009)	Total mortality	General population (174)	Doppler US
(Ilyas et al., 2009)	CV hospitalisation, mortality	Suspected carotid artery disease (284)	SphygmoCor
(Mitchell et al., 2010)	CV events	General population (2232)	SphygmoCor
(K.-L. Wang et al., 2010)	CV mortality	General population (1272)	Doppler US
(Verbeke et al., 2011)	CV events	Renal transplant recipients (512)	SphygmoCor
(Blacher et al., 2012)	CV mortality	Geriatric men (3310)	Complior
(Maldonado et al., 2011)	CV events	Population based (2200)	Complior
(Protogerou et al., 2011)	CV mortality	Elderly (259)	Complior
(Szeto et al., 2012)	CV mortality	Peritoneal dialysis (155)	Complior
(Verwoert et al., 2012)	CHD events	Elderly (2849)	Complior

CV: Cardiovascular, ESRD: End Stage Renal Disorder, CHD events: Coronary Heart Disease events, US: Ultrasound, PWV: Pulse Wave Velocity

2.7 Status quo of cardiovascular risk assessment in NZ

The WHO factsheet on CVDs states that it is the leading cause of global mortality, representing 31% of all global death in year 2016. Stroke and heart attack constituted 85% of these CVDs related deaths (WHO, 2016), which is expected to reach 23.6 million by 2030 (Mozaffarian et al., 2015). New Zealand is not far behind; reports indicate that CVD and stroke are a leading cause of mortality and morbidity (Statistics New Zealand, 2014). When considering the financial burden; stroke alone costs NZ about $NZ 450 million per year (Feigin et al., 2014). Moreover, the annual stroke cohort hospitalisation is at least $NZ 880 million; each stroke is associated with a cost of $NZ 60,000 to $NZ 99,000 over five years (Hogan & Siddharth, 2018). Cardiovascular risk factors such as physical inactivity, obesity, diet, smoking, diabetes, alcohol consumption, high cholesterol and hypertension are modifiable; controlling these would lower the incidence of CVD and stroke globally (O'Donnell et al., 2016; Truthmann et al., 2015). There are several population-based scoring systems, such as the Framingham Risk Score (Mitchell et al., 2007), CVD risk assessment, and the management decision support system (called 'PREDICT') (Wells et al., 2015), and the Heart Systemic Coronary Risk Evaluation (HeartSCORE) (Pereira et al., 2014) used to assess cardiovascular risk. These scores are based on five conventional cardiovascular risk factors (age, gender, systolic blood pressure, cholesterol and smoking). However, studies have shown that the conventional scoring systems do tend to overestimate the absolute CVD risk in the European population (Brindle et al., 2003). The Framingham Risk Score was modified to account for NZ's ethnic variability and newly identified risk factors from an ongoing PREDICT study cohort (Ministry of Health, 2018; Wells et al., 2015). The updated NZ guidelines for CVD risk assessment and management for primary care published in 2018

advised assessments to be based on new five-year CVD risk prediction equations published from the NZ PREDICT study (Ministry of Health, 2018).

Ethnicity in NZ

Ethnicity is considered to be an affiliation with specific social groups and the identity of an individual. Generally, ethnicity has been self-assigned by people as per the categories defined by the NZ specifications. The NZ's Ministry of Health website defines nine Level 1 ethnic group codes as: Europeans, Māori, Pacific Peoples, Asian, Middle Eastern/Latin American/African, Other Ethnicity and residual categories (Ministry of Health, 2010). The latest census data collected in 2018 has not been published yet. As per the 2013 NZ census report, the total population is on the rise, and so are the major ethnic groups (Statistics New Zealand, 2014). Figure 14 is a graphical representation and comparison between the 2006 and 2013 census for the NZ population. Europeans are still the largest ethnic group, with 74% of the total population, followed by Māori people with 14.9%. The Asian population is on the rise and has surpassed Pacific people with 11.8% vs 7.4%. Other ethnicities (1.2%) include Latin American, Middle Eastern, and African ethnic groups. The report also stated that Europeans are older than other major ethnic groups with a median age of 41 years compared to 38.1 years as reported in the 2006 census report (Statistics New Zealand, 2014).

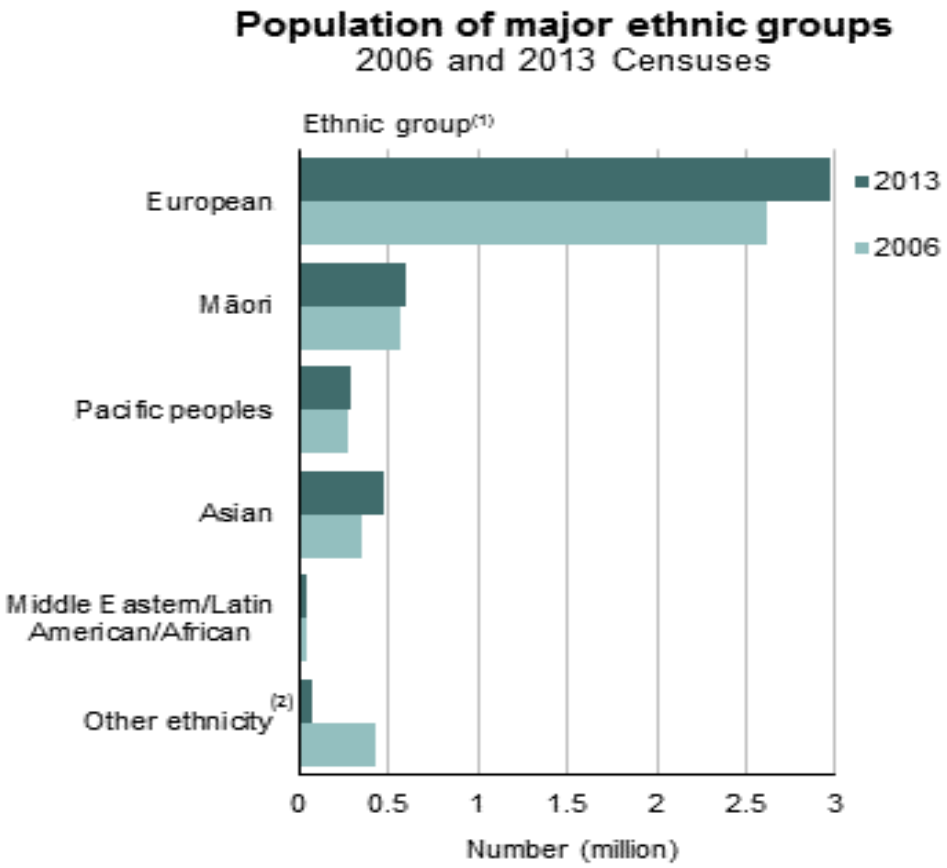

Figure 14: 2013 NZ census report on ethnicity compared with 2006 census

It has been reported that ethnic variability, genetic composition, cardiovascular risk factors, and diabetes are associated with overall arterial health. Reports published by the Ministry of Health, NZ show disproportionate CVD burden for Māori and Pacific people (Ministry of Health, 2016). Furthermore, there is a strong association between Māori ethnicity and type II diabetes, and a 30% higher risk of having a first cardiovascular event (Kenealy et al., 2008). Therefore, there is a good reason to expect a relationship between ethnicity and PWV. To gain a perspective of ethnic variability of arterial stiffness metrics around the world, a summarised view is presented in Table 3.

Table 3: Chronological summary of studies conducted around the world showing the association of ethnic variability with arterial stiffness

Reference	Ethnicity	Country	PWV/AIx measurement	Device	Study population (number)	Outcome
(Ferreira, Viana, Mill, Asmar, & Cunha, 1999)	African (blacks) and European (whites)	Brazil	cf-PWV	Complior	Europeans (49 normotensives, 33 untreated hypertensives) Africans (24 normotensives, 14 untreated hypertensives)	Whites had high PWV values in the normotensive group. On the contrary, blacks had high PWV in the hypertensive group. After adjusting with age, whites and blacks had significantly different mean PWV values.
(McEniery et al., 2005)	Caucasian, Asian, Far Eastern, Afro-Caribbean	UK and Australia	AIx, aortic PWV, brachial PWV (cr-PWV)	High-fidelity micromanometer (SPC-301), SphygmoCor	Healthy, normotensive subjects (4001)	AIx and brachial PWV increased significantly with age. AIx increased more in younger adults, while older adults had higher PWV scores.
(Shokawa et al., 2005)	Japanese Americans	Hawaii	cf-PWV	Transcutaneous transducer MCG400	Long term epidemiologic study on a baseline assessment of CV risk factors and diabetes, followed for ten years	Increased PWV was a predictor for future CVD mortality.

(Shiburi et al., 2006)	Africans	South Africa	cf-PWV	SphygmoCor	Normotensive subjects (347)	PWV threshold of 8 m/s to diagnose increases AS in young adults.
(Wojciechowska et al., 2006)	White Europeans	Poland, Belgium, the Czech Republic	PWA at Radial artery	SphygmoCor	Normotensive subjects (870)	All indices of AS were related to age, AIx was lower in men, over 40 years of age. The operational threshold must be adjusted for age and gender.
(Li et al., 2008)	Chinese	China	PWA at Radial artery	SphygmoCor	Normotensive subjects (924)	Men had higher PP and lower AIx than women. All indices showed a curvilinear relationship with age. Adjusting with confounding factors, the threshold for PP decreased while it remained the same for AIx.
(Nguyen, Srinivasan, Xu, Chen, & Berenson, 2008)	Bi-racial community (white and black)	Bogalusa, LA, USA	af-PWV	Echocardiographic Doppler US	Non-diabetic young adults (991)	Mean arterial pressure, age, smoking and HR were significant predictors of af-PWV in both races. Whereas, low adiponectin had an inverse relationship in blacks.

(Inoue et al., 2009)	Japanese	Hiroshima, Japan	cf-PWV	Automated device FCP-4731	Middle-age and elderly (3960), follow up for eight years	All-cause and CVD mortality were high with increased PWV.
(Julio A Chirinos et al., 2011)	British whites, Andean Hispanics, Chinese, black Africans	Data from different large population-based studies around the world	AIx at radial artery	SphygmoCor	Normotensive subjects (3497)	After adjustment for age, height, HR, and mean arterial pressure, higher central AIx was found in African blacks and Andean Hispanics than British whites. A lower AIx was observed in American Indians with no significant difference in Chinese and British whites.
(Santos et al., 2011)	General population (Caucasian, Mulatto, African descent), and Amerindian (native community)	Vitoria, Brazil	cf-PWV	Complior	Wider inclusion criteria to include socioeconomic, geographic and demographic data (1427)	PWV, SBP, DBP, MBP was higher in African descent compared to other groups, stays same after adjustment for age and mean arterial pressure. In normotensive people, PWV adjusted values were higher in African descent and the lowest in Amerindians.

(Farro et al., 2012)	Uruguayan	South America	cf-PWV	Mechanotransducers	Normotensive subjects (429)	Normal and reference PWV values were obtained from different algorithms for age and BP categories. Comparative PWV values in >60 years of age with European population.
(Magalhaes et al., 2013)	African	Angola, Brazil	cf-PWV	Complior	Normotensive group with CV risk factors (301), and healthy group (131)	The healthy group had low PWV scores (6.6±1.0 m/s) compared to the normotensive group (7.3±1.3 m/s). Age and plasma uric acid was a strong predictor of PWV in the healthy group, while, age, MBP, and gender were PWV predictors for the normotensive group.
(Al-Hashmi et al., 2014)	Omani Arab	Oman, Arab	AIx at radial artery and cf-PWV	SphygmoCor	Healthy subjects (120)	Mean AIx was 13±11%, and PWV value was 6.7±1.6 m/s. Women had higher AIx, whereas a higher PWV score was observed in men. Scores were comparable to the European, Uruguayan, and South African population.

(Diaz, Galli, Tringler, Ramirez, & Cabrera Fischer, 2014)	Urban and rural Argentinean	Argentina	cf-PWV	High-fidelity silicon piezo-resistive pressure sensors	Healthy asymptomatic and normotensive subjects (780)	Mean PWV was 6.84±1.65 m/s, linearly correlated with age (r^2= 0.61) in younger subjects.
(Silva et al., 2015)	Angolan (black)	Angola, Brazil	cf-PWV	Complior	Healthy prepubertal school children (157)	Mean PWV value of 5.73±0.68 m/s with no gender difference. Positive correlation with age, height, weight, BP. Height being the independent predictor in black children.

cf-PWV: Carotid-Femoral Pulse Wave Velocity, AS: Arterial Stiffness, AIx: Augmentation Index, cr-PWV: Carotid-Radial PWV, CV: Cardiovascular, CVD: Cardiovascular Diseases, PWA: Pulse Wave Analysis, PP: Pulse Pressure, af-PWV: Aorta-Femoral PWV, US: Ultrasound, HR: Heart Rate, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, MBP: Mean Blood Pressure

A scoping review of the literature was conducted to find articles testing the association of arterial stiffness with ethnicity. It was evident that ethnicity plays a significant role in estimating the normative values for PWV. There has been a keen interest in studying the association of arterial stiffness markers with cardiovascular risk factors all over the world. People from Asian (Inoue et al., 2009; Li et al., 2008; Shokawa et al., 2005), Arabian (Al-Hashmi et al., 2014) and African descent (Ferreira et al., 1999; Magalhaes et al., 2013; Santos et al., 2011; Shiburi et al., 2006; Silva et al., 2015) had lower PWV values compared to white Europeans in normotensive subjects. Hara et al. found higher PWV values in Japanese-Americans compared with the native Japanese (Hara, 1986). Later in the year 2005, a study with 10-year follow-up conducted with Japanese-Americans in Hawaii assessed the relationship between PWV and cardiovascular risk. They reported the optimal PWV cut-off of 9.9 m/s for predicting cardiovascular mortality (Shokawa et al., 2005). Inoue et al. investigated the association of PWV with all-cause and cardiovascular mortality in a large population from Japan. Comparing with previous studies, the baseline PWV values were relatively lower in participants from Japan than in Hawaii (8.3 m/s vs 9.7 m/s) (Inoue et al., 2009). A threshold of 8 m/s was observed in people with African descent, which, though not age-matched, was lower than the Framingham Heart Study Offspring cohort threshold of 12.7 m/s in men and 12.0 m/s (Mitchell et al., 2004; Shiburi et al., 2006). Li et al. studied limits of normality in Chinese population for pulse wave analysis (PWA) and found out that their thresholds were higher than the Europeans and Africans (Li et al., 2008). Latin American population subjected to PWV estimation showed comparative values with European counterparts showing different presentation of PWV values before and after 50 years of age with no gender difference (Diaz et al., 2014; Farro et al., 2012). PWV was observed to be linked with cardiovascular factors such as age, BP, smoking, CVD mortality, and variable results

for sex. Some studies have measured AIx along with PWV and found that AIx is a good predictor of arterial stiffness at a young age, whereas, PWV is higher in older adults (McEniery et al., 2005; Wojciechowska et al., 2006).

The clinical applicability of PWV depends on defining the normal and reference values in a defined population. A normal population would have normal or optimal BP with no other cardiovascular risk factors; whereas, the reference value population would present cardiovascular risk factors (Boutouyrie & Vermeersch, 2010; Elias, 2011). Several studies have been performed to estimate normal and reference values for aortic PWV in different age groups to overcome the absence of a reliable threshold to be used in clinical practice. A summary of the studies from different regions reporting normative values is shown in Table 4.

Table 4: Summary of studies estimating normal and reference PWV values

Reference	Country	Study population (number)	Age (years)	PWV values (m/s)
(Koivistoinen et al., 2007)	Finland	Healthy subjects and reference value population (799)	26-75	Male (M)= 8.9 (1.8) Female (F)=8.1 (2.0)
(Alecu et al., 2008)	France	Elderly, without hypertension or diabetes as reference value group (RVG) and with hypertension and diabetes group (HDG) (455)	60-75	RVG= 8.7 (2.3) HDG=10.2 (2.5)
(Boutouyrie & Vermeersch, 2010)	Europe	Normal and reference value population categorised as per age ranges (16,867)	All age ranges	<30=6.2 (4.7-7.6) 30-39=6.5 (3.8-9.2) 40-49=7.2 (4.6-9.8) 50-59=8.3 (4.5-12.1) 60-69=10.3 (5.5-15.0) ≥70=10.9 (5.5-16.3)

(Reusz, Cseprekal, Temmar, Kis, Cherif, Thaleb, Fekete, Szabo, et al., 2010)	Italy	Healthy children and teenagers; male (M) and female (F) (1008)	6.5-19.9	Q1, 6.55-9.91 years= M=4.39 (3.1-5.9) F=4.496 (2.8-5.8) Q2, 9.92-13.27 years= M=4.74 (3.2-6.3) F=4.77 (3.5-6.8) Q3, 13.28-16.63 years= M=5.24 (3.6-8.0) F=5.11 (3.9-6.9) Q4, 16.64-19.99 years= M=5.53 (3.7-7.9) F=5.33 (3.1-7.6)
(Elias, 2011)	Australia	Normotensive (N) group and Hypertensive (H) group (502)	40-93	N; R 40-49=8.1(1.3); 8.6 (1.5) 50-59=8.0 (1.3); 9.3 (1.5) 60-69=8.9 (1.9); 10.3(2.3) 70-79=10.1 (2.1); 12.3(3.1) 80-93=11.4(2.3);13.1(3.1)
(Fischer, Schreiver, Heimhalt, Noerenberg, & Haffner, 2012)	Germany	Healthy children and adolescent; boys (B) and girls (G) (314)	5-19.6	Q1, 5.0-8.3 years= B=4.1 (3.4-5.0) G=4.1 (3.4-5.4) Q2, 8.4-10.8 years= B=4.4 (3.6-5.1) G=4.2 (3.4-5.4) Q3, 10.9-14.1 years= B=4.7 (3.8-5.7) G=4.5 (3.4-5.9)

				Q4, 14.2-19.6 years= B=5.3 (4.2-6.2) G=4.8 (3.4-5.8)
(Hidvegi et al., 2012)	Hungary	Healthy children; boys (B) and girls (G) (1802)	3-18	B= age 3 years to 18 years mean PWV value raise from 5.5 (0.3) to 6.5 (0.3) with marked increase at age 12.1 years G= for the same age range of 3-18 PWV values got to 6.4 (0.3) from 5.6 (0.3); marked increase at 10.4 years
(Voges et al., 2012)	Germany	Healthy children and young adults (71)	2.3-28.3	M= 3.5 (0.6) F= 3.7 (0.9)

The reference data presented by these groups is based on prospective and retrospective data carried out in different countries, including different methodologies with no consideration of ethnicity. Extrapolating the same data for the NZ population could result in selection bias. For this reason, this current project aims to study the influence of ethnicity on PWV values at different age and BP groups. The study population consisted of European and non-European people, focusing particularly on Māori and Pacific people as a larger sample subset. Due to the limitation and restrictions on time and funds, the four ethnic groups were not included as separate subgroups in this study. Nonetheless, the presence of a positive correlation could be further investigated in a future study planned with a large sample size.

The European consensus document was first published in 2006 on the measurement of arterial stiffness using cf-PWV in an attempt to review the literature and recommended guidelines to measure arterial stiffness (Laurent et al., 2006). Consequently, PWV measurement was introduced as a recommended laboratory investigation for cardiovascular assessment in the 2007 ESH/ESC Guidelines for the management of arterial hypertension (Mancia et al., 2007). However, the guidelines did also note that this had limited availability in clinical practice because of a need for larger studies in different populations (Mancia et al., 2007; Mancia et al., 2013). In 2010, a task force systematically reviewed published literature to determine the normal and reference PWV values in the healthy and at-cardiovascular-risk European population. The report recommended a cut-off of PWV as 12 m/s using the direct distance method with 0.8 factor applied. The threshold value of 12 m/s has been revised to 10 m/s after a new standard distance method is applied (Van Bortel et al., 2012). In 2015, the AHA published a scientific statement regarding recommendations for improving and standardising

vascular research on arterial stiffness. The statement addressed issues such as defining arterial stiffness, methods of measurement, clinical utility, limitations and knowledge gaps (Townsend et al., 2015).

However, NZ official guidelines make no such recommendations for measuring arterial stiffness during CVD risk assessment, in comparison to the European or AHA guidelines (New Zealand Guidelines Group, 2012). Secondly, limited information on the normal and reference values of PWV in the NZ population has restricted the application of arterial stiffness in clinical settings. Estimating the normative values of arterial stiffness with a proactive approach to CVD risk assessment could help in attaining the long-term community health goals of the NZ government.

It has been stated in the ESC/ESH Guidelines for the management of hypertension that there is only a small fraction of people who would have high BP as the only reason for increased CVD risk. The majority will present with additional cardiovascular risk factors that will aggravate the total cardiovascular risk (Mancia et al., 2013). Elevated PWV values, but relatively normal BP in a clinical setting, could be a critical step to identify potential candidates for medical intervention. However, the clinical use of PWV should take into consideration other pathophysiological conditions affecting PWV, such as age and BP (Boutouyrie & Vermeersch, 2010). At present, reference PWV values have been reported from Asia, Europe, America and Australia (Diaz et al., 2014). There is no literature reporting the normal and reference PWV values in the healthy NZ population. In order to include PWV measures as a routine part of recommended cardiovascular risk assessment, an extensive dataset is required that covers the wide and unique ethnic make-up of the NZ population. This research project is the first step to formulate a data pool of PWV values in the NZ population.

2.8 Summary

This chapter provides a comprehensive review of the physiology of the arterial system and the concept of pulse wave reflection. The review establishes the role of arterial stiffness as a clinical predictor of stroke and cardiovascular risk. The clinical relevance and different ways to measure arterial stiffness were discussed. Highlighting the fact that cf-PWV has been acclaimed as a 'gold-standard' measure of arterial stiffness, the definition and mathematical equation for the assessment of PWV were reviewed. Methodological issues and expert consensus were identified for distance and transit time measurements to minimise the procedural variability. While stringent guidelines for the normal and reference PWV values are available for the European, Asian, African, and American population, the literature review identified a research gap, with no such guidelines written for the NZ population. The present study will help to establish normative values for the NZ population and assess the relationship of PWV with ethnicity and cardiovascular risk factors.

Chapter 3: Research Methods

3.1 Overview

This chapter outlines the research methods followed to scientifically address the study research questions. The chapter content is as follows: details of the study design; participant screening and recruitment; parameters measured; instruments; data collection; statistical analysis; location; and ethical considerations made during this study.

The study estimated the normal and reference values for aortic PWV in a selected sample of the NZ study population. The correlation of PWV values with associated cardiovascular risk factors was analysed. A descriptive research design was used, as it would describe the phenomenon of arterial stiffness. However, the study also included exploratory and predictive outcomes. For practical reasons, the area of research was restricted to the Auckland region. The researcher was responsible for screening, data collection and statistical analysis related to this research work.

The sample size or power calculation was discussed with the biostatistics team at the National Institute of Stroke and Applied Neurosciences (NISAN), Auckland University of Technology (AUT). It was concluded that with no literature or research study available on the reference values of PWV in the NZ population, the present study would be carried out as a pilot project to define the normative PWV values. People who responded to the study invitation flyers and who met the study criteria were contacted. The selected participants signed a written informed consent form and were grouped into three age groups, and the sample population was classified according to four blood pressure categories. With ten participants per age and BP group, and referring to the reported literature from Omani Arab population (n=120, healthy subjects) (Al-Hashmi et al., 2014)

and Brazil (Africa, n=120, normotensive and untreated hypertensive participants) (Ferreira et al., 1999) with similar objectives of estimating normal and reference values, a target sample size of 120 was determined. Ethnicity has not been considered as a subgroup for this study. However, the study sample was dichotomised for comparison as European and non-European population. Doppler ultrasound was chosen as the reference method to record PWV. It has been shown to be a feasible, reliable, and reproducible method to determine PWV based on comparative studies performed with invasive (Baguet et al., 2003) and non-invasive devices (Calabia et al., 2011; Jiang et al., 2008; Lehmann et al., 1998).

3.2 Research design

An observational cross-sectional design was used to study the correlation of aortic PWV values with age and mean blood pressure. In an attempt to obtain a representative sample within the Auckland region, the study population was screened based on the convenience sampling method where recruitment individuals was made based on the expression of interest received. Baseline participant data was used to divide the population into sub-groups of similar characteristics (e.g. age and BP as shown below in Section 3.3). Data collection was conducted from May 2018 to September 2018.

3.3 Study participants

Participants aged over 18 years and of both sexes with all ranges of BP were selected. The selected participants were categorised accordingly, as per the three age groups and four BP categories (AmericanHeartAssociation, 2017).

Age groups: 18-30, 30-60 and >60 years of age

BP categories:

1. *Normal: <120/80 mmHg,*
2. *Elevated: systolic 120-129 mmHg and diastolic <80 mmHg,*
3. *Stage 1 Hypertension: systolic 130-139 mmHg or diastolic 80-89 mmHg,*
4. *Stage 2 Hypertension: systolic at least 140 or higher or diastolic at least 90 mmHg or higher*

For screening and recruitment, an invitation flyer (See **Appendix F: Invitation flyer**) with the study information was circulated through online and offline portals. The AUT University campuses, General Practitioner (GP) clinics, and community/public places were all targeted. All efforts were made to recruit participants from a range of ethnic groups, including Māori, Pacific, European and Asian people, in order to incorporate a representative sample of the NZ population and to study any influence of ethnicity on PWV values.

In order to estimate the normal and reference aortic PWV values, the study population was sub-categorised into two groups, namely, the Normal Value Population (NVP) and the Reference Value Population (RVP). The first group, NVP, had normal BP with no additional cardiovascular risk factors (e.g. dyslipidemia and smoking). Additionally, the second group, RVP, expanded the normal value sub-set to include people with cardiovascular risk factors having no independent influence on PWV values. It has been reported that people with diabetes, and those on treatment for hypertension and dyslipidemia have significantly higher PWV values (Boutouyrie & Vermeersch, 2010). Type 2 diabetes negatively affects the aortic compliance and distensibility and alter the arterial wall structurally, thus affecting PWV (Cruickshank et al., 2002b; Taniwaki et al., 1999). It is known that PWV is dependent on systolic blood pressure; anti-hypertensive drugs such as angiotensin converting enzyme inhibitors or angiotensin receptor blockers

lower the PWV value (M. Liu, Li, Li, & Wang, 2013). Therefore, participants with these risk factors have been were excluded from the present study. A flowchart in Figure 15 depicts the selection criteria for the 'normal and reference value' study population.

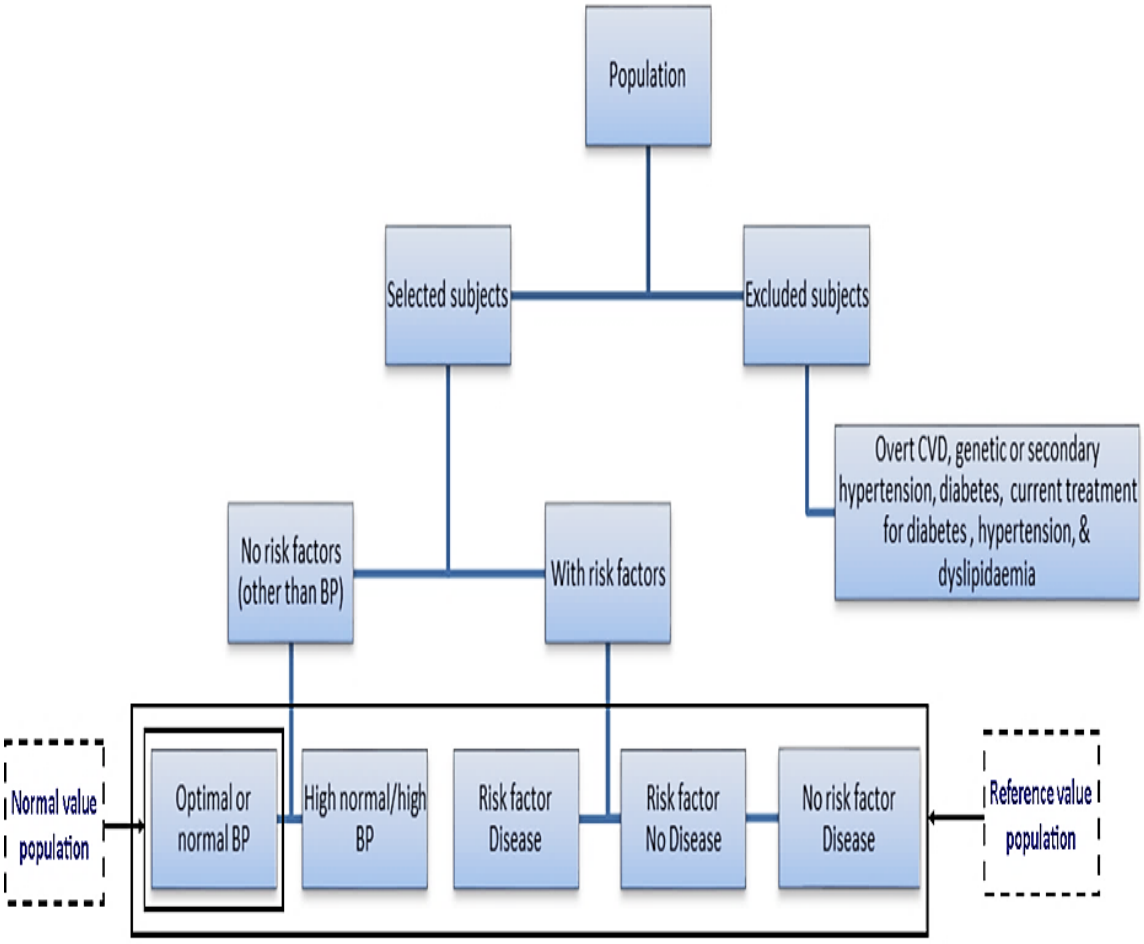

Figure 15: Selection criteria for the study population screening

3.4 Exclusion criteria

Self-reported participants were excluded regarding the presence of cardiovascular risk factors that have an independent effect on PWV, including overt/established CVD (myocardial infarction, angina pectoris, heart failure, stroke), secondary hypertension (due to kidney disease, endocrine diseases, side effect of medications, tumors), hypertensive crisis (>180/120 mmHg), diabetes, dyslipidemia, and ongoing

antihypertensive and antidyslipidaemic drug treatments (Boutouyrie & Vermeersch, 2010).

In case of identification of any abnormal findings such as irregular heartbeats or extremely high blood pressure (>160 mmHg), the researcher discussed the findings with the participant. If the participant agreed, the researcher did contact their GP.

3.5 Parameters measured

The demographic and clinical parameters were measured and recorded in the Case Report Form (See **Appendix J: Case Report Form**). The data collected from the study participants was age, sex, ethnicity, employment status, height, weight, Body Mass Index (BMI), peripheral and central SBP and DBP, mean BP, pulse rate (PR), augmentation index (AIx), smoking status, alcohol consumption, diabetes, high cholesterol, stages of hypertension, ongoing medications, and PWV with Doppler ultrasound.

3.6 Instruments

The study instruments were: ACUSON Sequoia c512 Echocardiography Ultrasound System, linear transducer probe (6L3), Uscom BP+ automated BP measurement device, weighing balance, height scale and measuring tape

3.7 Training

The researcher undertook training for performing the Doppler ultrasound at the common carotid and femoral artery from a certified sonographer (suggested by Dr Jenny Sim, Program Director, Department of Anatomy and Medical Imaging, University of Auckland, Auckland). The practice session was conducted on volunteers from the Institute of Biomedical Technology (IBTec) lab. The training consisted of understanding

the normal physiology of the arteries, recording the pulse waves, identifying normal and abnormal heart rhythms with electrocardiogram (ECG) patterns. Although, there were no inter-rater reliability test carried out, the researcher followed the standard protocol for Doppler ultrasound arterial measurement and was responsible for data collection (Baguet et al., 2003; J. Y. Hwang, 2017; Lee, 2014) and was responsible for all data recording. Measure such as proper rest time, ECG sensor placement, right positioning of transducers, and recording multiple cardiac cycles were taken to reduce inter-rater variability in the PWV measurement.

3.8 Procedure

The invitation flyers were circulated through online and offline portals at the University campus, medical practices, and around the city (See **Appendix F: Invitation flyer**). The interested participants were briefed about the study and the selection criteria. A half-hour session was scheduled, and at each session, the selected participants were provided with the Participant Information Sheet. The objectives, rationale and study processes were explained. Participants were given a chance to ask any questions or concerns they might have, before being asked to sign the Consent Form. A copy of the Participant Information Sheet and signed Consent Form was given to the participants for their record. See **Appendix G: Participant Information Sheet** and **Appendix H: Consent Form** for the respective templates. The Case Report Form as shown in **Appendix J: Case Report Form**, had the demographic and clinical data recorded for each participant. The demographic details included the date of assessment, date of birth, age, sex, nationality, ethnicity, and employment status. Participant data was de-identified in the Case Record Forms. Each participant was assigned with a 'Participant ID' to be used for identification. The participant's contact details and information regarding the

general practitioner were recorded in the Contact Details Form (See **Appendix I: Contact Details Form**). The signed Consent form and Contact Details Form were kept securely and separately from the Case Report Forms. All individual participant data was treated as confidential.

After the initial discussion on study information and recording the demographic data, a resting period of at least 10 minutes was given before measuring the clinical data. The clinical assessments were subdivided into four subsections as explained below.

3.8.1 Body mass index

As per the World Health Organization (WHO) fact sheet, BMI is used as an indication of whether a person is within a recommended healthy weight range for their height and is often used as a proxy measure for obesity (WHO, 2011). The calculation of BMI involves measuring the participant's weight and height. The universal formula for BMI is weight in kilograms (kg) divided by square of height in meters (BMI= kg/m^2). An electronic weighing scale was placed on a flat wooden surface, and a height-measuring tape was wall mounted (See Figure 16 and Figure 17). The participants were asked to step on the scale for the weight measurement barefoot and without their outer jacket, if possible, followed by standing against the wall for height measurement. The readings were recorded in the Case Report Form.

Figure 16: Electronic weighing scale

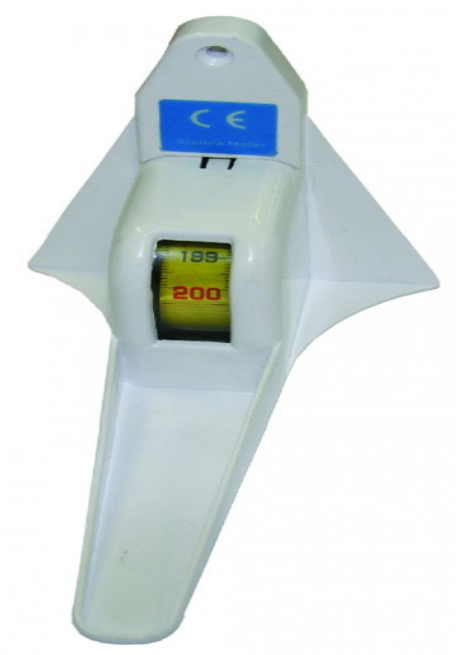

Figure 17: Wall mounted height-measuring tape

3.8.2 Blood pressure, pulse rate, and augmentation index

The blood pressure recordings were made in a seated position using the Uscom BP+ automated BP measurement device following the procedure detailed in the manufacturer's protocol (Figure 18) (C. M. Park et al., 2010). The participants were asked to sit in a chair and place the left arm on the table flexed to be at the heart level. An inflatable BP cuff of the appropriate size was wrapped around the upper left arm.

Participants were advised to not to speak during the measurement, sit straight, with their back against the chair and feet on the ground. The automated BP+ machine was started with the press of a button. The result screen on the BP+ monitor gave the information on the peripheral SBP and DBP, central SBP and DBP, PR, and AIx. Additionally, a graphical representation of the pressure wave and a rhythm strip were shown on the screen. The readings were repeated twice with an interval of two minutes and were averaged. If the difference between the consecutive readings was more than +/- 10 mmHg, the readings were repeated to have an average of three readings. The parameters values were recorded in the Case Report Form.

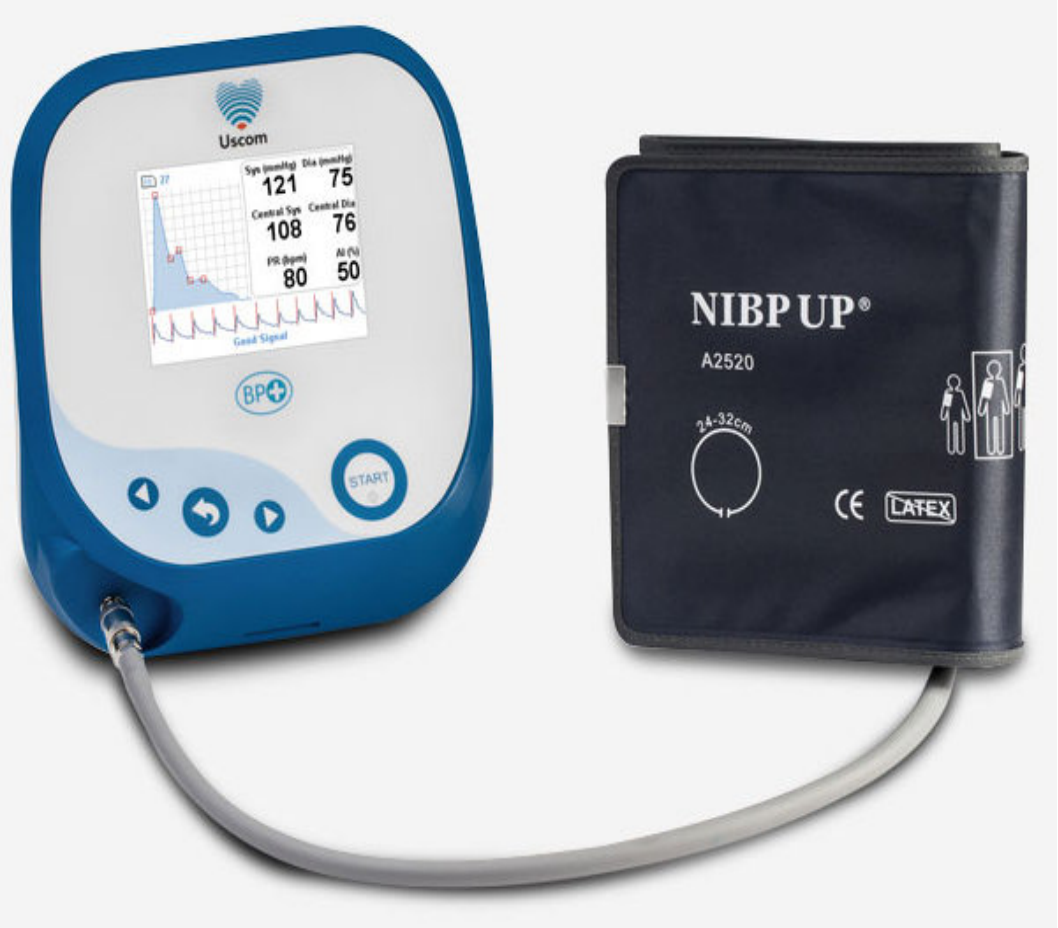

Figure 18: Uscom BP+ monitoring device

3.8.3 CV risk factors and medication information

The clinical data section of the Case Report Form had self-reported information relating to various cardiovascular risk factors such as smoking, alcohol consumption, diabetes, and dyslipidaemia. The stages of hypertension were marked based on the peripheral systolic and diastolic BP as normal (<120/80 mmHg), elevated (systolic 120-129 and diastolic <80 mmHg), stage 1 (systolic 130-139 or diastolic 80-89 mmHg), and stage 2 (systolic ≥140 or diastolic ≥90 mmHg). Information on any ongoing medications including the diagnosis, doses, frequency, starting date, and drug compliance was recorded. In instances during the initial part of the session, where it was revealed that the participant was on current medications belonging to the antihypertensive, antidiabetic or antidyslipidaemic categories, the participant was informed that they were ineligible for the study and their data was excluded from the study.

3.8.4 Pulse wave velocity

Doppler ultrasound (ACUSON Sequoia c512 Echocardiography Ultrasound System, Figure 19) was used for calculating the pulse wave velocity at the right common carotid artery and right common femoral artery. The imaging was carried out at the B-mode (2D mode) and PW-mode (Pulse Wave mode) in colour and spectral Doppler. A 6L3 linear vascular transducer probe with a frequency bandwidth of 6-3 MHz and three lead ECG were equipped with the ultrasound machine (Figure 20 and Figure 21). All readings were taken twice in succession across three to five cardiac cycles at the two arterial sites. The images were stored to the AUT's ELECTECH DICOM server and viewed through the RadiAnt Dicom Viewer 1.0.4 image viewer.

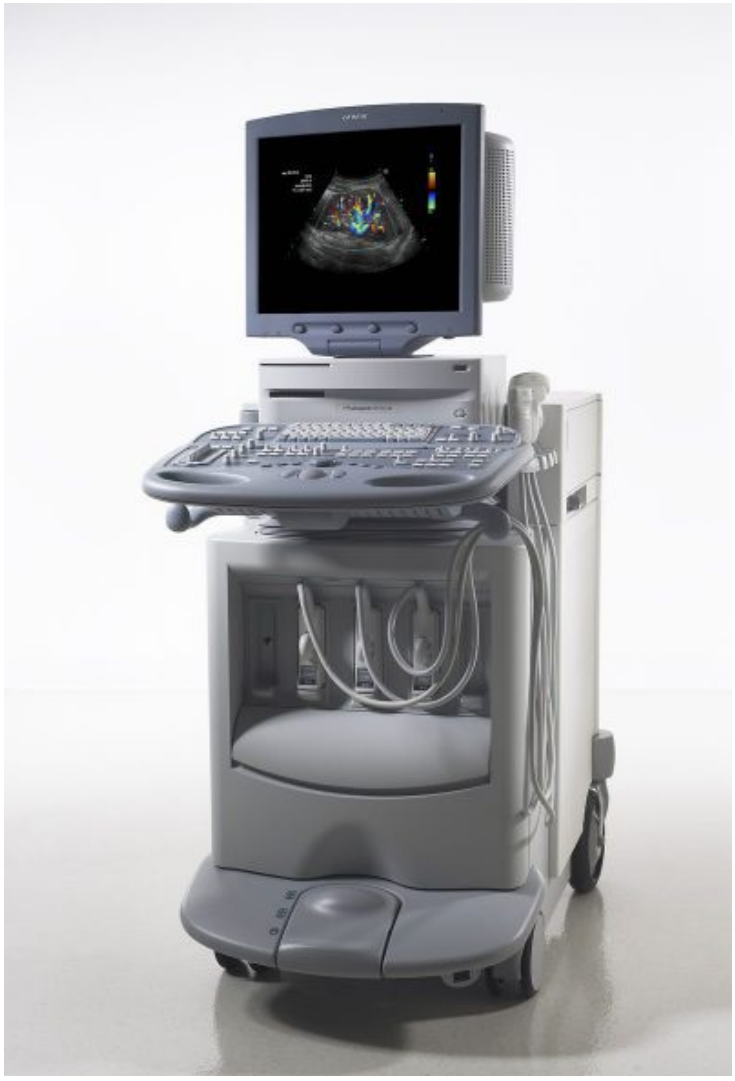

Figure 19: ACUSON Sequoia c512 Echocardiography Ultrasound system

Figure 20: ACUSON 6L3 transducer

Figure 21: Three lead ECG cable

The participants were asked to lie down on the bed in the supine position, head slightly raised over a pillow in a comfortable position. The ECG electrode patches were placed on the right and left wrist and right ankle after palpating for the pulse. The ECG leads were then connected to the respective electrodes. The participant's details were entered in the Doppler ultrasound and they were advised not to speak or to sleep during the testing. With the participant's neck tilted, so they were looking to the left, the common carotid artery was palpated at the supraclavicular level, and the transducer was placed transversely. The artery was examined proximally in transverse followed by distally to the bifurcation. Other factors, such as depth, gain, focal zone, angle, and scale were optimised. The pulse wave was imaged in the pulse wave spectral and colour mode by placing the transducer longitudinally 1-2 cm proximal to the carotid bifurcation. A similar procedure was repeated for the common femoral artery after identifying the location by placing the transducer at the inguinal crease (groin) in the transverse plane. The images with bi-phasic pulse wave at the common carotid artery and tri-phasic pulse waves at the common femoral artery gated against the ECG were saved for analysis.

The distance (m) between the two marked arterial sites was measured with a measuring tape using the direct distance method. The transit time was calculated using the foot-to-foot method or intersecting tangent approach (See Figure 10 for the transit time measurement) from the stored images retrieved from the ELECTECH. Using the digital callipers provided with the RadiAnt software, time was measured from the R wave of the PQRST wave to the foot of the pulse wave for three consecutive waveforms (as shown in Figure 22 and Figure 23). The average time from the common carotid artery subtracted from the average time calculated from the common femoral artery gave the transit time. As advised in the consensus document, 80% of the common carotid artery

to common femoral artery direct distance was used (Van Bortel et al., 2012). Therefore, PWV was estimated as the direct distance from carotid and femoral sites divided by the transit time times 0.8 as a factor.

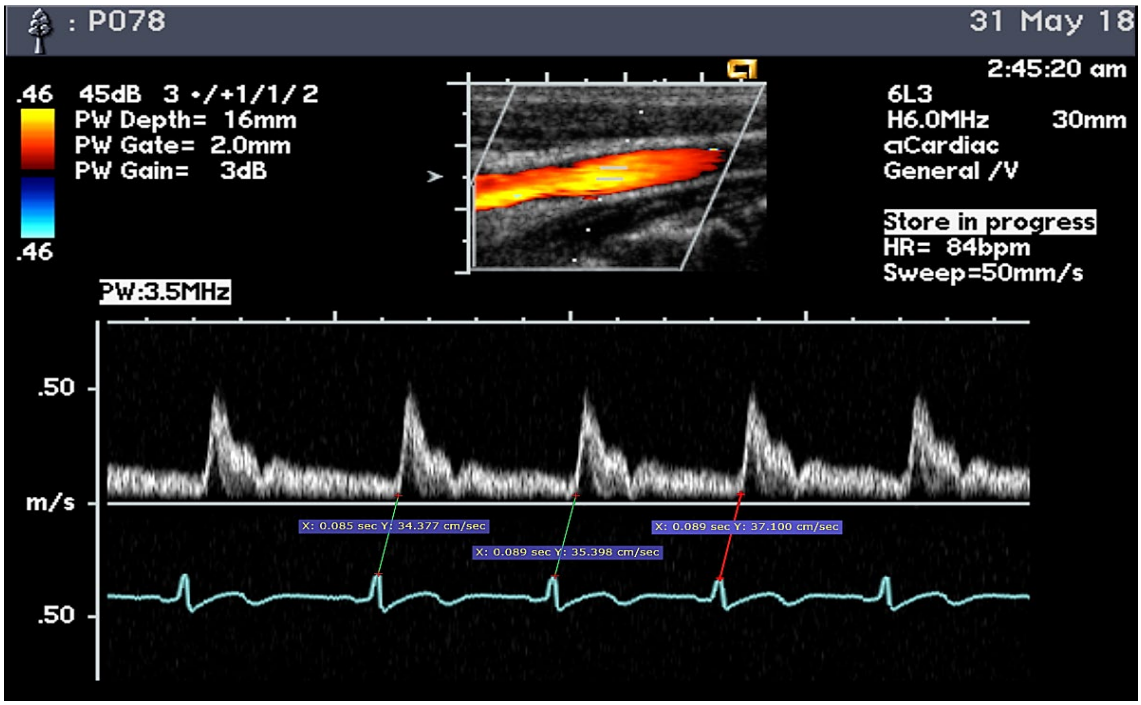

Figure 22: Doppler of the pulse wave at the common carotid artery

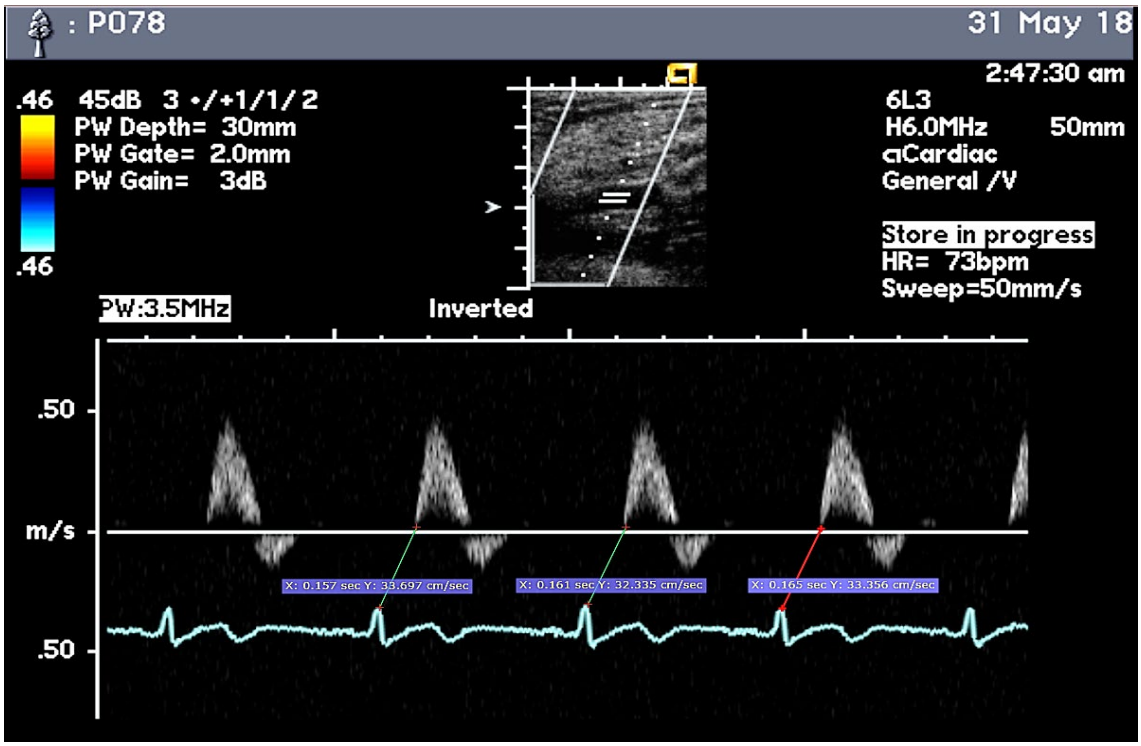

Figure 23: Doppler of the pulse wave at the common femoral artery

3.9 Statistical analysis

The statistical analysis was performed by the researcher using SPSS for Windows Version 24.0. Analysis was planned as per the following steps: (a) descriptive analysis for the demographic and clinical parameters, (b) correlational analysis to measure the relationship between the variables, (c) inferential analysis for comparing the means between the groups and judging the probability distribution and variance, and (d) regression analysis to fit a prediction model and regression equations.

A lack of NZ specific data meant that a sample size calculation was not possible. The sample size of 120 participants was based on the available literature from overseas. Based on the collected data, an SPSS dataset was prepared with the study data by defining the variables measures and their values. Variables with nominal measures were sex (1= Male, 2= Female), ethnicity (1= European population, 2= non-European population), and mean PWV. Age categories (1= 18-30 years, 2= 31-60 years, 3= >60 years), smoking (1= Yes, 2= No), alcohol consumption (1= Yes, 2= No), diabetes (1= Yes, 2= No), dyslipidemia (1= Yes, 2= No), hypertension (1= Yes, 2= No), BP categories (1= normal, 2= elevated, 3= stage 1, 4= stage 2), and NVP or RVP group (1= NVP, 2= RVP) were defined as ordinal measures. Finally, height, weight, BMI, peripheral and central SBP and DBP, PR, AIx, and MBP were categorised as scale measures.

3.9.1 Descriptive analysis

The descriptive data was expressed as numbers, frequencies, and percentages. Continuous variables with normal distribution were expressed as a mean (95% Confidence Interval), whereas, data that was not normally distributed was presented as median (range) and percentages for population or quantitative characteristics. The PWV values were represented as mean ± SD and median 25th and 75^{th} percentiles to identify

the extreme limits. The assumptions of the data for being parametric or non-parametric would be checked running the normality tests (Shapiro-Wilks test, skewness, normality probability plots: P-P plots), homogeneity of variance. A p-value of less than 0.05 was considered as statistically significant.

3.9.2 Correlation analysis

The relationship between the variables (PWV values and cardiovascular risk factors) was tested with the correlation analyses. A bivariate correlation calculating the Pearson's coefficient for the normally distributed data and Spearman's coefficient for non-normally distributed data was made. The effect size was defined as small (r=0.10), medium (0.30), and large (0.50).

3.9.3 Inferential analysis

An inferential analysis was performed on the data to study the degree of association to make generalisations about the whole population the sample was taken. The objective was to test the hypothesis that there is no difference in the mean PWV values in the different groups compared. The normal data were analysed by the t-test or analysis of variance (ANOVA) for the three age groups and four BP groups, whereas, the non-normal data were analysed using the Mann-Whitney U test or Kruskal-Wallis test.

The independent influence of age and BP on PWV was estimated while adjusting for other cardiovascular risk factors (overt/established CVD, secondary hypertension, diabetes, dyslipidaemia, and ongoing antihypertensive, antidyslipidaemic and hypoglycemic drugs treatment)s using the analysis of covariance (ANCOVA).

3.9.4 Regression analysis

The regression equations and graph were formulated by running the linear regression analysis to the data. By fitting a model to the data, predicting values of the PWV were determined from the predictor variables (cardiovascular risk factors: age and mean BP (MBP)). Following that, multiple regression analysis models were developed using the backward stepwise method to see the increase in the prediction of PWV after adding other variables.

3.10 Location

The research was carried out at the AUT, City campus. A clinic laboratory setup was staged with a Doppler ultrasound machine, table, chair, bed, and changing area at the WD301E, IBTec, AUT.

3.11 Ethical consideration

Ethical approval for research in human subjects was sought from the National Health and Disability Ethics Committee (HDEC) and the researcher's institutional Auckland University of Technology Ethics Committee (AUTEC). The participant's privacy and confidentiality was protected during and after the study. The study files and all other information provided by the participants remained strictly confidential. No material that could personally identify the person was used in any reports on this study. All future use of the information collected will be strictly controlled in accordance with the Privacy Act. Upon completion of the study, the case report files, and other records were stored in an external storage device in a secure place with Dr Rita Krishnamurthi at room AR416, Level 4, NISAN, AUT, North Shore Campus. All computer records were password protected. The signed consent forms were stored with Dr Andrew Lowe, WS305, School

of Engineering, Mathematical and Computer Sciences, AUT, City Campus. The study data will be stored for ten years before being destroyed.

3.12 Summary

Chapter 3 is about the research approaches and methodology used to conduct the study. Following on the information gathered in Chapter 2 from the literature review, a study protocol was designed to answer the study research question. Section 3.1 gives an overview of the thought process behind choosing the appropriate methods. The subsections of the protocol, including the design, study participants, screening procedure, which instruments were used and the procedure followed to collect data, are mentioned in the following sections. The rationale behind using different statistical approaches are provided in Section 3.9. The chapter summarises with information on the location and the ethical considerations made during the research project in Section 3.9 and 3.10, respectively. The subsequent Chapter 4 will contain the findings from the data analysed.

Chapter 4: Results

4.1 Overview

In this chapter, data is presented to support estimates of the normative values of aortic pulse wave velocity (PWV). The subsections will have descriptive information on the demographic and clinical characteristics of the cohort studied. It is followed by the outcomes of the inferential analysis exploring the correlation of cardiovascular risk factors and predicting models for the PWV.

4.2 Sample characteristics

The invitation flyers were emailed and placed around public places such as the University campus, and rest homes. A total of 195 responses were received after the invitation flyers were circulated. Of those participants interested in the study, 75 were excluded based on the criteria listed in chapter 3, section 3.4., page 76. Most of these excluded participants were on prescribed medications for hypertension, diabetes, and/or hypercholesterolemia (high blood cholesterol). However, three participants with recently diagnosed borderline dyslipidaemia (not on medication) were included in the reference group. Some of those interested had to be declined as the required numbers for that age group were met. The 120 participants that met the eligibility criteria were then categorised into two groups, namely, the Normal Value Population (NVP) and Reference Value Population (RVP) based on the presence of the risk factors. The distribution of the selected participants is shown in Figure 24.

The demographic and clinical characteristics of the study population are summarised in Table 5. The data is presented by two main groups, NVP and RVP, and as a whole participant population with an overall age range of 19-88 years. The mean age of the

NVP group was lower than that of the RVP group, aged 39.7 years and 56.5 years, respectively. The sex ratio was with 54:66 (male to female). The study population was categorised into two major groups as European (n=65) and non-European (n=55). The non-European group consisted mainly of participants who were Māori (1.7%), Asian (36.7%), Middle Eastern, American, and African ethnicity (MELAA) (36.7%). The distribution of participants as ethnic mix and age groups is shown in Table 6, while the percentage of each ethnic group in comparison with the Auckland region and New Zealand as per the 2013 census is represented in Table 7. The RVP group had a higher number of participants belonging to European descent (26 European vs 17 non-European). The Body Mass Index (BMI) calculated with the recorded height and weight was similar in both groups (NVP: 24.99; RVP: 25.85).

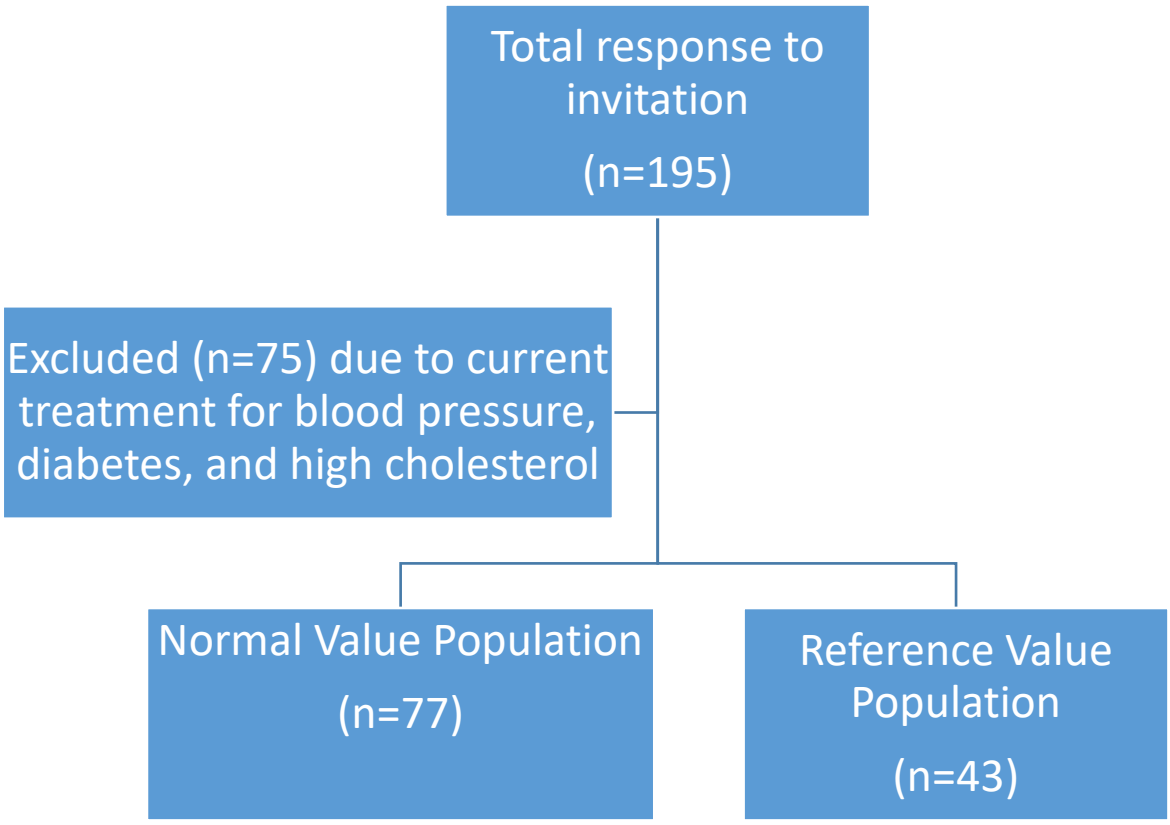

Figure 24: Flowchart showing the selection and classification of study participants.

Table 5: Demographic and clinical characteristics of the study population

Parameters	**Normal value population**	**Reference value population**	**Whole population**
N	77	43	120
Age [years, mean (± SD)]	39.69 (16.95)	56.52 (18.0)	45.58 (19.04)
Age range (years)	19-88	21-85	19-88
Sex (M/F)	37/40	17/26	54/66
Ethnicity (E/NE)	36/41	17/26	65/55
Height [m, mean (± SD)]	1.69 (0.11)	1.66 (0.10)	1.68 (0.10)
Weight [kg, mean (± SD)]	71.77 (14.46)	71.69(16.06)	71.74 (14.99)
BMI [kg/m^2, mean (± SD)]	24.99 (4.31)	25.85 (4.70)	25.30 (4.45)
SBP [mmHg, mean (± SD)]	111.90 (9.76)	138.80 (15.81)	121.54 (17.80)
DBP [mmHg, mean (± SD)]	72.88 (8.62)	86.33 (9.67)	77.70 (11.07)
CSBP [mmHg, mean (± SD)]	105.31 (10.09)	131.00 (15.73)	114.52 (17.48)
CDBP [mmHg, mean (± SD)]	73.42 (8.68)	86.87 (9.95)	78.24 (11.18)
MBP [mmHg, mean (± SD)]	88.44 (8.25)	107.32 (11.27)	95.20 (13.06)
PR [bpm, mean (± SD)]	76.78 (17.38)	71.58 (15.29)	74.92 (16.78)
AIx % [mean (± SD)]	63.09 (40.65)	106.48 (64.84)	78.63 (54.55)
Smoking [Yes, n (%)]	13 (16.88)	5 (11.63)	18 (15)
Alcohol consumption [Yes, n (%)]	47 (61.04)	32 (74.42)	76 (63.33)
Diabetes [Yes, n (%)]	-	0	0
Dyslipidaemia [Yes, n (%)]	-	3 (2.5)	3 (2.5)
Hypertension [Yes, n (%)]	-	30 (25)	30 (25)
Mean PWV [m/s, mean (± SD)]	5.40 (1.21)	6.73 (1.56)	5.88 (1.49)

SD: Standard Deviation, M: Male, F: Female, E: European population, NE: non-European population, BMI: Body Mass Index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, CSBP: Central Systolic Blood Pressure, CDBP: Central Diastolic Blood Pressure, MBP: Mean Blood Pressure, PR: Pulse Rate, AIx: Augmentation Index, PWV: Pulse Wave Velocity, n: number of participants

Table 6: Ethnic mix breakdown as per the age groups

Age group (years)	Number of participants					
	European	Māori	Pacific people	Asian	MELAA	Others
18-30	15	0	0	20	5	0
31-60	17	2	0	17	4	0
>60	33	0	0	7	0	0

Table 7: Ethnic groups in the study compared with Auckland region and NZ as per the 2013 census

Ethnic group	Auckland region (%)	New Zealand (%)	Study (%)
European	59.3	74.0	54.2
Māori	10.7	14.9	1.7
Pacific people	14.6	7.4	0
Asian	23.1	11.8	36.7
MELAA	1.9	1.2	7.5
Others (New Zealander, other ethnicity nec)	1.2	1.7	0
MELAA: Middle Eastern, Latin American, African; nec: not elsewhere classified			

4.3 Normal and reference PWV values

The mean PWV of the whole study population (n= 120) was 5.88 ± 1.49 m/s. Healthy participants with normal BP constituting the NVP group had the lowest mean PWV value at 5.40 ± 1.21 m/s, while in the RVP group with cardiovascular risk factors the mean PWV was 6.74 ± 1.56 m/s. The mean PWV values of the NVP and RVP groups were significantly different ($p<0.001$, $z= -4.986$) when compared using the non-parametric test (Mann-Whitney Test). After binning the data cases by age categories and the two population groups, namely, NVP and RVP, descriptive analysis and non-parametric tests, were conducted. The mean PWV values for each group in different age groups is shown in Table 8. The majority of participants aged 18-30 years were in the NVP group (n=34)

while only six were in the RVP group with no significant difference in their mean PWV values. For the older age group of over 60 years of age, though the number of RVP participants was more than that of NVP (n, RVP= 25, NVP= 15) the difference in the mean PWV values was not significant. The middle age group of 31-60 years showed that the mean PWV values for the RVP (n= 12) were significantly different from that of the NVP group (n= 28) with a p-value of 0.02.

Table 8: Mean pulse wave velocity in the normal and reference groups as per the age category

Age category (years)	**Mean PWV (± SD) (m/s)**				
	NVP	**n**	**RVP**	**n**	**P-value**
18-30	4.75 (0.75)	34	5.10 (0.22)	6	0.063
31-60	5.48 (0.97)	28	6.39 (1.32)	12	0.020*
> 60	6.72 (1.41)	15	7.30 (1.55)	25	0.171
All	5.40 (1.21)	77	6.74 (1.56)	43	0.001***

PWV: Pulse Wave Velocity, SD: Standard Deviation, NVP: Normal Value Population, RVP: Reference Value Population, *: p<0.05, **: p<0.01, ***: p<0.001

The mean and median PWV values, along with the 25^{th} and 75^{th} percentile for different age groups, are shown in Table 9. The mean PWV values compared within the three age categories were significantly different, the highest being the over 60 years of age group with 7.08 m/s [18-30 vs. 31-60 ($p<0.001$, $z = -3.26$), 18-30 vs. >60 ($p<0.001$, $z = -6.40$), 31-60 vs. >60 ($p<0.001$, $z = -4.16$)]. A box plot in Figure 25 shows the distributional characteristics and level of mean PWV scores of the three groups (n= 120). A box plot has got a box and two whiskers that divides the data into four quartiles. It helps in understanding the data distribution, variance, mean, and median at a glance. The box plot for 18-30 years group is comparatively shorter, suggesting that overall participants have a high level of agreement in PWV values. It can be concluded that 75% of the PWV

data for 18-30, 31-60, and > 60 years group is less than 5.22, 6.29, and 7.93, respectively. The box representing the inter-quartile range (50% of the scores) was biggest for the middle-aged group (31-60 years) indicating higher variation in the PWV values than the other two groups. The mean PWV value of the age groups was statistically different compared to within each group as shown in Figure 25.

Table 9: Pulse wave velocity distribution as per the age categories

Age category (years)	Mean PWV (± SD) (m/s)	Median PWV (25-75 percentile) (m/s)
18-30	4.81 (0.70)	4.87 (4.26-5.23)
31-60	5.75 (1.15)	5.52 (4.85-6.43)
> 60	7.08 (1.50)	6.49 (5.84-8.03)
All	5.88 (1.49)	5.56 (4.86-6.50)

PWV: Pulse Wave Velocity, SD: Standard Deviation

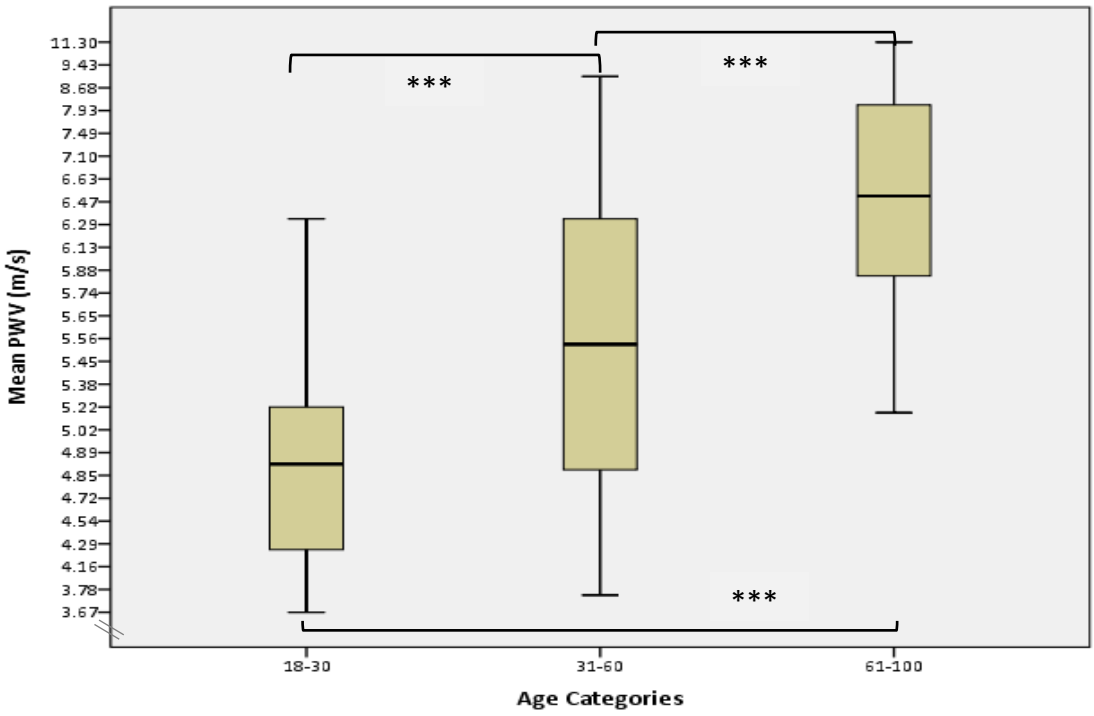

*Figure 25: Box plot distribution of the mean pulse wave velocity (PWV) in different age groups (***: p<0.001 indicates statistically significant difference of the respective age groups)*

Similarly, analysing the mean PWV values in males and females using a non-parametric Mann-Whitney Test showed no significant difference between them in any of the individual age groups. The outcome is shown in Table 10. The number of participants in the sex variable had a normal distribution; therefore, group means were compared using the independent t-test showing a significant difference in PWV overall between males and females (p= 0.03).

Table 10: Mean pulse wave velocity by sex and age category

Age category (years)	Mean PWV (± SD) (m/s)				P-value
	Male	n	Female	n	
18-30	4.91 (0.75)	23	4.67 (0.63)	17	0.35
31-60	5.48 (0.82)	19	6.00 (1.35)	21	0.54
> 60	6.92 (1.80)	12	7.15 (1.40)	28	0.44
All	5.56 (1.2)	54	6.15 (1.57)	66	0.03*

PWV: Pulse Wave Velocity, SD: Standard Deviation, NVP: Normal Value Population, RVP: Reference Value Population, *: p<0.05

The ethnic groups mentioned in Table 6 and Table 7 were grouped as European and non-European for comparing the mean PWV values. A non-parametric test (Mann-Whitney Test) analysing the two ethnic groups showed a significant difference (p= 0.004, z= -2.889) between the two groups. The European population (n= 65) had a mean PWV of 6.20 ± 1.5 m/s, while the non-European population group (n= 55) presented with a mean PWV value of 5.50 ± 1.4 m/s.

4.4 Cardiovascular risk factors and PWV – correlation analysis

To evaluate the degree of association between the mean PWV values and recorded cardiovascular risk factors, a non-parametric correlation analysis was performed by estimating the Spearman's rho (r). To interpret the r-values, the association could be positive or negative, where, r = ±0.1 represents a small effect, r = ±0.3 is a medium effect

and r = ±0.5 would be a large effect. Cardiovascular risk factors such as age (r = 0.71, p<0.01), mean blood pressure (MBP) (r = 0.49, p<0.01), blood pressure (BP) categories (r = 0.50, p<0.01), and being in the NVP or RVP group (r = 0.46, p<0.01) showed a positive significant correlation with PWV with a large effect. Height and weight were not significantly correlated; however, BMI (r = 0.21, p<0.01) shared a medium effect positive correlation with mean PWV. Overall, sex as a risk factor was positively correlated but with a small effect (r = 0.17, p = 0.02). The association between the mean PWV and BP categories as per the age groups is depicted in Figure 26. It is evident that for each age group, the elevated BP group had lower PWV scores compared to other BP categories. The youngest age group had the lowest PWV, which coincided with this group having the lowest BP. Moreover, within the youngest age group, PWV drops most dramatically with increasing BP, whereas PWV was higher in older age groups and did not change much except for a drop in the oldest age group of 'elevated BP category'.

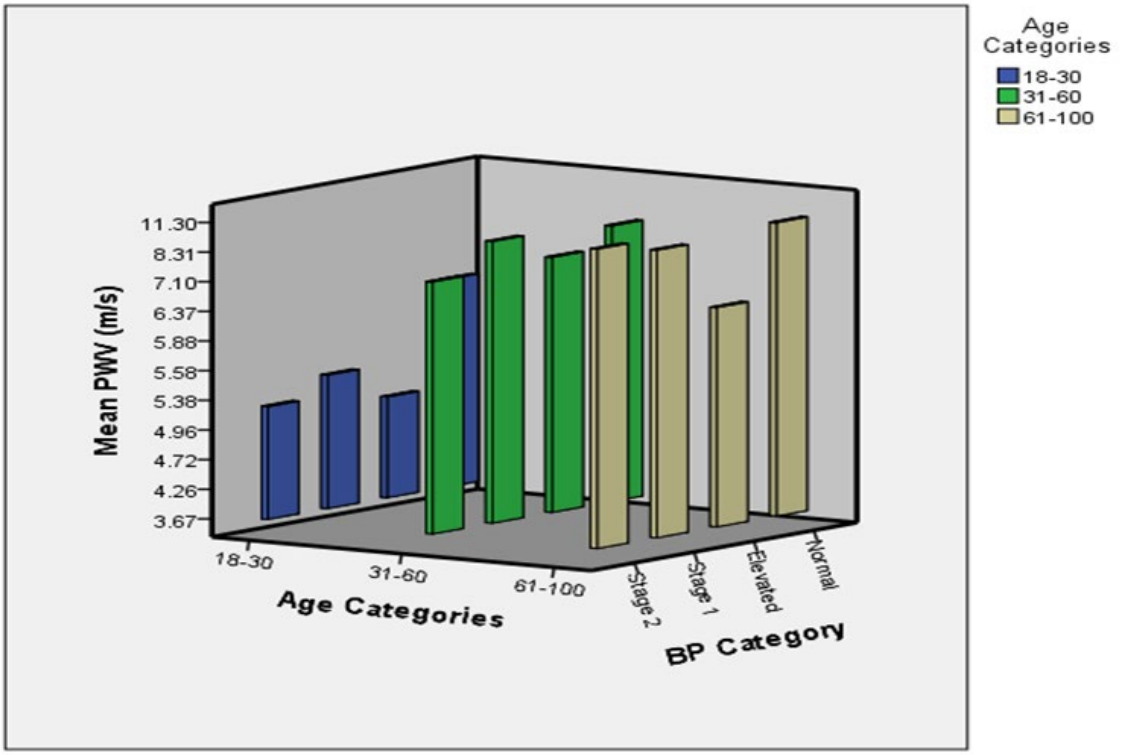

Figure 26: Mean pulse wave velocity (PWV) values according to the age and blood pressure (BP) categories

4.5 Influence of cardiovascular risk factors on PWV – regression analysis

From the correlation analysis, it was evident that age and BP were two major positively correlated cardiovascular risk factors. A linear regression model was conducted to determine the association of the outcome variable or dependent, i.e. mean PWV from a predictor or independent variable. As a cardiovascular risk factor, age accounts for 48.8% of the variability in mean PWV [R^2 = 0.49, F (1,118) = 112.32, p<0.001]. The regression equation having age as a predictor for mean PWV was as follows:

$$\boldsymbol{PWV = 3.391 + 0.05 \times Age}$$

It was evident that age significantly predicted mean PWV (β = 0.55, p<0.001). The graph in Figure 27 demonstrates the regression equation and the correlation between age and mean PWV. Addition of MBP as predictor variable into the age-centred regression model influenced mean PWV significantly (p<0.001). The enhanced prediction by MBP accounted for 54.5% variability in an age-centred mean PWV [R^2 = 0.54, F (2,117) = 70.17, p<0.001]. The adjusted regression model equation was expressed as:

$$\boldsymbol{PWV = 0.908 + 0.04 \times Age + 0.03 \times MBP}$$

A multiple linear regression was used to model a linear relationship between the positively correlated independent variables. Cardiovascular risk factors such as age, sex, BMI, MBP, smoking, alcohol drinking, BP categories, and being in NVP or RVP group were tested for their cumulative influence on the mean PWV. A stepwise backward method was used to enter the predictors in the model. The resultant model calculated the contribution of each variable based on the p-values of the t-test and removed the variables that did not fit. The four variables that showed association with the mean PWV were age (β = 0.04, p<0.001), BMI (β = 0.04, p<0.05), BP category (β = 0.69, p<0.001),

and NVP or RVP groups (PopuNR) (β = -0.81, p<0.05). All together, these four variables account for 57% of the variation in mean PWV scoring [R^2 = 0.57, F (4,115) = 39.09, p<0.001]. The linear model equation that fits the contributing variables is given as:

$$\boldsymbol{PWV = 2.708 + 0.04 \times Age + 0.04 \times BMI + 0.69 \times BP\ Category - 0.81 \times PopuNR}$$

The residual graphs are represented in Figure 28 and Figure 29. A histogram of the residuals in Figure 28 and residuals vs the predicted mean PWV in Figure 29 showed a pattern close to a normal distribution. A horizontal regression line at R^2 =0 affirms the hypothesis.

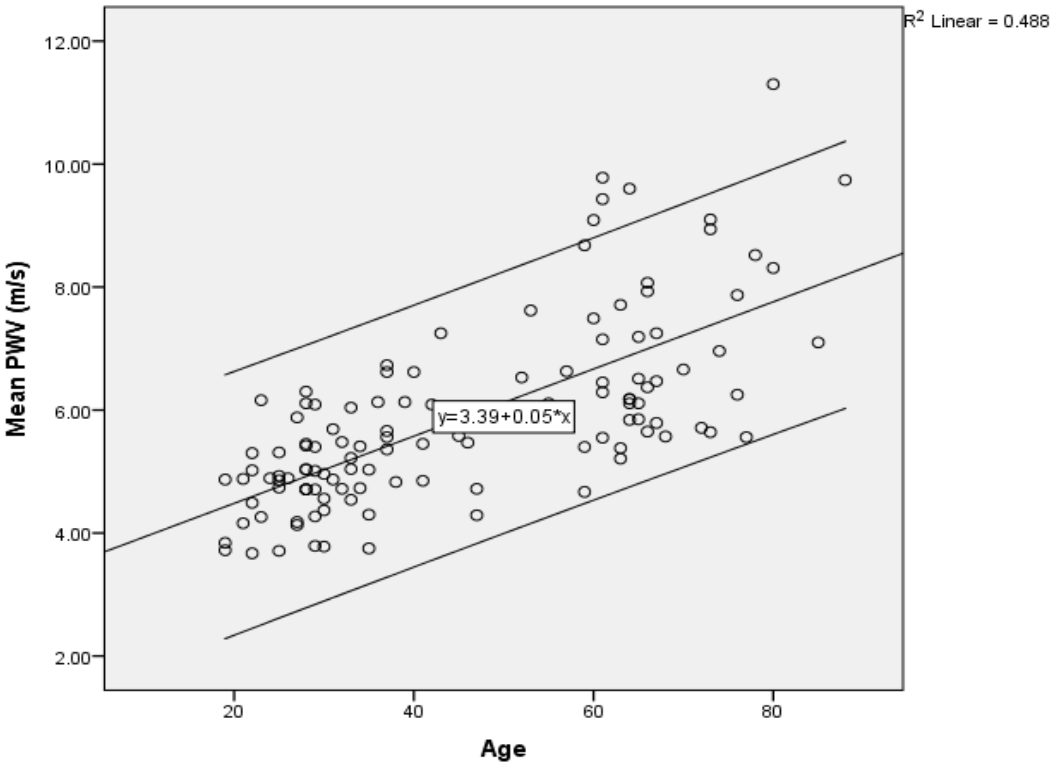

Figure 27: Mean pulse wave velocity (PWV) and age linear regression model

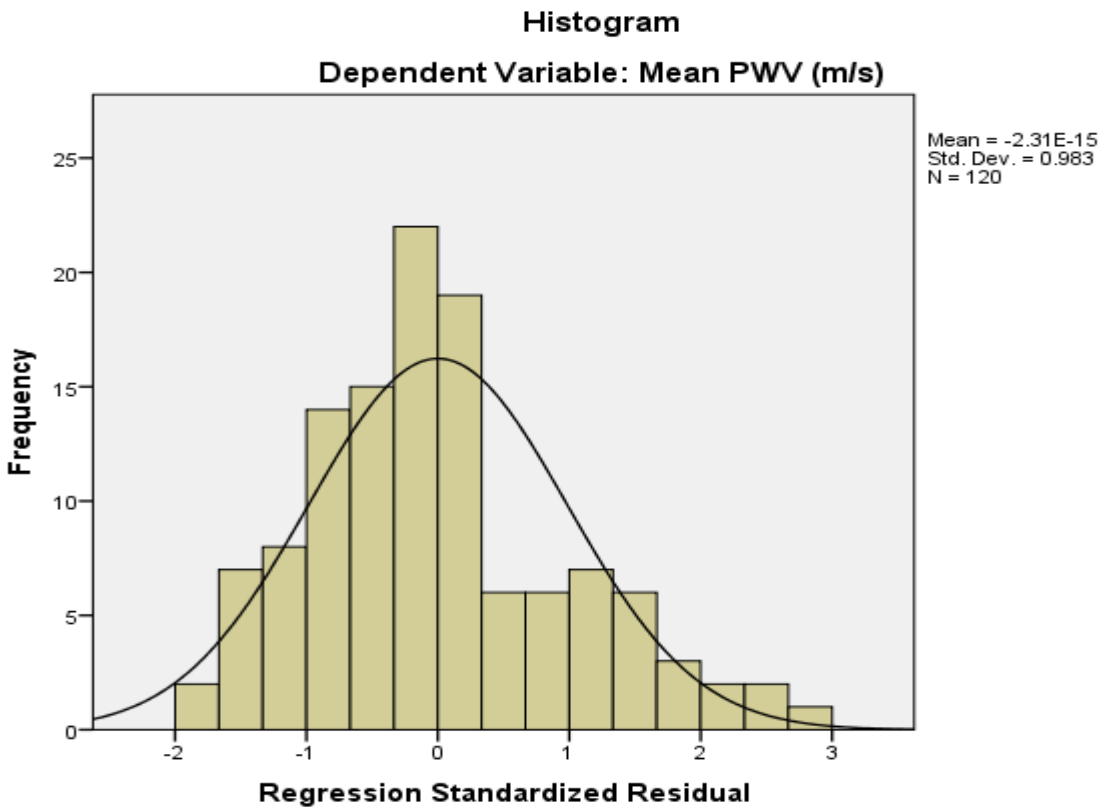

Figure 28: A histogram of the regression standardised residual

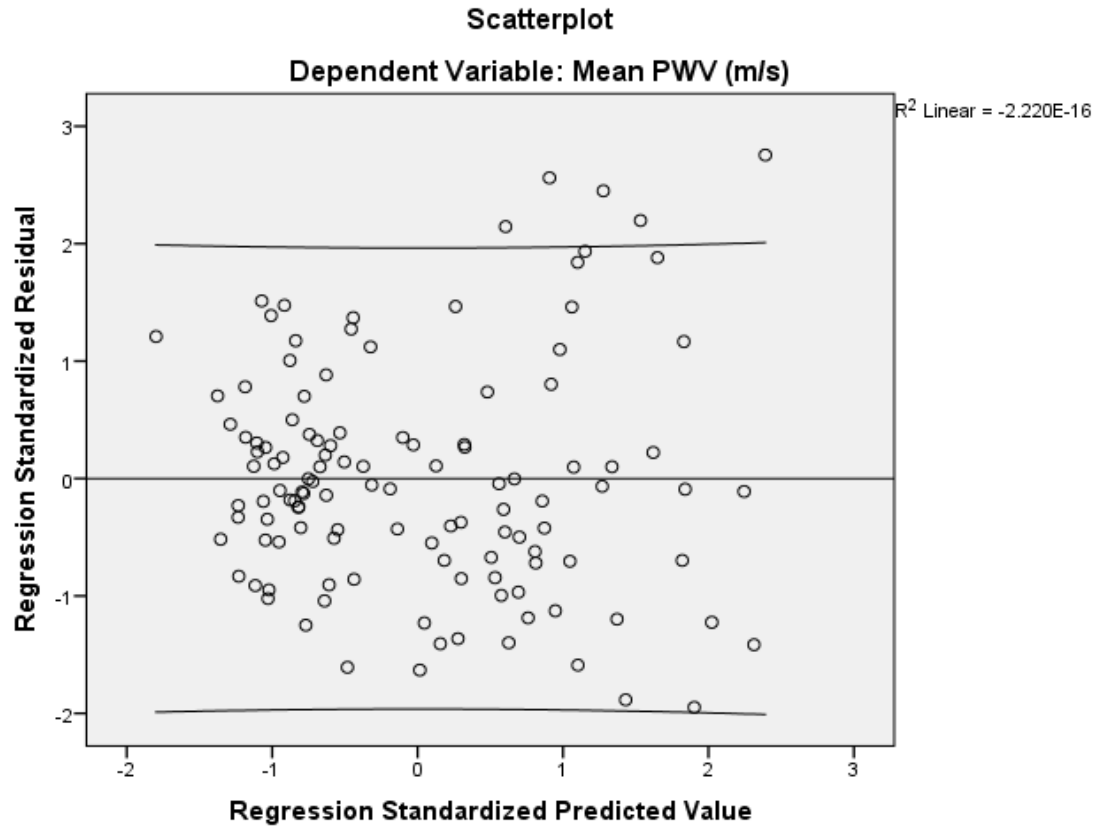

Figure 29: A scatterplot of the regression standardised residuals and predicted mean pulse wave velocity (PWV) values

Table 11 shows a comparative analysis of multiple regression analysis from the present study and data published by other published studies with the objective of assessing the correlation and predictability of PWV. These studies had similar age range with varied sample size. It was observed that the regression models form different regions had a mix of independent variables, including age, MBP, BP categories, height, BMI, HR, gender (female), and smoking. Comparing the regression equations from studies having European, American and African population, indicated lower mean PWV values in general (shown in Figure 30).

Table 11: Comparative analysis of regression analysis of the present study with other published literature

Reference	**Unstandardized coefficient**	**Study sample description**
(Ferreira et al., 1999)	PWV= 4.078 + 0.042 x age + 0.024 x SBP	120, 19-50 years, African and European
(Nguyen et al., 2008)	For white race, R^2= 17.5 MAP= 0.28, age= 0.22, smoking= 0.13, HR= 0.08, adiponectin= –0.006 For black race, R^2= 23.1 MAP= 0.32, age= 0.18, smoking= 0.14, HR= 0.12, adiponectin= – 0.13	991, 24-44 years, white and black American
(Elias, 2011)	PWV= 10.059 + 1.069 x age group + 0.447 x BP diagnostic category + 0.081 x age group x BP R^2= 0.38	626, 40-93 years, American
(Santos et al., 2011)	Ethnicity= 0.246, gender= 0.217, age= 0.071, MBP= 0.046, HR= 0.004, BMI= -0.041	1427, 25-64 years, Brazil
(McEniery et al., 2005)	Age= 0.078, MBP= 0.034, HR= 0.016, gender (female)= – 0.266 Adjusted R^2= 0.65	4001, 18-90 years, American

(Reusz, Cseprekal, Temmar, Kis, Cherif, Thaleb, Fekete, Szabo, et al., 2010)	PWV=1.129 + 0.049 x age + 0.008 x height + 0.024 x MAP	450, European, healthy school children and adolescents
(Magalhaes et al., 2013)	PWV= 1.899 + 0.065 x age + 0.057 x MBP – 0.504 x gender (female) R^2= 0.34	301, 22-72 years, African
Present study (Dahiya, 2019)	PWV= 2.708 + 0.04 × Age + 0.04 × BMI + 0.69 × BP Category - 0.81 × PopuNR	120, 19-88 years, New Zealanders

PWV: Pulse Wave Velocity, SBP: Systolic Blood Pressure, MAP: Mean Arterial Pressure, HR: Heart Rate, MBP: Mean Blood Pressure, BMI: Body Mass Index, PopuNR: being in the normal or reference value population

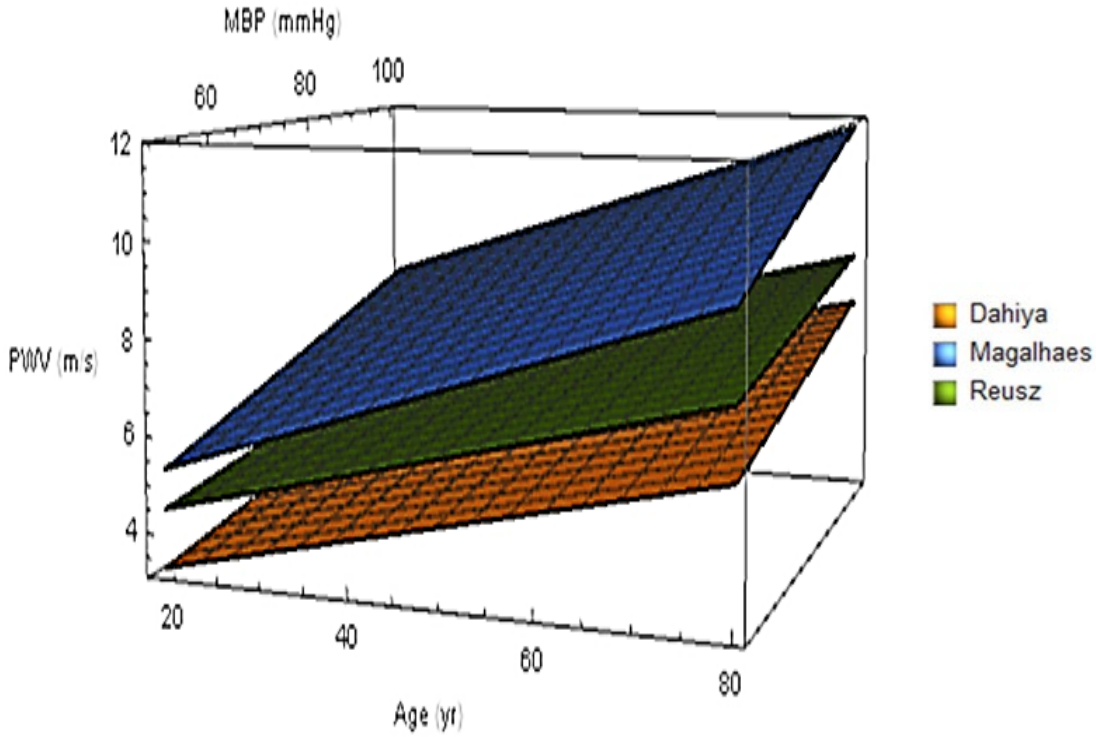

Figure 30: Graphical representation of regression equations involving age and mean blood pressure (MBP)
Regression equations from the studies with African (Magalhaes et al., 2013) and European (Reusz, Cseprekal, Temmar, Kis, Cherif, Thaleb, Fekete, Szabó, et al., 2010)

population plotted against the present study (Dahiya, 2019) multivariate regression model.

The multiple linear regression model had BP category as one of the predictor variables. As the BP Category was comprised of four different levels of blood pressure, the regression analysis was further refined by creating dummy variables. Three nominal dummy variables, namely, Normal vs Elevated, Normal vs Stage 1, and Normal vs Stage 2, were entered into the model. The regression of mean PWV on these dummy variables resulted in the following model:

$$\begin{aligned} \boldsymbol{Mean\ PWV} = \ & \mathbf{5.406} + \mathbf{2.42} \times (\boldsymbol{Normal\ vs\ Stage\ 2}) \\ & + \mathbf{1.27} \times (\boldsymbol{Normal\ vs\ Stage\ 1}) \\ & + \mathbf{0.33} \times (\boldsymbol{Normal\ vs\ Elevated}) \end{aligned}$$

Collectively, these dummy variables account for 30.6% of variation in the predictability of mean PWV values (R^2= 0.306, F (3,116) = 17.012, p<0.001). It was deduced from the model that mean PWV values increase significantly more in Stage 1 (β = 1.27, p<0.001) and Stage 2 (β = 2.42, p<0.001) compared to participants with normal BP. Furthermore, the changes in the PWV values are not significantly associated with having an elevated BP (β = 0.33, p=0.40).

4.6 Summary

To summarise, Chapter 4 focused on the findings obtained from statistically analysing the data from 120 study participants to estimate the normal and reference PWV values. The findings were represented as descriptive, correlational, inferential and regression model analyses. It was observed that certain cardiovascular risk factors such as age, BMI and BP categories were positively correlated to PWV. The overall mean PWV values

showed a significant difference by sex, with females having a higher mean PWV, whereas there was no difference in PWV found between the sexes of each age category. Mean PWV values for the reference group with cardiovascular risk factors were significantly higher than that of the normal BP group.

Additionally, the PWV values in the older age group of over 60 years of age and with stage 1 and stage 2 hypertension showed the highest scores. The multiple regression model suggested that age, BMI, BP category, and being in the NVP or RVP group collectively account for about 57% of the variability in predicting the mean PWV values. The following Chapter 5 would discuss the observed results in detail, supporting the findings with the reviewed literature.

Chapter 5: Discussion and Conclusion

5.1 Overview

This PhD study was a novel exploration of pulse wave velocity (PWV) normative values with the aim to inform the development of enhanced and more accurate cardiovascular disease (CVD) risk prediction in the New Zealand (NZ) setting. Cardiovascular disease is one of the foremost causes of morbidity and mortality in industrialised nations. Few topics have received as much consideration within the cardiovascular literature over the last five years as risk prediction. The appraisal of risk has been a key component in endeavours to characterise risk factors for CVD, to distinguish novel markers of risk for CVD, to distinguish and survey potential targets of therapy, and to upgrade the cost-effective usage of therapies for both primary and secondary prevention of CVD. The stiffness of large arteries plays a vital role in cardiovascular haemodynamics. Arterial stiffness can be evaluated or assessed non-invasively with regional and local methods (Briet et al., 2012; Cecelja & Chowienczyk, 2012; Palombo & Kozakova, 2016). The carotid-femoral pulse wave velocity (cf-PWV) is one of the methods used frequently to measure arterial stiffness (Laurent et al., 2006; Pereira et al., 2015) and is an acknowledged marker in stratifying personal cardiovascular risk in adults.

The present study focuses on obtaining new knowledge to benefit cardiovascular risk assessment in NZ utilising PWV. Risk estimates can hypothetically be utilised to raise population awareness of infections or diseases (such as CVD) that cause a noteworthy burden of morbidity and mortality, to communicate information about the health hazards to people and subgroups, and to encourage adherence to a healthier lifestyle (Prabhakaran, Jeemon, & Roy, 2016).

The main aim of the current research was to estimate the normative aortic PWV for the NZ population, thus creating an expandable dataset of PWV values for NZ for the very first time. In addition to this, the research also studies the association of PWV with cardiovascular risk factors. A descriptive research design was used to measure arterial stiffness; however, it also included exploratory and predictive outcomes for studying PWV associations.

The present study followed the standardised methodological procedure for measuring the PWV. The direct distance method was found to be practical in the sense that there is a single measurement to be taken, decreasing the chances of manual error that could occur with the indirect approach. Additionally, the distance measurement with the indirect distance method would not match the physiological distance between the arterial sites (Boutouyrie & Vermeersch, 2010; Van Bortel et al., 2012; Weber et al., 2009). Following the guidelines, 80% of the direct distance was taken by multiplying the PWV value by 0.8. The foot-to-foot method was used for transit time measurement obtained from the ultrasound images of the flow waveform gated with an electrocardiogram (ECG). The intersecting tangent algorithm was easily applied to locate the foot of the wave. The methods of PWV calculation used in this study allow consistency with the published literature and could help in extending the data pool to be reviewed in future.

5.2 Sample characteristics

A lack of literature available on reference values of PWV in the NZ population required references to the overseas data for sample size calculation. Most of the studies from American and European population were with large sample size ranging from 500-17,000 participants (Boutouyrie & Vermeersch, 2010; Mattace-Raso et al., 2006; Reusz,

Cseprekal, Temmar, Kis, Cherif, Thaleb, Fekete, Szabó, et al., 2010). Recommendations from the expert consensus documents pave their way to standardise PWV measurement (Mancia et al., 2013; Van Bortel et al., 2012). However, studies conducted in the Arab and African regions aimed to estimate reference PWV values for the first time had data collected from 120 participants (Al-Hashmi et al., 2014; Ferreira et al., 1999). After a literature review and a discussion with biostatistics team, a target sample size of 120 was determined. The results of the present study can be used to inform sample size and power calculations for future studies. For example, based on expected standard deviation (SD) of 1.5 m/s, a study powered (0.05 type 1 error rate, 0.2 type 2 error rate) to find a two-tailed difference of 0.5 m/s (the approximate mean difference in PWV between males and females, or between normal and stage 1 BP categories in the 18-30 age category, found in this study) would need a sample size of 284. In the case where one group is likely to be more populous (e.g. for ethnicity), the overall required sample size increases.

Participants were stratified across the three age groups and four blood pressure groups. Based on the presence or absence of cardiovascular risk factors participants were classified as members of the Normal Value Population (NVP) or Reference Value Population (RVP) groups. The classification was based on the sample demographics and clinical characteristics shared by the participants. The ages of the study population were normally distributed with an age range of 19-88 years which covered the three age groups: the young, middle age and older age. In this study, it was observed that the mean age of the NVP group was 40 years; whereas, it was 56.5 years in the RVP group. When compared to European data (Boutouyrie & Vermeersch, 2010), the NVP population with normal or optimal BP was slightly older (39.69 vs 33 years) and the RVP

group had a similar mean age (56.52 vs 50 years). The similar mean ages to the overseas study make the data more comparable.

Although the study population was not stratified by ethnic groups, participants were classified as either European or non-European. The European group constituted 54.2% of the whole study population and the majority of the study population of the RVP group (42.3%). The non-European group was made up of Māori, Chinese, Indian, and others including participants from Iran, Sri Lanka, Pakistan, the Philippines, and the Middle East. The NZ population is constituted of Europeans (74%), Māori (14.9%), Asians (11.8%), Pacific people (7.4%) and others (1.7%) (Ministry of Health, 2015; Statistics New Zealand, 2014). The present study attempted to recruit study population that resembles the ethnic make-up of Auckland with an understanding that this would not be the exact representation of the NZ population. The screening process was restricted to the Auckland region which has a diverse ethnic spread with Pacific Islanders (14.6%) and Asian people (23.1%) constituting the majority of the non-European ethnic population (Statistics New Zealand, 2014). The difference may be attributed to the recruitment process in this study. There was an open invitation for participation, and only people expressing interest could be contacted. Hence, the participants were randomised without targeting specific ethnic groups. A different recruitment approach that is directed towards the study population being stratified for different ethnic groups would provide a better outcome in terms of ethnic group representativeness.

5.3 Normal and reference PWV values

The primary objective of the research was to estimate normative PWV values in the NZ population. The mean PWV value of the whole study population was 5.88 ± 1.49 m/s with median of 5.56 m/s. Data were categorised into two population groups of having

normal BP with no cardiovascular risk (NVP) and with the presence of cardiovascular risk factors (RVP). A significant difference in the mean PWV values was found between these two groups (NVP: 5.40 m/s vs RVP: 6.74 m/s). The observed scores were expected to differ for the two groups, based on the reviewed literature that established a high PWV associated with the presence of cardiovascular risk factors (Ben-Shlomo et al., 2014; Cecelja & Chowienczyk, 2009, 2012). However, the mean PWV value obtained in this study population (5.88 ± 1.49 m/s) was lower than the published reference values from different regions worldwide with comparable age range (Alecu et al., 2008; Boutouyrie & Vermeersch, 2010; Elias, 2011; Wojciechowska et al., 2006). Reference PWV values in elderly participants of European descent (60-75 years) without the presence of hypertension and diabetes reported the mean aortic PWV value of 8.7 ± 2.3 m/s (Alecu et al., 2008). Another European study in healthy adults (25-76 years) with low cardiovascular risk estimated the reference PWV values for males and females as 8.9 ± 1.8 m/s and 8.1 ± 2.0 m/s, respectively (Koivistoinen et al., 2007). However, studies from the Arab (6.7 ± 1.6 m/s) and African (6.6 ± 1.0 m/s) region with comparable age range to that of our study had similar mean PWV values (Al-Hashmi et al., 2014; Magalhaes et al., 2013). Although in our study, mean PWV in the European population was significantly different from the non-European population group; mean PWV values for the European ethnic group was lower than reported literature in general (6.20 ± 1.5 m/s vs 8.7 ± 2.3 m/s) (Alecu et al., 2008). The observed dissimilarities point out a possible difference in the physiological make-up of large arteries between the study population and European dataset. Additionally, it suggests beside the physiological variability in age, sex, body size, and BP, ethnicity plays an important role in PWV predictability. Another possibility for the difference could be use of different commercial PWV measuring devices such as PulsePen (Alecu et al., 2008), SphygmoCor (Al-Hashmi et al.,

2014), CircMon (Koivistoinen et al., 2007), and Complior SP (Magalhaes et al., 2013) compare to the gold standard 'Doppler ultrasound' used in this study.

The European expert consensus recommendation of 10 m/s as a standard cut-off for cf-PWV was based on the direct distance measurement method. It represents about 4% risk for a first major cardiovascular event within the next eight years in the Framingham Heart Study cohort with a mean age of 63 years (Van Bortel et al., 2012). The closest values were seen in the oldest age group of over 60 years of age with a mean PWV value of 7.08 m/s. The low PWV scores could be linked to the study limitation of the recruitment process with skewed ethnicity and recruiting healthy people. Whether this implies certain ethnicities have lower PWV could not be deduced at this stage. Otherwise, it indicates a warning to focus on not recommending single threshold values of 10 m/s as an umbrella fit for all. Furthermore, it warrants designing a study with a normally distributed large and different population of diabetic, dyslipidaemic and hypertensive participants, which might provide a better understanding of the estimation of normative PWV values.

PWV shares a direct relationship with age; with increasing age arteries get stiffer and thus resulting in increased PWV values (Cavalcante et al., 2011; Milan et al., 2019a). As expected, the PWV values obtained in this study increased with each subsequent age group. The mean PWV values, when compared statistically, were found to be significantly different ($p<0.001$) between the three age groups. Although the published literature on estimating the reference PWV values had different age quartiles, this study followed the same trend of increasing PWV values (Boutouyrie & Vermeersch, 2010; Elias, 2011; Koivistoinen et al., 2007). It is believed that the difference of PWV values would be more when the RVP group would include other cardiovascular risk factors such

as diabetes, dyslipidaemia, and drug treatment for hypertension (Anderson et al., 2009; Dasgupta et al., 2017).

The age groups were analysed as per the NVP and RVP categories. As expected, the NVP group had younger participants overall, while there were more over 60 years age group participants in the RVP group. Unexpectedly, the mean PWV was statistically different only for the middle-aged group of 31-60 years of the NVP and RVP groups. The younger and older age groups showed no significant difference in the mean PWV values between the NVP and RVP groups. These findings are unique to the present study as it is expected that PWV values of the RVP group should be higher than the NVP group. Several studies have reported a significant difference between the normal and reference groups with a varied age range including children, young adults, and old-aged people as the study population (Al-Hashmi et al., 2014; Alecu et al., 2008; Boutouyrie & Vermeersch, 2010; Koivistoinen et al., 2007). The results from this present study indicates similar mechanical arterial wall properties from the RVP group and their NVP counterparts. Exclusion of cardiovascular risk factors such as being diabetic, having high cholesterol or being on medication for these conditions was reflected with low mean PWV values in the RVP group. The RVP group was defined to having low cardiovascular risk factors based on other published literature with similar objective (Alecu et al., 2008; Boutouyrie & Vermeersch, 2010; Diaz et al., 2014; Koivistoinen et al., 2007). As reported by several investigators, people with diabetes, dyslipidaemia and hypertension have higher PWV values than the healthy controls (Anderson et al., 2009; Cruickshank et al., 2002a; Dasgupta et al., 2017; Loehr et al., 2016).

Similar results were found when mean PWV values were compared for each sex instead of age categories. There was a significant difference in the mean PWV values of males

and females (p=0.03) for the whole study population, with females having higher PWV values than males (6.15 m/s vs 5.56 m/s, respectively), but not for individual age categories. The higher mean age of females (females: 49.7 years vs males: 40.5 years) could explain the difference in the mean PWV values. The observation is mirrored by the NZ's official guideline recommendation of beginning CVD risk assessment in men earlier than women (45 years vs 55 years, respectively) (Ministry of Health, 2018). As previously mentioned, when compared within each age group for sex, no difference was found between the mean PWV values. The reason for this finding could be that mean ages of both sexes for each age group were more similar. Our findings are in agreement with published literature finding no difference in the PWV values in males and females between the normotensive and hypertensive groups (Alecu et al., 2008; Boutouyrie & Vermeersch, 2010; Elias, 2011).

5.4 Correlation between cardiovascular risk factors and PWV

In this study, age, mean blood pressure (MBP), BP categories, and being in the NVP or RVP group were found to be positively correlated with PWV. There are several studies conducted around the world across a wide array of ages in the study population to estimate the normal and reference values for PWV. Almost every study investigated the association of cardiovascular risk factors with PWV. While age and BP consistently showed a positive correlation, other risk factors varied regionally. A European study found age and BP correlated with PWV with no correlation found with gender, smoking, or lipid status (Boutouyrie & Vermeersch, 2010). A study conducted in Germany in healthy children and young adults (range 2.3 to 28.3) overlaps the younger age group of 18-30 years in our study. It showed a significant positive correlation of aortic PWV with age, height, weight, and body surface area (r= 0.25 to 0.32, p= 0.047 to 0.009) with no

significant difference between the sexes (Voges et al., 2012). Another study published by Mitchell et al. (2007) studied the pooled sample from the Framingham Heart Study cohort. They found that after adjusting for age, PWV was correlated with MAP, Body Mass Index (BMI), diabetes, and dyslipidaemia (Mitchell et al., 2007). The findings of our study were consistent with the literature in terms of age and BP categories being significantly correlated. However, cardiovascular risk factors such as height and weight showed no significant correlation, whereas BMI and sex had a very small effect. The differences observed in our study could be due to the exclusion of some clinical parameters, such as diabetes and dyslipidaemia that are directly associated with high BMI scores at the time of diagnosis (Balkau et al., 2006; Tirosh et al., 2011). Additionally, the demographic difference in the NZ population might affect the association of less correlated risk factors.

Investigating the relationship of mean PWV values with age and BP categories, as expected, it was found that high PWV values in the middle and older age groups were associated with high BP. It was interesting to note that those in the elevated BP category compared to the Stage 1 and Stage 2 BP category had low PWV values in all three age groups. The PWV values were even lower than the normal BP group. Whether this anomaly with 'elevated BP category' is due to small sample size or ethnic mix cannot be inferred at this stage because the elevated and higher BP categories share the similar number of participants with a mix of only European and Asian ethnicities. Compared to normal BP, for the 18-30 years age group, higher BP categories had lower PWV values. The reasons for this finding are not clear but could be linked to the possible ethnic make-up of the youngest age group. The 18-30 years of age group was mostly college and University students with ≈50% belonging to NZ European ethnicity while the rest of the

non-European was of mixed ethnicity inclining towards the Asian group making this an Auckland region-specific finding. These findings suggest caution when investigating younger people presenting with high BP, as PWV may not be associated with BP in the same way as for middle and older age groups. The relationship of PWV in younger people across a range ethnic groups with high BP needs to be investigated further.

5.5 Influence of cardiovascular risk factor on PWV

Based on the correlation analysis made from the data and literature reviewed, age and BP were the two cardiovascular risk factors that were consistently associated with carotid-femoral PWV (cf-PWV). Linear regression models were analysed to study the association with the mean PWV from the independent variables. When assessed individually, age as a factor accounted for 48% of the variability in mean PWV. MBP showed a positive correlation with PWV, and when MBP was added to the model, the variability in age for PWV was significantly enhanced to 54.5% predictability. Since MBP is being derived from systolic and diastolic BP, it is an important risk factor to monitor. As per the reviewed literature, an increase in BP has been linked to PWV at any increase of age (Boutouyrie & Vermeersch, 2010; Millasseau et al., 2005). However, usually there will be only a small fraction of people for whom high BP is the only reason for raised PWV values, and our study results agree with it by presenting a positive correlation with whether a participant belongs to the NVP or RVP group besides the age, MBP, BP categories, and BMI as variables.

Multivariate linear regression analysis was performed for all the positively correlated risk factors. In our study, out of all the correlated factors, age, BMI, BP categories and being in NVP or RVP group contributed by influencing the predictability of PWV. The negative coefficient value for the NVP/RVP group implies an inverse relationship with

PWV. Collectively, the four independent variables contributed to 57% of the variation in the mean PWV values; they are already known to be important factors for assessing cardiovascular risk. The results were in line with other published findings. In an elderly population, aged 60-75 years presented determinants (age, BMI, diabetes, heart rate, MBP, and antihypertensive treatment) to the regression model explained 26.4% of the variance in PWV (Alecu et al., 2008). The lower percentage variance of this study compared to our study's estimated variance of 57% could be because of the different age range targeting the elderly population. Another study with adults (40-93 years) showed 48% of PWV variance with cardiovascular risk factors including age, hypertension status, height, weight, heart rate, mean arterial pressure, creatinine, and glucose (Elias, 2011). It was observed that the present study cohort had lower mean PWV values compared with the European, American and African population. The observation was supported by comparing the regression equations (unstandardized coefficients) from the literature reporting regression models. The reason behind such finding could be because of the unique mix of ethnicity of study population. However, this needs to be investigated further. The objective of the present study was to include healthy people; however, if other risk factors such as heart rate, diabetes, dyslipidaemia, drug treatment of hypertension, diabetes or dyslipidaemia had been included in the RVP group, it is likely that they would influence the PWV variance in the multivariate model.

Even though previous literature suggests that BP and age share a linear relationship with PWV, the findings in the present study have shown a more nuanced relationship. Notwithstanding that MBP is a significant, positive predictor variable in linear regression, low PWV values at high BP range in the younger age group and generally lower PWV values at elevated BP compared with stage 1 and stage 2 hypertension in all

age groups were study-specific findings. Because of these differences, BP categories were included in a regression model as dummy variables. Explaining 30.6% of the variation in mean PWV, stage 1 and stage 2 hypertension significantly increase mean PWV values compared with a normal BP. However, the model does not answer the observation of having low PWV values with high BP in the younger age group (18-30 years). This observation could be due to different ethnic make-up of this age group (mainly European or migrant Asians studying at the Universities) or is related to physiological build-up in the younger age group of this study. We could not find any study showing something similar in young adults. A study published a decade ago compared the effect of age and arterial pressure on PWV between African and European origins. Although, not an exact match with our study, the mean age of the study population was 26 ± 7 years. They reported that the normotensive black Africans had low mean PWV values, whereas, at high BP levels, blacks exhibited higher mean PWV values than their white Europeans counterparts (Ferreira et al., 1999). Different composition of arterial wall among the racial groups was thought to be the reason behind this finding.

5.6 Novel contributions

- The main objective of this research of estimating the normal and reference PWV values in the NZ population was a unique attempt. The NZ population is a mix of several different ethnic groups with different CVD risk assessment milestones for Māori, Pacific and South Asian populations. To have a NZ population specific data set for PWV values was a gap that needed to be addressed. Comparing the regression equations from the literature, our study indicated overall lower mean PWV values. The finding of this study contributes to highlighting the possible

demographic differences between the study population from NZ and reported literature. The findings of this study, therefore, indicate the need for NZ to establish its own reference values for PWV.

- The official NZ guidelines on CVD risk assessment does not mention measuring arterial stiffness as a routine part of cardiovascular risk assessment. To the best of the author's knowledge, this research was the first in collecting data for cf-PWV on healthy individuals using the standard methodological process with the Doppler ultrasound. Although there is a need for a large cohort study measuring PWV across the country, the normal and reference data obtained in this research can serve as a starting point for future investigation of the use of PWV to enhance cardiovascular risk prediction.
- The secondary objective was to study the association of PWV with cardiovascular risk factors. The statistical analysis of cardiovascular risk factors and PWV provides a snapshot of the study population. Correlation analysis showed a positive correlation of PWV with age, MBP, BP category, being in the NVP or RVP group and BMI. Moreover, the multivariate linear regression model had age, BMI, BP categories, and being in the NVP or RVP group as the contributing predictive variables. The current study has resulted in a different set of risk predictors with the presence of being in normal or reference group besides other cardiovascular risk factors for PWV indicates a population-specific finding that would help to identify and to stratify people at risk.
- A low PWV at higher BP levels in a younger study population is an unexpected finding of this research. High BP has been shown positively correlated with higher mean PWV. The reasons for this occurrence were not able to be identified within the limits of this study, and opens opportunities for further research. There is no

similar finding reported in the reviewed literature. The European and Asian ethnic mix of young University students and the physiological make-up of the 18-30 years age group could be the cause of this observation.

- The study has several benefits for future research. The study informs the method and protocol on how to design and conduct a full large-scale study by addressing the limitations of the present one. The findings of this study can be used to inform the sample size of a sufficiently powered observational study to determine the mean PWV values in the NZ population by age, and sex. It will work as an example with the dos and don'ts while planning further research work. Without the constraints on resources and time for this study, we would recommend, for future studies:
    - Enrolling participants through GP practices targeting areas of specific ethnicities
    - Retrieving medical history from patient records instead of relying on self-reporting
    - Request blood test results to determine other well-known cardiovascular risk factors
    - Collecting sufficient other data to fully assess the existing Framingham Risk Score and PREDICT scores to determine association with PWV
    - Longitudinal studies to determine the additive predictive value of PWV as a biomarker of CVD and events.

5.7 Study limitation

The study is a first attempt to estimate the normative PWV values in NZ; there was no national or literature data available for reference. However, the outcomes should be interpreted within the context of its limitations.

- Although, the sample size of 120 participants was chosen based on statistical grounds with a wide age range, there were insufficient participants to be stratified by ethnicity. The implications of present study findings would be limited for the whole NZ population as the study population did not represent the ethnic make-up of NZ. Specifically, there was an under-representation of Māori and Pacific people, who have the highest rate of cardiovascular diseases and poorest outcomes. Participants with European descent were over-represented constituting 54% of the study population. This participant distribution is likely to have moved the mean PWV values away from the actual representation of the NZ population. A larger sample size stratified by ethnicity would have provided a better understanding of the association of PWV and ethnicity.
- The study aimed to have a similar proportion of ethnicities across different age groups. However, due to the restricted timeframe of the doctoral research, the recruited participants could not be meaningfully stratified into ethnic subgroups and thus estimated normative values could not be categorised for ethnic subgroups. The open recruitment process means that participants were required to express interest to be enrolled in the study. The majority of participants were of European descent. Sufficient numbers of Māori and Pacific Islanders did not express interest and were not recruited. A different approach, (e.g. culturally tailored study documents, or having culturally specific study personnel), could be

required to convey the message of the study to solicit interested people, which should improve the recruitment ratio. We expect to find that ethnicity will be associated with PWV values.

- The study shortcomings are centred mainly around the methods. In order to estimate the normative PWV values, risk factors having an independent effect on PWV were excluded. Because of the defined inclusion/exclusion criteria of not including people with diabetes, dyslipidaemia, or being on medications for these conditions, the RVP group was not ethnically diverse. For the over 60 years age group, participants found to be matching the inclusion criteria were predominantly European. In contrast, participants of non-European ethnic groups such as Indian, Chinese and other Asian ethnicities frequently met the exclusion criteria of having established CVD, secondary hypertension, diabetes, high cholesterol, or having treatment for these conditions. These excluded people are at elevated risk of having a first CVD event. Assessment of PWV may have different significance in people with these conditions.
- Another potential limitation could be around the reliability of the ultrasound measurements. Measures such as recording performed by a single person following the standard Doppler ultrasound protocol were undertaken to mitigate the variability. However, as there were no inter and intra-reliability or intra-observer variability tests carried out, of the degree of inconsistency in the recorded data that might limit its applicability is unknown.

5.8 Future research work

- The objective of estimation of the reference PWV values can be extended further by including people at low and moderate CVD risk. A study design to assess the

influence of cardiovascular risk factors such as having diabetes, high cholesterol, treatment for hypertension on aortic PWV would provide better identification of people at risk with follow-ups.

- The present study had the screening process restricted to the Auckland region due to practical constraints. To get a sample population that is representative of NZ, the screening and recruitment process must be extended beyond Auckland. A nation-wide study at multiple centres estimating the normative PWV values would help in updating the NZ guidelines for cardiovascular risk assessment and CVD management.
- There are notable ethnic disparities in stroke/CVD incidence and outcomes in NZ and elsewhere, with Māori and Pacific people having consistently higher incidence rates and worse functional outcomes compared to NZ Europeans. Ethnic variability in NZ can be addressed by designing a study with a large population stratified by ethnic group.
- Stroke and CVD are preventable by controlling modifiable risk factors such as obesity, diet, and physical inactivity. The effect of interventions such as a nutritious diet, exercise, a smoke and alcohol-free life, reducing body weight in general and high-risk groups are hard to obtain. Future studies targeting modifiable risk factors could be designed targeted at measuring PWV as a biomarker for arterial stiffness, and as an objective measure of long-term reduced CVD risk.
- To overcome the limitations of using Doppler ultrasound, validated commercial devices such as a SphygmoCor, Complior or PulsePen could be used. Although Doppler ultrasound is an imaging technique, it has disadvantages of not being a portable device, needs specific training, and is comparatively costly to operate.

The commercial devices will provide a more practical approach to measuring PWV in the clinic or home setting.

In conclusion, this study was the first to measure PWV in a NZ sample as a platform to conduct future research on the importance of this measure in the prediction of CVD risk. It was a novel attempt to have a NZ specific dataset of normative PWV values and lays the foundation for future work. The research began with identifying the gap of having a dataset for NZ specific normative values of PWV as a predictive measure of arterial stiffness. The project was planned as two separate studies, starting with validating a novel bioimpedance device followed by estimation of normal and reference PWV values using the new device. However, because of some technical issues the device's validation did not work out and keeping in mind the timeframe it was decided to estimate PWV using the Doppler ultrasound. The findings from this pilot project presented mean PWV values that increased systematically with age. However, the overall mean values were lower compared to the European and overseas data. The data showed age and BP as being independently associated with PWV in agreement with the literature. There were some study specific findings that would need further investigation. This pilot project was limited by having a small sample size, exclusion of other CV risk predictors, and low ethnic diversity. Nonetheless, it gives us a starting point to have an evidence-based approach for including PWV in cardiovascular assessment, early identification, improving medical management, and reducing the overall CVD burden.

Abbreviations

Ag-AgCl	Silver-Silver Chloride electrodes
AHA	American Heart Association
AIx	Augmentation Index
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AUT	Auckland University of Technology
AUTEC	Auckland University of Technology Ethics Committee
BMI	Body Mass Index
BP	Blood Pressure
cf-PWV	Carotid-femoral Pulse Wave Velocity
CT	Computerised Tomography
CVD	Cardiovascular Diseases
DBP	Diastolic Blood Pressure
ECF	Extra Cellular Fluid
ECG	Electrocardiogram
EEG	Electroencephalogram
ESC	European Society of Cardiology
ESH	European Society of Hypertension
GP	General Practitioner
HDEC	Health and Disability Ethics Committee
HR	Heart Rate
IBTec	Institute of Biomedical Technology
ICF	Intra Cellular Fluid
MAP	Mean Arterial Pressure
MBP	Mean Blood Pressure
MRI	Magnetic Resonance Imaging
NISAN	National Institute for Stroke and Applied Neuroscience
NVP	Normal Value Population
NZ	New Zealand
PP	Pulse Pressure
PR	Pulse Rate
PWA	Pulse Wave Analysis
PWV	Pulse Wave Velocity
RVP	Reference Value Population
SBP	Systolic Blood Pressure
WHO	World Health Organisation

Appendix A: Study I HDEC Ethics Approval

Health and Disability Ethics Committees

Health and Disability Ethics Committees
Ministry of Health
133 Molesworth Street
PO Box 5013
Wellington
6011

0800 4 ETHICS
hdecs@moh.govt.nz

21 July 2017

Dr Ekta Singh Dahiya
AR425, Level 4
National Institute for Stroke and Applied Neurosciences (NISAN)
Auckland University of Technology (AUT)
Northcote, Auckland 0627

Dear Dr Dahiya

Re:	Ethics ref:	17/NTA/128
	Study title:	Validation of a new non-invasive arterial pulse wave velocity (PWV) monitor for determining reference PWV values for the New Zealand population

I am pleased to advise that this application has been *approved* by the Northern A Health and Disability Ethics Committee. This decision was made through the HDEC-Expedited Review pathway.
In relation to our earlier request (letter of 14 July 2017), the Committee apologises as an error was made by the secretariat in the reference used for ethnicity categories to be applied. Please see non-standard conditions below.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Northern A Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.

2. Before the study commences at *a given* locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

Non-standard conditions:

— Please use Statistics New Zealand's ethnicity classifications when collecting ethnicity data to ensure the options available are suitable for New Zealand participants. These classifications are: New Zealand European, Maori, Samoan, Cook Islands Maori, Tongan, Niuean, Chinese, Indian, Other (such as Dutch, Japanese, Tokelauan) please state.

Non-standard conditions must be completed before commencing your study. Non-standard conditions do not need to be submitted to or reviewed by HDEC before commencing your study.

If you would like an acknowledgement of completion of your non-standard conditions letter you may submit a post approval form amendment. Please clearly identify in the amendment that the changes relate to non-standard conditions and ensure that supporting documents (if requested) are tracked/highlighted with changes.

For information on non-standard conditions please see section 128 and 129 of the Standard Operating Procedures at http://ethics.health.govt.nz/home.

After HDEC review

Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

Your next progress report is due by 20 July 2018.

Participant access to ACC

The Northern A Health and Disability Ethics Committee is satisfied that your study is not a clinical trial that is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialled. Participants injured as a result of treatment received as part of your study may therefore be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,

Dr Brian Fergus
Chairperson
Northern A Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix B: Study I AUTEC Ethics Approval

AUTEC Secretariat

Auckland University of Technology
D-88, WU406 Level 4 WU Building City Campus
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E: ethics@aut.ac.nz
www.aut.ac.nz/researchethics

10 August 2017

Rita Krishnamurthi
Faculty of Health and Environmental Sciences

Dear Rita

Ethics Application: 17/265 **Validation of a new non-invasive arterial pulse wave velocity (PWV) monitor for determining reference PWV values for the New Zealand population**

I wish to advise you that a subcommittee of the Auckland University of Technology Ethics Committee (AUTEC) has **approved** your ethics application.

This approval is for three years, expiring 7 August 2020.

Standard Conditions of Approval

1. A progress report is due annually on the anniversary of the approval date, using form EA2, which is available online through http://www.aut.ac.nz/researchethics.
2. A final report is due at the expiration of the approval period, or, upon completion of project, using form EA3, which is available online through http://www.aut.ac.nz/researchethics.
3. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form: http://www.aut.ac.nz/researchethics.
4. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
5. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.

Please quote the application number and title on all future correspondence related to this project.

AUTEC grants ethical approval only. If you require management approval for access for your research from another institution or organisation then you are responsible for obtaining it.

You are reminded that it is your responsibility to ensure that the spelling and grammar of documents being provided to participants or external organisations is of a high standard.

For any enquiries please contact ethics@aut.ac.nz

Yours sincerely,

Kate O'Connor
Executive Manager
Auckland University of Technology Ethics Committee

Cc: ekta.dahiya@aut.ac.nz; Andrew Lowe; Valery Feigin

Appendix C: Study II HDEC Ethics Approval

Health and Disability Ethics Committees

Health and Disability Ethics Committees
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12 April 2018

Dr Ekta Singh Dahiya
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Auckland University of Technology (AUT)
Northcote 0627

Dear Dr Dahiya

Re:	Ethics ref:	18/STH/45
	Study title:	Determining the normative values of arterial pulse wave velocity using Doppler ultrasound for New Zealand population

I am pleased to advise that this application has been *approved* by the Southern Health and Disability Ethics Committee. This decision was made through the HDEC-Expedited Review pathway.

Summary of ethical issues (resolved)

The main ethical issues considered by the Committee and addressed by the Researcher are as follows.

1. Please note that unless required as a result of the Maori consultation undertaken, the Vision statement does not need to be included in the ICF and may be deleted.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Southern Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.

2. Before the study commences at *a given* locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

After HDEC review

Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

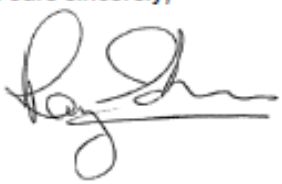
Your next progress report is due by 12 April 2019.

Participant access to ACC

The Southern Health and Disability Ethics Committee is satisfied that your study is not a clinical trial that is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialled. Participants injured as a result of treatment received as part of your study may therefore be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,



Ms Raewyn Idoine
Chairperson
Southern Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix D: Study II AUTEC Ethics Approval

AUTEC Secretariat

Auckland University of Technology
D-88, WU406 Level 4 WU Building City Campus
T: +64 9 921 9999 ext. 8316
E: ethics@aut.ac.nz
www.aut.ac.nz/researchethics

24 April 2018

Rita Krishnamurthi
Faculty of Health and Environmental Sciences

Dear Rita

Re Ethics Application: **18/157 Validation of a new non-invasive arterial pulse wave velocity (PWV) monitor for determining reference PWV values for the New Zealand population**

Thank you for providing evidence as requested, which satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 24 April 2021.

Standard Conditions of Approval

1. A progress report is due annually on the anniversary of the approval date, using form EA2, which is available online through http://www.aut.ac.nz/researchethics.
2. A final report is due at the expiration of the approval period, or, upon completion of project, using form EA3, which is available online through http://www.aut.ac.nz/researchethics.
3. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form: http://www.aut.ac.nz/researchethics.
4. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
5. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.

Please quote the application number and title on all future correspondence related to this project.

AUTEC grants ethical approval only. If you require management approval for access for your research from another institution or organisation then you are responsible for obtaining it. You are reminded that it is your responsibility to ensure that the spelling and grammar of documents being provided to participants or external organisations is of a high standard.

For any enquiries, please contact ethics@aut.ac.nz

Yours sincerely,

Kate O'Connor
Executive Manager
Auckland University of Technology Ethics Committee

Cc: ekta.dahiya@aut.ac.nz; Andrew Lowe

Appendix E: Study I AUTEC Amendment Approval

Auckland University of Technology Ethics Committee (AUTEC)

Auckland University of Technology
D-88, Private Bag 92006, Auckland 1142, NZ
T: +64 9 921 9999 ext. 8316
E: ethics@aut.ac.nz
www.aut.ac.nz/researchethics

28 November 2018

Rita Krishnamurthi
Faculty of Health and Environmental Sciences

Dear Rita

Re: Ethics Application: **17/265 Validation of a new non-invasive arterial pulse wave velocity (PWV) monitor for determining reference PWV values for the New Zealand population**

Thank you for your request for approval of amendments to your ethics application.

The amendment to the research protocol to allow early phase bioimpedance testing is approved. Recruitment for preliminary experiments are within the research team and data collection as per previous approved protocol.

Non-Standard Conditions of Approval

1. On the Consent Form please allow participants the opportunity to choose neck/groin testing.

Non-standard conditions must be completed before commencing your study. Non-standard conditions do not need to be submitted to or reviewed by AUTEC before commencing your study.

I remind you of the **Standard Conditions of Approval**.

1. A progress report is due annually on the anniversary of the approval date, using form EA2, which is available online through http://www.aut.ac.nz/research/researchethics.
2. A final report is due at the expiration of the approval period, or, upon completion of project, using form EA3, which is available online through http://www.aut.ac.nz/research/researchethics.
3. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form: http://www.aut.ac.nz/research/researchethics.
4. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
5. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.

Please quote the application number and title on all future correspondence related to this project.

AUTEC grants ethical approval only. If you require management approval for access for your research from another institution or organisation then you are responsible for obtaining it. If the research is undertaken outside New Zealand, you need to meet all locality legal and ethical obligations and requirements.

For any enquiries please contact ethics@aut.ac.nz

Yours sincerely,

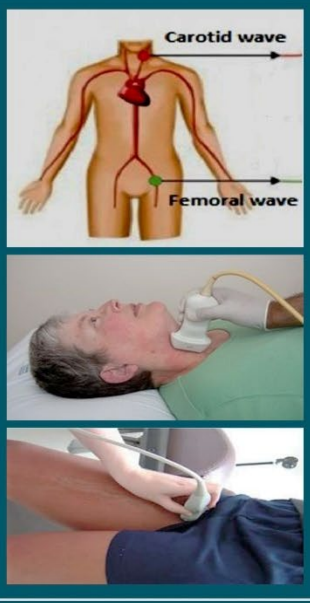
Kate O'Connor
Executive Manager
Auckland University of Technology Ethics Committee

Cc: ekta.dahiya@aut.ac.nz; Andrew Lowe; Valery Feigin

Appendix F: Invitation flyer

RESEARCH VOLUNTEERS NEEDED

Study on cardiovascular and stroke risk assessment



TO ASK QUESTIONS OR SIGN UP

Please contact:

Dr Ekta Singh Dahiya
Principal Investigator, National Institute for Stroke and Applied Neurosciences (NISAN), AUT
021 722 280, ekta.dahiya@aut.ac.nz

Dr Rita Krishnamurthi
Project Supervisor, Senior Lecturer, NISAN, AUT
09 921 9999 ext. 7809, rita.krishnamurthi@aut.ac.nz

AUT NATIONAL INSTITUTE FOR STROKE AND APPLIED NEUROSCIENCES

AUT INSTITUTE OF BIOMEDICAL TECHNOLOGIES

AUT

You are invited...

To qualify, you should:

- Be aged ≥ 18 years (18-30, 30-60, >60 years)
- Not have diabetes, high cholesterol, any known heart disease or taking medicines for these conditions

What is this study about?

The study is aimed to estimate the normal range of values of pulse wave velocity (a way of measuring how stiff our arteries are) to measure cardiovascular disease (heart health) and stroke risk in New Zealand population.

What will happen?

- A single visit for a 30 minutes session.
- Measurement of pulse wave velocity, blood pressure, ECG, height, weight (all non-invasive & painless).
- Measurement will be at the major arteries in your neck and groin as shown in the pictures on the left.
- **A $20 gift voucher/koha will be provided on the visit as a token of appreciation.**

Dr Ekta Singh Dahiya
021 722 280
ekta.dahiya@aut.ac.nz

Appendix G: Participant Information Sheet

Determining the normative values of arterial pulse wave velocity using Doppler ultrasound for New Zealand population

Estimating normative pulse wave velocity values (Study II)

Participant Information Sheet and Consent Form

An Invitation

Kia ora and hello, you are invited to take part in this research study which aims to establish the normal range of values of arterial pulse wave velocity for the New Zealand (NZ) population. Pulse wave velocity is a way of measuring arterial stiffness, and predicting a person's future risk of stroke or heart diseases (cardiovascular diseases). It will be measured using a routinely used machine known as Doppler ultrasound monitor. This research is a part of a doctoral study which is coordinated by the National Institute for Stroke and Applied Neurosciences (NISAN), Auckland University of Technology (AUT) in Auckland.

Your participation is entirely voluntary (your choice). You do not have to take part in this study. If you choose not to take part, any care or treatment that you are currently receiving will not be affected. If you do agree to take part, you are free to withdraw from the study at any time, without giving a reason. This Participant Information Sheet will help you decide if you would like to take part. You may take as much time as you like to consider whether to take part, we are happy to answer any questions you might have. If you agree to take part in this study, you will be asked to sign the Consent Form on the last page of this document. You will be given a copy of both this information sheet and to consent form to keep.

What is the purpose of this research?

This research is being done to find an improved way of measuring the risk of having stroke and heart disease. Earlier and more accurate detection of cardiovascular risk may lead to improved prevention of these diseases. Increased pulse wave velocity is a sign of stiffer blood vessels, which could lead to stroke and heart diseases. To measure this stiffness, pulse wave velocity is calculated by taking measurements from two sites on the body where the pulse can be easily detected. However, there are currently no guidelines on normal range of pulse wave velocity values in the NZ population to allow comparison between people at different levels of risk. For this reason, it is not being used as a routine test for heart diseases.

Therefore, this study will estimate the normative values of pulse wave velocity in different sub groups of the NZ population. The data from this study will help

determine how routine pulse wave velocity assessment can be used to measure cardiovascular risk in clinical settings. The results of the current study will be written up as part of a PhD thesis and will be published in scientific conferences and research journals.

Who can take part in the study?

You are being invited to participate in this study, as you are an adult aged over 18 years, living in the community and are available for the duration of the study.

However, those people who have had a heart attack (myocardial infarction), heart pain (angina), heart failure, or stroke; high blood pressure caused by drugs or other diseases; have diabetes or high cholesterol and/or who are taking medication for diabetes and high cholesterol levels will be excluded from the study.

A total of 120 people will be expected to participate in the study.

What happens if I do decide to take part?

If you decide to take part, your eligibility to enter the study will be assessed (you may not be eligible to participate in this study). If you meet the inclusion criteria, you will be invited to a one-time visit at the WD301, Institute of Biomedical Technology (IBTec), AUT City Campus (55 Wellesley St E, Auckland, 1010) on the day of your appointment arranged via phone/email. Exact details of the place for the appointment and a map will be provided. You will be attending one session that will last approximately an hour. You will be advised to wear loose fit clothes during the session and to bring any prescribed medications you are currently taking. There is no preparation required for this test. You may have a family member or friend with you in the exam room during the test. You may ask any questions regarding the study and would be asked to sign the consent form before beginning the test.

Your general and health information including details of your medications will be recorded into a case report form. You will be asked for information regarding blood pressure, and cholesterol and whether you have diabetes. However, you will not be required to provide any blood test results. There will be no blood samples collected for this study. After a resting period of 10 minutes, your blood pressure will be recorded using an automated BP machine. You will lie comfortably on a stretcher in the test room. The pulse wave velocity measurement will be made using Doppler ultrasound.

You will be prepared for electrocardiogram (ECG) to measure your heart's electrical waves by placing ECG electrode patches on your right arm, left arm, and right leg. A water-soluble gel will be applied at your neck and groin region before taking couple of measurements with the probe. Doppler ultrasound amplifies the sound of the blood flow, and you will hear a heartbeat like sound. The distance between the two

arterial sites will be measured using a measuring tape. The pictures in Figure 1 and 2 show how this measurement will take place.

The readings from Doppler ultrasound will be investigated to examine the relationship between pulse wave velocity and age and/or blood pressure amongst the different ethnic groups.

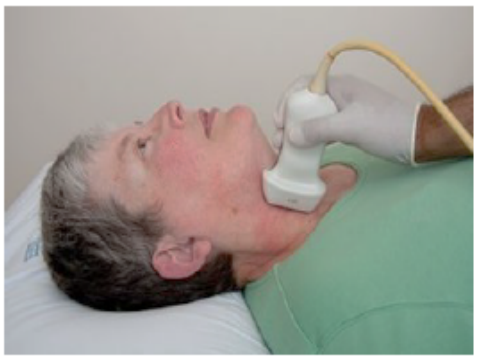


Figure 1: Position of ultrasound probe on common carotid artery

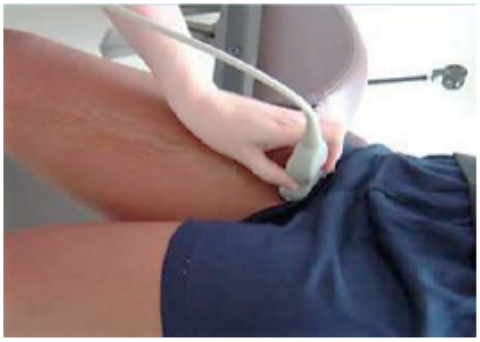


Figure 1: Position of ultrasound probe on common femoral artery

How will the study affect me?

This is a low risk study, to date, no adverse effects or risks have been reported by participants with Doppler ultrasound or any non-invasive PWV monitor. We will use standard ultrasound procedures that are well within safety guidelines for studying the blood vessels. There is a small chance of experiencing a prickling sensation or redness over the area that is in contact with the electrodes patches. If present, this sensation can only be felt during the first few seconds after peeling off the patch.

You will receive no direct benefit from participation in the study. However, you will obtain an individual assessment of your heart health with blood pressure and ECG monitoring. Furthermore, readings from this study will help to estimate the normal range values for pulse wave velocity in NZ population so it can be used in practice to more accurately measure the risk of stroke and heart disease in future.

In case of identification of any abnormal findings such as irregular heartbeats or extremely high blood pressure (>160 mmHg), the investigator will discuss this with you and, with your permission, ask to contact your GP.

What are the costs of participating in this research?

There are no monetary costs associated with participating in this research. A $20 gift voucher/koha will be provided on the visit as a token of appreciation.

How will my privacy be protected?

Your privacy and confidentiality will be protected during and after the study. The study files and all other information provided by the participants will remain strictly confidential. No material that could personally identify you will be used in any reports on this study. Upon completion of the study your records will be stored for 10 years in a secure place at NISAN and IBTec, AUT University in Auckland. All computer records will be password protected. All future use of the information collected will be strictly controlled in accordance with the Privacy Act.

Any image taken during the test will only show the experimental setup; your face and any other identifying characteristics cannot be seen.

What compensation is available for injury or negligence?

In the unlikely event of a physical injury because of your participation in this study, rehabilitation and compensation for injury by accident may be available from the Accident Compensation Corporation (ACC), providing the incident details satisfy the requirements of the law and the Corporation's regulations.

Will I receive feedback on the results of this research?

Yes, you are given an opportunity on the consent form to indicate if you would like feedback on the research project. If you answer "yes" to this, a short summary of the study findings will be sent to you on completion of the study at the contact details provided on the consent form.

What do I do if I have concerns about this research?

Please keep this Information Sheet and a copy of the Consent Form for your future reference. The study has received Ethical Approval from the Health and Disability Ethics Committee (HDEC) which oversees research studies to be sure that they are appropriate, respectful, and confidential with participants and their information. Reference number for this stud is 18/STH/45.

For more information, concerns or complaints about the study, please feel free to contact the Co-ordinating Investigator, Dr Ekta Singh Dahiya on 021 722 280 at National Institute for Stroke and Applied Neurosciences (NISAN), AUT or email: ekta.dahiya@aut.ac.nz.

Alternatively, you can contact the Project Supervisor, Dr Rita Krishnamurthi, Senior Lecturer, NISAN, AUT on 09-921-9999 ext. 7809 or email: rita.krishnamurthi@aut.ac.nz.

Or;

Dr Andrew Lowe, Senior Lecturer, School of Engineering, Mathematical and Computer Sciences, AUT on 09-921-9999 ext. 6303 or email: andrew.lowe@aut.ac.nz.

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

Free phone:	0800 555 050
Free fax:	0800 2787 7678 (0800 2 SUPPORT)
Email:	advocacy@hdc.org.nz

For Maori health support please contact:

Name, position:	John Perrott, Mātauranga Māori Engagement Manager
Phone:	09 921 9999 ext 8654
Email:	john.perrott@aut.ac.nz

You can also contact the health and disability ethics committee (HDEC) that approved this study on:

Phone:	0800 4 ETHICS
Email:	hdecs@moh.govt.nz

Approved by the Health and Disability Ethics Committee on 12/04/2018, HDEC Reference number18/STH/45.

Vision Mātauranga Statement

This study is designed to estimate the reference Pulse wave velocity (PWV) ranges for the NZ population using the golden standard Doppler ultrasound. Carotid and femoral arteries, being superficially located are commonly used sites to track regional pulse waves and has better predictability of AS. This study will include 120 people including healthy subjects and people at high risk of CVD (high blood pressure, and smoking). The PWV values recorded will be statistically correlated with age, blood pressure, ethnicity and other CV risk factors.

While this project does not specifically involve Māori knowledge, resources or people directly in its design and implementation, this project could provide important information relevant to Māori health and wellbeing. The Mātauranga Maori consultation provisions have been developed for participants who identify with their Māori ethnicity. Our Vision Mātauranga provisions are focused on developing a comprehensive consultation plan that supports Māori access to their own spiritual realm (Te ao wairua), their language (Te reo Māori) and protocols (Tikanga Māori), and we acknowledge the importance of how Māori link to their extended family structures (mana whanau) during all consultation meetings. These outcomes will ensure accessible and culturally safe services guaranteed to Māori under the Treaty of Waitangi, Education Act 1989, and AUT's Vision Mātauranga policies. All participants of Māori ethnicity within this study will be able to opt into or out of the Vision Mātauranga provisions.

Vision Mātauranga provisions include:

1. All participants will be given the choice of having karakia performed at the start and conclusion of all meetings.
2. Privacy will be sought for all consultations and decision making during the consultation, data collection, analysis, and reporting phases of the project.
3. Culture and Identity (measures of cultural recognition, cultural heritage, spirituality, use of te reo and tikanga Māori) will be valued and protected at all times. Maori participants will be made aware of the availability of Pou Kokiri (language interpreter and protocol advisor Dr Valance Smith) for this purpose.
4. During the consultation phase, all participants will be made aware of a designated kaitiaki responsible for keeping all personal data safe from misuse or use without consent.

Manaakitanga, a principle associated with reciprocation and hospitality, will underpin all interactions with participants and communities, acknowledging the mana and expertise of each participant and their whānau. Part of this is the acknowledgement of the imbalances of power that have often ensued between researcher and participant in the past, so that participants in this project are viewed as active contributors to the project and their input and 'data' treated as taonga (a treasured contribution).

Appendix H: Consent Form

Estimating normative pulse wave velocity values (Study II)

Consent Form

- ○ I have read and understood the information provided about this research project in the Information Sheet.
- ○ I have had an opportunity to ask questions and to have them answered.
- ○ I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without being disadvantaged in any way.
- ○ I understand that if I withdraw from the study, then I will be offered the choice between having any data that is identifiable as belonging to me removed or allowing it to continue to be used. However, once the findings have been produced, removal of my data may not be possible.
- ○ I have not had a heart attack (myocardial infarction), heart pain (angina), heart failure, or stroke; high blood pressure caused by drugs or other diseases; have diabetes or high cholesterol and/or am taking medication for diabetes and high cholesterol levels.
- ○ I agree to give my approval for contacting my health practitioner in case of any abnormal findings identified during the research.
- ○ I understand that my participation in this study is confidential and that no material that could identify me will be used in any reports on this study.
- ○ I agree to take part in this research.
- ○ I wish to receive a summary of the research findings (please tick one): Yes ○ No ○

Participant's signature : .....

Participant's name: .....

Participant's contact details:

.....

.....

For Administrative use:
Participant ID:
DOB:

Researcher's signature : .....

Researcher's name: **Dr Ekta Singh Dahiya**

Date: .....

Approved by the Health and Disability Ethics Committee on 12/04/2018, HDEC Reference number 18/STH/45.

Note: The Participant should retain a copy of this form.

Appendix I: Contact Details Form

CONTACT DETAILS FORM

Determining the normative values of arterial pulse wave velocity using Doppler ultrasound for New Zealand population

Estimating normative pulse wave values (Study II)

Principal Investigator: Dr Ekta Singh Dahiya
Project Supervisor: Dr Rita Krishnamurthi
Project Co-supervisor: Dr Andrew Lowe
HDEC Reference number: 18/STH/45

Participant ID		Date of Birth	DD	MM	Year

Form Number	
Data entered onto ART™ database	
Signed	Date

Participant ID		Date of Birth	DD	MM	Year

PARTICIPANT NAME DETAILS

Participant first name(s):	
Participant last name:	

PHONE NUMBERS

Mobile:		Home:		Work:	
Preferred phone number:	☐ Mobile	☐ Home	☐ Work		
Email address:					
Postal address:					
Do you have a preferred day and/or time for the researcher to contact you?					

ALTERNATIVE CONTACT

Alternative contact name:	
Best contact details: (Phone/email/address)	
Relationship to participant:	

GP CONTACT DETAILS

Name of GP:	
Name of Clinic/Surgery:	
Address:	

Appendix J: Case Report Form

CASE REPORT FORM

Determining the normative values of arterial pulse wave velocity using Doppler ultrasound for New Zealand population

Estimating normative pulse wave values (Study II)

Principal Investigator: Dr Ekta Singh Dahiya
Project Supervisor: Dr Rita Krishnamurthi
Project Co-supervisor: Dr Andrew Lowe
HDEC Reference number: 18/STH/45

Participant ID		Date of Birth	DD	MM	Year

Form Number	
Data entered onto ART™ database	
Signed	Date

Participant ID		Date of Birth	DD	MM	Year

CRF Completion Instructions

- Complete the CRF using a black ball point pen and ensure that all entries are complete and legible.
- Avoid the use of abbreviations and acronyms.
- The CRF should be completed as soon as possible after the scheduled visit.
- Do not use participant identifiers anywhere on the CRF, such as name, hospital number etc., in order to maintain the confidentiality.
- Ensure that all fields are completed on each page:
    - If a test is Not Done record ND in the relevant box(es)
    - Where information is Not Known write NK in the relevant box(es)
    - Where information is Not Applicable write NZ in the relevant box(es)
- Weights should be recorded to the nearest 0.1 kg.
- Medications taken by the participants should be recorded using the generic name whenever possible, except combination products which will be recorded using the established trade name.
- If a participant is identified with cardiac arrhythmia, a single line must be drawn across each uncompleted page.
- The protocol deviation/violation/serious breach should be logged and commented including reason for missed protocol or assessments etc.
- The Principal Investigator (PI) is responsible for the accuracy of the data reported on the CRF. The PI must sign and date the PI's Sign Off page to certify accuracy, completeness and legibility of the data reported in the CRF.
- The CRF documents should be stored in a locked, secure area with the PI when not in use where confidentiality can be maintained. Ensure that they are stored separately to any other documents (e.g. Consent Forms) that might reveal the identity of the participant.

Participant ID		Date of Birth	DD	MM	Year

Date of Assessment: DD MM Year

Informed Consent signed: ☐ Yes ☐ No

DEMOGRAPHIC DATA

Date of Birth:	DD	MM	YEAR		Age:	YEARS	MONTHS
Sex:	☐ Male	☐ Female	☐ Others		Nationality:		
Ethnicity:	☐ NZ European	☐ Maori	☐ Samoan	☐ Tongan	☐ Cook Island Maori		
	☐ Niuean	☐ Fijian	☐ Chinese	☐ Indian	☐ Others (specify)		
Comments:							
Employment Status:	☐ Student	☐ Currently employed	☐ Not-employed	☐ Retired			

CLINICAL DATA

Height:	m	Weight:	kg	BMI (kg/m²):	

Sys BP (mmHg)	Dia BP (mmHg)	Central Sys (mmHg)	Central Dia (mmHg)	PR (bpm)	AI (%)	MBP (mmHg)
Avg:	Avg:	Avg:	Avg:	Avg:	Avg:	Card S.no. :

Smoking:	☐ Non-smoker ☐ Ex-smoker ☐ Current smoker Average number of cigarettes smoked per day:
Alcohol consumption (>6 drinks/occasion):	☐ Never ☐ ≤ 1/month/Occasionally ☐ Weekly/Daily/Almost daily Standard unit consumed per occasion:
Diabetes:	☐ Yes ☐ No ☐ NK ☐ Type I DM ☐ Type II DM
Dyslipidaemia: (high cholesterol)	☐ Yes ☐ No ☐ NK

Hypertension: (high BP)	☐ Yes ☐ No	☐ Normal (< 120/80 mmHg)	☐ Elevated (sys 120-129 & dia <80 mmHg)
		☐ Stage 1 (sys 130-139 or dia 80-89 mmHg)	☐ Stage 2 (sys ≥140 or dia ≥90 mmHg)

Participant ID		Date of Birth	DD	MM	Year

Ongoing medications				
Medication (Record generic or trade name)	Reason for use (diagnosis)	Dose & units	Frequency	Start date
Do you regularly take your medications?		☐ Yes ☐ No		
Other information:				

Pulse Wave Velocity (PWV) measurement with Doppler ultrasound	
Direct distance, DD (m): (Common Carotid Artery, CCA to Common Femoral Artery, CFA)	
Average Transit time, TT (s): (CFA-CCA)	
Mean PWV (m/s):	
Comments:	

Body Mass Index (BMI): Weight/(Height)^2

Mean Blood Pressure (MBP): DBP + 0.4 (SBP-DBP)

Pulse Wave Velocity (PWV): Direct distance/Transit time * 0.8

Appendix K: Study Report Form

STUDY REPORT

Determining the normative values of arterial pulse wave velocity using Doppler ultrasound for New Zealand population

Estimating normative pulse wave values (Study II)

Participant ID		Name	

INDIVIDUAL DATA

Date of Birth:	DD	MM	YEAR		Age: years			Sex:	
Height:	m		Weight:	kg		BMI (kg/m²):			
Blood Pressure (mmHg):				Stage of Hypertension:					
Pulse Wave Velocity (PWV) measurement with Doppler ultrasound									
Mean PWV (m/s):									

GROUP DATA

Pulse Wave Velocity (PWV) measurement with Doppler ultrasound			
	Group 1 (18-30 years)	Group 2 (30-60 years)	Group 3 (Over 60 years)
Mean PWV (±SD) (m/s):	4.8 (0.70)	5.8 (1.14)	7.2 (1.47)

Remarks:
BMI (Body Mass Index):
Underweight: < 18.5; Normal weight: 18.5-25; Overweight: 25-30; Obese: > 30

Stage of Hypertension:
Normal: <120/80; Elevated: systolic 120-129 & diastolic <80; Stage 1: systolic 130-139 or diastolic 80-89; Stage 2: systolic ≥140 or diastolic ≥90 mmHg

Appendix L: Research output from thesis

Dahiya, E., Krishnamurthi, R., Lowe, A., & Feigin, V. (2016). Measuring Pulse Wave Velocity: Developing a New Approach. In V. Feigin & P. A. Barber (Chair), Symposium conducted at the meeting of the New Zealand Applied Neurosciences Conference, Auckland, New Zealand. Retrieved from http://www.karger.com/DOI/10.1159/000453097

P3 – Clinical Neuroscience and Neurorehabilitation Research

432

The Role of GH/IGF-I Axis and Neurorehabilitation in the Functional Improvement after Acquired Brain Injuries: FOLTRA's Method

Devesa, P.; Devesa, J.

Fundación FOLTRA, Teo, Spain

Currently it is well known that adult neurogenesis continuously occurs along life in any animal species, including humankind. Adult neurogenesis mainly takes place in two brain regions: Subgranular Zone of Dentate Gyrus (SGZ) and Subventricular Zone of the lateral ventricle (SVZ); other cerebral areas may produce differentiated neurons though. A number of different Growth Factors interact in these neurogenic niches regulating the proliferation-differentiation-migration and survival of neural precursors to repair brain injuries or for the acquisition of recent memory. Among these factors, the GH/IGF-I axis seems to play a key role, both acting directly or by inducing the expression of other neurotrophic factors. When damage occurs in the brain, the subsequent loss of neurons and astrocytes leads to a number of functional impairments, but also to an attempt of self-repair, that is usually not sufficient due to the severity of the injury. Previous studies from our group and others indicate that the exogenous administration of GH or IGF-I strongly helps neurorehabilitation therapies by increasing adult neurogenesis and brain plasticity. In this presentation we will show the results obtained with this therapeutic strategy in patients suffering from Cerebral Palsy (CP), Traumatic Brain Injury (TBI) and Stroke. Our data indicate that GH administration is safe and effective, regardless of whether the patient is GH-deficient or GH secretion is normal. Moreover, our results also indicate that the time elapsed since the injury occurred is not a negative conditioning factor, except when joint deformations exist.

451

Measuring Pulse Wave Velocity: Developing a New Approach

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Pulse wave velocity (PWV) is a gold-standard measure of arterial stiffness (AS) and has been acknowledged as an independent diagnostic marker of stroke and cardiovascular risk. PWV is defined as the rate at which a pulse travels between two sites along an arterial segment. The purpose of this study is to review existing methodologies and validate a new non-invasive monitor against Doppler Ultrasound. The normal and reference PWV ranges will be estimated for the New Zealand (NZ) population, overall and by major ethnic groups. Carotid and femoral arteries, being superficially located are commonly used sites to track regional pulse waves. Local assessment of stiffness in a short arterial segment is done by MRI or ultrasound. Invasive methods using catheterisation, though more accurate, are not routinely practiced. Commercially available non-invasive monitors are less expensive and easier to use. The majority of these monitors detect arterial pulse wave using applanation tonometry (e.g. Complior, SphygmoCor, Pulse Pen, Doppler Ultrasound) and record pulses either simultaneously or sequentially by gating using the R-wave of the ECG. Despite practical advantages of these non-invasive monitors, assessment of PWV still needs a controlled environment and trained personnel, and current devices don't allow continuous monitoring. The development of a new non-invasive monitor with wearable patch sensors would enable PWV and 24-hour ambulatory arterial stiffness index (AASI) measurement. Establishing a link between AS and cardio-cerebral-vascular risk factors using normative values for the NZ population will assist in developing more accurate risk assessment and management strategies.

453

Risk of Medical Comorbidities after Traumatic Brain Injury – Methods for a Case-Control Study

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Traumatic Brain Injury (TBI) has been found to affect up to 790 people per 100,000 in New Zealand every year. Previous research has highlighted a wide array of poor physical, emotional, cognitive and behavioural outcomes associated with TBI. It remains poorly understood as to whether people who have experienced a TBI have an increased risk of developing subsequent comorbidities. A greater understanding of the risk of developing comorbidities post TBI will therefore assist in intervention planning post-TBI. This age- and sex-matched case-control study aims to investigate the onset of post-injury medical comorbidities in the 1–4 years following injury. Eligible participants will be recruited retrospectively from the Waikato Trauma Registry. All TBI admissions (including mild, moderate and severe TBI) and orthopaedic admissions at Waikato Hospital that occurred between Jan 1st 2012 and Dec 31st 2014 will be contacted. An estimated 400 TBI and 400 orthopaedic participants are expected to be recruited. Information on the injury sustained will be collected from the registry. A brief telephone assessment on self-reported pre- and post-injury medical comorbidities will be conducted using the Cumulative Illness Rating Scale. This study will address

Dahiya, E., Krishnamurthi, R., Lowe, A., & Feigin, V. (2018). Estimating reference values of aortic pulse wave velocity for the New Zealand population Symposium conducted at the meeting of the New Zealand Medical Sciences Congress 2018, Queenstown, New Zealand.

M8. Estimating reference values of aortic pulse wave velocity for the New Zealand population

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Pulse wave velocity (PWV) is a gold-standard measure of arterial stiffness (AS) and has been acknowledged as an independent diagnostic marker of stroke and cardiovascular (CV) risk. New Zealand (NZ) has a high prevalence of people with cardiovascular disease (CVD) with ethnic disparities. However, the use of PWV for routine clinical assessment of CVD risk is not practiced due to a lack of reference values and official recommendations for the NZ population. In this research, aortic carotid-femoral PWV values were estimated (N=92/120) using Doppler ultrasound for a 'reference value population' (RVP, n=26) with CVD risk factors but free from diabetes, high cholesterol, any known heart disease or on medications for these conditions. Whereas, participants with normal BP without any CVD risk factors constituted the 'normal value population' (NPV, n=66). The screened participants were grouped by age (18-30, 30-60, > 60 years) and blood pressure (BP) (normal, elevated, stage 1, stage 2) categories. Peripheral and central systolic/diastolic BP, pulse rate, and augmentation index (AI) were measured by USCOM BP+ monitor.

The data collected so far shows that PWV shares a positive correlation with age (R^2=0.4) and blood pressure (R^2=0.2). The mean aortic PWV were significantly lower in the NVP (5.1 ± 0.92 m/s) compared to RVP (6.3 ± 1.26 m/s) ($p<0.001$). The mean PWV values for the three age categories were 4.8, 5.6, and 6.7 m/s respectively with higher values in the RVP. Effect of age, sex, body mass index (BMI), AI, mean BP, smoking, alcohol consumption, diabetes, dyslipidaemia, and hypertension on PWV was assessed. Multiple regression analysis showed a significant contribution to the prediction of the Mean PWV with age (β=0.5, p=<.001), and BMI (β=0.1, p=.03).

The preliminary results show PWV could have value in a proactive approach to CVD risk assessment that would help in attaining long-term community health goals of the NZ government. The final outcome will assist in establishing the normal and reference values of PWV in the NZ population.

Appendix M: Preliminary trials measuring PWV with bioimpedance

This appendix will report the background, methods and results of the experimental trials that were conducted during the PhD study as a "proof of concept" study. The sections in this appendix will describe the concept of measuring pulse wave velocity (PWV) with bioimpedance, and reports on the findings of the experiments conducted. These experiments were an additional objective for this doctoral research.

Overview

The research team at the Institute of Biomedical Technology (IBTec) was working on the concept of measuring aortic PWV with bioimpedance. A prototype bioimpedance-measuring device was designed in the lab, and it was planned to validate it against the gold standard 'Doppler ultrasound.' However, the initial tests with that monitor were not promising. The team decided to use another device that could measure bioimpedance and opted for 'PhysioFlow,' designed and marketed by Manatec Biomedical, Paris, France. It is a non-invasive device used to monitor haemodynamic parameters based on thoracic electrical bioimpedance ("Project's Origin," 2016). It has been shown in the previous literature that PhysioFlow can measure aortic stiffness both regionally and locally (Collette, Humeau, Chevalier, Hamel, & Leftheriotis, 2011).

Rationale

The objective was to measure the regional aortic PWV at the common carotid and femoral arteries with PhysioFlow, followed by comparing it against the standard Doppler ultrasound. Several experiments were designed and performed to study different electrode locations, subject positions, and replicating Collette's protocol for regional PWV measurement (Collette, Humeau, et al., 2011). The findings from these trials would be used in future to design and improve the initial prototype.

Literature review

Bioelectricity and bioimpedance

Every living organism, be it a cell, tissue or organ from plants, animals, or the human body has its own electrical phenomenon. The cell membrane transports the energy consumed by the cell through activated ion channels. Depolarisation (excitation) leads to the generation of current flowing within (intracellular fluid, ICF) and around (extracellular fluid, ECF) the cell (Martinsen & Grimnes, 2011).

Furthermore, the electrical properties of a cell can be classified into two types depending on the source of electricity, as an active or passive response. Any response that occurs because of the stimulation of the tissue inside the cells is bioelectricity or an active response; for example, electrocardiogram (ECG) or encephalogram (EEG). Whenever the stimulus is from an external electrical current source, the response observed is a passive response, such as used in impedance cardiography (Khalil, Mohktar, & Ibrahim, 2014).

The biological tissues act as a complex conductor, the characteristics of which are defined by the content of water and electrolytes. These determine whether it will behave as resistive or reactive. Normally, the cell membrane represents a reactive capacitor and the ICF/ECF as resistors resisting the electrical flow (Capela, 2013; Kyle et al., 2004; Martinsen & Grimnes, 2011). However, at different frequencies, tissue conducts current differently. At low frequencies (or at zero frequency), the cell membrane (also denoted as a capacitor), behaves as an insulator, making the current pass around the cells in the ECF. On the other hand, at very high or near infinity frequencies, the capacitor becomes conductive, and the current passes through the cells

and surrounds, including both the ICF and ECF (Capela, 2013; Kyle et al., 2004). The concept of *in vivo* current conductance is further depicted in Figure 31.

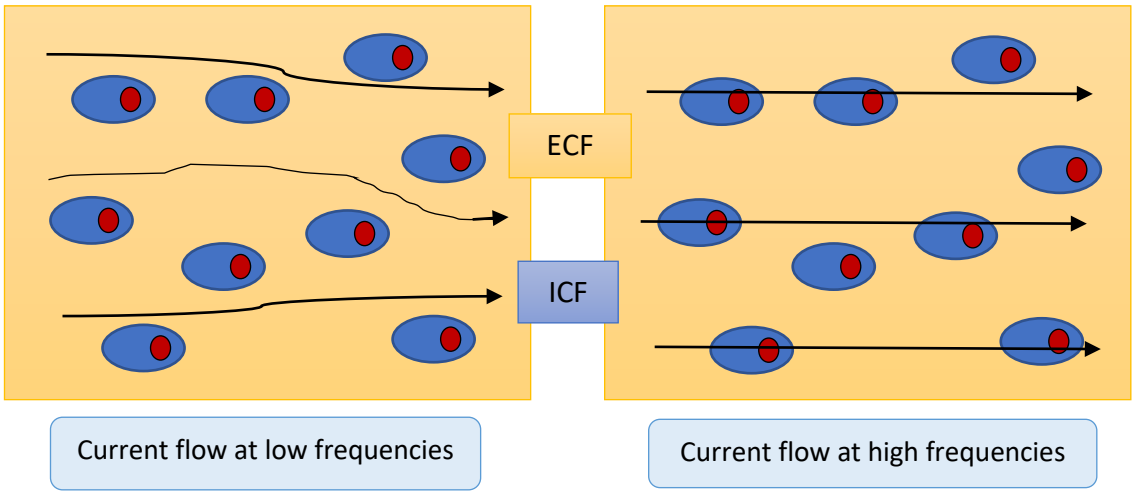

Figure 31: Electrical conductance of biological tissue (Capela, 2013)

When the abovementioned phenomena are to be represented as an electrical circuit, the Cole-Cole model is used for the biological tissue-equivalent system (also known as Fricke's electrical circuit) (Capela, 2013; Kyle et al., 2004). The circuit consists of a capacitor as a cell membrane (Cm), and two resistors, namely, ICF (Ri) and ECF (Re). The Ri and Cm are connected in series at one arm and parallel to the Re at the other arm of the circuit. The value of these parameters could be recorded at various frequencies. However, the majority of the analysis is carried out at a single frequency at 50 kHz (Kyle et al., 2004). The electrical circuit is shown in Figure 32.

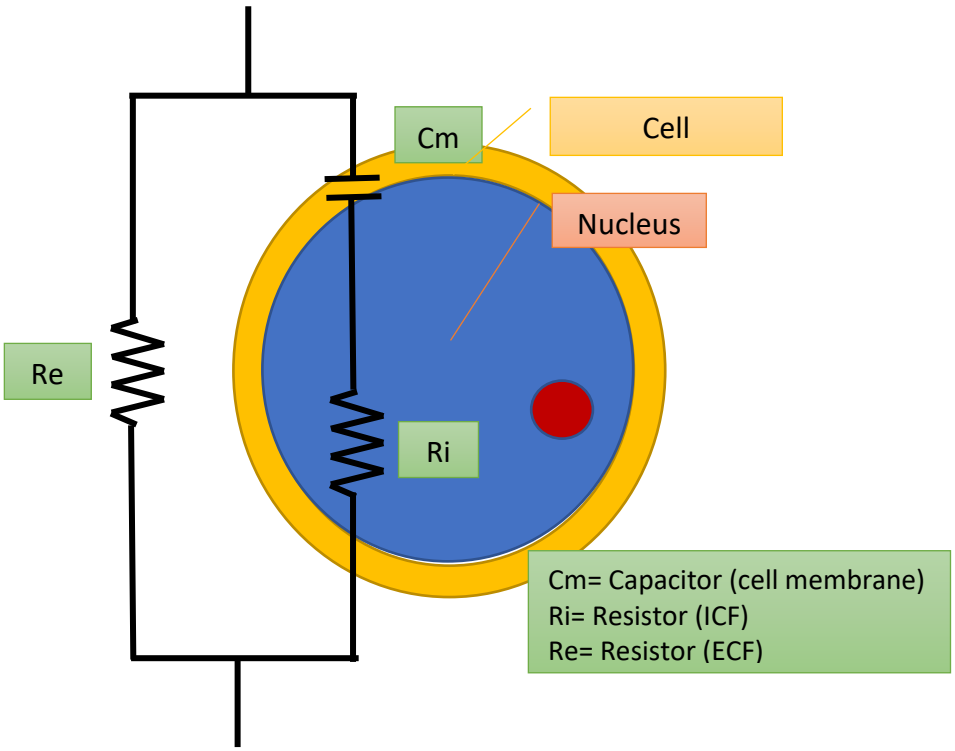

Figure 32: Fricke's electrical circuit for a cell

Impedance or bioimpedance is the measure of how much a living body impedes or obstructs the electrical current flowing through it. In other terms, it is a passive response to a current flow that has been applied externally (Capela, 2013). Impedance has the same SI unit as the resistance, Ohm (Ω) and is calculated by using Ohm's law. Mathematically, impedance is the ratio of the conductor's voltage to the current it carries between the two terminals (Capela, 2013; Kennelly, 1893).

$$\boldsymbol{Impedance\ (Z) = \frac{Voltage\ (V)}{Current\ (I)}}$$

There are two methods to measure the impedance depending on the number of surface electrodes used. These are the bi-polar (or two electrodes) method and tetra-polar (or four electrodes) methods. Due to the technical limitations around the two-electrode system, usually, the four-electrode method is used (Bera, 2014; Capela, 2013; Singh. G., Anand. S., Lall. B., Srivastava. A., & Singh. V., 2018). In this method, as shown in Figure

33, four surface electrodes are placed on the biological tissue. The outermost two electrodes (red colour) on either side would be used to apply alternating current from the current source. Whereas, the inner two electrodes (blue colour) would be used to detect the change in voltage.

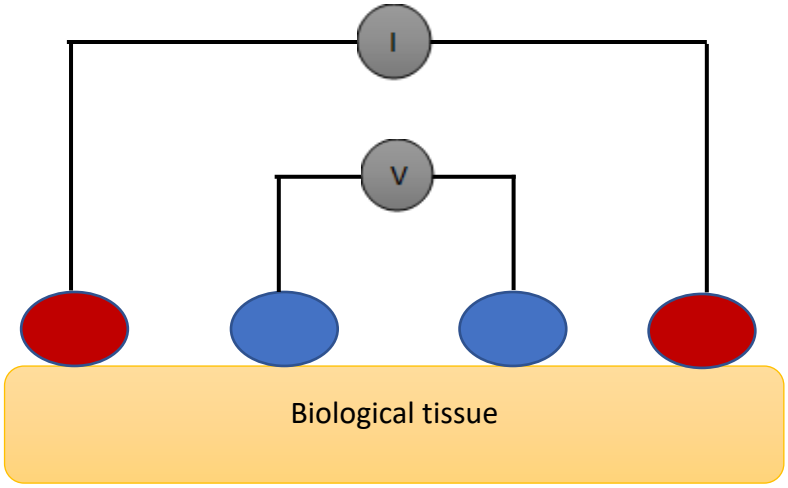

Figure 33: Tetra-polar method of bioimpedance measurement

The electrical properties of tissues were first reported back in 1871, which was further explored in a wide range of tissues (living or dead) for a large range of frequencies (Khalil et al., 2014; Kyle et al., 2004). By the mid-90s, the applicability of bioimpedance analysis was applied by Thomasset who used impedance to measure total body water via two needles inserted subcutaneously and Nyboer by using a hand-to-foot whole-body method (Nyboer, 1970; Thomasset, 1962). Different techniques were worked on for measuring body fat-free mass and total body water using four surface electrodes (Hoffer, Meador, & Simpson, 1969; Nyboer, 1970). The application of bioimpedance analysis extended from reading the biological tissues to the clinical bedside. Being non-invasive, portable, safe and reproducible facilitated the wide use of impedance in healthcare, disease diagnosis and monitoring, mechanical/chemical/civil engineering,

geosciences, and other industries (Singh. G. et al., 2018). A summary of the techniques with their application is given below in Table 12.

Table 12: Summary of clinical applications of bioimpedance

Techniques	Applications
Bioelectric Impedance Analysis	Study and evaluate body composition including total body water, fat-free mass, BMI, ICF, ECF
Electrical Impedance Spectroscopy	Measure the complex electrical impedance of the physiological state of the tissues
Electrical Impedance Plethysmography	Measure electrical impedance due to change of blood volume; detect blood clots, deep vein thrombosis, early-stage arteriosclerosis
Impedance Cardiography	Study changes in the electrical impedance trans-thoracic region; measures cardiovascular parameters such as heart rate, cardiac output, stroke volume, thoracic fluid content
Electrical Impedance Tomography	Diagnose and monitor pulmonary activity, gastric activity, brain function and others
Electrical Impedance Mammography	Detect breast cancer by imaging
Magnetic Induction Tomography	Study biological tissues, diagnostic magnetic field imaging without direct electrical content

BMI: Body Mass Index, ICF: Intracellular fluid, ECF: Extracellular fluid

PWV and bioimpedance

Summarising the information on PWV from chapter 2 section 2.4, page 29, PWV is defined as the velocity of the pressure wave generated at the heart as it travels along the arterial tree. PWV is a clinical biomarker considered the gold standard for measuring arterial stiffness to identify people at cardiovascular risk (Mancia et al., 2013). Estimation of PWV is done at two chosen arterial sites by measuring the distance and transit time (Cavalcante et al., 2011; Milan et al., 2019a). PWV assessments could be done locally at an arterial site using ultrasound or MRI, and regionally focusing on

calculating average arterial stiffness of a segment of the arterial tree (Pereira et al., 2015; Van Bortel et al., 2014). Regional PWV is measured using devices based on the principle of applanation tonometry (SphygmoCor, Pulse Pen) and piezo-electric technology (Doppler ultrasound, Complior, Arteriograph, Vicorder) (Milan et al., 2019a; Pereira et al., 2015; Rajzer et al., 2008). Based on expert consensus, assessment of PWV at the common carotid artery and common femoral artery is considered to be standard (Van Bortel et al., 2012).

The new methodological approach for impedance cardiography emerged in 1940, and later in the 1960s, the trans-thoracic impedance analysis method was developed to study the heart with cardiac haemodynamic and fluid status (Kerai, 2018). Parameters such as stroke volume, cardiac output, left ventricular end-diastolic volume, thoracic fluid volume, and heart rate are recorded using impedance cardiography (Bera, 2014; Capela, 2013; Singh. G. et al., 2018; Tan, Lai, & Hwang, 2006). However, the quest to record the pulse wave by a bioimpedance device began in the early 1920s. Kusche et al. presented a new device that could measure bioimpedance variations using a pulse wave for the very first time (Kusche, 2007). The article opened a range of possibilities to study the cardiac system. The pulse transit time is the time taken by a pulse to reach a peripheral arterial site from the heart (Boutouyrie et al., 2009; Boutouyrie & Vermeersch, 2010; Millasseau et al., 2005). The transit time of a pulse wave could be measured through Doppler ultrasound, ECG-photoplethysmography, and pressure sensors. A research team validated a new method using bioimpedance to measure the pulse transit time from elbow to wrist against EEG-photoplethysmography (correlation coefficient 0.93) (Bang et al., 2009). In the same year, another group worked on getting a continuous heart rate and PWV measurement from a small body surface area (1.5 x 7

cm) (Cho, Kim, & Cho, 2009). Moving forward, Collette et al. from France proposed a new mathematical model to measure the PWV locally with bioimpedance using the four-electrode approach at the large femoral artery (Collette, Leftheriotis, & Humeau, 2009). Later in 2011, the same group suggested that arterial stiffness measured locally in the thorax region by measuring local arterial resistance and distensibility is significantly correlated with the regional PWV measure (Collette, Lalande, Willoteaux, Leftheriotis, & Humeau, 2011). The work was followed by a study comparing the local arterial stiffness with the regional method (measured as carotid-femoral pulse wave velocity (cf-PWV) and Impedance wave velocity, IWV). The article concluded that local arterial stiffness is significantly correlated with regional PWV measurements ($r=0.75$, $p<0.0001$) measured with bioimpedance and tonometry (Collette, Humeau, et al., 2011).

General experimental setup

In this study, the gold standard for regional aortic PWV was measured with Doppler ultrasound (ACUSON Sequoia c512 Echocardiography Ultrasound System). Descriptions and protocol for measuring the PWV at the common carotid and femoral artery is discussed in detail in chapter 3, section 3.8.4, page 82. A similar protocol was followed for measuring the pulse wave at the radial artery. The linear transducer probes used for the carotid-femoral artery and radial artery were 6L3 and 17L5, respectively. Also, a measuring tape was used to measure the distance between the two arterial sites.

For the bioimpedance measurements, an impedance device named PhysioFlow (PhysioFlow PF05 Lab1, Manatec Laboratories, France) was used. The device comes with six electrode leads to record the bioimpedance and ECG. Originally, PhysioFlow was designed as a non-invasive device to monitor cardiac haemodynamic functions ("Project's Origin," 2016). The protocol for measuring trans-thoracic impedance and

location of electrodes has been explained in the literature (Tan et al., 2006; Tonelli, Alnuaimat, Li, Carrie, & Mubarak, 2011). The pre-gelled silver-silver chloride (Ag-AgCl) surface electrodes are used for recording the bioimpedance. Two of these electrodes would be placed around the left side of the neck (Z1 and Z2 at the common carotid artery), and another two would be at the thoracic area (Z3 and Z4+ECG3 at the xiphoid region). The remaining two electrodes (ECG1 and ECG2) would be on either side of the chest to record a single ECG signal. It works on the principle of tetra-polar (four-electrode) configuration. Therefore, the outer ones (Z1 and Z4) would act as transmitters of current, and the inner ones (Z2 and Z3) would sense the change in voltage. The leads would be connected to the specific electrodes that send a signal through an electronic processing unit (Digital to Analogue Converter, DAC) to a computer for data acquisition and analysis.

LabVIEW software (LabVIEW 2016, National Instruments, Texas, USA) was used to design a system for a visual output screen and was developed for recording the output from the PhysioFlow. The raw data was transferred and analysed in the Matlab software (Matlab 2015b, The Mathworks Inc., Massachusetts, USA). Figure 34, Figure 35 and Figure 36 show an Ag-AgCl ECG electrode, PhysioFlow setup and lead positions, respectively, for trans-thoracic bioelectrical impedance measurement. Additionally, a simplified flowchart of the working of bioimpedance measurement is shown in Figure 37.

Figure 34: A pre-gelled Ag-AgCl ECG electrode

Figure 35: PhysioFlow PF05 Lab1 bioimpedance device

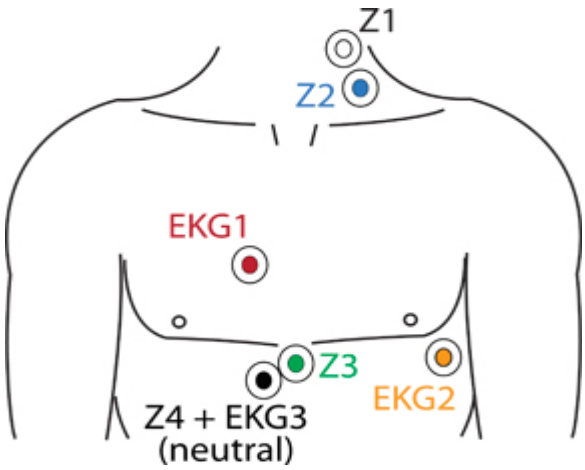

Figure 36: Placement of electrodes for trans-thoracic bioimpedance measurement

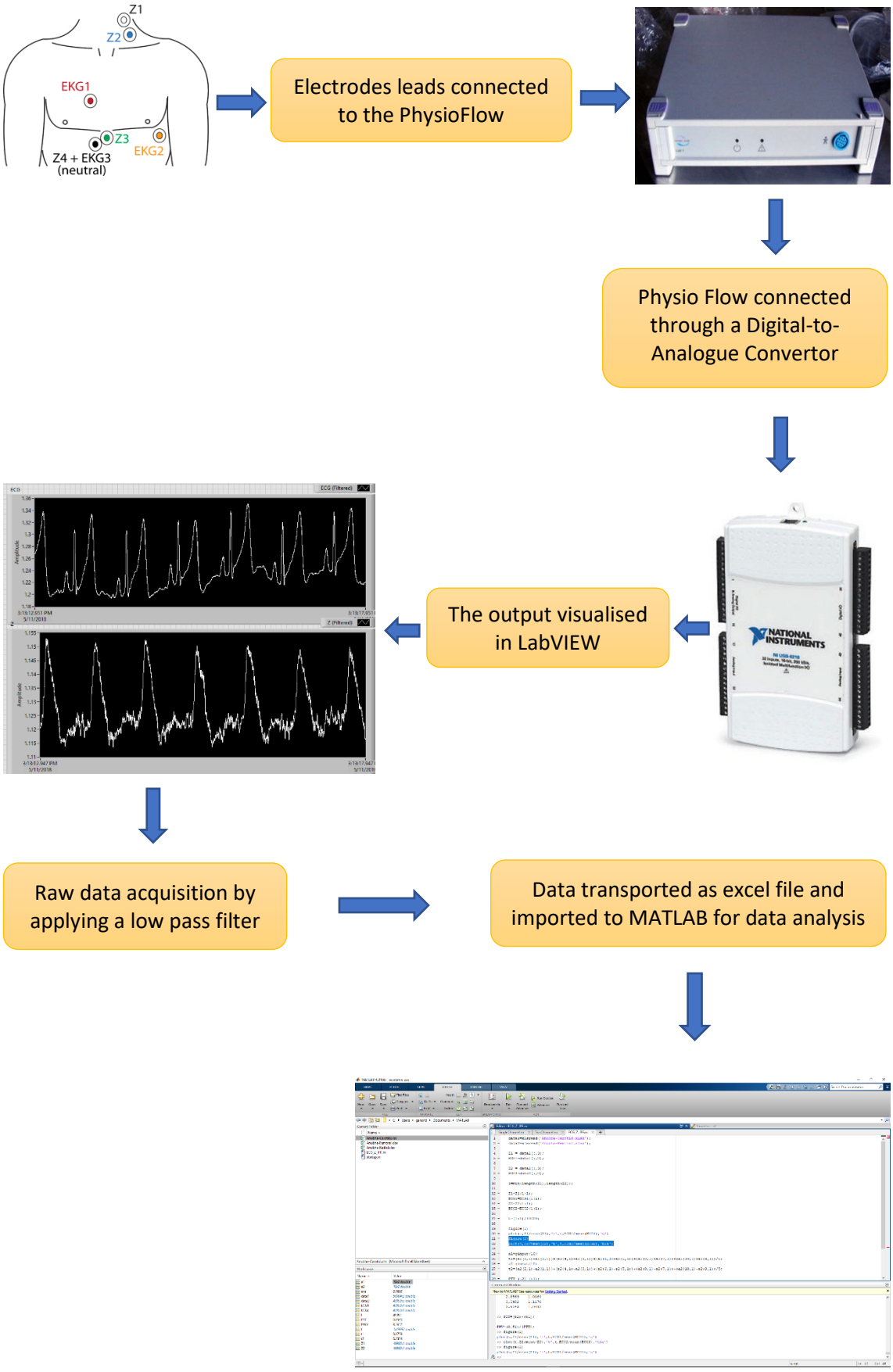

Figure 37: Flowchart of measurement of the bioimpedance

Experiment 1

Aim: To compare the transit time measured with the PhysioFlow and Doppler ultrasound.

Instrumentation: PhysioFlow, Doppler ultrasound, linear transducer probes– 17L5 and 6L3, surface ECG electrodes, LabVIEW and MATLAB software.

Background: The purpose of conducting this preliminary experiment was to compare the difference in transit time while measuring the regional PWV between the PhysioFlow and Doppler ultrasound. It is evident that the standard protocol to measure the PWV is to record the pulse wave at the common carotid and femoral artery (Laurent et al., 2006). Since recording with the PhysioFlow would need electrodes to be placed along the arteries compared to using a transducer probe for the ultrasound, it was decided to read the carotid and radial artery first. Additionally, we planned to look into the effect of the participant's positioning (supine vs sitting) at the time of recording.

Procedure: The PhysioFlow was set up and connected with the computer using LabView software, as mentioned earlier. The initial experiments were performed within the research team members (n=4) as participants. Each participant was prepped by placing the electrodes on the left common carotid and radial artery for the PhysioFlow to measure the bioimpedance for 10 seconds. The ultrasound was recorded on the right common carotid and radial artery for 3-5 full cardiac cycles. An illustration of the setup is shown in Figure 38. The readings were recorded from the common carotid artery and common radial artery sequentially gated with the ECG. We started with a sitting position for the PhysioFlow and sitting and supine positions with ultrasound following the steps stated in chapter 3, section 3.8.4, page 82.

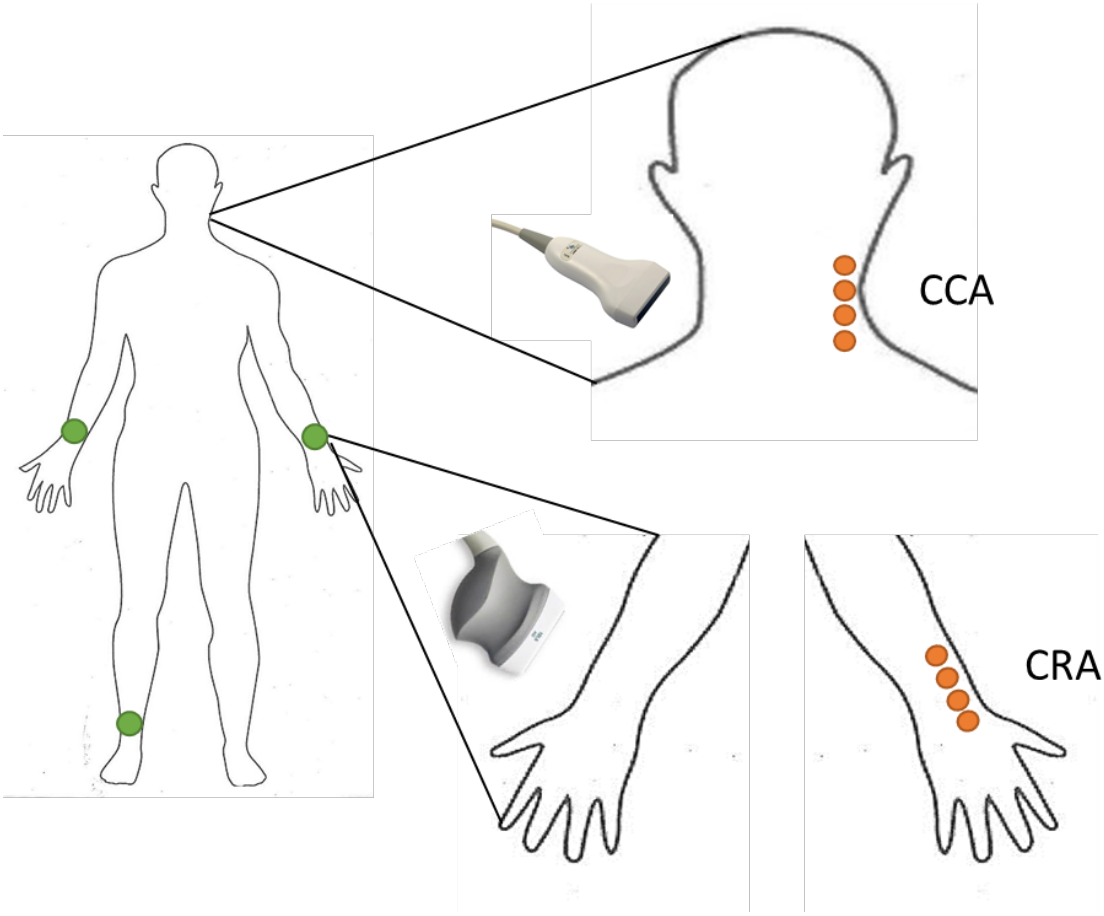

Figure 38: Position of the electrodes (bioimpedance-orange, ECG-green) and ultrasound transducer at the common carotid artery (CCA) and common radial artery (CRA)

The pulse transit time was calculated from carotid-radial ultrasound images, taking an average of two consecutive readings. The transit time was measured by using the 'foot-to-foot' method. Distance from the foot of the pulse wave to the peak of the ECG wave was recorded for each site, and the transit time was calculated by subtracting the values from the carotid artery from that of the radial artery. The same procedure was followed for the impedance wave gated with the R-wave of ECG to calculate the impedance transit time.

Findings: On comparing the pulse transit time, there was a difference in the readings obtained from the ultrasound measured from the supine and sitting positions. Furthermore, the PhysioFlow impedance waves appeared to be inverted for the supine

position to that when sitting upright. Table 13 summarises the pulse transit time calculated from the ultrasound and PhysioFlow.

Table 13: Pulse transit time from carotid-radial arteries using ultrasound and PhysioFlow in different positions

Participant ID	Transit time (s)		
	PhysioFlow (sitting)	**Ultrasound (supine)**	**Ultrasound (sitting)**
P01	0.064	0.092	0.056
P02	0.076	0.092	0.078
P03	0.067	0.088	0.072
P04	0.065	-	0.07

Based on the observations, further experiments were planned to investigate the reason behind getting varying results in the participant's different positions. The possibility of using different type/form of electrodes, such as suction electrodes, or a small patch designed with four electrodes, was discussed.

Experiment 2

Aim: To compare the transit time at the common carotid artery and common radial artery using PhysioFlow and Doppler ultrasound in a semi-supine position.

Instrumentation: PhysioFlow, Doppler ultrasound, linear transducer probes – 17L5 and 6L3, surface ECG electrodes, LabVIEW and MATLAB software.

Background: Based on the previous experiment findings, this experiment was aimed at recording the pulse transit time while having a position intermediate to supine and sitting. The Fowler's, or semi-supine position, is when the subject is sitting in a semi-upright position at an angle of 45-60 degrees from horizontal. It would help us

understand the role of position while recording and observing an inverted impedance wave with the PhysioFlow.

Procedure: The ultrasound and PhysioFlow were set up, and the participants (n=4) were asked to sit in a semi-supine position supported by a bed with the backrest inclined. The electrodes were placed in the same position, as described in Figure 38. The readings were recorded at the common carotid artery and radial artery using the ultrasound and PhysioFlow following the same protocol as discussed in the previous experiment. The average of pulse transit time was calculated and compared with the ultrasound and PhysioFlow scores.

Findings: It was observed that there was no difference in the transit time readings from the two methods. The readings from the ultrasound and PhysioFlow are shown in Table 14. The PhysioFlow impedance waves were not inverted this time. However, the reason for impedance pulse wave inversion in the supine position is still unclear. As per the current ultrasound protocol guidelines, spectral carotid analysis can be performed in a semi-supine, or Fowler's position (Tahmasebpour, Buckley, Cooperberg, & Fix, 2005). However, whether this position affects the measurement of the common femoral artery was still not clear.

The findings from this experiment were encouraging and supported the fact that having the assessment made in the semi-supine position produced similar values by both methodological approaches. The next question to be answered was whether these results would be replicated for the carotid and femoral arteries.

Table 14: Summary of pulse transit time at a semi-supine position measured with PhysioFlow and ultrasound

Participant ID	Pulse transit time					
	PhysioFlow			Ultrasound		
	Carotid (s)	Radial (s)	Transit time (s)	Carotid (s)	Radial (s)	Transit time (s)
P01	0.115	0.178	0.063	0.117	0.182	0.065
P02	0.11	0.18	0.07	0.119	0.206	0.087
P03	0.095	0.203	0.108	0.10	0.209	0.109
P04	0.10	0.19	0.09	0.11	0.20	0.09

Experiment 3

Aim: To test a regional assessment of PWV and impedance wave velocity (IWV) at the common carotid and femoral arteries with PhysioFlow and ultrasound.

Instrumentation: PhysioFlow, Doppler ultrasound, linear transducer probe – 6L3, surface ECG electrodes, LabVIEW and MATLAB software.

Background: It is evident from the literature that aortic PWV is a common measure of arterial stiffness and is a predictor of CVD (Cavalcante et al., 2011; Pereira et al., 2015; Sahani et al., 2016). Collette et al. reported measuring the local and regional arterial stiffness by an IWV using the PhysioFlow and compared it against the standard tonometry technique (Collette, Humeau, et al., 2011). Henceforth, the present experiment was planned to replicate the protocol at the common carotid and femoral arteries and comparing it to the readings from the Doppler ultrasound. The distance measured between the two arterial sites was taken as a direct distance method (from carotid to femoral), and impedance/pulse wave velocity was calculated by dividing the distance over transit time multiplied by a factor of 0.8.

Procedure: The PhysioFlow and ultrasound setup were kept ready and connected to the computer system. Participants (n=3) were prepped by placing the surface electrodes in a supine position. For the ultrasound, the standard protocol to measure the right cf-PWV was followed, as mentioned in chapter 3, section 3.8.4, page 82. Regional assessment of IWV was made by recording the bioimpedance signal first at the chest (carotid artery) followed by the thigh (femoral artery) for 10 seconds. The position of the electrodes for the chest or trans-thoracic bioimpedance was as shown in Figure 39, having two electrodes at the left carotid and another two at the xiphoid area separated by a distance of 20 cm. The two ECG electrodes were to record ECG signals simultaneously to gate the impedance wave. The process was repeated for the left thigh, placing two electrodes at the femoral artery and the other two at a distance of 20 cm alongside the femoral artery. The position of the impedance electrodes and ECG for the regional IWV assessment is shown in Figure 39.

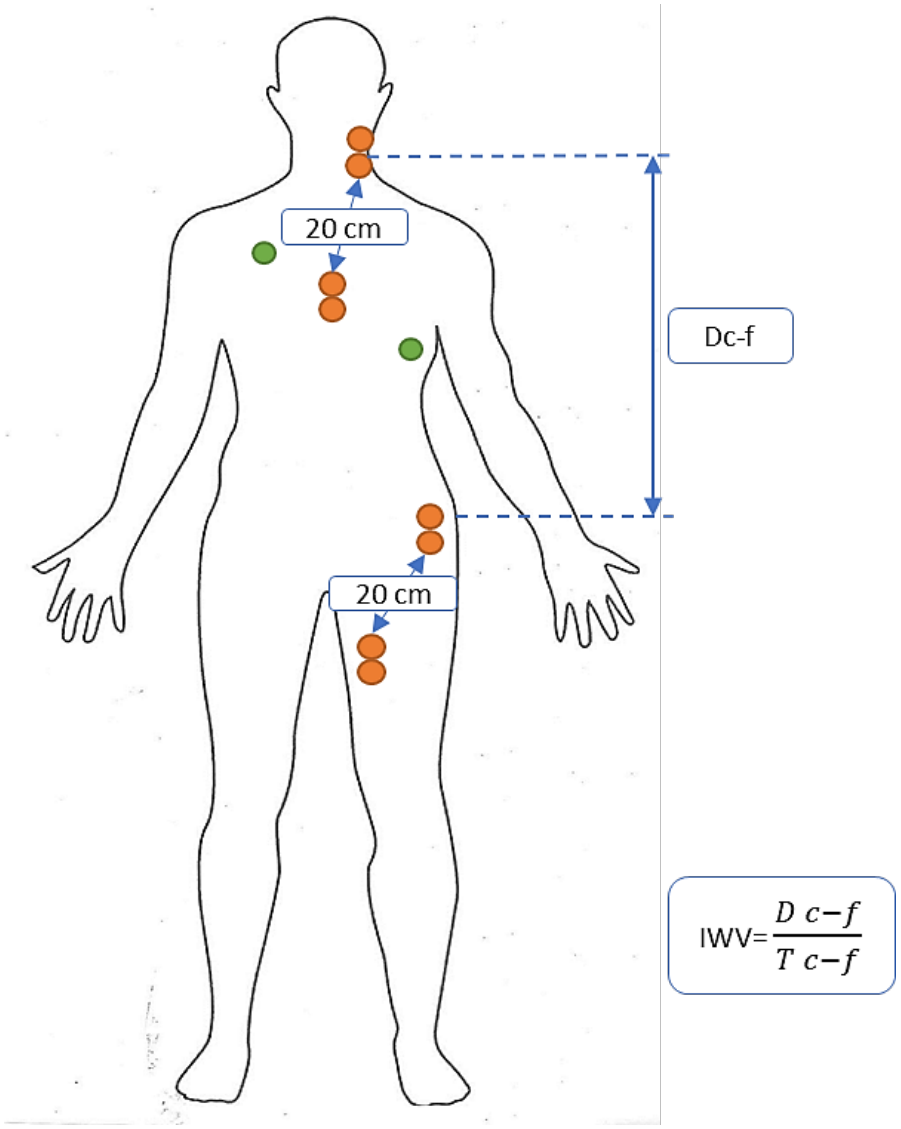

Figure 39: Position of the bioimpedance (orange) and ECG (green) electrodes to calculate impedance wave velocity (IWV)
Dc-f: Distance carotid to femoral; Tc-f: Transit time carotid to femoral

The pulse and impedance wave transit time was calculated following the principle of the foot-to-foot method. The pulse/impedance waves were gated against the peak of the R-wave of the ECG recording and the distance obtained from the carotid artery images was deducted from the distance of the femoral artery images. The PWV and IWV values were calculated using the formula of distance over time.

Findings: The data obtained after the filtering and processing of the bioimpedance signals from the PhysioFlow was compared with the ultrasound data. A summary of the results is given in Table 15.

Table 15: The impedance wave velocity and pulse wave velocity measured at the common carotid and femoral arteries using PhysioFlow and ultrasound

Participant ID	PhysioFlow IWV (m/s)	Ultrasound PWV (m/s)
P01	5.41	4.70
P02	5.36	4.96
P03	4.76	5.46

IWV: Impedance wave velocity, PWV: Pulse wave velocity

Due to the small sample size, we were not able to assess readings statistically; however, the impedance and pulse wave velocity obtained from the two devices were comparable. The result agrees with the Collette group who compared the regional aortic stiffness by measuring cf-PWV using a PulsePen and PhysioFlow (Collette, Humeau, et al., 2011). The placement of a pair of electrodes at the chest wall and the thigh provided better impedance waveforms than earlier experimental electrode placement setups. However, to lower the calculation error, there is still a need to refine the process of assessing the transit time from the foot of the waveform.

Summary

Bioimpedance analysis with the impedance cardiography being an easy, non-invasive, and reliable healthcare approach, has been widely used to assess and monitor the cardiovascular parameters. Using impedance for studying the changes in the arterial blood flow is a hot research topic. This chapter started with giving an overview of the practical issues and intention of the research team to explore the concept of bioimpedance to estimate PWV. Section 6.2 discussed the literature review on the topic beginning from the electrical properties of living tissues, defining impedance, and how to measure it. A review of research work done in the field of bioelectric impedance

analysis was made with its application of different techniques in the medical sector. A research gap of measuring the regional PWV or IWV with bioimpedance was identified. There were three experiments in total aimed at evaluating the effect of the following: the different placement of electrodes, the participant's positions, different arterial sites while recording, and comparing the results of PhysioFlow with an ultrasound. It was observed that measurement of bioimpedance at the common carotid artery in the supine position with a PhysioFlow gave inverted waves which were corrected by recording being in a semi-supine position. Additionally, the regional assessments of IWV (with PhysioFlow) and PWV (with ultrasound) were comparable at common carotid and femoral arteries. The observation made in experimental trials will assist the research team in reflecting on the various approaches and possible ideas that could be used in future research to validate a standard protocol of measuring PWV with a bioimpedance device.

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