A STUDY TO INVESTIGATE THE EFFECTS OF AEROBIC EXERCISE TRAINING (AET) ON THE LARGE INTRACRANIAL AND EXTRACRANIAL CEREBRAL ARTERIES AND THE COGNITIVE AND MOTOR FUNCTIONS IN POST STROKE PATIENTS.

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DEPARTMENT OF HEALTH TECHNOLOGY AND INFORMATICS

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

AUGUST 2024

Certificate of Originality

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written, nor material that has been accepted for the award of any other degree or diploma, except where due acknowledge has been made in the text

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Abstract

Stroke is a leading cause of morbidity and mortality worldwide, including in Hong Kong, where it ranks as the fourth leading cause of death, accounting for over 6.2% of deaths in 2020. To mitigate the socioeconomic burden due to stroke, robust preventative and treatment management strategies are required. Aerobic exercise training (AET), a non-invasive therapeutic intervention has potential to improve the deconditioned haemodynamic, motor, and cognitive functions associated with stroke. However, limited studies have assessed its effects on cerebrovascular status in post stroke patients. The present study assessed the effects of AET on cerebral arteries' morphological and haemodynamic features, and on cognitive and motor function in chronic post-stroke patients. The study also compared transcranial Doppler ultrasound (TCD) and transcranial color-coded Doppler ultrasound (with (cTCCD) and without (ncTCCD)) angle correction in assesing haemodynamic features of the middle cerebral arteries (MCAs). Furthermore, cerebral arteries structural and hemodynamic features of post-stroke patients and age-matched non-stroke individuals were compared.

Study One compared non-imaging transcranial Doppler ultrasound (TCD) and transcranial color-coded Doppler ultrasound (with (cTCCD) and without (ncTCCD)) angle correction in quantifying middle cerebral arteries (MCAs) haemodynamic parameters. This was a cross-sectional study involving 50 healthy adults aged $\geq$ 18 years old. The bilateral MCAs were insonated via three trans-temporal windows (TTWs-anterior, middle, and posterior) using TCD, cTCCD, and ncTCCD techniques. The MCA peak systolic velocity (PSV) and mean flow velocity (MFV) were recorded at proximal and distal imaging depths that could be visualised on TCCD with a detectable spectral waveform**.** A total of 152 measurements were recorded in 41 (82%) subjects with at least one-sided open TTW across the three techniques. The mean PSV measured by TCD, ncTCCD, and

cTCCD were 83±18 cm/s, 81±19 cm/s and 93±21 cm/s, respectively, and cTCCD yielded significantly higher PSV than TCD and ncTCCD (Bias =-10cm/s, p<0.001; Bias =-12cm/s, p=<0.001, respectively). In conclusion, the study validated TCCD as a practically applicable imaging technique in assessing MCAs blood flow velocities, whereas TCCD with angle correction (cTCCD) yielded higher and more accurate MCA blood flow velocities than non-imaging TCD and ncTCCD techniques.

Study Two compared the morphological and hemodynamic features of cerebral arteries between post-stroke patients and age-matched controls without a history of stroke. This was a cross-sectional study involving a total of 124 participants (57 post-stroke patients and 67 age-matched non-stroke controls) carried out at the Institutional laboratories. The study explored potential stroke risk biomarkers that could posit as post stroke AET rehabilitation efficacy indicators in the subsequent main RCT study based on novel ultrasound-based techniques, such as 3D arterial analysis, enhanced edge detection algorithms, and arterial stiffness measurements. Mean differences (MD) between post stroke and non-stroke cerebral arteries parameters represented main outcomes. The carotid β-stiffness index (CAS β), elastic modulus (CAS kPa), and pulse wave velocity (CAS PWV) were significantly higher for post stroke patients compared to non-stroke individuals (CAS β: 15.8±26.7 vs 9.3±7.7, p=0.013; CAS kPa: 208.9± 333 kPa vs 123.7± 112kPa, p=0.006, and CAS PWV: 7.8± 3.9m/s vs 6.5± 2.2 m/s, p=0.002), respectively. Conversely, carotid compliance (CAS CC) and distensibility coefficient (CAS DC) for post stroke group were lower compared to non-stroke individuals (0.476±0.27 vs 0.739±0.67, p<0.001; and 0.009 ± 0.006 vs 0.013±0.014, p=0.003). The 3D carotid lumen volume stenosis (%) did not differ between the two groups, indicating that hemodynamic failure due to stenosis is unlikely to be the primary mechanism of stroke occurrence in the study population. Furthermore, post stroke patients

exhibited reduced blood flow compared to non-stroke individuals, in both extra and intracranial cerebral arteries (all DCCA parameters, $p<0.05$; ICA EDV, $p=0.022$; MCA PSV, $p=0.001$; MCA EDV, $p<0.001$ and MCA MFV, $p<0.001$).

In conclusion, this study highlighted morphological features- CIMT, and all arterial stiffness indices to be significant biomarkers of stroke risk and indicators for monitoring treatment efficacy. Furthermore, population-based reference values for 3D ultrasound carotid lumen volume stenosis (%) and novel carotid arterial stiffness indices are provided for local non-stroke and post-stroke populations.

Study Three assessed the effects of AET on the large intracranial and extracranial cerebral arteries' morphological and haemodynamic features, and on cognitive and motor functions in chronic post-stroke patients. This was a single-blinded randomized controlled trial involving 42 post-stroke patients randomly assigned into either—36 sessions, 3times per week, 30 minutes duration, supervised cycling AET (n=21) or stretching (control) exercises (n=21). Pre and post interventional cerebral arteries' morphological and haemodynamic features were assessed using novel duplex carotid ultrasound (DCUS) applications and transcranial color-coded Doppler (TCCD) ultrasound, whereas Montreal cognitive assessment Hong Kong version (Moca-HK) tool assessed cognition. Mean differences (MD) between pre and post AET values represented study main outcomes. The study demonstrated that 36 sessions of cycling AET targeting high intensity HRR conducted over a 12-week period, elicited significant beneficial changes in cerebral arteries' morphological and functional features— carotid intima-media thickness (CIMT), mean difference (MD)=-0.069, $p<0.0001$; 3D carotid lumen volume stenosis (%), MD =-2.4, $p<0.001$; 3D plaque volume, MD =-60mm^3, $p=0.001$; 3D carotid vessel wall volume, MD=-55, 0.031*, and all arterial stiffness indices highlighted in Study 2, $p<0.05$). Additionally, modest improvements in

extracranial cerebral arteries' haemodynamic parameters— (DCCA EDV, MD=1.64, p=0.003; DCCA RI, MD=-0.035, p<0.001, DCCA PI, MD=-0.15, p=0.001); (ICA RI, MD=-0.05, p=<0.001 and ICA PI, MD=-0.2, p<0.001) were observed. Contrarily, no changes in MCA haemodynamic parameters (PSV, EDV, MFV, PI and RI) were observed, suggesting a well functional cerebral thermoregulation mechanism. The study further revealed significant medium effects size improvements on cognitive function (MoCA-Hk, MD=1.38, p=0.006, cohen d=0.63) and motor function (6MWT, mean difference=37m, p=0.002, cohen d=0.72 and TUG time, M. D= -2.84s, p=0.004, cohen d=0.53). Changes in cerebral arteries' morphological and haemodynamic features were observed not to be directly associated with the cognitive functional changes, except for DCCA EDV (Spearman's R=0.330, p=0.033*) suggesting the presence of some indirect mechanisms.

In summary, cycling AET elicited significant beneficial effects on cerebrovascular health, cognitive and motor function in chronic post stroke patients. Noteworthy, was improvement of cerebral arteries' features to values comparable or better than those of age-matched non-stroke individuals. The current study findings have significant clinical implications suggesting cycling AET as game changer in mitigating the deconditioned cerebral arteries vascular status and improving quality of life in post stroke patients. Further studies to explore possible indirect underlying mechanisms and investigate long-term effects of AET in mitigating against future stroke recurrences are recommended.

Keywords: aerobic exercise training (AET), cerebrovascular disease, cerebral arteries, duplex carotid ultrasound techniques, transcranial color-coded Doppler ultrasound, morphological features, haemodynamic, arterial stiffness, cognitive function, motor function, post-stroke

Publications, Clinical Trials and Conference Proceedings

Publications

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List of Abbreviations

AET	Aerobic exercise training
BDNF	Brain derived neurotrophic factor
BMI	Body mass index
BPd / DBP	Diastolic blood pressure
BPs / SBP	Systolic blood pressure
β	Beta
CAS	Carotid arterial stiffness
CBFV	Cerebrovascular blood flow
CC	Carotid Compliance
CEA	Carotid endarterectomy
CHP	Centre for Health Protection
CI	Confidence interval
CIMT	Carotid intima media thickness
CTA	Computed tomography angiography
cTCCD	Transcranial color-coded Doppler ultrasound (with angle correction)
CVD	Cerebrovascular disease
cVMR	Cerebral vasomotor reactivity
CVWV	Carotid vessel wall volume
DALYs	Death and disability adjusted life years
DC	Distensibility coefficient
DCCA	Distal common carotid artery
DCUS	Duplex carotid ultrasonography
DSA	Digital subtraction angiography
3D	Three dimensional
ECST	European carotid surgery trial
EDV	End diastolic velocity
FOV	Field of view
HR	Heart rate
HRR	Heart rate reserve
ICA	Internal carotid artery
ICD	International classification of diseases
ICH	intracerebral hemorrhage
ICS	Intracranial cerebral arteries stenosis
IRB	Institutional review board
kPa	Elastic Modulus
LIB	lumen intima boundary
LOA	Limits of agreement
MAB	Media adventitia boundary
MCA	Middle cerebral artery
MCID	Minimum clinically important difference
MD	Mean difference
MFV	Mean flow velocity

MoCA-HK	Montreal Cognitive Assessment Hong Kong version
MRA	Magnetic resonance angiography
NASCET	North American symptomatic carotid endarterectomy trial
ncTCCD	Transcranial color-coded Doppler ultrasound (without angle correction)
NO	Nitric oxide
PET	Positron emission tomography
PRF	Pulse repetitive frequency
PSV	Peak systolic velocity
PV	Plaque volume
PWV	Pulse wave velocity
PI	Pulsatility index
RCT	Randomised controlled trial
RI	Resistive Index
ROI	Region of interest
SAH	Subarachnoid haemorrhage
SCWT	Stroop color word test
SD	Standard deviation
SMD	Standardized mean differences
TAPV	Time averaged peak velocity
TCD	transcranial Doppler ultrasound
TCCD	Transcranial color-coded Doppler ultrasound
TGC	Time gain compensation
TIA	Transient ischemic attack
TTW	Trans-temporal window
TUG	Timed up and go test
6MWT	Six-minute Walk test
WASID	Warfarin-Aspirin symptomatic intracranial disease method
WHO	World Health Organization

Chapter 1

Introduction and Background

Stroke is a neurological medical condition defined by the World Health Organization (WHO) as the "rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin" (Coupland et al. 2017). It is reported to be the second leading cause of death and the third leading cause of mortality and disability combined worldwide (Feigin et al. 2021). In China, stroke is ranked the number three cause of death following malignant tumors and heart disease with a reported death rate of 149.5 per 100,000 and accounting for 1.57 million deaths in 2018 (Wang et al. 2020) whereas, in the local setup of Hong Kong, 6.2% of all registered deaths in 2020 were attributed to cerebrovascular disease (Centre for Health Protection, 2021).

Stroke occurs as a result of blood flow disturbance to the brain, due to either a blockage or rupture of the cerebral blood vessels feeding the brain, leading to brain death (Staessens et al. 2020; Bersano and Gatti 2023; Kuriakose and Xiao 2020). The middle cerebral arteries (MCAs) are accountable for as much as 80% of the cerebral blood flow (Nagata et al. 2016). Additionally, the majority of stenotic lesions are found to affect the MCAs (Kim 2019), making its interrogation of much interest in the wake up call to stroke. Based on the mechanisms of occurrence, stroke is broadly categorized into two types: ischemic and hemorrhagic, with ischemic stroke being the most prevalent (Kuriakose and Xiao 2020).

Due to its high mortality and morbidity rates, stroke therefore poses a serious public health and social-economic burden worldwide as alluded by (Rajsic et al. 2019; Kuriakose and Xiao 2020). Besides, the high prevalence of first time stroke, recurrence rates as high as 51.3% have also been

reported in studies summed up in a systematic review by (Kolmos, Christoffersen, and Kruuse 2021). To mitigate the challenges and burdens associated with stroke, there is a need to formulate holistic and robust stroke management approaches that address the multifaceted dimensions of stroke. The approaches should not only focus on addressing stroke prevention strategies but should more so provide standardized guidelines for its diagnosis and therapeutic recommendations at every stage of stroke.

The current guidelines for the primary prevention of stroke advocate the use of risk prediction equations, including the Pooled Cohort Equations for cardiovascular disease (CVD) or the Framingham Stroke Risk Profile for stroke (Chun et al. 2021). However, these models are based on traditional demographic and clinical risk factors such as age, sex, race, hypertension, diabetic status among others that may not fully capture the complexity of stroke aetiology (Khera et al. 2020). Moreover, the recent advancements in the medical imaging field, especially medical ultrasound imaging modality have seen the emerging of automated arterial analysis quantification programs capable of reliably and accurately assessing the cerebral arteries' morphological parameters such as the carotid stiffness indices (Pulse wave velocity (PWV), carotid compliance(CC) and distensibility coefficients(DC)) among others (Yuan et al. 2017; Li et al. 2017). Furthermore, semi-automated measurements of 3dimensional based carotid lumen volume % stenosis, carotid plaque volume and carotid vessel wall volume at ultrasonography is now a practical reality (Fresilli et al. 2022; Song et al. 2019; Johri et al. 2020). The structural and functional features of cerebral arteries identified through new technological applications could serve as potential stroke predictor biomarkers, enhancing the accuracy of current models or aiding in the development of more robust predictive models. To date, there are still paucity of studies that have assessed and established these new ultrasound-based parameters as independent predictor

variables of stroke. Ultrasound imaging provide a non-invasive, cost-effective, and readily accessible means to assess vascular health and detect subclinical atherosclerosis. In the clinical assessment of intracranial cerebral arteries haemodynamics, transcranial color-coded Doppler (TCCD) ultrasonography an advancement to the non-imaging transcranial Doppler Ultrasound (TCD) is also gaining much attention (Lovett and O'Brien 2022), although limited studies have assessed its interchangeability with non-imaging TCD and whether or not the technique of angle correction (cTCCD) provides accurate results compared to non-angle correction (ncTCCD). Furthermore, Hassan et al. (2024), decried imbalanced and missing data to be among the key challenges in the identification of stroke risk factors and in accurate stroke prediction. In chapter 3, Study One, was therefore aimed at comparing the non-imaging transcranial Doppler ultrasound (TCD) and Transcranial color-coded Doppler ultrasound (with (cTCCD) and without (ncTCCD)) angle correction in quantifying the MCAs haemodynamic parameters. This was then followed by a cross-sectional Study Two, presented in Chapter 4 that compared the cerebral arteries' morphological and haemodynamic features based on novel ultrasonography imaging applications between post stroke and non-stroke individuals in an attempt to ascertain whether the new biomarkers are independent stroke risk predictor variables.

Furthermore, apart from the above-mentioned preventative strategies to reduce the impact of stroke, efficient stroke treatment approaches are required (Kuriakose and Xiao 2020; Billinger et al. 2014). Since Ischemic stroke is a consequence of inadequate blood flow to the brain due to thromboembolic occlusion (Staessens et al. 2020), treatment approaches should thus target at improving the vascular status of both intracranial and extracranial cerebral arteries inorder to reduce stroke recurrence as alluded by (Ivey et al. 2011). Moreso, treatment should target at alleviating the stroke induced neurological damages in the various domains such as cognitive and

motor function, with an overall goal of improving survival and quality of life in post-stroke patients. Current treatment methods including, medical treatment methods that involve using drugs like aspirin and clopidogrel and endovascular treatments are not without their challenges, as they are limited to the acute phase of stroke (Markus et al. 2005; Cheng and Kim 2015), and moreover, they are invasive in nature with reported unsuccessful rates of up to 20% (Yoo and Andersson 2017). The need to interrogate alternative efficient, well tolerated, and non-invasive treatment strategies in post stroke patients is of growing interest.

Aerobic exercise training (AET) defined as "structured exercise programs that involve the rhythmical movement of large muscles for sustained periods"(Yugrakh 2021), is one such non pharmacological, therapeutic intervention that has the potential to restore the deconditioned hemodynamic, cognitive and motor function that characterizes the presence of stroke (Pang et al. 2013; Billinger et al. 2014; Latino and Tafuri 2024). Cycling AET is a common type of AET, with a potential application in post stroke patients due to its safety. However, in a recently conducted systematic review (Khan et al. 2024) decried the paucity of studies that have interrogated the potential effects of cycling AET on motor function in post stroke patients, hence the need for such studies cannot be overemphasised. The principal mechanism by which AET may improve cerebrovascular blood flow is through increased production of nitric oxide (NO), a known mediator of endothelial function (Szostak and Laurant 2011; Gambardella et al. 2020). NO is reported to have anti-atherogenic properties that may prevent plaque buildup in the blood vessels(Maiorana et al. 2003; Green et al. 2004), hence has the potential to promote positive cerebrovascular structural changes within the large intracranial and extracranial arteries in post stroke patients.

Despite, the available evidence demonstrating beneficial effects of AET on the systemic vascular system(Green and Smith 2018; Ivey et al. 2010; Billinger, Coughenour, et al. 2012; Desouza et al. 2000), only a few studies have focused on its long term effect on cerebrovascular haemodyamics and in particular among post stroke patients in the chronic phase with assessments mainly based on cerebral vaso motor reactivity (cVMR) (Ivey et al. 2011; Billinger et al. 2017). In general there is conflicting evidence on the impact of AET on cerebrovascular health with (Ivey et al. 2011; Billinger et al. 2017) reporting improved cVMR, whereas no improvement was observed by (Thomas et al. 2013). Furthermore, a recent study by (Reed et al. 2024) observed a paradoxical increase in the MCA pulsatility index (PI) despite improvements in central vasculature following AET. In contrast, (Braz et al. 2017) reported that cardiorespiratory fitness did not affect MCA mean flow velocity in non-stroke participants. Although, arterial stiffness is reported to have improved after exercise in recent studies, the focus was only in healthy non-stroke individuals, and assessment was done using traditional arterial stiffness index measures like the brachial–ankle pulse wave velocity (Huang et al. 2023). There is however limited information on the potential reduction in the atherosclerotic plaque size among chronic stroke patients undergoing AET, thus further interrogation is needed.

Duplex carotid ultrasound (DCUS) and transcranial Doppler ultrasound (TCD) are two non-invasive and non-ionizing techniques that can be used to assess the haemodynamic and morphological changes within the extracranial and intracranial cerebral arteries, respectively. As previously highlighted, in recent years these modalities have evolved with the emergence of new applications such as automated arterial analysis quantification programs and 3Dimensional arterial analysis capable of reliably and accurately assessing the cerebrovascular system (Fresilli et al. 2022; Song et al. 2019; Johri et al. 2020).

Furthermore, despite the available evidence demonstrating beneficial effects of exercises on neurocognition (Dishman et al. 2006), randomized trials have provided divergent findings with (Hoffman et al. 2008; Young et al. 2015) concluding that exercise does not confer clinically meaningful improvements in neurocognitive function. Additionally, the underlying mechanism resulting in the potential beneficial effects of exercises on cognitive function is still not clear, although several mechanisms are proposed. An increase in the expression of brain growth factors such as brain-derived neurotrophic factor (BDNF) associated with long term physical activity is believed to be a partial mediator of the enhancing effect of exercise on cognitive learning and memory (Dishman et al. 2006; Erickson et al. 2011) whereas (Liu et al. 2022) attributed the attenuation of immune inflammation to be the potential mechanism that exercises improved mental health. In a recent study (Reed et al. 2024) proposed the mechanisms to be vascular in origin that is linked to pulsatile cerebral blood flow. Moreover, although the mechanism through which exercise may influence vascular status is well established (Szostak and Laurant 2011; Bartel and Mosabbir 2021; Maiorana et al. 2003) there is still conflicting evidence on whether the changes in cerebral perfusion could explain the improvement in cognitive function.

Considering the above mentioned diversified and contradictory background, coupled with the emerging of advanced ultrasonography techniques, the need to further interrogate the possible value of AET in improving the deconditioned cerebral arteries' morphological and haemodynamic features as well as cognitive and motor function in post-stroke patients using robust study designs, incorporating evidence based prescription, whilst utilising a multi-parametric approach incorporating, novel DCUS techniques to assess the primary outcomes cannot be overemphasized. In Chapter 5, Study Three investigated the effects of cycling AET on the cerebral arteries' morphological and haemodynamic features as assessed by novel, multi-parametric Duplex Carotid

ultrasound (DCUS) techniques and transcranial color-coded Doppler (TCCD) ultrasound, and on the cognitive and motor functions in post-stroke chronic patients.

Chapter 2

Literature Review

2.1 Brief overview of the cerebrovascular system anatomy and physiology

The cerebrovascular system forms a part of the circulation system and is made up of large vessels connecting the aorta and terminating in the brain. It has two main portions, the intracranial and extracranial portions which can further be divided into the anterior and posterior circulation depending on whether the blood flow source is the internal carotid or vertebral arteries, respectively(Agarwal and Carare 2021). However, it is imperative to note that the middle cerebral artery (MCAs) a part of the anterior circulation, is responsible for as much as 80% of the cerebral blood flow (Nagata et al. 2016; Agarwal and Carare 2021).The bilateral internal carotid arteries arising from the common carotid artery and located within the carotid triangle of the neck region, feeds the MCAs and form the extracranial portion of cerebral arteries, and such knowledge of the anatomical arrangement is critical for the appropriate localisation of the cerebral arteries during medical procedures, such as carotid ultrasonography and other medical imaging techniques (Dungan and Heiserman 1996). Furthermore, at histological level, the arterial structural arrangement consists of two parts, the lumen and arterial wall, whereas the arterial wall is made up of three layers mainly: intima (smooth innermost layer), media (muscular middle layer) and the outer adventitia layer. The intima layer is composed of an elastic membrane and smooth endothelial lining that work together to provide a frictionless pathway hence allowing for efficient movement of blood through the vessels. The media layer on the contrary is primarily made up of thick smooth muscle cells and elastin that help to regulate blood flow and pressure through

alteration of the vessel lumen diameter, whereas the adventitia is the outer layer made of collagenous and elastic tissue whose main role is to attach the vessel to surrounding tissues (Tucker, Arora, and Mahajan 2024).

The cerebrovascular arterial system serves as the powerhouse responsible for supplying oxygen and nutrients rich blood to the brain tissue as well as draining deoxygenated blood from the brain (American Association of Neurological Surgeons, 2021), hence any compromise to the vessel lumen (in cases of stenosis) and arterial wall (in atherosclerosis disease) may results in diminished blood flow to the brain, and lead to brain death and the subsequent loss of neurological function. Maintenance of a healthy cerebrovascular architecture is thus critical to ensure normal brain perfusion and function, and a group of conditions affecting blood vessels and blood supply to the brain are broadly categorized as cerebrovascular disease (CVD) including stroke.

2.2 Stroke disease

Stroke has previously been classified as a circulatory system diseases, with symptoms lasting for at least 24hrs thus excluding, transient ischemic attack (TIA) from the stroke definition (Coupland et al. 2017). However, through various stakeholders engagements WHO in its latest ICD 11 reclassified stroke as a neurological diseases (Shakir 2018), and the stroke definition is now further expanded to include individuals with symptoms less than 24hrs previously treated as TIA, in the presence of confirmatory evidence of stroke on neuroimaging(Groff et al. 2024).

2.2.1 Epidemiology of stroke

Stroke poses a major public health and socio-economic burden worldwide due to its high morbidity and mortality rates(Feigin et al. 2021; Rajsic et al. 2019; Johnson et al. 2019). In Hong Kong, cerebrovascular disease is ranked the 4th commonest cause of death with 6.2% of all registered

deaths in 2020 attributed to cerebrovascular disease (Centre for Health Protection, 2021). A previous study by Zhou et al. (2016) reported an increasing trend in the number of provinces in China in which stroke was the number one cause of death and disability adjusted life years (DALYs). The provinces increased from 15 in 1990 to 27 in 2013. A recent systematic and meta-analysis study by Hu et al. (2020) that was aimed at establishing the trends in stroke incidence, prevalence, and accompanying deaths between 1980 and 2017 concluded that there has been an increase in stroke incidences since 2005. The crude death rates from stroke are reported to have been increasing with China deemed to have the highest increase (Wang et al. 2020). Ischemic stroke is reported to be the most prevalent subtype of stroke among the Caucasian populations accounting for approximately 80% of all strokes, whilst intracerebral hemorrhage (ICH) and subarachnoid haemorrhage (SAH) accounted for 10-15% and 5% respectively, and the rest is due to other causes. A study in Southwestern China similarly, reported high incidences of ischemic stroke accounting for 81.9% of the cases whilst haemorrhagic stroke accounted for only 18.1% among adults $\geq$ 40years of age (Yi et al. 2020). However, it should be noted that most of the reported incidence and prevalence rates are based on the previous ICD 10 classification of stroke where TIA was not considered as stroke as the symptoms lasted for less than 24 hrs. Although, a recent systematic review aimed at evaluating the impact of the ICD-11 change in stroke definition on incidence and outcomes reported a 13 % scan positivity rate for stroke in those previously diagnosed with TIA (Groff et al. 2024). Besides, first time stroke recurrence rates as high as 51.3% have been reported (Kolmos, Christoffersen, and Kruuse 2021) and the recurrent stroke was most frequent in large artery atherosclerosis (LAA) and cardioembolic (CE) stroke with recurrent stroke similar to index stroke subtype compared to small vessel occlusion (SVO).

2.2.2 Pathophysiology of stroke.

Stroke occurs primarily as a result of blood flow disturbances to the brain either due to an occlusion or due to rupture of the cerebral arteries responsible for supplying blood to the brain. Based on the mechanism of occurrence two main types of strokes exist, Ischemic and haemorrhagic stroke. In haemorrhage a susceptible blood vessel supplying the brain is ruptured and blood is pooled into the brain parenchymal in the case of intracranial haemorrhage or subarachnoid space. The bleeding results in increased intracranial pressure (ICP) which will have an overall negative effect on the cerebral blood flow (CBF). Haemorrhagic stroke is further classified into intracerebral, subarachnoid, subdural or intraventricular depending on the site of bleeding (American Association of Neurological Surgeons, 2021). It is when such disturbances occur that the neurological deficits and subsequent loss of quality of life experienced by the affected individuals are observed. Hypertension is the main risk factor of haemorrhagic stroke.

2.2.2.1 Causes of ischemic stroke

Ischemic stroke is reported to be the most prevalent subtype of stroke, accounting for more than 80% of all stroke cases (Yi et al. 2020), and occurs primarily due to a sudden interruption of blood flow to the brain resulting in loss of neurological function(Staessens et al. 2020).

The disturbance in blood supply in Ischemic stroke is deemed to be a primary consequence of stenosis or narrowing of either the intracranial or extracranial cerebral arteries or both, due to plaque build-up within the artery in atherosclerotic disease (Hu et al. 2017; Esposito et al. 2007; Heck and Jost 2021). Atherosclerosis is a chronic inflammatory disease caused by the deposition of modified lipoproteins, accumulation of immune cells, and formation of fibrous tissue within the

cerebral arteries vessel wall (Keeter et al. 2022) and is characterised by vascular wall hardening (arterial stiffness) (Saba et al. 2018). Carotid artery stenosis account for 10-20% of ischemic stroke (Brinjikji et al. 2016; Neira and Connolly 2022; Nicolaides et al. 2010; Flaherty et al. 2013) whilst in the Asian population intracranial cerebral artery stenosis (ICAS) is a key pathogenic factor accounting between 33% to 67% of stroke cases (Wang et al. 2014; Nguyen-Huynh et al. 2008).

However, notwithstanding the contributions of cerebral artery stenosis to ischemic stroke, recent evidence is pointing towards, vulnerable atherosclerosis plaque rupture as the main mechanism of ischemic stroke rather than haemodynamic failure following cerebral arterial stenosis (Heck and Jost 2021; Saba et al. 2018). Symptomatic carotid plaques have been associated with a 32% risk of suffering an ischemic stroke in comparison to about 2% risk attributable to asymptomatic carotid plaques (Sultan et al. 2022). The vulnerable atherosclerosis plaque represents a plaque that is susceptive to rupture hence is at a higher risk of predisposing the individual to complications such as arterial occlusion and/or distal embolism (Fresilli et al. 2022). Histological patterns such as a large lipid rich necrotic core, thin fibrous cap (portion of the plaque that faces the vascular lumen and maintains the integrity of the plaque)(Mughal et al. 2011), the presence of inflammatory infiltrate, intraplaque hemorrhage, ulcerations and intraplaque neovascularization are associated with the susceptibility of a plaque to rupture (Di Leo et al. 2018; Virmani et al. 2005; Rafailidis et al. 2020; Mughal et al. 2011) whereas carotid artery plaque volume, is equally indicated to be a critical determinant of the plaque vulnerability thus high chances of rupture (Saba et al. 2014). Plaque composition is therefore a key factor of plaque stability or vulnerability that requires monitoring.

2.2.2.2 Ischemic stroke prevention strategies

To mitigate the challenges and burden associated with ischemic stroke, preventative measures

pretext on the early identification of those at risk of stroke and advocating for healthy living to control the stroke risk factors have been suggested. Amongst the suggested healthy lifestyle changes that can be adopted are eating a healthy low fat, and high fibre diet, avoiding smoking and excessive alcohol consumption, and regular exercising. (Kalkonde et al. 2018). According to Li et al. (2019), the Chinese government has taken positive steps in mitigating the impact of stroke by investing in preventative measures. However, despite the presence of such measures, it cannot be argued that stroke occurrence and recurrence is inevitable, and this represents a serious global economic, social and public health challenge due to treatment and the care of post-stroke patients (Rajsic et al. 2019). Stroke survivors are at high risk for recurrent stroke with reported cumulative recurrence incidences of 5.4% and 11.3% at 1 year and 5 years respectively (Khanevski et al. 2019). The current guidelines for the primary prevention of stroke advocate the use of risk prediction equations, such as the Pooled Cohort Equations for cardiovascular disease (CVD) or the Framingham Stroke Risk Profile for stroke (Chun et al. 2021).

2.2.2.2.1 Limitations of current preventative strategies

The current models make use of traditional demographic and clinical risk factors such as age, sex, race, hypertension, diabetic status among others that may not fully capture the complexity of stroke aetiology (Khera et al. 2020). Moreover, medical imaging field has advanced, especially medical ultrasound imaging modality and this has seen the emerging of automated arterial analysis quantification programs capable of reliably and accurately assessing the cerebral arteries' structural and blood mechanics features (Yuan et al. 2017; Li et al. 2017). There is an urgent need to develop robust and efficient stroke prevention strategies that incorporate novel ultrasound techniques to prevent stroke recurrence and improve the survival and quality of life of post-stroke patients.

2.2.2.3 Ischemic stroke treatment and management

Since ischemic stroke is a consequence of inadequate blood flow and oxygen to the brain due to thromboembolic occlusion as alluded by Staessens et al. (2020), the treatment techniques should thus target to improve the health status of the cerebral arteries and restore the neurological function to achieve positive post-stroke outcomes. The medical treatment of carotid artery stenosis includes the use of anti-platelets drugs such as aspirin and clopidogrel with Markus et al. (2005) concluding that a combination of the two drugs provide better treatment outcomes. Carotid revascularization is reported to reduce the risk of ischemic stroke in patients with significant symptomatic stenosis (Park and Lee 2018). The endovascular surgeries targeting the restoration of normal blood flow in occluded vessels include carotid stenting, carotid endarterectomy (CEA) and trans-carotid artery revascularisation. These treatment method have been reported to benefit stroke patients and act as prophylaxis against stroke in International clinical trials such as the European carotid surgery trial (ECST) (Warlow 1993) and the North American symptomatic carotid endarterectomy Trial (NASCET) (Barnett et al.1991). However despite the clinical benefits of endovascular treatment methods, the methods are invasive and require strict eligibility selection criteria and procedural guidance from medical imaging modalities in order to achieve the best procedural safety and efficacy(Tarpley et al. 2013) and in some instances the coexistence of carotid artery stenosis and intracerebral aneurysm poses a therapeutic dilemma (Navaneethan et al. 2006). Therefore, the need to identify new therapeutic strategies in post stroke patients is of growing interest.

Aerobic exercise training in the form of cycling (AET) defined as “structured exercise programs that involve the rhythmical movement of large muscles for sustained periods”(Yugrakh 2021), is one such non-pharmacological, therapeutic intervention, designed to increase cardiorespiratory fitness, and has the potential to restore the lost function in the various domains such as the motor

and cognitive functions (Mkoba et al. 2021), as well as the deconditioned hemodynamic function in post stroke patients. Various cerebral arteries structural characteristics such as the degree of stenosis, plaque morphology, carotid intima media thickness and arterial stiffness in conjunction with the haemodynamic parameters can be used as indicators to assess the cerebrovascular health status and hence the progress of the AET.

2.3 Mechanisms through which AET may influence cerebrovascular health

The suggested principal mechanism by which AET may improve cerebrovascular health is through increased production of nitric oxide (NO). The mechanism of endothelial cell stimulation is triggered by the laminar shear stress activation that is experienced by the endothelial lining of the artery due to exercise (Szostak and Laurant 2011). The mechano-sensors proteins mainly the Syndecan-4 (Syn4), vascular endothelial growth factor (VEGF), and Krüppel-like Factor 2 (KLF2) in the endothelial cells then translate the physical force into a biochemical signal that stimulates the production of the potent vasodilator nitric oxide. NO is a known mediator of endothelial function with two major effects on the blood vessels which are the induction of endothelium-dependent vasodilation (Bartel and Mosabbir 2021; Gambardella et al. 2020) and the anti-atherogenic properties that prevent plaque buildup in the blood vessels (Maiorana et al. 2003). The anti-atherogenic phenomenon has the potential to promote positive cerebrovascular atherosclerotic changes within the large intracranial and extracranial arteries in ischemic stroke patients whilst the vasodilation effect may promote cerebral blood flow. In addition to the possible direct vasodilatation and anti-atherogenic effects of physical exercise on the cerebral arteries, improved cardiac contractility has been reported which in turn may have a positive indirect impact on the large cerebral arteries haemodynamics.

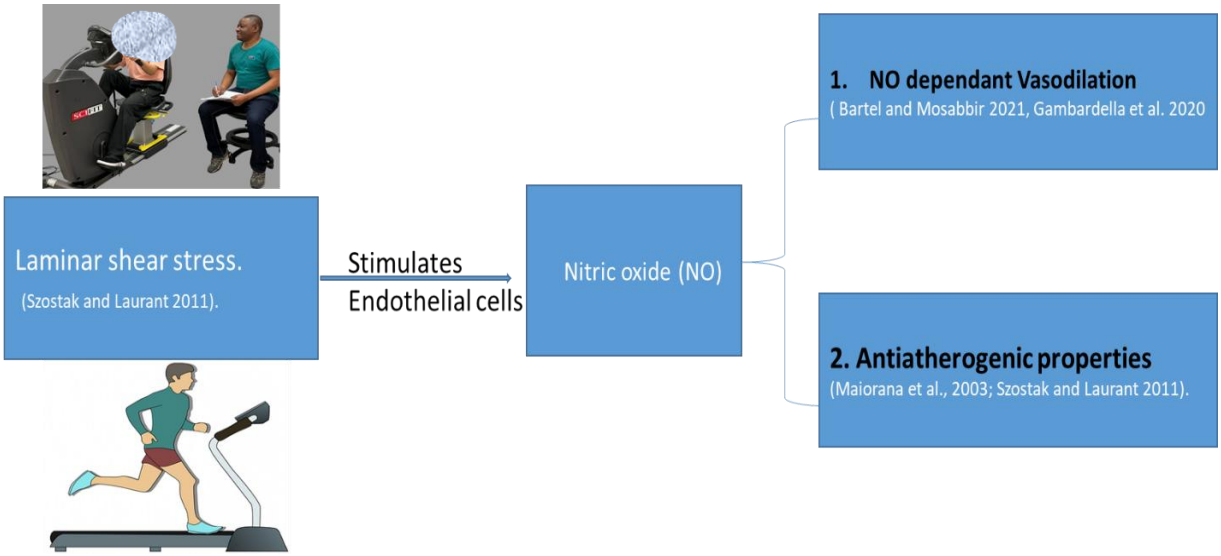


Figure 2.1: A schematic representation of the possible mechanisms through which AET may improve cerebrovascular system morphology & haemodynamic features.

2.3.1 Studies showing the effects of AET on the cerebrovascular system.

The beneficial effects of AET in systemic circulation and among the general population inclusive of stroke patients have been interrogated (Green and Smith 2018; Desouza et al. 2000; Billinger, Coughenour, et al. 2012; Ivey et al. 2010). Regular walking aerobic exercise was observed to "prevent the age-associated loss in endothelium-dependent vasodilation and restore levels in previously sedentary middle aged and older healthy men" (Desouza et al. 2000). Improved upper extremity brachial vasomotor reactivity of both arms was reported in subacute stroke patients following an 8 weeks recumbent stepper AET, prescription (3times per week, maximum duration=40mins, Intensity: moderate (50-59%) heart rate reserve (HRR) and high intensity (60-69%) HRR (Billinger, Mattlage, et al. 2012), although the study sample size was small consisting of only 10 participants. Similarly, Ivey et al. (2010) in a study that compared the effects of a 6-

month treadmill exercise in 53 chronic stroke patients with a control group undergoing stretching exercise, reported treadmill exercise to have a beneficial effect on peripheral haemodynamic functions in chronic stroke patients. Green et al. (2004) concluded that the degree to which physical exercise impacts vascular flow is dependent on the muscle mass under activation with generalized systemic changes observed following large leg muscle group activation whilst exercises involving forearm muscles resulted in only localized vascular changes.

However, despite evidence showing the beneficial effects of AET on systemic circulation limited studies have assessed the effects of AET on the cerebrovascular health system that has a direct contribution to the occurrence of Ischemic stroke, and in chronic post-stroke patients (> 6 months from stroke onset). The few studies on cerebrovascular health have reported contradictory findings. Ivey et al. (2011) observed significantly larger improvements for both the ipsilesional and contralesional cerebral vasomotor reactivity (cVMR) index ($P<0.05$) and contralesional cVMR percent ($P<0.01$) in the treadmill group when compared to the non-aerobic stretching exercise group. Contrarily, a 2 months rehabilitation program among 67 acute ischemic stroke subjects did not effect a change in both the ipsilateral and the contralateral mean flow velocity (MFV) of the middle cerebral artery (MCA) (Treger et al. 2010).

According to Szostak and Laurant (2011), exercise was reported to inhibit plaque development and induces the regression of coronary stenosis. Additionally, a modest but significant reduction of CIMT, from 0.5 ± 0.06 mm to 0.46 ± 0.10 mm (P = 0.04) was observed among healthy adults following 12 weeks of moderate intensity AET program (Glodzik et al. 2018) whereas a recent systematic review concluded that an exercise duration of >6 months was associated with a 0.02 mm reduction in CIMT in populations other than chronic post stroke patients (Wang et al. 2022). Contrary to these findings no change to the CIMT and CIMT/lumen ratio in response to walking

aerobic exercise intervention, among healthy sedentary participants was observed, in a (5.3 ± 0.3 days/wk, 42.3 ± 1.4 min/session, and at 73 ± 1% of maximum heart rate (H_{max}) exercise program (Tanaka et al. 2002). Similarly, a (3months, 2-3 times per week) indoor cycling in healthy premenopausal women did not yield a significant influence on the local intima-media thickness (Bjarnegård, Hedman, and Länne 2019). The effects of AET on vascular functional status as represented by arterial stiffness has been reported with decreased arterial stiffness observed in individuals with coronary artery diseases and haemodialysis after AET (Chen et al. 2017). In addition Bjarnegård, Hedman, and Länne (2019) study reported a significant improvement in the carotid artery distensibility in healthy middle aged women post a 3months, indoor cycling AET program($p<0.05$). In the chronic post stroke population, beneficial effects of a combination of various AET types including treadmill training (duration=2 hours per session, frequency=twice a week, overall duration= 3 months), on arterial function was reported (Takatori et al. 2012). Meanwhile, despite these findings, the potential reduction in the large intracranial and extracranial cerebral arteries morphological features such as atherosclerotic plaque and CIMT among chronic post stroke patients undergoing AET (cycling ergometry) still remains unclear hence it requires further interrogation.

2.3.2 Methods of assessing the cerebrovascular system's haemodynamic and morphological features.

Several imaging modalities can provide important information on large cerebral arteries' morphological and haemodynamic features. The imaging modalities include digital subtraction angiography (DSA), computed tomography angiography (CTA), magnetic resonance angiography (MRA), positron emission tomography (PET) and ultrasonography imaging (White and Nanapragasam 2018). Digital subtraction angiography (DSA) is the primary imaging modality for

the evaluation of cerebral arteries in the diagnosis of atherosclerotic stenosis (Wong and Wong 2010; Saba et al. 2018). However, this imaging technique is expensive, invasive and employs the use ionising radiation, thus rendering its clinical utility in treatment follow up monitoring unfavourable.

The clinical diagnostic utility of computed tomography angiography in the assessment of cerebrovascular disease is indisputable (White and Nanapragasam 2018; Nguyen-Huynh et al. 2008; Saba et al. 2018). Excellent diagnostic accuracy as high as 100% in both sensitivity and specificity, in the detection of large vessel occlusions have been reported, as well strong intra-class correlation of 0.98 (P0.001) between the DSA and CTA based degree of intracranial stenosis (ICAS) (Nguyen-Huynh et al. 2008). Furthermore, a recent study by Heck and Jost (2021), demonstrated that CTA was correlated to DSA in identifying stenosis. Despite the much benefits associated with CTA, its clinical use in the diagnosis and treatment follow up management of cerebrovascular disease is however hindered due to the associated high radiation doses delivered to the patients (Brenner and Hall 2007; Nickoloff and Alderson 2001). Additionally, the modality employs the use of contrast media hence there is risk of nephrotoxicity and other contrast media related risks. The diagnostic utility of CTA is reported to be further compromised in cases of heavy calcifications (Heck and Jost 2021).

Magnetic resonance angiography has long been demonstrated to be a useful imaging modality in atherosclerotic plaque characterisation, as it can distinguish the atherosclerotic plaque tissue components (lipid, fibrous, calcified, haemorrhage and thrombolytic components) in vivo(Takaya et al. 2005; Wasserman et al. 2005; Saba et al. 2018; Toussaint et al. 1996). The use of contrast-enhanced magnetic resonance imaging can further provide vital information regarding the plaque neovascularization. However, the main emerging limitation to the clinical utilisation of MRA is

the potential risk of side effects from the gadolinium-based contrast agent as studies have reported it to be associated with nephrogenic systemic fibrosis (Shamam and De Jesus 2022) hence its utility especially in stroke patients with severe nephropathy and for treatment monitoring follow ups is now under scrutiny. Additionally, MRA is a costly, not readily available imaging modality that uses strong magnetic fields, thus further limiting its clinical utility in the diagnosis, guiding and in follow up of treatment interventions involving patients with ferromagnetic implants such as cardiac pacemakers, bone fracture immobilisation rods.

Positron emission tomography using ^{18}F-fluorodeoxyglucose as a radiotracer is a modality that provides a non-invasive means to measure the biological processes linked to atherosclerosis disease in particular arterial inflammation (Joseph and Tawakol 2016). PET modality alone is limited in providing key anatomical information such as the degree of stenosis, an equally relevant parameter in stroke risk assessment and in guiding therapeutic interventions. However, novel techniques of image hybridisation have been developed where the molecular imaging modalities are combined with high spatial resolution modalities that can provide anatomical information, not to mention that there are still compatibility issues especially between the MRI magnetic field and PET components which are ferromagnetic. In addition, the commonly used radiotracer ^{18}FDG can accumulate in all cells that metabolise glucose thus posing a potential risk of overestimating the uptake in cases of high glucose metabolic backgrounds.

Ultrasonography is a noninvasive, non-ionizing, and an easily accessible imaging modality that enables the regular monitoring of haemodynamic and morphological features in both the intracranial and extracranial cerebral arteries without predisposing the patients to ionizing radiation. The cerebral arteries morphological features that can be interrogated using ultrasonography imaging may include but are not limited to the carotid artery stenosis, carotid

intima media thickness, and the carotid plaque vulnerability features whilst the haemodynamic features such as the mean flow velocity (MFV), peak systolic velocity (PSV), pulsatility index (PI) and resistive index (RI) of the cerebral arteries can be accurately measured at ultrasonography (Lee 2014). Duplex carotid ultrasound (DCUS) and transcranial Doppler (TCD) ultrasound techniques are the cornerstone techniques in the assessment of the extracranial and intracranial cerebral arteries haemodynamic and morphological features respectively (Naqvi et al. 2013b; Wang et al. 2021). Duplex carotid ultrasound imaging is a non-invasive, non-ionising, reliable and a readily available imaging technique that is based on the duplex ultrasound mode, a combination of two ultrasound modes the (grey scale and Doppler) modes. DCUS is capable of examining the blood flow mechanics and morphological features (luminal and vessel wall) of extracranial carotid arteries with high image resolution and without predisposing the patients to ionizing radiation (Choi 2021; Li et al. 2021). The ultrasound Doppler mode principles are underpinned on the doppler effect phenomena which states that a perceived frequency shift will occur when there is relative motion between an observer and a sound source, and the magnitude of the shift directly depends on velocity of reflector (moving red blood cells), transmitted frequency, and cosine of incident angle between ultrasound beam and blood flow direction, and is inversely proportional to speed of sound in the media as governed by the Doppler equation. Thus, the velocity of moving blood cells in cerebral arteries can be approximated given the other parameters.

Over the recent years novel imaging approaches to DCUS and TCD ultrasonography techniques that include, three dimensional (3D) vessel wall imaging (Pugliese et al. 2018, Johri et al. (2020)), semi-automated carotid arterial stiffness analysis, computer assisted image analysis (Li et al. 2021), and transcranial color-coded Doppler (TCCD) among others have emerged. These novel techniques have the potential to reliably and accurately provide a holistic assessment of the

changes that may occur in the cerebral arteries in post stroke patients undergoing cycling AET. Below is a description of the clinical utility of these techniques in assessing the various cerebral arteries morphological and haemodynamic features.

2.3.2.1 DCUS in carotid intima media thickness (CIMT) assessment.

CIMT a marker of vascular morphology is a well-established surrogate marker of subclinical atherosclerosis disease and is associated with increased risk for adverse cerebral events independent of other risk factors such as carotid plaques (Chambless et al. 2000; Rosvall et al. 2005; Bots et al. 1997). The advances in transducer technology has now enabled the performance of high resolution duplex Carotid ultrasound scans, and coupled with novel edge-detection algorithms the CIMT can now be automatically measured as the distance between the lumen intima boundary (LIB) and the media adventitia boundary (MAB) (Yuan et al. 2017; Wang et al. 2022) with high repeatability (Lau et al. 2012) in comparison to manual methods previously used.

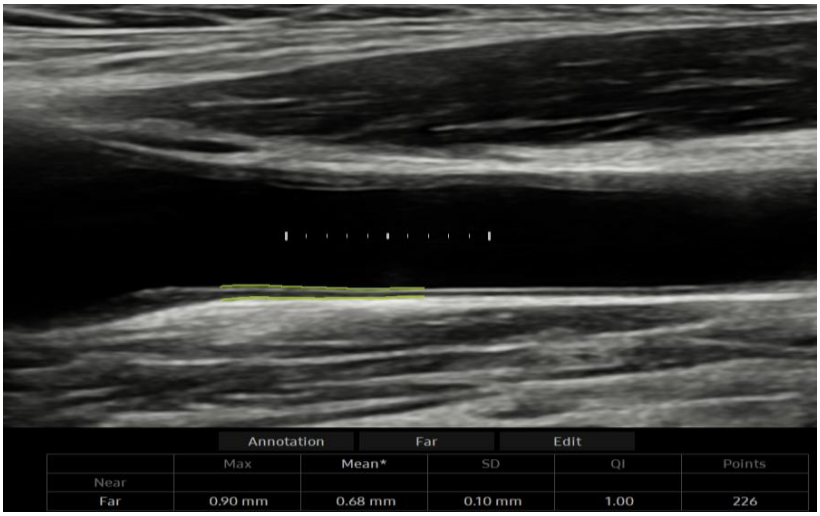


Figure 2.2: Image showing the measurements of CIMT at a 1 cm long segment ROI, starting at the inferior margins of the carotid bulb and in the far wall of the distal common carotid artery (DCCA). The mean CIMT for the segment is 0.68 ± 0.1mm

2.3.2.2 DCUS in carotid arterial stiffness assessment.

Arterial stiffness is defined as the decreased ability of an artery to contract and expand in response to pressure changes (Kim 2023; Chen et al. 2017), thus it represents the loss of arterial elasticity. This occurs mainly due to reduction in the elastin content and an increase in collagen, in the media layer leading to stiffening of the arteries (Kim 2023; Tsai and Hsu 2021). Moreover, arterial wall calcifications and endothelial dysfunction which results in decreased nitric oxide production and increased production of vasoconstrictive substances, contribute to increased vessel wall stiffness(Kim 2023; Lyle and Raaz 2017). Arterial stiffness is therefore an indicator of both arterial structural and functional alteration and is deemed a reliable surrogate marker of atherosclerosis disease(Chen et al. 2017) and an independent risk factor of cardiovascular events. Furthermore, arterial stiffness has been linked to being an independent risk factor of stroke. Changes in arterial stiffness can therefore be a useful outcome indicator of the therapeutic efficacy of aerobic exercise intervention in cerebrovascular disease patients.

Traditionally, the carotid-femoral pulse wave velocity assessed by Applanation tonometry (SphygmoCor) has been the common index of arterial stiffness(Tuttolomondo et al. 2010; Tsai and Hsu 2021). However, technological advancement in DCUS techniques now enable the detection of arterial stiffness, and novel indices apart from pulse wave velocity such as the Beta stiffness index, and carotid compliance can now be applied. Pulse wave velocity (PWV), is marker of the propagation speed at which the pressure wave propagates along the arteries, and is closely linked with artery elasticity, thickness, and radius (Li et al. 2017). The ability to provide physiological information give DCUS a leading edge over other imaging modalities in the assessment of carotid atherosclerosis (Heck and Jost 2021). However it should be noted that despite the emerging of these novel common carotid artery stiffness indices, there is still paucity

of information on the reference values across various populations and subject groups such as the adult population, and in particular post stroke patients as alluded by (Uejima et al. 2020; Tuttolomondo et al. 2010). In the local setup of Hong Kong limited studies have successfully evaluated the contemporary DCUS based arterial stiffness indices, in other populations apart from stroke patients(Yuan et al. 2017).

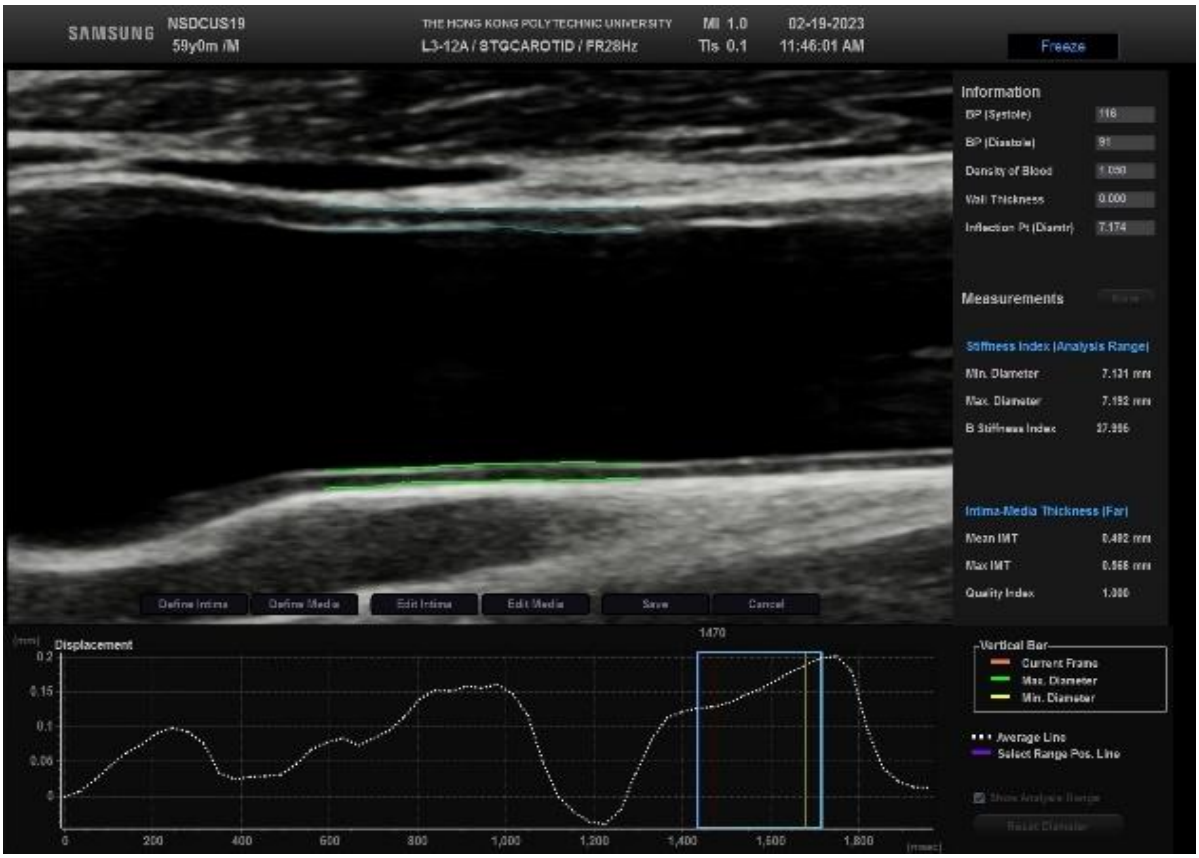
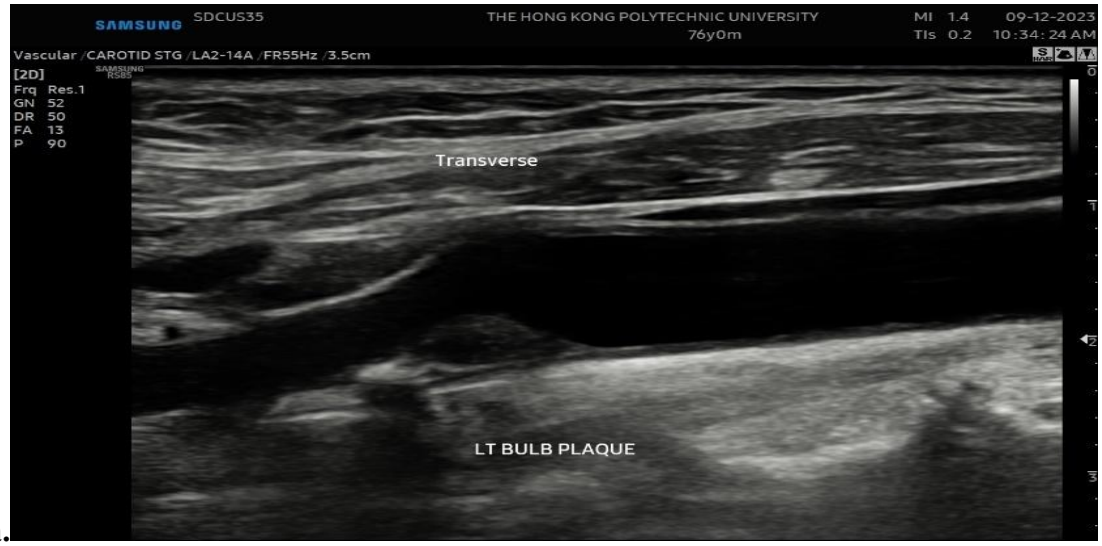


Figure 2.3: Image showing the measurements of Carotid arterial stiffness indices at a 1cm long segment ROI, starting at the inferior margins of the carotid bulb and in the far wall of the distal common carotid artery (DCCA).

2.3.2.3 DCUS in atherosclerotic plaque characterisation.

Atherosclerotic plaque defined as “a focal structure protruding into the arterial lumen by at least 0.5 mm or 50% of the surrounding carotid intima media thickness (CIMT), or having an CIMT of > 1.5 mm” (Choi 2021) plays a crucial role to the mechanism of ischemic (Heck and Jost 2021; Saba et al. 2018) hence its assessment is important. DCUS is able to determine a number of plaque characteristics such as plaque size, plaque surface, echogenicity, neovascularisation and the presence of intraplaque haemorrhage and calcifications or ulcerations). A study by Grønholdt (1999) observed that the DCUS based echo lucent plaques were associated with a higher risk of neurological events compared to the echogenic plaques. Similarly Nezu and Hosomi (2020), concluded that unstable plaques presenting with a hypoechoic echogenicity, ulceration and high mobility on DCUS are linked to ischemic stroke future events.

a.



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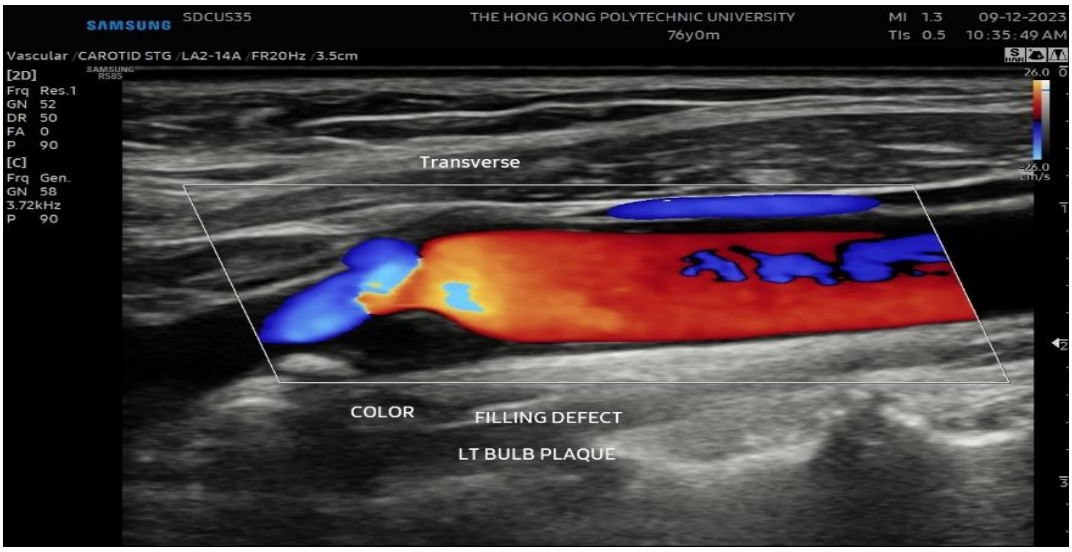


Figure 2.4: a.) Image showing the presence of a predominantly hypoechoic plaque in the carotid bulb encroaching into the ICA b.) corresponding color doppler image showing a filling defect.

2.3.2.4 Three-dimensional carotid ultrasonography (3DCUS) in atherosclerotic plaque

The recent technological developments in DCUS technology, now permit three dimensional (3D) volumetric characterization of the arterial wall anatomy with enhanced spatial resolution and assess stenosis over a vessel segment (Johri et al. 2020). 3DCUS is a technique introduced in the 1990s, targeted mainly at improving the accuracy of carotid artery disease diagnosis, and in

ensuring reproducibility of atherosclerotic plaques volumetric measurements in follow up scans (Delcker and Diener 1994). Although the technology of 3DCUS is not new as such, its clinical utility have remained low, despite early indications of promising diagnostic performance (Wessels et al. 2004), and this has been attributed mainly to the high transducer costs, time-consuming data reconstruction, and the requirement for dedicated laboratories that were associated with early 3DCUS techniques (Pugliese et al. 2018). In its infancy, the 3DCUS technique relied on the disk segmentation method, a cumbersome method involving mechanically moving the transducer along the patient's neck to acquire multiple 2D cross-sectional slices of the carotid artery at about 1 mm intervals. The plaque areas would then be summed up to calculate the plaque volume.

Recently there is a growing interest in the utilisation of 3DCUS necessitated by the advancements to the 3DCUS technology, that include the advent of a novel single sweep transducer technology and faster 3D data acquisition systems such as the S-3D Arterial Analysis available on Samsung RS80A ultrasound machine (Samsung Medison Co., Ltd., Republic of Korea). The advances in transducer design and fast data acquisition techniques have eliminated the requirement for dedicated laboratories and allowed for fast and accurate automatic quantification of the degree of carotid stenosis and plaque vulnerability (Fresilli et al. 2022). The 3D ultrasound technique have the advantages of enabling complete visualization of the plaque geometry and surface hence this provide a means for the differentiation between ulceration and gaps between contiguous plaques (Johri et al. 2020). In a study by Fresilli et al. (2022), 3D-Arterial Analysis (3DAA) was observed to be superior to carotid duplex ultrasonography (CDUS) and contrast enhanced ultrasonography (CEUS), with diagnostic accuracy values of (98.4%, 82.3%, and 94.5%) for 3DAA, CDUS, and CEUS respectively whilst using CTA as the reference standard. A study by Song et al. (2019), similarly concluded that 3DCUS using a single sweep technique was a feasible and accurate

method of detecting arterial plaques and assessing plaque volume when compared to CTA. Furthermore, to the validation of the diagnostic accuracy of 3DCUS in cerebrovascular disease, studies have validated 3DCUS to be a highly reliability technique in carotid stenosis assessment among stroke patients (Song et al. 2019; Pelz et al. 2015).

Due to its high reliability, the novel 3DCUS technique has found clinical application in the quantitative assessment of carotid plaque volume progression following statin therapy in stroke patients (Schminke et al. 2002). There is however limited evidence on the clinical utility of 3D carotid ultrasonography in following up post stroke patients undergoing aerobic exercise training (AET), despite the potential of 3DCUS to provide useful information on the volumetric stenosis burden, hence it is imperative for future research to interrogate its clinical applicability in post stroke subjects.

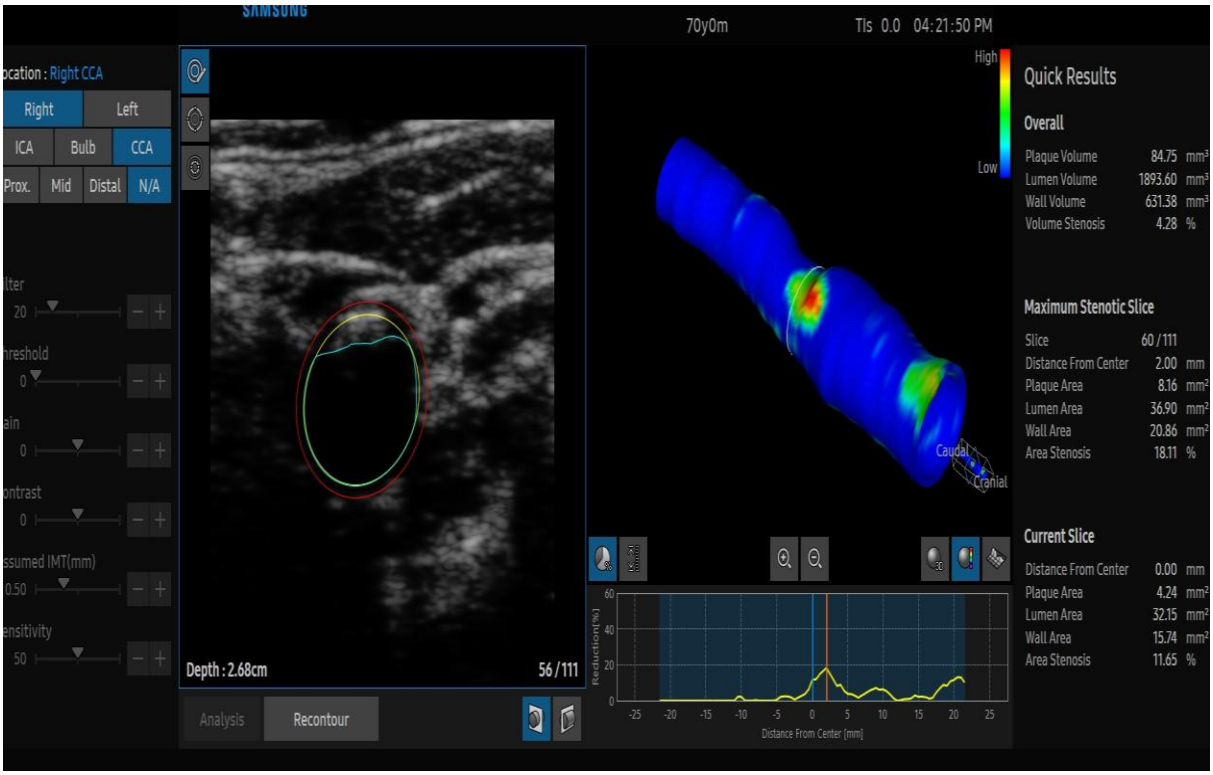


Figure 2.5: Image demonstrating 3d arterial analysis acquisition and output parameters

2.3.2.5 DCUS in Carotid arteries stenosis

Carotid artery stenosis, due to plaque build-up within the artery in atherosclerotic disease, represents the main parameter for ischemic stroke risk estimation (Esposito et al. 2007) and in addition the stenosis grade is key to inform clinical decision making about treatment such as the use of anticoagulant, or angioplasty (Naylor et al. 2018). It is recognised in the NASCET study that patients with symptomatic internal carotid artery (ICA) stenosis greater than 70%, benefit from surgical carotid endarterectomy (CEA) (Barnett et al. 1991), hence the effective detection and monitoring of intracranial atherosclerotic stenosis is critical.

Duplex carotid ultrasound (DCUS) has been reported to be a useful tool in the assessment of extracranial carotid arteries stenosis. In DCUS technique, carotid arterial stenosis is assessed based on two methods the flow velocity method and the diameter based method (Larsson and Rosfors 2021).

2.3.2.5.1 Flow velocity based carotid stenosis assessment method

This is the widely accepted preoperative method for grading carotid stenosis. In this method the degree of carotid artery stenosis is extrapolated from the internal carotid artery velocity values as recommended by the Society of Radiologists in Ultrasound (SRU) in 2003 (Grant et al. 2003). The reliability and accuracy of this DCUS flow velocity based carotid stenosis assessment method is reported (Grant et al. 2003; de Bray and Glatt 1995; Aburahma et al. 2011). The diagnostic utility of DCUS was additionally shown in a recent study by (Cui et al. 2017) who concluded that DCUS was a sensitive and specific imaging tool particularly in the diagnosis of severe carotid stenosis (sensitivity=100%; specificities=98.10%) although the accuracy levels were lower for mild stenosis compared to CE MRA. However, despite the reported diagnostic capabilities of DCUS,

there is an ongoing debate about the inter-method agreement between DCUS and the angiographic imaging modalities as alluded by (Pelz et al. 2020).

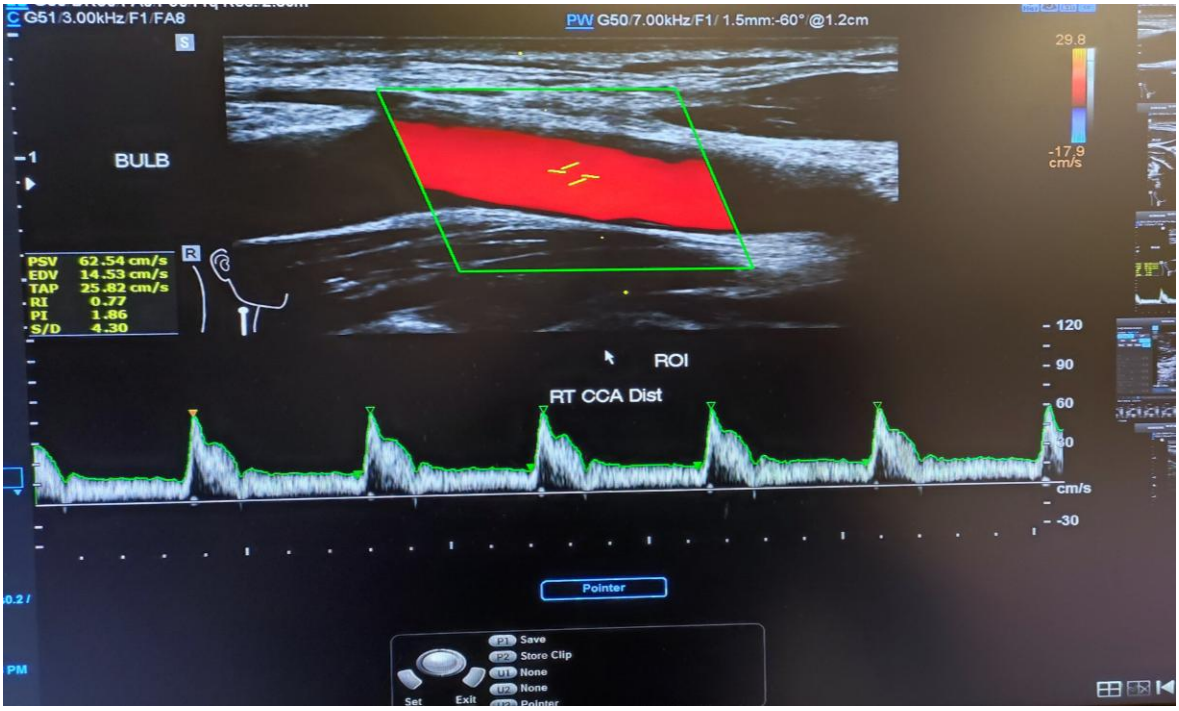


Figure 2.6: Image showing haemodynamic assessment of the Right distal CCCA. Adapted from: own images Hong Kong Polytechnic University ultrasound lab, 2022.

2.3.2.5.2 Diameter based method of assessing degree of carotid artery stenosis

In the diameter-based method the degree of vessel patency is assessed in B-mode by directly measuring the arterial luminal diameter at the narrowest portion in the carotid bulb and compare it with the maximum lumen diameter. The maximum lumen diameter is measured either at the same narrowest point (European carotid surgery Trial (ECST) carotid stenosis methods) or at a point distal to the narrowest point in the internal carotid artery where the vessel walls are parallel (North American symptomatic carotid endarterectomy trial (NASCET)) to determine the percentage (%) stenosis (Walker and Naylor 2006). The NASCET method is the commonly

utilised method of carotid stenosis grading. Although this method is not routinely used to inform clinical decision making with regards to selection of candidates for endarterectomy, a recent study by Larsson and Rosfors (2021) recommended its use to supplement the velocity-based grading method as it was observed to be performed with high reproducibility. The method is however limited in cases of severe calcifications where acoustic shadowing obstruct the visualisation of the lumen and vessel wall boundaries (Larsson and Rosfors 2021).

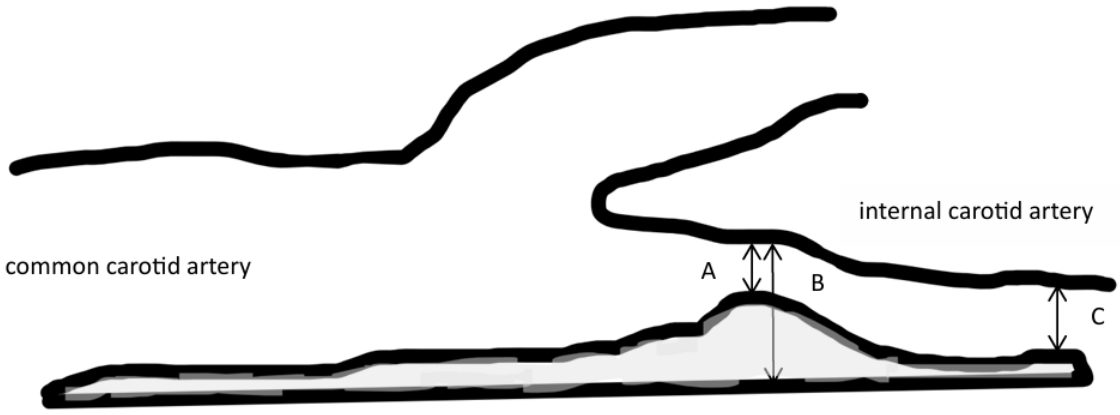


Figure 2.7: A schematic representation of North American symptomatic carotid endarterectomy trial (NASCET) and European carotid surgery Trial (ECST) carotid stenosis methods (own image).

NASCET= ((C-A)/C)*100 ECST= ((B-A)/B)*100

2.3.2.6 Transcranial Doppler (TCD) ultrasound in assessing intracranial cerebral arteries haemodynamic features

Transcranial Doppler (TCD) Ultrasound is a non-invasive, non-ionizing, portable, inexpensive, and safe technique that utilizes a low-frequency pulsed Doppler transducer(≤2 MHz) for the investigation of intracranial blood flow in the deeply located large intracranial arteries through the thin bone acoustic windows such as the trans temporal, suboccipital and the supraorbital windows (Sarkar et al. 2007; Naqvi et al. 2013b) that dates back to 1982 (Aaslid, Markwalder, and Nornes

1982). The middle cerebral artery (MCA) a terminal end of the intracranial carotid artery is the main intracranial cerebral artery evaluated on TCD as it is responsible for approximately 80% of the cerebral blood flow(Nagata et al. 2016). The intracranial blood flow parameters that can be examined with TCD ultrasound include the mean flow velocity (MFV), peak systolic velocity (PSV), pulsatility index (PI) and the resistive index (RI) among other parameters.

Clinically there are two TCD techniques 1.) the non-imaging TCD (commonly referred to as transcranial Doppler ultrasound (TCD)) and the imaging TCD (commonly known as transcranial color-coded Doppler ultrasound (TCCD) (Park et al. 2018). Despite the non-imaging TCD being a common ultrasound technique in assessing intracranial cerebral arteries haemodynamic, it is however limited due to the non-imaging nature of the technique as it cannot provide anatomical information to allow for the precise differentiation between individual vessels especially in the presence of anatomical variations (Bartels 2012). In TCD, the basal cerebral arteries are identified blindly based on indirect parameters such as the depth of the sample volume, transducer position, and the flow direction (Purkayastha and Sorond 2013).

TCCD on the other hand is an emerging technique that combines B-mode and color Doppler ultrasound imaging. This combination facilitates the precise identification of basal cerebral arteries through color-coding of the blood flow velocity on ultrasound image (Nedelmann et al. 2009), thus the ability to directly visualise the anatomic location of the blood vessels in relation to the brain stem structures in TCCD, gives it an advantage of correct identification of artery to be examined and accurate placement of Doppler sampling gate over the vessel for reliable measurement in comparison to non-imaging TCD. Furthermore, TCCD offers the window for angle correction, thus enabling a more accurate measurement of flow velocities whereas the assumption in TCD is that the insonation angle is less than 30 degrees (Nedelmann et al. 2009; Purkayastha and Sorond

2013). In addition TCCD has the potential to accurately determine the actual cerebral blood flow as the diameter of the blood vessel can be non-invasively measured, contrary to non-imaging TCD, where the mean blood velocity can only be a surrogate measure of cerebral blood flow, although limited studies have reported the cerebral diameters based on TCCD (Jarrett et al. 2020). Despite the technical inherent urge of TCCD over TCD, there is limited understanding of its application in intracranial cerebral artery assessment attributed mainly to its unavailability on most commercially available ultrasound machines, thus the majority of previous studies reporting on the ultrasonography evaluation of intracranial cerebral blood flow focussed on the non-imaging TCD (Purkayastha and Sorond 2013).

Recently, TCCD has increasingly become more availability on the ultrasound machines and thus gradually being adopted into clinical practice (Lovett and O'Brien 2022). The current trend in the acquisition of ultrasound machines capable of performing TCCD has not spared the study centre to which the non-imaging TCD system has been the sole TCD equipment available. Regardless of the surge in the utilisation of TCCD, limited studies have compared the agreement between TCD and TCCD (with or without) angle correction to justify for the possible inferences between the two techniques measurements. A study by Schöoning, Buchholz, and Walter (1993) reported a 10% to 15% difference in the peak systolic velocity (PSV) and time-averaged maximum velocity (TAP) between TCD and TCCD angle corrected measurements. Park et al. (2018) concluded that PSV can be measured more accurately by TCCD with angle correction (cTCCD) whilst Tsuchiya et al. (1991) echoed similar sentiments that cTCCD provides more reliable mean flow velocity (MFV) of the middle cerebral arteries (MCAs) in comparison to TCCD without angle correction (ncTCCD). The studies comparing TCD and TCCD limited the region of interest to where the blood flow velocity was interrogated to predetermined fixed depths informed by previous literature

from non-imaging TCD. Notwithstanding that TCCD enables the anatomic visualisation of the color-coded MCA, allowing the blood flow parameters to be assessed at a specific anatomical reference point with respect to the point of origin of the MCA for each individual subject such as the proximal portion, to date no study has provided such reference-based blood flow measurements. It is therefore imperative to assess the inter-method agreement between the non-imaging TCD and TCCD in assessing the intracranial cerebral arteries haemodynamic and ascertain whether the two available devices are interchangeable. It is important to note that in some instances the prevalence of inadequate acoustic windows is common thus limiting the use of TCD in the assessment of cerebral arteries haemodynamics (Seidel, Kaps, and Gerriets 1995).

The diagnostic accuracy of TCCD technique in stratifying intracranial cerebral arteries stenosis was reported in a systematic review and meta-analysis that the PhD student conducted. The systematic review and meta-analysis provided evidence that TCCD imaging technique exhibits high diagnostic performance in the stratification of intracranial steno-occlusions among patients presenting with CVD, when compared to DSA as a reference standard (sensitivity=90%, specificity=86%, AUC=0.96, and DOR=120.7(60.5-240.8)). TCCD thus has the potential to be used in stratifying ICAS in CVD patients, and could be considered in clinical cases where DSA is limited or contraindicated to patients (Gunda, Yip, et al. 2024). Furthermore, both TCCD diagnostic parameters (PSV and MFV) were observed to yield high and comparable diagnostic performance with similar AUC (96%), hence the two parameters were considered useful in stratifying ICAS among CVD patients. The results of the diagnostic accuracy of TCCD when compared to DSA as reference standard are shown in figure 2.8 (a-d), whilst the summary results of other subgroup analysis are provided in Table 2.1.

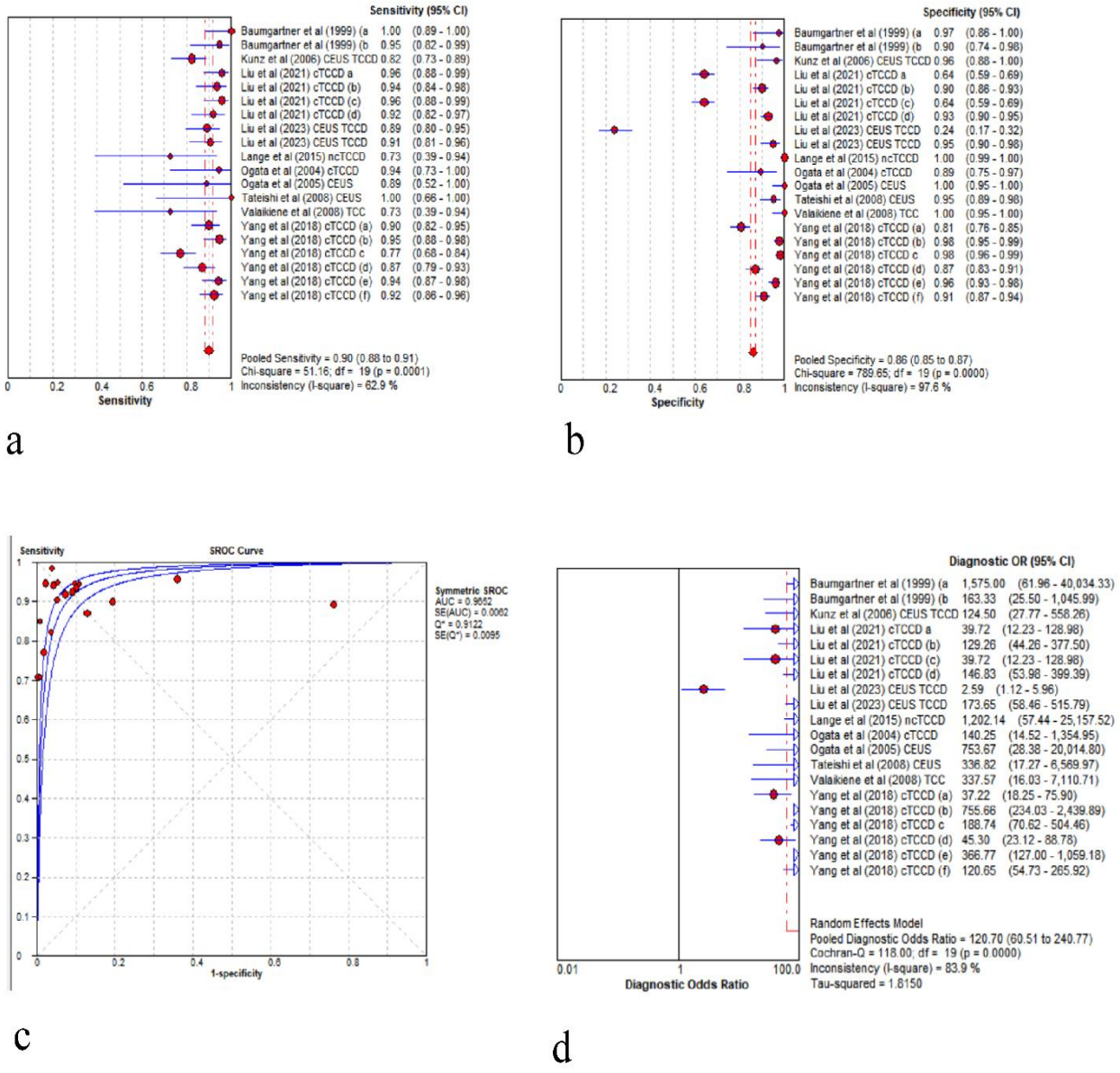
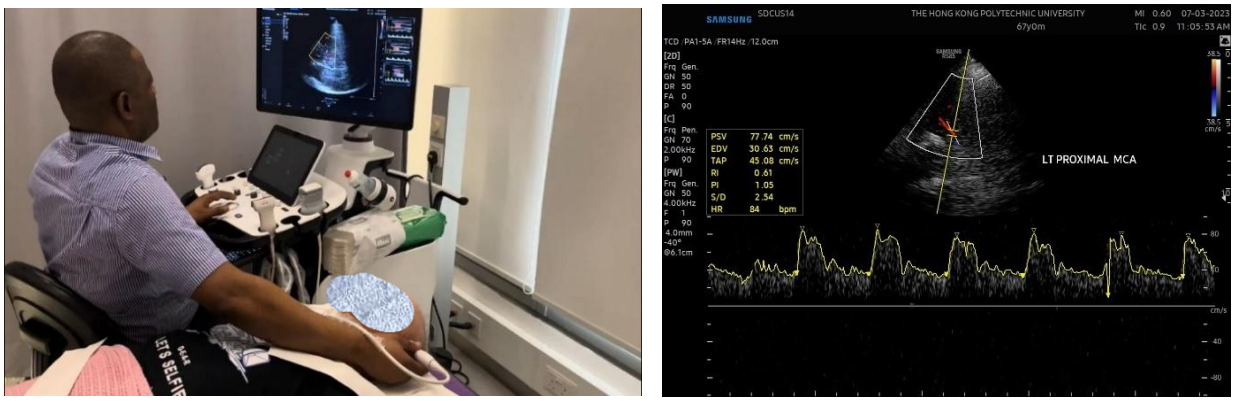


Figure 2.8: Diagnostic accuracy indicators of TCCD technique when compared to only DSA as the reference standard. (a) sensitivity, (b) specificity, (c) AUC, (d) DOR. The position of the red circles corresponds to the diagnostic accuracy indicator value for each individual study, whilst the position of the red diamond shaped box represents the pooled diagnostic accuracy indicator value

Table 2.1: Summary of pooled diagnostic performance of TCCD in stratifying ICAs according to various categories.

Category	Sensitivity (%) —95% CI	Specificity (%) —95% CI	AUC	DOR—95% CI
(All angiographies—DSA, MRA, CTA) as reference standards	83 (81–85)	87 (86–88)	0.96	98 (56–169)
DSA alone as reference standard	90 (88–91)	86 (85–87)	0.97	121 (61–169)
Stratifying stenosis ≥50% to near occlusion (all angiographies)	91 (89–93)	88 (87–89)	0.97	148 (84–262)
Total occlusions	92 (84–97)	98 (96–99)	0.98	148 (84–262)
PSV as diagnostic parameter	85 (82–87)	85 (84–87)	0.96	106 (39–288)
MFV as diagnostic parameter	84 (81–87)	87 (85–88)	0.96	79 (39–157)
non-contrast TCCD	82 (80–84)	88 (87–89)	0.98	94 (56–160)
contrast-enhanced TCCD	87 (83–91)	80 (76–83%)	0.95	87 (13–584)

DSA—digital subtraction angiography, CTA—computed tomography angiography, MRA—magnetic resonance angiography; PSV—peak systolic velocity; MFV—mean flow velocity, TCCD—transcranial color-coded Doppler ultrasound technique.



a. **b.**

Figure 2.9: (a) Image showing the patient positioning and probe orientation in performing TCCD and (b) subsequent haemodynamic parameters. Adapted from self.

2.4 Mechanism through which AET improves cognitive and motor function in post stroke patients

Recent studies in neuroscience have linked physical activity and exercise to have beneficial effects on cognitive and neuroprotective function ((Latino and Tafuri 2024; Erickson et al. 2011). Several possibly mechanisms to explain this relationship have been proposed. The brain's ability to sustain neuronal metabolism is reported to rely mainly on the cardiovascular supply of cerebral blood flow (CBF) (Tarumi and Zhang 2018). Cerebral hypoperfusion in unilateral carotid artery stenosis is reported to be associated with increased amyloid-β plaque accumulation which in turn results in neuronal dysfunction and loss of cognition function in stroke (Huang, Lin, et al. 2012) whereas upper-stream changes in cardiovascular function have been suggested to also influence CBF (Zhu et al. 2015). This implies that stroke could occur as a result of upper stream cardiovascular impairment, and this may be associated with the disturbance of neuronal homeostasis leading to loss of cognitive and sensory motor functions after stroke. Therefore, improvement in cognitive function requires a mechanism that improves cerebral perfusion and hence reduces amyloid accumulation and sustaining neuronal metabolism. It is against this background that aerobic

exercise training, an intervention that is designed to improve cardiorespiratory health, is hypothesized to have potential beneficial effects on the cognitive and motor functions in chronic stroke patients. Hence there is a need to study the effects of AET on the intracranial and extracranial haemodynamic and morphological features and its relationship to the cognitive processes in stroke patients.

Other possible mechanisms include the possible modulation of the human brain structure and function, through increases in the expression of brain growth factors such as brain-derived neurotrophic factor (BDNF) after exercise training (Dishman et al. 2006; Latino and Tafuri 2024). Aerobic exercise training was observed to increase the size of the hippocampus volume, leading to improvements in spatial memory.

2.4.1 Studies on the effects of aerobic exercise on cognitive and motor function

Post-stroke cognitive impairment (PSCI) is reported to be an underrated worldwide problem in stroke patients that may eventually lead to post-stroke dementia (PSD) (Kosgallana et al. 2019). Subclinical neurocognitive deficits have been linked to increased risk of dementia and mortality among the elderly population by Bassuk, Wypij, and Berkman (2000) whereas physical activity has been reported to be associated with reduced risks of cognitive impairment, Alzheimer disease and dementia of any type among the elderly (Laurin et al. 2001). There is overwhelming scientific evidence in observational studies demonstrating the beneficial effects of exercise on improving physical fitness and health. Exercise and physical activity have been associated with a decrease in the risk of developing cardiovascular disease, stroke, type 2 diabetes, colon and breast cancers (Garber et al. 2011). Lautenschlager et al. (2008) reported modest improvement in cognition among adults with subjective memory impairment post exercise. The outcome measures in this study was assessed using the Cognitive Subscale (ADAS-Cog) scores after undertaking a 6-month

physical activity program whilst Okumiya et al. (1996) reported similar positive benefits of exercise in improving neurobehavioral function, in adults greater than 75 years old. Smith et al. (2010) meta-analytic review of 29 randomized controlled trials which was aimed at determining the effects of aerobic exercise interventions on neurocognitive function also concurred with previous studies indicating the potential benefits of aerobic exercise training on cognitive function. The meta-analysis concluded that AET improved the attention and processing speed, executive function and memory cognitive domains as compared to the control non-aerobic groups. Studies that focused on adults greater than or equal to 18 years of age with a treatment duration greater than one month were included in the meta-analysis however none of the 29 reviewed studies assessed the effects of AET on cognitive function among post stroke patients. According to another systematic review by Cox et al. (2016), only limited evidences have shown physical activities to positively influence cognitive function in young to middle-aged adults, with a recommendation for further research in these subgroups. The cognitive function was assessed by validated platforms across the three cognitive domains: executive function, memory and processing speed. There are several other studies that have reported the same findings of improvement in neurocognitive functions following exercise training including studies by Kramer and Erickson (2007) and Cumming et al. (2012).

It is however important to note that despite the available evidence demonstrating beneficial effects of exercises on neurocognition, randomized trials have provided divergent findings with (Hoffman et al. 2008) concluding that exercise does not confer clinically meaningful improvements in neurocognitive function among clinically depressed adults. Hoffman et al. (2008) study involved a 4months treatment period with the various domains of cognitive function assessed. The executive function was assessed using Trail Making Test A and B, Stroop Color/Word, Ruff 2 & 7 Test,

Digit Symbol) before and after the treatment whereas, Verbal Memory was assessed with Logical Memory and Verbal Paired Associates), and the assessment of Working Memory was achieved using the animal naming and the controlled oral word association test. Similarly, Young et al. (2015) concluded that aerobic exercise have no cognitive benefit in healthy older adults when compared to a no intervention group and even in subgroups that had reported cardiorespiratory benefits from the aerobic exercise training. The subjects in this review were those who did not have cognitive impairment and aged above 55years. Despite these findings, Young et al. (2015) however did not rule out the possibility of achieving beneficial cognitive outcomes in different subgroups that were excluded in his review study. The subgroups that were excluded in his study included those with mild cognitive impairment (MCI) and subjects with other conditions that have the potential to be associated with cognitive impairment, such as stroke and depression. The primary outcome measurement was the cognitive function with the measures grouped into seven categories including attention, memory, perception, executive functions, cognitive inhibition, and cognitive speed and motor function. The motor function was also reported in two of the reviewed studies with the finger tapping and pursuit rotor task being the two tests undertaken to assess the motor function. Pang et al. (2013) in a systematic review and meta-analysis decried the presence of only a few studies relating to the efficacy of aerobic exercise in improving health outcomes in the physical, psychosocial and cognitive domains among chronic stroke patients. On the other hand Young et al. (2015) decried the lack of standardisation of the cognitive tests, and advocated for the design of smaller core set of cognitive tests that incorporate measures of key cognitive domains.

Literature has shown that the majority of stroke survivors eventually regain the ability to walk without continuous physical assistance, however only a handful of these post stroke survivors are

able to achieve adequate walking speed and endurance to resume independent living and normal community participation. Mayo et al. (2002) reported that approximately 50% of stroke survivors lived with the consequences of stroke and would require home assistance whilst Hill et al. (1997) similarly noted that only 7% of post stroke patients were able to regain their full dynamic balance and mobility with the rest of the patients having to leave with the stroke related limitations. This incapacitation has been attributed to impaired motor control (Flansbjer, Downham, and Lexell 2006). Post-stroke muscle weakness is reported to be a major contributor to post stroke mobility activity limitations with studies having demonstrated a significant association between muscle weakness after stroke and measures of gait performance. The need for a comprehensive rehabilitation prescription to mitigate the challenges brought by stroke with respect to the motor function is thus undebated.

The effects of AET on motor function in post stroke patients have been studied in an attempt to interrogate the extent to which this rehabilitation training improves the motor functional outcomes in post stroke patients. Significant improvements in walking capacity was reported in chronic stroke patients following a 3times per week, 8 week duration aerobic cycling intervention that was prescribed at 60% to 80% of heart rate reserve (Linder et al. 2021). The main outcome measure in this study was the change in walking capacity as measured by the six minute walk test 6MWT from baseline to the end of treatment. Boyne et al. (2017) study aimed at investigating the influence of dosing parameters and patient characteristics on the efficacy of AET in poststroke demonstrated larger effect sizes for walking speed and the 6-minute walk test in those patients undertaking the walking AET mode compared to those who undertook the seated AET mode. The study concluded that high-intensity aerobic exercise training improves locomotor function although future randomized studies to validate these findings were recommended and the need for task specific

AET to affect walking speed and endurance. Similarly, Madhavan, (2019) reported a 19% increase in walking speed and a 12 % increase in walking endurance as assessed by the 10m walk test and 6minutes walk test respectively however the study was limited to only 16 chronic stroke patients and did not have a control group.

Although, AET modalities have been observed to positively enhance the motor function in post stroke patients (Khan et al. 2024) in a recently conducted systematic review decried the presence of only a few studies to have specifically assessed the effects of cycling AET on the motor function which is a safer and more tolerated AET modality hence the current study aimed at assessing the effects of cycling AET on motor function.

2.4.2 Methods of assessing the post stroke cognitive and motor functions.

Despite the need for uniform post stroke cognitive and motor impairment quantification, Kosgallana et al. (2019) decried that no consensus on the preferred assessment tools has been reached yet. The complexity of the cognitive assessment tools ranges from the single domain tests, for example the verbal fluency test and multi-domain assessments (Montreal Cognitive Assessment (MoCA), to the comprehensive neuropsychological batteries. The 30 point Montreal Cognitive Assessment (MoCA) and the 30 point Mini Mental State Examination(MMSE) were both validated for post stroke global cognitive impairment screening with their scores evaluated against a diagnosis of cognitive impairment derived from a comprehensive neuropsychological battery (the criterion standard) (Cumming et al. 2013). However, the MoCA was developed in a bid to improve upon the MMSE through the assessment of additional cognitive domains, including executive functioning, visuospatial, immediate and delayed memory hence it offers additional assessment benefits compared to the MMSE. The MoCA tool is freely available to any clinician and has been translated into a number of languages including a Montreal Cognitive Assessment

Hong Kong version (MoCA-HK). Besides the Global cognitive assessment tools there exist tools that can be employed to test the individual cognitive functions like attention, executive functions, working memory and memory. These tools include but are not limited to trail making test and Stroop interference. The performance-based trail making test (TMT) is divided into TMT A and TMT B that can measure attention or processing speed and the executive function respectively (Smith et al. 2010).

The functional independence measure (FIM) is an 18-item scoring tool that measures both motor (13 items) and cognitive (5 items) independence on a 7-level ordinal scale (Linacre et al. 1994). According to Treger et al. (2010), the FIM "measures the amount of assistance needed in self-care, sphincter control, transfers, locomotion, communication, and social cognition", and hence this tool is suitable for the assessment of rehabilitation outcomes. The FIM total score is the sum of each individual item's score with each item scored on an ordinal scale of 1 to7 implying that the score ranges from 18 to 126 with higher scores reflecting increased independence in the performance of given tasks. The motor assessment scale (MAS) is another mobility measurement scale whereas the modified Rankin score was confirmed to be the most frequently used functional outcome measure in stroke trials and is mostly administered in 90-day post stroke patients (Quinn et al. 2009). The current study is however focused on chronic stroke patients who are 6 months and above post stroke. Despite these various scales ability to provide a general measure of mobility Low Choy et al. (2002) decried that most of these scales do not include many of the motor tasks retrained by physiotherapists hence the need to use other specific tools that allow for the setting out of targets that can be followed up to assess the progress of the motor tasks during rehabilitation. The six-minute walk test (6MWT) can provide information on endurance testing whilst the Timed up and go test can provide information on gait speed. The TUG test is a reliable, cost-effective,

safe, and time-efficient way to evaluate overall functional mobility (Kear, Guck, and McGaha 2017). This assessment tool according to Gladstone, Danells, and Black (2002) is the first quantitative evaluative instrument for measuring sensorimotor stroke recovery in the hemiplegic stroke patient and is viewed to be "a well-designed, feasible and efficient clinical examination method that has been tested widely in the stroke population".

In summary the effects of aerobic exercise training on neuro-cognition and motor function have been studied in various subgroups. The studies assessing the impact of AET on neurocognitive and motor function have reported diversified results ranging from varied magnitude of improvements in cognitive and motor function to no benefit. Beneficial variations among different subgroups were also reported (Cox et al. 2016). There are only a few of these studies that focused on post stroke patients (Pang et al. 2013). Some of the studies seeking to investigate the effects of AET on the cognitive and motor functions are reported to have used suboptimal assessment tools and exercise prescriptions although there is disagreement among the studies on whether the intensity of the exercise prescription have an impact on the outcomes. Angevaren et al. (2007) concluded that the average intensity of weekly physical activities and not the duration is positively and significantly associated with the overall cognitive function whereas no such association between intensity of physical activity and change in cognitive function was observed by Smith et al. (2010). Considering the above mentioned diversified background information on the findings pertaining to the effects of exercise on neuro-cognition and motor function and the contradictory results on the impact of the exercise prescription on the cognitive function outcome it is therefore imperative to assess the cognitive and motor functional changes in post-stroke patients undergoing AET using an evidence based AET prescription in stroke as informed by (Pang et al. 2013).

2.5 Correlation between DCUS and TCCD ultrasound-based features and post-stroke functional outcomes

Understanding the possible cerebral arteries' morphological and haemodynamic changes underlying the post stroke cognitive and motor functional outcomes is critical as such knowledge of the relationship has the potential to provide critical information to the physicians, therapists, patients and the family on the possible prognostic value of interventional modality. The assessment of such a relationship using DCUS and TCCD based ultrasound features has been interrogated in post-stroke patients mainly in the acute and subacute stages of stroke.Treger et al. (2010) demonstrated a significant association between MFV in the contralateral MCA and the functional and neurologic impairment change as measured by the FIM score indices (final FIM score, change in FIM score and the relative change in FIM score) in acute stroke patients. Furthermore studies have shown that other ultrasound parameters such as the MCA asymmetric index as assessed during the acute stages of stroke could predict functional and neurologic outcomes (Han et al. 2019). According to Carmo et al. (2021) a moderate correlation was observed between post stroke hemodynamic and respiratory responses during a 30 minute treadmill aerobic exercise although the haemodynamic parameters correlated to the motor and cognitive functions were not ultrasound based included training heart rate, arterial blood pressure, oxygen pulse saturation, heart rate, and respiratory rate.

Despite the possible complimentary benefits to current clinical methods in the prediction of post-stroke cognitive and motor functional outcomes the evaluation of such associations in chronic stroke patients who are undergoing AET using a multi-parametric DCUS and TCD approach is understudied.

2.6 Basis of the Study

Aerobic exercise training has been established to have some beneficial effects in enhancing cardiovascular health in diversified, non-neurological human population groups (Ivey et al. 2011), and has the potential to restore the deconditioned hemodynamic function in post-stroke patients. However, despite such notion, there are limited studies that have evaluated the effects of AET on the cerebral vasculature in chronic post stroke patients. The few studies have reported contradictory findings in which (Ivey et al. 2011; Billinger, Mattlage, et al. 2012) observed positive benefits, whereas Treger et al. (2010) and (Steventon et al. 2018) did not observe changes in the MCA MFV following exercise rehabilitation training program. The scope of the hemodynamic assessments in these studies have been limited to the MCA mean flow velocity as assessed by the non-imaging transcranial Doppler Ultrasound (TCD) notwithstanding the advancement in the TCD technique that have seen the emerging of TCCD. Although it cannot be argued that the MCA is responsible for 80% of the cerebral blood flow (Nagata et al. 2016), and the MCA MFV can reflect cerebral arterial perfusion, other parameters such as the Peak systolic velocity (PSV), Resistive index (RI), Pulsatility index (PI), could provide additional and diversified important information for comprehensive assessment of cerebrovascular resistance and intracranial compliance (Han et al. 2019). In addition the assessment of the response of the extracranial cerebral arteries to AET is equally important as previous studies have reported that stroke patients who suffered from severe carotid artery stenosis are predicted to have higher 5-year mortality rates than the others (Muscari et al. 2016; Huang et al. 2018).

Physical exercise has been linked to NO production according to Szostak and Laurant (2011), and the NO is reported to have anti-atherogenic properties that prevents plaque formation in blood vessels (Maiorana et al. 2003). Despite this notion suggesting the potential positive impact of

physical exercise on atherosclerotic plaque formation, in preclinical studies (Wu et al. 2018) there is paucity of information on the possible reduction in the carotid atherosclerotic plaque in post-stroke patients undergoing AET hence a detailed investigation is needed since Ischemic stroke is deemed to be primarily a consequence of carotid artery stenosis due to atherosclerotic plaque build-up as well as vulnerable plaque rupture (Hu et al. 2017; El-Barghouty et al. 1995; Esposito et al. 2007; Heck and Jost 2021).

Similarly the effects of AET on the neuro-cognition and motor function have been studied in various subgroups, with however limited studies focusing on post stroke patients (Pang et al. 2013). Diversified results ranging from varied magnitude of improvements in cognitive and motor function to no benefit have been reported with studies observed to have used suboptimal assessment tools and exercise prescriptions although there is disagreement among the studies on whether the intensity of the exercise prescription has an impact on the outcomes. Additionally, the association between the cerebrovascular system haemodynamic and morphological changes and the cognitive and motor functional changes following AET in post-stroke chronic patients has been understudied. This is despite the potential clinical value of establishing such associations as they can be complimentary to current clinical methods in the prediction of post-stroke cognitive and motor functional outcomes based on haemodynamic and morphological features.

Furthermore, novel advanced DCUS techniques such as arterial stiffness analysis, and 3-dimensional carotid ultrasound with a potential to improve the accuracy and reproducibility of follow up assessments of the cerebral arteries status have emerged and validated in other populations but their clinical utility for the treatment follow up assessments in post stroke patients still remains understudied. The non-imaging TCD technique is the technique currently being used

for the intracranial cerebral arteries haemodynamic assessment at the study site. However, a newly acquired Samsung RS85 ultrasound machine (Samsung Medison Co., Ltd., Republic of Korea) equipped with a phased array (PA1-5A) transducer has the capability to perform TCCD, thus it is imperative to assess the interchangeability of the non-imaging TCD technique and the newly acquired TCCD technique in the assessment of intracranial cerebral arteries haemodynamic parameters.

Based on the abovementioned background of the study, the triangulation between AET and large intracranial and extracranial cerebral arteries' haemodynamic and morphological changes and the cognitive and motor functional changes in post-stroke patients needs further interrogation using robust study designs such as the randomised controlled single blinded trial, incorporating evidence based prescription such as the one informed by Pang et al. (2013), whereas primary outcomes are assessed using a multi-parametric approach incorporating, novel DCUS techniques such as Arterial stiffness analysis, and 3D vessel wall assessment among other methods. In addition, it is imperative to compare the haemodynamic parameters as assessed by the non-imaging TCD and those assessed by the newly adopted TCCD technique at the study center in order to establish the inter-method agreement between the two techniques.

The current study therefore investigated the effects of aerobic exercise training (AET) on cerebral arteries' morphological and haemodynamic features as assessed by multi-parametric Duplex Carotid ultrasound (DCUS) and transcranial Doppler (TCD) ultrasound techniques, and on cognitive and motor functions in post-stroke patients. In addition, the present study compared cerebral arteries' haemodynamic parameters between non-imaging TCD and transcranial color-coded Doppler (TCCD) (with (cTCCD) and without (ncTCCD) angle correction) techniques.

Furthermore, morphological and hemodynamic features of cerebral arteries between post stroke patients and age-matched controls without stroke were compared. Finally, the association between the large intracranial and extracranial cerebrovascular system changes as determined by DCUS and TCCD ultrasound and the cognitive and motor function changes in post-stroke patients undergoing AET was assessed.

2.7 Project Significance and Value:

It is of paramount importance to interrogate and characterise the effects of the AET on the large intracranial and extracranial arteries haemodynamic and morphology since the artery stenosis and plaque morphology have been regarded to play a significant role in the pathophysiology of cerebrovascular events according to El-Barghouty et al. (1995), and this information is lacking in the literature. The large extracranial and intracranial arteries are responsible for supplying the nutrients and oxygen to the brain and impairment in the supply of the nutrients and oxygen results in brain cellular death (Bor-Seng-Shu et al. 2012), hence the need to investigate the extent to which AET enhances the blood flow in these large arteries. Such knowledge on the possible improvement in the large intracranial and extracranial arteries haemodynamic and morphological features in post stroke patients following AET can provide a measure of the effectiveness of the rehabilitation training in improving the health outcomes of post-stroke patients, and thus assist in the clinical management of these patients. The use of a non-invasive, non-ionizing and readily available multiparametric ultrasound-based approach will provide a holistic investigation of the cerebrovascular haemodynamics response to AET as compared to previous studies that relied on single ultrasound parameters.

The improvement of neurocognitive and motor functioning in post stroke patients has important public health implications as this reduce the socioeconomic burden associated with the further

deterioration of neurocognitive function that has been associated with increased risk of dementia and high mortality rates (Bassuk et al., 2000; Laurin et al.,2001).

Establishing the associations between the large intracranial and extracranial cerebral arteries' morphological and haemodynamic features changes and the cognitive and motor functional outcomes of stroke may provide a basis for establishing the causal relationship between these variables which can assist in future prediction of the cognitive and functional post-stroke outcomes. The ability to be able to predict the post-stroke cognitive and motor functional outcomes using non-invasive imaging methods serves an important role in informing the patients, family and physicians of the future prognostic outcome expectations.

Chapter 3

Study One: A comparative study of transcranial color-coded Doppler (TCCD) and transcranial Doppler (TCD) ultrasonography techniques in assessing the intracranial cerebral arteries haemodynamic features.

3.1. Introduction

The assessment of intracranial cerebral arteries' haemodynamic plays a crucial role in the diagnosis and treatment management of cerebrovascular diseases, a major public health concern due to its associated high morbidity and mortality worldwide (Feigin et al. 2021). Haemodynamic failure due to cerebral arteries stenosis and vulnerable atherosclerosis plaque rupture are the main mechanism of ischemic stroke (Wang et al. 2014; Heck and Jost 2021; Saba et al. 2018), although intracranial cerebral arteries stenosis (ICS) is deemed to account for most stroke cases (33% to 67%) especially in the Asian population (Wang et al. 2014). The early identification and accurate classification of ICS is critical to inform treatment management and enhance prognostic outcomes in CVD patients as selection of patients to undergo surgical revascularization and thrombolysis is informed by the degree of stenosis. ICS is directly assessed by establishing the percentage luminal reduction of intracranial cerebral arteries'(ICAs) using the Warfarin–Aspirin Symptomatic Intracranial Disease method (WASID) during angiographic techniques (Huang, Degnan, et al. 2012) or indirectly inferred from intracranial cerebral arteries' (ICAs) haemodynamic parameters (Tsivgoulis et al. 2007). The accurate assessment of intracranial cerebral arteries' (ICAs) haemodynamic is thus critical.

Digital subtraction angiography (DSA) is the primary imaging modality for diagnosing ischemic cerebrovascular disease (Saba et al. 2018). The imaging modality is, however, invasive and expensive. Other angiographic imaging modalities such as Computed tomography angiography

(CTA) and magnetic resonance angiography (MRA) are also useful in the assessment of intracranial cerebral arteries stenosis, but they are expensive and involve the administration of contrast agent. Transcranial Doppler ultrasound (TCD) is a portable, and non-invasive medical imaging technique that utilizes low-frequency ultrasound (≤2 MHz) to assess the intracranial cerebral artery haemodynamic through thin bone acoustic windows (Naqvi et al. 2013a). Clinically there are two TCD techniques, i.e. the non-imaging TCD – commonly referred to as transcranial Doppler ultrasound (TCD) and the imaging TCD – commonly known as transcranial color-coded Doppler ultrasound (TCCD). Despite the non-imaging TCD being a common, and validated technique in assessing ICAs haemodynamic (Yeo and Sharma 2010; Sharma, Wong, and Alexandrov 2016), it is still limited in routine clinical practice, as it cannot allow for the precise differentiation between individual vessels especially in the presence of anatomical variations (Bartels 2012). TCCD on the other hand combines B-mode and color Doppler ultrasound imaging which facilitates the correct identification of ICAs through color-coding of the blood flow velocity(Nedelmann et al. 2009). This allows for accurate placement of the Doppler sampling gate over the vessel for reliable measurement. TCCD also offers the window for angle correction enabling a more accurate measurement of blood flow velocities whereas TCD assumes the insonation angle is less than 30 degrees(Nedelmann et al. 2009; Purkayastha and Sorond 2013). Despite the technical inherent urge of TCCD over TCD, its application in ICAs assessment has been limited, mainly due to its unavailability.

Recent evidence is pointing towards a surge in the acquisition and adoption of TCCD into clinical practice(Lovett and O'Brien 2022). The study centre to which the non-imaging TCD system has been the sole TCD equipment available, has also not been spared in this acquisition trend. It is worth noting that most previous studies have reported the ICAs haemodynamic parameters based

on TCD measurements with paucity of data on TCCD (Purkayastha and Sorond 2013; Lien et al. 2001). Additionally, limited studies have assessed the inter-method agreement between TCD and TCCD to justify for the possible inferences between the two techniques' ICAs haemodynamic measurements, in follow up cases or serial monitoring during or after treatment that using both TCD and TCCD measurements may be needed(Schöoning, Buchholz, and Walter 1993; Tsuchiya et al. 1991). Moreover, the studies did not utilise the widely accepted and more accurate method of Bland Altman plot(Doğan 2018; Han et al. 2022; Ranganathan, Pramesh, and Aggarwal 2017).

As the middle cerebral arteries (MCAs) a terminal end of the intracranial carotid artery is reported to be responsible for 80% of the cerebral blood flow (Nagata et al. 2016), with most of the stenotic lesions involving the MCAs it is therefore the commonly interrogated ICAs in the clinical work up of CVD (Kim 2019). Traditionally the MCA blood flow velocity has been widely used as a surrogate marker of cerebral blood flow (Jarrett et al. 2020; Lucas et al. 2010). Given the above background, the present study aimed to compare non-imaging transcranial Doppler ultrasound (TCD) and Transcranial color-coded Doppler ultrasound (with (cTCCD) and without (ncTCCD)) angle correction in quantifying the MCAs haemodynamic parameters. It is hypothesized that TCCD with angle correction will provide different haemodynamic measurement when compared to TCD and TCCD without angle correction.

3.2. Materials and Methods

3.2.1. Compliance with ethical standards

This prospective cross-sectional study was approved by the Institutional Review Board of The Hong Kong Polytechnic University (HSEARS20220714001). All participants provided written informed consent prior to undertaking the ultrasound examinations.

3.2.2. Study population

A consecutive sample of 50 healthy volunteer adults of Chinese origin were enrolled via a public email call. The subject's inclusion criteria were, healthy adults, without previous history of stroke or transient ischemic attack (TIA) and aged 18 years or older. The exclusion criteria were, previous history of stroke or TIA, age < 18 years, and allergic to the ultrasound gel. The study sample size was informed by previous similar studies(Pelz et al. 2020).

3.2.3. Data collection methods and equipment

The Delica EMS-9PB TCD machine (Shenzhen Delica Medical Equipment Co., Ltd, Republic of China) in conjunction with a 1.6 MHz pulsed wave transducer was used for the non-imaging TCD assessment, whilst the Samsung RS85 ultrasound machine (Samsung Medison Co., Ltd., Republic of Korea) equipped with a 1-5 MHz phased array transducer was used for TCCD assessment. The TCD and TCCD ultrasound scans of the MCAs for each subject were performed by a single sonographer, with abundant experience in ultrasound scanning. Additionally, to cater for the possible physiological changes that may affect blood flow velocity over time such as blood pressure, cognitive and motor activity (Purkayastha and Sorond 2013), both examinations were performed on the same day, with subjects at rest.

The bilateral MCAs of the subjects were insonated via the trans-temporal window (TTW), at two standardised imaging depths (regions of interest, ROI): 1.) proximal MCA segment at the bifurcation, and 2.) distal portion of the MCA that could be visualised on TCCD with a detectable spectral waveform across the TCD and TCCD techniques. The ROIs were identified by first scanning using the TCCD technique, at three TTW locations (anterior, middle and posterior) with the subject lying in supine position as described in previous studies(Chan et al. 2023; Alexandrov et al. 2007). The TCCD protocol involved performing firstly, an axial B-mode scan of the head, which was followed by color and spectral Doppler scans respectively.

The ultrasound machine settings for the current study involved optimising B-mode ultrasound main parameters such as power output, frequency, overall gain, time gain compensation (TGC), focusing and depth. The optimisation was primarily achieved through the selection of a user defined optimized preset under the TCD Application available on the RS85 Samsung ultrasound machine; (power output=90%, frequency=General preset, Dynamic range=50, Frame average=8, Scan area=100%, Focus=1, and Gain=50). Minor adjustments to suit individual subjects were done, during the actual scanning. The depth would be increased to visualise the contra-lateral temporal skull bone and later adjusted to ensure that the ROI corresponding to ipisilateral proximal and distal MCA segments occupies at least 2/3 of the field of view (FOV) during velocity measurements as shown in figure 3.1(a-d). A single focal zone was utilised and set at the level of the ROI to enhance lateral resolution. Additionally, color and spectral Doppler settings were optimised throughout the study. The color gain was optimised by first increasing the color gain until noise signals appear, depicted as a vessel bleed artifact outside the MCA blood vessel, and then gradually decreased until the noise just disappears. The velocity scale or pulse repetitive frequency (PRF) was optimized by decreasing it until the aliasing artifact appears, and then

increased to an optimum point until aliasing artifacts just disappears. Furthermore, the sample volume was set at 4mm for both techniques TCCD and TCD techniques. The typical B-mode, color and spectral machine settings parameters used in this study are depicted in the Table 3.1.

In the B-mode axial scan plane the mesencephalic brain stem structures were identified as hypoechoic, butterfly-shaped structures surrounded by hyperechoic star-shaped basal cistern. Color Doppler imaging mode was then turned on to identify the ipsilateral MCA at the Circle of Willis. Once the ipsilateral MCA was ascertained on color Doppler mode, a set of three consecutive measurements of the haemodynamic parameters (PSV, and MFV) were performed at the two ROIs for the TCCD test methods 1.) ncTCCD, and 2.) cTCCD using an automated spectral Doppler waveform analysis. The median of the 3 measurements was considered for data analysis. In ncTCCD, blood flow velocities were measured without applying angle correction. In cTCCD, the cursor for angle correction was applied and aligned parallel to the direction of the color Doppler blood flow before measurement of the blood flow velocities, according to Polak et al.(Polak, Alessi-Chinetti, and Kremkau 2019)]. The same procedure was repeated by scanning the other side of the head. The MFV acquired in the TCCD techniques was represented by the time averaged peak velocity (TAP). After completing the TCCD examinations, participants then proceeded to undertake the TCD examinations. During the TCD examination, the subjects were insonated via the same TTW location used in the TCCD examination using a 1.6MHz hand- held probe. The Uni-channel mode on the Delica EMS-9PB TCD machine was used, with the following typical parameters (Probe frequency=1.6MHz, Power output=23.3, Gain- (8-18)). The sample volume depths were set at the two ROIs similar to those interrogated at TCCD, whereas a sample volume gate of 4mm was similarly maintained across the techniques. Open TTW was defined as the ability

to visualise the midbrain structures on the grayscale ultrasound image, and the ipsilateral MCA. The ROIs, and the Doppler signal acquisition techniques are shown in Figures 3.1(A-F).

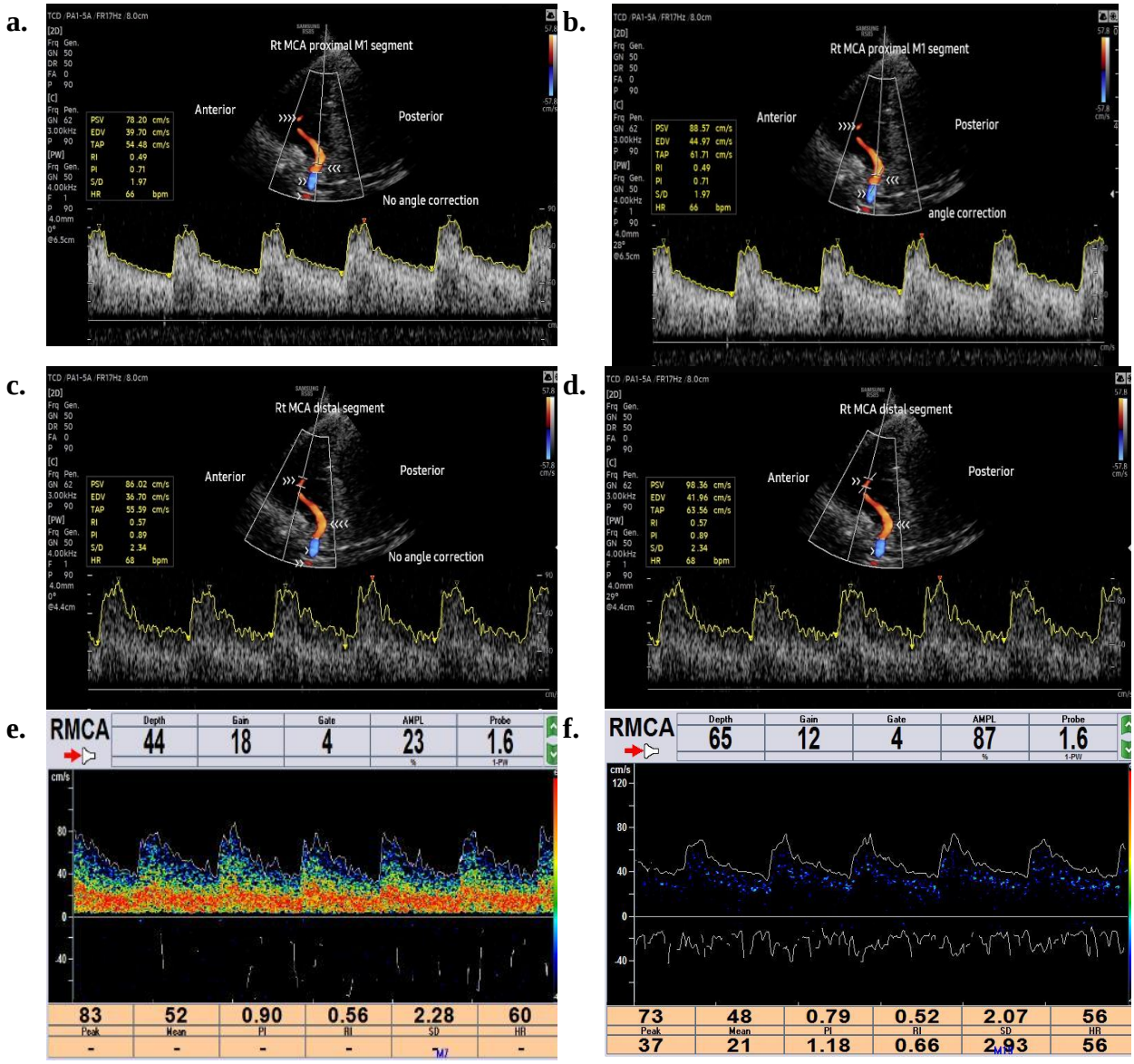


Figure 3.1: Images of a 40 year old healthy subject showing the ROIs and the Doppler signal acquisition techniques: (A.) ncTCCD measurement of PSV and TAP of proximal MCA (triple arrowheads), (B.) cTCCD measurement of PSV and TAP of proximal MCA (triple arrowheads), (C.) ncTCCD measurement of PSV and TAP of distal MCA (triple arrowheads), and (D.) cTCCD measurement of PSV and TAP of distal MCA (double arrowheads). (E.) TCD waveform showing measurement of PSV and MFV at distal depth of MCA, (F.) TCD waveform showing measurement of PSV and MFV at proximal depth of MCA. The long vessel color-coded in red is the right MCA whereas the ipsilateral anterior cerebral artery segment is color-coded in blue.

Table 3.1: Samsung RS85 ultrasound machine protocol settings.

2D mode	**Color Doppler mode**	**Spectral Doppler mode**
Power=90	Power=90	Power=90
Probe Frequency-General Preset (1-5Mhz)	Gain-variable (start with high gain to optimum	Frequency=General preset
Dynamic Range=50	Persistence-	Gain=50
Gray map=7	Color box- adjusted to cover ROI.	Wall filter=1
Frame Average=8		Scale/PRF=variable (optimized to avoid aliasing artifact, & reduced in low velocity settings)
Scan area=100%		Sample volume size=4mm
Focus number=1		
Depth- adjusted until ROI occupy ~2/3 FOV.		

3.2.4. Data Analysis

IBM SPSS version 25 statistical package was used for data analysis. The ncTCCD and cTCCD MCA haemodynamic parameters (PSV and MFV) were compared with the TCD measurements for concordance. Both descriptive and inferential statistics were utilized. One-way ANOVA and the non-parametric equivalent Kruskal Wallis, followed by Bonferroni post-hoc analysis were used to compare the means of the PSV and MFV between the three techniques respectively. The $p < 0.05$ was considered statistically significant. The mean difference (Bias), and percentage differences in the MCA haemodynamic parameters between the three techniques were determined to represent the main outcome measures. The percentage difference of the MCA PSV among the three techniques: 1.) TCD versus ncTCCD, 2) TCD versus cTCCD, and 3.) ncTCCD versus cTCCD was computed as the absolute value of the mean difference (Bias) divided by the average of the MCA PSV of the 2 measurement techniques using [equations 1, 2, and 3] respectively. Same comparisons were also applied to MCA MFV measurement, and the percentage differences in the MFV across the three techniques were computed using equations [4-6].

$$D_p(TCD\ vs\ ncTCCD, PSV) = \frac{|MD|}{\frac{TCD + ncTCCD}{2}} * 100\% \quad (1)$$

$$D_p(TCD\ vs\ cTCCD, PSV) = \frac{|MD|}{\frac{TCD + cTCCD}{2}} * 100\% \quad (2)$$

$$D_p(ncTCCD\ vs\ cTCCD, PSV) = \frac{|MD|}{\frac{ncTCCD + cTCCD}{2}} * 100\% \quad (3)$$

$$D_p(TCD\ vs\ ncTCCD, MFV) = \frac{|MD|}{\frac{TCD + ncTCCD}{2}} * 100\% \quad (4)$$

$$D_p(TCD\ vs\ cTCCD, MFV) = \frac{|MD|}{\frac{TCD + cTCCD}{2}} * 100\% \quad (5)$$

$$D_p(ncTCCD\ vs\ cTCCD, MFV) = \frac{|MD|}{\frac{ncTCCD + cTCCD}{2}} * 100\% \quad (6)$$

In equation 1, $D_p(TCD\ vs\ ncTCCD, PSV)$- represents the Percentage difference (%) in Peak Systolic Velocity (PSV) measurements, between the two ultrasound techniques; non-imaging transcranial Doppler ultrasound (TCD) and Transcranial color-coded Doppler ultrasound, without angle correction (ncTCCD). Equations 2-6 follow similar formating, whereas in equations 4-6, the PSV is substituted by the Mean Flow Velocity (MFV) as the measurement variable. D_p-represents Percentage difference (%); $|MD|$-represents absolute value of the mean difference or Bias of the variable measurement between two techniques.

Additionally, Bland-Altman plots provided a visual assessment of the agreement between the techniques, TCD and TCCD (with and without angle correction), in measuring the MCA haemodynamic parameters. The difference between the TCD and TCCD (with and without angle correction) haemodynamic parameters values were plotted on the Y-axis against the average of the two measurements (X-axis). The limits of agreement (LOA) were calculated as the (Bias ±1.96SD) and reflects the precision of the measurements. Two methods were deemed interchangeable when greater than 95% of the data points lie within the upper and lower LOA.

3.3. Results

3.3.1. Demographic characteristics of the study participants

A total of 50 participants (17 men and 33 women; mean age, 49±17 years; age range, 20 to 72 years) were enrolled between January and March 2023. All subjects underwent both TCD and TCCD examinations of MCAs. The demographic data of the study participants is presented in Table 3.2. The body mass index (BMI) of the subjects was (mean=23±4 kg/m^2, range=17 to 32 kg/m^2). Four subjects (8%) were underweighting with a BMI<19 kg/m^2. Four subjects (8%) had a systolic blood pressure of over 140mmHg indicative of hypertension.

Table 3.2: Demographic characteristics of the study participants (n=50).

	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	B.Ps (mmHg)	BPd (mmHg)	HR (BPM)
Mean	49	61	163	23	118	79	76
Standard deviation (SD)	17	12	8	4	14	9	12
Minimum	20	44	148	17	89	60	44
Median	57	60	164	22	116	82	75
Maximum	72	95	178	32	154	94	119
Normality test (KS)	0.223	0.144	0.096	0.116	0.114	0.126	0.128
Normality test *p*-value	0.014	>0.100	>0.100	>0.100	>0.100	>0.100	>0.100

BMI-body mass index; B.Ps-systolic blood pressure; BPd-diastolic blood pressure; HR-heart rate; BPM-beats per minute. The units of the demographic variables are written in brackets.

3.3.2. Trans-temporal window (TTW) status in the study population

A total of 41 subjects (82%) had at least one sided open TTW for assessing MCA in TCD and TCCD (36-bilateral open TTW, and 5-unilateral open TTW – 3 on left side and 2 on right side),

whereas 9 (18%) subjects had bilateral TTW failure to evaluate MCA. Finally, among the 50 subjects a total of 77 MCAs were visualised and haemodynamic parameters interrogated on both TCD and TCCD techniques. In one subject, despite the presence of bilateral open TTW, spectral Doppler signals could not be obtained at same distal depths for the TCD and TCCD techniques, on both sides of the head. A total of 152 MCA measurements were thus considered for blood velocity analysis (142 measurements from 36 subjects with bilateral open TTW, and 10 from 5 subjects with unilateral TTW). A higher percentage of the presence of at least one side of open TTW window was observed among male subjects in comparison to female subjects (94% versus 76%, respectively), and a corresponding higher percentage of TTW failure was observed in females compared to males' counterparts (24% and 6%, respectively) (Figure 3.2). The observed gender-based differences in the TTW status was however statistically insignificant (χ^2 -test statistics= 2.562; df=1; p=0.109). The majority of the participants had middle TTW, 27 (66%), and the remaining 14 (34%) were posterior TTW.

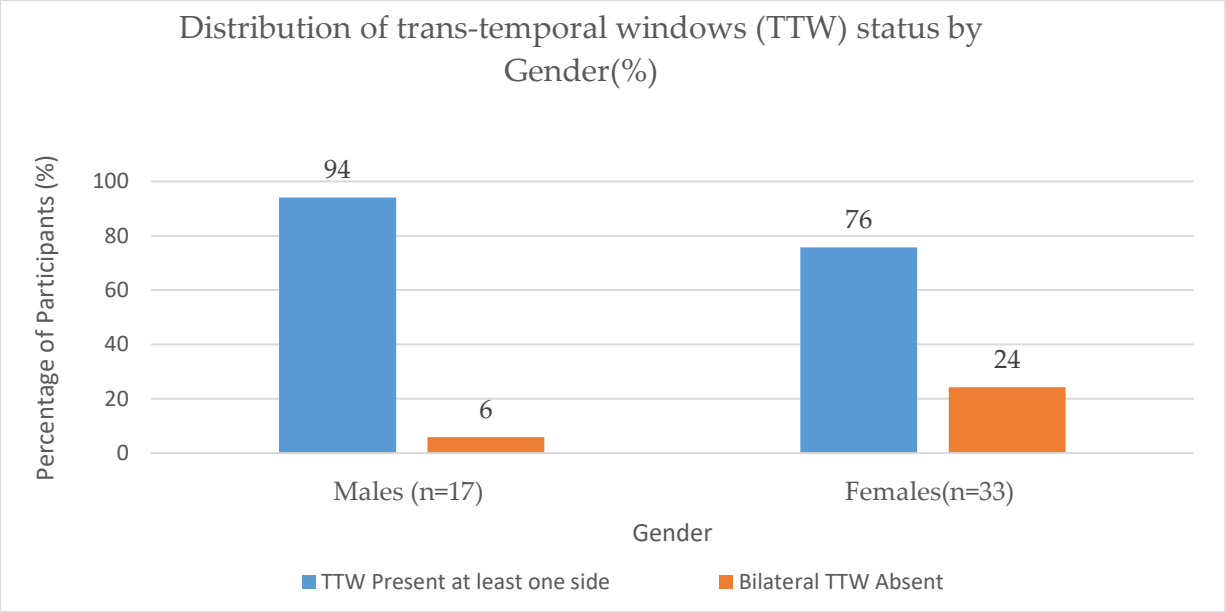


Figure 3.2: Histogram showing the trans-temporal window (TTW) status by Gender.

3.3.3. MCA depths (proximal and distal) interrogated and Doppler angles

The mean proximal depth of the MCAs that could be visualised on TCCD was 59± 3 mm (range = 49-68 mm), whilst the mean distal depth was 44±5 mm (range = 35-63 mm). The mean Doppler angles observed in the study was 24±15 degrees.

3.3.4. Comparison of All 152 MCA PSV measurements across the 3 techniques (TCD, ncTCCD and cTCCD)

The mean MCA PSV measured by the TCD, ncTCCD and cTCCD were 83±18 cm/s, 81±19 cm/s and 93±21 cm/s, respectively (Figure 3.3). One-way ANOVA results were significant (F-stats=16.62; $p<0.001$) and subsequent Bonferroni post hoc tests showed insignificant difference in PSV measured between TCD and ncTCCD (t stat=0.8245; $p=1.000$), whilst a significant difference was observed between TCD and cTCCD ($t=4.53$; $p<0.001$). Additionally, MCA PSV measured by cTCCD was significantly higher than that measured using ncTCCD ($t=5.36$; $p<0.001$). The percentage differences in the PSV between TCD versus ncTCCD, and TCD versus cTCCD techniques were 2% and 11% respectively, whilst a 14% increase in the ncTCCD, PSV was observed following angle correction (Figure 3.4).

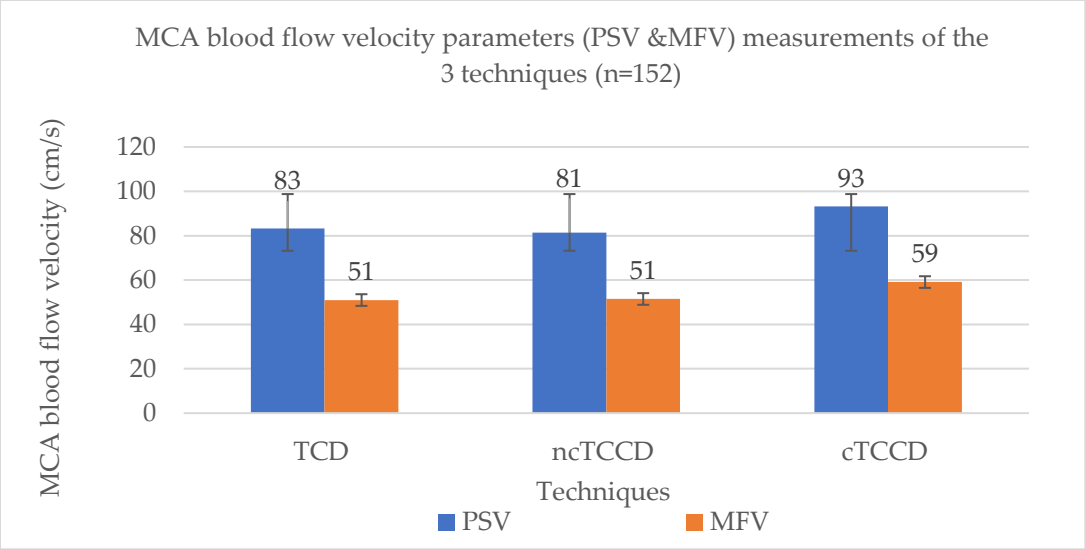


Figure 3.3: Histogram showing the mean MCA PSV& MFV (cm/s) across the 3 techniques

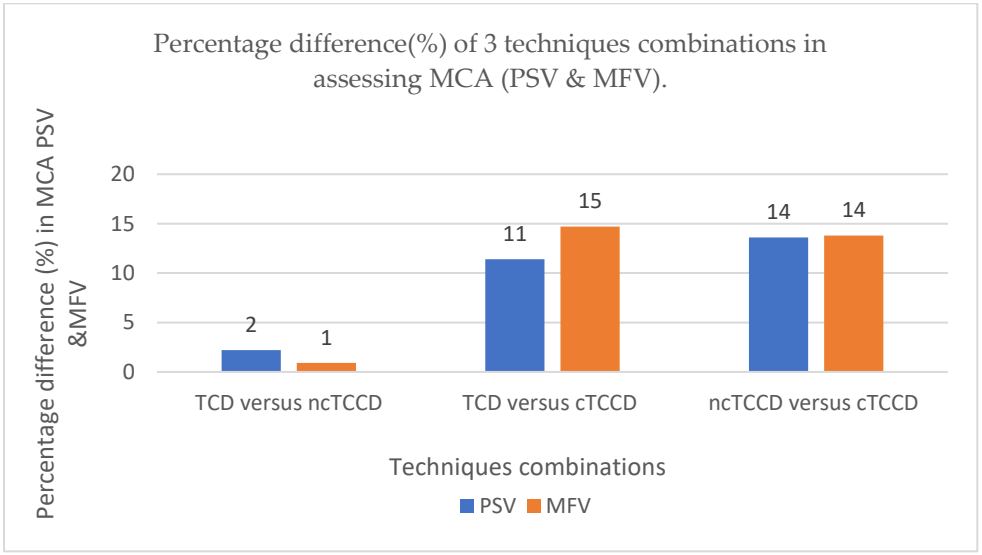


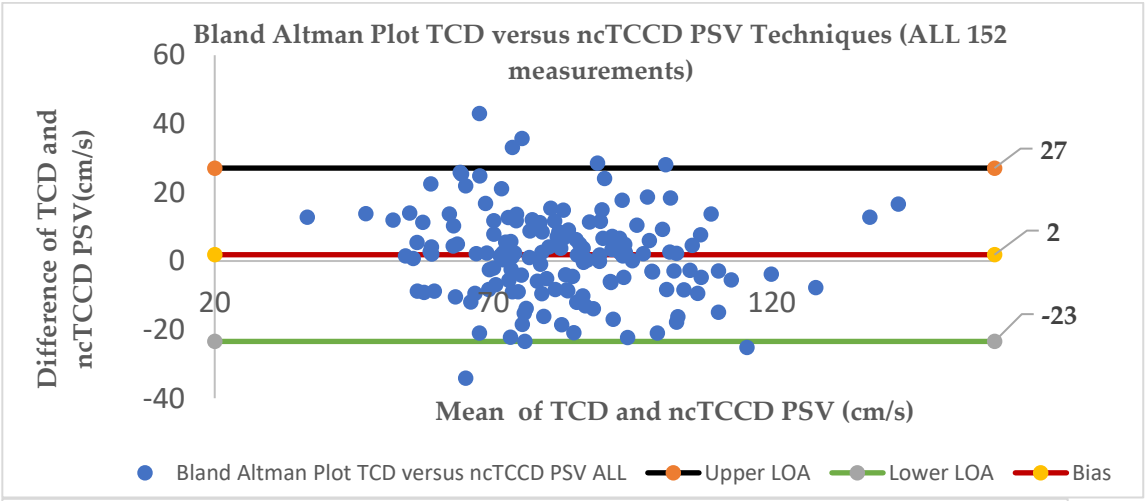
Figure 3.4: Histogram showing the Percentage Differences (%) in MCA PSV & MFV between the 3 Techniques combinations.

The Bland-Altman plot demonstrated a bias of 2 cm/s in PSV measurements between TCD and ncTCCD techniques (Fig 3.5A). The small positive value of the bias reflects that on average ncTCCD technique minimally gives a lower PSV measurement in comparison to TCD. The LOA were 27 and -23 cm/s. A good agreement was observed between the TCD and ncTCCD techniques,

as 95% of the data points lie within the LOA, hence the two techniques are considered interchangeable in measuring the MCA PSV.

The bias in the PSV measurements between TCD and cTCCD techniques was -10 cm/s (Figure 3.5B). The relatively large negative value of the bias reflects that cTCCD technique substantially yields higher PSV measurement in comparison to TCD technique. The limits of agreement were 20 and -40 cm/s. The Bland Altman plot showed only 92% of the data points to lie within LOA, hence the PSV measured by cTCCD technique may not be interchangeable to that of TCD.

a.



b.

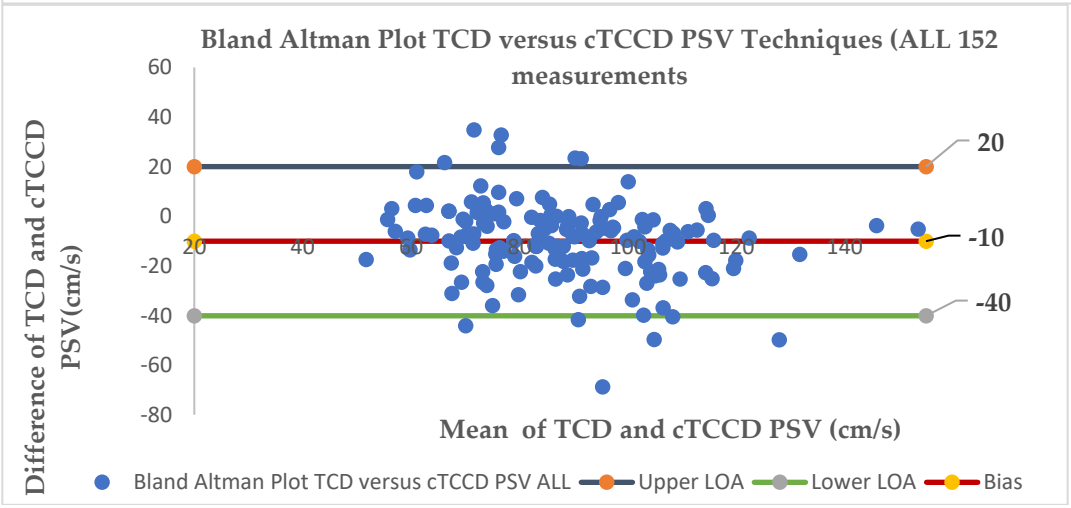


Figure 3.5: Bland-Altman plots for agreement between three techniques, (TCD, ncTCCD, and cTCCD) in assessing MCA PSV (ALL 152 measurements). (a) TCD versus ncTCCD techniques, (b) TCD versus cTCCD techniques. The red solid lines in A and B, represents the mean of the difference (bias) in the MCA PSV measurement, between the TCD versus ncTCCD, and TCD versus cTCCD techniques. The black and green lines represents the upper (ULA) and lower (LLA) limits of agreement, respectively. The ULA is given as the bias+1.96* standard deviation (SD), and the LLA is given as (bias-1.96* SD).

3.3.4.1. Comparison of the Proximal and Distal MCA PSV in each of the 3 techniques

The mean MCA PSV measurements of the proximal and distal MCA segments of each of the three imaging techniques are shown in Table 3.3. In the three imaging techniques, the PSV measured at the proximal MCA was significantly higher than that measured at the distal MCA ($p<0.05$). The mean proximal MCA PSV for the TCD, ncTCCD and cTCCD techniques were 90±15 cm/s, 88±15 cm/s and 100±17 cm/s respectively whilst the corresponding mean distal MCA PSV were 76±18 cm/s, 75±21 cm/s and 87±22 cm/s respectively. For both proximal and distal MCA, a significant difference in PSV between TCD and cTCCD, and between ncTCCD and cTCCD techniques was observed ($p<0.05$). The MCA PSV between TCD and ncTCCD was however, not significantly different from each other regardless of the interrogated depth ($p=1.00$).

Table 3.3: Descriptive statistics of the proximal and distal MCA PSV measurements in the three techniques.

	PSV (cm/s)			
	Minimum	Maximum	Mean	Std. Deviation
*Proximal MCA (n=77) **				
TCD	66	151	90[#]	15
ncTCCD	55	134	88[†]	15
cTCCD	60	156	100[#†]	17
*Distal MCA (n=75) **				
TCD	43	144	76[#]	18
ncTCCD	30	131	75[†]	21
cTCCD	52	153	87[#†]	22

* Significant difference ($p<0.05$) between proximal and distal MCA in the three techniques; # significant difference ($p<0.05$) between TCD and cTCCD; † significant difference ($p<0.05$) between ncTCCD and cTCCD.

The Bland Altman plots between TCD and ncTCCD in the measurement of proximal and distal MCA PSV are shown in Figure 3.6 A, and B respectively. Totally 95% of the proximal, and 96% of the distal MCA measurements data points are within the LOA, hence the two techniques are interchangeable regardless of the imaging depth. In the Bland Altman plots between TCD and cTCCD, 94% and 93% of the data points are within the LOA for the proximal and distal MCA PSV measurements, respectively (Figure 3.6 C, and D respectively).

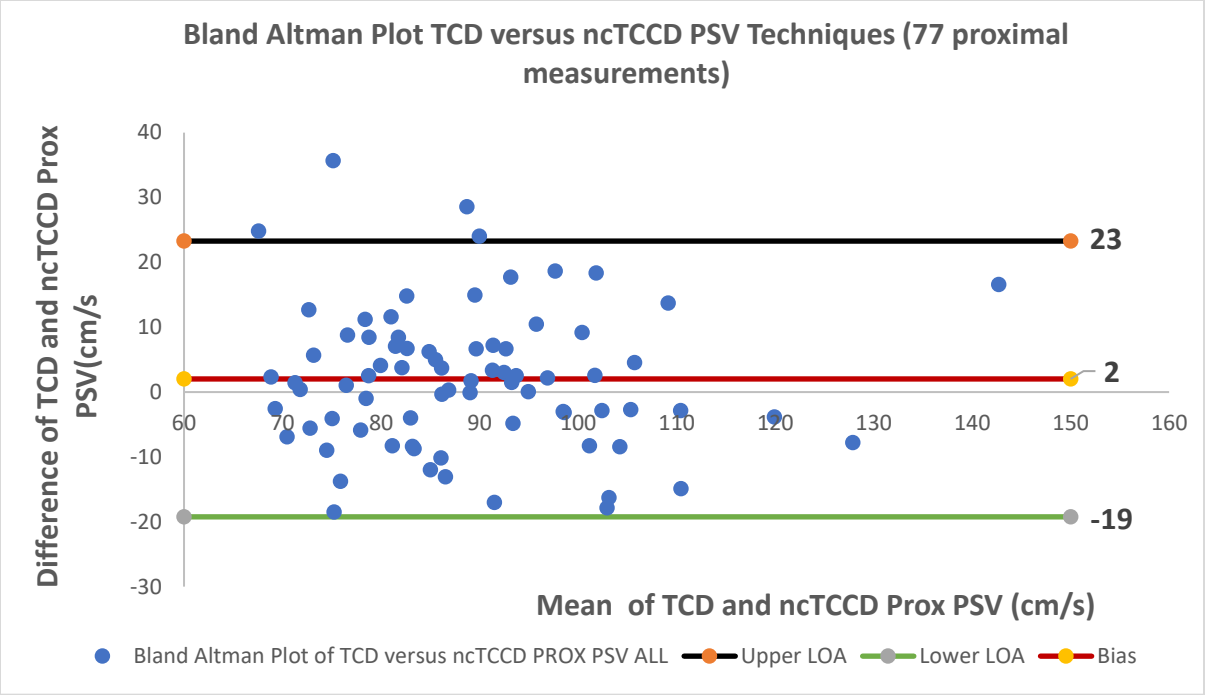


Figure 3.6A. Bland Altman plot TCD versus ncTCCD MCA PSV (proximal measurements)

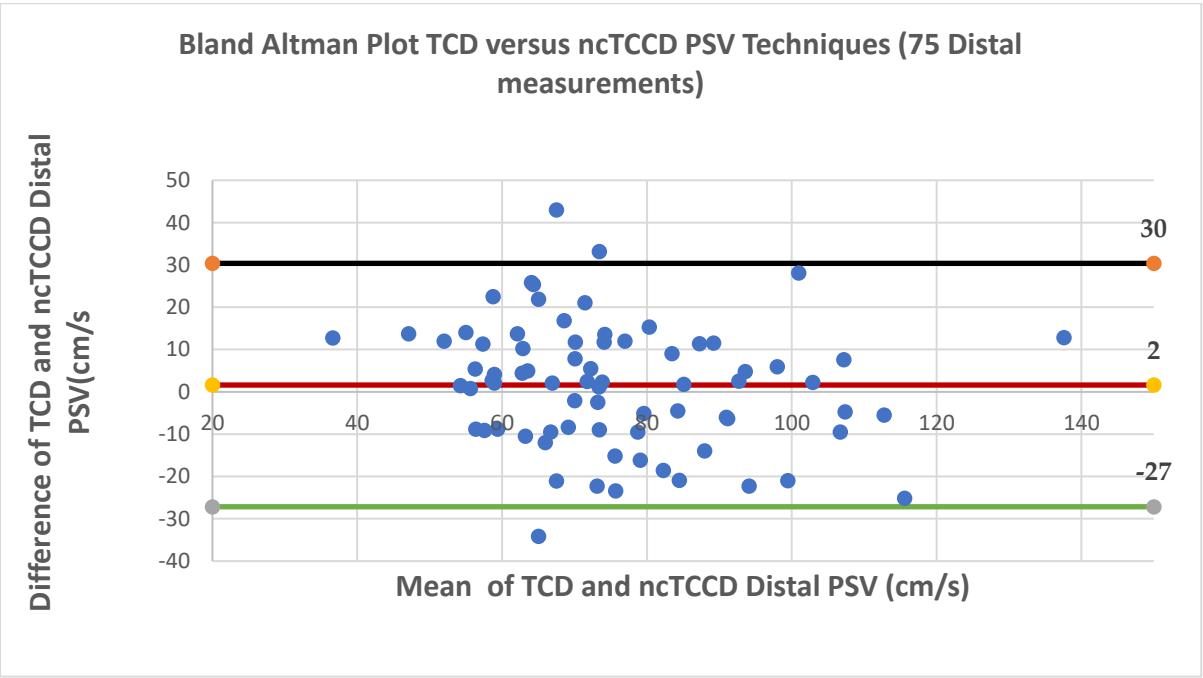


Figure 3.6B. Bland Altman plot TCD versus ncTCCD MCA PSV (Distal measurements).

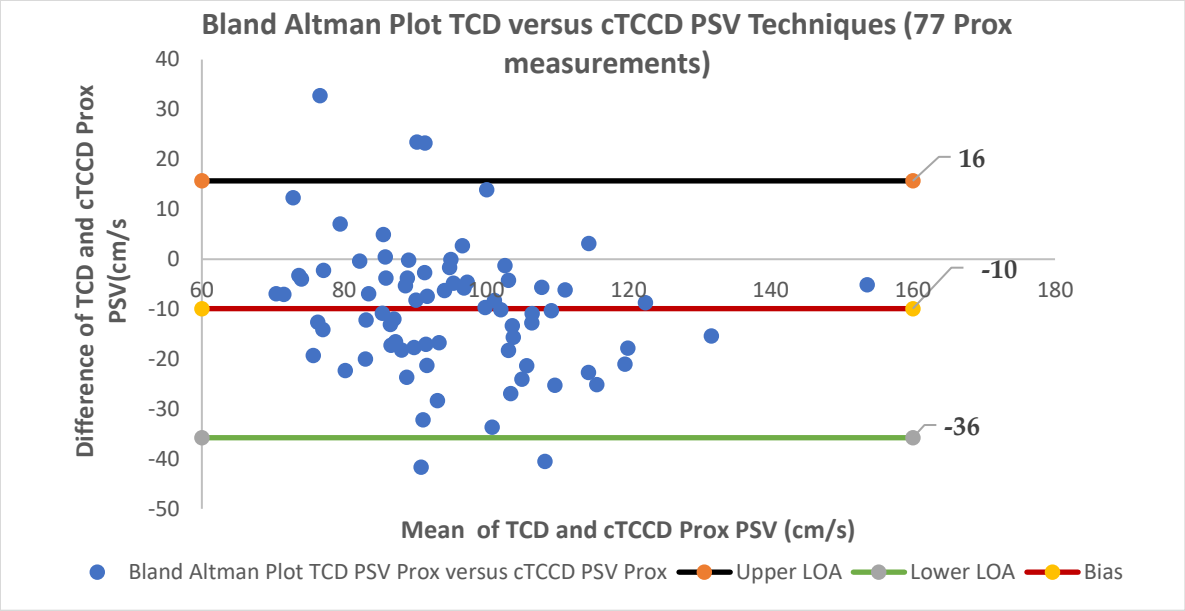


Figure 3.6 C. Bland Altman plot TCD versus cTCCD MCA PSV (Proximal measurements)

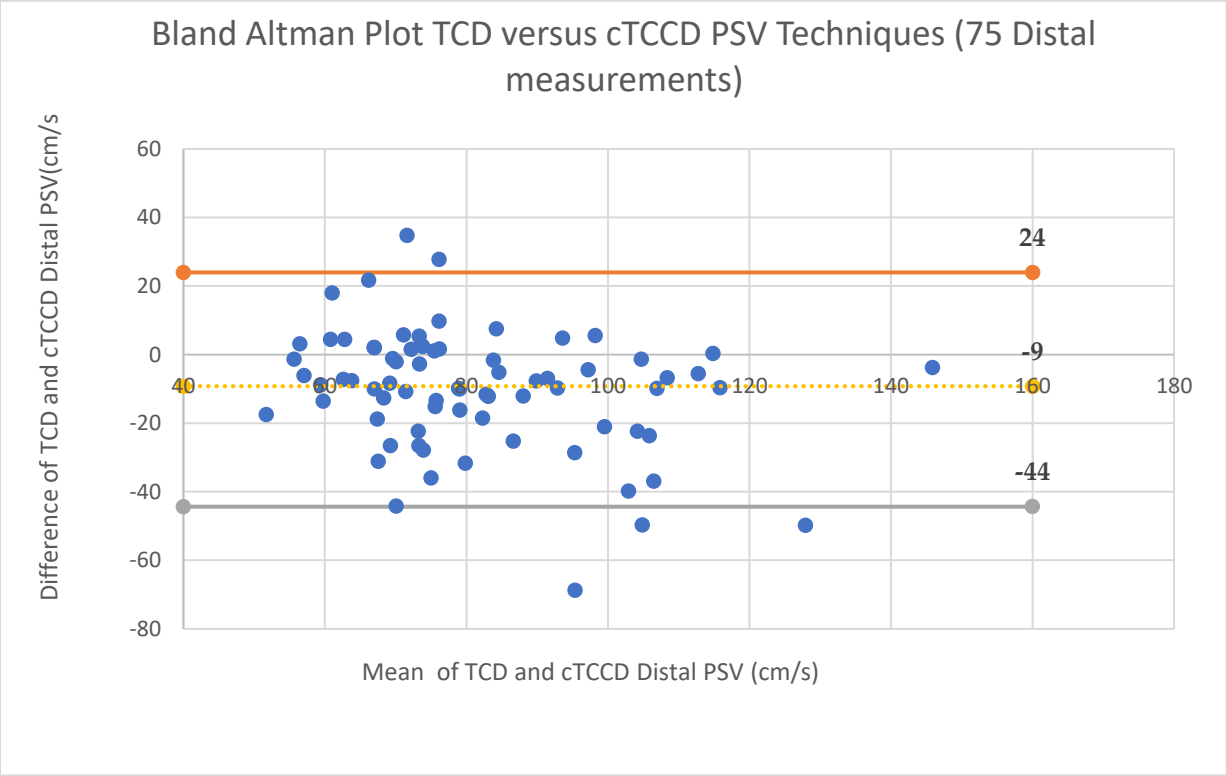


Figure 3.6 (a-d): Bland Altman plot showing the comparison of the proximal and distal MCA PSV in each of the 3 techniques.

3.3.5. Comparison of MCA MFV across the 3 techniques (TCD, ncTCCD and cTCCD).

In the 152 measurements, the mean MCA MFV measured using TCD, ncTCCD, and cTCCD were 51±11 cm/s, 51±12 cm/s and 59±14 cm/s respectively (Figure 3.3). The MFV measured by ncTCCD was not significantly different from that of TCD (stats=-0.529; p=1.000), whereas there was a significant difference in the MFV between TCD versus cTCCD (stats= -5.142; p<0.001), and ncTCCD versus cTCCD (stats= -4.613; p<0.001) techniques. The percentage differences in assessing the MCA MFV between TCD versus ncTCCD, TCD versus cTCCD, and ncTCCD versus cTCCD were 1%, 15%, and 14% respectively (Figure 3.4).

The Bland Altman plots for the comparison of the 3 techniques in assessing the MCA MFV measurements are shown in Figure 3.7. A bias of -0.5 was observed between the TCD and ncTCCD techniques, and the LOA were 18 and -19 cm/s, whereas 91% of the data points are lying within the LOA. cTCCD, exhibited higher MCA MFV when compared to TCD technique, bias=-8 cm/s, and the LOA was 13 and -29 cm/s, whereas 93% of data points are within the LOA.

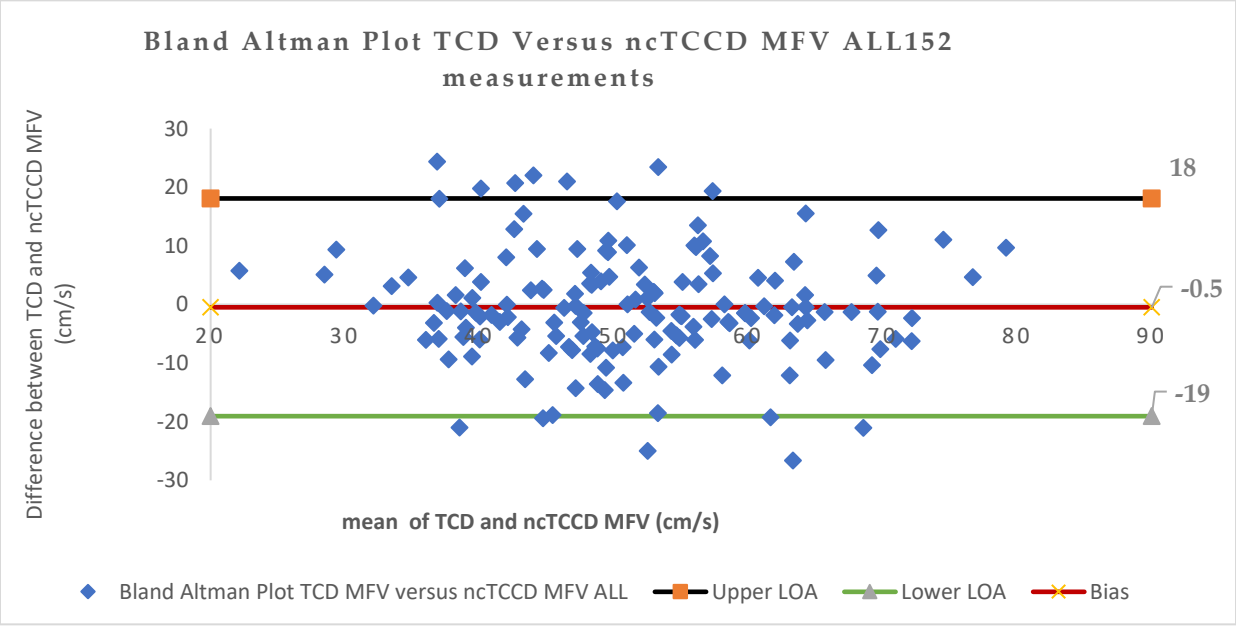
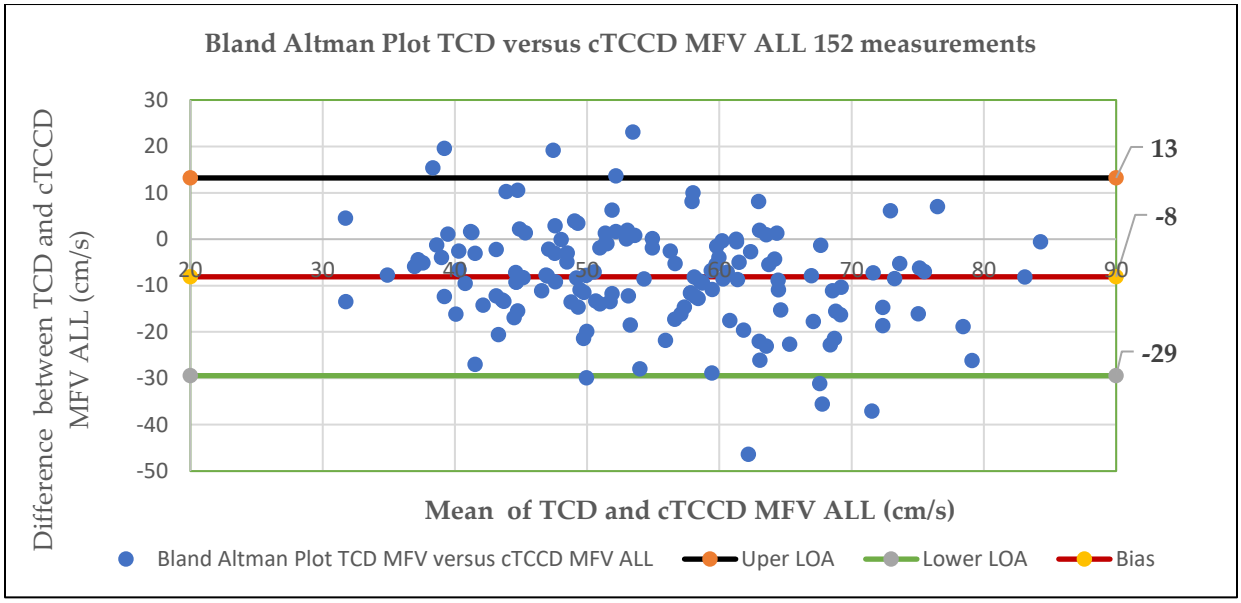


Figure 3.7. a: TCD versus ncTCCD techniques.



b.

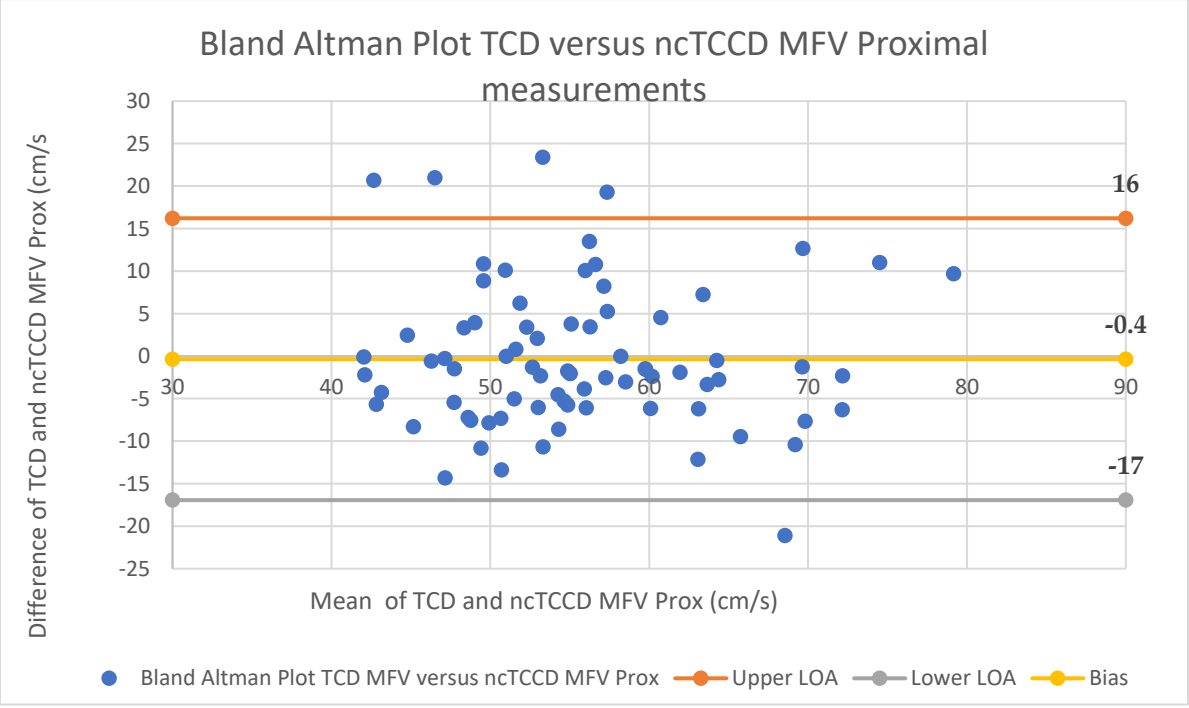
Figure 3.7(a-b): Bland Altman plots for agreement between three techniques (TCD, ncTCCD, and cTCCD) in assessing MCA MFV (ALL 152 measurements). (a) TCD versus ncTCCD techniques, (b) TCD versus cTCCD techniques. The red solid lines in A and B, represents the mean of the difference in the MCA MFV measurement, between the TCD versus ncTCCD, and TCD versus cTCCD techniques. The black and green lines represent the upper (ULA) and lower (LLA) limits of agreement, respectively. The ULA is calculated as the bias+1.96* standard deviation (SD), and the LLA is given as bias-1.96* SD. The limits of agreement (LOA) were calculated as the (Bias ±1.96SD) and reflects the precision of the measurements.

3.3.5.1. Comparison of the Proximal and Distal MCA MFV in each of the 3 techniques.

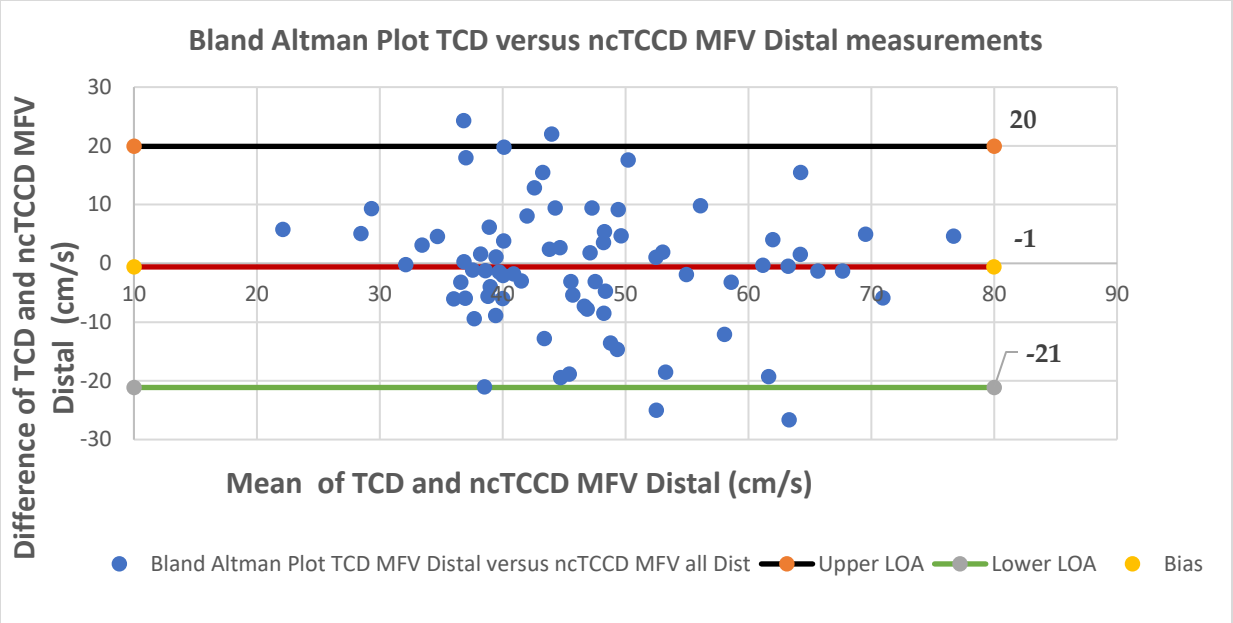
The mean proximal MCA MFV measured using TCD, ncTCCD and cTCCD were 55±9 cm/s; 56±9 cm/s and 63±11 cm/s respectively, and the corresponding distal MCA MFV were 46±11 cm/s; 47±13 cm/s and 55± 14 cm/s respectively. The observed mean differences between TCD

and ncTCCD techniques' MFV measurements in both the proximal and distal MCA were not statistically significant (Bias=-0.4 cm/s; p=1.000, and Bias= -0.6; p=1.000 respectively). Contrarily a significant difference was observed in the MFV measurements in both the proximal and distal MCA between TCD and cTCCD (Bias=-8 cm/s; p<0.001, and Bias= -8; p<0.001 respectively), and ncTCCD and cTCCD (Bias=-8 cm/s; p<0.001, and Bias= -8; p<0.001 respectively).

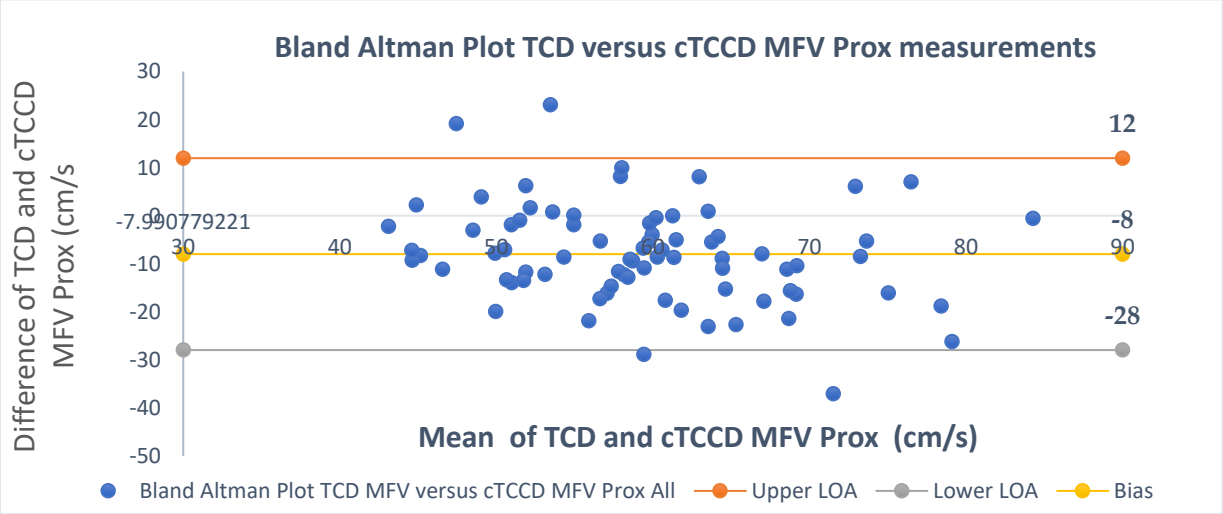
The Bland Altman plots for the comparisons of the 3 techniques in the assessment of the proximal and distal MCA MFV are shown in Figure 3.8 (A, B, C and D.) A marginal systematic bias was observed between the TCD and ncTCCD techniques proximal and distal MFV (-0.4 cm/s; and -0.6 cm/s respectively), whilst cTCCD, yielded higher MFV when compared to TCD for both proximal and distal measurements (bias=-8, and 8cm/s respectively).



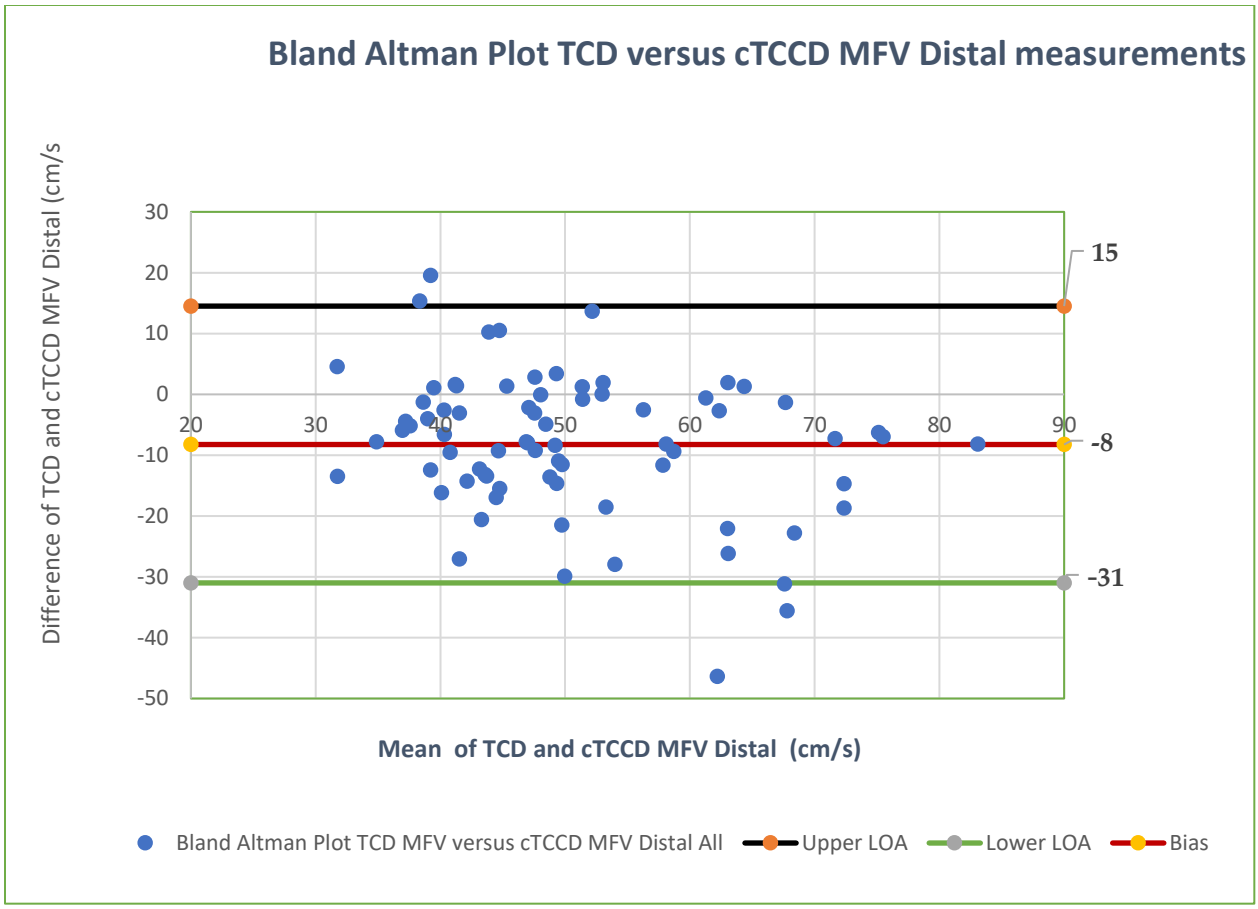
a.


Bland Altman Plot TCD versus ncTCCD MFV Distal measurements
Difference of TCD and ncTCCD MFV Distal (cm/s)
Mean of TCD and ncTCCD MFV Distal (cm/s)
20
-1
-21
Bland Altman Plot TCD MFV Distal versus ncTCCD MFV all Dist
Upper LOA
Lower LOA
Bias

b.


Bland Altman Plot TCD versus cTCCD MFV Prox measurements
Difference of TCD and cTCCD MFV Prox (cm/s
Mean of TCD and cTCCD MFV Prox (cm/s)
12
-8
-28
-7.990779221
Bland Altman Plot TCD MFV versus cTCCD MFV Prox All
Upper LOA
Lower LOA
Bias

c.



d.

Figure 3.8(a-d): Bland Altman plots for agreement between three techniques (TCD, ncTCCD, and cTCCD) in assessing MCA MFV (ALL 152 measurements) at the proximal and distal depth-

a.) TCD versus ncTCCD MCA MFV (77 Proximal measurements), b.) TCD versus ncTCCD MCA MFV (75 Distal measurements), c.) TCD versus cTCCD MCA MFV (77 Proximal measurements), d.) TCD versus cTCCD MCA MFV (75 Distal measurements)

3.4. Discussion

This study compared TCD and TCCD with or without angle correction techniques in quantifying the MCA haemodynamic parameters and ascertain whether the techniques are interchangeable. The present study demonstrated a significantly higher number (n=41, 82%) of subjects with at least one side of open TTW for TCD and TCCD examinations. In addition, the current study found that, majority of the open TTW were middle TTW ($\chi 2$ -test statistics=4.122; p=0.042). The higher prevalence of middle TTW observed in the present study, reaffirms the need to use middle TTW as the initial location to focus the transducer when performing TCD scan as reported in a recent study by Chan, et al. (Chan et al. 2023), as this has the potential of reducing the scan times.

Based on the definition of TTW failure representing the number of MCA vessels that could not be visualised to allow for the interrogation of the haemodynamic parameters, a TTW failure rate of 23% was observed in this study, which is lower than that reported in previous studies (28.8% – 37%)(Lien et al. 2001; Kwon et al. 2006; Lin, Fu, and Tan 2015). As Asian subjects were used in the present and previous studies, the difference in the TTW failure rates could probably be attributed to the age difference, between our study and previous studies. It has been reported that age is a significant factor of TTW failure rates, with higher failure rates observed in older adults than in younger population due to increased temporal bone thickness associated with ageing (Kwon et al. 2006; Lin, Fu, and Tan 2015; Kenton et al. 1997). In the present study, the mean age of the subjects was 49±17 years which was lower than that in previous studies (64.5±13.2 to 65.1±11.9 years)(Lien et al. 2001; Kwon et al. 2006; Lin, Fu, and Tan 2015). As anticipated, subjects with bilateral TTW failure were demonstrated to have higher mean age of (58±15years) compared to mean age of (46±18 years) in those with bilateral open TTW, thus further reaffirming that older age is linked to TTW failure. Previous studies have shown that bilateral TTW failure is

more common in females (Kwon et al. 2006; Lin, Fu, and Tan 2015; Hoksbergen et al. 1999). In the present study, most subjects with bilateral TTW failure were females (n=8, 89%). A further gender versus TTW status cross tabulation considering the significant gender inhomogeneity present in the current study, revealed a statistically insignificant difference in the TTW status across gender (Chi-squared statistics= 2.562; df=1; p=0.109). The significantly higher incidence of subjects with open TTW, independent of gender, implies that TCCD can be a practical imaging tool to use in the Chinese population where intracranial artery stenosis is indicated to be the most common cause of ischemic stroke accounting for 33% to 67% of stroke cases(Wang et al. 2014; Nguyen-Huynh et al. 2008).

The current study established the MCA PSV and MFV values measured by TCD, ncTCCD, and cTCCD techniques among the healthy Chinese adults. In the measurement of MCA MFV using the TCCD techniques, our results were lower than those reported in a study by Tsuchiya et al.(Tsuchiya et al. 1991), and a greater discrepancy was observed for the angle correction technique (ncTCCD = 51±12 cm/s vs 55±16 cm/s; cTCCD = 59±14 cm/s vs 87±16 cm/s, respectively). The wider variations in the mean doppler angles of (24±15 vs 49) degrees, for our study and Tsuchiya et al.(Tsuchiya et al. 1991) respectively, explains the observed discrepancy in the MFV.

The previous studies that compared TCD and TCCD techniques in assessing MCA haemodynamic parameters, have not utilised the widely accepted and accurate method of Bland Altman plot to establish whether the techniques are interchangeable(Schöoning, Buchholz, and Walter 1993; Bartels and Flügel 1994). Based on the results of the Bland Altman plots, the present study has established the existence of good agreement hence interchangeability between the TCD and ncTCCD in assessing the MCA PSV. We observed greater than 95% of the data points to lie within the limits of agreement, with no evidence of fixed bias. The mean difference of 1.83 cm/s, and a p

value of 1.000 between the TCD and ncTCCD techniques implies that ncTCCD yields MCA PSV which is lower but not statistically different from the TCD PSV. Contrarily, cTCCD technique was demonstrated to yield significantly higher PSV values, and not interchangeable with the TCD technique in assessing the MCA PSV (< 95% of data points were within limits of agreement; bias= -10 cm/s; $p<0.001$). Similar to our study, previous studies have observed significant differences in MCA PSV derived between the TCD and cTCCD, with percentage differences ranging between 5-15%(Bartels and Flügel 1994; Klötzsch et al. 2000) whereas in the current study a percentage difference of 11% was observed.

Additionally, a marginal statistically insignificant bias of 0.48 cm/s between the TCD and ncTCCD MCA MFV measurements ($p=1.000$) was noted in our study, although it could not reaffirm the interchangeability of the two techniques. In contrast, a significant difference in the MFV was observed between TCD versus cTCCD and ncTCCD versus cTCCD ($p<0.05$). Our findings concur to those observed in Martin, et al. (Martin, Evans, and Naylor 1995). TCD and ncTCCD apply a Doppler angle of zero degree in computing the blood flow velocities, however we observed a mean Doppler angle of 24±15 degrees between MCA and ultrasound beam direction in the current study. Hence, this probably explains the significantly higher velocities derived using the cTCCD technique in comparison to both TCD and ncTCCD in the current study. cTCCD may therefore provide more accurate measurements of the MCA blood flow velocities, and where comparisons to previous TCD and TCCD without angle correction is required, caution should be taken to consider the observed biases between the techniques. The Doppler angle corrections, reported in our study were less than the mean insonation angles reported in previous studies by Bartels and Flugel (Bartels and Flügel 1994) and Eicke (Martin Eicke et al. 1994). Furthermore, in previous studies the Doppler measurements were mainly performed along the horizontal section of the

MCAs M1 segment, whereas in the current study we interrogated the distal and proximal portions of the MCA, which did not necessarily correspond to the MCA M1 horizontal portion. The variations in the ROI interrogated between the current and previous studies may further explain the differences between the studies.

In the current study the distal and proximal MCA reference depths that could be interrogated by TCCD based on the direct visualisation of the color-coded MCA among healthy Chinese adults were reported for the first time. Previous studies that have compared the two methods TCD and TCCD, interrogated the blood flow velocities at predetermined fixed depths, without taking into considerations the possible anatomical variations in the subjects MCAs. As one's proximal depth may actually corresponds to another subject's distal segment depth, using the fixed depth approach may result in measuring flow velocities derived at anatomically different positions of the MCAs across the participants. The current study provided a more anatomically based standardised methodological approach by focussing at the proximal and distal reference portions of the MCAs. In one study, a cut off depth ranging between 45 mm and 60 mm was used as the criteria to identify the MCA blood flow (Kwon et al. 2006), whereas the current study reaffirmed these cut off values, when the mean of the distal and proximal depths are used (44mm and 59 mm) respectively. However, the depth at which MCA signals could be observed ranged between 35mm and 68 mm in the present study. Furthermore, the proximal and distal MCA haemodynamic parameters in all the three techniques were observed to be significantly different from each other, with higher velocities recorded proximally than the distal MCA portion. The observed high proximal MCA velocities compared to distal MCA may be explained by anticipated high blood pressure at the proximal MCA segment, close to bifurcation, which in turn is reported to be associated with increase in MCA velocities(Lucas et al. 2010). Due to the significant difference in the MCAs

haemodynamic parameters measurements between the proximal and distal portions, it is imperative for clinicians to state the interrogation depth in reporting the MCAs haemodynamic measurements regardless of the TCD/TCCD technique employed.

The use of a single sonographer to perform both the TCD and TCCD examinations has the potential to introduce some recall bias. To mitigate this possible limitation, a set of three consecutive measurements were taken and the median values considered. Furthermore, no flow calibration study was undertaken to ascertain the actual blood flow velocities. Additionally, the present study findings are applicable to the Chinese population as this study recruited Chinese subjects. Further studies are needed to verify the applications of TCCD in other ethnic populations. However, as a strength the imaging depth was successfully standardised between the techniques to ensure that velocities from the same blood flow samples are interrogated and compared between the TCD and TCCD techniques. Furthermore, the TCD and TCCD examinations were both performed on the same day and time to cater for any possible physiological changes that may occur over time.

3.5. Conclusions

This study validated TCCD as a practically applicable imaging technique. TCCD with angle correction is a more accurate technique that tends to yield higher MCAs blood flow velocities than non-imaging TCD and ncTCCD. Furthermore, ncTCCD is comparable to non-imaging TCD and should be considered in clinical cases that using both TCD and TCCD measurements are needed, such as in follow up cases or serial monitoring during or after treatment, where a patient baseline results are undertaken by another method. Finally, the study reaffirmed the importance of reporting the interrogation depth in MCAs haemodynamics assessment as significant differences between proximal and distal blood flow velocities exist.

Chapter 4

Study Two- A cross-sectional comparative study of cerebral arteries' morphological and hemodynamic features between post-stroke adult patients and age-matched non-stroke individuals.

4.1 Introduction

Stroke remains a leading global health concern, due to its high mortality and morbidity rates worldwide (Feigin et al. 2021), thus it poses a significant burden on the healthcare systems (Rajsic et al. 2019). This underscores the need for robust stroke prevention, early detection, and treatment intervention measures in a bid to curb the associated detrimental public health and economic burden associated with stroke. The early detection and prevention of stroke is pretexted on the accurate identification of independent stroke risk biomarkers and coming up with robust stroke predictive models. The independent biomarkers could additionally mediate as potential treatment efficacy and prognostic indicators. As stroke is a neurological condition mainly attributed to disturbances in the blood supply to the brain, cerebral arteries which are responsible for supplying blood to the brain are therefore key in stroke pathogenesis (Bersano and Gatti 2023).

Although, traditional risk prediction models, such as the Pooled Cohort Equations for cardiovascular disease (CVD) and the Framingham Stroke Risk Profile, have been instrumental in guiding clinical decision-making(Chun et al. 2021), it cannot be refuted that these models often rely on conventional risk factors such as age and hypertension status (Khera et al. 2020) to mention a few, which may not fully capture the complexity of stroke aetiology. Furthermore, Hassan et al. (2024), decried imbalanced and missing data to be among the key challenges in the identification of stroke risk factors and stroke treatment outcome indicators.

Despite medical imaging based cerebral arteries' morphological and hemodynamic characteristics such as vulnerable plaques characteristics and haemodynamic failure due to stenosis being established features with potential to significantly influence the risk of ischemic stroke (Esposito et al. 2007; Saba et al. 2014; Saba et al. 2024; Heck and Jost 2021), recent advancements in medical imaging modalities, particularly ultrasonography techniques coupled with machine learning algorithms have emerged as a promising way forward with potential to revolutionize and enhance stroke prediction, treatment efficacy monitoring and treatment prognostic prediction. Ultrasonography imaging provides a non-invasive, cost-effective, and readily accessible means to assess cerebrovascular health. In Chapter two of this thesis various emerging ultrasonography techniques and applications such as arterial stiffness analysis (Yuan et al. 2017; Yin et al. 2021), edge detection algorithms and 3-dimensional arterial analysis (Fresilli et al. 2022; Johri et al. 2020) that can offer reliable assessments of the cerebral arteries' morphological and haemodynamic status compared to manual methods are discussed. However, despite the potential usefulness of the new ultrasonography based cerebral arteries' structural and haemodynamic parameters as independent predictors of stroke risk and treatment efficacy indicators, there is still paucity of information on their clinical utility. The need to further explore the potential role of these variables as independent stroke predictor biomarkers and their subsequent use as indicators of aerobic exercise training efficacy in eliciting cerebral arteries' structural and haemodynamic features in post stroke patients cannot be overemphasised. By comparing the structural and functional features of cerebral arteries between post stroke patients and healthy non-stroke individuals, potential independent stroke risk biomarkers that could also posit as stroke treatment efficacy indicators in our main study highlighted in Chapter 5 could be derived. Additionally, establishing population-based stroke biomarkers is a priority.

Given this background, in an attempt to identify possible stroke risk biomarkers based on novel ultrasound-based applications such as 3D-carotid arterial analysis, enhanced edge detection carotid arterial stiffness analysis, and transcranial color-coded Doppler ultrasound (TCCD), the current study therefore sought to compare the morphological and hemodynamic features of the large intracranial and extracranial cerebral arteries between post stroke patients and age-matched controls without stroke Asians of Chinese origin. It was hypothesized that cerebral arteries' morphological and hemodynamic features between post stroke patients and age-matched non-stroke control subjects are significantly different from each other. By identifying additional potential stroke predictor and treatment efficacy indicator variables based on non-invasive ultrasonography techniques, the findings of this study have potential to contribute to the advancement of stroke prediction and treatment efficacy monitoring, thus ultimately improving patient outcomes and reducing the global burden of stroke-related morbidity and mortality.

4.2 Materials and Methods

4.2.1 Study design

This was a cross-sectional study aimed at comparing the large extracranial and intracranial cerebral arteries' morphological and haemodynamic features between community dwelling post-stroke patients and age-matched healthy non-stroke subjects carried out at the Y612, Ultrasound laboratory of The Hong Kong Polytechnic University. Ethical approval for this research project was obtained from the Institutional Review Board (IRB) of The Hong Kong Polytechnic University (Reference: HSEARS20220714001) (Appendix 1), and informed consent was obtained from the patients before the data collection process. Furthermore, a coding system was used to ensure confidentiality and anonymity of the research subjects.

4.2.2 Population and sampling technique

The study recruited consecutive post stroke adults' patients and healthy adults without stroke, TIA or any stroke mimics. The participants were recruited via an email call and posters send via social media platforms. The inclusion criteria in this study for both groups were consenting adults aged 50 years and above, dwelling in the Hong Kong community, whereas for the post stroke patients' group those in the chronic phase (≥ 6months) time of stroke onset were included. The exclusion criteria were subjects < 50 years old, not willing to give informed consent, and those allergic to ultrasound gel. Additionally, patients with TIA or stroke mimics conditions were excluded from the study.

4.2.2.1 Sample size calculation

Assuming a medium effect size of 0.5, α of 0.05, statistical power of 80%, this study targeted a sample size of 128 subjects (64 in each group) as calculated from the G* Power software using the t- test (Difference between two independent means).

4.2.3 Data collection methods and tools

The participant's demographics, anthropometric and medical history and data on the large intracranial and extracranial arteries haemodynamic and morphological features were collected at one period as follows:

4.2.3.1 Patient's demographic, anthropometric and medical history

The demographic and medical history data were obtained by completing the data collection sheets which were provided to the subjects, whereas data on anthropometric features weight, and height was measured by the investigator, before undertaking the ultrasound scanning procedures. The participants' blood pressure and heartrate were measured using an Omron (HEM-8712), automatic

blood pressure monitor (Omron healthcare manufacturing Vietnan Co.,Ltd.,Binh Duong, Vietnam). The participant was classified as hypertensive when either the systolic blood pressure (BPs) exceeded 140mmHg or had a clinical diagnosis of hypertension and receiving anti-hypertensive treatment, despite BPs being below 140mmHg.

4.2.3.2 Large intracranial and extracranial arteries haemodynamic and morphological features

Duplex carotid ultrasonography (DCUS) and transcranial color-coded Doppler ultrasound techniques were employed to examine the intracranial and extracranial cerebral arteries haemodynamic and morphological features, respectively. The ultrasonography scanning was performed by a sole experienced sonographer (S.TG) using a Samsung RS85 ultrasound machine (Samsung Medison Co., Ltd., Republic of Korea). The ultrasound scanner was equipped with a high frequency linear array transducer (2-14 MHz), and a single sweep volumetric transducer (3D Linear Probe, LV3-14A (sweep angle, 30 degrees, 3-14 MHz with the centre frequency at 6.8 MHz), the two probes which enabled the acquisition of both 2D and 3D images of the carotid arteries, respectively. In addition, the ultrasound system had an automated arterial analysis quantification program used for the automated assessment of CIMT, and semi-automated evaluation of carotid arterial stiffness indices. Moreso, a low frequency (1-5 MHz) phased array transducer accompanied the scanner and was utilized for the TCCD ultrasound protocol. Standardization of the ultrasound machine settings was done prior to conducting the study, and a user defined preset was generated to ensure consistency in the ultrasound scan settings throughout the study period. The parameters included, scan depth, overall gain, time gain compensation (TGC), and focusing, among others, and were standardized for pre- and post-intervention measurements (Appendix 2.)

The carotid ultrasound protocols involved the assessment of bilateral common carotid arteries (CCA), the carotid bulbs, and internal carotid arteries (ICA) in both the longitudinal and transverse scans using grayscale ultrasound. This was followed by colour, spectral Doppler analysis and finally 3-Dimensional carotid arterial analysis to evaluate the carotid plaque volume, vessel wall volume as well as the degree of carotid arteries 3D based lumen volume percentage (%) stenosis. The carotid arteries morphological features assessed apart from the 3D arterial analysis features highlighted above, included the carotid intima media thickness (CIMT), arterial stiffness parameters, whereas the carotid arteries and MCAs pulsed wave Doppler analysis parameters mainly- PSV, EDV, MFV, RI and PI were assessed.

Both the left and right carotid arteries of the subjects were examined with the patient in a supine position and the neck turned towards the contralateral side, whilst a pillow was placed under their shoulders to allow for neck hyperextension as illustrated in figure 4.1. Optimization of Doppler Imaging Parameters is presented in Appendix 2, the degree of carotid artery stenosis DCUS flow velocity-based protocol.

The specific technical specifications and protocols for each individual morphological and haemodynamic parameter are further discussed below.

Figure 4.1: Image showing the equipment setup and patient positioning whilst performing duplex carotid ultrasound examination and displayed on the screen is the Spectral doppler waveform of the Rt extracranial internal carotid artery (Rt ICA) of a 59year old male.

4.2.3.2.1 Carotid Intima-Media Thickness (CIMT)-2D arterial analysis

The automated arterial analysis quantification program in the Samsung RS85 ultrasound machine was used to measure carotid intima-media thickness (CIMT). The CIMT was measured as the distance between the media-adventitia boundary (MAB) and the Lumen-Intima boundary (LIB) of a 1cm long segment in the distal common carotid artery, located at the far wall and at carotid bulb inferior margin. To ensure consistency in the location of the arteries, this region of interest (ROI) was maintained across all the CIMT measurements. Each of the two common carotid arteries (Left and Right) was scanned three times and the median of the three measurements was used to represent the CIMT for each of the arteries. The CIMT was measured as shown in Figure 4.2.

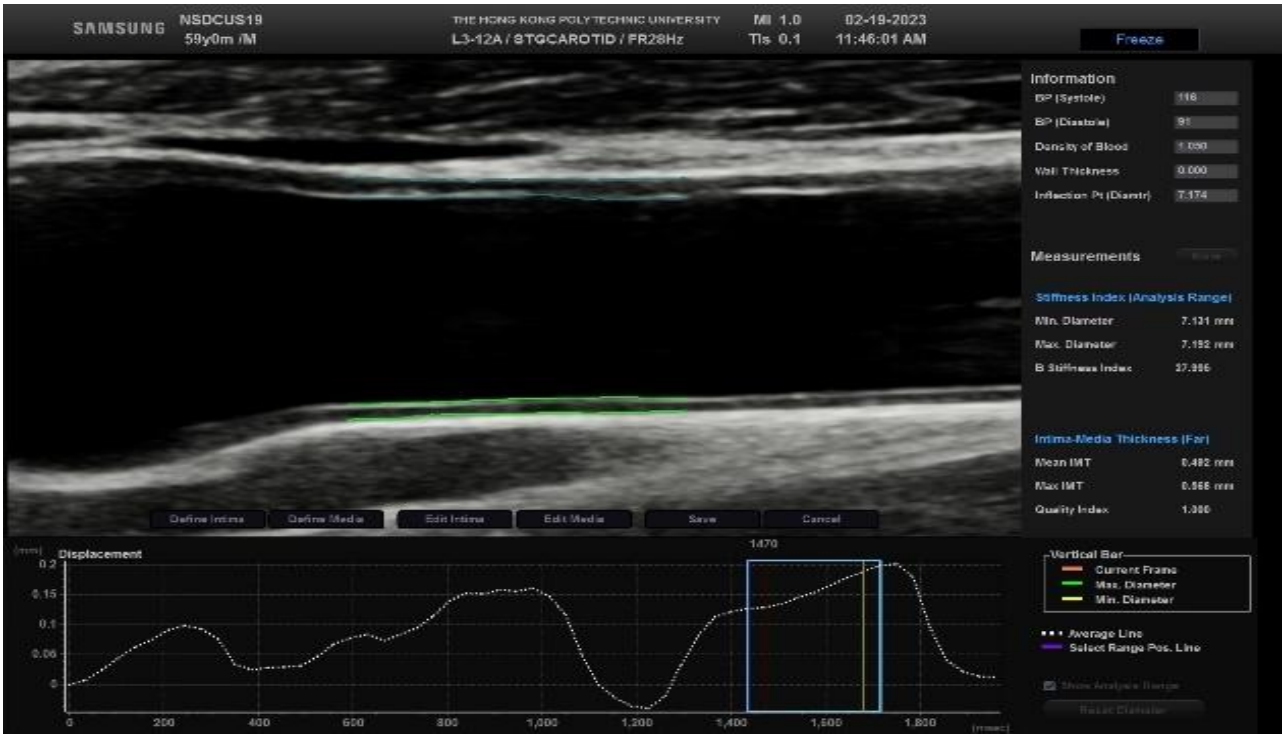


Figure 4.2: Gray scale DCUS longitudinal section image of the Rt distal common carotid artery (Rt DCCA) of a 59year old male demonstrating the measurement of CIMT, in the far field (between two parallel green lines) using an Automated Arterial Analysis softwares on a Samsung ultrasound machine, Adapted from. Own images The Hong Kong Polytechnic University ultrasound laboratory, 2023.

4.2.3.2.2 Carotid arterial stiffness (CAS)

This was measured using the semi-automated arterial analysis software on the Samsung RS85 ultrasound machine. The ultrasound transducer probe was placed with its upper end at the inferior margin of the carotid bulb in the distal common carotid artery (similar to CIMT), and the CAS was evaluated by contouring two parallel lines over the far and near wall of a 1cm long segment as shown in Figure 4.3a. The various CAS parameters including the Beta stiffness index (CAS β-stiffness index), Pulse wave velocity (CAS PWV), elastic modulus of elasticity (CAS kPa), carotid compliance (CAS CC) and distensibility coefficient (CAS DC) were automatically computed and

displayed on the ultrasound machine (Figure 4.3b), after initially inputting the systolic and diastolic blood pressure measurements of the subjects. This method assumed a constant blood density (ρ) of 1.050, and the mathematical representation of the various CAS parameters was informed by (Yuan et al. 2017) as shown in equations below. Higher values of the CAS β-stiffness index, PWV, kPa, represents stiffer arteries (Li et al. 2017) and contrarily, higher CAS CC and DC values define better arterial elasticity.

β- Beta stiffness index=$\frac{\mathrm{Dd.ln(SBP/DBP)}}{(\mathrm{Ds}-\mathrm{Dd})}$ $eqn1.$

α- Alpha stiffness index= $\frac{\mathrm{Ad.ln(SBP/DBP)}}{(\mathrm{As}-\mathrm{Ad})}$ $eqn\ 2.$

CC-Carotid compliance = $\frac{\Delta \mathrm{D}}{\Delta \mathrm{P}}\left(\frac{\mathrm{mm}}{\mathrm{kPa}}\right)$ $eqn\ 3;$

DC-Distensibility Coefficient= $\frac{\Delta \mathrm{A}/\mathrm{Ad}}{\Delta \mathrm{P}}$(1/KPa) eqn 4.

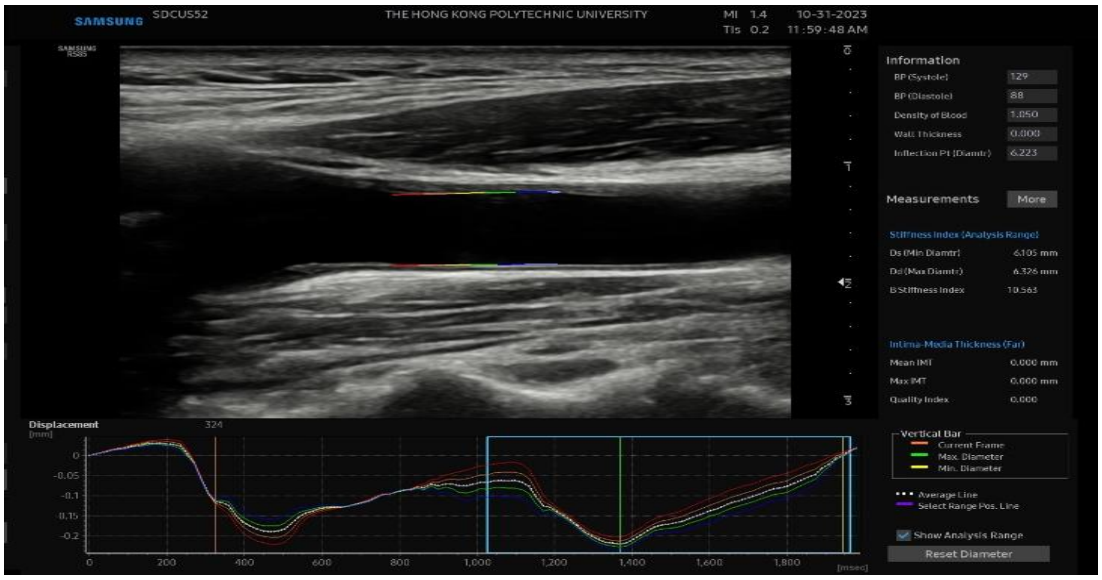
E -Elastic modulus (kPa)= stress/strain $\frac{P}{\Delta \mathrm{D}/\mathrm{Dd}}$ eqn 5.

PWV-Pulse wave velocity = $\sqrt{\frac{\alpha.\mathrm{DBP}}{\rho}}$ (m/s) eqn 6.

Strain = $\frac{\Delta \mathrm{D}}{\mathrm{Dd}} x100(\%)$ $eqn\ 7;$ where

ρ -blood density constant (1.050); ΔD, ΔA, ΔV, and ΔP are the stroke change in the lumen diameter, area, volume, and blood pressure respectively. Min. D-Minimum vessel diameter Max. D-Maximum vessel diameter; SBP-Systolic blood pressure; DBP-diastolic blood pressures; Dd-Diastolic diameter; Ds-Systolic diameter; As-Systolic lumen area; Ad-Diastolic lumen area.

a.



b.

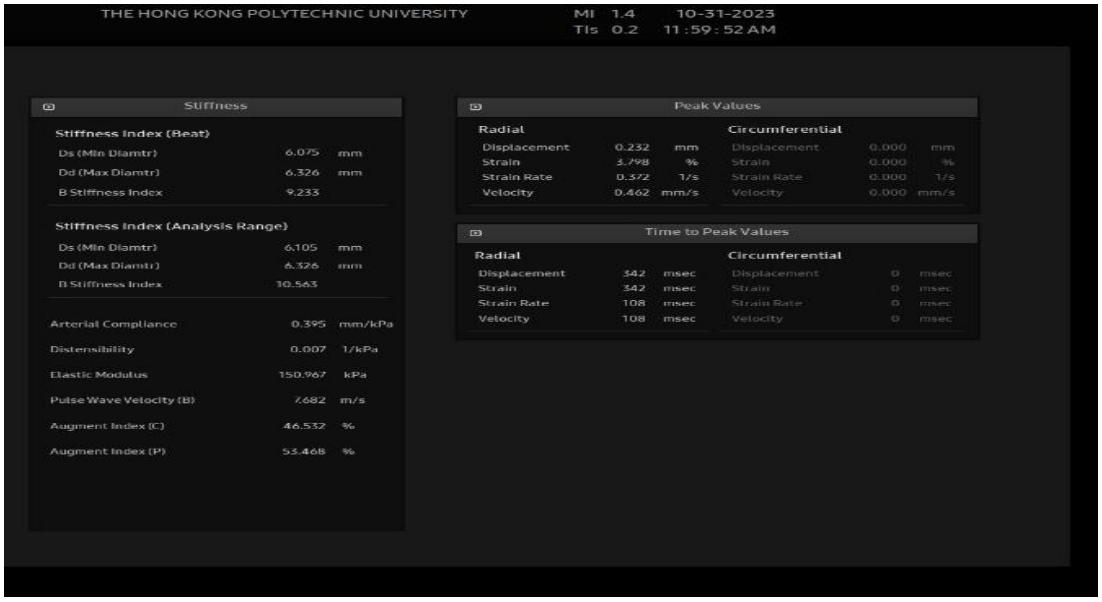


Figure 4.3: a.) Image showing the measurements of Rt Carotid arterial stiffness at a 1cm long segment ROI, starting at the inferior margins of the carotid bulb and between the near and far walls of distal common carotid artery (DCCA), b.) The corresponding Carotid arterial stiffness indices full report, SDCUS52

4.2.3.2.3 Carotid Plaque Characterisation

Carotid plaques are defined as focal structures that encroach on the arterial lumen by 50% of the surrounding CIMT or structures > 1.5mm (Johri et al. 2020). The plaque characteristics assessed using DCUS included the (plaque size (height, area & volume), plaque surface, echogenicity, intraplaque haemorrhage, calcifications and ulcerations).

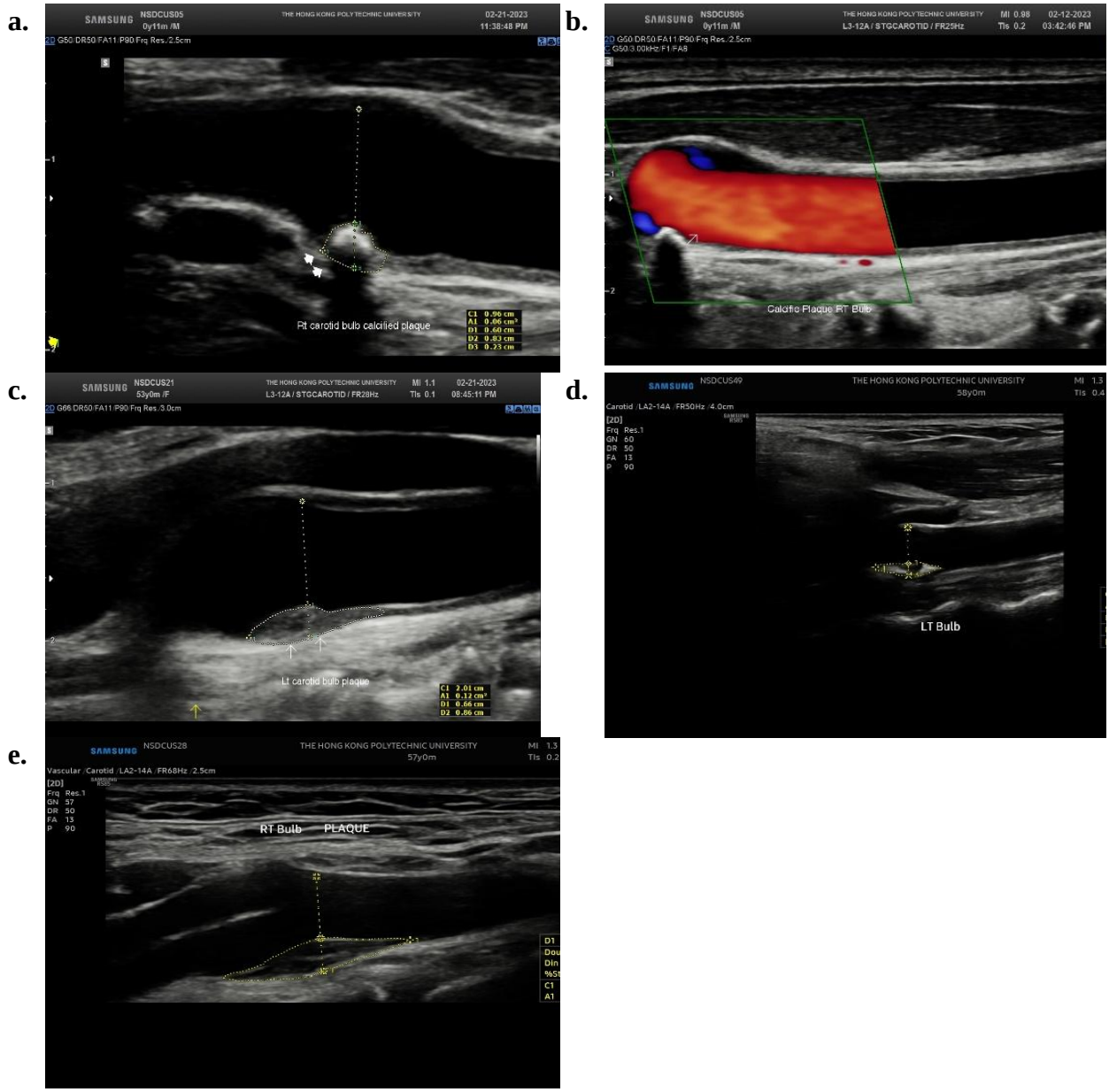


Figure 4.4(a-e): a.) Longitudinal section of the RT carotid bulb showing a calcified plaque with posterior shadowing in one of the subjects with posterior shadowing. b.) color doppler showing a

filling defect due to the plaque. c. suspected intraplaque haemorrhage in the lt bulb plaque d.) suspected ulceration with irregular fibrous cap. e.) image showing a predominantly hypoechoic plaque in the Rt bulb.

4.2.3.2.4 Three-dimensional arterial analysis

The 3D single-point acquisition protocol was used to acquire the 3D datasets, including the 1.) overall percentage volume stenosis, being the percentage reduction in lumen volume, 2.) vessel wall volume, and 3.) plaque volume. This protocol involved holding the 3D Linear transducer, LV3-14A still at a single fixed position corresponding to the inferior margins of the carotid bulb in the distal common carotid segment for about 5 seconds while the subject was in arrested inspiration. The 3D image acquisition was done using a 0.5mm slice thickness selection, a sweep angle of 30 degrees, and transducer centre frequency at 6.8 MHz).

This protocol facilitates land-marking and identification of an individual lesion that can be serially tracked with repeated measurements. The selection of the inferior margin of the carotid bulb was informed by previous studies which concluded that the bulb represented the major site of involvement in atherosclerotic stenosis (Mughal et al. 2011) and whereas for consistence the inferior margin can be easily and consistently visualised. In the event of visible plaque, a second sweep centered at the plaque area was undertaken. The patient positioning is like the one shown in figure 4.1, whilst the resultant 3D-arterial analysis output is presented in figure 4.5. The image quality optimization strategy was also the same as in grey scale imaging of the CIMT, however the gain was lowered slightly by about 10% from that used during CIMT acquisition to avoid the reverberation artifact being contoured as plaque region.

a.

b.

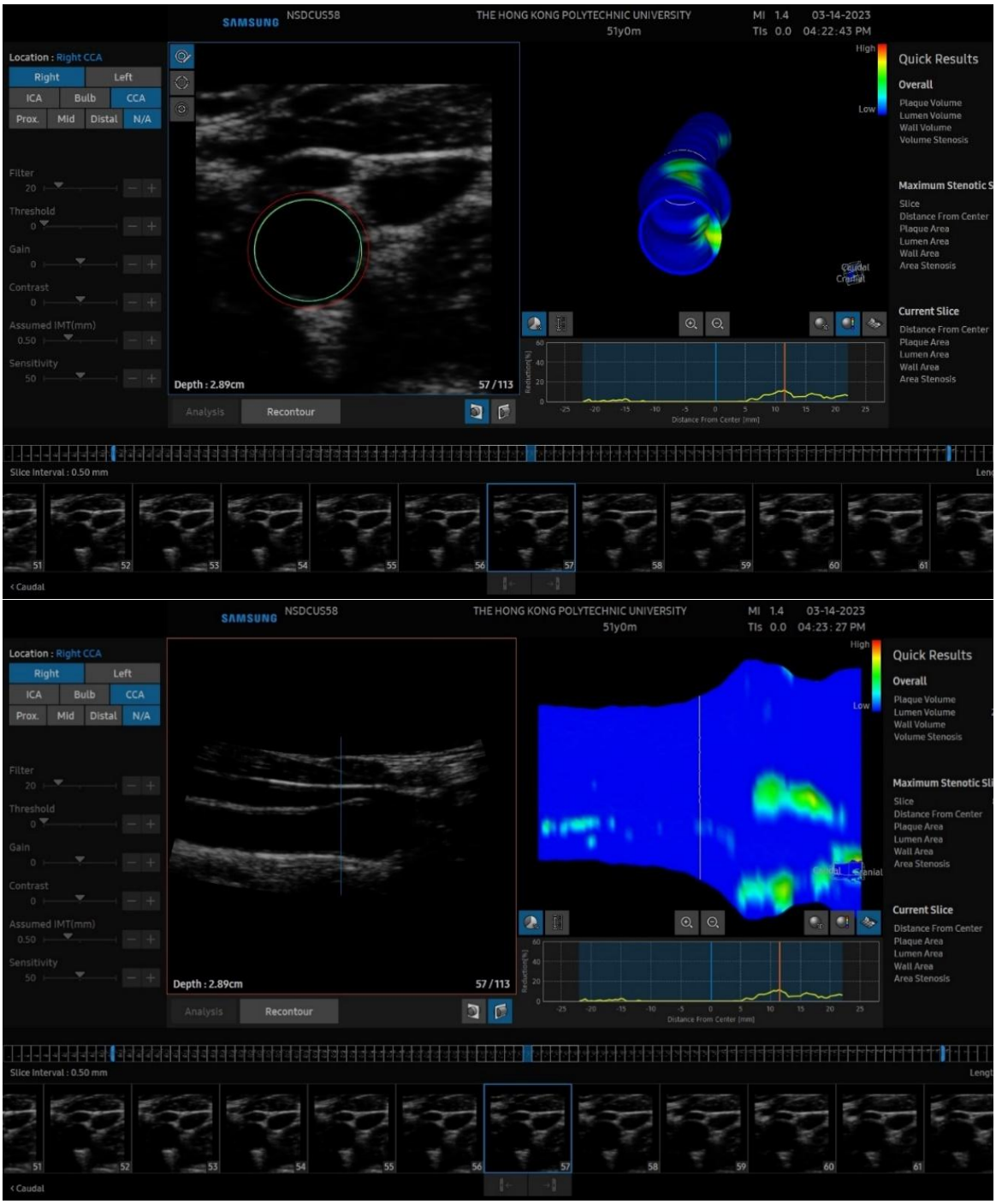
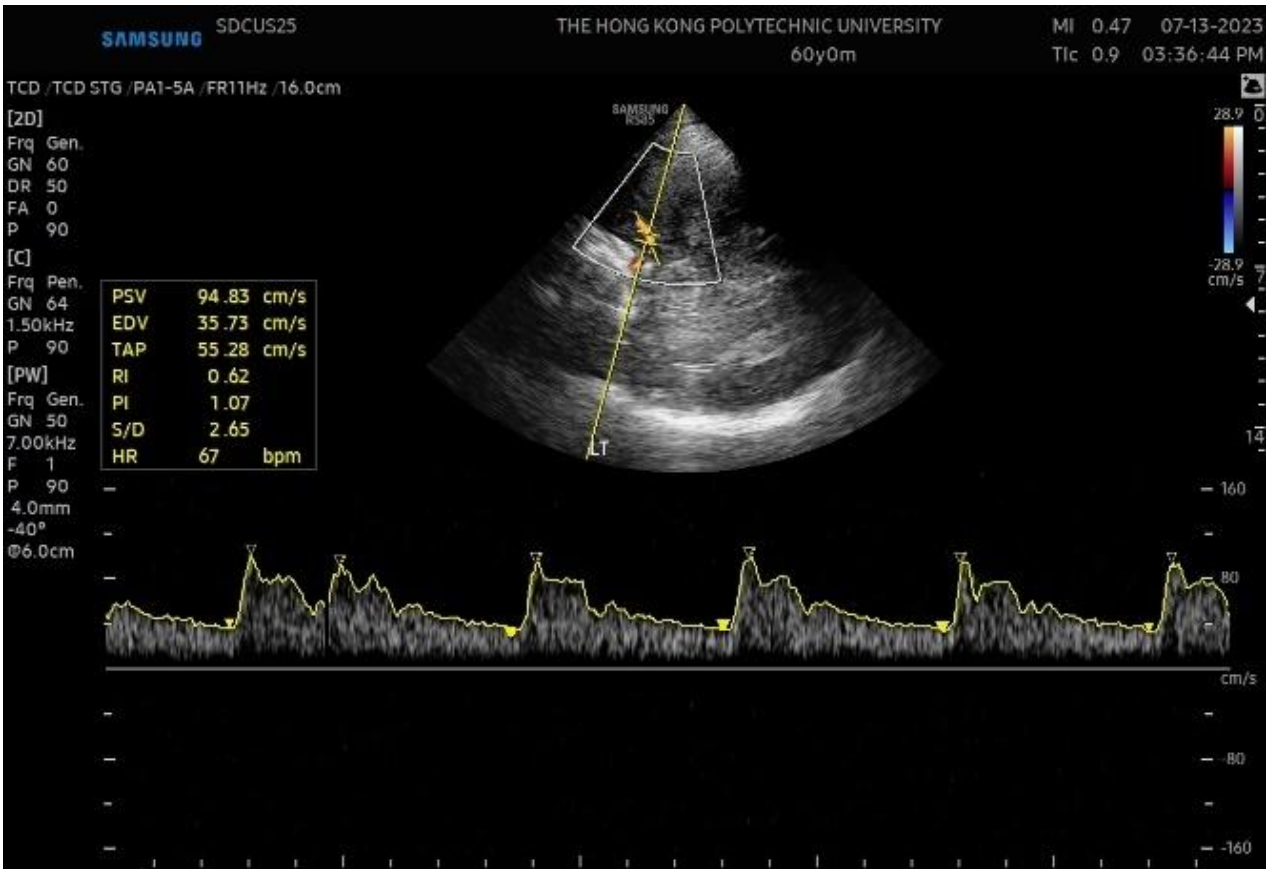


Figure 4.5: Ultrasound images of a 51year old adult non-stroke subject demonstrating the level at the single point acquisition protocol was applied during the 3D arterial analysis acquisition and the corresponding colorimetric map, a. Transverse section, b.) longitudinal section

4.2.3.2.5 Transcranial color-coded Doppler (TCCD) Ultrasonography

The TCCD protocol involved the scanning of the middle cerebral arteries through the transtemporal windows as described in a previous study highlighted in chapter 3 conducted by our research group (Gunda, Ng, et al. 2024). In the present study only TCCD with angle correction (cTCCD) technique was adopted for the measurement of MCA haemodynamic parameters as it was reported to be more accurate in the previous study one highlighted in Chapter 3, which was conducted by our research group. To ensure the MCA haemodynamic parameters were measured whilst the study participants were in a resting state, all participants were allowed at least 10 minutes of rest whilst seated on a comfortable chair before the TCCD ultrasound examination. The patient positioning and probe orientation during transcranial color-coded Doppler ultrasound scanning of the MCAs is shown in Figure 4.6

a.)



b.

Figure 4.6: a.) A picture showing the patient positioning and probe orientation during transcranial color coded doppler ultrasound scanning of the middle cerebral arteries on a post stroke subject, b.) resultant spectral Doppler waveform and haemodynamic parameters

4.2.4 Data analysis

An inter-group comparison between the post stroke and age matched non-stroke adults' cerebral arteries haemodynamic and morphological features was performed using SPSS version 26 software. Continuous data were expressed as means ± standard deviation (SD) and categorical data was expressed as frequencies (%). The data normality and homogeneity of variance were checked using the Kolmogorov Smirnov test and Levene's test respectively. The Independent t-test or non-parametric equivalent Mann-Whitney U test were used to assess any significance in differences

between continuous variables in the two groups, depending on whether assumptions of normality and homogeneity of variance were met. The mean difference (MD) in the continuous variables between the two groups and corresponding 95% confidence intervals (CI) were calculated to represent the main outcome measures, and the corresponding p-values. The Chi-squared test ($\chi 2$) was utilised to compare differences for categorical data or Fishers Exact test when more than 20% of cells had frequency count less than 5. Statistical significance level was set at $p < 0.05$, and all tests were two sided. The results were displayed in either tabular, or graphical formats.

4.3 Results

4.3.1 Demographic characteristics of the study participants (Post stroke versus non-stroke Group).

A total of 124 consecutive participants consisting of 57(46%) post-stroke patients (male=29, females=28, p=0.895) and 67 (54%) non-stroke healthy adults (male=22, female=45, *p=0.005) met all the inclusion criteria and were enrolled in this study between January and February 2024 (Figure 4.7).

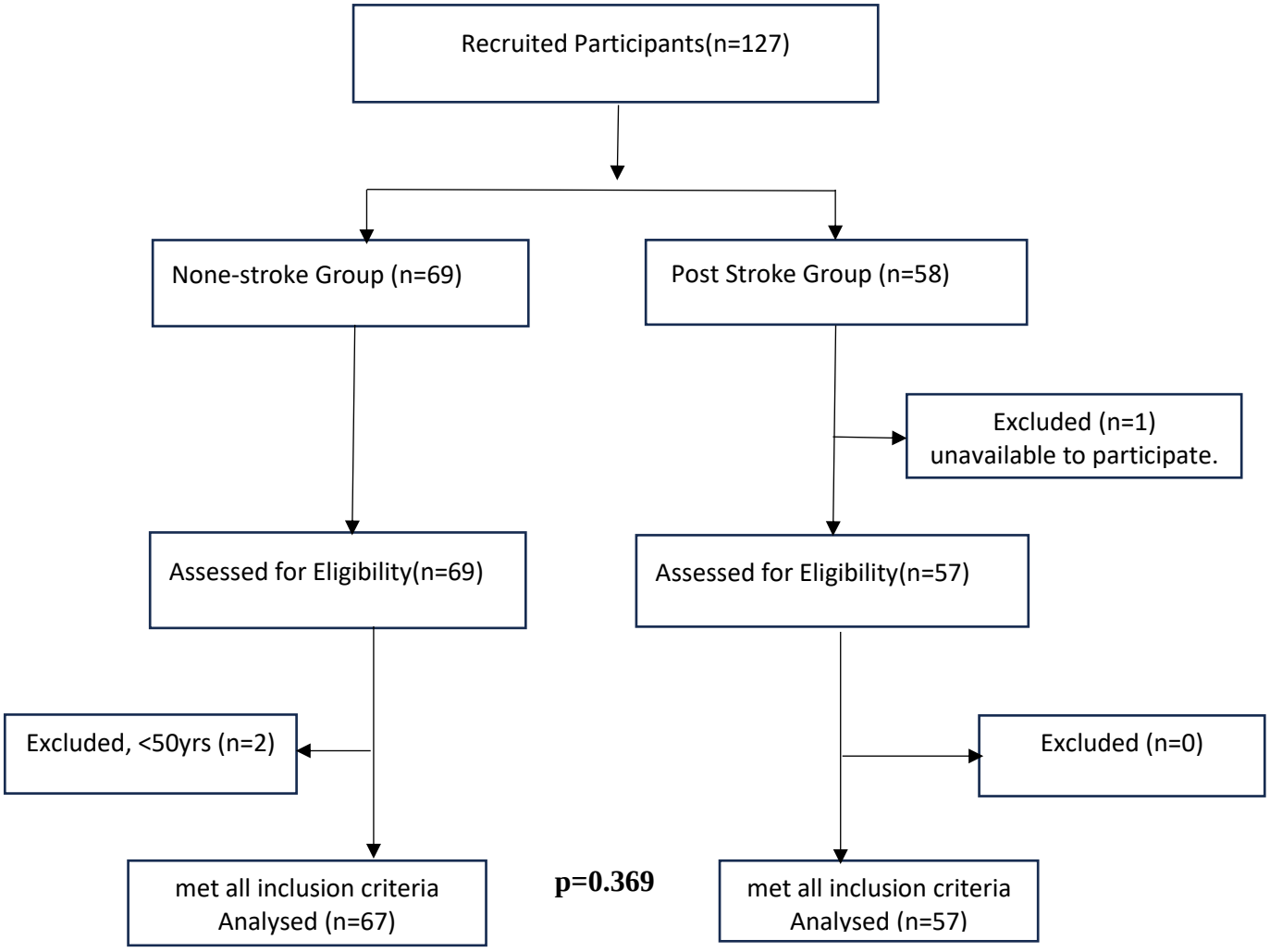


Figure 4.7: Study Participants Selection Process.

Additionally, the proportions of post stroke subjects, and non-stroke subjects enrolled were not significantly different from each other (Chi-squared test statistics=0.806; df=1; p=0.369), however a significant gender difference was observed between the two groups p=0.042 (Table 4.1). The combined groups' participants' age ranged from (50-84) years, and the mean age was 62.5 ±6.9 years. Despite enrolling participants 50years and above, the post stroke group's participants were significantly older compared to the non-stroke participants with mean age of (64.5 ± 7.2 years and 60.8 ± 6.1 years, p=0.002) respectively. There was however no significant difference between the two group's remaining continuous demographic characteristics mainly weight, height, BMI, BPs, BPd and HR (Table 4.1).

Furthermore, it was observed in this study that amongst all hypertensive(n=51) participants across the two groups, majority of them 41(80%) were from the post-stroke group whereas the remaining 10 (20%) were non stroke individuals. The between group difference in the hypertensive status was significant (χ2-stats=41.33; df=1; *p<0.001). The within group percentages of hypertensive participates in the non-stroke and post stroke groups were 15% (n=10, p<0.001) and 72% (n=41, p=0.001), respectively. Similarly, significant differences in the proportions of those who had hyperlipidaemia was observed between the two groups with a greater percentage of the hyperlipemia participants associated with the post stroke patients 35(74.5%) versus 12(25.5%) in non-stroke, (χ2stats=24.753; df=1; *p<0.001). The observed within group percentages of those with hyperlipidemia in the post stroke group was 35(61.4%) versus 12(18%) in the non-stroke group. In addition, the post stroke group contributed the largest percentages of all the diabetic participants 17(74%) versus 6(26%) in the non-stroke group (χ2 - stats=8.88; df=1; *p=0.003), although they were generally lower within group percentages of those with diabetes which were 17(30%) in post stroke versus 6(9%) in non-stroke individuals. The incidence of previous smoking

history was rare with only a single participant reported to in each group to have a previous history of smoking. The combination of hypertension and hyperlipidemia had the highest count in the post stroke group consisting of 26(45.6%) participants compared to only 1(1.5%) participant in the non-stroke group. Similarly, the presence of the 3 factors (hypertension, diabetes and hyperlipidemia) was higher in the post stroke group 10(17.5%) compared to only 1(1.5%) in the non-stroke group. The results of these demographic and clinical characteristics are presented in Table 4.1.

Table 4.1: A comparison of Demographic characteristics of the study participants (Post stroke versus non-stroke Group)

Baseline characteristic		Group		
		Post stroke (n=57)	**Non-stroke (n=67)**	**Between Group, p values**
Gender	Male	29 (51), p=0.895	22(33), p=*0.005	χ2–stats4.14, p=0.042
	Female	28(49)	45(67	
Age (years)		64.5 (7.2)	60.8(6.1)	0.002*
Weight		61.9(12.1)	61.2(10.6)	0.750
Height (cm)		161.7(9.1)	162.5(7.3)	0.566
BMI ((kg/m^2)		23.6(3.7)	23.1(3.0)	0.368
BPs (mmHg)		127(16.3)	124(15)	0.502
BPd(mmHg)		78(9)	78(10)	0.742
HR (bpm)		73(11)	77(11)	0.294
Hypertension	Yes	41(72)	10(15)	<0.001*
	No	16(28)	57(85)	
Hyper-lipidemia	Yes	35(61)	12(18)	<0.001*
	No	22(39)	55(82)	
Diabetes mellitus	Yes	17(30)	6(9)	0.003*
	No	40(70)	61(91)	
Smoking history	Yes	1(2)	1(1)	1.00
	No	56(98)	66(99)	
Type of stroke	Ischemic	37(65)		0.024*
	Hemorrhagic	20(35)		
Stroke Onset time (yrs)		6.5(6.9)		
Age at stroke onset (yrs)		58 (9.8)		
Recurrence	Yes	3(5.3)		<0.001*
	No	54(94.7)		
Affected Side	Left side	28(49)		<0.001*
	Right side	26(45)		
	Bilateral	3(5)		

BPs—Systolic Blood Pressure; BPd—Diastolic Blood Pressure, BMI— Body Mass Index, HR—Heart rate, Age of stroke onset (yrs) —age at which the first stroke occurred, Stroke Onset time (yrs) —stroke onset to ultrasound assessment time , *p <0.05—significant level, Continuous data is expressed as mean (S.D) and independent t-test p-values are displayed, categorical data is expressed as frequencies (%), and p-values are based on Pearson χ2 -Chisquared test or Fishers Exact test when more than 20% of cells have frequency count less than 5.

In the current study Ischemic stroke was observed to be the most prevalent stroke type accounting for 37(65%) of all the strokes, whereas haemorrhagic stroke accounted for the remaining 20(35%) and the differences in proportions was significant (Chi-squared test statistics=5.07; df=1; p=0.024). Although there were more males 29(51%) with stroke compared to women n=28(49%), the prevalence of stroke type was not significantly different across gender (Chi-squared test statistics=2.459a; df=1; p=0.117) (Figure 4.8). The mean age at stroke onset was 58±9.8years, and ranged between 19 to 74 years, whilst the stroke onset to the ultrasound assessment time ranged from 0.58 to 41 years, with a mean time of 6.5±6.9 years. In this study, nearly half of the patients, 28 (49%), exhibited hemiparesis on the left side, while 26 (45%) demonstrated right-sided hemiparesis. A small fraction, 5%, experienced bilateral effects from their stroke. There was however no significance side difference to the occurrence of hemiparesis observed (p=0.785). Furthermore, stroke recurrence was observed in a minimal portion of the cohort, with only 3 (5.3%, p<0.001) patients experiencing a second stroke. These findings align with previous research indicating a relatively balanced distribution of hemiparesis on either side of the body among stroke patients, and a low incidence of stroke recurrence within a certain timeframe post-initial stroke.

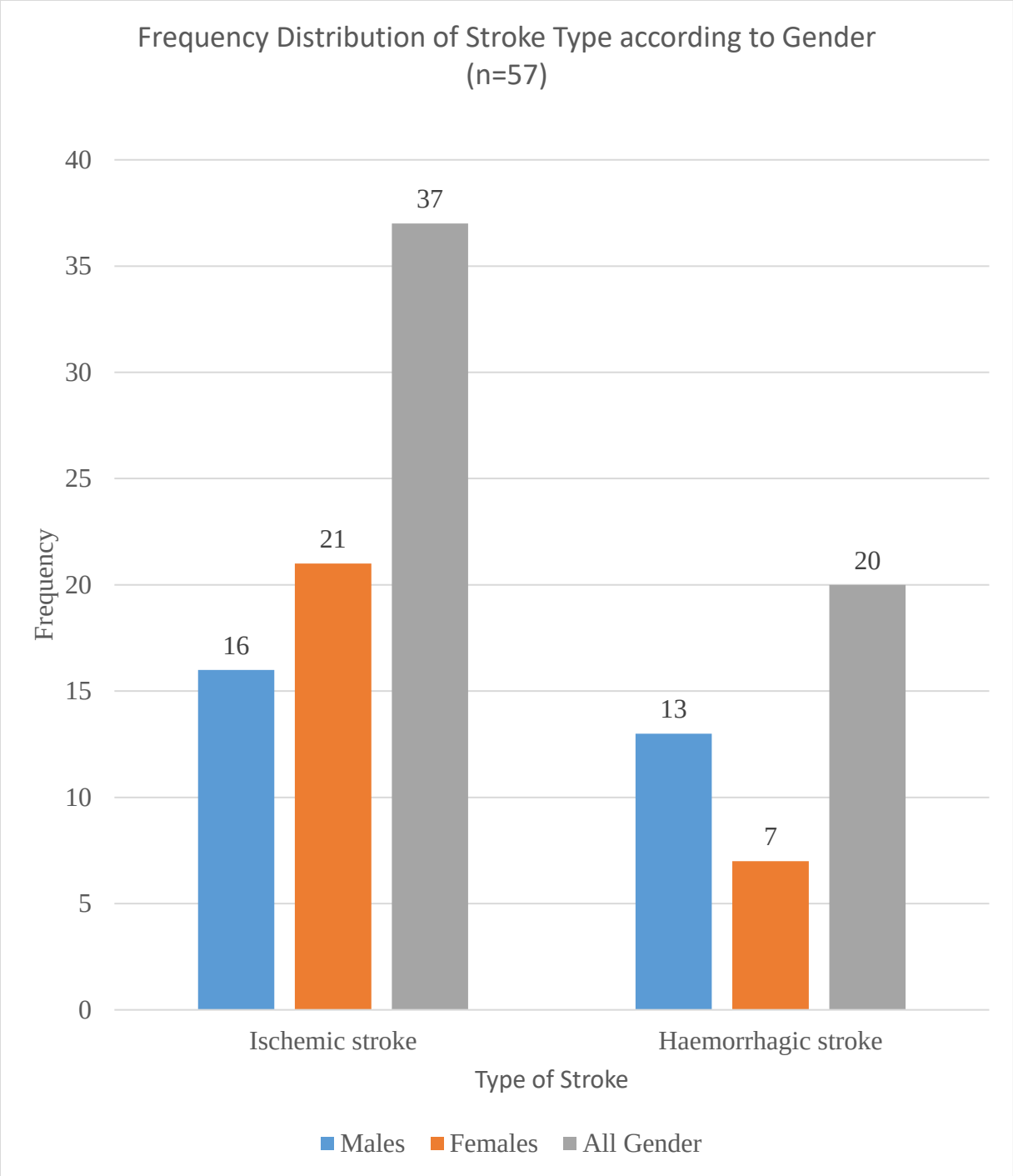


Figure 4.8: Histogram showing the Frequency Distribution of Stroke Types according to Gender.

4.3.2 Comparison of Morphological Features between Post stroke vs Non-Stroke Groups.

4.3.2.1 Carotid Intima media thickness (CIMT).

The CIMT measured over a 1cm long region of interest (ROI) in all the 134 distal common carotid arteries (DCCA) segments in the individuals without stroke (n=67) ranged from 0.47 to 1.6 mm, with a mean CIMT of 0.69±0.15mm. The corresponding range and mean CIMT values when all Post stroke patients were included (n=57) across the 114 DCCA segments were 0.54 to1.7mm and 0.83±0.2mm, respectively. There was a significant difference in the observed mean CIMT between the two groups, with higher CIMT values reported in the post stroke group compared to those without stroke (Mann-Whitney U, p<0.001) (Figure 4.9).

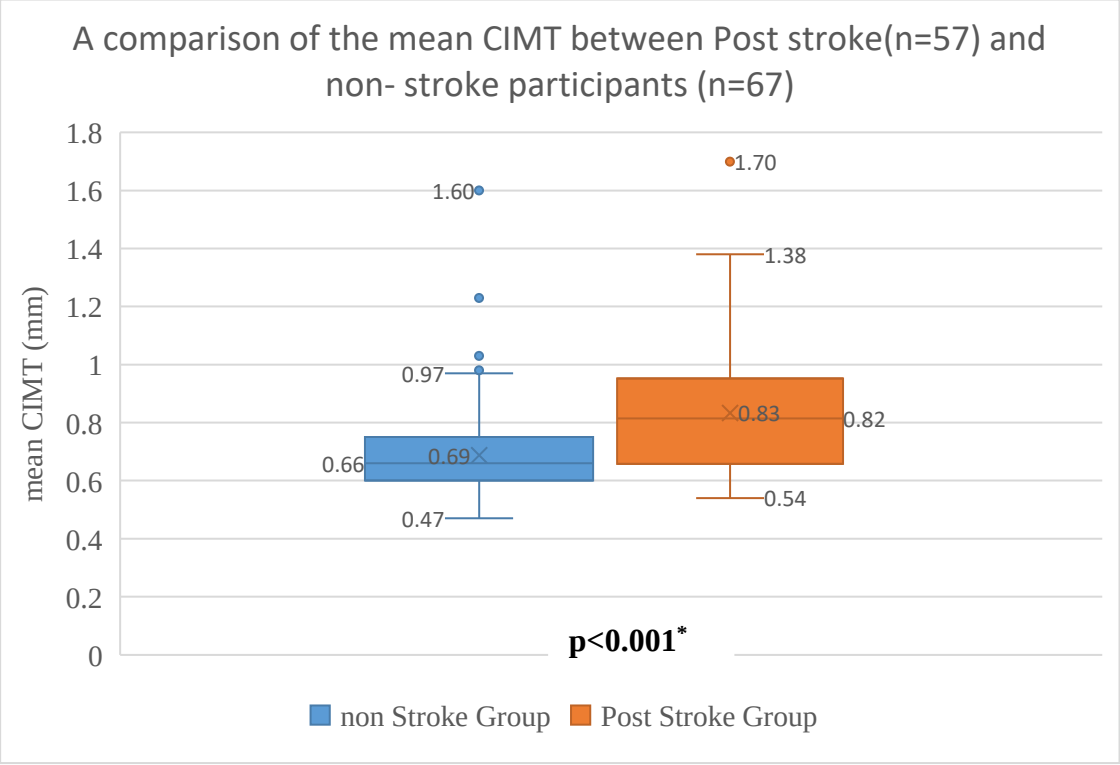


Figure 4.9: Box Plots showing carotid intima media thickness (CIMT) between the Post stroke and non-stroke participants Groups

The study results on CIMT based on a gender subgroup analysis within each group showed that the mean CIMT for males (n=58 carotid arteries') and females (56 carotid arteries') in post stroke group were 0.88 ±0.22 and 0.78± 0.17mm, whilst in individuals without stroke the corresponding mean CIMT for males (n=44 carotid arteries') and females (90 carotid arteries') were 0.72±0.13 and 0.68 ±0.18mm, respectively. The mean difference between the mean CIMT of the two gender groups in the post stroke group was statistically significant (MD= 0.1mm; p=0.004*), whereas the non-stroke group males and female had marginally different mean CIMT values (MD=0.05, p=0. 201) (Table 4.3). A significant between group difference was observed in mean CIMT across gender when post stroke and those without stroke groups were compared, in both males to males, and female to female comparisons the Mann Whitney U, $^{+}$p values were less than 0.001(Table 4.2). Furthermore, when the CIMT was analysed based on laterality, the observed mean CIMT for the left side in post stroke patients and those without-stroke was 0.85± 0.22, and 0.7± 0.17, p<0.001) respectively, whilst the right sided CIMT in the respective post stroke patients and the non-stroke groups were (0.81±0.18, and 0.68± 0.13, p<0.001).

Moreover, the CIMT was further analysed according to the stroke subtype. The results demonstrated that the mean CIMT in the ischemic stroke patients (n=74 vessels) was higher compared to those with haemorrhagic stroke (n=40 vessels) (0.84±0.22mm versus 0.82±0.17mm), however the difference was not statistically significant(p=0.505). The results for the subgroup analysis are summarised in Table 4.2 and box plots in Figure 4.10 (a—d).

Table 4.2: Summary of the subgroup analysis comparing the mean CIMT across Gender, Laterality, and Stroke Type.

Carotid Intima Media Thickness-CIMT (mm)							
			Non-Stroke Group (n=67, Males=22, Females=45)		**Post Stroke Group** (n=57, Males=29, Females=28)		**Between Grp**
				Within Grp		**Within Grp**	**p-value**
Mean CIMT (mm)	**Gender**	Male	0.72(0.13)	MD=0.05	0.88(0.22)	MD=0.1	$^{+}p<0.001^{*}$
		Female	0.67(0.15)	p=0.201	0.78(0.17)	p=0.004*	$^{+}p<0.001^{*}$
	Laterality	Left	0.7(0.17)	MD=0.02	0.85(0.22)	MD=0.0	$p<0.001^{*}$
		Right	0.68(0.13)	p=0.373	0.81(0.18)	4 p=0.255	$p<0.001^{*}$
	Type of Stroke	Ischemic			0.84(0.22)		
		Haemorrhagic			0.82(0.17)	p=0.505	

MD— mean difference (male -female) CIMT values, +p—Mann Whitney U, *p<0.05—significant level, The CIMT (mm) is expressed as mean (SD)

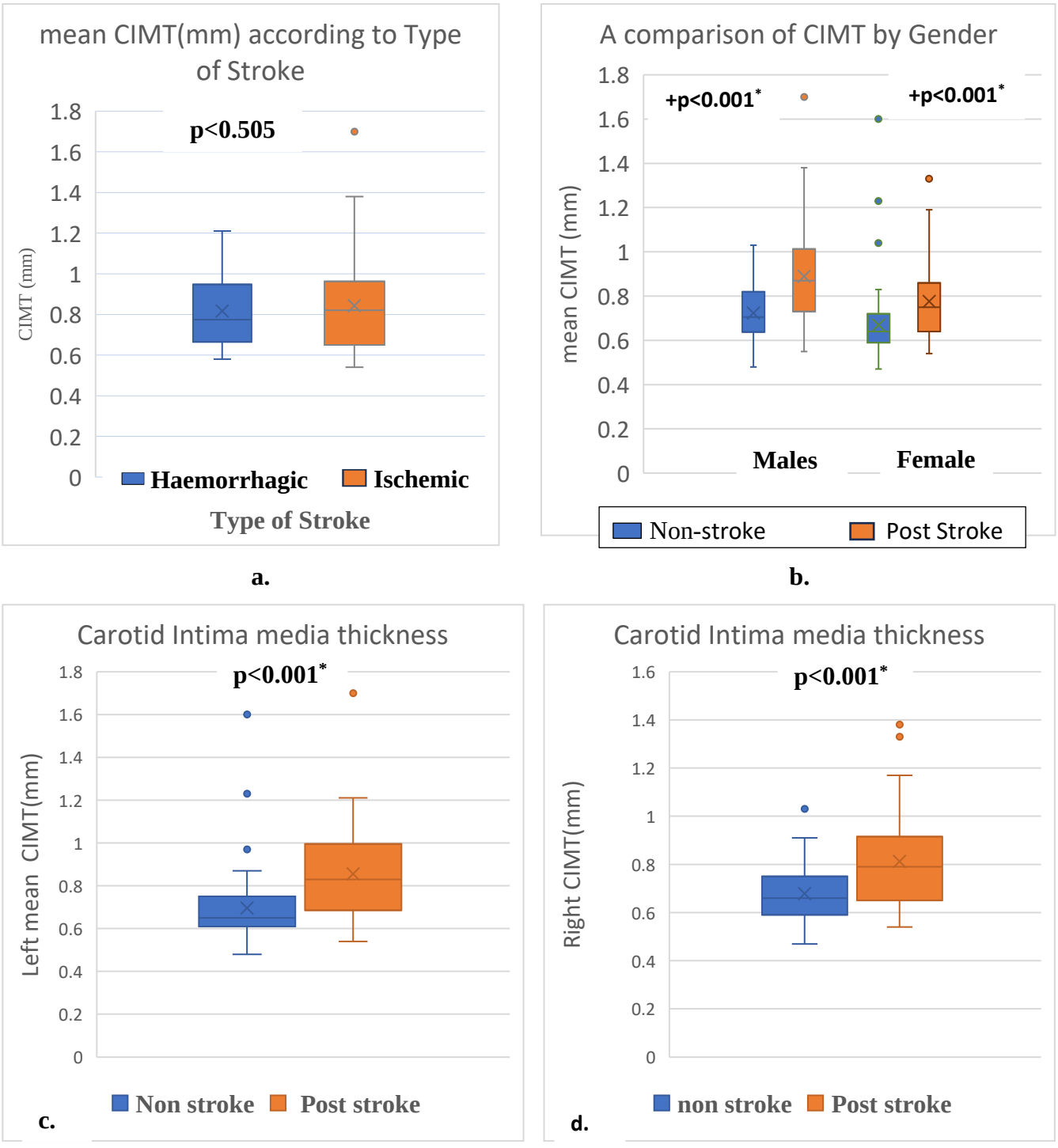


Figure 4.10: Box plots showing the mean CIMT for the post stroke and non-stroke participants groups a.) CIMT according to stroke type, all Participants, b.) mean CIMT by Gender c.) a comparison between mean CIMT of post stroke and non-stroke by laterality (left side), d.) comparison between mean CIMT of post stroke and non-stroke by laterality (right side)

4.3.2.2 Carotid arterial stiffness.

The research findings on the comparison of Carotid arterial stiffness (CAS) parameters between the post stroke and those without stroke are displayed in Table 4.3. The Carotid arterial stiffness indices measurements across 134 carotid arteries were taken from a similar ROI as the CIMT previously described. The β-stiffness index, Elastic modulus (KPa), and Pulse wave velocity for post stroke compared to those without stroke were (15.8±26.7 versus 9.3±7.7, p=0.013*; 208.9± 333 kPa versus 123.7± 112kPa, p=0.006*, and 7.8± 3.9m/s versus 6.5± 2.2 m/s, p=0.002*), respectively. The significantly higher values of these 3 indices in post stroke patients compared to those individuals without stroke are indicative of increased carotid arterial wall stiffness in the post stroke group compared to individuals without stroke.

The carotid compliance (CC) and distensibility coefficient (DC) for post stroke group compared to those without stroke were (0.476±0.27 versus 0.739±0.67, p<0.001*; and 0.009 ± 0.006 versus 0.013±0.014, p=0.003*), and similarly these results demonstrated higher elasticity properties in individuals without stroke in comparison to the post stroke patients. In all CAS parameters the assumption of homogeneity of variance was not met hence all p-values are based on non-parametric Mann-Whitney U Test) as depicted in Table 4.3.

Table 4.3: A comparison of Carotid arteries' stiffness indices between the Post Stroke patients and non-stroke individuals.

Carotid arteries Stiffness Parameters (Post Stroke Group n=114, Non-stroke Group n=134, vessels)				
	Stroke Status Grouping	**Mean (SD)**	**MD 95%, CI**	**p-values**
CAS β	Post Stroke	15.8 (26.7)	6.51, (1.7; 11.3)	0.013*
	Non- Stroke	9.3 (7.7)		
CAS CC (mm/kPa)	Post Stroke	0.476 (0.27)	-0.26, (-0.4; -0.13)	<0.001*
	Non- Stroke	0.739 (0.67)		
CAS DC(1/kPa)	Post Stroke	0.009 (0.006)	-0.004(-0.007; -0.001)	0.003*
	Non-Stroke	0.013(0.014)		
CAS kPa	Post Stroke	208.9(333)	85.2, (24.8;145)	0.006*
	Non- Stroke	123.7(112)		
CAS PWV(m/s)	Post Stroke	7.8(3.9)	1.3, (0.5;2.1)	0.002*
	Non- Stroke	6.5(2.2)		

n —number of carotid arteries, CAS β— Carotid artery β-stiffness index, CAS CC—Carotid artery compliance (mm/kPa), CAS DC —Distensibility Coefficient (1/kPa), CAS kPa —Elastic modulus (KPa), CAS PWV(m/s) — Pulse wave velocity (m/s), MD —represents Mean Difference i.e (Post stroke minus without stroke) values, *Positive MD in CAS β, CAS kPa, and PWV values represents higher arterial stiffness in post stroke patients compared to individuals without stroke, whilst a negative MD in Carotid CAS CC (mm/kPa) and CAS DC(1/kPa), correspondingly implies a higher stiffness or lower elasticity values in post stroke compared to non-stroke. p-values in all stiffness parameters are based on non-parametric Mann Whitney U test.

4.3.2.2.1 Carotid artery stiffness across Gender across in the post stroke and non- stroke group

The carotid arteries' stiffness indices across gender in the post stroke and non- stroke groups are displayed in Table 4.4. The gender-based analysis reviewed no significant gender difference across all the CAS parameters within the post stroke and non-stroke groups (p>0.05), (Table 4.4)

Table 4.4: A comparison of the Carotid artery stiffness parameters across Gender in post stroke and non- stroke group.

		Non-Stroke Group (n=134, Males=44, Females=90)	**Within Grp, p**	**Post Stroke Group** (n=114, Males=58 Females=56)	**Within Grp, p**	**Between Grp p-value**
CAS β	Male	10.2(10.30	0.369	14.2(15.8)	0.522	0.141
	Female	8.9(6.2)		17.4(34.7)		0.023
CAS CC (mm/kPa)	Male	0.79(0.54)	0.535	0.47(0.27)	0.797	0.001
	Female	0.71(0.73)		0.48(0.28)		0.025
CAS DC(1/kPa)	Male	0.014(0.016)	0.648	0.008(0.005)	0.233	0.023
	Female	0.013(0.012)		0.001(0.007)		0.059
CAS kPa	Male	136.4(137)	0.360	195.6(228)	0.668	0.131
	Female	117.5(97)		222.7(417)		0.031
CAS PWV(m/s)	Male	6.8(2.6)	0.262	7.9(3.3)	0.814	0.083
	Female	6.3(1.9)		7.7(4.5)		0.039

n—number of vessels

4.3.2.2.2 Comparison of the carotid arterial stiffness indices according to Type of stroke.

The carotid arterial stiffness indices within the post stroke group were further analysed according to stroke type and the subgroup analysis results are shown in Table 4.5. The mean differences in most of the stiffness indices (Cas β index, Cas cc, Cas dc, and Pwv) were not significantly different between the two stroke types except for the modulus of elasticity (kPa), which was higher in the ischemic stroke type group, M.D (95%, CI) =99.1[-29;228, p=0.048*].

Table 4.5: A comparison of the carotid arteries' stiffness parameters in the Post stroke Group across Stroke Type.

Carotid Stiffness Parameters according to Stroke Type (Ischemic, n=74; Haemorrhagic, n=40)				
	Type of Stroke	**Mean (SD)**	**M.D [95%, CI]**	**p-values**
CAS β	Ischemic	18.4 (32.1)	7.4 [-0.5; 5.2]	0.067
	Haemorrhagic	11.0 (7.5)		
CAS CC (mm/kPa)	Ischemic	0.477(0.31)	0.001[-0.1;0.1]	0.984
	Haemorrhagic	0.476 (0.22)		
CAS DC(1/kPa)	Ischemic	0.009 (0.007)	0.000[-0.002;0.003]	0.655
	Haemorrhagic	0.009 (0.004)		
CAS kPa	Ischemic	244 (405)	99.1[-29;228]	0.048*
	Haemorrhagic	145 (94.1)		
CAS PWV(m/s)	Ischemic	8.1 (4.6)	0.9[-0.6;2.5]	0.229
	Haemorrhagic	7.16 (2.1)		

MD—Mean Difference (Ischemic stroke-haemorrhagic stroke values); [95%, CI] —95% confidence interval. The significance level was set at $p<0.05^{*}$. n—number of vessels

4.3.3 Carotid arteries' Haemodynamic parameters in the post stroke and non-stroke groups.

The haemodynamic parameters for the post stroke patients and age matched non-stroke individuals are displayed in Table 4.6. The mean ICA and DCCA PSV in the non-stroke group were 65.5± 17 and 66.3± 15 cm/s, respectively. The corresponding mean ICA and DCCA PSV in the post-stroke group were respectively, 62 ± 20 cm/s and 57.8± 14 cm/s, and these values were lower than those for the non-stroke group, although the difference was only statistically significant for DCCA PSV($p<0.001^{*}$). Similarly, the non-stroke individuals' group had significantly higher ICA and

DCCA EDV values of 23± 7.7 cm/s and 20.8± 5.8 cm/s, respectively compared to the post stroke group ICA and DCCA EDV values of 20.7± 7.9 cm/s and 16.2± 4.7 cm/s, respectively. The p values for the ICA and DCCA EDV between the two groups were 0.022^{*} and $<0.001^{*}$, respectively. Furthermore, the resistive index was observed to be generally higher in the post stroke group in comparison to those without stroke, for both the ICA and DCCA segments. The ICA RI values for the post stroke group and non-stroke group were 0.66± 0.1, and 0.64 ± 0.08, p=0.252, respectively whereas the DCCA segment RI values for the post stroke group and non-stroke group were 0.73± 0.2 and 0.68± 0.06; p=0.011*, respectively.

The maximum ICA PSV observed in the non-stroke group was 116 ±17.6cm/s, whereas in the post stroke group it was 150cm/s. None of the non-stroke group individuals had ICA PSV greater than 125cm/s, below which the degree of stenosis is classified as zero in the absence of carotid plaques based on the Society of Radiologists in Ultrasound Consensus Conference recommendations as alluded by (Grant et al. 2003) whereas in 2 post stroke patients the ICA PSV was greater than 125cm/s. However, among those with ICA PSV < 125cm/s, 24(35.8%) in the non-stroke group and 33(57.9%) in the post stroke group had carotid plaques hence were classified to have mild stenosis **(**Table 4.7).

Table 4.6: A comparison of carotid arteries' haemodynamic parameters between post stroke and non-stroke groups.

	Gender	Non-Stroke Group (n=134, M=44, F=90)	within Grp, p	Post Stroke (n=114, M=58 F=56)	within Grp, p	Between Grp, p
ICA PSV (cm/s)	Male	60.6(14)	0.022*	58.5(19)	0.058	0.551
	Female	67.9(18)		65.6(21)		0.508
	ALL	**65.5(17)**		**62 (20)**		**0.147**
ICA EDV (cm/s)	Male	21.1(6.9)	0.040*	18.6(7)	0.003	0.074
	Female	24(7.9)		23(8.2)		0.468
	ALL	**23(7.7)**		**20.7(7.9)**		**0.022***
ICA TAPV (cm/s)	Male	33(9)	0.002*	31.2(10)	0.003	0.332
	Female	39.2(11.2)		37.7(12.7)		0.463
	ALL	**37.1(10.9)**		**34.4(11.8)**		**0.055**
ICA PI	Male	1.24(0.41)	0.795	1.31(0.5)	0.108	0.454
	Female	1.2(0.64)		1.18(0.48)		0.673
	ALL	**1.22(0.6)**		**1.24(0.4)**		**0.741**
ICA RI	Male	0.65(0.09)	0.735	0.67(0.12)	0.422	0.431
	Female	0.65(0.07)		0.65(0.09)		0.579
	ALL	**0.64(0.08)**		**0.66(0.1)**		**0.252**
ICA/DCCA PSV Ratio	Male	0.96(0.24)	0.104	0.97(0.3)	<0.001	0.792
	Female	1.04(0.29)		1.24(0.36)		<0.001*
	ALL	**1.0(0.3)**		**1.1(0.4)**		**0.023***
DCCA PSV (cm/s)	Male	64.9(14)	0.444	61.1(15)	0.013	0.218
	Female	67(15)		54.4(12)		<0.001*
	ALL	**66.3(15)**		**57.8(14)**		**<0.001***
DCCA EDV (cm/s)	Male	18.8(4.9)	0.007*	16.1(4.9)	0.821	0.006*
	Female	21.7(6.0)		16.3(4.6)		<0.001*
	ALL	**20.8(5.8)**		**16.2(4.7)**		**<0.001***
DCCA TAPV (cm/s)	Male	31.7(7.3)	0.016*	28(6.9)	0.638	0.012*

	Female	35.8(9.8)		28.7(7)		<0.001*
	ALL	**34.4(9.3)**		**28.4(6.9)**		**<0.001***
DCCA PI	Male	1.48(0.4)	<0.001*	1.63(0.4)	0.001	0.059
	Female	1.25(0.21)		1.37(0.4)		0.043
	ALL	**1.3(0.3)**		**1.5(0.4)**		**<0.001***
DCCA RI	Male	0.7(0.07)	0.014*	0.73(0.07)	0.941	0.048*
	Female	0.68(0.06)		0.73(0.29)		0.063
	ALL	**0.68(0.06)**		**0.73(0.2)**		**0.011***

ALL – represents all participants (males and females combined), n—number of vessels, ICA—Internal carotid artery, PSV—peak systolic velocity; EDV—end diastolic velocity; TAPV—time averaged peak velocity, PI—pulsatility index, RI—resistive index, DCCA—distal common carotid artery.

Table 4.7: Degree of stenosis status by velocity-based method in post stroke and non-stroke groups.

Degree of stenosis	Frequency (%)	
	Non-Stroke Group	Post- Stroke Group
0	43 (64.2)	22(38.6)
< 50 (mild)	24(35.8)	33(57.9)
(50-69) %	0 (0)	2(3.5)
≥70% to near occlusion	0(0)	0(0)
Near occlusion	0(0)	0(0)
Total occlussion	0(0)	0(0)

4.3.4 Carotid plaque incidence and location in the post stroke and non-stroke groups.

A total of 93 plaques (56—Left side, 37—Right side) were observed in 35(61.4%) post stroke patients whereas in the non-stroke group there were a total of 34 plaques (23=Lt side, 11=Right side) in 24(35.8%) of the participants (Table 4.8). There was a significant difference in the plaque incidence between the post stroke and non-stroke group, with greater number of plaques observed in the post stroke patients (Chi squared=8.082, p=0.004). In both groups majority of the carotid plaques were in the carotid bulb, 31 (91.2%) and 66 (71%) in the non-stroke and post stroke groups respectively, additionally, the post stroke group had higher percentages (17%) of plaques located in the ICA compared to the non-stroke individuals (6%).

Table 4.8: carotid arteries plaques incidence and location in the post stroke and non-stroke groups.

Plaque Incidence and Location in the post stroke and non-stroke groups							
	Number of Participants- n (%)			Plaque numbers	Plaque Location-n (%)		
	Plaque absent	Plaque present	Between Grp p-value		Bulb	ICA	Other
Non-stroke (n=67)	43(64.2)	24(35.8)	0.004	34	31(91)	2(6)	1(3)
	Within Grp p=0.027						
Post stroke (n=57)	22(38.6)	35(61.4)		93	66(71)	16(17)	11(12)
	Within Grp p=0.085						

4.3.4.1 Carotid plaque characteristics based on grey scale carotid ultrasound.

There was generally a comparable high incidence of hyperechoic plaques in both the non-stroke and post stroke adults' groups 25(73.5%) and 67(72%), respectively, whereas among the hyperechoic plaques, calcifications were more prevalent in the post stroke group (30.1%) versus 14.7% in the non-stroke group. Additionally, a higher percentage of hypoechoic plaques was observed in the post stroke group in comparison to the non-stroke group. The incidence of calcified plaques was 5(14.7%) of the total plaques in the non-stroke group whilst in post stroke group it was 28(30%). There was a higher percentage of plaques exhibiting some haemorrhagic changes suggestive of plaque vulnerability in the post stroke (6.5%) compared to non-stroke group (2.9%). Two plaques showed ulcerative changes and one plaque had suspicion of intraplaque haemorrhage.

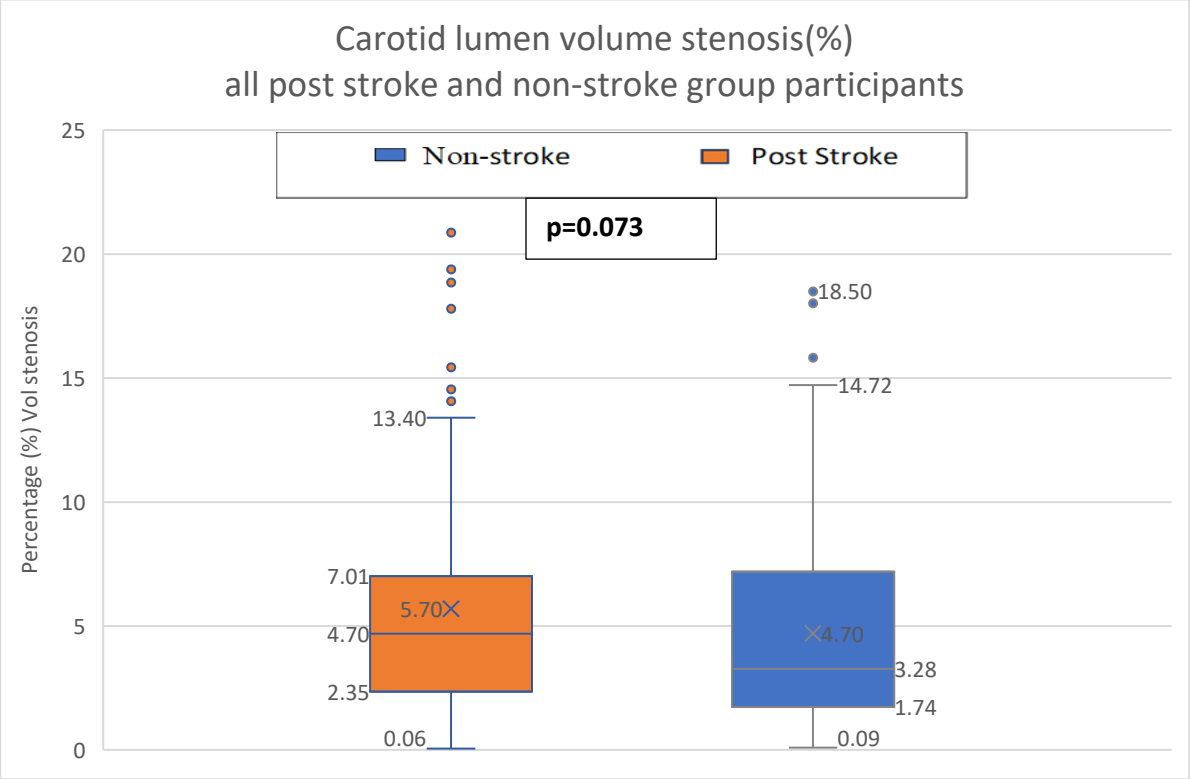
Table 4.9: Ultrasound-based plaque characteristics in the post stroke and non-stroke groups.

Plaque characteristic	**Frequency n (%)**	
	Non-Stroke	**Post Stroke**
Hyperechoic	25(73.5)	67 (72)
calcified	5 (14.7)	28 (30.1)
Ulcerations	2(5.9)	4(4.3)
hypoechoic	4(11.8)	23 (24.7)
Isoechoic	5(14.7)	0(0)
Intraplaque haemorrhage	1(2.9)	6(6.5)
Irregular Plaque outline	3(8.8)	9(9.7)

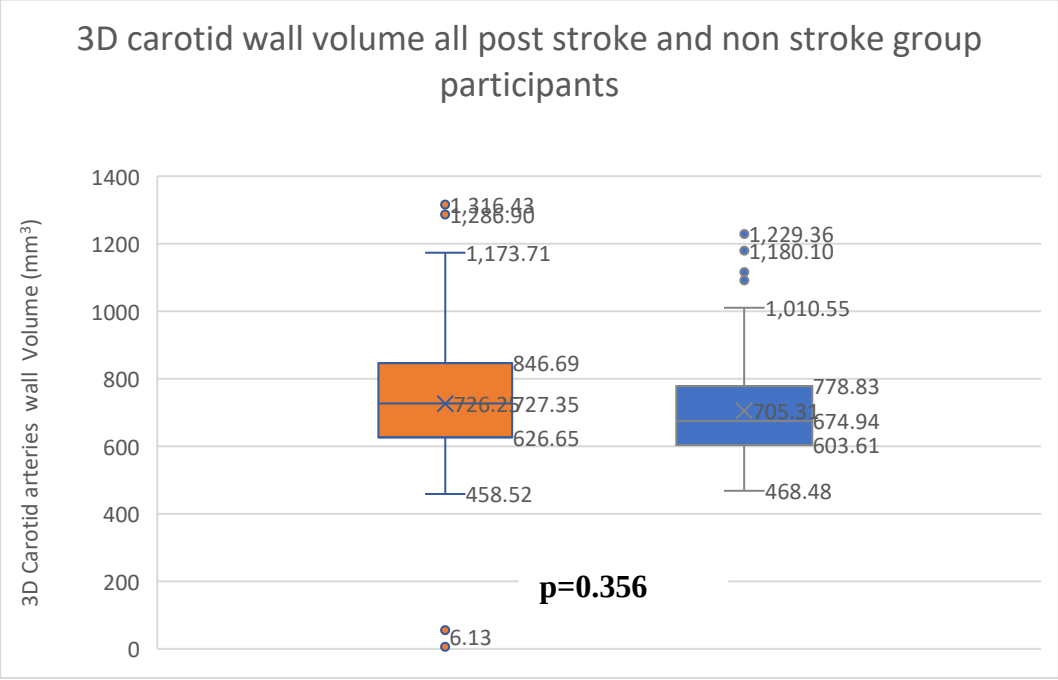
4.3.5 Three-dimensional carotid arterial analysis

The results of 3d carotid arterial analysis parameters based on a single point acquisition technique for all study participants are shown in the box plots presented in figures 4.11 (a-c). The lumen volume stenosis (%) observed in all the 114 (58 male, 56 female) carotid arteries segments for post stroke adults' patients and 134 (44 male, 90 female) age matched non-stroke individuals were respectively (5.7±4.8, and 4.7± 4.0, p=0.073), and no statistically significant difference was noted, although the post stroke group had marginally higher % volume stenosis values than the non-stroke. Furthermore, the 3d carotid vessel wall volume (CVWV) in post stroke patients was marginally higher than in individuals without stroke, although not significantly different (726±200mm^3, versus 705±145, p=0.356), respectively. However, post stroke patients showed a

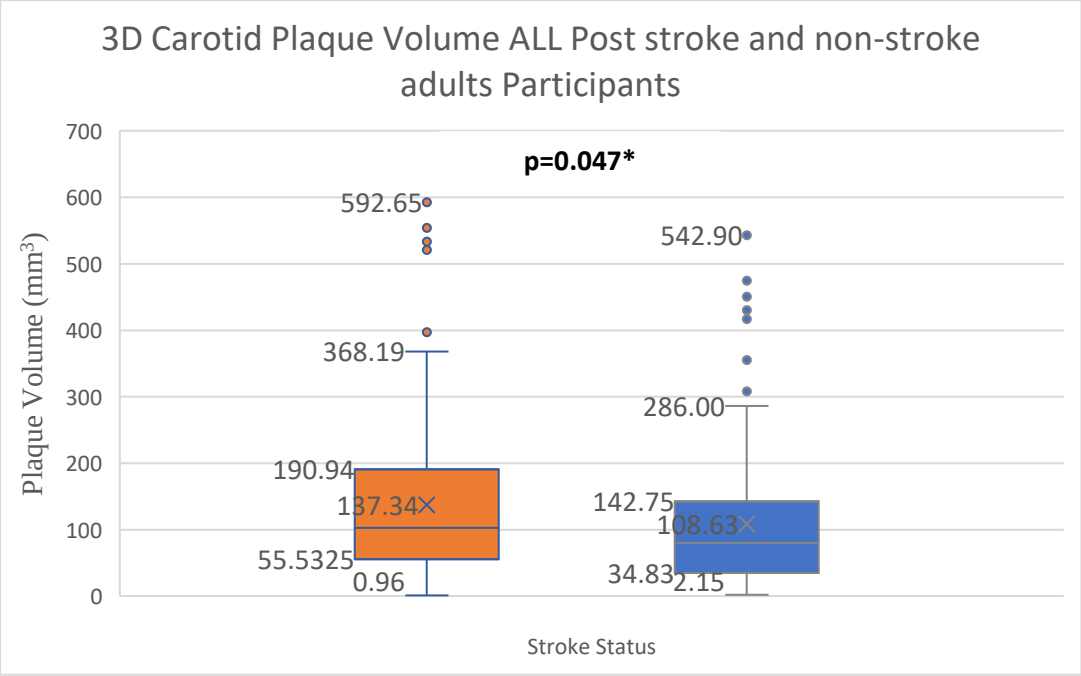
significantly higher 3d plaque volume (PV) in comparison to the non-stroke counterparts, (137±122, versus 108±103, p=0.047*.) respectively.



a.



b.



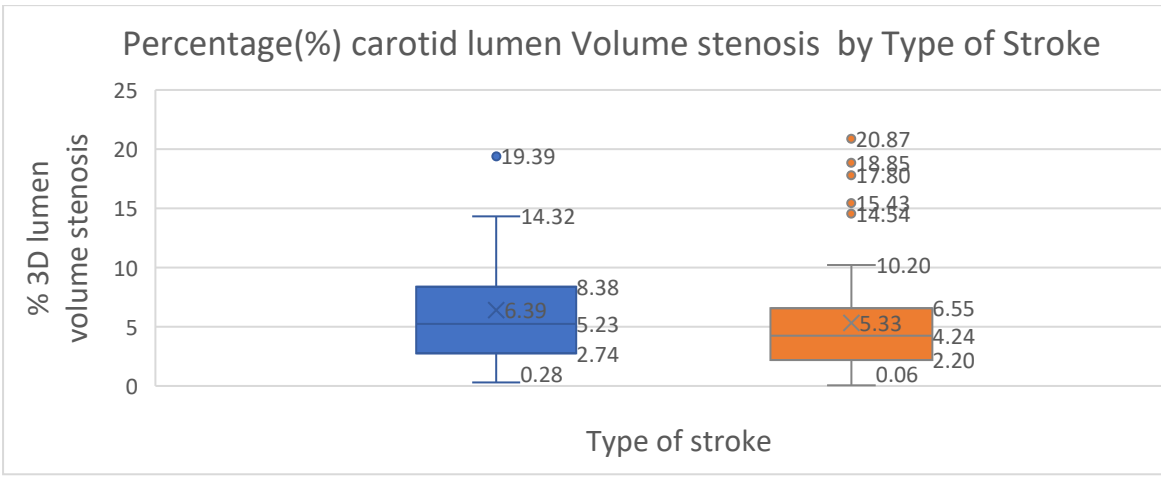
c.

Figure 4.11: Box Plots showing 3D carotid arterial analysis parameters based on single point acquisition technique in ALL Post stroke versus non-Stroke adults a.) carotid lumen volume stenosis (%), b.) carotid wall volume; c.) plaque volume.

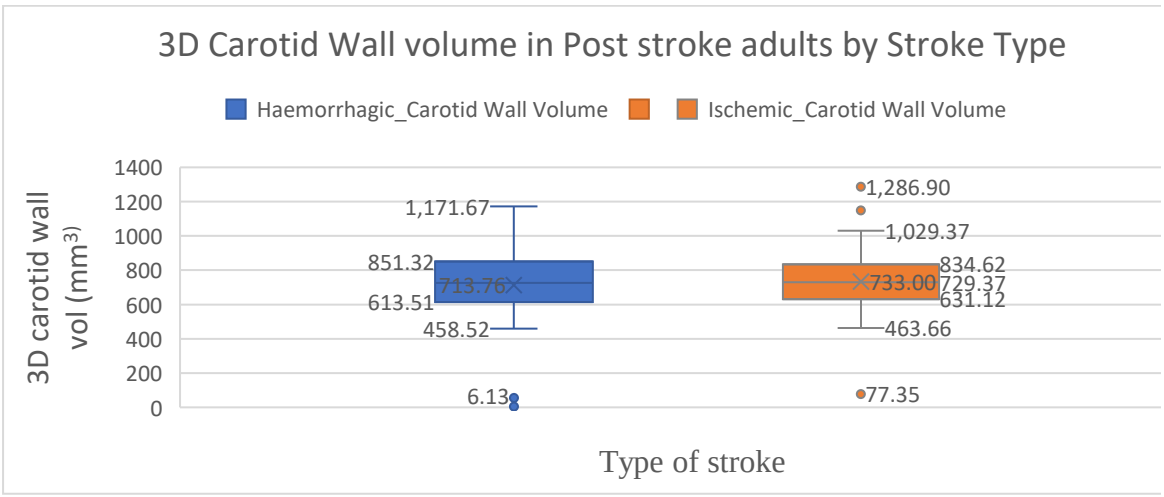
4.3.5.1 Three-dimensional carotid arterial analysis parameters according to type of stroke

Further subgroup analysis according to type of stroke indicated that only the 3d carotid wall volume is higher in ischemic stroke than in haemorrhagic stroke. Ischemic stroke patients had a higher carotid wall volume in compared to haemmorrhagic stroke group (733mm^3 versus 713mm^3). However, the lumen volume stenosis (%) and plaque volume were higher for haemorrhagic stroke compared to ischemic stroke types. The results are shown in figure 4.12 (a-c).

a.



b.



c.

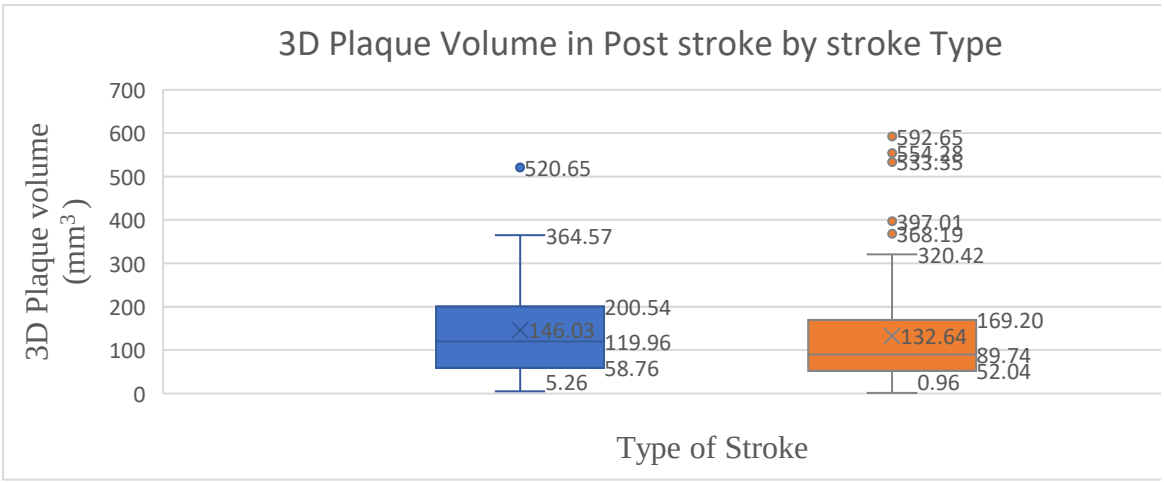
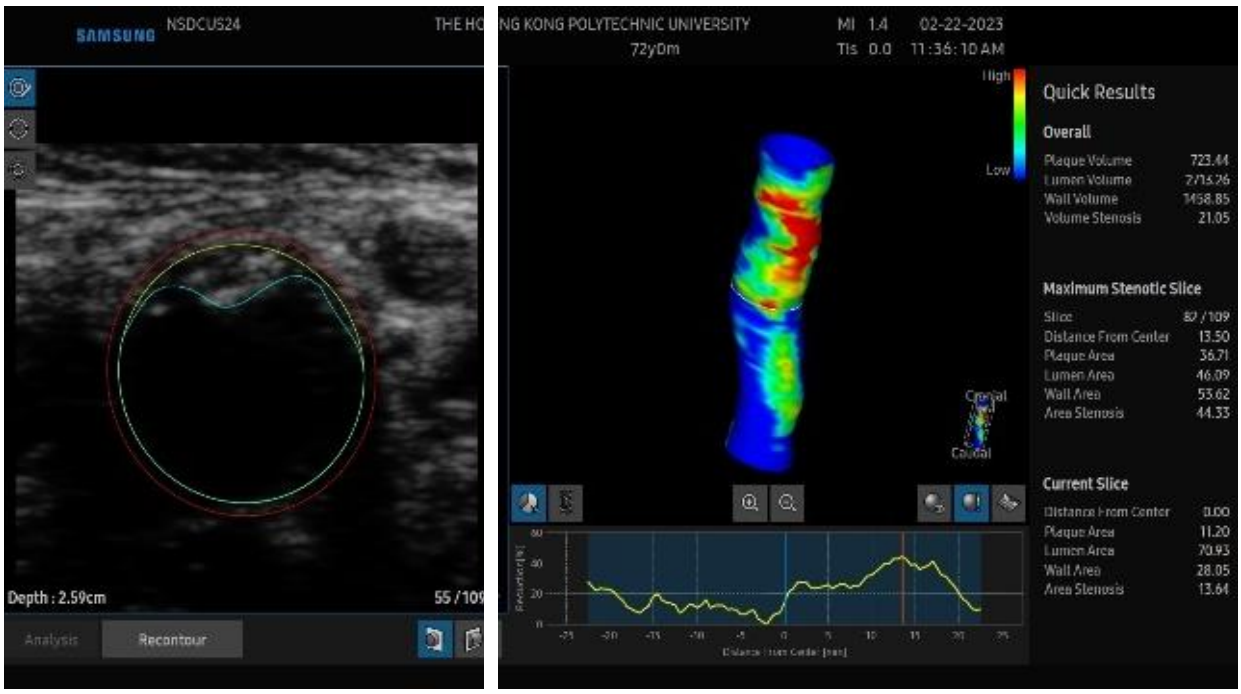


Figure 4.12: Box plots showing 3D-carotid arterial analysis parameters based on single point acquisition technique in post stroke adults by stroke type-ischemic (orange color) and haemorrhagic (blue color) a.) carotid lumen volume stenosis (%), b.) carotid wall volume; c.) plaque volume.

4.3.5.2 Three-dimensional plaque vulnerability arterial analysis.

Based on the 3D arterial analysis, plaque colorimetric map vulnerability criteria, suggested by (Fresilli et al. 2022) were vulnerability is considered as a major presence (>50%) of red areas compared to blue areas none of the plaques were classified as vulnerable plaques in both groups (Figure 4.13).



a. **b.**

Figure 4.13: Images showing 3D volumetric assessment of carotid artery stenosis using automated arterial analysis software on Samsung RS85 ultrasound machine. (a) is a transverse section demonstrating a plaque and (b) 3D acquisition image showing the more vulnerable areas depicted by the red color. Adapted from: own images Hong Kong Polytechnic University ultrasound lab, (2022).

4.3.5 MCA haemodynamic parameters of the post stroke and non-stroke groups based on cTCCD.

4.3.5.1 Demographic characteristics and trans-temporal window (TTW) status of study participants.

In all the post stroke and non-stroke study participants (n=124), at least one side open TTW for assessing the MCA using TCCD was observed in 91(73.4%) participants with mean age of 61.1± 6.2 years, whilst the remaining 33(26.6%) participants had bilateral TTW failure. Furthermore, when split according to stroke status grouping, a total of 39 (68.4%) post stroke patients (n=57) had at least one sided open TTW for assessing the MCA using TCCD (35-bilateral open TTW, and 4-unilateral open TTW - 3 on left side and 1 on right side), whereas 18 (31.6%) subjects had bilateral TTW failure to evaluate MCA. In the non-stroke group (n=67), bilateral TTW failure was observed in 15(22.4%) individuals whilst a total of 52(77.6%) had at least one sided open TTW for assessing the MCA (42-bilateral open TTW, and 10-unilateral open TTW - 3 on left side and 7 on right side). In two post stroke patients, despite the presence of bilateral open TTW, no spectral Doppler signals was registered unilaterally due to suspected total vessel occlusion, thus, a total of 72 MCAs measurements in post stroke patients were included for analysis (68 measurements from 35 patients with bilateral open TTW, and 4 from patients with unilateral TTW). Similarly, no spectral Doppler signal was registered in 1 non-stroke group subject with bilateral open TTW, hence a total of 93 MCAs measurements (83 measurements from 42 patients with bilateral open TTW, and 10 from patients with unilateral TTW) in non-stroke group were considered for haemodynamic parameters comparisons with the post stroke group.

The male participants had a higher within group percentages of at least one side of open TTW compared to female participants, 45(88.2%) versus 46(63%), respectively), whereas a corresponding higher percentage of TTW failure was observed in females compared to males'

counterparts (27(37%) and 6(11.8%), respectively). The observed gender-based differences in the TTW status was statistically significant (χ^2 -test statistics= 9.779; df=1; p=0.002*) (Table 4.10).

Table 4.10: TTW status according to gender crosstabulation-All groups' participants

		TTW status all Study Participants (N=124, mean age= 61.1± 6.2 yrs)			
		Bilateral TTW absent	**at least one side TTW present**	**Total**	**p-value**
Male	Count	6	45	51	0.002*
	Expected Count	13.6	37.4	51.0	
	% within Males	11.8%	88.2%	100.0%	
Female	Count	27	46	73	
	Expected Count	19.4	53.6	73.0	
	% within Females	37.0%	63.0%	100.0%	
Total	Count	33	91	124	
	Expected Count	33.0	91.0	124.0	
	% within all Participants	26.6%	73.4%	100.0%	

N—number of Participants

4.3.5.2 MCAs haemodynamic parameters between post stroke and non-stroke groups

The results for the MCAs haemodynamic parameters for the two comparison groups are shown in Table 4.11. The mean MCA PSV, EDV and MFV in the non-stroke group were 93.4± 21.4 cm/s, 38.0±12.5cm/s and, 59.7±14.2cm/s, respectively. The corresponding mean MCA PSV, EDV and

MFV in the post-stroke group were 80.6 ± 27 cm/s, 29.2±14.6 cm/s, and 50.2±18.4 cm/s respectively and these were significantly lower than those for the non-stroke group($p<0.05$*).

Contrarily, the non-stroke individuals' group had significantly lower RI and PI values of 0.59± 0.01 and 0.94 ± 0.2, respectively compared to the post stroke group RI and PI values of 0.64± 0.15 and, 1.07±0.4 respectively. The MCA interrogation depths for the non-stroke group and post stroke patients of 57.7±3.82mm and 58.4±5.18, respectively were not significantly different p=0.15.

Table 4.11: MCAs haemodynamic parameters of post stroke and non-stroke groups.

MCA haemodynamic Parameters	**Group**	**n**	**Mean**	**Std. Deviation**	**p-values**
PSV (cm/s)	non-Stroke	93	93.4	21.4	0.001*
	Post Stroke	72	80.6	27.7	
EDV (cm/s)	non-Stroke	93	38.0	12.5	<0.001*
	Post Stroke	72	29.2	14.6	
MFV (cm/s)	non-Stroke	93	59.7	14.2	<0.001*
	Post Stroke	72	50.2	18.4	
RI	non-Stroke	93	0.59	0.01	0.161
	Post Stroke	72	0.64	0.15	
PI	non-Stroke	93	0.94	0.2	0.115
	Post Stroke	72	1.07	0.4	
Depth (mm)	non-Stroke	93	57.7	3.82	0.15
	Post Stroke	72	58.4	5.18	

n—number of MCA vessels, p—values are based on Mann-Whitney U Test as assumptions of normality and homogeineity of variance were not met in all parameters. Depth—represents the proximal depth MCA Depth(mm) at the ICA/MCA bifurcation.

4.3.5.2.1 Comparison of MCA haemodynamic parameters according to gender.

There were significant gender differences in the MCA haemodynamic parameters across the two groups with females exhibiting higher values of PSV, EDV and MFV, and correspondingly showed reduced values of RI and PI indices, although the differences in both RI and PI were not statistically significant. This observation is indicative of enhanced MCA blood flow within the adult female population regardless of whether one had stroke or not (Table 4.12)

Table 4.12: Gender based comparison of MCAs haemodynamic parameters between post stroke and non-stroke group

MCA haemodynamic Parameter		**Non-Stroke Group (nM=38, F=55)**		**Post Stroke Group (n- M=48 F=24)**	
	Gender		**p-values**		**p-values**
PSV (cm/s)	Male	88.8(18)	0.090	70.1(23)	<0.001*
	Female	96.5(23)		101(24)	
EDV (cm/s)	Male	34.4(14)	0.020*	24.7(14)	<0.001*
	Female	40.5(10.8)		38(11)	
MFV (cm/s)	Male	55.9(13.4)	0.028*	43(16)	<0.001*
	Female	62.4(14.3)		64.5(14)	
PI	Male	1.0(0.3)	0.354+	1.1(0.4)	0.303+
	Female	0.9(0.16)		1.0(0.4)	
RI	Male	0.62(0.13)	0.650+	0.65(0.17)	0.327+
	Female	0.58(0.07)		0.62(0.12)	

n—number of MCA vessels, p—independent t test, p+—Mann Whitney U test

4.3.5.2.2 MCA haemodynamic parameters according to type of stroke

The mean PSV, EDV and MFV in the ischemic stroke group were significantly higher compared to the haemorrhagic patient's subgroup, whereas no significant difference was observed for the RI

and PI, although lower values for both RI and PI were observed in the ischemic stroke group. The results are shown in Table 4.13.

Table 4.13: Comparison of MCA haemodynamic parameters according to Type of stroke.

MCA Parameters	Type of stroke	n	Mean	Std. Deviation	p-values
PSV (cm/s)	Ischemic	43	90.1	27.7	<0.001*
	haemorrhagic	29	66.5	21.3	
EDV (cm/s)	Ischemic	43	32.4	14.1	0.020*
	haemorrhagic	29	24.3	14.2	
MFV (cm/s)	Ischemic	43	56.5	18.0	<0.001*
	haemorrhagic	29	40.9	14.8	
RI	Ischemic	43	0.64	0.14	0.852
	haemorrhagic	29	0.65	0.17	
PI	Ischemic	43	1.04	0.30	0.440
	haemorrhagic	29	1.11	0.49	
Depth (mm)	Ischemic	43	58.7	5.1	0.513
	haemorrhagic	29	58.1	5.4	

n—number of MCA vessels,

4.3.6 Stroke status prediction models based on individual and combined stroke risk factors.

Binary Logistic regression was further used to generate a stroke status prediction model based on observed significant independent predictors (age and categorical variables-hypertensive,

hyperlipidemia and diabetes mellitus, all p<0.05). The predictive performance of the individual demographic and clinical factors and the combined factors is shown in Table 4.14. The combined factors model (cModel 1) summary and classification table is further are shown in table 4.15. The model combining the 4 independent stroke occurrence demographic-clinical predictor variables (age, hypertension, hyperlipidemia, and diabetes) was significant (Chi-square=63.322, df=4, p<0.001), with an optimal prediction performance compared to models incorporating only single factors, AUROC (0.88), sensitivity of 78.9%, specificity of 82.1% and overall accuracy was 80.6% (Figure 4.14).

Table 4.14: Diagnostic performance of the stroke status prediction models based on individual and combined stroke risk factors

Model variable/s	Sensitivity (%)	Specificity (%)	DA (%)	AUROC (95% CI)	p-value
Age	47.4	76.1	62.9	0.66(0.563; 0.757)	0.002
Hypertension	71.9	85.1	79.0	0.79 (0.7; 0.87)	<0.000
Hyperlipidaemia	61.4	82.1	72.6	0.72(0.62; 0.81	<0.000
Diabetes	29.8	91	62.9	0.60(0.50; 0.71)	0.046
cModel 1	78.9	82.1	80.6	0.88(0.83; 0.94)	<0.000

cModel 1—represents model with combined variables, Age, Hypertension, Hyperlipidemia, Diabetes mellitus. The cut-off age (years) is set at the default mean age of post stroke group (64.5years)

Table 4.15: cModel 1 summary and classification table

Model Classification Table[a] & summary						
	Predicted			Model summary		
Observed	Non Stroke	Post Stroke	Percentage Correct	Cox & Snell R Square	Nagelkerke R Square	p-value
Non Stroke	55	12	82.1	0.400	0.534	<0.001
Post Stroke	12	45	78.9			
Overall Percentage			80.6			
a. The cut value is 0.500						

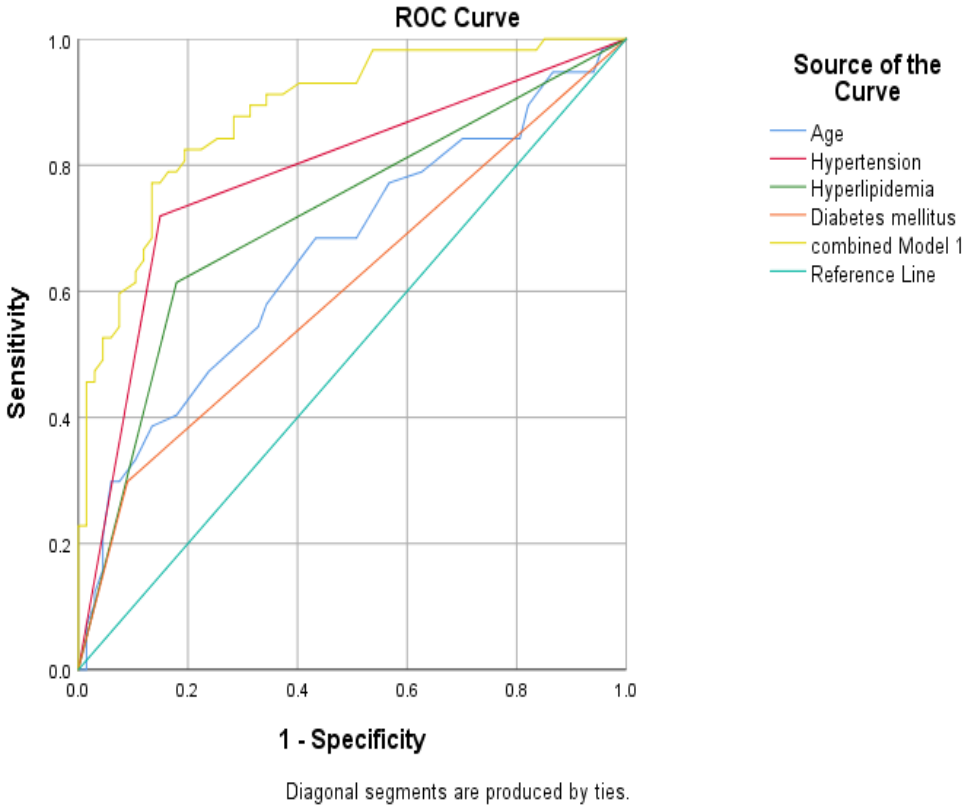


Figure 4.14: ROC curve demonstrating the predictive performance of the models based on the 4 individual demographic-clinical stroke risk factors (age, hypertension, hyperlipidemia, and diabetes) and when combined at the default setting. The cutoff value for the predicted probabilities was set at default 0.5 and the cutoff age (years) was set at default post stroke group mean age (64.5years).

4.4 Discussion

This cross-sectional study compared the morphological and hemodynamic features of cerebral arteries between post stroke patients and non-stroke controls using duplex carotid ultrasound techniques and transcranial color-coded Doppler ultrasound to elucidate stroke predictor biomarkers based on demographic characteristics and the new ultrasound applications. These biomarkers could also potentially serve as rehabilitation efficacy indicators in the subsequent study highlighted in chapter 5.

4.4.1 Demographic characteristics.

In the current study we observed that age, and categorical variables mainly, hypertensive, hyperlipidaemia and diabetes mellitus status were significantly different between the two groups, with post stroke patients exhibiting higher mean age, and greater proportions of these patients were hypertensive, hyperlipidaemic and had diabetes mellitus all ($p<0.001$) and these variables are independent primary predictors of stroke risk. However, the remaining continuous demographic characteristics of weight, height, BMI, BPs and BPd were not significantly different between the two groups, hence may not be primary predictors of stroke risk. The observation of significant age differences between the two groups ($p=0.002$) as shown in table 4.1, despite an earlier attempt to match non-stroke controls with post stroke cases for age by enrolling participants 50years and above is consistent with previous literature. Previous studies have also shown age to be a significant stroke risk factor(O'Donnell et al. 2010). The higher prevalence of hypertension, diabetes mellitus, and hyperlipidemia in the post-stroke group underscores the critical role of these systemic conditions in stroke pathogenesis and their potential role as independent predictors of stroke status in the aged population 50years and above. Similar findings were also reported in which these three clinical variables-hypertension, diabetes mellitus, and hyperlipidemia, in

addition to smoking history were demonstrated to be strongly associated with the risk of ischemic and intracerebral haemorrhagic stroke (Kumar et al. 2020). However, in the current study the prevalence of individuals with a smoking history in both the comparison groups was low. Although the post stroke individuals exhibited marginally higher systolic blood pressure (126mmHg) in comparison to non-stroke individuals (124mmHg), the BPs between the two groups was not significantly different (p=0.502). This could probably be attributed to the observation that most clinically diagnosed hypertensive patients in the two groups were on anti-hypertensive drugs which may have probably worked to control and lower the BPs effectively.

We further observed that majority of post stroke patients were ischemic (65%) versus haemorrhagic (35%). The observed distribution in stroke type occurrence is comparable to that reported by (Sultan, Khayat, et al. 2023), where ischemic and haemorrhagic stroke represented 68%, and 32% of stroke cases and in a study by (Kolmos, Christoffersen, and Kruuse 2021), ischemic stroke also accounted for as high as 65.3% of stroke type. There was no significant side to side differences in the affected side as 28(49%) and 26(45%) of the patients were affected on the left and right side respectively, whereas only 3(5.3%) were bilaterally affected. The percentage stroke recurrence rate of 5.3% observed in the current study is lower than previously reported recurrence rates of between 5.7-17% in the first year (Kolmos, Christoffersen, and Kruuse 2021). This result could probably be attributed to the advancement in secondary stroke prevention strategies in recent years as the mean stroke onset time in the current study was only 6.5±6.9 years). Similarly, Kolmos, Christoffersen, and Kruuse (2021), systematic review also observed a reduction in the recurrence rates among first-ever strokes after the year 2010 compared to prior years to which the observations were also attributed to be probably due to enhanced secondary prevention strategies.

4.4.2 Carotid arteries morphological and functional features.

4.4.2.1 Carotid intima media thickness (CIMT)

The current study observed a statistically significant difference in the mean CIMT between individuals with and without a history of stroke, with the post stroke patients exhibiting higher mean CIMT (mean difference, 95% CI =0.15, (0.1 to 0.19), Mann-Whitney U, $p<0.001$). This finding suggests that mean CIMT, could be a predictor variable of stroke outcome and concurs with previous findings(Tessitore et al. 2010; Kumar et al. 2020). Furthermore, CIMT could thus be used as a potential treatment efficacy indicator variable. In the current study males were observed to generally exhibit higher CIMT values compared to their females' counterparts regardless of whether the individuals had stroke or are without stroke, although the differences were statistically significant in the post stroke group ((mean difference =0.1, p=0.004) and non-significant in the non-stroke group (mean difference =0.05, p=0.201) Table 4.2. Previous studies that have compared the CIMT across gender have also reported similar findings in which the males generally observed to have higher CIMT values, although the study populations were different (Mazurek et al. 2014). However, in Mazurek et al. (2014) study the subjects were non-stroke young adults in contrast to an adult population involved in the current study. The results from this present study thus reaffirmed the need for stroke occurrence prediction models incorporating CIMT to take cognisance of the observed gender differences. In addition, the findings may also suggest that it takes greater changes in males CIMT, for them to be affected by stroke in comparison to female counters parts, thus when using CIMT as a marker of therapeutic efficacy gender consideration is required. Additionally, the mean CIMT between post stroke patients and individuals without stroke had been observed to differ significantly in the current study regardless of the side compared, with thicker CIMT values recorded in post stroke patients across all sides. However, a within group

analysis demonstrated no significant side-to-side differences in mean CIMT in both groups. Our current study evaluated the CIMT over a 1cm long segment and not a single point which tends to give a general overview of the atherosclerotic burden.

4.4.2.2 Carotid arterial stiffness

This study observed that β-stiffness index, elastic modulus (KPa), and pulse wave velocity which are the direct measures of the resistance to deformity of the arteries (arterial stiffness), were significantly higher in post stroke patients compared to those without stroke, whereas carotid compliance and distensibility coefficient had a positive mean difference in favour of the age matched non-stroke individuals ($p<0.05^{*}$, in all stiffness parameters), and these results are clearly indicative of a significantly decreased elasticity of carotid arteries in post stroke patients when compared to age matched individuals without stroke. Moreso, the findings of this current study concur with the recent growing evidence that has linked arterial stiffness to be an independent predictor of stroke occurrence hence a potential stroke treatment efficacy indicator (Chen et al. 2017; Miyagi et al. 2023), although in the current study more reliable techniques were employed compared to previous studies. Previous studies have mainly reported systemic arterial stiffness measures with paucity of studies focusing on regional stiffness measures, and moreover these arterial stiffness measures were based mainly on, PWV(m/s). The current study has provided additional ultrasound based, direct and indirect carotid arterial stiffness reference values inclusive of 1.) beta stiffness index, 2.) elastic modulus, 3.) carotid compliance, and 4.) carotid distensibility) apart from PWV for both post stroke adults, and healthy age matched individuals without stroke. The paucity of such ultrasound based carotid artery stiffness reference values has hindered, the widespread clinical use of the technique. The current study findings is consistent with that reported

in study by (Vriz et al. 2017) who equally observed no gender based difference in the carotid stiffness indicators (PWV, elastic modulus, and β- stiffness).

4.4.2.3 Carotid plaques burden and characteristics.

There was a significant difference in the plaque incidence between the post stroke and non-stroke group, with greater number of plaques observed in the post stroke patients (Chi squared=8.082, p=0.004). Majority of plaques had a regular outline, intact fibrous cap and hyperechoic echogenicity in comparison to the surrounding CIMT in both groups, and most plaques were located in the carotid bulb. According to Mughal et al. (2011) the bulb represents the major site of involvement in atherosclerotic stenosis and the current study findings are consistent with previous literature, hence detailed analysis of the carotid bulb is recommended during carotid ultrasonography imaging. Despite the high incidence rates of carotid plaques in both the post stroke and non-stroke groups, only a few 2(3.5) of the plaques yielded haemodynamically significant stenosis, whereas in the non-stroke individuals all the plaques caused low grade stenosis less than 50%, probably explaining that haemodynamic failure due to plaque build-up may not be the main mechanism of stroke in the current study population.

The study further demonstrated no significant clear disparity in carotid plaque hyper-echogenicity between adults' individuals with and without a history of stroke as equally higher percentages of the plaques were hyperechoic across the two groups 73.5% and 72% for the post stroke and non-stroke groups respectively. However, our findings indicated that predominantly hypoechoic plaques were associated with the presence of stroke as a greater percentage of hypoechoic plaques was observed in the post stroke group (24.7%) compared to the non-stroke individuals (11.8%). These findings concur with previous studies in which predominantly hypoechoic and hyperechoic carotid plaques were significantly associated with the presence and absence of cerebrovascular

symptoms, respectively (Sultan, Khayat, et al. 2023).

4.4.2.4 3-dimensional carotid arterial analysis

Although the post stroke group had marginally higher, 3D-based lumen volume % stenosis compared to the non-stroke, the mean difference between the two groups was not statistically significantly (MD=1[-0.09; 2.1], p=0.073). This observation of a non-significant difference in 3D-based lumen volume % stenosis, between the post stroke group compared to those without stroke may possibly explain the assertion that haemodynamic failure due to stenosis may not be the most favourable mechanism of stroke occurrence in our study population. Similarly, although notwithstanding the contributions of cerebral artery stenosis to ischemic stroke, the current study findings concurs with recent evidence that is pointing towards, vulnerable atherosclerosis plaque rupture as the main mechanism of ischemic stroke rather than haemodynamic failure following cerebral arterial stenosis (Heck and Jost 2021; Saba et al. 2018). Further studies to interrogate the possible, plaque characteristics that may be able to differentiate between post stroke patients and age matched non-stroke adults are recommended.

4.4.3 Carotid arteries' haemodynamic parameters in the post stroke and non-stroke groups.

In the current study, statistically significant differences were observed in all the distal common carotid arteries (DCCA) ultrasound based haemodynamic parameters between the post stroke patients and age matched non-stroke groups (all $p<0.05$), whereas for the internal carotid arteries parameters, only the ICA EDV (cm/s) was significantly different between the two groups. The ICA blood supply is responsible for supplying the MCA which in turn supplies as much as 80% of the cerebral blood flow(Nagata et al. 2016). Significantly different haemodynamic parameters between the post stroke and non-stroke individuals in these arteries could probably act as useful

indicators of monitoring the rehabilitation efficacy in remodelling the cerebral vascular status in post stroke patients and could as well be independent predictor variables of stroke status. However, in the current study there was no significant differences between the two groups with respect to the existence of clinically relevant ICA PSV, and EDV (cm/s) thresholds for intervention (Grant et al. 2003) as only 2 participants in the post stroke had stenosis between 50-69%. This observation could probably be attributed to the possible haemodynamic remodelling that may have occurred over the time course as the subjects involved in the current study were in the chronic phase of stroke. Moreso, significant gender differences in the carotid arteries' haemodynamic parameters mainly, PSV and EDV were observed in the current study with women exhibiting higher values compared to men. Similar, gender differences have been previously reported, (Comerota et al. 2004).

4.4.4 MCAs haemodynamic parameters of the two groups (post stroke versus non-stroke groups).

Although it cannot be argued that the MCA mean flow velocity (MFV) can reflect cerebral arterial perfusion, other haemodynamic parameters such as the peak systolic velocity (PSV), resistive index (RI), pulsatility index (PI), could also provide additional and diversified important information for comprehensive assessment of cerebrovascular resistance and intracranial compliance (Han et al. 2019). In this current study several other haemodynamic parameters were assessed and compared between post stroke and non-stroke groups. We observed a significantly higher prevalence of at least one sided open transtemporal window (TTW) (73.4%) and bilateral TTW failure of (26.6%) in the present study. The study results implies that TCCD is a practical imaging modality in the adult's population, and the prevalence rates of open TTW observed in this study concur with our previous findings reported in chapter 3, although the TTW status was

marginally higher (82%) than for the present study. This may be attributed to the age difference between present study and previous studies mean age of (62.5 ±6.9 years, and 49±17 years) respectively (Gunda, Ng, et al. 2024) as age has been reported to be a significant factor of TTW failure rates(Kwon et al. 2006; Lin, Fu, and Tan 2015)**.** Moreover, female participants had higher bilateral TTW failure compared to males (χ^2 -test statistics= 9.779; df=1; p=0.002), and these findings agree with our previous findings discussed in Chapter 3, where female participants had higher TTW failure.

The study demonstrated that non-stroke individuals had significantly higher mean MCA PSV, EDV and MFV compared to the post-stroke group whereas the corresponding RI and PI values were observed to be marginally higher in the post stroke group, although not statistically significant (Table 4.11). These findings are indicative of enhanced MCA blood flow in non-stroke adult individuals when compared to post stroke patients. In addition, the MCA haemodynamic parameters PSV, EDV and MFV could therefore be independent predictor variables of stroke status and poses as rehabilitation efficacy monitoring indicators.

Furthermore, a comparison of the haemodynamic features across the stroke subtype showed higher PSV, EDV and MFV in the ischemic group compared to the haemorrhagic stroke patients, and subsequently lower RI and PI indices in the respective groups. This observation of enhanced cerebral blood flow in the ischemic stroke patients could probably be attributed to the generally acceptable notion that haemorrhagic stroke are generally larger and more disabling than ischemic strokes(O'Donnell et al. 2010) hence ischemic stroke patients tend to recover earlier than those who had suffered haemorrhagic stroke. Moreso, the participants with haemorrhagic stroke in the current study presented with similar features associated with ischemic patients such as plaque incidence.

The mean MCA interrogation depths for the non-stroke group and post stroke patients of 57.7±3.82mm and 58.4±5.18, respectively, were not significantly different from each other p=0.15. Moreover, the observed interrogation depths were similar to those reported in our previous study presented in chapter 2 where a mean proximal depth of 59±3 mm was observed. As interrogation depth is reported to influence MCA haemodynamic parameters in our previous study (Gunda, Ng, et al. 2024) in chapter 2, the non significant difference in the MCA depth between post stroke and non-stroke groups in the current study enables a fair comparison of the haemodynamic parameters due to minimised possible bias associated with interrogation depth differences.

4.4.5 Stroke status prediction models based on individual and combined stroke risk factors.

The accurate prediction and addressing hidden risk factors for stroke can be difficult, especially when dealing with imbalanced and missing data (Hassan et al. 2024). Based on binary logistic regression, preliminary stroke prediction models incorporating demographic and clinical variables were presented in this study. The model combining four variables age, hypertension, hyperlipidemia, and diabetes demonstrated optimal prediction performance, in comparison to single factors model (Auroc=0.88, sensitivity=78%, specificity=82.1%, overall accuracy=80.6%) table 4.14. These preliminary findings point towards a promising future, where the probable incorporation of the observed ultrasound-based stroke status prediction independent parameters and utilising other machine learning algorithms have potential to yield improved performance levels in stroke risk prediction.

4.4.6 Limitations of the study

This study is not without limitations. Firstly, the cross-sectional study design focussing on post stroke patients in the chronic phase (>6 months of stroke onset) limits the ability to infer causality between observed vascular features and stroke occurrence, as vascular adaptations may have occurred between stroke onset to assessments time, thus longitudinal studies may be needed to elucidate the temporal relationship between arterial changes and stroke risk. Moreover, precaution should be taken in interpretation of gender-based stratification results as proportions of males and females were significantly different in the non-stroke group.

4.4.7 Conclusion.

In conclusion, this study revealed significant differences in several key demographic, morphological, and haemodynamic parameters including carotid intima-media thickness, carotid arterial stiffness indices- pulse wave velocity, beta stiffness elasticity modulus, carotid compliance, and distensibility coefficient, 3d-plaque volume, ICA EDV, MCA PSV, EDV and MFV between the post stroke adults' patients and age-matched non-stroke individuals. These features could serve as valuable independent biomarkers for stroke risk assessment and rehabilitation efficacy monitoring indicators in subsequent study three highlighted in chapter 5.

Furthermore, haemodynamic failure due to plaque build-up may not be the main mechanism of stroke occurrence in the current study population, thus future studies to interrogate plaque characteristics biomarkers are recommended. Moreso, the current study provided reference 3d ultrasonography based carotid arteries lumen volume stenosis (%) and carotid arterial stiffness values stratified by gender in the local adult's population with and without stroke, which are critical for the clinical practice utilisation of these novel ultrasonography techniques.

Chapter 5

Study Three- The effects of Aerobic exercise training (AET) on the cerebral arteries' haemodynamic and morphological features and the cognitive and motor functions in post stroke patients in post-stroke patients

5.1 Introduction

Stroke is the second leading cause of death and long-term disability worldwide (Feigin et al. 2021), and in Hong Kong, 6.2% of all registered deaths in 2020 were due to cerebrovascular disease (CVD) (Centre for Health Protection, 2021). The medical condition of stroke occurs primarily as a result of disturbances in the blood supply to the brain due to deconditioned vascular status (structural and functional status) of the cerebral arteries (Staessens et al. 2020; Esposito et al. 2007; Wang et al. 2014), leading to impaired brain functions and the subsequent manifestations of neurological deficits observed in the affected individuals, such as the motor, and cognitive functions deficits (Bersano and Gatti 2023; Kuriakose and Xiao 2020). Post-stroke cognitive impairment (PSCI) is reported to be an underrated worldwide problem, that may progress to post-stroke dementia (Kosgallana et al. 2019).

Due to the crucial role the cerebrovascular system plays in the maintenance of adequate blood perfusion to the brain hence preservation of normal brain function, with as high as 80% of cerebral blood flow emanating from the middle cerebral arteries (MCA), which is supplied by internal carotid arteries (Nagata et al. 2016; Staessens et al. 2020), it can be posited that improvements in the cerebral arteries' structure and function have the potential to significantly influence post stroke recovery in the various domains including the cognitive and motor function domains. Aerobic exercise training (AET), through the probable mechanism of endothelial cells stimulation due to

the laminar shear stress during exercise training that result in increased production of nitric oxide (NO), a known mediator of endothelial function (Szostak and Laurant 2011; Gambardella et al. 2020) has been shown to induce beneficial effects on systemic vascular health in the general population (Green and Smith 2018; Desouza et al. 2000; Billinger, Coughenour, et al. 2012; Ivey et al. 2010). However, despite the current available evidence on the possible role of AET in enhancing systemic vascular health status, only limited attempts to interrogate the underlying morphological and haemodynamic changes in the cerebral arteries (intracranial and extracranial) that may be brought about by AET in particular among post-stroke patients have been made to date. The few studies that have assessed the effects of AET on cerebral vascular health have reported contradictory findings (Ivey et al. 2011; Treger et al. 2010). Besides the contradictory findings the effects of AET reported in these studies were based only on a few cerebral function indicators, mainly 1.) cerebral vasomotor reactivity (cVMR) and 2.) mean flow velocity (MFV) of the MCAs based on non-imaging transcranial Doppler ultrasound (TCD). There is also paucity of information on the effects of AET on chronic post -stroke patients' extracranial cerebral arteries from which the MCAs derive its blood supply hence further interrogation is required (Nagata et al. 2016; Agarwal and Carare 2021).

Medical ultrasonography a non-invasive, non-ionising and readily available imaging modality has evolved over the recent years, and this has seen several duplex carotid ultrasonography novel applications not limited to, enhanced edge detection, three-dimensional(3D) vessel wall imaging (Fresilli et al. 2022; Johri et al. 2020; Song et al. 2019; Sultan, Bashmail, et al. 2023), quantitative arterial stiffness analysis(Yuan et al. 2017) and transcranial color coded Dopppler ultrasound (TCCD), (Nedelmann et al. 2009; Lovett and O'Brien 2022) emerging. The new applications have been validated to be capable of assessing the cerebral arteries vascular status reliably and

accurately, thus can monitor and provide a holistic assessment of the possible cerebral arteries morphological and vascular changes that may occur in post stroke patients undergoing aerobic exercise training. In addition, previous studies conducted by the PhD candidate research group, highlighted in Chapter 2 and 3, also validated TCCD, which is an advancement to non-imaging TCD as an accurate technique in assessing cerebral arteries haemodynamic features and in stratifying cerebral arteries stenoses in patients presenting with CVD.

Besides, our previous study highlighted in Chapter 4 has additionally interrogated and identified significant differences in several morphological and hemodynamic features of the cerebral arteries between post stroke patients and age-matched non-stroke controls. These features based on novel ultrasonography applications and not limited to carotid stiffness parameters, and 3D ultrasound-based plaque volume are potential stroke rehabilitation methods efficacy and prognostic predictor indicators.

Furthermore, there is still paucity of evidence on the association between the possible AET induced cerebral arteries' morphological and haemodynamic changes and the probable changes in the motor and cognitive function in post stroke patients. Although cycling AET has particularly gained attention among the various AET modalities with potential application in post stroke patients due to its safety, (Khan et al. 2024) decried the paucity of studies that have interrogated the potential benefits of cycling AET in modulating the deconditioned vascular, and cognitive functions in post stroke patients. The current study thus sought to assess whether aerobic exercise training (AET) in the form of cycling ergometry, could enhance the large intracranial and extracranial cerebral arteries' morphological and haemodynamic features and the cognitive and motor function in post-stroke patients. Furthermore, the study aimed to assess the link between the possible AET modulated vascular changes and the cognitive and motor functional changes in post-stroke

patients. It was therefore hypothesised that, cycling AET improves the large intracranial and extracranial cerebral arteries morphological and haemodynamic features as well as the cognitive and motor function among post stroke patients.

5.2 Materials and Methods

5.2.1 Methodological approach.

The present study assessed the effects of cycling AET on the large intracranial and extracranial cerebral arteries as assessed by transcranial color-coded Doppler and duplex carotid ultrasonography techniques, respectively. It further assessed the effects of cycling AET on the cognitive and motor functions in post-stroke patients using a quantitative methodological approach. Finally, the associations between the changes in the morphological and haemodynamic parameters and those changes observed in the cognitive and motor function were investigated, hence this present study consisted of three main sections as highlighted above.

5.2.2 Study Design.

This current study was a single blinded, randomised controlled trial (RCT) involving community dwelling chronic post-stroke patients undergoing a supervised cycling AET program, undertaken at Y611 and Y612 laboratories of the Hong Kong Polytechnic University. Although, the assessors were not blinded to the participants group allocation, the stretching group participants were blinded to the fact that they were in the control group. Moreover, the assessors who performed the motor and cognitive function tests were not the same as the one who assessed the haemodynamic and morphological features. Furthermore, all pre-interventional data was stored on a computer and not assessed until the protocol completion. The quantitative data on the large extracranial and intracranial cerebral arteries' haemodynamic and morphological features as determined by DCUS

and TCCD ultrasonography, and the quantitative data on the motor and cognitive function of the patients was collected and analysed at two different times in post-stroke patients who were randomly assigned into two groups consisting of a cycling AET (interventional) group and a stretching (control)

Prior to conducting this RCT study, in an effort to have an in-depth understanding of the study area and validate the data collection tools, two preliminary studies were conducted. The two studies included, 1.) A systematic review and meta-analysis aimed at assessing the diagnostic performance of the recent advanced ultrasonography imaging techniques in cerebrovascular disease diagnosis whose results are highlighted in Chapter 2, and 2.) A comparative study conducted to establish the inter-method agreement of an emerging TCCD technique available on the recently acquired Samsung RS85 ultrasound machine (Samsung Medison Co., Ltd., Republic of Korea) equipped with a phased array (1-5 MHz) transducer, and the traditional non-imaging TCD technique in assessing the intracranial cerebral arteries haemodynamic. Prior to the acquisition of the TCCD equipment, the status quo at the study site involved the use of the traditional non-imaging TCD technique for intracranial cerebral arteries haemodynamic evaluation hence it was critical to validate the new technique among the local population. The study is presented in Chapter 3, as study one.

5.2.2.1 Ethical Considerations

Ethical approval for this research project was obtained from the Institutional Review Board (IRB) of the Hong Kong Polytechnic University (Reference: HSEARS20220714001). Additionally, the Clinical trial research protocol was registered according to the WHO recommendations for conducting clinical trials (ClinicalTrials.gov Identifier: NCT05706168). Informed consent was obtained from the patients before the AET, DCUS, and TCCD ultrasound examinations and the

cognitive and motor function assessments. A coding system was used to ensure confidentiality and anonymity of the research subjects. The Ethical approval letter and informed consent forms designed for this project are attached in Appendix 1, and 2, respectively.

5.2.3 Population and sampling technique.

The post-stroke patients who participated in our previous Study two, highlighted in Chapter 4 were recruited as participants of this current study. The recruited post stroke subjects were further purposively selected to recruit only those who meet the inclusion/exclusion criteria for participant recruitment as shown below. The enrolled participants meeting all the inclusion criteria were then randomly assigned into either a cycling AET group or stretching control group such that each group had an equal number of subjects. The randomization of participants was conducted using an online software available at: (https://www.graphpad.com/quickcalcs/randomize1.cfm).

5.2.3.1 Inclusion and Exclusion criteria for Post stroke Patients.

Post stroke patients of Chinese origin with mild to moderate disability who could undertake the cycling AET and were not participating in any structured aerobic exercise training were targeted in the study. Alawieh, Zhao, and Feng (2018) cited some factors that are reported to influence the prognosis of stroke outcomes such as socioeconomic (age and race) and clinical factors for example Rehabilitation therapeutics, time to treatment, and stroke subtype among others hence such factors were catered for in the current study. Since age and stroke onset time are reported to be confounding variables in post-stroke recovery, only patients greater than 50 years old, with > 6months from time of stroke onset (chronic stroke patients) were included. Although, all post stroke subjects regardless of stroke type were included, to cater for the clinical factor of stroke subtype a subgroup analysis based on stroke type was further performed. Excluded from the study

were non-consenting post stroke patients, non-Chinese nationals, those participating in a structured AET, and patients who were severely non- ambulatory and to perform the cycling AET and stretching activities. Moreso, post stroke patients allergic to ultrasound gel were excluded from the current study, whereas those with insufficient bone windows preventing for TCCD assessment were only excluded for the TCCD analysis.

5.2.3.2 Sample size calculation

A previous meta-analysis on the effects of AET in stroke patients reported a medium effect size of 0.59 (SMD) (Pang et al. 2013). Based on an effect size of 0.59, α of 0.05, statistical power of 80%, the current study targeted a minimum sample size of 94 subjects (~47 in each of the two post stroke groups- cycling AET and stretching-control) as calculated from the G* Power software using the t test (Difference between two independent means).

5.2.4 Data collection methods and tools.

Three sets of data were collected from the post-stroke patients in the two assigned groups (cycling AET and stretching control groups). This data included the patient's 1) demographics, anthropometric and medical history; 2) large intracranial and extracranial cerebral arteries' haemodynamic and morphological features and 3) the post-stroke cognitive and motor functions (Appendix 2). Although, the heart rate was the main target parameter during cycling AET, additional data pertaining to cycling progression was also recorded during throughout the 36 sessions performed by each post stroke participant (Appendix 3).

5.2.4.1 Patient's demographic, anthropometric, and medical history.

The demographic (sex, age, education level, and income) and medical history were obtained from the questionnaire on the data collection sheets completed by the participants (Appendix 2), and where clarity was required, direct interviews were conducted on the subjects. Data on anthropometric features such as weight, and height was measured by the investigators, before undertaking the various procedures for the study. The collection of such clinical and socioeconomic data enables subgroup analysis to rule out any confounding effect of some of these factors that are reported to influence the prognosis of post stroke patients as alluded by (Alawieh, Zhao, and Feng 2018). The definition of the various clinical data collected is highlighted as follows

5.2.4.1.1 Hypertension

The participants' blood pressure and heartrate were measured using an Omron (HEM-8712), automatic blood pressure monitor (Omron healthcare manufacturing Vietnan Co.,Ltd.,Binh Duong, Vietnam). A participant was classified as hypertensive when the systolic blood pressure (BPs) was ≥ 140mmHg, diastolic blood pressure (BPd) ≥90 mmHg or had a clinical diagnosis of hypertension and receiving anti-hypertensive treatment, despite BPs and BPd being below 140mmHg and 90mm Hg respectively (Huang et al. 2023).

5.2.4.1.2 Hyperlipidaemia

The subject was hyperlipidaemic, when he or she had a clinical diagnosis and is undertaking cholesterol lowering medications.

5.2.4.1.3 Diabetes mellitus

A participant was defined as having a history of diagnosed diabetes or (and) taking hypoglycaemic drugs. There was no confirmation of for fasting blood glucose done at the study site.

5.2.4.1.4 Smoking history

This was identified when the participant had a previous history of smoking or was a current smoker having consumed at least 10o cigarettes in his or her lifetime National Centre for Health statistics., 2017).

5.2.4.1.5 Body mass index

The body mass index was calculated as the measured body weight (kg) divided by the square of height (m^2).

5.2.4.2 Cerebral arteries' morphological and haemodynamic features

Several morphological features such as carotid intima-media thickness, arterial stiffness, 3-dimensional lumen volume % stenosis, as well as both intra/extracranial cerebral arteries' haemodynamic parameters were assessed among others. Duplex carotid Ultrasound novel applications including, enhanced edge detection, 3Dimensional arterial analysis, quantitative arterial stiffness analysis and transcranial color coded Dopppler ultrasound were used for the assessments, and these techniques are reliable, non-ionising and non-invasive hence enables easy follow ups without any radiation induced risks. The cerebral arteries' morphological and haemodynamic features for both the cycling AET group and stretching control group were measured using similar ultrasound scanning protocols, and under the same patient preparation and positioning conditions as previously described in Study two highlighted in chapter 4. 2.3.2. The only major difference between the ultrasound protocols of the two studies is that in the current study cerebral arteries' morphological and haemodynamic features were established at two points in time, baseline (before the AET program) and at the end of the AET program i.e after 36 sessions of either cycling AET or stretching exercises instead of a cross-sectional approach adopted in the former study. Additionally, the pre and post TCCD imaging depths were standardised for each

participant as the MCA haemodynamic parameters were reported to be influenced by the interrogation depth in our previous Study One in Chapter 3. A single experienced operator performed all the ultrasound examinations for the two subject groups.

5.2.4.2.1 Reliability of the DCUS and TCD measurements.

To ensure the validity and reliability of the DCUS and TCCD ultrasound measurements, a pilot study was undertaken during which the ultrasound scanning parameters that influence the image quality metrics in the evaluation of the carotid and cerebral arteries were optimised. The parameters included the depth of view, overall gain, Time gain compensation (TGC), focusing, and Doppler angle correction among others. A new preset of the optimized parameters was set on the ultrasound machine before the commencement of the study and was used throughout the study for both pre and post intervention measurements. Random errors associated with the measurement processes were minimized during the scanning procedure by undertaking 3 sets of measurements and making use of the average value of the measured parameters.

5.2.4.3 Post stroke cognitive and motor functions assessment.

The cognitive and motor functions of the post-stroke subjects were assessed at two points in time, 1.) prior to AET program (baseline), and 2.) after completing 36 sessions (12 weeks) of the exercise program for the cycling AET and stretching (control) groups. These assessments were conducted in the same laboratory where ultrasonography assessments were done. Trained healthcare assesors fluent in the local Cantonese language performed the motor and cognitive assessments. Despite the assessors not blinded to the group allocations, they were however not involved in the interventional program as well as not responsible for performing the ultrasonography examinations. Prior to performing the cognitive and motor function assessments, assessors were trained to improve reliability and validity of the assessment results.

5.2.4.3.1 Cognitive Function assessment

The Montreal Cognitive Assessment Hong Kong version (MoCA-HK) was used to assess global cognition and Stroop color-word Test (SCWT) assessed specific cognitive domains. The data collection form is shown in Appendix 2. In figure 5.1, the administration of the MoCA-Hk version test setup is shown.

Figure 5.1: Image showing an assessor administering the MoCA-HK version test

5.2.4.3.1 Motor Function assessment.

5.2.4.3.1.1 Six-minute Walk Test (6MWT) procedure

The 6MWT assessment was conducted in the laboratory passageway, by an assessor not involved in the patient's exercise protocol. The test was performed firstly on the recruited participants before engaging in the cycling AET or stretching exercise training programme to test the initial endurance of the participants. Those with a 6MWT speed <0.28m/s were be deemed not fit and excluded

from participating in the study according to a recommendation by (Lee et al. 2015)). The test was further repeated at the end of the exercise program (after 36 sessions, 12wks).

Upon arrival the post stroke participants were made to seat and relax for about 10mins. The resting pulse rate and Blood Pressure (BP) were measured and recorded using a Heartrate monitor previously described in this chapter. During administration of the 6MWT patients were requested to walk safely and as fast as he/she could for 6 minutes along a 9m long unimpeded walkway, marked at 1m intervals (marked by tape and cone on floor). A wireless pulse monitor was placed on the participants' wrist and connected to a remote monitor via Bluetooth to record the pulse rate for safety purposes. The distance travelled during the 6 minutes was measured by the investigator and gait velocity (m/s) was calculated by dividing the distance walked by the 6minutes. The Pre and Post AET results of the 6MWT were then compared to assess for any changes in the walking distance.

5.2.4.3.1.2 Timed Up and Go Test procedure.

The original TUG test protocol by (Podsiadlo and Richardson 1991) was performed by a trained health care assessor in the current study. During administration of the TUG (Timed Up and Go) test, patients were requested to stand up from a standardised chair with an armrest, height set to 43cm, walk a 3 meters distance (marked by tape and cone on floor), turn around the cone, and return to sit down on the chair, at their regular pace and as safely as possible. The duration required to complete the test rounded to nearest seconds was timed by the assessor from the command word "Go" to the time the patient was seated again. Patients were permitted to use their personal mobility aids; however, no physical assistance was permitted. Additionally, post stroke subjects were required to maintain a consistent turning direction, towards the affected side for both pre- and post-TUG tests as the turning direction has been reported to influence the TUG test results with less

time observed upon turning in the direction of the affected side (Son and Park 2019). The setup for this current study is shown in figure 5.2

Figure 5.2: Image showing an assessor administering the Six-minute walk test (6MWT)

5.2.5 The AET program equipment and exercise prescription

The AET modes of treadmill and cycle ergometer were observed as the two commonly used AET modalities (Pang et al. 2013; Madhavan et al. 2019). In the current study, cycling AET was considered as it is safer and more convenient to the participants since it does not require extra safety precautions such as the use of overhead harness system to prevent patient falls unlike treadmill training. The AET protocol used in this study was further informed by previous studies and is a modification to (Laursen, Kitic, and Jenkins 2011; de Lucas et al. 2013), incorporating some recommendations from a review by Pang et al. (2013) which concluded that a frequency and duration of 3–5 days per week and 21–30 minutes per day, respectively with an overall program

duration ranging between 3 weeks to 6 months were the most common exercise prescription.

5.2.5.1 Cycle ergometer Equipment.

A cycle ergometer, ISO1000R Isokinetic Recumbent Bike (Scifit, USA), offering forward resistance in conjunction with a Polar T31-coded, chest strap- heartrate monitor (China) was used for delivering 36 sessions of aerobic exercises, over a 3months period for each post stroke participant randomly assigned to the cycling group. The heart rate program that is freely available on the recumbent bike was selected and the chest strap which was linked to the recumbent bike via a Bluetooth connection was used to continuously monitor the heart rate during the throughout the exercise sessions.

5.2.5.2 Exercise program dosage:

Since the dosage of aerobic exercise is a function of the (frequency (F), intensity (I), and duration (time) (Centre for Health Protection, 2012), the current study therefore targeted *an* exercise dosage consisting of 1.) a session duration=30mins, 2.) frequency=3times/week and 3.) high intensity= (60-84% heart rate reserve (HHR), as calculated using the Karvonen formulae 4.) type=cycling ergometry, 5.) overall program duration=12 weeks.

5.2.5.3 Exercise Intensity prescription.

There are two methods for prescribing exercise intensity relative to the heart rate. The methods are the %HRR (Heart Rate Reserve) and the %HR $_{max}$. The %HRR (Heart Rate Reserve) method is the most preferred method as it considers the resting heart rate (resting HR) unlike the maximum heart rate (HR_{max}) method. In this method, the exercise intensity is expressed as a percentage of the Heart rate reserve (HRR) in which a 1.) light intensity aerobic physical activity is defined as (20-39%HRR, 2.) moderate intensity (40-59%HRR), and 3. high intensity=(60-

84%HRR)(Sanders and Medicine 2018; Pang et al. 2013). In the current study the moderate and high intensities were used, and the target heart rate for performing at these intensities was calculated based on the Karvonen formula as follows:

Target heart rate = [(age-predicted HRmax – resting HR) × % intensity desired] + resting HR

1) **Calculation of moderate-intensity target HR zone**

Lower target HR= [0.4 (age-predicted HRmax − resting HR)] + resting HR

Upper target HR= [0.59 (age-predicted HRmax − resting HR)] + resting HR

2) **Calculation of high intensity target HR zone.**

Lower target HR= [0.6 (age-predicted HRmax − resting HR)] + resting HR.

Upper target HR= [0.84 (age-predicted HRmax − resting HR)] + resting HR.

5.2.5.4 Exercise dose administration and progression.

In the current study, the cycling AET program targeted a high-intensity exercise training mode that was approached in two phases (Phase 1 and 2) described below. The exercise progression involved gradually increasing the exercise intensity from moderate to high intensity following an initial two-weeks acclimatization period for the first 6 sessions. Thereafter a high-intensity protocol was adopted for the remaining (3-12weeks, 30sessions), although consideration to the tolerance levels of the individual participant was made during the progression.

5.2.5.4.1 Phase one exercise protocol (weeks 1 and 2)

This phase included the first 6 exercise sessions (1-6), targeting a moderate exercise intensity (40-59%HRR). The 30 minutes session duration consisted of a 2-minutes warm-up cycling at 25watts power, which was then followed by 23 minutes of continuous cycling within the moderate-

intensity target heart rate zone, and lastly 5 minutes unloaded, cool down exercise (zero watts). The cool down time of 5 minutes was inclusive of between continuous cycling recovery periods, where applicable. During the continuous cycling period, the power output was automatically adjusted as the Heart rate program was used until the target HR zone was reached and the participants were asked to cycle within the target heart rate zone, at their self-selected cadence. The power output in cycling is a product of the resistance and the pedaling rate (cadence/ rpm) given by Power (watts) = Resistance (kg) *cadence (rpm). The first exercise session targeted the lower target HRR value for moderate-intensity of (+-40%HRR) across all participants. The intensity would then be gradually increased by about 10% of the target % HHR towards the upper end of the target HR zone in the proceeding sessions, at the participants' comfort. The participants were dismissed from the site after their HR returned to pre-exercise levels as a safety precautionary measure.

5.2.5.4.2 Phase two exercise protocol (3-12wks)

In this phase of the exercise program the same exercise protocol as in phase one was retained, except that a high exercise intensity (60-84%HHR) was targeted for sessions 7 to 36 in phase two.

5.2.5.5 Exercise Safety and Compliance Issues

The safety of AET as a rehabilitation intervention technique in stroke patients has been reported, and persons living with a mild to moderate stroke are encouraged to engage in AET routinely (Pang et al. 2013). The consideration of a stationary bike compared to a moving bike in this study, further reduce the risks of falling hence no need for an overhead harness. During the entire training session and in both exercise phases as safety measures, a close monitoring of the study participants heartrate to check for any cardiovascular intolerance and checking for any signs of fatigue or discomfort was done by the training coordinator. More so, participants were encouraged to hold

onto the bike handrails for both support and heartrate monitoring in addition to the use of a separate chest strap heartrate monitor, whilst their legs were securely fastened on the footrest. As a safety precaution the exercise session would be terminated in the following situations 1.) Heartrate increase above the high intensity exercise zone, 2.) HR fails to restore back to warm-up values during the recovery period, 3.) observed signs of severe fatigue, paleness, excessive sweating or confusion or if the participant indicated that they couldn't continue due to fatigue. Apart from these safety issues additional information pertaining to the progression of each participant was recorded at every session according to the data sheet in Appendix 3.

To ensure subject compliance to the AET the subjects were asked to come to The Hong Kong Polytechnic University relevant laboratories where the AET was conducted. Comprehensive reassurance of the possible clinical benefits that the AET may bring, and continued researcher professional conduct was ensured to continuously motivate the participants. A representation of the cycling AET administration setup is shown in figure 5.3.

Figure 5.3: Image showing the cycling AET administration setup.

5.2.6 Stretching exercises (control)

The participants in the control group were engaged in simple non-aerobic stretching exercises over the same duration as the interventional cycling group, which were conducted at Y611, Laboratory of The Hong Kong Polytechnic University. They were also allowed to receive their usual care (allowed participation in unplanned, unstructured, daily exercises).

5.2.7 Data analysis.

IBM SPSS version 26 statistical package was used to perform all statistical analyses and the intention to treat analysis protocol was adopted in this current RCT. The continuous data was expressed as mean ± SD, whereas categorical data was presented as frequencies (%). Kolmogorov Smirnov and Levene's homogeneity of variance tests checked the data for normality and homogeneity of variance respectively. The paired t-tests or non-parametric equivalent Wilcoxon signed rank test was used to perform an intra-group analysis to assess for any statistically significant within group differences in the cerebral arteries' morphological and haemodynamic features, as well as the motor and cognitive function test scores measured at two periods that is pre and post interventional periods. The Independent t test or non-parametric equivalent Mann Whitney U test were used to compare, firstly the baseline demographic and clinical variables data between the two exercise groups, and more so it was further used to compare the mean differences (MD) of the various outcome measures, between the cycling AET and stretching control groups to establish the overall Mean differences. The statistical significance was considered at $p<0.05$.

As an example, the absolute mean differences values between the pre- and post-AET MoCA-HK scores and pre and post-AET SCWT scores represented the cognitive function within group change scores (Δ MoCA-HK and ΔSCWT score respectively. This was further expressed as standardized mean difference (SMD) representing the overall effect size and was given by Cohen D= overall

Groups M.D/pooled S.D as there were equal sample sizes in the interventional cycling and control stretching groups. The effects size was categorized as small (0.2–0.5), medium (0.5–0.8) or large (≥0.8) as informed by (Andrade 2020; Cohen 2013).

A positive ΔMoCA-HK score represented an improvement in the cognitive whereas a negative change score was deemed a deterioration, whereas a decrease in SCWT time represented an improvement whilst an increase was a deterioration. Similarly, the absolute mean differences values between pre- and post-AET Six-minute Walk test distance (Δ6MWT-distance), Timed up and go test speed (ΔTUG-speed) and TUG time(ΔTUG-time) quantified the within group changes in motor function. A positive (Δ6MWT-distance) and (ΔTUG-speed) represented an improvement in motor function, whereas reduced and increased TUG times were defined as an improvement and deterioration respectively. The cognitive and motor functional changes were also expressed as percentage changes.

Multivariable linear regression analysis was used for the second part of this study to establish the associations between the exercise induced cerebral arteries' morphological and haemodynamic changes and the motor and cognitive functional changes in the study participants of the two post stroke patients' groups. The mean differences between the post and pre-AET cerebral arteries' morphological and haemodynamic features were correlated to the following cognitive and motor functional outcome measures 1.) changes in the Montreal Cognitive Assessment-Hong Kong version score (ΔMoCA-HK) and 2.) changes in the Stroop color word score (ΔSCWT score), 3.) changes in the 6MWT distance (Δ6MWT-distance), 4.) changes in the Timed up and go test speed (ΔTUG-speed).

5.3 Results

5.3.1 Study participants selection process.

In this current study chronic post stroke patients > than 6months from stroke onset, who had participated in our previous study highlighted in Chapter 4 that compared between post-stroke patients and non-stroke healthy adults' cerebral arteries' morphological and haemodynamic features were recruited. A total of 57 participants were thus recruited, and among the recruited participants 8 declined to participate in the interventional study. The remaining 49 patients were assessed for eligibility, and 7 were excluded for not meeting the inclusion criteria previously described. The main rejection reason was being completely non-ambulatory to be able to perform the required motor function tests and undergo 36 sessions of the exercise program. Forty-two post stroke patients met all the inclusion criteria and were randomized into either a cycling AET group (n=21) or stretching (control) group (n=21).

The interventional adherence rate in this current study was high (41)98% with all participants completing the 36 exercise sessions as per protocol, except one cycling AET participant. Based on the intention to treat analysis, data for all forty-two randomised patients was considered for final analysis. The study participants selection process is shown in the consort flow diagram (Figure 5.4)

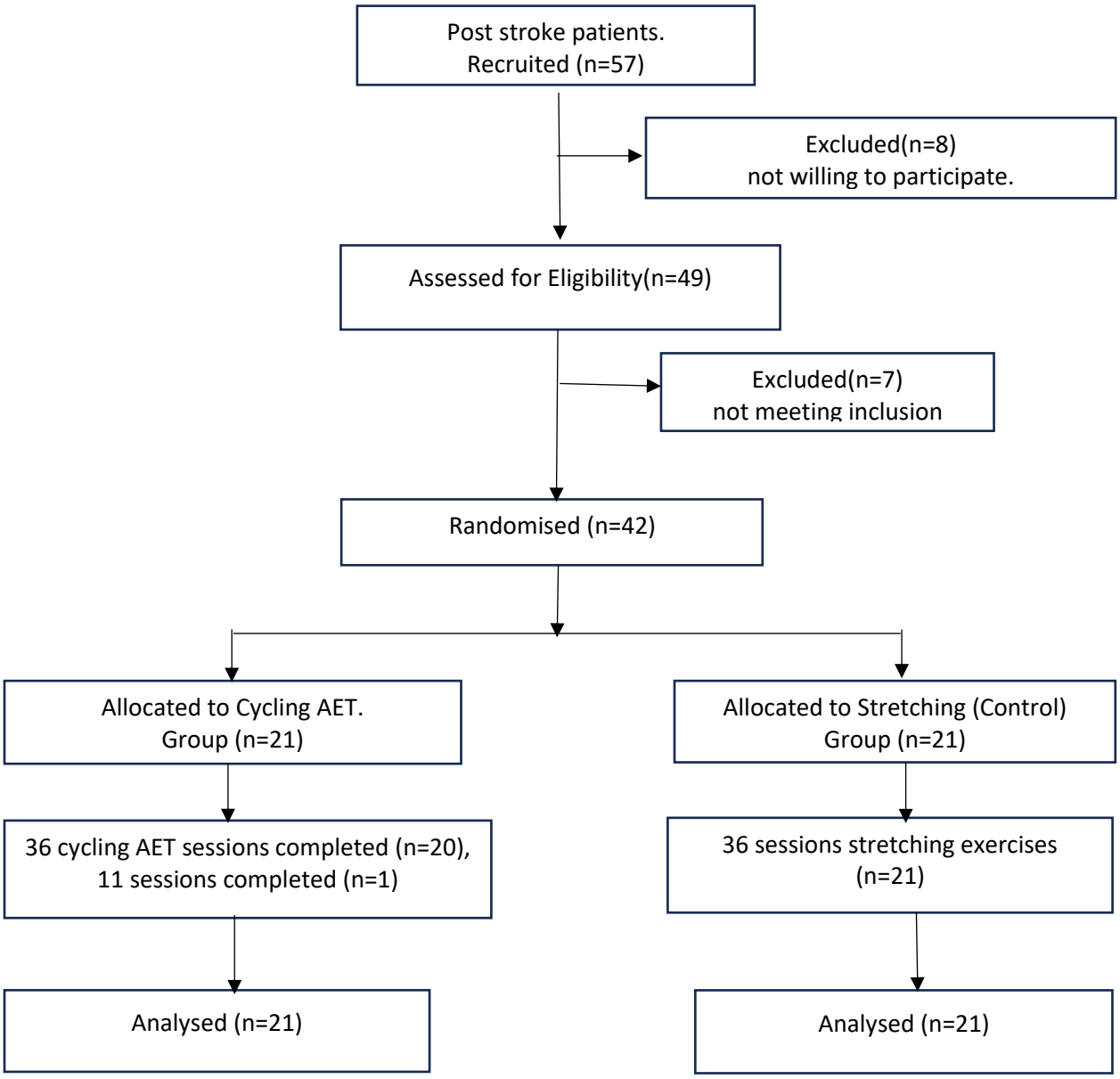


Figure 5.4: Post Stroke Participants Selection Process— Consort Flow Diagram.

5.3.2 Demographic characteristics and clinical history data for the post stroke patients in cycling AET (interventional) and stretching (control) groups.

The demographic and clinical characteristics of the participants in the two groups—cycling AET (interventional) and stretching (control) groups are presented in Table 5.1. There was no statistically significant gender discrepancy in the entire study participants (males=22, and female=20) (χ^2- test statistics=0.095, p=0.758). The mean age of all the participants was 64.4 ±7.6 years, whereas mean age between the cycling AET group (62.1±6.9years)-age range (50-74) and control groups (66.7±7.7years)-age range of 54-83 years, was not statistically different from each other (p=0.051). All the data for continuous demographic variables were normally distributed, Kolmogorov Smirnov (p>0.05) and Levene's homogeneity of variance assumption was met (p>0.05). The mean BMI was 24.3±3.8 kg/m^2, and none of the subjects were underweight BMI<18.5m^2, whilst the maximum BMI was 38.3. Although only 26% (11) of the subjects had systolic blood pressure above or equal to 140mmHg indicative of hypertension, the total percentage of those who were classified as was 73.8%. In this study hypertension was classified based on either a systolic blood pressure (BPs) exceeding 140mmHg or if one had a clinical diagnosis of hypertension and receiving anti-hypertensive treatment, despite BPs being below 140mmHg (Table 5.1).

A greater proportion of patients 27(64%) had Ischemic stroke type compared to those with haemorrhagic stroke, however the proportions of the stroke type were not significantly different across the two exercise groups (χ^2- statistics=0.933, p=0.334), respectively. The incidence rate of those with a smoking history was low as only a single subject in the stretching group had a smoking history. There were no statistically significant differences observed in the baseline values of all the categorical demographic characteristics, as assessed using the Chi-squared test (all p>0.05). However, it should be noted that the age of stroke onset was the only baseline parameter

significantly different between the two-exercise groups ($p=0.021^{*}$)**.** Additionally, no statistically significant differences were observed in the baseline 6MWT, TUG times, TUG speed, SCWT score, and MoCA-Hk version scores between the two comparison groups in this study.

Table 5.1: Baseline demographics and clinical characteristics of the cycling AET and stretching control groups.

Baseline characteristic	**Groups**			**Between group baseline differences**
	All (n=42)	**cycling AET (n=21)**	**stretching control (n=21)**	**p values**
Gender (male/female)	22/20 (52/48)	12/9	10/11	0.537
Age (years)	64.4(7.6)	62.14 (6.9)	66.7(7.7)	0.051
Weight	63.2(12.2	66.3(13.9)	60.1(9.5)	0.098
Height (cm)	161(8)	163(8.6)	158.9(8.9)	0.133
BMI ((kg/m^2)	24.3(3.8)	24.97(4.6)	23.57(2.7)	0.241
BPs (mmHg)	126(15.9)	125(17)	127(14)	0.662
BPd(mmHg)	78(8.7)	77(8)	79(9)	0.371
HR (bpm)	74(11.2)	74.1(11.9)	73.3(10.7)	0.819
Hypertension/ No (%)	31/11(74/26)	14/7	17/4	0.292
Hyperlipidemia/ No (%)	26/16 (62/38)	12/9)	14/7	0.525
Diabetes mellitus/ No (%)	12/30 (29/71)	7/14	5/16	0.495
Type of stroke *Ischemic/ hemorrhagic (%)	*27/15(*64/36)	*15/6	*12/9	0.334
Stroke Onset time (yrs)		7.6(9.1)	4.9(4.5)	0.222
Age of stroke onset		54.5(11.7)	61.8(7.5)	0.021*
Smoking history/No	1/41(2/98)	0/21	1/20	0.311
6 MWT distance (m)		261.7(106)	215.5(117)	0.189
TUG time(s)		17.5(9.1)	21.2(13)	0.169
MoCA-HK (score)		25.71(3.4)	24.9(4.2)	0.491
SCWT score		9.24(1.5)	8.9(2)	0.477

BMI— body mass index (kg/m^2), BPs—Systolic Blood pressure, BPd—Diastolic Blood pressure, HR— Heartrate, bpm—Beats per minute, MD—mean difference Type of stroke-*Ischemic / haemorrhagic, 6MWT—Six-minute walk test; TUG—Timed up and go test, MoCA-Hk—Montreal cognitive assessment Hong Kong version; SCWT—Stroop colour word Test, m=meters, s=second, continuous data is expressed as mean (S.D) and independent t-test p-values are displayed, categorical data is expressed as frequencies (%), p-values χ^2 -Chi squared test.

5.3.2.1 Demographic characteristics mean differences (post-pre) and effect sizes for the cycling AET and stretching (control) groups.

The within group mean differences in (weight, height, and BMI) was not normally distributed. There was a significant increase in weight of (1.1±2.1kg, p=0.029) in stretching group, with however no significant difference in the group's BMI (M. D=0.53±1.18 kg/m^2, p=0.054). BMI ((kg/m2). Although cycling intervention did not significantly reduce the BMI, the overall between group mean difference in BMI was statistically significant (M. D= -0.67, p=0.016) implying that The within and between exercise group change scores for the study participants demographic characteristics are shown in the table below. The within group change scores in body mass indices for cycling AET and stretching-control group were -0.14 (0.67) (kg/m^2) and 0.53 (1.18) (kg/m^2) respectively. A marginal non-significant decrease in the systolic blood pressure of approximately 2mmHg was observed in the cycling group and an overall effect size cohen d= (11.35). There are no statistically significant changes in the remaining demographic characteristics for both within and between groups changes, with small effects sizes noted.

Table 5.2: Demographic characteristics mean differences (Post-Pre) and effect sizes for the cycling AET and stretching (control) groups.

Outcome	**Groups**						**Effect sizes**	
	Cycling AET— Interventional (n=21)			**Stretching— Control** (n=21)			**Between Group Mean Difference.**	
	Pre-Cycle	Post-Cycle	Cycle M.D (Post-Pre), p-value	Pre-Stretch	Post-Stretch	Control M.D (Post-Pre), p-value	Overall M.D (95% C.I),	P-value
Weight (kg)	66.3(13.9)	66.4(13.24)	0.07(1.87), p=0.356	60.1(9.5)	61.19(10.18)	1.09(2.13), p=0.029*	-1.03 [0.224, -2.27]	++p=0.092
BMI (kg/m^2)	24.97(4.6)	(24.83(4.66)	-0.14(0.67), +p=0.221	23.57(2.7)	24.1(2.51)	0.53 (1.18), p=0.054	-0.67[-0.07, -1.27]	++p=0.016*
BPs(mmHg)	125(17)	123(14)	-2.38(11.35)	127(14)	126(12.75)	-1.19(17.9)	-1.19[8.16, -10.54]	p=0.798
BPd(mmHg)	77(8)	77(9.45)	0.38(6.76)	79(9)	79(8.57)	0.19(9.3)	0.19 [5.27, -4.89]	p=0.94
HR (bpm)	74(11.9)	75(11.7)	0.95(10)	73(10.7)	75(11.5)	2.(9.12)	-1.14 [4.87, -7.15]	p=0.703

Overall change score— represents the absolute values of the between Group Mean Difference = (cycle change score- stretch change score) values; (95 % CI) —95% confidence interval; continuous data is expressed as mean (S.D); statistical significance is set at *p<0.05,
All between group mean Differences p values are based on independent t test assuming equal variance (Levenes test > 0.05) uless specified as ++p -Mann Whitney U test and All within group p-values are based on Paired sample T-test unless specified as +p —Wilcoxon signed rank test,

SMD —standardized mean difference is represented by (cohen d) = $\frac{overall\ M.D}{pooledSD}$ equation 1, where pooled S.D— pooled standard deviation = $\sqrt{((SD_{cycling\ group}{}^2 + SD_{stretching\ group}{}^2)/2)}$

5.3.3 Pre and Post-interventional carotid arteries' morphological and functional features for the cycling and stretching groups

The pre and post carotid arteries' morphological and arterial stiffness parameters of the cycling AET and stretching control groups together with their mean differences are shown in Table 5.3. Baseline comparisons of the carotid arteries' morphological and functional features between the cycling AET and stretching-control groups demonstrated between groups homogeneity across all the evaluated parameters, all p-values >0.05. The study results showed significant improvements in all the carotid arteries' morphological and functional features between the pre and post cycling AET phases ($p<0.005$), whereas in the stretching control group significant improvements were only noted in carotid intima media thickness and carotid lumen volume stenosis (%), with the stretching group mean differences observed to be lower than those in the cycling AET group as shown in Table 5.3. The pre and post cycling AET cIMT values were 0.85 ± 0.25 and 0.78± 0.2 with a significant mean difference (95% CI) of -0.07 (-0.01; -0.04), p=<0.001*. The pre and post cycling AET Cas β stiffness index values were (17.6±28.7 and 8.6±6.9.) respectively whereas the mean difference (95% CI) of -9.0 (-16.2; -1.7), $p=0.017^{*}$ was significant representing an improvement in carotid arterial stiffness. The overall effects of cycling AET on carotid arteries morphological and arterial stiffness features are shown in figure 5.5. In all the assessed features a medium effect size was realised except in the 3d carotid lumen volume stenosis (%) where the effect size was small, Cohen d=0.23.

Table 5.3: Carotid arteries' morphological and functional features for the cycling AET (interventional) and stretching (control) groups and the mean differences (M.D).

Carotid arteries morphological and stiffness outcomes	**Exercise Groups**								
	Cycling AET— Interventional (n=42)				**Stretching— Control (n=42)**				**Between groups baseline comparisons**
	Pre-cycle	Post-cycle	cycle M.D, 95% CI	within cycle, p values	Pre-stretch	Post-stretch	Control M.D, 95%CI	within stretch, p values	p-values
cIMT (mm)	0.85 ± 0.25	0.78± 0.2	-0.07 (-0.01, -0.04)	<0.001*	0.82±0.18	0.79±0.17	-0.03 (-0.05, -0.007)	0.012*	0.497
CAS PWV (m/s)	8.02± 4.3	6.17± 1.7	-1.85 (-2.9, -0.8)	0.001*	7.39±4.0	6.57±1.86	-0.82 (-2.1, 0.5)	0.213	0.490
CAS β	17.6±28.7	8.6±6.9	-9.0 (-16.2, -1.7)	0.017*	9.7±5.0	10.1±10.4	0.5 (-15.1, 5.1)	0.839	0.641
CAS kPa	234.4±389	114±91.3	-120 (-219, -19.8)	0.02*	130±72	129±107	-61 (-173, 51)	+0.277	0.552
CAS CC (mm/kPa)	0.48±0.3	0.62±0.3	0.14(0.06, 0.23)	0.001*	0.52±0.3	0.55±0.2	0.03(-0.04, 0.1)	0.393	0.514
CAS DC (1/kPa)	0.009±0.005	0.01±0.006	0.003 (0.001, 0.005)	0.001*	0.01±0.007	0.01±0.004	0(-0.002, 0.002)		0.484
3D carotid lumen volume stenosis (%)	5.9±5.1	3.5±3.5	-2.4 (-3.5, -1.3)	<0.001*	5.36 ± 4.1	4.16 ±3.2	-1.2 (-2.3, -0.1)	0.032*	0.590
3D carotid plaque volume ($mm^{3)}$	148.7± 142	88.7±93.1	-60.0 (-95.2, -24.7)	0.001*	129.3±103	103±91	-26.3 (-53.5, 0.9)	0.058	0.478
3D carotid wall volume (mm^3)	764.9±185	709.6±132	-55 (-105, -5.2)	0.031*	723.8±180	715.4±145	-8.4 (-58.8, 420)	0.738	0.308

n—number of vessels (left and right side) from 21 subjects in each group; M.D—mean difference (post-pre); cIMT (mm) —mean carotid Intima media thickness of 1cm long ROI in the distal common carotid artery; CAS PWV (m/s) —carotid arteries pulse wave velocity; CAS β—Beta stiffness index; CAS kPa—Elastic modulus, CAS CC (mm/kPa); carotid compliance; CAS DC (1/kPa)-distensibility coefficient, $p<0.05$* significance level

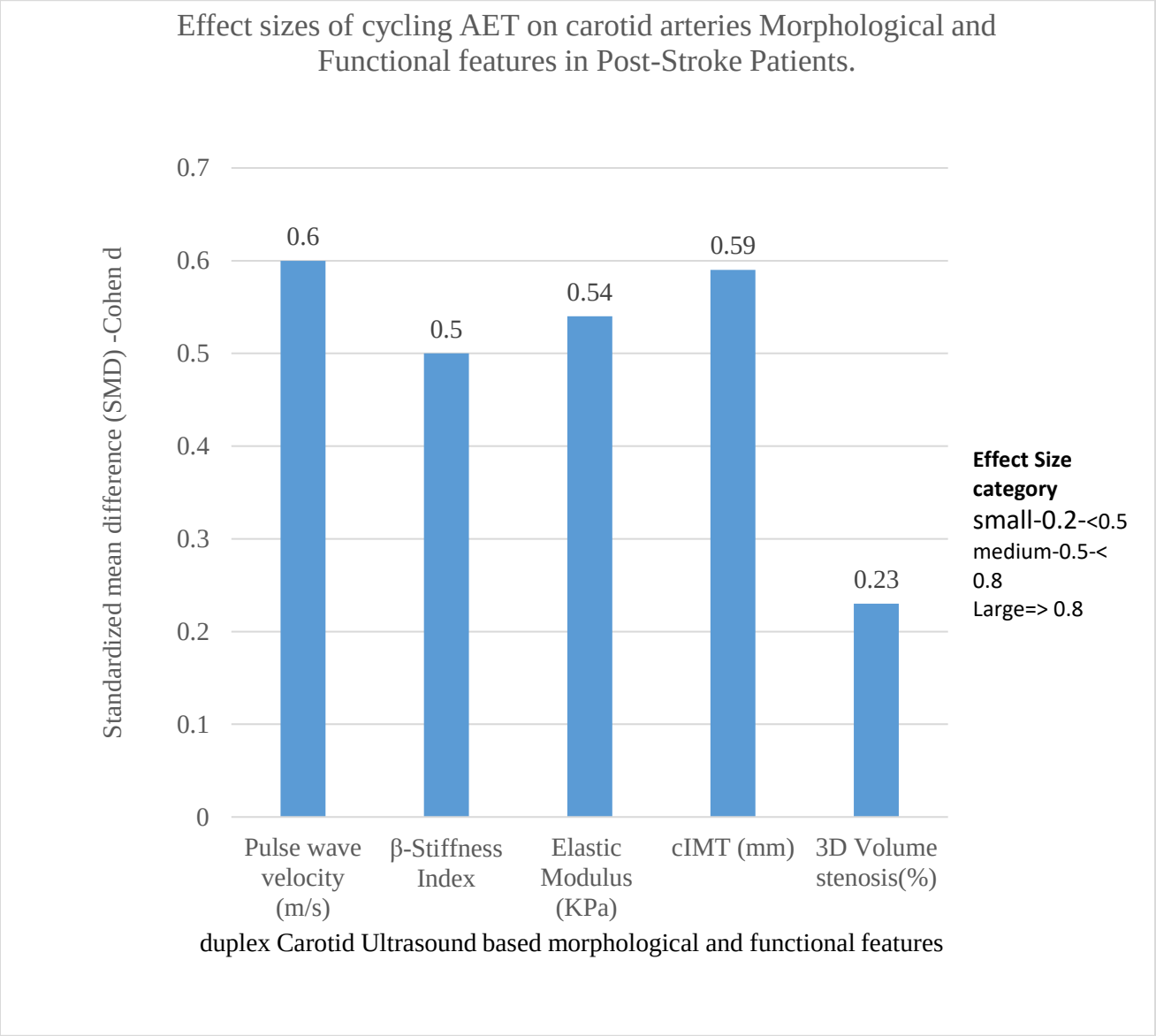


Figure 5.5: Histogram showing the standardised mean differences between cycling AET and control group on duplex carotid Ultrasound based morphological and functional features.

Furthermore, an analysis of the percentages (%) of carotid arteries exhibiting improvements in carotid arterial stiffness indices in the two groups revealed that cycling AET group had a greater % of participants with arterial stiffness improvements than the stretching groups. Decreases in PWV, and CASβ stiffness index was observed in 35(83%) of 42 cycling group' vessels assessed

whilst the elastic modulus was reported to decrease in 34(81%) of the vessels. The corresponding stretching group' percentages of vessels in which the PWV, CASβ stiffness index, and elastic modulus decreased were each 24(57%), respectively and lower than those in the cycling group. Similarly, greater percentages of post cycling AET group' participants had increases in the elasticity measures-distensibility coefficient 32(76%) and carotid compliance 33(79%) when compared to the stretching group where improvements in the two parameters were observed in 23(54.8%) and 21(50%) respectively. These results are shown in figure 5.6.

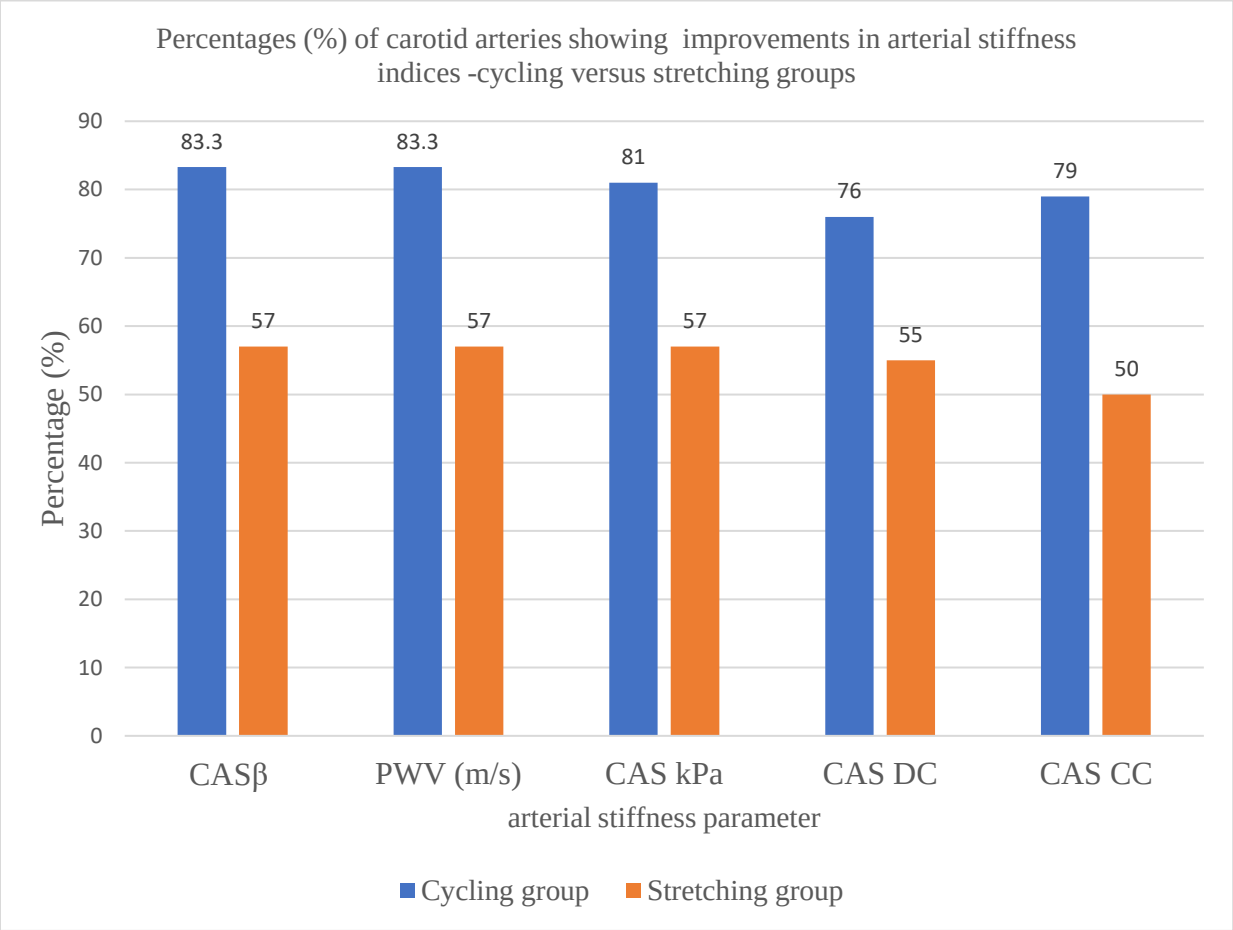


Figure 5.6: Histogram showing percentages (%) of carotid arteries with improvements in arterial stiffness indices for the cycling and stretching groups.

5.3.4 Carotid arteries haemodynamic parameters for the cycling AET (interventional) and stretching (control) groups (pre versus post) and the within groups mean differences (M.D)

The pre and post carotid arteries ICA and DCCA segments haemodynamic parameters for cycling AET and stretching- control groups and their respective mean differences are shown in Table 5.4 The cycling and stretching groups baseline values were observed to be similar in most of the haemodynamic parameters, except for ICA PSV, DCCA PSV and DCCA TAPV where the cycling group generally exhibited higher baseline values.

The cycling AET group showed significant reductions in the measures of vascular resistivity (RI) and pulsatility (PI) in both the DCCA and ICA segments whereas in the stretching group such changes were only observed in the ICA segment. The cycling AET group mean difference (95% CI) for ICA PI was -0.2 (-0.3; -0.09), p <0.001* whereas in the control group the ICA PI mean difference and 95% CI was -0.1 (-0.18; -0.02), p=0.018*. The corresponding ICA RI mean differences and 95% CI for the cycling AET interventional group and stretching-control group were -0.05 (-0.08; -0.3), <0.001* and -0.03(-0.05; -0.006), p=0.013 respectively. Although a decrease in both the ICA PI and RI values was observed in both groups, higher mean differences were noted in the cycling group compared to the control group for both parameters, PI and RI. The observed decreases in RI and PI are indicative of improvements in vascular bed resistance and pulsatility.

Furthermore, a significant increase in the DCCA EDV was observed in both cycling and stretching groups with higher mean differences observed in the cycling group compared to the stretching group. The cycling AET group mean difference (95% CI) for DCCA EDV was 1.6(0.58;2.7, p=0.003*) whilst the respective stretching group DCCA EDV mean difference was 1.5(0.43; 2.55, p=0.007*). Contrarily, no significant differences between the pre and post ICA haemodynamic

parameters, PSV, EDV, TAPV values in both the cycling AET and stretching-control group (all p values >0.05). The pre and post cycling group ICA PSV, DCCA PSV and DCCA TAPV were (67.7± 25.6 versus 63.0±18.0, p=0.148); (62.4±16.3 versus 61.1±18.1, p=0.489) and (30.0±6.8 versus 30.6±7.7, p=0.454), respectively.

Table 5.4: Carotid arteries haemodynamic features for the cycling AET (interventional) and stretching (control) groups and the mean differences (M.D).

Haemodynamic Parameter	**Groups**								**Effect sizes**			
	Cycling AET— Interventional (n=42)				**Stretching— Control** (n=42)				**Btwn Grps baseline**	**Btwn Grps M.D, p-values**		**SMD**
	Pre-Cycle	Post-Cycle	Cycle M.D (95% CI),	within cycle p-value	Pre-Stretch	Post-Stretch	Control M.D	within stretch p-value	p-value	Overall M.D (95% C.I),	p-value	Cohen d	Category
ICA PSV (cm/s)	67.7± 25.6	63.0± 18.0	-4.7 (-11.2;1.7)	0.148	57.9±14.7	58.8± 13.4	0.95 (-3.4;5.3),	0.660	**0.034***	-5.7 (-13.4;1.99)	0.144	0.32	small
ICA EDV (cm/s)	21.8±7.9	23.6±7.8	1.7(-0.4;3.8)	0.106	19.7±8.0	21.2±7.2	1.6 (-0.4; 3.5)	0.120	0.214	0.16 (-2.68; 2.99)	0.914	0.07	negligible
ICA TAPV (cm/s)	36.4±13.2	37.3±11.6	0.99 (2.36;4.24)	0.542	32.7±10.8	34.0±9.2	1.3 (-1.42;4.0),	0.342	0.173	-0.3 (-4.46;3.86)	0.887	0.03	negligible
ICA PI	1.3±0.4	1.1±0.3	-0.2 (-0.3; -0.09)	<**0.001***	1.26±0.48	1.16±0.4	-0.1 (-0.18; -0.02,	**0.018***	0.869	-0.1(-0.23;0.03)	0.125	0.34	small
ICA RI	0.68±0.1	0.63±0.09	-0.05 (-0.08; -0.3)	<**0.001***	0.67±0.09	0.64±0.08	-0.03 (-0.05; -0.006)	0.013*	0.435	-0.03 (-0.06;0.005)	0.099	0.38	negligible
DCCA PSV (cm/s)	62.4±16.3	61.1±18.1	-1.3 (-5.04; 2.4)	0.489	55.4±12.1	56.3±12.7	0.9 (-2.29; 4.0)	0.559	**0.027***	-2.2 (-6.98;2.59)	0.364	0.20	small
DCCA EDV (cm/s)	17.0±5.2	18.6±5.2	1.6 (0.58; 2.7)	**0.003***	15.3±3.8	16.8±4.4	1.5 (0.43;2.55)	**0.007***	0.087	0.14 (-1.33;1.62)	0.847	0.04	negligible
DCCA TAPV (cm/s)	30.0±6.8	30.6±7.7	0.6 (-1.03;2.3)	0.454	26.7±6.1	28.8±6.9	2.1 (0.53;3.65)	0.01	**0.023***	-1.47 (-3.7;0.76)	0.194	0.29	small
DCCA PI	1.54±0.5	1.39±0.4	-0.15 (-0.23; -0.07)	**0.001***	1.54±0.4	1.55±1.03	-0.013 (-0.3; 0.34)	0.935	0.982	-0.16 (-0.49;0.17)	0.337	0.21	small
DCCA RI	0.72±0.09	0.68±0.08	-0.04 (-0.05; -0.02)	<**0.001***	0.77±0.33	0.74±0.3	-0.03 (-0.17;0.12	0.720	0.331	-0.009 (-0.15;0.13	0.902	0.03	small

Abbreviations-Grp-groups, Btwn-between,SMD-standardised mean difference.

n-number of vessels, M.D-mean difference= (post-pre interventional values), Cohen d was used in cases of similar S.D between the two groups, otherwise Glass‘s delta was reported.

5.3.5 MCA haemodynamic parameters for the cycling AET and stretching group

5.3.5.1 trans-temporal window (TTW) status of the study population.

In all the cycling and stretching groups study participants (n=42), at least one side open TTW for assessing the MCA using TCCD was observed in 30 (71.4%) whilst the remaining 12(28.6%) participants had bilateral TTW failure. Furthermore, when split according to randomised assigned exercise grouping, a total of 19 (90.5%) cycling patients had at least one sided open TTW for assessing the MCA using TCCD (15-bilateral open TTW, and 3-unilateral open TTW - 2 on left side and 1 on right side), whereas 2 (9.5%) subjects had bilateral TTW failure to evaluate MCA. In the stretching exercise group, a total of 9(42.9%) patients had bilateral TTW failure and 12(57.1%) had at least one sided open TTW for assessing the MCA (10-bilateral open TTW, and 2-unilateral open TTW - 1 on left side and 1 on right side). Although one of the cycling group patients, had bilateral open TTW, no spectral Doppler signals was registered unilaterally due to vessel occlusion, hence, in total 32 MCAs were included for analysis for the cycling group (29 measurements from 15 patients with bilateral open TTW, and 3 from patients with unilateral TTW). In the stretching group haemodynamic parameters analysis was possible in 22 MCAs measurements (10 measurements from 10 patients with bilateral open TTW, and 2 from patients with unilateral TTW).

The mean age of all the participants in the two groups whose measurements were used in the analysis was 62.6 ±7.4 years, range 50-78years, and no significant differences in the participants' age between the stretching and cycling groups, mean age of 64.5±7.9 years versus 61.3±7years, p= 0.128, respectively.

5.3.5.1 Pre and post MCAs haemodynamic parameters of the cycling AET and stretching (control) groups.

The study results on the MCA haemodynamic parameters shown in table 5.5 demonstrated no significant differences in baseline haemodynamic parameters between the cycling and stretching groups (all $p<0.05$). Moreso, there were no significant differences between the pre and post MCA haemodynamic parameters (PSV, EDV, MFV, RI and PI) in both the cycling and stretching groups. However, the mean MCA interrogation depth for the cycling group was significantly deeper than the stretching group (60.2±4.8mm versus 56.4± 4.9mm, $p=0.008$) respectively.

Table 5.5: A comparison of the pre and post MCA haemodynamic parameters of the cycling versus stretching group

MCA haemodynamic parameter	**Exercise Groups**								
	Cycling AET— Interventional (n=32)				**Stretching— Control (n=22)**				**Between groups baseline p-values**
	Pre-cycle	Post-cycle	cycle M.D,95% CI (post-pre)	within cycle group p-values	Pre-stretch	Post-stretch	Control M.D, 95%CI (post-pre)	within stretch group p-values	p-values
PSV	81.4 ± 26.5	78.2±24.7	-3.2 (-7.43;1.04)	0.133	78.8±32.8	75.0±32.1	-3.8(-8.7; -1.6)	0.120	0.750
EDV	31.5± 13.3	30.4±13.8	-1.15(-4.1;1.79)	0.432	26.5±16.4	24.7±14.2	-1.8(-6.81;3.18)	0.459	0.223
MFV	51.5±17.7	48.3±16.6	-3.23(-6.5;0.004)	0.05	47.8±20.4	47.4±20.7	-0.41(-3.8;3.0)	0.804	0.480
RI	0.61±0.12	0.62±0.14	0.003(-0.03,0.04)	0.868	0.67±0.19	0.66±0.19	-0.01(-0.06;0.03)	0.555	0.208
PI	1.00±0.27	1.06±0.48	0.067(-0.6;0.20)	0.303	1.18±0.56	1.09±0.47	-0.09(-0.21;0.04)	0.179	0.151
Depth	60.2±4.8 -				56.4±4.9				0.008*

n-number of vessels that could be interrogated with TCCD

5.3.6 Comparison of post cycling AET cerebral arteries' morphological and haemodynamic parameters and those of age matched non-stroke adults.

A one-sample test was used to compare between post stroke cycling AET groups' morphological and haemodynamic features, and those of age matched non-stroke adults from the previous study two highlighted in chapter 4 and the study findings are shown in Table 5.6. Despite a significant reduction in cIMT after cycling AET, mean difference (95% CI) of -0.07 (-0.01; -0.04), p<0.001* the post cycling AET cIMT values were still significantly higher than those observed in age matched non- stroke subjects (p=0.004). Similarly, although significant improvement in carotid arteries' compliance was observed post cycling AET, the post stroke patient's carotid compliance value of 0.62±0.3 was still lower compared to 0.74±0.67, reported in chapter 4 for age matched non-stroke individuals (p=0.018). Contrarily, 3d ultrasound based carotid arteries' lumen volume stenosis (%) was significantly lowered to below non-stroke individuals following cycling AET (post cycle=3.5±3.5 versus non stroke=4.7±4.0, p=0.035), whereas post cycling AET carotid plaque volume (mm3) was now comparable to the non-stroke individuals' values.

The post cycling AET cerebral arteries' haemodynamic parameters (ICA RI, DCCA PI, and DCCA RI) decreased and were now comparable to non-stroke individuals' values, p values were 0.548, 0.142, and 0.748), respectively whereas the ICA PI significantly improved to values lower than those of the non-stroke individuals (p=0.001). Despite the notable improvements in carotid arteries' haemodynamic parameter DCCA EDV, following cycling AET (mean difference (95% CI) of 1.6(0.58; 2.7), p=0.003*), the post cycling AET values are still lower than those of the non-stroke group, thus there maybe still room for further improvements. The current study findings highlighted the possible utility of cycling AET in mitigating the potential stroke recurrence risk factors such as cIMT and arterial stiffness. Moreso, this is the first study to assess the effects of cycling AET on post stroke patient's lumen volume stenosis (%).

Table 5.6: A comparison between cycling AET and age matched non-stroke adults' cerebral arteries' morphological and haemodynamic features- (One-Sample Test)

	Subjects Group			**Between group differences (cycle AET versus non-stroke)**	
	pre-cycle AET	post-cycle AET	non-stroke adults	Precycle vs non- stroke, p values	post cycle vs non-stroke, p-values
Morphological features					
cIMT (mm)	0.85± 0.25	0.78± 0.2	0.69±0.15	<0.001	0.04
CAS PWV (m/s)	8.02± 4.3	6.17± 1.7	6.5±2.2	0.026	0.216
CAS β	17.6±28.7	8.6±6.9	9.3±7.7	0.069	0.526
CAS kPa	234.4±389	114±91.3	123.7±112	0.072	0.524
CAS CC (mm/kPa)	0.48±0.3	0.62±0.3	0.74±0.67	<0.001	0.018
CAS DC (1/kPa)	0.009±0.005	0.01±0.006	0.013±0.014	<0.001	0.228
Carotid lumen vol stenosis (%)	5.9±5.1	3.5±3.5	4.7±4.0	0.133	0.035
carotid plaque vol (mm^3)	148.7±142	88.7±93.1	108±103	0.071	0.186
carotid wall vol (mm^3)	764.9±185	709.6±132	705±145	0.043	0.821
Haemodynamic features					
DCCA EDV	17.0±5.2	18.6±5.2	20.8±5.8	<0.001	0.010
ICA PI	1.3±0.4	1.1±0.3	1.22± 0.6	0.390	0.001
ICA RI	0.68±0.1	0.63±0.09	0.64± 0.08	0.007*	0.548
DCCA PI	1.54±0.5	1.39±0.4	1.3± 0.3	0.002	0.142
DCCA RI	0.72±0.09	0.68±0.08	0.68± 0.006	0.008	0.748

post-cycling AET (n=42 vessels), non-stroke adults (n=134 vessels)

5.3.7 Pre and post interventional motor and cognitive function tests scores and effects sizes for the cycling AET and stretching groups

The pre and post interventional cognitive and motor function tests results for both the cycling (AET) and stretching groups are presented in Table 5.7. Additionally, the interventional effects sizes of cycling AET on cognitive and motor function expressed as absolute values of the mean differences (M.D.) and standardized mean differences (SMD), represented by Cohen's d are also detailed in Table 5.8. The comparisons of pre-interventional cognitive and motor function test values between the cycling AET and stretching groups revealed no significant differences in the baseline values for the 1.) 6 MWT distance, 2.) TUG time, 3.) MoCA-HK score and 4.) SCWT scores as highlighted in Table 5.1.

5.3.7.1 Motor function tests-Six-minute walk test (6MWT) and Timed up and go (TUG)

There were significant improvements in the 6MWT and TUG time after 12 weeks of high intensity cycling AET whereas no significant improvements in either the 6MWT distance or TUG time were observed for the stretching group post stroke patients. The pre and post cycling 6MWT distances of 261.7±106.7m and 298.7±133.5m, respectively were significantly different from each other (mean difference=36.95±48.4m, p=0.002*) whereas the corresponding pre and post stretching 6MWT distances were 215.5±117.0m versus 223.3± 121.2m, respectively and mean difference=7.74± 31.4m, p=0.273. These results indicated a significant increase in the 6MWT distance after cycling AET in contrast to a marginal increase in the 6MWT distance within the stretching group. Additionally, the cycling groups' TUG time significantly reduced from the pre-cycling AET value of 17.5±9.1s to 14.6±7.8s in the post cycle phase (mean difference= -2.84±4.0s, p=0.04*) whereas the corresponding calculated TUG speed increased by 0.07m/s from 0.34m/s to 0.41m/s. Contrary, in the stretching group the TUG time marginally increased from pre stretching

value of 21.2±13s to 21.9±17.5s, p=0.725, mean difference=0.7±8.6s in the post stretching phase, resulting in a 0.01 decrease in TUG speed. TUG speed was calculated as the TUG distance (6m) divided by the average time taken by all the participants to complete this fixed distance.

The overall between groups difference in the 6MWT distance was statistically significant 29.2 (t=2.32, p=0.027*), and translated to a standardized mean difference (SMD) of 0.72 representing a medium effect size of cycling AET on walking endurance as indicated by the 6 MWT distance in chronic post stroke patients. The overall between group TUG time mean difference was -3.54s, p=0.095) representing a medium effect size (Cohen d=0.53).

Furthermore, an analysis of the percentages (%) of post stroke patients who demonstrated improvements in motor function tests for the cycling and stretching-control group are shown in Figure 5.7. Improvements in 6MWT distance were defined as a positive mean difference (M.D) whereas a negative mean difference or decrease in TUG time represented an improvement in TUG time. The study results revealed that in 19 (90.5%) of the patients undergoing cycling AET an increase in the 6MWT distance was observed whereas in the stretching group only 13 (62%) of the patients correspondingly showed marginal increases in the 6MWT distance. Similarly, a greater percentage of post cycling AET subjects demonstrated a reduction in the TUG time 15(71.4%) compared to 12(57%) in post stretching group.

Table 5.7: Summary Pre- and Post-cognitive & motor function test scores and effect sizes for the cycling AET (interventional) and stretching (control) groups

Outcome	**Groups**						**Effect sizes**		
	Cycling AET— Interventional (n=21)			**Stretching— Control** (n=21)			**Between Group.**		**SMD**
	Pre-Cycle	Post-Cycle	Cycle M.D (Post-Pre), p-value	Pre-Stretch	Post-Stretch	Stretch M.D (Post-Pre), p-value	Overall M.D Score (95% C.I)	Cohen d	Category
Motor Function									
6MWT distance(m)	261.7(107)	298.7(133)	36.95(48.4), p=0.002*	215.5 (117.0)	223.3(121)	7.74(31.4) (p=0.273)	29.21 (p=0.027*)	0.72	Medium
TUG time	17.5(9.1)	14.6(7.8)	-2.84(4.0), p=0.004*	21.2(13)	21.9(17.5)	0.7(8.6), p=0.725	-3.54 (p=0.095)	0.53	Medium
TUG speed m/s	0.34	0.41	0.07	0.28	0.27	-0.01	0.08 (p=0.129)	0.48	Small
Cognitive Function									
MoCA-Hk score	25.71(3.4)	27.1(2.0)	1.38(2.21), p=0.006*	24.90(4.2)	24.76(5.12)	-0.143(2.7) (p=0.81)	1.52 (p=0.046*)	0.63	medium
SCWT score	9.24(1.4)	9.3(2.1)	0.05(1.4), p=0.87	8.86(2)	8.86(2)	0(1.5), p=1.000	0.05 (p=0.913)	0.03	negligible
SCWT time (s)	18.5(12.6)	16.4(10.1)	-2.08(5.0), p=0.074	21.7(16.3)	20.1(14.8)	-1.62(10.8), p=0.500	-0.46 ($^{+}$p=0.890)	0.05	negligible

AET—aerobic exercise training; M.D — mean difference (Post-Pre) values; cycle M.D — represents the within cycling AET Group mean difference; stretch M.D — represents the within control Group mean difference; Overall M.D— represents the absolute values of the between Group Mean

Difference = (cycle M.D- stretch M.D) values; (95 % CI) —95% confidence interval, 6MWT—Six-minute walk test; TUG —Timed up and go test, MoCA-HK—Montreal Cognitive Assessment Hong Kong Version; SCWT—Stroop colour word test, m=meters, s=second; continuous data is expressed as mean (S.D); $^{+}$p—Mann-Whitney U Test, p—Independent T-Test,statistical significance is set at $^{*}p<0.05$, and SMD —standardized mean difference is represented by (cohen d) = $\frac{overall\ Mean\ Difference}{pooledSD}$ equation 1, where pooled S.D— pooled standard deviation = $\sqrt{((SD_{cycling\ group}{}^{2} + SD_{stretching\ group}{}^{2})/2)}$

Effect size Categories: Small— 0.2 to <0.5, Medium —0.5 to < 0.8, Large — => 0.

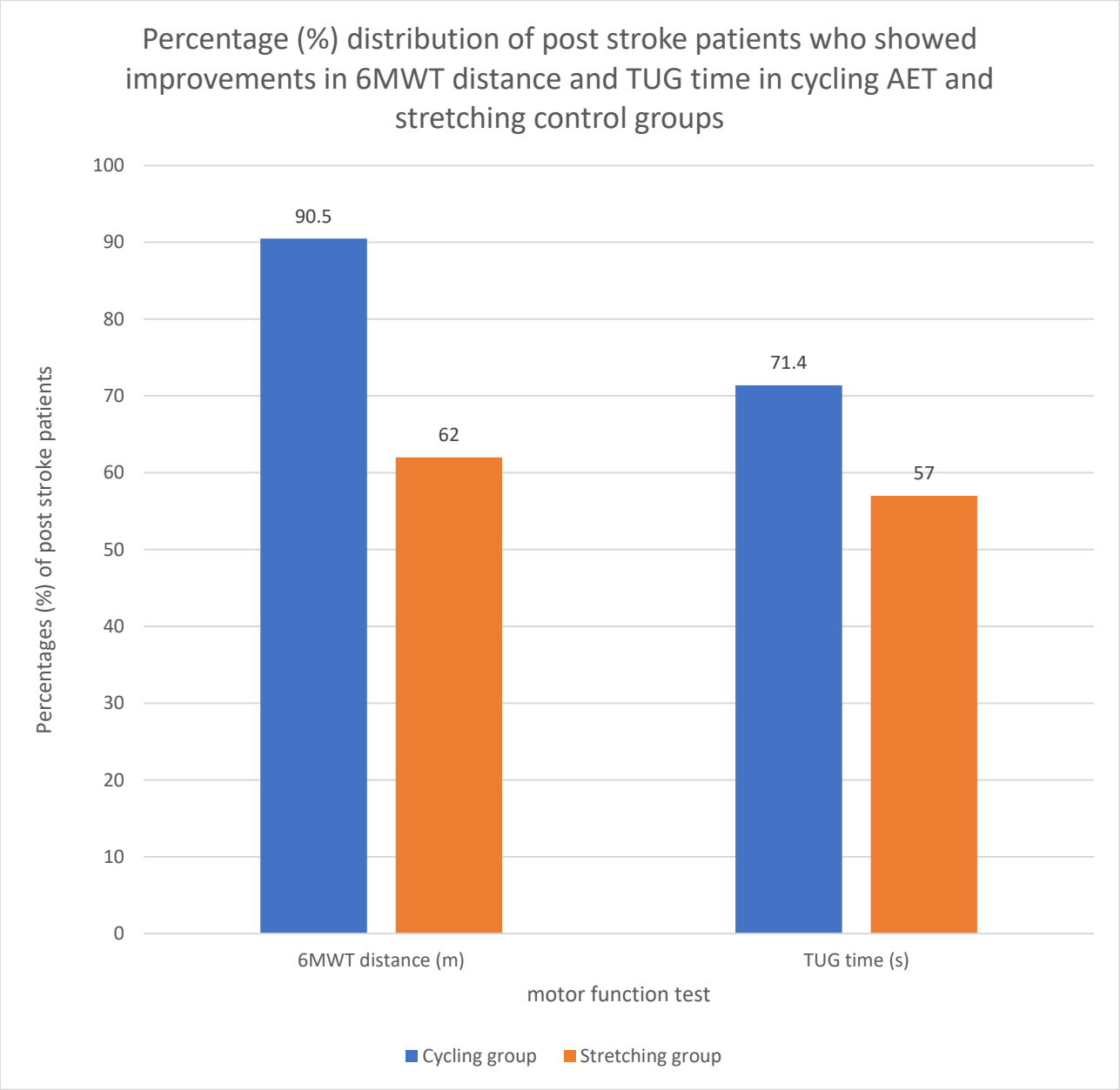


Figure 5.7: Histogram showing percentages (%) distribution of post stroke patients demonstrating improvements in the motor function tests (6MWT and TUG time) for the cycling and stretching-control group.

5.3.7.2 Cognitive function tests

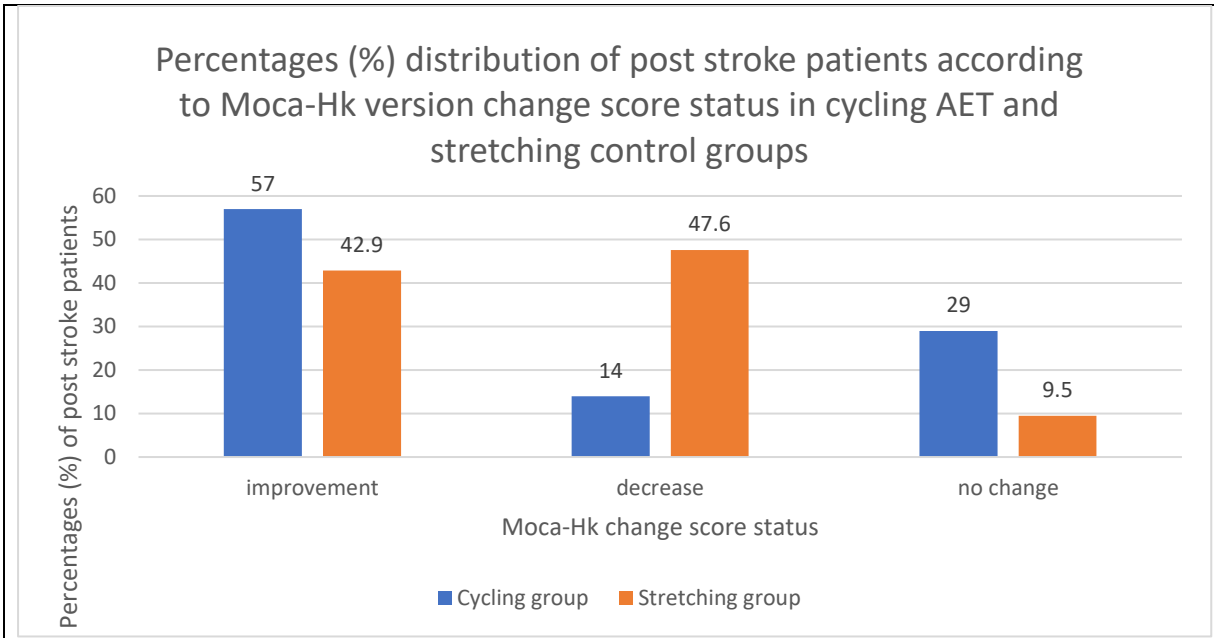
5.3.7.2.1 Montreal cognitive assessment Hong Kong version test (Moca-Hk version)

There were significant differences observed between the pre and post cycling AET MoCA-Hk version test scores. The pre and post cycling AET Moca-Hk version test scores were 25.7±3.4 and 27.1±2.0, p=0.006*, respectively and a positive change score or mean difference of 1.38±2.21 was reported (Table 5.6). The standardized mean differences, Cohen d=0.63 represented a medium positive effect size of cycling AET on cognitive function as assessed by the Moca-Hk version test. Contrarily, the stretching control group exhibited no significant changes between the pre and post interventional phases. The corresponding pre and post stretching Moca -Hk version scores were 24.9±4.2 and 24.76±5.12, p=0.81. Moreover, a higher percentage 10(47.6%) of the stretching group patients had marginal decreases in Moca-Hk test scores compared to only 3(14.3%) in cycling AET group (figure 5.8a).

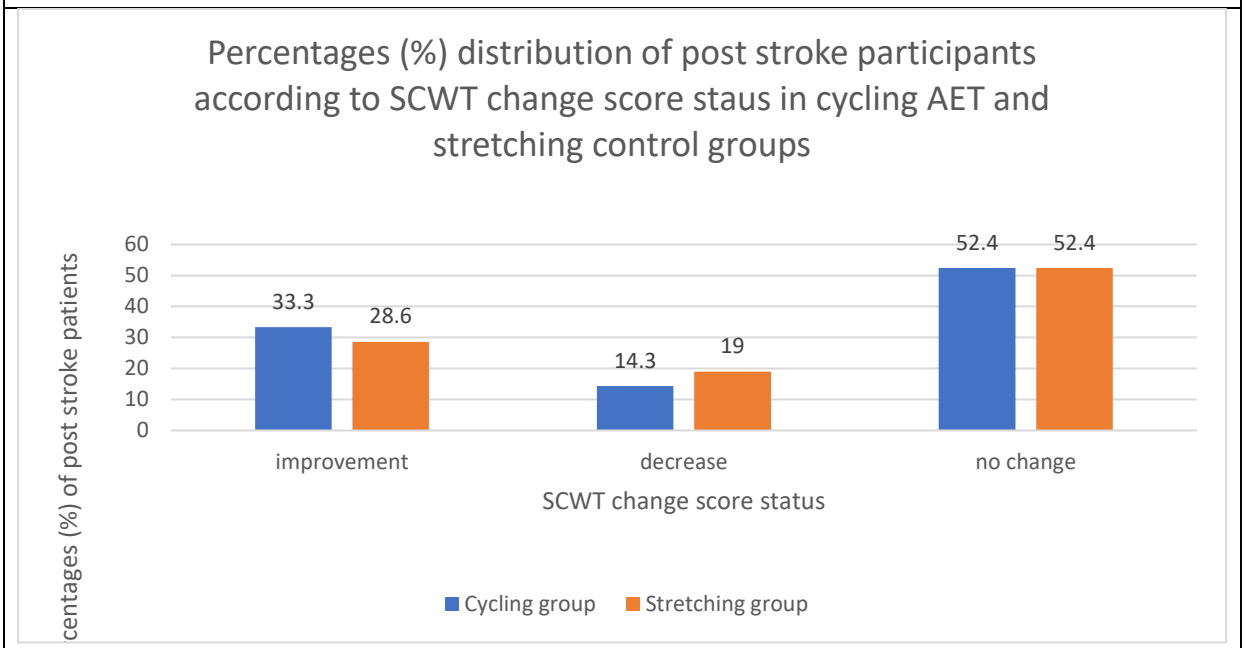
5.3.7.2.2 Stroop color word Test (SCWT).

The majority of post stroke patients in both the cycling and stretching control groups reported no changes in the Stroop color word test score. The percentage of those who showed no changes were 52.4% in each of the two post stroke patients' groups (figure 5.8b). The pre and post cycling AET SCWT scores of 9.24±1.4 and 9.3±2.1, respectively were not significantly different from each other (mean difference=0.05, p=0.87), whereas the corresponding pre and post stretching SCWT scores were similar each 8.86±2, p=1.000, mean difference=0(1.5). In both cycling AET and stretching control groups the pre interventional SCWT scores were noted to be generally high reaching averaging scores above 8.5. Furthermore, no notable reductions in the SCWT time were observed in both cycling AET and stretching groups, although the cycling group had more reductions than the stretching group, mean differences=-2.08 ±5.0s, p=0.074 and -1.62 ±10.8s,

p=0.500, respectively.



a.



b.

Figure 5.8: (a-b): Histogram showing percentages (%) distribution of cycling AET and stretching post stroke patients who demonstrated improvements in cognitive function tests a.) Moca-HK version b.) SCWT

5.3.8 Correlations between the changes in cerebral arteries' morphological and haemodynamic features and the changes in cognitive and motor function for the cycling AET group

The results on the correlations between changes in cerebral arteries' morphological and haemodynamic features and changes in cognitive and motor function for the cycling AET group are shown in Table 5.8. Significant correlations between cerebral arteries' morphological and haemodynamic features changes and MoCA-Hk version score changes were only observed and positively correlated to the changes in the haemodynamic parameter DCCA EDV (spearman's rho=0.330*, p=0.033). The DCCA PI (R=0.334, p=0.031*) and DCCA RI (R=0.320, p=0.039*) were the only haemodynamic parameters significantly correlated to the 6MWT distance change. No significant associations between the mean differences in the remaining morphological and haemodynamic features and changes in cognitive and cognitive function were observed. The current results suggest the absence of a direct effect of the exercise induced changes in the morphological features to the cognitive and motor function.

Table 5.8: Correlations between changes in cerebral arteries' haemodynamic features and changes in cognitive and motor function

cognitive and motor function mean differences (MD)		ICA PI	ICA RI	DCCA EDV	DCCA PI	DCCA RI
Cycle MoCA-Hk version	Correlation coefficient	0.040	0.233	0.330	-0.066	-0.107
	Sig. (2-tailed)	0.801	0.137	**0.033***	0.677	0.500
Cycle SCWT score	Correlation coefficient	0.003	0.025	-0.092	0.178	0.199
	Sig. (2-tailed)	0.985	0.877	0.563	0.259	0.207
Cycle 6MWT distance	Correlation coefficient	-0.074	0.116	0.127	0.334	0.320
	Sig. (2-tailed)	0.643	0.463	0.424	**0.031***	**0.039***
Cycle TUG time	Correlation coefficient	-0.012	-0.103	0.301	-0.084	-0.246
	Sig. (2-tailed)	0.937	0.518	0.052	0.595	0.117

5.4 Discussion

5.4.1 Study design

Our previous study highlighted in Chapter 4, identified significant differences in several morphological and hemodynamic features of cerebral arteries between post stroke patients and age-matched non-stroke controls, and these features posit as potential stroke rehabilitation or treatment method efficacy indicators. The present single blinded randomised controlled trial (RCT) examined whether aerobic exercise training (AET) improved these cerebral arteries' morphological and haemodynamic features in post stroke patients as assessed by multi-parametric duplex carotid ultrasound (DCUS) and transcranial color-coded Doppler (TCCD) ultrasound techniques. Moreso, the effects of AET on the cognitive and motor functions in post-stroke patients were also assessed. The current study additionally evaluated associations between cerebral arteries' structural and haemodynamic vascular changes and the cognitive and motor functional changes in a bid to explore the possible underlying mechanisms between AET induced cerebral arteries' haemodynamic changes and the improvement in cognitive and motor processes.

The exercise prescription targeting high intensity exercise mode was informed by a previous study in which this protocol was observed to prevent mental health issues and reduce immune inflammation, although in non-stroke young adults (Liu et al. 2022). Furthermore, a recent systematic review reiterated the strength of adopting the same high intensity AET mode that was used in the current study as the greatest improvement in walking function were realized in protocols that utilized this exercise mode (Khan et al. 2024). To further strengthen the study methodology, all assessments (motor, cognitive, and ultrasound examinations) and interventions were performed by trained healthcare professionals. Moreso, an intention to treat analysis contrary to per protocol analysis was used in this current. This approach enabled the preservation of the

original group assignment and sample size thus avoiding potential bias arising due to patients' exclusions hence it provides more conservative measures on the effect size as reiterated by (Santos-Gallego, Requena-Ibanez, and Badimon 2022). Moreover, the interventional protocol adherence rate in the current study was significantly high with all except one cycling AET group patient completing the study protocol's 36 exercise sessions of 41(98%), and this further catered for the potential limitations of using the intention to treat analysis related to adherence variabilities. During the study period no cases of injuries or related health problems arising from the interventional exercise were reported further alluding the notion that cycling AET is a practical and safe exercise protocol. The post interventional assessments were performed within a 10-day period after completing the last exercise session, and a mean duration of 6 days to cater for possible post interventional changes that may occur.

5.4.2 Effects of cycling AET on ultrasound based carotid arteries' morphological and haemodynamic features

5.4.2.1 Cycling AET effects on carotid intima-media thickness (cIMT)

cIMT has been recognized as a surrogate indicator for atherosclerosis and is independently associated with the likelihood of developing cardiovascular diseases. However, inconsistent findings on the impact of physical activity on cIMT have been previously reported (Wang et al. 2022). In the current study a significant reduction in the cIMT of 0.067±0.1mm, p=0.001*, with an overall medium effect size, Cohen d=0.59 was observed. The current study results demonstrating an improvement in the CIMT concur to those reported by (Glodzik et al. 2018) in which a significant reduction in CIMT, from 0.5 ± 0.06 mm to 0.46 ± 0.10 mm (p = 0.04) was observed among healthy adults following 12 weeks of moderate intensity AET program, however a higher magnitude of change was reported in the current study compared to previous studies.

Similarly, a recent systematic review by (Wang et al. 2022) concluded that an exercise duration of >6 months was associated with a 0.02 mm reduction in CIMT in populations other than chronic post-stroke patients. In another study by Bjarnegård, Hedman, and Länne (2019), a 3month indoor cycling in healthy premenopausal women did not yield a significant influence on the CIMT contrary to the current study findings. The post cycling AET CIMT values were further compared to those from our previous study discussed in chapter 4 on age matched adults without stroke. It was observed that despite a significant reduction in CIMT (mean difference (95% CI) =-0.07(-0.01;0.004, $p<0.001$) after cycling AET, the post cycling AET CIMT value of 0.78±0.2 mm was still higher than 0.69±0.15 reported in healthy non-stroke adults ($p=0.04$). These results suggest the existence of further room for improvement in reducing the CIMT in the post stroke group.

5.4.2.2 Cycling AET effects on ultrasound based carotid arterial stiffness

The study findings demonstrated that cycling AET had a positive effect on all evaluated carotid arterial stiffness indices (pulse wave velocity, β stiffness index, elastic modulus, carotid compliance, and carotid distensibility, all $p< 0.05$) thus it resulted in the improvement of arterial stiffness in chronic post stroke adults (Table 5.3). There are limited studies that have reported the effects of cycling AET on carotid arteries' stiffness based on novel ultrasound parameters, and moreover in post stroke patients. A study by Bjarnegård, Hedman, and Länne (2019), concluded that a 3month indoor cycling improved carotid artery distensibility in healthy premenopausal women, results which are similar to this current study in which a significant (post-pre) mean difference in carotid compliance of 0.14mm/kPa, $p=0.001^{*}$ was observed although in post stroke patients. The remarkable reduction in carotid arterial stiffness in post stroke patients following cycling AET point towards a potential reduction in future stroke recurrence as carotid arterial stiffness is reported to be an independent factor linked to stroke occurrence, beyond the influence

of cardiovascular (CV) factors and the stiffness of the aorta (van Sloten et al. 2015; Mitchell et al. 2010).

Furthermore, a comparison between the arterial stiffness indices of post-stroke patients undergoing cycling aerobic exercise training (AET) and those of age-matched non-stroke individuals, as discussed in Chapter 4, revealed noteworthy observations. After 36 sessions of cycling AET, the arterial stiffness parameters—pulse wave velocity, β stiffness index, and elastic modulus were significantly reduced to ranges similar to those of age-matched non-stroke individuals (all $p>0.05$). Additionally, the distensibility coefficient increased to 0.01±0.006, closely matching the non-stroke values of 0.013±0.014 ($p=0.228$) (Table 5.6). The post cycling AET carotid compliance was the only stiffness index that still remained significantly lower than those in non-stroke group ($p=0.018^{*}$) despite a notable increase from the pre interventional values of 0.48± 0.3 to 0.62±0.3, M.D (95% CI) =0.14 (0.06;0.23), $p=0.001^{*}$ following cycling AET. The higher standard deviation in the MD, suggest that a significantly varied magnitude of the changes in the arterial stiffness index across the subjects was observed hence further studies to establish the possible demographic factors behind different magnitudes of changes is suggested.

5.4.2.3 Cycling AET effects on 3d-ultrasound based arterial analysis parameters

In the current study significant reductions in the 3D ultrasound based arterial analysis parameters (carotid lumen volume stenosis (%), carotid plaque volume, and carotid wall volume) in post stroke patients following cycling AET were reported for the first time, p values were ($<0.001^{*}$, 0.001, and 0.031), respectively Table 5.3. The observed reductions in these morphological features in post cycling AET patients yielded comparable values to those of non-stroke individuals. These results set a notion for further assessments and validation of these novel ultrasound applications-based rehabilitation efficacy indicators and the translational benefits of the observed improvements

in mitigating stroke recurrences in post stroke patients.

5.4.2.4 Cycling AET effects on the cerebral arteries' haemodynamic parameters

The current study demonstrated that a 12-week cycling AET targeting a high intensity heart rate reserve (HRR) elicited a modest improvement in the extracranial cerebral arteries' haemodynamic; (DCCA EDV, MD=1.64, p=0.003*; DCCA RI, M.D=-0.035, p<0.001*, DCCA PI, MD=-0.15, p=0.001*); (ICA RI, MD=-0.05, p=<0.001* and ICA PI, MD=-0.2, p<0.001*). Although there were modest improvements in the DCCA haemodynamic features (EDV, RI and PI), paradoxical no significant changes were observed in the MCA haemodynamic parameters (PSV, EDV, MFV, PI and RI). Similar to the present study findings, recent studies assessing the potential effects of exercise training on cerebral haemodynamics involving non-stroke participants have demonstrated high intensity AET to either stabilize or reduce cerebral blood flow possibly to support thermoregulation (Oliva et al. 2023; Reed et al. 2024; Steventon et al. 2018). In a study by Reed et al. (2024), non-significant increases in cerebral pulsatility were observed, despite some notable improvements in central vasculature (aortic compliance) after 12 weeks of AET in non-stroke middle-aged adults, whereas Oliva et al. (2023) observed no changes in cerebral blood flow in healthy adults who underwent high intensity interval training, although moderate intensity demonstrated improvement in only the PI and RI haemodynamic parameters. Furthermore, a study by Steventon et al. (2018) reported exercise training not to have any effect on cerebrovascular function, more precisely on cerebrovascular blood flow (CBFV) and cerebral vasoreactivity (cVMR) in both (intracranial or extracranial) cerebral arteries (all $p > 0.05$).

The present study also employed a high-intensity exercise protocol, suggesting that its results may align with those reported in previous studies using the same approach (Oliva et al. 2023; Reed et al. 2024; Steventon et al. 2018), hence the current study may therefore propose a well working

cerebral autoregulation mechanism in post stroke patients capable of maintaining cerebral blood flow within normal ranges after cycling AET. To mitigate the potential influence of the interrogation depth on MCA haemodynamic measurements, standardisation of these depths between pre and post measurements across each group was ensured, as MCA haemodynamic parameters have been observed to vary with the interrogation depth in our previous study highlighted in chapter 3.

5.4.3 Effects of cycling AET on cognitive and motor function in post stroke patients

5.4.3.1 Motor function-Six-minute walk test (6MWT)

The present study demonstrated that 30 minutes of cycling AET, performed three times per week over a 12-week overall program duration targeting high intensity heart rate reserve (HRR) exercise intensity improved the motor function performance with respect to the 6MWT distance in chronic post stroke patients with a medium effect size (Cohen d=0.72) as shown in Table 5.7. In the present study majority of post cycling patients 19(90.5%), demonstrated improvements in the 6MWT distance whereas only 2(9.5%) of the cycling patients reported reduced values of the 6MWT distance, and among those who experienced decreased 6MWT distance was one participant who could not complete 36 cycling AET sessions as per protocol. The observed 6MWT distance of 37m was above the MCID of 34.4 m reported by (Tang, Eng, and Rand 2012). The significant improvements in walking capacity observed in this present study could probably be attributed to an optimized cycling AET protocol that was adopted coupled with a high protocol adherence rate. Although our study did not interrogate the potential improvement in the aerobic capacity several studies have shown that AET improves aerobic capacity (Pang et al. 2013) and the possible improvements in aerobic capacity could probably be attributed to be behind the observed improvements in the walking distance following cycling AET. In addition to the potential impact

of aerobic capacity on walking ability, several studies have indicated that cycling aerobic exercise training (AET) can enhance muscle strength. However, there are conflicting studies that have reported a decline in muscle strength due to AET (Markov et al. 2022). The relationship between muscle strength, muscle tone, and motor function is well established. Post-stroke muscle weakness is recognized as a major factor contributing to mobility limitations after a stroke, with research demonstrating a significant association between post-stroke muscle weakness and gait performance measures. The current study has thus further added to the growing body of knowledge supporting the use of cycling AET in improving the walking capacity in post stroke patients, as only in 3 studies was the MCID value of 34.4m achieved as alluded by (Khan et al. 2024) in a recently conducted systematic review.

5.4.3.2 Motor function- Timed up and go test (TUG test)

Our study observed a 16.2% reduction in TUG times from 17.5±9.1s to 14.6±7.8s, $p=0.004^{*}$ following cycling AET, and an overall medium effect size (Cohen d=0.53). The enhanced improvement in TUG time in this study is similar to previous studies' observations, although different exercise protocols were used (Kim et al. 2015). The mean difference between the pre and post cycling TUG time of -2.84±4s, $p=0.004^{*}$ observed in the current study was however significantly lower than that reported in a study by Kim et al. (2015), who reported TUG time changes of -8.4 ± 4.35s, $p<0.001$. Discrepancies in magnitude of observed improvements between the two studies could probably be attributed to the differences in pre-interventional-baseline TUG times between the current study and Kim et al. (2015) as post stroke participants in the current study exhibited better pre interventional mobility compared to those subjects reported in Kim et al. (2015) study. The pre interventional TUG times of the current study and Kim et al. (2015) study were 17.5 ±9.1s versus 25.11±5.40s, respectively and mean difference (95%CI)=-7.64(-11.8;-

3.5),p=0.001) hence the current study subjects may have been closer to reaching a ceiling effect. Despite notable improvements in TUG times following cycling AET, the post cycling AET TUG times in the current study were still significantly higher than reported normative TUG times(s) in apparently healthy non-stroke elderly 9.4 (8.9;9.9) (Bohannon 2006), (Svinøy et al. 2021) (mean difference=5.24 (1.7;8.8)s, p=0.06. These results suggest that there may be still room for further improvements in the gait speed among the post-stroke participants. As the turning direction was reported to influence the TUG test results with less time observed upon turning in the direction of the affected side (Son and Park 2019), all post stroke patients in our study were required to maintain a consistent turning direction towards the affected side for both pre and post-TUG tests.

5.4.3.3 Cognitive function-Moca-Hk version and SCWT

Despite cognitive function being a key outcome of interest in post stroke patients it remains understudied (Saunders et al. 2020). Our current study therefore investigated the effects of cycling AET on cognitive function in chronic post stroke subjects to complement the existing evidence on the benefits of physical activity. The study demonstrated that cycling AET had a positive medium effect size on cognitive function as assessed by the Moca-Hk version test (Cohen d=0.63). The current study findings of improved cognition concur with those reported in studies by (Lautenschlager et al. 2008; Kramer and Erickson 2007; Cumming et al. 2012) where modest improvements in cognition was observed, nonetheless the studies did not utilise the Moca-Hk version, which was used in the current study. The MoCA -HK version is a well validated tool that is suitable for the current study participants as these were limited to Asians of Chinese origins. Contrarily, to present study findings, Hoffman et al. (2008); (Young et al. 2015) concluded that exercise does not confer clinically meaningful improvements in neurocognitive function, although study population in these two previous studies involved non-stroke individuals. Despite, a medium

effect size of cycling AET on Moca-Hk version score, a negligible effect size was observed on SCWT. The small effect size of cycling AET on SCWT could probably be attributed to a ceiling effect phenomena as pre interventional SCWT scores in both cycling AET and stretching control groups reached an average score above 8.5/10. Thus, the assessment tool may not have been the ideal in this study population.

5.4.4 Correlations between the changes in cerebral arteries morphological and haemodynamic features and cognitive health outcomes

This study further examined whether the potential mechanisms of cognitive and motor changes were of cerebral vascular origin by assessing the associations between cerebral arteries' morphological and hemodynamic changes and the cognitive and motor function changes in post-stroke patients undergoing AET. The study results demonstrated no significant associations between changes in cerebral arteries' morphological features and changes observed in cognitive function, thus suggesting absence of a direct effect of AET induced morphological features changes to changes in cognitive and motor function. Although only changes in the haemodynamic parameter DCCA EDV were significantly associated with Moca-Hk version change scores the current study findings possibly explain that AET modulated morphological and haemodynamic changes may not be a direct underlying mechanism of AET in improving cognitive and motor function in post stroke patients. There is therefore a need for future studies to interrogate the possible indirect mechanisms through which the improved cerebral arteries' morphological and haemodynamic features could have influenced the notable improvements in cognitive and motor functions particularly in post stroke.

Nonetheless, in a few recently conducted studies (Oliva et al. 2023; Erickson et al. 2011; Liu et al. 2022), efforts have been made to interrogate the probable mechanisms through which exercise

may provoke improvements in cognitive function, although the population in these studies were mainly non-stroke. In Oliva et al. (2023) study, despite no notable changes in cerebrovascular haemodynamic among clinically healthy adults, as similarly observed in the current study, high-intensity AET was reported to provoke significant improvements in executive function. In addition, increases in lactate was observed to significantly correlate with cognition improvement. In another study AET has been reported to increase brain derived neurotrophic factor (BDNF) levels which is critical for neurogenesis and neuroplasticity(Pereira Oliva et al. 2020), hippocampal perfusion, and volume and this has been postulated to be behind the enhancing effect of exercise on learning and memory, although this was in older non-stroke adults (Erickson et al. 2011). Similar to the current study, improvement in mental health status among young adults was observed to be independent of haemodynamic changes, but was rather attributed to the attenuation of inflammation factors in the immune system post exercise training (Liu et al. 2022).

5.5 Limitations of the study.

The research study was without limitations. Firstly, it should be noted that some treatment methods are confounding factors to stroke rehabilitation functional outcomes. In this current study those taking medication that is known to alter blood flow mechanisms e.g. vasodilators, were however not controlled as majority of the post stroke patients enrolled were on such medication and this may have had an influence on the cerebral arteries' haemodynamic features changes observed in this study. Additionally, the sample size involved a total of 42 post stroke patients randomly assigned to cycling AET and stretching (control) groups (21 in each group), and this translated to a total of 42 cerebral arteries which fell short of the anticipated minimum sample size of 47 required to achieve a statistical power of 80% as highlighted in section 5.2.3.3 of this study, hence multicentre studies involving larger sample sizes may be recommended for future studies. Moreso,

no long-term follow ups in this study were performed to reaffirm the sustainability of observed cognitive and motor functional benefits after the end of the cycling AET an area of future research.

5.6 Conclusions

The present study revealed that 36 sessions of high intensity cycling aerobic exercise training (AET) conducted over a 12-week period significantly improved the ultrasound based morphological features of cerebral arteries, including carotid intima-media thickness (cIMT), 3D carotid lumen volume stenosis (%), carotid plaque volume, and carotid vessel wall volume. Furthermore, it positively affected arterial stiffness indices, including pulse wave velocity, β stiffness index, elastic modulus, carotid compliance, and carotid distensibility. Modest improvements were also observed in the hemodynamic parameters of the extracranial cerebral arteries —ICA RI, ICA PI, DCCA EDV, DCCA RI, and DCCA PI. Conversely, no significant changes were noted in the hemodynamic parameters of the middle cerebral artery (MCA), including peak systolic velocity (PSV), mean flow velocity (MFV), pulsatility index (PI), and resistance index (RI), thus the finding suggests a well-functioning cerebral thermoregulation mechanism in post-stroke patients. Noteworthy, was the potential of cycling AET in improving the structural and functional features of the cerebral arteries, reaching values comparable or better to those of age-matched individuals without a history of stroke, and this has potential clinical implication of possibly mitigating against potential future stroke recurrences in post stroke patients.

Additionally, cycling AET elicited beneficial changes in motor function as reflected by the 6MWT distance, and TUG test time, and exhibited a medium effect size on global cognition in post-stroke patients. The absence of significant associations between changes in the morphological features of cerebral arteries and changes in cognitive and motor function indicate that the improvements in

cognitive and motor function observed in post-stroke patients may not be directly attributable to the morphological and hemodynamic changes induced by AET, hence future studies to interrogate the possible indirect mechanisms through which the improved cerebral arteries' morphological and haemodynamic features could have influenced the notable improvements in cognitive and motor functions in post stroke are recommended.

This current study thus highlights the significance of cycling AET in enhancing the deconditioned cerebrovascular health, cognitive and motor function in the post stroke adult population and therefore contributed to the growing body of evidence supporting the role of AET in stroke rehabilitation and can help inform clinical practice guidelines.

Chapter 6

Summary of Thesis

Stroke is a significant global health issue, being a leading cause of morbidity and mortality worldwide, including in Hong Kong, where it ranks as the fourth leading cause of death. Despite existing preventative measures aimed at reducing stroke risk factors, the occurrence and recurrence of strokes remain prevalent. This highlights the need for comprehensive stroke management that encompass prevention, diagnosis, and rehabilitation strategies. Aerobic exercise training (AET) has the potential to improve the deconditioned hemodynamic, motor, and cognitive functions associated with stroke.

The current randomised controlled trial (RCT) study thus investigated the effects of AET on cerebral arteries' morphological and haemodynamic features as assessed by multi-parametric Duplex Carotid ultrasound (DCUS) and transcranial color-coded Doppler (TCCD) ultrasound, and also its impact on cognitive and motor functions in chronic post-stroke patients. Furthermore, the study compared morphological and hemodynamic features of cerebral arteries between post stroke patients and age-matched controls without stroke in an attempt to identify new biomarkers of stroke risk and indicators for monitoring treatment efficacy based on novel ultrasound applications including automated enhanced edge detection algorithms, and 3Dimensional arterial analysis. In addition, ultrasound techniques of non-imaging transcranial Doppler ultrasound (TCD) and transcranial color-coded Doppler ultrasound (with (cTCCD) and without (ncTCCD)) angle correction were compared in MCAs haemodynamic assessment.

The main RCT, Study Three presented in Chapter 5 of this thesis demonstrated that 12 weeks of cycling AET targeting high intensity HRR significantly improved the ultrasound based

morphological features of cerebral arteries' including (CIMT, M.D=-0.069, p<0.0001; lumen volume stenosis (%), MD=-2.4, p<0.001, 3D plaque volume, MD=-60mm^3, p=0.001) and all arterial stiffness indices (p<0.05). Additionally, modest improvements in extracranial cerebral arteries' haemodynamics; (DCCA EDV, MD=1.6, p=0.003*; DCCA RI, M.D=-0.04, p<0.001, DCCA PI, MD=-0.15, p=0.001); (ICA RI, MD=-0.05, p=<0.001 and ICA PI, MD=-0.2, p<0.001) were observed. Furthermore, cycling AET elicited significant medium effect size improvements on cognitive function Moca-Hk version change score (M.D=1.38, p=0.006, cohen d=0.63) and on motor function (6MWT, M.D=37m, p=0.002, cohen d=0.72 and TUG time, M. D= -2.84s, p=0.004, cohen d=0.53). In Study Two, novel ultrasound applications based, morphological features, (CIMT, 3D plaque volume, β-stiffness index, elastic modulus (kPa), pulse wave velocity (PWV), carotid compliance and distensibility coefficient) were identified as potential stroke risk and treatment efficacy monitoring biomarkers. Moreover, TCCD was validated as a practical and accurate imaging modality in assessing intracranial cerebral arteries' haemodynamics, whereas TCCD with angle correction (cTCCD) tends to yield higher peak systolic velocity than ncTCCD.

In conclusion, this current study supports the role of cycling AET in stroke rehabilitation, demonstrating its beneficial effects on the deconditioned cerebrovascular health, cognitive and motor functions critical indicators of quality of life in post-stroke patients. Furthermore, biomarkers of stroke risk and treatment efficacy monitoring based on novel ultrasound applications were established. Moreso, population-based reference values of 3d ultrasound based carotid lumen volume stenosis (%), and novel carotid arterial stiffness indices stratified according to gender are provided for the non-stroke and post stroke local population. Further research to assess long-term effects of AET in mitigating against future strokes and underlying mechanisms is recommended.

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Appendix 1: Polyu institutional review board ethical approval letter

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

To	Ying Tin Cheung (Department of Health Technology and Informatics)		
From	Pang Marco Yiu Chung, Chair, PolyU Institutional Review Board		
Email	marco.pang@	Date	24-Aug-2022

Application for Ethical Review for Teaching/Research Involving Human Subjects

I write to inform you that approval has been given to your application for human subjects ethics review of the following project for a period from 15-Aug-2022 to 30-Aug-2024:

Project Title:	A study to investigate the effects of aerobic exercise training (AET) on the large intracranial and extracranial cerebral arteries and the cognitive and motor functions in post-stroke patients.
Department:	Department of Health Technology and Informatics
Principal Investigator:	Ying Tin Cheung
Project Start Date:	15-Aug-2022
Project type:	Human subjects (clinical)
Review type:	Expedited Review
Reference Number:	HSEARS20220714001

You will be held responsible for the ethical approval granted for the project and the ethical conduct of the personnel involved in the project. In case the Co-PI, if any, has also obtained ethical approval for the project, the Co-PI will also assume the responsibility in respect of the ethical approval (in relation to the areas of expertise of respective Co-PI in accordance with the stipulations given by the approving authority).

You are responsible for informing the PolyU Institutional Review Board in advance of any changes in the proposal or procedures which may affect the validity of this ethical approval.

Pang Marco Yiu Chung

Chair

PolyU Institutional Review Board

Page 1 of 1

Appendix 2: Post stroke patient's demographic and clinical history data collection sheet

Please kindly complete **ALL** the questions below by ticking √ or putting an **X** in the appropriate box

Patients Name…… Patients' code: SDCUS…… Contact number……

Gender…….. ☐ M ☐ F

Race…… Age…….

Educational level: ☐ Primary ☐ Secondary ☐ Tertiary

Employment history: ☐ Employed ☐ Non-employed ☐ Pensioner

Weight (kg)…… Height (cm)…….

Blood Pressure (B.P) …… Age predicted (HR_{max})…… Resting Heart rate (HR_{rest})…….

Stroke onset time……. Stroke subtype: …… Affected side……..

Do you have any clinical history of:	**Yes**	**No**	
Allergy to ultrasound gel. …….	☐	☐	
Hypertension……	☐	☐	
Dementia……..	☐	☐	
Previous stroke/ TIA……	☐	☐	
Diabetes mellitus……..	☐	☐	
Hyper-lipidemia……	☐	☐	
Do you smoke cigarettes……	☐	☐	If Yes how many daily….
Are you undergoing any Rehabilitation therapy?	☐	☐	If Yes explain further…
Did you have any heart surgery……	☐	☐	If Yes explain further
heart transplantation/ Cardiac Pacemaker	☐	☐	
Do You experience chest discomfort with exertion?	☐	☐	
Do You experience unreasonable breathlessness?	☐	☐	
Do You experience dizziness, fainting, or blackouts?	☐	☐	

Please note that the information you fill in is confidential. Participants' names will not be disclosed in any way. For further clarification please feel free to contact The Principal Investigator Professor Michael Ying (phone: 34008566, email: michael.ying@).

病人人口統計和個人病史資料收集表

請填寫以下資料並在適當的格子內加√或者打 **X** 以完成所有問題

病人姓名…….

病人編號…….

聯絡電話…….

性別…… ☐ M ☐ F

族裔…….

年齡…….

教育水平： ☐ 小學 ☐ 中學 ☐ 大專或以上

個人收入……. ☐ 受僱人士 ☐ 非受僱人士 ☐ 養老金領取者

體重 (kg) ……

高度 (cm) ……

血壓 (B.P) …… 上壓…….. 下壓…….

年齡預估最大心跳率 (HR_{max})…… 靜止心跳率 (HR)……(bpm)

體溫……..

中風發病時間……. 中風類型……

你有沒有以下病史：	**有**	**沒有**
對超聲波導電凝膠 ultrasound gel 過敏. …….....	☐	☐
高血壓……..	☐	☐
失智症 Dementia……..	☐	☐
過去曾有中風/ 短暫性腦缺血發作 (*transient ischaemic attack*) (*TIA*)….	☐	☐
糖尿病……..	☐	☐
高血脂症……..	☐	☐
你現在有沒有接受中風康復治療？……	☐	☐

如有請解釋……

你填寫的所有資料都會保密，參加者的姓名不會向任何人公開。如果需要進一步澄清，歡迎聯絡項目負責人 Michael Ying 教授 (電話：34008566, 電郵：michael.ying@).

Duplex carotid ultrasound (DCUS) morphological and haemodynamic Parameters

Scan Date…. Start Time… End Time… Scan duration……

1. **Carotid Intima-Media Thickness (CIMT)-2D arterial analysis**

The automated quantification program (arterial analysis) in the Samsung ultrasound machine was used to measure the carotid intima-media thickness (CIMT). The CIMT was measured as the distance between the media-adventitia boundary (MAB) and the Lumen-Intima boundary (LIB) in the far field of a 1cm long distal common carotid artery segment. To ensure consistency in the location of the region of interest (ROI), measurements are taken with the distal tip of the cursor placed at the inferior margin of the carotid bulb where the two vessel walls are parallel to each other. Each of the two common carotid arteries (Left and Right) is scanned three times and the average of the three measurements is computed to give the CIMT for each of the arteries. The overall mean CIMT was taken as the average of the left and right CIMT measurements.

Carotid intima-media thickness CIMT (mm) Region of interest (ROI); Far-field, 1cm long DCCA segment, proximal to the inferior margin of the carotid bulb.						
		Max	**mean**	**SD**	**QI**	**POINTS**
Pre-AET-(1st) Scan Date…….	**LT**					
	RT					
Post-AET-(2rd) Scan Date…….	**LT**					
	RT					
Change score						

2. Carotid arterial stiffness (CAS)

The CAS was evaluated over the far and the near wall of a 1cm long segment of the DCCA starting from the inferior margin of the carotid bulb using the semi-automated arterial analysis software on the Samsung ultrasound machine (RS85)

		Carotid artery stiffness Parameters											
		Min. D (mm)	Max. D (mm)	B-stiffness	CC (mm/kPa)	DC (1/kPa)	Elastic modulus (kPa)	PWV (m/s)	AIC %	AIP %	Peak Strain %	Peak Strain rate (1/s)	Peak Radial Displacement (mm)
Pre-AET Scan Date….....	**LT**												
	RT												
Post-AET Scan Date….....	**LT**												
	RT												
Change score													

β- Beta stiffness index $=\frac{Dd.\ln(SBP/DBP)}{(Ds-Dd)}$; α- Alpha stiffness index= $\frac{Ad.\ln(SBP/DBP)}{(As-Ad)}$; CC-Carotid compliance = $\frac{\Delta D}{\Delta P}$ (mm/kPa); DC-Distensibility Coefficient= $\frac{\Delta A/Ad}{\Delta P}$(1/KPa); E -Elastic modulus (kPa)= stress/strain $\frac{P}{\Delta D/Dd}$ PWV-Pulse wave velocity = $\sqrt{\frac{\alpha.DBP}{\rho}}$ (m/s); Strain = $\frac{\Delta D}{Dd}x100(\%)$

ρ -blood density constant (1.050) P_e-early diastolic pressure P_l-late diastolic pressure

ΔD, ΔA, ΔV, and ΔP are the stroke change in the lumen diameter, area, volume, and blood pressure respectively.

Min. D-Minimum vessel diameter Max. D-Maximum vessel diameter

SBP-Systolic blood pressure; DBP-diastolic blood pressures; Dd- Diastolic diameter; Ds-Systolic diameter; As-Systolic lumen area Ad-Diastolic lumen area

3. Degree of carotid artery stenosis DCUS flow velocity-based Protocol.

The degree of stenosis is extrapolated from the internal carotid artery velocity values as recommended by the Society of Radiologists in Ultrasund (SRU) (Grant et al. 2003).

Pre-AET Scan Date…….			Pulsed Wave Doppler Parameters						
LT	Arterial Segment	PSV (cm/s)	EDV(cm/s)	TAMVM FV (cm/s)	TAPV	PI	RI	ICA/CCA PSV Ratio	Degree of stenosis
	DCCA								
	ICA								
	Stenotic region								
	Pre-stenosis								
	Post stenosis								
RT	DCCA								
	ICA								
	Stenotic region								
	Pre-stenosis								
	Post- stenosis								

Post-AET, Scan Date……..			Pulsed Wave Doppler Parameters						
LT	Arterial Segment	PSV (cm/s)	EDV(cm/s)	TAMVM FV (cm/s)	TAPV	PI	RI	ICA/CCA PSV Ratio	Degree of stenosis
	DCCA								
	ICA								
	Stenotic region								
	Pre-stenosis								
	Post stenosis								
RT	DCCA								
	ICA								
	Stenotic region								
	Pre-stenosis								
	Post- stenosis								

Optimization of Doppler Imaging Parameters.

Scale/PRF=3.0khz; Doppler angle=60; Depth: 2.5cm ; Sample volume=1.0mm ; Frequency Res; Dynamic range 50; Grayscale map-7; Color Gain=51 Overall Gain=50; ECA-external carotid artery; ICA-Internal carotid artery; PSV-Peak systolic velocity (cm/s); End diastolic velocity (cm/s); MFV-mean flow velocity**;** RI-Resistive index; PI-Pulsatility Index

4. Three dimensional (3D) single region Protocol carotid artery stenosis assessment.

Region of Interest	Parameter	**Pre-AET Scan Date**		**Post AET Scan Date**	
		LT DCCA Dist from center ……mm, slice no…..	Rt DCCA Dist from center …mm, slice no…..	LT Dist from center ……mm, slice no…..	RT Dist from center ……mm, slice no…..
Overall segments (all acquired slices) PCCA, DCCA, Bulb, ICA, ECA	Plaque Volume				
	Lumen volume				
	Wall volume ($mm^{3)}$				
	Volume stenosis %				
Maximum stenotic slice	Plaque area (mm^2)				
	Lumen area				
	Wall area				
	Area stenosis %				

Centre @inferior margin of the carotid bulb; Scan angle=30 degrees, Frequency Resolution, slice interval=0.5mm, to minimise reverberation artifacts on 3D acquisition the gain was set about 10% lower than the one we employed for 2D arterial analysis.

4. Plaque characteristics and 2D stenosis.

Pre-AET Scan Date…….		**Presence Of carotid Plaque/No**	**Plaque height**	**Plaque area**	**Lumen diameter (D1)**	**Lumen diameter (D2)**	**% Stenosis** $(1-(\frac{D1}{D2}))*100$	**calcifications**	**ulcerations**	**Echogenicity**			**GSM**
										hypo	**hyper**	**Iso**	
LT	PCCA												
	DCCA												
	BULB												
	ICA												
	ECA												
RT	PCCA												
	DCCA												
	BULB												
	ICA												
	ECA												
Post-AET Scan Date……		**Presence Of carotid Plaque/No**	**Plaque height**	**Plaque area**	**Lumen diameter (D1)**	**Lumen diameter (D2)**	**% Stenosis**	**calcifications**	**ulcerations**	**Echogenicity**			**GSM**
										hypo	**hyper**	**Iso**	
LT	PCCA												
	DCCA												
	BULB												
	ICA												
	ECA												
RT	PCCA												
	DCCA												
	BULB												
	ICA												
	ECA												

Abbreviations: PCCA-Proximal common carotid artery (≥ 2 cm proximal to carotid bifurcation); DCCA-Distal common carotid (< 2 cm proximal to carotid bifurcation); D1- narrowest Lumen diameter at the most stenotic site; D2- lumen diameter at stenotic area including the plaque diameter; hypo-hypoechoic; hyper-hyperechoic; Iso-isoechoic. overall gain=50%, dynamic range=50, TGC slopping in tissues and vertical in vessel lumen, persistence is set on low, frame rate 15, depth =2.5cm, Plaque occupies most of the images. **Steps:** 1. Take longitudinal and transverse images of the Plaques.

6. Transcranial color-coded duplex sonography (TCCD) Data collection sheet

Patient Name…… Code: STCD…….Date...…….Time ……
Age (Yrs)…..…… Gender……Weight (kg)……Height (cm)……BMI……. Blood Pressure……Heart rate……
Any History of stroke…….Transient ischemic attack (TIA)……Hyperlipdemia…… Cardiovascular risk…….

Doppler Parameters	**Pre-AET (cTCCD)** Scan Date…….		**Post-AET (cTCCD)** Scan Date…….		**Pre-AET (ncTCCD)** Scan Date…….		**Post- AET (ncTCCD)** Scan Date…….	
	Doppler Angle=	Doppler Angle=	Doppler Angle=					
1.LT MCA	**Proximal depth=**	**Distal depth=**	**Proximal=**	**Distal depth=**	**Proximal=**	**Distal depth=**	**Proximal=**	**Distal depth=**
PSV								
EDV								
TAPV								
RI								
PI								
S/D								
2.RT MCA								
PSV								
EDV								
TAP								
RI								
PI								
S/D								

1. Pre-AET (cTCCD)-Baseline angle corrected TCCD technique values, Pre-AET (ncTCCD)-Baseline no angle correction TCCD technique values,
2. Post-AET (cTCCD)-Post aerobic exercise training (angle corrected TCCD technique) values, Post-AET (ncTCCD)-Post aerobic exercise training (no angle correction TCCD technique) values,
3. Sample volume=4mm TCCD, TCD, visualise sphenoid greater wing, contralateral inner skull table, Angle correction parallel to color, sweep speed=300hz.

Steps (Acquired Images)

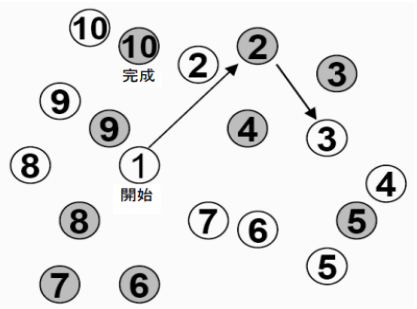
1. Lt Proximal MCA angle correction 2.) Lt Proximal MCA no angle correction, 3.) Lt Distal MCA angle correction, 4.) Lt Distal MCA distal no angle correction 5.) Rt Proximal MCA angle correction, 6.) Rt Proximal MCA no angle correction, 7.) Rt Distal MCA angle correction, 8.) Rt Distal MCA distal no angle correction

7. **Montreal Cognitive Assessment Hong Kong version (HK-MoCA)-Pre-AET Test**

Montreal Cognitive Assessment Hong Kong version (HK-MoCA)
蒙特利爾認知評估香港版

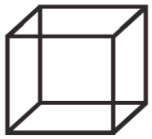
姓名:
教育程度:
姓別/年齡: 日期:

視覺空間/執行性



[ ]

複製圖形



[ ]

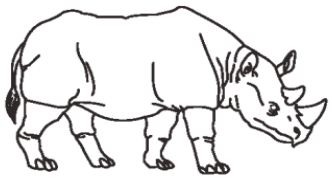
畫時鐘 (十一點十分)
(3 分)

[ ] 輪廓 [ ] 數字 [ ] 時分針

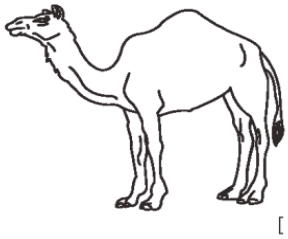
分數 ___/5

命名

[ ]



[ ]



[ ]

___/3

記憶

讀出詞語再由病者重複
以上步驟做兩次
5分鐘後回憶

	面孔	絲絨	教堂	雛菊	紅色
第一次嘗試					
第二次嘗試					

不用計分

專注

讀出數字 (每秒一個)

病者須把數字向前重複[ ] 2 1 8 5 4
病者須把數字向後重複[ ] 7 4 2

___/2

讀出數字: 當數字 '1' 出現時病者必須用手敲打桌面
(如≥2錯誤便不給予分數) [ ] 5 2 1 3 7 4 1 1 8 0 6 2 1 5 1 7 4 5 1 1 1 4 1 7 0 5 1 1 2

___/1

由100開始連續 7減算 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65
4 或 5 個正確減算得 **3 分**, 2 或 3 個正確得 **2 分**, 1個正確得 **1 分**, 沒有正確得 **0 分**

___/3

語言

重複: 姨丈買魚腸[ ]
西施四十四歲[ ]

___/2

流暢: 一分鐘內能說出的動物名稱的數目 [ ] (N ≥ 11 個名稱)

___/1

抽象

相似點: 例如: 香蕉 – 橙 =生果 [ ] 火車 – 單車 [ ] 手錶 – 間尺

___/2

___/5

定向

[ ] 日 [ ] 月 [ ] 年 [ ] 星期 [ ] 地點 [ ] 地區

___/6

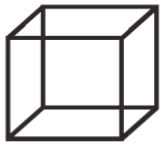
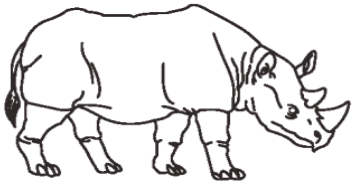
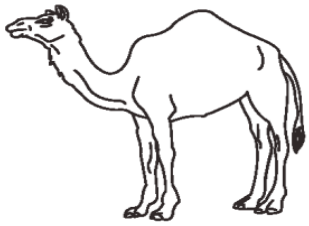
總分 ___/30
如≤6年教育加1分
22分或以上為正常

Administered by sign ……Date……Start time…… End time……..**Score** /30
Comments……

8. **Montreal Cognitive Assessment Hong Kong version (HK-MoCA)-Post-AE**

Montreal Cognitive Assessment Hong Kong version (HK-MoCA)
蒙特利爾認知評估香港版

姓名:
教育程度:
姓別/年齡: 日期:

視覺空間/執行性	複製圖形 	畫時鐘 (十一點十分) (3 分) [ ] 輪廓 [ ] 數字 [ ] 時分針	分數 ___/5
命名	   [ ] [ ] [ ]		___/3
記憶 讀出詞語再由病者重複以上步驟做兩次 5分鐘後回憶	第一次嘗試: 面孔 絲絨 教堂 雛菊 紅色 / 第二次嘗試		不用計分
專注	讀出數字 (每秒一個)	病者須把數字向前重複[ ] 2 1 8 5 4 病者須把數字向後重複[ ] 7 4 2	___/2
	讀出數字: 當數字 '1' 出現時病者必須用手敲打桌面 (如≥2錯誤便不給予分數)	[ ] 5 2 1 3 7 4 1 1 8 0 6 2 1 5 1 7 4 5 1 1 1 4 1 7 0 5 1 1 2	___/1
	由100開始連續 7減算	[ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65 4 或 5 個正確減算得 **3 分**, 2 或 3 個正確得 **2 分**, 1個正確得 **1 分**, 沒有正確得 **0 分**	___/3
語言	重複:	姨丈買魚腸 [ ] 西施四十四歲 [ ]	___/2
	流暢:	一分鐘內能說出的動物名稱的數目 [ ] (N ≥ 11 個名稱)	___/1
抽象	相似點: 例如: 香蕉 – 橙 =生果	[ ] 火車 – 單車 [ ] 手錶 – 間尺	___/2
			___/5
定向	[ ] 日 [ ] 月 [ ] 年 [ ] 星期 [ ] 地點 [ ] 地區		___/6

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Translated by Wong A and Mok V
http://www.mocatest.org

總分 ___/30
如≤6年教育加1分
22分或以上為正常

Administered by sign ……Date……Start time…… End time……..**Score** /30
Comments……

9. Stroop Color-Word Test (SCWT) 斯特魯普顏色與文字實驗- Pre AET score

對照組	兼容的	非兼容的
狗	紅	紅
凳	黃	黃
船	綠	綠
窗	藍	藍
座	紅	紅
扇	藍	藍
輪	黃	黃
盤	綠	綠
樽	藍	藍
欄	紅	紅

Time= Correct Answers= Time= Correct Answers= Time= Correct Answers=
Date……Start time……End time…….. Administered by sign…
Comments……

- 參加者需要看著一堆與實際顏色不同意思的字，請參考下表。
- 然後參加者需要說出字的實際顏色而不是字的意思。
- 完成這個任務的時間會被記錄下來。

10. Stroop Color-Word Test (SCWT) 斯特魯普顏色與文字實驗- Post AET score

對照組	兼容的	非兼容的
狗	紅	紅
凳	黃	黃
船	綠	綠
窗	藍	藍
座	紅	紅
扇	藍	藍
輪	黃	黃
盤	綠	綠
樽	藍	藍
欄	紅	紅

Time= Correct Answers= Time= Correct Answers= Time= Correct Answers=
Date……Start time……End time…….. Administered by sign……
Comments……

- 參加者需要看著一堆與實際顏色不同意思的字，請參考下表。
- 然後參加者需要說出字的實際顏色而不是字的意思。
- 完成這個任務的時間會被記錄下來。

11. Motor Function Tests (6MWT and TUG test (Pre –Post AET scores)

Tick where applicable: ☐ **6MWT** ☐ **TUG test**

Patients Name:……. DOB…… Age (Yrs)…… Gender ☐M ☐ F Patients code:…….. Weight……..(kg) Height….. (cm) Date……..			
Start: Heart rate (HR)……. B.P……..…...	End test HR ……. End test B.P……..		Maximum HR (HR_{max}) (220-Age)……
Any medication taken before test (dose & time)…… Assistive Device and/or Bracing Used:……			Test stopped/paused before test end ☐ No ☐ Yes, if yes reason e.g fatigue, Dysnea e.t.c……..
Test	Parameter	**Pre-AET- Baseline** Date:----------	**Post -AET (12 weeks)** Date:----------
1.) TUG test 3m walkway	walking time (seconds)		
	Walking speed (m/s)		
	walking distance (m)	6m	6m
2.) 6MWT	Total walking distance (m)	1st Min last Min	
	Walking speed (m/s)		
	Walking Time	(6mins/ 360s)	(6mins/ 360s)

1. Timed Up and Go test (TUG) -Walking speed.

The Timed Up and Go (TUG) test is a reliable, cost-effective, safe, and time-efficient way to evaluate overall functional mobility. (Kear, Guck, and McGaha 2017). The general information and instructions are derived from (Podsiadlo and Richardson, 1991)

Set-up:

A 3-meter (9.8 feet) walkway is measured and marked with a tape placed at the end. A standard height chair (seat height 46cm, arm height 67cm) is placed at the beginning of the walkway. A stopwatch to time is required. The participant wears regular footwear and may use a walking aid.

Instructions to the Patient.

- The patient is instructed to seat in the chair with his/her back against the chair back, arms resting on the armrests. The upper extremities should not be on the assistive device (if used for walking), but it should be nearby
- The patient is given the instructions that when the instructor says “Go” he/she will be asked to stand up out of the chair, walk 3 m, turn around, walk back to the chair, and sit down at a comfortable pace.
- The test is then demonstrated to the patient and when the patient is ready the instructor will say “Go”
- The timing of the test begins at the word “Go,” and ends when the participant’s bottom touches the seat at the end.
- The procedure is repeated 3 times and an average time is calculated.
- 參加者可以著平時著開的普通鞋，如果有需要，亦可以使用行路輔助工具例如拐杖。
- 請參加者背靠椅背，手放在椅子扶手，舒服地坐在椅子上。參加者雙手不能扶著輔助工具 (如行路時需要使用)，但輔助工具需要在附近。
- 在聽到指示說‘去’的時候，請站起來，行 3 米，轉身，然後返回座位舒服地坐下。
- 我會先示範一次，示範完後，如果你準備好，我就會說‘去’。
- 當說了‘去’之後就會開始計時，到參加者返回座位坐下就會停止計時。
- 這個計時會做三次，然後取平均值。

2. Six-minute Walk test (6MWT).

This was conducted before engaging in the cycle ergometer training programme to test the initial endurance. Those with a 6MWT speed <0.28m/s were be deemed not fit and are excluded from participating in the study (Lee et al. 2015)). The test was further repeated at the end of AET at (12wks). The distance travelled during the 6 minutes was recorded. The Pre and Post AET results of the 6MWT are compared to assess for any changes in the walking distance.

Equipment Required:

Wireless pulse monitor, sphygmomanometer, stopwatch, tape measure, unimpeded walkway, marked at 1m intervals, and cones to mark the distance that needs to be covered.

General Procedure.

- Before the 6min walk test, the patient was made to seat and relax for about 10mins. The resting pulse rate and Blood Pressure (BP) were measured and recorded.
- The maximum heart rate (HRmax) was calculated by subtracting 220 from the patient's age (220-Age) bpm.
- The Heart rate reserve was calculated as the difference between the HRmax and the resting Heart rate (HRrest) i.e HRR= (HRmax-HRrest).
- The patient was then asked to walk safely and as fast as he/she could for 6 minutes whilst a wireless pulse monitor placed on the wrist and connected to a remote monitor via Bluetooth to record the pulse rate for safety purposes.
- The distance walked (m) was measured by the investigator and gait velocity (m/s) calculated by dividing the distance walked by the 6 to obtain the gait velocity (m/s).
- 開始測試前，參加者需要坐下放鬆 10 分鐘，並量度記錄心跳和血壓。

- 測試時，參加者需要配戴無線心跳監測器以確保安全，然後以安全而又最快的步速在這個走廊裡圍繞著雪糕筒來回走動，並在 6 分鐘內走最大的距離。
- 6 分鐘的時間頗長，參加者可能會感到喘氣或者疲累。你可以在中途減慢速度或者停下休息，甚至可以靠在牆上休息，但請務必在體力休息足夠後繼續走動以完成測試。
- 請注意，你要在雪糕筒的一邊走路，然後圍繞雪糕筒轉圈往另一邊繼續走，不斷來回，直至 6 分鐘時間停止，過程中不需要猶豫。
- 過程中我們會在不同時段鼓勵你，並提示你還有多少時間剩，例如 1 分鐘後會話你還剩下 5 分鐘，然後每隔 1 分鐘會鼓勵一次，另外最後 15 秒都會有提示話快要結束，直至 6 分鐘完成後就會話時間到，請停止繼續行。
- 現在我會先示範一次，你看著我如何轉圈，不需要猶豫。

Instructions to the patients.

- "The purpose of this test is that you walk safely, as far as possible for 6 minutes with a pulse monitor placed around your thorax (wrist or thorax) to record the pulse rate. You will walk back and forth in this hallway. Six minutes is a long time to walk, so you will probably get out of breath or become exhausted. You are permitted to slow down, stop, and rest as necessary. You may lean against the wall while resting but resume walking as soon as you are able. You will be walking back and forth around the cones. You should pivot briskly around the cones and continue back the other way without hesitation. Now I'm going to show you. Please watch the way I turn without hesitation."
- During the different periods the following encouragements are utilised:

1 min “You are doing well. You have 5 minutes to go.”

2 min “Keep up the good work. You have 4 minutes to go.”

3 min “You are doing well. You are halfway to go.”

4 min “Keep up the good work. You have 2 minutes to go.”

5 min “You are doing well. You have 1 minute to go.”

15 seconds to go: “Very soon I’m going to tell you to stop where you are.

6 min “Please stop where you are, It's time up.”

II. 研究同意書

研究題目: 一個探討有氧運動訓練對中風後的病人的顱內和顱外大動脈、認知功能及運動功能的影響的研究。

我，_____ 特此同意進行本研究內容書中所列的程序。

研究人員已經向我解釋了這項研究的程序和性質，我已經理解。 我瞭解研究數據將保存在安全的地方，並且只有研究人員才能存取。

我亦清楚參與完全是自願的，因此我可以隨時退出研究，並可以聯繫香港理工大學機構審查委員會秘書進行任何澄清或投訴。

_____	_____
研究人員簽署	參加者簽署
_____	_____
研究人員姓名	參加者姓名
_____	_____
日期	日期

Appendix 3: Cycle ergometer training protocol data collection sheet.

Tick where applicable: ☐ **Cycle ergometer**

Patient Name:……. DOB…… Age (Yrs)…… Gender ☐M ☐ F Patient code:…….. Weight……..(kg) Height….. (cm) Date…….. Start time:…… End time….	
Start B.P…… End B.P……	1. Maximum HR (HR_{max})= (220-Age).....….. 2. resting Heart rate (resting HR) baseline…….(60-100bpm normal) 3. Heart rate reserve= [% intensity (age-predicted HRmax − resting HR)] + resting HR- Karvonen formula 4. Lower target HR……. 5. Upper target HR ……..
	Test stopped/paused before 30mins ☐ No ☐ Yes, if yes Reason e.g *signs and symptoms: Pain, dizziness, discomfort in (the chest, arms, jaws), fatigue, Dyspnea, arrhythmias

a.Exercise Dosage:

1.)Duration=30mins, 2.)frequency=3times/wk and 3.) Intensity=High=(60-84%HHR), 4.) Type=cycling, overal program duration=12 weeks

Prescription: Use Heart rate Protocol on ISO1000R recumbent bike

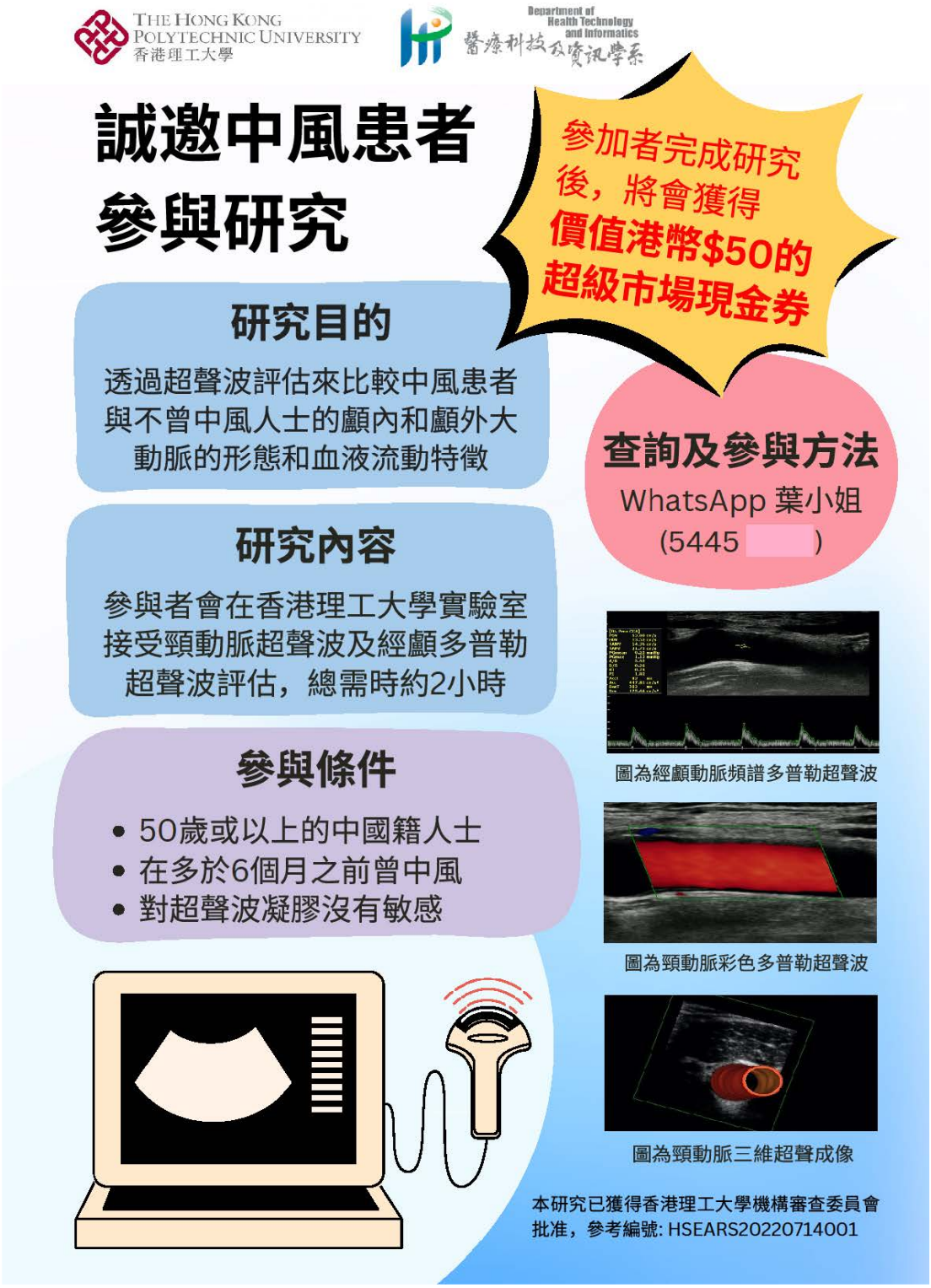
2mins warmup @ 25watts Power, 23mins-Continuous cycling @ THR zone, 5mins cool down (inclusive btwn interval)

***Power (W**)= [Resistance (Res) (kg) *cadence (Cad) (rpm)]

*All adjustments in intensity will be informed by the session target heart rate zone and the individual patient's tolerance

Session	Date	Cad (rpm)	Power Watts Avg /Peak	Distance/ km	**Target HR**	**Actual HR Avg / Peak**	METS Min /Max	Avg METS	Calories	Total Distance	**Comments**

Appendix 4: Subject recruitment poster



Appendix 5: Post exercise interview consent form and questions

The Hong Kong Polytechnic University

Department of Health Technology and Informatics

Post Interventional -Interview Consent form

Research Project Title: A study to investigate the effects of Aerobic Exercise training (AET) on the cognitive and motor functions and the associated cerebral arteries blood flow changes in post stroke patients.
Project Chief Supervisor: Professor Michael Tin-cheung Ying.
Department of Health Technology and Informatics, The Hong Kong Polytechnic University.
Tel: +852 3400 8566; email address: michael.ying@
Co-Supervisors:

1. Professor Marco Yiu-chung Pang.

Department of Rehabilitation Sciences, The Hong Kong Polytechnic University.
Tel: +852 2766 7156; email address: marco.pang@

2. Dr. Fiona Xiangyan Chen.

Department of Health Technology and Informatics, The Hong Kong Polytechnic University. Tel: +852 3400 8891; email address: fiona.chen@
Student Researcher: Mr Gunda Simon Takadiyi.
Department of Health Technology and Informatics, The Hong Kong Polytechnic University. Tel: +852 9547 ; email address: simon.gunda@

Nature of the Interview:
The interview will provide a platform for sharing the benefits you have experienced or noticed during or after participating in the above study and is anticipated to last approximately 2 minutes. Audio recording and video taking of the interview will be made. Additionally, we will capture videos and photos of you while you perform aerobic or stretching exercises and undergo cognitive and motor assessments, as well as a carotid ultrasound examination. The whole procedure will take around 10 minutes. The collected materials may be used for presentation to a third party.

Confidentiality: All materials collected will be stored securely in appropriate file formats on servers belonging to The Hong Kong Polytechnic University and/or its authorized agents.
Rights of withdrawal or refusal: Consent to the interview is strictly voluntary. You are free to withdraw your consent and discontinue participation at any time without any penalty.
Can I get more information on the study?

Yes, you may contact Professor Michael Tin-cheung Ying on Tel: 3400 8566 or via email address michael.ying@_____ for any enquiries.
This research project has received ethics approval from the Institutional Review Board of The Hong Kong Polytechnic University, and any comments or complaints regarding the conduct of the study should be addressed to Ms Cherrie Mok, Secretary of the Institutional Review Board of The Hong Kong Polytechnic University (Tel: 2766 6378; email: cherrie.mok@). By signing this document, I, _____ hereby consent:

1. To undergo the procedures as listed
2. To allow the recording of my image and voice (e.g., audio and video)
3. To grant permission for the presentation of my image and recording to a third party.

Investigator's Signature: _____	Subject's Signature: _____
Investigator's Name: _____	Subject's Name: _____
Date: _____	Date: _____

THE HONG KONG POLYTECHNIC UNIVERSITY 香港理工大學

Department of Health Technology and Informatics 醫療科技及資訊學系

香港理工大學

醫療科技及資訊學系

訪談同意書

研究題目:

一個探討有氧運動訓練對中風後病人的認知功能、運動功能及大腦動脈血流的影響的研究。

研究導師:

應天祥教授

香港理工大學醫療科技及資訊學系

電話: +852 3400 8566; 電郵: michael.ying@

研究副導師:

1. 彭耀宗教授

香港理工大學康復治療科學系

電話: +852 2766 7156; 電郵: marco.pang@

2. 陳向燕博士
香港理工大學醫療科技及資訊學系
電話: +852 3400 8891; 電郵: fiona.chen@
研究生:
Mr Gunda Simon Takadiyi.
香港理工大學醫療科技及資訊學系
電話: +852 9547 ; 電郵: simon.gunda@

訪談的性質:
訪談目的為了解您在參與上述研究期間或之後，所得到或留意到研究帶來的幫助。整個訪談歷時約 2 分鐘，形式為音頻錄製和視頻拍攝。此外，我們會拍攝您進行有氧運動或伸展運動、接受認知評估、運動評估以及頸動脈超聲檢查的片段，有關視頻和照片將被記錄。整個拍攝過程歷時約 10 分鐘，而上述所有資料有機會向第三方展示。
保密
所有資料會安全地儲存在香港理工大學及/或其授權代理的伺服器。

撤回或拒絕的權利
參與訪談完全是自願的。您可以隨時撤回您的同意並停止參與而不會受到任何處罰。

我能獲得更多關於這項研究的資訊嗎?
能，如有任何查詢，您可以致電 3400 8566 或電郵 michael.ying@ 聯絡應天祥教授 。
本研究項目已獲得香港理工大學機構審查委員會的倫理批准，如對研究的進行有任何意見或投訴，請聯繫香港理工大學機構審查委員會秘書莫雪麗女士 （電話：2766 6378；電郵：cherrie.mok@ ）。

我，_____特此同意:
1 . 接受所列的活動
2 . 允許研究人員錄製我的圖像和聲音（如用於音頻和視頻）
3. 授權研究人員將我的圖像和錄音呈現給第三方。

研究人員簽署：_____ 參加者簽署：_____

研究人員姓名：_____ 參加者姓名：_____

日期：_____ 日期：_____

Interview Questions structure-English

1. Can you please start by sharing your name and a little bit about yourself?
2. What motivated you to participate in this study?
3. What benefits have you noticed or experienced during or after your participation in the cycling/ stretching exercises?
4. Can you share any specific instances where you felt these benefits?
5. How has participating in this study impacted your daily life?
6. Would you recommend others to participate in such studies? Why or why not?
7. Is there anything else you would like to share about your experience with this study?

Interview Questions structure-Cantonese version

1. 請簡單介紹一下自己
2. 請問是甚麼驅使您參與這項研究呢?
3. 在進行自行車測力計運動／伸展運動期間或之後，您從中感受到甚麼幫助呢?
4. 您能分享您有感受到以上幫助的具體情況?
5. 參與這項研究為您的日常生活帶來甚麼幫助?
6. 您會否推薦其他人參與這項研究呢?為什麼?
7. 關於您參與這項研究的體驗，您還有想要分享的事情嗎?