PREMATURE VASCULAR AGING IN PRECIPITOUS STROKE IN A MIDDLE-INCOME COUNTRY.

Philanathi Mabena

A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science in Medicine.

Johannesburg, 2020.

Declaration

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Signature of candidate)

13 day of November 2020 at Johannesburg

Declaration: Student's contribution to article(s) and agreement of co-author(s)

I, Philanathi Mabena, student number 828413, declare that this Dissertation Report is my own work and that I contributed adequately towards research findings published in the article(s) stated below which are included in my Dissertation Report.

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Name of Primary Supervisor Professor Gavin Norton and Professor Angela Woodiwiss

Signature of Primary Supervisor

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Article 1: Title: Carotid intima-media thickness, but not chronic kidney disease independently associates with noncardiac arterial vascular events in South Africa.

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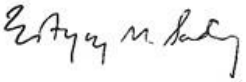
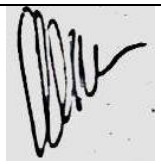
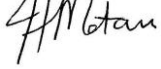
4th author	Sadiq E		5/5/20
5th author	Robinson C		07/05/2020
6th author	Motau TH		05/05/2020
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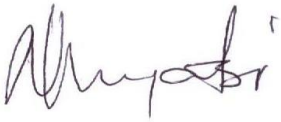
9th author	Manyatsi N		05/05/2020
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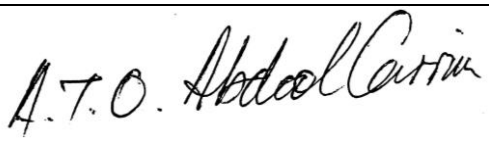
11th author	Abdool-Carrim T		07/05/2020
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Article 2: Title: Complementary impact of carotid intima-media thickness with plaque in associations with noncardiac arterial vascular events.

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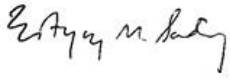
4th author	Sadiq E		5/5/20

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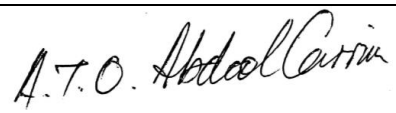
12th author	Abdool-Carrim T		07/05/2020
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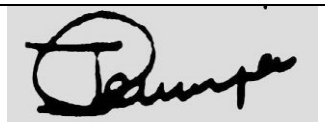
14th author	Cassimjee I		19/05/2020
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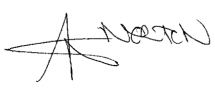
16th author	Norton GR		25/05/2020

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Abstract

Stroke often occurs over a younger adult age range in low to middle-income countries. As standard risk prediction strongly depends on age, the ability to detect the risk of stroke over this younger adult age using this approach alone may therefore be limited. Although the assessment of carotid intima media thickness (IMT) or plaque presence may add to risk prediction, the efficacy of these assessments in detecting stroke risk at a younger adult age is unknown. In the present study, I compared carotid IMT and plaque (B-mode ultrasound employing a linear array 7.5MHZ probe) in 164 patients of African ancestry (age ≥18 years) with a new stroke to that in 430 age-and-sex matched-controls (age ≥16 years) of African ancestry from a community sample. 96 (58.5%) of the stroke sample were of an age where the event would be considered as premature (<55 years in women and <50 years in men). Although hypertension and a decreased HDL cholesterol concentration were associated with stroke at a younger adult age, more than 50% of participants with stroke at this age had either no risk factor or a single risk factor. As compared to at an older age, a lower prevalence of small-vessel disease, higher prevalence of stroke of other determined aetiology and lack of detection of atherosclerotic strokes was noted at a younger age. Cardioembolic stroke accounted for 20% of all strokes with a similar prevalence in both young and old age categories. In unadjusted or multivariate adjusted models, carotid IMT was increased over most of the adult lifespan in those with stroke as compared to controls at the same age. However, in stepwise regression models, carotid IMT was independently associated with stroke at an older ($p<0.0001$) but not younger ($p=0.17$) age, whereas plaque was independently associated with stroke at both a younger ($p<0.0001$) and older ($p<0.0001$) age. Moreover, in the same regression model, plaque ($p<0.0001$) but not IMT ($p=0.82$), was independently associated with stroke at a younger age while IMT ($p<0.0001$) and plaque ($p=0.004$) were both independently associated with stroke at an older age. Moreover, plaque showed a greater performance than IMT for stroke detection over a younger age range. In conclusion, stroke is frequently premature in groups of African

ancestry in South Africa and this is often associated with an unremarkable conventional risk factor score. While non-invasive assessments of carotid artery plaque are strongly and independently associated with stroke over the young adult age range, IMT is not. The assessment of carotid plaque may be an essential tool for predicting the risk of premature stroke in Africa.

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Dedication

In memory of my aunt

Christina Nompendulo Mabena

1946-2019

This work is dedicated in-part to my aunt, Nompendulo Christina Mabena, who for many years grappled with health-related issues but still managed to share a smile despite her suffering. She recently had a stroke that left her partially disabled and shortly after being readmitted to hospital, finally passed on. She was an inspiration. This work is also dedicated to my great uncle, Sikhumbuzo Columbus Mabena, who has similarly struggled with unending health-related issues and now faces the challenges of living with kidney failure. Both my aunt and great uncle have provided constant encouragement to seek answers to the numerous remaining questions surrounding the medical conditions from which our people suffer. It is through their encouragement that I continue to commit myself to search for the answers that will ultimately allow our people to enjoy lives free from suffering.

“Romans 8:28: And we know that in all things God works for the good of those who love him, who have been called according to his purpose.”

Presentations arising from this study

As indicated below I have delivered several oral presentations of my Masters' work at 2 national conferences and 1 symposium. All of these presentations were titled: *"Increases in Carotid Intima-Media Thickness Are Not as Extensive in Younger as Compared to Older Individuals with Stroke in Africa."* (**Authors**: **Mabena Philanathi,** Sadiq Eitzaz, Gazwa Pichou, Modi Girish, Majane Olebogeng, Woodiwiss Angela & Norton Gavin).

List of conferences:

1. 45th Physiology Society of Southern Africa 2017 Conference in Pretoria, 27-31 August 2017.
2. SATA International Conference on Clinical and Basic Sciences Research in Cape Town, 27-29 November 2017.

List of symposiums:

1. Wits 8th Postgraduate Symposium in Parktown, Johannesburg, 25th October 2017.

List of manuscripts contributed to:

1. Kolkenbeck-Ruh, A., Woodiwiss, A.J., Naran, R., Sadiq, E., Robinson, C., Motau, T.H., Monareng, T., **Mabena, P.**, Manyatsi, N., Gazwa, P.Z., Abdool-Carrim, T., Majane, O.H.I., Veller, M., Modi, G. and Norton, G.R. (2019). Carotid intima-media thickness, but not chronic kidney disease independently associates with noncardiac arterial vascular events in South Africa. Journal of Hypertension, 37(4):795-804.
2. Kolkenbeck-Ruh, A., Woodiwiss, A.J., Monareng, T., Sadiq, E., **Mabena**, **P.**, Robinson, C., Motau, T.H., Stevens, B., Manyatsi, N., Tiedt, S., Dembskey, R.,

Abdool-Carrim, T., Veller, M., Cassimjee, I., Modi, G. and Norton, G.R. (2020). Complementary impact of carotid intima-media thickness with plaque in associations with noncardiac arterial vascular events. Angiology, 71(2):122-130.

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Statement of contribution to work

Carotid artery imaging using B-mode ultrasound on patients with stroke was in-part conducted by myself under the guidance of an experienced professional sonographer (Belinda Stevens) and in-line with approaches employed in control participants. The analysis and interpretation of the data was conducted by myself with the guidance of my supervisors.

List of Abbreviations

APS	Antiphospholipid antibody syndrome
APOE	Apolipoprotein E
AUC	Area under the receiver operating characteristic curves
BMI	Body mass index
CADASIL	Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CARASIL	Cerebral recessive arteriopathy with subcortical infarcts and leukoencephalopathy
C-IMT	Carotid intima-media thickness
CCA	Common carotid artery
CI	Confidence interval
CLI	Critical limb ischemia
COL4A1	Collagen type IV alpha 1
CPGRU	Cardiovascular Pathophysiology and Genomics Research Unit
CSF	Cerebrospinal fluid
CT	Computerized tomography
CVD	Cardiovascular disease

DBP	Diastolic blood pressure
ECA	External carotid artery
ESUS	Embolic stroke of undetermined source
FD	Fabry disease
HbA1c	Haemoglobin A1c
HDAC9	Histone deacetylease 9
HDL	High density lipoprotein
HIV/AIDS	Human immunodeficiency virus/Acquired immunodeficiency syndrome
ICA	Internal carotid artery
IMT	Carotid intima-media thickness
LDL	Low density lipoprotein
MRI	Magnetic resonance imaging
NCD	Non-communicable diseases
NHLS	National Health Laboratory Services
NOTCH3	Neurological locus notch homolog protein 3
OR	Odds ratio
PDE4D	Phosphodiesterase 4D
PITX2	Paired-like homeodomain transcription factor 2
RHD	Rheumatic heart disease
ROC	Receiver operator characteristic

SAS	Statistical Analysis System
SBP	Systolic blood pressure
SEM	Standard error of the mean
SOWETO	South West Township
SSA	Sub-Saharan Africa
TG	Triglyceride
TOAST	Trial of Org 10172 in Acute Stroke Treatment
X-Ray	Roentgenogram
ZFHX3	Zinc finger homeobox 3

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Preface

Over 80% of deaths attributed to stroke worldwide occur on the African continent, most of which are low-to-middle-income countries such as South Africa. In South Africa alone, stroke is responsible for over 30 000 deaths per year. In this regard, stroke burden doubles in ages over 55 years regardless of gender. However, stroke occurrence over a younger age (below ages of 45 years), has been noted to be more frequent in those from low-to-middle income countries, where 19-30% of all strokes may occur over this age range. In contrast, in high-income countries only 5% of all strokes occur in this age range. In this regard, the adverse impact of stroke on disability adjusted life years is far greater when occurring at a younger as opposed to older adult age. As age is the strongest risk factor for stroke, risk prediction over a younger adult age using standard risk approaches is markedly limited. The question therefore arises as to how best to improve risk prediction in young adults. The work performed in the present dissertation therefore arose in-part in order to identify better approaches to risk prediction over a younger adult age range in Africa. In the present study, I aimed to compare the extent to which carotid intima-media thickness (IMT) and plaque, indices of atheroma formation, are associated with stroke beyond conventional risk factors over a younger as compared to older adult age in South Africans of African ancestry.

In chapter 1 of the present dissertation, I first highlight the evidence to indicate that stroke frequently occurs at a young adult age in developing countries and that the burden of chronologically early (premature) stroke poses a major challenge to healthcare systems. I subsequently discuss the possible evidence or the lack thereof to support a role for a dominance of specific stroke subtypes and risk factors that may explain these events at a young adult age. As I highlight that identification of conventional risk factors is likely to be self-limiting when attempting to select those at risk for an early stroke for more careful management, I thus argue that an important approach to identifying young adults with risk factors who are at particular risk of stroke may be to assess the presence of end-organ

changes, in particular the extent of atheroma using non-invasive approaches. I nevertheless also review the evidence to show that there is controversy as to which index of carotid atheroma best predicts events but underscore that the evidence over a younger adult age is lacking. I therefore highlight the novelty of the study described in the present dissertation. In chapter 2 of the present study, I then describe the methods employed to conduct the present study and in chapter 3 of the present study, the results obtained. In chapter 4 I then discuss the relevance of our results in the context of findings of other studies and draw several conclusions from the data obtained.

Chapter 1

Introduction

1.1 Introduction

Globally, non-communicable diseases (NCD) are considered to be the main causes of death and disability. Indeed, in 2012, 36 out of 56 million (68%) deaths that occurred worldwide were attributed to NCDs (Aryal et al., 2015). Where previously in low and middle-income countries, including most countries in Sub-Saharan Africa, infectious diseases and diseases of poverty were the main causes of death and disability, NCDs are now considered to be equally as important (Allen, 2017). In this regard, more than 85% of deaths attributed to NCDs in the world occur in low-to-middle-income countries, including South Africa (Van Heerden et al., 2017) and in 2012, 30% of deaths in Africa were from NCDs (Van Heerden et al., 2017). In South Africa alone, a middle-income country, the burden of NCDs has doubled over the past 20 years (Rheeder et al., 2017). Of the NCDs, which include cancer, respiratory disease, cardiovascular disease (CVD) and diabetes mellitus, CVD is by far the most important cause of morbidity and mortality in the world and in low and middle-income countries (Al-Mawali, 2015). Importantly, in 2013, CVD accounted for 1 million deaths in Sub-Saharan Africa alone (Keates et al., 2017).

Cardiovascular disease includes myocardial infarction, heart failure, stroke and renal failure. In this regard, globally stroke was noted in 2013 to be the third most common cause of death after HIV/AIDS (an infectious disease) and ischaemic heart disease and the second leading cause of physical disability (Bertram et al., 2013; Feigin et al., 2017). In the same year (2013), ischaemic stroke claimed the lives of approximately 6.5 million people, and of the 25.7 million (71%) people that survived a stroke, 11.3 million were young persons, who subsequently lost the most exciting and productive years of their lives due to physical disabilities and cognitive dysfunction (Feigin et al., 2017). In that study (Feigin et al., 2017) about 10.3 million new strokes were recorded. Importantly, 80% of all stroke deaths that occur in the world are noted in developing countries such as South Africa (Bertram et al., 2013). About 87% of the global stroke incidence occurs in Africa and developing countries (Adeloye, 2014). Stroke is responsible for approximately 30 000 deaths every year in South

Africa alone, and this is a more frequent occurrence in rural South Africa where most inhabitants are of a low socio-economic status (Maredza et al., 2016). Stroke is the second most frequent cause of death after infectious diseases (HIV/AIDS) and is the leading cause of physical disability in South Africa (Maredza and Chola, 2016).

The increasing incidence of stroke in the developing world including South Africa is attributed to an aging population and an epidemiological transition (Connor et al., 2009). Although the high prevalence rates of uncontrolled risk factors for stroke are likely to account for the high incidence in Africa, stroke may have an ethnic distribution that contributes to these high incidence rates. Indeed, stroke is far more common in those of African origins residing in other parts of the world such as the United Kingdom as compared to those of European origin residing in the same environment (Whincup et al., 2012).

In the present dissertation I explored the possibility of enhancing the prediction (risk) of stroke in Africa at a young adult age. Thus, in the present chapter I critically review the evidence and provide arguments to support the importance and highlight the novelty of this question. In this regard in the present chapter I first highlight the evidence to indicate that stroke frequently occurs at a young adult age in developing countries and that the burden of chronologically early (premature) stroke poses a major challenge to healthcare systems. I subsequently discuss the possible evidence or the lack thereof to support a role for a dominance of specific stroke subtypes and risk factors that may explain these events at a young adult age. As I highlight that identification of conventional risk factors is likely to be self-limiting when attempting to select those at risk for an early stroke for more careful management, I thus argue that an important approach to identifying young adults with risk factors who are at particular risk of stroke may be to assess the presence of end-organ changes, in particular the extent of atheroma using non-invasive approaches. However, I further suggest that the assessment of indices of atheroma formation may be limited, and hence argue for a need to perform further studies as part of the present dissertation to better understand their use.

1.2 Age distribution of stroke

Above the age of 55 years, stroke burden doubles, regardless of gender (Chen et al., 2010). In most countries stroke is therefore considered to be largely a disease of the elderly and stroke in younger individuals is regarded as being a rare occurrence (Terni et al., 2015). In fact, 89% of all strokes occur in the elderly, with 50% of these strokes affecting those above the age 75 years and 23% those over 85 years of age (Chen et al., 2010). Thus, the average age of stroke is 60-65 years or older. The average age of ischaemic stroke in 19 countries that formed 4 global regions namely, Europe with highest recruitment sites (9 countries), North America (2 countries), Latin America (3 countries) and East Asia/Pacific (5 countries) in the Embolic Stroke of Undetermined Source (ESUS) study performed in 2144 ischaemic stroke patients was noted to be 67 years (Perera et al., 2016). Nevertheless, the age distribution of stroke differs according to gender where younger women (age 20-24 years old) and women above age 85 years are more likely to have a stroke than men of the same age whilst middle aged (50 years) men are more likely to have a stroke than middle aged women (Yu et al., 2015). The frequency of stroke occurrence has nevertheless been reported to be decreasing in the elderly (Chen et al., 2010; Putaala, 2016).

Although stroke is not common in the young, in clinical practice the young frequently present with acute stroke (Smajlovic, 2015), A definition of “young stroke” is still debatable, but the majority of studies define “young stroke” as those individuals who are below 45 years of age at the onset of stroke (Griffiths and Sturm, 2011). However, premature cardiovascular events are in general thought to occur in women <55 years of age and in men<50 years of age (Maas and Appelman, 2010). Stroke may occur both in adolescents (average age of 13 years) and in young adults (age 20 years to 50 years) (Komolafe et al., 2015). Although only 15% of ischaemic strokes occur at a younger age (Komolafe et al., 2015) with only 10% in those less than 50 years of age (Putaala, 2016; Saeed et al., 2014), ischaemic stroke in younger people from either low-middle or high-income countries is becoming more common (Cabral et al., 2017). Of all that have stroke at a young age, men between ages of 35-44

years have a higher incidence of stroke as compared to women, but women who are below the age of 30 years have a greater stroke incidence than men (Griffiths and Sturm, 2011).

In contrast to high-income countries, in low and middle-income countries, the average age of stroke is considerably lower. In low-to-middle-income countries approximately 19-30% of all strokes occur in those less than 45 years of age as compared to only 5% of all strokes occurring in this age group in high-income countries (Fahmi and Elsaid, 2016). In this regard, South Africa is no exception with the average age of patients hospitalized for stroke in tertiary care hospitals, being 45 years of age (Connor et al., 2009). However, this age distribution shows geographic variations and in rural areas of Africa the average age of stroke may not be as young as that noted in urban areas (Tomari et al., 2017). African countries often report a quite unusual prevalence rate of stroke below age 45 years. The average age of stroke in one study was 31 years and most of these patients were men (73%) (Owolabi and Ibrahim, 2012). Importantly, in that study 59.2% were ischaemic strokes whilst haemorrhagic strokes accounted for 40.8% (Owolabi and Ibrahim, 2012). The early onset of stroke in African countries is thought to be particularly through haemorrhagic stroke (Deresse and Shaweno, 2015), but the prevalence rates of ischaemic stroke at a younger age are increasing in developing countries.

1.2.1 Impact of stroke at a younger versus older age

Those who experience a stroke at a young age have an increased chance of survival and a better outcome as compared to older persons (Ezejimofor et al., 2017). However, the impact of stroke at a young as compared to older age on disability adjusted life years is far greater. Indeed, the occurrence of a premature stroke (below age 50 years) results in considerable loss of potential and productivity with notable effects on personal wealth and the wealth of the country (Nakibuuka, 2015). Stroke is well recognized as resulting in a decreased physical activity and major emotional and psychological effects with a considerable reduction in the quality of life. Stroke at any age markedly reduces the chance

of being fully employable (Muli and Rhoda, 2013). Only 25% of patients with ischaemic stroke eventually return to their jobs after the stroke, whilst 70% of patients remain disabled and are unemployable (Reshi et al., 2017). The loss of jobs at a young age to those with families has a major impact on social structures limiting the ability of families to generate income, and feed and educate children. Hence, the disabling impact of stroke in the young affects not only the individual but is a major threat to immediate dependents such as children or spouses and to the economy due to loss of effectiveness and productivity at a working age (Smajlovic, 2015).

1.3 Types of stroke and their risk factors at different ages

It is well recognized that the risk factors and the aetiology of stroke in young adults differs from those seen in the older person (Putaala et al., 2009). Nevertheless, as will be highlighted differences in risk factors between studies are inconsistent and the aetiology of stroke in younger persons remains unclear. As previously indicated, in the present dissertation I evaluated the possibility of improving risk prediction of younger persons who develop a stroke. Consequently, in the present chapter I will discuss the age distribution for the various types of stroke that occur and the evidence to indicate that the risk factors involved may differ in younger as compared to older strokes. In so doing I will highlight any potential differences that cast light on the pathophysiology of stroke at a younger age as compared to an older age and hence posit a thesis as to how to improve risk prediction for stroke at a younger age.

1.3.1 Ischaemic versus haemorrhagic stroke

Stroke is defined as a rapid onset of focal neurological signs that last for more than 24 hours after the event with a presumed vascular cause (Markus, 2012). The commonest form of stroke anywhere in the world is ischaemic stroke which is caused by an obstruction

to a blood vessel (located either intracranially or extracranially) by a thrombus, embolus or by atherosclerotic plaque (Baidya et al., 2015). The other major form of stroke is haemorrhagic stroke, which is caused by bleeding into the cranium due to a ruptured blood vessel (Steiner et al., 2014) and may be primarily intracerebral or subarachnoid (Hisham and Bayraktutan, 2013). Ischaemic stroke accounts for approximately 85% of all stroke cases (Musuka et al., 2015). However, the burden of haemorrhagic stroke may be far greater on the African continent (low-middle income countries) as compared to high-income countries where the incidence of haemorrhagic stroke is lowest (Lee et al., 2015).

In those below 45 years of age, subarachnoid haemorrhage occurs at a rate of 3-6/100 000/year and intracerebral haemorrhage at a rate of 2-7/100 000/year (Smajlovic, 2015). Haemorrhagic stroke is generally reported to account for more stroke (40 to 45%) in those below the age of 45 years as compared to stroke in older persons (15 to 20% are haemorrhagic) (Smajlovic, 2015). This nevertheless is likely to depend on the characteristics of aging populations as in the elderly, anticoagulants are a major cause of haemorrhagic stroke (Lindley, 2018). Moreover, the general age distribution of haemorrhagic stroke may differ in developing countries such as South Africa, where haemorrhagic stroke may be determined more by socio-economic status and ethnicity than age (Connor et al., 2009). In South Africa, older persons are more likely to develop haemorrhagic stroke than younger persons but the bigger difference is between black and white persons with younger black versus white (21 vs 9%) persons and older black versus white (31% vs 16%) persons developing haemorrhagic stroke (Connor et al., 2009). Thus, haemorrhagic stroke may be a key determinant of stroke at a younger age, but this will depend on the characteristics of populations, including socio-economic class, ethnicity and the use of oral anticoagulants.

1.3.2 Ischaemic stroke subtypes

Ischaemic strokes involve either small or large blood vessels (Jordan et al., 2007; Krishnamurthi et al., 2018). In order to better understand and manage ischaemic stroke,

several classification systems have emerged of which the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification is frequently employed. The TOAST classification of ischaemic stroke includes five subtypes, namely, large-artery atherosclerosis, cardio-embolism, small-artery occlusion (lacunar), stroke of other determined aetiology and strokes of undetermined aetiology (Adams et al., 1993). Large and small vessel strokes are responsible for approximately 3% to 42.5% of all strokes, cardioembolic strokes for approximately 12.6% to 54% and undetermined strokes are responsible for approximately 5% to 44% of all strokes (Kes et al., 2012). However, there is an age distribution of the prevalence of these subtypes which will be discussed below, and which cast some light on pathophysiological mechanisms at different ages. There is also a geographic distribution where for example in the United States of America and European countries, small-vessel disease is responsible for about 15 to 26% of ischaemic strokes, whilst in Asia, small-vessel disease causes 25 to 54% of ischaemic strokes (Choi, 2015). Importantly, as indicated above, both in developed and developing countries, stroke incidence is increasing in the young (those below the ages of 45 years old) with ischaemic strokes (66%) being more common than haemorrhagic strokes which in some regions of the world show no significant increase in incidence (Cabral et al., 2017).

1.3.2.1 ***Atherosclerotic (large artery) stroke***

To identify a stroke of large-artery atherosclerotic origin, stenosis of either intracranial or extracranial arteries of more than 50% must occur (Adams et al., 1993). Atherosclerosis is the process that leads to narrowing of the arterial lumen in large artery atherosclerotic stroke (Wu et al., 2017). It begins in childhood with the development of fatty streaks that are found in almost every systemic artery (Dawson et al., 2009). This process of atherosclerosis eventually causes the formation of arterial plaque (more advanced form of atherosclerosis). The atherosclerotic process is caused by several risk factors discussed below and involves arterial inflammation (Jashari et al., 2018). Of the large-artery atherosclerotic strokes, 25.4%

of these patients may have extracranial stenosis (occlusion of the vessel lumen more than 50%) and 31.5% intracranial stenosis (Chutinet et al., 2012). Importantly, large-artery atherosclerotic strokes account for only 18.6% of all strokes (Putaala, 2016) and may only explain 10% of ischaemic strokes at a young adult age (Smajlovic, 2015). However, this varies with some authors indicating that in high-income countries, atherosclerotic and cervical artery dissection, are the most common forms of strokes at a young age (Chraa et al., 2014). These discrepancies may be explained by differences in the number of concomitant risk factors that occur, with the presence of more than two (multiple) stroke risk factors accounting for more large-artery atherosclerotic strokes at a younger age (Smajlovic, 2015).

1.3.2.2 ***Small artery (lacunar) stroke***

A stroke caused by small-artery occlusion is considered to have occurred in patients with a stroke associated with lacunar infarcts with at least one or more risk factors for lacunar syndromes (history of diabetes mellitus and hypertension) (Adams et al., 1993). Approximately 25% of all ischaemic strokes are due to small-artery occlusions or lacunar infarcts. Small artery strokes are a result of low perfusion from penetrating arterioles within the brain. However, these small-artery lacunar strokes may not be as fatal as compared to other ischaemic stroke subtypes within the TOAST classification (Bailey et al., 2012). Two pathological mechanisms explain small-artery occlusive strokes. First, thickening of the arterial wall (tunica intima-media thickness) due to the deposition of fibrinoid within the intima-medial layer of the wall and hypertrophy of smooth muscle cells resulting from hypertension, decrease blood flow to penetrating arteries (Caplan, 2015 and Pantoni, 2010). Second, obstruction of the proximal part of penetrating arteries as a result of plaque within larger arteries that narrows the lumen and eventually affects blood supply to penetrating arteries (Caplan, 2015). Importantly, embolism from the heart cannot explain lacunar strokes. About 8 to 28% of strokes that occur in the elderly (50 to 75 years) are lacunar

strokes (Shi and Wardlaw, 2016). However, lacunar strokes are relatively uncommon in the young (Shi and Wardlaw, 2016), but the distribution relative to alternative causes of stroke in the young, is nevertheless unclear. Therefore, further studies are required to determine the distribution of lacunar stroke at a younger as compared to older age relative to alternative stroke subtypes.

1.3.2.3 ***Cardioembolic stroke***

Cardioembolic strokes occur following an embolus that originates from the heart and occludes arteries supplying the brain (Adams et al., 1993). In the elderly, major underlying causes of ischaemic strokes are atrial fibrillation or cardioembolic diseases of the heart (Lindley, 2018). In fact, cardioembolic strokes have been reported to be the most frequent cause of stroke amongst the very elderly (80 years or more) (Lindley, 2018). A major source of the emboli responsible for cardioembolic strokes is from the heart of those with dilated cardiomyopathy (ischaemic or idiopathic). Indeed, 75% of those with cardioembolic stroke have ischaemic heart disease (Aquil et al., 2011) and many have overt myocardial infarction associated with a reduced ejection fraction (13%) which is generally accompanied by a dilated cardiomyopathy (ischaemic dilated cardiomyopathy) (Habib et al., 2018). Importantly, atrial fibrillation may affect up to 50% of those with stroke (Aquil et al., 2011), but the contribution varies considerably with some studies demonstrating that atrial fibrillation contributes to only 5.6% of stroke (Habib et al., 2018) and in some studies, up to 10% (McIntyre and Healey, 2017).

As ischaemic dilated cardiomyopathy is uncommon below age 43 years, cardioembolic stroke is generally considered not to be a major cause of stroke in the young (Maaijwee et al., 2014). However, as cardiomyopathy is more common in males than females, when stroke occurs in the young, it is more frequently cardioembolic in young males (15.5%), than young females (6.1%) (Maaijwee et al., 2014). Importantly, although ischaemic cardiomyopathy and hence cardioembolic stroke is generally considered to be

uncommon in the young, the prevalence of cardioembolic stroke varies within the younger age group. Indeed, some studies (Putaala et al., 2009) have reported that younger individuals (15 to 44 years of age) have a greater (21.9%) chance of cardioembolic stroke as compared to those who are older (17%) (45 to 49 years of age). This may depend on the prevalence rates of atrial fibrillation and cardiomyopathy of alternative causes (idiopathic dilated cardiomyopathy) or the presence of rheumatic heart disease (with a valvular origin of the embolus) in different populations and at different ages. Of note, dilated cardiomyopathy may occur at any stage in a person's life but is more frequent in the third and fourth decades of an individual's life with men affected more commonly than women (Sliwa et al., 2005). In fact, the average age of stroke of cardioembolic origin may be as low as 28 years in low-to-middle income countries (Zühlke et al., 2016). Furthermore, atrial fibrillation affects younger individuals in Sub-Saharan Africa (SSA) from ages as early as 15 years old and progresses with age, especially women in their reproductive years (below 45 years old) (Sliwa et al., 2010). Contrary, in affluent countries, atrial fibrillation only affects the elderly populations (those above the ages of 60 years old) (Sliwa et al., 2010). However, the occurrence of atrial fibrillation in the SSA region is coupled with the concurrent diagnosis of heart failures of any form (56%) and valvular diseases (43%) (mainly due to rheumatic heart diseases) (Sliwa et al., 2010). In part, the presence of rheumatic heart diseases (RHD) in younger individuals residing in low-to-middle income countries, may be responsible for cardioembolic related strokes (Rheumatic Heart Disease in Africa, 2014). This is because Sub-Saharan African countries are the major hotspot of rheumatic heart diseases in the world, where the incidence is 30 per 100 000 yearly in those aged 14 to 19 years and continues to increase to 53 per 100 000 yearly in those aged 60 years or older (Rheumatic Heart Disease in Africa, 2014). Moreover, rheumatic heart diseases are the third most frequent cause of heart failures in adults (50 years old) in Africa, responsible for 14.3% cases of heart failures after hypertensive heart failure (45.4%) and idiopathic dilated cardiomyopathy (18.8%) (Damasceno et al., 2012; Rheumatic Heart Disease in Africa, 2014). However, in South

Africa, rheumatic heart diseases are the most common cause of acute heart failure (Damasceno et al., 2012).

1.3.2.4 ***Other causes of stroke***

Stroke of “other” determined aetiology include haematological, hypercoagulability and non-atherosclerotic disorders (Adams et al., 1993). Notable “other” causes of stroke, some of which are particularly important in the younger population, include procoagulation states associated with thrombophilias (see discussion on risk factors), venous infarction, substance abuse, vascular dissections, vasculitis, neurosyphilis, single neck vessel occlusion, Takayasu’s disease, and haematological disorders including sickle cell disease (Kes et al., 2012; Komolafe et al., 2015). Approximately 20-30% of strokes may be of “other” determined aetiology in younger people (Smajlovic, 2015). Indeed, in 1008 ischaemic strokes, 25% of the patients were at a younger age and were identified as having strokes of “other determined aetiology” (Putaala et al., 2009).

1.3.2.5 ***Stroke of undetermined aetiology***

Strokes of undetermined aetiology account for 5% to 44% of all ischaemic strokes (Kes et al., 2012). Strokes of undetermined aetiology may occur more frequently in younger individuals and generally more than 50% of these strokes affecting those younger than 50 years of age (Putaala, 2016). In this regard, strokes of undetermined aetiology may account for 28.1% of strokes in the young as compared to 15.7% of strokes in older persons (Putaala et al., 2009). These findings may explain why it has been difficult to fully account for the increased prevalence of ischaemic stroke in younger people in recent years.

1.3.3 Risk factors for stroke

Modifiable risk factors for stroke include hypertension, diabetes mellitus, dyslipidemia, regular smoking, regular alcohol use, physical inactivity, and obesity (Romero et al., 2008; Willey et al., 2017) whilst non-modifiable risk factors include increasing age, sex, race or ethnicity and genetic characteristics (Boehme et al., 2017). In the following I will discuss the role of these risk factors in different types of stroke at different ages.

1.3.3.1 Risk factors for haemorrhagic stroke

Hypertension has consistently been reported to be the most common risk factor for haemorrhagic stroke. Up to 75% of patients with haemorrhagic stroke have hypertension (Sindhartha et al., 2015). Importantly the prevalence of hypertension amongst older and younger individuals with haemorrhagic stroke is similar (Fu et al., 2015). Hypertension is therefore the strongest risk factor for haemorrhagic strokes in both younger and older age groups (Smajlovic, 2015). Although hypertension is as much the cause of haemorrhagic stroke in those below 45 years of age as it is in the older population, there are a few exceptions which include aneurysms, drug abuse, bleeding disorders, and cavernomas, which may more frequently account for haemorrhagic stroke at a younger age. Of these, aneurysms and drug (cocaine, amphetamine) abuse have a significant role to play in causing haemorrhagic stroke in the young (Griffiths and Sturm, 2011; Smajlovic, 2015). Importantly, in the elderly a frequent cause of haemorrhagic stroke is the use of anticoagulants.

1.3.3.2 Risk factors for ischaemic stroke

1.3.3.2.1 *Hypertension*

Not only is hypertension the most important risk factor for haemorrhagic stroke but is also considered a major risk factor for ischaemic stroke, in particular atherosclerotic and

small vessel stroke (Lv et al., 2016). Approximately 60% of all patients suffering from ischaemic stroke present with hypertension (Wu et al., 2016) and hypertension is similarly often the most common risk factor for ischaemic stroke in younger persons (Baidya et al., 2015). African countries are no exception with reports in for example, Ethiopia, demonstrating that hypertension accounts for 65.6% of all strokes, the majority of which are ischaemic stroke (Deresse and Shaweno, 2015). In South Africa, the importance of hypertension as a major cause of either haemorrhagic or ischaemic stroke has rendered the opinion that managing hypertension can prevent up to 45% of all strokes (Thomas, 2013). The increasing prevalence of stroke and increasing mortality rates at a younger age in developing countries are often attributed to an increasing prevalence of hypertension (Cano-Gutierrez et al., 2015). Most young people in low-middle-income countries who present with a stroke are unaware of the presence of hypertension (Ezejimofor et al., 2017).

1.3.3.2.2 ***Diabetes mellitus***

Second to hypertension, diabetes mellitus is the next most frequently cited risk factor for both large-artery atherosclerotic and small-artery (lacunar) stroke (Aquil et al., 2011). Indeed, diabetes mellitus is associated with intracranial atherosclerosis (Choi et al., 2017) and diabetes mellitus is often detected in the young at the time of a stroke with a high rate of unawareness (Rutten-Jacobs et al., 2014). Diabetes mellitus is often associated with an unusually high preponderance of atherosclerotic stroke with almost half the diabetics with stroke in one study having atherosclerotic stroke and the others largely having a stroke of unknown aetiology (Rutten-Jacobs et al., 2014). However, the contribution of type II diabetes mellitus to the development of ischaemic stroke is not fully understood (Larsson et al., 2017). Whether controlling blood glucose levels in diabetes mellitus prevents or reduces the development of atherosclerosis in intracranial arteries is unclear (Choi et al., 2017). Importantly, the frequency of diabetes mellitus in stroke has been shown to differ only to a small degree at different ages. In Brazilians with stroke, diabetes mellitus was noted to be

responsible for 25% of those younger than 65 years of age, 32% between the ages of 65-79 years, and 20% in those over 80 years of age (Pieri et al., 2008). However, alternative studies have demonstrated that a strikingly high proportion of young persons with stroke have diabetes mellitus (Habib et al., 2018). Indeed, in that study (Habib et al., 2018) diabetes mellitus accounted for 87.3% of ischaemic stroke in those between 18 to 49 years of age with hypertension (44.4%), dyslipidemia (23.5%) and smoking (31%) playing a much smaller role (Habib et al., 2018). Of the large-artery atherosclerotic strokes that occur at a young age, 30.3% are in individuals who are also obese (Hauer et al., 2017). Irrespective of the age group considered, in those with large-artery atherosclerotic strokes hypertension is noted in more than 50% (Hauer et al., 2017), but diabetes mellitus is almost double (25.7% versus 15.7%) in older (between 65 to 75 years) as compared to younger (below 55 years) persons with stroke (Hauer et al., 2017). Nevertheless, in Africa the role of diabetes mellitus may be less important where in for example, Ethiopia, diabetes mellitus may account for only 8.5% of all strokes (Deresse and Shaweno, 2015).

1.3.3.2.3 ***Dyslipidaemia***

Dyslipidemia is one of the important risk factors for ischaemic stroke and is more strongly associated with extracranial artery atherosclerosis but less associated with intracranial artery atherosclerosis (Kim et al., 2012; Qian et al., 2013). Dyslipidaemia may be a particularly important cause of stroke in the young. In fact, dyslipdaemia may be the leading cause of stroke in the young in Finland (60%) (Putaala et al., 2009). Moreover, in a large study (n=3944) conducted across 12 countries in Europe including Finland, Norway, Austria, Belgium, France, Germany, Hungary, Netherlands, Switzerland, Greece, Italy and Turkey, dyslipidaemia was the second leading cause of stroke (46%) at a young age (Putaala et al., 2012).

1.3.3.2.4 ***Smoking***

Smoking is also a well-recognized modifiable risk factor for ischaemic (mainly atherosclerotic) stroke (Lee et al., 2015) with either active smoking or secondary exposure playing a role (Oono et al., 2011). Not only does smoking produce atherosclerotic effects, but it increases the chances of thrombus formation through an increased viscosity and platelet cell activity (adhesion and aggregation). Smokers therefore have an increased risk of ischaemic strokes (Mackay et al., 2013). Smoking may also be a particularly important cause of stroke in the young. In the aforementioned study conducted in Finland (Putaala et al., 2009) smoking was the second leading cause of stroke in the young (44%) after dyslipidaemia (60%) and hypertension (39%). Moreover, in the aforementioned large study (n=3944) conducted across 12 countries in Europe including Finland, Norway, Austria, Belgium, France, Germany, Hungary, Netherlands, Switzerland, Greece, Italy and Turkey (Putaala et al., 2012) smoking was the leading risk factor (49%) followed by dyslipidaemia (46%) and hypertension (36%) in those with stroke at a young age.

1.3.3.2.6 ***Sex differences***

With respect to sex as a risk factor for stroke, this is age-specific where over 85 years of age, the incidence of stroke is more common amongst women than men, possibly because there is a reduction in life expectancy in men (Haast et al., 2012). However, in the general population (between the ages of 45 to 74 years), men have an increased stroke incidence and mortality as compared to women (with black women having a reduced stroke mortality by 25% to 35% and approximately 20% for white women) (Reeves et al., 2008). Indeed, in 5211 patients with acute ischaemic stroke (average age = 79 years) there were more men (57.1%) than women (42.9%) with stroke (Yu et al., 2015). Nevertheless, these sex differences may simply be accounted for by differences in traditional risk factors. In that study (Yu et al., 2015) the proportion with traditional risk factors was greater for men than

women (hypertension, 76.4 vs 66.4%; dyslipidemia, 30.6 vs 22.8%; obesity, 18.4 vs 9.3%). Moreover, differences in stroke mortality between young men and young women are not seen in those below the ages of 45 years old; although women have poor stroke outcomes with a reduced quality of life, as compared to men (Reeves et al., 2008). In addition, recent data do not support sex differences in ischaemic stroke subtypes (atherothrombotic, 63.1% and 60.2%, small-artery disease, 19.5% and 19.7%, cardioembolism, 10.1% and 13.1%, other determined aetiology, 0.7% and 0.8%, and undetermined aetiology, 6.6% and 6.3%) (Yu et al., 2015).

1.3.3.2.7 ***Ethnic disparities***

With regards to ethnicity as a risk factor for stroke, African Americans have double the risk of developing a stroke (either ischaemic or haemorrhagic) compared to non-Hispanic white Americans (Boehme et al., 2017; Johnson et al., 2017). Moreover, as compared to non-Hispanic white Americans, African Americans have more severe strokes (Johnson et al., 2017) with the loss of physical and cognitive function being highest amongst blacks as compared to whites (Burke et al., 2014; Burke et al., 2015). Importantly, an association of traditional risk factors including hypertension does not fully explain these racial disparities.

1.3.3.2.8 ***Genetic factors***

Irrespective of which risk factor dominates the pathogenesis of stroke, many who suffer from either atherosclerotic or small vessel ischaemic stroke at a young age do not have traditional risk factors, although the trends for early vascular changes and the development of atherosclerosis in the arterial vasculature are noted (Putaala, 2016). There is therefore an increasing awareness that genetic or alternative unidentified factors may play an important role in determining stroke. In this regard, a family history of ischaemic stroke increases the chances of an ischaemic stroke. Indeed, ischaemic stroke in younger persons

either from large or small vessel disease is strongly associated with a family history of stroke (Markus, 2010). Associations between several gene variants and stroke have been reported, including relations between stroke and variations on phosphodiesterase 4D (PDE4D) genes (Lindgren, 2014), large vessel ischaemic stroke and variations in the histone deacetylease 9 (HDAC9) gene, cardioembolic stroke and variations in the paired-like homeodomain transcription factor 2 (PITX2) and zinc finger homeobox 3 (ZFHX3) genes (Lindgren, 2014), small vessel stroke and variations in the notch 3 (NOTCH3) gene (Markus, 2011), and haemorrhagic stroke and variations in the apolipoprotein E (APOE) gene (e2 and e4 alleles) or the collagen type IV alpha 1 (COL4A1) gene (Lindgren, 2014). Additional examples of the variety of genetic causes of stroke include gene variants for small-vessel disease. These include cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), cerebral recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL), collagen type IV alpha 1 chain (COL4A1) and Fabry disease (FD), (Choi, 2015).

1.3.3.2.9 ***Thrombophilia***

There is a possibility that those who experience cardiovascular events (such as stroke) when they are young, have thrombophilia (Janssen et al., 2011). Thrombophilia may be hereditary or acquired (Ng et al., 2011). Hereditary thrombophilia predisposes to hypercoagulability due to several gene mutations that affect coagulation. In hereditary thrombophilia, genes (such as factor V Leiden, prothrombin G20210A, protein C, S) coding for a normal coagulation, when mutated, lead to changes in the coagulation process. Mutation of factor V Leiden causes resistance to proteolytic action of protein C, resulting in the elevated production of thrombin which is needed to convert fibrinogen from its inactive state into a soluble fibrin. The prothrombin G20210A mutation leads to more production of thrombin in the blood (Torres and Saddi, 2015). Factor V Leiden is the most common inherited disorder amongst other disorders (protein C, protein S, prothrombin G20210G/A

gene mutation) and accounts for most thrombophilia cases. Factor V Leiden mutation affects Caucasians more than any other race group including those of African descent. Importantly, hereditary thrombophilia affects those who are younger than 45 years of age (Khan and Dickerman, 2006; Soare and Popa, 2010) and can manifest either as venous or arterial thrombosis (Soare and Popa, 2010). Nevertheless, inherited thrombophilia has a predisposition to thrombosis of the venous system (deep vein thrombosis) as opposed to the arterial system and is thought to account for only 1-4% of ischaemic stroke (Ng et al., 2011).

As opposed to hereditary thrombophilia which involves a variety of coagulation abnormalities, acquired thrombophilia is associated with antiphospholipid antibody syndrome (APS) and is more common in young women (Ng et al., 2011). Acquired thrombophilia is mainly characterized by arterial and venous thrombosis. The most common antiphospholipid antibodies that acquired thrombophilia is associated with, include lupus anticoagulant antibodies and anticardiolipin antibodies (Ng et al., 2011). Between these two types of antiphospholipid antibodies, lupus anticoagulant antibodies have a 5 to 16 times higher risk of thrombosis as compared to anticardiolipin antibodies (Ng et al., 2011).

Although thrombophilia contributes to stroke, markers of thrombus formation are more useful for stroke prediction in older as compared to younger persons (Tamura et al., 2007). Thus, thrombophilia is unlikely to explain the increasing prevalence of stroke in the young. Whether activation of coagulation cascade through alternative mechanisms such as thrombin protein (Maino et al., 2015) contributes to stroke in the young remains to be determined.

1.3.3.2.10 ***Variable relative contribution of risk factors***

Importantly, the relative contribution of different risk factors to different types of stroke is variable. For example, in one study hypercholesterolemia (≥6.2 mmol/L) was noted to be the leading risk factor for large-artery atherosclerotic strokes, followed by hypertension and then smoking (Song et al., 2005). In contrast, in this same study (Song et al., 2005)

hypertension was the dominant risk factor for small artery stroke followed by hyperglycaemia (≥7.0 mmol/L) and then an increased alcohol consumption.

1.4 A need to enhance risk prediction of stroke in the young

The higher burden of stroke at a younger as compared to older age and reports that stroke occurs in populations of African origins and in Africa at a much lower mean age than in the rest of the world, raises the question of how best to prevent these events at such a young age. Without question risk prediction and subsequent appropriate intervention is a well-established approach to prevention of vascular events. However, almost without exception studies that have contributed to knowledge on how best to risk predict have been conducted in those at an older age where the risk for events is high. Consequently, there is little knowledge on whether similar approaches as those established in older groups apply equally well to younger groups. Of importance, those with moderate risk factors at a young age seldom achieve a strikingly high-risk score as the strongest risk factor in several risk-scoring systems, that is age, places them at an intermediate risk. Thus, risk prediction in the young to middle aged is fraught with uncertainty. In the presence of risk factors that are severe, such as severe or refractory hypertension, risk prediction at a young adult age is much more obvious. However, those with stroke at a young adult age seldom present either as a hypertensive emergency or as refractory hypertensives. To appropriately risk predict at a younger age consideration must be given to what may account for differences in the age of events.

If stroke in younger groups is attributed to different risk factors or their combinations, then these risk factors require early identification and management. However, as highlighted in the above, the risk factors for stroke in the young remain unclear although the conventional risk factors in some studies cast some light on the occurrence of stroke in the young. Alternatively, stroke in younger groups may be attributed to the same risk factors which have been more severe, have occurred from an earlier age and have not been

controlled. If this is indeed the case, then appropriate approaches to detecting those who have had these risk factors from an earlier age and with little control require identification. In this regard, the presence of preclinical cardiovascular damage (end organ changes) is generally employed to detect those who have had these risk factors for longer and with little control. As will be discussed several non-invasive indices of vascular end-organ changes are available, but to the best of my knowledge few studies have reported on end-organ vascular abnormalities in stroke in the young (Paul et al., 2012; Saeed et al., 2014). In these studies (Paul et al., 2012; Saeed et al., 2014) the authors report on the presence of vascular pathology in those with young stroke determined using ultrasonic imaging of the carotid artery. However, the authors of these studies (Paul et al., 2012; Saeed et al., 2014) failed to show the extent to which carotid imaging added to risk prediction and failed to assess vascular pathology according to guidelines. In the next section of this chapter I will therefore highlight the strengths and limitations of this measurement and identify the limitations of the published data on this measure in patients with stroke at a young age (Paul et al., 2012; Saeed et al., 2014).

1.4.1 Carotid intima-media thickness (IMT) as a measure of vascular pathology

For several decades increases in the thickness of the intima plus media in the carotid artery (carotid IMT) measured 1 cm proximal to the flow divider (junction of internal and external carotid), has been used as a marker of subclinical atherosclerosis, the underlying cause of many cardiovascular diseases (Gomez-Marcos et al., 2012). This is based on the impact of atherosclerosis on the intimal layer, with resultant combined thickening of the intima and the media. Presently, the assessment of carotid IMT can be performed using non-invasive, linear B-mode imaging with border detection software and a semi-automated approach (Bartels et al., 2012). The use of carotid IMT as a measure of early vascular damage is indeed a good predictor of future cardiovascular events such as myocardial infarction and ischaemic stroke (Saxena et al., 2017). However, exactly what it is an index

of, is now questionable and in more recent years, the use of IMT as an index of atheroma has been challenged.

One of the key factors that contributes to vascular pathology is hypertension which is an important cause of medial hypertrophy and hyperplasia of vascular smooth muscle and which may reflect compensatory changes in the media in response to a high shear stress rather than pathological changes in the intima (Diaz et al., 2018). Importantly, it is common for atherosclerotic plaque to develop in areas of low shear stress such as the bulb of the bifurcation of the common carotid artery, areas where medial thickening does not occur. Hence, hypertension is often strongly associated with increases in carotid IMT in areas other than where atherosclerotic plaque occurs (Herder et al., 2012) and the main factors responsible for the carotid IMT progression are increasing age and hypertension (Baroncini et al., 2015). Nevertheless, as hypertension is the main risk factor for stroke (see above discussion) it is still possible that the measurement of IMT predicts the risk of stroke and that stroke risk is predicted in young persons. Indeed, a family history of stroke is strongly associated with an increased carotid IMT (Fromm et al., 2014). The question of the value of IMT measurements in risk prediction has largely arisen from studies comparing the ability of carotid IMT versus that of identifying the presence of carotid atherosclerotic plaque in risk prediction. How is carotid plaque identified and how does it compare to the use of IMT in risk prediction?

The most common sites for the development of atherosclerotic plaque in the carotid artery include the distal common carotid, carotid bulb and extracranial part of the internal carotid artery (Thapa et al., 2013). The identification of carotid plaque is also performed using non-invasive, linear B-mode imaging. This assessment does nevertheless require more skill than that required for IMT assessment and there is large degree of subjectivity involved in identification. Consequently, IMT may be more practically feasible for risk assessment. Of the locations for formation of carotid plaque, the internal carotid artery (within 2cm from the level of carotid bifurcation) is thought to be the commonest site for plaque development. However, in order of frequency of plaque detection, carotid plaque is

noted in the common carotid artery 37% of the time, followed by the carotid bulb (33%) and then the internal carotid artery (23%) (Thapa et al., 2013). Importantly, atherosclerotic plaque is much more common in those above 60 years of age (37%), as compared to those younger than 40 years of age (11%), (Thapa et al., 2013). As there is no question that the presence of plaque is an atherosclerotic process, the question is whether IMT and plaque predict risk to a similar degree has arisen. In this regard, the presence of carotid plaque is a much stronger risk predictor for coronary heart disease than IMT (increased risk of 52% vs 15-17%) (Naqvi and Lee, 2014). However, possibly as stroke, in many communities is driven to a large extent by hypertension, an increased carotid IMT is a strong predictor for stroke whilst carotid plaque mainly predicts ischaemic heart disease (Naqvi and Lee, 2014). In fact, as compared to a carotid IMT increase less than 0.6 mm, a 1 mm increase in the carotid IMT confers an 8-fold greater risk of having a stroke amongst women and a 3.3-fold increase risk of stroke in men, (Zielinski et al., 2007). It may be argued that when predicting stroke that IMT is more robust for small vessel or haemorrhagic stroke which are more closely associated with hypertension and carotid plaque, with atherosclerotic (large vessel) stroke. However, in both small-vessel stroke and large-vessel atherosclerotic stroke, carotid atherosclerotic plaque is more frequent and carotid IMT increased (Jung et al., 2012). Moreover, IMT is increased as much in haemorrhagic as it is in ischaemic stroke (Ohira et al., 2011).

1.4.2 Carotid IMT in young stroke

To the best of my knowledge only a few previous studies have assessed IMT in young persons with stroke and demonstrated marked increases (Paul et al., 2012; Saeed et al., 2014). However, the assessments in these studies (Paul et al., 2012; Saeed et al., 2014) were not conducted according to the Mannheim consensus (Touboul et al., 2012). In this regard, they assessed areas where plaque occurs, which includes the carotid bifurcation and internal carotid artery (Paul et al., 2012) or IMT only was employed to identify plaque (Saeed

et al., 2014). Moreover, comparisons of IMT were made with assumed normal values rather than with age-matched controls (Saeed et al., 2014). Furthermore, whether beyond risk factors IMT was as effective at identifying stroke in younger as compared to older groups was not determined (Paul et al., 2012; Saeed et al., 2014). In addition, whether beyond risk factors IMT was as effective at identifying stroke as carotid plaque was not evaluated (Paul et al., 2012; Saeed et al., 2014). Thus, although current evidence supports the notion that measuring carotid IMT in the young could assist in identifying those who might develop a stroke beyond risk factors (Oygarden et al., 2016), and that increases in carotid IMT in younger persons may be one of the strongest predictors of vascular events (Oren et al., 2003), substantial further work is required to assess the value of this assessment in the young.

1.5 Problem statement

In economically developed countries, stroke mainly affects the elderly (average age of 67 years), and rarely occurs in younger persons (Perera et al., 2016; Terni et al., 2015). In this regard, 89% of all strokes occur in those older than 55 years of age (Chen et al., 2010). Only 15% of ischaemic strokes occur at a younger age (Komolafe et al., 2015) with only 10% in those less than 50 years of age (Putaala, 2016; Saeed et al., 2014). However, ischaemic stroke is becoming more common in younger persons (Cabral et al., 2017) and in low and middle-income countries (such as South Africa), the average age of stroke is considerably lower than that noted in high-income countries with approximately 19-30% of all strokes occurring in those less than 45 years of age as compared to only 5% of all strokes occurring in this age group in high-income countries (Fahmi and Elsaid, 2016). Importantly, the impact of stroke on disability adjusted life years is far greater when occurring at a younger as compared to older age (Nakibuuka, 2015). The importance of predicting an imminent premature stroke and intervening to prevent the stroke therefore cannot be over-emphasized. Although there is some evidence to suggest a dominance of some risk factors

over others in determining stroke at a young adult age, generally the risk factors are the same. A focus on selective risk factors or identifying risk factors that are unique to the young therefore does not enhance risk prediction. As age is the strongest risk factor for stroke, the use of standard risk charts may be limited in identifying those at imminent risk of a stroke at a young age. Thus, the use of end-organ vascular assessments, may be an important method of enhancing risk prediction for stroke at a young adult age. In this regard, indices of carotid atheroma, including IMT and plaque are well established predictors of stroke. However, as argued in the aforementioned sections, the ability of either alone or combination to predict stroke at a young adult age is unknown.

1.6 Aims

The aim of the present study was therefore to compare the extent to which carotid IMT increases and plaque formation occurs beyond conventional risk factors in patients with stroke as compared to age- sex- and ethnicity-matched controls in younger as compared to older persons of African ancestry.

Chapter 2

Methods

2.1 Study participants

The present study took place in the Charlotte Maxeke-Johannesburg Academic Hospital, Division of Neurology, and in the School of Physiology, University of Witwatersrand, Medical School. The study was approved by the University of the Witwatersrand Committee for Research in Human Subjects (clearance certificate number: M1704125). Informed, written consent was obtained from the healthy controls and from the patient or from the next of kin in case of impaired cognitive functions.

2.1.1 Case group

164 patients ≥18 years of age of African ancestry (black patients only) consecutively admitted to the Neurology Unit as inpatients with a new stroke, defined as a focal neurological deficit of vascular origin, were evaluated. Participant with cerebral meningitis based on cerebrospinal fluid (CSF) examination, or the presence of intracranial malignancy or intracranial mass lesions on radiographic imaging, were excluded. These data were collected in collaboration with the Department of Neurology and specialist neurologists who decided on the diagnosis based on clinical, laboratory and imaging data. The Trial of Org 10172 in Acute Stroke (TOAST) Classification was used to classify each patient (courtesy of Dr Eitzaz Sadiq).

2.1.2 Control group.

Case data were compared with data obtained in 430 age and sex-matched individuals from participants of a community sample older than 16 years of age. The community sample was derived from randomly recruited nuclear families of black African descent (mainly the Nguni [Zulu, Xhosa, Ndebele, Swati], Sotho [South, North Sotho and Tswana] and Venda chiefdoms) with at least two parents or two siblings and living in the

South West Township (SOWETO) of Johannesburg, South Africa using the population census figures of 2001. No subjects of mixed, Asian, or European ancestry were recruited. Random recruitment of community participants was based on the following approach: Street names and addresses of households were obtained from the department of home affairs, 2001 census. These households were allocated numbers, and numbers were selected from a random number generator. People residing in informal dwellings or institution/homes were not recruited.

2.2 Demographic and clinical data

Demographic and clinical information including each patient's medical history, the use of medication or tobacco and alcohol use was obtained by use of a questionnaire together with extraction of data from patient files. Clinical data included onset, character, and severity of symptoms of stroke, previous diagnosis of cardiovascular diseases (e.g. previous stroke, myocardial infarction), diabetes mellitus (present or absent), hypertension (systolic blood pressure>140 mmHg or diastolic blood pressure >90 mmHg), dyslipidaemia, or any other clinically important disease or risk factor and the therapy thereof. Information on the nature of the stroke was extracted from the patient files and included information on stroke severity, chest roentgenogram (X-Ray), electrocardiogram, lumbar puncture and brain imaging (CT or MRI Scan) data.

For the control sample, the questionnaire was explained to the families at the first home visit and then subsequently completed in the presence of trained study assistants at an office visit where questions could be answered. In order to avoid translational errors, the questionnaire was not translated into an African language, but study assistants familiar with all languages spoken in these townships assisted with the completion of each questionnaire when assistance was requested. At a second home visit, answers relative to date of birth, gender, previous medical history, the presence of hypertension, diabetes mellitus, prior and current drug therapy, smoking status (including the number of cigarettes smoked in the past

and at the present time), daily alcohol consumption (beer, traditional beer or other forms of alcohol and the daily quantity) were obtained. For the patients, data obtained on the questionnaire were confirmed from the clinical file or from family members if the patient was uncertain.

2.3 Blood analysis

Data of standard laboratory blood tests of fasting renal function, blood glucose, lipid profiles and percentage glycated haemoglobin (HbA1c), performed as part of the clinical work-up, were extracted from the patient's files. In the control sample, blood analysis was performed for research rather than clinical purposes. Blood samples were obtained on the day of the clinic visit, transported to the laboratory in a cooler box, on ice and sent to the South African National Health Systems Laboratories (NHSL) to obtain a lipid profile (total cholesterol, low density lipoprotein [LDL] cholesterol, high density lipoprotein [HDL] cholesterol and triglyceride [TG] concentrations), a blood glucose measurement, and glycated haemoglobin (HbA1c). Lipid profiles were determined by an automated method for direct measurement and the standard thresholds (total cholesterol <4.10 mmol/l, LDL cholesterol <3.0 mmol/l and HDL cholesterol ≥1.5 mmol/l) were determined according to the South African dyslipidemia guidelines 2012 (SAMJ, 2012). Participants were considered as having diabetes mellitus or an abnormal glucose control if they had a fasting plasma glucose concentration ≥ 7mmol/l, an HbA1c>7.0%, or in whom glucose-lowering agents are prescribed. Dyslipidemia was identified from standard thresholds of total, LDL or HDL cholesterol concentrations.

2.4 Anthropometry

Anthropometric data including body height, weight and waist circumference was determined using standard approaches. Obesity was defined as a body mass index (BMI)

≥30 kg/m^2. Central (abdominal) obesity was defined as an enlarged waist circumference (≥ 88 cm in women and ≥ 102cm in men).

2.5 Blood pressures

Blood pressures were determined by trained observers who measured brachial artery BP using a mercury sphygmomanometer according to guidelines. After 10 minutes of rest (patient sitting), five consecutive BP readings followed by a pulse rate count were measured. Standard cuffs with an inflatable bladder with a length of 12 cm and a width of 12 cm were used unless the arm circumference exceeded 31 cm, then larger cuffs with a 31 x 15 cm bladder were employed. The five readings were averaged to obtain single systolic, diastolic and mean arterial BP reading. Participants were considered as having hypertension if the average of the mean values for the clinic readings was ≥140/90 mm Hg or when receiving antihypertensive medication.

2.6 Carotid intima-media thickness and plaque

Carotid intima-media thickness (Carotid IMT) was determined by using high resolution B-mode ultrasound (SonoCalc IMT, Sonosite Inc, Bothell, Washington) employing a linear array 7.5MHZ probe (Figure 2.1). Assessments were performed in the supine position with the participants head turned slightly away from the side on which the measurements were taken (Figure 2.2). Images of at least 1 cm length of the far wall of the distal portion of the right and left common carotid artery from an optimal angle of incidence (defined as the longitudinal angle of approach where both branches of the internal and external carotid artery are visualized simultaneously) at least 1 cm proximal to the flow divider was obtained. Carotid IMT measurements were determined from the far wall of the carotid using semi-automated border-detection and quality control software (Figure 2.3) as the use of this software improves IMT reproducibility and reduces observer bias

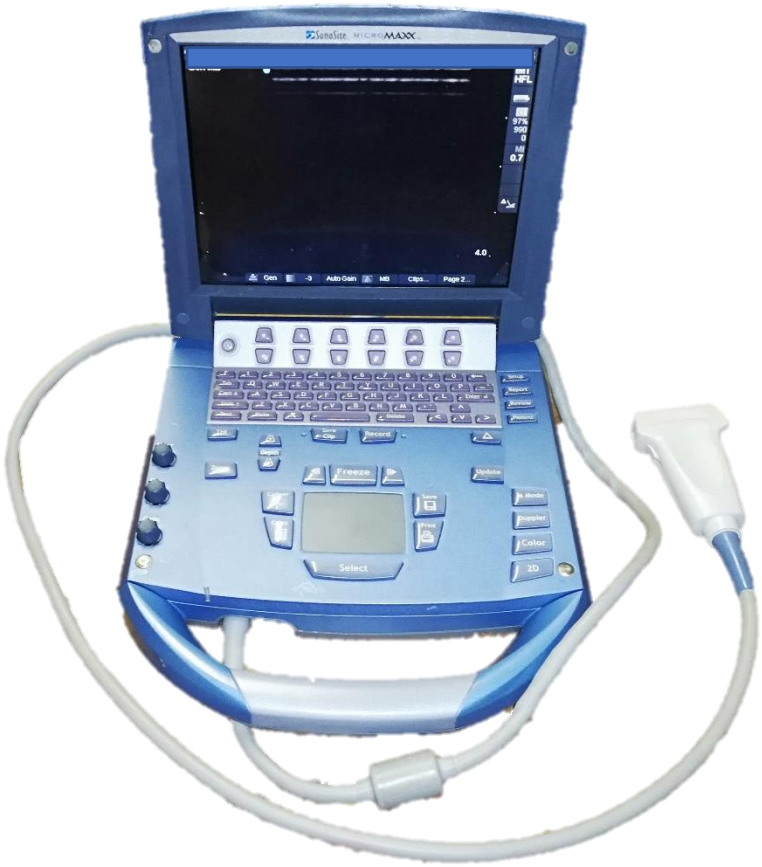

Figure 2.1. A semi-automated B-mode ultrasound employing a linear array 7.5 MH ultrasound probe.

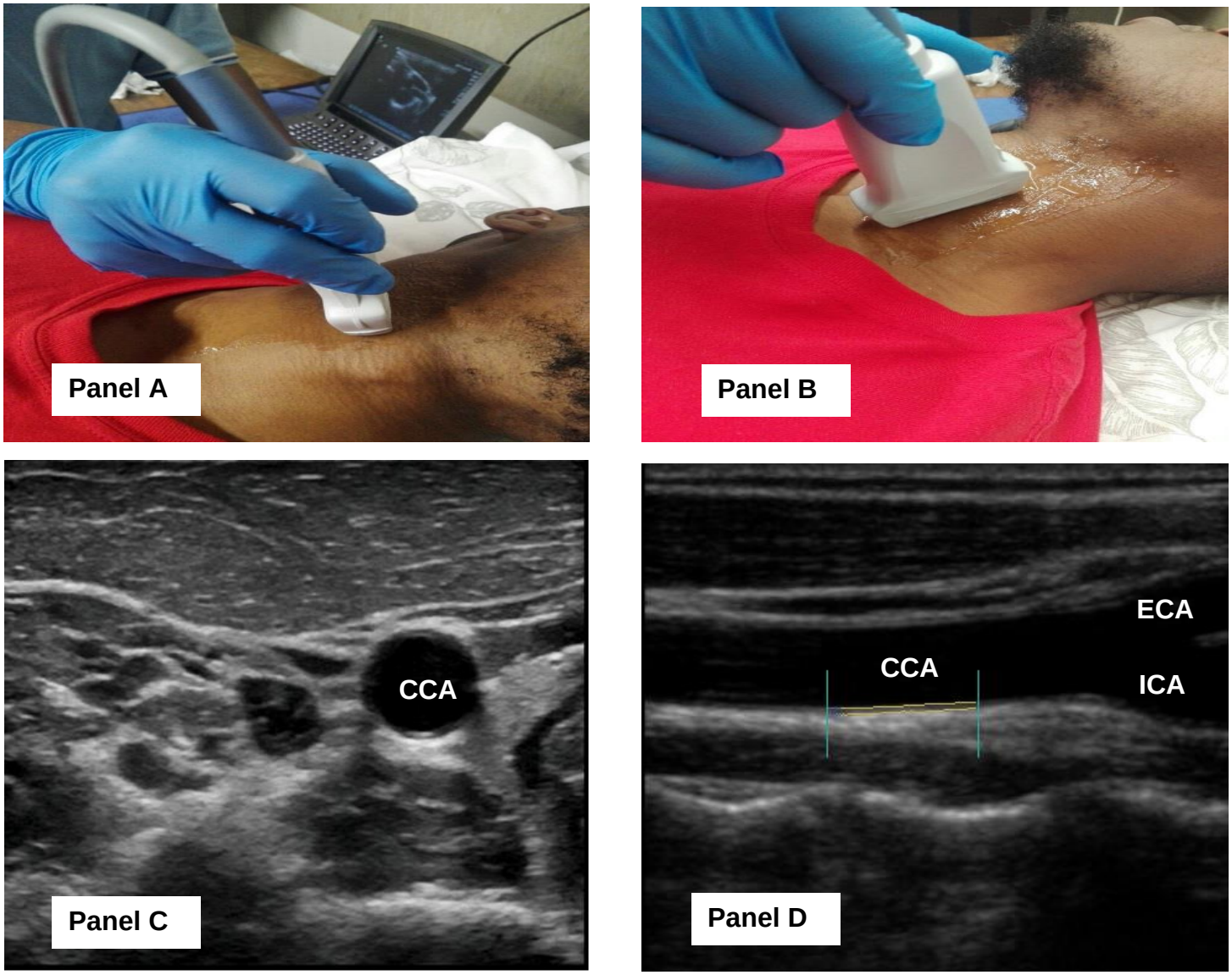

Figure 2.2. A transverse view (as shown by panel A and panel C ultrasound images) and a longitudinal view (shown by panel B and panel D ultrasound images) of the carotid artery. The two yellow lines parallel to the lumen shown in panel D, represent IMT measurements using semi-automated border-detection and quality control software. (CCA: Common carotid artery, ICA: Internal carotid artery, ECA: External carotid artery).

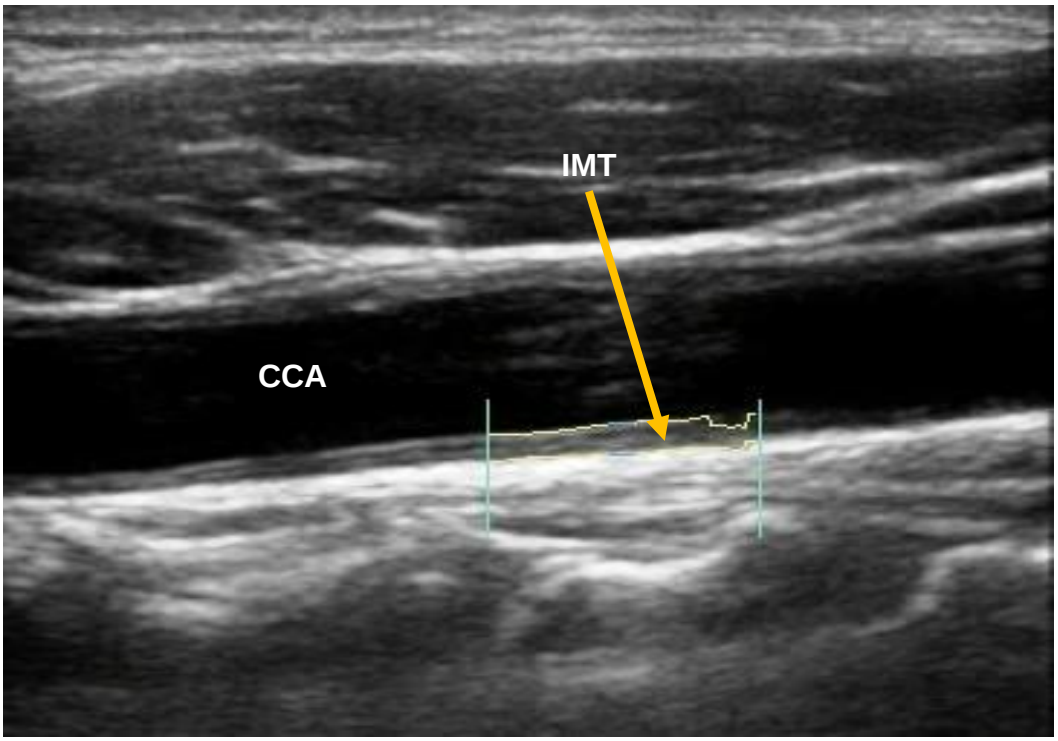

Figure 2.3. An ultrasound image of the common carotid segment and IMT measurements represented by the two yellow lines parallel to the lumen using semi-automated border-detection and quality control software. (CCA: Common carotid artery, IMT: Intima-media thickness).

compared with manual techniques. The average of three IMT measurements from the optimal angle and from each carotid artery was recorded as the IMT for each participant. A carotid IMT ≥ 0.8 mm or an IMT >95th percentile for age of normotensive, non-diabetic and non-obese participants from the community sample was considered to be pathological. Carotid ultrasound was also employed to assess the extent of plaque from both longitudinal and cross-sectional images of the carotid artery. Carotid artery plaque was identified as a focal structure that encroaches into the arterial lumen of at least 0.5 mm (Figure 2.4) or 50% of the surrounding intima-media thickness value or demonstrates a thickness of ≥ 1.5 mm as measured from the media-adventitia interface to the intima-media lumen interface (Touboul et al., 2012). All the assessments were evaluated by an experienced ultrasonographer who assessed the quality of images and identify the presence of plaque independent of myself.

2.7 Data analysis

For database management and statistical analysis, SAS software version 9.4 (SAS Institute) was employed. To assess whether relations between carotid IMT and plaque presence are associated with stroke independent of conventional cardiovascular risk factors, multivariate adjusted logistic regression analysis was performed, and regression relations were adjusted for age, gender, hypertension, diabetes mellitus/impaired blood glucose control (HbA1c>7.0%), smoking and HDL cholesterol concentrations. To compare the performance of carotid IMT versus plaque presence for stroke prediction, area under the receiver operating characteristic curves (AUC) was calculated. Sensitivity and specificity were calculated using standard approaches.

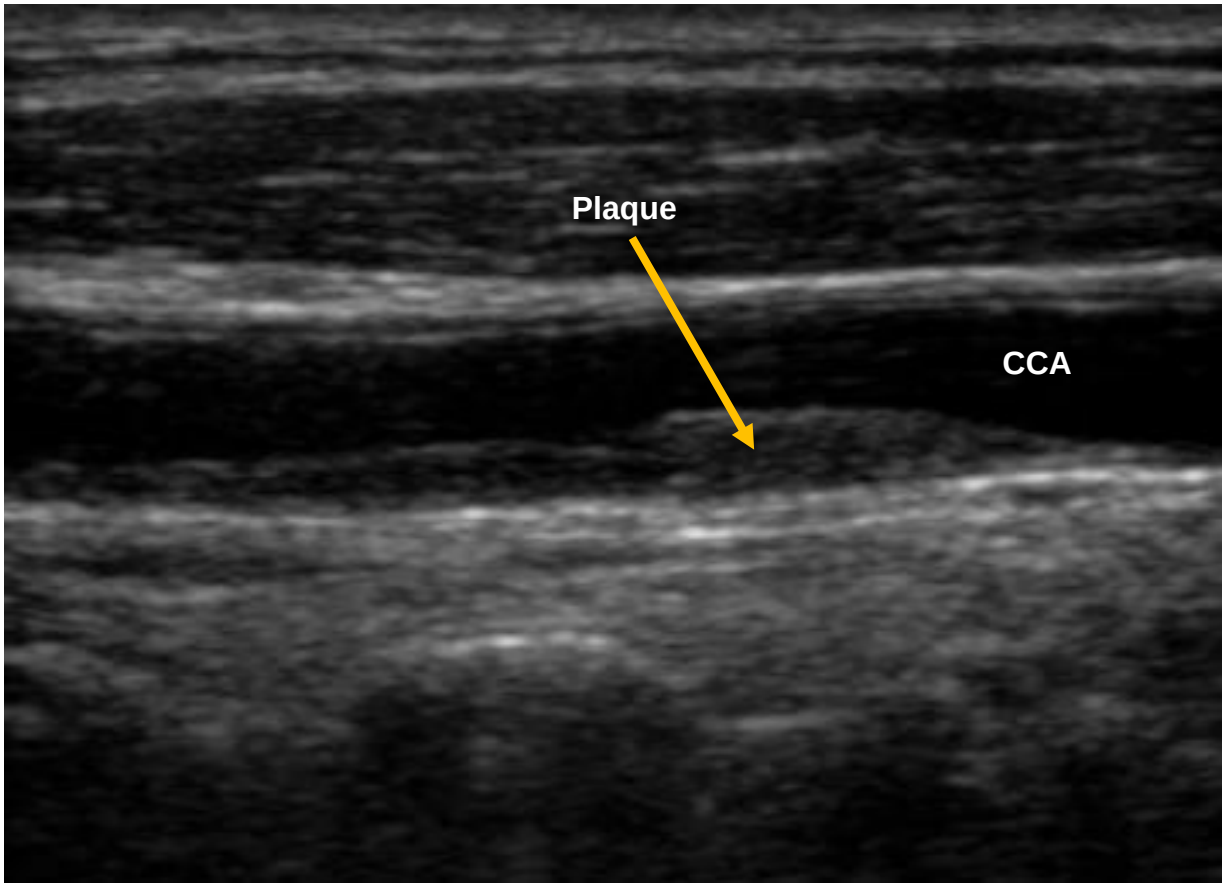

Figure 2.4. A longitudinal view of an ultrasound image of the common carotid artery showing the presence of plaque. (CCA: Common carotid artery).

Chapter 3

Results

3.1 Participant characteristics and risk factors

The characteristics of the participants with stroke and the age and sex-matched control sample are given in table 3.1. The characteristics of those with a premature event (below ages of 50 years for males and 55 years for females) as compared to an event at an older age are shown in the table (Table 3.1). Importantly, stroke occurred at a premature age in 58.5% of patients hospitalized for this event. As compared to age- and sex-matched controls, irrespective of whether patients had a stroke at a younger or older age, those with a stroke had a greater prevalence of hypertension, but no difference in the prevalence of diabetes mellitus or the proportion of those who smoked regularly. Diastolic blood pressure was nevertheless lower in the younger cases as compared to the younger controls, an effect most likely attributed to the use of antihypertensive therapy. Although BMI was not increased in the younger participants with stroke as compared to age and sex matched controls, the older participants with stroke had a higher BMI than the age and sex matched controls. Total cholesterol concentrations were lower in both the younger and older cases as compared to their respective age and sex-matched controls. Moreover, although LDL cholesterol concentrations were similar in the younger cases versus controls, they were lower in the older cases as compared to controls. The lower concentrations of total and LDL cholesterol in those with a stroke are likely attributed to the use of lipid-lowering agents. Importantly however, HDL cholesterol concentrations, which are not affected by lipid lowering therapy, were markedly lower in participants with stroke as compared to controls irrespective of age. In short, no marked differences in risk factors distinguished stroke at a younger as compared to older age. Importantly, as compared to older cases, in whom only 28% had either only one or no major risk factors, more than 50% of participants with stroke at a younger adult age had either no risk factors or a single risk factor (Table 3.2). Importantly, none of these participants had refractory or severe hypertension, few had diabetes mellitus, and none had had a previous event. Thus, prior to the event, in the absence of end organ measures, the overall risk assigned to 50% of younger patients who developed a stroke, would therefore

Table 3.1. Characteristics of the stroke and age and sex-matched control participants.

	Age<50 or 55 years [a]			**Age≥50 or 55 years [a]**		
	Controls (n=273)	Cases (n=96)	p value	Controls (n=157)	Cases (n=68)	p value
Age (years)	41.7±9.3	40.1±9.0	=0.16	63.1±7.4	63.3±9.8	=0.87
Sex (% male)	40.3	37.5	=0.63	45.9	47.1	=0.87
Body mass index (kg/m^2)	30.2±8.5	28.8±8.14	=0.18	27.8±4.8	31.6±8.1	=0.0014
% Hypertensive	34.8	46.9	=0.036	69.7	88.2	=0.003
% Diabetes mellitus	3.7	7.3	=0.14	20.4	30.9	=0.12
% Regular smokers	19.4	27.0	=0.15	16.6	21.7	=0.38
% Regular alcohol intake	25.6	Not recorded	__	22.9	Not recorded	__
Systolic blood pressure (mm Hg)	126±18	125±20	=0.44	137±18	136±18	=0.88
Diastolic blood pressure (mm Hg)	85±12	79±16	=0.001	85±10	82±13	=0.06
Total cholesterol (mmol/l)	4.58±0.99	4.03±0.99	<0.0001	5.11±0.94	4.34±1.06	<0.0001
LDL cholesterol (mmol/l)	2.60±0.73	2.53±0.82	=0.40	3.00±0.76	2.62±0.95	=0.004
HDL cholesterol (mmol/l)	1.42±0.43	1.04±0.33	<0.0001	1.35±0.35	1.16±0.42	=0.0007

[a] Refers to men < or ≥50 years of age or women < or ≥55 years of age. LDL, low density lipoprotein; HDL, high density lipoprotein; IMT, intima-media thickness.

Table 3.2. Number of risk factors in those with either premature stroke or stroke at an older age.

	Age<50 or 55 years [a]		**Age≥50 or 55 years [a]**	
Number of risk factors present	Control % (n=273)	Case % (n=96)	Control % (n=157)	Case % (n=68)
0	39.2	12.5	18.5	2.9
1	42.9	38.5	44.6	25.0
2	14.6	39.6	25.5	47.1
3	3.3	8.3	10.8	22.1
4	0	1.0	0.6	2.9

[a] Refers to men < or ≥50 years of age or women < or ≥55 years of age. Risk factors include, the presence of hypertension, diabetes mellitus or reduced HDL cholesterol concentrations (defined according to guidelines as HDL cholesterol <1.5 mmol/l) or regular smoking.

have been entirely unremarkable.

3.2 Stroke subtypes (TOAST classification)

Figure 3.1 shows the prevalence of stroke subtypes in those with a premature event as compared to an event at an older age. Haemorrhagic stroke was uncommon in either age category with no striking differences noted between age categories. The most frequent stroke subtype in either age category was stroke of undetermined aetiology. In the older age group, the next most common stroke subtype was stroke associated with small-vessel disease, whilst in the younger age group stroke associated with small-vessel disease was uncommon occurring with a similar prevalence as haemorrhagic stroke. The second most common stroke subtype at a younger age was that of other determined origins (n=25) with the most common cause being a vasculitis in a third of these patients, but with varying alternative causes contributing to the remaining patients (Figure 3.2). Cardioembolic stroke accounted for approximately 20% of all strokes with a similar prevalence noted in those with an event occurring at a younger as compared to an older age category (Figure 3.1). Atherosclerotic stroke could only be identified at an older age and accounted for a very small proportion of total stroke (Figure 3.1). In short, the main differences in stroke subtypes at a younger as compared to an older age was the lower prevalence of small vessel disease, the higher prevalence of other determined etiology, and the lack of detection of atherosclerotic stroke. Nevertheless, the similar and relatively high prevalence (22%) of cardioembolic stroke in the young as compared to the older age group was unexpected.

3.3 Carotid intima-media thickness and plaque

As indicated in Figure 3.3, in either unadjusted or multivariate adjusted models, across most of the adult age range, except in those with stroke <40 years of age, carotid

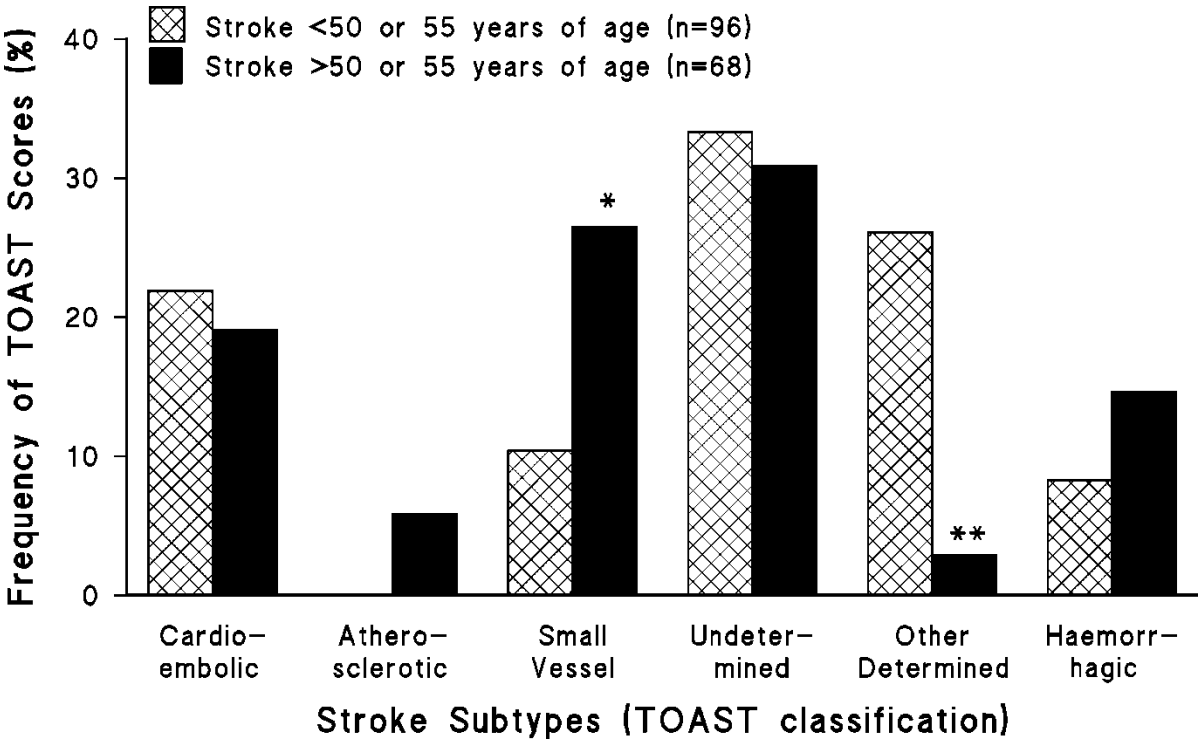

Figure 3.1. Prevalence of stroke subtypes (TOAST scores) in patients either with a premature stroke event (males<50 years and females<55 years of age), or an event at an older age. *p<0.05, **p<0.0001 versus young stroke patients.

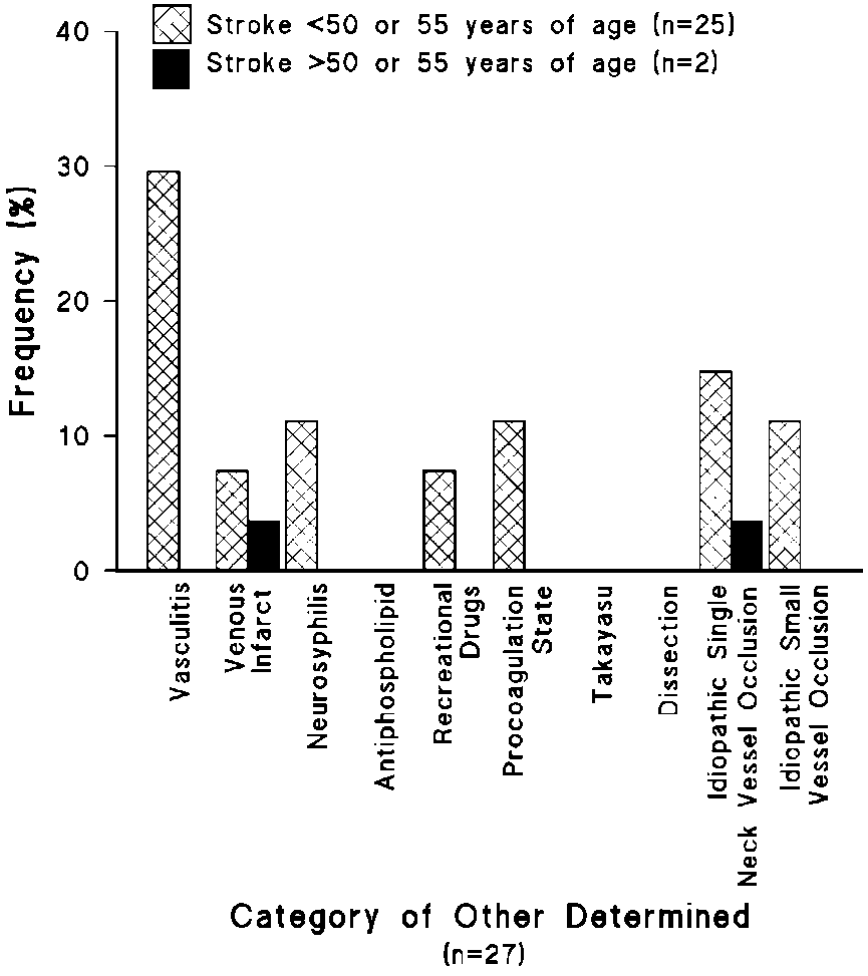

Figure 3.2. Causes of stroke of other determined aetiology in patients with either a premature stroke (males<50 years and females<55 years of age), or stroke at an older age (males≥50 years and females≥55 years of age).

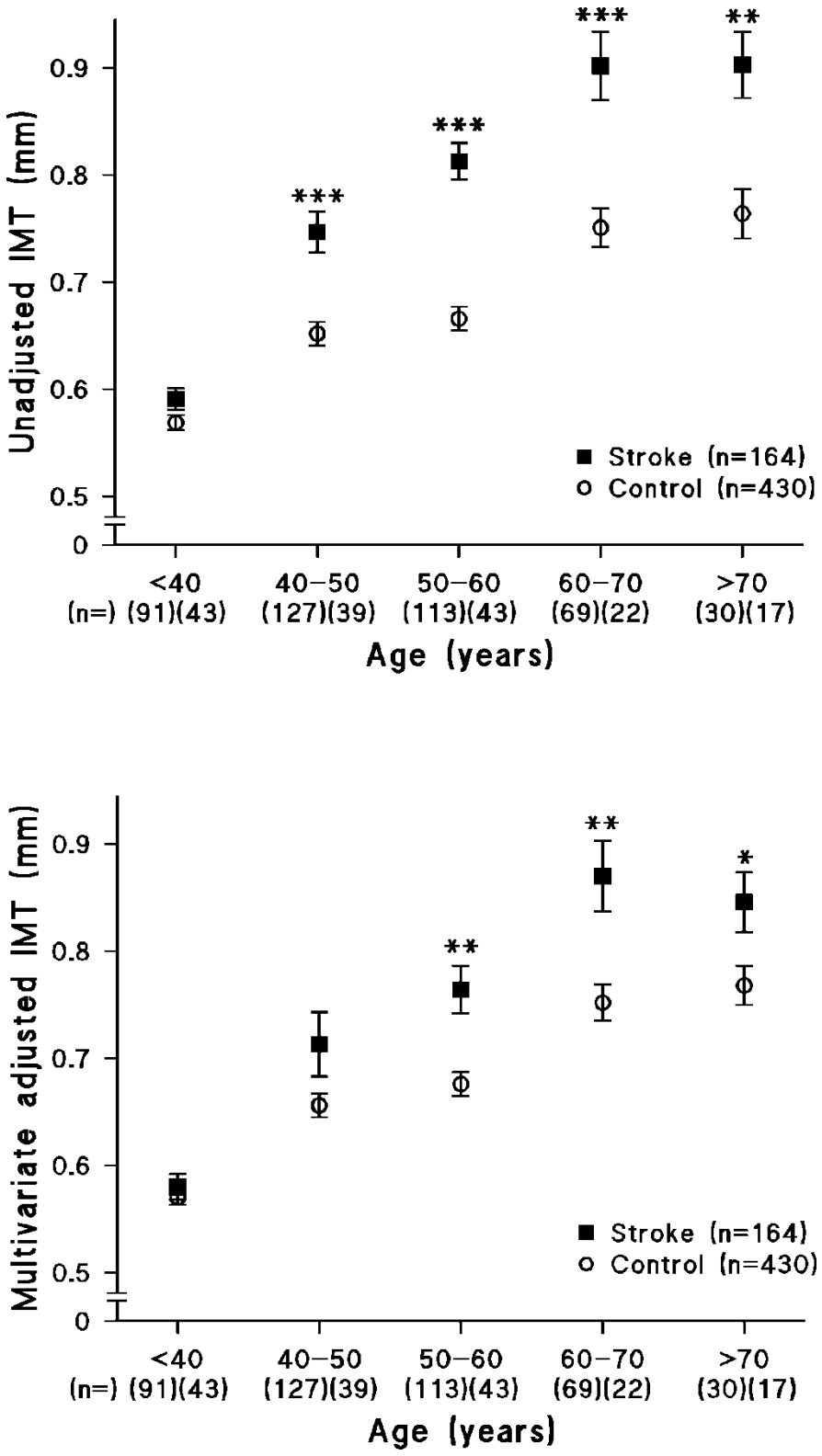

Figure 3.3. Unadjusted (upper panel) and multivariate-adjusted (lower panel) carotid intima-media thickness (IMT) across the adult lifespan in patients with a stroke as compared to age and sex-matched controls. Adjustments are for age, sex, BMI, hypertension, diabetes mellitus, smoking, HDL cholesterol. *p<0.05, **p<0.001, ***p<0.0001 versus controls.

IMT was increased in those with stroke as compared to age and sex-matched controls. Moreover, irrespective of whether thresholds of 0.80 mm or threshold (cut-point) values >95th percentile for age of normotensive, non-diabetic and non-obese participants was evaluated, the prevalence of those with an increased IMT was greater in those with stroke than in controls across most of the adult lifespan except in those with stroke <40 years of age (Figure 3.4). However, the prevalence of those with carotid plaque was markedly increased across all of the adult lifespan, including in those with stroke <40 years of age (Figure 3.4). This translated into an increased chance (odds) beyond conventional risk factors of a stroke with an increased IMT or the presence of plaque across most of the younger adult lifespan except in those <40 years of age where only plaque presence was associated with stroke (Figure 3.5). Importantly, neither an increased IMT nor the presence of plaque were consistently associated with stroke beyond conventional risk factors in the older age group (Figure 3.5).

3.4 Relative contribution of carotid intima-media thickness or plaque versus risk factors

In stepwise regression models, the impact of carotid IMT or plaque on stroke beyond conventional risk factors was at least as strong as individual risk factors (Tables 3.3 and 3.4). Whilst IMT was independently associated with stroke in the older age group (Table 3.3), plaque was independently associated with stroke in both the younger and older age groups (Table 3.4). When included in the same regression models, plaque, but not IMT was independently associated with stroke in the younger age group, whilst IMT and plaque were both independently associated with stroke in the older age group (Table 3.5).

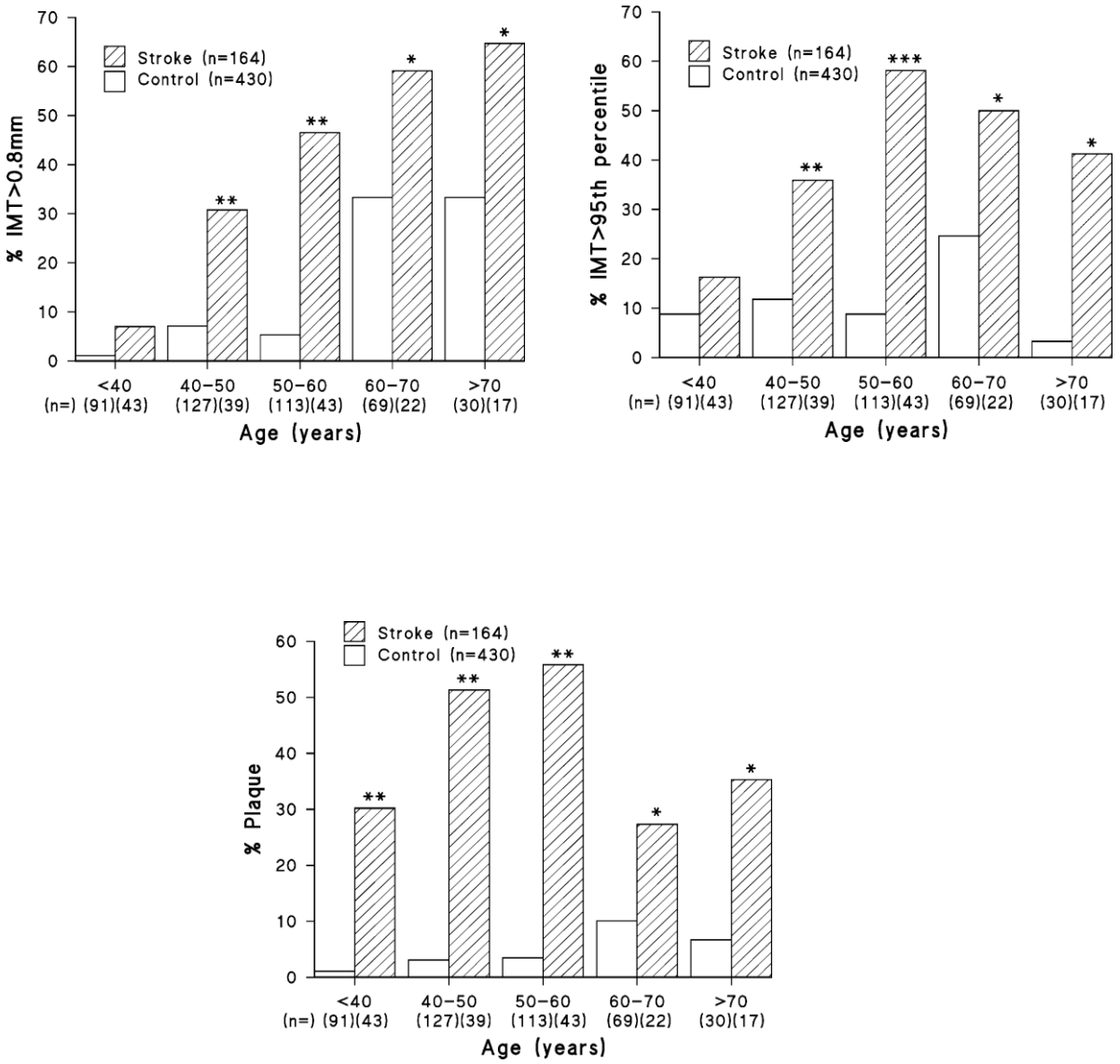

Figure 3.4. Prevalence of an increased carotid intima-media thickness (IMT) (defined as a value above 0.80 mm or the 95th percentile for age) (upper panels) and the presence of carotid plaque (lower panel) across the adult lifespan in patients with a stroke event and age and sex-matched controls.*p<0.05, **p<0.001, ***p<0.0001 vs controls.

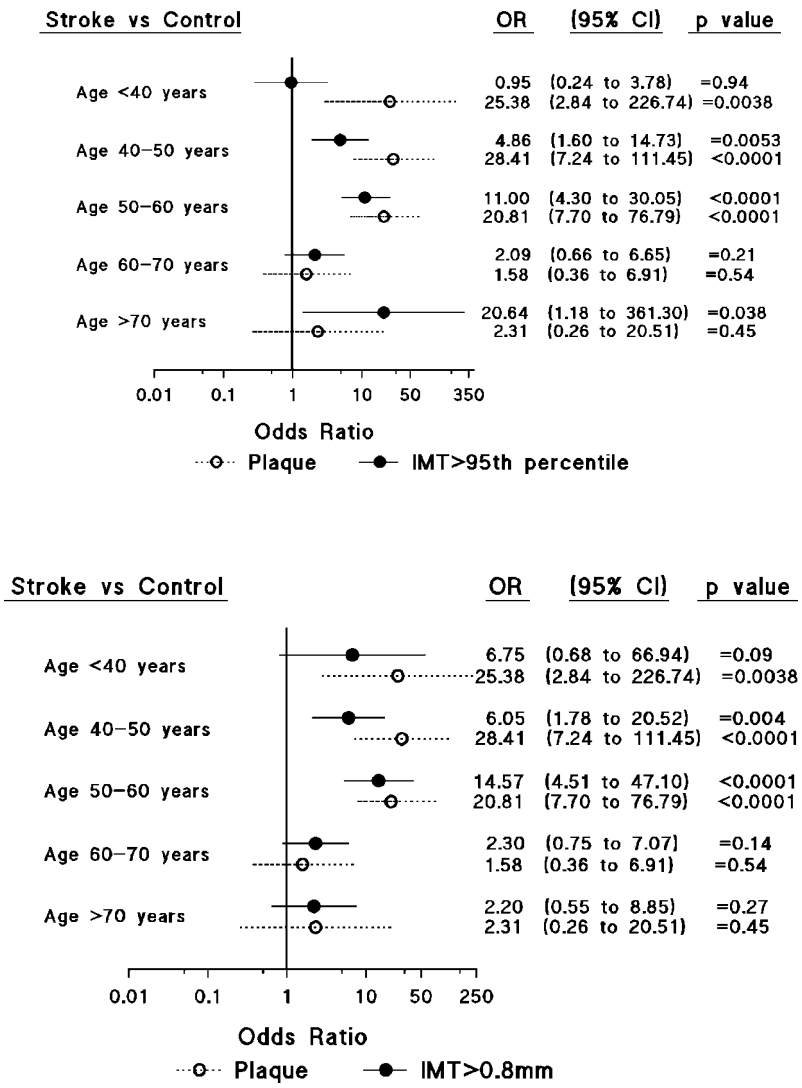

Figure 3.5. Multivariate adjusted odds (Odds ratio [OR] and 95% confidence interval [CI]) of stroke event across the adult lifespan with presence of carotid plaque (open circles) or an increased carotid intima-media thickness (IMT) (closed circles) as defined by a carotid IMT above the 95th percentile for age. Adjustments are for age, sex, BMI, hypertension, diabetes mellitus, smoking, HDL cholesterol and carotid plaque (for IMT only).

Table 3.3. Relative contribution (standardized slopes [β-coefficients]) of carotid intima-media thickness (IMT) versus conventional risk factors in associations with premature strokes or strokes occurring at an older age.

	Age<50 or 55 years [a]		**Age≥50 or 55 years** [b]	
IMT vs Risk Factor	Std β±SEM	p value	Std β±SEM	p value
IMT	0.075±0.055	=0.17	0.376±0.068	<0.0001
Hypertension	0.057±0.050	=0.25	0.099±0.063	=0.12
Diabetes	0.019±0.050	=0.71	0.004±0.063	=0.95
Smoking	0.065±0.048	=0.18	0.011±0.062	=0.85
HDL	-0.345±0.047	<0.0001	-0.223±0.062	=0.0004

[a]Refers to men <50 years of age or women <55 years of age; [b]Refers to men ≥50 years of age or women ≥55 years of age. β-coeff., standardized β-coefficient; IMT, carotid intima media thickness; HDL, high density lipoprotein.

Table 3.4. Relative contribution (standardized slopes [β-coefficients]) of carotid plaque versus conventional risk factors in associations with premature strokes or strokes occurring at an older age.

	Age<50 or 55 years [a]		**Age≥50 or 55 years** [b]	
Plaque vs Risk Factor	Std β±SEM	p value	Std β±SEM	p value
Carotid plaque	0.462±0.049	<0.0001	0.290±0.068	<0.0001
Hypertension	0.018±0.043	=0.68	0.188±0.063	=0.003
Diabetes	0.016±0.045	=0.71	0.016±0.065	=0.804
Smoking	0.028±0.043	=0.51	0.060±0.062	=0.336
HDL	-0.284±0.042	<0.0001	-0.182±0.064	=0.0051

[a]Refers to men <50 years of age or women <55 years of age; [b]Refers to men ≥50 years of age or women ≥55 years of age. β-coeff., standardized β-coefficient; HDL, high density lipoprotein.

3.5 Carotid intima-media thickness versus plaque in risk detection

Both carotid plaque and IMT showed significant performance (area under the receiver operator characteristic curve) for the detection of stroke at any age category (Table 3.6). However, carotid plaque showed a markedly greater performance than IMT for stroke detection over a younger age range (Table 3.6 and Figure 3.6). This was largely attributed to a greater sensitivity at a given specificity of plaque than IMT for stroke detection (Table 3.6).

3.6 Carotid atheroma in different stroke subtypes

Except in patients identified as having an atherosclerotic stroke, who had an increased IMT as compared to alternative stroke subtypes, no differences in either IMT or plaque in the carotid artery were noted between different stroke subtypes (Table 3.7).

Table 3.5. Relative contribution (standardized slopes [β-coefficients]) of carotid intima media thickness (IMT) and carotid plaque in the same regression models versus conventional risk factors in associations with premature strokes or strokes occurring at an older age.

	Age<50 or 55 years [a]		**Age≥50 or 55 years** [b]	
IMT + Plaque vs Risk Factor	Std β±SEM	p value	Std β±SEM	p value
IMT	0.012±0.050	=0.82	0.315±0.070	<0.0001
Carotid plaque	0.460±0.050	<0.0001	0.200±0.070	=0.004
Hypertension	0.015±0.050	=0.74	0.114±0.062	=0.07
Diabetes	0.017±0.045	=0.71	0.014±0.03	=0.82
Smoking	0.029±0.043	=0.49	0.004±0.061	=0.94
HDL	-0.283±0.043	<0.0001	-0.189±0.062	=0.003

[a]Refers to men <50 years of age or women <55 years of age; [b]Refers to men ≥50 years of age or women ≥55 years of age. β-coeff., standardized β-coefficient; IMT, carotid intima media thickness; HDL, high density lipoprotein.

Table 3.6. Comparison of performance (area under the receiver operator characteristic curve-AUC), sensitivity and specificity of various factors for the detection of premature strokes (<50 and 55 years of age in men and women respectively) and strokes at an older age (≥50 and 55 years of age in men and women respectively).

	Age<50 or 55 years [a]			**Age≥50 or 55 years** [a]		
	AUC±SEM	Sensitivity	Specificity	AUC±SEM	Sensitivity	Specificity
IMT	0.6291±0.0351	-	-	0.7697±0.0333	-	-
IMT >0.8 mm	0.5908±0.0225	22.92	95.24	0.6606±0.0347***	54.41	77.71
IMT >95th percentile	0.5961±0.0254	30.21	89.01	0.6905±0.0334**	51.47	86.62
Carotid plaque	0.7044±0.0257*†‡	42.71	98.17	0.6645±0.0320*	41.18	91.72

[a]Refers to men <50 years of age or women <55 years of age; [b]Refers to men ≥50 years of age or women ≥55 years of age. IMT, carotid intima media thickness. *p<0.05, **p<0.005, ***p<0.0001 versus IMT; †p<0.0001 versus IMT>0.8 mm; ‡p<0.0005 versus IMT>95th percentile.

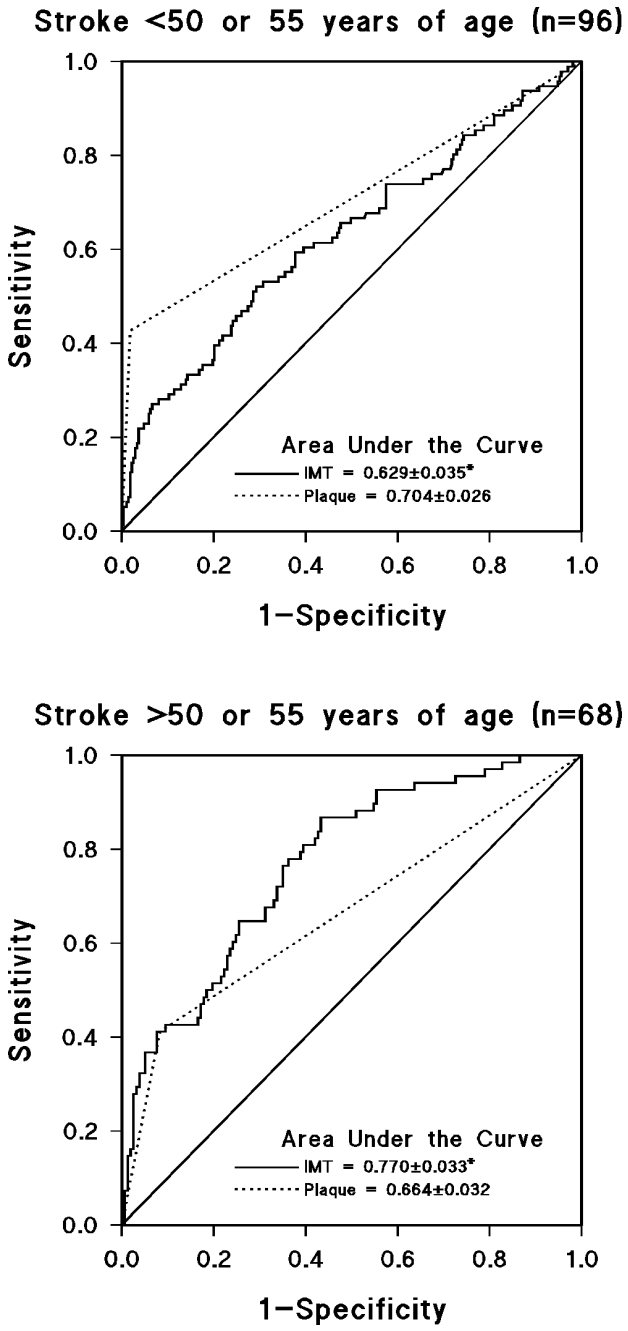

Figure 3.6. Comparison of performance (area under the receiver operator characteristic curve) of carotid IMT (assessed continuously) and plaque for the detection of premature stroke (<50 and 55 years of age in men and women respectively) and stroke occurring at an older age. *p<0.001 versus plaque.

Table 3.7. Age and sex adjusted carotid intima-media thickness (IMT) and prevalence of plaque in different stroke subtypes based on the TOAST classification.

	IMT (mm)	Plaque (%)
Haemorrhagic (n=18)	0.798±0.032	44.4
Atherosclerotic (n=4)	1.009±0.069*	50.0
Small vessel (n=28)	0.775±0.026	50.0
Cardio-embolic (n=34)	0.750±0.023	47.1
Other determined (n=27)	0.707±0.029	29.6
Undetermined (n=53)	0.769±0.019	39.6

*p<0.01 versus all other sub-types.

Chapter 4

Discussion

4.1 Summary of main findings

The main findings of the present study are as follows: In a large case-control analysis of patients admitted with stroke across the full adult lifespan versus age and sex-matched controls derived from a randomly selected community sample, I evaluated whether differential interaction exist between carotid plaque or IMT and stroke in different age categories. Importantly, no significant risk factors were associated with stroke at an early adult age (premature stroke) as compared to those with stroke occurring at an older age. Furthermore, over a half of patients with stroke at a young adult age would have been considered as being at low risk prior to the event (none or 1 risk factor only, no severe or refractory hypertension, no diabetes mellitus, and no prior event). Although stroke of "other" determined aetiology was more common and small vessel disease less common in the young, no striking differences in stroke subtypes differentiated stroke at a younger as compared to an older age. Nevertheless, a high prevalence of stroke of cardioembolic origin (22%) was noted at a young adult age, similar to the prevalence at an older age (19%). With respect to the extent of end organ changes as indexed by carotid imaging, in separate models, both carotid plaque and IMT were independently associated with stroke. However, whilst independent relations between plaque and stroke were consistent across a younger adult age, IMT failed to show consistent independent relations in the younger age category. Although relations between IMT and stroke were noted beyond conventional risk factors in the older adult age, IMT values above thresholds (cut-points) showed independent relations with stroke in those between 40-60 years, whilst above 60 years of age unconvincing independent relations were noted.

4.2 Novelty of findings

Whilst a number of studies have compared the ability of carotid IMT and plaque to predict stroke, almost without exception these studies report on events over an older adult age group when these events commonly occur in most populations in developed nations (Amato et al., 2017; Gardin et al., 2015). In this regard, arterial events that occur at a younger age may be associated more with thrombotic rather than atherosclerotic occlusion (Chang et al., 2014; Ferro et al., 2010). Indeed, atherosclerotic stroke occurs infrequently in the young, only accounting for approximately 10% of ischaemic strokes at this age (Smajlovic, 2015). In this regard, as highlighted in subsequent discussion, atherosclerotic occlusion was not detected in those with stroke at a young adult age in the present study. Thus, arterial events such as stroke with extensive arterial occlusion caused by atherosclerosis (>50% occlusion) are more difficult to identify in the young, suggesting that the atherosclerotic effect that causes the stroke is less extensive and rather results more frequently in thrombotic occlusion. In support of this notion, recent evidence provided by our group describe a higher frequency of the occurrence of pathologically thrombotic as compared to atherosclerotic occlusion (histological evidence) in the lower limb at a younger age and that thrombotic occlusion of lower limb arteries is associated with markedly less extensive carotid atheroma (Kolkenbeck-Ruh et al., 2019). If these observations apply to stroke as well as lower limb ischaemia, in these circumstances there is the possibility that the atherosclerotic change responsible for stroke may be less extensive and hence that plaque may not be as readily detected at a young age. In contrast, IMT, which is in-part a marker of early atherosclerotic changes, may be more useful to detect the risk for thrombotic arterial occlusion in the young. In this regard, whilst carotid plaque fails to independently associate with thrombotic occlusion in the lower limb, carotid IMT does indeed show an independent relationship (Kolkenbeck-Ruh et al., 2019). However, the same may not hold true for stroke. In fact, one previous study has reported on a high prevalence of carotid plaque in young patients with stroke (Saeed et al., 2014). Nevertheless, that study (Saeed et al., 2014) did not identify plaque following the Mannheim consensus document (Touboul et al.,

2012) using only an IMT thickness beyond 1.5 mm to define the presence of plaque. Moreover, that study (Saeed et al., 2014) failed to compare the prevalence of plaque in those with stroke to an age-and sex-matched control group, and hence could not report on relationships independent of conventional risk factors. Furthermore, that study (Saeed et al., 2014) did not assess carotid IMT in an area away from regions of plaque formation. Furthermore, one prior study (Saxena et al., 2017) demonstrated that as compared to age-matched controls, IMT was markedly increased in both younger and older persons with stroke. However, in that study whether carotid plaque was associated with stroke at a younger age was not determined (Saxena et al., 2017). Thus, the role of plaque in assessing the risk of stroke in young adults beyond risk factors is unclear and the relative role of plaque versus IMT in associations with stroke over a young age is unknown. In the present study I provide clear evidence that whilst plaque is consistently and independently associated with stroke beyond conventional risk factors over a young age range, independent relations between IMT and stroke are inconsistent over this age group. Moreover, over a younger adult age range, plaque showed a greater performance (area under the receiver operator characteristic curve) for stroke detection than IMT. Thus, in younger individuals, plaque is likely to have more value for the risk prediction of a premature stroke than IMT.

4.3 Dissociation between carotid plaque and IMT

The role of carotid plaque versus IMT in risk prediction is controversial (Amato et al., 2017). Although IMT predicts atherosclerotic cardiovascular events (Centurion, 2016; Ruijter et al., 2012), there is nevertheless considerable uncertainty as to the role of IMT in risk prediction beyond the identification of atherosclerotic plaque. As highlighted in the introduction to the present dissertation, marked dissociations exist in carotid IMT and plaque between ethnic groups (Mackinnon et al., 2010), and this suggests that IMT and plaque index different

pathophysiological changes. In this regard, IMT may not only index pathological alterations, but also reflect non-atherosclerotic arterial wall changes such as compensatory medial hypertrophy in response to a high wall stress (Touboul et al., 2012), or early fatty streaks which regress rather than progress to atherosclerotic plaque (Insull, 2009; Wannarong et al., 2013). Indeed, whilst traditional risk factors for stroke only explain 15 to 17% of variations in carotid IMT, they nevertheless explain 52% of carotid plaque (O'Leary et al., 1996). Meta-analyses and large studies have demonstrated that carotid plaque may be more predictive of cardiovascular events than IMT (Inaba et al., 2012; Jeevarethinam et al., 2018; Johnsen and Mathiesen, 2009; Mitchell et al., 2018; Ren et al., 2015; Sillesen et al., 2018; Spence, 2012; Yoon et al., 2017). However, some studies suggest that IMT is superior to plaque at predicting events (Barakoti, 2018; Ren et al., 2015), but this could be attributed to IMT measurements obtained in regions of plaque. In fact, when comparing the carotid IMT measured in areas free of plaque, plaque is the best predictor of cardiovascular events (Ravani et al., 2015). In this regard, although an increase in the carotid IMT is indeed a good predictor of both stroke and myocardial infarction (Khan et al., 2011, Naqvi et al., 2014), many studies have assessed IMT in areas that are not plaque-free, have often assessed IMT on just one side, have not obtained multiple measurements of IMT to ensure reproducibility, have not assessed common carotid IMT in a standard position proximal to the flow divider; or have not assessed IMT on the far wall of the carotid to obtain an optimal angle of incidence (Naqvi et al., 2014). In this regard, all of these criteria are required to obtain valid assessments of IMT (Touboul et al., 2012). Hence, the possibility that relationships between IMT or the lack thereof and events reflect inappropriate assessment approaches requires consideration. In this regard, in the present study we determined the average of 3 IMT measurements in each carotid artery and employed appropriate approaches to assessing IMT. Despite these approaches in the present study whilst plaque was consistently and independently associated with stroke at a younger adult age, IMT was not. In contrast, although not reported in the present dissertation, our group, using the same approaches as applied to

patients with critical limb ischaemia have demonstrated an independent relationship between IMT, but not plaque and critical limb ischaemia over a young adult age range (Kolkenbeck-Ruh et al., 2019). Thus, our approaches to assessing IMT are unlikely to have limited our ability to show consistent relations between IMT and stroke beyond risk factors.

4.4 Possible reasons for the limited relations between IMT and stroke at a younger Age

There are several possible reasons that may explain the lack of consistent independent relations between IMT and stroke in the young in the present study. The fact that carotid plaque was so consistently and independently associated with stroke over the younger adult age range indicates that atherosclerosis was indeed a strong cause of stroke even if atherosclerotic stroke was not an identified TOAST classification subtype of stroke at a younger age. In this regard, as discussed in the introduction, atherosclerosis may account for a significant proportion of stroke of undetermined aetiology (if occlusion was pathologically more thrombotic than atherosclerotic and hence a vessel demonstrating >50% occlusion could not be detected), cardio-embolic (if coronary artery disease is the cause) or even small vessel (where small artery flow is limited by atheroma in distal portions of more proximal arteries) stroke. Importantly, no differences in carotid plaque or IMT were noted across subtypes of stroke except for increased IMT in atherosclerotic strokes. The most plausible explanation for the inconsistent independent relationship between IMT and stroke is that given in aforementioned discussion and that is that IMT not only indexes pathological atherosclerotic changes, but also compensatory medial changes (Diaz et al., 2018; Touboul et al., 2012) or fatty streaks that are able to regress (Insull, 2009). However, this should not exclude IMT as a good predictor of events at an early age, because as pointed out in aforementioned discussion, our group has also demonstrated that increases in IMT are consistently and independently associated with critical limb ischaemia over

a young adult age range, whilst carotid plaque is not (Kolkenbeck-Ruh et al., 2019). Indeed, the disparity between these two measurements could be due to the fact that they have different predictive power for cardiovascular events rather than that one is superior to another (Liviakis et al., 2010). In fact, the predictive power of carotid IMT and plaque is greater when used in combination rather than when employed separately (Ravani et al., 2015).

4.5 Haemorrhagic stroke and premature events

Importantly, a remarkably low prevalence of haemorrhagic stroke was noted in the present study (7% in the young and 14% in the older age group) as compared to the proportions noted in a hospital audit conducted at the same centre a decade prior to the present study (Connor et al., 2009). In that study (Connor et al., 2009) haemorrhagic stroke was noted in 21% of younger and 32% of older patients admitted with stroke. The reduced prevalence rates of a disorder largely attributed to hypertension, may reflect better management of hypertension at a population level, although we cannot exclude the possibility that many patients with haemorrhagic stroke may have succumbed prior to admission and hence may not have been included in the present or the prior (Connor et al., 2009) study. The tendency for a higher prevalence of haemorrhagic stroke in older rather than younger persons in the present and a prior (Connor et al., 2009) study is in contrast to what is generally reported to occur. In this regard, haemorrhagic stroke accounts for more stroke (40-55%) in those below the age of 45 years as compared to stroke in older persons (15 to 20%) (Smajlovic, 2015). As highlighted in the introductory chapter this is nevertheless likely to depend on the characteristics of aging populations as in the elderly, anticoagulants are a major cause of haemorrhagic stroke (Lindley, 2018). However, the possibility also exists that the higher prevalence of hypertension in the elderly results in a higher proportion of those not treated to target who develop haemorrhagic stroke. Irrespective of the age distribution of haemorrhagic stroke in the present study, the low

overall prevalence suggests that seeking those at risk for hypertensive bleeds (undetected hypertension or very poorly controlled BP levels) is unlikely to improve risk detection and prevention in the young.

4.6 Subtypes of ischaemic stroke and premature events

In keeping with most studies which show that large-artery atherosclerotic strokes are uncommon in the young and may only account for approximately 10% of ischaemic stroke at this age (Smajlovic, 2015), in the present study atherosclerotic stroke was not detected at a younger adult age. This is likely to be attributed to the overall low prevalence of atherosclerotic stroke noted in general in the present study as even in the elderly a prevalence of only 6% was noted. As atherosclerotic stroke is more likely to occur with multiple risk factors and in particular with diabetes mellitus and dyslipidaemia, the absence of atherosclerotic stroke in the young in the present study suggests that strategies to identify diabetics, dyslipidaemics, or those with multiple risk factors is unlikely to improve risk detection and prevention in the young.

In keeping with the 8 to 28% of strokes that occur in the elderly (50 to 75 years of age) being lacunar strokes (Shi and Wardlaw, 2016), in the present study we note that in the elderly 27% had lacunar stroke. However, also in keeping with reports that lacunar strokes are relatively uncommon in the young (Shi and Wardlaw, 2016), we note a prevalence of only 11% in those with stroke at a younger age. Thus, strategies to identify lacunar syndromes which are frequent consequences of hypertension and diabetes mellitus are unlikely to improve risk detection and prevention in the young.

In contrast to what is noted in many countries where cardioembolic stroke occurs mainly in the elderly (Maaijwee et al., 2014), in the present study we show a similarly important contribution of cardioembolism to stroke (19-22%) in both the young (22%) and the elderly (19%). These findings are remarkably consistent with alternative studies (Putaala et al., 2009)

that have reported that younger individuals (15 to 44 years of age) have a slightly greater (21.9%) chance of cardioembolic stroke as compared to those who are older (17%) (45 to 49 years of age). This may depend on the prevalence rates of atrial fibrillation and cardiomyopathy in low-to-middle-income countries where the average age of stroke of cardioembolic origin may be as low as 28 years (Zühlke et al., 2016). The high prevalence of cardioembolic stroke in the young in the present study therefore underscores one possible approach to stroke prevention in the young and that is to identify those with underlying cardiac pathology that will benefit from anticoagulants. As ischaemic heart disease is an infrequent finding in young persons of African origins, these patients are nevertheless more likely to have cardiomyopathy of non-ischaemic origins or atrial fibrillation. Peripartum cardiomyopathy, one of the non-ischaemic cardiomyopathies which may be a cause for embolic strokes, accounts for 20% of admissions in young women of reproductive age with an incidence of 1 to 1000 cases in South Africa and 1 to 100 cases in Nigeria (Sliwa et al., 2005). Furthermore, in South Africa, peripartum cardiomyopathy is the fifth most common cause of heart failure and identified as endemic (Damasceno et al., 2012). Of interest, 47% of those with cardioembolic stroke had carotid plaque, a finding which suggests that a significant cause of cardioembolic stroke is associated with standard risk factors. A decade ago, hypertensive heart diseases were reported to be the most frequent cause of heart failure, accounting for 45.4% cases with 20% of admissions due to uncontrolled hypertension in young people from the Sub-Saharan Africa; after idiopathic dilated cardiomyopathy (18.8%) and rheumatic heart disease (14.3%) (Damasceno et al., 2012; Rheumatic Heart Disease in Africa, 2014). In part, these studies (Damasceno et al., 2012; Rheumatic Heart Disease in Africa, 2014) address the question on whether hypertensive or ischaemic heart disease relates with stroke attributed to cardioembolic diseases but whether these strokes occur with the presence of carotid plaque remains unclear. Therefore, further studies are warranted to determine whether hypertensive or ischaemic heart disease associates with stroke caused by cardioembolic disease and the presence of carotid plaque.

Consistent with estimates of approximately 20-30% of strokes being of “other” determined aetiology in younger people (Putaala et al., 2009; Smajlovic, 2015), in the present study we note that 26% of stroke in younger persons was of “other” determined aetiology. In this regard a wide range of causes were documented in the present study with the only possibly remarkable finding being that a relatively high proportion were noted to have a vasculitis. Whether this relates to underlying infections with the human immunodeficiency virus, a common finding in South Africa, requires further study and is the topic of Dr Eitzaz Sadiq’s PhD. Of interest, those with stroke of “other” determined origins also had a high prevalence of carotid plaque, despite the causes being largely unrelated to atherosclerosis. Whether this represents a greater chance of vascular damage caused by alternative pathophysiological mechanisms in the presence of underlying atheroma, is unknown.

In keeping with what is generally noted (Kes et al., 2012), in the present study stroke of undetermined aetiology accounted for approximately 31% of stroke. The prevalence of 33% of stroke of undetermined aetiology at a younger adult age is consistent with reports on the age distribution of stroke subtypes, where stroke of undetermined aetiology has been shown to account for 28.1% of strokes in the young (Putaala et al., 2009). However, in the present study, a greater proportion of strokes of undetermined aetiology were noted at an older age (30%) as compared to previous reports of 15.7% in older persons (Putaala et al., 2009). Of importance, 40% of those with stroke of undetermined aetiology showed carotid plaque. It is therefore likely that atheroma plays an important role in many of these patients, but that stroke occurs without being able to detect striking occlusion of intra or extracranial arteries and in the absence of lacunes.

4.7 Risk factors and premature stroke

In keeping with the majority of studies world-wide and as highlighted in the introduction, in the present study hypertension was the major modifiable risk factor for stroke in both the young and the elderly. However, in striking contrast to many studies which show that several additional conventional risk factors also contribute to stroke, in the present study the only other risk factor that accounted for differences between cases and controls was HDL cholesterol concentrations. No differences in either diabetes mellitus, nor regular smoking were noted between cases and controls and a strikingly low proportion of those with stroke at a young age were noted to have diabetes mellitus (7.3%). This is in direct contrast to studies that demonstrate that diabetes mellitus accounts for 87.3% of ischaemic stroke in those between 18 to 49 years of age with hypertension (44.4%) only playing a secondary role (Habib et al., 2018). However, the low prevalence of diabetes mellitus in the present study in young patients with stroke is in support of other data obtained in Africa where for example, in Ethiopia, diabetes mellitus may account for only 8.5% of all strokes (Deresse and Shaweno, 2015).

Although in the present study regular smoking was not independently associated with stroke in either the elderly or the young, a relatively higher proportion of younger and older patients with stroke were smokers as compared to age and sex-matched controls. Consequently, given a larger study sample, smoking may have been an independent risk factor for stroke. Importantly, previous studies have demonstrated that smoking may be the second leading cause of stroke in the young (44%) after dyslipidemia (60%) and hypertension (39%) (Putaala et al., 2009). Moreover, in a large study (n=3944) conducted across Europe (Putaala et al., 2012), smoking was the leading risk factor (49%) followed by dyslipidemia (46%) and hypertension (36%) in those with stroke at a young age. As a significant proportion of patients with stroke in both the younger (27%) and older (21.7%) age groups in the present study smoked, this represents an important target for preventing stroke across the adult age range.

An essential finding of the present study was the marked differences in HDL concentration in patients with stroke as compared to controls at either a younger or older age. In

this regard, dyslipdaemia may be the leading cause of stroke in the young in Finland (60%) (Putaala et al., 2009). Moreover, in a large study (n=3944) conducted across 12 countries in Europe, dyslipidaemia was the second leading cause of stroke (46%) at a young age (Putaala et al., 2012). To avoid the effect of statins on cholesterol concentrations, in the present study HDL cholesterol concentrations were nevertheless employed to identify dyslipidaemia. Whether differences in LDL cholesterol concentrations, which can be targeted with interventions, between those with stroke as compared to controls occurs in black Africans, requires further study.

4.8 Clinical Implications

There are several clinical implications of the present study. The most important implication is that even when overall conventional risk factor scores are unremarkable, that imminent premature stroke may occur. Importantly, this may have been predicted with carotid imaging for the detection of plaque and hence prevented with aggressive risk management if plaque was noted. Thus, the present study suggests an urgent need for large scale clinical studies to assess whether carotid plaque does indeed predict stroke at a younger age and to identify who would most benefit from these assessments. In the meanwhile, young adults with risk factors should be screened. Whether this is possible at a public health level in middle-income countries is nonetheless unclear as resources may be limited. As B-mode ultrasound may be expensive and requires a degree of expertise to reliably perform, alternative approaches should be sought. This is feasible considering the ease of assessing certain vascular measures (including carotid-femoral pulse wave velocity) which have more recently been identified by our group to increase over a young adult age in those with stroke (Motau et al., 2020). A second implication of the present study is that a considerable number of strokes that occur at a younger

adult age are attributed to cardioembolic causes. Hence, better screening is required to detect those at risk of stroke caused by underlying cardiac pathology.

4.9 Limitations

There are several limitations of the present study that require consideration. As the study design was not longitudinal, conclusions regarding the predictive nature of IMT and plaque are limited. Nevertheless, bearing in mind that increases in IMT and the presence of plaque are slow structural changes with limited reversibility, the increases in IMT and the presence of plaque noted in those with stroke, compared to age and sex-matched controls, are likely to reflect structural changes that have occurred well prior to the event. Moreover, it is difficult to conceive of patients without increases in IMT or the presence of plaque at the time of the assessment having increases in IMT or plaque prior to the event which have then resolved. Nevertheless, the lack of independent impact of IMT on stroke over a younger age range could be accounted for by the incorporation of controls from the community sample with high IMT values and hence at a high risk of events. This would indeed bias against showing independent relations with stroke. Nevertheless, IMT was independently associated with stroke over an older age range and hence it is unlikely that the random selection procedures of controls resulted in recruitment of controls specifically at a high risk.

Importantly, case-control analysis is limited by the use of non-random selection of control samples with a small sample size, resulting in population stratification. To limit population stratification, in the present study however we employed a large study sample and age-, sex- and ethnically-matched subjects from the same socio-economic background of a large, randomly selected community sample as control subjects. In this regard, the consistency of the case-control findings across several categories of age suggests a high level of reproducibility and hence a limited degree of population stratification. Last, to limit population

stratification, and to ensure that a significant number of events were captured at a younger adult age, only one ethnic group was studied. Hence, further studies in different populations are required to evaluate whether the present findings are unique to developing populations.

4.10 Conclusion

In conclusion, stroke is frequently premature in groups of African ancestry in South Africa and this is often associated with an unremarkable conventional risk factor score. While non-invasive assessments of carotid artery plaque are strongly and independently associated with stroke over the young adult age range, IMT is not. The assessment of carotid plaque, but not IMT may therefore be an essential tool for predicting the risk of premature stroke in Africa. The present results highlight the need to better assess the risk for stroke at an age where this event has a marked effect on disability adjusted life years.

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APPENDIX I

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

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26 October 2017
Person No: 828413
TAA

Mr P Mabena
Erf 261 Mandzolwandle
Newtownship
Lusikisiki
4820
South Africa

Dear Mr Mabena

Master of Science in Medicine: Change of title of research

I am pleased to inform you that the following change in the title of your Dissertation for the degree of **Master of Science in Medicine** has been approved:

From:	**Carotid atheroma in young people of African ancestry with stroke**
To:	**Premature vascular aging in precipitous stroke in a middle income country**

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

APPENDIX II

R14/49 Mr Philanathi Mabena et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M1704125

NAME: (Principal Investigator)	Mr Philanathi Mabena et al
DEPARTMENT:	Physiology University of the Witwatersrand Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Carotid Atheroma in Young People of African Ancestry with Stroke. (A Component of the Study entitled: Risk Predictors for Stroke in Human Immunodeficiency Virus infected South Africans - M140429)
DATE CONSIDERED:	Adhoc
DECISION:	Approved unconditionally
CONDITIONS:	Sub-Study (M140429)
SUPERVISOR:	Prof Gavin Norton and Prof Angela Woodiwiss
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	08/05/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review in April and will therefore be due in the month of April each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES