Prevention of Stroke: Risk Stratification and Targeted and Novel Therapies

A thesis by

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Declaration

The work described in this thesis was performed during my period as a Clinical Research Fellow in the University Division of Cardiovascular and Medical Sciences at the Western Infirmary, Glasgow.

The complex statistical analyses in chapter two were performed by Karen Lamb now from the Department of Statistics at the University of Strathclyde. All other statistical analyses were performed by me. Carotid and transcranial Doppler ultrasound scans were performed by Mrs Karen Shields, Mrs Linda Malcolm or I.

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This work has been presented at various national and international meetings including the European Stroke Congress in Brussels (2006), Glasgow (2007) and Stockholm (2009), the World Stroke Congress in Cape Town (2005), the International Stroke Congress in San Francisco (2007), the European Society of Hypertension in Milan (2007), and the Scottish Society Physicians in Peebles (2007).

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1. Dawson J, Walters MR. New and Emerging Treatments for Stroke. British Medical Bulletin 2006;77:87-102.
2. Dawson J, Walters MR. Uric Acid and Xanthine Oxidase: Future Therapeutic Targets in the Prevention of Cardiovascular Disease? British Journal of Clinical Pharmacology 2006;62:633-644.
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7. Quinn TJ, Dawson J, Lees KR. Acute stroke: we have the treatments and we have the evidence – we need to use them. Critical Care 2007;11:148.
8. Muir S, Harrow C, Dawson J, Lees KR, Weir CJ, Sattar N, Walters MR. Allopurinol Use Yields Potentially Beneficial Effects on Inflammatory Indices In Those With Recent Ischaemic Stroke; a Randomised, Double Blind Placebo Controlled Trial. Stroke 2008;39:3303-07.
9. Quinn TJ, Dawson J, Lees KR Past, present and future of Alteplase for ischaemic stroke. Expert Review of Neurotherapeutics 2008;8:181-192.

Chapter 2

10. Dawson J, Lamb E, Horvers M, Verrijth MJ, Lees KR, Walters MR. A Recognition Tool for Transient Ischaemic Attack. Quarterly Journal of Medicine 2009;102:43-49.

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11. Dawson J, Lees KR, Weir CJ, Quinn TJ, Ali M, Hennerici M, Walters MR, and the VISTA collaborators. Baseline Serum Urate and 90-Day Functional Outcomes Following Acute Ischemic Stroke. Cerebrovascular Diseases, in press.
12. Dawson J, Quinn TJ, Harrow C, Lees KR, Weir CJ, Cleland SJ, Walters MR. Allopurinol and nitric oxide activity in the cerebral circulation of those with diabetes. Diabetes Care 2009;32:135-37.

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1. Dawson J, Lees JR, Chang TP, Walters MR, Ali M, Davis SM, Diener HC, Lees KR. Relation Between 3-month Modified Rankin Scale Score, Duration Of Institutional Stay And Estimated Cost of Care. Poster presentation at the International Stroke Conference, San Francisco 2007. Abstract published in Stroke.
2. Muir S, Harrow C, Dawson J, Walters M. Effect of allopurinol on serum urate and inflammatory markers in patients with recent ischaemic stroke. Oral presentation at the European Association of Clinical Pharmacology, Amsterdam 2007.

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Chapter 3

4. Dawson J, Quinn TJ, Rafferty M, Lees KR, Ray G, Walters MR. Aspirin Resistance in Patients With Recent Stroke; a Case-Control Study. Oral presentation at the European Stroke Conference, Stockholm 2009.

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5. Dawson J, Nalci Y, Walters MR, D Hole. Sloan W, Radziwonik S, McInnes GT. Serum Uric Acid and Stroke in Hypertension: Influence of Gender. Oral presentation at the European Hypertension Society Meeting, Milan 2007. Poster Presentation at the World Stroke Congress, Cape Town 2006. Abstract published in International Journal of Stroke.

6. Dawson J, Quinn TJ, Harrow C, Lees KR, Walters MR. Allopurinol and the Cerebral Vasculature of Patients with Subcortical Stroke; a Randomised Trial. Oral presentation at the European Stroke Conference, Stockholm 2009.

7. Abbreviations

ACCORD – The action to control cardiovascular risk in diabetes trial

ACE Inhibitor – Angiotensin converting enzyme

ACTIVE - Atrial fibrillation clopidogrel trial with irbesartan for prevention of vascular events

ADVANCE – The action in diabetes and vascular disease trial

AF – Atrial fibrillation

AI – Augmentation index

ALLHAT - Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial

APTT – Activated partial thromboplastin time

ARB – Angiotensin receptor blocker

ARR – Absolute risk reduction

ARU – Aspirin responsive units

ASCOT – The Anglo-Scandinavian cardiac outcomes trial

AUC – Area under the curve

BMI – Body mass index

BNP – Brain natriuretic peptide

CAFÉ – The conduit artery function endpoint study

CAPRIE – The clopidogrel versus aspirin in patients at risk of ischaemic events study

CARESS - The clopidogrel and aspirin for reduction of emboli in symptomatic carotid stenosis trial

CAS – Carotid artery stent

CAVATAS – Carotid angioplasty and stenting in high-risk patients with severe symptomatic carotid stenosis study

CBF – Cerebral blood flow

CCB – Calcium channel antagonist

CEA – Carotid endarterectomy

CEPI – Collagen / Epinephrine

CHARISMA – The clopidogrel for high atherothrombotic risk and ischaemic stabilization, management and avoidance trial

CHD – Coronary heart disease

CHF – Congestive heart failure

COX – Cyclo-oxygenase

CPSS - Cincinnati pre hospital stroke scale

CREST – The carotid revascularization endarterectomy versus stenting trial

CRP – C reactive protein

CT – Computed tomography

CVD – Cerebrovascular disease

CVR – Cerebrovascular reactivity

DBP – Diastolic blood pressure

DM – Diabetes mellitus

ELISA – Enzyme-linked immunosorbent assay

ESPRIT – European/Australian stroke prevention in reversible ischaemia trial

ESPS 2 – The second European stroke prevention study

EVA-3s – Endarterectomy versus stenting in patients with symptomatic severe carotid stenosis

EXPRESS – The existing preventative strategies for stroke study

FAST - Face arm speech test

FBF – Forearm blood flow

G.P – General practitioner

GREACE – The Greek atorvastatin and coronary heart disease evaluation study

HPLC - High performance liquid chromatography

HPS – Heart protection study

HR – Hazard ratio

ICA – Internal carotid artery

ICH – Intracranial haemorrhage

IS – Internal standard

ISRCTN – International standard randomised controlled trial number

LAPSS - Los Angeles pre hospital stroke screen

LIFE – Losartan intervention for endpoint reduction in hypertension study

L-NMMA – NG-monomethyl-L-arginine

MASS - Melbourne ambulance stroke screen

MATCH – The aspirin and clopidogrel compared with clopidogrel alone after recent

ischaemic stroke of transient ischaemic attack in high-risk patients trial

MCA – Middle cerebral artery

MES – Microembolic signals

MI – Myocardial infarction

MOSES – The morbidity and mortality after stroke, eprosartan compared with nitrendipine for secondary prevention trial

MRI - Magnetic resonance imaging

mRs – Modified Rankin scale

NHANES – The national health and nutrition examination survey

NIHSS – National institute of health stroke scale

NNT – Number needed to treat

NO – Nitric oxide

NPV – Negative predictive value

OPT-CHF – The impact of oxypurinol in patient with symptomatic heart failure study

OR – Odds ratio

PFA-100 – Platelet Function Analyser 100

PIUMA – The progetto iperetensione Umbria monitiraggio ambulatorial study

PPV – Positive predictive value

PROactive – The prospective pioglitazone clinical trial in macrovascular events trial

PRoFESS – The prevention regimen for effectively avoiding second strokes trial

PROGRESS – The preventing stroke by lowering blood pressure in ischaemic stroke study

PT – Prothrombin time

PVD – Peripheral vascular disease

PWA – Pulse wave analysis

PWV – Pulse wave velocity

ROC – Receiver operating characteristic

ROS – Reactive oxygen species

ROSIER Scale – The recognition of stroke in the emergency room scale

RPFA – Rapid platelet function analyzer

RRR – Relative risk reduction

rt-PA – Tissue plasminogen activator

SAPPHIRE – The stenting and angioplasty with protection in patients at high risk for endarterectomy trial

SBP – Systolic blood pressure

SD – Standard deviation

SHEP – Systolic hypertension in the elderly program

sICAM – Soluble intercellular adhesion molecule

SITS – The safe implementation of thrombolysis in stroke monitoring study

SOD – Superoxide dismutase

SPACE – The stent-protected angioplasty versus carotid endarterectomy in symptomatic patient's trial

SPARCL – The stroke prevention by aggressive reduction of cholesterol trial

SPIRIT – The stroke prevention in reversible ischaemia trial

SSS – Scandinavian stroke scale

STICH – The surgical trial in intracerebral haemorrhage

TCD – Transcranial Doppler ultrasound

TEG – Thromboelastograph

TIA – Transient ischaemic attack

UK – United Kingdom

UKPDS – United Kingdom prospective diabetes study

VEGF – Vascular endothelial growth factor

VISTA – Virtual international stroke trials archive

WARSS – The warfarin and aspirin recurrent strokes study

WASID – The warfarin-aspirin symptomatic intracranial disease study

XO – Xanthine oxidase

Abstract

Stroke is a common disorder with dire consequences for the patient and for society and will increase in prevalence over the coming years. Following stroke, many patients unfortunately suffer a further stroke, and recurrent strokes account for approximately 25% of the total. Considerable scope therefore exists to improve both primary and secondary stroke prevention. This thesis has addressed several areas at key stages in the prevention of stroke by developing strategies to better identify those at highest risk, attempting to better target pre-existing anti-platelet therapy and by beginning the evaluation of xanthine oxidase reduction and uric acid lowering therapy in the prevention of stroke.

A clinical scoring system to aid diagnostic recognition in those with suspected transient ischaemic attack (TIA) was successfully developed and has the ability to reduce the referral of those without cerebrovascular disease to busy TIA clinics. The score was developed on data from 3216 patients and included 9 clinically useful predictive variables. After adjustment to reflect the greater seriousness of missing true TIA patients, 97% of TIA and 22% of non-TIA patients were accurately identified. The results were confirmed during prospective validation. Use of the score could have a substantial effect on waiting times for assessment; there is potential double the numbers seen within the timeframe recommended by guidelines with no other change to services. This would be an important advance given the recent evidence that rapid assessment and treatment of those with TIA greatly reduces stroke risk.

Aspirin resistance was found to be higher in those with cerebrovascular microembolic signals (MES) and carotid disease compared to those with equivalent carotid disease and no

MES. This study included sixty-two patients who mostly had symptomatic carotid disease. Approximately a quarter had MES. The rate of aspirin resistance on at least one test was 25.8% (16 patients), with 13 (21%) resistant on PFA-100 testing, 8 (12.9%) using the Verify-Now system and 5 (8.1%) resistant on both. Aspirin resistance was more common in patients with MES (50% compared to 17.4% without, p=0.018 on Fishers exact test). This provides a link to a well established and robust surrogate marker of outcome and thus a useful model to further study the benefits of guided anti-platelet strategies. An interventional clinical anti-platelet trial based upon individual aspirin responsiveness in high risk individuals such as those with MES is now warranted. Aspirin resistance was also confirmed to be a common phenomenon in a case-control study of 180 patients. It was present in 34% of those with recent stroke and in 18% of those with risk factors but no established disease. However, the role of poor compliance with therapy as a cause in a substantial number of cases was established; it accounted for approximately half of those labelled resistant in the stroke group. Further, when only those with objective evidence of recent aspirin ingestion were considered, the prevalence of aspirin resistance was similar in both groups (at 26%). This suggests that objective measures to confirm compliance with aspirin therapy should be mandatory in future studies of aspirin resistance.

Increasing serum uric acid was found to be a predictor of poor functional outcome following acute stroke but not in an independent fashion. In total, 852 patients were included in this study and greater uric acid levels were associated with increased odds of poor outcome on univariate but not multivariate analysis (OR 1.3, 95% CI 0.73-2.31). However, there was no evidence of an association with favourable outcome as other groups have found. Increasing serum uric acid was also shown to be predictive of increased risk of stroke, total, vascular and coronary mortality in treated hypertensive patients but

interestingly, the relationship between stroke mortality and serum uric acid appears J-shaped and most apparent in females.

A study of the use of allopurinol in those with diabetes showed that xanthine oxidase inhibition improves cerebral nitric oxide bioavailability suggesting a beneficial effect of allopurinol on cerebrovascular health. This study included 14 participants who had impaired baseline cerebrovascular nitric oxide bioavailability. Allopurinol led to a significant improvement in responses to NG-monomethyl-L-arginine (L-NMMA) when compared to placebo ($p=0.032$, median improvement in ICA flow reduction following L-NMMA of 3144 (95% CI 375 to 7143)) mls). L-NMMA is an inhibitor of endothelial nitric oxide synthase which reduces cerebral blood flow in healthy volunteers; the bigger the reduction, the greater the endothelial health. However, a study of the effect of allopurinol treatment on cerebrovascular reactivity (as measured by response to acetazolamide infusion) in a group of patients with recent subcortical stroke revealed no positive effect. Cerebrovascular reactivity was unchanged by treatment with allopurinol. This raises interesting questions regarding the longevity of any positive effect of allopurinol as this, and other studies of 3 month duration, have revealed no benefit. Further, subjects in this study did not, on balance, have elevated serum uric acid and it has recently been suggested that only those with significantly elevated levels benefit in the setting of congestive cardiac failure. Whether this is also true in those with stroke also requires to be clarified. A large study of the effect of allopurinol on carotid intima-media thickness, a robust and modifiable marker of vascular risk, in those with recent stroke is planned to address these questions.

The studies in this thesis therefore include a number of pragmatic findings which could improve care at all stages in the prevention of stroke. The TIA scoring system could

improve recognition of the high risk condition TIA, a useful model has been developed in which to study a population of patients truly resistant to aspirin and important lessons have been learned to aid further evaluation of xanthine oxidase inhibition; a promising therapy for the prevention of stroke.

CHAPTER ONE

INTRODUCTION

A stroke is defined by the World Health Organization (WHO) as a syndrome of "rapidly developing clinical symptoms and/or signs of focal (or at times global) disturbance of cerebral function lasting more than 24 hours (unless interrupted by surgery or death), with no apparent cause other than of vascular origin." The majority are ischaemic, secondary to arterial occlusion by in-situ thrombus or embolus (1), with the remainder being due to intracerebral haemorrhage or sub-arachnoid haemorrhage.

1.01 Burden of Stroke / Recurrent Stroke

The WHO estimates that 17 million people die annually from heart disease and stroke. In the United Kingdom, stroke remains the single biggest cause of major disability (2) and is the third leading cause of death. The condition has a profound effect on patients and relatives and is associated with a vast economic burden (2;3). In the United Kingdom and other countries, these costs are on the rise and consistently consume around 5% of health care resources (4), costing the NHS and wider economy approximately £7 billion per year (5).

The incidence of stroke appears to be similar or perhaps higher in Scotland than in other parts of the United Kingdom and in other European and Western countries (6-11). The Scottish Borders Stroke Study (11) attempted to ascertain all stroke events in this area over a two year period (from 1998-2000). The crude annual incidence rate per 100,000 per year was 280 (95% CI 258-304) for all stroke subtypes with 197 (95% CI 179–217) due to cerebral infarction, 24 (95% CI 17–31) due to intracerebral haemorrhage, 11 (95% CI 7–16) due to subarachnoid haemorrhage and 49 (95% CI 40–59) of undetermined subtype. Incidence rates were higher in males and increased dramatically with age, particularly

above the age of 65 years, who accounted for 80% of the first ever in a lifetime strokes. In total, 790 strokes occurred during the study, 194 (24.7%) of which were recurrent strokes.

The MONICA Project provides further extensive data on stroke incidence, case fatality and mortality rates across 16 European countries and China during the period 1985-87. The incidences of first-ever and recurrent stroke were greater among men than women in all populations. The incidence (age standardized) of stroke was higher in China than in West or Central Europe apart from in Finland (for example, incidence was 242 per 100,000 in China compared with 361 in Kuopio, Finland and 130 per 100,000 in Göteborg, Sweden) (12). Rates were higher in Eastern Europe than in West or central Europe. Approximately 20% of stroke events recorded in the MONICA project were recurrent events. The incidence of recurrent stroke was highest in men and women from Beijing and men in Russia (Novosibirsk) but was lowest in Sweden (12).

Stroke is, of course, a global problem and that the incidence and impact of stroke differs among ethnic groups (13). The majority of stroke deaths occur in low and middle income countries and among non-Western populations (14) and Black, Chinese, South Asian and Japanese populations have a higher incidence of stroke compared with Caucasians (2). In some Asian populations (excluding India), the burden of cerebrovascular disease exceeds that of coronary heart disease (CHD). In China, for example, stroke is the second leading cause of death in urban areas (15) and it is estimated that three times as many Asian people die from stroke than from CHD (16).

Trends in stroke incidence (age standardised) were also evaluated in the MONICA cohort (between 1985 and 1990). Incidence fell in most MONICA centres except in several

Eastern European regions (17;18). Other studies also support the notion that age standardised stroke rates and mortality are decreasing (19;20). However, the age of the population in most developed nations, including the UK is projected to increase considerably in the next half century – the proportion of individuals aged over 65, who account for approximately 80% of strokes, is likely to increase by near two-fold (from 20 to 35%) (21). It is estimated that the number of individuals in the UK aged over 65 years will increase from approximately 9,684,000 to 16,528,000 from 2005 to 2050. The number of stroke events will therefore increase. Even if age standardised rates of stroke remain stable or decrease slightly it is projected that the incidence of stroke per annum in the European region will rise by over 30% in the next 25 years (10).

It is of interest that those who have suffered stroke have a higher risk of recurrent stroke than of myocardial infarction (MI), whereas the opposite applies for those who have suffered MI. Data from large scale observational studies suggest that nearly 80% of recurrent vascular events in those with previous stroke are stroke and that only 20% are MI (22). In the Cardiovascular Health Study, the stroke rate was double that of MI in those whose index event was stroke (23), whereas in the CAPRIE study it was 7 times higher (24). Approximately 25% of strokes are recurrent events (25) and approximately 25% of stroke patients will suffer recurrence within 5 years. The risk of recurrence may be highest early, with approximately 4% suffering recurrence within one month and 12% at one year (26). Thereafter, the risk of recurrence after one year has been estimated at 4-5% per annum (27) and while risk may be highest early, the majority of recurrent strokes do of course occur after one year (28).

It is important also to consider the incidence of transient ischaemic attack (TIA). While many now regard stroke and TIA as one condition, they are currently managed in a different fashion in the UK and beyond. A TIA is an episode of temporary and focal neurological symptoms caused by cerebral artery occlusion, with symptom resolution in less than 24 hours (29). Patients with a recent transient ischaemic attack (TIA) or minor stroke require rapid investigation and treatment initiation to minimize the risk of future vascular events, and in particular, the early risk of stroke. Between 15 and 30% of patients with stroke give a history of preceding TIA (30) and the seven day risk of stroke following TIA may exceed 30% in high risk groups (31) and risk is highest in the first 48 hours (32). These patients represent a group at extremely high risk of stroke and accordingly, guidelines recommend that patients are assessed and investigated within 1 week (33;34) and that fast track or rapid access neurovascular clinics are established (35).

Data from the Oxford Community Stroke Project suggested a crude annual incidence of TIA of 35 per 100,000 population per year in a southern English population, with a slightly higher age and sex standardized rate. Other studies have revealed age and sex standardized rates of 68 and 83 per 100,000 per year (36;37) and more recent data from Oxfordshire showed a higher incidence than previously (38), perhaps reflecting better recognition of TIA.

Summary of the Burden of Stroke

Stroke is clearly a common disorder with dire consequences for the patient and for society and will increase in prevalence over the coming years. Following stroke, many suffer a further stroke, and recurrent strokes account for approximately 25% of the total. Considerable scope therefore exists to improve both primary and secondary stroke

prevention. TIA is also common and represents a high risk condition which must be addressed as an emergency. Those with TIA represent an excellent group in which to study new treatments and care strategies given their high risk of events.

1.02 Treatment of Acute Stroke

1.02.1 Therapeutic Targets

Infarct volume increases in the first few hours after onset of ischaemic stroke, with the ischaemic penumbra gradually being subsumed into the area of infarction. The penumbra is the region of brain where blood supply is significantly reduced but energy metabolism is maintained because of collateral flow (39). Its viability depends on the severity and duration of ischaemia and, if blood flow is swiftly restored, some penumbral tissue may be saved. However, during the time lag to reperfusion, a physiological cascade occurs which places penumbral tissue at further risk. Cellular metabolism becomes anaerobic leading to acidosis and sodium-potassium transporters become dysfunctional causing a rise in intracellular osmolarity and cytotoxic oedema. Intracellular calcium increases and ultimately the process becomes self-perpetuating with further rises in intracellular calcium, possibly because of release of intracellular glutamate (40). Increased calcium concentration within cells enhances injury through activation of lipase, protease and free radical generation. Blood brain barrier integrity is reduced (41) and if breached, blood components can enter the interstitial space causing vasogenic oedema and increasing the risk of haemorrhagic transformation of infarcted tissue. Furthermore, even if reperfusion occurs, the penumbra is vulnerable to the effects of reperfusion injury (42) as further free radical formation and release of harmful neurotransmitters may be provoked. It has been estimated

that up to 2 million neurons are lost each minute from stroke onset from this area every minute that treatment is delayed (43).

In the context of intracerebral haemorrhage, it has recently been recognised that early haemorrhage growth occurs in a significant number of patients (44;45). In a study of 103 patients, all of whom presented within three hours of onset and underwent serial brain imaging with x-ray computed tomography (CT), substantial haemorrhage growth occurred in 28% of patients (45). Haemorrhage growth was defined as a 33% increase in volume, occurred particularly within the first hour, and was associated with clinical deterioration. Haemorrhage growth may be increased in those with hypertension although data do conflict (46;47).

Thus, for both ischaemic and haemorrhagic stroke there are therapeutic targets, which are likely to exist only in the early hours after stroke. This of course necessitates rapid assessment and investigation. However, the National Sentinel audit (5) (of England and Wales) found that only 30% of those suspected stroke patients who were deemed to require urgent brain imaging (within 30 minutes) received a scan on the same day. Also, many ambulance services do not treat potential stroke patients as the highest category of emergency. Reducing delays to assessment and imaging to increase the numbers presenting within the first hours after onset of stroke is therefore crucial and will have a significant impact on patient outcomes. Such delays are multi-factorial and will require comprehensive strategies to increase public awareness and improve ambulance, emergency medicine and radiology services, whilst ensuring all patients have equity of access to stroke unit care.

1.02.2 Current Management Strategies

Once a patient has arrived at hospital and received a diagnosis of acute stroke, management serves a dual purpose; to reduce the high risk of recurrent stroke and to reduce the burden of disability in established stroke. Secondary preventative measures continue to improve and will be outlined later. Acute treatment of stroke involves control of physiological variables, strategies to reperfuse the ischaemic area, measures to reduce growth of primary intracerebral haemorrhage (haemostatic therapy, currently unproven), protection of the vulnerable, yet salvageable, ischaemic penumbra (neuroprotectant therapy, currently unproven) and surgery. All patients should be cared for in a dedicated acute stroke unit, which in itself saves lives and significantly improves functional outcomes (48). By definition, a stroke unit is an area and environment of organised and multidisciplinary care which ensures access to specialist medical, nursing and allied staff and treatment.

All patients with suspected acute stroke or TIA should undergo emergency brain imaging to help confirm the diagnosis, distinguish infarct from haemorrhage and to identify important differential diagnoses such as tumour. The choice of brain imaging modality lies between computed tomography (CT) and magnetic resonance imaging (MRI). An urgent brain scan enables rapid differentiation of ischaemic from haemorrhagic stroke and there is no rationale for waiting to image patients – it delays treatment initiation and is the least cost effective approach (49). A non-contrast CT brain is the most widely available test and has excellent sensitivity for identification of haemorrhage early after onset, while MRI has similar sensitivity for identification of haemorrhage and significantly greater sensitivity for detection of ischaemia (50). Newer CT and MRI based techniques are available which have the potential to increase both diagnostic sensitivity and specificity and increase the numbers of patients receiving treatments such as thrombolysis (51).

1.02.3 Control of Physiological Variables

Arterial hypertension occurs in as many as 80% of patients following acute stroke (52). It is associated with a poor outcome (53) but it may represent a protective response - falls in blood pressure may lead to infarct extension because of impaired cerebrovascular autoregulation following acute stroke (54). There is some evidence that a U-shaped relationship exists with arterial hypotension also being associated with a poor outcome (52). Such uncertainty also surrounds intracerebral haemorrhage where hypertension may contribute to haemorrhage growth. Current guidelines suggest lowering blood pressure in the presence of hypertensive encephalopathy, aortic dissection, severe cardiac failure and if blood pressure is extremely high on repeated measurement (>220 mmHg systolic or > 120 mmHg diastolic blood pressure) (55). This leaves a great deal of uncertainty in the majority of patients and it is unclear whether prior antihypertensive therapy should be discontinued in the acute phase and at what thresholds of blood pressure we should intervene and the treatment targets that we should aim for. Fortunately, several large clinical trials are now underway and should address these issues (Controlling Hypertension and Hypotension Immediately Post-Stroke Trial [CHHIPS trial] (56), Continue or Stop post-Stroke Antihypertensives Collaborative Study [COSSACS] (57) and Efficacy of Nitric Oxide in Stroke Trial [ENOS trial] (58)).

Elevated blood glucose is common in the acute phase following ischaemic stroke (59) and is associated with a poor outcome (60), regardless of the presence of pre-existing diabetes. In a recent systematic review of non-diabetic patients, stress hyperglycaemia defined as blood glucose of greater than 6 or 7.1 mmol/l was strongly predictive of increased hospital mortality (RR 3.28, 95% CI 2.32-4.64) and poor functional outcome (RR 1.41, 95% CI 1.16-1.73) (59). This may be because elevated blood glucose increases brain lactate

production, which is associated with increased infarct size (61), may reduce the efficacy of thrombolytic therapy (62;63) and may increase the risk of haemorrhagic transformation of infarcted tissue. However, whether routine lowering of hyperglycaemia with insulin improves outcome after acute stroke is as yet unproven. The recent GIST-UK (Glucose Insulin in Stroke) trial (64) enrolled 933 patients and found no evidence of benefit for insulin therapy in terms of mortality (OR 1.14, 95% CI 0.86 to 1.51) or secondary outcomes. Significantly lower levels of both blood pressure and glucose levels were seen following treatment although it is likely the trial was underpowered and further randomized controlled trials are required. At present, current guidelines suggest insulin titration in those with serum glucose of > 10 mmol/l (55).

Fever has also been associated with a poor outcome following acute stroke (65), possibly because of a detrimental effect on intracerebral metabolism, increased free radical production (66) or changes in blood brain barrier function (67). Guidelines suggest the use of fanning and paracetamol if temperature rises above 37.5°C and that this should prompt a search for infection (55). It is again unclear whether this improves clinical outcome but trials are in progress to evaluate this (68).

1.02.4 Reperfusion Strategies for Acute Ischaemic Stroke

Intravenous Thrombolytic Therapy

Thrombolytic therapy with tissue plasminogen activator (rt-PA) is the only licensed treatment for acute ischaemic stroke in Europe where it must be administered within three hours of symptom onset. First evidence of efficacy was published in 1995 and license was granted for use in the United States of America in 1997. In Europe, however, a conditional license was first granted in 2002 and uptake has remained disappointingly slow.

Approximately two thirds of otherwise eligible patients miss out on treatment because of delay in presentation and/or early misdiagnosis (69). The United Kingdom performs particularly badly and currently holds 15th place in the European league table of thrombolysis use; only around 0.2% of stroke patients were treated in 2005 and access to treatment across the nation is patchy (70).

Intravenous thrombolytic therapy is clearly beneficial as shown in a recent pooled meta-analysis of the major thrombolysis studies (71). The analysis considered 2775 patients treated within six hours of ictus. The odds of favourable outcome (defined as no disability) were 2.8 (95% CI 1.8-9.5) for treatment within 90 minutes and 1.6 (95% CI 1.1-2.2) for treatment between 91 and 180 minutes. Benefit was still apparent for patients treated between 181 and 270 minutes (odds ratio 1.4, 95% CI 1.1-1.9). The rate of significant intracerebral haemorrhage was 5.9% in those treated with rt-PA compared to 1.1% in those treated with placebo. However, only a small number of these were of clinical significance, and this risk of haemorrhage is already accounted for in the calculation of odds of favourable outcome. Thus, the chance of being free of handicap after stroke is increased nearly 3 fold by thrombolytic treatment provided it is administered within 90 minutes of onset. Smaller but still significant benefits are seen up to 4.5 hours. The number needed to treat (NNT) to achieve an excellent outcome (and avert one case of death or dependency) following treatment is approximately 7 (71) while the NNT to achieve a reduction in disability is approximately 3 (72). The number needed to harm is 30 and these figures compare favourably to other interventions such as thrombolysis for acute MI.

A rigorous audit of outcomes in several thousand European patients (The Safe Implementation of Thrombolysis in Stroke-Monitoring Study (73)) treated within license

and in routine clinical practice has confirmed that safety is at least as good as in clinical trials, with low rates of symptomatic significant haemorrhage. Efficacy also appears to be similar (table 1.1). The risk of haemorrhage should not deter use of thrombolytic therapy but precautions must be taken to minimise its risk. The factors associated with an increased risk of haemorrhage are increasing age, extensive early infarct change on brain imaging, diabetes mellitus (DM), elevated blood glucose, DM and a history of previous stroke, and a low platelet count (74). Those with higher baseline stroke severity may also have a higher risk of haemorrhage but equally, may derive greatest benefit from treatment. Exclusion criteria are similar to those for thrombolysis in myocardial infarction, although patients with a blood pressure of >180/110 mmHg, or those who require treatment to attain a satisfactory blood pressure, are generally not treated. There are also concerns regarding its use in those with DM and a history of previous stroke. Mild systemic bleeding can also occur and there is a risk of angio-oedema of approximately 1%, which is typically mild (75).

	Mortality (at 3/12)	**Independent** (at 3/12)	**S-ICH** (Per SITS-MOST)*	**S-ICH** †
Trials	17.3%	49%	N/A	8.6%
SITS-MOST	11.3%	54.8%	1.7%	7.3%
Placebo	18.4%	30.2%	N/A	1.9%
* Bleed large enough to cause symptoms and accompanying neurological deterioration. † Any bleed with any alteration in neurological status regardless of severity.				
Table 1.1. Summary of outcomes in thrombolysis studies and the SITS register				

Very recent data show that intravenous thrombolytic therapy delivered within 3 to 4.5 hours of onset but otherwise within the terms of the license is also effective and has a similar

safety profile to treatment within 3 hours of onset. This was tested in a recent randomized, placebo controlled double blind trial, the ECASS III study (76). It included 821 participants with presumed ischaemic stroke and revealed an increase in the odds of an excellent outcome (OR 1.34, 1.02 to 1.76, p=0.04). The rate of symptomatic intracerebral haemorrhage was 2.4 % in the treatment and 0.2% in the placebo group (p=0.008).

Intra-arterial Thrombolysis / Mechanical Reperfusion

These therapies are not widely available in the UK. Intra-arterial thrombolysis involves direct catheterisation of an occluded artery and local administration of thrombolytic agents (rt-PA or urokinase (not available in UK)). This appears to be an effective therapy for confirmed middle cerebral artery (MCA) or basilar artery occlusion. For example, in the PROACT II (Prolyse in Acute Cerebral Thromboembolism) trial (77), where treatment was initiated within 6 hours of onset, reperfusion rates in those with confirmed MCA occlusion were significantly higher following intra-arterial thrombolysis with urokinase and intravenous heparin (66% compared to 18% in heparin treated controls, p<0.001). Clinical outcomes were also significantly improved with more patients living independently at day 90 (40% compared to 25% of controls, p=0.043, relative risk reduction 58%).

Basilar artery occlusion carries a grave prognosis with a high mortality rate – perhaps in excess of 70%. While randomised controlled trial evidence are lacking, several case series have been published which suggest reduced mortality rate following intra-arterial thrombolysis (78;79). However, a recent systematic analysis suggested reperfusion rates and clinical outcomes were similar with both intra-arterial and intravenous treatment emphasising the need for administration of either form of thrombolysis in this devastating condition (79).

Catheter based techniques may allow lower systemic doses of thrombolytic agents to be used but also the use of mechanical clot disruption and retrieval. The later approach would be a particular advantage in those with a significant haemorrhage risk or in those unsuitable for intravenous rt-PA. As yet, no randomized data confirm efficacy of mechanical embolectomy. However, in a study of 151 patients with ischaemic stroke and any treatable intracranial or extracranial artery occlusion who were deemed unsuitable for rt-PA (80;81) presenting within 8 hours of onset, a high rate of vascular recanalisation was seen (46% compared to 18% in a historical control sample). Those who exhibit recanalisaton also have better outcomes than would be expected for their baseline stroke severity (81). These strategies therefore represent a real alternative for those with major stroke who are unsuitable for rt-PA but the limited availability and need for specialist neuro-radiology staff may hinder introduction of these techniques into routine practice.

Trials are ongoing to assess efficacy of thrombolytic therapy in ischaemic stroke up-to 6 hours after onset of symptoms (82) and to establish whether newer imaging techniques can better guide thrombolytic therapy and perhaps allow treatment up to 9 hours after onset in selected patients. Results of small phase 2 studies of this later approach have been conflicting (83-85), although it remains a promising strategy.

1.02.5 Aspirin Treatment

Patients with ischaemic stroke should be treated with aspirin. The effect size is modest but important; a combined analysis of the CAST (86) and IST (87) revealed 9 fewer deaths or fatal strokes for every for every 1000 patients treated with aspirin in the acute phase (88). If

patients receive thrombolytic treatment, it is generally advised that aspirin should be deferred for 24 hours.

1.02.6 Anticoagulant Therapy

Early anticoagulation has not been shown to yield benefit early after stroke. In a meta-analysis of 22 trials, anticoagulation within 24-48 hours after ischaemic stroke onset led to a reduction in early recurrent stroke (OR 0.76, 95% CI 0.65 to 0.88) which was offset by an increase (of similar magnitude) in the number of symptomatic intracranial haemorrhages (OR 2.52, 95% CI 1.92 to 3.3) (89). Very early treatment with unfractionated heparin has been linked with better functional outcome and lower early recurrence rate (90;91) but again at the cost of increased haemorrhagic complications. Early anticoagulation is therefore not recommended as a treatment for acute ischaemic stroke (55) although is recommended by some for those with atrial fibrillation (92).

1.02.7 Neuroprotectant Strategies

Neuroprotectant drugs aim to save ischaemic brain tissue by damping down the potentially harmful molecular processes in the penumbra. This may prolong the life of the penumbra, maintain blood brain barrier integrity, reduce oedema and haemorrhagic transformation, and reduce reperfusion injury. Such strategies may also be of benefit in the peri-haemorrhage region in intracerebral haemorrhage. Unfortunately, no neuroprotectant strategy has yet been proven effective in phase III clinical trials or reached clinical practice, although at the time of writing several agents are the subject of clinical trials (93).

1.02.8 Therapeutic Hypothermia

Therapeutic hypothermia is arguably the most promising novel therapy for acute stroke (94). There are several mechanisms by which hypothermia may convey a neuroprotectant mechanism. It will decrease cellular metabolism (95), limit cytotoxic and excitatory cascades, reduce free radical formation and suppress blood brain barrier breakdown (96). Mechanisms of cooling include surface cooling which, while able to attain reductions in temperature (97), has disadvantages of patient discomfort and may necessitate sedation and paralysis. The preferable, yet more invasive approach, is to use intravascular cooling via a central venous heat exchange catheter and infusion of cold saline (98). It also allows greater control over rewarming, which may be associated with increases in intracranial pressure if performed rapidly (99).

A wealth of data show therapeutic hypothermia is effective in animal models of cerebral ischaemia, particularly with transient ischaemia where cooling was initiated within one hour (100-102) Therefore, like thrombolytic therapy, it is likely that therapeutic hypothermia will only prove effective if initiated early after onset and in patients with reperfusion (either spontaneous or iatrogenic).

Evidence of efficacy in humans is lacking, although a recent case report (103) and some human feasibility studies (104) provide prima facie evidence of efficacy. Further research is required.

1.02.9 Specific Treatment for Intracerebral Haemorrhage

Supportive treatment is indicated as for all types of stroke. Approximately 15% of cases of intracerebral haemorrhage are associated with warfarin. These patients have a higher risk of

death and disability (105) and require rapid reversal of anticoagulation (106). No specific licensed treatment exists for the majority with spontaneous intracerebral haemorrhage. Recombinant activated factor VII (rFVIIa) is a licensed treatment for bleeding in haemophiliacs who are resistant to factor VIII replacement and is a powerful initiator of haemostasis even in patients with normal coagulation. It is also utilized in the setting of major haemorrhage (107) and peri-operative bleeding (108). Adminstration early after stroke limits early haemorrhage growth (109;110) but has not been proven to improve clinical outcome (110). Initial data in this regard were highly encouraging when, in a study of 399 patients (109), mortality was reduced in the treatment group and the odds of improvement in disability in survivors was nearly doubled. Unfortunately a larger phase III trial did not confirm these benefits (110). This lack of efficacy may relate to randomization imbalances in terms of poor prognostic factors such as intraventricular haemorrhage rate (111;112) and this does remain a promising strategy. A minimal increase in risk of thromboembolic events was seen in these studies.

1.02.10 Surgical Treatment of Acute Stroke

Complete MCA infarction is associated with brain oedema, increased intracranial pressure and a risk of transtentorial herniation and death. Medical therapy does little to improve mortality rates, which may be as high as 80% (113). Decompressive hemicraniectomy has recently been shown to be effective in this scenario in a pooled analysis of 3 recent European trials (114), both in terms of improved mortality and improved functional outcome. Compared to best medical treatment, numbers needed to treat to prevent death, severe disability or moderate disability are 2; 2 and 4 respectively. However, in these studies, participants were relatively young (mean age 43, maximal age 60) and important questions remain concerning optimal timing of treatment and patient selection. Further,

while this is a viable treatment for the most feared subtype of stroke, this treatment is thankfully only relevant for a minority of stroke patients.

Surgery is also a potentially effective therapy for ICH. The recently completed STICH trial (115) enrolled 1033 patients with ICH and no benefit was seen from early surgery (OR 0.89, 95% CI 0.66 to 1.19 for favorable outcome). Pre-specified subgroup analysis suggested there may be benefit in lobar ICH located less than 1 cm from the brain surface and a further randomized trial is underway to test this hypothesis (116).

Summary of Acute Treatments

Despite the clear and significant burden of acute stroke, there are depressingly few effective treatments. For ischaemic stroke, the only proven effective interventions are stroke unit care, aspirin and reperfusion therapy with intravenous thrombolytic therapy and intra-arterial thrombolysis in a small subset of patients with proximal middle cerebral artery occlusion. No specific licensed treatment exists for ICH (except for stroke unit care). There are promising therapies in development but this lack of available treatment, the increasing number of strokes due to the ageing population and the burgeoning burden of stroke in the developing world makes optimal and cheap preventative therapy vitally important.

1.03 Preventative Treatments for Stroke

Preventative therapies can be considered as either primary (where the individual may be at risk of but has no established cardiovascular disease) or secondary (where the individual has established cardiovascular disease). Current preventative strategies include anti-platelet therapy and lipid lowering therapy to prevent ischaemic stroke, anticoagulant therapy to

prevent cardioembolic stroke and blood pressure reduction to prevent all stroke subtypes. It is also recommended that patients undergo screening for and treatment of prevalent diabetes mellitus, smoking cessation therapy and lifestyle modification (if required). Many of these strategies will also reduce risk of other cardiovascular events such as MI.

1.03.1 Anti-platelet Strategies

Aspirin is the only licensed strategy for the primary prevention of stroke. There are four licensed strategies for anti-platelet therapy to prevent recurrent stroke in the UK. These are aspirin, clopidogrel, dipyridamole and the combination of aspirin and extended release dipyridamole. A summary of the mechanism of action, licensed indication and dosage for each of these drugs is shown in table 1.2. Aspirin (acetylsalicylic acid) prevents platelet aggregation by irreversible inhibition of cyclooxygenase-1 (COX-1). COX-1 is a constitutive enzyme present in most body cells and is irreversibly acetylatylated by aspirin, thereby reducing production of prostaglandins and of thromboxane A_2 by platelets. Platelets, given their lack of nuclei, are unable to synthesise additional cyclo-oxygenase meaning the inhibitory effect of aspirin on thromboxane production will last for the lifespan of the platelet. Thromboxane A2 has prothrombotic properties and stimulates platelet activation and aggregation.

Drug	**Mechanism of Action**	**Dose***	**Licensed Indications**				***Major Side Effects***
			PP	**SP CVD**	**SP CHD**	**SP PVD**	
A	Inhibition of cyclooxygenase-1	75mg	Y	Y	Y	---	*Risk of PUD*
D	Inhibition of platelet re-uptake of adenosine and of phosphodiesterase 5 activity	200mg BD	---	Y	---	---	*Headache GI Upset*
C	Inhibition of the adenosine triphosphate receptor	75 mg	---	Y	Y	Y	*GI Upset*

*A = aspirin. C = clopidogrel. D = Extended release dipyridamole. * = recommended dose in Scotland. PP = primary prevention of stroke and myocardial infarction. SP = secondary prevention. CVD = cerebrovascular disease. CHD = coronary heart disease. PVD = peripheral vascular disease. PUD = peptic ulcer disease. GI = gastrointestinal upset.*

Table 1.2. Anti-platelet drugs available for prevention of stroke and cardiovascular disease in the UK.

Several trials have shown aspirin to be effective in reducing the risk of a first cardiovascular event (117-121) in apparently healthy men and men and women with adverse cardiovascular risk. Meta-analysis of these trials (122), including over 55000 patients, of whom 11446 were women, showed a 32% relative risk reduction in risk of first MI (RRR 0.68, 95% CI 0.59 to 0.79) and a 15% reduction in risk of any important vascular event (RRR 0.85, 95% CI 0.73 to 0.93). However, no reduction in incidence of non-fatal stroke was seen (RRR 1.06, 95% CI 0.87 to 1.29). Subgroup analyses in the individual trials suggested benefit in females although it did not reach statistical significance in one (119;121). The recent Women's Health Study involved 39876 initially healthy women aged

> 45 years and evaluated 100 mg aspirin versus placebo (123). No reduction in major cardiovascular events, death or MI was seen, although incidence of stroke was reduced (RR 0.83, 95% CI 0.69 to 0.99). A recent sex specific meta-analysis of primary prevention trials (124) including 95456 individuals (51342 women) confirmed a differing effect of aspirin between the sexes. It suggested that in women, cardiovascular events and stroke rates (OR 0.83, 95% CI 0.7 to 0.97) were reduced by aspirin treatment but there was no reduction in rate of MI while in men, MI was reduced but there was no reduction in rate of stroke (OR 1.13, 95% CI 0.96 to 1.33). Current local guidelines recommend that individuals whose 10 year cardiovascular risk exceeds 20% and those with diabetes who are aged over 40 years are given 75 mg of aspirin. However, even this can be questioned given recent data from the POPADAD study where no benefit was seen following aspirin use in 1276 individuals with diabetes (125). There is no clear evidence to support any alternative anti-platelet strategy in the primary prevention setting.

Aspirin is the most commonly prescribed anti-platelet agent for secondary prevention after stroke and prevents up to a fifth of recurrent strokes (126). Most studies suggest an approximate relative risk reduction of 15% in comparison with placebo (86;127;128) for both recurrent stroke and composite vascular endpoints. For example, in the UK-TIA study (127), 2435 subjects were enrolled and aspirin treatment (either 300mg or 1200 mg per day) led to a 15% RRR in a composite endpoint of vascular death, nonfatal MI and nonfatal stroke). It is also effective in reducing death and recurrence in the immediate period after stroke (86;87).

At the time of writing, aspirin monotherapy has been compared with placebo, dipyridamole, clopidogrel, the combination of aspirin and clopidogrel, the combination of aspirin and

extended released dipyridamole and with ticlopidine (as well as other drugs that are in development or not commonly used). Clopidogrel monotherapy has also been compared to the combination of aspirin and clopidogrel and to the combination of aspirin and dipyridamole.

Ticlopidine is a member of the thienopyridine family and is an ADP receptor inhibitor. It is superior to placebo following stroke in terms of a composite endpoint of stroke, MI or vascular death (129) while studies have suggested marginal superiority (130) over or equivalence (131) to aspirin. It is rarely used and is not licensed in the UK because of a risk of severe neutropaenia and thrombotic thrombocytopenia.

The CAPRIE trial (24) compared clopidogrel monotherapy to aspirin in 19195 patients with symptomatic cardiovascular disease (recent stroke, MI or symptomatic peripheral vascular disease). Overall, clopidogrel use linked with a small but statistically significant reduction in the rate of the primary composite endpoint of ischaemic stroke, MI or vascular death (5.32% with clopidogrel compared to 5.83% with aspirin, p=0.043). This small 8.7% RRR appeared predominantly driven by an effect in those with PVD (RRR 23.8%, p=0.0028) while in the stroke and MI subgroups, no difference was seen (RRR 7.3%, 95% CI -5.7 to 18.7% in those with recent stroke).

Dipyridamole monotherapy is superior to placebo in terms of recurrent stroke prevention, but not in prevention of myocardial infarction or vascular death and may have similar efficacy to aspirin but again without proven benefit in prevention of MI (132;133). This similar efficacy is at the cost of a significantly increased trial drop out rate because of side

effects such as headache. Aspirin and dipyridamole in combination have also been shown to be superior to placebo (134).

The recent MATCH (135) and CHARISMA trials (136) showed that, in the secondary prevention of stroke, dual therapy with clopidogrel and aspirin is no better than either alone. The MATCH trial included 7599 with recent stroke or TIA and additional risk factors for recurrence and compared clopidogrel with the combination of aspirin and clopidogrel. The combination did not reduce the occurrence of a composite endpoint of ischaemic stroke, MI and vascular death (15.7% vs 16.7%, RRR 6.4%, p=0.244) but did lead to a two-fold increase in life-threatening bleeding episodes (2.6% vs 1.3%, p<0.0001). The CHARISMA trial compared aspirin and clopidogrel therapy versus aspirin alone amongst 15,603 patients, the majority of whom had established cardiovascular disease. In 27% of patients the trial entry criterion was previous cerebrovascular disease. Clopidogrel was no more effective than placebo in aspirin treated patients in the entire cohort (occurrence of primary composite endpoint of 6.8% with combination compared to 7.3%, RRR 7%, p=0.22). In the secondary prevention cohort there was a significant reduction in the primary endpoint (6.9% vs 7.9%, RRR 12%, p=0.046). Trends toward benefit were apparent in those who entered the trial on account of stroke, and all-cause stroke appeared lower in the population as a whole. However, benefits only bordered on statistical significance before adjustment for multiple comparisons and bleeding complications were again increased nearly two-fold in with combination therapy (2.1% vs 1.3%, RR 1.62, p<0.001). Among those with multiple risk factors but no established disease, the primary endpoint was non-significantly increased on those on dual therapy (RR 1.2, 95% CI 0.91 to 1.59).

The CARESS trial compared dual anti-platelet therapy with aspirin and clopidogrel with aspirin in patients with recently symptomatic > 50% carotid stenosis and microembolic signals (MES). The study was randomized, double blind and the primary endpoint was the proportion of patients who were MES positive at seven days and the secondary endpoint included recurrent stroke or TIA. The primary endpoint was significantly reduced in those who received both aspirin and clopidogrel (43.8% compared to 72.7%, RRR 39.8%, 95% CI 13.8 to 50%). There was a strong trend towards a reduction in rate of stroke or TIA although numbers were small (4 recurrent strokes and 7 TIAs in the monotherapy group compared to 4 TIAs with dual therapy). These promising results suggest that those with large vessel atherosclerosis and recent stroke may benefit – in the same way as those with recently symptomatic large vessel coronary disease – from early dual anti-platelet therapy (137).

The recent ESPRIT trial (138) involved 2763 patients with recent stroke or TIA and revealed, during a mean follow-up of 3.5 years, that the combination of aspirin and slow-release dipyridamole afford a 20% relative risk reduction (HR 0.8, 95% CI 0.66 to 0.98, ARR 1% per year) in the rate of vascular death or non-fatal stroke or MI compared to aspirin alone. The surprising lack (and indeed reduction) of increased bleeding complications is curious and further claims of anti-inflammatory and non-platelet mediated benefits of dipyridamole have emerged (139). The results of ESPRIT confirm those of ESPS 2 (132) and have consolidated the position of aspirin and dipyridamole combination therapy as preferable to aspirin alone in the secondary prevention of stroke.

The recent PRoFESS study compared aspirin and dipyridamole with clopidogrel and included 20332 patients with recent stroke (140). The primary endpoint was recurrent

stroke and there was no difference between the treatment groups (9% following aspirin and dipyridamole vs 8.8% following clopidogrel, HR 1.01, 95% CI 0.92 to 1.11). Unlike in ESPRIT and ESPS-2, the combination of aspirin and dipyridamole led to an increase in rate of major haemorrhagic events including haemorrhagic stroke in comparison to its comparator which offset the slight reduction in ischaemic stroke.

Thus, aspirin, clopidogrel and dipyridamole monotherapy appear to provide similar efficacy in the secondary prevention of stroke, although clopidogrel may be slightly superior. The combination of aspirin and clopidogrel poses unacceptable risks for little gain, except perhaps in certain high risk subgroups. Aspirin and extended release dipyridamole has been shown to be superior to aspirin in two studies but recent data suggest this strategy offers similar yield to clopidogrel monotherapy, which is better tolerated. In certain high risk groups aspirin and clopidogrel combination therapy may be indicated but further study is required to confirm efficacy and establish optimal treatment duration. At present therefore, a reasonable strategy would be to employ aspirin and dipyridamole combination therapy as first line and, where patients are intolerant to dipyridamole to consider either aspirin or preferably clopidogrel monotherapy. For the primary prevention of stroke, aspirin appears to be of benefit but perhaps only in women, whereas in men, most evidence suggests a reduction in risk of MI.

1.03.2 Anticoagulant Therapy (non-Cardioembolic Stroke)

The WARSS study enrolled 2206 patients with recent (within 30 days) ischaemic stroke without evidence of cardioembolic source. It compared aspirin 325 mg with warfarin with a target INR of 1.4 to 2.8 (141). There were no significant differences between the groups in terms of efficacy or bleeding risk. The WASID trial (142) compared warfarin and aspirin in

those with recent stroke and documented 50 to 99% intracranial stenosis (who had no evidence of significant carotid or cardio-embolic disease) and revealed no benefit from warfarin in terms of stroke recurrence which was similar in both groups (HR 1.04, 95% CI 0.73 to 1.48). However, there was significantly worse protection from cardiac events and increased haemorrhage rate and increased mortality. The SPIRIT trial was also terminated early because of increased bleeding risk, although did involve a high target INR (3 to 4.5) (143). It included 1316 participants with minor stroke or TIA. The ESPRIT trial (144) compared aspirin with warfarin (target INR 2 – 3) in 1038 individuals with recent ischaemic stroke. There was no difference in rate of the primary outcome between treatment groups although ischaemic events were lower following warfarin (HR 0.73, 95% CI 0.52 to 1.01) but major haemorrhage rate was higher (HR 2.56, 95% CI 1.48 to 4.43). Anticoagulation is therefore not recommended for prevention of stroke in those with non-cardioembolic stroke (55).

1.03.3 Statin / Cholesterol Lowering Therapy

The link between serum cholesterol levels and coronary artery disease is well established and the evidence that cholesterol lowering therapy with HMG-CoA reductase inhibitors (statins) reduces coronary morbidity and mortality in those with and without established CHD is unequivocal (145). Debate however continues concerning the association between serum cholesterol levels and stroke. Such a link would seem likely given the strong link between it and CHD, while the heterogeneity of stroke causes and epidemiological evidence that lower serum cholesterol may link with increased risk of intracerebral haemorrhage suggests such a link cannot be assumed (146;147). However, statin therapy is now an accepted strategy following ischaemic stroke (55). Further, in a large meta-analysis of over 90000 patients (mostly with manifest CHD) who were enrolled in statin trials, statin

treatment significantly reduced the risk of incident stroke (OR 0.79, 95% CI 0.73-0.85) (148). Each 10% reduction in LDL cholesterol appears to afford a 15.6% RRR of stroke (95% CI 6.7% to 23.6%). Further compelling evidence arose following the Heart Protection Study which enrolled over 20000 patients with CHD or risk factors and compared simvastatin 40 mg with placebo (149). Simvastatin reduced the risk of first stroke by 25% (95% CI 15% to 34%, $p<0.0001$). The ASCOT trial lipid lowering arm (ASCOT-LLA) (150), which included those with hypertension, revealed a similar magnitude of stroke risk reduction. Reduction in stroke risk did not accompany the modest reduction in serum cholesterol seen in the ALLHAT study (151) but, on balance,, the evidence shows that for those with and without CHD, statin therapy reduces both risk of MI, mortality and stroke risk. Approximately 16% of patients in the HPS study had suffered previous stroke, just over half of whom had no documented CHD. In this group with stroke, statin therapy yielded no reduction in recurrent stroke (HR 0.98, 95% CI 0.79 to 1.22).

Until very recently therefore, it was not clear whether statin therapy reduced the risk of recurrent stroke following an index cerebrovascular event. The SPARCL trial (152) randomised 4731 patients to high dose (80 mg) atorvastatin or placebo. Treatment led to a 16% relative risk reduction of recurrent stroke and to reductions in the rates of most other vascular complications. These results, subsequently confirmed in a meta-analysis (153), are in contrast to those from the HPS, and confirm that statin therapy can reduce risk of recurrent stroke. The effect was however less than would be expected in terms of secondary prevention of MI.

An important consideration is the epidemiological link between low serum cholesterol and incidence of haemorrhagic stroke, raising the important possibility that statin therapy may increase risk of ICH. Data from randomized controlled trials conflicts but the pivotal SPARCL trial (152) did show an increased risk of haemorrhagic stroke in those in the treatment arm (HR 1.67, 95% CI 1.09 to 2.56). When these data were combined with those from the HPS in a meta-analysis, risk remained elevated in those receiving statin treatment (HR 1.73, 95% CI 1.19 to 2.5) (152). However, the same meta-analysis revealed a 20% reduction in the risk of the more common recurrent ischaemic stroke (HR 0.8, 95% CI 0.7 to 0.92) and a smaller but significant reduction in overall recurrent stroke burden (HR 0.88, 95% CI 0.78 to 0.99).

Thus, statin therapy is clearly indicated and recommended as a preventative strategy in those who have suffered ischaemic stroke or who have CHD (55;154). Whether statins should be given to those who have suffered ICH and have no other indication for their use is at the time of writing unclear.

1.03.4 Blood Pressure Lowering Therapy

Hypertension is the most significant risk factor for stroke and the relationship between increasing blood pressure and increasing stroke risk even extends to within the normal blood pressure range (155;156). Treatment of hypertension is of unequivocal benefit in reducing stroke risk in the primary prevention setting (157) and evidence suggests that newer antihypertensive agents, such as amlodipine and angiotensin receptor blockers (ARBs), offer greater protection than “older” atenolol and diuretic based regimens (158). In a recent meta-analysis (158), calcium channel antagonist (except verapamil), angiotensin converting enzyme inhibitor (ACEI) and ARB based therapy was superior to diuretic or ß-

blocker therapy, affording 7% greater protection against stroke (OR 0.93 (95% CI 0.89-0.98). This may however be due to the small differences in achieved blood pressure control rather than specific ancillary properties of any drug (159).

Treatment of hypertension in those who have stroke has now also been shown to reduce recurrent stroke risk. In a recent meta-analysis (158), odds of recurrent stroke were reduced by 24% (OR 0.76, 95% CI 0.63-0.92), but interestingly, the strongest evidence exists for diuretic based therapies, as opposed to ACE inhibitor or ß blocker based regimens (OR 0.63, 95% CI 0.55 to 0.73 vs placebo compared to a non-significant 8% reduction in the odds on ACEI based therapy). In the PROGRESS trial (160), which included 6105 patients with prior stroke, treatment with either perindopril or a combination of perindopril and indapamide led to a 26% RRR (95% CI 16 to 34%) in major vascular event rate and a 28% RRR (95% CI 17 to 38%) in risk of recurrent stroke during follow up. On sub-analysis, combination of perindopril and indapamide led to a 43% (95% CI 30 to 54%) RRR in risk of recurrent stroke compared to placebo, a 40% (95% CI 29 to 49%) RRR in the risk of major vascular events and a 12/5 mmHg blood pressure reduction. Perindopril monotherapy afforded only a 5/3 mmHg reduction in blood pressure and a non-significant 4% RRR in stroke risk. Benefits were similar regardless of history of hypertension and the mean blood pressure at trial entry was 147/86 mmHg suggesting that those within the normal blood pressure range may also benefit from blood pressure reduction after stroke.

The MOSES trial (161) suggested that an ARB based regimen using eprosartan was superior to the calcium channel antagonist nitrendipine. This data supported a new and popular hypotheses that drugs which increased angiotensin II production would, via increased activation of the AT2 receptor, increase neuronal resistance to anoxia via

recruitment of collateral vessels and thus provide protection to ischaemic brain (162). However, a number of issues surround interpretation of the MOSES trial including the inclusion of multiple events and the use of a comparatively low dose of nitrendipine (although blood pressure reduction was similar in both treatment arms). Further against this hypothesis, the PRoFESS trial (163) showed no significant reduction in recurrent stroke, any recurrent vascular events or diabetes following telmisartan use. The trial involved 20332 patients with recent stroke and 2.5 years follow up. Benefit was not apparent despite a 3.8/2 mmHg reduction in blood pressure, although use of concomitant blood pressure lowering therapy was higher in the placebo group.

Antihypertensive therapy is therefore recommended to both reduce risk of first and recurrent stroke and risk of other vascular events following ischaemic stroke, TIA and intra-parenchymal haemorrhage. Therapy should be considered in all after stroke, although target blood pressure levels are not well defined. Data support the use of diuretic treatment or a combination of diuretic and ACE inhibitor therapies in the secondary prevention setting and newer antihypertensive agents such as amlodipine or ACEI / ARB regimens in the primary prevention setting.

1.03.5 Diabetes

Diabetes is a risk factor for stroke (164-166) and is an increasingly prevalent condition found in as many 33% of patients with ischaemic stroke (167). In data from Oxfordshire (168), diabetes was shown to be an independent risk factor for recurrent stroke (HR 1.85%, 95% CI 1.18 to 2.9, $p<0.01$) and a similar relationship has been found in other analyses (169).

It is recommended that for the primary prevention of stroke that a lower blood pressure target is employed in those with diabetes (130/80 mmHg) and hypertension and this is effective in reducing stroke risk (170;171). In the UKPDS (172), blood pressure control (to a mean of 144/82 mmHg) yielded a 44% RRR in risk of stroke and a smaller but similar reduction in a combined vascular endpoint. Additive beneficial effects on progression to macroalbuminuria means that ACE inhibitor or ARB based regimens are recommended as first-line agents in those with diabetes (173).

Glycaemic control is effective in reducing the risk of microvascular complications, in both type 1 and type 2 diabetes (174;175). These include diabetic retinopathy, nephropathy and neuropathy. Data concerning the impact of glycaemic control on risk of macrovascular complications including stroke are less convincing. Trends toward a reduction in cardiovascular event rates have been seen but this remains unproven (176;177). Furthermore, data from two recent randomised controlled trials were disappointing. The ADVANCE trial (178) included 11,140 patients and compared an intensive strategy of gliclazide ± other drugs with standard treatment and found a 10% reduction in incidence of microvascular or macrovascular disease during follow-up (HR 0.9, 95% CI 0.82 to 0.98, p=0.1). This was predominantly due to a reduction in rate of nephropathy with no difference in macrovascular event rate (HR 0.94, 95% CI 0.84 to 1.06, p=0.32). The ACCORD trial (179) was of similar magnitude and included 10,251 patients. It compared efficacy of an intensive strategy intended to achieve a glycated haemoglobin level of below 6% and a standard therapy strategy aiming for a level of 7 to 7.9%. The rate of primary outcome of non-fatal MI or stroke or cardiovascular death was similar between the groups (HR 0.9, 95% CI 0.78 to 1.04, p=0.16) but mortality was higher (HR 1.22, 95% CI 1.01 to 1.46, p=0.04) leading to premature termination of the trial. Significant hypoglycaemia and

weight gain were also more common. Sub-analysis showed no suggestion of benefit with specific regard to stroke risk in either of these trials.

Recent European guidelines suggest those with type 2 diabetes who do not require insulin and have suffered stroke be commenced on pioglitazone therapy (55). However, this recommendation is based on data from subgroup analysis from the PROactive trial (180) which included 5238 patients with type 2 diabetes and known macrovascular disease and compared pioglitazone with placebo. In the cohort with previous stroke (≈500 in each group) there was a trend toward a reduction in major vascular events and death and a significant reduction in both fatal and non-fatal stroke (HR 0.53, 95% CI 0.34 to 0.85) and CV death, non-fatal MI and stroke (HR 0.72, 95% CI 0.52 to 1, p=0.0467) (181).

1.03.6 Cardioembolic Stroke / Atrial Fibrillation

Approximately 20% of strokes are cardioembolic with the majority of cases being due to atrial fibrillation. Left ventricular mural thrombus and valvular heart disease are other important causes (182) with the majority of left ventricular mural thrombi being associated with acute myocardial infarction. Atrial fibrillation is increasingly common and the incidence rises dramatically with age. It is estimated that its prevalence roughly doubles with each advancing decade from 0.5% at age 50-59 years to nearly 9% in the ninth decade of life (183).

Presence of atrial fibrillation predicts both first stroke and recurrent stroke and those with concurrent increased age, diabetes, congestive cardiac failure, previous stroke and hypertension are at the highest risk. Concurrent increased left atrial size, spontaneous echo contrast and left ventricular dysfunction on echocardiography are also predictive factors

(184). Scoring algorithms are now commonly employed to help predict stroke risk and identify those with atrial fibrillation most likely to benefit from anticoagulant treatment. An example is the CHADS2 score (185) where the variables presence of recent congestive cardiac failure, hypertension, age > 75 years and diabetes mellitus are assigned 1 point and history of stroke or TIA two points. Those with a score of 2 or more (assuming no treatment) have a stroke risk of approximately 4% per annum rising to 8.5% per annum in those with a score of 4. The most widely used and studied anticoagulant is warfarin which inhibits synthesis of vitamin K dependent clotting factors.

In the primary prevention setting, warfarin is hugely effective. Pooled analysis from the major placebo controlled trials reveals a highly beneficial RRR of 68% for ischaemic stroke (186) compared to placebo. In absolute terms, the stroke rate fell from 4.5% per annum to 1.4% with warfarin treatment. Maximum benefit requires the INR to be above 2 (187;188) and the recommended INR range is 2 to 3.

Pooled analysis from large placebo controlled trials (189) also confirms that primary prevention with aspirin provides a reduction in stroke risk compared to placebo (RRR 21%, 95% CI 0 to 38%). However, it is clearly an inferior strategy when compared to warfarin (190). Meta-analysis revealed a RR of 0.36 (95% CI 0.26 to 0.51) for those treated with warfarin compared to aspirin. Similar benefit is also seen in the elderly (191;192).

In the secondary prevention of stroke, warfarin is also highly effective in those with atrial fibrillation. In the European Atrial Fibrillation Trial (188), the annual rate of stroke, MI, systemic embolism or vascular death was 8% with warfarin in comparison to 15% with placebo (HR 0.60, 95% CI 0.41 to 0.87). The timescale of initiation of anticoagulation in

this setting is unclear. It is clear that early unselected use of anticoagulation (with heparin) in those without cardioembolic stroke is ineffective (89-91) but whether it is of benefit in those with AF is unclear (92). In the European Atrial Fibrillation Trial (188), nearly one half of patients commenced anticoagulation within 14 days, although their neurological deficits were minor. Thus, it is generally recommended that anticoagulation be initiated within two weeks in those with AF and stroke, although delays of one week are commonly employed and guidelines suggest the decision regarding timing be individualised (55). Dual anti-platelet therapy with aspirin and clopidogrel was compared to warfarin in the ACTIVE W trial (193). This included 6706 patients and suggests that dual anti-platelet therapy offers inferior stroke protection at the cost of a similar burden of bleeding complications.

Regarding safety and risk of haemorrhage, warfarin can be considered safe in comparison to aspirin, with a major bleeding rate of 1.3% compared to 1% on aspirin or placebo.

There are a number of newer anticoagulant agents in development, many of which are now in phase 3 study. These include direct thrombin inhibitors (such as ximelagatran and dabigatran etexilate (194;195) and factor Xa inhibitors such as Rivaroxaban (196;197). The potential benefits of these drugs include a lack of requirement for dose adjustment and regular monitoring but as yet, none has been shown to be as safe or effective as warfarin for the prevention of stroke in those with atrial fibrillation. Ximelagatran was shown to be as efficacious as warfarin with a similarly low risk of bleeding complications in both primary and secondary prevention in two large trials (198). The drug was however removed from the market because of concerns over hepatic toxicity (199).

In the ACTIVE-A study, clopidogrel was compared to placebo in those with atrial fibrillation taking aspirin who were deemed unsuitable for anticoagulation (200). The rate of vascular events was significantly lower in those treated with clopidogrel and aspirin (RR 0.89, 95% CI 0.81 to 0.98) and in particular, the risk of stroke was reduced (RR 0.72, 95% CI 0.62 to 0.83). The risk of major haemorrhage was however increased with dual antiplatelet therapy (from 1.3% per year to 2% per year, RR 1.57, 95% CI 1.29 to 1.92).

Thus, in summary, anticoagulation with warfarin is the treatment of choice for prevention of recurrent stroke in those with cardioembolic stroke. It is also the treatment of choice for primary prevention of stroke in those with atrial fibrillation whose risk of stroke exceeds the likely risk of haemorrhage. Risk stratification algorithms can be employed to aid this decision. In those deemed unsuitable for anticoagulation, aspirin is effective and therapy with aspirin and clopidogrel leads to significantly fewer strokes at the cost of increased haemorrhage risk.

1.03.7 Carotid Artery Intervention

Approximately 10-15% of ischaemic strokes are thought to be due to large artery atherosclerosis (201), most of which are associated with a stenosis of the ipsilateral extracranial carotid artery. This stenosis can be remedied by carotid endarterectomy (CEA), where, under local or general anaesthesia, the carotid artery is dissected free, opened and the atheromatous stenosis removed. Percutaneous placement of a stent via the femoral artery is an alternative approach. Large randomized controlled trials have shown superiority of carotid endarterectomy in comparison to medical therapy (202-204) in those who have symptomatic severe carotid stenosis. A pooled analysis of the major symptomatic stenosis trials (205) included data on over 6000 patients. In those with symptomatic severe (≥70%)

stenosis, surgery afforded a 16% absolute risk reduction (ARR) over 5 years and a smaller 4.5% ARR (p=0.04) in those with moderate (50-69%) stenosis. Surgery slightly increased risk (by 2.2%) of stroke in those with less than 30% stenosis (p=0.05) but no effect on risk was seen in those with 30-49% stenosis or near occlusion.

Further analysis from a pooled dataset of the largest trials shows that early surgery, despite its inherent increased risk, is associated with greater absolute and relative risk reduction than delayed surgery (206). In those with severe stenosis, surgery in those who were randomized to treatment within 2 weeks of symptoms afforded an ARR of 23% (95% CI 13.6 to 32.4%) compared to only 7.4% (95% CI -3.3 to 18.1%) if randomized after 12 weeks. In those with moderate stenosis randomized there was a 14.8% ARR (95% CI 6.2 to 23.4%) following surgery if randomized within 2 weeks but no significant benefit if randomized thereafter.

Thus, if treated early, benefit is also apparent for those with a symptomatic 50-69% stenosis. It is also possible to further refine the risk-benefit ratio. Of those with symptomatic moderate stenosis, men, those aged over 75, those with evidence of infarction and those with hemispheric (rather than retinal) symptoms appear to benefit most (207-210). Co-morbidity must also be considered as this will increase peri-operative risk and thus adversely refine the risk benefit ratio. Peri-procedural risk is a key factor in determining benefit from carotid endarterectomy; the lower the absolute benefit from carotid endarterectomy (such as in younger female patients or asymptomatic disease), the lower the surgical complication rate requires to be to ensure benefit. It is generally accepted that this must be <6% for those with severe stenosis but < 3% in those with moderate stenosis (55) and those with asymptomatic disease.

Carotid artery stenting (CAS) has not yet been proven to be superior or even equivalent to carotid endarterectomy. Several trials have been performed, 2 of which have been terminated early because of poor results in the stenting arm and 1 of which because of slow recruitment. The Wallstent trial (211) involved 219 patients with symptomatic stenosis and found a higher peri-procedural stroke rate and a higher rate of major stroke or death at 1 year when compared to endarterectomy (12.1% vs 4.5% (p=0.049) and 3.7% vs 0.9% (p=0.204) respectively). It has since been criticized for allowing relatively inexperienced stent operators to participate – a criticism also levied at the recent EVA-3S study (212).

EVA -3S included patients with at least 60% carotid stenosis and ipsilateral stroke within 120 days. It was terminated prematurely after inclusion of 527 patients; the 30 day incidence of stroke or death was 3.9% following carotid endarterectomy and 9.6% after stenting (RR 2.5, 95% CI 1.2 to 5.1). When only disabling stroke or death were considered, it remained more common in the stenting group (3.4% vs 1.5%, RR 2.2, 95% CI 0.7 to 7.2) and outcomes were significantly worse at 6 months.

A further recent trial (213) also failed to prove non-inferiority of carotid stenting. The SPACE trial included 1200 patients with severe symptomatic (within 6 months) carotid stenosis. Outcomes were similar. The rate of death or ipsilateral ischaemic stroke within 30 days was 6.84% following stenting and 6.34% following endarterectomy (OR 1.09, 95% CI 0.69 to 1.72). Despite these similar outcomes, the pre-specified non-inferiority margin was missed. The reasons for these differing results are not clear but in SPACE, interventional radiologists were required to show greater levels of experience than in EVA-3S before being included in the study.

Some trials have however showed similar outcomes following stenting. The CAVATAS trial enrolled 504 patients with symptomatic carotid disease and compared CEA with carotid angioplasty (only ¼ of patients received stents) (214). The trial also included participants with vertebrobasilar stenotic disease. The 30 day risk of stroke or death was similar (9.9% with CEA and 10% with CAS) with no difference in recurrent stroke rate at 1 year. The peri-surgical risk was however higher than that suggested as the maximum accepted level in consensus guidelines (55). CAVATAS does however provide important long-term follow up data in those who have undergone percutaneous carotid intervention and while restenosis appears more common in those who underwent angioplasty (215), it may not translate into worse clinical outcome and may not afflict those who also received stenting (216).

In the SAPPHIRE trial (217), 334 patients deemed at high risk were randomized to either CEA or CAS with emboli detection. Patients either had at least 50% symptomatic stenosis or asymptomatic 80% stenosis. The endpoint was a composite of death, stroke or MI within 30 days after the event or ipsilateral stroke between 31 days and 1 year. This was less frequent following stenting (12.2% vs 20.1%, absolute difference of 7.9%, 95% CI -0.7 to 16.4%) and the non-inferiority criteria were met (p=0.004 for non-inferiority). The benefit was largely driven by a reduction in MI in those treated with CAS. Importantly, the peri-procedural rate of stroke, MI or death following CAS was only 4.8% in this study in comparison to the higher rates in other trials (212;213). The peri-procedural complication rate following endarterectomy was high at 9.9%. However, long-term follow up data are now available (218) and further support the authors initial assertions that, in this patient group, stenting can afford similar outcomes compared to CEA.

At the time of writing, the Carotid Revascularization Endarterectomy vs Stenting Trial (CREST) (219) is ongoing and aims to recruit 2500 patients with symptomatic stenosis and will hopefully clarify the relative benefits or otherwise of carotid stenting.

Carotid endarterectomy in the primary prevention setting (those with asymptomatic stenosis) has also been the subject of large randomized controlled trials (220-222). In general, these trials support benefit in those with at least moderate stenosis although the absolute benefit appears small and thus mandates a low peri-procedural complication rate to give benefit. For example, in the most recent trial, the Asymptomatic Carotid surgery Trial (223), 3120 patients were randomized to either immediate endarterectomy or indefinite deferral of endarterectomy. The peri-operative rate of stroke or death was 3.1% following CEA. Even when these events were considered, the 5 year risk of stroke or death was lower following CEA (6.4% vs 11.8%, ARR 5.4%, 95% CI 3 to 7.8). Similar benefits were seen when only fatal events were considered. On subgroup analysis, benefit was not apparent in those aged over 75 years.

1.03.8 Utility of Microembolic Signals

As well as the parameters mentioned, there are further means of risk stratifying individuals with carotid disease. Asymptomatic microembolic signals (MES) can be detected using transcranial Doppler ultrasound scanning (TCD) (223). The prevalence of MES has varied in studies and they are thought to represent thrombi and platelet fibrin aggregates (224;225). They are found in those with carotid artery disease, and are more common in those with recent symptoms (226;227), in those with atrial fibrillation and they arise during cardiac and aortic surgery and can be found thereafter in those with cardiac valve prostheses, and in

particular those with metallic valves (228). In those with recently symptomatic carotid stenosis, they are typically seen in approximately 40% of individuals (227;229), although higher and lower prevalence has been seen.

In those with carotid disease, their occurrence has been shown to be predictive of an increased stroke risk (226;227;230;231) in a series of small studies. For example, in one study (226) of 64 asymptomatic individuals with unilateral 70% to 90% internal carotid artery stenosis, a microembolic rate of ≥ 2 per hour was associated with increased risk of suffering ischaemic stroke. However, only five strokes occurred, hence the uncertainty of size of the association (odds ratio 31, 95% CI 3 to 302, p=.005). In a further study, microembolic signals at study entry were predictive of TIA and stroke risk in both symptomatic and asymptomatic patients with an adjusted combined odds ratio of 8.1, although again there was uncertainty regarding the actual magnitude of the association (95% CI, 1.58 to 41.5, p=0.01 (231;232).

Microembolic signals (MES) are responsive to anti-platelet therapy; aspirin has some effect (233) but more marked effects are seen with addition of clopidogrel (234), with the platelet glycoprotein inhibitor tirofibran (235), and with S-Nitrosoglutathione (a nitric oxide donor) therapy (236). The feasibility of using MES as a surrogate endpoint in clinical trials was recently studied (234). As outlined earlier, the CARESS investigators showed that they could use MES to detect treatment differences between groups using much smaller numbers than trials with traditional clinical endpoints.

Thus, in those with recent stroke or TIA and carotid artery stenosis, carotid endarterectomy should be performed if the stenosis is greater than 70% but not sub-totally occluded and if

the peri-procedural complication rate in that centre is lower than 6% (55). It should also be performed in some high risk individuals with moderate stenosis but not in stenosis of less than 50% and only if the predicted peri-procedural complication rate is acceptably low. If indicated, surgery should be performed as soon as possible and preferably within 2 weeks of symptoms. CAS is a viable alternative strategy, particularly if endarterectomy is not possible provided the peri-procedural complication rate is in the region of 4-6% (170). In those with asymptomatic disease, guidelines therefore suggest that those with high risk of stroke such as men with severe stenosis who have a life expectancy of > 5 years can be considered for surgery where the peri-operative risk is low at 3% or less (55). Further, presence of microembolic signals may help identify a particularly high risk group with carotid disease.

1.03.9 Behavioural Risk Factors

Smoking Cessation

Cigarette smoking is an independent risk factor for both stroke and recurrent stroke (237). In a meta-analysis performed nearly 20 years ago (238), smoking linked with a doubling of risk of ischaemic stroke following adjustment for other risk factors. There are no randomised controlled trials to evaluate the efficacy of smoking cessation therapy after stroke as it is generally regarded as an important strategy given compelling data from observational studies and the other health benefits associated with stopping. Observational data suggest that stopping smoking affords a reduction in stroke risk and that risk returns to that of a non-smoker after 5 years (239). Further, data show that rates of admission for acute coronary syndrome fell significantly after the smoking ban in Scotland (240), although no data are available to show effect on stroke risk. Guidelines currently suggest

that a combination of pharmacological and behavioural therapy is used, an approach for which there is some evidence (241;242).

Excess Alcohol Intake

The relationship between alcohol intake and stroke risk is complex, unproven and somewhat controversial. It is generally accepted that the relationship is J-shaped with heavy drinkers having an increased risk and likewise those who consume no alcohol (243). Those who consume small or moderate amounts of alcohol, have the lowest risk. In a recent meta-analysis (244), those who consumed more than 5 drinks per day had a RR of 1.69 for stroke compared to non-drinkers while those who drink less than one or one to two drinks per day had a RR of 0.8 and 0.72 respectively. The relationship probably holds with regard to the risk of recurrent stroke (245). Guidelines suggest that those who are heavy drinkers should be advised to eliminate or reduce their consumption of alcohol.

Weight Reduction / Physical Exercise

Obesity is also a risk factor for stroke and (246), while it is linked to several major risk factors such as blood pressure, presence of diabetes and cholesterol level (247-249), the relationship appears independent (248). The relationship between risk and centri-pedal obesity (measured by the waist-hip ratio) may be the strongest with risk tripling in the highest quartile of the distribution (250). As yet, no data exist to suggest benefit from weight reduction in obese individuals with stroke but given the benefits on metabolic parameters such as lipid levels and blood pressure, guidelines suggest weight management is encouraged through lifestyle measures (154). Pharmacotherapy to aid weight reduction is a growing area and several agents are undergoing phase III clinical trials at the time of writing, some of which include clinical cardiovascular endpoints.

Moderate intensity physical exercise is also encouraged for those who area able and links with improvement in cardiovascular risk parameters (154). No evidence exists to confirm clear benefit on stroke risk but in those who have suffered stroke, exercise can improve mobility and fitness (251).

There are thus a number of effective preventative therapies for both the primary and secondary prevention of stroke. Strategies include anti-platelet therapy and lipid lowering therapy to prevent ischaemic stroke, anticoagulant therapy to prevent cardioembolic ischaemic stroke and blood pressure reduction, treatment of prevalent diabetes, lifestyle therapy and behavioural modification to prevent all stroke subtypes.

Summary

It is difficult to accurately predict the impact of currently available therapies but it is clear from recent randomised controlled trials and epidemiological data that first and recurrent stroke remain common and that they will become increasingly so. Using the recent PRoFESS trial as an example, approximately 9% of patient suffered recurrent stroke despite high uptake of preventative therapies. It is therefore clear that both the optimal and rapid use of current strategies and the development of novel therapies are required to ensure best possible stroke prevention.

This programme of research and thesis aimed to make improvements in several of these areas, with emphasis on improving access to care (chapter 2), improving use of current therapies (chapter 3) and the development of new preventative strategies (chapter 4).

CHAPTER TWO

A RECOGNITION TOOL FOR TRANSIENT ISCHAEMIC ATTACK

2.01 Difficulties in Delivering Effective Therapies

Those with TIA represent a high risk group whose risk of stroke is highest in the first 48 hours (32). UK guidelines therefore recommend that patients with suspected TIA are assessed as soon as possible (and at least within 1 week) (33;34) in fast track or rapid access neurovascular clinics (35). However, at the time of writing the sad reality is that TIA is poorly managed in the UK and guideline recommendations are not widely met. Recent reports from the UK National Audit Office and the House of Commons Public Accounts Committee (5) found that approximately half of patients with suspected TIA were seen within 14 days, 58% had a scan outside an effective time window and the majority waited 12 weeks or more for a carotid ultrasound scan. This must be improved as more rapid management is effective in reducing stroke risk (252). It may even be that current guidelines are not stringent enough and that assessment on the day of the event should be the aim. The recent EXPRESS study provides compelling evidence (252) that urgent (same day) assessment of those with TIA dramatically reduces risk of early stroke. This was a very well conducted observational study, where outcomes in an initial standard management phase were compared to those of an urgent same day appointment system. The risk of early stroke in the early phase was 10.2% compared to 2.1% in the later phase. There are two important points worth mentioning. Firstly, even the initial phase represents a significantly more acute and advanced system than exists in most of the UK. Secondly, the main difference between the study phases was the time lag to therapy initiation; treatment protocols were similar (although anti-platelet therapy was more aggressive in the

later phase), the geographical area was identical and the background incidence of stroke and TIA was similar throughout.

Such clinics should therefore be the aim but will increase pressure on scare clinical and radiology resources, require more frequent availability of specialist staff and are costly to implement and run and a number of barriers preclude their effective introduction. Current delays to assessment include delay to presentation by the patient, delay to referral to the TIA service by a General Practitioner (G.P), delays to TIA clinic appointments, delay to appropriate investigations and a time lag to initiation of appropriate treatment, including both medical therapy and therapy for carotid artery stenosis. Public awareness campaigns to improve patient recognition of stroke symptoms are underway, as are programmes to improve knowledge of TIA assessment and management among G.Ps. Several strategies could be employed to improve hospital TIA services. The number of rapid access out-patient clinics could be increased and access to imaging services could be improved. For example, stroke services could be supported to develop their own imaging services.

All of these strategies will prove costly and it is likely that improvements and streamlining of current systems will be most readily achieved.

2.01.1 Strategies to Improve Care of Those with TIA – Rapid Diagnosis

An obvious target for improvement is to reduce the number of non-cerebrovascular referrals to TIA clinics, thereby freeing up existing clinic time. Difficulties in accurate diagnosis of patients with suspected TIA are well documented (253-255); inter-observer agreement has been shown to be as low as 50% (256), and between 31 and 62% of patients referred with suspected TIA are deemed to have a non-cerebrovascular diagnosis by a

stroke specialist (255;257-259). This is also a significant problem in Glasgow (257) where a recent analysis of referrals over a one year period revealed that just under 50% had an alternative diagnosis. Such a large number will make it more difficult for services to meet their aim of rapid assessment of those with TIA.

Improvements in the diagnostic accuracy during assessment of suspected TIA by non-specialists (G.Ps for example) could be achieved if simple diagnostic algorithms were available. Stroke assessment tools have been developed to aid in the rapid and accurate identification of those with stroke and to ensure appropriate ambulance dispatch of the patient. Most have been designed for use by paramedical staff and include the Los Angeles Pre hospital Stroke Screen (LAPSS) (260), the Cincinnati Pre hospital Stroke Scale (CPSS) (261), a combination of the two, the Melbourne Ambulance Stroke Screen (MASS) (262) and the Face Arm Speech Test (FAST) (263). The CPSS and the FAST involve a screen for clinical signs commonly seen in stroke, while the LAPSS and MASS encompass both components of the history which make stroke less likely and clinical signs commonly seen in stroke. If all the history items are absent and the patient has appropriate signs, stroke is deemed to be present. Use of these scales leads to reasonable diagnostic accuracy - 80% diagnostic accuracy for the LAPSS, 84% for the CPSS and 86% for the MASS (262) with similar rates likely with FAST (263). A further study showed that paramedic diagnostic accuracy using the LAPSS improved after a period of training (264). The commonest stroke mimics with these tools are cardiac events, seizures, hypoglycaemia, subdural haematoma and fracture. Missed strokes (10% of 73 using the MASS for example) usually occurred because of presence of a history item or where a visual field defect was the only clinical sign (not scored in scale). The more recently developed ROSIER scale (265) extends the concept of the stroke assessment tool from the field into the emergency room

and is intended for use by emergency room staff. The clinical skill possessed by emergency room physicians, in comparison to paramedical staff, applied in an environment more conducive to thorough assessment should allow the use of more complex assessments and thereby yield better diagnostic accuracy. The main differences between ROSIER and the paramedic stroke scales are a specific question regarding syncope (which should increase specificity) and an examination to identify visual field defects (which should increase sensitivity). Early use of ROSIER has yielded promising results: sensitivity was 93% during the prospective validation phase, comparing favourably with the paramedic stroke recognition instruments. Specificity was broadly similar to previous scales, although fewer stroke mimics were referred during the validation phase of the study, suggesting that specificity may improve as experience grows.

Nearly all (6/7) of the missed stroke patients during testing of ROSIER had mild symptoms with an NIHSS score of <3, whom the authors would not have considered for thrombolytic treatment. This does, however, highlight a potential weakness of the ROSIER scale and also, posterior circulation stroke was more likely to be missed. The authors state that the inclusion of assessment of eye movements into the ROSIER scale would have led to detection of 2 more strokes and increased sensitivity further to 95% and this merits further consideration.

Lessons from these scales and a recent systematic review of the predictive value of various symptoms and clinical signs encountered when evaluating suspected stroke patients (266) helps identify those variables likely to be of use in an assessment algorithm for suspected TIA. It is clear that unilateral weakness, in particular of the arm and face, and a language disorder strongly suggest that a stroke or TIA has occurred, while the absence of such signs

and the presence of loss of consciousness or seizure activity point toward an alternative diagnosis. Diplopia, vertigo and sensory loss, while consistent with stroke or TIA, are of less value in making a clear clinical diagnosis but may contribute. As yet however, there are no such diagnostic algorithms in use for suspected TIA, although clinical prediction tools have been developed to aid risk stratification following confirmed TIA. An example is the "ABCD" scoring tool and its refinement "ABCD2", both of which were developed and validated in cohorts with confirmed TIA. Using only clinical parameters, the ABCD/ABCD2 scores describe ordinal hierarchical scales from 0 – 6/7 (table 2.1). Those with the highest scores have significantly increased risk of early stroke (31;32).

A	Age	≥ 60 years	*1 point*
B	Blood pressure	≥ 140/90 mmHg	*1 point*
C	Clinical features	Unilateral weakness	*2 points*
		Speech impairment without weakness	*1 point*
D	Duration	≥ 60 minutes	*2 points*
		10-59 minutes	*1 point*
D	Diabetes	Presence of Diabetes Mellitus	*1 point*

Table 2.1. ABCD2 Scoring Criteria. When using the ABCD score, no point is added for prevalent Diabetes Mellitus.

There remain some controversies regarding widespread use of these tools. Attempts to validate these systems in independent populations have been generally (32), but not universally successful (267) and some groups have questioned the utility of a score that does not incorporate significant carotid disease or other potential cardio-embolic source (268). Most importantly, in the original and subsequent ABCD validation studies, presentation with true TIA was assumed. This renders suitability of the tool by referring practitioners (or using information from them) on all those referred to TIA services

unsupported; TIA assessment services consistently see a high rate of non-cerebrovascular pathologies (257). Further, given that the majority of studies testing the properties of the ABCD system did not utilise brain imaging to support ischaemic brain damage and the known difficulties with diagnosis, it is possible the cohorts described included a substantial proportion of patients without a true TIA. It could be argued the very low rate of early stroke in those with a low ABCD score (32) supports this assertion and that part of the utility of these tools is to distinguish true TIA from other more benign pathologies. Regardless, the utility of these scores could be seen to justify attempts to develop better and more encompassing diagnostic tools.

Thus, data from the field of acute stroke and from risk stratification instruments for confirmed TIA suggest that development of an algorithm or clinical scoring system for diagnosis of TIA should be possible. If it were, and the number of patients referred with alternate diagnoses was reduced, this could reduce burden on services and improve care for those with genuine TIA.

2.02 Chapter Aim / Hypothesis

The hypothesis was that a diagnostic algorithm and clinical scoring system could be developed to aid accurate diagnosis of TIA and minor stroke in the community, that its use would reduce the numbers referred with non-cerebrovascular diagnoses and that this would reduce clinic waiting times and facilitate rapid assessment. The aim was to develop and rigorously test such a system.

2.03 Methods

The algorithm was developed using data contained in the West Glasgow Stroke Registry and was tested on an independent prospective data set from the same source. The impact of the projected reduction in non-cerebrovascular referral rate was then assessed using real data on referral rates and clinic availability during the prospective validation study.

The West Glasgow Stroke Registry

The Western Infirmary serves a catchment population of approximately 225,000 people and receives approximately 500-600 outpatient referrals per year (predominantly (>95%) from GPs). The fast track TIA clinic is held twice weekly. Baseline demographic data, a history of presenting complaint, relevant examination findings and diagnosis and management plans are prospectively recorded at the time of clinic review. All patients are discussed with a Consultant Stroke Physician (with at least 10 years experience). Data are entered into an electronic database (the West Glasgow Stroke Registry) which currently includes all patients who attended the Fast Track clinic between March 1992 and January 2005. At a follow up visit, data regarding investigation results, final diagnosis and treatment plans are gathered.

Development of the Diagnostic Tool

The diagnosis ("cerebrovascular" versus "non-cerebrovascular") as determined at clinic visit was used as the reference standard in the study. This included patients with TIA and those with minor stroke symptoms lasting 24 hours who had not been referred to the in-patient service. Variables felt likely to be useful in the diagnosis of TIA, including those thought to be suggestive of an alternative diagnosis (table 2.2) were identified in advance.

Included variables were symptoms used in stroke diagnostic algorithms and those previously shown to predict a stroke diagnosis (261-263;265;266). Logistic regression models were used to identify discriminatory variables and to develop a clinical scoring system.

Statistical Analysis

Analyses were performed using S Plus version 6.2. First, univariate analysis was used to identify variables predictive of diagnosis. Logistic regression models were used to identify independently discriminatory variables. Stepwise selection procedures were employed to identify significant explanatory variables using Akaike's Information Criterion (269). In a forward stepwise selection procedure, the initial model involves no explanatory variables and the most significant variable of all the explanatory variables is added to the model at each step until all significant variables are included. In backward selection, the initial model involves all possible explanatory variables and at each step the least significant variable is omitted from the model until the final model selected involved only significant variables. Two way interaction variables were also considered. The interactions considered were all feasible two way interactions within each subcategory of predictive variables (as shown in table 2.2). The final model was internally validated using three-fold cross validation. During three-fold cross-validation, the data were split into three groups of equal size and the model was fitted to the data of two of the groups to predict the class of the remaining group. This was repeated for all group combinations and for two further random splits of the data.

Risk Factors / Miscellaneous	**Predictive of Cerebrovascular Diagnosis**	**Predictive of Non Cerebrovascular Diagnosis**
Smoker	Unilateral Face Weakness *	Headache *
Hypertension *	Unilateral Arm Weakness *	Seizure *
Diabetes Mellitus *	Unilateral Leg Weakness *	Loss of Consciousness *
Hyperlipidaemia *	Unilateral Face Sensory Disturbance *	Pre-syncope
Atrial Fibrillation	Unilateral Arm Sensory Disturbance *	
History of Stroke or TIA *	Unilateral Leg Sensory Disturbance *	
Other Vascular Disease *	Sudden True Vertigo	
Duration of Symptoms *	Diplopia *	
	Sudden True Ataxia *	
	Language Disorder *	
	Hemianopic Visual Disturbance *	

Table 2.2. Pre-identified Variables Felt Likely to be Predictive of Clinic Diagnosis. * = variables predictive on univariate analysis, all p<0.001.

Variables that showed discriminatory power were considered for inclusion in a clinical scoring system. Non-weighted (where explanatory variables were assigned a value of 1), weighted and rounded weighted scoring systems (based upon the regression co-efficient) were then developed. Receiver operating characteristic curves were used to determine optimal cut-off scores; and sensitivity, specificity and positive and negative predictive values were calculated. Following this two 'costs of misclassification' models were developed to reflect the presumed greater importance of failing to identify cerebrovascular

events compared to incorrectly labelling mimics. A cost of 2:1 is considered when it is assumed that the cost of misclassifying a cerebrovascular patient as non-cerebrovascular is twice as much as the cost of misclassifying in the opposite direction. Similarly, a cost of 3:1 is where the cost of misclassifying a cerebrovascular patient as non-cerebrovascular is deemed to be three times as great. Essentially it is a penalty assigned for making a mistake, taking into account that a failure to treat a life-threatening condition is more serious than undertaking unnecessary but generally safe investigations. By raising or lowering the cost of a misclassification, decisions are biased in different directions, as if there were more or fewer cases in a given class. These ratios were arbitrarily chosen. The Hosmer-Le Cessie test (270) was used to evaluate fit of the model.

A formal power calculation was not performed for the multivariable logistic regression models; this is notoriously complex. However, the data includes a large number of outcomes for each variable included (35 for the least common variable (seizure) with others in the hundreds). Further, the small standard errors, small p values and large sample size give further evidence that power was sufficient for the plan to proceed to multivariate modeling.

Prospective Validation of the Diagnostic Tool

Data on all referrals to the Fast Track TIA clinic were gathered during the period October 2005 to June 2006. The clinical scoring system scores were not used to aid clinical diagnosis and were later calculated by independent observers who were not involved with the patients care or development of the scoring system.

Assessment of the Impact of the Tool

Delays to clinic appointment during the prospective validation phase were calculated (expressed as median and inter-quartile range). The effect of the projected reduction in non-cerebrovascular referral rates was then established via a model based upon the actual number of referrals and clinic availability during the study period. During early modeling it was predicted that the scoring system would reduce the number of non-cerebrovascular referrals by approximately 50% or 25% with the unadjusted weighted system and 2:1 costs model respectively. The median delay to clinic appointment was then recalculated first with every second then every fourth non-cerebrovascular patient being removed.

The advice of a Multi-centre Research Ethics Committee was sought and formal ethics committee approval and formal informed consent were deemed unnecessary.

2.04 Results

Development of the Scoring System

The development cohort included 3230 patients. Mean age was 65 years (SD 12.8). Other baseline characteristics are shown in table 2.3. Sufficient data were available for 3216 patients, of whom 2215 (69%) had a diagnosis of TIA or minor stroke.

Variable	Development Sample n=3230	Validation Sample n=237
Age	65 years (SD 12.8)	65 years (SD 15.7)
Smoker	1893 (58.6%)	84 (35.45%)
Hypertension	1484 (45.96%)	111 (46.84%)
Diabetes	261 (8.08%)	29 (12.24%)
Hypercholesterolaemia	637 (19.73%)	56 (23.63%)
Atrial Fibrillation	161 (4.99%)	18 (7.59%)
Previous Stroke or TIA	937 (29.02%)	55 (22.36%)
Other Vascular Disease	879 (27.22%)	60 (25.32%)

Table 2.3 – Baseline Characteristics of Samples Used to Develop The Scoring System.

Variables predictive of diagnosis on univariate analysis are shown in table 2.2. Three risk factors (history of stroke or TIA, hypertension and diabetes) and 17 clinical features were significantly predictive of clinician diagnosis on logistic regression analysis. The predictive clinical features were headache, vomiting, loss of consciousness, seizure (all predictive of non-cerebrovascular diagnosis), age, duration of symptoms, visual loss, diplopia, ataxia, speech disorder, dysphasia, unilateral arm or leg weakness, unilateral facial weakness, unilateral sensory disturbance, other pattern of weakness and other pattern of sensory disturbance (predictive of a cerebrovascular diagnosis). During stepwise selection, 8 variables were rejected from the model leaving twelve explanatory variables. These were history of stroke or TIA, headache, diplopia, loss of consciousness, seizure, age, duration of symptoms, speech disorder, unilateral leg weakness, unilateral upper motor neuron (UMN) facial weakness, unilateral lower motor neuron (LMN) facial weakness and other weakness. No interaction terms remained in the model.

Three variables were removed as it was felt they would not be useful in the scoring system; LMN facial weakness was removed as there were few cases and each occurrence was associated with a diagnosis of Bell's palsy (a rare occurrence in the TIA clinic). UMN facial weakness was then renamed unilateral facial weakness. Duration of symptoms was removed. This was recorded as < 1 hour, 1-24 hours, 1-3 days or > 3 days in the database and it was felt none of these boundaries was sensitive enough to influence decision making; many TIAs last less than 1 hour, while on the other hand, those lasting longer convey greater risk (31;32). "Other weakness" was also removed. This was essentially defined as non unilateral weakness, so was already being accounted for in the model and it was felt this may lead to confusion during use of the score. Unilateral leg weakness was included in the final model but unilateral arm weakness was not; this was because the vast majority of patients with unilateral arm weakness had unilateral leg weakness and vice versa meaning one of these variables was removed during stepwise regression. The variable unilateral leg weakness was therefore replaced by unilateral limb weakness. This left 9 predictive variables: 6 positive indicators of cerebrovascular disease and 3 indicators of a non-cerebrovascular diagnosis. The final regression coefficients (when only these 9 variables were included and rounded to one decimal place) are shown in table 2.4 and all had p values of <0.001. Removal of the unsuitable variables did not affect the performance of the final model (assessed using linear discriminant analysis).

Explanatory Variable	Coefficient	Standard Error
History of Stroke (or TIA)	0.51	0.1 ($3.5x10^{-7}$)
Headache	0.46	0.11 ($7.1x10^{-5}$)
Diplopia	1.23	0.28 ($2.7x10^{-6}$)
Loss of Consciousness	1.06	0.21 ($1.9x10^{-7}$)
Seizure	1.58	0.43 ($1.4x10^{-4}$)
Speech Abnormalities	1.29	0.14 ($<1x10^{-10}$)
Unilateral Limb Weakness	1.72	0.10 ($<1x10^{-10}$)
UMN Facial Weakness	0.63	0.15 ($9.5x10^{-8}$)
Age	0.04	0.004 ($<1x10^{-10}$)

Table 2.4. – The final regression co-efficients for variables predictive of clinic diagnosis. UMN = upper motor neurone.

The Weighted Scoring System

The weighted scoring system is shown in table 2.5. Predictive variables were allocated the value of the regression coefficient for that variable if the variable was present or absent as outlined in the table. ROC curves identified a score of >6.1 as the optimal cut-off for prediction of cerebrovascular diagnosis (figure 2.1). This accurately identified 84% of cerebrovascular diagnoses and 60% of non-cerebrovascular diagnoses with a positive predictive value (PPV) of 82% and negative predictive value (NPV) of 62% (table 2.6).

With the 2:1 cost ratio, an optimal cut-off score of > 5.4 was used (figure 2.2) and 97% of TIA and 24% of non-TIA patients were accurately identified with a positive predictive value of 73% and 78% (table 2.7). With a 3:1 cost ratio, an optimal cut-off score of > 5 was used and 98% of TIA and 15% of non-TIA patients were accurately identified. With a 5:1 cost ratio, an optimal cut-off score of > 4 was used and 100% of TIA and 2% of non-TIA

patients were accurately identified. As these later two scores yielded only minor increases in sensitivity with significant falls in specificity, they were not considered further.

TIA		
Variable	**Score if Yes**	**Score if No**
History of Stroke or TIA	0.5	0
Headache	0	0.5
Diplopia	1.2	0
LOC / Pre-syncope	0	1.1
Seizure	0	1.6
Speech Abnormalities	1.3	0
Unilateral Limb Weakness	1.7	0
UMN Facial Weakness	0.6	0
Age	Multiply by 0.04	
To calculate the score, all values should be summed. If total score >6.1, classify as TIA. For "2:1 cost ratio," if total score >5.4, classify as TIA.		
Table 2.5. – The Weighted TIA scoring system. All values reflect the regression coefficients seen.		

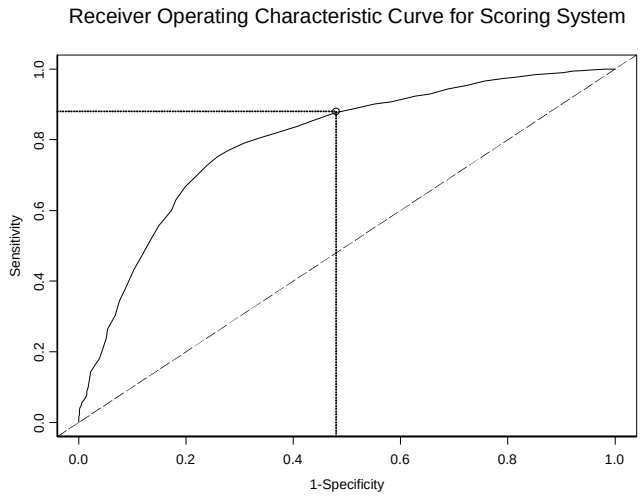

Figure 2.1. Receiver Operating Characteristic Curve for Weighted Scoring System

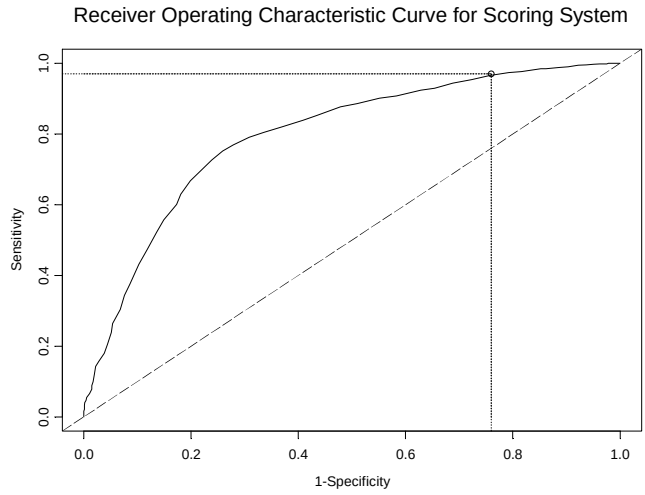

Figure 2.2. Receiver Operating Characteristic Curve for Weighted Scoring System With 2:1 Cost Ratio

Clinic Diagnosis	**Predicted Diagnosis**		***Row Total***
	CVD	**Non-CVD**	
CVD	1852	363	*2215*
Non-CVD	397	604	*1001*
Column Total	2249	967	*3216*

Table 2.6. 2*2 Table for Weighted Scoring System During the Development Phase. Calculated sensitivity 84%, specificity 60%, PPV 82%, NPV 62%.

Clinic Diagnosis	**Predicted Diagnosis**		***Row Total***
	CVD	**Non-CVD**	
CVD	1852	363	*2215*
Non-CVD	397	604	*1001*
Column Total	2249	967	*3216*

Table 2.7. 2*2 Table for Weighted Scoring System with 2:1 Cost Adjustment During the Development Phase. Calculated sensitivity 97%, specificity 24%, PPV 73%, NPV 78%.

The Un-weighted Scoring System

The un-weighted scoring system is shown in table 2.8. Predictive variables were allocated a value of one to be added if variable was present or absent as outlined in the table. ROC curves identified a score of >5.5 as the optimal cut-off for prediction of cerebrovascular diagnosis (figure 2.3). This accurately identified 82% of cerebrovascular diagnoses and 60% of non-cerebrovascular diagnoses with a positive predictive value of 82% and NPV of 60% (table 2.9).

Variable	**Score if Yes**	**Score if No**
History of Stroke or TIA	1	0
Headache	0	1
Diplopia	1	0
LOC / Pre-syncope	0	1
Seizure	0	1
Speech Abnormalities	1	0
Unilateral Limb Weakness	1	0
UMN Facial Weakness	1	0
Age	Multiply by 0.03	
To calculate the score, all values should be summed. If total score >5.5, classify as TIA. For "2:1 cost ratio," if total score >4.5, classify as TIA.		
Table 2.8. – The Un-weighted TIA scoring system.		

With the adjustment to reflect the greater seriousness of missing true cerebrovascular patients (the 2:1 cost ratio), an optimal cut-off score of > 4.5 was used (figure 2.4) and 98% of TIA and 18% of non-TIA patients were accurately identified (table 2.9).

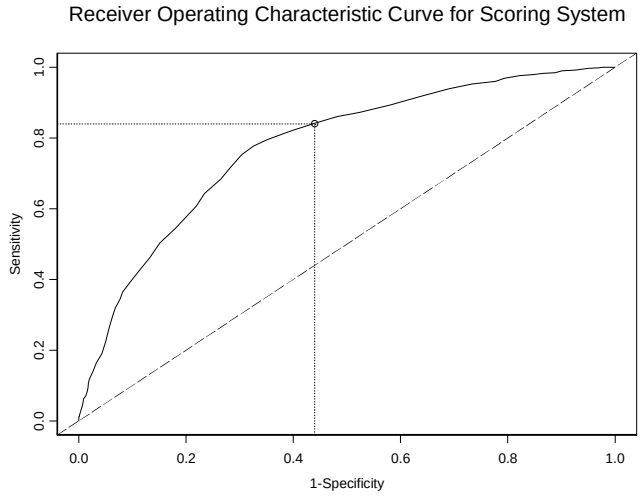

Figure 2.3. Receiver Operating Characteristic Curve for Un-weighted Scoring System

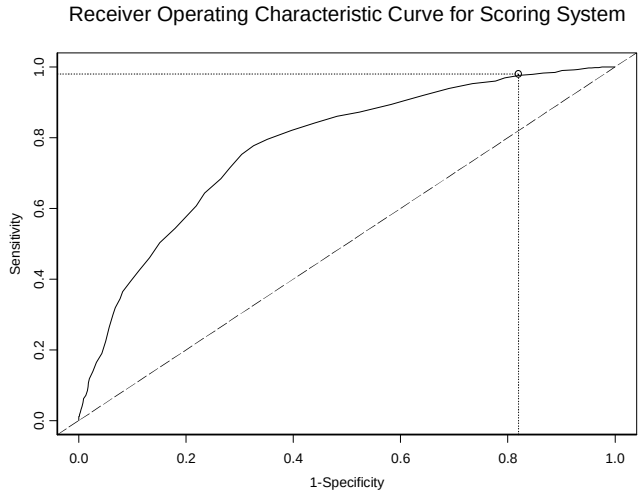

Figure 2.4. Receiver Operating Characteristic Curve for Un-weighted Scoring System With 2:1 Cost Ratio.

Clinic Diagnosis	Predicted Diagnosis		*Row Total*
	CVD	**Non-CVD**	
CVD	1822	393	*2215*
Non-CVD	401	600	*1001*
Column Total	2223	993	*3216*

Table 2.9. 2*2 Table for Un-Weighted Scoring System During the Development Phase. Calculated sensitivity 82%, specificity 60%, PPV 82%, NPV 60%. Using the 2:1 Cost Adjustment, calculated sensitivity 98% and specificity 18%.

The Rounded Scoring System

The rounded weighted scoring system is shown in table 2.10. Predictive variables were allocated a value of one to be added if variable was present or absent as outlined in the table. ROC curves identified a score of >6.6 as the optimal cut-off for prediction of cerebrovascular diagnosis (figure 2.5). This accurately identified 85% of cerebrovascular diagnoses and 55% of non-cerebrovascular diagnoses with a positive predictive value of 81% and NPV of 63% (table 2.11).

With the 2:1 cost ratio, an optimal cut-off score of > 5.7 was used (figure 2.5) and 97% of TIA and 23% of non-TIA patients were accurately identified.

Variable	**Score if Yes**	***Score if No***
History of Stroke or TIA	0.5	*0*
Headache	0	*0.5*
Diplopia	1	*0*
LOC / Pre-syncope	0	*2*
Seizure	0	*1.5*
Speech Abnormalities	1	*0*
Unilateral Limb Weakness	2	*0*
UMN Facial Weakness	0.5	*0*
Age	*Multiply by 0.04*	
To calculate the score, all values should be summed. If total score >6.6, classify as TIA. For "2:1 cost ratio," if total score >5.7, classify as TIA.		
Table 2.10. – The Rounded TIA scoring system. All values reflect the regression coefficients seen rounded to the nearest half integer.		

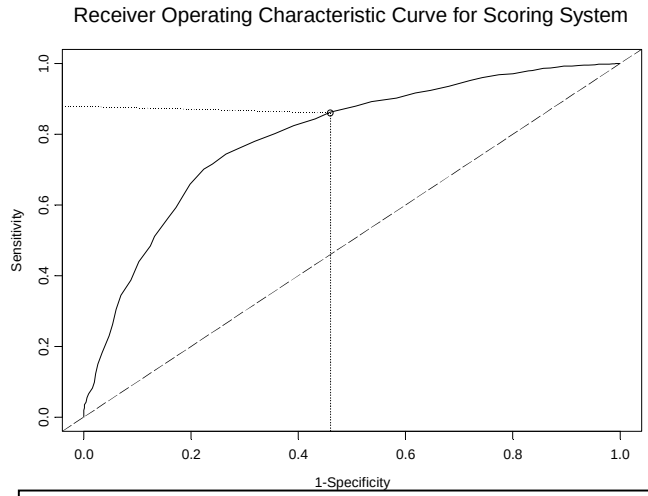

Figure 2.5. Receiver Operating Characteristic Curve for Rounded Weighted Scoring System.

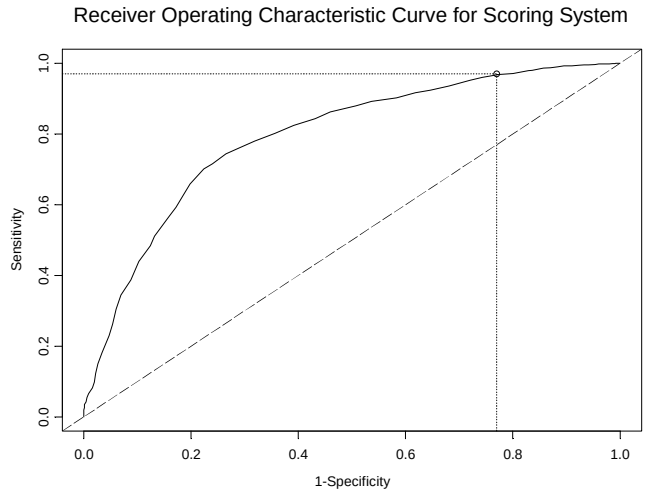

Figure 2.6. Receiver Operating Characteristic Curve for Rounded Weighted Scoring System with 2:1 Cost Ratio.

Clinic Diagnosis	**Predicted Diagnosis**		***Row Total***
	CVD	**Non-CVD**	
CVD	1891	324	*2215*
Non-CVD	450	551	*1001*
Column Total	2223	993	*3216*

Table 2.11. 2*2 Table for the Rounded Weighted Scoring System During the Development Phase. Calculated sensitivity 85%, specificity 55%, PPV 81%, NPV 63%. Using the 2:1 Cost Adjustment, calculated sensitivity 97% and specificity 23%.

The Prospective Validation Set

237 patients were included of whom 143 (60.3%) had a diagnosis of TIA or minor stroke. Further baseline characteristics are shown in table 2.3.

The weighted scoring system correctly identified 85% of patients with a cerebrovascular diagnosis and 54% of those with a non-cerebrovascular diagnosis with a PPV of 74% and a NPV of 70%. Using the 2:1 misclassification score, 93% of patients with a cerebrovascular diagnosis and 34% of those with a non-cerebrovascular diagnosis were correctly identified

with a PPV of 68% and NPV of 76% (table 2.12). With the 2:1 cost adjustment, the rounded weighted scoring system gave a sensitivity of 93% and a specificity of 37% with a PPV of 69% and NPV of 78% (table 2.13). Also with the 2:1 cost adjustment, the un-weighted scoring system gave a sensitivity of 94% and a specificity of 26% with a PPV of 66% and NPV of 73% (table 2.14). The confidence intervals for all parameters greatly overlap those found during the development phase suggesting similar performance of the model in each data set.

Clinic Diagnosis	**Predicted Diagnosis**		***Row Total***
	CVD	**Non-CVD**	
CVD	133	10	*143*
Non-CVD	62	32	*94*
Column Total	195	42	*237*

Table 2.12. 2*2 Table for the Weighted Scoring System with 2:1 Cost Adjustment During the Prospective Validation Phase. Calculated sensitivity 93%, specificity 34%, PPV 68%, NPV 76%.

Clinic Diagnosis	**Predicted Diagnosis**		***Row Total***
	CVD	**Non-CVD**	
CVD	133	10	*143*
Non-CVD	59	35	*94*
Column Total	192	45	*237*

Table 2.13. 2*2 Table for the Rounded Weighted Scoring System with 2:1 Cost Adjustment During the Prospective Validation Phase. Calculated sensitivity 93%, specificity 37%, PPV 69%, NPV 78%.

Clinic Diagnosis	Predicted Diagnosis		*Row Total*
	CVD	**Non-CVD**	
CVD	134	9	*143*
Non-CVD	70	24	*94*
Column Total	195	42	*237*

Table 2.14. 2*2 Table for the Un-Weighted Scoring System with 2:1 Cost Adjustment During the Prospective Validation Phase. Calculated sensitivity 94%, specificity 26%, PPV 66%, NPV 73%.

It was felt that the 2:1 cost adjustment models performed best and that the weighted and rounded weighted scoring systems performed equally well but were better than the un-weighted system. Given that the scores would require formal calculation because age was used as a continuous variable, the weighted scoring system with 2:1 cost adjustment was chosen as the final model (table 2.5, figure 2.2) as it most closely represents the data and an on-line tool was developed to allow automated calculation of the score (http://www.stams.strath.ac.uk/~karenl/tia/). The Hosmer-Le Cessie test gave a p value of 0.02 for fit of the final chosen model.

Impact on Clinic Waiting Times

During the period of the prospective validation set the median waiting time from referral date to clinic attendance was 15 days (IQR 8-23). Removal of every fourth and every second non-cerebrovascular patient had potential to reduce the median waiting time to 8 and 7 days respectively. The proportion of those seen within 7 days (and thus within the minimum standard of current guidelines) was 21.6%. This increased to 42.9 and 50% respectively following omission of every fourth and every second non-cerebrovascular

patient representing a 21.3 and 28.4% increase in those assessed within the framework of current guidelines. This is summarized in table 2.15.

	Current Clinic Performance	**25% ↓ in Non-TIA Referral Rate**	**50% ↓ in Non-TIA Referral Rate**
Median Delay to Clinic	15	8	7
Assessed Within 7 Days	21.6%	42.9%	50%

Table 2.15. Projected Improvements in Delays to Clinic Presentation

2.05 Discussion

The results of this study show that a clinical scoring system could be utilized to reduce the number of non-cerebrovascular referrals to a fast track TIA service. A scoring system that employs a 2:1 cost of misclassification adjustment would have little adverse impact on recognition rates of bona fide TIA and could reduce the number of non-cerebrovascular referrals by a clinically meaningful amount.

TIA is poorly managed in many countries, including in the UK as the above data from the UK National Audit Office and the House of Commons Public Accounts Committee (5) clearly show. UK consensus guidelines suggest that patients with suspected TIA are assessed and investigated within 1 week (33;34) while European guidelines appropriately suggest assessment "without delay" (55). Fulfilling these aims will be costly and will present challenges. For example, the clinic at the Western Infirmary was established (and was able) to assess patients within days but experienced a progressive increase in non-

cerebrovascular referrals to approximately 50% (257). This “overloaded” the system, such that the median delay to clinic assessment in the prospective validation period of this study was an unsatisfactory 15 days. Reducing the number of non-cerebrovascular referrals is feasible and will free up existing resources and facilitate urgent assessment of those with TIA.

However, accurate identification of stroke and TIA patients is notoriously difficult (255;256) and a variety of conditions, such as seizure, migraine or systemic upset can mimic TIA (266). Assessment algorithms exist but are rarely employed out with the clinical trial setting (271;272) and in practical terms represent little more than descriptions of a typical TIA which give little practical guidance to aid decision making by non-specialists. A recent systematic review of the predictive value of various symptoms and clinical signs (266) and the available stroke assessment tools (260-263;265) show that symptoms such as unilateral weakness and a language disorder suggest that stroke has occurred, while loss of consciousness or seizure activity point toward an alternative diagnosis. Diplopia, vertigo and sensory loss are also, but more weakly, consistent with stroke. In this study, similar variables were unsurprisingly found to be predictive of TIA.

Unilateral limb weakness, unilateral facial weakness, speech disorder, diplopia, history of stroke or TIA and increasing age were predictive of TIA. There are obvious deficiencies of a scoring system involving only these variables. Patients with amaurosis fugax, sensory lacunar and some posterior circulation events would be missed. It is unrealistic, given the heterogeneity of TIA symptoms, to expect that a clinical scoring system could detect all types of event. Amaurosis fugax for example has an entirely different set of differential diagnoses than other types of TIA. It is imperative that such systems are introduced in

conjunction with improved user education, with clear direction as to their limitations and clear advice that clinician concern and acumen override the score.

The recently developed ABCD and ABCD^2 scores are risk stratification instruments that deserve comment (31;32). They seem able to identify those with a high risk of stroke early after TIA but were developed on data from patients with a confirmed diagnosis of TIA and are thus unproven as diagnostic instruments. They would also suffer similar, or perhaps greater, weaknesses as the score developed in this study in terms of failure to detect amaurosis fugax, sensory lacunar and posterior circulation events. A recent analysis from our unit suggests this may be the case (273) but whether this score would add further to the increasingly used ABCD scores is unclear and under investigation.

There are limitations to this study. The clinic diagnosis, rather than final diagnosis (such as that supported by brain imaging) was used as the reference standard. Essentially therefore this study has assessed whether a scoring system can distinguish those whom a specialist feels require further investigation for cerebrovascular disease from those who do not. This is an appropriate aim for a diagnostic tool, in particular one to aid the clinical diagnosis of TIA. However, levels of inter-observer agreement in the unit from which the study data arises have not been assessed but hopefully the standardized review process, the large number of subjects and limited number of experienced observers used will limit these. Further, only three patients with suspected stroke had a final diagnosis which differed from the original clinic diagnosis during the prospective validation phase. Although it was reassuringly consistent with the development sample, the prospective validation sample was small and cannot replace external validation.

The Hosmer-Le Cessie test yielded a p value of 0.02 suggesting the favoured model is not a good fit to the data (p-values of greater than 0.05 suggest a good fit). The fit of the model could perhaps have been improved by selecting different weights for the scores involved in the system or by selecting a different cut-off point for the model but this would render the tool less clinically useful; a greater importance was assigned to sensitivity in order to ensure the maximum number of true TIA cases would be referred for specialist assessment.

This tool could be used by colleagues in primary care. However, it was developed using data generated during assessment by stroke specialists. It is not certain that GPs would reach the same conclusions regarding what constitutes a particular symptom, for example, pre-syncope or unilateral limb weakness. A sensitivity of 85%, as seen with the weighted scoring system, represents a failure rate of TIA detection too great for safe use in clinical practice. The 2:1 misclassification scoring system yielded a sensitivity of 97% and specificity of 24% during the development phase and 93% and 34% during the prospective validation phase. This reduction in non-cerebrovascular referral rate was shown to be clinically meaningful; it would greatly reduce delay to assessment in this service. The weighted system performed best, although the non-weighted system would be easiest to use in clinical practice. The rounded weighted system may appear easiest to use but age is incorporated as a continuous variable so formal calculation would be required so it was opted to use the weighted system which most accurately reflects the data as the final model. This makes the score seem cumbersome and perhaps too difficult to use but many centers are moving toward an electronic referral process which would allow scores to be calculated automatically from information given, without the need for manual calculation. An example of this can be found at http://www.stams.strath.ac.uk/~karenl/tia/. This allows

maximum information and discriminatory ability to be retained, and in particular to avoid dichotomizing continuous variables such as age.

The false negatives or "missed TIAs" are of interest. In the prospective validation phase there were 10 patients with a presumed cerebrovascular diagnosis who were not identified by the 2:1 costs scoring system. Of these, three were felt at final clinic review not to have had a TIA or minor stroke and only one of the remainder had brain imaging supportive of ischaemia in the relevant territory. This patient presented with a pure hemianopic visual disturbance. Two had possible transient ataxia, one had a transient language disorder and the remainder pure sensory symptoms. These patients should still be identified if the score was used in conjunction with basic clinical acumen; use of the score itself will improve knowledge of presentation of stroke and TIA and may thus increase detection of TIA at the population level.

Chapter Summary

This study suggests that a reduction in the rate of referral of non-cerebrovascular diagnoses could significantly improve performance of TIA services and that a simple clinical scoring system could be used to achieve this. Further work to evaluate use of the score in clinical practice is ongoing. Independent external validation and perhaps comparison to the ABCD scores would be of use.

CHAPTER THREE

ASPIRIN RESISTANCE AND RISK OF CEREBROVASCULAR EVENTS

Aspirin Resistance and Risk of Cerebrovascular Events

3.01 Potential Significance of Aspirin Resistance

As outlined in the introduction, aspirin based anti-platelet strategies are the most commonly prescribed and are likely to remain so. However, regardless of the chosen anti-platelet strategy, it is clear that the effect is modest and recurrent event rates remain high. Methods to identify the most suitable regimen for individual patients are attractive as recent data suggest that intra-individual response to anti-platelet agents differs.

It is increasingly recognised that some have a degree of "aspirin resistance." A recently published meta-analysis revealed a prevalence of 28% (274) but included studies covering a variety of patient groups and study methodologies. In the context of patients with a history of acute stroke, a "resistance" rate of 25.5% (and 8.1% to 1300mg aspirin per day) has been quoted (275). Recent meta-analyses have also shown resistant patients to have a near four fold increase in risk of suffering a vascular event compared (274;276) with aspirin responders. For example, across 20 studies of 2930 individuals, 810 (28%) were classified as aspirin resistant and 39% of these suffered a cardiovascular event during follow-up, compared to only 16% of those who were aspirin sensitive (OR 3.85, 95% CI 3.08 to 4.8). The OR for a new cerebrovascular event in those with aspirin resistance was 3.78 (95% CI 1.25 to 11.41); the wide confidence intervals reflect the small number of studies and subjects included. A high rate of aspirin resistance has been seen in those with stroke recurrence despite aspirin (277) and an increase in total vascular events (278) has been seen in aspirin non-responders with documented cerebrovascular disease. A recent study showed a far higher rate of aspirin resistance in those who suffered a repeat stroke during follow up in comparison to those who did not (OR 14.25, 95% CI 8.5 to 23.7) (279). It

seems therefore that lack of response to aspirin may link to increased stroke risk in both the primary and secondary preventative setting.

However, it is important to recognize that a variety of issues beset research in this field; as yet no consensus exists concerning the definition of aspirin resistance or its mechanism and no evidence exists to suggest that its presence should guide therapy.

3.01.1 Definition of Aspirin Resistance / Measuring Platelet Function

Aspirin resistance can be defined either clinically (vascular events in patients taking aspirin, perhaps better termed treatment failure) or in terms of laboratory measures (such as failure to inhibit platelet aggregation or thromboxane A2 production). The available laboratory measures are numerous.

The platelet count is not affected by anti-platelet therapy so is unsuitable as a means of assessing aspirin responsiveness. Likewise, the bleeding time, while it is affected by aspirin (280;281) and can show differences between action of anti-platelets (281), is not well-suited to widespread clinical use as it involves a skin incision and is unlikely to be well tolerated by patients.

The level of response to aspirin therapy can be determined by measuring the extent by which aspirin inhibits platelet production of thromboxane A_2. Thromboxane A_2 is metabolised to various stable thromboxane B_2 products which can be detected in the plasma or urine as simple and inexpensive indicators of platelet activity. Thromboxane A_2 is metabolised to thromboxane B_2 (detectable in plasma), and 11-dehydrothromboxane B_2 (detectable in urine). These metabolites can be measured by ELISA techniques and have

been used in studies of aspirin resistance which have shown an association between resistance and increased cardiovascular event rate (282). There are important issues concerning specificity of these techniques. For example, urinary 11-dehydrothromboxane B_2 levels reflect thromboxane production, which by in large will be inhibited by aspirin but extra-platelet sources of thromboxane A2 exist and may arise in those with recently symptomatic cardiovascular disease (283) and thus lead to overestimates of resistance levels.

There are a number of other means of measuring thromboxane-dependent platelet function. Light or optical aggregation techniques have traditionally been used and measure the increase in light transmittance through platelet suspensions when platelets are aggregated by an agonist such as thromboxane A_2, adenosine diphosphate or collagen. This requires the preparation of a suspension of platelet-rich plasma and while often considered the gold standard, is time consuming. Other drawbacks include the potential for artificial activation of platelets during centrifugation. Electrical impedance aggregometry (284) is a similar technique which measures changes in electrical impedance between two electrodes when platelets are aggregated by an agonist. Conveniently, this method can be performed on whole blood samples and obviates some of the drawbacks of optical aggregometry. However, the accuracy and reproducibility of both techniques is somewhat poor due to the impact on aggregation of factors such as age, sex and race. Furthermore, in-vitro techniques for measuring platelet function do not reflect the complex in-vivo interactions of platelets with other circulating blood elements.

Semi-automated cartridge based analysers are now available and have the advantage of being simple and quick to use and can be performed on whole blood samples. Two

commonly used systems are the PFA-100 system (Dade Behring, Leiderbach, Germany) and the RPFA system (Verify Now, Accumetrics). The PFA-100 (285) device measures platelet function under controlled shear stress. A citrated blood mix is placed in an analyser and is vacuum aspirated through a capillary system with a membrane coated with platelet agonists (collagen and epinephrine or adenosine diphosphate cartridges are available). Provided levels of von Willebrand factor and platelet glycoprotein receptors are sufficient, platelets will attach and aggregate upon contact with the membrane. Platelet adhesion, activation and aggregation is then expressed as the 'closure time' – the time taken for a platelet plug to form and occlude the analyser aperture. Only the collagen and epinephrine assay is sensitive to the action of aspirin. The device has been shown to be more sensitive than traditional optical aggregation techniques (286) and labels a higher proportion of individuals as resistant (274). The maximum time measured is 300 seconds, after which the closure time is given as >300 seconds. The RPFA system utilizes a slightly different method and is a form of whole blood aggregometry. A citrated blood mix and 'aspirin assay' are placed into an analyser and platelet induced aggregation is measured as an increase in light transmittance. The aspirin assay contains fibrinogen coated beads and arachidonic acid. Arachidonic acid causes platelet aggregation and binding to the fibrinogen beads follows causing platelet agglutination. The extent of this is proportional to the changes in light transmission and results are expressed as the number of aspirin response units and percentage inhibition of platelets by aspirin. The RPFA system can also give measures of clopidogrel and platelet-glycoprotein inhibitor responsiveness if different assays are used. Anecdotally, results on use of the PFA-100 device are also user dependent and a learned effect exists. Specific data are limited, although many laboratories report that reproducibility of results and the number of errors reduces with experience.

Thromboelastography can also be performed using a point of care device (TEG, Haemoscope Corp, Niles, IL, USA). The TEG system measures a variety of variables as blood is induced to clot under low shear stress conditions. The pattern of changes in visco-elastic properties of blood mirror those of clot formation and allow assessment of the kinetics of clot formation and growth and strength and stability of the clot. A coagulation profile is then displayed and while this is most commonly used to evaluate clot formation during cardiopulmonary bypass, it can be used to assess response to anti-platelet therapy. A recent development is the TEG Platelet Mapping assay (Haemoscope Corp, Niles, IL, USA) which eliminates the contribution of thrombin and fibrin to clot strength (via addition of heparin, reptilase and factor XIIIa) and thus allows the impact of platelet pathways to be studied by the addition of relevant agonists (287). Addition of arachidonic acid allows measurement of thromboxane A2 mediated platelet aggregation.

Flow cytometry techniques may also be used to determine levels of platelet inhibition by aspirin. Surface expression of numerous platelet activation markers such as P-selectin (288) and activated glycoprotein IIb/IIIa receptors (289) on platelet membranes can be induced and measured as indicators of aspirin activity. However, these techniques require expensive equipment, are not widely available and are time-consuming.

There are thus a variety of techniques to assess aspirin responsiveness and there are advantages and drawbacks with each. Most available methods are limited by the need for local analysis; whether whole blood or plasma based techniques, transportation to central laboratories, a standard feature in most clinical trials, is not possible as transportation of samples will increase likelihood of false results. The cartridge based analysers are attractive because they could function as point of care devices but have limitations in that they do

they do not directly measure platelet thromboxane A2 production and agreement between them has previously been shown to be poor (290). On the other hand, there is attraction to measuring platelet function as a whole rather than one means of platelet activation. Further, it is important to note that approaches that more directly measure thromboxane A2 production have other drawbacks, such as influence from extra-platelet or COX mediated production.

3.01.2 Mechanism of Aspirin Resistance

The mechanism of aspirin resistance is likely to be multi-factorial, or at least may be different in different individuals. It is also true that the presence of apparent aspirin resistance in the laboratory setting may signify increased platelet activation which reflects more severe atherosclerosis; aspirin resistance may therefore simply be a marker of more severe disease which could explain the apparent link with increased cardiovascular events. Putative mechanisms however include lack of patient adherence, insufficient dosing, reduced absorption, non COX-1 mediated thromboxane A2 synthesis, increased activity of alternate platelet activation pathways and interference of aspirin action by other drugs.

Poor compliance is perhaps the most important factor to consider. A flaw of existing work is that many studies have not reliably assessed recent aspirin ingestion and patient compliance (which may itself be a factor in some of these events (291)). If a patient has not taken aspirin, they will appear aspirin resistant and changing an anti-platelet regimen from aspirin to a newer anti-platelet may be unnecessary and costly. Approximately 10-40% of patients do not take prescribed anti-platelet tablets in the secondary prevention setting (292-296) and this could confound aspirin resistance work. It is now possible to measure aspirin (or its salicylate metabolites) in urine using high performance liquid chromatography

techniques, thus allowing objective determination of compliance. This has not been adequately performed in studies to date and it is possible that rates of resistance reported in the literature reflect a proportion of participants with poor therapy compliance.

Aspirin is hydrolysed to inactive salicylate in the gut through the actions of esterases which may reduce its bioavailability and effect (297;298). It is reported that use of proton pump inhibitors can increase mucosal hydrolysis of aspirin (299) which could cause apparent resistance but this is by no means proven (300). It is well known that concurrent intake of certain non-steroidal anti-inflammatory drugs (such as ibuprofen and naproxen) can interfere with the anti-platelet effect of aspirin by preventing aspirin binding to COX-1 (301) and thus prevent a reduction in inhibition of thromboxane production. Interestingly, use of ibuprofen alongside aspirin has been shown to link with increased mortality during follow-up in comparison to aspirin alone and aspirin with diclofenac (302).

Thromboxane A_2 may be produced from non-platelet sources, in particular in pro-inflammatory states where COX-2 activity is increased in monocytes and macrophages and can yield thromboxane A_2 production. This may be relevant in atherosclerosis which is an inflammatory condition (303) and may explain why those with recurrent events or more severe atherosclerosis appear more likely to be aspirin resistant. Renal production of thromboxane may also contribute to platelet activation and several cardiovascular risk factors can increase thromboxane synthesis via alternate pathways such as via isoprostane production (304). Likewise, other pathways of platelet activation exist and include direct stimulation of platelet glycoprotein receptors and platelet shear stress and will be less likely to respond to aspirin; perhaps in this scenario alternative drugs such as clopidogrel will prove of use (305).

Genetic polymorphisms may impact on the inter-individual variation in response to aspirin. A variety of candidate genes have been suggested (306). Polymorphisms in the COX enzymes, platelet receptors and factor XIII have all been postulated as potential mechanisms (307). However, the overall contribution of genetic variation to aspirin resistance has not yet been clearly determined and in a recent systematic review, only the PIA1/A2 polymorphism in the GPIIIa platelet receptor linked with aspirin resistance (OR 2.36, 95% CI 1.24 to 4.49) (308).

Summary

Thus, aspirin resistance appears to link with increased risk of cardiovascular events in those who take it. However, no firm evidence exists to suggest that routine screening for aspirin resistance and subsequent tailored anti-platelet regimens will improve outcome. Also, the size of the effect of aspirin resistance is unclear; confidence intervals around available estimates are wide and, given that aspirin prevents approximately 1/5 of recurrent strokes, it seems unlikely that resistance would lead to a three or four fold increase in event rate unless resistance reflected, at least in part more severe disease.

There is also no consensus concerning its definition, disagreement as to the best means of measuring it and no clear answer as to its cause. These questions are likely to remain until large scale prospective clinical trials are conducted or until aspirin resistance is associated with a robust and easy to study surrogate marker. A link with asymptomatic microemboli in patients with carotid disease for example would prove useful in stimulating and allowing further study; they are a high risk group, MES are easy to measure and are anti-platelet therapy responsive.

3.02 Chapter Aims / Hypothesis

First, as recommended, a brief study was performed to establish the local reference range for normal platelet function using the PFA-100 device (study one). The chapter hypothesis was that aspirin resistance would be associated with the presence of microembolic signals in aspirin treated patients with carotid disease and that the prevalence of aspirin resistance in those who had proven recent aspirin ingestion would be lower than the rates reported in previous literature. The aim was thus to compare the rate of aspirin resistance in those with and without MES (study two) and to quantify the prevalence of aspirin resistance in those with cerebrovascular disease whilst addressing concerns surrounding patient compliance (study three).

3.03 Study One - Development of a Local Normal Range for Use of the PFA-100 Device

It is recommended by the manufacturer and expert guidelines that each institution clarifies its own reference range using healthy controls and establishes a control group for further quality control analyses prior to interpretation of results when using the PFA-100 device. The aim of this study was to establish this range.

3.03.1 Study One Methods

Forty healthy volunteers were recruited. The study was performed in the University Division of Cardiovascular and Medical Sciences at the Western Infirmary, Glasgow and was approved by West Medical Research ethics committee. Inclusion criteria for this study were presumed normal platelet function and age > 18 years. Exclusion criteria were any anti-platelet or anti-coagulant use (within past 1 month), known haematological disorder or family history of haematological disorder and recent non-steroidal anti-inflammatory drug ingestion (within past 2 days).

Approximately 7 mls of blood was drawn using a 21 G needle. With the PFA-100 device, a citrated blood mix is placed into the analyser and platelet adhesion, activation and aggregation is expressed as the 'closure time' – the time taken for a platelet plug to form and occlude the analyser aperture after exposure to platelet agonists. Epinephrine and collagen cartridges are used and are pre-warmed to room temperature. Those with a closure time of between 110-160 seconds were considered for inclusion in a donor control

group and had testing performed in duplicate; if the repeat result was within a range of ±15% of the first sample, they were asked to be part of the control group.

The normal range was then expressed as the 95 % reference range of closure times in seconds. When performing quality control measurements, a closure time within the reference range was deemed satisfactory. The coefficient of variation of closure times has been reported as 10% in the literature (309).

3.03.2 Study One Results

In total 40 participants were recruited between May 2005 and October 2008. All were healthy with no past medical history, no family or personal history of haematological disorders and were taking no regular medications. The majority (24, 60%) were female. Only 37 individuals were included in the analyses. Of those who were excluded, one subsequently admitted to taking warfarin, one suffered factor V Leiden deficiency (both these individuals had abnormal results) and in one further case, no result could be obtained.

The mean closure time was 134 seconds (standard deviation 22.8). The range of values was 95 to 182 seconds and the 95% reference range was 89.4 to 178.9 seconds. Individual test results are shown in figure 3.1. Ten individuals had testing performed in duplicate and the mean coefficient of variation was 7.5%. A closure time below the upper limit of the 95% reference range in the context of recent aspirin ingestion was used to define aspirin resistance (rounded up to a closure time < 179 seconds for analyses).

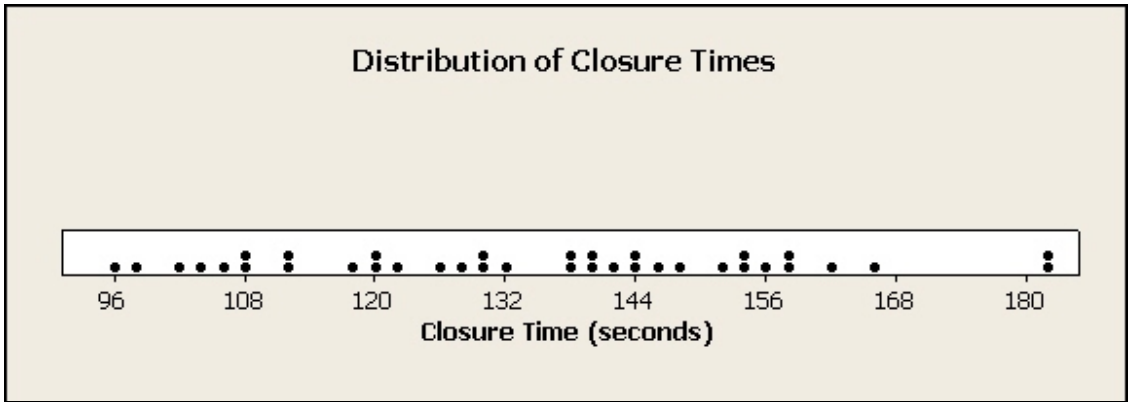

Figure 3.1 Distribution of Closure Times in 37 Healthy Volunteers Not Taking Regular Medication

3.03.3 Study One Discussion

The definition of aspirin resistance used here is consistent with that used in other studies (277;286;309-324) (table 3.1). It is important to note the length of time taken to complete this study and develop the local reference range – this was done alongside other studies rather than before which is in retrospect the optimal approach. However, this did not affect conduct of the other studies and the reference was developed before analyses were performed.

Authors	**Reference**	***Definition of Aspirin Resistance***
Jilma et al	(309)	*< 193 seconds*
Kundu et al	(310)	*< 193 seconds*
Christiaens et al	(311;315)	*< 186 seconds*
Cohen et al	(312)	*<192 seconds*
Pamukcu et al	(321)	*< 186 seconds*
Narvaez et al	(314)	*< 173 seconds*
Gum et al	(286)	*< 193 seconds*
Grundmann et al	(277)	*< 165 seconds*
Wong et al	(316)	*< 158 seconds*
Coma-Canella et al	(317)	*<161 seconds*
Fateh-Moghadam et al	(320)	*< 165 seconds*
Andersen et al	(318)	*< 193 seconds*
Lordkipanidze et al	(319)	*< 193 seconds*
Di Chiara et al	(322)	*< 193 seconds*
Gurbel et al	(323)	*< 193 seconds*
Angiolillo et al	(324)	*< 193 seconds*

Table 3.1 Definition of Aspirin Resistance Employed in Previous Studies Using the PFA-100 Device

3.04 Study Two - Microembolic Signals and Aspirin Resistance in Patients with Carotid Stenosis

The study hypothesis was that aspirin resistance would be associated with the presence of microembolic signals in aspirin treated patients with carotid disease. An observational cohort study was perfomed to test this.

3.04.1 Study Two Methods

The study was performed in the University Division of Cardiovascular and Medical Sciences at the Western Infirmary, Glasgow. The study was approved by the local research ethics committee.

Patients aged > 18 years taking current aspirin therapy and who had significant carotid artery disease (defined as at least a moderate internal carotid artery (ICA) stenosis or symptomatic ulcerating plaque disease) were included. Those taking clopidogrel, dipyridamole or anti-coagulant therapy and who had poor compliance with aspirin therapy were excluded. Further exclusion criteria included absence of a bony temporal window on TCD imaging, atrial fibrillation or other cardiac source of embolism (defined as valvular heart disease, recent myocardial infarction or known patent foramen ovale) and thrombolytic therapy within the past 2 weeks. Compliance was assessed by questioning and, where the participant was an in-patient, from drug prescription charts and discussion with nursing staff. The presence of a temporal window for TCD imaging was assessed during carotid Doppler scanning as part of routine clinical care. Carotid ultrasound imaging utilised an Acuson Aspen 128 with a 5-Mhz linear transducer (Acuson, Mountain View,

CA, USA). In the Doppler laboratory, a moderate ICA stenosis is defined as being present if peak systolic velocity (PSV) is >1.5 m/s and a severe stenosis if PSV is >2 m/s.

Participants were identified during out-patient attendance at the hospital Fast Track TIA service or during an in-patient stay in the Acute Stroke Unit. All gave full written informed consent to participate unless hemiplegia or visual disturbance precluded writing, where witnessed oral consent was accepted.

Study Assessments

Patients were reviewed at baseline where TCD monitoring for MES and blood testing for platelet function analyses were performed. Those who still met the eligibility criteria had repeat TCD monitoring and platelet function analysis performed as near to 7 days as possible. The purpose of this was to evaluate whether aspirin resistance was a stable phenomenon. At day 30, all patients were contacted by telephone or seen at clinic (or reviewed on the ward if still an in-patient) to screen for any signs of recurrent TIA or stroke using a standardised set of clinical questions. This marked the end of their involvement in the study. Where patients could not be contacted by telephone, this information was gathered at the next scheduled clinic review.

TCD recordings were made from the middle cerebral artery ipsilateral to the stenosis using a TCD 100M device (Spencer Technologies, Seattle, WA, USA) in–line with consensus guidelines (325). Recordings lasted one hour and were made by sonographers with extensive experience in TCD techniques. Where there was bilateral carotid disease, simultaneous bilateral TCD recordings were made. Recordings were monitored on-line by the sonographer and potential MES were also automatically identified using the emboli

detection function. All recorded potential MES were reviewed and independently verified by the sonographer and one of the study investigators. Both were blinded to the aspirin responsiveness status. The presence of MES was defined as ≥1 MES on TCD scanning.

Approximately 12 mls of blood was drawn using a vacutainer system and a 21 G needle into 3.8% citrate blood tubes. Well validated point of care cartridge based platelet analysers (PFA-100, Dade-Behring, Miami, FL (310;326) and Verify Now, Accumetrics (327;328)) were used to assess platelet function. Samples were analysed at between 30 minutes and 4 hours of being taken. The PFA-100 device was used as described above. The coefficient of variation when using the PFA-100 device is typically 10% (309) and as described above, similar was seen in study one. With the Verify Now device, a citrated blood mix and 'aspirin assay' which contains fibrinogen coated beads and platelet agonists, are placed into the analyser and platelet induced aggregation is measured as an increase in light transmittance. Results are expressed as the number of aspirin response units. The coefficient of variation when using the RPFA device is reported to range from 2.6 to 4.5% (information contained in Verify Now ASA product information sheet).

Both devices are simple to use and give results within 10 minutes. Using the PFA-100 analyser, aspirin resistance was defined as a closure time below the in-house upper limit of the normal range for healthy non-aspirin treated individuals (<179s, as obtained in study one of this chapter). Using the Verify Now system, aspirin resistance is defined as the presence of >550 aspirin reaction units (329). Participants were deemed to be aspirin resistance if found to be so on either test. Platelet function tests were performed blinded to results of TCD imaging and MES status.

Statistical Analysis

The primary endpoint was the proportion of aspirin resistant patients in the MES and non-MES group. This was assessed using Fishers exact test. Pre-specified secondary endpoints were the number of aspirin resistant patients in the MES and non-MES group according to each individual test, agreement between the first and second visit aspirin resistance status, agreement between the two platelet function tests and the rate of stroke or TIA according to aspirin status. Agreement between tests was assessed using attribute agreement analysis and described with kappa statistics. It was estimated that the prevalence of aspirin resistance would be 30% (274;275) in the whole study group and that the rate would be higher at approximately 40% in those with MES (277) (similar to the rate in patients with recurrent stroke in other studies) compared to a lower rate of approximately 10% in those without MES (277;278). A sample size of 31 patients in each group would allow detection of such a 30% difference in the rate of aspirin resistance between the MES and non-MES group with 80% power and a significance of 0.05. It was estimated that at least 40% of screened patients would have MES (223;229;234) but in order to ensure blinding to MES status could be maintained, it was planned to recruit the first 62 eligible patients.

P values of <0.05 were deemed to denote statistical significance and standard definitions of degree of agreement based on kappa statistics were used (330).

3.04.2 Study Two Results

Sixty-two patients between June 2006 and January 2008. All had baseline MES and aspirin responsiveness status recorded. In total, 37 patients (59.7%) remained eligible and had the day 7 visit performed. Of those who did not, twelve had undergone carotid endarterectomy,

one was commenced on clopidogrel, two went for scheduled cardiac surgery, two were moved to an off-site rehabilitation facility and could not attend, four did not attend and four withdrew consent for the second visit stating it was too soon after discharge.

The mean age was 69.7 (± 11.8) years. The majority (53 patients, 85.5%) had symptomatic carotid disease and a severe stenosis (50 patients, 80%). Half (31 patients) were in-patients in the acute stroke unit; the remainder were out-patients attending the department. Of those who were symptomatic, 21 (39.6%) were evaluated within 48 hours of onset, 9 (17%) within 7 days, 12 (22.6%) within one month and 11 (20.8%) within 6 months. Further baseline demographic details, including stroke subtype, drug therapy, risk factor burden, renal function and platelet levels are shown table 3.2.

Baseline aspirin responsiveness and platelet function data are shown in table 3.3. In total, 16 patients (25.8%) exhibited evidence of aspirin resistance on one or more test. Of these patients, 13 (21%) were resistant on PFA-100 testing, 8 (12.9%) were resistant on RPFA testing and 5 (8.1%) were resistant on both. Sixteen (25.8%) had evidence of MES, with a range of 1-39 signals during monitoring. Only one patient (who was aspirin resistant on all modalities and at both visits) suffered a recurrent vascular event during follow up.

Variable	***Value***
Age	*69.7 (11.8)*
No Stroke Symptoms	*6 (9.7%)*
Total Anterior Circulation Stroke	*1 (1.6%)*
Partial Anterior Circulation Stroke	*28 (45.2%)*
Lacunar Stroke	*12 (19.4%)*
Posterior Circulation Stroke	*3 (4.8%)*
Amaurosis Fugax only	*12 (19.4%)*
Symptomatic Carotid Disease	*53 (85.5%)*
< 50% stenosis / plaque disease only	*3 (4.8%)*
>50% stenosis	*9 (14.5%)*
> 70% stenosis	*50 (81%)*
Smoker	*20 (32.3%)*
Ischaemic Heart Disease	*14 (22.6%)*
Diabetes Mellitus	*9 (14.5%)*
Hyperlipidaemia	*19 (30.7%)*
Hypertension	*31 (50%)*
ACE I or ARB Therapy	*24 (38.7%)*
Diuretic Therapy	*23 (37.1%)*
Statin Therapy	*43 (69.4%)*
Baseline Platelet Count	*255.3 (101.7) *10^9/L*

Table 3.2 – Study Two Baseline Demographic Variables. Expressed as n (%) for categorical variables and mean (SD) for continuous variables. ACE I = ACE inhibitor, ARB = Angiotensin Receptor Blocker.

MES and Aspirin Responsiveness Status

Aspirin resistance on at least one test was present in 8/16 (50%) of those with microembolic signals and in 8/46 (17.4%) of those without MES (p=0.018 on Fisher's exact test).

PFA-100 CEPI-CT	*254.2 (72.6) seconds*
Aspirin Resistance on PFA-100	*13 (21%)*
RPFA	*442.3 (90.3) ARU*
Aspirin Resistance on RPFA	*8 (12.9%)*
Aspirin Resistance on Both Tests	*5 (8.1%)*
Aspirin Resistance on ≥ 1 Test	*16 (25.8%)*
Presence of MES	*16 (25.8%)*
Number of MES (when present)	*1-39 (range)*

Table 3.3 – Study Two Baseline Platelet Function Analysis Results
PFA-100 = Platelet Function Analyser 100. RPFA = Rapid Platelet Function Analyser. ARU = Aspirin Responsive Units. MES = microembolic signals. Values expressed as n (%) for categorical variables and mean (SD) for continuous variables unless stated.

Aspirin resistance was present in 5 (31.3%) of those with MES on PFA-100 testing compared to 8 (17.4%) without (p=0.29 on Fisher's exact test). On RPFA testing, aspirin resistance was present in 5 (31.3%) of those with MES and 3 (6.5%) of those without (p=0.02 on Fisher's exact test).

Agreement between Platelet Function Tests

Agreement between the aspirin responsiveness status measured by the different platelet function tests and at the two study visits is shown in table 3.4. At the baseline visit, agreement between the two tests was moderate (κ=0.40). A scatter plot of the individual patient data is shown in figure 3.2. At the second study visit, agreement between the tests was also moderate (κ=0.41). Agreement between aspirin responsiveness status at the first and second visit was moderate on PFA-100 (κ=0.53) testing, moderate on RPFA testing (κ=0.44) but was fair for presence of resistance on one or more test (κ=0.36). At the second study visit, 11 (29.7%) of patients exhibited evidence of aspirin resistance on one or more

test. All of these were resistant on PFA-100 testing and 4 (10.8%) were also resistant on RPFA testing.

Visit 1 (PFA-100 vs RPFA)	κ= 0.4	*P<0.001*
Visit 2 (PFA-100 vs RPFA)	κ= 0.41	*p=0.006*
Aspirin Resistance on ≥ 1 Test (Visit 1 vs Visit 2)	κ= 0.36	*p=0.014*
PFA-100 (Visit 1 vs Visit 2)	κ= 0.53	*P<0.001*
RPFA (Visit 1 vs Visit 2)	κ= 0.44	*p=0.004*

Table 3.4. Measures of Agreement Between Tests and Study Visits in Study Two. PFA-100 = Platelet Function Analyser 100. RPFA = Rapid Platelet Function Analyser. κ= kappa statistic. p values shown are for test of κ > 0.

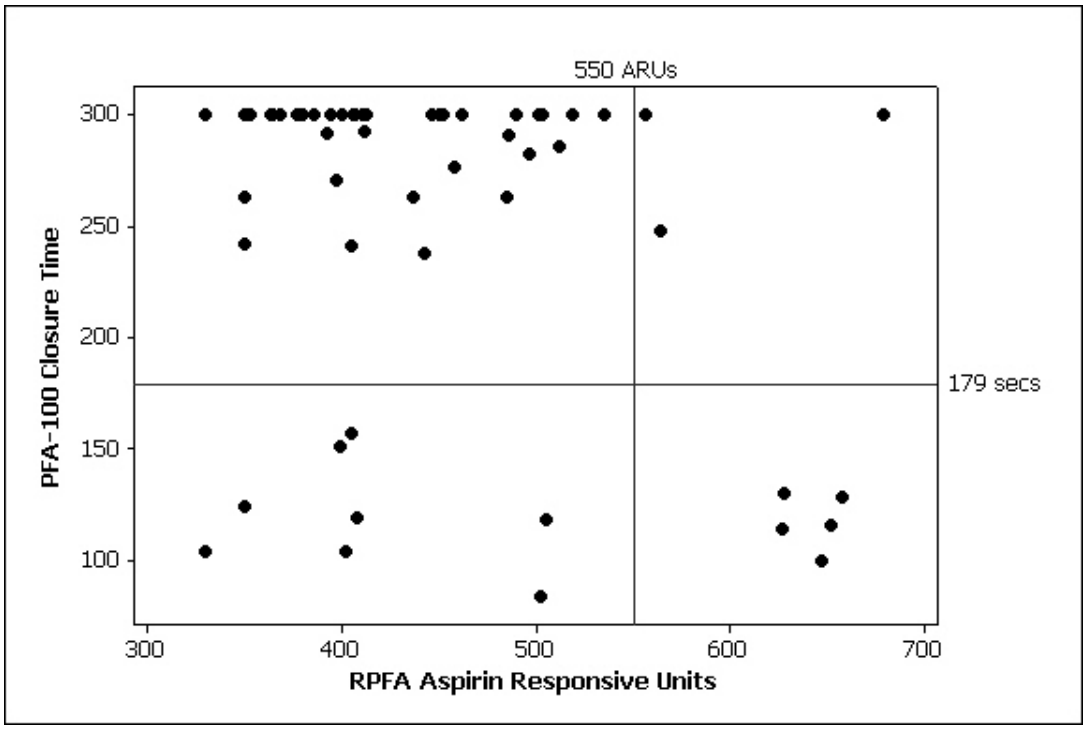

Figure 3.2. Scatterplot of PFA-100 Measured versus RPFA Measured Aspirin Responsiveness in Study Two. Each data point represents one individual. Values to the right of the vertical reference line represent resistance on RPFA testing while values below the horizontal reference line represent resistance on PFA-100 testing. ARU = Aspirin responsiveness units.

3.04.3 Study Two Discussion

Data from this study show that aspirin resistance is more common in those with significant carotid disease and MES compared to those with equivalent carotid disease and no MES. This ties increased prevalence of aspirin resistance to a readily measurable and robust marker of stroke risk. Importantly, the utility of MES exceeds their predictive value regarding future stroke risk; their presence and their response to treatment can be used to identify differences between anti-platelet strategies with small numbers (234). The findings from this study provide a useful model for further work which should aim to establish whether screening for aspirin responsiveness and subsequent changes to therapy reduces the rate of MES and stroke risk in those with carotid disease. For example, this strategy could be compared to other regimens such as unselected use of aspirin and clopidogrel (234).

Aspirin remains the most widely prescribed anti-platelet agent after stroke and attempts to better target its use, especially in high risk groups, are valid. Resistance to aspirin effect has received much attention over the recent years and does appear to be associated with an increased risk of cardiovascular events (274;276). However, several issues beset the field: as yet no consensus exists concerning the definition of aspirin resistance or its mechanism and no evidence exists to suggest that its presence should guide therapy. The size of recent anti-platelet trials (140) suggests that clarifying these issues with clinical endpoint studies may be costly and impractical. A link between aspirin resistance and a readily measurable surrogate marker of stroke risk would therefore be useful to establish as it would allow (with relative ease) further study of tailored anti-platelet strategies.

Aspirin resistance can be defined either clinically (vascular events in patients taking aspirin, perhaps better termed treatment failure) or in terms of laboratory measures (such as failure to inhibit platelet aggregation or thromboxane A2 production). Given the lack of consensus concerning the optimal definition two simple point of care analysers were used to assess aspirin responsiveness in this study. A wide definition of aspirin resistance (the presence of normal platelet function on one or more test) was also used; it was hoped that the simplicity of these methods will allow further research to proceed whilst ensuring any findings can be readily translated into routine clinical practice – the same could not be said for the more time consuming and cumbersome techniques of flow cytometry or traditional methods of platelet aggregometry. There are however drawbacks to the tests used: neither is truly specific to the effect of aspirin therapy nor do they do directly measure platelet thromboxane A2 production; and agreement between them has previously been shown to be poor (290). Attempts were made to limit the lack of specificity for aspirin action by ensuring those taking alternative anti-thrombotic / anticoagulant therapy or those with a known haematological disorder were excluded. Also, as mentioned, it is important to note that approaches that directly measure thromboxane A2 production have other drawbacks: extra-platelet sources of thromboxane A2 may arise in those with recently symptomatic cardiovascular disease (283) and thus lead to false overestimates of resistance levels.

The PFA-100 has been shown to be more sensitive than traditional optical aggregation techniques (286) and labels a higher proportion of individuals as resistant (274). Similar supportive trends were seen in this study: 13 (21%) were resistant on PFA-100 testing compared to only 8 (12.9%) on RPFA testing. Interestingly, resistance on RPFA testing appeared more closely linked to presence of MES than resistance on PFA-100 testing, although the numbers resistant on each technique were small and a firm conclusion as to

whether RPFA testing detects clinically more relevant aspirin resistance cannot be drawn. The prevalence of resistance using the combined definition in this study was 25.8% which is consistent with that seen in meta-analyses (274) and studies post stroke (275).

Only moderate agreement between the methods used to define resistance and between aspirin responsiveness status at the first and second visits in this study. While the agreement seen between the different platelet function tests is superior to that seen in other studies (290) in the post stroke period, it remains too low to allow a patient to be deemed resistant on the results of a single test and the 95% CI for the estimate of agreement in this study overlaps with previous work (290). Until the optimal technique of measuring resistance is clarified, it is likely that a battery of tests should be used. Other authors have suggested that aspirin resistance may have a temporal component (331) and although the rates of aspirin resistance were similar at the first and second visits here, agreement between visits was only moderate suggesting that the resistance status of some individuals changed. There are several potential explanations for this: compliance may have differed between visits in some individuals, as may the degree of platelet activation which can change following acute cardiovascular events (283). Importantly, it is unlikely that compliance has influenced the main results of this study; all patients verbally confirmed compliance and half were in-patients who had compliance objectively confirmed from hospital prescription charts and discussion with nursing staff.

Patients who were taking dipyridamole were excluded. The trial commenced before the ESPRIT trial (138) revealed incremental reduction in recurrent stroke with combined aspirin and dipyridamole therapy. Whether more widespread dipyridamole use will reduce early stroke risk or the likelihood of MES in those who are aspirin resistant is unclear.

Available data suggest its addition does not alter the response to aspirin treatment measured by either the PFA-100 or RPFA (332) and it may be that the association seen would hold. Two patients toward the end of the study were commenced on dipyridamole prior to their second visit and neither had a change in their MES or aspirin resistance status.

Only one patient in this study suffered a recurrent event, which, given the baseline risk of the group, appears low. This patient was aspirin resistant but no useful information can be drawn from this observation. The low recurrent stroke risk may in part reflect the number who proceeded to rapid carotid endarterectomy (7 of those with MES, 16 patients in total, 12 of whom within one week of entering the study) or had aggressive anti-platelet therapy (3 with MES received aspirin and clopidogrel).

A lower rate of MES than had been anticipated was seen. It was estimated that a rate of 40% (223) would arise in the study group as a whole but MES were seen in only 25.8% (16) patients. Given that some patients in the study (9, 14.5%) had asymptomatic carotid disease, this probably is a representative figure. Furthermore, recent data (333) suggest that prevalence of MES falls with time after stroke (from 49% at 24 hours to 29% at 48 hours, although this was not a specific evaluation of subjects with carotid disease), and only ≈ 40% of patients were recruited and assessed within 48 hours of symptoms.

In conclusion, increased prevalence of aspirin resistance is linked to the presence of MES in patients with carotid disease. This provides a useful model to further study the benefits of guided anti-platelet strategies and further work should establish whether screening for aspirin responsiveness and subsequent adjustment to anti-platelet therapy reduces the rate of MES and stroke risk in those with carotid disease.

3.05 Study Three - Aspirin Resistance and Cerebrovascular Events; the Importance of Participant Compliance with Aspirin

The study hypothesis was that the aspirin resistance would remain a common phenomenon, even after compliance was adequately assessed but that the rate would be lower than the rates previously reported in the literature. A prospective case-controlled study was performed to test this.

3.05.1 Study Three Methods

The study was performed in the University Division of Cardiovascular and Medical Sciences at the Western Infirmary, Glasgow. The study was approved by the West Medical Research Ethics Committee. The study incorporated an analysis of all participants and a urine sub-study where only those who had evidence of aspirin metabolites in the urine were included.

Cases

Cases were aged > 18 years taking current aspirin therapy who had a clinical diagnosis of ischaemic stroke or transient ischaemic attack. It was required that recruitment and platelet function analysis be performed within 24 hours of onset of symptoms and that participants would be in theory able to submit a urine sample. Those with known poor compliance with aspirin therapy, those taking clopidogrel or anti-coagulant therapy or who had received thrombolytic therapy within the past 2 weeks and those with brain imaging which identified intracerebral haemorrhage were excluded. Compliance was assessed by questioning.

Cases were identified on admission to the Acute Stroke Unit at the Western Infirmary Hospital. Where possible, all gave full written informed consent to participate unless hemiplegia or visual disturbance precluded writing, where witnessed oral consent was accepted. Where potential participants were unable to consent, assent from the nearest relative or welfare guardian was accepted. Ethical approval was given for this as exclusion of those unable to consent because of their stroke would bias the study sample. It is important these patients are included as it is at least as relevant to establish the role of aspirin resistance in larger, more severe strokes.

Controls

Controls comprised individuals aged over 18 years of age taking current aspirin therapy for at least one year who had never suffered a cardiovascular event on aspirin. Those with known poor compliance with aspirin therapy, those taking clopidogrel or anti-coagulant therapy or who had received thrombolytic therapy within the past 2 weeks were excluded. Compliance was assessed by questioning. All gave full written informed consent.

Controls were mostly identified during out-patient attendance at cardiovascular risk factor clinics at the Western Infirmary.

Study Assessments

Cases were reviewed at baseline and at 7 days or on discharge from depending on what was soonest. Controls only underwent baseline assessment.

Baseline Assessment

Demographic and diagnostic data were gathered. A blood sample was drawn (ca 10 mls) for platelet function analyses and attempts were made to obtain a urine sample for measurement of aspirin metabolites.

Measurement of Platelet Function and Aspirin Responsiveness

The same well validated cartridge based platelet analysers (PFA-100, Dade-Behring, Miami, FL (310;326) and Verify Now, Accumetrics (327;328)) were used as outlined above in study two. The definitions of as aspirin resistance were also the same as outlined previously (closure time of < 179 with the PFA-100 device and the presence of >550 "aspirin reaction units" when using the Verify Now ^{TM} system). Investigators were blinded as to levels of urinary aspirin metabolites when performing platelet function analysis (results were not available at the time of platelet function analysis).

3.05.1.1 Measurement of Aspirin Metabolites (Detection of Urinary Salicylates)

Where able, participants submitted a single urine sample (the next urine sample following recruitment). High performance liquid chromatography analysis was performed. All tests were performed at Divisional Labs at the Western Infirmary Hospital. Other methods such as the method of Trinder (the principle of the test is a direct colour reaction on urine - a violet colour indicates the presence of salicylates) are available but relatively insensitive techniques (334); studies have revealed urinary concentrations in excess of the limit of detection for the Trinder method (20mg salicylate/L urine) 10 hours after a single 600 mg dose (335) but it is unclear whether this threshold will be reliably reached after a single 75 mg aspirin dose, and for what period thereafter.

High performance liquid chromatography (HPLC) assays have been developed to identify aspirin and its salicylate metabolites in both plasma and urine (336-341). HPLC involves a column which holds material (the stationary phase), a pump to inject and move the mobile phase, which acts as a carrier for the analysis sample, through the column and a detector which shows the retention times of the molecules. The retention times, or the time taken to elute from the column, depend upon the chemical interactions between the stationary phase and the mobile phase, and thus the properties of the analysis sample. The stronger the interaction with the stationary phase, the longer the retention time. There a variety of types of HPLC analysis but the most widely employed is reverse-phase HPLC, which utilizes a non-polar stationary phase and compounds are separated on the basis of hydrophilicity and lipophilicity. There are also different types of mobile phase and the environment of the mobile phase can be manipulated to ensure better separation of the sample. Detector systems can also vary and can be based, for example, upon measurement of the refractive index, the ability of samples to absorb ultra-violet light, or a variety of other techniques.

HPLC techniques are highly sensitive; detection of concentrations as low as 0.1 mg/l of aspirin metabolites are possible with some assays (339). Such methods have been used in many studies of aspirin pharmacokinetics and of salicylate levels in healthy volunteers and data suggest that salicylates can be detected several hours, and perhaps days, after low dose aspirin ingestion (summarized below).

Aspirin and Measurable Metabolites

Acetylsalicylic acid is hydrolyzed in the stomach and in blood to salicylic acid and acetic acid (342). Both acetylsalicylic acid and salicylic acid are rapidly absorbed. Several studies suggest that plasma concentrations of acetylsalicylic acid peak within one hour of ingestion

(339;343;344) and that the elimination half life is approximately 20 to 40 minutes. Levels of approximately 1000 ng/ml are often seen (343). Plasma salicylate levels peak somewhat later at approximately 1 to 2 hours, are generally higher, and also have a longer elimination half-life of approximately 2 to 4.5 hours. Several studies confirm that salicylates can be detected in the plasma several, and up to 10 hours (339;345), after single oral dose ingestion. Following daily dosing of low dose aspirin, time – concentration curves suggest levels of acetylsalicylic acid and salicylic acid remain detectable for longer with high (near peak) levels of salicylic acid seen for approximately 6 hours after ingestion (343) on HPLC analysis. How long they remain detectable thereafter is unclear.

Salicylates are predominantly excreted by the kidneys, mostly following hepatic conjugation, as salicyluric acid (75%), free salicylate (10%), salicylic phenol (10%), acyl glucuronides (5%) and gentisic acid (<1%) (346). Studies suggest that the salicylate load from low dose aspirin is mostly excreted in the urine within 24 hours (340;343), although others suggest that only 10% is excreted within 10 hours after a single dose (335). Levels of salicylic acid of over 30 µg/ml have been seen in urine 10 hours after ingestion of a 600mg dose (335), while some more sensitive techniques allow detection of salicylates up to 48 hours after ingestion of a single 80 mg dose of aspirin (347). Otherwise, little data exist to suggest actual concentrations of salicylate metabolites in the urine in the hours after ingestion of low dose aspirin.

Importantly, salicylates are found in both the plasma and urine of those who do not take aspirin or other salicylate drugs. For example, in one study using HPLC analysis, salicylic acid was detected in the plasma of all 76 subjects studied with higher levels in vegetarians (0.11 µmol/l (range 0.04 to 2.47) compared to 0.07 µmol/l (range 0.02 to 0.2)) in non-

vegetarians (348). However, levels were significantly, and approximately 100 times higher, in those taking aspirin (10.03 µmol/l (range 0.23 to 25.4)). There was however some overlap with between vegetarians and those on aspirin; eight vegetarians had salicylic acid levels above the lowest level seen in aspirin treated patients. However, this level was surprisingly low and it is possible, or perhaps probable, that this individual was not compliant with aspirin therapy and that the vegetarian with very high levels had ingested some medicinal salicylate. In the urine, 24 hour excretion of salicyluric acid of 3.91 µmol per 24 hours (range 0.87 to 12.23) in non-vegetarians and 11.01 µmol per 24 hours in vegetarians (4.98 to 26.6) has been seen, compared to levels of 170.7 µmol per 24 hours (range 13.15 to 377.18) in those who have taken 75 mg of aspirin (349). Again there was some overlap between values seen in vegetarians and those on aspirin with a small number of vegetarians having levels above the lowest in the aspirin group. Interestingly, excretion of salicylic acid did not differ between vegetarian, non-vegetarians and those taking aspirin suggesting that salicyluric acid measurements are most meaningful. Levels are also increased following consumption of cranberry juice (350). In a further study using HPLC analysis (341), the median urinary concentration of salicylic acid was 0.56 µmol/L (range 0.07-0.89 µmol/L), while that of salicyluric acid was 3.20 µmol/L (range 1.32-6.54 µmol/L) in 10 individuals who did not take aspirin. For salicyluric acid, a level of > 6.54 µmol/L equates to > 1.3 µg/ml while for salicylic acid, a level of > 0.89 µmol/L equates to > 0.123 µg/ml.

Thus, although salicylates are present in the plasma and urine of those who do not take aspirin, levels are significantly higher in those who take even low dose aspirin and remain detectable in both plasma and urine for several hours and may be detectable in the urine for up to 48 hours after a single 80 mg dose (347). This suggests that salicylate load due to

aspirin ingestion can readily be distinguished from that of the normal dietary intake, particularly in non-vegetarians, and that sensitive techniques that measure salicylate metabolites, and those in the urine in particular, can be used to confirm recent ingestion in those meant to be taking low dose aspirin.

3.05.1.2 HPLC Analysis Used in This Study

Urine concentrations of salicylic acid and salicyluric acid levels were determined using an established reverse-phase HPLC method (339). The materials used included stock solutions (at a concentration of 1000 µg / ml) of salicylate and salicyluric acid in distilled H2O and 1.0M oxalic acid. The internal standard (IS) was 200ug m-hydroxybenzoic acid / ml distilled H2O. The mobile phase consisted of 25% H_2O, 25% acetonitrile, and 50% phosphate buffer (0.2M pH 2.5). The solution was degassed by bubbling Helium through it for approximately 10 minutes.

Standards comprised the stock solutions of salicylate and salicyluric acid. The standard curves for salicylate ranged from 3.125 to 25 µg / ml while those for salicyluric acid ranged from 25 to 200 µg / ml. As salicylate and salicyluric acid are measured simultaneously, each prepared standard contained both metabolites at their chosen concentration. There were seven prepared standards (table 3.5).

Std. No.	Conc. SA (µg/ml)	Conc. SU (µg/ml)	Volume Working Std. (µL)	Volume H_2O (µl)
1	0	0	0	1000
2	3.125	25	125	875
3	6.25	50	250	750
4	9.375	75	375	625
5	12.5	100	500	500
6	18.75	150	750	250
7	25	200	1000	0

Table 3.5. Prepared Standards Used in HPLC Analysis. SA = salicylic acid. SU = salicyluric acid.

The urine sample for analysis was then prepared as outlined below;

a) Pipette 200µl of urine for analysis into 5ml round-bottomed tube.

b) Add 50µl internal standard (IS) and briefly vortex mix.

c) Add 200µl 1M oxalic acid.

d) Add 2ml hexane / diethyl ether mixture; ratio1:1.

e) Mix on rotator for approximately 5 minutes.

f) Centrifuge for 5 mins at 2000 rpm.

g) Remove as much hexane / diethyl ether mixture as possible into clean 12ml tube.

h) Evaporate to dryness in centrifugal evaporator at minimum temperature setting (approximately 15 minutes).

i) Dissolve desiccant in 200µl Mobile Phase and briefly vortex mix.

j) Carefully transfer as much volume as possible to HPLC vial and cap.

The HPLC system consisted of a Shimadzu SCl-10A VP system controller, Shimadzu Lc-10AT VP pumps, a Shimadzu SIL-10AD VP sample injector, a Shimadzu SPD-6A variable

wavelength UV detector and a computer with Jones JCL 6000 data acquisition software. The column used was a Phenomenex Synergi 4um Hydro-RP column (dimensions 15cm x 4.6mm). The guard column is packed with Lichroprep RP-18, particle size 25-40um. The column and other system components are maintained at ambient temperature.

HPLC conditions were a flow rate of 0.5 mls / min, a detector set at UV, λ 303 nm and absorbance 0.02 and the injection volume was 50 µl. Retention times were approx. 5.4 minutes for IS, 6.9 minutes for salicyluric acid and 11.8 minutes for salicylic acid.

Limits of detection were not pre-specified. Salicylate metabolites are endogenous compounds with levels above the likely limit of detection seen in many individuals.

Definition of Evidence of Recent Aspirin Ingestion Used

Little data exist to inform levels of salicyluric acid or salicylic acid at differing times after aspirin ingestion and in those not taking aspirin. In a small sample in the local laboratory, levels of 0 to 5.25 µg/ml of salicyluric acid have been seen, with most values < 5 µg/ml in those taking no aspirin. Levels of > 5 µg/ml were seen 24 hours after single dose aspirin ingestion. Although direct comparisons are not possible, this threshold is broadly similar to that outlined by others (341) and a salicyluric acid of > 5 µg/ml was therefore used to define evidence of recent aspirin ingestion. These individuals were included in the urine sub-study. Given that it is also know that aspirin metabolites are predominantly cleared from the urine within 24 hours, evidence of no recent aspirin ingestion was defined as stated last dose of aspirin within 24 hours from submission of urine sample and a salicyluric acid level of < 5 µg/ml. Importantly, given that the platelet mediated effects of aspirin last longer than 24 hours, it is expected that some participants whose urine sample

was received after 24 hours would have evidence of aspirin mediated platelet dysfunction but a salicyluric acid level of < 5 µg/ml.

Final Assessment - At the final assessment the final diagnosis and patient outcome were established for cases.

Statistical Analysis

It was planned to study 90 cases and 90 controls. The primary endpoint was the proportion of individuals showing resistance to aspirin on any test. Secondary endpoints were defined as resistance to both tests, to the PFA-100 test and to the RPFA test. The rate of the primary (and secondary) endpoint(s) were compared between cases and controls. It was estimated that the rate of aspirin resistance would be approximately 30% in the general population but that it would be higher, at approximately 40%, in cases (277;351) and lower in controls. It was estimated that approximately 20% in the control population would have no evidence of recent aspirin ingestion but there are little data to help estimate the rate in those with a vascular event. Even after exclusion of these patients from the analyses (approximately 20% of all subjects), 90 patients per group will allow detection of a ~ 20% difference in aspirin resistance rate between the two groups with 80% power and a significance of 0.05 (for example 40% in cases vs 20% in controls).

Differences between cases and controls were assessed by the Student's t test for means and the chi-square test for proportions or Fisher's exact test. Agreement between the platelet function and salicylate detection testing was assessed using kappa statistics. A p value of <0.05 was used to determine statistical significance and standard definitions of degrees of

agreement were used. Analyses were performed separately for each of the two methods of determining aspirin responsiveness. Cases and controls were not matched on a 1:1 basis.

3.05.2 Study Three Results

Ninety cases were recruited between the 19th February 2007 and the 4th November 2008. Ninety controls were recruited from 29th June 2007 to 4th November 2008. Baseline characteristics are shown in table 3.6 and 3.7. There were several statistically significant differences between the groups. All participants verbally confirmed regular compliance with aspirin.

All cases (for the exception of one) and all controls were prescribed 75 mg per day of aspirin. Baseline levels of routine biochemistry and haematology indices including platelet count and coagulation parameters are shown in table 3.7. There were no significant differences. Coagulation screens were not routinely performed in controls and this comparison is thus based on a small number (n=7) of controls. The time from stroke symptoms to blood sampling was 16 (IQR 4.5 to 22.5) hours in cases.

Variable	**Cases**	**Controls**	***P Value***
Baseline Platelet Count	244.8 (8.1)	254.5 (14.1)	*0.55*
Baseline Haemoglobin	13.4 (0.2)	13.7 (0.3)	*0.39*
Baseline APTT	31.2 (0.5)	33.1 (1.9)	*0.35*
Baseline PT	11.9 (0.16)	12.1 (0.6)	*0.72*
Baseline Creatinine	96.8 (4.23)	97.7 (3.6)	*0.99*

Table 3.6. Study Three Baseline levels of Blood Parameters in Cases and Controls.

Variable	Cases	Controls	P Value
Age	71.1 (10.5)	66.7 (11)	0.007
Male Sex	43 (47.8%)	47 (52.2%)	0.55
Stroke	60 (66.7%)	---	---
TIA	30 (33.3%)	---	---
Total Anterior Circulation Stroke	11 (12.2%)	---	---
Partial Anterior Circulation Stroke	44 (48.9%)	---	---
Lacunar Stroke	22 (24.4%)	---	---
Posterior Circulation Stroke	11 (24.4%)	---	---
Amaurosis	2 (2.2%)	---	---
NIHSS Score	5.6 (5.4)	---	---
Smoker	27 (30%)	10 (11.1%)	0.001
Ischaemic Heart Disease	25 (27.8%)	27 (30%)	0.74
Previous CVA	24 (26.7%)	1 (1.1%)	<0.001
PVD	4 (4.4%)	4 (4.4%)	1
Diabetes Mellitus	6 (6.67)	18 (20%)	0.015
Hyperlipidaemia	22 (24.4%)	35 (38.9%)	0.054
Hypertension	52 (57.8%)	80 (88.9%)	<0.001
Atrial Fibrillation	13 (14.4%)	3 (3.3%)	0.008
Dipyridamole Therapy	13 (14.4%)	1 (1.1%)	<0.001
ACE I or ARB Therapy	45 (50%)	52 (57.8%)	0.37
Diuretic Therapy	35 (38.9%)	51 (56.7%)	0.025
Beta-blocker Therapy	32 (35.6%)	43 (47.7%)	0.13
CCB Therapy	28 (31.1%)	41 (45.6%)	0.065
Alpha-blocker Therapy	2 (2.2%)	20 (20.2%)	<0.001
Statin Therapy	66 (73.3%)	67 (74.4%)	1
NSAID Therapy	2 (2.2%)	4 (4.4%)	0.682

Table 3.7. Study Three Baseline Characteristics

Aspirin Responsiveness Status in Cases

In total , 55 (61.1 %) had blood sampling for platelet function testing performed within 24 hours of last stated aspirin ingestion. PFA-100 and RPFA tests were not successful in three individuals. In total, 30 cases (33.7 %) exhibited evidence of aspirin resistance on one or more test. Of these, 28 (32.1 %) were resistant on PFA-100 testing, 16 (18.4 %) were resistant on RPFA testing and 12 (14 %) were resistant on both (table 3.8).

	Cases	**Controls**	**P Value**	***95 % CI for Difference***
PFA-100 CEPI-CT	272 (142 to 300)	293 (205 to 300)	0.068 #	*0.01 to 23.99* #
Aspirin Resistance on PFA-100 *	28 (32.1 %)	15 (18.1 %)	0.031	*-27 to - 12.7 %*
RPFA	465 (396 to 530)	452 (399 to 481)	0.33 #	*-34.5 to 9.99*#
Aspirin Resistance on RPFA *	16 (18.4 %)	12 (13.8 %)	0.54	*-15.5 to 6.3 %*
Aspirin Resistance on Both Tests	12 (14 %)	5 (5.9 %)	0.07	*-16.9 to 1 %*
Aspirin Resistance on ≥ 1 Test	30 (33.7 %)	21 (24.7 %)	0.19	*-22.4 to 4.4 %*

Table 3.8. Study Three Platelet Function Analysis Results. * Only 87 participants had results available. P values and 95 % CI shown are for the difference between two proportions except for # where they are for the difference on Mann-Whitney testing.

Aspirin Responsiveness in Controls

All controls had blood sampling for platelet function testing performed within 24 hours of last stated aspirin ingestion. In total, 21 controls (24.7 %) who exhibited evidence of aspirin resistance on one or more test. Of these patients, 15 (18.1 %) were resistant on PFA-100

testing, 12 (13.8 %) were resistant on RPFA testing and 5 (5.9 %) were resistant on both (table 3.8).

Comparison Between Cases and Controls

The rate of aspirin resistance as defined by resistance on PFA-100 testing or resistance on both modalities was significantly higher in cases than control (table 3.8). There were also trends towards higher rates of resistance on RPFA testing and on resistance defined as resistance on at least one modality.

The Urine Sub-study - Cases

A urine sample was submitted by 66 cases (73.3%). The mean concentrations of salicyluric acid and salicylic acid seen in the urine were 50.1 (SD 64.5) µg/ml and 1.2 (SD 2.56) µg/ml respectively. In total 40 (60.1 %) had levels of salicyluric acid above the pre-specified level used to define evidence of recent aspirin ingestion (> 5 µg/ml).

The median time from drawing of blood to submission of the urine sample was 20 minutes (IQR 0 to 60 minutes). However, only 24 (36.6%) submitted their urine sample on the same day as their last aspirin dose, while 37 (54.6%) submitted their urine sample within 24 hours of their last stated aspirin dose. In total 12 of these (32.4 %) had no evidence of recent aspirin ingestion (sample submitted within 24 hours of last stated aspirin dose and levels of salicyluric acid of < 5 µg/ml).

When considering only those with evidence of recent aspirin ingestion, 10 (26.3 %) were aspirin resistant on at least one platelet function test. Of these, 9 (23.7 %) were resistant on

PFA-100 testing, 5 (13.4 %) were resistant on RPFA testing and 4 (10.5 %) were resistant on both (table 3.9).

Of those who submitted a urine sample within 24 hours of last ingestion and were aspirin resistant on at least one test, 5 of 10 (50 %) had salicyluric acid levels of < 5 µg/ml. For PFA-100, RPFA testing or resistance on both tests, this figure was 5 of 9 (55.6 %), 4 of 6 (66.7 %) and 4 of 5 (80 %) suggesting that poor compliance accounted for a significant number of the cases of resistance seen.

	Cases (n=39)	**Controls (n=32)**	**P Value**	***95 % CI for Difference***
PFA-100 CEPI-CT	283.5 (202.8 to 300)	276 (186 to 300)	0.89 #	*-8.01 to 14.98* #
Aspirin Resistance on PFA-100 *	9 (23.7 %)	6 (18.8 %)	0.77	*-23.7 to 15.1 %*
RPFA	485 (404.5 to 522.5)	451 (395.3 to 498.5)	0.49 #	*-54.9 to 20* #
Aspirin Resistance on RPFA *	5 (13.5 %)	5 (15.6 %)	1	*-14.6 to 18.8 %*
Aspirin Resistance on Both Tests	4 (10.5 %)	3 (9.7 %)	1	*-15.1 to 13.4 %*
Aspirin Resistance on ≥ 1 Test	10 (26.3 %)	8 (25.5 %)	1	*-21.3 to 20.3 %*

Table 3.9. Platelet Function Analysis Results in Those With Evidence of Recent Aspirin Ingestion. P values and 95 % CI shown are for the difference between two proportions except for # where they are for the difference on Mann-Whitney testing.

The Urine Sub-study - Controls

A urine sample was submitted by 37 controls (41.1%). The mean concentrations of salicyluric acid and salicylic acid seen in the urine were 110.1 (SD 90.1) µg/ml and 1.6 (SD 2.74) µg/ml respectively. In total 32 (86.5 %) had levels of salicyluric acid above the pre-specified level used to define evidence of recent aspirin ingestion (> 5 µg/ml).

All controls who submitted urine samples did so within the hour. All participants stated compliance with their aspirin up to the day of recruitment and thus all were assumed to have submitted their urine sample on the same day as their last aspirin dose. Thus, only 5 individuals (13.5 %) had no evidence of recent aspirin ingestion (sample submitted within 24 hours of last stated aspirin dose and levels of salicyluric acid of < 5 µg/ml).

When considering only those with evidence of recent aspirin ingestion, 8 (25.8 %) were aspirin resistant on at least one platelet function test. Of these, 6 (18.8 %) were resistant on PFA-100 testing, 5 (15.6 %) were resistant on RPFA testing and 3 (9.7 %) were resistant on both (table 3.9).

No individual with salicyluric acid levels of < 5 µg/ml had evidence of aspirin resistance.

The Urine Sub-study - Comparison Between Cases and Controls

The rate of aspirin resistance, regardless of definition used was similar in both cases and controls, although confidence intervals were wide because of the smaller sample size (table 3.9).

Agreement Between Platelet Function Tests

Agreement between the aspirin responsiveness status measured by the different platelet function tests is shown in table 5.6. Overall, agreement between the two tests was fair (κ=0.35). On sub analysis agreement was also fair in both the case and control population (table 3.10). A scatter plot of the individual patient data is shown in figure 3.3.

	Kappa	***P Value***
Cases (PFA-100 vs RPFA)	0.39	*<0.001*
Controls (PFA-100 vs RPFA)	0.27	*0.007*
Cases and Controls (PFA-100 vs RPFA)	0.35	*<0.001*

Table 3.10. Measures of Agreement Between Platelet Function Tests in Study Three. PFA-100 = Platelet Function Analyser 100. RPFA = Rapid Platelet Function Analyser. κ= kappa statistic. p values shown are for test of κ > 0.

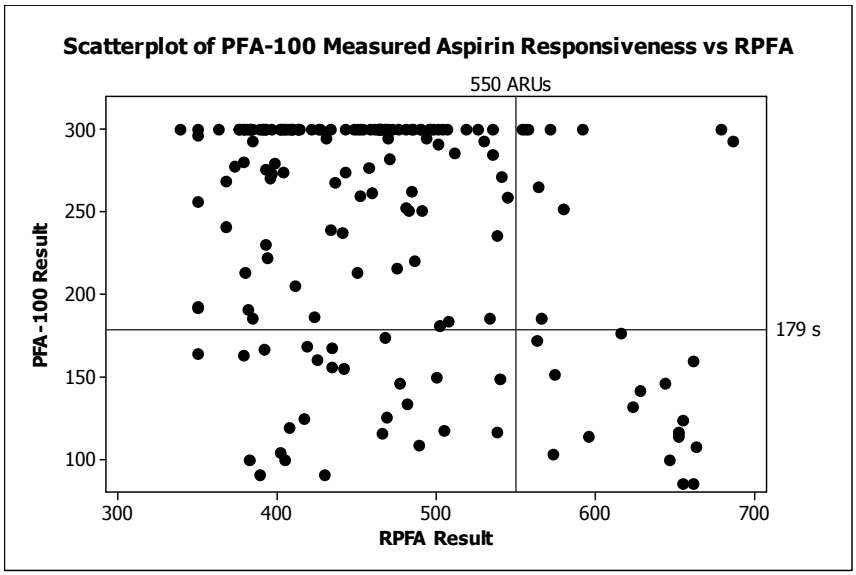

Figure 3.3. Scatterplot of PFA-100 Measured Aspirin Responsiveness versus RPFA Measured Aspirin Responsiveness in Study Three. Each data point represents one individual. Values to the right of the vertical reference line represent resistance on RPFA testing while values below the horizontal reference line represent resistance on PFA-100 testing. ARU = Aspirin responsiveness unit.

3.05.3 Study Three Discussion

Data from this study revealed a prevalence of aspirin resistance of 34 % in cases with recent stroke or TIA compared to 25 % in controls where resistance on one or more test was used as evidence of resistance. This difference did not reach statistical significance although resistance defined by PFA-100 testing or resistance on both tests was significantly higher in cases with recent stroke or TIA. However, when only those with objectively confirmed evidence of recent aspirin resistance were considered, the prevalence of resistance was similar in both groups. A high proportion of cases who were resistant to aspirin had no evidence of recent aspirin ingestion despite stating they regularly took aspirin, suggesting that compliance accounts for some of the apparent difference seen between cases and controls.

The fact that aspirin resistance appeared higher in cases than controls would fit with recent data suggesting that aspirin resistance links with a higher rate of cardiovascular events (274;276). However, previous case control studies have not yielded such differences between groups (352-354), although this is the first such study to examine participants with stroke and is the largest case-control study to date. Although the rate of aspirin resistance was higher in cases than controls, both were compatible with previous figures given in the literature (276-278) and in particular with previous studies using similar techniques (318;355-361). Thus, aspirin resistance, regardless of the definition used, is a common finding. There were some significant differences between the case and control groups. For example cases were older which links with a higher prevalence of resistance (362) although fewer were female or diabetic which is also linked with increased resistance (286;363). These factors could confound this analysis as these are also risk factors for cerebrovascular disease.

The majority of studies have attempted to assess participant compliance, including 17 of the 20 studies in a recent meta-analysis (274). However, as well as the three studies which made no mention of compliance assessment (277;321;364), 3 relied upon verbal confirmation of compliance (as done in this study) (359;365;366) and the remainder involved hospital in-patients or supervised ingestion of aspirin (278;355-357;360;367-373) at baseline. However, it is well know that verbal means of compliance assessment may not accurately reflect whether medication has actually been taken and an objective means of assessment is preferable (374) but few studies have employed such methods to date (354).

The importance of participant compliance cannot be overestimated. Approximately 10-40% of patients do not take prescribed anti-platelet tablets in the secondary prevention setting (292-296). These individuals will be highly likely to appear aspirin resistant on testing. Poor compliance has also been shown to be a major cause of aspirin resistance in previous studies; for example, just over half (367;375) to all (291) of apparent aspirin resistance has previously been shown to be due to poor participant compliance with therapy.

This study utilized direct measurement of aspirin metabolites in the urine as an objective means of ensuring recent aspirin ingestion in a secondary analysis. However, there are also difficulties with this approach; aspirin is rapidly metabolized and metabolites may be fully excreted in the urine at around 24 hours after a single dose, although this is less likely to occur in those on long term therapy. Further, salicylates are endogenous compounds, although levels are much higher then normal following aspirin ingestion. A cut-off level of salicyluric acid of 5 µg/ml was used in this study with higher levels used to confirm recent ingestion and lower levels used to suggest poor compliance provided the urine sample was

submitted within 24 hours of the last stated aspirin dose. It is hoped, given that participants were approached and recruited during a single attendance at a clinic or on admission for an acute vascular event that their compliance status reflects their normal behaviour; they could not have expected to participate in the study before attendance. The rate of non-compliance seen may be an overestimate as individuals who rapidly metabolise and excrete aspirin and its metabolites could have undetectable levels of salicyluric acid and salicylate before 24 hours has elapsed since last aspirin ingestion. However, results were similar when analysis was restricted to those who claimed aspirin ingestion within 12 hours of submitting a urine sample and those who take aspirin regularly (as all claimed in this study) excrete metabolites for longer in the urine.

Despite the fact that all individuals in this study claimed compliance (and would thus have been recruited to many previous studies published in the literature), approximately 1/3 of cases met the definition of poor compliance with aspirin therapy. This further suggests that verbal statements of compliance are not reliable and that more objective measures should be mandatory in studies of aspirin resistance. For example, in this study, over half of cases who submitted a urine sample within 24 hours of last ingestion and were aspirin resistant had salicyluric acid levels of < 5 µg/ml. This suggests that poor compliance was an important cause of aspirin resistance in this group. However, even when this was considered, resistance remained common and as in other studies, was highest with the PFA-100 device at approximately 20%.

Again in this study agreement between tests was only fair. This is in-line with work from other authors and has been discussed in chapter four.

In conclusion, data from this study suggests that aspirin resistance is a common phenomenon that merits further study but that poor compliance may contribute in a substantial number of cases of apparent resistance suggesting that objective measures to account for compliance should be mandatory in future studies of aspirin resistance.

Chapter Summary

The studies in this chapter have shown that the rate of aspirin resistance is higher in those with cerebrovascular microembolic signals (MES) and carotid disease compared to those with equivalent carotid disease and no MES. Approximately a quarter had MES and those with MES had a resistance rate of 50% compared to 17.4% in those without ($p=0.018$ on Fishers exact test). This provides a link to a well established and robust surrogate marker of outcome and thus a useful model to further study the benefits of guided anti-platelet strategies. Aspirin resistance was present in 34% of those with recent stroke and in 18% of those with risk factors but no established disease in a case-controlled study. However, the role of poor compliance with therapy as a cause in a substantial number of individuals was established; it accounted for approximately half of those labelled resistant in the stroke group. Further, when only those with objective evidence of recent aspirin ingestion were considered, the prevalence of aspirin resistance was similar in both groups (at 26%) and it remained a common phenomenon. Objective measures to confirm compliance with aspirin therapy should be mandatory in future studies of aspirin resistance.

CHAPTER FOUR

SERUM URIC ACID REDUCTION, XANTHINE OXIDASE INHIBITION AND RISK OF STROKE

4.01 Potential Novel Therapies – Lowering Serum Uric Acid

The role of serum uric acid in the development of cardiovascular disease has been the subject of controversy for many years. Many studies concerning the association between increasing serum uric acid level and event rate and mortality in a variety of cardiovascular disease states have been published. However, the presence of a causal relationship is less clear and elevated levels of uric acid may reflect a separate underlying disease process, atherosclerosis itself, or increased xanthine oxidase activity; all of which may influence vascular risk.

4.01.1 Biochemistry of uric acid in man

Uric acid is a breakdown product of ingested and endogenously synthesised purines (figure 4.1). DNA and RNA are degraded into purine nucleotides and bases, which are then metabolized, via the action of xanthine oxidase, to xanthine and uric acid. These later steps are irreversible and generate superoxide anions. Uric acid undergoes no further metabolism in humans and is excreted by the kidneys and intestinal tract. In the kidney, it is filtered and can be subsequently reabsorbed or further excreted in the proximal tubule, predominantly under the action of a urate transporter (URAT1) (376).

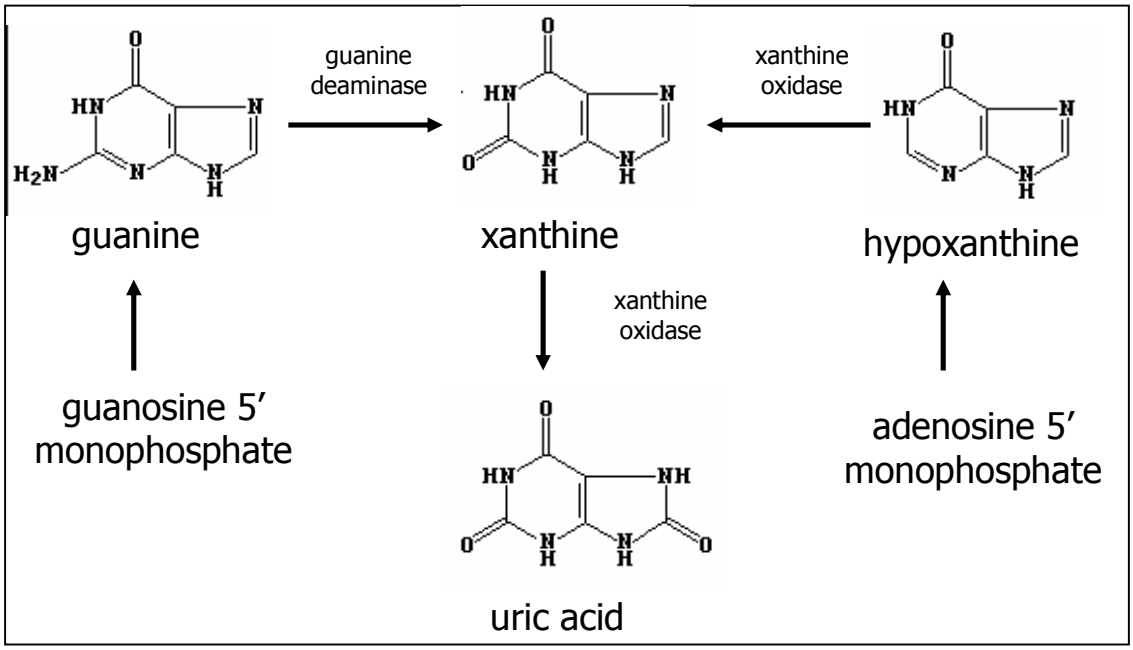

Figure 4.1. Uric Acid Metabolism in Man

Serum levels are governed by the balance of production and excretion. Production can be increased by several mechanisms including rare enzymatic defects, states of high cell turnover and alcohol ingestion (partly because of purines contained in alcoholic drinks (377;378)). However, the majority of cases of elevated serum uric acid result from impaired renal excretion, possibly because of inter individual differences in function of the URAT1 transporter.

4.01.2 Uric Acid as a Cardiovascular Risk Factor

This has been evaluated in a number of studies. Studies have typically utilised data from randomised control trials or epidemiological databases and expressed results as change in relative risk per increment of uric acid or as relative risk across uric acid quintiles (379).

Cardiovascular Risk States

In patients with diabetes, serum uric acid levels above the median (295µmol/l) have been associated with near double an increased risk of stroke (HR 1.91, 95 % CI 1.24-2.94) (380), while increasing uric acid levels were associated with increased prevalence of peripheral arterial disease in both a Taiwanese (381) and Australian cohort (382) (OR 1.004, 95% CI 1.001-1.008 and 1.03, 95% CI 1-1.06, per additional 0.1mmol/l in serum uric acid respectively).

In patients with essential hypertension, serum urate in the highest quartile was associated with increased cardiovascular event rate (HR 1.73, 95% CI 1.01-3), cardiovascular mortality and all cause mortality (HR 1.63, 95% CI 1.02-2.57) (383). A similar positive association was seen in the SHEP trial, where serum uric acid in the highest quartile conveyed an increased risk of cardiovascular events (HR 1.32 95% CI 1.03-1.69) but not of all cause mortality or stroke (384). Other large studies have shown that small increments in serum uric acid are associated with increasing incidence of cardiovascular disease and with cardiovascular death (385;386). These associations persist despite adequate adjustment for confounding factors and other risk factors. However, in the Syst-Eur study of hypertensive patients, a trend but no association was seen with cardiovascular events or mortality (HR 1.06, 95% CI 0.99-1.13 and HR 1.03, 95% CI 0.93-1.14 per 50µmol/L increment in serum urate respectively) (387). While the event rate was similar to that in other studies, the proportion of females was far greater, meaning it is possible that a gender effect has attenuated the association.

Cardiovascular Disease

In patients with stroke, increasing serum uric acid levels have been associated with a reduced likelihood of a favourable outcome and an increased risk of recurrent vascular events (OR 0.78, 95% CI 0.67-0.91 and RR 1.27, 95% CI 1.18-1.36 per additional 0.1mmol/l in serum uric acid respectively) (388). This association was more prominent in diabetic patients (389). Elevated levels of serum uric acid in the early stages after acute stroke have also been associated with early clinical deterioration (390). In patients with angiographically defined coronary artery disease, serum uric acid in the highest quintile (391) and quartile (392) has been shown to be predictive of all cause mortality (HR 1.5, 95% CI 1.02-2.1 and HR 1.23, 95% CI 1.11-1.36 respectively). Increasing levels of serum uric acid have also been shown to strongly predict mortality, need for cardiac transplant and in-hospital mortality in those with cardiac failure (393;394). Conversely, it has been shown that increased serum uric acid in the acute phase following stroke is associated with a good outcome (395); a finding that is addressed later.

Healthy Populations

The NHANES study (396) did show an association with each 59.5 µmol/l increment in serum uric acid leading to increased risk of cardiovascular events (HR 1.09, 95% CI 1.02-1.18 in men) and cardiac mortality (HR 1.17, 95% CI 1.06-1.28 in men). A yet more significant association was seen in women. However, a large analysis from the Framingham Heart study cohort found no association with cardiovascular mortality after adjustment for diuretic therapy (397) and a similar lack of association has been reported in other large studies of healthy individuals (398-400). Lower event rates in such healthy populations may explain this inconsistency; larger sample sizes are required and some may have lacked power to detect an independent association

For An Association		
Reference	**Population**	***Change in Outcome Measure***
(380)	DM	*HR 1.91 (1.24-2.94) (serum uric acid >295umol/l) for risk of stroke*
(389)	DM / stroke	*HR 1.49 (1.21-1.84)* [a] *for recurrent CV event*
(388)	Acute stroke	*RR 1.27 (1.18-1.36)* [a] *for recurrent CV events*
(390)	Acute stroke	*Serum uric acid ↑ in those with early clinical deterioration (p=0.001)*
(391)	CAD	*HR 1.5 (1.02-2.1)* [b] *for all cause mortality*
(392)	CAD	*HR 1.23 (1.11-1.36)*[c] *for all cause mortality*
(384)	↑ BP	*HR 1.32 (1.03-1.69)*[b]*, for CV events*
(401)	↑ BP	*HR 1.22 (1.11-1.35)*[d] *for development of CV disease*
(386)	↑ BP	*HR 1.14 (1.02-1.27)*[e] *for CV mortality,* *HR 1.34(1.14-1.57)*[e] *for fatal stroke*
(383)	↑ BP	*HR 1.73 (1.01-3)* [b] *for CV event rates*
Against An Association		
(395)	Acute stroke	*OR 1.12, 95% CI 1-1.25 per additional mg/dl uric acid for good outcome*
(387)	↑ BP	*HR 1.03 (0.93-1.14)*[e] *for CV mortality* *HR 1.06 (0.99-1.13)*[e] *for all CV events*
Results expressed as ratio and 95% CI. DM = Diabetes Mellitus, PVD = Peripheral Vascular Disease, CV = Cardiovascular, CAD = angiographic coronary disease, [a] *= per additional 0.1mmol/l in serum uric acid,* [b] *= for highest vs lowest quintile / quartile,* [c,d] *= per additional 0.6 and 0.86mmol/l in serum uric acid respectively,* [e] *= for each 50µmol/L increment in serum urate*		
Table 4.1: Summary of Studies Examining Relationship Between Serum UA and Outcome		

Summary / Gaps in the Epidemiological Evidence

Most large, well-conducted epidemiological studies support the hypothesis that elevated serum uric acid is a powerful predictor of increased vascular event rate and mortality in patients with hypertension, diabetes and in those with known cardiovascular disease (table 4.1). The results in healthy populations are less consistent. Despite this, the relationship with stroke rate and mortality is less clear; most studies have not specifically evaluated stroke mortality or have been limited by relatively low stroke rates (383;396). Where it has been evaluated, only one Chinese study has shown a strong association (386) while others have not (384;387). An association has been seen with stroke risk in patients with diabetes (380) and in a large observational cohort of patients aged > 55 years of age, although the association was less prominent in hypertensive individuals (402). The impact of serum uric acid on outcome in the acute period after stroke is also unclear. In a study of 2498 patients with acute stroke, increasing serum UA levels were associated with a reduced likelihood of favourable outcome at 90 days (OR 0.78, 95% CI 0.67-0.91 per additional 0.1 mmol/L UA) and an increased risk of recurrent vascular events (388), which were more prominent in those with diabetes (389). Some have suggested increased risk of early clinical deterioration following ischaemic stroke (390). However, a further study reported conflicting results: in 800 patients with acute ischaemic stroke, increasing UA levels were associated with a good outcome (OR 1.12, 95% CI 1-1.25 per additional mg/dl UA) at seven days (395).

4.01.3 An Innocent By-stander?

Against a Direct Causal Association

It is argued that elevated serum uric acid in those with cardiovascular disease simply reflects the presence of other risk factors such as hypertension or diabetes, diuretic treatment, impaired renal function, atherosclerosis itself or increased oxidative stress.

Worsening renal function is associated with increased serum uric acid levels and increased burden of cardiovascular disease. Markers of oxidative stress are increased in patients with chronic renal disease and are predictive of increased cardiovascular mortality (403;404). Successful renal transplantation (405-407) improves such markers. Therefore, the association may simply reflect impaired renal function and the associated oxidative stress and cardiovascular risk. While most studies have adequately adjusted for renal impairment, it cannot be excluded that raised uric acid levels reflect or contribute to sub clinical levels of renal impairment which contribute to the association in as yet undefined ways.

Even in those with normal renal function, higher levels of uric acid may reflect higher levels of xanthine oxidase activity and oxidative stress. This would also explain why serum uric acid levels rise after an ischaemic insult as discussed below. The action of xanthine oxidase leads to generation of superoxide anions, and is one of the principle sources of reactive oxygen species (ROS) in the human vasculature (408;409). The molecular effects and importance of ROS in cardiovascular disease has already been extensively reviewed (410-413). In summary, once formed, superoxide anions can inactivate nitric oxide, leading to formation of peroxynitrite, which is also a strong oxidant. This inhibits endothelium-dependent vasorelaxation, limits the favourable effects of nitric oxide on platelet aggregation and vascular smooth muscle proliferation and causes oxidation of DNA and

lipids; all factors integral to development of atherosclerosis (414). Under normal circumstances ROS production is usually countered by antioxidant defence mechanisms, including the action of superoxide dismutase (SOD) (415).

As well as this putative role in atherosclerosis development, ROS production increases acutely following cerebral or cardiac ischaemia and may contribute to the degree and extent of tissue damage (416-418). This hypothesis is supported by animal models of ischaemic stroke where SOD knockout mice exhibit greater lesion volumes after temporary middle cerebral artery occlusion (419-421), whereas SOD over expressing mice exhibit reduced lesion volume (422). Infusion of SOD and catalase have led to a reduction in stroke lesion volume in a murine stroke model (423). Human studies have also shown that locally increased oxidative stress and reduced peripheral antioxidant activity are associated with increased stroke lesion volume and a greater neurological deficit (424;425). Similar work in animal models of myocardial infarction has suggested oxidative stress is associated with increased development of heart failure (426;427). It is also likely that oxidative stress predisposes to development of heart failure after acute myocardial infarction in humans (428;429). Thus, increased uric acid levels may simply reflect increased xanthine oxidase activity which may directly contribute to the development of atherosclerotic disorders and predispose to more severe vascular events.

There is evidence that uric acid has antioxidant activity and that levels may rise after an ischaemic insult. This has led to an alternate hypothesis that elevated serum uric acid represents a physiological, and perhaps protective, response to the oxidative stress that characterises many vascular disease states (430;431). In a rat model of cerebral ischaemia, brain uric acid levels increased (432;433) and in a transient ischaemia model, infusion of

uric acid led to a reduction of infarct volume and improved behavioural outcome (433). This is further supported by data from a rat model of traumatic brain injury and a mouse model of multiple sclerosis, where uric acid was found to reduce formation of peroxynitrite radicals (434;435). In healthy human volunteers, uric acid administration has been shown to increase serum antioxidant capacity (436). In a study of 800 patients with ischaemic stroke, admission serum uric acid was higher in those with a good outcome and increasing levels associated with a good outcome (OR 1.12, 95% CI 1-1.25 per additional mg/dl uric acid) (395). This finding is in direct contrast to those of other studies (388-390).

While these findings warrant consideration, the potential antioxidant properties of uric acid in vitro or in vivo should not be over-interpreted. Increased local tissue levels in animal models of ischaemia and brain injury may simply reflect the levels of oxidative stress and xanthine oxidase activity, and not an innate protective response. The substance itself may well have antioxidant properties but its generation and associated superoxide anion production may be of much greater significance and detriment. Even the intriguing findings that uric acid infusion may be protective in animal models of brain ischaemia do not imply that uric acid lowering strategies, and particularly those involving xanthine oxidase inhibition, could not have favourable effects in vivo.

For a Causal Association

Data from animal and in-vitro studies do raise the possibility of a direct causal mechanism for uric acid in cardiovascular disease which provides further support for the convincing epidemiological associations. Monosodium urate crystals have been shown to stimulate release of the platelet constituents serotonin, ATP and ADP (437), while uric acid has been shown to stimulate rat vascular smooth muscle production in vitro (438). Uric acid may

also increase oxygenation of LDL (439) and may have a causative role in the development of hypertension (440-442). This is summarised in figure 4.2.

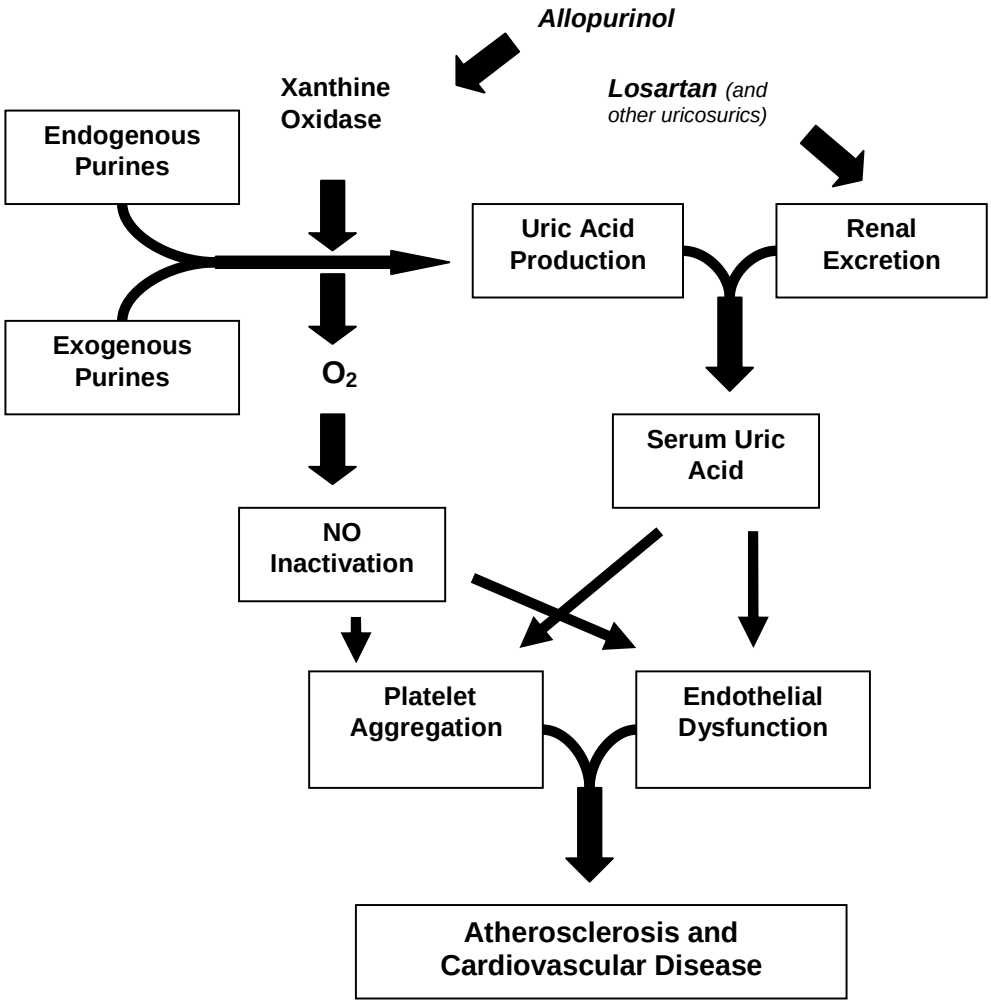

Figure 4.2 Potential Mechanisms by Which Elevated Serum Uric Acid is Related to Cardiovascular Disease and Potential Methods of Risk Reduction. Drugs and sites of action are shown in italic font,

4.01.4 Pharmacological intervention to Lower Uric Acid

Several drugs are known to lower uric acid. These either increase uric acid excretion (urosuric drugs), block the final step in uric acid production via xanthine oxidase inhibition or lead to uric acid breakdown (rasburicase). The most effective urosuric drugs are

probenecid and sulfinpyrazone, while fenofibrate (a fibrate) (443) and losartan (an angiotensin II antagonist) (444;445) also have urosuric activity. Rasburicase is a recombinant urate-oxidase enzyme which converts uric acid to allantoin. It is used in association with some anti-cancer treatments and is unsuitable for repeated dosing. There are two commercially available xanthine oxidase inhibitors, allopurinol and oxypurinol. Allopurinol is rapidly metabolised to oxypurinol, which is an analogue of xanthine and preferentially binds to xanthine oxidase thereby inhibiting its activity (446). Because of its action on both uric acid levels and xanthine oxidase activity, allopurinol is a logical drug to study in trials of cardiovascular risk reduction (figure 4.2).

Allopurinol is generally well tolerated with few side effects. Its major indication is in the prophylaxis of gout (447). Side-effects typically comprise gastro-intestinal upset and rashes. A rash develops in approximately 2 percent of patients and typically subsides after treatment is discontinued. More serious side effects, such as generalised hypersensitivity, occur in less than 1 in 1000 cases and include exfoliative dermatitis, often with vasculitis, fever, liver dysfunction, eosinophilia, and acute interstitial nephritis (448). The rate of adverse reaction is highest in patients with renal dysfunction and rashes are more common with concurrent amoxicillin therapy (449;450). There is a known interaction with azathioprine and 6-mercaptopurine therapy and some rare reports of cytopenia. This side-effect profile is comparable to that of commonly used secondary preventative agents such as HMG-CoA reductase inhibitors (451) and angiotensin converting enzyme inhibitors (452;453).

4.01.5 Uric Acid Lowering Drugs and Cardiovascular Risk

Three drugs known to reduce cardiovascular mortality have been shown to reduce serum uric acid. This may explain some of their beneficial effect but changes in other risk factors such as renal function and blood pressure may explain both the beneficial effects and changes in uric acid levels.

Fenofibrate is a fibric acid derivative known to reduce total and LDL cholesterol by approximately 15% with a similar increase in HDL cholesterol and with larger reductions in triglyceride levels (454). Fibrates reduce incidence of cardiac events in dyslipidaemic patients (455) and in those with coronary disease (456). These benefits may be greatest in type two diabetics (457) where they have been shown to reduce atherosclerosis progression (458) and total vascular events (459). Fenofibrate reduces serum uric acid levels (via increased renal excretion) by as much as 46% in healthy volunteers, hypertensive and diabetic patients and those with gout on specific urate lowering therapy (460-462). This may provide adjunctive efficacy in the treatment of gout when combined with allopurinol (463;464) and may contribute to the reduced vascular risk associated with fibrate therapy. This effect is not seen with other fibrates (465) and its mechanism is elusive although unlikely to be mediated by improvements in renal function (466).

Losartan is an angiotensin II receptor antagonist which is superior to atenolol in the prevention of cardiovascular events when given to hypertensive patients with left ventricular hypertrophy (467). Losartan is known to increase renal uric acid excretion (468) (by as much as 30%), thereby causing significant reductions in serum uric acid (469). This mediated via effects on the urate/anion transport mechanism in the renal proximal tubule (470). This is not a class effect; other angiotensin II antagonists have little or no

effect on serum uric acid excretion (471). Up to 29% of the 13% relative risk reduction seen with losartan use in the LIFE study has been attributed to its effect on serum uric acid (472). Serum uric acid increased with both atenolol and losartan use in the LIFE study, but the increase was significantly less with losartan. Whether this does underpin much of the benefit of losartan and whether it can attenuate the possibly detrimental effects of diuretics on uric acid requires further clarification. Although the effect was independent of measures of renal function, treatment with ACE blockade or angiotensin II antagonists do reduce levels of oxidative stress (473) which could itself account for this added benefit.

Statin therapy has also been shown to lower serum uric acid. In the GREACE study, atorvastatin was associated with a fall in serum uric acid (by 8.2%), whereas serum uric acid increased in those patients allocated to the placebo group (by 3.3%). After extensive adjustment of several risk factors including change in renal function, each 60µmol/l reduction in serum uric acid was associated with a reduction in vascular event rates (HR 0.76, 95% CI 0.62-0.89) (474). A fall in serum uric acid has been mirrored in other studies of statin therapy (149;475) but typically in association with improvements in serum creatinine. Atorvastatin however, has repeatedly been shown to lower uric acid (by 6.4%) in other studies (476) even after adjustment for renal function, possibly because of decreased uric acid production (477). As yet, this has not been adequately studied and cannot be assumed to explain some of atorvastatin's effects, although it may represent a further beneficial effect.

Specific Intervention to Lower Serum Uric Acid and Xanthine Oxidase Inhibition

The effect of xanthine oxidase inhibition on measures of endothelial and cardiovascular function has also been tested in a number of small studies, performed in the context of

diabetes, hypercholesterolaemia, hypertension, elevated 10 year cardiovascular risk, sleep apnoea, angiographically confirmed cardiovascular disease, stroke and heart failure. A recent systematic review performed by myself has identified 22 such studies (478-498). A summary of the findings is given below and in table 4.2.

Study design has varied using either oral or intravenous xanthine oxidase inhibition with differing treatment duration. For example, seven studies (478;480;486-488;493;495) utilised a single dose of allopurinol or oxypurinol. In other studies duration of therapy was one week (480), two weeks (491;492;498), one month (481;483;484;490;494;497), six weeks (489), two months (479), three months (482;496) and six months (485). Some studies were open label (478;480;486-488;493;496), others employed a cross over design (479-482;490;494;495;498) and the remainder were randomised controlled trials (483-485;489;491;492;497).

The populations studied have varied. There have been nine studies in heart failure or cardiomyopathy (478-486), two in those with established cardiac disease (487;488), three in those with diabetes (490-492), one in those with stroke (489), two in those with hypercholesterolaemia (493;494), one in those with the metabolic syndrome (497), one in smokers (495), one in those with hyperuricaemia and elevated Framingham risk score (496) and one in those with sleep apnoea (498).

The endpoints assessed also varied. Six studies reported changes in forearm blood flow responses following acetylcholine infusion (480;483;490;493-495) with similar measures of limb blood flow responses assessed in a further four studies (480;496-498). Three studies assessed changes in blood markers of oxidative stress (491;492) and inflammatory

activity (489) while further studies evaluated change in BNP levels (482), exercise tolerance (482;484) and myocardial efficiency, contractility and function (486) (478;484;488). Others reported effects on heart rate variability parameters (479), coronary vasoconstrictor activity (487) and in one study, a clinical endpoint of change in clinical status in those with chronic heart failure was assessed (485).

Improvements in endothelial function following allopurinol have been shown in patients with type II diabetes and mild hypertension (490). Endothelial function, expressed as percentage change in ratio of infused forearm blood flow (FBF) compared to non-infused arm FBF in response to intra-arterial acetylcholine infusion, was found to return to near normal following one month of 300mg allopurinol treatment. No effect was seen on control patients. Using similar methods, a single oral dose of allopurinol was found to improve peripheral endothelial function towards normal in smokers but had no effect in the non-smoking control group (495). The rapidity of the improvement strongly suggests that xanthine oxidase is a key contributor to the endothelial dysfunction seen in smokers. Further study in those with diabetes has suggested improvement in markers of oxidative stress (491;492).

Improvement in forearm endothelial function has also been seen in patients with hypercholesterolaemia following intra-arterial administration of oxypurinol (493) but this was not replicated in a similar but smaller study of 4 weeks oral allopurinol treatment (494). It is possible this later study was underpowered; the power calculation was based upon the effect size seen following 3 months of simvastatin treatment (499). The anticipated treatment effect was larger than that seen in other studies of allopurinol use, while the effect seen in this study at one month, was more similar and approximately half this.

Forearm blood flow responses, expressed as ischaemia induced change in brachial artery diameter, were also improved by a three month course of allopurinol in a group of patients with elevated 10 year cardiovascular risk (496) and hyperuricaemia. A recent study in patients with stable coronary artery disease (487) showed that intravenous administration of oxypurinol improved both peripheral endothelial function and coronary endothelial function (expressed as the coronary vasoconstrictor response to acetylcholine and changes in coronary blood flow) in those with impaired baseline function. A similar study showed an improvement in left ventricular function in those with IHD (488).

Intra-coronary infusion of oxypurinol has been shown to reduce myocardial oxygen consumption, probably via preserved nitric oxide bioactivity, and to significantly increase myocardial efficiency measurements in those with heart failure (478). In a further study a single intravenous infusion (486) and a one month course of 600 mg allopurinol (484) improved left ventricular ejection fraction. Further, both an intravenous infusion (480), a one month 300 mg oral course (481) and a six month 600 mg oral course (483) of allopurinol have been shown to improve a variety of measures of peripheral endothelial function in patients with heart failure. Recent work has shown that B-type natriuretic peptide concentrations are reduced by a three month course of oral allopurinol (482) but no improvements in exercise tolerance were demonstrated, while in previous study no change in heart rate variability parameters was seen (479).

Results from the largest study to date were disappointing. In the OPT-CHF trial (485), 405 participants with heart failure were randomised to receive either oxypurinol 600 mg or placebo for 6 months. The primary endpoint was defined as a change in clinical status

based upon changes in a variety of parameters; mortality, heart failure related hospitalisations, need for therapy escalation, New York Heart Association functional class and Patient Global Heart Failure Clinical Status. There was no difference in the proportion of patients who improved or worsened between treatment groups (43.3% improved with allopurinol compared to 45%) with placebo while 32% and 35.6%) remained the same and 24.6% and 19.3% worsened ($p = 0.42$)). However, post-hoc analyses suggested that in those with elevated serum uric acid, oxypurinol improved clinical status, whereas the opposite occurred in those with lower uric acid levels. In the oxypurinol cohort as a whole, those who improved had significantly greater reductions in serum uric acid levels than those who worsened. These findings are difficult to interpret. On the one-hand the post-hoc analyses suggest benefit in those with high uric acid levels and that the greater the fall following oxypurinol in such patients, the better the outcome, they also raise the possibility that oxypurinol can cause harm in some.

Potential Significance of These Studies

Abnormalities in peripheral and coronary arterial responses are accepted to signify endothelial dysfunction and are associated with other markers of cardiovascular disease (500). Improvements in these parameters have been shown to follow treatment with thiazolidinediones (501;502) and agents such as ACE inhibitors (503-505), HMG COA reductase inhibitors (502,505-509), and amlodipine (503). Most of these agents have been shown to be effective in reducing cardiovascular event rates and mortality. While direct comparisons are flawed and difficult, the magnitude of the changes effected by xanthine oxidase inhibition seems comparable to those caused by these agents (bar the exception outlined above) (472;505;506;510). Further, the reductions in BNP levels seen were comparable to those induced by ACE inhibition, angiotensin II antagonists (511), beta-

blockade (512) and spironolactone (513). It is therefore possible, but entirely speculative, that allopurinol could have a similar impact on clinical outcomes as these agents. The side effect profile of allopurinol is also comparable to other treatments but the cost is not; allopurinol is cheap making it an attractive preventative treatment.

Data have shown that following stroke, each additional 0.1 mmol/l increase in serum uric acid is associated with a 27% increased relative risk of a recurrent cardiovascular event (388). Data also show that following stroke, 300 mg allopurinol causes a sustained reduction in serum uric acid from a mean of 0.35 mmol/L (SD 0.09) to 0.22 mmol/L (SD 0.05) (unpublished data). Using a secondary prevention stroke trial as an example, it would therefore be reasonable to expect a 27% relative risk reduction in cardiovascular event rate with this treatment. With a predicted cardiovascular event rate of 6/100 patient years, or 16% over 3.5 years (as used to design and seen in the recent ESPRIT trial (138)), approximately 3000 patients would be required to be followed for a mean of 3 years to confirm this benefit. It is important to remember that this figure may actually be less because the beneficial effects of allopurinol on endothelial function would be expected to contribute to the treatment effect regardless of changes in uric acid. For example, in those studies involving a single dose of allopurinol or oxypurinol which showed benefit, the results presumably reflect xanthine oxidase inhibition rather than change in serum uric acid.

Ref.	Population	Intervention	*Change Following Treatment*
(480)	CHF, ↑ uric acid	Intra-arterial infusion of allopurinol	*Improvement in FEF **
(480)	CHF, ↑ uric acid	1 week allopurinol 300mg †	*Improvement in lower limb endothelial function.*
(482)	CHF	3 months allopurinol 300mg †	*Fall in BNP levels from baseline. No change in exercise tolerance*
(481)	CHF	1 month 300mg allopurinol †	*Improvement in FEF **
(484)	CHF	1 month oxypurinol 600 mg	*Increase in LVEF*
(486)	CHF	Single intra-venous oxypurinol infusion	*Increase in LVEF*
(485)	CHF	6 months oxypurinol 600 mg	*No change in functional status*
(479)	CHF	2 months allopurinol 300 mg	*No change in heart rate variability parameters*
(478)	Idiopathic DCM	Intra-coronary infusion of allopurinol	*Reduction in MVO2. Increase in myocardial efficiency*
(488)	CHD and LVSD	Single intra-venous oxypurinol infusion	*Increase in LVEF and reduction in end systolic ventricular volume*
(487)	Coronary Heart Disease	Single intra-venous oxypurinol infusion	*Attenuation of coronary vasoconstrictor response * and increase in coronary blood flow*

*CHF = congestive cardiac failure. FEF = forearm endothelial function. BNP = brain natriuretic peptide. DCM = dilated cardiomyopathy. MVO_2 = Myocardial O_2 consumption. LVSD = left ventricular systolic dysfunction. * = in response to intra-arterial Ach infusion, † = cross over design, § = control arm, || = ischaemia induced % change in BA diameter.*

Table 4.2. Studies of uric acid lowering therapy in those with or at risk of cardiovascular disease.

Ref.	Population	Intervention	*Change Following Treatment*
(489)	Acute ischaemic stroke	6 weeks allopurinol (300 or 100 mg) §	*Reduction in ICAM-1 levels following 300 mg allopurinol*
(490)	Type 2 DM	1 month 300mg od allopurinol †§	*Improvement in FEF **
(491)	Type 1 DM	2 weeks allopurinol 300 mg	*Reduction in GSSG/GSH*
(492)	Type 2 DM	2 weeks allopurinol 100 mg	*Reduction of lipid peroxidation and total antioxidant power in blood / saliva*
(493)	↑ cholesterol, ↑ BP	Single infusion of oxypurinol §	*Improvement in FEF * in ↑ cholesterol patients*
(494)	↑ cholesterol	4 weeks 300mg allopurinol †	*No change in FEF **
(495)	Smokers	Single 600mg po dose allopurinol †§	*Improvement in FEF **
(496)	↑ uric acid, ↑ CV risk.	3 months 300mg allopurinol §	*Improvement in FEF ‖*
(497)	Metabolic Syndrome	1 month allopurinol 300 mg	*Increase in FBF ¥ respective to baseline (3.8±0.1 %, p<0.01)*
(498)	OSA	2 weeks allopurinol 300 mg	*Improvement in FEF *,ǂ*

DM = diabetes mellitus. FEF = forearm endothelial function. = in response to intra-arterial Ach infusion, † = cross over design, FBF = forearm blood flow, § = control arm, ‖ = ischaemia induced % change in BA diameter. OSA = obstructive sleep apnoea.*

Table 4.2 cont. Studies of uric acid lowering therapy in those with or at risk of cardiovascular disease.

Gaps in the Clinical Trial Data

There are no adequately powered clinical endpoint trials of uric acid lowering strategies such as xanthine oxidase inhibition and there are no small scale studies in patients with stroke or that examine markers of cerebrovascular health.

Summary

The epidemiological evidence to support a role of elevated serum uric acid in cardiovascular disease is cogent. These associations are seen across healthy populations (albeit less consistently), those with cardiovascular risk factors and in those with established cardiovascular disease. While a clear pathophysiological role for uric acid in the development of cardiovascular disease has yet to be established, there are data to support detrimental and prothrombotic effects on platelet and endothelial function. Post-hoc analyses suggest that some of the beneficial effects of proven treatments for cardiovascular disease may be due to changes in serum uric acid level study. (458;472;474). Furthermore, xanthine oxidase mediated oxidative stress is likely to have a significant role in the development of atherosclerosis and several small studies have shown that xanthine oxidase inhibition improves endothelial function and markers of oxidative stress in a variety of disease states (478;480-482;487;488;490;493-496), although not yet with regard to markers of stroke risk or in those with stroke. Thus, even if serum uric acid is simply a marker of oxidative stress, there is a wealth of epidemiological, animal and now clinical data to suggest the benefits of strategies to lower uric acid and inhibit xanthine oxidase. Data from the OPT-CHF study raise the possibility that benefit may only be seen in those with elevated serum uric acid and that treatment may be detrimental in those with low serum uric acid. Large scale trials with clinical endpoints are justified to address this

important question in the context of heart failure, coronary disease and in broader categories of cardiovascular risk. Despite being an old drug, allopurinol may prove to be a cheap, effective and novel preventative therapy for the 21st century.

4.02 Chapter Aims and Hypothesis

The hypothesis was that elevated serum uric acid would be associated with a poor outcome following acute stroke; that it would be associated with increased stroke risk in those with hypertension; and that use of allopurinol would improve cerebrovascular health in a primary and secondary prevention cohort. The aim was therefore to evaluate the association between serum uric acid and functional outcome after stroke (study one) and the association between serum uric acid and stroke risk in hypertensive patients (study two) and to test whether allopurinol, the most widely used uric acid lowering drug, improves cerebrovascular health in those with diabetes (study three) and recent subcortical stroke (study four).

4.03 Study One - Baseline Serum Uric Acid and 90-Day Functional Outcomes Following Acute Ischaemic Stroke

The study hypothesis was that elevated serum uric acid would be associated with a poor outcome following acute stroke. A retrospective analysis of clinical trial data was performed to test this.

4.03.1 Study One Methods

The relationship between serum uric acid measured early after stroke and 90-day outcome were evaluated using data from the Virtual International Stroke Trials Archive (VISTA) resource. Full details of the VISTA resource are described elsewhere (514). Briefly, VISTA represents an archive of either complete datasets or the placebo component from clinical trials in acute stroke. The original data can be searched to select trials and/or patients fulfilling certain selection criteria or with fields containing certain information, such as serum uric acid level or functional outcome. Analyses on natural history are encouraged but exploration of treatment effects is prevented; in some cases trial datasets were donated under confidentiality rules that preclude identification of the trial in published analyses. The VISTA dataset continues to expand, but at the time of access for this study it contained data from 15 trials and over 15,000 patients.

Data were extracted from the VISTA dataset, where serum uric acid had been measured during conduct of an acute stroke trial within 6 hours of symptom onset. Only a single trial in VISTA routinely measured baseline uric acid. All patients had ischaemic stroke confirmed by brain imaging and baseline stroke severity (Scandinavian Stroke Scale (SSS)) (515) and demographic details were recorded. Follow up was for 3 months at which point

functional outcome assessment (using the mRs and Barthel index) and repeat SSS scores were performed.

Statistical Analysis

Descriptive statistics were used to describe the population with mean (±SD) and or number (%) used. Poor outcome was defined as death or dependency at 90 days (modified Rankin scale (mRs) (516) score > 2 or death within 90 days). Uric acid levels were converted to mmol/l. The relationship between baseline differences (including uric acid) and poor outcome was assessed and univariate differences between groups were explored. The χ^2 test (binary variables) and Student's t test (continuous variables) were used. Univariate and then multivariate logistic regression analysis was performed to assess the relationship between uric acid and outcome and to control for variables that differed significantly between outcome groups or that are known to predict stroke outcome. Natural logarithmic transformations of data were employed when exploratory analyses showed these to be merited. The linearity assumption was verified using the Box-Tidwell test.

Patients known to be alive at day 90 but for whom the mRs grading was unknown were excluded from the regression analyses. Sensitivity of the findings to this assumption was investigated by repeating the analyses twice, with missing scores entered as good and poor outcomes respectively. Mean values were imputed where data were missing for continuous variables.

All statistical analyses were performed using Minitab version 15 (Minitab inc. PA, USA).

4.03.2 Study One Results

In total, 852 patients were included. Mean age was 68 (±12) years and 524 participants (61.5%) were male. Mean baseline SSS was 27 (±9.2), mean systolic blood pressure was 160 (±27) mmHg and mean diastolic blood pressure 88 (±15) mmHg. Mean baseline uric acid was 0.35 (±0.11) mmol/l. These and other baseline characteristics are shown in table 4.3.

Age, years	*68.3 (12.1)*
Male	*524 (61.5)*
SSS, units	*27 (9.2)*
SBP, mmHg	*160.5 (27)*
DBP, mmHg	*88.4 (14.9)*
Creatinine (µmol/l)	*92.7 (24.3)*
Cholesterol (mmol/l)	*5.6 (1.2)*
Glucose (mmol/l)	*7.57 (2.9)*
Uric Acid (mmol/l)	*0.35 (0.11)*
Ischaemic Heart Disease	*165 (19.4%)*
Diabetes Mellitus	*164 (19.3%)*
Hypertension	*467 (54.8%)*
Atrial Fibrillation	*156 (18.3%)*
Congestive Cardiac Failure	*74 (8.7%)*

Table 4.3 – Study One Baseline Demographic Variables. Expressed as n (%) for categorical variables and mean (SD) for continuous variables.

Outcomes

Eleven patients could not be assigned to an outcome group as they were known to be alive but had no day 90 mRs score recorded. Four patients had no age recorded, 8 had no baseline measurement of heart rate and 2 had no baseline blood pressure measurement. At 90 days, 434 patients (51.6%) had suffered a poor outcome, 141 of whom had died.

Univariate Analysis

On univariate analyses (table 4.4), greater age, lower baseline SSS, higher systolic blood pressure, higher serum glucose, higher serum creatinine, female sex, a history of hypertension, atrial fibrillation, diabetes mellitus and cardiac failure were associated with increased odds of a poor outcome, as were greater serum uric acid levels (OR 1.57, 95% CI 1.02-2.42). Natural logarithmic transformation of data was utilized for serum uric acid, creatinine and glucose levels.

Variable	**Coefficient**	**OR**	**CI**	***P***
Uric Acid *	0.451001	1.57	1.02-2.42	*0.042*
Age	0.0381584	1.04	1.03-1.05	*< 0.001*
Male	-0.307936	0.73	0.56-0.97	*0.015*
BSSS	-0.136462	0.87	0.85-0.89	*< 0.001*
Creatinine *	0.784949	2.19	1.26-3.82	*0.006*
SBP	0.0065294	1.01	1-1.01	*0.012*
Glucose *	1.03505	2.82	1.8-4.41	*< 0.001*
Hypertension	0.309976	1.36	1.04-1.79	*0.026*
AF	0.697322	2.01	1.4-2.89	*< 0.001*
CHF	0.511970	1.67	1.02-2.74	*0.04*

Table 4.4. Relationship Between Baseline Variables and Stroke Outcome on Univariate Analysis. * = natural logarithmic transformation employed.

Multivariate Analysis

Multivariate analysis revealed that only greater age, lower baseline SSS and higher serum glucose and systolic blood pressure were independently associated with a poor outcome (table 4.5). Uric acid level was not independently associated with poor outcome (OR 1.3, 95% CI 0.73-2.31 (for log transformed data)).

Variable	**Coefficient**	**OR**	**CI**	***P***
Age	0.0273775	1.03	1.01-1.04	*0.001*
Uric Acid *	0.234900	1.3	0.7-2.31	*0.42*
Male	-0.215531	0.81	0.56-1.15	*0.24*
BSSS	-0.135167	0.87	0.85-0.89	*< 0.001*
Creatinine *	0.303908	1.36	0.65-2.84	*0.42*
SBP	0.0084116	1.01	1-1.01	*0.01*
Glucose *	0.775222	2.17	1.29-3.66	*0.004*
Hypertension	-0.0091985	0.99	0.7-1.4	*0.96*
AF	0.217369	1.24	0.8-1.92	*0.33*
CHF	0.0750884	1.08	0.6-1.95	*0.80*

Table 4.5. Relationship Between Baseline Variables and Stroke Outcome on Multivariate Analysis. * = natural logarithmic transformation employed.

Results were similar whether the 11 missing mRs scores were substituted by good or poor outcomes on both univariate and multivariate analysis (table 4.6).

Variable	OR #	CI #	OR (*)	*CI (*)*
Age	1.03	1.01-1.04	1.02	*1.01-1.04*
Uric Acid *	1.41	0.8-2.49	1.28	*0.73-2.26*
Male	0.84	0.59-1.19	0.79	*0.56-1.13*
BSSS	0.88	0.86-0.9	0.87	*0.85-0.89*
Creatinine *	1.19	0.57-2.48	1.45	*0.69-3.02*
SBP	1.01	1-1.01	1.01	*1-1.01*
Glucose *	1.10	1.04-1.16	1.95	*1.18-3.24*
Hypertension	0.97	0.69-1.37	0.99	*0.71-1.4*
AF	1.18	0.77-1.82	1.3	*0.84-2*
CHF	1.09	0.61-1.96	1.08	*0.6-1.94*

Table 4.6. Relationship With Poor Outcome (mRs > 2) – Multivariate Sensitivity Analysis. Missing outcome data assumed to represent poor (#) or good (*) outcome respectively. * = natural logarithmic transformation employed.

4.03.3 Study One Discussion

This study failed to demonstrate an independent association between serum uric acid and day 90 functional outcome after acute ischaemic stroke. An association was seen between increasing uric acid levels and odds of poor outcome on univariate analyses but not on multivariate analysis. It is however possible given the upper limit of the 95% confidence intervals that such an association does exist.

This study used a robust dataset from a large, rigorously monitored randomized controlled trial with near complete ascertainment (98.7%) of 90-day functional outcomes. Data collection was restricted to studies where uric acid was measured acutely within a known time frame and where modified Rankin scale scores were measured. All patients were

recruited and enrolled with a mean time of 4.5 hours from ictus and all those included in this analysis had baseline serum uric acid and stroke severity measured. The group included a wide range of ages and outcomes similar to those seen in other large trials (517). Reassuringly, variables such as increasing age, blood pressure, serum creatinine and worse baseline stroke severity predicted poor outcome as has been shown previously. By using day 90 mRs as the outcome measure, results of this study can be readily interpreted in a fashion that is clinically relevant and meaningful. However, the fact this was a clinical trial population is also a potential weakness - the patients are naturally highly selected and thus not guaranteed to be truly representative of the wider population. Log transformations of data were utilized because exploratory analyses suggested it appropriate and, while this makes direct comparison with other work difficult, it was appropriate. A further potential weakness is that the analysis was based upon a single measurement of uric acid and small intra-individual variation in uric acid levels is known to occur (518). This will reduce the apparent strength of any association. However, in a study of the potential role of uric acid in the acute setting, a spot measurement is the most relevant.

The literature to date overwhelmingly supports an association between elevated serum uric acid and increased incidence of cardiovascular events and mortality (519). More uncertainty surrounds the relationship between levels of serum uric and likelihood of recovery after stroke. As mentioned, it has been previously demonstrated that higher uric acid measured early after stroke is independently associated with reduced likelihood of a favourable outcome, an increased risk of recurrent vascular events (388) and an increased risk of early clinical deterioration (390). Others have shown increasing uric acid to be independently associated with increased odds of good outcome at 7 days (395). This study showed an association between increasing serum uric acid and increased odds of poor

outcome on univariate analysis but, given the results of multivariate analysis, does not support any of the previous findings.

These differences are intriguing. This and previous studies have used similar techniques, first exploring univariate differences between outcome groups then using multiple logistic regression to control for factors known to influence outcome. There were, however, differences in trial methodology that may explain this apparent discrepancy. Previous studies were retrospective analyses of registry datasets with differing endpoints. For example, one previous analysis utilized placement at 90 days (alive, placed in own home or care or dead) as the outcome measure whereas work suggesting benefit of elevated uric acid utilized day 7 Mathew scale score. Both these have potential drawbacks; placement at 90 days could be confounded by socioeconomic factors, while the Mathew scale is poorly validated and day 7 outcomes are less informative than those at day 90. The present study is the first analysis from a clinical trial utilizing the most widely accepted and utilized measure of functional outcome available. These data raise the possibility that no such independent association exists, although the estimated odds ratio is similar to that found in previous work and the confidence intervals overlap; it may be that the study had limited statistical power to detect an independent effect of the magnitude found in previous studies.

Regardless, these findings do not discount the possibility that uric acid reduction is a viable and promising strategy to reduce cardiovascular risk. Uric acid contributes to several key stages of the atherosclerotic process and may have detrimental effects on platelet, endothelial and smooth muscle cell function (438;440;520;521) and may have a role in the development of MRI detected ischaemic cerebral white matter hyper-intensities (522). These findings support the notion that elevated serum uric acid will increase cardiovascular

event rate and could link with worse outcome after stroke. On the other hand, uric acid is widely accepted to have antioxidant activity and some speculate that elevated levels may be a beneficial response to the oxidative stress that characterises vascular disease states (430). This may apply in ischaemic stroke: brain uric acid levels increase in a rat model of permanent cerebral ischemia (432;433) and infusion of uric acid reduces infarct volume and improves behavioural outcome in a rat transient ischemia model (434). Human data also support the antioxidant properties, where uric acid administration has been shown to increase serum antioxidant capacity in healthy volunteers (436) and reduce markers of oxidative stress in those treated with thrombolytic therapy for acute ischaemic stroke (523). Temporary iatrogenic hyperuricaemia may afford the beneficial antioxidant effects of uric acid without the detrimental effects of chronically elevated levels on the vasculature.

In summary, this study found no significant independent association between serum uric acid level soon after ischaemic stroke onset and 90-day outcome. Prospective trials are needed to clarify the role of uric acid management in the post stroke period and as a preventative measure in cardiovascular disease.

Study Two - Serum Uric Acid and Stroke Mortality in Patients with Hypertension

The study hypothesis was that elevated serum uric acid would be associated with increased stroke risk in those with hypertension. A retrospective analysis of a large registry dataset was performed to test this.

4.04.1 Study Two Methods

The relationship between serum uric acid and mortality in a group of hypertensive patients domiciled in the West of Scotland was evaluated. Data from the Glasgow Blood Pressure Clinic were used. This is a secondary and tertiary referral centre for patients from the West of Scotland area. Patients are reviewed at least annually. Demographic and clinical data are routinely gathered on all patients and entered into a computer database, which currently holds data on those who have attended since November 1967. The study design was evaluated by and approved by the West Medical Research Ethics Committee, who felt formal application and review were unnecessary.

Subjects are followed up by record linkage (524) to death records held and supplied by the Registrar General of Scotland, which provide accurate causes of death. Causes of death are described according to the ninth and tenth revisions of the World Health Organisation International Classifications of Diseases (ICD-9 and ICD-10). The primary outcome event was defined as death due to stroke (ICD-9 430-438). Secondary outcomes were total mortality, coronary heart disease mortality (ICD-9 410-414.9) and total vascular mortality (stroke, coronary (defined above) and peripheral vascular disease deaths (440-447.6).

Statistical Analysis

Descriptive statistics were used to describe the population with mean (±SD) or number (%) used. Baseline demographic variables and outcomes were compared between those who had uric acid levels measured compared to those who did not. The relationship between baseline differences (including uric acid) and the primary outcome was assessed and univariate differences between groups were explored. The χ^2 test (binary variables) and Student's t test (continuous variables) were used. The average serum uric acid throughout the study was used for analyses. Average levels of blood pressure and other blood parameters were also used.

A Cox proportional hazards model was used to assess the relationship between last measured uric acid and primary outcome after adjustment for differences between the groups or other factors which may influence occurrence of the primary outcome. All statistical analyses were performed using SPSS software – version 11.5. Results of the Cox proportionally hazard modeling are expressed as hazard ratios with 95% confidence intervals. The same statistical procedures were followed for the secondary outcomes. The risk between uric acid and stroke mortality appeared J-shaped so further analysis based upon uric acid quintiles was performed.

4.04.2 Study Two Results

Data from 11,399 hypertensive patients who attended the clinic between 6th November 1968 and 23rd April 2003 were analysed. Uric acid results were available for 6202 patients (54.4%). The date of the last record linkage was 10th July 2004. Mean follow up duration was 184 (SD 93.7) months with a median of 175 months. Baseline demographic variables

are shown in table 4.7. Those who had serum uric acid levels measured were less likely to be smokers, more likely to be diabetic, hyperlipidaemic, have established vascular disease and to be taking anti-hypertensive therapy. Mean blood pressures and mean cholesterol levels were however lower.

Variable	**Whole Group n = 11399**	**Uric Acid Measured n = 6202**	**No Uric Acid Measured n = 5197**	***P Value***
Age; years	49.3 (13.7)	49.1 (13.4)	49.5 (14)	*0.06*
Male Sex	5482 (48.1%)	2949 (47.5%)	2533 (48.7%)	*0.21*
Smoker	3313 (29.1%)	1728 (27.9%)	1585 (30.4%)	*0.002*
Cholesterol > 5 mmol/l	7444 (65.3%)	4355 (70.2%)	3089 (59.7%)	*<0.0001*
Diabetes Mellitus	545 (4.8%)	363 (5.9%)	182 (3.5%)	*<0.0001*
IHD	523 (4.6%)	301 (4.9%)	222 (4.3%)	*0.14*
CVA	345 (3%)	204 (3.3%)	141 (2.7%)	*0.07*
PVD	470 (4.1%)	273 (4.4%)	197 (3.8%)	*0.10*
Any CVD	1191 (10.4%)	686 (11.1%)	505 (9.7%)	*0.019*
Mean SBP; mmHg	157.8 (22.4)	156.5 (19.5)	159.3 (25.3)	*<0.0001*
Mean DBP; mmHg	93.9 (11.2)	92.3 (9.7)	95.9 (12.5)	*<0.0001*
BMI; kg/m^2	27.7 (5.4)	27.8 (5.4)	27.6 (5.5)	*0.08*
Mean Cholesterol; mmol/l	6.04 (1.15)	6.1 (1.1)	5.9 (1.1)	*<0.0001*
Mean Creatinine; µmol/l	99.1 (54.5)	98 (46.6)	102.5 (70.9)	*<0.0001*
ACE Inhibitor	2648 (23.2%)	1796 (29%)	852 (16%)	*<0.0001*
Beta Blocker	6104 (53.5%)	4071 (65.6%)	2033 (39.1%)	*<0.0001*
Calcium Channel Blocker	3512 (30.8%)	2427 (39.1%)	1085 (20.9%)	*<0.0001*
Diuretic	7120 (62.5%)	4250 (68.5%)	2870 (55%)	*<0.0001*

P values represent those obtained on comparison of those who had uric acid levels measured compared to those who did not. Two sample t-tests were employed for continuous variables, while chi-squared tests were used for binary variables.

Table 4.7. Study Four Baseline Demographic Variables

Uric Acid Data

The mean first serum uric acid was 0.34 mmol/l (SD 0.14). The mean last serum UA was 0.35 mmol/l (SD 0.18) while the mean average serum uric acid level during the study was 0.35 mmol/l (SD 0.14). These data and uric acid quintiles are shown in table 4.8. In total, 2431 (39.2%) of individuals had only one serum uric acid measured, 1039 (16.8%) had two, 660 (10.6%) had three and 2072 (33.4%) had four or more uric acid measurements.

Uric Acid Data		
Whole Group	***Mean (SD); mmol/l***	
First Uric Acid	*0.34 (0.14)*	
Last Uric Acid	*0.35 (0.18)*	
Mean Uric Acid	*0.35 (0.14)*	
Uric Acid Quintiles	**Range; mmol/l**	***N (%)***
1st Uric Acid Quintile	0.01 to 0.25	*1242 (20.1%)*
2nd Uric Acid Quintile	0.26 to 0.30	*1235 (19.9%)*
3rd Uric Acid Quintile	0.31 to 0.35	*1278 (20.6%)*
4th Uric Acid Quintile	0.36 to 0.41	*1207 (19.5%)*
5th Uric Acid Quintile	0.42 to 5.5	*1240 (19.9%)*

Table 4.8. Study Four Uric Acid Data. Uric acid quintiles are based upon mean serum uric acid levels.

Higher serum uric acid was significantly associated with male sex, beta-blocker, diuretic and calcium channel antagonist therapy, established cardiovascular disease and hyperlipidaemia, and increases in age, serum creatinine concentration, blood pressure, total serum cholesterol concentration and body mass index (table 4.9).

Continuous Variables	**Regression Coefficient**		***P value***
Age; years	0.001		*<0.0001*
Mean SBP; mmHg	0.001		*<0.0001*
Mean DBP; mmHg	0.001		*<0.0001*
Mean Cholesterol; mmol/l	0.007		*<0.0001*
Mean Creatinine; µmol/l	0.001		*<0.0001*
BMI; kg/m^2	0.003		*<0.0001*
Binary Variables	**Present**	**Absent**	***P value***
Male Sex	0.39	0.31	*<0.0001*
Smoker	0.34	0.35	*0.55*
Cholesterol > 5 mmol/l	0.35	0.33	*<0.0001*
Diabetes Mellitus	0.35	0.35	*0.44*
IHD	0.36	0.35	*0.07*
CVA	0.36	0.35	*0.21*
PVD	0.36	0.35	*0.19*
Any CVD	0.36	0.35	*0.009*
ACE Inhibitor	0.35	0.35	*0.87*
Beta Blocker	0.35	0.34	*<0.0001*
Calcium Channel Blocker	0.35	0.34	*0.007*
Diuretic	0.35	0.33	*<0.0001*

For continuous variables linear regression analysis was employed and the regression coefficients refer to the increase in serum uric acid seen for each unit increase in the variable. For binary variables, the levels shown are the mean average serum uric acid according to whether the variable was present or absent and the p values refer to those obtained on 2 sample t testing.

Table 4.9. Relationship Between Baseline Variables and Increasing Serum Uric Acid in Hypertensive Patients

Outcome Data

In the total study group there were 713 (6.3% of patients) stroke deaths, 1462 (12.8%) IHD deaths, 2532 (22.7%) vascular deaths and 4137 (36.3%) deaths in total. In those who had serum uric acid measured, there were 354 stroke deaths (5.7%), 723 (11.7%) IHD deaths, 1290 (20.8%) vascular deaths and 2124 (34.2%) deaths in total table 4.10).

Outcome Data				
Variable	**Whole Group n = 11399**	**UA Measured n = 6202**	**No UA Measured n = 5197**	***P Value***
Death	4137 (36.3%)	2124 (34.2%)	2013 (38.7%)	*<0.0001*
IHD Death	1462 (12.8%)	723 (11.7%)	739 (14.2%)	*<0.0001*
Stroke Death	713 (6.3%)	354 (5.7%)	359 (6.9%)	*0.008*
Any Vascular Death	2532 (22.7%)	1290 (20.8%)	1302 (25.1%)	*<0.0001*

Table 4.10. Outcome in the Whole Hypertensive Patients Study Group and Where Serum Uric Acid was Measured. P values refer to Chi-squared testing.

4.04.2.1 Relationships with Stroke Mortality

On univariate analysis male sex, smoking, diabetes, peripheral vascular disease, stroke, any established vascular disease, diuretic therapy and increases in age, SBP, DBP, serum cholesterol concentration, serum creatinine concentration and serum uric acid were associated with a significant increase in stroke mortality. Increasing BMI was associated with a reduction in stroke mortality (table 4.11).

Variable	**Hazard Ratio (HR)**	**95% Confidence Interval for HR**	***p-value***
Age	1.093	1.081 to 1.104	*<0.001*
Male Sex	1.273	1.033 to 1.568	*0.024*
IHD	1.517	0.976 to 2.359	*0.08*
CVA	5.093	3.663 to 7.082	*<0.001*
PVD	2.386	1.611 to 3.534	*<0.001*
Smoker	1.388	1.116 to 1.726	*0.004*
Any CVD	2.939	2.287 to 3.776	*<0.001*
Diabetes Mellitus	1.630	1.115 to 2.383	*0.018*
BMI; kg/m^2	0.972	0.951 to 0.994	*0.01*
Mean SBP; mmHg	1.029	1.026 to 1.033	*<0.001*
Mean DBP; mmHg	1.032	1.023 to 1.041	*<0.001*
Mean Creatinine; µmol/l	1.004	1.002 to 1.005	*<0.001*
Mean Uric Acid; µmol/l	1.861	1.19 to 2.911	*0.006*
Mean Cholesterol; mmol/l	1.413	1.042 to 1.254	*0.005*
ACE Inhibitor	0.775	0.595 to 1.008	*0.051*
Beta-Blocker	1.163	0.922 to 1.468	*0.20*
Calcium Channel Blocker	0.984	0.789 to 1.227	*0.89*
Diuretic	1.877	1.438 to 2.451	*<0.001*

Table 4.11. Risk of Stroke Death (Unadjusted) in Hypertensive Patients.

Following adjustment for the predictive variables, male sex, smoking, diabetes, stroke, and increases in age, SBP, DBP and serum creatinine were associated with a significant increase in stroke mortality. Serum uric was not associated with increased stroke risk (table 4.12).

Variable	**Hazard Ratio (HR)**	**95% Confidence Interval for HR**	***p-value***
Age	1.086	1.071 to 1.101	*<0.001*
Male Sex	1.552	1.21 to 1.99	*0.001*
CVA	3.156	2.232 to 4.465	*<0.001*
PVD	1.312	0.866 to 1.988	*0.20*
Smoker	1.52	1.203 to 1.921	*<0.001*
Diabetes Mellitus	1.623	1.085 to 2.426	*0.018*
BMI	0.983	0.96 to 1.007	*0.17*
Mean SBP	1.015	1.008 to 1.022	*0.001*
Mean DBP	1.025	1.011 to 1.039	*<0.001*
Mean Creatinine	1.002	1.001 to 1.004	*0.006*
Mean Uric Acid	0.947	0.278 to 3.222	*0.93*
Mean Cholesterol	1.015	0.919 to 1.120	*0.77*
Diuretic	1.144	0.843 to 1.554	*0.39*

Table 4.12. Risk of Stroke Death (Adjusted) in Hypertensive Patients.

On exploratory analysis, the relationship between uric acid and stroke mortality appeared J-shaped (figure 4.3) with risk lowest in the second quartile of serum uric acid levels (figure 4.4). Compared to those in the second quintile of uric acid levels, risk was significantly increased in those in the lowest quintile and increased from the third to the fifth quintile (table 4.13).

Following adjustment for other significant variables, a significant J-shaped relationship remained across the quintiles of uric acid with stroke mortality (table 4.14).

Variable	**Hazard Ratio (HR)**	**95% Confidence Interval for HR**	***p-value***
Mean Uric Acid	1.861	1.19 to 2.911	*0.006*
Uric Acid First Quintile	1.652	1.135 to 2.404	*0.009*
Uric Acid Second Quintile	1	-----	-----
Uric Acid Third Quintile	1.79	1.235 to 2.595	*0.002*
Uric Acid Fourth Quintile	1.948	1.347 to 2.817	*<0.001*
Uric Acid Fifth Quintile	2.207	1.531 to 3.181	*<0.001*

Table 4.13. Risk of Stroke Death (Unadjusted for Other Variables) in Hypertensive Patients.

Variable	**Hazard Ratio (HR)**	**95% Confidence Interval for HR**	***p-value***
Age	1.085	1.071 to 1.1	*<0.001*
Male Sex	1.555	1.206 to 2.006	*0.001*
CVA	3.192	2.253 to 4.522	*<0.001*
PVD	1.285	0.848 to 1.948	*0.24*
Smoker	1.519	1.202 to 1.920	*<0.001*
Diabetes Mellitus	1.651	1.104 to 2.469	*0.015*
BMI	0.984	0.96 to 1.008	*0.19*
Mean SBP	1.015	1.008 to 1.022	*<0.001*
Mean DBP	1.024	1.010 to 1.038	*0.001*
Mean Creatinine	1.002	1.001 to 1.004	*0.006*
Mean Cholesterol	1.016	0.920 to 1.122	*0.76*
Diuretic	1.143	0.841 to 1.555	*0.39*
UA First Quintile	1.777	1.186 to 2.661	*0.005*
UA Second Quintile	1	---	---
UA Third Quintile	1.468	0.993 to 2.169	*0.054*
UA Fourth Quintile	1.521	1.028 to 2.251	*0.036*
UA Fifth Quintile	1.434	0.953 to 2.159	*0.08*

Table 4.14. Risk of Stroke Death (Adjusted) in Hypertensive Patients.

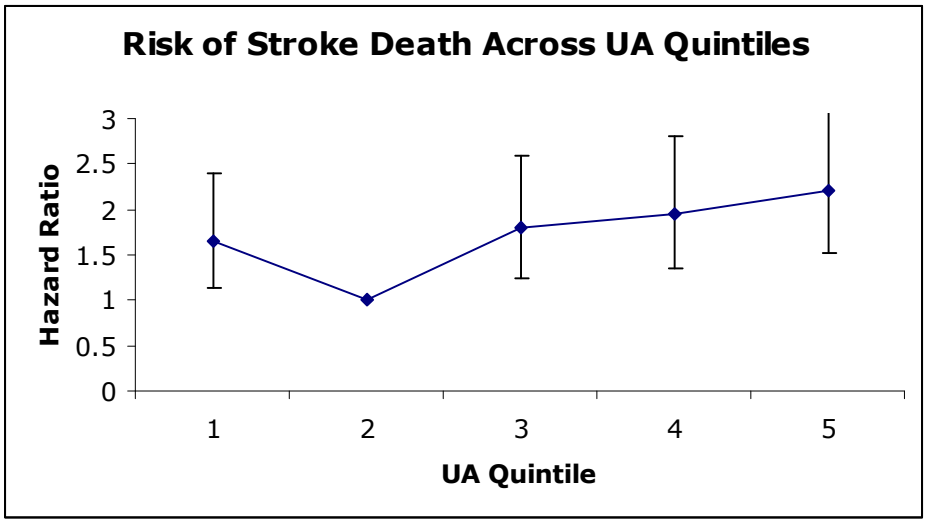

Figure 4.3. Risk of Death Across Serum Uric Acid Quintiles in Hypertensive Patients.

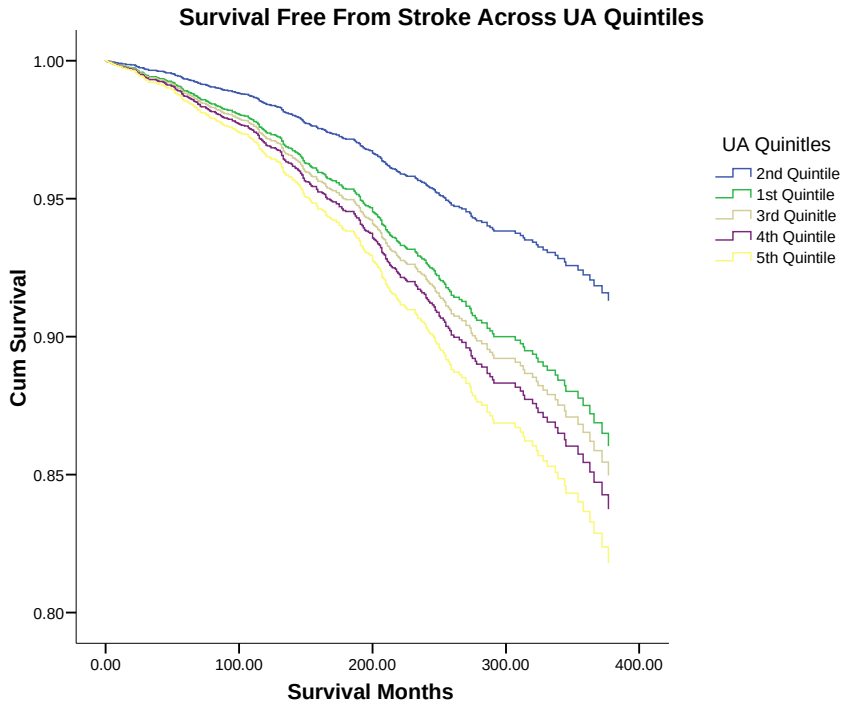

Figure 4.4. Survival Free From Stroke Across Uric Acid Quintiles in Hypertensive Patients.

4.04.2.2 Relationships with Total Mortality

On univariate analysis male sex, smoking, diabetes, ischaemic heart disease, peripheral vascular disease, stroke, any established vascular disease, beta-blocker, calcium channel and diuretic therapy and increases in age, SBP, DBP, serum cholesterol concentration, serum creatinine concentration and serum uric acid were associated with a significant increase in total mortality. Increasing BMI was associated with a reduction in total mortality (table 4.15).

Variable	**Hazard Ratio (HR)**	**95% Confidence Interval for HR**	***p-value***
Age; years	1.075	1.07 to 1.079	*<0.001*
Male Sex	1.431	1.314 to 1.559	*<0.001*
IHD	1.927	1.638 to 2.267	*<0.001*
CVA	2.357	1.957 to 2.839	*<0.001*
PVD	2.593	2.221 to 3.027	*<0.001*
Smoker	1.589	1.456 to 1.734	*<0.001*
Any CVD	2.306	2.065 to 2.574	*<0.001*
Diabetes Mellitus	1.603	1.371 to 1.874	*<0.001*
BMI; kg/m^2	0.987	0.979 to 0.996	*0.004*
Mean SBP; mmHg	1.024	1.022 to 1.026	*<0.001*
Mean DBP; mmHg	1.026	1.022 to 1.030	*<0.001*
Mean Creatinine; µmol/l	1.004	1.003 to 1.004	*<0.001*
Mean Uric Acid; µmol/l	2.052	1.759 to 2.394	*<0.001*
Mean Cholesterol; mmol/l	1.138	1.096 to 1.183	*<0.001*
ACE Inhibitor	0.904	0.815 to 1.002	*0.05*
Beta-Blocker	1.236	1.123 to 1.361	*<0.001*
Calcium Channel Blocker	1.14	1.044 to 1.245	*0.004*
Diuretic	1.888	1.692 to 2.105	*<0.001*

Table 4.15. Risk of Death (Unadjusted) in Hypertensive Patients.

Following adjustment for the predictive variables, male sex, smoking, diabetes, ischaemic heart disease, peripheral vascular disease, stroke, and increases in age, SBP, DBP, serum cholesterol concentration, serum creatinine concentration and serum uric acid were associated with a significant increase in total mortality. Calcium channel antagonist use was associated with a reduction in total mortality (table 4.16).

Variable	**Hazard Ratio (HR)**	**95% Confidence Interval for HR**	***p-value***
Age	1.075	1.069 to 1.081	*<0.001*
Male Sex	1.574	1.424 to 1.740	*<0.001*
IHD	1.248	1.049 to 1.486	*0.012*
CVA	1.44	1.18 to 1.757	*<0.001*
PVD	1.571	1.323 to 1.864	*<0.001*
Smoker	1.721	1.564 to 1.893	*<0.001*
Diabetes Mellitus	1.508	1.274 to 1.784	*<0.001*
BMI	0.995	0.986 to 1.005	*0.36*
Mean SBP	1.008	1.004 to 1.011	*<0.001*
Mean DBP	1.019	1.013 to 1.025	*<0.001*
Mean Creatinine	1.003	1.002 to 1.003	*<0.001*
Mean Uric Acid	1.437	1.015 to 2.035	*0.041*
Mean Cholesterol	1.05	1.008 to 1.094	*0.019*
Beta-Blocker	1.04	0.933 to 1.158	*0.48*
Calcium Channel Blocker	0.9	0.815 to 0.994	*0.038*
Diuretic	1.069	0.942 to 1.212	*0.30*

Table 4.16. Risk of Death (Adjusted) in Hypertensive Patients.

There was no evidence that the relationship between serum uric acid and total mortality was J-shaped (figure 4.5).

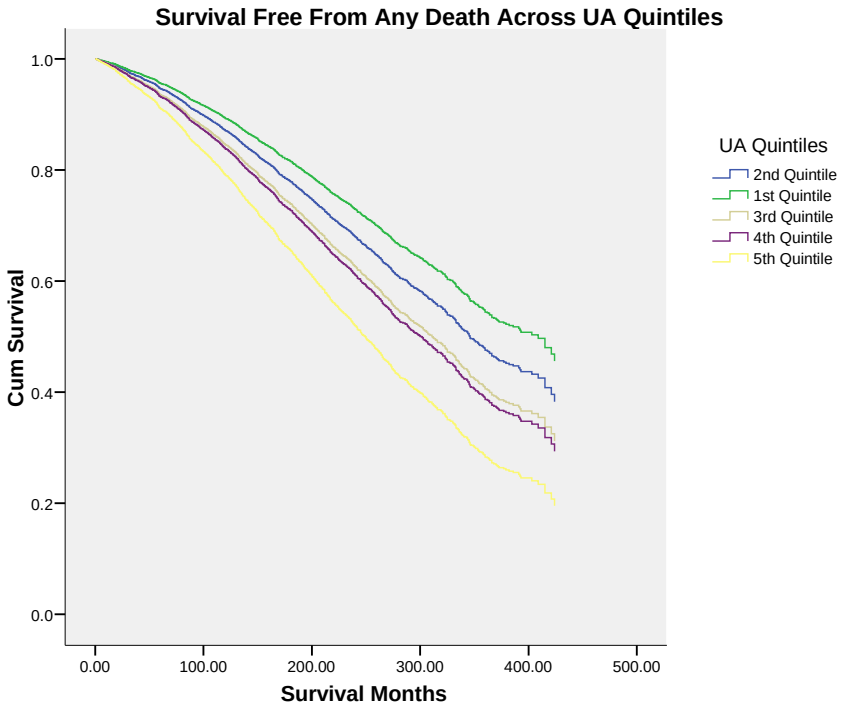

Figure 4.5. Survival Free From Any Death Across Uric Acid Quintiles in Hypertensive Patients.

4.04.2.3 Relationships with Vascular Mortality

On univariate analysis male sex, smoking, diabetes, ischaemic heart disease, peripheral vascular disease, stroke, any established vascular disease, beta-blocker and diuretic therapy and increases in age, SBP, DBP, serum cholesterol concentration, serum creatinine concentration and serum uric acid were associated with a significant increase in vascular mortality (table 4.17)

Variable	**Hazard Ratio (HR)**	**95% Confidence Interval for HR**	***p-value***
Age	1.077	1.071 to 1.082	*<0.001*
Male Sex	1.494	1.339 to 1.668	*<0.001*
IHD	2.251	1.852 to 2.735	*<0.001*
CVA	2.688	2.149 to 3.363	*<0.001*
PVD	2.701	2.223 to 3.282	*<0.001*
Smoker	1.655	1.48 to 1.85	*<0.001*
Any CVD	2.64	2.307 to 3.023	*<0.001*
Diabetes Mellitus	1.715	1.412 to 2.084	*<0.001*
BMI; kg/m^2	0.991	0.980 to 1.002	*0.12*
Mean SBP; mmHg	1.027	1.025 to 1.029	*<0.001*
Mean DBP; mmHg	1.033	1.028 to 1.037	*<0.001*
Mean Creatinine; µmol/l	1.004	1.003 to 1.004	*<0.001*
Mean Uric Acid; µmol/l	2.148	1.8 to 2.562	*<0.001*
Mean Cholesterol; mmol/l	1.183	1.127 to 1.241	*<0.001*
ACE Inhibitor	0.894	0.783 to 1.020	*0.09*
Beta-Blocker	1.369	1.207 to 1.554	*<0.001*
Calcium Channel Blocker	1.104	0.985 to 1.237	*0.09*
Diuretic	2.114	1.829 to 2.443	*<0.001*

4.17. Risk of Vascular Death (Unadjusted) in Hypertensive Patients.

Following adjustment for the predictive variables, male sex, smoking, diabetes, ischaemic heart disease, peripheral vascular disease, stroke, diuretic therapy and increases in age, SBP, DBP, serum cholesterol concentration, serum creatinine concentration and serum uric acid were associated with a significant increase in vascular mortality (table 4.18).

Variable	**Hazard Ratio (HR)**	**95% Confidence Interval for HR**	***p-value***
Age	1.077	1.069 to 1.084	*<0.001*
Male Sex	1.702	1.496 to 1.935	*<0.001*
IHD	1.465	1.190 to 1.803	*<0.001*
CVA	1.636	1.287 to 2.079	*<0.001*
PVD	1.585	1.277 to 1.967	*<0.001*
Smoker	1.787	1.581 to 2.019	*<0.001*
Diabetes Mellitus	1.566	1.27 to 1.931	*<0.001*
BMI	1.001	0.989 to 1.014	*0.85*
Mean SBP	1.011	1.007 to 1.015	*0.001*
Mean DBP	1.025	1.017 to 1.033	*<0.001*
Mean Creatinine	1.003	1.002 to 1.003	*<0.001*
Mean Uric Acid	1.793	1.233 to 2.607	*0.002*
Mean Cholesterol	1.091	1.036 to 1.149	*0.001*
Beta-Blocker	1.111	0.965 to 1.280	*0.14*
Diuretic	1.216	1.028 to 1.439	*0.023*

Table 4.18. Risk of Vascular Death (Adjusted) in Hypertensive Patients.

There was no evidence that the relationship between serum uric acid and vascular mortality was J-shaped (figure 4.6).

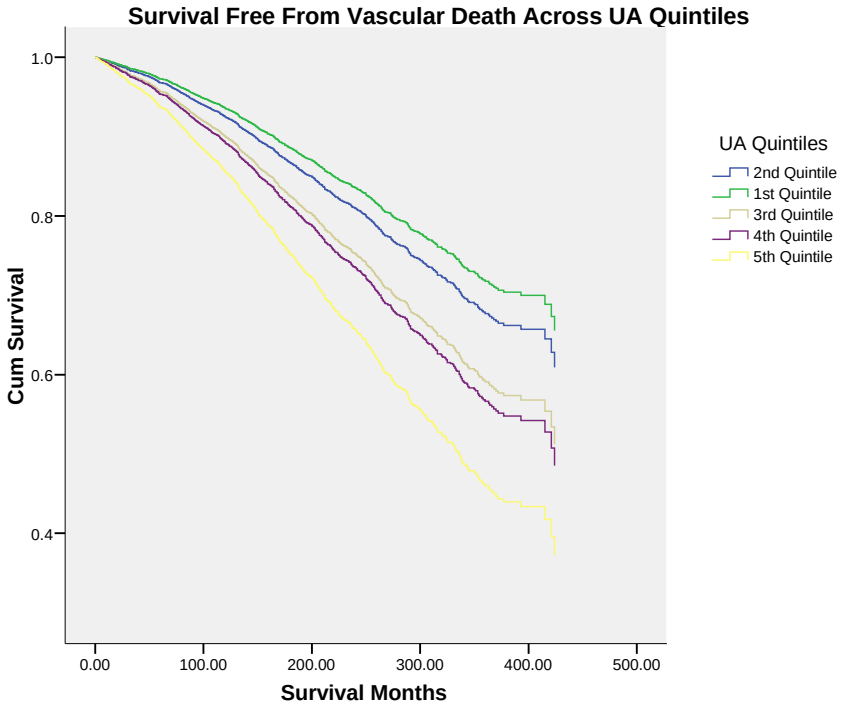

Figure 4.6. Survival Free From Vascular Death Across Uric Acid Quintiles in Hypertensive Patients.

4.04.2.4 Relationship with Ischaemic Heart Disease Mortality

On univariate analysis male sex, smoking, diabetes, ischaemic heart disease, peripheral vascular disease, stroke, any established vascular disease, beta-blocker, calcium channel antagonist and diuretic therapy and increases in age, SBP, DBP, serum cholesterol concentration, serum creatinine concentration and serum uric acid were associated with a significant increase in coronary mortality (table 4.19).

Variable	**Hazard Ratio (HR)**	**95% Confidence Interval for HR**	***p-value***
Age	1.066	1.059 to 1.073	*<0.001*
Male Sex	1.692	1.459 to 1.961	*<0.001*
IHD	2.847	2.248 to 3.607	*<0.001*
CVA	1.709	1.186 to 2.464	*0.008*
PVD	2.736	2.112 to 3.544	*<0.001*
Smoker	1.778	1.533 to 2.063	*<0.001*
Any CVD	2.591	2.161 to 3.107	*<0.001*
Diabetes Mellitus	1.949	1.523 to 2.494	*<0.001*
BMI; kg/m^2	1.002	0.988 to 1.017	*0.767*
Mean SBP; mmHg	1.025	1.023 to 1.028	*<0.001*
Mean DBP; mmHg	1.033	1.027 to 1.04	*<0.001*
Mean Creatinine; µmol/l	1.004	1.003 to 1.004	*<0.001*
Mean Uric Acid; µmol/l	2.146	1.696 to 2.714	*<0.001*
Mean Cholesterol; mmol/l	1.227	1.151 to 1.309	*<0.001*
ACE Inhibitor	0.958	0.806 to 1.140	*0.63*
Beta-Blocker	1.607	1.348 to 1.916	*<0.001*
Calcium Channel Blocker	1.206	1.038 to 1.401	*0.015*
Diuretic	2.166	1.782 to 2.632	*<0.001*

Table 4.19. Risk of IHD Death (Unadjusted) in Hypertensive Patients.

Following adjustment for the predictive variables, male sex, smoking, diabetes, ischaemic heart disease, peripheral vascular disease, diuretic and beta-blocker therapy and increases in age, SBP, DBP, serum cholesterol, creatinine and uric acid concentrations were associated with a significant increase in coronary mortality (table 4.20).

Variable	**Hazard Ratio (HR)**	**95% Confidence Interval for HR**	***p-value***
Age	1.066	1.056 to 1.077	*<0.001*
Male Sex	1.896	1.594 to 2.257	*<0.001*
IHD	1.996	1.55 to 2.57	*0.012*
CVA	1.045	0.703 to 1.555	*0.83*
PVD	1.693	1.269 to 2.259	*<0.001*
Smoker	1.859	1.579 to 2.189	*<0.001*
Diabetes Mellitus	1.746	1.339 to 2.276	*<0.001*
BMI	1.012	0.996 to 1.029	*0.15*
Mean SBP	1.01	1.004 to 1.015	*0.001*
Mean DBP	1.022	1.012 to 1.033	*<0.001*
Mean Creatinine	1.002	1.001 to 1.004	*<0.001*
Mean Uric Acid	1.7	1.004 to 2.878	*0.048*
Mean Cholesterol	1.159	1.082 to 1.241	*<0.001*
Beta-Blocker	1.28	1.05 to 1.561	*0.015*
Calcium Channel Blocker	0.9	0.76 to 1.067	*0.22*
Diuretic	1.267	1.008 to 1.593	*0.042*

Table 4.20. Risk of IHD Death (Adjusted) in Hypertensive Patients.

There was no evidence that the relationship between serum uric acid and coronary mortality was J-shaped (figure 4.7).

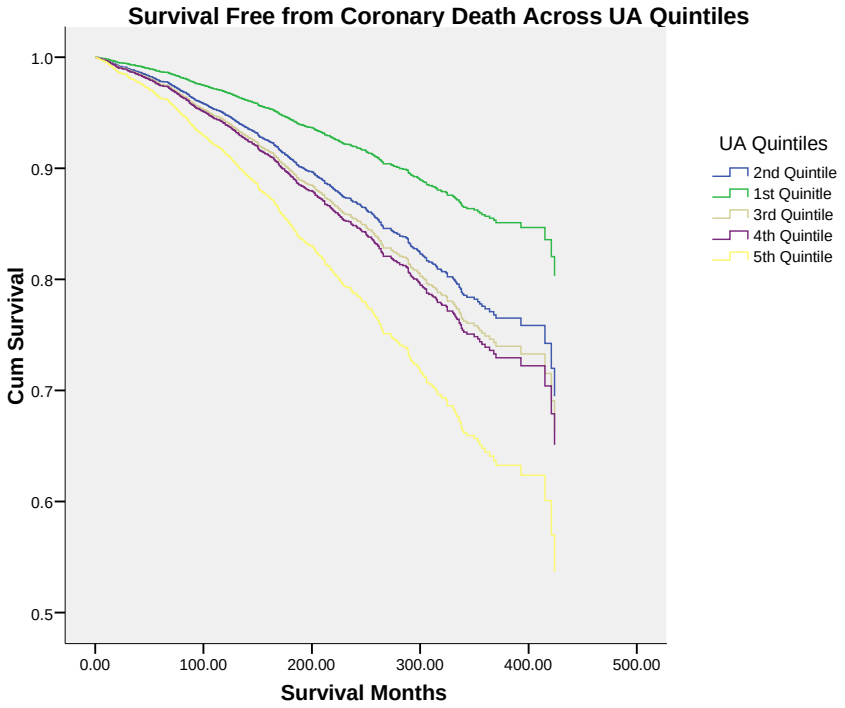

Figure 4.7. Survival Free From Coronary Heart Disease Death Across Uric Acid Quintiles in Hypertensive Patients.

4.04.2.5 Sex Specific Analysis

Serum uric acid level was strongly associated with total mortality in females but not males on exploratory univariate analysis. Similar was found for CHD mortality and vascular mortality and this sex difference remained apparent on multivariate analysis (table 4.21).

With regard to stroke mortality a significant J shaped relationship was observed in females. This was not observed in males where only the lowest quintile of uric acid levels linked with increased risk (table 4.21). On multivariate analysis, only the highest quintile of uric acid linked with increased stroke risk in females, while the lowest linked with increased risk in males (table 4.22).

	Males			***Females***		
	HR *	**95% CI for HR**	**p-value**	**HR ***	**95% CI for HR**	**p-value**
Total Mortality	1.16	0.77 to 1.74	0.48	8.84	6.06 to 12.91	*<0.001*
CHD Mortality	1.20	0.64 to 2.256	0.58	10.31	5.64 to 18.87	*<0.001*
Vascular Mortality	1.25	0.79 to 1.97	0.36	10.71	6.97 to 16.46	*<0.001*
Stroke Mortality						
1st Quintile	2.72	1.48 to 4.99	0.001	1.61	0.99 to 2.61	*0.05*
2nd Quintile	1	--	--	1	--	--
3 rd Quintile	1.35	0.78 to 2.33	0.29	2.11	1.27 to 3.51	*0.04*
4th Quintile	1.34	0.79 to 2.29	0.28	2.56	1.52 to 4.32	*<0.001*
5th Quintile	1.37	0.81- to 2.32	0.24	3.74	2.21 to 6.34	*<0.001*

Table 4.21. Risk of Total, Vascular, Coronary and Stroke Death on Univariate Analysis According to Sex in Hypertensive Patients.. HR = hazard ratio. Hazard ratios shown are per mmol/l increase in serum uric acid, except for stroke where they are relative to the 2nd quartile.

	Males			***Females***		
	HR *	**95% CI for HR**	**p-value**	**HR ***	**95% CI for HR**	***p-value***
Stroke Mortality						
1st Quintile	2.64	1.343 to 5.182	0.005	1.45	0.87 to 2.40	*0.15*
2nd Quintile	1	--	--	1	--	--
3 rd Quintile	1.43	0.78 to 2.61	0.25	1.51	0.90 to 2.54	*0.12*
4th Quintile	1.49	0.83 to 2.69	0.19	1.68	0.98 to 2.90	*0.060*
5th Quintile	1.22	0.67 to 2.24	0.51	2.03	1.14 to 3.62	*0.017*

Table 4.22. Risk of Stroke Mortality on Multivariate Analysis According to Sex in Hypertensive Patients. Adjustment variables are as shown in table 4.14. HR = hazard ratio. Hazard ratios shown are relative to the 2nd quartile.

4.04.3 Study Two Discussion

The results of this study suggest that increasing serum uric is associated with increased risk of stroke, total, vascular and coronary mortality in treated hypertensive patients but that the relationship between stroke mortality and serum uric acid appears J-shaped. Sex specific analyses also suggest that such a relationship with stroke risk is only apparent in females.

The relationship between uric acid and cardiovascular risk is well documented and increasingly accepted (521). However, many studies have not specifically evaluated stroke mortality (383;396;525;526) and, in studies where this has been examined, there has often been little evidence of an association, even when it was apparent for all cardiovascular endpoints. It is important to note however that all studies to date have been limited by relatively low stroke rates. For example, in an analysis of data from the SHEP trial (384), there were only 243 strokes. In an analysis from the Syst-Eur trial (387), there were only 128 strokes, of which only 36 of these were fatal. In the Syst-China trial (386), there were only 87 strokes, although these did make up the majority of cardiovascular endpoints. In analysis of data from the NHANES study (396) there were only 111 strokes and in the PIUMA study analysis, there were only 48 strokes (383).

The population studied here (those with treated hypertension) represent a group where the relationship between stroke rate and mortality is particularly unclear. Some studies have shown an association (380;386;402) while others have not (384;387). In the Syst-China study (386), a 50 µmol/l increase in serum uric acid afforded a relative hazard ratio of 1.28 (95% CI 1.1 to 1.5) for stroke mortality, although there was no significant association with all stroke events. Whether results from a Chinese population with a disproportionately high haemorrhagic stroke risk and low serum cholesterol can be widely generalised is questionable. An association has been seen with stroke risk in patients with diabetes (380), where serum uric acid levels above the median were associated with increased stroke risk (HR 1.91, 95% CI 1.24 to 2.94) although the number of strokes was again small (114 in total). In a large observational cohort of patients aged > 55 years of age (Rotterdam study data (402)) an association was seen with stroke incidence (HR 1.18, 95% CI 1.06 to 1.3). In

total, there were 381 stroke events; the largest number to date. However, subgroup analysis suggested this association was only present in females and in those with no previous hypertension.

There were 354 stroke deaths in this study; the highest number in any study to date, and the largest study of those with hypertension. This allows thorough analysis of any relationships. The impact of sex in many studies is difficult to assess but those who have addressed it suggest the association exists only in females, or is at least more prominent (402;525;526), although other studies show no such difference (387). In the Rotterdam study for example, no association was seen in males but an association was present in females. This study showed similar with a highly significant relationship was present in females but not males. There are known differences in uric acid metabolism between the sexes; women have a lower total body uric acid pool, presumably due to oestrogen mediated differences in renal excretion (527). Despite uric acid being higher in post menopausal women (528), menopausal status has little effect on the association between uric acid and cardiovascular disease (396). It is therefore possible, but speculative, that raised serum uric acid in females reflects a different mechanism than in males; perhaps increased xanthine oxidase activity and the associated superoxide anion production. It is also possible that changes in uric acid have less impact on those with higher baseline risk and higher rates of traditional risk factors; such as the males studied here. The possibility of a sex specific effect of uric acid lowering strategies on cardiovascular outcomes should be considered in the design of future clinical trials, although because of higher baseline risk, measures such as allopurinol use may prove most effective in males because of ancillary effects such as xanthine oxidase inhibition.

A J-shaped relationship between serum uric acid and cardiovascular risk has also been seen in many previous studies (383;385;396;525) and also with regard to stroke risk (380;386). This study found similar with regard to stroke risk but not total, vascular or coronary mortality. Why these differences exist is unclear. There are plausible explanations. It has been speculated that this J-shaped relationship reflects the putative detrimental effects of elevated uric acid on endothelial and platelet function and vascular smooth muscle and the association between lower serum uric acid and low plasma antioxidant activity. It is also possible that some of the increased risk seen with lower uric acid is due to poor nutrition or other illnesses which may contribute to cardiovascular risk - hypouricaemia is more common in hospitalised patients, with concomitant medications and in those with liver disease or neoplasia (529). Why this should be particular to stroke risk here is not clear.

There are strengths to this study including its size, the prospective nature of the data collection and the large number of fatal strokes. There are however, some weaknesses. The long study period means several of these events may have occurred before aggressive preventative strategies became commonplace and treatment regimens for hypertension have changed over the years. For example, there is now less emphasis placed upon diuretic therapy which increases serum uric acid. Whether this impacts upon the contemporary relevance of the results is unclear but possible.

In summary, data from this study suggest that serum uric acid is associated with increased stroke mortality and that this relationship is J-shaped, unlike that between uric acid and total, vascular and coronary mortality.

4.05 Study Three - Allopurinol and Basal Nitric Oxide Production in the Cerebral Circulation of those with Diabetes; a Randomised Trial

The study hypothesis was that the XO inhibitor allopurinol would enhance nitric oxide bioavailability in the cerebral circulation of patients with type 2 diabetes. This would be reflected by enhancement of the effects of L-NMMA, which produces transient reductions in cerebral blood flow (CBF). A randomized, double blind, placebo controlled crossover study was performed to test this.

4.05.1 Study Three Methods

The study was performed in the University Division of Cardiovascular and Medical Sciences at the Western Infirmary, Glasgow. The study was approved by the local research ethics committee and was registered in the ISRCTN database (ISRCTN 68849312).

Male patients aged over 40 years old with type 2 diabetes of duration less than 5 years and stable glycaemic control (HbA1c below 9.0%) were studied. Oral biguanide therapy was permitted, although those on insulin or sulphonylurea therapy were excluded. Further exclusion criteria were: greater than 70% extra-cranial internal carotid artery (ICA) stenosis, known coronary arterial disease, significant co-morbidity or frailty likely to cause either death or difficulty with protocol adherence within 3 months, significant renal impairment (defined as serum creatinine concentration over 250 µmol/L) and contra-indication to or indication for administration of allopurinol. Patients taking sulphonylurea therapy were later included following a protocol amendment. Exclusion criteria were chosen to minimise the theoretical risks of sub-endocardial myocardial ischaemia following L-NMMA, to

minimise potential risks of allopurinol treatment and to ensure carotid flow readings were not falsely elevated because of intrinsic carotid disease.

Patients were identified during out-patient attendance at hospital clinics. All participants gave written informed consent to participate and underwent full Bruce protocol exercise tolerance testing prior to randomisation to exclude sub-clinical coronary arterial disease. If evidence of this was found, further participation was not permitted. All drug treatments remained unchanged throughout the study period.

Study Assessment

Study visits took place in the Investigations Ward of the Acute Stroke Unit in the Western Infirmary and were supervised by one of the study investigators. The participant schedule is shown in figure 4.8.

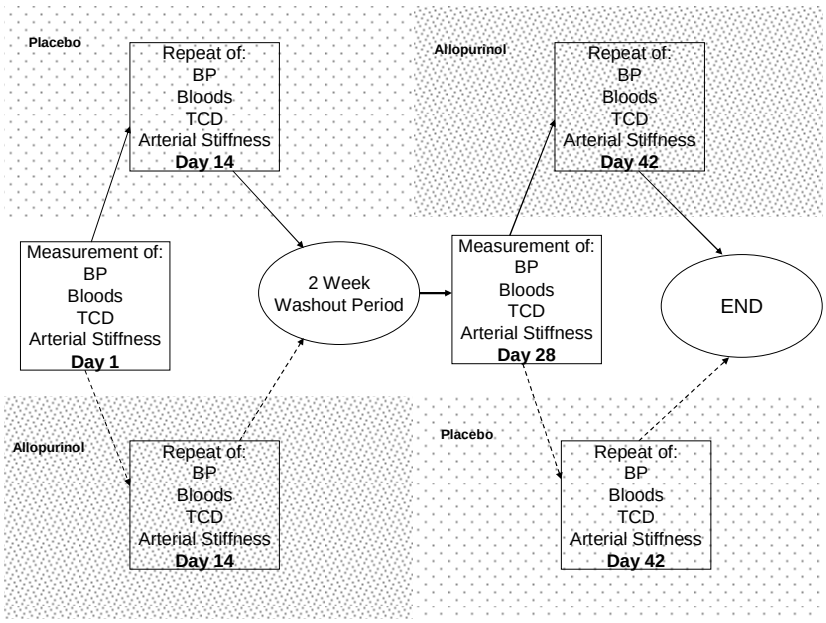

Figure 4.8. Study Three Participant Study Schedule

Baseline assessment of mean flow velocity in the ICA and middle cerebral artery (MCA) was performed, along with measures of ICA diameter and area. Peripheral (radial) pulse wave analysis (PWA) and (carotid-radial) pulse wave velocity (PWV) measurements were also performed. On completion of these measurements, a 45 ml infusion of 0.8 µmol/kg/min of L-NMMA dissolved in normal saline was given intravenously over 15 minutes. L-NMMA was obtained from Clinalfa. Assessment of ICA and MCA parameters was repeated upon cessation of infusion and at 10, 20 and 30 minutes thereafter. PWA and PWV measurements were repeated upon cessation of infusion and at 15 and 30 minutes thereafter. Carotid insonation was performed using an Acuson Aspen 128 with a 5-Mhz linear transducer (Acuson, Mountain View, CA, USA) and measurements were taken 1 cm distal to the carotid bifurcation. MCA insonation was performed using a TCD 100M (Spencer Technologies, Seattle, WA, USA) and readings were taken at, or as near as possible to, a depth of 50 cm. Ultrasound examinations were performed by a sonographer with several years' experience of neurovascular ultrasound. PWA and PWV measurements were made using a SphygmoCor device (Atcor Medical, Sydney, Australia) by operators with several years experience.

Following baseline assessment, participants were randomised to receive either 300 mg of allopurinol or matching placebo orally once daily. Dosing began on the day of baseline assessment and continued for two weeks after which participants returned for further assessment as described above. A two-week washout period then ensued after which repeat assessment, a two week dose of the other agent (placebo or allopurinol) and the final assessment occurred. All haemodynamic studies were conducted as near to midday as feasible and subjects were asked to refrain from caffeine for 24 hours prior to each study.

At each study visit, blood was drawn to allow measurement of routine biochemistry and haematology parameters and circulating markers of endothelial/inflammatory function (VEGF, sICAM, eSelectin and CRP). For these tests, serum was frozen and stored for subsequent analysis using ELISA techniques.

Randomisation and Treatment Allocation

Allopurinol tablets were manufactured by Alpharma. Placebo tablets were manufactured by Penn Pharmaceuticals. Both were encapsulated to appear identical, in the Pharmacy Production Unit at the Western Infirmary Hospital. Study treatments were collected by one of the investigators on the day of the baseline and the first post-washout study visit. Concordance with therapy was assessed by questioning and pill counts where available. Investigators remained blinded to serum uric acid data until the end of the study to prevent unmasking of treatment allocation. Randomisation was performed by the pharmacy department and the randomisation code was held by an independent study pharmacist. The code was not broken until all follow up was complete and all data were prepared for analysis.

Statistical measures

The primary endpoint was change in ICA flow induced by the L-NMMA infusion expressed as area under flow / time curve (AUC) measured from the start of infusion to 20 minutes after its completion (530;531). Based on previous pilot data (530), it was calculated that a sample size of 20 patients would enable detection of a clinically significant improvement in L-NMMA responsiveness (to approximately 75% of that seen in non-diabetic individuals), with 90% power (alpha = 5%). Secondary endpoints were the intra-individual percentage change in ICA flow following L-NMMA infusion (from baseline to

10 minutes post infusion), change in systolic and diastolic blood pressure (SBP and DBP) and middle cerebral artery flow velocity (MCAv) induced by the L-NMMA infusion (expressed as AUC), change in augmentation index (AI, measured during PWA) and PWV, and change in circulating markers of endothelial/inflammatory function.

AUCs were calculated using the trapezoidal method with the aid of NCSS software (Utah USA). A negative AUC signifies a reduction in flow (or blood pressure for example) following L-NMMA infusion and a positive area under the curve indicates an elevation in flow. The difference between the AUCs pre and post each intervention period were calculated and compared (allopurinol minus placebo) using paired non-parametric tests (Wilcoxon signed rank test). A positive difference in AUCs represents an improvement in basal NO activity during that study phase and a positive difference between the study periods represents improvement in favour of allopurinol. For the AI and PWV, pre infusion values and the change immediately following infusion were compared between study periods using the same approach as taken for AUCs. The difference between pre and post treatment values was compared for the circulating markers of endothelial/inflammatory function. As a sensitivity analysis, a mixed effects model was generated to adjust for any effect of treatment ordering and to incorporate information from patients who did not complete all of the study visits.

4.05.1.1 Rationale for Use of L-NMMA Responsiveness a Measure of Cerebrovascular Function

Endothelial dysfunction is key to the development of cardiovascular disease with maintenance of normal endothelial function dependant upon production of endothelial nitric oxide (NO). Studies have also shown this to be involved in maintenance of resting cerebral

blood flow (CBF) (532) and a dynamic effect of NO on cerebral autoregulation has been demonstrated via studies of infusion of NG-monomethyl-L-arginine (531) (L-NMMA). L-NMMA is an analogue inhibitor of endothelial nitric oxide synthase (eNOS) and thus basal NO activity; in healthy volunteers, L-NMMA lowers CBF (531) and has also been shown to reduce forearm blood flow (533), to increase large artery stiffness (534) and to increase blood pressure (535). The magnitude of the response is dependent upon basal NO activity and thus endothelial health; for example, the bigger the fall in CBF following L-NMMA, the better the cerebrovascular endothelial function.

This CBF response is reduced in those with established type 2 diabetes, where little effect of L-NMMA is seen (530) and this may predispose them to hypoxic or ischaemic cerebrovascular insult. In a recent prospective case-controlled study (530), L-NMMA produced a mean reduction in ICA flow (expressed as the area under the flow-time curve) of 12.8% (SD 17.8) in healthy volunteers compared to only 2.1% (SD 21.7) in those with diabetes. The response to L-NMMA is therefore a valid measure and improvements in peripheral responses have been shown following treatment with agents proven to impact on cardiovascular morbidity and mortality, such as statins and ACE inhibitors (536;537). Whether therapy can improve the impaired response of the cerebrovasculature to L-NMMA in diabetes or other conditions is as yet untested.

The mechanism of the impaired response seen in diabetes is unclear. Superoxide anions ($\cdot O_2^-$) inactivate NO (538), inhibit endothelium-dependent vasorelaxation (539) and are implicated in diabetes related endothelial dysfunction. While many sources of $\cdot O_2^-$ exist in the vascular endothelium, xanthine oxidase (XO) activity has been shown to be important (408) and, as outlined, inhibition of this enzyme (with allopurinol) improves peripheral and

cardiac endothelial function in many cardiovascular disease states (521). If pharmacological attenuation of XO activity improved cerebrovascular NO bioavailability in patients with diabetes, this would suggest a role for XO activity in the pathogenesis of diabetic cerebrovascular disease and provide a potential means to improve stroke prevention.

4.05.1.2 Rationale for Use of Pulse Wave Analysis and Pulse Wave Velocity as Measures of Arterial Function and Health

The relationship between brachial cuff blood pressure and risk of cardiovascular disease (and in particular of stroke) is clear and could be described as one of the few certainties in medicine. At all ages and across the range of blood pressure down to at least 115/75mmHg, the risk of death from stroke and cardiac disease increases in a log-linear fashion (155). In a recent meta-analysis of data from over 1 million adults, a doubling of rate of death from stroke and cardiac disease was seen with each 20mmHg higher systolic blood pressure (or 10 mmHg higher diastolic blood pressure). The simplicity of measurement of cuff brachial blood pressure and the legion of evidence (157) showing that a reduction in these values leads to reduction in morbidity and mortality means this will likely remain a cornerstone of cardiovascular risk prediction and in particular for stroke where the evidence for a link between other risk factors such as cholesterol and incidence is less compelling.

However, evidence has emerged that measures of central blood pressure parameters, which are the values to which target organs are exposed, and measures of stiffness of arteries are also important and linked, independently of brachial cuff blood pressure, to risk of cardiovascular disease (540;541). Some support for the use of these parameters can be gained from brachial cuff measurements; analysis of Framingham data suggest that cardiac

events are more closely related to systolic brachial cuff pressure and that events may actually be inversely related to diastolic pressure for any given systolic level (542). This suggests that pulse pressure, and thus increasing stiffness of large arteries, is in important factor in the development of cardiovascular disease.

It is important to note that in normal healthy individuals the central and peripheral pulse pressures differ with pulse pressure rising more peripherally because of an increase in systolic pressure (543). This however alters with age; in young adults, the brachial pulse pressure pulse may be 50% greater than in the ascending aorta while they are similar in the elderly. This presumably reflects increasing vascular stiffness and is not best quantified by brachial cuff measures. Measures to directly quantify central pressure parameters and arterial stiffness are naturally invasive but non-invasive estimation of central pressures can now be achieved.

The most widely employed methods are those of pulse wave analysis and measurement of pulse wave velocity which employ the use of arterial applanation tonometry. Such an example is the Sphygmocor system which incorporates a thin probe with a high-fidelity strain gauge transducer with a small pressure-sensitive ceramic sensor area linked to a computer and software to aid automated calculation of indices. The probe is held over the skin at the point of maximal arterial pulsation. For PWA, this is done at a single site, most readily the radial artery at the wrist, and at two sites during measurement of PWV (for example at the radial and carotid artery or carotid and femoral artery). In all individuals the aortic pulse wave is augmented by the reflection of blood at arterial bifurcation points, so called points of impedance discontinuity. The sum of all these reflected waves will behave like one single wave at each large artery origin in the aorta. In the young, or those with

highly elastic aortas, this wave returns during late systole or diastole, whereas in the elderly, it arrives in early systole and augments the pulse pressure. The augmentation index is a measure of the degree to which the reflected wave contributes to the pulse pressure and is defined as the magnitude of this augmented pressure divided by the pulse pressure. A rise in the augmentation index and thus early arrival of the reflected wave should therefore reflect the increasing arterial stiffness that occurs with age and cardiovascular risk factor states. Ideally, this would also be measured directly in the aorta but measures from the radial site have been shown to correlate well with aortic and carotid values (544;545) when a transfer function is employed to allow their approximation. The transfer function is also utilized to estimate the central systolic, diastolic blood and pulse pressures; all from the radial site.

A variety of studies have linked these parameters to cardiovascular risk factor states. For example, a recent study of over 12000 individuals in England and Wales (546) found that the pulse pressure ratio (aortic pulse pressure : peripheral pulse pressure, increases in which should link to increased arterial stiffness) and augmentation index were higher in those with hypertension, hypercholesterolaemia, diabetes and in smokers and in those with established cardiovascular disease in comparison to healthy controls. These findings remained consistent across the age range. Studies have also linked increasing augmentation index to increases in cardiovascular mortality and morbidity in those with end-stage renal disease (547) and in those undergoing percutaneous coronary intervention (548;549) although only one of these utilized transfer function based measurements from the radial site (551) and not all studies yielded positive associations, including a study of nearly 500 individuals with hypertension (550). Date also exist to suggest that transfer function based estimation

of central blood pressure values can better predict outcomes in comparison to peripheral measures (551), although again data conflict (552).

It is therefore of interest to ask whether differences in central parameters explain observed differences in cardiovascular event reduction between drug classes which do not appear explained by differences in brachial blood pressure level. Also, it would provide encouraging evidence of benefit if any new or novel therapy, not aimed directly at lowering blood pressure, improved some of these measures of arterial stiffness. The CAFÉ study investigators addressed the first question. The CAFÉ study (553) was a sub-study of the ASCOT trial (554), where an amlodipine ± perindopril based regimen led to fewer cardiovascular events or procedures in comparison to an atenolol ± bendrofluazide based regimen (HR 0.84, 95 % CI 0.78 to 0.9, p <0.0001). This was not completely accounted for by differences in blood pressure in a post-hoc analysis (555). In the CAFÉ study, which included 2199 participants from 5 ASCOT centres, there was little difference in brachial systolic BP (0.7 mmHg, 95% CI -0.4 to 1.7, p=0.2) but substantial reductions in the amlodipine arm were seen regarding central aortic systolic (4.3 mmHg, 95% CI 3.3 to 5.4 mmHg, p<0.0001) and pulse (3 mmHg, 95% CI 2.1 to 3.9 mmHg, p<0.0001) pressures. These indices also linked to clinically relevant endpoints with 10mmHg increments in central pulse pressure associated with an 11% increase in risk of events (HR 1.11, 95% CI 1 to 1.23 per 10 mmHg increment).

The pulse wave velocity is the measurement of speed of the pressure waves traveling along an arterial segment, usually calculated from measurements at a proximal (for example carotid) and distal (for example radial) site. The distance between these sites is measured with a tape measure and using ECG gating, the time taken for the arterial pulse to transmit

this distance between can be determined allowing the velocity of the pulse wave to be calculated. A higher pulse wave velocity suggests increased arterial stiffness has been shown to link with increased mortality in a cohort with severe renal disease (556), hypertension (557), established cardiac disease (558) and in a healthy cohort (559). For example, in a cohort from the Rotterdam study revealed a hazard ratio of 2.45 (95% CI 1.29 to 4.66) for development of coronary disease and 2.28 (95% CI 1.05 to 4.96) for stroke in those in the highest tertile of pulse wave velocity compared to those in the lowest. The mean PWV in the whole cohort was 13.3 (SD 2.9), although individual tertile ranges were not given. In a hypertensive cohort (557), mean PWV in those who suffered a coronary event was significantly higher at 12.8 (SD 3.2) compared to 11.4 (SD 3.1) in those who did not ($p<0.0001$) and each SD increment (3.5 m/s) conveyed a RR of 1.39 (95% CI 1.08 to 1.79, $p=0.01$) for coronary events. As yet, there is no data to confirm that reductions in PWV lead to reductions in cardiovascular events.

It is also of interest that an increase in both the AI and PWV has been seen following L-NMMA seen in a previous study of healthy volunteers (534). This study incorporated a range of doses of L-NMMA (from 0.1 to 3 mg/kg/minute) and effect was only seen with the higher doses, which were higher than used here. This response has not yet been evaluated in those with diabetes and this is important to establish as it is possible that there are regional differences in basal NO production between different vascular beds and that responses to treatment may similarly differ (560).

Thus, it is relevant and rational to measure these parameters in studies of putative new preventative strategies in those at risk of stroke. However, there is an important note of caution when interpreting the results of such studies; if an agent were to yield similar

benefits to those seen in the CAFÉ study in terms of the AI or to give a 50% improvement toward values in healthy controls in those with diabetes, 135 and 388 participants per group respectively would need to be recruited to ensure 90% power (alpha 0.05). Benefit is therefore unlikely to be seen in small pilot studies, or at least good statistical power is unlikely.

4.05.2 Study Three Results

Fourteen patients were recruited between September 2006 and August 2007. Nineteen suitable patients were identified and approached and 5 declined participation. Eleven of those recruited completed follow up. One patient was deemed unsafe to continue as he had significant ST segment shift on his exercise tolerance test, one failed to attend for further assessment after visit one and one withdrew consent after attendance for exercise tolerance testing. One patient was unable to receive L-NMMA during his last study visit (because of lack of supply) leaving 10 who completed the study protocol. Data from this participant were included in analyses of blood markers and non L-NMMA dependent parameters. No other adverse event occurred during the study. The study was terminated early due to a national lack of availability of L-NMMA.

Of these recruited, mean age (SD) was 53.1 (10.8) years. Demographic details, including baseline drug therapy, risk factor burden, renal function and uric acid levels are shown table 4.23.

Smoker	4 (28.6)
Hyperlipidaemia	5 (35.7)
Hypertension	12 (85.7)
Oral Hypoglycaemic Agent	11 (78.6)
Anti-platelet Therapy	9 (64.3)
ACE Inhibitor or ARB Therapy	11 (78.6)
Diuretic Therapy	5 (35.7)
Lipid Lowering Therapy	13 (92.3)
Calcium Channel Antagonist Therapy	6 (42.9)
Baseline Serum Creatinine	88 (16.9) µmol/l *
Baseline Serum Glucose	9.9 (4.5) mmol/l *
Baseline Serum Uric Acid	0.33 (0.07) mmol/l *
Baseline Internal Carotid Artery (ICA) Flow	619 (SD 162) ml/min *
Baseline Change in ICA Flow (ml)	-789 (IQR -4194 to 7233) ml †
Baseline SBP	154 (SD 11) mmHg *
Baseline Change in SBP (mmHg*min)	-133 (IQR -205 to 94) †
Baseline DBP	85 (SD 6.8) mmHg *
Baseline Change in DBP (mmHg*min)	66 (IQR -60 to 276) †
Baseline MCAv	39.8 (SD 4.4) *
Baseline Change in MCAv (m/s*min)	14 (IQR -29 to 53) †
Baseline Augmentation Index (AI)	23.9% (SD 6.86) *
Baseline Change in AI	1.7% (SD 10.6) *
Baseline Pulse Wave Velocity (PWV)	8.3 (SD 2.25) m/s *
Baseline Change in PWV	-0.025 (0.89) m/s *

Table 4.23. – Study Three Baseline Variables (at first study visit). Expressed as n (%) for categorical variables and mean (SD) for continuous variables * except for area under curve measurements where median values and interquartile ranges (IQR) are given †. These values (†) refer to changes following L-NMMA infusion. ARB = angiotensin receptor blocker. MCAv = middle cerebral artery flow velocity.

Baseline Visit Response to L-NMMA Infusion

Mean (SD) ICA flow rate pre L-NMMA infusion was 619 (162) ml / min. The median area under the change in ICA flow/time curve following L-NMMA infusion was -789 ml (IQR -4194 to 735) (p=0.16 for test of median difference less than zero). ICA flow fell by a median of 4.8% (95% CI -17.3 to 9.4, p=0.25) following L-NMMA infusion. This, and baseline values for the other parameters, are shown in table 4.24. There was no change in blood pressure parameters where AUCs were considered, although systolic and diastolic blood pressures were raised immediately post infusion (by 3.6 mmHg, 95% CI -0.8 to 8.0 mmHg and 5.6 mmHg, 95% CI 1.2 to 9.9 mmHg respectively). No change was seen in PWA or PWV parameters following LNMMA.

Effect of Allopurinol on Response to L-NMMA Infusion

Allopurinol treatment significantly augmented the reduction in ICA (expressed as area under the flow time curve) following L-NMMA infusion (p=0.032, median improvement in ICA flow reduction following LNMMA of 3144 (95% CI 375 to 7143) mls). Allopurinol did not significantly influence response of other measured parameters to L-NMMA (4.24). ICA flow fell by a median of 11.9% (95% CI -2.5 to 23.3, p=0.04) following LNMMA after treatment with allopurinol, The effect of allopurinol on the group and intra-individual changes in ICA flow following LNMMA are shown in figures 4.9 and 4.10.

Variable	Following Placebo	Allopurinol	Difference Between Periods *	P value †
Change in ICA Flow (ml)	-1314 (-3359 to 419)	1134 (-964 to 7872)	3144 (375 to 7143)	0.032
Change in SBP (mmHg*min)	20 (-260 to 158)	116 (-248 to 501)	14 (-461 to 588)	0.92
Change in DBP (mmHg*min)	-55 (-220 to 86)	-162 (-206 to 94)	-56 (-218 to 90)	0.48
Change in MCA MFV (m/s*min)	-41 (-112 to 108)	-38 (-146 to -9)	-35 (-174 to 45)	0.36

Table 4.24 – Change in Haemodynamic Parameters / Response to L-NMMA During Study Three. N = 10 included. All values refer to the change in area under the time curve. * = estimated difference in medians and the 95% CI on Wilcoxon signed rank test. † = for paired Wilcoxon signed rank test.

A mixed effects model sensitivity analysis adjusted for the order in which participants received allopurinol and placebo. The results were consistent with the main analysis (p=0.046 for allopurinol versus placebo) and found no significant order effect (p=0.51). Baseline ICA flow level also did not explain the reduction in ICA flow following L-NMMA infusion.

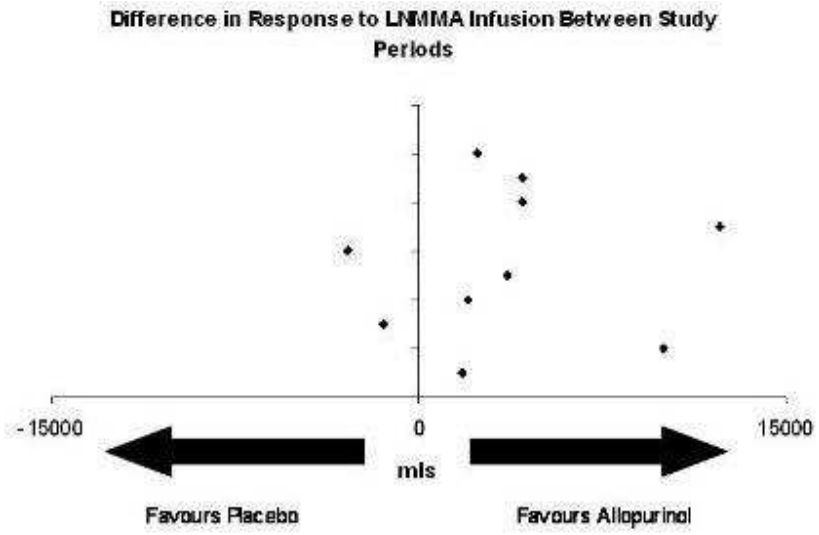

Figure 4.9. Intra-patient Change in ICA Flow Following L-NMMA Infusion in Study Three. Each data point represents the difference in response to LNMMA between study periods for one individual (in mls). For each study phase, values were calculated as the difference between the pre and post intervention area under the flow-time curve. The difference between study phases was then calculated (allopurinol minus placebo) and positive values (to the right) represent improvement in response following allopurinol and negative values improvement following placebo.

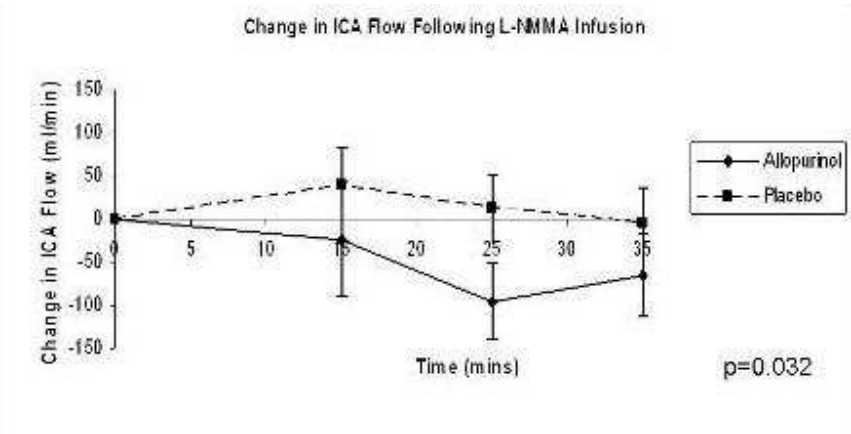

Figure 4.10. Change in ICA Flow Following L-NMMA Infusion in Study Three. The values shown represent the median (± 1 SE) change in ICA flow following intervention with allopurinol or placebo. The p value refers to between group comparisons on non-parametric testing (Wilcoxon signed rank test). A greater reduction in ICA flow following L-NMMA represents an improved response.

Effect of Allopurinol on Peripheral Vascular Reactivity

The pre-infusion AI or PWV did not differ significantly between the treatment periods (estimated median difference between placebo and treatment periods of -4.3% (95% CI -11 to 7%, p=0.56) and -0.32 m/s(95% CI -2.1 to 7.8, p=0.39) respectively). The response to L-NMMA did not differ significantly between the treatment periods for both the AI (estimated median difference between placebo and treatment periods of 7%, 95% CI -11.5 to 23%, p=0.54) and PWV (estimated median difference of -0.7 m/s, 95% CI –7.6 to 1.85, p=0.68) (table 4.25).

Variable	**Baseline Visit** ‡	**Change Post Placebo**‡	**Change Post Allopurinol** ‡	**Difference Between Periods** §	***P value*** ||
AI (%)†	23.5 (18.5 to 29.5)	-3 (-14.5 to 4.5)	2 (-6.00 to 10)	-4.3 (-11 to 7)	*0.56*
PWV (m/s) †	9 (7.6 to 9.6)	0.8 (0.1 to 10.2)	0.75 (-0.85 to 2.3)	-0.32 (-2.1 to 7.8)	*0.39*

Table 4.25. Change in Measures of Peripheral Vascular Reactivity During Study Three. † = values refer to difference between pre-infusion values. ‡ = values shown are median and interquartile range. § = values shown are estimated difference in medians and the 95% CI on Wilcoxon signed rank test. \|\| = for paired Wilcoxon signed rank test.

Effect of Allopurinol on Circulating Markers of Inflammation/Endothelial Function

No significant differences were seen in any marker (table 4.26). The VEGF level rose during allopurinol treatment, although this did not reach statistical significance and s-ICAM levels seemed to be lower following allopurinol treatment. However, with all blood markers, the confidence intervals for the differences between study periods were large.

Variable	Placebo	Allopurinol	Difference Between Periods *	*P value †*
VEGF (pg/ml)	-14 (-67 to 59.1)	66.9 (5.9 to 119.6)	91 (-53 to 151)	*0.08*
s-ICAM (ng/ml)	73.6 (-85.3 to 96.5)	9 (-7.8 to 65.3)	-51.7 (-81 to 91.9)	*0.69*
e-Selectin (ng/ml)	0.01 (-14.6 to 10.3)	2.4 (-12.9 to 17.1)	16 (-19 to 26)	*0.45*
CRP (mg/l)	0.09 (-0.3 to 0.75)	0.195 (-0.01 to 1)	0.33 (-0.48 to 1.29)	*0.40*

Table 4.26. Change in Markers of Endothelial Function During Study Three. N = 11 included. Median values (interquartile range) are given for each study period. A negative difference in medians represents improvement in favour of allopurinol. * = estimated difference in medians. † = for paired Wilcoxon signed rank test.

4.05.3 Study Three Discussion

L-NMMA can be used as a pharmacological tool to assess the extent of underlying NO activity. In the cerebral vasculature, L-NMMA reduces CBF through restriction of NO activity and the higher the basal NO activity, the larger the effect. In this study, treatment with allopurinol enhanced this effect implying that it improves basal levels of NO activity and raises the possibility that use of allopurinol could improve cerebrovascular health. The response to L-NMMA improve toward the previously published (530) 12.8% (95% CI 3.29 to 22.31%) fall in ICA flow seen in healthy volunteers (from a non-significant 4.8% (95% CI -17.3 to 9.4) reduction at baseline to an 11.9% (95% CI -23.3 to 2.5) fall following allopurinol treatment).

The potential beneficial effects of allopurinol on the vasculature are two fold; it reduces XO mediated $\cdot O_2^-$. production and serum uric acid. The XO system has been shown to be an important source of $\cdot O_2^-$. in the vasculature (408) but this is the first human study to show that inhibition of XO improves cerebral NO bioavailability. Most large, well-conducted epidemiological studies suggest that increasing serum uric acid is associated with increased cardiovascular event rate and mortality (519), possibly via detrimental effects on platelet, smooth muscle and endothelial function (521), and UA reduction may impact favourably on cardiovascular risk (472).

Previous studies, typically also utilising cross over design and brief treatment duration, have shown a beneficial effect of XO inhibition on measures of forearm or coronary endothelial function in patients with or at high risk of vascular disease (521) - including in patients with diabetes. One previous small study (492) failed to show benefit of allopurinol on markers of oxidative stress (such as the lipid peroxidation assay) in patients with diabetes, although there were large differences in the levels of baseline HbA1c and cardiac risk factors such as smoking with levels worse in those randomised to allopurinol. This study is the first to show benefit of allopurinol on cerebrovascular endothelial function. Whether these improvements are driven by change in uric acid or XO activity is unclear but previous work suggests the later (483), although as mentioned, uric acid reduction may bring other benefits in the longer term.

There are strengths to this study; it utilised a rigorous protocol, with which there is considerable experience, and a similar baseline response to L-NMMA was found compared to that seen in previous work (530). The response to L-NMMA is a valid measure; it is an analogue inhibitor of eNOS and infusion has previously been shown to reduce both CBF

(531) and forearm blood flow (533), to increase large artery stiffness (534) and to increase blood pressure (535). Also, improvements in these responses have been shown following treatment with other agents proven to impact on cardiovascular morbidity and mortality (536;537).

There are limitations to the study and areas that require further study. Fewer patients were included than intended because of the lack of ongoing availability of L-NMMA, reducing the statistical power of the study. The unfortunate supply problem affected several centres in the UK. Even so, the consistency of effect seen is reassuring; 8 of 10 patients who completed the protocol experienced an improvement in cerebrovascular NO availability during the allopurinol phase. The dosing period was short (a limitation of many previous studies) and it is possible that any changes in vascular function afforded by a reduction in XO activity and $\cdot O_2^-$. production may be short lived and soon by-passed by other sources of $\cdot O_2^-$. The effect of more prolonged XO inhibition requires clarification. Also, while it is an appropriate measure to study, this study has only examined the effect on eNOS activity and other forms of NO and $\cdot O_2^-$. production may be important in both disease and health.

No improvement was seen in blood markers or measures of peripheral arterial stiffness in this study. Pre-infusion measures of arterial stiffness did not differ significantly between the study periods and neither did the response to L-NMMA. These results contrast with a previous study where forearm blood flow responses were improved following allopurinol (490) but a different technique was employed here and the study was not specifically powered to detect differences in these parameters. Also, at the baseline visit, the increase in AI or PWV following L-NMMA seen in previous studies of healthy volunteers (534) was not replicated. This is perhaps because the response is impaired in those with diabetes or

because of the comparatively low dose of L-NMMA used. Study to further evaluate this response in those with diabetes would be of use. Importantly, it is possible that there are regional differences in basal NO production between different vascular beds and that responses to treatment may similarly differ (560).

In summary, this study showed that XO inhibition with allopurinol improves cerebral NO bioavailability and suggest a beneficial effect of allopurinol on cerebrovascular health. Further investigation to establish the use of allopurinol in stroke prevention is required.

4.06 Study Four - The Effect of Allopurinol on the Cerebral Vasculature of Patients with Subcortical Stroke

The hypothesis was that allopurinol would improve cerebrovascular reactivity in a cohort of patients with recent subcortical stroke. A randomised, blinded, placebo controlled trial was performed to test this hypothesis.

4.06.1 Study Four Methods

The study was performed in the University Division of Cardiovascular and Medical Sciences at the Western Infirmary, Glasgow. The study was approved by the West Medical research ethics committee and was registered in the ISRCTN database (ISRCTN 06452574).

Patients aged over 18 years old with subcortical stroke between two weeks and six months prior to randomisation were studied. Exclusion criteria were: >70% extra-cranial internal carotid artery stenosis, known coronary arterial disease, significant co-morbidity or frailty likely to cause death within 3 months or likely to make adherence to study protocol difficult for patient, contra-indication to or indication for administration of allopurinol (as detailed in summary of medicinal product characteristics), concurrent azathioprine or 6-mercaptopurine therapy, contra-indication to administration of acetazolamide, serum creatinine > 250μmmol/l, enrolment in another clinical trial and women of childbearing potential. Exclusion criteria were chosen to minimise the potential risks of allopurinol treatment and acetazolamide infusion and to ensure carotid flow readings were not falsely elevated because of intrinsic carotid disease.

Patients were identified during admission to the Acute Stroke Unit at the Western Infirmary and out-patient attendance at hospital clinics. All participants gave written informed consent to participate. All drug treatments remained unchanged throughout the study period, except in one participant who was commenced on dipyridamole and perindopril during the study.

Study Assessment

Study visits took place in the Investigations Ward of the Acute Stroke Unit in the Western Infirmary and were supervised by one of the study investigators. The participant schedule is shown in figure 4.11.

Assessment of mean flow velocity in the ICA and middle cerebral artery (MCA) was performed, along with measures of ICA diameter and area. Peripheral (radial) pulse wave analysis (PWA) and (carotid-radial) pulse wave velocity (PWV) measurements were also performed. On completion of these measurements, a 15 mg/kg ml infusion of acetazolamide was given intravenously over 3 minutes. Assessment of ICA and MCA parameters and PWA and PWV measurements were repeated at 15 thereafter. Carotid insonation was performed using an Acuson Aspen 128 with a 5-Mhz linear transducer (Acuson, Mountain View, CA, USA) and measurements were taken 1 cm distal to the carotid bifurcation. MCA insonation was performed using a TCD 100M (Spencer Technologies, Seattle, WA, USA) and readings were taken at, or as near as possible to, a depth of 50 cm. Ultrasound examinations were performed by a sonographer with several years' experience of neurovascular ultrasound. PWA and PWV measurements were made using a SphygmoCor device (Atcor Medical, Sydney, Australia) by operators with several years experience.

Following baseline assessment, participants were randomised to receive either 300 mg of allopurinol or placebo orally once daily (on a 1:1 basis). Dosing began on the day of baseline assessment and continued for 90 days after which participants returned for further assessment as described above. All haemodynamic studies were conducted as near to midday as feasible and subjects were asked to refrain from caffeine for 24 hours prior to each study.

At each study visit, blood was drawn to allow measurement of routine biochemistry and haematology parameters and circulating markers of endothelial/inflammatory function (VEGF, sICAM, eSelectin and CRP). For these tests, serum was frozen and stored for subsequent analysis using ELISA techniques.

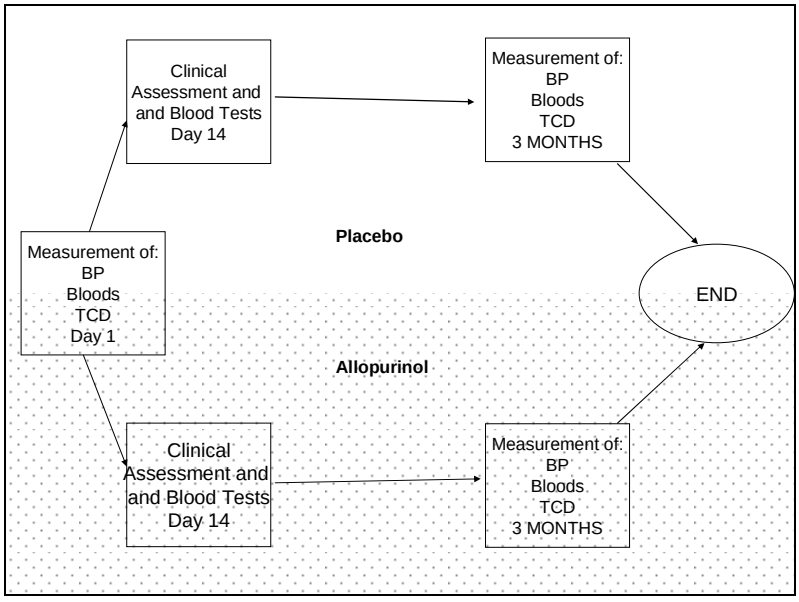

Figure 4.11. Study Four Participant Study Schedule.

4.06.1.1 Rationale for Use of Response to Acetazolamide as a Measure of Cerebrovascular Function

Impaired regulation of the subcortical microvasculature has been proposed as a pathogenic mechanism in first and recurrent lacunar cerebral infarction and in vascular dementia (561). This impaired regulation can be assessed using a standard non-invasive ultrasound protocol which measures cerebral vasomotor reactivity (CVR), the compensatory dilatatory capacity of cerebral resistance vessels in response to increased arterial carbon dioxide concentration, which can be manipulated by an acetazolamide infusion (562-564). The magnitude of the change in cerebral blood flow is the measure of CVR and is a functional assessment of the cerebral vasculature (562). Impaired CVR occurs in a variety of cardiovascular risk states (565;566), correlates with the severity of leucoencephalopathy seen on magnetic resonance imaging (567) and confers an increased risk of ischaemic stroke, possibly due to increased rigidity in the arteriolar wall and to failure of the cerebral vasculature to compensate for fluctuations in perfusion pressure (566;568). Importantly, CVR can be improved with statin (569) and ACE inhibitor (562) therapy, both strategies known to reduce incidence of stroke in high-risk individuals and is thus a useful therapeutic target upon which to test new treatments.

CVR as measured by response to acetazolamide is thus a valid measure and a 15mg/kg or 1g dose of acetazolamide leads to maximal vasodilatory effect (570) with a peak effect at approximately 15 minutes (571). Acetazolamide is a reversible inhibitor of the enzyme carbonic anhydrase which catalyses the reaction;

$CO_2 + H_20 = H_2CO_3 = H^+ + HCO_3^-$.

It is presumed that the effect of acetazolamide on cerebral blood flow is mediated by the increase in extracellular pCO_2 and decrease in extracellular pH it affords. Both of these changes lead to dilatation of small cerebral blood vessels (572). However, it is important to note that acetazolamide may have a direct effect on smooth muscle of the blood vessel wall (573) and thus acts through both endothelium dependent and independent mechanisms. It is possible therefore that changes in CVR assessed by this means may not truly reflect benefits or otherwise of a treatment such as allopurinol, whose vascular effects are mediated by a reduction in oxidative stress and improved endothelial function. Assessment of effect on measures such as L-arginine reactivity or infusion on L-NMMA may have thus be a more appropriate measure (574;575) but is unsuitable in this population due to concerns with its administration in those with established cardiovascular disease.

Randomisation and Treatment Allocation

Allopurinol tablets were manufactured by Alpharma. Placebo tablets were manufactured by Penn Pharmaceuticals. Both were packaged in identical fashion in the Pharmacy Production Unit at the Western Infirmary Hospital. Study treatments were collected by one of the investigators on the day of the baseline and the first post-washout study visit. Concordance with therapy was assessed by questioning and pill counts where available. Investigators remained blinded to serum uric acid data until the end of the study to prevent unmasking of treatment allocation. Randomisation was performed by the pharmacy department and the randomisation code was held by an independent study pharmacist. The code was not broken until all follow up was complete and all data were prepared for analysis.

Statistical measures

The primary endpoint was the change in MCA flow velocity induced by the acetazolamide expressed as the percentage change measured at 15 minutes after the infusion compared to the 0 minute value (termed CVR). Based on previous data (562), it was calculated that a sample size of 25 patients per group would enable detection of a clinically meaningful 10% improvement in CVR following allopurinol treatment with 90% power (alpha = 5%, assumed standard deviation 10%). Secondary endpoints were change in augmentation index (AI, measured during PWA) and PWV, and change in uric acid levels and circulating markers of endothelial/inflammatory function during the study. Approval was sought and obtained to recruit 80 participants, which would allow detection of a smaller yet potentially significant 3.7% improvement in CVR with 90% power.

The difference in CVR values between the follow-up and baseline visits were compared between treatment groups. For the AI and PWV, 0 minute values at the baseline visit and follow-up visit were compared. The difference between pre and post treatment values was compared for the circulating markers of endothelial/inflammatory function. As data were non-normally distributed, the Mann-Whitney test was used for all between group comparisons. Minitab Software (version 15, California USA).

4.06.2 Study Four Results

Fifty participants were recruited between March 2006 and May 2008. Forty-five of those recruited completed the protocol. Two participants withdrew consent prior to the baseline visit, one felt unwell following acetazolamide infusion so did not wish to continue, one had an unreliable TCD window so that MCA velocities could not be reproducibly measured and

one participant (who had stopped study medication) did not attend on two occasions for the follow-up visit. Data from this latter participant were included in analyses of baseline levels of blood markers and CVR. All these participants were randomised to the allopurinol group. There were two serious adverse events, both of which occurred in the allopurinol group but were deemed unrelated to treatment; one participant developed tonsillitis and required hospital admission while another developed acute appendicitis. One participant in the placebo group suffered gout. The study was terminated after recruitment of 50 participants.

Of these recruited, mean age (SD) was 58.3 years (SD 10.4). Demographic details, including baseline drug therapy, risk factor burden, renal function and uric acid levels are shown table 4.27. Baseline variables were broadly similar although the proportion established on diuretic and ACE inhibitor or angiotensin receptor blocker therapy was higher in the placebo group.

Baseline Response to Acetazolamide Infusion

At the baseline visit, MCAv increased by a mean of 15.5 m/s (SD, 12) and by 36.3% (SD 26) following acetazolamide infusion in the whole group and by 36.5% (SD 27.9) and 36.2% (SD 4.9) in the allopurinol and placebo groups respectively. The AI and PWV did not alter following acetazolamide infusion.

Change in CVR Following Allopurinol Treatment

CVR did not change following treatment with allopurinol (median change in CVR 0.89% in the allopurinol group and -0.68% in the placebo group, p=0.64, (95% CI for estimated difference in medians -13.4 to 25.5%)) (figure 4.12).

Variable	**Allopurinol Group**	**Placebo Group**	***All Participants***
Completed Protocol	20 (80%)	25 (100%)	*45 (90%)*
Age	59.4 (9.3)	57.3 (11.5)	*58.3 (10.4)*
Smoker	9 (37.5%)	13 (52%)	*22 (44.9%)*
Hyperlipidaemia	8 (33.3%)	7 (28%)	*15 (30.6%)*
Hypertension	13 (54.2%)	16 (64%)	*29 (59.1%)*
Previous CVA	4 (16.7%)	0	*4 (8%)*
Diabetes	2 (8.3%)	3 (12%)	*5 (10.2%)*
Anti-platelet Therapy	23 (95.8%)	24 (96%)	*47 (95.9%)*
ACE Inhibitor or ARB Therapy	7 (29.2%)	20 (80%)	*27 (55.1%)*
Diuretic Therapy	6 (25%)	11 (44%)	*17 (34.7%)*
Calcium Channel Antagonist Therapy	1 (0.04%)	3 (12%)	*4 (8.2%)*
Beta-Blocker Therapy	5 (20.1%)	2 (8%)	*7 (14.3%)*
Lipid Lowering Therapy	23 (95.8%)	23 (92%)	*46 (93.9%)*
Oral Hypoglycaemic Agent	2 (8.3%)	3 (12%)	*5 (10.2%)*
Serum Creatinine (µmol/l)	91.1 (19.2)	90.4 (21.8)	*90.8 (20.4)*
Serum Glucose (mmol/l)	6 (1.8)	6.6 (2.4)	*6.3 (2.2)*
Serum Uric Acid (mmol/l)	0.35 (0.1)	0.33 (0.09)	*0.34 (0.09)*
SBP (mmHg)	133.9 (17.1)	142.9 (24.1)	*138.6 (21.3)*
DBP (mmHg)	74.4 (11.8)	78.8 (11.4)	*76.7 (11.7)*
MCAv (m/s)	42.6 (10.8)	44.6 (12.5)	*43.7 (11.6)*
Augmentation Index (AI, %)	24.4 (12.3)	22.4 (11.5)	*23.4 (11.8)*
Pulse Wave Velocity (PWV, m/s)	7.6 (1.6)	8.1 (1.6)	*7.8 (1.6)*
Any Serious Adverse Event	2 (8.3%)	0	*2 (4%)*

Table 4.27 – Study Four Baseline Variables

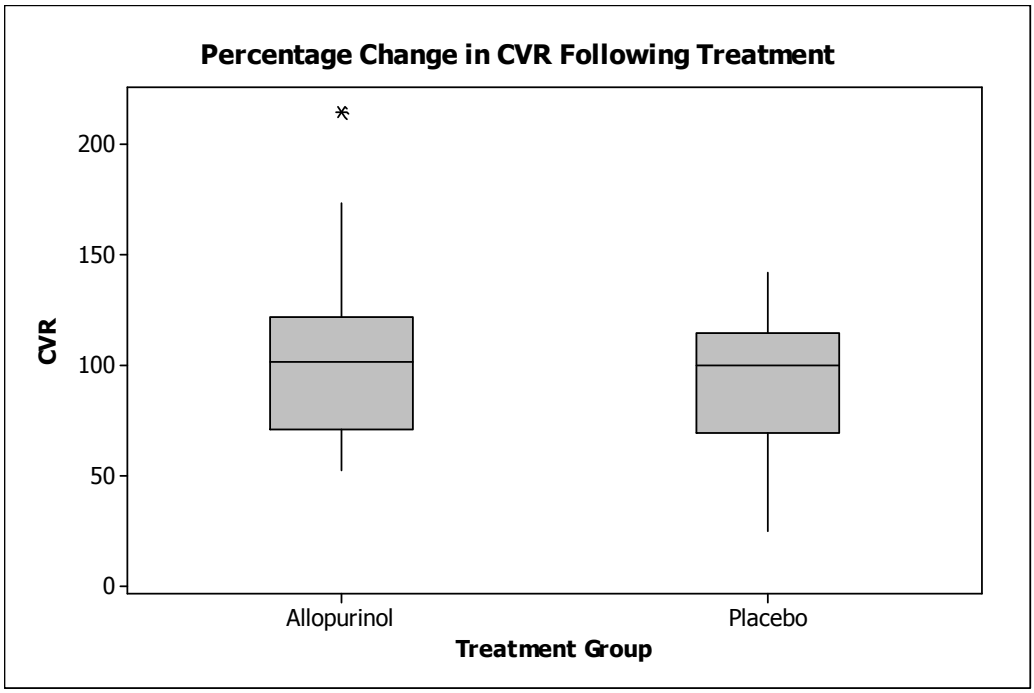

Figure 4.12. Percentage Change in Cerebrovascular Reactivity (CVR) Following Treatment in Study Four

Secondary Endpoints

The AI did not change following treatment with allopurinol (median change 0% in the allopurinol group and 2 % in the placebo group, p=0.51). There was also no change in PWV (median change -0.3 m/s in allopurinol group compared to -0.35 m/s in the placebo group, p=0.42) (table 4.28). Uric acid level was significantly reduced by allopurinol treatment but there were no significant changes in the other blood parameters or markers of endothelial function (table 4.29).

Variable	**First Visit ‡**	**Change Post Placebo‡**	**Change Post Allopurinol ‡**	**Difference Between Groups §**	***P Value*** ‖
AI (%)	23.35 (14.25 to 52)	2 (-3.75 to 35)	0 (-6 to 6)	-1.5 (-7 to 4)	*0.5*
PWV (m/s)	7.82 (6.6 to 8.9)	-0.35 (-1.35 to 0.35)	-0.3 (-1.25 to 1.4)	0.35 (-0.5 to 1.5)	*0.42*

Table 4.28 - Measures of Peripheral Arterial Stiffness in Study Four. All values refer to the baseline pre-infusion values. ‡ = values shown are median and interquartile range. § = values shown are estimated difference in medians and the 95% CI on Mann-Whitney testing. ‖ = for Mann-Whitney testing.

Variable	**Placebo**	**Allopurinol**	**Difference Between Periods ***	***P value †***
Uric Acid (mmol/l)	0.02 (-0.02 to 0.06)	-0.12 (-0.2 to -.05)	-0.15 (-0.2 to -0.09)	*<0.0001*
VEGF (pg/ml)	11.8 (-60.6 to 157.4)	-11.4 (-50 to 10.8)	-27.3 (-12.5 to 42)	*0.23*
s-ICAM (ng/ml)	12.8 (-13.3 to 93.7)	10 (-68 to 56.7)	-32.7 (-142.4 to 25.1)	*0.3*
e-Selectin (ng/ml)	-4.71 (-13.35 to 11.72)	-6.43 (-21.51 to 8.06)	-2.85 (-19.64 to 11)	*0.72*
CRP (mg/l)	0.29 (-2.12 to 0.76)	0.03 (-0.41 to 0.93)	0.09 (-0.72 to 1.44)	*0.87*

Table 4.29 – Change in Blood Parameters and Markers of Endothelial Function During Study Four

4.06.3 Discussion

This study found no effect of allopurinol treatment on CVR, as measured by response to acetazolamide infusion in patients with recent subcortical stroke.

The lack of effect of allopurinol on CVR is not in-keeping with data which support the beneficial effect of allopurinol on the vasculature. The effect of XO inhibitors on measures of endothelial and cardiovascular function has been tested in many small studies (521). Trials have variously used oral or intravenous drug and typically involved a cross over design, with changes in arterial responsiveness as outcome measures, which have been shown to improve following treatment with CV risk-modifying drugs (510). Improvements following XO inhibition are seen in patients with type II diabetes and hypertension, hypercholesterolaemia, smokers, in those with elevated 10 year CV risk and hyperuricaemia, in stable coronary disease and in the context of heart failure (521), although beneficial effects were not seen in some studies in heart failure and hyperlipidaemia. This is the first study of the effect of allopurinol on a measure of cerebrovascular health in patients with previous stroke. Previous work from this unit has shown that allopurinol use is associated with a potentially beneficial attenuation of the rise in ICAM-1 levels after stroke (489) and improvements in cerebrovascular function in those with type 2 diabetes (576) hence the wish to evaluate whether allopurinol improves cerebrovascular health after stroke.

This study employed a rigorous protocol, with which there is considerable local experience, and CVR measured in this fashion has previously been shown by this group and others to improve following treatment with statins and ACE inhibitors (561;569). This study may

have failed to see benefit for several reasons. Firstly, the study size would allow detection of a 10% improvement in CVR with 90% power. This is the same magnitude of benefit that was previously seen with perindopril use but this study has limited power to detect smaller yet potentially beneficial improvements. This is compounded by the large variability seen in the change and measures of CVR. Also, by chance, all patients who withdrew before or early after starting medication had been randomised to the allopurinol group. Further, in this study, 94% of patients were established on statin therapy, compared to only 30% in similar studies of ACE inhibition (562), and over half were receiving ACE inhibition or ARB therapy, while 70% were on some form of anti-hypertensive therapy. The rate of concurrent ACE I / ARB therapy was more than double in the placebo group compared to those who received allopurinol. It may be that, in such a well treated cohort, the potential beneficial effect of allopurinol is less than originally anticipated. The potential limitations of CVR measured in this way have already been discussed. It remains, however, a valid measure given the concerns surrounding other measures such as response to L-NMMA in those with established cardiovascular disease.

Further, while the baseline levels of CVR in this study are comparable to previous studies in this patient group, there is considerable overlap in the literature between values reported in healthy volunteers (577), those with elevated cardiovascular risk, previous stroke and occlusive carotid disease (563). It is therefore difficult to estimate to what degree baseline CVR was impaired in this group and thus the degree of improvement that one would expect to see from effective therapy.

Many previous studies have investigated the effect of a short treatment duration, and even single dose, of allopurinol treatment and have consistently revealed benefit on measures of

vascular function (521). It is of interest that 2 of the 3 published studies (487) (482;496) testing a longer treatment duration have failed to yield benefit. Any beneficial effects of xanthine oxidase inhibition on the vasculature may be short lived and by-passed by other sources of oxidative stress. It may also be that up-regulation of xanthine oxidase occurs with time, meaning any improvements may not be sustained for a prolonged period. Uric acid levels were not elevated in this population and studies in the setting of heart failure suggest that the vascular effects of allopurinol are demonstrable only where xanthine oxidase activity is up-regulated and uric acid levels are increased (578), although the effects may not be mediated by uric acid reduction per se (483).

In summary, this study did not confirm a beneficial effect of allopurinol on cerebrovascular reactivity. It may therefore be that previous encouraging findings will not therefore translate into important clinical benefits. Further study is required and results of this trial suggest this should focus on those with increased serum uric acid levels and aim to clarify whether the previous positive effects of allopurinol are sustained over a prolonged period.

Chapter Summary

These studies suggest uric acid is an important risk factor for adverse vascular outcome. Allopurinol may also be a promising preventative strategy. Serum uric acid predicts poor functional outcome following acute stroke but not in an independent fashion. There was no evidence of an association with favourable outcome as other groups have found. Increasing serum uric acid was also shown to be predictive of increased risk of stroke, total, vascular and coronary mortality in treated hypertensive patients but interestingly, the relationship

between stroke mortality and serum uric acid appears J-shaped and most apparent in females.

Allopurinol was shown to improve cerebral nitric oxide bioavailability suggesting a beneficial effect of allopurinol on cerebrovascular health. However, allopurinol did not improve cerebrovascular reactivity (as measured by response to acetazolamide infusion) in a group of patients with recent subcortical stroke. This raises interesting questions regarding the longevity of any positive effect of allopurinol as this, and other studies of 3 month duration, have revealed no benefit. Further, subjects in this neutral study did not, on balance, have elevated serum uric acid and it has recently been suggested that only those with significantly elevated levels benefit in the setting of congestive cardiac failure. Further work should establish whether this is also true in those with regard to stroke prevention.

CHAPTER FIVE

FUTURE DIRECTIONS

Stroke is a common disorder with dire consequences for the patient and for society and will increase in prevalence over the coming years. Following stroke, many patients unfortunately suffer a further stroke, and recurrent strokes account for approximately 25% of the total. Considerable scope therefore exists to improve both primary and secondary stroke prevention. This thesis has addressed several areas at key stages in the prevention of stroke by developing strategies to better identify those at highest risk, attempting to better target pre-existing anti-platelet therapy and by beginning the evaluation of xanthine oxidase reduction and uric acid lowering therapy in the prevention of stroke.

A clinical scoring system to aid diagnostic recognition in those with suspected transient ischaemic attack (TIA) was successfully developed and has the ability to reduce the referral of those without cerebrovascular disease to busy TIA clinics. This would have a substantial effect on waiting times for assessment; a key aim given the recent evidence that rapid assessment and treatment of those with TIA greatly reduces stroke risk. Further work to evaluate and introduce the score to routine clinical practice is required. It is planned to incorporate use of the scoring system into the referral process to our TIA clinic. It will be established whether, when used by General Practitioners, the scoring system can identify those without TIA whilst safely identifying those with true TIA. The potential impact on services will also be assessed.

Aspirin resistance was confirmed to be a common phenomenon, although the role of poor compliance with therapy as a cause in a substantial number of cases was established. This suggests that objective measures to confirm aspirin ingestion should be mandatory in future studies of aspirin resistance. Aspirin resistance was also higher in those with cerebrovascular microembolic signals (MES) and carotid disease compared to those with

equivalent carotid disease and no MES. This provides a link to a well established and robust surrogate marker of outcome and thus a useful model to further study the benefits of guided anti-platelet strategies. An interventional clinical anti-platelet trial based upon individual aspirin responsiveness in high risk individuals such as those with MES is now warranted and funding will be sought for this in the near future.

Increasing serum uric acid was found to be a predictor of poor functional outcome following acute stroke but not in an independent fashion. However, there was no evidence of an association with favourable outcome as other groups have found. Increasing serum uric acid was also shown to be predictive of increased risk of stroke, total, vascular and coronary mortality in treated hypertensive patients but interestingly, the relationship between stroke mortality and serum uric acid appears J-shaped and most apparent in females. A study of the use of allopurinol in those with diabetes showed that XO inhibition improves cerebral NO bioavailability suggesting a beneficial effect of allopurinol on cerebrovascular health. However, a study of the effect of allopurinol treatment on cerebrovascular reactivity (as measured by response to acetazolamide infusion) in a group of patients with recent subcortical stroke revealed no positive effect. This raises interesting questions regarding the longevity of any positive effect of allopurinol as it, and other studies of 3 month duration, have revealed no benefit. Further, subjects in this study did not on balance have elevated serum uric acid and it has recently been suggested that only those with significantly elevated levels benefit in the setting of congestive cardiac failure. Whether this is also true in those with stroke also requires to be clarified. A large study of the effect of allopurinol on carotid intima-media thickness, a robust and modifiable marker of vascular risk, in those with recent stroke is planned to address these questions.

The planned study is a randomised, double-blind placebo controlled pilot study to investigate benefit of 2 years allopurinol 300 mg on change in carotid intima-media thickness in those with recent stroke. Levels of endothelial progenitor cells and circulating markers of endothelial function and safety and clinical endpoint data will also be gathered assessed. The trial will require approximately 500 participants to be followed for a two year period to ensure adequate power. The study hypothesis is that allopurinol will reduce IMT progression rate, increase levels of EPCs and reduce levels of circulating endothelial markers with no increase in serious adverse events. The study will begin in May 2009 and take five years to complete.

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