Current Atherosclerosis of Intracranial and Extracranial Vessels in Ischemic Stroke Patients

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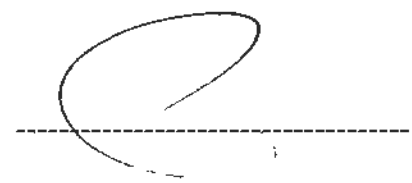
Declaration of Originality

I hereby declare that all studies that are contained in part II of this thesis are completely original and have not been submitted to any university for a degree or diploma. There are four original studies that are included in this thesis. All the studies were conducted in the Prince of Wales Hospital, Shatin, Hong Kong SAR. I am responsible for the design, acquisition, analysis, and interpretation of the data of all the 4 studies.



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Précis

Racial differences in the distribution of cerebrovascular occlusive disease are well documented. Extracranial stenosis is more common in Caucasian while intracranial stenosis is more common in Asian, Hispanic and African-American. The prevalence of asymptomatic intracranial stenosis in middle age and elderly general population in China was about 7 %. The frequency of intracranial atherosclerosis among patients with stroke and TIA is 40 to 50% in Chinese populations. Concurrent extracranial and intracranial stenoses is common in Asian, the incidence range from 10 to 39% in patients with stroke. The current population of China is 1.3 billion and it was estimated that 30% of the population will be aged 60 and above by 2050 in China. The incidence of stroke in China is 215 per 100000 which is one of the highest among the world and this burden is expected to escalate in the coming decades. However, studies of concurrent stenoses among Chinese are scarce. The aim of this précis is to present my studies that were conducted mainly among Chinese stroke patients on this particular field. The scope of the studies covers the following 4 areas: (1) Identification of Long-term prognosis of patients with concurrent stenoses; (2) Long-term prognosis of patients with concurrent stenoses and ischemic heart disease; (3) Lesion pattern and stroke mechanisms in concurrent stenoses; and (4) genetic polymorphisms of ischemic stroke patients with concurrent stenoses. The background,

objectives, subjects, methods, results, and conclusions of these studies will be presented in this précis.

Background

Study 1: Use of MRA to Predict Long-term Outcomes of Ischemic Stroke Patients with Concurrent Stenoses

Concurrent atherosclerosis of intracranial and extracranial vessels is common in Asian. However, the long-term prognosis of this group of patients is unclear. Although conventional contrast angiography provides excellent visualization of the craniocervical vasculature, the invasive nature of the procedure and the risk of perioperative complications hinder its widespread use. Magnetic resonance angiography (MRA) is a safe and reliable modality in studying intracranial circulation. The objective of this study is to determine the long-term outcomes of patients with concurrent atherosclerosis of intracranial and extracranial vessels after acute stroke using MRA.

Study 2: Long-term Outcomes of Ischemic Stroke Patients with Concurrent Intracranial, Extracranial Stenoses and Ischemic Heart Disease

Coexisting ischemic heart disease (IHD) is common in patients with stroke and it is

associated with increased risk of cardiac death. Previous studies in Caucasian and Korean showed that IHD was more common in patients with extracranial carotid artery disease. Concurrent atherosclerosis of intracranial and extracranial vessels is common in Asian and it is associated with poor outcomes. The impact of coexisting IHD in this group of patients was unknown. This study aims to investigate the long-term outcomes of ischemic stroke patients with concurrent stenoses and IHD.

Study 3: Lesion Patterns and Stroke Mechanisms in Concurrent Atherosclerosis of Intracranial and Extracranial Vessels

The stroke mechanisms and the lesion patterns of patients with concurrent stenoses are unclear. Diffusion-weighted imaging (DWI) is the most sensitive diagnostic modality in detecting acute ischemic lesions. This study aimed to investigate the ischemic lesion patterns in concurrent stenoses using DWI, and to identify the mechanisms of stroke.

Study 4: Genetic Polymorphisms affecting Homocysteine and Lipid metabolism in Ischemic Stroke Patients with Concurrent Stenoses in Hong Kong

The etiology of concurrent stenoses is poorly understood and hereditary factors are believed to play important roles. The objective of this study is to determine whether

genetic polymorphisms affecting homocysteine and lipid metabolism are associated with concurrent stenoses.

Objectives

Study 1: To determine the long-term outcomes of patients with concurrent atherosclerosis of intracranial and extracranial vessels after acute stroke using MRA.

Study 2: To determine the long-term outcomes of ischemic stroke patients with concurrent stenoses and IHD.

Study 3: To determine the ischemic lesion patterns in concurrent stenoses using DWI.

Study 4: To determine whether genetic polymorphisms affecting homocysteine and lipid metabolism are associated with concurrent stenoses.

Subjects and Methods

Subjects were patients who were admitted to the acute stroke unit of the Prince of Wales Hospital (PWH) because of stroke or transient ischemic attack (TIA) from June 2001 to December 2004. Brain computed tomography (CT) was performed on all

patients within 24 hours of admission to rule out intracranial hemorrhage. Carotid Doppler, Magnetic resonance imaging and magnetic resonance angiography were arranged within 1 week of stroke. We excluded patients less than 18 years old, those who had atrial fibrillation, intracranial hemorrhage, vascular malformations, active cancer, myocardial infarction, liver and renal failure, prothrombotic tendency such as underlying active lupus disease, antiphospholipid syndrome, factor C/S deficiency and those who were pregnant. Those who are unfit for MRI study because of unstable medical conditions and claustrophobia were excluded.

Study 1:

A prospective cohort study conducted at the Prince of Wales Hospital. We recruited consecutive patients admitted with acute cerebral ischemia, including transient ischemic attack (TIA) and cerebral infarct within 7 days of symptom onset during June 2001 to December 2003.

Study 2:

A prospective cohort study conducted at the Prince of Wales Hospital. We recruited consecutive patients admitted with acute cerebral ischemia, including transient ischemic attack (TIA) and cerebral infarct within 7 days of symptom onset during

January 2002 to June 2004.

Study 3:

A cross-sectional study conducted at the Prince of Wales Hospital. We recruited consecutive patients admitted with acute cerebral ischemia, including transient ischemic attack (TIA) and cerebral infarct within 7 days of symptom onset from January 2002 to December 2003.

Study 4:

A case-control study conducted at the Prince of Wales Hospital. We recruited patients admitted with acute cerebral ischemia, including transient ischemic attack (TIA) and cerebral infarct from January 2002 to December 2004. Controls were patients without history of cardiovascular diseases including stroke and myocardial infarction. All cases and controls were ethnic Han Chinese and they were genotyped for the following polymorphisms: Paraoxnase 1 (PON1) c. 192 Q-> R, Methylenetetrahydrofolate reductase (MTHFR) c.222 A->V, glutamate-cysteine ligase polymorphism in catalytic-subunit (GCLC) C->T and oxidized low-density-lipoprotein receptor (OLR) C->T.

Results

Study 1:

Totally 343 patients with acute ischemic stroke were included, of whom 104 (30%) had concurrent intracranial and extracranial lesions. The follow-up period was up to 76 months (mean 44.5 months). Overall, fifty-three patients (15.5%) died of any cause and 91 patients (26.5%) suffered a further non-fatal vascular event. The overall 5-year cumulative rates of mortality, restroke and poor outcomes (combined death and further vascular events) were 18%, 27% and 37% respectively. In patients with concurrent lesions, these rates were 31%, 41% and 51% respectively. The corresponding rates were 13%, 22%, and 31% in patients without concurrent lesions. The risks were highest in the first year after stroke. More deaths (log rank, 16.3; p=0.0001), restrokes (log rank, 9.71; p=0.002) and poor outcomes (log rank, 13.87; p=0.0001) were found among patients with concurrent lesions. The presence of concurrent vascular lesions, advanced age, smoking, hyperlipidemia and previous history of stroke were independent predictors of poor outcomes.

Study 2:

Totally 428 patients were included. The mean follow-up period was 65 months (up to 87 months). Ninety-three patients (22%) died of any cause and 104 patients (22%)

suffered from non-fatal vascular events. Fifty-four patients (13%) had ischemic heart disease. Among them, 27 patients (50%) had concurrent stenoses. In patients with concurrent stenoses and IHD, only 3 (11%) were free of death and recurrent vascular events. Eight (30%) had recurrent non-fatal stroke, 7 (26%) had non-fatal myocardial infarct, 11 (41%) died and 4 (22%) due to fatal myocardial infarct. The overall 5-year cumulative rates of mortality, recurrent vascular events and combined poor outcomes were 21%, 23% and 43% respectively. In patients with concurrent stenoses and IHD, these rates were 40%, 50% and 83% respectively. More deaths (log rank, 6.56; $p=0.01$), recurrent vascular events (log rank, 25.24; $p<0.001$) and poor outcomes (log rank, 27.50; $p<0.001$) were found among patients with concurrent stenoses and IHD.

Study 3:

Totally 251 patients were included in the analysis. Of these, 109(43%) had concurrent stenoses. Patients who had concurrent stenoses, as compared with those without concurrent stenoses, had more symptomatic stenoses (84% vs. 58%; odd ratio, 4.0; 95% confidence interval [CI], 2.1 to 7.3 ; $p<0.001$) , more concomitant perforating artery infarct (PAI), pial infarct (PI) and borderzone (BZ) infarct (14% vs. 4%; odd ratio, 3.6; 95% CI, 1.4 to 9.7 ; $p=0.007$), more multiple DWI lesions (55% vs. 37%; odd ratio, 2.1; 95% CI, 1.3 to 3.4 ; $p=0.005$) and more infarcts in the territory of the

leptomeningeal branches of middle cerebral artery (MCA) (26% vs. 13%; odd ratio, 2.2; 95% CI, 1.2 to 4.3 ; p=0.01). In multivariate regression analysis, smoking, prior stroke, the presence of concomitant PAI, PI, and BZ infarcts, multiple DWI lesions and symptomatic stenoses were significantly associated with concurrent stenoses. Among patients with concurrent stenoses, those who had tandem lesions, as compared with those who had non-tandem lesions, had more PAI and BZ infarcts (27% vs. 8%; odd ratio, 4.3; 95% CI, 0.9 to 19.8; p=0.04), more concomitant PAI, PI and BZ infarcts (18% vs. 0%; p=0.02), and more multiple DWI lesions (65% vs. 23%; odd ratio, 6.2; 95% CI, 2.2 to 17.2; p<0.001). Infarcts in the territory of MCA leptomeningeal branches and symptomatic stenoses were more common in patients with tandem lesions.

Study 4:

A total of 191 patients with acute ischemic stroke were included, of whom 47 (25%) had concurrent stenoses. The genotype distributions of PON1 Q192R and MTHFR A222V, which affect lipid and homocysteine metabolism, were significantly different between patients with stroke and controls. The presence of at least one R allele in PON1 Q192R and TT allele in OLR rs1050283 were associated with concurrent stenoses. There was also a tendency toward association between the presence of at

least one V allele in MTHFR A222V and concurrent stenoses.

Conclusions

Study 1:

The long-term prognosis of ischemic stroke patients with concurrent atherosclerosis of intracranial and extracranial vessels is poor. They are at high risks of further vascular event or death

Study 2:

Ischemic stroke patients with concurrent stenoses and IHD had high risks of death and recurrent vascular events. Future studies on aggressive medical therapy and early cardiac interventions in this high-risk group of stroke patients are warranted.

Study 3:

Concomitant PAI, PI and BZ infarcts, multiple DWI lesions and infarcts in the leptomeningeal branches of MCA were more common in patients with concurrent stenoses, especially those with tandem lesions. This study suggested that the combination of hemodynamic compromise attributable to concurrent stenoses and artery-to-artery embolization is a common stroke mechanism in these patients.

Study 4:

This study showed that genetic polymorphisms affecting homocysteine and lipid metabolisms are possible risk factors for stroke and concurrent stenoses.

Summary

In summary, the present studies (1) emphasized the poor outcomes of Chinese stroke patients with concurrent stenoses; (2) showed that Chinese stroke patients with concurrent stenoses and ischemic heart disease are at high risks of recurrent vascular event and death; (3) suggested that the combination of hypoperfusion and artery-to -artery embolization may be a common stroke mechanism in patients with concurrent stenoses; and (4) showed genetic polymorphisms affecting homocysteine and lipid metabolism are possible risk factors for concurrent stenoses.

The present studies have laid foundation for future researches in concurrent stenoses among Chinese subjects. Potential preventive treatments, such as aggressive blood pressure, homocysteine, or lipid lowering therapies, the role of early cardiac interventions, and the use of extracranial and intracranial stenting in patients with concurrent stenoses should be explored in future clinical trials. It is my hope that all these studies will contribute in improving the long-term outcomes in this high-risk

group of patients and reduce the burden of stroke in our aging society.

Publications that were included in this Thesis

(1) **BL Man**, YP Fu, YY Chan, Wynnie Lam, CF Hui, WH Leung, KS Wong. Use of MRA to Predict Long-term Outcomes of Ischemic Stroke Patients with Concurrent Stenoses in Hong Kong. Cerebrovascular Disease 2009; 28(2):112-8.

(2) **BL Man**, YP Fu, YY Chan, Wynnie Lam, CF Hui, WH Leung, V. Mok, KS Wong. Long-term Outcomes of Ischemic Stroke Patients with Concurrent Intracranial and Extracranial Stenoses and Ischemic Heart Disease. Cerebrovascular Disease 2010; 29(3):236-41.

(3) **BL Man**, YP Fu, YY Chan, Wynnie Lam, CF Hui, WH Leung, V. Mok, KS Wong. Lesion Patterns and Stroke Mechanisms in Concurrent Atherosclerosis of Intracranial and Extracranial Vessels. Stroke. 2009 Oct; 40(10):3211-5.

(4) **BL Man**, L. Baum, YP Fu, YY Chan, Wynnie Lam, CF Hui, WH Leung, V. Mok, KS Wong. Genetic Polymorphisms affecting Homocysteine and Lipid metabolism in Ischemic Stroke Patients with Concurrent Stenoses in Hong Kong. Journal of Clinical Neuroscience 2010 Oct; (10):1244-7.

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List of Abbreviations

ACA	Anterior cerebral artery
AF	Atrial fibrillation
ANCOVA	Analaysis of covariance
BI	Barthel index
BZ	Borderzone
CD	Carotid Doppler
CEA	Carotid endarterectomy
CI	Confidence interval
CT	Computed tomography
DM	Diabetes mellitus
DWI	Diffusion-weighted imaging
ECST	The European Carotid Surgery Trial
GCLC	Glutamate-cysteine ligase catalytic-subunit
HT	Hypertension
HR	Hazard ratio
IADL	Instrumental activities of daily living
ICA	Internal carotid artery
IHD	Ischemic heart disease
MCA	Middle cerebral artery
MI	Myocardial infarct
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
MTHFR	Methylenetetrahydrofolate reductase
NASCET	The North American Symptomatic Carotid Endarterectomy Trial
NIHSS	National institute of health stroke scale
OLR	Oxidized low-density lipoprotein receptor
PAI	Perforating artery infarct
PI	Pial infarct
PCA	Posterior cerebral artery
PCR	Polymerase chain reaction
PON1	Paraoxonase 1
PWH	Prince of Wales Hospital
TCD	Transcranial Doppler
TIA	Transient ischemic attack
PCA	Posterior cerebral artery

PART I LITERATURE REVIEW

CHAPTER 1 Introduction

Atherosclerosis of cerebral vessels is a common cause of stroke. Racial differences in the distribution of cerebrovascular occlusive disease are well documented. Extracranial stenosis is more common in Caucasian while intracranial stenosis is more common in Asian, Hispanic and African-American.[1-4] Concurrent atherosclerosis of extracranial and intracranial vessels is common in Chinese. Its incidence ranges from 10 to 39% in patients with symptomatic cerebrovascular disease.[2, 5-8]The current population of China is 1.3 billion and it was estimated that 30% of the population will be aged 60 and above by 2050.[9] The incidence of stroke in China is one of the highest in the world[10] and this burden is expected to escalate in the coming decades in China. However, studies of concurrent stenoses in Chinese are scarce.

Part I of this thesis is literature review. The epidemiology of atherosclerosis of craniocervical vessels, the current understanding of concurrent stenoses, imaging modalities, and medical and surgical treatments of extracranial and intracranial stenosis will be laid out. Following this, studies of Chinese stroke patients with concurrent stenoses are presented in Part II.

1.1 Racial difference in the distribution of cerebrovascular occlusive diseases

Stenosis of extracranial vessels is a major cause of stroke in Caucasians[1,2]. The prevalence of intracranial stenosis is very low. [4-6] However, intracranial atherosclerosis has been shown to be more common in Asian and African-American populations[7-24].

In studies with predominant Caucasian population, Fisher[11, 12] found that in his necropsy series of patients with cerebrovascular disease, extracranial carotid artery stenosis was the major cause of ischemic stroke. He could not find a single example of thrombosis of the middle cerebral artery. Blackwood et al. [13] and Lhermitte et al.[14] also found the rarity of atherosclerotic occlusion of the middle cerebral artery(MCA) in England and France. Middle cerebral arterial lesions were attributed to either embolization from the heart or from plaques in the proximal carotid arteries or arch of aorta. Harvard Stroke Registry [15]also suggested that most ischemic lesions were due to either cerebral emboli or occlusive disease of the internal carotid artery. The data imply that true intrinsic disease of the intracranial arteries is rare and that most intracranial vascular occlusions are due to emboli. Cerebral angiography [16] findings also confirmed the high incidence of lesions of extracranial carotid arteries in patients with ischemic stroke in Caucasian populations.

In studies of Asian and African-American populations, the prevalence of intracranial stenosis was much higher. Bauer et al[17] first reported the racial differences in the distribution of cerebral atherosclerosis in an angiographic study of patients admitted

to a single hospital. Caucasians were more likely to have occlusive lesions of extracranial carotid arteries, whereas African-Americans tended to have tortuous or dilated vessels with diffuse, non-occlusive atherosclerosis. Heyden et al [18] found a ratio of middle cerebral artery occlusions to internal carotid artery occlusive lesions of 13/84 in Caucasians, whereas in African-Americans, it was reversed at 10:1. In the Joint Study of Extracranial Arterial Occlusion[19], African-Americans had more occlusive intracranial diseases and fewer transient ischemic attacks. Gorelick et al[20, 21] studied the determinants of extracranial versus intracranial atherosclerosis in a series of patients with angiograms of the anterior and posterior circulations. Caucasians were more likely to have extracranial carotid lesions, transient ischemic attacks (TIAs) and lesions of the vertebral artery origin. African-Americans were more likely to have lesions involving the intracranial carotid artery, MCA main stem and distal basilar artery. In a review of stroke and races, Caplan et al[1] pointed out that African-Americans were more likely to have atherosclerosis involving the intracranial cerebral vessels, whereas Caucasians more commonly had diseases of the extracranial vessels, particularly the cervical carotid artery. Inzitari et al[22] used discriminant function analysis of data from the Extracranial/Intracranial Bypass Study to study the influence of race and risk factors on the location of cerebral atherosclerosis in 1367 patients with cerebral angiography. Race was the only factor that remained an independent determinant of location of atherosclerosis. The effect was much greater in Asians than in African-Americans, with both groups having more intracranial lesions and fewer extracranial lesions than Caucasians did. Witky et al [3] found that the distribution of cerebral atherosclerosis is influenced by race and sex but not by other vascular risk factors. Caucasians were more

likely than African-Americans to have extracranial carotid artery lesions. Studies in Japanese patients also found a high incidence of disease of the middle cerebral artery.[23-28] Intracranial stenosis is common in Chinese. Wong et al [29] examined 66 acute ischemic stroke patients by Transcranial Doppler (TCD), in which 22 patients (33%) had intracranial occlusive diseases and just 3 (6%) had extracranial carotid stenosis. They also found that about 7% of asymptomatic community subjects over 40 years of age have intracranial stenosis in mainland China[30]. MCA occlusive disease is more common than intracranial carotid disease in other studies of Chinese population.[31, 32]

Concurrent extracranial and intracranial stenosis is common especially in Asians. There is little data on the prevalence of concurrent stenoses in general population. Wong et al. found that 21% the stroke patients had concurrent stenoses in Hong Kong.[33] Liu et al. found that 18% of the stroke patients in Taiwan got significant concurrent stenoses.[34] Lee et al. reported that 48% of patients with more than 30% extracranial carotid stenosis had concurrent intracranial stenoses in Korea.[35]

1.2 Risk factors associated with extracranial and intracranial atherosclerosis

Caucasians with extracranial atherosclerosis have a high frequency of coronary and peripheral vascular disease, hypertension, hyperlipidemia, and TIA.[1] Hennerici et al[36] found that 32.8% of patients with symptomatic peripheral vascular disease had significant associated extracranial arterial occlusive disease.

Risk factors for intracranial stenosis may be categorized as non-modifiable, well

documented and modifiable, and less well documented regardless of whether modifiable.[37] Non- modifiable risk factors include age[38-40], male sex[38-45], Chinese population[29], Hispanic population[4], African American population[42, 46], angiotensin-converting enzyme polymorphism[47], plasma endostatin/ vascular endothelial growth factor ratio[48], glutathione S-transference omega-1 gene polymorphism[49] and plasma homocysteine levels[50]. Well-documented and modifiable risk factors include hypertension[30, 38-40, 43, 51], serum beta lipoprotein[50], total serum cholesterol[50, 52], serum LDL cholesterol[52], serum apolipoprotein (a)[53, 54], serum HDL-cholesteral[53], sickle cell disease[55, 56] and meningitis[57]. Less well documented regardless of whether modifiable risk factors include diabetes mellitus[6, 38-40, 48, 51, 54], metabolic syndrome[58, 59], Alzheimer's disease[60, 61], aortic plaques[62], radiotherapy[63], tuberculous and cryptococcal meningitis[64] and family history of stroke.[30]

Most of the studies for risk factors of intracranial stenosis were conducted in symptomatic patients. However, intracranial stenosis is also common in patients with vascular risk factors. In asymptomatic Chinese patients, Wong et al found that elderly, hypertension, diabetes, and hyperlipidemia were associated with MCA stenosis.[65] The prevalence rose with increasing number of these associated factors from 7.2% for one, to 29.6% for four associated factors.

There is little information on the risk factor profile of patients with concurrent stenoses. Lee et al found that diabetes mellitus is the only significant factor associated with concurrent stenoses in Korean patients.[35]

1.3 Natural history of extracranial and intracranial stenoses

1.3.1 Natural history of extracranial carotid stenosis

In the United States, extracranial carotid stenosis is a major cause of stroke. It has been estimated that carotid artery disease is responsible for 20% to 30% of stroke.[66] In the North American Symptomatic Carotid Endarterectomy Trial (NASCET)[67], the cumulative risk of any ipsilateral stroke at two years were 26% in the 331 medically treated patients with severe (70-99%) stenosis. The risk of major or fatal ipsilateral stroke was 13.1% in this medical group. In the European Carotid Surgery Trial (ECST)[68], the 3-year risk of ipsilateral ischemic stroke was 16.8% for patients with severe (70-99%) stenosis in the control group. Three-year total risk of surgical death, surgical stroke, ipsilateral ischemic stroke, or any other stroke was 21.9%. In patients with symptomatic moderate (50-69%) or severe stenosis[69], the five-year rate of any ipsilateral stroke was 22.2% among those treated medically, and the rate of disabling ipsilateral stroke was 7.2%. For those with less than 50% stenosis, the five-year rate of any ipsilateral stroke was 18.7% and the rate of disabling ipsilateral stroke was 4.7%. The annual stroke event rate for asymptomatic patients with hemodynamically significant carotid artery stenosis ranges from 2% to 5%.[70-73] Progression of asymptomatic carotid artery stenosis to occlusion is unpredictable and can be disastrous. At the time of occlusion, disabling stroke may occur in 20% of patients, and thereafter in 1.5% to 5% annually.[74-76]

1.3.2 Natural history of intracranial stenosis

In the Warfarin versus Aspirin Symptomatic Intracranial Disease Study for Stroke (WASID) trial, patients with asymptomatic intracranial stenosis had annual stroke risk of 3.5%.[77] Impaired vasoreactivity on computed tomographic perfusion, magnetic resonance perfusion, and single-positron emission computed tomography, and increased oxygen extraction fraction on positron emission tomography[78-81] may have a role in predicting future stroke risk in patients with asymptomatic intracranial stenosis.

In symptomatic patients, Wong et al showed that patients with intracranial vascular lesions have higher risks of death or further vascular event than those without intracranial vascular lesions. The annual recurrent stroke rates during the first and second year were 10.9% and 7.5% in patients without vascular lesion, and 17.1% and 8.6% respectively in those with intracranial atherosclerosis.[82] Other studies suggested that the annual stroke risk in patients with intracranial stenosis is 3 to 15%.[83-86] More than 70% of patients with occlusive disease in the middle cerebral artery had persistent disease after 6 months.[87] Persistent TCD abnormalities would be more likely to be due to intracranial atherosclerosis. Progression of MCA occlusive diseases, as evidenced by increased velocity in TCD study, is associated with an increased risk of vascular events.[87] Patients with symptomatic intracranial atherosclerosis who fail antithrombotic therapy have extremely high rates of recurrent TIA/stroke or death (up to 50%). Recurrent ischemic events typically occur within a few months after failure of standard medical therapy.[88]

In Caucasians, survivors usually die of coronary events rather than recurrent strokes.[89] In Chinese patients, patients usually die of recurrent strokes than of ischemic heart disease.[90] This mirrors the predominance of cerebrovascular disease over coronary artery disease in Chinese. Stroke outnumbered acute myocardial infarction by 5 to 1 in a previous study of Chinese patients.[90]

1.3.3 Natural history of concurrent stenoses

The information on the long-term outcome of patients with concurrent stenoses is scarce. Wong et al. found that the annual risk of death in patients with concurrent stenoses was 14.1% in the first year and 12.3% in the second year. The annual risk of cerebrovascular event was 24.3% and 7.7 % respectively.[91]

Chapter 2. What are concurrent stenoses?

Concurrent stenoses are concurrent atherosclerosis of intracranial and extracranial vessels and it is common in Chinese. In this study, we define concurrent stenoses as presence of more than 30% stenoses in both extracranial and intracranial vessels, including lesions in the same vascular territories (tandem lesions) and lesions in different vascular territories (non-tandem lesions). In this Chapter, the fundamental aspects of concurrent stenoses, which include anatomy, etiologies, plaque pathology and genetics, will be discussed. Knowledge on these is crucial for understanding the clinical outcomes and treatments of concurrent stenoses.

2. 1 Anatomy of extracranial and intracranial vessels

The major craniocervical vessels in neck include the carotid and the vertebral arteries. The aortic arch gives rise to the brachiocephalic, the left common carotid, and the left subclavian arteries. The brachiocephalic artery in turn gives rise to the right subclavian and the right common carotid arteries. The two common carotid arteries run upward lateral to the trachea to approximately the level of the fourth cervical vertebra, where each bifurcates into the external and internal carotid arteries. The two vertebral arteries arise from their respective subclavian arteries. Each vertebral artery has four anatomical segments. It runs from its origin towards the C6 vertebra (first segment), traverses upwards through the foramina transversaria from C6 to C2 vertebra (second segment), loops around the atlanto-occipital joint (third segment), and finally pierces the

dura passing through the foramen magnum to enter the intracranial cavity (fourth segment). The two vertebral arteries join at the pontomedullary junction to form the basilar artery[92].

The major cerebral large arteries include intracranial internal carotid artery (ICA), middle cerebral artery (MCA), anterior cerebral artery (ACA), posterior cerebral artery (PCA) and basilar artery (BA). The pial plexus consists of the terminal branches of the ACA, MCA, and PCA, forming a network of arteries on the surface of the hemispheres. In general, the deep perforators supply structures such as thalamus and basal ganglia while the superficial perforators supply the white matter of the centrum semiovale.[27] The diameter of the small vessel ranges from 40μm to 1mm while the diameter for MCA varies from 3-5mm.

2.2 Etiologies

The main underlying mechanism of concurrent stenoses is atherosclerosis which typically affects large or medium sized arteries. It affects larger vessels ranging from 200-850μm and is characterized by accumulation of subintimal foam cells.[93] Atherosclerosis is a systemic disease and tends to affect different parts of body at the same time.[94, 95] In the carotid artery, high-risk plaques tend to be severely stenotic.[96] Compared with extracranial vessels of a similar size, the adventitia and media of the intracranial arteries are thinner, and their internal elastic lamina are fenestrated differently and thicker.[97] Study of intracranial vessels showed that the luminal stenosis, lipid area,

presence of neovasculature and inflammatory cells were independent risk factors of ischemic stroke in the MCA territory.[98]

2.3 Plaque pathology

2.3.1 Pathology of carotid plaques

The earliest pathological studies described the occurrence of atherosclerosis near branch ostia, bifurcations, and bends, suggesting that flow dynamics play an important role in its induction. It has been demonstrated that laminar flow is disturbed at carotid bifurcation regions.[99] The greatest atherosclerotic plaque accumulation typically occurs on the outer wall of the proximal segment of the sinus of the internal carotid artery, in the region of the lowest wall shear stress. The intimal thickness is the least on the flow divider side at the junction of the internal and external carotid arteries where wall stress is the highest.[100]

Fewer pathological studies have correlated carotid and aortic plaque morphology with cerebral findings, and, as a result, the mechanisms by which carotid atherosclerosis results in cerebrovascular symptoms are less well understood than those linking coronary disease and myocardial symptoms. Several studies, one of which analyzed 526 symptomatic carotid plaques, have demonstrated that the pathology of symptomatic plaques is similar to that of culprit coronary plaques.[101, 102] Furthermore, these studies have demonstrated that occlusive thrombus triggered by plaque rupture is one of the major determinants of ischemic stroke in patients affected by carotid atherosclerotic

disease.[101, 102] However, there are important differences between the coronary and carotid vascular beds. For example, unlike the myocardial circulation, the carotid vascular bed is subject to high blood flow. The majority of ischemic strokes seem to result from embolization from an atherosclerotic plaque or acute occlusion of the carotid artery and propagation of the thrombus distally rather than static occlusion.[103]

A study by Spagnoli et al. [102] proposed that thrombosis associated with plaque rupture is one of the major determinants of ischemic stroke in patients affected by carotid atherosclerotic disease. Thrombotically active plaques were defined by the presence of an acute thrombus composed of platelets or fibrin on the plaque surface with or without interspersed red and white blood cells. A thrombotically active plaque was observed in 74% of plaques from patients with ipsilateral major stroke. Of these, 90.1% were associated with plaque rupture and 9.9% with luminal surface erosion. In contrast, 35.2% of patients with transient ischemic attack and 14.6% of asymptomatic patients were found to have thrombotically active plaque. In the group of patients with transient ischemic attack, erosion was approximately twice that of patients with stroke. Total thrombotic occlusion was found in 40.8% of cases with a thrombotically active plaque as documented by angiographic stenosis of more than 95%. These results demonstrated a major role of carotid thrombosis and inflammation in ischemic stroke in patients affected by carotid atherosclerotic disease. Moreover, the study demonstrated that the severity of clinical events correlated significantly with the degree of inflammation in ruptured plaques, suggesting that inflammatory cells originating from the inflamed plaque may contribute to cell and tissue injury in ischemic brain disease.

While reports highlight significant differences in the frequency of plaque rupture between symptomatic and asymptomatic patients, other factors have also been associated with ischemic stroke. These include surface irregularity[104], plaque vascularity[105], ulceration[103], fibrous cap thinning, and infiltration of the fibrous cap by macrophages and T cells[101]. Carr et al.[105] reviewed 44 carotid endarterectomy specimens (from 25 asymptomatic and 19 symptomatic patients). The asymptomatic and symptomatic patients had similar mean percent stenosis (77% versus 74%, respectively). It showed that symptomatic carotid artery disease is more frequently associated with plaque rupture (74%) than that in asymptomatic disease (32%).[105] The observations suggest critical differences in plaque morphology between patients with symptomatic and asymptomatic disease.

In a study of carotid endarterectomy specimens from symptomatic high-grade stenotic lesions and asymptomatic autopsy specimens without high-grade carotid artery stenosis, Bassiouny et al.[106] showed that high-grade carotid stenotic plaques were associated with a significantly higher incidence of ulceration, thrombosis, and lumen irregularity compared with non-stenotic asymptomatic plaques. Although these features were more prominent in symptomatic patients, they were also present in 80% of the stenotic bifurcations and did not distinguish between symptomatic endarterectomy and asymptomatic autopsy lesions. However, the study of Bassiouny et al.[106] failed to show distinct morphological differences between asymptomatic and symptomatic carotid lesions. The reason may be related to the degree of stenosis in the varying patient populations. In a subsequent report from the same group, examination of the proximity of

the necrotic core to the lumen showed that it was twice as close to the lumen in symptomatic versus asymptomatic plaques. The percent area of necrotic core or calcification was similar for both groups. The number of macrophages infiltrating the fibrous cap was three times greater in the symptomatic plaques compared with the asymptomatic plaques. [104]

Disruption or ulceration of the fibrous cap was more common in the symptomatic than asymptomatic plaques. The mean fibrous cap thickness in carotid plaque rupture was nearly three times greater than coronary plaque rupture. In addition, there are fewer macrophages in the fibrous cap of carotid plaque ruptures than coronary plaque ruptures [99]. Similarly, in carotid vulnerable plaques, the number of macrophages is fewer than coronary vulnerable plaques .[107] Plaque vascularity has been shown to correlate with intraplaque hemorrhage and the presence of symptomatic carotid disease [108]. The role of vasa vasorum in provoking acute coronary syndromes and aortic plaque disruption is the focus of ongoing research. Imaging techniques for detection of vasa vasorum in carotid plaques may be important in future evaluation of carotid stenosis.

The classification of atherosclerotic plaque devised for coronary arteries and aortas is well suited for use in the carotid circulation. There are, however, unique features of carotid plaque morphology because of the high flow rates and the shear forces caused by the bifurcation of the common carotid artery into the internal and external carotids. Most importantly, the ulcerated plaque, which is rare in the coronary artery circulation, is relatively common in the carotid and other elastic arteries. Ulcerated plaque is a term

used when the thrombus and a portion of the plaque have embolized, leaving an excavation in the remaining lesion. Another feature of carotid atherosclerosis is the infrequency of total occlusion relative to the coronary circulation. Occlusive carotid disease is reported in 3% of patients with posterior circulation infarcts, 14% in those with partial anterior circulation infarcts, and 29% in patients with total anterior circulation infarcts[103]; however, in coronary circulation, the incidence of chronic total occlusion in patients dying suddenly is 40%.[109] The explanation for the low rate of total occlusions in carotid plaques is most likely related to high flow rates that limit thrombotic occlusions, unless there is severe luminal narrowing caused by repeated plaque ruptures.[99]

Plaque hemorrhage in the carotid artery is far more frequent than in the coronary arteries and may be related to high flow rates and pressures in the lumen and the vasa vasorum. The maximum frequency of hemorrhage is observed in arteries with 50 to 75% cross sectional area luminal narrowing.[110] In coronary plaques, intraplaque hemorrhage is responsible for necrotic core enlargement and excessive foamy macrophages in the fibrous caps.[111] Red blood cell membranes are the richest source of cholesterol as compared with any other cell in the body. Free cholesterol in fibroatheromas, thin cap fibroatheromas, and plaque ruptures is derived from erythrocytes that become trapped in the necrotic core when intraplaque hemorrhages occur.[111] Takaya et al. [112] reported that patients with carotid intraplaque hemorrhage at 18 months follow-up had larger necrotic cores as well as accelerated plaque progression as compared with patients without intraplaque hemorrhage. The frequency of calcification is similar in coronary and carotid arteries, with maximum calcification seen in carotid arteries narrowed greater than 70%

cross sectional area. However, the frequency of calcified nodules, a form of calcification that results in irregular nodules of calcium, is higher in carotid disease (6-7%), as compared with 1 to 2% in coronary artery disease.[99] In contrast, plaque erosion, while common in the coronary circulation, is somewhat less frequent in the carotid artery. In carotid arteries, percent stenosis was highest in healed plaque ruptures and was greater than thin cap atheromas and acute plaque ruptures.[99]

2.3.2 Correlation of risk factors to carotid plaque morphology

Several studies have correlated plaque morphology to risk factors in the carotid and coronary circulation. Spagnoli et al. [113] have shown that the fibrous carotid plaque correlated with aging and diabetes, the granulomatous plaque with hypertensive females, and the foam cell rich xanthomatous plaque exhibiting extensive alcianophilia with hypercholesterolemia. In smokers, plaques were frequently complicated by mural thrombosis. Mauriello et al. [114] studied carotid endarterectomy specimens and showed that patients with the highest tertile of fibrinogen had a high incidence of thrombosis compared with plaques of subjects with the lower and middle tertile. Plaque rupture was significantly associated with high fibrinogen level. Hyperfibrinogenemia was an independent predictor of fibrous cap thickness, macrophage foam cell infiltration of the cap, and thrombosis[114].

2.3.3 *Correlation of recurrent carotid disease to plaque pathology*

The rate of recurrent carotid stenosis after carotid endarterectomy varies from 4 to 10% and usually occurs more than 3 months after surgery.[115, 116] In a series of 1726 endarterectomies performed at the Cleveland Clinic from 1983 to 1997, 65 (3.8%) patients were re-operated on for recurrent carotid stenosis occurring 3 to 194 months after the initial procedure. Of these patients, approximately half were symptomatic with neurological symptoms. The recurrence interval was 57 months in specimens with atherosclerotic disease, whereas in specimens with myointimal hyperplasia, the recurrence interval was 21 months. In recurrent disease, the myointimal hyperplasia consisted of smooth muscle cells in a proteoglycan matrix interspersed with fibrin; the collagen and elastin representing organization of the thrombus is sparse. Neovascularity may be present but is usually not extensive and surface thrombi tend to be platelet rich.[117]

A review of the literature by Ecker et al. [118], representing a collection of more than 500 carotid endarterectomies that reported restenosis with follow-up periods varying from 18 to 82 months, shows a recurrence rate ranging from 0.7 to 7.9% during an average of 3.5 years. The follow-up study of 975 patients, however, yielded a restenosis rate of only 0.1%.[118] Another study with collection of recurrent endarterectomy specimens up to 36 months post-procedure showed that these lesions typically contain myointimal hyperplasia, and beyond this interval, atherosclerotic lesions are more common.[119] Seventy-four percent of specimens with atherosclerotic lesions contain fibrin-rich surface thrombi, which are in continuity with an intraplaque thrombus.[119] Extensive neovascularity in lesions with atherosclerosis is common. The plaque components include foam cells, cholesterol clefts, abundant collagen with focal areas of

necrosis and calcification. Some cases may show myointimal hyperplasia in the deep intima, but it is usually interspersed with atherosclerotic plaque. Although all the components of atherosclerosis are present in primary and recurrent lesions, the atherosclerotic elements are arranged in a less orderly manner in the latter.[119] Primary plaques demonstrate a central necrotic core with cholesterol clefts beneath a fibrous cap, whereas in recurrent lesions, the necrotic core is superficial and often unsupported by a dense layer of collagen. In recurrent lesions, the thrombus is contained within the plaque, whereas in primary lesions, it is usually associated with intraplaque hemorrhage, which is rarely observed in recurrent lesions.[115, 119] Pauletto et al. [120] reported that examination of primary endarterectomy lesions may be predictive of maximum intimal-medial thickness of revascularized vessels. Plaques with an abundance of smooth muscle cells, mostly of the fetal-type were more likely to develop greater neointimal growth after surgery compared with lesions rich in macrophages and lymphocytes.

2.3.4 Pathology of middle cerebral artery plaque

Compared with extracranial vessels of a similar size, the adventitia and media of the intracranial arteries are thinner, and their internal elastic lamina is fenestrated differently and thicker.[97] Although not established, this histological difference may contribute in part to the difference in atherosclerosis. Chen et al. [98]investigated 152 middle cerebral arteries (MCA). Atherosclerotic plaques of more than 40% cross-sectional area luminal narrowing stenosis were found in 69 MCA (45.4%). The degree of luminal stenosis was higher among the plaques associated with infarct than those not

associated with infarct. Plaques associated with infarct had a higher extent of lipid core compared with those not associated with infarct, which also had a higher prevalence of neovasculature and thrombus than those not associated with infarct. This study found that the luminal stenosis, lipid area and presence of neovasculature were independent risk factors of ischemic stroke in the MCA territory. The results suggested that luminal stenosis and lipid core within plaques may play a collaborative role in leading to ischemic events.

Both artery-to-artery embolism [121] and hemodynamic impairment[122] distal to a stenotic MCA have been proposed as mechanisms for ischemia, and it is probable that both mechanisms are involved.[123] The luminal stenosis and plaque composition are important parameters to reflect subsequent ischemic stroke. Plaque burden or composition is thought to stratify the risk of an ischemic event more accurately than luminal stenosis, which may not reflect the true burden of disease as outward expansion of the vessel wall can disguise large in situ plaques.[124, 125] As for neovasculature within plaques, its effect on ischemic events has been reported in coronary and carotid arteries.[126-129]

The inflammatory process favors the onset and development of atheroma plaque which contributes to the appearance and evolution of atherothrombosis.[129] The cascade of inflammatory reactions related with atherosclerosis involves monocytes, macrophages, T lymphocytes and vascular smooth muscle cells.[130, 131] Chen et al.[98] showed increased infiltration of both CD45RO and CD68 in the plaques associated with infarct, which

suggests that both T lymphocytes and macrophages may play an important role in the ultimate occurrence of ischemic stroke. In unstable or ruptured coronary plaques, a key pathological feature is infiltration of inflammatory cells, consisting of activated macrophages, T lymphocytes and mast cells.[132] Such cellular infiltrates have also been found in advanced carotid plaques.[133, 134]

Table 2.1 Comparison of coronary, internal carotid artery and middle cerebral artery plaques

	Coronary plaques	ICA plaques	MCA plaques
Ulcerated plaque	rare	common	common
Plaque hemorrhage	infrequent	frequent	frequent
Plaque erosion	frequent	infrequent	infrequent
Total occlusion	common	infrequent	common
Calcification	frequent	frequent	infrequent
Fibrous cap	thinner	thicker	N/A
Adventitia and media	thicker	thicker	thinner

ICA, internal carotid artery; MCA, middle cerebral artery; N/A, not applicable.

2.4 Genetics

The cause of most common strokes is multi-factorial and involves both genetic variants and environmental factors. Studies in families have estimated that the relative risk of stroke in a first-degree relative of a patient who has had a stroke is between 1.5 and 2.5. Common stroke is extremely heterogeneous and most likely results from the additive or multiplicative effect of a wide spectrum of pathogenic alleles, each of which confers a small degree of risk. Some of these alleles may predispose persons to specific subtypes of stroke and some gene variants may modulate the severity of stroke.[135] Genetic polymorphisms of pathways, including lipid metabolism[136-138], methylene tetrahydrofolate reductase [139], coagulation[140], blood pressure regulation[141, 142], cellular adhesion and inflammatory processes[143-147] are reported to associate with stroke. Genome wide association showed that two intergenic single-nucleotide polymorphisms on chromosome 12p13 and within 11 kb of the gene Nerve Injury Induced Protein 2(*NINJ2*) were associated with stroke. *NINJ2* encodes an adhesion molecule expressed in glia and shows increased expression after nerve injury [148] and it promotes neurite outgrowth from primary dorsal root ganglion neurons.[149]

Chapter 3. Imaging modalities of craniocervical vessels

3.1 Digital subtraction angiography

Digital subtraction angiography (DSA), as used in NASCET and ECST, is still considered to be the most accurate method for assessment of carotid stenosis.[69, 150] DSA is an invasive technique that requires femoral-artery puncture and direct intra-arterial injection of contrast medium, usually in the common carotid arteries. High-resolution images of the stenotic lumen can be obtained, as well as estimation of flow dynamics, such as slow and delayed blood flow. Intracranial views are usually also obtained to depict tandem lesions, for example at the carotid siphon. The most common method to measure carotid stenosis is that undertaken in NASCET, which used the diameter of the healthy distal internal carotid artery as the denominator.[69] There was some initial confusion with regard to measurement techniques as the method chosen in ECST used the diameter of the unseen estimated carotid bulb at the site of maximal stenosis as the denominator[150]. Both NASCET and ECST have since been shown to be mostly consistent with each other, and the mathematical relation between the two methods of measurement (with 50% and 70% stenosis in NASCET being equivalent to about 70% and 82% stenosis in ECST) is now well understood.[151, 152]

Conventional DSA is a two-dimensional modality and multiple orthogonal projections are necessary to depict the tightest stenosis because of the occurrence of non-circular lumens.[153] Although three-dimensional rotational DSA is now possible with modern angiographic machines, the extent of stenosis can be overestimated compared

with results from conventional DSA, which therefore remains the gold-standard method.[154]

DSA has several disadvantages—it is invasive, labor intensive, time intensive, expensive, and requires a period of bed rest.[155] The risk of groin hematoma has been reported to be up to 8% in large series, although these hematoma rarely cause considerable morbidity or delay hospital discharge.[156] Moreover, DSA requires skilled operators and is usually done in specialist neurovascular centers. Therefore, it is much less readily available, particularly compared with alternative non-invasive tests, and this can delay definitive management. This poor access is becoming more of a problem given the impetus to treat patients with transient ischemic attacks or minor strokes rapidly in the first few weeks after the event, when the risk of subsequent cerebrovascular accident is the highest[157].The main concern with DSA, however, is a small, but not negligible, risk of neurological complications.[156, 158-173] Even in current times, the rates of permanent neurological complications still probably range from 0.09 to 0.50% and those of transient neurological complications from 0.45 to 1.90%.[156, 165, 170, 173] When DSA is undertaken for suspected carotid stenosis, results from some studies have suggested that complication rates might, in fact, be higher because of tortuosity and atherosclerotic disease at the aortic arch and proximal vessels. In a meta-analysis by Cloft and co-workers[174], the overall combined neurological complication rate was significantly higher for patients with transient ischemic attacks or strokes compared with patients with subarachnoid hemorrhages, aneurysms, or arteriovenous malformations (3.9 *vs.* 0.8%), as was the risk of permanent neurological complications (0.70 *vs.* 0.07%). In the most recent and largest

series to date, the overall rate of neurological complications was, however, only slightly higher in the subgroup with stroke or carotid stenosis than in the whole population studied (1.8 *vs.* 1.3%), as was the rate of permanent stroke (0.6 *vs.* 0.5%).[156]

Some authors have suggested that arch aortography (where a pump injection is done with a pigtail catheter in the ascending aorta) might be safer than common carotid catheterizations.[173] This idea is intuitive, but separation of the diseased artery from overlapping vessels can occasionally be difficult. Despite advances in catheter technology and expertise, DSA remains associated with a small, but important, risk of neurological complications. Such risks potentially reduce any net benefits gained by revascularization procedures, and these concerns have generated substantial interest in non-invasive techniques for carotid imaging such as Doppler ultrasound, CT angiography, and magnetic resonance angiography (MRA).

3.2 Carotid Doppler ultrasound

Doppler ultrasound is well established as an accurate, non-invasive method to assess carotid stenosis. This technique is inexpensive and portable, and provides reliable information on the localization and extent of stenosis, flow dynamics, plaque structure, and vessel-wall characteristics. Several studies have shown the accuracy of this technique[170, 175], but have also indicated its limitations: high operator variability[176], inter-hospital variability[177], susceptibility to artifacts from calcified plaques, and difficulty in distinguishing a subtotal occlusion from a total occlusion.[178] Additionally, Doppler

ultrasound relies on extrapolating changes in flow measurements to an anatomical stenosis and does not provide an overview of the vascular anatomy and surrounding structures. Stenosis is mainly graded by assessing the Doppler spectral waveforms in the stenotic region and in the common carotid artery where specific measurements are usually made, such as the peak-systolic velocity, end-diastolic velocity, and peak-systolic velocity ratio.[179]

Accurate measurement of flow velocity is dependent on several factors such as accurate placement of the sampling volume within the area of greatest stenosis, adequate size of sampling volumes, appropriate use of Doppler angles, as well as correct cross-checking with color flow ultrasound to prevent diagnostic errors.[179] These factors introduce much inter-observer variability in the assessment of carotid stenosis.[180] Doppler ultrasound is reliable when used by those experienced in this technique, with sensitivities of 85–92% and specificities of 77–89% for severe stenosis reported in some systematic reviews, and is used as the sole investigative technique in some centres.[181] Worrying rates of misclassification have, however, been reported in some large series, and, in many centers, there is lack of confidence with regard to the use of Doppler ultrasound as the sole preoperative imaging technique.[182, 183] The low false-negative rate of Doppler ultrasound, however, makes it an ideal screening test before use of a confirmatory test such as MRA or CT angiography, and some centers are willing to accept two repeat independent Doppler ultrasound studies without any further investigations.

3.3 Magnetic Resonance Imaging and Angiogram

MRA has emerged as one of the non-invasive methods of choice for vascular imaging.[184-186] The core advantage of MRA is the possibility of portraying blood vessels in a format analogous to DSA. Multiple projections of the carotid arteries can be generated by post-processing techniques, such as maximum intensity projections, where the brightest pixels along a user-defined projection are extracted to create a projection image. MRA encompasses several techniques: the two main techniques for the assessment of carotid disease include time-of-flight and contrast-enhanced MRA.

Before the advent of contrast-enhanced MRA, time-of-flight MRA was the main method, which involves the use of the properties of blood flow to generate vascular signal.[187] However, time-of-flight MRA is limited by long imaging times of 10–15 minutes, which increases susceptibility to artifacts due to motion, and swallowing, and susceptibility to turbulent flow and dephasing artifacts, which tend to produce flow voids in regions of tight stenosis[188]. Although clinical assessments have shown good agreement with DSA, with regions of flow voids correlating particularly well with severe stenosis, these flow gaps can sometimes be seen in moderate stenosis, which can reduce confidence in the technique.[188-192] High sensitivities ranging from 86% to 90% for detection of severe stenosis are generally reported.[185] The controversial issue remains that stenosis can be overestimated with time-of-flight MRA, with specificities as low as 64% being reported in some series.[193] This purported inaccuracy might mean that some patients are inappropriately referred for surgery. Despite these limitations, time-of-flight MRA remains a useful option for patients who have contraindications to magnetic

resonance contrast medium, such as those with renal failure, and image quality can be excellent, particularly at high field strengths (e.g. 3 Tesla).

Contrast-enhanced MRA is thought to be a substantial technical improvement over time-of-flight MRA and is the MRA technique of choice for carotid stenosis.[194] Contrast-enhanced MRA relies on injection of a paramagnetic agent such as gadolinium to reduce the T1 relaxation time of tissue and to generate contrast between the intravascular lumen and surrounding tissues. Unlike time-of-flight MRA, vascular contrast is therefore relatively independent of flow dynamics, and problems associated with saturation effects are substantially reduced. This results in a lumen that is much less sensitive to dephasing artifacts, enabling accurate delineation of the true diameters of vessels, even in high-grade stenosis and in morphologically complex ulcerated plaques[194]. A large volume from the aortic arch to the circle of Willis can be obtained in less than 1 min[195].The multi-segmental vascular coverage enables potential assessment of tandem stenosis that is proximal or distal to the bifurcation.[196, 197] Contrast-enhanced MRA can also be more sensitive than time-of-flight MRA and Doppler ultrasound in depicting plaque ulceration.[189, 198, 199] Several studies have shown the technical advantages of contrast-enhanced MRA over time-of-flight MRA techniques.[200-202] A systematic review found contrast-enhanced MRA to be the most accurate of non-invasive diagnostic techniques, with sensitivities of 88–97% and specificities of 89–96% for detection of severe stenosis. The main problem with contrast-enhanced MRA is its relatively low spatial resolution, which despite being submillimeter, is still two-to-three times less than that offered by conventional DSA or CT angiography. The resolution of contrast-enhanced MRA is,

however, set to improve with new technological developments, such as high field strengths, parallel imaging techniques, or blood-pool contrast agents that stay in the circulation for increased duration and can enable high-resolution steady-state angiographic acquisitions.[203, 204]

3.4 CT angiography

CT angiography (CTA), undertaken with modern spiral CT machines, enables a volumetric acquisition through continuous radiograph source rotation by simultaneous continuous table movement. This method enables rapid examination with little discomfort by peripheral injection of a contrast agent. CTA is reliable in detecting both intracranial and extracranial stenoses.[205-210] With the recent advent of multi-slice technology, high spatial and contrast resolution images of the lumen of arteries from the aortic arch to the circle of Willis can be obtained rapidly in the pure arterial phase. From the axial source images, post-processing that uses multi-planar reformats, maximum intensity projections, or three-dimensional volume-rendering algorithms can be undertaken to produce angiographic images similar to those produced from DSA and to enable stenosis measurements in accordance with NASCET or ECST criteria.[194]

Limitations of CT angiography include ionization radiation dose and artifacts caused by plaque calcium. Extensive plaque calcification, particularly when circumferential, can obscure a clear image of the lumen of the diseased carotid artery and thus affect exact assessment of the extent of stenosis.[194] There are relatively fewer

published data with regard to CT angiography for carotid stenosis, particularly multi-slice CT angiography, when compared with the abundant published work on MRA or Doppler ultrasound. Some reports have suggested that the extent of stenosis can be underestimated with CT angiography compared with DSA, although this could be due to limitations of post-processing techniques such as volume-rendering algorithms.[211,212]

In a meta-analysis, the sensitivity of CT angiography ranged from 68% to 84% and the specificity from 91% to 97%.[213] There is, however, little doubt that, with the constantly improving CT technology such as dual-energy CT machines or 320-slice CT machines, CT angiography is likely to become increasingly popular as the non-invasive technique of choice for carotid stenosis. This expectation is mainly due to the accessibility of CT angiography (it is more accessible than MRA in many parts of the world), the excellent coverage from arch to skull vertex, and the high-quality anatomical images produced.[194] The high-quality images of CT angiography tend to inspire confidence in clinicians and radiologists, so rigorous comparative studies with DSA are generally perceived as unnecessary. With the notable decline in the use of DSA worldwide, studies that compare non-invasive tests to DSA are a less frequent occurrence in current published works, and, in the future, these comparisons will become increasingly difficult to do from both a practical and an ethical standpoint.[194]

3.5 Transcranial Doppler

Transcranial Doppler (TCD) allows measurements of blood flow velocity to be

made from the basal intracerebral vessels. Although Doppler ultrasound was first applied to patients in the 1960s[214], it was not appreciated for many years that sufficient ultrasound could pass through the skull to allow recording from intracerebral vessels. It was only in the 1980s that successful insonation of the middle cerebral artery was described by Aaslid *et al*.[215] To enable sufficient transmission of ultrasound through the skull, a low frequency transducer (usually 2 MHz) is used. This has the consequence that the spatial resolution is poor and, therefore, the technique is primarily useful for giving Doppler information on blood flow velocity. Even using state-of-the-art TCD ultrasound equipment, it is impossible to successfully insonate the intracerebral vessels in approximately 25% of Chinese due to the lack of an acoustic window.[5] A number of acoustic windows are used to provide access to different intracerebral vessels[216]. Most commonly, a temporal window above the zygomatic arch is used, through which the terminal internal carotid artery, middle cerebral artery, anterior cerebral artery, and proximal posterior cerebral artery can be insonated. The distal vertebral arteries and basilar artery can be insonated via an occipital window. Access can be obtained to the distal internal carotid artery and the ophthalmic artery via the orbit.

Duplex ultrasound machines have been adapted for transcranial imaging. The B-mode modality does allow some delineation of structure, and lesions such as intracranial hemorrhage and mid-line shift have been identified; however, the spatial resolution is much inferior to computed tomography or magnetic resonance imaging.[217] Nevertheless, this imaging modality does have advantages for studying intracerebral vessels, primarily due to the use of the color coded modality. It can be easier to identify certain intracranial

arteries, and this can help in determining whether they are absent or merely difficult to identify due to a poor acoustic window. It allows the sample volume to be placed m the vessel of interest and the Doppler angle to be adjusted manually so that angle corrected flow velocity can be determined.[218]

A major problem with TCD remains the lack of an acoustic window in approximately 25% of Chinese. The use of ultrasonic contrast agents can overcome this problem. An intravenous injection is given of an agent containing stabilized microbubbles. This passes into the intracranial arterial circulation, and results in increased back-scattering and signal intensity. Using this technique in combination with color flow duplex imaging the anatomy of the complete circle of Willis can be visualized.[219]

TCD is non-invasive and less expensive test. It is good for continuous monitoring of hemodynamic changes in intracranial circulation. However, it is operator-dependent and its use is limited in patients with poor temporal windows.[220] In the SONIA trial, the positive predictive values of TCD was 36% (95% CI: 27 to 46) and negative predictive values was 86% .[221] TCD is reliable in excluding lesion with less than 50% stenosis. However, abnormal findings in TCD need to be confirmed by other tests such as MRA, CTA and DSA.[222]

3.6 Carotid plaque imaging

The benefits of carotid endarterectomy in recently symptomatic patients with severe carotid stenosis are well established. However, there are subgroups of patients, particularly those with moderate stenosis or who are asymptomatic, in whom the choice between revascularization or medical intervention is less clear and for whom better methods of risk stratification are needed.[223] This has led many investigators to research other factors associated with carotid plaques that might be markers of increased risk of stroke. The concept of the vulnerable or high-risk plaque, initially derived from coronary studies, has been increasingly shown to apply in the carotid circulation.[224, 225] Glagov and co-workers[226] showed that the diseased vessel could compensate for a large plaque by means of outward expansion of the adventitial boundary. Consequently, a large ulcerated high-risk plaque might not substantially compromise the lumen and yet such lesions can cause thromboembolic complications.[224, 227] Histopathological studies have shown that certain morphological features of carotid atheroma, such as a large lipid core (occupying at least 25% of plaque area) separated from the lumen by a thin or ruptured fibrous cap (which can be weakened and infiltrated by macrophages) and the presence of intraplaque hemorrhage, are associated with increased stroke risk.[224] These features are key targets in the development of new atheroma imaging techniques and are potential markers of vulnerability to stroke and luminal stenosis.

Angiographic techniques such as DSA, CT angiography, and MRA all primarily depict the lumen but can provide some indirect information about plaque morphology through the presence or absence of plaque ulceration. Irregular or ulcerated carotid plaque on DSA is a powerful independent predictor of stroke in patients receiving

medical treatment[228], it correlates well with plaque histology[229], and is an important determinant of benefit from endarterectomy.[230]

Although CT angiography can provide some information on plaque constituents on the basis of analysis of attenuation values, factors such as plaque calcification, inability to detect plaque hemorrhage, and substantial overlap between the attenuation values of plaque components limit clinical use.[231]

B-mode ultrasound can potentially be used to characterize plaque morphology, with heterogeneous echolucency associated with vulnerability characteristics such as intraplaque hemorrhage and lipid cores compared with echo-rich plaques, which are primarily fibrous. However, limitations include reproducibility issues and inter-observer variability.[232] Transcranial Doppler ultrasound is a useful technique for the detection of silent microemboli, and absence of microemboli could indicate patients with asymptomatic carotid stenosis who are at low risk of stroke.[233] Features of vulnerable carotid plaques have also been associated with increased microemboli on transcranial Doppler ultrasound.[234]

High-resolution MRI has emerged as one of the most promising techniques for in vivo imaging of human carotid atheroma. Carotid MRI can be done by use of specific surface coils on commercial magnetic resonance machines, and, with the help of different contrast weightings, can provide excellent soft-tissue differentiation to distinguish between different plaque constituents. Several groups have validated the robustness of

MRI as a tool to qualitatively and quantitatively characterize plaque features, such as intraplaque hemorrhage or large lipid cores[235], with histology of excised carotid endarterectomy specimens as the gold standard.[236-238] Although objective precise measurement of fibrous-cap thickness is currently difficult, as these measurements (to the order of 200–500 μm) exceed the spatial resolution achieved by MRI[239], MRI can enable subjective characterization of the fibrous cap as thick or as thin or ruptured.[235, 236] MRI can also be used to classify intermediate to advanced atherosclerotic plaques accurately in vivo in accordance with the histopathological classification of the American Heart Association.[240] Moreover, with modifications to conventional sequences, magnetic resonance direct thrombus imaging (a strongly fat-suppressed T1-weighted coronal three-dimensional volume acquisition with high sensitivity for methemoglobin) has shown excellent diagnostic accuracy for detection of intraplaque hemorrhage in vivo, with sensitivity and specificity of 84%[241].

Additionally, MRI provides an ideal template for the use of more specific contrast agents that can be used to provide information about biological processes within the plaque. For example, preliminary technical reports have shown that plaque macrophages can be imaged in vivo in human carotid atheroma with MRI and with the use of macrophage-specific contrast agents, such as ultra-small particles of iron oxide.[242] Macrophages are the key cellular mediators of plaque inflammation, which is thought to be the fundamental process that underlies the initiation and progression of atherosclerosis[117]. The potential clinical use of in vivo MRI as a diagnostic tool has been well proven. In asymptomatic patients with moderate carotid stenosis, Takaya and co-

workers[243] showed that the presence of thinned or ruptured fibrous caps, intraplaque hemorrhage, or large lipid cores was associated with an increased occurrence of subsequent neurological complications over a mean follow-up period of 38 months. Case–control studies have also shown that plaques of symptomatic patients were more likely to have similar vulnerable features, as defined by MRI, than were plaques of asymptomatic patients with a similar level of carotid stenosis.[244]

Although promising and well established as a robust research tool, more clinical data from randomized controlled trials, as well as improved histological reporting methods in carotid morphology studies[244], will be necessary before plaque imaging and MRI become routinely used in clinical practice for risk stratification. MRI is also of use as a non-invasive tool to monitor the effect of drug interventions such as high-dose statins on atheroma, and might have a major role in the setting of future anti-atheroma drug development trials.[245]

Another promising research approach is the use of PET, which can be combined with CT or MRI for structural information. 18F-fluorodeoxyglucose (FDG) is a PET contrast agent that competes with glucose for uptake into metabolically active cells, where it accumulates in proportion to metabolic activity. By use of CT-FDG-PET, Rudd and colleagues[246] assessed uptake of FDG within carotid-artery atherosclerotic plaques in patients with recently symptomatic severe carotid stenosis, with substantially less uptake being shown in contralateral asymptomatic arteries. Combined use of FDG-PET with MRI is also possible to provide both structural and metabolic information about the

plaque status.[247, 248] Furthermore, 18F-FDG-PET has been used to monitor the therapeutic effect of statins on the stabilization of vulnerable atherosclerotic plaques, which is of potential use in future anti-atheroma drug trials.[173] Consecutive patients randomly assigned to receive either simvastatin or dietary management underwent FDG-PET for cancer screening and had FDG uptake in the thoracic aorta or carotid arteries[249]. Results from repeat PET examinations at 3 months showed that simvastatin, but not diet alone, attenuated plaque uptake of FDG, which correlated with increases in plasma HDL cholesterol concentration.

3.7 Characterization of intracranial atherosclerotic plaque

Arterial wall characteristics studied with spin-echo MRI with contrast[250] demonstrated definite and thick enhancement in 37% of patients with intracranial vertebral artery disease and 21% of patients with intracranial internal carotid artery disease.

There is also increasing interest in the use of intravascular ultrasonography (IVUS) in intracranial arteries[251, 252] to better understand plaque morphology and progression based on data derived from coronary arteries.[253] IVUS provides accurate real-time dynamic measurements and virtual histology maps of the plaque, and detects inflammation within plaques.[254] IVUS can also provide two-dimensional images, which are then post-processed into longitudinal three-dimensional or volume reconstructions[255] resembling angiographic images that can be viewed as three dimensional images in

longitudinal axis.

Microbubble contrast agents with specific ultrasound signal can identify adventitial microvessels such as vasa vasorum.[256] Microbubbles coated with albumin or lipid shells bind with tissue leukocytes and can identify leukocyte activation and expression of adhesion molecules in regions of inflammation within the plaque by generating an oscillatory ultrasound contrast effect.[256] Thermographic guidewires[257] can detect intra-arterial temperature increases between 0.1°C and 0.3°C detecting inflammatory plaques at high risk for rupture. Vulnerable plaques can also be identified by radiotracer such as 18Ffluorodeoxyglucose because of higher metabolic activity of the inflamed cap.[258]

Intravascular MRI has demonstrated feasibility in in-vitro models for reliably identifying plaque composition and size in arteries deep within the body[259] and can adequately visualize inner and outer plaque boundaries in all arteries.

3.8 Quantification and validation of plaque regression

The treatment with statins to promote plaque regression of intracranial stenosis is largely inferred from data in the coronary literature. High-intensity statin therapy (atorvastatin 80mg/day) compared with moderate-intensity statin therapy (pravastatin 40mg/day) for 18 months was shown to reduce atherosclerotic plaque area in the coronary arteries by IVUS in the Reversal of Atherosclerosis with Aggressive Lipid Lowering Therapy (REVERSAL) trial.[260] Similarly, high-dose rosuvastatin (40mg/day)

reduced atherosclerotic plaque volume as measured by serial IVUS in the ASTEROID trial.[261] These results correlated with a large reduction in the mean LDL cholesterol from 130.4 to 60.8mg/dl. An analysis of these and similar trials show a linear relation between LDL cholesterol reduction and plaque regression.[261] However, IVUS is not easily advanced into the intracranial circulation, which limits its application to intracranial stenosis. Therefore, new ways to assess intracranial stenosis are warranted to evaluate the efficacy of statins and other therapies that induce plaque regression.

3.8 Trends and controversies in non-invasive imaging

Although there were already many published studies dealing with non-invasive carotid imaging at the start of the 21st century, there was much controversy, even among experts, on whether non-invasive tests were accurate enough to replace DSA.[262, 263] Some authors argued that non-invasive tests were not safer than DSA if substantial misclassification of stenosis resulted in inappropriate selection of patients for surgery.

Part of the problem was that many of the available studies were undermined by factors such as poor design, inadequate sample size, and inappropriate analysis and presentation of data. As a consequence, clinicians were often confused by measures of diagnostic accuracy for non-invasive tests, which ranged from perfect to worryingly poor[264].

Imaging research often has publication bias—initial technical and early clinical

assessments of new imaging technologies tend to be excessively enthusiastic and positive, but might become less positive as use of the technology becomes more widespread outside well controlled research settings. This moderation might partly account for conflicting reports in the literature.[194] For example, before carotid endarterectomy, some centers base their decision making on the results of Doppler ultrasound alone, and several reports have advocated the safety of this approach.[265, 266] However, Johnston and co-workers found that Doppler ultrasound had a specificity of 46% for detection of only severe stenosis and that DSA would have altered the decision to proceed to surgery in up to 28% of cases when compared with Doppler ultrasound alone.[267] Similar confusing and conflicting reports can be found for both MRA[267] and CT angiography.[212, 268]

Not surprisingly, these uncertainties have resulted in a wide range of different practices between centers and between countries.[184-186, 269] Some practices rely on one Doppler ultrasound study alone, whereas others use screening with Doppler ultrasound plus a further confirmatory test such as CT angiography, MRA, DSA, or even a repeat independent Doppler ultrasound examination. Although the variability in practices might be partly due to availability of local expertise, the lack of robust evidence on the optimum imaging strategy could also be a contributing factor. Nonetheless, the fact that the routine use of DSA for carotid imaging worldwide has substantially decreased since its peak in the 1980s is undeniable.[194] In 1997, Dawson and colleagues[184] surveyed members of the Peripheral Vascular Surgery Society in the USA and found that up to 82% of respondents still used DSA as a confirmatory test after screening with Doppler ultrasound. In France, a 2002 survey showed that, although the use of DSA was declining, up to 51.5% of

centers still routinely used this technique.[186] In the UK, results from a 2005 postal survey showed that nearly 100% of centers use Doppler ultrasound as a first-line technique and, for up to 64% of centers, CT angiography or MRA were the preferred methods for confirming equivocal Doppler ultrasound results.[270]

Many centers now place emphasis on reducing the misclassification rates by use of combinations of non-invasive strategies; for example, decision making is based on Doppler ultrasound and MRA if the results are concordant, but confirmatory DSA is then used if the results are discordant.[271] Although several small studies could potentially be combined into a meta-analysis to increase precision, uniformity and high standard of reporting are essential. Such uniformity could be ensured by, for example, adhering to guidelines such as those developed by the Standards for Reporting of Diagnostic Accuracy (STARD) steering group.[272] A meta-analysis of studies published between 1980 and 2004 included 41 studies (2541 patients, 4876 arteries) that met the STARD criteria, and concluded that non-invasive tests could replace DSA if used with caution with appropriate radiological expertise and local governance.[181] Contrast-enhanced MRA was more sensitive (0.94, 95% CI 0.88–0.97) and specific (0.93, 0.89–0.96) for 70–99% stenosis than Doppler ultrasound, time-of-flight MRA, and CT angiography (sensitivities 0.89, 0.88, 0.76; specificities 0.84, 0.84, 0.94, respectively). Data for 50–69% stenosis and combinations of non-invasive tests were, however, sparse and unreliable.[194]

DSA was used in the large randomized controlled trials of carotid endarterectomy and is thus the gold standard method against which non-invasive techniques have to be

validated.[273] DSA is, however, an imperfect gold standard, as this technique is subject to a substantial amount of inter-observer and intra-observer variability.[185] Therefore, no test will ever be completely consistent with DSA, and all tests will have a level of misclassification rate associated with them. Therefore, the core of the problem is whether the misclassification rates associated with non-invasive tests outweigh the benefits of more accurate imaging with DSA. Such types of analysis have been done in the context of cost-effectiveness modeling, and such studies have confirmed that the neurological risks incurred with DSA were outweighed by the use of non-invasive tests, despite the occurrence and consequences of the associated misclassification.[274, 275]

Additionally, when the true economic effect of the use of non-invasive strategies from a societal and hospital perspective were incorporated into the analysis, routine DSA was no longer cost-effective in the assessment of carotid disease.[274] DSA, however, might still have a role in selected cases when there is disagreement between non-invasive techniques.[274] Despite the initial controversy, most centers now consider non-invasive techniques, alone or in combination, to be sufficiently accurate to replace DSA in the routine assessment of carotid disease. This belief is lent support from the recent meta-analysis of the available evidence by the Health and Technology Assessment review on carotid imaging in the UK.[276] Use of non-invasive diagnostic strategies also enable patients to receive endarterectomy more quickly, and prevent more strokes and produce greater net benefit compared with DSA.[276] The use of DSA continues to decline across the world and is mostly replaced by contrast-enhanced MRA and CT angiography. With the continuing improvement of non-invasive technology, DSA will probably soon be

done only as a prelude to carotid angioplasty and stenting, rather than as a pure diagnostic test.[194]

Another consideration is the extent to which non-invasive techniques can be used to distinguish complete occlusion from near-occlusions. A near-occlusion is a critical stenosis with characteristic reduction in the distal internal carotid artery caliber and slow flow on DSA, and is difficult to image reliably with Doppler ultrasound or time-of-flight MRA techniques. CT angiography and contrast-enhanced MRA can both be used to reliably image near-occlusions, although CT angiography is probably more robust because of its higher spatial resolution.[271, 277] Re-analysis of data from the large carotid surgery trials, however, suggests that the benefit of surgery for near-occlusions might be attenuated because these occlusions might be associated with lower rates of stroke than previously thought.[278]

Chapter 4. Treatment of atherosclerosis of craniocervical vessels

4.1 Medical treatment

4.1.1 *Management of atherogenic risk factors*

Subset analyses[279] from the WASID study demonstrated that systolic blood pressure more than 140mm Hg and cholesterol more than 200mg/dl were associated with an increased risk for stroke, myocardial infarction, or vascular death. Ischemic stroke risk increased with increasing mean systolic and diastolic blood pressures.[279] Another analysis[280] from the WASID study found that the time to the first of ischemic stroke, myocardial infarction, or vascular death was shorter among patients with metabolic syndrome during the follow-up period.

The Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial showed that in patients with recent stroke or TIA and without known coronary heart disease, high dose atorvastatin reduced the overall incidence of future strokes and cardiovascular events.[281] Based on the SPARCL trial, the AHA/AS[282] recommends statin therapy with intensive lipid-lowering effects for patients with atherosclerotic ischemic stroke or TIA and without known coronary artery disease to reduce the risk for stroke and cardiovascular events. For those patients with atherosclerotic ischemic stroke or TIA and a history of coronary artery disease, statins are recommended with a target of low-density lipoprotein (LDL)-cholesterol level of less than 100mg/dl, and an LDL-cholesterol level of less than 70mg/dl for very-high-risk patients with multiple risk factors. In view of relatively high rate of concurrent existence coronary artery disease, the Councils of the

AHA/ASA Coronary Risk Evaluation recommends routine testing for coronary artery disease in patients with carotid or other large-vessel disease.[283]

4.1.2 Prevention of intraluminal thromboembolism

The initial retrospective Warfarin–Aspirin Symptomatic Intracranial Disease (WASID) Study suggested that warfarin was more effective than aspirin in preventing recurrences.[84] However, the prospective WASID trial was stopped early owing to safety concerns about a significant increase of hemorrhagic complications in the warfarin-treated group.[77] In Hong Kong, Wong et al. compared the efficacy of a low-molecular-weight heparin (LMWH), nadroparin, with aspirin in Asian acute stroke patients with mainly intracranial large artery occlusive disease.[284] The study found no significant benefit of LMWH over aspirin, except that secondary outcome measures showed a benefit in outcome for LMWH versus aspirin on the modified Rankin scale.[284]

4.1.3 Plaque stabilization and regression

Atherosclerosis includes atherosis caused by intracellular and extracellular lipid infiltration, and sclerosis secondary to connective tissue deposition and endothelium dysfunction, leading to reduced arterial compliance.[285, 286] Cilostazol, a phosphodiesterase inhibitor, reduces restenosis rate after percutaneous coronary intervention. A Korean study[287] randomized 135 patients with acute symptomatic intracranial stenosis on aspirin 100mg/day to either cilostazol 200mg/day or placebo for 6 months and found that the

progression of intracranial stenosis on 6-month MRA was significantly less in the cilostazol group than that in the placebo group (7 vs. 29%). Regression of lesion was seen in 24% and 15% of the patients in the cilostazol and placebo groups, respectively.

4.1.4 Enhancement of collateral flow

In patients with multisegmented intracranial stenoses with no other therapeutic option, angiogenic growth factors may represent a new venue. Angiogenic growth factors can stimulate new blood vessel growth and restore perfusion in animal models of myocardial ischemia.[288] Vascular endothelial growth factor (VEGF), particularly highly diffusible VEGF121 (VEGF A), may be the best suited for cardiac proangiogenesis gene therapy.[289] In the REVASC study[289], direct intramyocardial injections of replication-deficient adenovirus-containing AdVEGF121 resulted in objective improvement in exercise-induced ischemia in patients with severe angina caused by coronary artery disease and no conventional options for revascularization. In the EUROINJECT-ONE double blind, randomized trial[290], injections of phVEGF A165 plasmid improved the stress-induced myocardial perfusion abnormalities[290] and regional wall motion, although there was no clear improvement in symptoms.[291] The Angiogenic Gene Therapy (AGENT) trial[292], which delivered an adenoviral vector containing fibroblast growth factor-4 via intracoronary infusion, failed to show statistically significant clinical benefit in the treated group, presumably because of inefficient uptake of the viral vector by the myocardium. The use of angiogenic growth factors in stroke is still investigational.

4.1.5 Recommendations on medical treatment from Professional Organizations

In patients with "noncardioembolic" ischemic stroke and TIA, the American Heart Association (AHA)/American Stroke Association (ASA) [293]recommend aspirin monotherapy, aspirin/extended-release dipyridamole combination, and clopidogrel monotherapy (rather than oral anticoagulants) as acceptable options. The aspirin/extended-release dipyridamole combination is recommended over aspirin alone. Clopidogrel may be preferentially considered over aspirin alone by direct-comparison trials and is also a reasonable alternative for patients allergic to aspirin.[293]

Medical treatment for patients with symptomatic intracranial stenosis is often unsatisfactory. In the Prospective Study of Symptomatic Atherothrombotic Intracranial Stenosis (GESICA Study)[294], despite optimal medical treatment, 38.2% of patients experienced a recurrent cerebrovascular event in the territory of the stenotic artery at 2-year follow-up (13.7% ischemic stroke and 24.5% TIAs). Recurrent cerebral ischemic events occurred in a median of 2 months following the first event.

4.2 Surgical treatment

4.2.1 Carotid Endarterectomy

After Eastott showed in 1954 that carotid surgery was possible[295], the popularity of the procedure increased and two landmark studies, NASCET and ECST, have confirmed the role of carotid endarterectomy (CEA) in patients with severe stenosis.

In the North American Symptomatic Carotid Endarterectomy Trials (NASCET) [67, 69], the 2-year cumulative risks of any ipsilateral stroke in severe symptomatic carotid stenosis >70% diameter reduction were 26% in the medical group and 9% in the surgical group. In other words, the number needed to treat to prevent one ipsilateral stroke over 2 years by carotid endarterectomy in severe symptomatic stenosis was 6. For a major or fatal ipsilateral stroke, the corresponding estimates were 13.1% and 2.5% in medical and surgical groups respectively, with an absolute risk reduction of 10.6 +/- 2.6%.

Among patients with moderate stenosis of 50 to 69%, the five-year rate of any ipsilateral stroke was 15.7% among patients treated surgically and 22.2% among those treated medically. To prevent one ipsilateral stroke with moderate stenosis during the five-year period, 15 patients would have to be treated with carotid endarterectomy. Among patients with less than 50% stenosis, the failure rate was not significantly lower in the group treated with endarterectomy (14.9%) than in the medically treated group (18.7%).

They concluded that CEA is highly beneficial to patients with recent hemispheric and retinal transient ischemic attacks or non-disabling strokes and ipsilateral high-grade stenosis (70 to 99%) of the internal carotid artery. Endarterectomy in patients with symptomatic moderate carotid stenosis of 50 to 69% yielded only a moderate reduction in the risk of stroke. Patients with stenosis of less than 50% did not benefit from surgery.

In MRC European Carotid Surgery Trial (ECST) [150], the frequency of a major stroke or death at 3 years was 26.5% in the control group and 14.9% in the surgical group. The absolute benefit from surgery was 11.6% when the stenosis was greater than about 80% in diameter. For the combined outcome of surgical complications, ipsilateral major ischemic strokes and other major strokes, there was no net benefit if the stenosis was below about 70–80% in diameter. They concluded that CEA is indicated for most patients with a recent non-disabling carotid-territory ischemic event when the symptomatic stenosis is greater than about 80%.

The problem is that ECST and NASCET used different methods for measuring carotid artery stenosis, thus confusing the issue of the relation between carotid stenosis and clinical outcomes. The ECST group used an approximation of the carotid bulb diameter as the denominator, with the measured stenosis as the numerator. The NASCET group used the distal internal carotid artery as its reference denominator. For example, 75% stenosis in ECST is equivalent to approximately 50% stenosis by the NASCET criteria[296].

A meta-analysis[273] has shown that CEA increases the 5-year risk of ipsilateral ischemic stroke in patients with less than 30% stenosis, has no effect in patients with 30–49% stenosis, has marginal benefit in those with 50–69% stenosis, and is highly beneficial in those with 70% stenosis or greater without near-occlusion (if the surgical risk is low). There was a trend towards benefit from surgery in patients with near-total occlusion at 2 years' follow-up but no benefit at 5 years.

4.2.2 Carotid Artery Stenting

As compared with endarterectomy, stenting avoids the need for general anesthesia and an incision in the neck that could lead to nerve injury and wound complications. The costs may be less than those of surgery, mainly because the hospital stay is shorter. Several trials have done to compare the efficacy and safety of carotid artery stenting with carotid endarterectomy.

4.2.2.1 CREST study

The multicenter randomized Stenting versus Endarterectomy for Treatment of Carotid-Artery Stenosis study (CREST)[297] included symptomatic patients with carotid stenosis greater than 50% and asymptomatic patients with stenosis greater than 60% (NASCET criteria). Two thousand five hundred and two patients were randomized between stenting and surgery with a median follow-up period of 2.5 years. There was no significant difference in the estimated 4-year rates of the primary end point between the stenting group and the endarterectomy group. There was no differential treatment effect with regard to the primary end point according to symptomatic status or sex. The 4-year rate of stroke or death was 6.4% with stenting and 4.7% with endarterectomy; the rates among symptomatic patients were 8.0% and 6.4%, and the rates among asymptomatic patients were 4.5% and 2.7%, respectively. Peri-procedural rates of individual components of the end points differed between the stenting group and the endarterectomy group: for death (0.7% vs. 0.3%, P = 0.18), for stroke (4.1% vs. 2.3%, P = 0.01), and for

myocardial infarction (1.1% vs. 2.3%, P = 0.03). After this period, the incidences of ipsilateral stroke with stenting and with endarterectomy were similarly low (2.0% and 2.4%, respectively; P = 0.85).

The study concluded that among patients with symptomatic or asymptomatic carotid stenosis, the risk of the composite primary outcome of stroke, myocardial infarction, or death did not differ significantly in the group undergoing carotid-artery stenting and the group undergoing carotid endarterectomy. During the peri-procedural period, there was a higher risk of stroke with stenting and a higher risk of myocardial infarction with endarterectomy.

4.2.2.2 EVA-3S study

The multicenter randomized Endarterectomy versus Angioplasty in patients with Severe carotid Stenosis Study (EVA-3S) included symptomatic patients with carotid stenosis greater than 70% (NASCET criteria). [298] Later the threshold was reduced to more than 60%. Symptoms had to have occurred within 120 days before randomization. Five hundred and twenty-seven patients were randomized between stenting and surgery. The trial was put on hold 3 years after the start of randomization for a short period of time, and the use of cerebral protection devices was then made mandatory. The 30-day risk of any stroke or death was significantly higher after stenting (9.6%) than after endarterectomy (3.9%) and 6 months after treatment was 11.7% versus 6.1%, respectively. It was stopped early for reasons of both safety and futility.

4.2.2.3 SAPPHIRE trial

The Stenting and Angioplasty with Protection in Patients at High Risk for Endarterectomy (SAPPHIRE) trial included only patients with a high surgical risk (334 patients).[299] The eligible degree of stenosis depended on whether a patient was symptomatic (more than 60%) or asymptomatic (more than 80%). The degree of stenosis was based on ultrasound flow velocity criteria. The trial used cerebral neuroprotection devices in the stenting group. The 30-day incidence of stroke after stenting was 3.6%. The cumulative incidence of a major cardiovascular event at 1 year after the procedure was lower in the stenting group (12.2% versus 20.1%). The trial was terminated early because of a slowdown in recruitment. Most patients (70%) included in the SAPPHIRE trial had asymptomatic stenosis, which carries a lower risk of stroke during carotid repair than does symptomatic stenosis.[300, 301] Subsequently, it emerged that SAPPHIRE's Principle Investigator received undeclared royalties from sales of the protection device used in the trial.[302]

4.2.2.4 SPACE trial

The multicenter Stent-Protected Angioplasty versus Carotid Endarterectomy in symptomatic patients (SPACE) trial is a multinational, prospective, randomized study to test the hypothesis that carotid artery stenting is not inferior to carotid endarterectomy for treating patients with severe symptomatic carotid artery stenosis. It enrolled only patients with symptomatic carotid stenosis greater 70% (measured using NASCET criteria). One

thousand and two hundreds patients were randomized between stenting and surgery. It did not prove non-inferiority of carotid artery stenting compared with carotid endarterectomy for the 30-day complication rate. However, the study showed that ipsilateral ischemic strokes up to 2 years after the procedure and any peri-procedural stroke or death do not differ between the carotid artery stenting and the carotid endarterectomy groups. Recurrent stenosis of 70% or more is significantly more frequent in the carotid artery stenting group compared with the carotid endarterectomy group, with a life-table estimate of 10.7% versus 4.6% (p=0.0009).[303, 304]

4.2.3 Carotid endarterectomy vs. stenting

A recent meta-analysis of 11 randomized trials performed through 2009 (not including CREST) showed that carotid endarterectomy was superior to carotid-artery stenting with regard to short term outcomes but not longer-term outcomes.[305] Clearly, more long-term data are needed before a full appreciation of the relative risks and benefits of the two procedures can be made.[306] In addition, more information concerning the intriguing effect of age, which may indicate greater equipoise in the choice of interventions in younger patients, is needed. Local complications, particularly cranial-nerve palsies, are mainly associated with carotid endarterectomy. Though it appears that the increased risk of stroke with carotid-artery stenting is offset by an increased risk of myocardial infarction with carotid endarterectomy, stroke has greater long term health consequences than myocardial infarction. The risk–benefit issue is complex and should be discussed with patients.

Cerebral-protection devices have been developed to reduce embolization of plaque fragments during stenting. Uncontrolled studies [300, 307, 308]suggest that these devices may reduce the risk of procedural stroke. However, the protection devices may cause additional adverse events in some patients and increase the costs. Larger randomized control trials are needed to determine its efficacy.

In conclusion, stenting carries a significant risk of stroke and local complications, and the long-term efficacy of this technique is not well known. Long-term follow- up study and a larger number of patients are required to provide definite answers about the risk–benefit profile of stenting as compared with endarterectomy.

4.2.4 Extracranial-intracranial arterial bypass for intracranial stenosis

The WASID trial[77] and subset analyses[309] demonstrated a relatively high risk for stroke among certain medically treated subgroups of patients with intracranial stenosis. The high rate of recurrent stroke despite antithrombotic agents highlights the need to consider surgical treatment as a therapeutic option.[310-312]

Though there was tremendous enthusiasm for the extracranial–intracranial bypass initially, it was quickly tempered by the results of the International Extracranial–Intracranial (EC–IC) Bypass trial[313] in 1985. Initially, the trial demonstrated relatively acceptable rates or perioperative mortality and major stroke morbidity, 0.6% and 2.5%, respectively with bypass patency rates of 96%. [314] However, with long-term follow-up, it was determined that those patients who were operated on had significantly higher rates of

fatal and nonfatal strokes than those patients who were treated medically.[314] In short, the trial demonstrated that EC–IC bypass resulted in no benefit with regards to stroke prevention over best medical management. This immediately led to a dramatic decrease in the use of the EC–IC bypass.[315] Further analysis of the EC–IC bypass study, however, revealed considerable deficiencies with regard to study design and implementation.[315]

Recent studies have demonstrated that PET measurement of oxygen extraction fraction (OEF) can identify patients at high risk for recurrent stroke based on assessment of hemodynamic compromise.[316-318] Specifically, the 2-year rate of ipsilateral ischemic stroke is 5% in patients with a normal OEF and 26% in patients with increased OEF distal to a symptomatically occluded carotid artery. Additionally, superficial temporal to middle cerebral artery (STA–MCA) anatomizes have been shown to return OEF values to normal on PET studies.[313]

The Carotid Occlusion Surgery Study (COSS) trial, initiated to assess the relationship between cerebral hemodynamics as defined by OEF and cognitive function in patients undergoing treatment for unilateral carotid artery occlusion with EC-IC bypass. [317-319] The study was stopped prematurely in 2011 because of slow recruitment and because of a very low incidence of ipsilateral symptomatic ischemic events in patients assigned to the medical arm. An unrealistic high number of patients would have been required to show any difference between the 2 groups[320].

Additionally, the Japanese EC–IC Bypass Trial (JET study) is also in progress to

determine the ability of STA–MCA bypass to prevent stroke caused specifically by intracranial stenosis, based on evaluations of hemodynamic ischemia.[321, 322] The interim analyses of the JET study suggest that in those patients with intracranial stenosis, previous stroke, and hemodynamic ischemia, surgical intervention by way of EC–IC bypass is superior to medical management in terms of stroke prevention, although final results are pending.[322]

While new clinical trials are underway to reassess the efficacy of EC–IC bypass within select populations, new operative techniques have also been developed to reduce the rates of surgical morbidity. Specifically, near-infrared indocyanine green (ICG) video angiography has been used to evaluate intraoperative vascular patency for both intracranial aneurysm and bypass surgeries.[323, 324] In each of these cases, this technology has proven to be simple, reliable, and effective in identifying significant intraoperative vascular occlusions and stenoses that required immediate surgical intervention.[323, 324]

In addition to ICG video angiography, the excimer laser-assisted nonocclusive anastomosis (ELANA) technique represents a useful novel bypass surgical technique. Though the main utility of this technique, thus far, is for complex intracranial aneurysms, it could also have some utility in atherosclerotic intracranial disease. Essentially, the ELANA technique allows for the creation of high-flow EC–IC bypasses without the use of temporary occlusion. This is achieved through the use of a suction laser catheter which produces an arteriotomy only after the anastomosis is created.[325]

The final results of both the COSS trial and the JET study have yet to be published; however, the principles upon which they are founded make them attractive potential solutions to the current shortcomings in the medical management of both symptomatic internal carotid artery occlusion and intracranial stenosis, respectively. The original EC–IC bypass trial demonstrated no benefit in terms of stroke reduction in the surgical arm as compared to the medical arm, with respect to intracranial stenosis. However, critical analysis of the EC–IC bypass trial reveals several limitations, including poor patient selection[313]. It is postulated that because the JET study selects for patients with high risk lesions (as measured by hemodynamic ischemia), this study will be able to identify a particular subgroup of patients with intracranial stenosis that will benefit from surgical intervention. Moreover, it is precisely that group of patients who are at highest risk for recurrent stroke, and preliminary JET study results do suggest lower rates of recurrent stroke in the surgical arm as compared to the medical arm.[322]

Additionally, although the COSS trial does not specifically select for patients with intracranial versus extracranial internal carotid artery occlusion, its examination of the utility of EC–IC bypass in the context of increased OEF parallels the work of the JET study. Furthermore, once the results of the COSS trial are published, subgroup analyses may shed considerable light on the specific treatment of intracranial disease, as opposed to general internal carotid artery occlusion.[313]

4.2.5 Endovascular treatment of intracranial stenosis

The increasing enthusiasm for intracranial intervention was dampened somehow by the neurologic peri-procedural complication rate, estimated at 5.3 to 28%.[326-330] In the SSYLVIA multicenter trial[329], a similar stroke recurrence after intracranial stenting was reported, with a rate of 7.3% at 1 year. This raises the important issue of whether the proposed treatment of intracranial stenting improves the natural history of this disease. In the past few years, the morbidity and mortality associated with intracranial angioplasty and stenting have decreased with the development of new intracranial specific devices. In 2005, the Food and Drug Administration approved the Gateway balloon/Wingspan stent system (Boston Scientific, Watertown, MA) under a Humanitarian Device Exemption providing the first "on-label" treatment for treatment of intracranial stenosis.[331]

Cruz-Flores and Diamond[332] reported a rate of 8% perioperative stroke, 3% perioperative death, 10% perioperative stroke or death, and 10% other perioperative complications (such as groin hematoma and arterial dissection) based on a systematic review of 79 studies (1,999 cases of intracranial stenosis). In studies with at least 1 year follow-up, the 1 year risk of stroke or death was estimated at 6%. The self-expanding nitinol Wingspan Stent System was approved based on a 45-patient Wingspan Humanitarian Device Exemption Safety Study with intracranial stenosis more than 50%[331]. The mean severity of angiographic stenosis was reduced from 75 to 32% in 44 treated patients (lesion could not be traversed in 1 patient). The study reported a procedural success rate of 98% and a 30-day rate of death or ipsilateral stroke of 4% after procedure. Among the 43 patients with 6-month follow-up, the rate of death or ipsilateral

stroke was 7.0%. Further lesion reduction was observed in 24 of the 40 patients who underwent follow-up DSA at 6 months.

In the US Wingspan registry[333], intra-arterial stenting treatment resulted in successful treatment in 99% of 78 patients. There were 5 (6%) major periprocedural neurological complications (4 deaths) within 30 days. Follow-up imaging[334] demonstrated in-stent restenosis in 29 (30%) patients (more frequent within the anterior circulation); 8 were symptomatic (4 with stroke, 4 with TIA), and 15 required retreatment. In the National Institutes of Health multicenter Wingspan IAS registry study[335], technical success rate was 97% (less than 50% residual stenosis) among 129 patients with symptomatic more than 70% intracranial stenosis. The frequency of any stroke or death within 30 days or ipsilateral stroke beyond 30 days was 14% at 6 months. The frequency of more than 50% restenosis on follow-up DSA was 25% in 52 patients.

The Pharos Vitesse stent (Micrus Endovascular Corporation, San Jose, California, USA) is preparing to join the armamentarium of treatments for intracranial stenosis.[336] The balloon-expandable stent has been assessed in a prospective, single-center German case series of patients with symptomatic intracranial stenosis of 50% or more[337]. Of the 21 patients treated in the study, seven received treatment with the stent for the purpose of recanalization during acute stroke. One patient did not meet technical success criteria when the stent could not be placed, and a second patient failed to meet treatment success criteria with a residual stenosis of more than 50%. Two strokes (9.5%) occurred within the first 30 days, one in a patient with subacute stent thrombosis associated with

discontinuation of aspirin and the other a pontine infarct occurring in the setting of a basilar stent. Two of the patients treated during their acute ischemic events had recurrent strokes, one of which was ipsilateral (4.8%). This ipsilateral stroke was due to stent restenosis at 17 months. An additional patient died of unknown causes at 3 months. The Pharos Vitesse stent has received the Conformite Europeene mark authorization for commercial distribution in the European Union, and the company has submitted an investigational device exemption application to the FDA for the prospective, randomized Vitesse Intracranial Stent Study for Ischemic Therapy (VISSIT).[336]

The Stenting vs. Aggressive Medical Management for Prevention Recurrent Stroke in Intracranial Stenosis (SAMMPRIS) trial , initiated to compare intensive medical therapy alone to intracranial angioplasty and stenting combined with intensive medical therapy in patients with symptomatic stenosis (70-99 percent) of a major intracranial artery[338], has been halted. The trial was stopped by the National Institutes of Health (NINDS) after the researchers reported that five stroke-related deaths had occurred within the 30-day enrollment in the stenting arm. In fact, the most recent review by the Data Safety monitoring Board found that 14 percent of the patients within the angioplasty and stenting group experienced a stroke or died within the first 30 days of enrollment. In comparison, this rate of stroke or death in the patient arm that was medically managed was 5.8 percent. The SAMMPRIS committee, along with the NINDS and Data Safety Monitoring Board, has determined that medical therapy alone is superior to angioplasty with stenting for these patients.

The most important factor that prompted the early termination of the SAMMPRIS trial was the unexpectedly high complication rate in the stenting arm. Two studies concerning the safety of intracranial stenting were published after SAMMPRIS. Fiorella et al [339] published the largest series of Wingspan in the United States to treat >50% symptomatic stenoses. The immediate perioperative stroke and death rates were 5.7% and 2.5%, respectively. Over a mean follow-up period of 14.2 months, the primary end point (any perioperative stroke or death and any ipsilateral stroke thereafter) was noted in 15.7% of patients or approximately 13.2%/year. Seventy-six percent of the ipsilateral strokes occurred within the first 6 months with no events beyond 12 months.

Jiang and colleagues[340] reported on 100 consecutive patients from a single center with a 99% stent placement success rate. The 30-day perioperative stroke and death rate of 5%. Over a mean follow-up of 21.4+/- 8.2 months, there were an additional 4 ipsilateral strokes giving a cumulative primary event rate of 9% or approximately 5.04%/year. The secondary event rate (non-ipsilateral stroke, intracerebral hemorrhage, non-stroke mortality, TIA, or target lesion restenosis) was 15%. Angiographic follow-up was available in 44% of the patients at a mean of 8.6 months, 26.7% had an in-stent stenosis with approximately 43% symptomatic.

There is another ongoing study that is quite similar to the SAMMPRIS trial in Hong Kong. The Early Stent-assisted Angioplasty in Symptomatic Intracranial Stenosis (ESASIS) trial[341] aims to study the benefit of stenting in reducing the risk of ipsilateral stroke. With the announcement of the safety concern and early termination of the

SAMMPRIS trial, the Data Safety Monitoring Board of ESASIS study reviewed the 30-day safety data (combined stroke and death) for 77 patients randomized and found the safety data for the medical group is comparable to the 6% reported for the medical arm of the SAMMPRIS study, whereas the safety data for the stenting group is not comparable to the 14% reported for the stent arm of the SAMMPRIS study and they recommended that the ESASIS trial continues recruitment but with close monitoring of safety.

The jury is still out on the benefit/risk ratio of angioplasty and stenting for intracranial arterial stenosis and we are waiting for more evidence to support the use of intracranial stenting in patients with significant intracranial stenosis.

4.2.5.1 Periprocedural Management after intracranial stenting

A meta-analysis of 25 trials comparing antiplatelet therapies after percutaneous intervention (PCI) demonstrated that clopidogrel and aspirin combination are superior to aspirin alone or warfarin and aspirin combination in preventing major cardiac adverse events.[342] Other medications before stent placement such as cilostazol and dipyridamole have shown some benefit over single agent alone in preventing thrombotic events after stent placement.[342] Based on cardiac data, practitioners are continuing dual antiplatelet agents for a total of 3 months after the intracranial stenting, although the range of 4 to 12 weeks is common and accepted practice. Clopidogrel 75mg daily with aspirin (81–325mg) for at least 72 hours before the intervention is recommended. If not feasible, loading dose of 300mg within 6 and 24 hours or 600mg within 6 hours is reasonable[343]. In patients

allergic to clopidogrel, ticlopidine is a reasonable alternative at 250mg twice daily or a 500mg loading dose within 24 hours of the procedure. Placing a drug eluting stent (DES) during PCI requires 1 year of dual antiplatelet therapy[342], which is reasonable in patients with intracranial DES placement.

4.2.5.2 Risks of intracranial stent restenosis

Relatively high restenosis rates for these uncoated intracranial stents were found in the SSYLVIA trial, 35%, and the two registries, 25 and 29.7%, despite the lower rate reported in the original Wingspan study.[64, 329, 333-335] The identification of risks for restenosis could help with patient selection or even the prevention of restenosis. Using a regression model to query a list of possible risk factors in the 37 patients with intracranial stenosis treated with the Neurolink stent who had 6-month angiography, diabetes, postprocedure stenosis at least 30%, and smaller pretreatment vessel diameter contributed to the prediction of stenosis at 6 months.[329]

Comparison of clinical risk factors in 78 patients with and without restenosis in the US Wingspan Registry suggested that smoking might be associated with a weak trend toward higher restenosis rates, but other risk factors, including diabetes, were not important contributors.[334] Although not noted in previous studies of intracranial stents, the US Wingspan Registry found that restenosis occurred more frequently in the anterior circulation (42%) than in the posterior circulation (13%).[334] Effects of lesion location and age on restenosis were further explored by this registry group.[344] In the population of

patients 55 years of age or younger, 79.5% of treated lesions were in the anterior circulation, whereas in those older than 55 years of age, 50.5% were in the anterior circulation. Follow-up was available in a limited sample of 93 lesions, of which 33.3% were in younger patients. Restenosis developed in 45.2% of lesions in the younger group and in 24.2% of the older patients. The majority of the restenoses occurring in the younger group was in the anterior circulation, 50% vs. 20% in the posterior circulation. Lesions in the supraclinoid segment of the internal carotid artery seemed to be particularly prone to restenosis, occurring in 88.9% at imaging follow-up, half of which were symptomatic. In the older group, restenoses occurred in 31% of anterior circulation stents and in 18.2% of posterior circulation stents. Although intriguing, the results are limited by the incomplete imaging follow-up of all patients and by the use of modalities other than angiography in some cases.[344]

Within the cardiac literature, significant resources have been devoted to the development of drug-eluting stents (DES), which are coated with either sirolimus or paclitaxel.[345] These DES have reportedly reduced the rate of short-term in-stent restenosis from approximately 30% to less than 10%, as compared to bare metal stents.[345] However, it has been demonstrated that DES are associated with incomplete intimal healing and significantly increased rates of late stent thrombosis, such that patients must stay on dual antiplatelet therapy for several years following stent placement, an important consideration in patients with intracranial disease.[313] Recently, the neurosurgical experience with DES has been published and it is, thus far, concordant with the cardiac literature. That is, in short-term and midterm analyses (<12 months), rates of in-stent

restenosis with DES were significantly reduced as compared to bare metal stents.[346, 347] However, the long-term neurosurgical experience with DES has yet to be published, as the field is still in its relative infancy and the true safety and efficacy of neurosurgical DES cannot be fully evaluated until this long-term data is available. Thus, though having the endovascular option is exciting, an appropriate level of caution should be taken prior to the use of intracranial stents[313]. Long-term studies that assess the ability of endovascular therapy to reduce overall stroke risk are needed.

4.2.5.3 Primary angioplasty vs. stent placement

In a multicenter review[348], there was no difference in follow-up survival between the 95 angioplasty-treated versus the 98 stent-treated groups after adjusting for age, gender, and center. The stroke-free survival at 2 years for the angioplasty group and the stent-treated group was 92 % and 89%, respectively. Significant restenosis-free survival at 12 months was 68% for the angioplasty-treated patients and 64% for the stent-treated patients with DSA follow-up. In a systematic review[349] of 69 studies (1,027 primary angioplasty-treated patients and 1,291 stent-treated patients), 91 and 104 patients experienced stroke, death, or both in the angioplasty-treated and stent-treated groups, respectively, during the 1-month period. A greater rate of 1-year stroke and death was observed in angioplasty-treated patients (20%) compared with stent-treated patients (14%). The pooled restenosis rate was 14 and 11% in the angioplasty-treated and stent-treated groups, respectively.

4.2.6 Recommendations from professional organizations

Endovascular therapy of intracranial stenosis has increased in prevalence since the introduction of the Wingspan stenting system. Preliminary data suggest high rates of initial technical success; however, rates of peri-procedural morbidity and mortality, including in-stent restenosis, are not insignificant. Furthermore, data regarding impact on overall stroke risk are currently unavailable due to the lack of long-term follow-up. Because of the preliminary nature of this research and significant peri-procedural complications, these interventions should be reserved for high risk patients as previously identified[313]. Long-term outcome studies on endovascular therapies should be conducted to fully assess the utility of this treatment strategy in intracranial stenosis.

The American Society of Interventional and Therapeutic Neuroradiology, Society of Interventional Radiology, and American Society of Neuroradiology[350] recommend that intracranial stenting should be offered to symptomatic patients with intracranial stenoses more than 50% who have not responded favorably to medical therapy. The Brain Attack Coalition[351] considers intracranial stenting an optional component for a comprehensive stroke center, although there are selected cases in which such techniques may be of value.

The AHA/ASA guidelines[352] consider intracranial stenting of uncertain benefit and therefore investigational in patients with hemodynamically significant intracranial stenosis who have symptoms despite medical therapies.

The Practice Guidelines Committee of the American Society of Neuroimaging and

the Society of Vascular and Interventional Neurology[353] recommend for physicians requesting privileges for performing intracranial stenting, a minimum of 50 procedures requiring microcatheter and microwire placement in intracranial vessels beyond intracranial internal carotid artery or vertebral artery and at least 25 intracranial stenting performed under supervision in addition to meeting the training period and overall case volume requirements set by the Accredited Council of Graduation Medical Education for endovascular surgical neuroradiology residency education.

4.2.7 Trials in progress

There are two randomized medical management trials using Cilostazol, which is a phosphodiesterase type 3 inhibitor, to look at progression of intracranial stenosis as the primary outcome measure. The first is the Trial of Cilostazol in Symptomatic intracranial Arterial Stenosis II in Asia, which is a double-blind, randomized trial comparing aspirin (75–150mg per day) in combination with cilostazol (100mg twice a day) with a combination of clopidogrel (75mg per day) in patients with significant MCA or basilar artery stenosis[354] . The second is the open-label Cilostazol–Aspirin Therapy Against Recurrent Stroke With Intracranial Artery Stenosis in Japan, comparing open-label aspirin and cilostazol with aspirin alone in patients with symptomatic 50–99% stenosis of the supraclinoid internal carotid artery, MCA, or basilar artery.[355] The trials will help to optimize medical and surgical management of patients with intracranial stenosis.

PART II STUDIES ON CONCURRENT STENOSES

Chapter 5 Use of MRA to Predict Long-term Outcomes of Ischemic Stroke Patients with Concurrent Stenoses in Hong Kong (Study 1)

5.1 Abstract

Background and Purpose: To determine the long-term outcomes of ischemic stroke patients with concurrent intracranial and extracranial atherosclerosis using MRA.

Method: A prospective cohort of patients in Hong Kong with acute ischemic stroke was studied with magnetic resonance angiography of brain and carotid duplex. All patients were followed up regularly for the development of recurrent stroke, cardiac event, or death.

Results: Totally 343 patients with acute ischemic stroke were included, of whom 104 (30%) had concurrent intracranial and extracranial lesions. The follow-up period was up to 76 months (mean 44.5 months). Overall, fifty-three patients (15.5%) died of any cause and 91 patients (26.5%) had a further non-fatal vascular event. The overall 5-year cumulative rates of mortality, restroke and poor outcomes (combined death and further vascular events) were 18%, 27% and 37% respectively. In patients with concurrent lesions, these rates were 31%, 41% and 51% respectively. The corresponding rates were 13%, 22%, and 31% in patients without concurrent lesions. The risks were highest in the first year after stroke. More deaths (log rank, 16.3; p=0.0001), restrokes (log rank, 9.71; p=0.002) and poor outcomes (log rank, 13.87; p=0.0001) were found among patients with concurrent lesions. The presence of concurrent vascular lesions, advanced age, smoking,

hyperlipidemia and previous history of stroke were independent predictors of poor outcomes.

Conclusion: The long-term prognosis of ischemic stroke patients with concurrent atherosclerosis of intracranial and extracranial vessels is poor. They are at high risks of further vascular event or death.

5.2 Introduction

Concurrent atherosclerosis of intracranial and extracranial vessels is common in Asian.[2, 5-8] However, the long-term prognosis of this group of patients is unclear. Given the difference in vessel involvement, concurrent stenoses may have different prognosis from pure intracranial or extracranial stenosis. Although conventional contrast angiography provides excellent visualization of the craniocervical vasculature, the invasive nature of the procedure and the risk of perioperative complications [356] hinder its widespread use. Magnetic resonance angiography (MRA) is a safe and reliable modality in studying intracranial circulation.[221, 357] It is particularly useful in Chinese as about 25% of the patient has poor temporal windows in TCD.[5] The objective of this study is to determine the long-term outcomes of patients with concurrent atherosclerosis of intracranial and extracranial vessels after acute stroke using MRA.

5.3 Methods

5.3.1Study design and patients

This is a prospective cohort study conducted at the Prince of Wales Hospital in Hong Kong. The study was approved by local ethnics committee. We recruited consecutive patients admitted with acute cerebral ischemia, including transient ischemic attack (TIA) and cerebral infarct within 7 days of symptom onset during June 2001 to December 2003. We excluded patients less than 18 years old, those who had atrial fibrillation, intracranial hemorrhage, vascular malformations, active cancer, myocardial

infarction, liver and renal failure, prothrombotic tendency such as underlying active lupus disease, antiphospholipid syndrome, factor C/S deficiency, post-interventions including carotid endarterectomy (CEA) and stenting and those who were pregnant. Those who are unfit for MRI study because of unstable medical conditions and claustrophobia were excluded.

All patients underwent computer tomography (CT) and magnetic resonance imaging (MRI) of brain to establish the diagnosis of acute ischemic stroke, and vascular imaging including MRA of brain and carotid duplex to look for vascular stenosis. Baseline data, including age, sex, medical history, and physical examination, were collected on admission. Scoring of pre-stroke disability was estimated with the modified Rankin Scale (mRS), and severity of neurological deficits of index stroke was assessed with the National Institutes of Health Stroke Scale (NIHSS). Vascular risk factors were noted, in particular any history of smoking, hypertension, diabetes mellitus, ischemic heart disease and previous TIA or stroke. Blood biochemistry, full blood count, ECG, and chest x-ray were checked routinely.

5.3.2 MRI and MRA

All patients were scanned within one week of symptom onset with a Siemens Sonata (Erlanger, Germany) with a head coil. The three-dimensional time-of-flight images were acquired with a repetition time of 40 ms, time to echo of 7.15 ms, flip angle of 25°, 20-cm field of view, 192x512 acquisition matrixes, and one signal average for a

total imaging time of 5 minutes 35 seconds. The MRI and MRA films were read by experienced neuroradiologists blinded to the results of the clinical examination. Acute infarcts on Diffusion-weighted imaging (DWI) were diagnosed when these lesions were shown to be hyperintense on the DWI integrated for the 3 diffusion sensitivity directions and hypointense on the apparent diffusion coefficient map. For images showing motion artifact in 1 diffusion sensitivity direction, infarct or subacute infarct was diagnosed only if the lesion showed all of the following features: (1) it had a much higher signal than on the image map with b=0, (2) it was not caused by normal anisotropy of diffusion or artifact, and (3) it was seen on the DWIs in both of the remaining orthogonal diffusion sensitivity directions. Symptomatic infarct was defined as infarct in the vascular territory of the stenotic vessel. Intracranial stenosis was graded according to the following criteria: grade 1, normal or mild stenosis (0% to 29% diameter stenosis); grade 2, moderate stenosis (30% to 69% diameter stenosis); grade 3, severe stenosis (70% to 100% diameter stenosis). The percentage of stenosis of MCA and other intracranial vessels was measured by visual inspection by investigator (W. Lam) who was blinded to the clinical findings.

5.3.3 Carotid Duplex

For the Duplex ultrasound examination of the extracranial carotid arteries, we used a Philips SD800 ultrasound machine and a 7.5-MHz transducer. The diagnostic criteria for 70% stenosis of the internal carotid artery required a peak systolic velocity ratio of 2.4. The above diagnostic criteria in the neurovascular laboratory were based on laboratory

references, which had a quality assurance program with supplementary angiographic studies.

Concurrent stenoses were defined as presence of more than 30% stenoses in both extracranial and intracranial vessels, including lesions in the same vascular territories (tandem lesions) and lesions in different vascular territories (non-tandem lesions).

5.3.4 Outcome measures

All survivors were followed up regularly by telephone or personal interviews, in addition to routine medical outpatient clinic attendance. If patients could not be reached, we found their medical records and tried to contact their relatives for more information about the patient's condition. All telephone and personal interviews were performed by a stroke specialty nurse using a standardized questionnaire.

The specified outcomes were the occurrence of a further vascular event (including TIA, stroke, or acute coronary syndrome) or death. Poor outcome was defined as the occurrence of either death or a further vascular event. If death and a further vascular event were recorded as occurring simultaneously, one event was categorized into the analysis. The nature of the vascular event and the cause of death were based on medical records and death certificates when possible. Recurrent events were based on the diagnosis on the discharge summary according to the treating physician. The definite and detailed description of the cause of death by the patient's relative was used if the cause

could not be obtained by other means. Obscure or indefinite information about cause of death was classified as unknown cause.

5.3.5 Statistical analysis

All data were entered into the SPSS software (version 13.0 for Windows) for storage and analysis. Statistical significance was considered at p =0.05 and was 2-sided. In the present study, age was recorded as continuous variables. Sex, vascular risk factors, presence of symptomatic stroke and end points of long-term outcome were used as categorical variables. In Univariate analysis, independent-sample t test and X^2 test or Fisher's exact test was used. In analysis of predictors of the long-term clinical outcome, Cox proportional-hazards regression function was used to estimate impact in terms of risk ratios of possible determinants of survival and further vascular events, taking the time variable into consideration. Hazard ratio (HR), 95% confidence interval (CI), and probability value were observed. In survival analyses, the Kaplan-Meier product-limit method was used to estimate survival condition. The log-rank test was used to compare rate estimates.

5.4 Results

From June 2001 to December 2003, a total of 343 consecutive patients were recruited into the study. Among them, 108 (32%) had normal vessels, 76 (22%) had

intracranial stenosis only, 55 (16%) had extracranial stenosis only, 83 (24%) had concurrent intracranial stenosis and <70% extracranial stenosis, 21 (6%) had concurrent intracranial stenosis and >70% extracranial stenosis. In the group with intracranial stenosis only, 109 vessels were involved. Twenty-four vessels (22%) had mild stenosis, 30 (28%) had moderate stenosis and 55 (50%) had severe stenosis. In patients with concurrent intracranial and extracranial lesions, 154 intracranial vessels were involved. Thirty-two vessels (21%) had mild stenosis, 49 (32%) had moderate stenosis and 73(47%) had severe stenosis. The baseline characteristics, stroke severity, use of anticoagulation during hospitalization, use of statins, anti-thrombotics, anti-hypertensives and warfarin after index stroke were summarized in Table 5.1.

5.4.1 Mortality

We followed up this cohort for a mean period of 44.5 months (up to 76 months) after stroke onset. A total of 53 patients (15.5%) died of any cause and 91 patients (26.5%) had a non-fatal vascular event as shown in Table 5.2. The 5-year mortality was significantly higher in patient with concurrent vascular lesions than those without concurrent lesions (Figure 5.1). The patients with concurrent lesions and more than 70% extracranial stenosis had the highest mortality (Table 5.3). In Cox proportional-hazard regression model, the adjusted hazard ratio for death in the presence of concurrent vascular lesions was 2.53(95% CI 1.43 to 4.51, p=0.002). Other independent risk factors of long-term mortality were advanced age, hyperlipidemia and smoking.

5.4.2 Occurrence of further vascular event

In the 343 patients, further vascular events occurred in 106 patients (31%). These included 84 TIAs/strokes and 22 acute coronary syndromes. The cumulative risks of cerebrovascular event were shown in Table 3. The risk was significantly higher in patients with concurrent vascular lesions (log rank, 9.71; p=0.002) and those with a previous history of stroke (log rank, 14.12; p=0.0001), hyperlipidemia (log rank, 6.69; p=0.01), and advanced age (log rank, 7.82; p=0.005). In a Cox proportional-hazards regression model, the presence of concurrent vascular lesions, advanced age, hyperlipidemia, smoking and previous history of stroke were independent predictors of recurrent cerebrovascular event.

5.4.3 Combined poor outcomes

Both death and further vascular event represent poor outcomes after stroke. When these were combined, the cumulative rates were shown in Table 3. The occurrence of poor outcomes was significantly higher in patients with concurrent vascular lesions (log rank, 13.87; p=0.0001) and those of male sex (log rank, 3.88; p=0.04), history of previous stroke (log rank, 10.49; p=0.001), hyperlipidemia (log rank, 11.81; p=0.001), and advanced age (log rank, 14.05; p=0.0001). In Cox proportional-hazards regression model, the presence of concurrent vascular lesions, advanced age, hyperlipidemia, smoking and previous history of stroke were independent predictors of combined poor outcomes.

5.4.4 Effect of multiple intracranial stenoses in patients with concurrent lesions

Among 104 patients with concurrent lesions, 71 had stenosis of one intracranial vessel and 33 had stenosis of 2 or more intracranial vessels. The mortality was significantly higher in patients with multiple intracranial stenoses than those with 1 intracranial vessel involvement (p= 0.022).

5.4.5 Presence of symptomatic stroke in the stenotic vascular territory

Among 131 patients with either intracranial or extracranial stenosis only, 96 (73.3%) had symptomatic stroke while 94 out of 104 (90.4%) patients with concurrent stenoses had symptomatic stroke (p=0.001).

5.5 Discussion

Racial differences in the distribution of cerebrovascular occlusive disease are well documented. Extracranial stenosis is more common in Caucasian while intracranial stenosis is more common in Asian, Hispanic and African-American[1-4]. The prevalence of asymptomatic carotid stenosis in the white population was about 2 to 8% in the middle age and elderly general population[358] and the prevalence of asymptomatic intracranial stenosis in similar population in China was about 7 %[30]. The frequency of intracranial atherosclerosis among patients with stroke and TIA is 40 to 50% in Chinese populations[31] and around 8% in North America. Concurrent extracranial and intracranial stenoses is

common especially in Asian, the incidence range from 10 to 39% in patients with symptomatic cerebrovascular disease.[2, 5-8].

This is the first relative large MRA-based prospective cohort study to take into consideration the clinical behavior of concurrent intracranial and extracranial atherosclerosis. We attempted to determine the long-term prognosis and significance of concurrent occlusive disease on mortality and recurrence of vascular events. In this study, we recruited 343 patients and among them, 104 patients (31%) had concurrent intracranial and extracranial stenoses. The patients with concurrent vascular lesions had higher mortality, higher risk of restroke and higher incidence of combined poor outcomes. Fifty-one percent of these patients had further vascular events or death by 5 years, which almost doubled the risk of those without concurrent lesions.

Concerning the risk factor profiles, previous history of stroke and higher premorbid modified Rankin scale were associated with concurrent vascular lesions. There were non-significant trends for patients with concurrent vascular lesions to be older, to have other vascular risk factors including ischemic heart disease, hypertension and diabetes, and to have a more severe stroke. Apart from the presence of concurrent lesions, advanced age, smoking, hyperlipidemia and previous history of stroke were independent predictors of death and poor outcomes among our patients. Our data agreed with previous studies that environmental risk factors play an important role in the development of concurrent vascular lesions.[5-7, 30, 65, 82, 359]

The long-term outcomes of ischemic stroke patients with < 70% extracranial stenosis and concurrent intracranial stenosis are unclear in the literature. Our study showed that this group of patients had poor prognosis. Forty-nine percent of the patients had further vascular event or death by 5 years. The patients with concurrent intracranial and > 70% extracranial stenoses had the worst outcomes. Their 5-year cumulative mortality was 53%. The risks were highest in the first year as 41% of had restroke and 29% died. However, the number of patient was small and most of them could not undergo CEA due to poor premorbid state or total occlusion of the extracranial carotid arteries, which might partly explain the poor outcomes in this group.

The poor outcomes of patients with concurrent vascular lesions may be attributed to the burden of atherosclerosis and synergistic effect of concurrent stenoses. When compared with the group with no vascular stenosis, patients with intracranial, extracranial or concurrent lesions had more death, restroke and combined poor outcome events. In patients with concurrent lesions, those with multiple intracranial stenoses also had higher mortality. This agreed with previous findings that the number of occlusive vessel is an independent predictor of death and recurrent vascular events in patients with intracranial stenosis.[5, 82]

Symptomatic stroke was significantly more common in patients with concurrent stenoses. Although this may be a simple reflection of bigger disease burden in this group of patients, the combination of hypoperfusion and high risk of artery-to -artery embolization is a possible explanation. Previous study showed that patients with high-

grade stenosis or occlusion of MCA had a higher risk of artery-to-artery embolization than those with milder stenosis. An autopsy series revealed that embolic materials were observed frequently within border-zone areas that contained infarcts.[360, 361] Caplan and Hennerici [123]had postulated that the combination of embolism and hypoperfusion can lead to impaired clearance of emboli and produce infarcts in border-zone areas where perfusion is most impaired, especially in patients with severe internal carotid stenosis. The exact mechanism and the lesion patterns of stroke in concurrent stenoses need to be determined by future study.

In view of the poor prognosis, patients with concurrent vascular lesions deserve more medical attention to optimize the management of their vascular risk factors.[362, 363] However, specific evidence-based treatment to improve their prognoses is still unknown and they should constitute the target group for future randomized trials.

5.6 Conclusion

This is the first relative large MRA-based prospective cohort study. Ischemic stroke patients with concurrent atherosclerosis of intracranial and extracranial vessels have high risks of death and recurrent vascular events. Although the event rates are highest in the first year after stroke, the risks remain significant over the subsequent years. Our findings provide important data for planning future randomized clinical trials for this high-risk group of stroke patients.

5.7 Strengths and limitations

The strengths of this study are the large number of patients and the small number lost to follow-up. The weakness is the lack of conventional cerebral angiogram which is the 'gold standard' to confirm the diagnosis of occlusive disease. However, it would be impossible to conduct a large study with catheter conventional angiography because of the cost and risks associated with this procedure. MRA and carotid duplex are acceptable alternatives since their role in detection of occlusive vascular lesions is well determined and they are safe and non-invasive.

Table 5.1 Basic Characteristics of the 343 patients

Characteristics	Total (N=343)	Vascular study results: Concurrent vascular lesion absent (N=239)	Vascular study results: Concurrent vascular lesion present (N=104)	p
Age, y	68.6+/-11.6	66.8+/-12.2	72.6+/-8.8	0.36
Male sex, n (%)	195(57)	139 (58)	56 (54)	0.46
Risk factors, n (%)				
Ischemic heart disease	25(7)	14(6)	11(11)	0.12
Diabetes	102(30)	69(29)	33(32)	0.59
Hypertension	211(62)	140(59)	71(69)	0.09
Ever smoker	164(48)	113(47)	51(49)	0.36
Hyperlipidemia	198(58)	135(57)	63(61)	0.09
Prior stroke	79(23)	46(19)	33(32)	0.01
Prior TIA	12(4)	8(3)	4(4)	0.82
Pre-stroke Rankin scale, n (%)				0.001
0–1	322(94)	229 (96)	93(89)	
2–5	21(6)	10(4)	11(11)	
NIHSS in hospital, n (%)				0.56
0–1	31(9)	24(10)	7(7)	
2–8	230(67)	163(68)	67(64)	
>9	82(24)	52(22)	30(29)	
Acute treatment with				
Anticoagulation, n (%)	40(12)	25(11)	15(15)	0.29
Mean SBP at FU,mmHg(SD)	138(20.2)	137 (20.8)	139 (19.0)	0.54
Mean DBP at FU,mmHg (SD)	74(12.6)	75(12.9)	71(11.3)	0.01
Use of Statins	171(50)	117(49)	57(55)	0.29
Use of Anti-hypertensives	305(89)	208(87)	97(93)	0.1
Use of Anti-thrombotics	329(96)	229(96)	100(96)	0.76
Use of warfarin	10(3)	6(3)	4(4)	0.50

Values are mean +/- SD or as indicated. NIHSS indicates National Institutes of Health Stroke Scale.

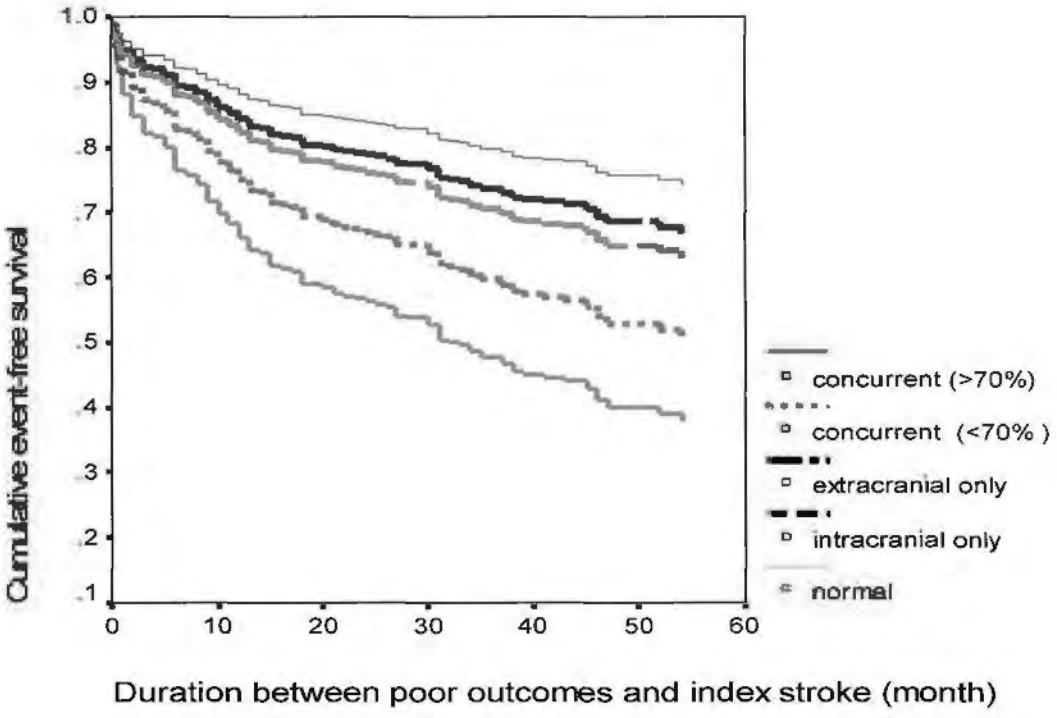
Table 5.2 Causes of Further Vascular Events or Death

Outcome	total (N=343)	Concurrent vascular lesions absent (N=239)	Concurrent vascular lesions present (N=104)
Survival free of vascular events, n (%)	224 (65.3)	170(71.1)	54 (51.9)
Nonfatal vascular events, n			
TIA/stroke	73(21.3)	44(18.4)	29(27.9)
Acute myocardial infarction	18(5.2)	10(4.2)	8(7.7)
Death, n (%)	53(15.5)	25(10.5)	28(26.9)
Vascular			
Index stroke	3(0.9)	1(0.4)	2(1.9)
Recurrent stroke-ischemic	8(2.3)	3(1.3)	5(4.8)
Recurrent stroke- hemorrhagic	3(0.9)	2(0.8)	1(1.0)
Ischemic heart disease	4(1.2)	2(0.8)	2(1.9)
Ruptured aortic aneurysm	0(0)	0(0)	0(0)
Nonvascular			
Gastrointestinal bleeding	1(0.3)	1(0.4)	0(0)
Renal failure	3(0.9)	1(0.4)	2(1.9)
Congestive heart failure	0(0)	0(0)	0(0)
Carcinoma	3(0.9)	2(0.8)	1(1.0)
Pneumonia/Sepsis	21(6.1)	11(4.6)	10(9.6)
Unknown	7(2.0)	2(0.8)	5(4.8)

Table 5.3 Cumulative risks of death, cerebrovascular event and combined poor outcomes

	Total, % (N=343)	Concurrent lesions absent, % (N=239)	Concurrent lesions present, % (N=104)	Concurrent lesion(<70% extracranial stenosis), % (N=83)	Concurrent lesions (>70% extracranial stenosis), % (N= 21)
Cumulative Mortality					
Year 1	8	4	16	13	29
Year 2	11	6	22	17	39
Year 3	16	12	27	22	45
Year 4	18	13	31	25	53
Year 5	18	13	31	25	53
Cumulative risks of cerebrovascular event					
Year 1	19	16	27	23	41
Year 2	23	19	34	33	41
Year 3	26	22	36	35	41
Year 4	27	22	41	41	41
Year 5	27	22	41	41	41
Cumulative Combined poor outcomes					
Year 1	24	19	36	33	48
Year 2	30	24	46	43	58
Year 3	36	30	49	47	58
Year 4	37	31	51	49	58
Year 5	37	31	51	49	58

Figure 5.1 Cumulative event-free survival in patients with different intracranial and extracranial lesions.



Concurrent(<70%), concurrent lesions with <70% extracranial stenosis; concurrent (>70%), concurrent lesions with >70% extracranial stenosis; extracranial only, extracranial stenosis only ; intracranial only, intracranial stenosis only ; normal, normal craniocervical vasculature.

Chapter 6: Long-term Outcomes of Ischemic Stroke Patients with Concurrent Intracranial, Extracranial Stenoses and Ischemic Heart Disease (study 2)

Chapter 6.1 Abstract

Background and Purpose: Coexisting ischemic heart disease (IHD) and concurrent atherosclerosis of intracranial and extracranial vessels is common in Asians. This study aims to investigate the long-term outcomes of ischemic stroke patients with concurrent stenoses and IHD.

Method: This was a prospective cohort study in Hong Kong. Consecutive Chinese patients with acute ischemic stroke underwent MRI, MRA and carotid duplex.

Results: Totally 428 patients were included. The mean follow-up period was 65 months (up to 87 months). Ninety-three patients (22%) died of any cause and 104 patients (22%) had non-fatal vascular events. Fifty-four patients (13%) had ischemic heart disease. Among them, 27 patients (50%) had concurrent stenoses. In patients with concurrent stenoses and IHD, only 3 (11%) were free of death and recurrent vascular events. Eight (30%) had recurrent non-fatal stroke, 7 (26%) had non-fatal myocardial infarct, 11 (41%) died and 4 (22%) due to fatal myocardial infarct. The overall 5-year cumulative rates of mortality, recurrent vascular events and combined poor outcomes were 21%, 23% and 43% respectively. In patients with concurrent stenoses and IHD, these rates were 40%, 50% and 83% respectively. More deaths (log rank, 6.56; p=0.01), recurrent vascular

events (log rank, 25.24; $p<0.001$) and poor outcomes (log rank, 27.50; $p<0.001$) were found among patients with concurrent stenoses and IHD.

Conclusion: Ischemic stroke patients with concurrent stenoses and IHD had high risks of death and recurrent vascular events. Future studies on aggressive medical therapy and early cardiac interventions in this high-risk group of stroke patients are warranted.

6.2 Introduction

Coexisting ischemic heart disease (IHD) is common in patients with stroke[364-368] and it is associated with increased risk of cardiac death.[369, 370] Previous studies in Caucasian[371, 372] and Korean[367] showed that IHD was more common in patients with extracranial carotid artery disease. Concurrent atherosclerosis of intracranial and extracranial vessels is common in Asian[373-378] and it is associated with poor outcomes.[373, 374] The impact of coexisting IHD in this group of patients was unknown. Given the difference in vessel involvement, concurrent stenoses with ischemic heart disease may have different prognosis from pure intracranial or extracranial stenosis. This study aims to investigate the long-term outcomes of ischemic stroke patients with concurrent stenoses and IHD.

6.3 Methods

6.3.1 Patients

This was a prospective cohort study conducted at the Prince of Wales Hospital in Hong Kong. The study was approved by local ethics committee. We recruited consecutive patients admitted with acute cerebral ischemia, including transient ischemic attack (TIA) and cerebral infarct within 7 days of symptom onset during January 2002 to June 2004. We excluded patients less than 18 years old, those who had intracranial hemorrhage, vascular malformations, active cancer, liver and renal failure, prothrombotic tendency such as underlying active lupus disease, antiphospholipid syndrome, factor C/S

deficiency and those who were pregnant. Those who were unfit for MRI study because of metallic implants, unstable medical conditions and claustrophobia were excluded.

All patients underwent computer tomography (CT) and magnetic resonance imaging (MRI) of brain to establish the diagnosis of acute ischemic stroke, and vascular imaging including magnetic resonance angiography (MRA) of brain and carotid duplex to look for vascular stenosis. Baseline data, including age, sex, medical history, and physical examination, were collected on admission. Vascular risk factors were noted, in particular any history of smoking, hypertension, diabetes mellitus, IHD and previous TIA or stroke. Blood biochemistry, full blood count, ECG, and chest x-ray were checked routinely. The diagnosis of coexisting IHD was made in patients with documented myocardial infarction (MI) or typical angina pectoris in whom there was also a positive exercise test, evidence of myocardial ischemia revealed by radionuclide study or at least 60% stenosis of one major coronary artery on coronary angiogram.[379]

6.3.2 MRI and MRA

All patients were scanned within one week of symptom onset with a Siemens Sonata (Erlanger, Germany) with a head coil. The three-dimensional time-of-flight images were acquired with a repetition time of 40 ms, time to echo of 7.15 ms, flip angle of 25°, 20-cm field of view, 192x512 acquisition matrixes, and one signal average for a total imaging time of 5 minutes 35 seconds. The MRA films were read on a designated station by experienced neuroradiologists (W. Lam) who was blinded to the results of the

clinical examination. Acute infarcts on Diffusion-weighted imaging (DWI) were diagnosed when these lesions were shown to be hyperintense on the DWI integrated for the 3 diffusion sensitivity directions and hypointense on the apparent diffusion coefficient map. For images showing motion artifact in 1 diffusion sensitivity direction, infarct or subacute infarct was diagnosed only if the lesion showed all of the following features: (1) it had a much higher signal than on the image map with b=0, (2) it was not caused by normal anisotropy of diffusion or artifact, and (3) it was seen on the DWIs in both of the remaining orthogonal diffusion sensitivity directions. Symptomatic infarct was defined as infarct in the vascular territory of the stenotic vessel. Intracranial stenosis was graded according to the following criteria: grade 1, normal or mild stenosis (0% to 29% diameter stenosis); grade 2, moderate stenosis (30% to 69% diameter stenosis); grade 3, severe stenosis (70% to 100% diameter stenosis). The percentage of stenosis of MCA and other intracranial vessels was measured by visual inspection.

6.3.3 Carotid Duplex

For the Duplex ultrasound examination of the extracranial carotid arteries, we used a Philips SD800 ultrasound machine and a 7.5-MHz transducer. Extracranial stenosis was graded as mild (<50%), moderate (50-69%) and severe (70-99%) according to the principles established by the NASCET study [380]. The diagnostic criteria for 70% stenosis of the internal carotid artery required a peak systolic velocity ratio of 2.4. The above diagnostic criteria in the neurovascular laboratory were based on laboratory references, which had a quality assurance program with supplementary angiographic studies.

Concurrent stenoses were defined as presence of more than 30% stenoses in both extracranial and intracranial vessels, including lesions in the same vascular territories (tandem lesions) and lesions in different vascular territories (non-tandem lesions).

6.3.4 Outcome measures

All survivors were followed up regularly by telephone or personal interviews, in addition to routine medical outpatient clinic attendance. If patients could not be reached, we found their medical records and tried to contact their relatives for more information about the patient's condition. All telephone and personal interviews were performed by a stroke specialty nurse using a standardized questionnaire.

The specified outcomes were the occurrence of a further vascular event (including TIA, stroke, or acute coronary syndrome) or death. Poor outcome was defined as the occurrence of either death or a further vascular event. If death and a further vascular event were recorded as occurring simultaneously, one event was categorized into the analysis. The nature of the vascular event and the cause of death were based on medical records and death certificates when possible. Recurrent events were based on the diagnosis on the discharge summary according to the treating physician. The definite and detailed description of the cause of death by the patient's relative was used if the cause could not be obtained by other means. Obscure or indefinite information about cause of death was classified as unknown cause.

6.3.5 Statistical Analysis

All data were entered into the SPSS software (version 13.0 for Windows) for storage and analysis. Statistical significance was considered at p =0.05 and was 2-sided. In the present study, age was recorded as continuous variables. Sex, vascular risk factors, and end points of long-term outcome were used as categorical variables. In univariate analysis, independent-sample t test and X^2 test or Fisher's exact test were used. In analysis of predictors of the long-term clinical outcome, Cox proportional-hazards regression function was used to estimate impact in terms of risk ratios of possible determinants of survival and recurrent vascular events, taking the time variable into consideration. Hazard ratio (HR), 95% confidence interval (CI), and probability value were observed. In survival analyses, the Kaplan-Meier product-limit method was used to estimate survival condition. The log-rank test was used to compare rate estimates.

6.4 Results

From January 2002 to June 2004, 1024 patients were screened and 648 met inclusion criteria. Forty three patients declined the MRI procedure and 98 withdrew consent due to claustrophobia. A total of 507 participants underwent cerebral MRI. Thirty-eight patients had no consent and 41 defaulted follow-up and were excluded from the analyses, leaving a total of 428 patients available for analysis. Among them, 102 (24%) had normal intracranial and extracranial vessels, 105 (26%) had intracranial stenosis only, 49 (11%) had extracranial stenosis only, 172 (40%) had concurrent stenoses.

Fifty-four patients had ischemic heart disease. Among them, 7 (13%) had normal intracranial and extracranial vessels, 16(30%) had intracranial stenosis only, 4 (7%) had extracranial stenosis only, 27 (50%) had concurrent stenoses. Twenty-eight (52%) had undergone coronary angiogram and 12 (22%) had coronary stenting done. The basic characteristics of the patients were summarized in Table 6.1.

We followed up this cohort for a mean period of 65 months after stroke onset (up to 87 months). A total of 93 patients (22%) died of any cause and 104 patients (22%) had non-fatal vascular events. In patients with concurrent stenoses and IHD, only 3 (11%) were free of death and further vascular event. Eight (30%) had further non-fatal stroke, 7 (26%) had non-fatal MI, 11 (41%) died and 6 (22%) due to fatal MI. (Table 6.2)

6.4.1 Mortality

The overall 5-year mortality was 21%. The mortality of patients with concurrent stenoses and IHD was 40% which was higher than other groups of patients (Figure 6.1). Concurrent stenoses and IHD was associated with increased mortality (log rank, 6.56; p=0.01). Other risk factors include previous history of stroke (log rank, 7.46, p=0.006), hypertension (log rank, 4.81; p=0.03) and smoking (log rank, 4.96; p=0.03). In Cox proportional-hazard regression model, the adjusted hazard ratio of mortality in patients with concurrent stenoses and IHD was 1.73(95% CI 1.29 to 2.32, p<0.001).

6.4.2 Occurrence of recurrent vascular events

In the 428 patients, recurrent vascular events occurred in 155 patients (36%) which included 167 TIAs/strokes and 39 MIs. The overall 5-year cumulative risk of recurrent vascular events was 23% (Figure 6.2). Patients with concurrent stenoses and IHD had more recurrent vascular events and their 5-year event-free survival was only 50%. The risk of recurrent MI was much higher in patients with concurrent stenoses and IHD (Figure 6.3) and their risk of recurrent stroke was also higher. Concurrent stenoses and IHD was associated with higher risk of recurrent vascular events (log rank, 25.24; $p<0.001$). Other risk factors include previous history of stroke (log rank, 10.16, $p=0.001$) and hypertension (log rank, 7.61; $p=0.006$). In Cox proportional-hazard regression model, the adjusted hazard ratio of recurrent vascular events in patients with concurrent stenoses and IHD was 2.68 (95% CI 1.66 to 4.32, $p<0.001$).

6.4.3 Combined poor outcomes

Both death and further vascular event represent poor outcomes after stroke. When these were combined, the overall 5-year cumulative risk of combined poor outcomes was 43 % (Figure 6.4). The occurrence of combined poor outcomes was higher in patients with concurrent stenoses and IHD and their 5-year event-free survival was only 17%. Concurrent stenoses and IHD was associated with higher risk of combined poor outcomes (log rank, 27.50; $p<0.001$). Other risk factors include male sex (log rank, 7.25; $p=0.007$), previous history of stroke (log rank, 23.19, $p<0.001$), smoking (log rank, 5.97; $p=0.02$)

and hypertension (log rank, 5.61; p=0.02). In Cox proportional-hazard regression model, the adjusted hazard ratio of combined poor outcomes in patients with concurrent stenoses and IHD was 2.57 (95% CI 1.64 to 4.03, p<0.001).

6.5 Discussion

This is the first relative large MRA-based prospective cohort study to investigate the long-term outcomes of ischemic stroke patients with concurrent stenoses and IHD. We recruited 428 patients and among them, 27 (6%) got concurrent stenoses and IHD. These patients had higher mortality, higher rate of recurrent vascular events and combined poor outcomes. Their 5-year event-free survival was only 17%. The poor outcomes of patients with concurrent stenoses and IHD may be attributed to the burden of atherosclerosis and synergistic effect of concurrent stenoses and IHD. This agreed with previous findings that the number of vessel involved was an independent predictor of death and recurrent vascular events in ischemic stroke patients. [373, 374]

Concerning the risk factor profiles, the presence of concurrent stenoses and IHD, smoking, hypertension, male sex and previous history of stroke were independent predictors of death and poor outcomes. Our data agreed with previous studies that environmental risk factors play an important role in the development of concurrent vascular lesions and IHD. [367, 373, 375, 381-383]

The risk of recurrent MI was much higher in patients with concurrent stenoses and

IHD. Twenty-five percent of them had recurrent non-fatal MI and 22% of them were died of fatal MI. This can be explained by the high prevalence of coronary artery disease in this high-risk group of stroke patients. Future studies on aggressive medical therapy and early cardiac interventions in this high-risk group of stroke patients are warranted.

6.6 Conclusions

This was the first relative large MRA-based prospective cohort study to investigate the long-term outcomes of ischemic stroke patients with concurrent stenoses and IHD. Ischemic stroke patients with concurrent stenoses and IHD had high risks of death and recurrent vascular events. Future studies on aggressive medical therapy and early cardiac interventions in this high-risk group of stroke patients are warranted.

6.7 Strengths and limitations

The strengths of this study are the large number of patients and the small number lost to follow-up. The limitations are firstly, the lack of conventional cerebral angiogram which is the 'gold standard' to confirm the diagnosis of occlusive disease. However, MRA and carotid duplex are acceptable alternatives since their role in detection of occlusive vascular lesions is well determined and they are safe and non-invasive. Secondly, coronary angiogram was not done in all patients with IHD to confirm the diagnosis. However, it is difficult to have coronary angiogram in all patients especially those with renal impairment and poor premorbid states. Thirdly, the small absolute

number of patients with concurrent intracranial, extracranial and ischemic heart disease may cause potential bias.

Table 6.1 Basic Characteristics of the 428 patients

Characteristics	Total (N=428)	No concurrent lesions and IHD[#] (N=401)	Concurrent lesions with IHD[##] (N=27)	p
Age, y+/-SD	73.1+/-11.4	73.0+/-11.5	76.5+/-9.5	0.13
Male sex, n (%)	248 (58)	228(57)	20 (74)	0.08
Risk factors, n (%)				
Diabetes	140(33)	129(32)	11(41)	0.36
Hypertension	281(66)	264(66)	17(63)	0.74
Ever smoker	191(45)	175(44)	16(59)	0.12
Hyperlipidemia	248(58)	233(58)	15(56)	0.78
Prior stroke	88(21)	81(21)	7(26)	0.50
Prior TIA	13(3)	13(3.2)	0(0)	0.34
Treatment after stroke				
Antihypertensives	381(89)	354(89)	27(100)	0.06
Antithrombotics	409(96)	382(96)	27(100)	0.27
Statins	235(55)	217(54)	18(67)	0.21

* p=<0.05

TIA indicates transient ischemic attack; IHD, ischemic heart disease.

[#] included patients with normal vessels, intracranial stenosis only and extracranial stenosis only
[##] included patients with stenoses in both intracranial and extracranial vessels

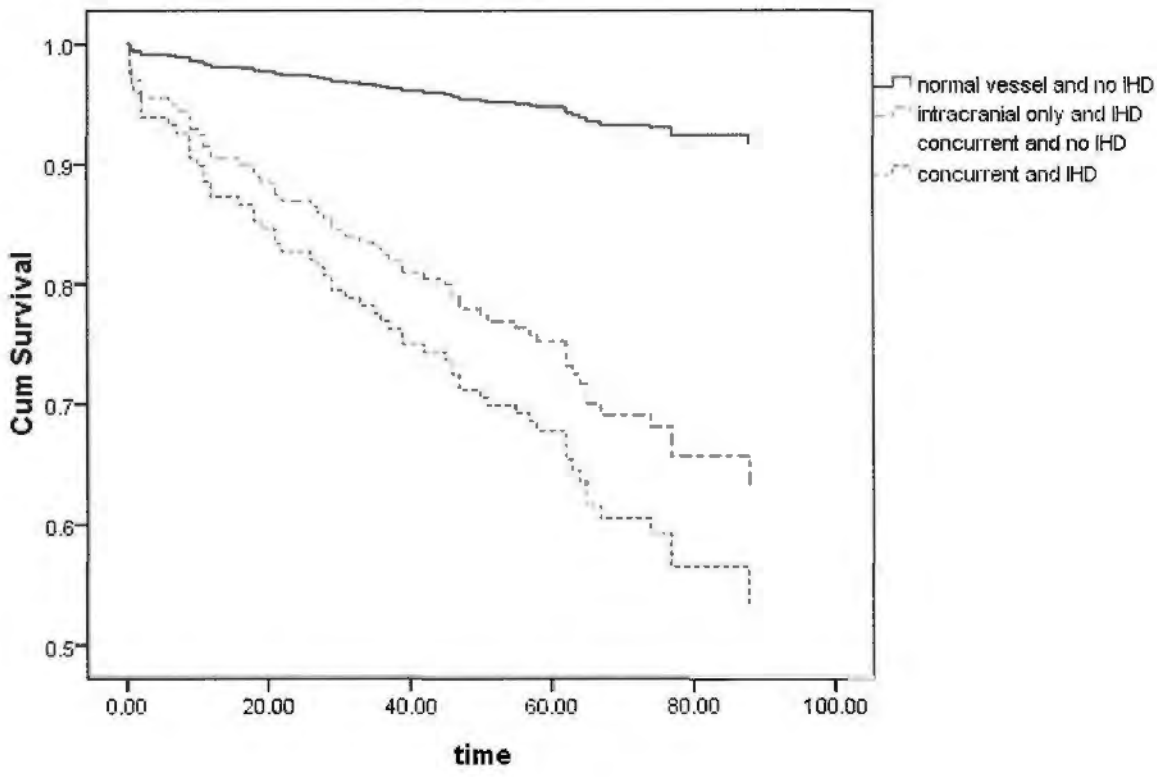
Table 6.2 Causes of Further Vascular Events or Death

Outcome	Total (N=428)	No Concurrent lesions and IHD[#] (N=401)	Concurrent lesions and IHD[##] (N=27)
Survival free of vascular events, n (%)	231 (54.2)	229(57.1)	3 (11.1)
Nonfatal vascular events, n			
TIA/stroke	91(21.3)	83(20.7)	8(29.6)
Acute myocardial infarction	22(5.1)	15(3.7)	7(25.9)
Death, n (%)	93(21.7)	82(20.4)	11(40.7)
Vascular			
Index stroke	5(1.2)	4(1.0)	1(3.7)
Recurrent stroke-ischemic	9(2.1)	9(2.2)	0(0)
Recurrent stroke- hemorrhagic	7(1.6)	7(1.7)	0(0)
Ischemic heart disease	10(2.3)	6(1.5)	6(22.2)
Ruptured aortic aneurysm	1(0.2)	1(0.2)	0(0)
Nonvascular			
Gastrointestinal bleeding	2(0.5)	2(0.5)	0(0)
Renal failure	3(0.7)	3(0.7)	0(0)
Carcinoma	10(2.3)	9(2.2)	1(3.7)
Pneumonia/Sepsis	31(7.2)	30(7.5)	1(3.7)
Unknown	15(3.5)	13(3.2)	2(7.4)

TIA indicates transient ischemic attack; IHD, ischemic heart disease

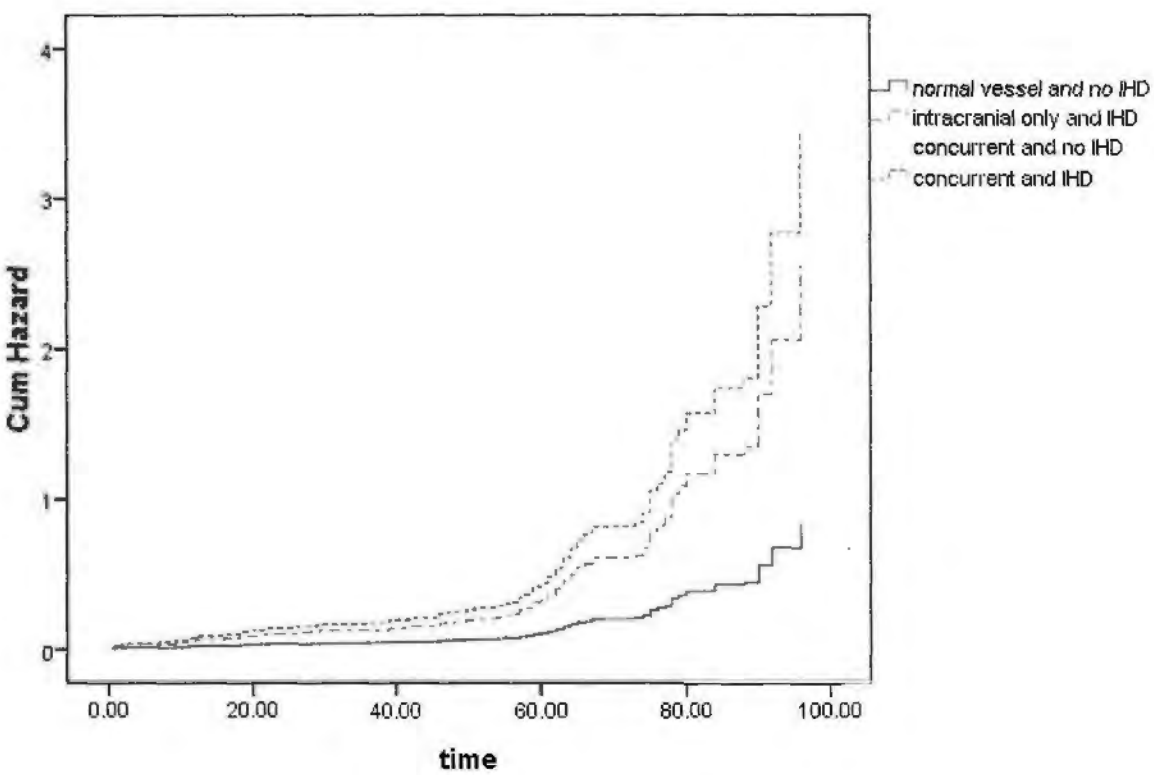
[#] included patients with normal vessels, intracranial stenosis only and extracranial stenosis only
[##] included patients with stenoses in both intracranial and extracranial vessels

Figure 6.1 Cumulative survivals of different groups of patients



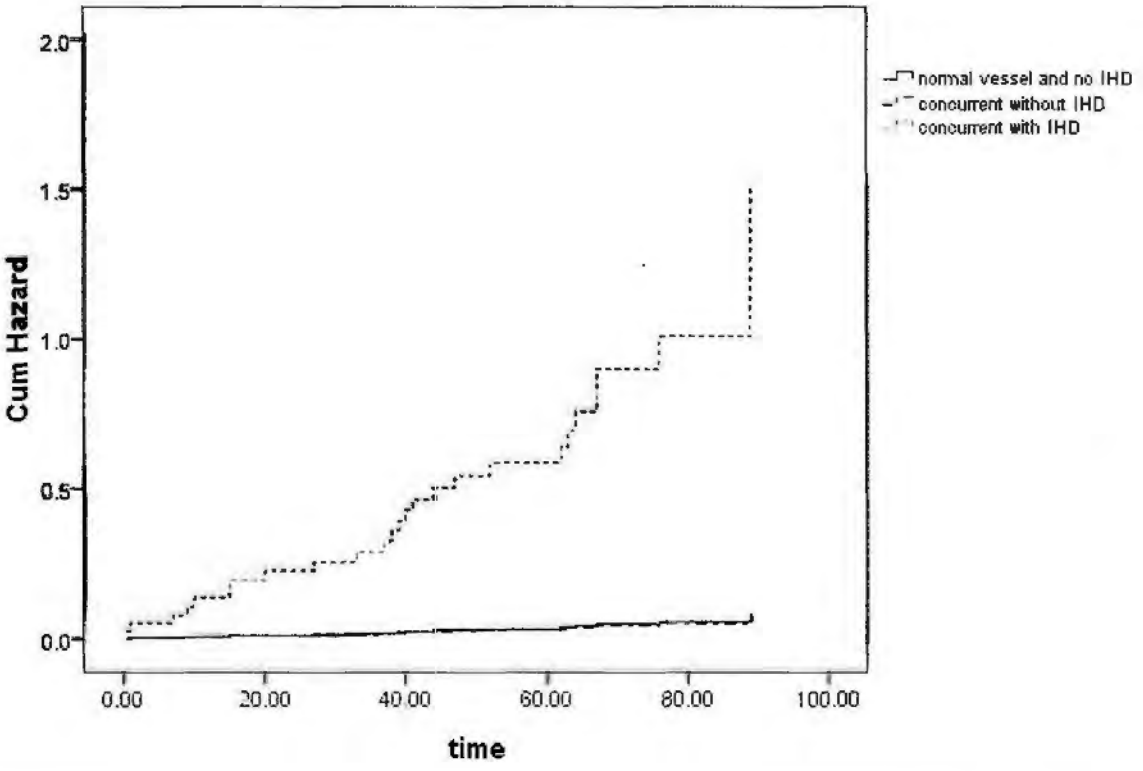
Normal vessel indicates normal craniocervical vasculature; intracranial only, intracranial stenosis only; concurrent, concurrent stenoses; IHD, ischemic heart disease.

Figure 6.2 Cumulative hazards of further vascular events of different groups of patients



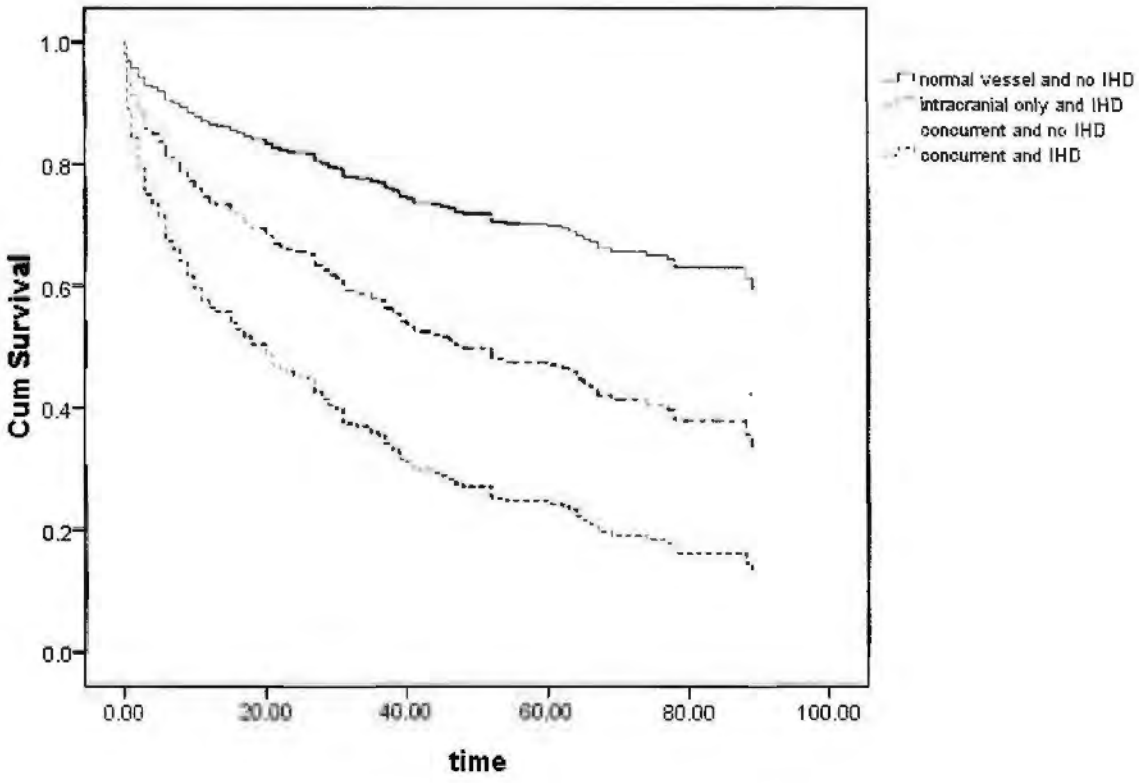
Normal vessel indicates normal craniocervical vasculature; intracranial only, intracranial stenosis only; concurrent, concurrent stenoses; IHD, ischemic heart disease.

Figure 6.3 Cumulative risks of recurrent myocardial infarction of different groups of patients



Normal vessel indicates normal craniocervical vasculature; concurrent, concurrent stenoses; IHD, ischemic heart disease.

Figure 6.4 Cumulative event-free survival of combined poor outcomes of different groups of patients



Normal vessel indicates normal craniocervical vasculature; intracranial only, intracranial stenosis only; concurrent, concurrent stenoses; IHD, ischemic heart disease.

Chapter 7: Lesion Patterns and Stroke Mechanisms in Concurrent Atherosclerosis of Intracranial and Extracranial Vessels (Study 3)

7.1 Abstract

Background and Purpose: Concurrent atherosclerosis of intracranial and extracranial cerebrovascular system is common in Asian. The typical lesion patterns and the mechanisms of stroke in patients with concurrent stenoses are unclear. This study aimed to determine these stroke features of such patients in Hong Kong.

Method: We conducted a cross-sectional cohort study in a university hospital from January 2002 to December 2003. Consecutive Chinese patients with acute ischemic stroke underwent CT brain, MRI brain (with MRA and DWI sequences) and carotid duplex.

Results: Totally 251 patients were included in the analysis. Of these, 109(43%) had concurrent stenoses. Patients who had concurrent stenoses, as compared with those without concurrent stenoses, had more symptomatic stenoses (84% vs. 58%; odd ratio, 4.0; 95% confidence interval [CI], 2.1 to 7.3 ; $p<0.001$) , more concomitant perforating artery infarct (PAI), pial infarct (PI) and borderzone (BZ) infarct (14% vs. 4%; odd ratio, 3.6; 95% CI, 1.4 to 9.7 ; $p=0.007$), more multiple DWI lesions (55% vs. 37%; odd ratio, 2.1; 95% CI, 1.3 to 3.4 ; $p=0.005$) and more infarcts in the territory of the leptomeningeal branches of middle cerebral artery (MCA) (26% vs. 13%; odd ratio, 2.2; 95% CI, 1.2 to 4.3 ; $p=0.01$). In multivariate regression analysis, smoking, prior stroke, the presence of

concomitant PAI, PI, and BZ infarcts, multiple DWI lesions and symptomatic stenoses were significantly associated with concurrent stenoses. Among patients with concurrent stenoses, those who had tandem lesions, as compared with those who had non-tandem lesions, had more PAI and BZ infarcts (27% vs. 8%; odd ratio, 4.3; 95% CI, 0.9 to 19.8; p=0.04), more concomitant PAI, PI and BZ infarcts (18% vs. 0%; p=0.02), and more multiple DWI lesions (65% vs. 23%; odd ratio, 6.2; 95% CI, 2.2 to 17.2; p<0.001). Infarcts in the territory of MCA leptomeningeal branches and symptomatic stenoses were more common in patients with tandem lesions.

Conclusion: Concomitant PAI, PI and BZ infarcts, multiple DWI lesions and infarcts in the leptomeningeal branches of MCA were more common in patients with concurrent stenoses, especially those with tandem lesions. This study suggested that the combination of hemodynamic compromise attributable to concurrent stenoses and artery-to-artery embolization is a common stroke mechanism in these patients.

7.2 Introduction

Concurrent atherosclerosis of intracranial and extracranial cerebrovascular system is common in Asian[374, 384-387] and is associated with poor outcomes.[373] The stroke mechanisms and the lesion patterns of patients with concurrent stenoses are unclear. Given the difference in vessel involvement, concurrent stenoses may have different lesion patterns from pure intracranial or extracranial stenosis. Diffusion-weighted imaging (DWI) is the most sensitive diagnostic modality in detecting acute ischemic lesions.[388, 389] DWI has been used in several studies to explore the pathomechanism of ischemic stroke in patients with atherosclerotic middle cerebral artery (MCA) disease.[388, 390-393] However, data on concurrent lesions are scanty. This study aimed to use DWI to investigate the ischemic lesion patterns and stroke mechanisms in patients with concurrent stenoses.

7.3 Methods

7.3.1 Patients

This was a cross-sectional study conducted at the Prince of Wales Hospital in Hong Kong. The study was approved by local ethics committee. We recruited consecutive patients admitted with acute cerebral ischemia, including transient ischemic attack (TIA) and cerebral infarct within 7 days of symptom onset from January 2002 to December 2003. We excluded patients less than 18 years old, those who had atrial fibrillation, intracranial hemorrhage, vascular malformations, active cancer, myocardial infarction, liver and renal failure, prothrombotic tendencies such as underlying active lupus disease,

antiphospholipid syndrome, factor C/S deficiency and those who were pregnant. Those who were unfit for MRI study because of unstable medical conditions and claustrophobia were excluded.

All patients underwent computer tomography (CT) and magnetic resonance imaging (MRI) of brain with DWI to define the patterns of acute infarcts, and vascular imaging including magnetic resonance angiography (MRA) of brain and carotid duplex to look for vascular stenoses. Baseline data, including age, sex, medical history and physical examination, were collected on admission. Vascular risk factors were determined, in particular any history of smoking, hypertension, diabetes mellitus, ischemic heart disease and previous TIA or stroke. Blood biochemistry, full blood count, electrocardiogram and chest x-ray were checked routinely.

7.3.2 MRI, DWI and MRA

All patients were scanned within one week of symptom onset with a Siemens Sonata (Erlanger, Germany) with a head coil. The three-dimensional time-of-flight images were acquired with a repetition time of 40 ms, time to echo of 7.15 ms, flip angle of 25°, 20-cm field of view, 192x512 acquisition matrixes, and one signal average for a total imaging time of 5 minutes 35 seconds. Acute infarcts on DWI were diagnosed when these lesions were shown to be hyperintense on the DWI integrated for the 3 diffusion sensitivity directions and hypointense on the apparent diffusion coefficient map. For images showing motion artifact in 1 diffusion sensitivity direction, infarct or subacute

infarct was diagnosed only if the lesion showed all of the following features: (1) it had a much higher signal than on the image map with b=0, (2) it was not caused by normal anisotropy of diffusion or artifact, and (3) it was seen on the DWIs in both of the remaining orthogonal diffusion sensitivity directions. DWI lesion patterns were analyzed by an investigator (W. Lam) who was blinded to clinical data. The topography of ischemic lesions was determined using published templates.[394] The vascular territories were divided into perforator, pial, and borderzone regions. Perforating artery infarct (PAI) included striatocapsular infarct or perforating vessel infarct of the cerebral arteries. Pial infarct (PI) was defined as an infarct occurring in the vascular territories supplied by the main leptomeningeal branches of the cerebral arteries. Borderzone (BZ) infarcts were defined as anterior or posterior cortical BZ or internal BZ. Multiple DWI lesions referred to multiple noncontiguous hyperintense lesions occurring in the vascular territories described above. DWI lesions were allocated to 1 of the following 10 patterns (Table 2): Single lesion [(1) small PAI (diameter <2 cm), (2) large PAI (diameter >2 cm), (3) PI, (4) large territorial infarct, (5) BZ infarct] and multiple lesions [(6) PAI and PI, (7) PAI, PI, and BZ, (8) PAI and BZ, (9) PI and PI, (10) PI and BZ]. The MRA films were read by experienced neuroradiologists (W. Lam) who was blinded to the results of the clinical examination. Intracranial stenosis was graded according to the following criteria: grade 1, normal or mild stenosis (0% to 29% diameter stenosis); grade 2, moderate stenosis (30% to 69% diameter stenosis); grade 3, severe stenosis (70% to 100% diameter stenosis). The percentage of stenosis of MCA and other intracranial vessels was measured by visual inspection.

7.3.3 Carotid Duplex

For the Duplex ultrasound examination of the extracranial carotid arteries, we used Philips SD800 ultrasound machine and a 7.5-MHz transducer. Extracranial stenosis was graded as mild (<30%), moderate (30-69%) and severe (70-99%). The diagnostic criteria for 70% stenosis of the internal carotid artery (ICA) required a peak systolic velocity ratio of 2.4. The above diagnostic criteria in the neurovascular laboratory were based on laboratory references, which had a quality assurance program with supplementary angiographic studies.

Concurrent stenoses were defined as presence of more than 30% stenoses in both extracranial and intracranial vessels, including lesions in the same vascular territories (tandem lesions) and lesions in different vascular territories (non-tandem lesions).

7.3.4 Statistical Analysis

All data were entered into the SPSS software (version 13.0 for Windows) for storage and analysis. Student's t test, χ^2 test or Fisher's exact test were used for comparing continuous and dichotomous variables between patients with or without concurrent stenoses. Statistical significance was considered at p =0.05 and was 2-sided. Univariate linear regression analysis was used to find the variables that accounted for concurrent stenoses. Variables that were identified as significant in the univariate analysis

($p< 0.05$) were entered into the forward stepwise multivariate linear regression analysis to examine their independent contributions to the variance of concurrent stenoses.

7.4 Results

From January 2002 to December 2003, 834 patients were screened, 523 were eligible and 119 patients declined the MRI procedure. A total of 404 participants underwent cerebral MRI. One hundred and fifty-three patients had no evidence of acute infarct in DWI and were excluded. Totally 251 patients were included in the analyses. Among them, 47 (19%) had normal intracranial and extracranial vessels, 73 (29%) had intracranial stenosis only, 22 (9%) had extracranial stenosis only, and 109 (43%) had concurrent stenoses. Patients who had concurrent stenoses, as compared with those without concurrent stenoses, were older, more likely hypertensive and had more symptomatic stenoses (84% vs. 58%; odd ratio, 4.0; 95% CI, 2.1 to 7.3; $p<0.001$). (Table 7.1)

7.4.1 Ischemic Lesion Patterns

Among patients with concurrent stenoses, multiple concomitant lesions (53%) were most commonly seen. Single small PAI was less frequent (35%). Patients who had concurrent stenoses, as compared with those without concurrent stenoses, had more concomitant PAI, PI and BZ infarcts (14% vs. 4%; odd ratio, 3.6; 95% CI, 1.4 to 9.7 ; $p=0.007$), and more multiple DWI lesions (53% vs. 37%; odd ratio, 2.1; 95% CI, 1.3 to 3.4 ; $p=0.005$) . In contrast, single small PAI was more common in patients with non-

concurrent stenoses (51% vs. 35%; odd ratio, 1.9; 95% CI, 1.2 to 3.2; p=0.01). (Figure 7.1) (Table 7.2)

7.4.2 Vascular Territory of Stroke

Infarcts in the territory of MCA leptomeningeal branches were more common in patients with concurrent stenoses (26% vs. 13%; odd ratio, 2.2; 95% CI, 1.2 to 4.3; p=0.01) but MCA perforating branch infarcts were more common in patients with non-concurrent stenoses (47% vs. 35%; odd ratio, 1.7; 95% CI, 1.0 to 2.8; p=0.05). (Table 7.3)

After adjustment of age and sex, univariate linear regression analysis showed that smoking (R^2=0.302, p<0.0001), prior stroke (R^2=0.046, p=0.01), the presence of concomitant PAI, PI and BZ infarcts (R^2= 0.044, p=0.01), multiple DWI lesions (R^2= 0.044, p=0.01) and symptomatic stenoses (R^2= 0.12, p<0.0001) were associated with concurrent stenoses. In multivariate regression analysis, all these factors were significantly associated with concurrent stenoses.

7.4.3 Tandem vs. Non-tandem Lesions

Among 109 patients with concurrent stenoses, 83(76%) had tandem lesions. Symptomatic stenoses occurred more frequently in patients with tandem lesions than those with non-tandem lesions (89% vs. 69%; odd ratio, 3.7; 95% CI, 1.2 to 10.8; p=0.02). Most patients (64%) with tandem lesions had multiple concomitant infarcts and

almost half of them (49%) had BZ infarcts in any combination. Infarcts in the territory of MCA leptomeningeal branches were more common in patients with tandem lesions than those with non-tandem lesions (33% vs. 4%; odd ratio, 12.1; 95% CI, 1.6 to 93.7; p=0.003). In contrast, single small PAI was the most common infarct pattern in patients with non-tandem lesions (73% vs. 23%; odd ratio, 9.1; 95% CI, 3.3 to 25.0; p<0.001). Patients who had tandem lesions, as compared with those who had non-tandem lesions, had more PAI and BZ infarcts (27% vs. 8%; odd ratio, 4.3; 95% CI, 0.9 to 19.8; p=0.04), more concomitant PAI, PI and BZ infarcts (18% vs. 0%; p=0.02), and more multiple DWI lesions (64% vs. 19%; odd ratio, 6.2; 95% CI, 2.2 to 17.2; p<0.001). (Table 7.4)

7.4.4 Severity of Tandem Lesions

Among 83 patients with tandem lesions, 34 % had non-significant (<70%) intracranial and extracranial stenoses, 48% had a significant (>70%) stenosis in one vessel, and 18% had significant stenoses in both vessels. Patients who had significant stenoses of both vessels, as compared with those who had no significant stenosis, had more concomitant PAI, PI and BZ infarcts (47% vs. 7%; odd ratio, 11.4; 95% CI, 2.0 to 66.1; p=0.002) and more multiple DWI lesions (93% vs. 50%; odd ratio, 14.0; 95% CI, 1.6 to 121.4; p=0.004). Single small PAI was more common in patients who had no significant stenosis than those who had significant stenoses of both tandem lesions (36% vs. 0%, p=0.01).

7.5 Discussion

Concurrent atherosclerosis of intracranial and extracranial vessels is common in Asian.[385, 395-398] It is associated with higher mortality and risks of recurrent cerebrovascular events.[373, 374] The underlying mechanisms are thought to be burden of atherosclerosis and hemodynamic compromise attributable to concurrent stenoses of intracranial and extracranial arteries.[374, 399] However, the exact mechanism remains unclear. DWI has been used for exploration of stroke mechanisms in previous studies of ICA, MCA stenosis and BZ infarcts.[388-391, 393, 400] Our study was the first MRI study using DWI to define stroke mechanisms in concurrent stenoses. Concerning the risk factor profiles, patients with concurrent stenoses were older and more likely hypertensive. There were non-significant trends for patients with concurrent stenoses to have other vascular risk factors including diabetes, hyperlipidemia, smoking and more prior stroke. Our data agreed with previous studies which suggest a possible role for diabetes mellitus, metabolic syndrome and other cardiovascular risk factors in the development of larger artery disease.[374, 386, 401, 402]

Concomitant PAI, PI and BZ infarcts were more common in patients with concurrent stenoses while a single small PAI was more common in patients with non-concurrent stenoses. This can be accounted by the difference in vascular territory involved as patients with concurrent stenoses had more infarcts in the leptomeningeal branches of MCA while patients without concurrent stenoses had more infarcts in the perforating branches of MCA. Previous study suggested that internal BZ infarcts are

caused mainly by hemodynamic compromise[389]. We found that pure BZ infarct was rare (only 0.8% of the cohort). Most BZ infarcts were accompanied by multiple concomitant infarcts. Most of the patients with concurrent stenoses, especially those had severe tandem lesions, got concomitant PAI, PI and BZ infarcts. The multiple DWI lesions may be markers of embolism. Our findings suggested that the combination of hemodynamic compromise attributable to concurrent stenoses and artery-to -artery embolization is a common stroke mechanism in concurrent stenoses. The results of an autopsy series revealed that embolic materials were observed frequently within borderzone areas that contained infarcts.[403, 404] Caplan and Hennerici had postulated that the combination of embolism and hypoperfusion can lead to impaired clearance of emboli and produce infarcts in BZ where perfusion is most impaired, especially in patients with severe internal carotid stenosis.[405] Previous study also suggested that patients with high-grade stenosis or occlusion of MCA have a higher risk of artery-to-artery embolization than those with milder stenosis.[391] There was an experimentally proven case of border-zone infarct induced by microemboli.[406] Another study had shown that the number of microembolic signals on transcranial Doppler predicted the number of acute infarcts on DWI.[393] All these evidence support our hypothesis of the stroke mechanism in patients with concurrent stenoses.

7.6 Conclusion

Infarcts in the leptomeningeal branches of MCA, concomitant PAI, PI and BZ infarcts, as well as multiple DWI lesions were significantly associated with concurrent

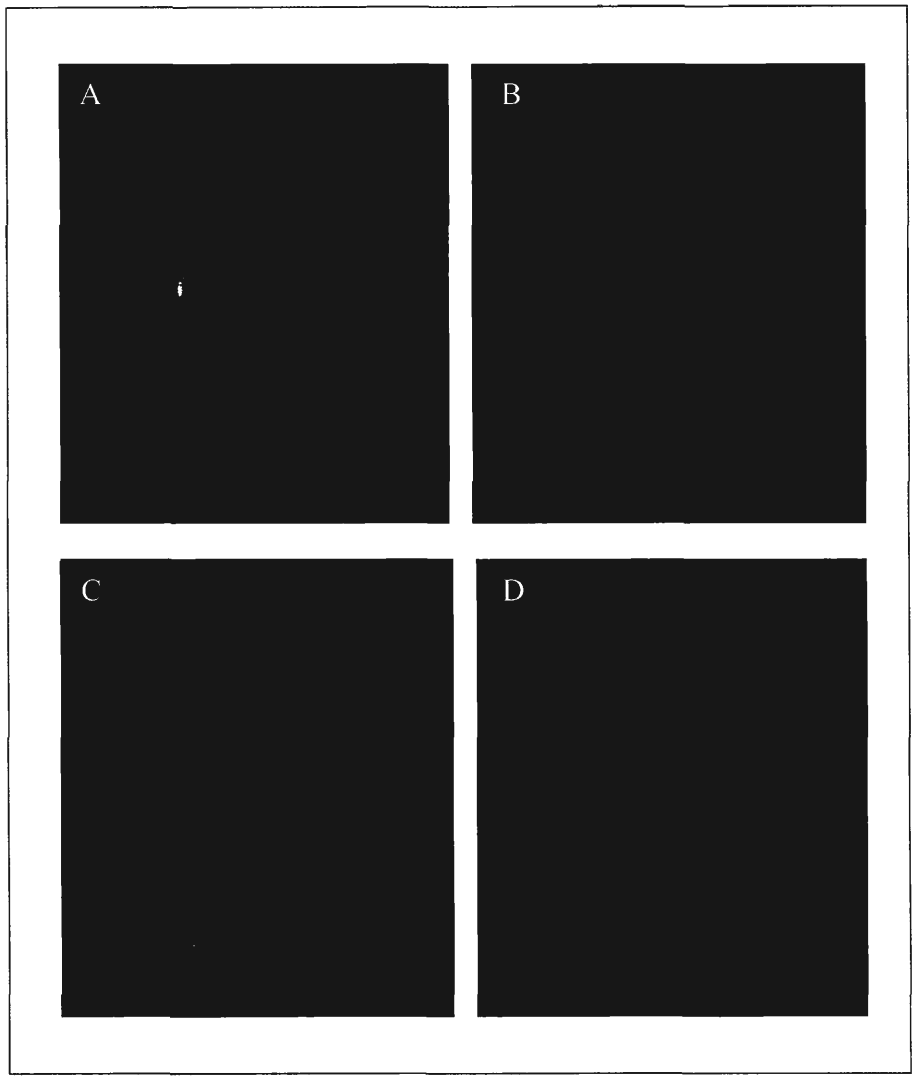
stenoses. This study suggested that the combination of hemodynamic compromise attributable to concurrent stenoses and artery-to -artery embolization is a common stroke mechanism in patients with concurrent stenoses. A more precise elucidation of the pathophysiological mechanisms leading to concurrent stenoses helps to plan future clinical trials for prevention and treatment of this disorder.

7.7 Strengths and limitations

The strength of this study included that, firstly, MRI and MRA which are safe and non-invasive imaging were used to evaluate stroke mechanism and vascular etiology. Secondly, a relatively large number of patients were recruited.

Limitations of this study included that, firstly, the samples were hospital-based and might not be representative of all patients with concurrent stenoses. Secondly, differentiating multiple pial infarcts from multiple BZ infarcts can sometimes be difficult. Thirdly, conventional cerebral angiogram which is the 'gold standard' to confirm the diagnosis of occlusive disease was not used. However, it would be impossible to conduct a large study with catheter conventional angiography because of the cost and risks associated with this procedure.

Figure 7.1 Diffusion-weighted images of different lesion patterns



A, Small perforating artery infarct; B, Internal borderzone infarct;

C, Pial infarcts; D, Multiple diffusion-weighted image lesions

Table 7.1 Basic Characteristics of the 251 patients

Characteristics	Total (N=251)	Vascular study results: Non-Concurrent stenosis[#] (N=142)	Vascular study results: Concurrent stenoses[##] (N=109)	p
Age, year +/-SD	68.0+/-11.3	66.2+/-11.9	70.2+/-10.0	0.005 *
Male sex, n (%)	142(57)	78(55)	64 (59)	0.55
Risk factors, n (%)				
Ischemic heart disease	16(6)	20(7)	6(6)	0.62
Diabetes	86(34)	44(31)	42(39)	0.21
Hypertension	171(68)	89(63)	82(75)	0.03*
Ever smoker	108(43)	60(42)	48(44)	0.78
Hyperlipidemia	146(68)	79(56)	67(62)	0.35
Prior stroke	42(17)	23(16)	19(18)	0.76
Prior TIA	7(3)	3(2)	4(4)	0.46
MRI findings, n (%)				
Presence of microbleeds	66(26)	37(26)	29(27)	0.92
Number of microbleeds +/- SD	1.2+/-3.4	1.2+/-3.6	1.1+/-3.0	0.88
Presence of hemorrhage	27(11)	15(11)	12(11)	0.91
Symptomatic stenosis	174(69)	82(58)	92(84)	0.0001*

* p=<0.05

[#] included patients with normal vessels, intracranial stenosis only and extracranial stenosis only
[##]included patients with stenoses in both intracranial and extracranial vessels

Table 7.2 Association between Ischemic Lesion Patterns and the Presence of Concurrent Stenoses

Lesion Patterns	Total (%) N= 251	Non-concurrent stenosis (%)[#] N= 142	Concurrent stenoses (%)[##] N= 109	p
Single				
1. Small PAI	110(44)	72(51)	38(35)	0 01[*]
2. Large PAI	15(6)	10(7)	5(5)	0.42
3. PI	4(2)	1(1)	3(3)	0.20
4. Large Territorial	10(4)	5(4)	5(5)	0.67
5. BZ	2(1)	2(1)	0(0)	0.21
Multiple				
6. PAI + PI	16(6)	7(5)	9(8)	0.29
7. PAI + PI + BZ	21(8)	6(4)	15(14)	0.007[*]
8. PAI + BZ	49(20)	25 (18)	24(22)	0.38
9. PI + PI	18(7)	11(8)	7(6)	0.69
10. PI + BZ	6(2)	3(2)	3(3)	0.74
Presence of multiple DWI lesions	113(45)	52(37)	58(53)	0.009[*]

[*] $p<0.05$

PAI indicates Perforating artery infarct; PI, Pial infarct; BZ, Borderzone infarct; Large territorial, Large cortical infarct.

[#] included patients with normal vessels, intracranial stenosis only and extracranial stenosis only
[##] included patients with stenoses in both intracranial and extracranial vessels

Table 7.3 Association between Vascular Territory of Stroke and the Presence of Concurrent Stenoses

Vascular Territory	Total (%) N= 251	Non-concurrent stenosis (%)[#] N= 142	Concurrent stenoses (%)[##] N= 109	p
1. Leptomeningeal branches of ACA	8(3)	5(4)	3(3)	0.73
2. Leptomeningeal branches of MCA	47(19)	19(13)	28(26)	0.01*
3. Leptomeningeal branches of PCA	27(11)	12(9)	15(14)	0.18
4. Basilar Artery	49(20)	30(21)	19(17)	0.46
5. Perforating branches of ACA	1(0.4)	0(0)	1(1)	0.25
6. Perforating branches of MCA	105(42)	67(47)	38(35)	0.05
7. Perforating branches of PCA	2(1)	2(1)	0(0)	0.21

* $p<0.05$

ACA indicates Anterior cerebral artery; MCA, Middle cerebral artery; PCA, Posterior cerebral artery.

[#] included patients with normal vessels, intracranial stenosis only and extracranial stenosis only
[##] included patients with stenoses in both intracranial and extracranial vessels

Table 7.4 Association between Ischemic Lesion Patterns and the presence of Tandem Lesions in Concurrent Stenoses

Lesion Patterns	Total (%) N= 109	Tandem lesions (%) # N= 83	Non-tandem lesions (%) ## N= 26	p
Single				
1. Small PAI	38(35)	19(23)	19(73)	< 0.0001*
2. Large PAI	5(5)	5(6)	0(0)	0.25
3. PI	3(3)	2(2)	1(4)	0.56
4. Large Territorial	5(5)	4(5)	1(4)	0.66
5. BZ	0(0)	0(0)	0(0)	NA
Multiple				
6. PAI + PI	9(8)	7(8)	2(8)	0.63
7. PAI + PI + BZ	15(14)	15(18)	0(0)	0.02*
8. PAI + BZ	24(22)	22 (27)	2(8)	0.04*
9. PI + PI	7(6)	6(7)	1(4)	0.54
10. PI + BZ	3(3)	3(4)	0(0)	0.44
Presence of multiple DWI lesions	58(53)	53(64)	5(19)	<0.0001*

* $p<0.05$

PAI indicates Perforating artery infarct; PI, Pial infarct; BZ, Borderzone infarct; Large territorial, Large cortical infarct; NA, not applicable.

included patients with stenoses in intracranial and extracranial vessels of the same vascular territories.
##included patients with stenoses in both intracranial and extracranial vessels of different vascular territories.

Chapter 8: Genetic Polymorphisms of Ischemic Stroke Patients with Concurrent Stenoses in Hong Kong (Study 4)

8.1 Abstract

Background and Purpose: The etiology of concurrent stenoses is poorly understood, and hereditary factors are believed to play important roles. This study aims to determine whether genetic polymorphisms affecting homocysteine and lipid metabolism are associated with concurrent stenoses.

Methods: Han Chinese stroke patients and controls in Hong Kong were genotyped for the following polymorphisms: Paraoxonase 1 (PON1) Q192R, methylenetetrahydrofolate reductase (MTHFR) A222V, glutamate-cysteine ligase catalytic-subunit (GCLC) -129C>T, and oxidized low-density lipoprotein receptor (OLR) 3' UTR C>T (rs1050283).

Results: A total of 191 patients with acute ischemic stroke were included, of whom 47 (25%) had concurrent stenoses. The genotype distributions of PON1 Q192R and MTHFR A222V, which affect lipid and homocysteine metabolism, were significantly different between patients with stroke and controls. The presence of at least one R allele in PON1

Q192R and TT allele in OLR rs1050283 were associated with concurrent stenoses. There was also a tendency toward association between the presence of at least one V allele in MTHFR A222V and concurrent stenoses.

Conclusions: This study showed that genetic polymorphisms affecting homocysteine and lipid metabolisms are possible risk factors for stroke and concurrent stenoses.

8.2 Introduction

Concurrent stenoses of extracranial and intracranial vessels is common, especially in Asians, where the incidence ranges from 10 to 39% in patients with symptomatic cerebrovascular disease.[407-409] The etiology of concurrent stenoses is poorly understood, but hereditary factors are believed to play important roles.[410] Given the difference in vessel involvement, concurrent stenoses may have different genetic polymorphisms from pure intracranial or extracranial stenosis. Both hyperhomocysteinemia and hyperlipidemia promote atherosclerosis[411, 412]. The objective of this study is to determine whether genetic polymorphisms affecting homocysteine and lipid metabolism are associated with concurrent stenoses. Paraoxonase 1 (PON1) Q192R, methylenetetrahydrofolate reductase (MTHFR) A222V, glutamate-cysteine ligase catalytic-subunit (GCLC) 129C>T, and oxidized low-density lipoprotein receptor (OLR) 3' UTR C>T (rs1050283) polymorphisms have been associated with cardiovascular disease and stroke[413-419], therefore we genotyped these polymorphisms in 167 control subjects and 191 stroke patients, of whom 47 had concurrent stenoses.

8.3 Methods

8.3.1 Study Design and Patients

This case-control study was conducted at a university hospital, the Prince of Wales Hospital, in Shatin, Hong Kong and was approved by the local ethics committee. Since most stroke patients in Shatin are treated at the Prince of Wales Hospital, our stroke unit

population is fairly representative of the general community. We recruited consecutive patients admitted with acute cerebral ischemia, including transient ischemic attack (TIA) and cerebral infarct. The following exclusion criteria were used: intracranial hemorrhage, pregnancy, unstable medical conditions, unfit for MRI study and claustrophobia. Controls were non-cardiovascular disease patients, such as those with migraine and Parkinson disease, who were unrelated to the cases. All cases and controls were ethnic Han Chinese.

All stroke patients underwent computer tomography (CT) and magnetic resonance imaging (MRI) of brain to establish the diagnosis of acute ischemic stroke, and vascular imaging including MRA of brain and carotid duplex to look for vascular stenosis.

8.3.2 MRI and MRA

All stroke patients were scanned within one week of symptom onset with a Siemens Sonata (Erlanger, Germany) with a head coil. The three-dimensional time-of-flight images were acquired with a repetition time of 40 ms, time to echo of 7.15 ms, flip angle of 25°, 20-cm field of view, 192x512 acquisition matrixes, and one signal average for a total imaging time of 5 minutes 35 seconds. Intracranial stenosis was graded according to the following criteria: grade 1, normal or mild stenosis (0% to 29% diameter stenosis); grade 2, moderate stenosis (30% to 69% diameter stenosis); grade 3, severe stenosis (70% to 100% diameter stenosis). The percentage of stenosis of MCA and other intracranial vessels was measured by visual inspection by one of the authors (W. Lam), who was blinded to the clinical findings.

8.3.3 Carotid Duplex

For the Duplex ultrasound examination of the extracranial carotid arteries, we used a Philips SD800 ultrasound machine and a 7.5-MHz transducer. Extracranial stenosis was graded as mild (30-50%), moderate (50-69%) and severe (70-99%). The diagnostic criteria for 70% stenosis of the internal carotid artery required a peak systolic velocity ratio of 2.4. The above diagnostic criteria in the neurovascular laboratory were based on laboratory references, which had a quality assurance program with supplementary angiographic studies[380].

Concurrent stenoses were defined as presence of more than 30% stenoses in both extracranial and intracranial vessels, including lesions in the same vascular territories (tandem lesions) and lesions in different vascular territories (non-tandem lesions).

8.3.4 Genetic study

We genotyped 4 polymorphisms: glutamate-cysteine ligase catalytic-subunit (GCLC) 129C>T, oxidized low-density lipoprotein receptor (OLR) 3' UTR C>T (rs1050283), paraoxnase 1 (PON1) Q192R, and methylenetetrahydrofolate reductase (MTHFR) A222V. All were genotyped by restriction fragment length polymorphism methods using the conditions described below:

A set of primers was used to amplify a 613-base pair (bp) fragment of the GCLC promoter by polymerase chain reaction (PCR). After restriction enzyme digestion, subjects with the CC genotype were identified by the presence of 500-, and 113-bp products on 3% agarose gel electrophoresis, while those with the CT genotype were identified by the presence of 500-, 302-, 198-, and 113-bp fragments; and those with the TT genotype were identified by the presence of 302-, 198-, and 113-bp fragments.

A set of mismatch-forward and reverse primers was used to amplify a 314-bp fragment of the OLR1 gene in order to establish a restriction enzyme site. The amplified fragment contains the C>T polymorphic site in the 3'UTR of the non-coding exon 6, 188-bp from the stop codon. After restriction enzyme digestion, the CC genotype was identified by the presence of a 314-bp band on 3% agarose gel electrophoresis, while the CT genotype was identified by 314- and 280-bp bands; the TT genotype was identified by a 280-bp band.

For PON1 genotyping[416], the Q192 allele remained uncut at 99 bp, while the R192 allele was cut.

For MTHFR genotyping[414], the A222 allele remained uncut at 245 bp, while the V222 allele was cut to 173 bp.

8.3.5 Statistical Analysis

All data were analyzed using SPSS software (version 16.0 for Windows). Age, blood pressure and lipid profiles were recorded as continuous variables. Sex, vascular risk factors and genetic polymorphisms were recorded as categorical variables. In univariate analysis, independent-sample t test and either χ^2 test or Fisher's exact test were used. P-values ≤ 0.05 (two-sided) were considered statistically significant. Where noted, the Bonferroni correction was used to adjust for multiple comparisons.

8.4 Results

A total of 191 stroke patients were recruited into the study. Among them, 55 (29%) had normal vessels (no stenosis), while the rest had at least some degree of stenosis (>0% diameter): 44 (23%) had intracranial stenosis only, 45 (24%) had extracranial stenosis only, 40 (21%) had concurrent intracranial and <70% extracranial stenoses, and 7 (4%) had concurrent intracranial and >70% extracranial stenoses. One hundred and sixty-seven control subjects without history of cardiovascular diseases were recruited. The baseline characteristics of patients and controls are summarized in Table 8.1.

The genotype distribution of PON1 Q192R was significantly different between patients with stroke and controls (χ^2=4.43, p=0.036) (Table 8.2). The presence of at least one R allele of PON1 Q192R was significantly associated with stroke (χ^2=5.96, p=0.015).

The genotype distribution of MTHFR A222V was significantly different between

patients with stroke and controls (χ^2=4.03, p=0.031). There was a significant association between the presence of at least one V allele of MTHFR A222V and stroke (χ^2=4.92, p=0.023).

There was no significant difference in genotype distribution of OLR rs1050283 and GCLC -129T between the two groups. Since four polymorphisms were analyzed, Bonferroni correction for multiple comparisons would require a significance level of 0.05/4=0.0125, a test which none of the above associations passed.

8.4.1 Concurrent stenoses and controls

There was no significant difference in genotype distribution of PON1 Q192R between patients with concurrent stenoses and controls (p=0.12) (Table 8.3). The presence of at least one R allele was significantly associated with concurrent stenoses (χ^2=7.34, p=0.007).

There was no significant difference in genotype distribution of MTHFR A222V between patients with concurrent stenoses and controls (p=0.22) (Table 8.3). There was a tendency toward association between the presence of at least one V allele and concurrent stenoses (χ^2=3.79, p=0.053).

The genotype distribution of OLR rs1050283 was no significantly different between patients with concurrent stenoses and controls (p=0.07) (Table 8.3). There was a

significant association between the presence of TT allele and concurrent stenoses (χ^2=4.03, p=0.015).

There was no significant difference in genotype distribution of GCLC -129C>T between patients with concurrent stenoses and controls (p=0.78) (Table 8.3).

One of the above associations remained significant after correction for multiple comparisons (p<0.0125): the presence of at least one R allele in PON1 Q192R between patients with concurrent stenoses and controls.

8.5 Discussion

Concurrent extracranial and intracranial stenoses are common in Asians, with the incidence ranging from 10 to 39% in patients with symptomatic cerebrovascular disease[407-409]. This is the first study to investigate the association of genetic polymorphisms with concurrent stenoses. In this study, we recruited 191 stroke patients and 167 controls of Han Chinese origin. Among them, 47 patients (25%) had concurrent stenoses. The genotype distributions of PON1 Q192R and MTHFR A222V, which affect lipid and homocysteine metabolism, were significantly different between patients with stroke and controls. The presence of at least one R allele in PON1 Q192R and TT allele in OLR rs1050283 were associated with concurrent stenoses. There was also a tendency toward association between the presence of at least one V allele in MTHFR A222V and concurrent stenoses.

The paraoxonase (PON) enzyme family is comprised of three members, PON1, PON2, and PON3, whose genes are located adjacent to each other on chromosome 7q21-22[420, 421]. The enzymes inhibit low-density lipoprotein oxidation [422]. Human PON1, 2, or 3 expression inhibits, or reverses, the development of atherosclerosis via mechanisms involving the reduction of oxidative stress, the promotion of cholesterol efflux from macrophages, and the normalization of vascular endothelium function.[423-425] The PON1 Q192R polymorphism modulates enzyme activity. Paraoxon hydrolytic activity is lowest in PON1 RR subjects, and this is associated with less protective capacity.[426] PON1 polymorphisms are associated with coronary heart disease[137, 427, 428] and stroke.[138]

The oxidized low-density lipoprotein receptor (OLR) gene is located on chromosome 12.[429] Binding of oxidized low-density lipoprotein to OLR increases expression of cellular adhesion molecules and inflammatory mediators and activation of pro-apoptotic pathways.[430, 431] OLR is found at high concentrations in human atherosclerotic lesions[429, 432], and individuals with the OLR 3' UTR T allele may have greater risk for coronary artery disease.[418, 433-435] The 3' UTR polymorphism affects binding of nuclear proteins and is in complete linkage disequilibrium with polymorphisms that affect OLR mRNA splicing.[415, 434] The T allele is associated with lower soluble LOX-1 plasma levels, with the lowest levels in individuals carrying the TT genotype and intermediate levels with the CT genotype.[418]

Methylenetetrahydrofolate reductase (MTHFR) is a cytosolic enzyme that catalyzes the conversion of methylenetetrahydrofolate to methyltetrahydrofolate, which serves as the carbon donor for methylation of homocysteine to methionine.[436] Polymorphisms in genes involved in homocysteine metabolism have been shown to associate with cardiovascular diseases[437], stroke, aneurysms[438, 439], and dissection.[440] Our study showed an association of the MTHFR A222V polymorphism with concurrent stenoses, and this may be related to the accumulation of asymmetric dimethylarginine[441, 442], which is a major endogenous inhibitor of nitric oxide and is strongly predictive of premature cardiovascular disease and death.[443]

A strength of this study is the relatively large number of patients with concurrent stenoses. The limitations include the lack of direct measurement of enzyme activities and homocysteine level. As with any other association study, future study to replicate the results in at least one other separate population is required before the results can be accepted unreservedly. The association between single nucleotide polymorphism and concurrent stenoses would make the statistical analysis less reliable. Future genome-wide association study is needed to define the genetic variations in Chinese patients with concurrent stenoses.

8.6 Conclusions

This study showed that genetic polymorphisms affecting homocysteine and lipid metabolisms are possible risk factors for stroke and concurrent stenoses. Our findings

provide important information for future treatment trials in this high-risk group of patients.

Table 8.1 Basic characteristics of stroke patients and controls

Characteristics	Total (N=358)	Controls (N=167)	Stroke patients (N=191)	p
Age, y (SD)	70.9(9.8)	71.8(6.8)	70.1(11.7)	0.12
Male sex, n (%)	183(51)	80(48)	103 (54)	0.26
Risk factors, n (%)				
Diabetes	76(21)	25(15)	51(27)	0.01*
Hypertension	203(57)	78(47)	125(65)	<0.001*
Mean systolic BP, mmHg (SD)	158.8 (27.9)	144.1(20.1)	166.4(28.3)	<0.001*
Mean diastolic BP, mmHg (SD)	81.2(17.7)	72.3(12.0)	85.8(18.4)	<0.001*
Mean total cholesterol, mmol/L (SD)	5.5(1.0)	5.5(0.9)	5.6(1.1)	0.51
Mean LDL, mmol/L (SD)	3.5(0.9)	3.5 (0.7)	3.5(0.9)	0.85
Mean TG, mmol/L (SD)	1.7 (1.0)	1.5(0.8)	1.8(1.0)	0.047*
Mean HDL, mmol/L (SD)	1.3(0.4)	1.3 (0.4)	1.4 (0.4)	0.19

BP, blood pressure; LDL, low-density lipoprotein; TG, triglycerides; HDL, high-density lipoprotein.
* $p<0.05$

Table 8.2 Genetic polymorphisms in patients with stroke and controls

Genotype	Stroke patients	Controls	p
PON 1 Q192R, n	191	162	0.036*
QQ, n (%)	22(11.5)	34(21.0)	
QR, n (%)	95(49.7)	75(46.3)	
RR, n (%)	74(38.7)	53 (32.7)	
MTHFR A222V, n	191	153	0.031*
AA, n (%)	101(52.9)	99 (64.7)	
AV, n (%)	73(38.2)	45(29.4)	
VV, n (%)	17 (8.9)	9 (5.9)	
GCLC -129C>T, n	189	162	0.48
CC, n (%)	150(79.4)	127(78.4)	
CT, n (%)	37(19.6)	27(17.9)	
TT, n (%)	2(1.1)	6(3.7)	
OLR1 rs1050283, n	190	163	0.59
CC, n (%)	108(56.8)	91(55.8)	
CT, n (%)	69(36.3)	68(41.7)	
TT, n (%)	13 (6.8)	4 (2.5)	

* $p<0.05$
PON 1, paraoxonase 1; MTHFR, methylenetetrahydrofolate reductase; GCLC, glutamate-cysteine ligase catalytic-subunit; OLR1, oxidized low-density lipoprotein receptor 1.

Table 8.3 Genetic polymorphisms in patients with concurrent stenoses and controls

Genotype	Concurrent stenoses	Controls	p
PON 1 Q192R, n	47	162	0.12
QQ, n (%)	2(4.3)	34(21.0)	
QR, n (%)	29(61.7)	75(46.3)	
RR, n (%)	16(34)	53 (32.7)	
MTHFR A222V, n	47	153	0.22
AA, n (%)	23(48.9)	99 (64.7)	
AV, n (%)	23(48.9)	45(29.4)	
VV, n (%)	1 (2.1)	9 (5.9)	
GCLC -129C>T, n	47	162	0.78
CC, n (%)	35(74.5)	127(78.4)	
CT, n (%)	11(23.4)	29(17.9)	
TT, n (%)	1(2.1)	6(3.7)	
OLR1 rs1050283, n	47	163	0.07
CC, n (%)	22(46.8)	91(55.8)	
CT, n (%)	20(42.6)	68(41.7)	
TT, n (%)	5 (10.6)	4 (2.5)	

* $p<0.05$
PON 1, paraoxonase 1; MTHFR, methylenetetrahydrofolate reductase; GCLC, glutamate-cysteine ligase catalytic-subunit; OLR1, oxidized low-density lipoprotein receptor 1.

PART III CONCLUSION

Chapter 9 Summary and Clinical Implications

The current population of China is 1.3 billion and it was estimated that 30% of the population will be aged 60 and above by 2050 in China. The incidence of stroke in China is one of the highest among the world and this burden is expected to escalate in the coming decades. Concurrent extracranial and intracranial stenoses are common in Chinese. However, studies of concurrent stenoses among Chinese are scarce. The series of studies that were presented in this thesis had addressed 4 important aspects of concurrent stenoses among Chinese: (1) long-term prognosis of patients with concurrent stenoses; (2) long-term prognosis of patients with concurrent stenoses and ischemic heart disease; (3) lesion pattern and stroke mechanisms in concurrent stenoses; and (4) genetic polymorphisms of ischemic stroke patients with concurrent stenoses. The key findings and clinical implications of the studies in each of this aspect are summarized as follow:

1. Predict long-term outcomes of ischemic stroke patients with concurrent stenoses (Study 1)

The objective of this study is to determine the long-term outcomes of patients with concurrent stenoses after acute stroke using MRA. Thirty percent of the stroke patients had concurrent stenoses. The overall 5-year cumulative rates of mortality, restroke and poor outcomes in patients with concurrent lesions were 31%, 41% and 51% respectively. The corresponding rates were 13%, 22%, and 31% in patients without concurrent lesions. The risks were highest in the first year after stroke. The presence of concurrent vascular lesions, advanced age, smoking, hyperlipidemia and previous history of stroke were independent predictors of poor outcomes. The long-term prognosis of ischemic stroke

patients with concurrent stenoses is poor. They are at high risks of further vascular event or death. Our findings provide important data for planning future randomized clinical trials for this high-risk group of stroke patients.

2. *Long-term outcomes of ischemic stroke patients with concurrent stenoses and ischemic heart disease (Study 2)*

Coexisting ischemic heart disease (IHD) is common in patients with stroke and it is associated with increased risk of cardiac death. This study aims to investigate the long-term outcomes of ischemic stroke patients with concurrent stenoses and IHD. Totally 428 patients were included. Fifty-four patients (13%) had ischemic heart disease. Among them, 27 patients (50%) had concurrent stenoses. In patients with concurrent stenoses and IHD, only 3 (11%) were free of death and recurrent vascular events. Eight (30%) had recurrent non-fatal stroke, 7 (26%) had non-fatal myocardial infarct, 11 (41%) died and 4 (22%) due to fatal myocardial infarct. The overall 5-year cumulative rates of mortality, recurrent vascular events and combined poor outcomes in patients with concurrent stenoses and IHD were 40%, 50% and 83% respectively. Ischemic stroke patients with concurrent stenoses and IHD had high risks of death and recurrent vascular events. Based on findings of current study, we suggested that future studies on aggressive medical therapy and early cardiac interventions in this high-risk group of stroke patients are warranted.

3. *Lesion patterns and stroke mechanisms in concurrent atherosclerosis of intracranial and extracranial vessels (Study 3)*

This study aimed to investigate the ischemic lesion patterns in concurrent stenoses using DWI, and to identify the mechanisms of stroke. Forty-three percent of the patients had concurrent stenoses. Concomitant PAI, PI and BZ infarcts, multiple DWI lesions and infarcts in the leptomeningeal branches of MCA were more common in patients with concurrent stenoses, especially those with tandem lesions. This study suggested that the combination of hemodynamic compromise attributable to concurrent stenoses and artery-to-artery embolization is a common stroke mechanism in these patients.

4. Genetic polymorphisms affecting homocysteine and lipid metabolism in ischemic stroke patients with concurrent stenoses (Study 4)

The etiology of concurrent stenoses is poorly understood and hereditary factors are believed to play important roles. The objective of this study is to determine whether genetic polymorphisms affecting homocysteine and lipid metabolism are associated with concurrent stenoses. Twenty-five percent of the patients had concurrent stenoses. The genotype distributions of PON1 Q192R and MTHFR A222V, which affect lipid and homocysteine metabolism, were significantly different between patients with stroke and controls. The presence of at least one R allele in PON1 Q192R and TT allele in OLR rs1050283 were associated with concurrent stenoses. There was also a tendency toward association between the presence of at least one V allele in MTHFR A222V and concurrent stenoses. This study showed that genetic polymorphisms affecting homocysteine and lipid metabolisms are possible risk factors for stroke and concurrent stenoses.

Chapter 10 Strengths and Limitations of the Studies

Most of the strengths and limitations of the studies had been mentioned already in the thesis. Some of the more important strengths and limitations of the studies will be highlighted here. First, the strength of our studies included that consecutive patients admitted to our acute stroke unit were being recruited into the studies. This reduces selection bias and hence increases the validity of our findings. Second, our cohort consisted of a large homogenous group of Chinese patients and the drop-out rate was low. Third, we used MRI, MRA and DWI in the evaluation of stroke and its vascular etiology. MRI with DWI is very sensitive in detecting acute infarct.

Limitations of the studies included that the samples were hospital-based and may not be representative of all patients with concurrent stenoses. Secondly, conventional cerebral angiogram which is the 'gold standard' to confirm the diagnosis of occlusive disease was not done in most of our patients. However, MRA and carotid duplex are acceptable alternatives since their role in detection of occlusive vascular lesions is well determined and they are safe and non-invasive. Thirdly, coronary angiogram was not done in all patients with IHD in Study 2 to confirm the diagnosis. However, it is difficult to have coronary angiogram in all patients especially those with renal impairment and poor premorbid states.

Chapter 11 Future Research Directions

The present studies have laid foundation for future researches in concurrent stenoses among Chinese population. Given the high risk of death and recurrent vascular events in patients with concurrent vascular lesions, early identification of the condition is important for prognosis and possible intervention.

11.1 Lipid lowering agents

In our study, hyperlipidemia is a strong predictor of death, restroke and poor combined outcome. The genetic polymorphisms affecting homocysteine and lipid metabolisms are possible risk factors for stroke and concurrent stenoses. More studies on the association on lipid profiles and concurrent vascular lesion as well as vascular outcomes are warranted. Study on aggressive treatment of hyperlipidemia in this group of patients is needed.

11.2 Intracranial stenting

Recent studies showed that stenting may reduce the risk of recurrent stroke in patients with moderate to severe intracranial stenosis[444, 445]. Patients with intracranial stenosis and concurrent extracranial stenoses especially those with symptomatic moderate to severe stenosis should constitute the target group for a future randomized trial comparing stenting with medical therapy.

11.3 Carotid Endarterectomy

Carotid endarterectomy is the definitive treatment in patients with > 70% carotid stenosis. Patients with < 70% extracranial stenosis and concurrent intracranial stenoses are at high risk of death and recurrent vascular events and should constitute the target group for a future randomized trial comparing carotid endarterectomy with medical therapy.

11.4 Screening and early cardiac intervention

Ischemic stroke patients with concurrent stenoses and ischemic heart disease had high risks of death and recurrent vascular events. Future studies on screening of ischemic heart disease in stroke patients, aggressive medical therapy and early cardiac interventions in this high-risk group of stroke patients are warranted.

It is my hope that findings of present studies and future research endeavors will contribute in reaching the ultimate goal of relieving the stroke burden upon individual and upon our aging community.

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