Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

Risk markers for acute stroke in Ireland – association of sodium-lithium countertransport (SLC) with vascular disease.

Submitted for the degree of M.D. to the University of Dublin, Trinity College September 2001

By

Daniel Ronan Collins

Accepted April 2002

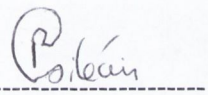
Declaration

I declare that the work contained in this thesis is wholly my own.

It has not been submitted as an exercise for a degree to this or to any other university.

I grant permission to Trinity College Dublin to loan or copy this work as appropriate and in accordance with library regulations of the university.

Signed



Daniel Ronan Collins MB MRCP(UK)

Dedication

To my wife Nicola for her love, patience and encouragement.

To my parents for having the foresight and generosity to encourage my education.

To my brothers and sister, my best friends.

Summary

The work in this thesis is divided into two parts, an epidemiological study of the risk markers for stroke in an Irish population and a laboratory study of the possible association between sodium lithium countertransport (SLC) and vascular disease. Prior to starting this work no dedicated acute stroke service was in existence in an Irish teaching hospital and no data had been published on the incidence of major risk factors for stroke in an Irish population. Chapter one of this thesis sets out the background theory to my work and I have set myself a number of objectives in the form of questions which are summarised in chapter two. The methodology employed, and techniques and equipment used are detailed in chapter three.

Stroke is a devastating illness which places a huge personal burden on the patient and carer, and a great financial burden on the health service. Approximately 2,000 new strokes occur per million of population in the western world, half of whom will have died within one year. Identification of risk factors can explain differences between stroke incidence in geographical areas and is fundamental in planning effective secondary prevention strategies. In chapter four of this thesis I present a three-year audit of a stroke service which initially looks at stroke type, demographic pattern, outcome and length of stay trends from year to year in a newly formed stroke service.

A number of trends emerge from this study and, in particular, I found an encouraging trend towards a greater number of patient referrals to the stroke service of similar stroke severity with reduced mortality and need for institutional care at outcome, without increasing length of hospital stay. While some of this trend is, perhaps, explained by a younger mean patient population from year to year, and while chi squared analysis was not significant for differences in death or length of stay from year to year, the results are at least encouraging and support an organised approach to stroke care.

My study of the risk markers for stroke in this population reveals that hypertension is the commonest risk factor for all stroke types, but that it rarely occurs in isolation and most stroke patients have at least 2 major risk factors for their illness. Other risk factors for stroke occur with similar frequency to data published internationally, although some increased rates of ischaemic heart disease and atrial fibrillation were noted. These were comparable however, to incidences reported from other Irish centres after this work had begun.

The second part of my thesis looks at the possible association between sodium-lithium countertransport (SLC) and vascular disease such as stroke and myocardial infarction (MI). SLC is a cation transporter which exists across the red cell membrane and has been shown to be independently associated with essential hypertension. In addition some studies have shown a greater propensity towards end-organ damage in hypertensive patients with elevated SLC activity. This may suggest that SLC could represent a broader risk marker of vascular disease.

In chapter five of this thesis I examined the inter-assay and intra-assay variation of the SLC assay and found it to be similar to previous work, thus verifying the previously reported reliability and robustness of the technique even when

performed by different operators. This chapter also looks at the potential influence of smoking, an important vascular risk factor, on SLC. While no difference was readily evident in SLC activity between smokers and non-smokers, there was an acute promotory effect of smoking on the transporter. This suggests at least that smoking may be an important variable in any large study of SLC and warrants further work. This is the first such report of the effect of smoking on SLC.

Chapter six of this thesis looks at the potential association between SLC and stroke, and, SLC and myocardial infarction. The opening section of the chapter examines the potential effect that sample-timing post-stroke might have on SLC activity and how this compares to the standard inter-assay variation. Analysis of these results show that SLC variation is significantly greater than the normal inter-assay in patients immediately post stroke with most patients showing a decline in levels over a ten-day period which is mirrored by falling cholesterol levels.

The main body of the chapter focuses on the comparison of SLC activity between normal healthy controls and hypertensive patients, and between controls and patients with stroke (cerebral infarction) and myocardial infarction. These patients were matched for age and sex. A significant difference in SLC activity was seen in hypertensive patients over controls, concurring with previous reports in the literature. Similarly significantly elevated SLC activity was noted in stroke patients compared to controls, although no such association was noted in the myocardial infarction group. While the numbers in this study are relatively small (limited by the manual nature of the assay), and significant differences were not apparent when stroke patients with a history of hypertension were removed from analysis, the trend observed is interesting, particularly as stroke patients had similar blood pressure levels to controls and lower cholesterol levels which would be expected to lower SLC activity. This observation is the first reported association of SLC with stroke and, if replicated, would imply a possible role for SLC as a broader marker of vascular disease.

Publications

The following publications have resulted from this work.

1. Stroke: Non-motor sequelae, medical co-morbidity and patterns of intervention after referral to a special interest service.
   D.R.Collins, D.O'Neill.
   Irish Journal of Medical Science. Mar.1998; 33-34.

2. Potential for treatment with thrombolysis in an Irish stroke unit.
   D.R.Collins, D.O'Neill, P.M.E.McCormack.
   Irish Med J 1999: 92(1); 236-238.

3. An acute stroke service: Potential to improve patient outcome without increasing length of stay.
   D.R.Collins, D.McConaghy, A.McMahon, D.Howard, D.O'Neill, P.M.E. McCormack.
   Irish Med J 2000: 93(3);84-86.

4. Association of cardiac co-morbidity with outcome in stroke.
   D.R.Collins, P.McCormack, D.O'Neill.
   Age & Ageing 1999:28(supp 1);43.

5. Acute promotory effect of smoking on sodum-lithium countertransport (SLC).
   D.R. Collins, K.Scott, P.O'Kelly, P.M.E. McCormack, J.Feely.
   Irish Journal of Medical Science (abstract-in press).

6. Could sodium-lithium countertransport (SLC) serve as a risk marker for vascular risk in patients with hypertension ?
   D.R. Collins, K. Scott, P.O'Kelly, P.M.E. McCormack, J. Feely.
   Age and Ageing (abstract-in press).

7. An acute stroke service: encouraging trends in outcome without increasing length of stay over a three year period.
   D.R. Collins, D. McConaghy, A. McMahon, D.O'Neill, P.M.E. McCormack.
   Irish journal of Medical Science (abstract-in press).

8. Role for lipid lowering therapy in primary and secondary prevention of stroke.
   D.R. Collins, L.Kelly, D.O'Neill, P.M.E. McCormack.
   Irish Medical Journal (submitted Aug 01).

9. Incidence of operable carotid artery stenosis in a stroke population.
   D.R. Collins, F.Dunne, S.Tierney, M.Feely, P.M.E. McCormack.
   Irish Journal of Medical Science (abstract-in press).

Acknowledgements

No work of this nature is possible without the advice, help, encouragement and occasional 'cracking of the whip' by colleagues. In this regard I am indebted to a great many people.

I wish to thank Dr. Desmond O'Neill and Dr. Patricia McCormack for their constant encouragement and advice in planning and executing this work, without their help this thesis would not be possible. I am also grateful for the help and assistance of all the doctors, nursing staff and clerical staff of the stroke-service at the Adelaide & Meath Hospital, Tallaght.

I am extremely grateful to Professor John Feely, my supervisor, for giving me the opportunity to study for this M.D. at the Department of Clinical Pharmacology & Therapeutics, Trinity College Dublin. Throughout he has not only advised and critically reviewed my work, but also provided me with every assistance to perform the laboratory work contained herein. In this vane, I also wish to thank Mr. Ken Scott not only for his technical expertise in the laboratory, but also for his friendship and companionship and his 'never say die' attitude on the lonely evenings at the SLC assay. He may not be the best 'tipster in the world', but he has improved under my tuition ! I am also indebted to Mr. Kevin Foran and Dr. Pearse Kavanagh for their advice and help in obtaining the necessary laboratory supplies and everyone at the department for making 'this outsider' welcome.

I cannot thank Dr. David Doff and The Dept. of Geology TCD enough, for providing the most crucial of pieces of equipment, the atomic absorption machine, for his patience in teaching me to use it and his great enthusiasm for scientific work in other fields.

I am also indebted to Mr. Peter Gaffney for his help in analysing samples at the Dept. of Biochemistry at the Adelaide and Meath Hospital, and all the haematology laboratory staff at St. James' Hospital.

For their patience with my feeble mathematical brain, I wish to thank Mr. Patrick O'Kelly and Dr. Alan Kelly, of the Dept. of Community Health and Statistics TCD, for taking the time and effort to explain and advise on statistical methods.

Abbreviations used in this thesis

ATP – Adenosine triphosphate.

BADL – Barthel index of activities of daily living.

CI – Confidence interval.

CT – Compterised tomography.

DCU – District care unit.

ECG – Electrocardiogram.

ESR – Erythrocyte sedimentation rate.

HDL – High-density lippoprotein.

IDDM - Insulin dependent diabetes mellitus.

LACI – Lacunar infarction syndrome.

LDL – Low-density lippoprotein.

LVH – Left ventricular hypertrophy.

MI – Myocardial infarction.

MRI – Magnetic resonance imaging.

NIDDM – Non-insulin dependent diabetes mellitus.

PACI – Partial anterior circulation infarct syndrome..

POCI – Posterior circulation infarct syndrome.

SLC – Sodium lithium countertransport.

SSNS – Scandinavian stroke neurological scale.

TACI – Total anterior circulation infarct syndrome.

TIA – Transient ischaemic attack.

VLDL – Very low-density lippoprotein.

Table of Contents

Figures:

Chapter One

Introduction

Chapter 1 - Introduction

Contents

1.1 Stroke

1.1-1: The Impact of Stroke

Stroke is the third leading cause of death after ischaemic heart disease and cancer and represents a major burden in terms of personal suffering, disability and health economics. Stroke disease is the leading cause of disability in adults and the second most common cause of dementia. It has been estimated that stroke caused 4.4 million deaths world-wide in 1990 (Murray & Lopez, 1997). Within most western countries, 2,000 new strokes occur per million of the population each year (Sudlow & Warlow, 1996), of whom half will have died or remain disabled at one year (Bamford *et al.*, 1991).

The economic impact of stroke is also considerable with direct costs of stroke care accounting for 5% of the total health services budget annually in the UK (Isard & Forbes, 1992), and over 3% of the total budget in Holland (Evers *et al.*, 1997). With the prevalence of stroke survivors living with disability estimated at 6 per 1,000 population, the real cost, in terms of caring for disabled survivors, loss of earnings where carers have chosen to take on this challenge and in personal suffering is likely to be huge. About 6,000 new strokes occur in Ireland every year and stroke accounts for 10% of all mortality (Central Statistics Office [Ireland] 1998). It is estimated that 30,000 people are living with some form of disability post-stroke. In personal terms stroke is a devastating event for patient and carer, often described as the most significant event of their lives.

1.1-2: Pathophysiology of Stroke

Stroke can be loosely defined as 'a focal neurological deficit caused by vascular disease which persists for longer than 24 hours' (Bonita 1992). This however, does not include global neurological deficits such as coma, or sudden death, which can also have a vascular cause. A more encompassing definition is: 'A stroke is a clinical syndrome characterised by rapidly developing clinical symptoms and/or signs of focal, and at times global loss of cerebral function, with symptoms lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin' (Hatano 1976).

The brain is an 'end-organ', lying at the end of the circulatory system composed of the heart and vascular tree, from which it receives a fixed blood supply, of 50-55 mls/100g tissue. In the normal adult brain (average weight 1300-1400 g) this results in a cerebral blood flow of 800mls/minute, representing 15-20% of the total cardiac output. This disproportionate share of total cardiac output reflects the brain's relatively high metabolic rate and its reliance on glucose as the sole substrate to meet this demand (approx. 75-100g glucose / minute). Interruption of this supply results in cerebral dysfunction and the clinical manifestation of stroke. In pathological terms, stroke occurs by one of two principal mechanisms, thrombosis or haemorrhage. Thrombosis, either *in-situ* or by embolism accounts for approximately 90% of all strokes, with haemorrhage (most usually intracerebral and less commonly subarachnoid) accounting for the rest. The brain itself is merely the 'end-organ' of these processes and it is diseases of the heart and vascular tree that largely predispose an individual to stroke.

Infarction

Historically cerebral infarction or 'softening' of brain tissue was first recognised pathologically as a distinct lesion by Leon Rostan a parisien physician in the 19th century. For many years cerebral infarction was felt to be the result of inflammation and it was Abercrombie who first drew the analogy between gangrene of the foot and such lesions, being caused by 'failure of the circulation' secondary to 'ossification of the arteries' (Abercrombie 1836). Virchow clarified the disease processes involved in such circulatory failure and ossification. He clearly established fatty infiltration of artery walls rather than primary inflammation, as being the principal pathological process of arterial thrombosis and appled the term 'arteriosclerosis' to this process (Virchow 1847). Virchow was also first to introduce the idea that such thrombi could originate from distant sites and travel within the circulation to produce blockage, which he termed 'embolus' (Virchow 1856). From analogy, again with the gangrenous foot, he extrapolated that such a mechanism could lead to cerebral infarction.

While the concept of cerebral embolism from the heart gained early acceptance, it was not until relatively recently that the concept of embolism from within the arterial system (i.e. artery to artery), and in particular from the carotid artery, has been widely accepted. Chiari initially recognised the frequency with which atherosclerosis affected the carotid bifurcation, and suggested that emboli from this site could account for cerebral infarction (Chiari 1905). However, it was not until the advent of modern imaging, initially with angiography (Seldinger 1953) and later with ultrasound, that such lesions could clearly be demonstrated *in vivo*. More recently the aorta has been recognised as an important site of atherosclerotic lesions causing cerebral embolism

where no clear source has been found in the heart or neck vessels (Soloway & Aronson 1964; Amarenco *et al.,* 1994).

Thus it is disease of the heart and arterial tree rather than any inherent disease of the brain, that results in most strokes. In terms of cerebral infarction, risk markers for stroke are essentially those conditions that result in, accelerate, or are the end product of athero- and arteriosclerosis of the coronary and cerebral circulation leading to atherothrombo-embolic disease. This process begins at a young age with insults to the endothelial lining of the vessel, breaching its' integrity and leading to the accumulation of a lipid pool within the arterial intima, ("lipid streaks"), and smooth muscle proliferation. This process aggravated by hypertension, diabetes, hyperlipidaemia, smoking and alcohol excess, results in thickening of the arterial wall and narrowing of the lumen. The arterial wall initially repairs itself, through the action of fibroblasts forming a fibrous cap but this can result in platelet adherence and thrombus formation causing further narrowing of the lumen and predisposition to embolus. Lipid within the arterial wall also excites inflammation with chemotactic and cytokine release and the migration of macrophages to the intima resulting in oedema, haemorrhage, platelet activation and thrombosis and leading to eventual ulceration of the overlying fibrous cap (Warlow *et al.,* 1996a). This promotes both further thrombus formation *in-situ* which can lead to occlusion in an already critically stenosed artery, or the subsequent embolism of either lipid or thrombus material to occlude a more distal vessel. Ischaemic stroke therefore generally occurs by one of three mechanisms, the formation of thrombus in situ occluding the involved vessel, embolisation of material from a cardiac source or embolisation from within the arterial tree.

In terms of risk markers for ischaemic stroke some are causal factors of atherosclerosis, while others are stigmata of this process, but all increase the odds ratio of stroke. While this in itself may be important in individual settings, the full significance of any given risk factor depends on 1) the relative risk of the factor; 2) the prevalence of the risk factor in the population; and 3) the effect of therapeutic intervention. Differences in risk markers amongst populations have been shown to be important in explaining differences in incidence of stroke (WHO MONICA project 1997). A knowledge of risk markers for stroke in a given population would be helpful both in terms of predicting the likely future incidence of stroke and therefore the level of resource needed, and in formulating better primary and secondary prevention strategies.

Risk markers associated with atherosclerosis are hypertension, hyperlipidaemia, diabetes mellitus, smoking and excess alcohol intake. Such factors also cause disease in the coronary arteries and ventricular muscle resulting in a compromised coronary circulation and often left ventricular dysfunction. This may manifest itself symptomatically as angina or actual myocardial infarction, and more insidiously as atrial fibrillation. Both are important risk markers for stroke, partly because the relative health of the coronary arteries reflects the state of the arterial system as a whole, and also because left ventricular dysfunction and atrial fibrillation both predispose to thrombus formation within the heart chambers, substantially increasing the risk of embolism and stroke. Carotid artery stenosis is similarly the end result of atherosclerosis, but an important risk marker for stroke, as it is a common site of origin of emboli and surgical treatment reduces risk of further stroke. Similarly other

'indirect' risk markers for ischaemic stroke are age and previous stroke or transient ischaemic attack (TIA).

Ischaemic stroke occasionally may result from a primary vasculitis where auto-antibodies are directed against components of the arterial wall resulting in inflammation, thrombosis and occlusion of the involved vessel. This process can occur in a variety of medical conditions such as giant-cell arteritis, rheumatoid arthritis or systemic lupus erythematosis, and in general such vasculitides tend to affect medium to smaller arteries. The resultant strokes are therefore often localised producing isolated motor or cranial nerve deficits, but, because of the underlying disease process, are also likely to be progressive and recurrent without appropriate immunosuppressive therapy. Alternatively a coagulopathy such as an inherited protein C or S deficiency, factor V leiden etc. can result in a disordered coagulation cascade and recurrent venous and arterial thrombosis (Bushnell & Goldstein, 2000). Perhaps becoming increasingly recognised as a risk factor, is elevated homocysteine levels in coronary and cerebral thrombosis (Stampfer *et al.,* 1992; Pery *et al.,* 1995). While not common, these conditions are occasionally the primary risk factor in an individual stroke. Though their presence would increase the odds ratio of ischaemic stroke significantly in the individual, they are encountered infrequently (i.e. low prevalence) and as such not important in the attributable risk to the population as a whole.

Cerebral Venous Thrombosis

Rarely a venous thrombosis can be the cause of stroke, resulting in congestion of brain tissue and a haemorrhagic infarct. Such thrombosis, of the sagittal sinus for example, can result from hypercoaguable states (referred to above), or systemic disorders such as dehydration, infection or non-metastatic complication of a

malignancy. In clinical terms the resulting strokes have a presentation characterised by headache, cranial nerve palsies and seizures, consequent on the increased intracerbral pressure generated (Bousser *et al.* 1985) and typical CT appearances.

Intracerebral Haemorrhage

Extravasation of blood into brain tissue (intra-cerebral haemorrhage), causing death had been recognised much earlier than cerebral infarction (Wepfer 1658; Morgagni 1761). While its aetiology remained unclear at the time, it is interesting to note one early post-mortem series of patients with brain haemorrhages and chronic renal disease. In it, Kirkes demonstrated hypertrophy of the heart in 17/22 patients, suggesting blood pressure as the common aetiological factor but long before blood pressure was measurable or understood (Kirkes 1855). Later work was to demonstrate the importance of miniature aneurysms in the small blood vessels of the brain as a post-mortem finding in cases of cerebral haemorrhage (Charcot & Bouchard 1868). While their observations were subsequently disputed, they were later confirmed and the important link between such abnormalities, hypertension and age in cases of cerebral haemorrhage was discovered (Russell 1963; Cole & Yates 1967).

In terms of cerebral haemorrhage, stroke is generally caused by one of two main mechanisms, haemorrhage from deep small perforating arteries resulting in an intra-cerebral and occasionally intra-ventricular haemorrhage, or by haemorrhage from arteries in the sub-arachnoid space. The former is strongly associated with hypertension, lipohyalinosis of the arterial wall and the development of micro-aneurysms ("Charcot-Bouchard" aneurysms). Current theories suggest leakage from lipohyalinotic vessels produce a 'false' aneurysm which under the influence of hypertension can subsequently rupture (Takebayashi & Kaneko 1983).

Amyloid deposition in the adventitia and media of small leptomeningeal and superficial cortical vessels, is becoming increasingly recognised as a factor in intracerebral haemorrhage, so-called cerebral amyloid angiopathy (Vinters 1987). The resulting deposition of amyloid causes fibrinoid degeneration, segmental dilatation and aneurysm formation. The pattern of haemorrhage is usually characteristic and identifiable in such cases, producing multilobar peripheral haemorrhages particularly in the parietal and frontal lobes.

The taking of antiplatelet agents such as aspirin or anti-coagulants such as warfarin can also result in intracerebral haemorrhage (Juvela *et al.*, 1995; Swedish Aspirin Low-Dose Trial Collaborative Group 1991.), particularly if there is co-existent, uncontrolled hypertension or where the prothrombin time is prolonged above therapeutic range. Less commonly a primary bleeding disorder such as that associated with deficiency of clotting factor viii (haemophillia A), or deficient von Willebrand factor may be the precipitant risk factor, although these are not common causes of cerebral haemorrhage in the population as a whole. Other rarer causes of intracerebral haemorrhage include arteritis, neoplasm and arterio-venous malformations.

Sub-Arachnoid Haemorrhage

Arterial aneurysms known as 'berry' aneurysms are the chief aetiological factor in sub-arachnoid haemorrhage. Although the exact aetiology of their origin is unknown they are generally considered to be acquired. Histologically the aneurysm is composed of a thin layer of connective tissue covered by normal endothelium, but the normal muscle and elastic layer are absent. The majority arise at the points of arterial division in the vessels that form the circle of Willis, with most occurring in the

distribution of the internal carotid artery (anterior circulation) and only 15% occurring in the vertebrobasilar territory. Important contributory factors are age, atherosclerosis and hypertension (Crompton 1966). Ironically, such haemorrhages are not infrequently proceeded by arterial spasm and subsequent infarction, usually involving the vessel close to the aneurysm at day 4-7 post onset (Crompton 1964).

Occasionally a larger aneurysm may form in the internal carotid or basilar artery as a result of severe atherosclerosis in elderly people. Replacement of smooth muscle by fibrous tissue due to atherosclerosis results in stretching and dilatation of the vessel, in turn producing a tortuous aneurysm. These aneurysms rarely rupture but may calcify or thrombose.

1.1-3: Risk factors and prevention of stroke.

A number of important advances in stroke prevention have been made in the 1990's, principally as a result of a series of randomised controlled trials. Principles of primary and secondary prevention of stroke have been clarified through a better understanding of the nature and significance of risk factors, and sound evidence for therapeutic strategies. Traditionally age, history of previous transient ischaemic attack (TIA) or stroke, hypertension, diabetes mellitus, ischaemic heart disease and atrial fibrillation, and smoking have been regarded as the principle risk factors for cerebral infarction, and hypertension and use of anticoagulant / antiplatelet agents as the major risk markers for cerebral haemorrhage. Recent large population studies and therapeutic trials have uncovered new potential risk factors such as hyperlipidaemia, homocysteine and oestrogen therapy. Identifying new risk markers is not only important in terms of increasing our undertsanding of the aetiology of stroke, but also

in terms of prevalence of such risk markers and the possibility of therapeutic intervention in stroke prevention. The more important advances in this regard are reviewed here.

Hypertension

Hypertension is an important modifiable risk factor for both ischaemic and haemorrhagic stroke, increasing the relative risk by a factor of 2. Epidemiological evidence from the Framingham study has described the logarithmic relationship between diastolic blood pressure and risk of stroke (Wolf *et al.*, 1991.b). Furthermore the variation in stroke incidence among different populations can at least in part be explained by differences in diastolic blood pressure (WHO MONICA project.1997). Our understanding of what is an acceptable level of diastolic blood pressure has greatly increased from recent work, showing the benefit and safety of reduction of pressure to approximately 80 mmHg (Hansson *et al.*, 1998).

Less was known about the importance of systolic blood pressure as a risk factor, until evidence of efficacy of treatment of isolated systolic hypertension in stroke prevention was published (SHEP Cooperative research group 1991). There is clear unequivocal evidence from these and other trials to suggest treatment of hypertension, both diastolic and systolic prevents stroke. In addition, while the relative risk of stroke is increased by a factor of 2 in hypertensive patients, the attributable risk to the population, as a whole, is far greater given the high prevalence of hypertension in the population. (Whisnant 1997). In terms of intracerebral haemorrhage, hypertension is the single most important risk factor, with up to 50% of cases directly related to it in one study (Hsiang *et al.*, 1996). Among known hypertensive patients, poor

compliance with treatment and inadequate control of blood pressure has been attributed to 20% of cases of intracerebral haemorrhage (Hsiang *et al.*, 1996). and up to a quarter of all incident strokes (Klungel *et al.*, 1999).

Diabetes Mellitus

Epidemiological evidence from the Framingham Study and others (Kannel & McGee, 1979; Barrett-Connor & Khaw, 1988) has long established diabetes as an independent risk factor for cardiovascular disease and stroke. As with hypertension the odds ratio of stroke with diabetes is approximately 2-3 (Sacco 1995; Currie *et al.*, 1997), and both would appear synergistic in terms of vascular risk with dramatic reductions in major cardiovascular events observed with aggressive blood pressure control in diabetic patients (Hansson *et al.*, 1998). Diabetes is not as prevalent as hypertension however in most populations, and so the attributable risk to the population as a whole is somewhat lower (Whisnant, 1997). But this risk would appear to be modifiable making diabetes an important target in primary preventative strategies, particularly as its true prevalence in the community is probably underestimated. The exact pathophysiology of this risk is still poorly understood but important associations between diabetes, hypertension, microalbuminuria and accelerated atherosclerosis have been recognised. In addition there is evidence of a greater prevalence of carotid artery disease among diabetics, and a positive relationship with the duration of illness, higher insulin levels and poor glycaemic control has been established (Wagenknecht *et al.*, 1997; Bonora *et al.*, 1997).

Good diabetic control reduces the incidence and progression of long-term microvascular complications in patients with insulin dependent diabetes mellitus –

IDDM (type 1 diabetes), (Diabetic Control and Complications Trial Research Group, 1993). This finding was confirmed in a study of non-insulin dependent diabetes mellitus - NIDDM (type 2 diabetes) recently, when tight control of blood sugar levels by diet and oral hypoglycaemic agent, or diet and insulin significantly reduced microvascular complications (UK Prospective Diabetes Study [UKPDS] Group, 1998). A specific extrapolation that good diabetic control would reduce macrovascular complications was unsupported however. Subsequent work with the oral hypoglycaemic agent metformin in obese patients however, did show significant reductions in diabetic endpoints, including stroke and myocardial infarction (UK Prospective Diabetes Study [UKPDS] Group, 1998.b). Again this was related to better control and lower haemoglobin A_{1C} levels. This suggests, at least, that the mode of action by which blood sugar is reduced and the effect on baseline insulin levels may also be important, given that metformin reduces fasting insulin levels and results in less weight gain than other oral hypoglycaemic agents.

Atrial fibrillation

Atrial fibrillation has long been recognised as a very significant risk factor for ischaemic stroke. While it may occasionally result from previous rheumatic heart disease, cardiomyopathy (hypertrophic and dilated), or *de novo*, it is usually associated with underlying coronary artery disease. For this reason and the risk of intra-atrial thrombosis with subsequent embolism, it is very strongly linked with cerebral infarction. Our knowledge of stroke prevention in the setting of atrial fibrillation has been greatly improved by a number of clinical trials examining the effect of aspirin versus warfarin in stroke prevention (Stroke Prevention in Atrial Fibrillation Study Group 1991; European Atrial Fibrillation Trial Study Group 1993).

The accumulated results from these studies demonstrate a 60-70% risk reduction with warfarin versus a 20-35% reduction with aspirin in patients with atrial fibrillation. In addition these trials have also determined the therapeutic range (in terms of Internationalised prothrombin Ratio [INR]) over which warfarin is effective and at which side effects of haemorrhage are at an acceptable level. This has been one of the most important advances in our approach to the clinical management of atrial fibrillation in both primary and secondary prevention of stroke.

Carotid Artery Disease

The effectiveness in treating symptomatic carotid artery stenosis in primary and secondary prevention of stroke was clearly established in the 1990's. While its role in the aetiology of ischaemic stroke was postulated in the early part of the century (Chiari 1905), its importance as a risk factor could not be fully appreciated until the efficacy of treatment by surgical endarterectomy was demonstrated. What is clear from the clinical trials to date, is that treatment of a symptomatic moderate or severe stenosis (defined as a 50-69% and 70-99% stenosis respectively), with surgery results in significant reduction in stroke over medical management alone (North American Symptomatic Carotid Endaerterectomy Trial Collaborators, 1991; Barnett *et al.*, 1998; European Carotid Surgery Trialists Collaborative Group, 1998). The beneficial effect is greatest with higher grades of stenosis (absolute risk reduction of 17% at 2 years), the number needed to treat to prevent one stroke during a five-year period being half that of lower grade stenosis. Benefit is also dependent on good surgical technique with low post-operative complication rates. Indeed, at a 2% perioperative mortality and stroke rate, any benefit accruing to surgical intervention of symptomatic 50-69% stenosis would disappear.

Hyperlipidaemia

Unlike the situation with coronary artery disease, strong correlation between plasma levels of cholesterol, low-density lipoprotein (LDL) and the risk of stroke have remained unsubstantiated. Indeed lower total cholesterol levels have been linked to an increased risk of cerebral haemorrhage (Yano *et al.*, 1989). Furthermore, there is dispute as to an inverse relationship between high-density lipoprotein (HDL) levels and the risk of stroke, as is the case with coronary artery disease. While such a relationship in stroke was thought not to exist initially (Prospective studies collaboration 1995), more recent work has claimed an association between low HDL and risk of non-fatal stroke (Wanmathee *et al.*, 2000).

Our understanding however, of the role of cholesterol and its interplay with ischaemic heart disease and risk of stroke has been dramatically increased through a series of large secondary prevention trials of lipid-lowering therapy with 3-hydroxy-3—methylglutaryl-co-enzyme A (HMG-CoA) reductase inhibitors (or 'statins') in patients with coronary artery disease (Scandinavian Simvastatin Survival Group [4S] 1994; Long-term Intervention with Pravastatin in Ischaemic Disease [LIPID] Group 1998). A meta-analysis of these trials indicate a consistent reduction in risk of stroke by about 30% when LDL levels are reduced in patients with coronary artery disease, although the benefit does not extend to stroke mortality (Herbert *et al.*, 1997; Blauw *et al.*, 1997).

Treatment aimed at reducing LDL levels may have a further role in stroke prevention where disease of the carotid artery can be substantiated. A number of ultrasonography studies have clearly established carotid artery intimal thickening as a predictor of

stroke risk (O'Leary *et al.*, 1999). Trials of statins to produce lowering of LDL levels by at least 25% in both asymptomatic patients and those with coronary artery disease, have shown benefit in prevention of progression of such thickening and the development of new lesions (McMahon *et al.*, 1998). There is also increasing evidence that statins may have important neuroprotective, anti-inflammatory and anti-oxidant properties, and have been shown to limit infarct size in animal models of acute ischaemia (Vaughan & Delanty 1999).

It seems sensible from this evidence to recommend lipid lowering therapy to all hyperlipidaemic patients with a history of ischaemic heart disease or where there is proven carotid artery disease, in primary and secondary prevention of stroke. Future research may well expand such therapeutic recommendations for statin therapy both in the treatment and prevention of stroke, independent of lipid levels.

Oral contraception and hormone replacement therapy

Case controlled analysis has established an increased risk of ichaemic stroke with oral contraceptive (OCP) use with an odds ratio of 1.5 adjusted for smoking (Hannaford *et al.*, 1994). This increased risk was directly related to the oestrogen dose per pill, and disappeared in former users when adjusted for smoking habits. A similar increased risk of haemorrhagic stroke with current OCP use, independent of previous use, dose or type of preparation, has been found in developing countries in women over 35 years, where both hypertension and smoking are strong synergestic factors (WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception, 1996). Other studies of current versus never users of low dose contraceptive preparations have failed to find any such association, although when a history of

migraine was considered, current use of OCP did increase the risk of both ischaemic and haemorrhagic stroke in this subset of patients (Schwartz *et al.,* 1998). A recent meta-analysis of case controlled studies confirms an association between current OCP use and risk of ischaemic stroke, adjusting for age, hypertension and smoking habits (Gillum *et al.,* 2000). The observed increased risk is lower with low-oestrogen preparations.

A similar diversity of results has been reported with post-menopausal hormone replacement therapy (HRT). Some cohort studies have reported an increase risk of non-fatal ischaemic stroke with HRT (Fung *et al.,* 1999; Hart *et al.,* 1999). Meta-analysis of the issue has also found conflicting evidence that HRT reduced risk of fatal stroke but may have increased risk of non-fatal stroke and subarachnoid haemorrhage (Paganini-Hill 1995), although most reviews of the evidence have found no compelling association either way (Kittner & Bousser, 2000; Tavani & La Vecchia 1999). Some of this disparity in results may be explained by interaction with other risk factors and selection bias in studies of HRT (women on HRT are likely to be more health conscious). The recently observed neutral effect of oestrogen replacement on cardiac events in women with coronary artery disease, contradicting previously positive results in observational studies, may be an example of the latter phenomemon (Hulley *et al.,* 1998).

Smoking & Alcohol

Smoking, both active and passive exposure to environmental tobacco smoke (ETS), is an important risk factor for stroke. Active smoking is associated with a four-fold increase in the risk of of ischaemic stroke and this risk is even greater if ETS exposure is also a factor (Bonita *et al.,* 1999). Passive ETS exposure to spousal smoking in non-

smokers is also associated with a doubling of the risk of stroke (You *et al.*, 1999). Smoking accelerates athersclerosis and may be even a more potent risk factor in carotid occlusion models of cortical ischaemia (You *et al.*, 1993). Nicotine has also been shown to increase endothelial expression of, the pro-thrombotic, plasminogen activator inhibitor-1 in cultures of human brain endothelial cells, thus theoretically at least, promoting in-situ thrombosis (Zidovetzki *et al.*, 1999). Smoking cessation reduces the excess risk of ischaemic stroke, with a similar risk to life-long non-smokers being observed after 10 years cessation in the above studies.

Moderate alcohol consumption appears to be protective although heavy usage ($\geq$ 7 drinks a day) is associated with significantly increased risk of ischaemic stroke (Sacco *et al.*, 1999). Other investigators have similarly found trends to reduced ischaemic stroke mortality with moderate alcohol intake, particularly in women, although no such association was observed in relation to haemorrhagic stroke (Hansagi *et al.*, 1995). Several studies have implicated heavy alcohol as a risk factor for intracerbral haemorrhage (Juvela *et al.*, 1995; Monforte *et al.*, 1990).

Homocysteine

Much interest has surrounded the role of homocysteine in the development of atherosclerosis and coronary artery disease. Its role as a vascular risk factor is still not fully understood, but may be significant given that levels of homocysteine can be reduced by folate therapy (Homocysteine Lowering Trialists' Collaboration, 1998) and as such would be amenable to treatment. Positive associations have been reported between elevated homocysteine levels and risk of myocardial infarction (Stampfer *et al.*, 1992) and stroke (Pery *et al.*, 1995), though other studies have failed to find such

a relationship (Evans *et al.,* 1997; Alfthan *et al.,* 1994). Such disparity probably reflects differences in trial design and homocysteine assays used (Hankey & Eikelboom, 1999) and while, lowering homocysteine has been shown to retard atherosclerosis progression (Peterson & Spence, 1998), its effect on vascular end-points remains unproven.

1.1-4 Recent Advances in treatment of acute stroke

Stroke-Units

Perhaps the most important advance in the management of stroke has been the evidence that organised stroke unit care reduces mortality and morbidity following a stroke (Langhorne *et al.,* 1993; Stroke Unit Trialists Collaboration 1997). Although trials of stroke unit care tend to be heterogenous in their design, the latter, a meta-analysis of all available data provides compelling evidence for their effectiveness. The core principal of stroke unit care is a specialist led multidisciplinary team management of all stroke patients from admission to discharge. Locating stroke patients within a geographical ward area could be beneficial, by developing local skills and ensuring the rehabilitation needs of stroke patients are not overlooked in favour of more acutely ill patients, but other patterns of organised stroke care based on the mobile team may also be effective (Woods-Daupinee *et al.,* 1984; Stone 1987).

Why stroke unit management is more effective than general medical care is probably multifactorial. The use of standardised protocols by stroke teams, ensuring the administration of aspirin (where appropriate), avoidance of detrimental hypoxaemia, hyperglycaemia and pyrexia, and prevention of early fatal complications (e.g. pulmonary embolus, aspiration pneumonia) would have obvious benefit. Indeed

examination of the mortality figures from the stroke unit meta-analysis shows divergence in rates between controls and stroke unit management beginning to occur at one week after the index event when many of these complications would occur (Langhorne & Dennis 1998). In addition there is also evidence that recognition of neuro-medical complications and rehabilitation needs might be enhanced by such a special interest service (Kalra *et al.* 1995; Collins & O'Neill 1998), and this may explain the improved outcome of stroke unit care in terms of reduced dependency and need for institutional care. While impressive results were observed in many trials (Stevens *et al.,* 1984; Kaste *et al.*, 1995) others were less convincing in this regard. A summary result of an odds ratio of 0.75 % (95% CI 0.65-0.87 $2p<0.0001$), was reproducible, however, when both a random effects model of statistical analysis was used to assess the different trials, and when only trials including a long term follow-up (six months to one year) were analysed (Langhorne & Dennis 1998).

Much concern has focused on organised stroke care increasing length of hospital stay and costs. Clearly a reduction in those needing long-term institutional care is of economic benefit and a reduction in mortality and dependency also serve to reduce the indirect costs of stroke to society e.g. lost working days, earnings etc. Many health managers however are likely to focus on the direct cost of an acute stroke and this will be inextricably linked to length of stay. The evidence on length of hospital stay is inconclusive. A pooled analysis of all the trials suggested a relative reduction of 8% with stroke unit management but in absolute terms a non-significant reduction of 0.3 days (Langhorne & Dennis 1998). It would seem reasonable to conclude, however, that stroke unit management does not increase length of hospital stay on the available evidence, and may even reduce it. This compelling evidence that stroke unit care reduces death and dependency and is likely to have long-term economic benefits to

both patient and society, should encourage this model of management of stroke in all hospitals where acute stroke patients are admitted.

Cerebrovascular Thrombolysis

The recannulisation of occluded coronary arteries with thrombolytic therapy has revolutionised the treatment of acute myocardial infarction in terms of reduced mortality (GISSI-1 1986; ISIS-2 1988), and it has long been hoped that similar acute treatment would improve outcome in cerebral infarction. Initial trials with streptokinase were all unsuccessful however, and abandoned due to excess intracerebral haemorrhage and early mortality (Donnan *et al.*, 1995; Multicentre Acute Stroke Trial-Italy [MAST-1], 1995).

The demonstration that treatment within three hours with tissue plasminogen activator (t-PA) improved global outcome with similar mortality to placebo at six months, has renewed hope in thrombolysis as a potential effective early treatment for cerebral thrombosis (National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995). The treatment has substantial limitations however, as defined thus far, as the time of onset to treatment must not exceed three hours and there is still an excess of intracerebral haemorrhage and death in the treated group in the early stages.

A similar trial has shown equitable mortality at six months between treatment and placebo when the therapeutic window was extended to six hours, though without demonstrating clear benefit in the primary end-point (European Cooperative Acute Stroke Study II Group, 1998). It has been shown that a secondary analysis of death and dependency versus independence in the two groups lends support to the efficacy

of t-PA up to six hours post stroke, and if further verified may make thrombolysis more available as an acute treatment for cerebral infarction. Ethical and practical difficulties will still remain however for doctor, patient and family in teasing out the early risk versus delayed benefit with such treatments.

Antiplatelet Agents

The role of aspirin as an effective treatment in acute myocardial infarction has been well demonstrated (ISIS-2, 1988). In this trial aspirin produced a 23% odds reduction in death over placebo and when used in conjunction with thrombolysis doubled the odds reduction of vascular death from 25% to 42% over placebo. The theory that aspirin might fulfil a similar role in cerebral infarction was vindicated by two large randomised controlled trials, the International Stroke Trial (International Stroke Trial Collaborative Group 1997) and the Chinese Acute Stroke Trial (Chinese Acute Stroke Trial [CAST] Collaborative Group 1997). Both trials, including over 30,000 patients with acute cerebral infarction and aspirin started within 48 hours of onset, were associated with significant reductions in recurrent events and death compared to placebo. Although the number needed to treat (NNT) is high, at 100 patients treated to prevent one excess death over placebo, aspirin is an inexpensive and easily administered treatment and as such has an everyday relevance in the treatment of stroke. While other antiplatelet agents such as Dipyrimadole (ESPS 2 Group, 1997) and Clopidogrel (CAPRIE Steering Committee 1996)) have demonstrated benefit in the secondary prevention of stroke their benefit in the acute setting has yet to be demonstrated.

1.2 Sodium-Lithium Countertransport (SLC)

1.2-1: Ion Transport systems in the Human Erythrocyte:

The human erythrocyte membrane is a semi-permeable, complex organisation of phospholipids and cholesterol within which operates a number of well-defined physiological ion transport systems, which maintain cell volume and pH. The membrane is unique not only for it's bi-concave morphology (which serves to increase it's surface area, a factor vital in the primary red cell function of binding and releasing oxygen), but also in it's apparent internal fluidity and ability to alter cell shape as it passes through capillaries and splenic sinusoids. The membrane allows for the diffusion of oxygen and transport of both anions and cations in and out of the red cell.

Cation Transport

The major cation pathways in the human erythrocyte are summarised in **Fig 1.a** and are briefly outlined here.

Sodium-Potassium (Na^+-K^+) ATPase pump

As with any semi-permeable membrane, ions small enough to pass through, will freely move along their concentration gradient from one side to the other. However clearly there is another factor at work in the case of the red cell membrane. Given the normal plasma levels of potassium (K^+) range from 3.3 –5.0 mMol/L and those of sodium (Na^+) 133-150 mMol/L, one would expect similar values within the red cell as ions diffuse along their concentration gradient across the semi-permeable membrane. This is not the case however, as the average intracellular level of K^+ is some 20 times

greater at 102.4 mMol/L and the level of Na^+ only 6.2 mMol/L in healthy subjects (Fortes-Mayer & Starkey 1977). Some factor obviously opposes the simple diffusion gradient to maintain this imbalance on both sides of the membrane and that is the Na^+-K^+ ATPase pump. Originally described in 1975, the transporter consists of an intramembranous protein which transports Na^+ and K^+ against their concentration gradients (Glynn & Karlish 1975). To achieve this, hydrolysis of adenosine triphophate (ATP) by the magnesium-dependent enzyme, adenosine triphosphatase, occurs to provide a source of energy, thus the "ATPase" in the transporter name. The characteristics of this transport mechanism have been well defined. An unequal movement of Na^+ and K^+ ions occurs, so that two K^+ ions are transported inwards to the three Na^+ ions extruded. In addition the ATPase enzyme can be inhibited by the cardiac glycoside 'ouabain'. For this reason it is sometimes referred to as the 'ouabain sensitive pump'.

This unequal movement of ions creates an electrical imbalance as there is a net cation loss from the red cell membrane, and normal stoichiometric considerations dictate that there must be a corresponding flow of anions from the red cell to maintain 'neutrality'. This net loss of ions from the erythrocyte would be accompanied by osmotically driven water and hence result in loss of cell volume. This does not occur in the normal physiological setting however as the Na^+-K^+ ATPase pump is balanced in it's activity by a range of other ion fluxes and transporters such as sodium-hydrogen (Na^+-H^+) exchange, sodium-sodium countertransport (Na^+-Na^+), sodium-potassium (Na^+-K^+) co-transport and calcium -hydrogen (Ca^{++}-H^+) exchange.

These transport mechanisms are not influenced by the effect of ouabain and have been referred to as the 'ouabain-resistant fluxes'. These fluxes essentially serve as a 'brake' on the activity of Na^+-K^+ ATPase so that electric neutrality is maintained without undue loss of cell volume.

Sodium-proton (Na^+-H^+) exchange

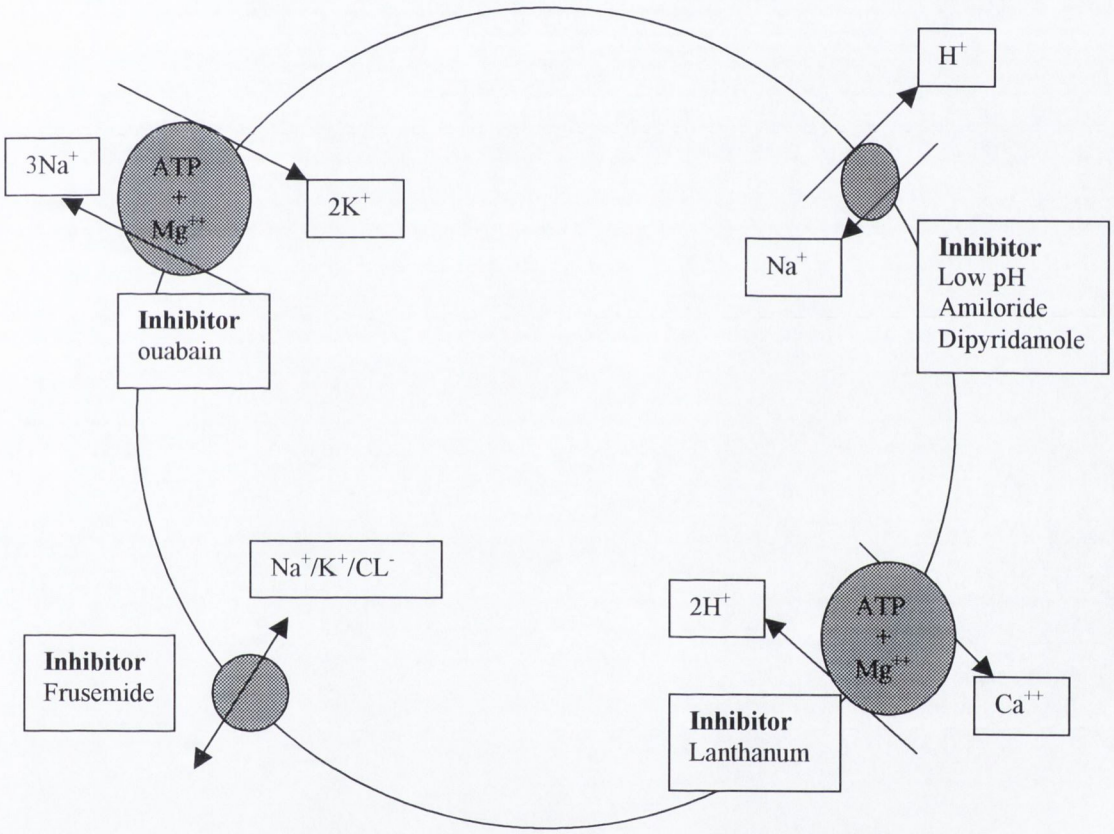
Na^+-H^+ exchange was only recently identified as being present in the human erythrocyte (Escobale & Canessa 1986), although it's presence on other nucleated human cells was well known (Siebens & Kregnow 1978). The transporter exchanges sodium for hydrogen ions and appears to be only intermittently active, being stimulated in particular by intracellular acidosis. Conversely it can be inhibited by suspension in an acidic medium (Parker, 1986) and therefore would appear to be important in regulation of cell pH.

A number of inhibitors of the transporter have being identified including, the anti-platelet agent dipyrimadole and pyrazine diuretic amiloride. However complete inhibition with either agent is not achievable raising the possibility of two separate Na^+-H^+ exchange mechanisms. In addition the Na^+-H^+ transporter in other non-erythroid human cells has being linked with renal tubular re-absorption of sodium, and cellular response to insulin and angiotensin (Seifter & Aronson, 1986; Mahnensmith & Aronson 1985). This would suggest a possible multi-faceted role for this transporter, being involved in different, important homeostatic cellular responses in different cell-lines.

Sodium-potassium (Na^+-K^+) co-transport

The Na^+-K^+ co-transport mechanism, which co-transports sodium and potassium in the same direction, was first demonstrated on human erythrocytes by Wiley & Cooper (1974). They demonstrated the interdependence of the influx of these two cations in ouabain containing media (thus effectively blocking the Na^+-K^+ ATPase pump), and that the co-transporter is inhibited by frusemide, as it is by other loop diuretics. Animal models suggest that this mechanism is involved in the regulation of cell volume as in birds, for example (Brand & Whittam, 1984) but its' role in human red cell function is unclear. The co-transporter is dependent on chloride ions, which not only stimulate the transporter carrier protein, but whose concurrent movement is also essential in order to maintain electric neutrality. In addition, the co-transporter is dependent on the functional integrity of the Na^+-K^+ ATPase mechanism, as the concentration gradients generated by the latter provide the driving force for the former (Porzig, 1983). Its' activity can be measured experimentally as the fraction of ouabain-insensitive Na^+ and K^+ movement that is inhibited by loop diuretics.

Fig 1a. Cation transport systems in the human erythrocyte.



Calcium (Ca^{++}) transport.

Calcium is the only divalent cation for which a specific transport mechanism has been established on the erythrocyte membrane. The red cell is normally permeable to extracellular calcium, but uncontrolled cellular uptake from plasma would not only result in an electric imbalance across the membrane, but would also lead to a compensatory massive potassium 'leak' with consequent disruption of red cell and membrane function (Chiu *et al.*, 1983). To counterbalance this permeability an ATP-magnesium-dependent calcium extrusion pump exists which exchanges one Ca^{++} ion for two H^+ ions, thus effectively acting as a neutral Ca++/H+ countertransporter. Indeed the activity of this transport system would appear to be double of the rate of calcium uptake (1-10 mMol/L cell.hr. versus. 20 mMol/L cell.hr.) (Schatzman, 1983), thus suggesting the importance of regulation of uncontrolled passive uptake of calcium for cell function and integrity. The transporter is stimulated by Na^+ , K^+ and a Ca^{++}-Calmodulin complex and can be inhibited by lanthanum (Szaz *et al.*, 1978), or by inducing a state of ATP or magnesium depletion.

Anion transport systems

The transport of monovalent ions such as bicarbonate (HCO_3^-) and chloride (Cl^-) is fundamental for the respiratory function of the human erythrocyte, whereby carbon dioxide (CO_2) is transported from the tissues to the lungs for excretion. CO_2 is not carried in the gaseous state but rather as an anion, through binding with water initially to form carbonic acid and subsequent (almost immediate) dissociation into a proton and bicarbonate ion:-

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

The bicarbonate ion is then transported passively along its concentration gradient through the anion channel into the red cell and transported to the lungs, where the reverse process occurs and the CO_2 is expired. Fundamental to this process is the transport of Cl^- ions in the opposite direction to maintain electric neutrality. In addition to this very essential function the anion channel serves to maintain electro-neutrality in the face of net cation fluxes which are continuously occurring. This anion channel is governed by an integral membrane glycoprotein, capnophorin which occurs abundantly on the red cell membrane. It can be identified on sodium dodecylsulphate polyacrymide gel electrophoresis (SDS-PAGE), (Cabantchik *et al.*, 1978). Once charged by an anion its binding sites can move freely from one side of the membrane to the other. While chiefly and most rapidly transporting small monovalent anions the transporter also transports phosphate (PO_4^{2-}) and sulphate (SO_4^{2-}) to a lesser and slower degree. It is also probable that cations are carried through this channel, particularly at a higher pH. In this setting, bicarbonate ions dissociate making carbonate ion available in solution i.e.

$HCO_3^- \leftrightarrow CO_3^{2-} + H^+$ (at high pH).

In this scenario highly hydrated cations such as sodium and lithium can bind with the carbonate ion forming a negatively charged ion-pair and be transported through the anion channel i.e.

Na^+(or Li^+) + $CO_3^{2-} \leftrightarrow Na^+$(or Li^+)CO_3^-.

This hypothesis would not apply theoretically to less hydrated cations such as K^+. Some experimental evidence for this mechanism does exist however, with the

demonstration of increased Na^+ and Li^+ fluxes in the red cell in the presence of bicarbonate ions (Wieth 1970), and, as I shall discuss later (see section 3.2-2), in the mechanism of lithium-loading red cells.

Section 1.2-2: Pathways of lithium transport in human erythrocytes.

Pathways of lithium transport in the human erythrocyte are summarised diagramatically in **Fig.1b**

Influx

Lithium is transported in and out of the human erythrocyte by a number of mechanisms. In the first instance influx of lithium occurs by passive diffusion of the ion along its concentration gradient. This is probably the most important in terms of quantity and what normally happens in vivo. Furthermore, by complexing with bicarbonate ion to form $LiCO_3^-$, lithium can be transported passively as a monovalent anion through the erythrocyte anion channel (Funder & Wieth 1974). This can account for up to 25% of lithium uptake when suspended in bicarbonate-rich medium (Pandey *et al.*, 1978) and is technically important in the method of lithium loading of erythrocytes used in this thesis to assay SLC. In experimental processes this mechanism can be inhibited by the anti-platelet agent dipyrimadole.

Lithium uptake can also occur through the Na^+-K^+ ATP 'pump' when erythrocytes are suspended in a Na^+ and K^+ free medium. This process has a maximum activity of approximately 0.5 mMol Li^+/Litre cells/hour. Electroneutrality is maintained by

exchange for either efflux of a Na^+ or K^+ ion, and again like the original Na^+-K^+ exchange mechanism this process can be inhibited by ouabain (Pandey *et al.*, 1979).

Efflux

Lithium-loaded cells will allow passive diffusion of lithium ions along their concentration gradient if suspended in lithium-free media, a so-called passive 'leak' of ions. However a ouabain sensitive efflux mechanism has also been recognised and it is generally accepted that the ouabain sensitive Na^+-K^+ ATP pump can move lithium ions in a bi-directional fashion across the erythrocyte membrane (Pandey *et al.*, 1978). This mechanism favours lithium efflux in 'loaded' cells however, given the affinity at the external binding site favours $K^+ > Li^+ > Na^+$ and at the internal site favours $Na^+ > Li^+ > K^+$. This arrangement also means that at normal physiological conditions or where intracellular sodium levels are at least equal to lithium levels, that lithium efflux would be negligible. This is indeed the case, and in patients taking lithium therapeutically where concentrations of lithium in the plasma range between 0.5-1.2 mMol/l, the result is negligible movement of lithium ions in the normal physiological operation of the Na^+-K^+ ATP pump (Pandey *et al.*,1978).

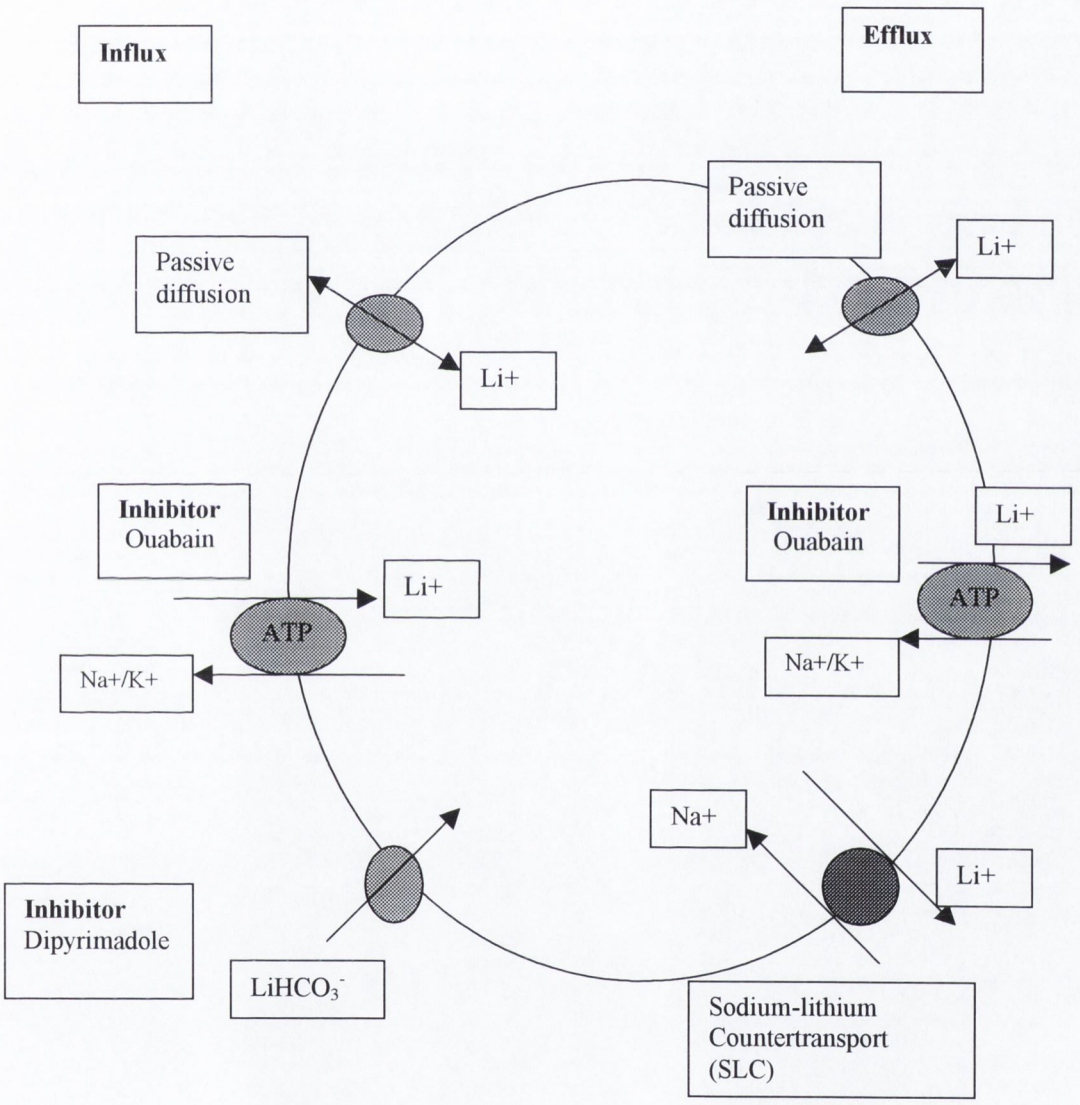
However there is also a ouabain-insensitive lithium efflux pathway originally described by Haas *et al.* (1975), where lithium appears to be extruded against its concentration gradient in exchange for an external Na^+ ion. Indeed the process is stimulated by external sodium ions and inhibited by internal sodium in lithium-loaded cells. This discovery resulted from the key observation earlier by Mendels & Frazier (1973), that in patients taking lithium for treatment of manic-depression, that the intracellular lithium level was only one third of the plasma concentration, a fact that

could not be explained by known transport mechanisms. Clearly a mechanism existed which transported lithium against its concentration gradient. Haas's discovery was subsequently confirmed (Duhm *et al.*, 1976), and forms the basis of what is now known as sodium-lithium countertransort (SLC).

1.2-3: Characteristics of Sodium-Lithium Countertransport (SLC).

Sodium-lithium countertransport (SLC) is one of many pathways for cation transport across the red cell membrane, which as a whole, play an important role in regulating red cell volume and pH. The ATP-independent, 'ouabain- insensitive' pathway once stimulated mediates a 1:1 stochiometric exchange of Na^+ and Li^+. The carrier molecule involved transports ions in a "ping-pong" manner binding a cation on one side of the membrane and transporting it as a complex across the cell membrane, releasing it by dissociation and then binding to the other cation to transport it in the opposite direction. The carrier interacts with no other cations but is capable of binding either cation (Na+ or Li+) on either side of the membrane. The affinity of the carrier molecule is greater, however, for lithium on either side of the membrane but is weighted in such a fashion as to favour binding of internal Li^+. The process is activated by external Na^+, and because of the asymmetry of affinity Na^+ stimulated Li^+ efflux is 2-3 times faster than Li^+ stimulated Na^+ efflux. The former is amenable to more accurate measurement and referred to as sodium-lithium countertransport (SLC).

Fig 1b. Pathways of lithium transport in the human erythrocyte.



1.2-4: Determinants of SLC activity

SLC and hypertension

SLC has received much attention over the last twenty years or so in the medical literature. Much of this attention has focused on the observation that SLC activity is significantly increased in individuals with essential hypertension compared with non-hypertensive controls (Canessa *et al.*, 1980). Moreover, accelerated SLC appeared to be familial and it was elevated in normotensive first-degree relatives of hypertensive patients (Woods *et al.*, 1982, Houtman *et al.*, 1993). This effect has been shown to be independent of gender, age, body mass and plasma lipids in a subsequent study (Turner & Michels, 1991).

The relationship between SLC activity and hypertension is, however, by no means clear-cut and while, higher levels of transport activity have been documented in hypertensive patients compared to controls, an overlap in the range of values between the two groups has been a consistent finding (Morgan *et al.*, 1986; Diez & Arrazola, 1991). In addition considerable overlap of values have been found when secondary hypertensives are compared with essential hypertensives (Duhm & Behr, 1986; Petrov *et al.*, 1994). Hence, SLC activity does not appear to be specific for essential hypertension, and the suggestion that activity is raised in a subset of such patients only, has found support in the observation that patients with a family history of hypertension are most likely to have increased SLC activity (Houtman *et al.*, 1993; Wilson & Meyer, 1981). However other studies have failed to show this association (Canali *et al.*, 1987; Turner *et al.*, 1987). The suggestion has been made that increased

SLC activity is most likely to be found in hypertensive patients with a family history of both hypertension and cardiac events (Wilson & Meyer, 1981) and the independent effects of these two variables on SLC activity has been demonstrated (Carr *et al.*, 1989). This view is further supported by the demonstration that both hypertension and hyperlipidaemia (both cardiovascular risk factors) are independently associated with high SLC activity (Corrocher *et al.*, 1985) and that the greatest activity is found in individuals with both conditions (Carr *et al.*, 1990). Hence, while SLC may not be a specific marker for hypertension in itself, it may represent a broader marker of cardiovascular risk.

The role of plasma lipids and erythrocyte membrane lipid composition on SLC

SLC activity has been shown to be positively correlated with plasma cholesterol, triglycerides and very low density lippoprotein (VLDL), (Corrocher *et al.*, 1985; Strazzullo *et al.*, 1986; Engelmann *et al.*, 1993) and negatively associated with plasma HDL and HDL: cholesterol ratio (Adragna *et al.*, 1985). Changes in SLC have also been shown to correlate with alterations in plasma triglyceride and HDL levels over a two-year period (Hunt *et al.*, 1990), and a fall in SLC has also been demonstrated with alterations in plasma lipid profile induced by exercise, dietary modification or by therapeutic means (Carr *et al.*, 1991; Rutherford *et al.*, 1992). This is unlike the situation with hypertension where there is a poor correlation between SLC and blood pressure readings and high SLC levels in hypertensive patients have not responded to control of blood pressure (Beuckelmann & Erdmann, 1986). SLC activity may therefore, depend more on the patients' lipid status rather than hypertension although both have been shown to have independent influence.

The mechanism by which plasma lipids influence SLC activity remains unclear. One suggestion, is that a dynamic equilibrium exists between plasma and erythrocyte membrane lipids and that the activity of the transport carrier molecule is influenced by the physico-chemical properties of the membrane including its viscosity (Cooper 1977). However, while SLC activity is increased by increased plasma cholesterol, the converse is true when the erythrocyte membrane is cholesterol enriched either naturally or artificially (Engelmann & Duhm, 1991; Lijnen *et al.*, 1992). SLC activity also correlates positively with plasma triglyceride levels but triglycerides are not normally found in the red cell membrane. The phosphatidylcholine:sphingomyelin ratio of the red cell membrane has been shown to correlate positively with SLC activity, however and this ratio in turn correlates with plasma triglyceride levels (Engelmann *et al.*, 1993). The fact that triglyceride levels may play a crucial role in SLC activity is further supported by an association between a recessive gene and elevated triglyceride and SLC activity (Hasstedt *et al.*, 1994).

SLC and Cardiovascular Disease

SLC activity has been shown to positively correlate with hypertension (particularly in individuals with a family history of same and cardiac events) and hyperlipidaemia both independent risk factors for cardiovascular disease. There is also strong evidence for the role of heredity in determining SLC activity just as in risk of cardiovascular disease (Williams *et al.*, 1987; Hardmann *et al.*, 1992). In addition important positive correlates exist between SLC and body mass index (Trevisan & Laurenzi, 1991), and high alcohol intake (Winocour *et al.*, 1992), both known risk factors for cardiovascular disease.

Convincing evidence exists for an association between elevated SLC activity and Type I / Type II diabetes (Canessa *et al.*, 1992). In particular there appears to be a subset of such patients with a propensity to hypertension and diabetic nephropathy, who have markedly raised levels of SLC (Fujita *et al.*, 1994; Mangili *et al.*, 1993; Viberti & Earle 1992). This is an important observation, suggesting that SLC may serve as a marker for end-organ damage in diabetic macrovascular complications.

High SLC activity is also correlated with the development of left ventricular hypertrophy in hypertensive patients (Yap *et al.*, 1989), showing equally that it may be useful as a marker of cardiac end-organ damage. One study of african-american women found a positive association between SLC, left ventricular mass and insulin resistence before the onset of clinical hypertension and diabetes, suggesting it might serve as a useful predictive marker (Sherif *et al. 2000).* To date little has been reported on the relationship between SLC activity and the development of actual myocardial infarction or cerebral end-organ damage (i.e. stroke) in patients. There is good rationale for suggesting that patients with stroke and myocardial infarction (MI) may have higher SLC activity than controls. The relationship between SLC activity and cardiac (in the form of hypertrophy) and renal end-organ damage in hypertensive patients has already been established. In addition, SLC activity correlates positively with many other vascular risk factors and displays an hereditary component, as does the risk of stroke and MI with a positive family history.

SLC activity has also been shown to be influenced by red cell membrane lipid composition, being increased in membranes with increased phosphatidylcholine: sphingomyelin ratio, and increased micro-viscosity of the red cell membrane is

associated with such changes in SLC activity in hypertensive patients with a family history (Carr *et al.*, 1995). Other studies have demonstrated alterations in the lipid make-up of the red cell membrane in normolipaemic hypertensives associated with increased microviscosity and macroviscosity (Villar *et al.*, 1996). It seems plausible therefore that SLC activity could theoretically at least, be associated with the pathogenesis of MI and certain types of stroke (e.g. lacunar infarction), through associations with altered cell membrane structure and whole blood viscosity.

However it is unlikely that SLC in itself is pathogenic. It's accepted normal range of activity of 0.1-0.39 mMol/L red cells /hr. is dwarfed by the magnitude of other sodium fluxes in the red cell (take for example the Na^+/H^+ amiloride sensitive transporter with activity of 25-60 mMol/L red cells /hr when activated - Corry *et al.*, 1993). Nevertheless however important associations between SLC activity and vascular disease states do exist, and it may be that SLC represents a sensitive indicator of changes in the red cell membrane structure, repeated in other cell membranes such as the kidney or smooth muscle cell. Whether such changes in membrane function or structure are the cause or result of vascular disease is speculative. Nevertheless it would be useful to increase our knowledge of the effect of environmental and medical disease states on SLC and it's kinetics, as this will increase our understanding of likely biochemical mechanisms at play.

Chapter Two

Aims

Chapter two – Aims

Contents:

Section 2.1-1: Introduction

The title of my thesis encompasses the two main objectives of this work, which is in part epidemiological and in part laboratory based. The two objectives have a very different methodological approach, yet are intrinsically linked as they concern the fundamentals of pathophysiology of stroke and how this can be better understood by our knowledge of risk markers for vascular disease. This in turn would help identify those at greatest risk of stroke and, through detailed individual risk factor profiling, lead to effective secondary prevention. In this vein I have set myself two main questions which are outlined below.

Section 2.1-2: Question one

"What are the risk markers for stroke amongst an Irish stroke population?"

In chapter one, I have already discussed why knowledge of such risk markers is important, both in terms of predicting outcome after a stroke and planning strategies for secondary prevention. On a population basis there appears to be differences in risk marker prevalence which can, at least, partly explain geographic variations in stroke incidence. In this regard it would be informative to compare prevalence of risk factors in an Irish population with international figures. Greater knowledge of the prevalence of risk markers in a given stroke population would also, in general, help plan for the resources needed for ongoing stroke care and secondary prevention strategies within that population.

It would also be informative to examine the interaction between risk markers and different subtypes of stroke, as different stroke subtypes have different degrees of

mortality and morbidity and are likely to have different underlying pathological mechanisms as the primary cause. In addition it is clear from the recent advances in stroke treatment and prevention, that best medical practice now requires detailed individual risk factor profiling, and attention to modifiable risk.

With greater knowledge of, the effect of modification of smoking, hypertension, hypercholesterolaemia and diabetes on vascular risk, the benefit of anticoagulation in atrial fibrillation and the advent of successful surgical procedures for carotid artery disease, comes a responsibility to identify these important modifiable risk markers in all stroke patients.

Section 2.1-3: Question two

"Could sodium lithium countertransport (SLC) represent a broad risk marker of vascular disease? ".

The second aim of this thesis is to elucidate the role of sodium-lithium counter-transport activity (SLC) as a marker in ischaemic stroke and ischaemic heart disease. I have already discussed the potential and limitations of SLC as a useful biological marker of vascular risk in chapter one, and have concentrated my work on adding to our knowledge of SLC in the context of vascular disease. In this regard I proposed two further questions:-

"Does smoking affect SLC?"

Smoking is clearly an important risk factor in the genesis of atherosclerotic arterial disease leading to myocardial infarction and stroke. Little is known about this very

important vascular risk factor as a variable in SLC measurement. Another important atherosclerotic risk factor and commonly consumed environmental factor, alcohol has been shown to affect SLC (Adebayo *et al.*, 1994), and it seems plausible that smoking could have a similar effect. The higher carbon monoxide levels in smokers leading to an increased percentage of carboxyhaemoglobin within the erythrocyte could in theory at least, affect this membrane transporter. As this effect, could be acute and transient like that observed with alcohol however, my study sought to determine the rate of SLC in smokers versus non-smokers and to determine the acute effect of smoking on the transporter. In order to limit the effect of variables extraneous to the question at hand, I recruited smoking and non-smoking volunteers from an otherwise "healthy" population of young male doctors working at the hospital. While these are likely to be a select group within the normal population, such an approach is justified in this study of smoking and SLC, given the limitations the assay imposes on the sample size and thus the implausability of a multivariate model of analysis to control for external factors.

"Is there a possible association between SLC activity and development of cardiovascular disease other than hypertension, such as stroke and myocardial infarction ? "

As outlined in chapter one, SLC is independently associated with essential hypertension. In addition there is some suggestion from the available literature, that those with higher SLC rates are those most likely to sustain "end-organ" damage, at least with regard to nephropathy in diabetic patients and left ventricular hypertrophy

(LVH) in patients with hypertension. If this effect could be observed with regard to patients with stroke or myocardial infarction, then SLC could represent a useful broad marker of cardiovascular risk.

In order to answer this question, or at least observe a trend, I proposed to study four groups of patients and volunteers matched as closely for age, sex, and environmental factors as possible. These include patients who had suffered a stroke or MI, hypertensive patients without such end-organ damage, and a control population of healthy volunteers.

Section 2.1-4: Summary

- My thesis is, in part, epidemiologically based and partly laboratory based and aimed at a better understanding of risk markers of vascular disease, and stroke in particular, in an Irish setting.

- The first part of this thesis aims to identify the risk markers for stroke in an Irish population.

- The second part of this thesis aims to increase our understanding of sodium-lithium countertransport (SLC) as a potential marker of vascular disease by asking two questions: " Does smoking affect SLC?" and "Is SLC elevated among people with stroke and myocardial infarction when compared with people without such disease?"

Chapter Three

Methodology

Chapter three – methodology

Contents:

Section 3.1 Risk Markers for Stroke

3.1-1: Design of the Stroke Service.

Stroke unit care has been shown to be effective in reducing the mortality and morbidity of stroke as outlined in chapter one. Organised stroke care may differ in aspects of admission policy and geographical location but all espouse the multidisciplinary approach to patient treatment and rehabilitation. In this regard our stroke service, set up in 1996, is no different. It consists of a consultant-led medical team, nurse specialist, occupational therapist, physiotherapist, speech and language therapist and social worker dedicated to the management of stroke patients. Ideally this team would be full-time dedicated to stroke care but as medical manpower is limited to two consultant sessions, one full-time registrar and one half-time intern in our case, an initial policy of accepting all acute stroke patients irrespective of age, type or severity on 3 out of 5 nights of medical take, has been adopted. All acute stroke patients on these nights have their care taken over by the stroke service the next working day. This accounts on average for two-thirds of all acute stroke patient admissions to our hospital in a year. Where possible these patients are admitted to the Age-Related Health Care ward, which, not only helps define the service geographically, but also 'taps –in' to existing rehabilitation expertise and helps to develop new skills.

The service is under-pinned by a consultant-led weekly ward round in which each patient is fully evaluated. Current neuro-medical issues are discussed with the patient and medical team and therapeutic options explored. The stroke service has the full support of radiological expertise at a weekly meeting in which all computerised

tomography (CT) images and other relevant investigations are reviewed and discussed with the consultant radiologist. This is important particularly, where the cause or nature of a stroke is unclear and where further neuro-imaging, such as magnetic resonance imaging (MRI), may be necessary. In addition important working relationships have been established between other medical specialties, most notably psychiatry of later life, cardiology, gastroenterology and neurology.

Essential to the ongoing treatment and rehabilitation of individual patients', is the multi-disciplinary meeting involving all the stroke-service disciplines, which takes place weekly after the ward round. This meeting allows for inter-disciplinary exchange of information and ideas, so that a holistic approach to the patients' current needs, future goals and discharge planning can be formulated. The timing of discharge to home, where possible, is also an important decision taken at this meeting. Clearly there is a balance to be made, which recognises the financial implications of prolonged hospital stay, and the need for daily rehabilitation input to ensure the best possible recovery.

Discharge home is facilitated, therefore, by an active community rehabilitation programme. In this respect the stroke service has forged important links with the District Care Unit (DCU) which operates a home-delivered physiotherapy, occupational therapy and nursing service to the patient. The service operates primarily for over 65's and is contacted routinely prior to the patients discharge, often accompanying the stroke service occupational therapist on a home visit with the patient weeks in advance of discharge, thus anticipating the equipment and rehabilitative needs of individual patients. We have also established an important

working relationship with the stroke rehabilitation unit at Baggot Street Community Hospital, which unlike the DCU, operates an all-age policy and in addition has medical and speech and language therapy support, which are not available through the DCU. Our service is further enhanced by a weekly out-patient session at which patients' progress at home can be reviewed, and ongoing investigations and results analysed as an out-patient. **Fig.3a**

3.1-2: Data Collection

This was a prospective study of the risk factors for stroke in an Irish patient population over a 3-year period from July 1, 1996 to June 30, 1999. After admission all patients were assessed by the stroke service registrar and details regarding clinical examination, stroke type and severity, computerised tomography (CT) of brain, previous medical history and known risk factors were recorded on a standard pro-forma (**Appendix.1**). Investigations for underlying risk factors were recorded and the results noted. Patients were followed throughout their hospital stay and any new neuro-medical sequelae of their stroke documented. In addition progress in rehabilitation and eventual outcome were also recorded.

Clinical Stroke Type

Ischaemic stroke type was documented according to the Bamford classification of clinical sub-type on initial assessment (Bamford *et al.*, 1991). This classification bases identification of different clinical stroke syndromes on the likely vascular territory in which they would occur. It divides anterior circulation (distribution of internal carotid artery supplying the anterior and middle cerebral arteries) infarction syndromes into

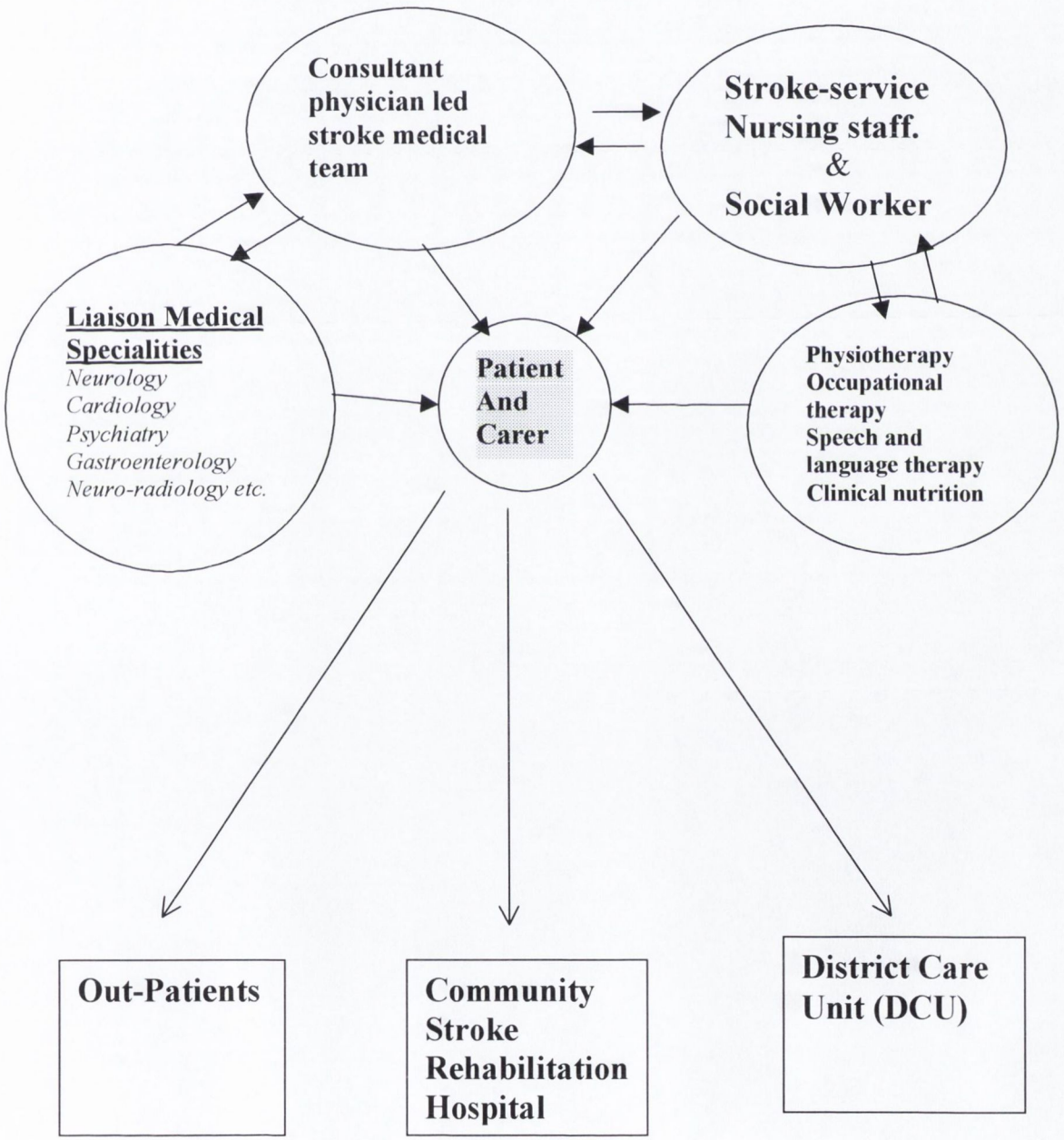
total anterior circulation infarct (TACI) and partial anterior circulation infarct (PACI), while smaller vessel syndromes are classified as lacunar infarction (LACI). All clinical syndromes of occipital lobe, cerebellar or brainstem involvement are classified as posterior circulation infarctions (POCI). [**Appendix 2**].

The classification is useful as it can be clinically applied to all stroke patients initially, (even those with haemorrhage as the primary event which are then subsequently excluded), and identifies the likely anatomical site of lesion prior to availability of CT. It also describes the functional deficit caused by the stroke and to a certain degree, helps identify the likely underlying pathological mechanism causing the stroke. For example a TACI is usually caused by total or near total occlusion of the middle cerebral artery or large branch thereof, and such strokes are usually embolic in nature, the thrombus originating in the chambers of the heart, or from an atherosclerotic plaque in the ascending aorta or carotid artery. Conversely a LACI is typically caused by occlusion of a small perforating artery supplying the region of the internal capsule, and would be more in favour of hypertension or diabetes causing small vessel disease, as the chief aetiological factor.

Radiological Imaging

CT brain (model: Toshiba; Xpress Gx) was performed as soon as possible after admission and reviewed by a consultant radiologist in conjunction with the medical team at a weekly radiological conference. Thus all strokes were also described according to radiological appearance as primary intracerebral/intraventricular haemorrhage, sub-arachnoid haemorrhage, cerebral infarction, normal or "other cause" (such as tumour) where this was evident. All normal CTs in patients, with a

Fig. 3a: Schematic representation of the stroke –service at the Adelaide and Meath Hospital Dublin.



history of an acute onset of a neurological deficit in a pattern consistent with an insult to a vascular territory, and which lasted more than 24 hours were presumed infarcts. This is not uncommon as the changes of infarction, particularly smaller insults, often take several days to manifest themselves radiologically on CT and, if done "too soon", a CT will appear normal in the presence of evolving infarction. Several studies have shown a greater infarct visibility on CT with larger lesions and increased interval from stroke onset. At 48 hours post-stroke approximately half of all infarcts will be detectable on CT (Warlow *et al.* 1996.b).

A repeat examination was not undertaken unless clinically indicated by deterioration, development of new neurological signs etc. Haemorrhage, however, will show up acutely in almost every case of clinical stroke and is the chief reason for seeking an early CT, as the presence of haemorrhage will greatly affect subsequent management of the patient. In the presence of a normal scan and where a clinical doubt existed as to the vascular aetiology of a deficit, a Magnetic Resonance Imaging (MRI) scan was obtained.

Stroke severity and outcome

A number of scales have been validated for quantifying stroke severity. Most are based on a combination of neurological and functional assessment. In our service, stroke severity was quantified by using the modified Barthel index of activities of daily living (BADL) at day 10-14. The BADL is a numerical score, which is based on patient function in the context of everyday activities such as, washing, dressing, feeding etc. (**Appendix 3**). It grades ability in each of the functional activities as

unable, able with assistance or independent. In its' modified form, a total score of 20 indicates independence in all activities of daily living, with lesser scores indicating mild, moderate or severe dependency ratings. It has been validated as a reproducible index of disability and in the setting of stroke as an indicator of severity (Wade & Collin 1988; Collin & Wade 1988)). The BADL has usually been performed in this setting at an interval of 1-2 weeks after admission, in order to allow the patient adapt to the new surroundings of the hospital ward, overcome the acute effect and malaise of the stroke, and to be properly assessed in all the listed activities. For these reasons the BADL was assessed in all patients at days 10-14 post stroke and again at 6 weeks (or discharge from hospital if sooner) to monitor rehabilitation progress and recovery. The BADL does have its limitations, however, and the exact nature of neurological deficit caused by a stroke is not described.

Because of such limitation, the Scandinavian Stroke Neurological Scale (SSNS) was introduced into our assessment from year three onwards. This was performed at admission and discharge on all patients to supplement the BADL in describing both initial stroke severity and final outcome after rehabilitation. The SSNS is a numerical scale, which assesses patients' state of awareness, cognition, limb and facial muscle function. It gives a total score of 58 indicating normal, to zero indicating coma with compromised brainstem function. The lower the score the more severe the stroke. It is validated for assessing the severity of all strokes irrespective of aetiology, and is commonly used in clinical trials of stroke treatments as a baseline measure of severity and to monitor changes in patient condition (Scandinavian Stroke Study Group 1985).

Stroke outcome was assessed by a grading system of 1-5 approximating the Oxford Handicap Scale itself a modification of the Rankin scale (Rankin 1957). This scale basis degrees of handicap on patients symptoms, dependency and lifestyle and is widely used because of its relative simplicity and reliability, even when assessed by over-the-phone interview (Bamford *et al.*, 1989; Candelise *et al.*, 1994). In this system grade 1 indicated a return to home with independent function and grade 2 a discharge home with need of support for some activities of daily living. Grade 3 and 4 indicated a need for long-term care with the former meaning preservation of some mobility and functional ability (generally referred to as "light" long-term care) and the latter implying total nursing care and bed-ridden dependency (generally referred to as "heavy" long-term care). Grade 5 indicated mortality. While this scale was easy to use as an outcome measure it does mix function and placement. While placement considerations were generally based on functional outcome, other factors such as pre-existing morbidity, social circumstances etc. could influence decisions independently of functional recovery from stroke. Outcome applied to the index event only and therefore was assessed as discharge grade or death.

3.1-3: Documenting Risk Markers for Stroke

The risk markers for stroke were defined on the basis of the commonly accepted major factors that increase the relative risk of cerebral infarction and haemorrhage. These included in the case of infarction, valvular heart disease and atrial fibrillation, previous stroke or transient ischaemic attack (TIA), history of myocardial ischaemia or infarction, carotid artery disease, hypertension, diabetes mellitus and smoking. Other possible risk factors for stroke such as hyperlipidaemia and excessive alcohol intake were also documented. In the case of cerebral haemorrhage, hypertension, a

history of clotting disorder, concurrent use of anticoagulants and antiplatelet agents, history of arterio-venous malformation or family history of intracerebral / subarachnoid haemorrhage were the major risk markers sought. Where a risk marker was already known from the patients' history, this was documented. All subsequent investigations were performed on the basis of clinical judgement and protocol aimed at uncovering major factors contributing to the risk of stroke. These investigations can broadly be grouped under routine bloods, cardiac and vascular.

Initial investigation

All patients on admission had routine bloods taken for full blood count, coagulation screen (prothrombin time and activated partial thromboplastin time), erythrocyte sedimentation rate (ESR), renal, liver and bone profiles. Diabetes mellitus was excluded by two fasting blood glucose tests, a week apart, < 7.0 mMol/L (American Diabetes Association 1997). Where a result was equivocal a formal glucose tolerance test (fasting < 7.8 mMol/L and two hour post-prandial < 11.1 – WHO 1980) and haemoglobin A_{1c} was performed to establish the diagnosis. Hyperlipidaemia was excluded by a fasting lipid profile done within 48 hours of the event, to minimise the possible reduction effect of a vascular event on total cholesterol levels, although such changes post stroke are less pronounced than is the case with myocardial infarction (Kargman *et al.*, 1998). Reference ranges complied with the local department of clinical chemistry guidelines of total cholesterol ≤ 5.2 mMol/L and low density lippoprotein (LDL) ≤ 3.6 mMol/L ((Laboratory Standardisation Panel of the National Cholesterol Education Program 1998). This may be 'generous' in terms of more recent guidelines recommending levels of total and LDL cholesterol to be below 5 and 3.0 mMol/L respectively in those with co-existing cardiovascular disease

(Recommendations of the Second Joint Task Force of the European and Other Socities on Coronary Prevention 1998), but in practical terms this made little difference to numbers identified as hyperlipidaemic in this study.

Apart from these routine tests, thrombophilia (e.g. Anti-phospholipid syndrome, Factor V leiden, Hyperhomocysteinaemia) and vasculitic (Systemic Lupus Erythematosis, Systemic Rheumatoid Arthritis etc.) screening was performed for known risk factors where there was a clinical suspicion (usually in the case of relatively young female patients, where a paucity of vascular risk factors to explain the stroke existed).

On admission all patients had blood pressure and heart rate measured by nursing staff and a chest x-ray, electrocardiogram (ECG) and cardiac auscultation performed. The ECG was read, by stroke service registrar, for evidence of atrial fibrillation, myocardial ischaemia or hypertrophy indicative of longstanding hypertension. Myocardial ischaemia was defined as a history of current or previous infarction or angina and on ECG according to the Minnesota codes, as presence of pathological Q-waves (1.1-1.3), ST segment depression (4.1-4.4) or the presence of T-wave changes (5.1-5.4) when compared to previous ECG's (Prineas *et al.*, 1982). Ischaemic heart disease was thus defined by ECG appearance or a history of previous or current angina or myocardial infarction.

If the patient was in sinus rhythm a 24- hour "holter" monitor (Reynolds Pathfinder model 213) was put in place to exclude the possibility of intermittent atrial fibrillation. This portable device electronically records the patients heart rhythm on a continuous basis and automatically selects variations from sinus rhythm on

subsequent computer analysis. Presence or absence of atrial fibrillation was confirmed by analysis of the "holter" in the Department of Cardiology at the Adelaide and Meath Hospitals Dublin incorporating The National Children's Hospital.

In addition where a patient had an audible heart murmur, history of rheumatic heart disease, recent myocardial infarction or significant left ventricular dysfunction clinically or radiologically, a trans-thoracic echocardiogram (Acuson Sequoia c256) was performed by the department of cardiology, to evaluate any possible valvular lesion or mural thrombus.

While in hospital patients had their blood pressure measured and recorded twice daily by professional nursing staff with a calibrated mercury sphygmomanometer (Accuson mercury sphygmomanometer). Hypertension was defined as use of concurrent anti-hypertensive medication or where there were persistent readings of systolic pressure $\geq$ 160 mmHg or diastolic $\geq$ 90 mmHg in patients, beyond the acute-phase response readings. These values were chosen as most of our patients were elderly, clinical evidence exists for the efficacy of treating isolated systolic blood pressure at this level in this age group and because it is one of the most commonly cited ranges for hypertension in studies of risk factors in stroke (WHO MONICA project 1997; ARIC investigators1989; Kalra *et al,* 1998). Since commencing this study however, WHO guidelines have recommended lower target levels for systolic blood pressure (WHO-International Society of Hypertension, 1999), though these have not gained universal acceptance (O'Brien & Staessen, 2000).

Where clinical suspicion existed, either from the patients' history of a pre-existing problem, the presence of a bruit on auscultation (though generally accepted as a poor marker), a clinical pattern of previous strokes or TIA's in the same vascular territory,

or absence of any more obvious risk marker, formal carotid artery duplex doppler examination was performed (model: ATL; HDI 5000). In practical terms this was carried out on the majority of patients, as resources allowed, by the Department of Radiology at the hospital. Doppler ultrasound is a non-invasive and reliable technique for assessing the degree of stenosis, if any, in the carotid artery. Significant stenosis was taken as stenosis $\geq$ 70% of the internal carotid artery, in line with evidence showing the benefit or operative intervention on this degree of stenosis (European Carotid Surgery Trialists Collaborative Group 1998).

Data was compiled on the standard pro-forma, prospectively during the patients' admission and completed on death or discharge. Initially this has been a written exercise requiring data manipulation by hand. I have subsequently with the aid of the Dept. of Community Health & Statistics, Trinity College Dublin, designed a database on Microsoft Access © (Microsoft corp., USA) to allow for easier archiving and manipulation of the on-going data collection.

Section 3.2 Determination of Sodium-Lithium Countertransport (SLC)

A proposal outlining the study design and objectives was submitted to the Joint Research and Ethics Committee of the Federated Dublin Voluntary Hospitals and ethical approval was obtained before commencing work. A written consent form was supplied to all patients and volunteers based on the standard ethical guidelines set out by the committee.

Section 3.2-1: Patient Details and Blood Sampling:

A detailed medical history was taken from all participants with regard to personal and family history of stroke, cardiovascular disease and hypertension. Any use of medication was recorded and personal tobacco consumption was also noted.
Patient weight and height (where practical) was recorded. A blood pressure reading was taken on 3 occasions, five minute apart, in a sitting position prior to phlebotomy using a calibrated mercury sphygmomanometer (Accuson). The average reading was recorded.

SLC activity exhibits diurnal variation and is affected by plasma lipid levels. To standardise results, all samples were taken with the participant fasting, from midnight the night before, and between 08.00 –10.00. A sample of venous blood (approx. 25 mls) was taken into three 7ml sodium-heparin tubes (Becton & Dickinson) for SLC determination and one 5ml Lithium-Heparin tube (Becton & Dickinson) for fasting lipid profile analysis. All samples were processed within 1-2 hours to determine SLC activity at the research laboratory, Department of Pharmacology & Therapeutics, Faculty of Health Sciences, St. James' Hospital Dublin. Fasting lipid profile

measurement was carried out by the Department of Biochemistry at the Adelaide and Meath Hospital Dublin, incorporating The National Children's Hospital.

Section 3.2-2: Sample Preparation for SLC Determination.

Washing the red cells

Blood samples was transferred to a 50ml Falcon tube and centrifuged at 2,500 rev/min at 4^0C for 10 minutes in a Sorvall RT600 refrigerated centrifuge (DuPont). Plasma and buffy coat were removed by pipetting and the sample was washed 5 times in a NaCl-free washing solution of physiological osmolarity (290-310 mOsm) and pH (7.4 at 37°C). The aim of this procedure is to ensure that plasma and its constituents are adequately removed from the red cells.

The washing solution comprised 140mMol Choline chloride, 10mMol glucose, 4mMol Magnesium chloride, 10mMol Tris and 10mMol MOPS. With all solutions used in this study osmolarity was checked on an 'Advanced Micro-Osmometer Model 3300 (Advanced Instruments Inc., USA)' and pH on a 'Mettler Toledo MP230 pH meter (Mason technology).

Lithium Loading

After washing the sample of red blood cells was incubated in a Lithium loading solution in order to achieve intracellular levels of between 6-10 mMol Li^+ /L cells. This is the generally accepted value of intracellular cation to ensure a valid assay (Canessa, 1989). Lithium solution saturates the internal binding sites for the cation with a Km of 0.5mMol/Lcell (Sarkadi *et al.*, 1978) and it is important that this value

is exceeded by 10 fold. This is to ensure complete saturation of the transporter binding sites throughout the kinetic experiment, as sodium-stimulated lithium efflux will be superimposed on the passive lithium efflux along its concentration gradient. To ensure a valid assay therefore, it is important that the internal binding site for lithium remains saturated in the face of the unavoidable passive 'leak'.

To achieve this adequate level of lithium-loading a number of methods have been used. The most efficient method involves loading with lithium carbonate solution, and intracellular levels of 5 mMol/L cells has been reported within 20 minutes (Ibsen *et al.*, 1982; Jensen *et al.*, 1990). In this solution Li^+ ions enter the red cell passively along its concentration gradient and also in ion pair formation as $LiCO_3^-$, through the anion channel. The method is complicated however by the need for a thorough washing of the red cells to ensure removal of the carbonate ion and avoid a change in intracellular pH that would otherwise result. Such a change would affect the transporter activity, being stimulated by extracellular alkalosis and inhibited by acidosis. This occurs at lower pH due to extracellular H^+ competing with Na^+ ions for binding sites and transporter activity falls off dramatically at pH below 7.4. The converse is also true in that intracellular acidosis stimulates activity (Canessa *et al.*, 1988).

An alternative approach is to incubate red cells in a Lithium chloride solution of pH 7.4 for 3 hours at 37°C. Lithium loading occurs by passive diffusion of lithium ions along the concentration gradient and also through the action of the Na^+/K^+ pump. This was the original method used by Canessa (Canessa *et al.*, 1980), and has also been used by many other workers (Mangili *et al.*, 1988; Walker *et al.*, 1990).

In this study lithium loading was achieved using a combined lithium carbonate and lithium chloride solution, utilising the efficiency of the former approach without undue pH change by encompassing the latter. This method has already proven to be effective (Adebayo 1995). The loading solution 140mMol Lithium Chloride, 10mMol Lithium Carbonate, 10mM glucose, 10mMol MOPS, 10mMol Tris, at physiological osmolarity (290-310 mOsm) and pH 7.4. Approximately 7mls of washed red cells were added to 25 mls of lithium loading solution in a falcon tube. Incubation was for 2 hours at 37°C in a water bath with gentle agitation (Grant Instruments, Cambridge Ltd.).

After loading the lithium rich red cells were washed with washing solution and centrifuged for 10 minutes at 2,500 rev/min at 4°C and the supernatant removed. This step was repeated 5 times to ensure adequate removal of extracellular lithium prior to the kinetic study. A Li^+ level of 30uMol/L or less in the supernatant of the final wash has been recommended to meet this criterion (Canessa *et al.*, 1980). After the final wash a 2ml sample of supernatant was kept and subsequently measured to ensure this standard was met.

Kinetic Study

After washing a 500 µl aliquot of lithium rich red cells was deproteinised by addition of 1ml of 10% trichloroacetic acid in an eppendorf tube and vortexed. This was then centrifuged at 990*g* for 3 minutes in a model 5415C Eppendorf centrifuge and the supernatant removed for determination of intracellular cations.

Analysis of $Li^+/K^+/Na^+$ ions was performed by flame photometry (Instrumentation Laboratory 943) in the Department of Biochemistry, Adelaide and Meath Hospital

Dublin incorporating The National Childrens Hospital. A 1 ml sample of the washed red cells was also a taken for determination of haematocrit and red cell count by the Department of Haematology, St. James' Hospital Dublin. Calculation of intracellular $Li^+/K^+/Na^+$ was by the method reported by Fortes-Meyer & Starkey (1977).

The remaining washed red cells were then made up to a 50% solution with washing solution. From this a 1ml sample was added to 5ml solutions containing zero, 35, 50, 70, 100, 140 mMol/L of Na^+. The Na^+ free sample was of the same constitution as the washing solution except in addition it contained 0.1mMol ouabain to inhibit the Na^+/K^+ ATP pump. The constitution of the Na^+ rich medium was140mMol NaCl, 10mMol glucose, 4mMol Magnesium chloride, 10mMol Tris, 10mMol MOPS and 0.1 mMol ouabain. Again solutions were of physiological osmolarity (290-310 mOsm) and pH (7.4 at 37°C). The process of preparation of samples is summarised in **Fig. 3b.**

The six solutions were incubated at 37°C with gentle agitation and at 20, 40, and 60 minutes 1.5 ml aliquots were drawn, cooled on ice for 2 minutes in eppendorf tubes and then centrifuged at 990g for 2 minutes. 1 ml of supernatant was then pipetted into fresh, labeled eppendorf tubes and stored at 4°C for lithium determination. After kinetic sampling a 1.5 ml sample was taken from the efflux media and the red cell count and haematocrit determined. This value was used in later calculations to express the SLC value per litre of red cells.

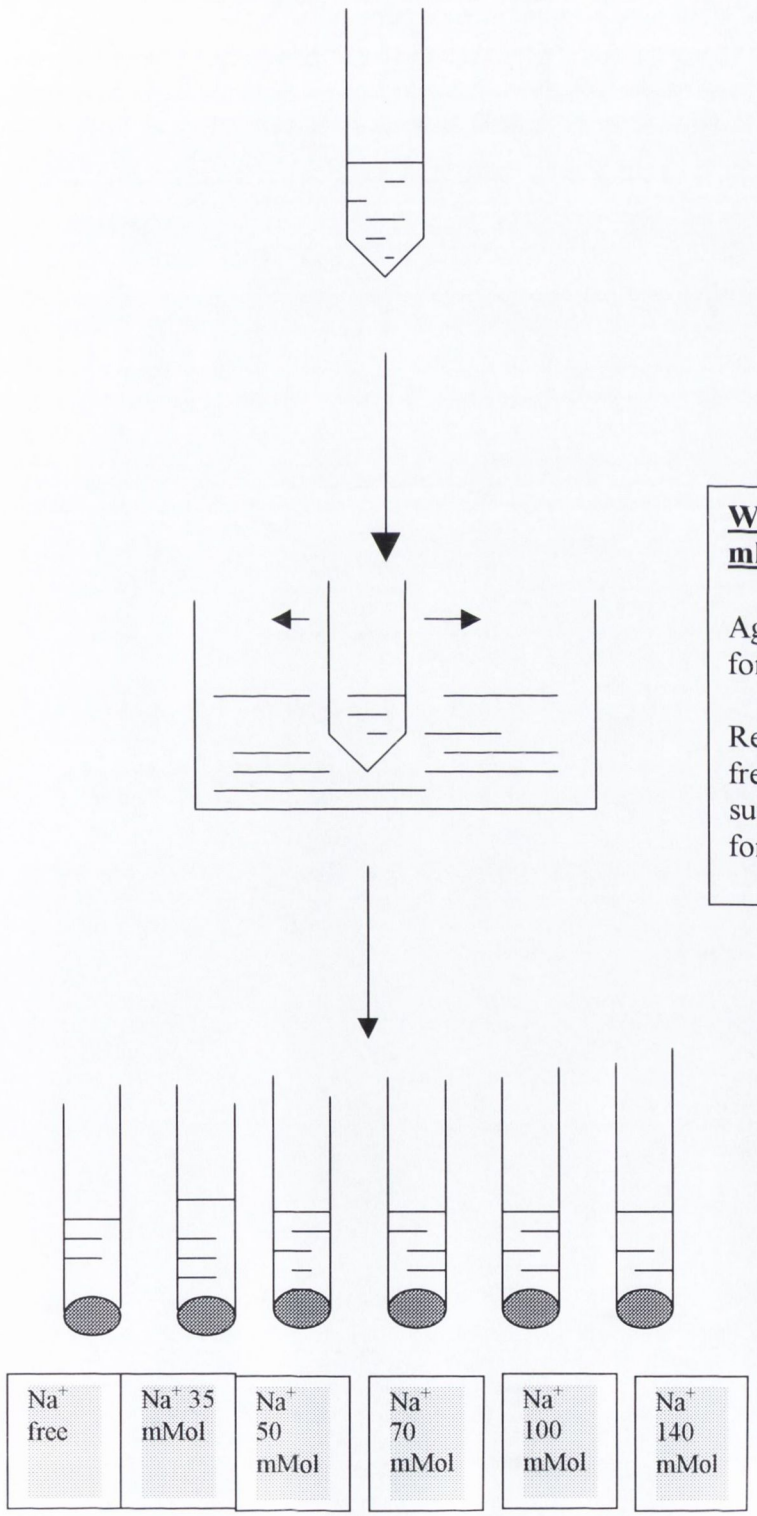
Determination of lithium concentration

Final determination of Lithium levels in the supernatant of the final wash and kinetic samples was carried out on a Model IL257 Atomic Absorption spectrophotometer in

the Department of Geology, Trinity College Dublin. The machine was set at a suction rate of 5.0 ml per minute with a current of 5mA. Lithium content of the supernatants was determined at a wavelength of 670.4 nm and a bandwidth of 0.5. Potassium chloride (KCl) was added to all standards and samples to achieve a concentration of 2mg/ml prior to analysis, to prevent the ionisation of lithium. To prevent unnecessary dilution of the samples this was achieved using a highly concentrated solution of KCl (160mg/ml), so that 50µL of KCL solution added to the 1ml samples would provide the required K^+ ions.

The machine was firstly calibrated with standards of 14.5, 29, 58 and 87 µmol Li^+/L in efflux media. From this a standard curve was obtained and all subsequent readings were translated into lithium ion concentration by reference to this standard curve. After each five samples the standard solutions were re-read to minimise the effect of 'machine drift'. Throughout these experiments the maximum observed drift was 5%.

Fig. 3b: Schematic representation of sample preparation for SLC assay.



Section 3.2-3: Calculation of SLC

Fundamentally SLC is the difference in lithium concentration at 60 minutes, between the sodium free and sodium rich (140mMol/L) efflux media. Expressed another way, it is the difference between passive diffusion and passive diffusion + sodium stimulated lithium efflux at 60 minutes.

Conventionally it is expressed in mMol Li^+ per litre cells per hour (mMol Li^+/l cells/hour), thus relating the rate of lithium efflux to the volume of red cells from which it was extruded. Two principal methods exist by which this calculation is achieved. The original method of Canessa & Tosteson (1979) and the method used in all these experiments, involved a correction factor (Fa) based on direct measurement of the volume of red cells from which the Li^+ was extruded. Thus the value of lithium in the supernatant at each time point in µMol/l solution is converted into µMol/l cells by multiplying with this factor Fa.

$$Fa = (1 - Hct \times d/c) / (Hct \times d/c)$$

Where Hct. is the haematocrit of the washed lithium-loaded red cell suspension added to each efflux medium, 'c' its' haemoglobin concentration and 'd' the haemoglobin concentration of the lithium-loaded red cell suspension in each efflux medium.
This can be calculated as such or alternatively "Hct x d/c" in the above equation actually represents the haematocrit of the lithium-loaded red cell suspension in each efflux medium and can be measured directly in the laboratory. This is what was done in all these experiments.

Values of lithium concentration multiplied by Fa were then plotted against the time points 20, 40, and 60 minutes and the value at 60 minutes determined from the equation of the line obtained. This in turn was converted into mMol/l cells /hour by dividing by 1000. The difference in values so obtained between the sodium free and sodium rich (140 mMol/l) solutions represents the SLC value for that experiment.

There is another principal method (Smith *et al.*, 1982) which seeks to estimate the final red cell volume, given a known volume of washed lithium-loaded red cells of known haematocrit, added to a known volume of efflux medium. In this method lithium values in the supernatant of each efflux medium (expressed in μMol/l solution) were plotted against sampling time. The slope of this plot (μMol//l solution/minute) was then converted into the conventional unit of mMol/l cells/hour by multiplying with a factor (F) where

$$F = (a + b / a \times Hct.) \times 60 \times .001$$

Where 'a' is the volume of washed lithium-loaded red cell suspension of haematocrit ('Hct.') added to volume 'b' of each efflux medium.

While both methods have been used in the literature, that of Canessa & Tosteson is likely to be the more accurate given that it calculates rather than estimates the haematocrit of the final red cell / efflux medium suspension. Indeed previous work in this laboratory has shown that both methods, although strongly correlated, do not produce identical results and that the intra-assay and inter-assay variation is smaller with Canessa's method (Adebayo & Feely 1992).

Kinetic Analysis

Like most specific transport processes and enzyme systems in man, SLC obeys saturation kinetics. The first step in such a process is the binding of free catalyst [E] with free substrate [S]. This step can be rate limiting at low concentrations of substrate where the overall rate of transport will be determined by the [E] + [S] interaction, and each ES complex will have time to breakdown before another forms. Alternatively the rate of transport will be enhanced at high concentrations of [S] where all available catalyst sites are bound to. In essence the process of transport requires the breakdown of interacting complexes, the rate of which will be proportional to the concentration of [E] and [S] and therefore cannot exceed a maximum rate when all the carrier is complexed. Systems that obey saturation kinetics characteristically give a curved plot of velocity (v) against substrate concentration [S] and conform to the Michaelis-Menten equation:

$v = Vmax.[S] / (Km + [S])$.

In order to allow for a more detailed analysis of the SLC result obtained, it is necessary to determine the Vmax. and Km of the transporter. The Vmax. is the theoretical rate at which the transporter would operate at an infinite substrate concentration and as such can only be determined by graphic extrapolation. It does serve however, as a measure of the rate of transport molecule movement, in this case across the red cell membrane. The Km is the concentration of substrate at which the velocity of the transporter divided by Vmax. = 0.5, and is a measure of the affinity of the transporter for binding its substrate, in this case external sodium. Thus for example a dramatic increase in SLC value could be mediated by an increase in Vmax.

and thus increased rate of carrier molecule movement across the red cell membrane, or alternatively by a decrease in Km and hence greater saturation of substrate binding sites at lower concentrations of external sodium. Obviously the converse also applies.

Data conforming to the Michaelis-Menten equation can be linearised using a Hanes-Woolf plot. So that for SLC the following equation is valid

$$SLC = Vmax.[Na^{+}_{0}] / Km + [Na^{+}_{0}]$$

Where SLC is as defined previously and corresponds to velocity (v), and $[Na^{+}_{0}]$ is the concentration of sodium corresponding to substrate concentration [S]. Using the linear form of this equation chosen for data analysis i.e. Hanes-Woolf plot, this equation is transformed thus:

$$[Na^{+}_{0}] / SLC = [Na^{+}_{0}] / Vmax. + Km /Vmax.$$

It follows therefore that a plot of $[Na^{+}_{0}]$ / SLC against $[Na^{+}_{0}]$ yields a straight line graph whose slope is 1/Vmax. and intercept Km / Vmax. Thus a graph of sodium concentrations divided by SLC versus the sodium concentration allows us to calculate the Vmax. and Km and hence the reason for determining the SLC value at various concentration of sodium

Section 3.3: Summary of chapter three

- The design of the stroke-service at the Adelaide and Meath Hospital Dublin is described. All patients admitted to the service had data prospectively collected on a standard pro-forma. This data included information on patient medical history, stroke type and severity, investigations and patient outcome. Stroke type was classified according to the Bamford classification in the case of cerebral infarction and by computerised tomography appearance in cases of haemorrhage. Severity was assessed using the Barthel Index of Activities of Daily Living at day 10-14. Outcome was categorised by a Modified Rankin scale. Investigations were aimed at uncovering major risk markers of acute stroke relevant to the individual clinical situation, and utilised standard hospital equipment as described.
- The method of SLC assay is described. All subjects had standardised sampling of blood for the SLC assay and lipid determination between 08.00 and 10.00 in a fasting state. Preparation of the red blood cells and lithium-loading procedures followed that reported in the literature with modification by work carried out previously by investigators at our intstituion. Calculation of SLC was by the method of Canessa and utilised different sodium concentrations to allow for a more in-depth kinetic analysis of the transporter.

- SLC obeys saturation kinetics and therefore conforms to the Michaelis –Menten equation. This data can be linearised by a number of acceptable plots including Hanes-Woolf, which was used in these experiments. This enables the plotting of data in linear form of which the slope represents 1/Vmax and whose intercept represents Km /Vmax. This allows to determine not only the absolute value of SLC but also the transporter activity (represented by Vmax.) and its affinity for the substrate it transports (Km).

Chapter 4

Results I

Chapter four – Results I

Contents:

4.1 : Three year audit of the stroke service:

4.1-1 Introduction

Stroke unit care has been shown to reduce mortality and improve outcome as outlined in chapter one, section 1.1. In light of this finding our stroke service was set up in 1996 with the aim of defining stroke care through an organised dedicated multidisciplinary team. It was envisaged that by organising, largely existing resources, into a cohesive unit that stroke care could be improved through implementation of treatment protocols, regular multidisciplinary team meetings, improved liaison with other medical specialities and through the forging of strong relationships with community-based rehabilitation teams. The design of this service has already been discussed in chapter three, section 3.1.

4.1-2 Objectives

The initial objective of this three-year study was to audit the stroke-service and compare patient demographics, stroke subtypes and mortality figures from year to year and compare such data with that already published in the international literature.

4.1-3 Methodology

This has been outlined in chapter three, section 3.1. In brief all strokes referred to the stroke service over a three-year period had data concerning, age, sex, stroke type and severity, length of hospital stay and outcome recorded on a prospective basis on a standard pro-forma. This information was then entered on a computerised database (Microsoft Access ™) and expressed as mean figures ± standard deviation for

variables. A comparison of mortality, length of stay and rates of institutionalisation was made from year to year by chi squared analysis using JMP software on an IMAC© computer (Apple Macintosh).

4.1-4: Results

One hundred and ninety-three patients, with a clinical picture of acute stroke, were admitted to the stroke service over a three-year period. On CT appearance 79 % had cerebral infarction and 16% had intracerebral haemorrhage, 5% were of other aetiology (tumour, sub-arachnoid haemorrhage, multiple sclerosis). Patient demographics, stroke type and severity, and patient outcomes are summarised for the individual years below:

Year 1:

Thirty-one patients were admitted to the service with acute stroke, 42% were men. CT brain was consistent with infarction in 25 (81%) patients (with haemorrhagic transformation in one patient); 6(19%) had primary intracerebral bleeds. One patient with intracerebral haemorrhage was found to have a co-existant cerebral tumour. Mean BADL at day 10-14 was 8.33 $\pm$ 7.32. Of this population 17(55%) were discharged home independently or with support; 8(26%) required nursing home care and 6(19%), excluding tumour, died. The mean length of stay was 34 +/- 26 days.

Year 2:

Seventy-two patients with acute stroke were admitted to our service of which 57% were men. CT brain was consistent with infarction in 49(69%) of patients (two of whom had haemorrhagic transformation); 20(27%) had primary intracerebral

haemorrhages, while 3(4%) patients had evidence of a tumour. Mean BADL at day 10-14 was 9.5 $\pm$ 7.67. Of this population 46(64%) were discharged home independently or with support; 9(12%) required residential long-term care; 4(6%) required nursing home care; 2 (3%) patients were transferred to neurosurgery at another hospital and 11(15%), excluding tumour, died. The mean length of stay was 31 $\pm$ 27 days.

Year 3:

Ninety patients with acute stroke were admitted to the stroke service of which 51% were men. Among this population, 79(88%) patients had a CT consistent with infarction (one with haemorrhagic transformation), 9(10%) had primary intracerebral bleeds, there was one case (1%) of sub-arachnoid haemorrhage, and one case of acute neurological deficit was found to have probable multiple sclerosis on further evaluation. Mean BADL at day 10-14 was 11.2 $\pm$ 6.89.

Of this population 61(68%) were discharged home independently or with support; 1 (1%) patient required residential long-term care; 18(20%) required nursing home care; 2(2%) patients were transferred to neurosurgey at another hospital and 8(9%) died. The mean length of stay was 34 $\pm$ 33 days.

4.1-5 Analysis

Kruskal-Wallis chi-squared analysis was performed, using JMP software, for stroke severity (BADL), death, institutionalisation and length of stay from year to year. There was no significant difference in stroke severity from year to year ($p=0.082$) although a trend towards less severity did exist which was significant at the 10% level. While there was no significant difference in mortality observed from year to year ($p=0.209$), there was a trend towards less people needing institutional care.

Again this was not significant at the 5% level (p=0.098) although was at a 10% level. This was also encouraging given that there was no significant difference in length of stay from year to year (p=0.547).

4.1-6 Discussion

A number of trends emerge from this audit. In the first year the relatively low number of patients admitted to the service reflects the difficulty in changing patterns of admission towards a new service. This had largely corrected itself by the second year as medical personnel within the hospital became more familiar with the service. The dramatic fall in mean age between years 2 & 3 reflects the move of our hospital from it's original city centre site in Dublin 8, where 14.1% of the population were over 65 years to a new suburban site in Dublin 24, with a greater proportion of younger people and only 5% over 65 (From 1996 census of Ireland -personal communication –Dr. J.B.Walsh, MedEl Directorate, St. James' Hospital, Dublin). One might anticipate a fall in stroke admissions accompanying this change in age profile, though this does not appear to be the case (official HIPE data awaited), perhaps reflecting the influence of lifestyle in this largely lower socio-economic population.

Trends in stroke- type also reflect this change of catchment area in our audit. There was a high incidence of intracerebral haemorrhage in years 1 and 2 of 19% and 27% respectively and an overall rate of 16.1% over the three years. This is much higher than international trends. Data from the Malmo stroke registry (Sweden) quoted an incidence of intracerebral haemorrhage of 9.6% (Elneihoum *et al.*, 1998), and data in studies of male patients with first ever stroke has variously quoted the incidence of intracerebral haemorrhage to be 10% in a swedish population (Welin *et al.*, 1987) and

6% in Framingham population (Kannel *et al.*, 1983). The incidence of intracerebral haemorrhage among our male stroke patients over the three years was 21% again much higher than quoted figures (**Table 4.4**). This may reflect a rising incidence of such strokes with age and their predominance in elderly hypertensive patients, given our elderly population in years one and two. Our population was certainly older than the study by Welin *et al.* where patients were recruited at the age of 54 and followed for 18.5 years, although the incidence of all stroke types in this study rose steadily with age. A drop off in incidence of intracerebral haemorrhage to more traditionally accepted rates is seen with the move of our hospital in year 3 (10%), as our catchment population became younger. Alternatively this higher rate of haemorrhage may reflect a bias in referral of cerebral haemorrhage to a specialist service particularly, in the initial years. The high rate of haemorrhage may also account for the slightly lower mean BADL scores and higher mortality in the first 2 years, as such patients are more likely to have impaired consciousness and cerebral oedema at stroke onset. The trend towards improved BADL scores over the three years however, could also reflect improved early treatment of stroke rather than a true reduction in stroke severity.

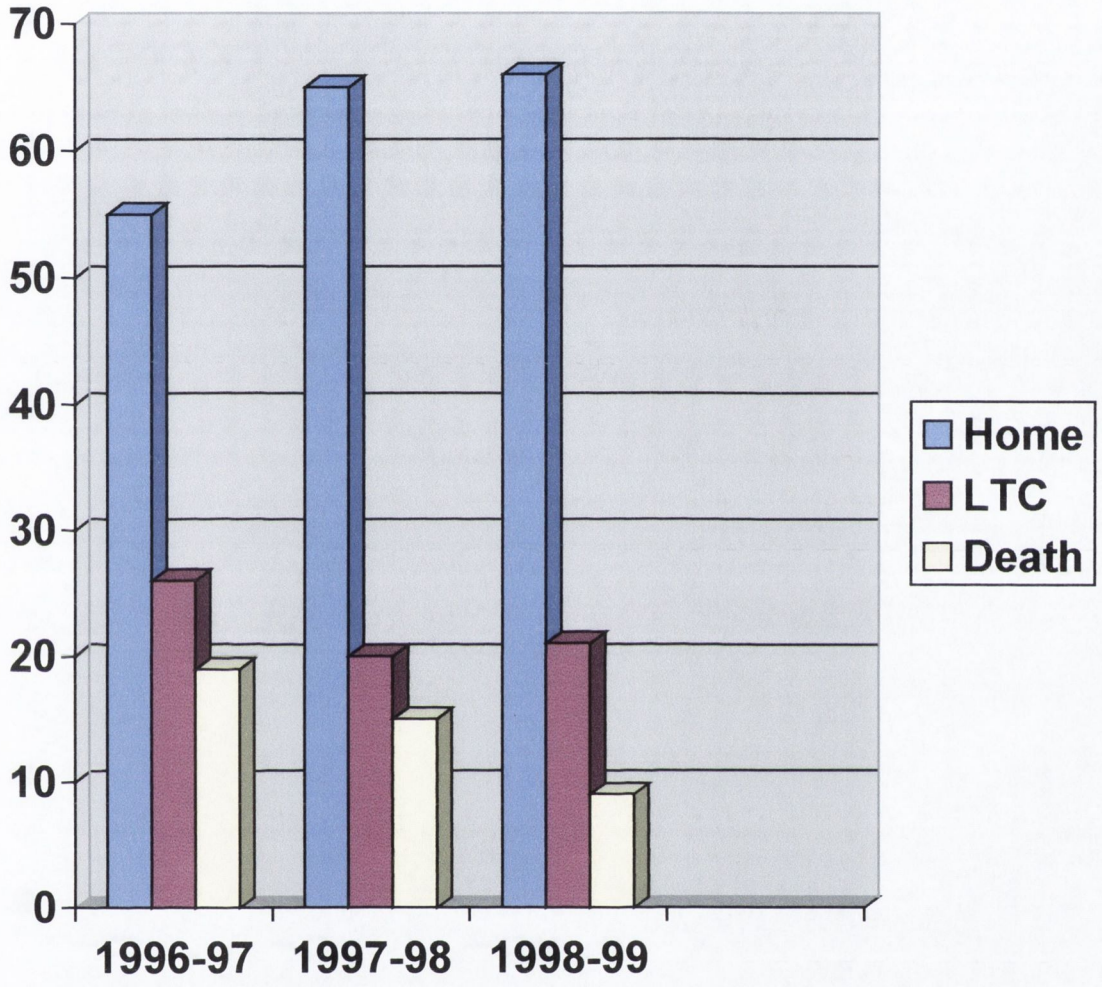
The consistent trend towards lower mortality and improved outcome is encouraging. It is probably multifactorial in nature. As well as a fall in incidence of cerebral haemorrhage and a younger patient population, development of stroke-care skills within the team probably accounts for some of this trend. The total mortality up to discharge or death from all stroke types was 13.5%. This compares well with previously published data on stroke outcome in three Irish general hospitals, which showed a mortality of 26.1 % during hospital stay after the initial stroke (Fan *et al.*, 2000). Most international studies of mortality post-stroke have looked at longer term follow-up, however the Rochester stroke project reported a 30 day (similar to the

mean hospital stay in this cohort) mortality of 17% (Meissner *et al.*, 1988). The Dubbo study of ischaemic stroke in an elderly (defined as greater than 60 years) Italian population, reported a 31% mortality after the index event (Simons *et al.*, 1998) which is much higher than the 11% mortality from ischaemic stroke observed in this cohort. However no data is presented on stroke severity and the case mixes are likely to be different.

It was also heartening to see a genuine increase in rates of patients going home in this audit, and that lower mortality is not merely reflected by increasing demand on long-term care facilities (**Fig. 4a**). Although the numbers requiring residential or nursing home care at outcome did not differ significantly from year to year, there was a consistent trend towards lesser numbers, which was significant at the 10% level. This would appear encouraging, but is difficult to fully analyse given the difference in patient numbers admitted to the stroke service between years 1 and 2, possibly reflecting a bias to referral of the more severe strokes in the initial year of the service. The issue is further complicated by a likely difference in case-mix and the younger population seen with the change in catchment area in year 3. In this regard it can be seen that stroke severity, as reflected by mean BADL at day 10-14, was less from year to year (**Table 4.1**), though there was a wide distribution in all years and it did not differ significantly (p=0.082).

Although a pooled analysis of stroke unit care suggests a non-significant reduction in hospital stay (Langhorne & Dennis 1998) and some studies of organised stroke care point to a reduction in hospital costs (Wentworth & Atkinson 1996), one fear of organised stroke care has been that it would increase length of hospital stay and costs appreciably. Our audit does not support this theory and despite the lower mortality

Fig. 4a: Outcome In Stroke Patients Year By Year.

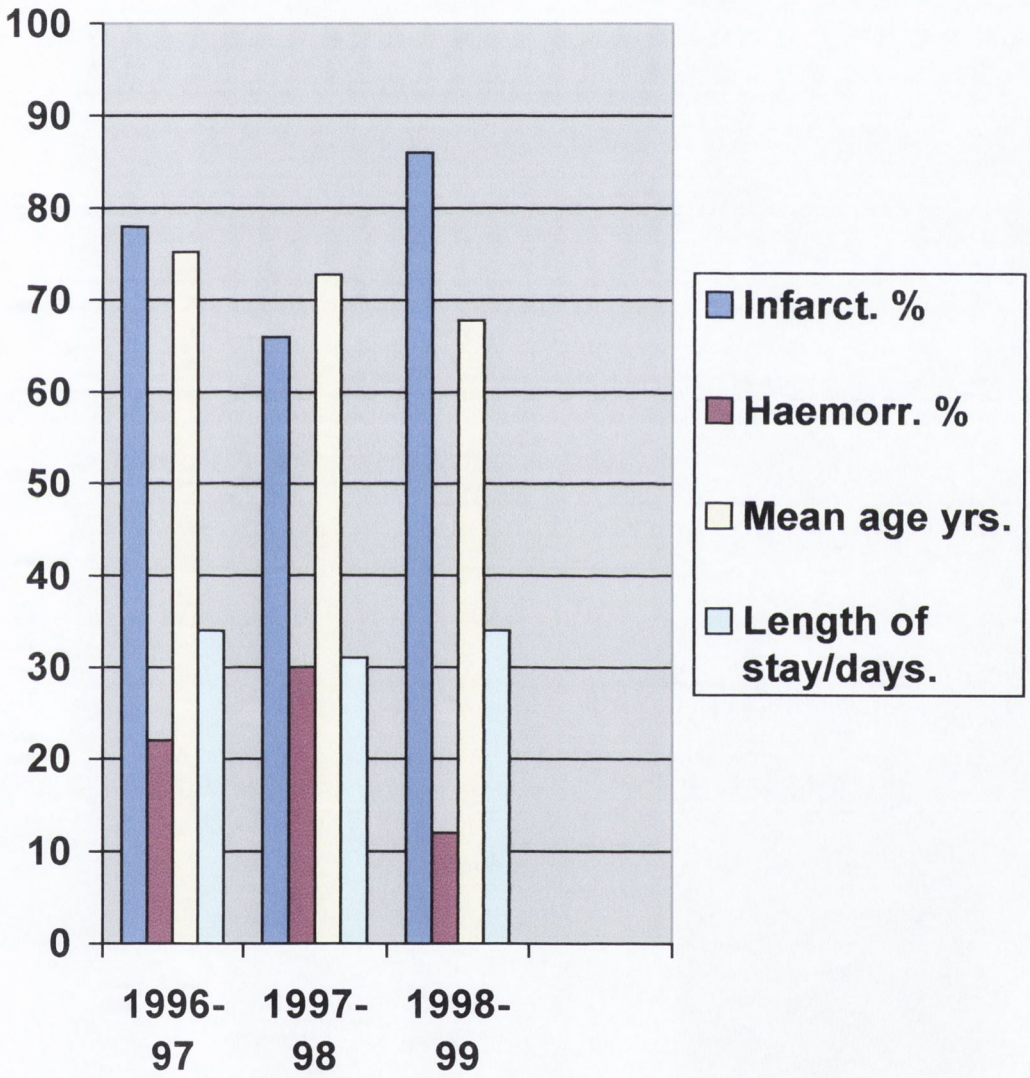


and increasing patient numbers without change in staffing levels, there has been no significant change in length of stay from year to year (p=0.547) **[Fig. 4b]**. This is a limited observation however, both in terms of patient numbers and in changing patterns of stroke aetilogy and age patterns, from year to year in this study.

Our stroke service was developed within an existing model of comprehensive, multidisciplinary acute and rehabilitative geriatric medical care. It is an inclusive "all-age" service, and its efficacy depends on strong links with other disciplines (Anaesthetics, Cardiology, Diabetology, Gastroenterology, Neurology, Neurosurgery, Psychiatry of Later Life and Vascular Surgery) to meet the often, complex challenge of stroke patients.

Our stroke-service is supported by strong links to community based rehabilitation units and visiting multidisciplinary teams. This is important in facilitating early discharge and maintaining patient progress on leaving the hospital setting. At least one randomised controlled trial of organised stroke care ecompassing a home-based rehabilitation team, has shown significantly earlier discharge rates and improved outcome at 26 weeks over hopsital-based stroke unit care alone (Indredavik *et al.*, 2000). While it is easy to understand how such a model would lead to earlier discharge, the factors relating to improved outcome are not clear. The study showed significance at 26 but not 6 weeks, suggesting that at some time post-stroke rehabilitation in the home setting might be more efficacious. This improved outcome has not been borne out by review of other similar 'early supported discharge services' (Early Supported Discharge Trialists, 2000), but strong links with community based services would intuitively, at least, seem important for ongoing patient care.

Fig. 4b: Stroke Type and Length of Stay Year by Year.



This audit shows the developing nature of the service with year to year trends towards lower mortality and improved outcome. Demographic details of patients and stroke severity are summarised in **Table 4.1**.

Table 4.1: Stroke-Service Patient Population and stroke severity 1996-1999.

	1996 / '97	**1997 / '98**	**1998 / ' 99.**
No. Patients	31	72	90
Male / Female	13 / 18	41 / 31	46 / 44
Mean Age (range) (75 th Percentile)	75.2 (50 – 90 yrs.) (75 yrs.)	72.8 (31-90 yrs.) (66 yrs.)	67.8 (35-93 yrs.) (63 yrs.)
Mean BADL @ day 10-14	8.33 $\pm$ 7.32	9.5 $\pm$ 7.67	11.2 $\pm$ 6.89

4.2 Risk markers for stroke

4.2-1 Introduction

a) Cerebral infarction:

Some 200 plus risk factors for coronary artery disease have been described and as our understanding of stroke increases knowledge regarding risk factors has led to greater numbers of reported associations. Many of these associations are causal while others may be confounding and their causal link more difficult to substantiate. It is generally accepted that the primary risk markers for ischaemic stroke are increasing age, previous stroke or transient ischaemic attack (TIA), hypertension, cardiac disease including atrial fibrillation, carotid artery stenosis, diabetes mellitus and cigarette smoking. With the exception of age most are modifiable and hence the importance of

their recognition and treatment in both primary and secondary prevention strategies. Rarer but significant specific risk markers such as arteritis are also important in assessing individual risk though their effect on the population as a whole maybe negligible due to low prevalence.

Other important associations with ischaemic stroke exist but their relationship is less well defined and their causality more difficult to prove. Such associated risk markers include hyperlipidaemia, excess alcohol intake (or conversely total abstinence), hyperhomocysteinaemia, and fibrinogen levels. Data regarding both primary and possibly significant risk markers are presented, with the exception of homocysteine and fibrinogen levels which are not routinely assessed at our hospital.

b) Cerebral haemorrhage

The primary risk markers for cerebral haemorrhage include chiefly hypertension, family history of such events, arteriovenous malformation and use of anti-platelet or anticoagulant agents. Other factors such as cerebral amyloid angiopathy have gained increasing recognition as a cause of cerebral haemorrhage, but are not easy to confirm in the living patient although the pattern of haemorrhage can usually lead to a high degree of clinical suspicion where present (see section 1.1-3). Less common primary causes include arteritis or cerebral neoplasm. Results in this thesis focused on primary identifiable risk markers.

4.2-2 Objectives

The objective of this study is to determine the primary risk markers for cerebral infarction and haemorrhage in an acute stroke patient population. I also sought to

compare the incidence of such risk markers in patients with recurrent versus first-ever cerebral infarction, and the differences in incidence of hypertension between cases of cerebral infarction and haemorrhage.

4.2-3 Methodology

This has been described in detail in section 3.1. In brief all patients admitted to the stroke-service of the Adelaide and Meath Hospital, Dublin over a three-year period had data collected prospectively on stroke type and severity, presence of existing risk factors, investigations aimed at uncovering risk markers and outcome collected over a three year period. This data was collected on a standard pro-forma and subsequently entered on computer database (Microsoft Access ©).

4.2-4 Results

a) Cerebral infarction

Patient demographics

Overall one hundred and fifty-three cases of acute cerebral infarction were admitted to the service over the three-year period. Of these 79 were female and 74 male, of mean age 69.97 years (range: 31 to 93 years). According to clinical stroke subtype 48.8 % were lacunar infarction syndromes, 18.6 and 27.1% were total and partial anterior circulation infarction syndromes respectively and 5.4% were posterior circulation infarct syndromes. Mean BADL at day 10-14 was 10.99 $\pm$ 7.06. Average length of stay was 37.3 days $\pm$ 32.93. Of this population 11.1 % died in hospital and 14.4%

required long-term institutional care, 27.3% of which was residential long-term care with patients remaining mobile, with assistance at least.

Primary risk markers for stroke

Of the recognised primary risk markers for cerebral infarction there was on average 2.66 per patient (range 0-6). Only 4% had no detectable risk factor for stroke, 11.1% had one, 28.1% had two, 31.4% had three, 22.9% had four, 2% had five and a single individual (0.6%) had six demonstrable risk factors for cerebral ischaemia. (Table 4.2)

Table 4.2: ***Number of risk factors prevalent in patient population of ischaemic strokes.***

No. Risks	0	1	2	3	4	5	6
n=153 (%)	6 (4%)	17 (11.1%)	43 (28.1%)	48 (31.4%)	35 (22.9%)	3 (2%)	1 (0.6%)

Hypertension

This was the most prevalent risk marker for cerebral infarction in our patient population with a total of ninety-nine patients (64.7%) with either a history of, or clinically evident hypertension requiring treatment during admission. Eighty-Four (55%) patients had known hypertension, while fifteen (10%) were newly diagnosed, requiring treatment after the acute phase of stroke. Admission systolic and diastolic blood pressures were similar for both hypertensive groups [Systolic: 169 $\pm$ 25.5 mmHg {95% CI 163.6 – 174.4} versus 175 $\pm$ 31 mmHg {95% CI 158.1 – 191.9}; $p>0.1$ & Diastolic: 91.9 $\pm$ 14.2 mmHg {95% CI 88.9 – 94.9} versus 92.3 $\pm$ 14.6

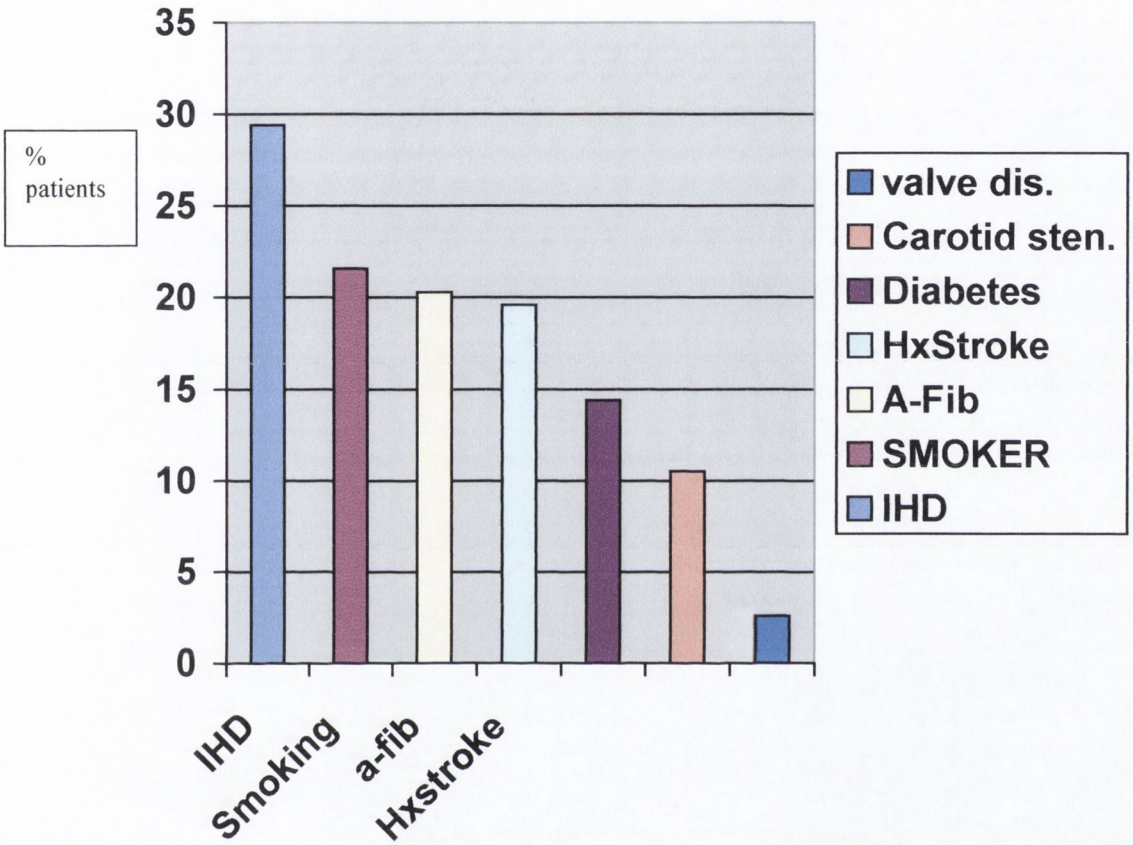
mmHg {95% CI 84.4 – 100.2}; p>0.1 for established and newly diagnosed groups respectively). Both figures were significantly higher than admission systolic [143 ± 22 mmHg {95% CI; 136.7 – 149.3} p< 0.001] but not diastolic [80.5 ± 11.5 mmHg {95% CI; 77.2 – 83.7} p>0.05] blood pressures in the non-hypertensive group though, the small numbers make meaningful comparison in the 'new' group difficult.

As can be seen from **table 4.2**, the majority of patients had two or more risk factors and hypertension never occurred in isolation in our study. 45 patients (29.4%) had evidence of ischaemic heart disease in addition to hypertension; 33 patients (21.6%) were current smokers; 31 patients (20.3%) had co-existent atrial fibrillation; 30 patients (19.6%) a previous history of a stroke or TIA; 20 patients (13.1%) also had diabetes; 16 patients (10.5%) had concomitant carotid artery disease; 4 (2.6%) had evidence of valvular heart disease. This is summarised in **Fig 4c.**

Atrial fibrillation

Among this population, 52 (34%) patients had either evidence of existing atrial fibrillation on admission ECG or were subsequently discovered to have intermittant atrial fibrillation on 24-hour cardiac ("holter") monitor analysis. 75% (n=85) of patients with sinus rhythm on baseline ECG had a 24-hour cardiac monitor and intermittant atrial fibrillation was detected in 13 cases, a positive pick-up rate of 15%. Thirty-one patients with atrial fibrillation had concomitant hypertension as a risk factor. Five patients had known rheumatic mitral valve disease accounting for 9.6% of cases of atrial fibrillation.

Fig. 4c: Incidence of Risk Factors Occurring with Hypertension in Ischaemic Stroke.



Coronary Artery Disease

Patients in my study were classified as having ischaemic heart disease on the basis of a clinical history of diagnosed coronary artery disease or myocardial infarction, or on ECG appearance using the Minnesota code. Patients with new onset angina pectoris or recent myocardial infarction were diagnosed on the basis of cardiac chest pain accompanied by ST segment depression on an electrocardiogram, or ST elevation with a corresponding rise in cardiac enzymes (see methodology section 3.1).

In total, 64 patients (41.8%) had evidence of coronary artery disease. Of these, 30 (46.8% of those with ischaemic heart disease) were in atrial fibrillation, 17 (26.6% of those with ischaemic heart disease) had clinical and radiological or echocardiographic evidence of left ventricular failure.

Prosthetic Heart Valves

Four patients (2.6%) in this population of ischaemic strokes had a prosthetic heart valve (all aortic and of artificial non-biological type), one of whom had clinically demonstrable infective endocarditis with vegetations visible on trans-thoracic echocardiography.

Carotid Artery Disease

Overall 103 patients with cerebral infarction (67%) had a carotid duplex scan performed after their stroke. Clinically relevant disease of the carotid artery i.e. stenosis of $\geq$ 70 % of the internal carotid artery ipsilateral to the side of the infarct,

was demonstrable on carotid duplex scanning in 23 patients (15%). Lesser degrees of stenosis (50-69%) was evident in a further 30 patients (19%). A partial audit of patients with clinically relevant disease showed that 40% of patients were operable, based on functional recovery and anaesthetic risk.

Previous Stroke / Transient Ischaemic Attack (TIA)

In 122 patients (79.7% of cases) admitted with ischaemic stroke it was their first-ever stroke or evidence of cerebrovascular symptomatology. 31 patients (20.3%) had had a previous history of a stroke and a further 16 patients (10.5%) had had a TIA prior to the index stroke. 17 (11.1%) patients had a family history of ischaemic stroke in a first-degree relative.

Diabetes

All patients admitted with ischaemic stroke were screened for diabetes with two fasting blood samples and using American Diabetes Association guidelines of fasting blood sugar > 7.0 mMol/L being diagnostic. Where results were equivocal a formal glucose tolerance test was performed and the two-hour post prandial glucose assayed. 28 patients (18.3%) were found to be diabetic, 67% of whom had pre-existing diabetes and 33% being newly diagnosed. All patients had late-onset diabetes, initially managed by dietary measures and oral hypoglycaemic agents, however 2 patients (1.3%) subsequently became insulin dependent.

Smoking

Smoking was defined as current if a patient was regularly smoking cigarettes within the previous year or ex-smoker if the patient was previously a regular smoker but had not smoked within the 12 months preceeding the stroke. 52 patients (34%) were current smokers and 46 (30.1%) were ex-smokers. Cigarette smoking is strongly

associated with all types of stroke with a relative risk of 1.5 overall, and 2.0 for ischaemic stroke (Donnan *et al.*, 1993). This risk declines after cessation, strongly supporting a causative role but trials are difficult to design given the addictive nature of the habit.

Primary risk factors for cerebral infarction in our stroke population are summarised in **Fig. 4d.**

Possible Risk Markers for Ischaemic Stroke

Hypercholesterolaemia

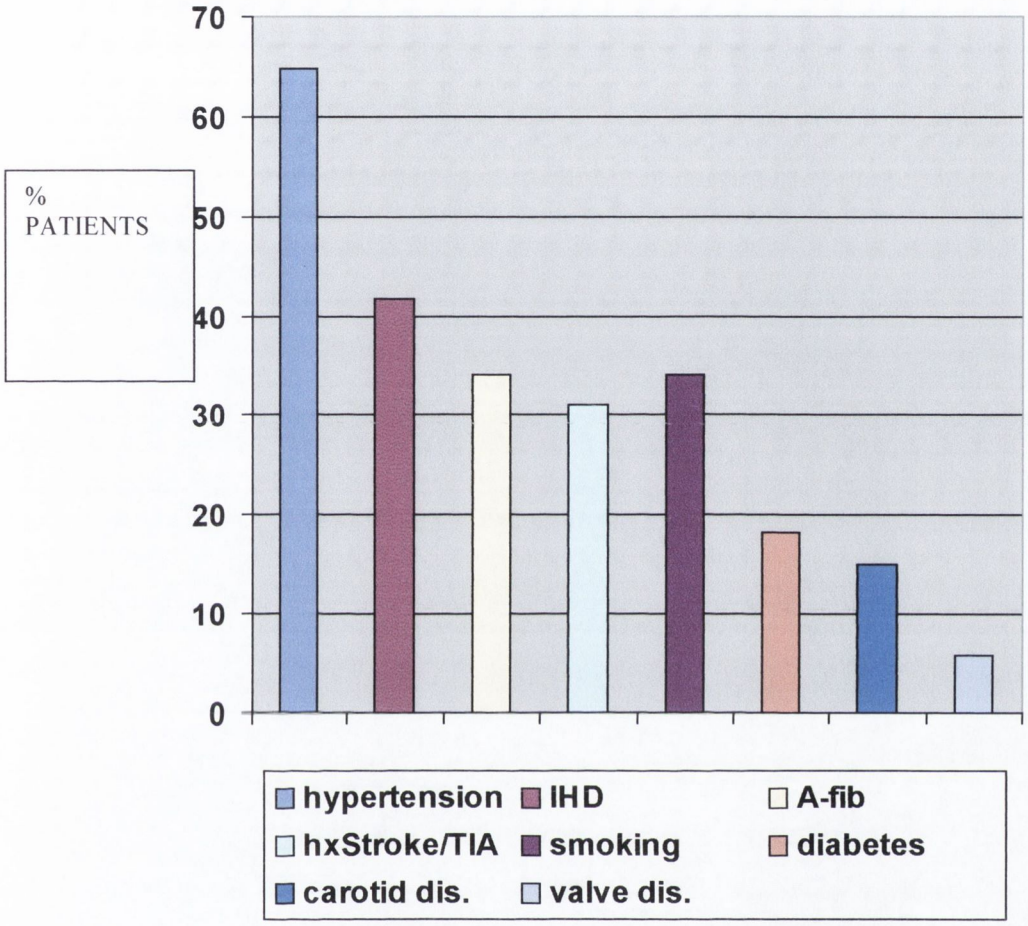
Fasting lipid profile was available in all but 12 patients and 65 of these (46%) had total cholesterol or low density lippoprotein (LDL) levels above those recommended, 5.2 mMol/L for total cholesterol and 3.6 Mmol/L for LDL (Laboratory Standardization Panel of the National Cholesterol Education Program 1998). The role of cholesterol in stroke is controversial however, and in this regard the co-existence of ischaemic heart disease or carotid artery stenosis may have an important bearing on treatment decisions. We found 16% of patients also had co-existant ischaemic heart disease and a further 7% significant carotid stenosis ($\geq$ 70%).

These figures are summarised in **Fig. 4e.**

Alcohol

10 patients (6.5%) regularly consumed alcohol in daily quantities associated with an increased risk of stroke i.e. more than 7 drinks a day (Sacco *et al.*, 1999) and regularly exceeded the recommended safe weekly intake as recommended by the British

Fig. 4d: Primary Risk Markers for Ischaemic Stroke



Medical Association (i.e. 22 units for a male and 14 units for a female). Almost all these patients had a prior history of, or current clinical evidence of alcohol dependency.

Miscellaneous Vascular Disease

A small number of patients had specific vascular disease predisposing to risk of cerebrovascular infarction. 4 patients (2.6%) had a diagnosis of giant cell arteritis and one patient had newly diagnosed hyperhomocysteinaemia.

b) First versus recurrent cerebral infarct

It would seem plausible that any study of a population of patients with acute cerebral infarction would yield different risk factor profiles between patients suffering their first-ever versus recurrent stroke. Indeed similar studies elsewhere have suggested different pathological mechanisms for recurrent stroke (Yamamoto & Bogousslavsky, 1997; Hankey *et al.*, 1998).

A sub-analysis of my patient population over the three years revealed 31 cases of recurrent cerebral infarction as opposed to 122 first-ever events. 55% of the recurrent group were female and mean age was 73.2 years, while 50% of the first-ever group were female and mean age was 69.2 years. Incidence of the primary risk markers for cerebral infarction of atrial fibrillation, hypertension, valvular heart disease / ischaemic heart disease, carotid artery disease, diabetes and smoking were compared and are summarised below in **table 4.3.**

Fig. 4e: Proportion of acute cerebral infarction patients with elevated cholesterol levels and concomitant ischaemic heart disease or carotid artery stenosis.

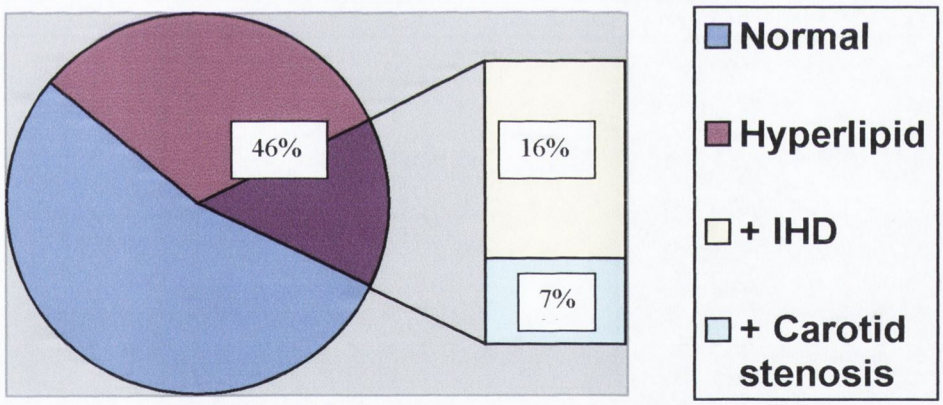


Table 4.3 Comparison of major risk factors between first-ever cerebral infarct and recurrent event.

	sex	mean age	A-fib.	BP	valve disease	IHD	Carotid stenosis	diabetes	smoker
Recur. N=31	55 % fem.	73.2 years	48.3%	65.5%	10.3%	48.2%	20.7%	20.7%	20.6%
First-ever N=124	50 % fem.	69.2 years	29.8%	65.3%	4.8%	40.3%	13.7%	18%	37.9%

A-Fib – *Atrial Fibrillation*

BP – *Hypertension.*

IHD – *Ischaemic Heart Disease*

As one might expect the incidence of atrial fibrillation was greater in the recurrent group – 48.3% versus 29.8 %, as was the incidence of valvular heart disease (10.3 % versus 4.8%). This pattern was also evident in the incidence of ischaemic heart disease (48.2% versus 40.3 %) and carotid artery stenosis (20.7% versus 13.7%). The incidence of hypertension was similar with 65.5% of the recurrent group, with clinical hypertension, versus 65.3% of the first-infarct group. Neither was there an appreciable difference in incidence of diabetes between the two groups (20.7 % versus 18 %). Smoking was more prevalent in the 'first-ever' stroke group with 37.9 % being current smokers versus 20.6 % of the recurrent group, and perhaps not suprisingly after a previous stroke, there were a greater number of ex-smokers in the recurrent group 41.4 % versus 27.4 %.

c) Cerebral Haemorrhage

The primary risk markers for cerebral haemorrhage are generally considered to be hypertension, family history, cerebral arterial malformation and use of antiplatelet or

anticoagulant agents. Thirty-one patients with primary intracerebral haemorrhage were admitted to the stroke service over the three-year period, representing 16.8% of all strokes. Six patients had previously had a cerebral infarct and all but one of these recurrent strokes were on either antiplatelet or anticoagulant agents. An additional four cases (including one sub-arachnoid haemorrhage) were transferred to neurosurgical care at another institution and lost to follow-up for data purposes and are not included in statistical analysis, and one case of haemorrhage was secondary to tumour. Of the 31 patients with intracerebral haemorrhage, 20 of these patients were male and 11 female of mean age 74.8 years (range: 48-91 years). Average length of in-hospital stay was 34.6 $\pm$ 29.6 days and mean BADL at day 10-14 was 7.1 $\pm$ 7.5. Of this population 22.5% died and 16.3% required institutional long-term care. Patients had on average one recognised primary risk marker for intracerebral haemorrhage each.

Hypertension

Eighteen patients (58%) had a prior history of (n=13), or clinically newly diagnosed hypertension requiring treatment during their hospital stay.

Antiplatelets /Anticoagulation

8 patients (25.8%) were using antiplatelet agents (all aspirin in doses of 75-300 milligrammes daily) while 2 patients (6.5%) were on the anticoagulant agent warfarin prior to their stroke. One of these patients had a prior history of cerebral infarction and was on prophylactic warfarin for underlying atrial fibrillation. No patient with a cerebral haemorrhage in my study population had a prior history of a similar event.

Family History / Arterial malformation

One patient (3.2%) had a definite family history of cerebral haemorrhage of unknown type in a first-degree relative (sister), and one patient was subsequently shown to have an arterial aneurysm on cerebral angiography that necessitated surgical intervention.

A comparative summary of the major differences between the cerebral infarction and intracerebral haemorrhage groups is listed in **table 4.4** below.

Table 4.4 Major comparative points between cerebral infarction and haemorrhage group.

Type	m/f	age (mean)	BADL @day 10-14	Mortality %	Institutional Care %	BP. %
Infarct N=153	74/79	69.97	10.9 ± 7.1	11.1	14.4%	58
Haem. n=31	20/11	74.8	7.1 ± 7.5	22.5	16.3%	64.7

BADL – Barthel Index of Activities of Daily Living.

BP – Blood pressure (signifying hypertension).

4.2-5 Discussion

This study provides an assessment of risk markers of acute stroke in an Irish population over a three-year period. All data was collected using a standard pro-forma (**see appendix 2**) on a prospective basis from time of admission to discharge and subsequently transferred to a computerised database (Microsoft Access ©). Such data is important in assessing future risk and in focussing secondary preventative strategies

in the individual. It is also useful in comparing stroke case-mix with international trends as differences in risk markers are associated with different patterns and incidence of stroke amongst different populations (WHO MONICA project. 1997).

The initial trend of interest in such a study is whether our population demographics and incidence of stroke type are significantly different when compared with other studies. Overall the mean age of patients presenting to our stroke unit was 70.7 ± 12.9 years. The mean age of patients presenting with cerebral infarction was 70 years and slightly higher at 74 years in the haemorrhagic group. This is slightly older than trends observed elsewhere particularly in terms of haemorrhagic stroke. Data from the Framingham study showed a mean a mean age of 65 years for all stroke-types and similarly Foulkes *et al.* (1988) in a large study of 1, 805 american stroke patients found an overall median age of 65 years, with a median range of 66-70.5 years for the subtypes of cerebral infarction, and a median age of 61 years for patients with intracerebral haemorrhage. Clearly there was a trend in this study for an older patient particularly in terms of cerebral haemorrhage, and may be accounted for by trends in stroke type and age profile of our hospital catchment area in years one and two, where both the age of patient and incidence of haemorrhage was appreciably higher than year three when our hospital moved to a new site. Another factor is possibly ethnically related and, whereas all our patients were caucasian, over half the patients in the quoted studies were african-american. Incidence of risk factors are likely to be different in such mixed populations as is their effect on cardiovascular disease (Anand *et al.*, 2000). A recent report from a comparable Irish centre has produced a similar age profile to that observed here, with 80% of the ischaemic stroke population being greater than 65 years (McDonnell *et al.*, 2000).

In terms of sex difference my data showed 48 % of patients with cerebral infarction were male, comparable to the 52 % observed in the Leigh Valley stroke register (Min Lai *et al.*, 1994) and the 45% observed in Framingham (Wolf *et al.*, 1991). Some difference was evident among those with intracerebral haemorrhage with roughly equal sex distribution reported by Wolf et al. in their cohort and 57% male in other studies (Foulkes *et al.*, 1988), while our data showed 66% of patients with intracerebral haemorrhage were male. It is clear however, that in comparison to such databases, numbers quoted in this study are small.

In terms of risk factor incidence for stroke type, data from this study suggests strongly that hypertension is the most prevalent factor amongst patients with both cerebral infarction (64%) and haemorrhage (58%), similar to incidences reported in the U.S. and Australia for all stroke types (Alter *et al.*, 1994; Hankey *at al.*, 1998), and somewhat higher than the 30% reported in the Lausanne stroke registry, Switzerland (Bogousslavsky *et al.*, 1988). It was also the most prevalent risk factor for ischaemic stroke in a recent comparable Irish study (McDonnell *et al.*, 2000*)* which quoted a lower prevalence of 45%, although hypertension was not defined in this study. Hypertension is strongly associated with risk of stroke independent of variables such as age and sex. The relationship between diastolic blood pressure and risk of stroke is log-linear throughout the range of normal physiological pressures and almost doubles with every 7.5 mmHg increase in pressure (Collins & MacMahon 1994). Although misunderstood as a risk factor until relatively recently, the relationship between systolic blood pressure and risk of stroke is at least as strong, and isolated systolic hypertension in the presence of normal diastolic pressure is also associated with increased risk of stroke (SHEP co-operative research group 1991). Hypertension

increases the risk of all the major pathological classifications of stroke (ischaemic and haemorrhagic). Its association with ischaemic stroke relates to increased severity of atheroma of large vessels and accelerated intracranial small vessel disease (Homer *at al.*, 1991; Sutton-Tyrell *et al.,* 1993). Indeed progression of large vessel stenosis can be slowed by treatment of concomitant hypertension (Sutton-Tyrell *et al.*, 1994). This may be important in preventing stroke recurrence. Several studies have reported the significant association between hypertension (and diastolic blood pressure in particular) and stroke recurrence (Alter *at al.*,1987; Sacco *et al.*, 1982), and that good control lessened the odds of recurrence significantly (Alter *et al.*, 1994). Other studies have however failed to show this trend (Meissner *et al.*, 1988; Hankey *et al.*, 1998), though their populations may be different. Frequency of hypertension varies with different ethnicity (Friday *et al.*, 1989) and likewise the effect of good control on recurrence rates may differ in different races.

As hypertension is the most frequent risk factor observed in this cohort and a common aetiological factor in both cerebral infarction and haemorrhage, it is useful to analyse occurrence of other risk factors with hypertension in stroke. The presence of other risk factors in addition to hypertension may have an additive effect. Atrial fibrillation approximately doubles the risk of stroke in hypertensive patients of all ages (Whisnat *et al.*, 1996) and other controlled studies of hypertensive populations have shown higher incidences of atrial fibrillation, diabetes mellitus and lower HDL levels in those with stroke as an end-point (Makino *et al.*, 2000). Clearly identifying such patients at risk could be helpful in intensifying secondary prevention measures. Because such measures are often costly and inconvenient for patients, there has been an increasing trend towards the use of 'risk tables' for the assessment of individual

risk based on the presence or absence of multiple factors, when deciding on appropriate treatment. The co-existence of risk factors may also help explain the ocurrence of different stroke types. Hypertension accelerates both large and small artery atheroma, but why different stroke types occur may depend on the presence of other risk factors. Bogousslavsky *et al.* (1996) previously reported that age > 67 years, smoking, hypercholesterolaemia and family history of stroke and ischaemic heart disease were independent predictors of ischaemic rather than haemorrhagic stroke in the presence of hypertension, when cardioembolic strokes were excluded. In addition diabetes in women was identified as an additional factor.

As a single risk marker, atrial fibrillation probably represents the most significant risk for the individual patient, increasing the risk of ischaemic stroke by a factor of six in the non-rheumatic setting (Hart & Halperin 1994) and to even a greater extent in rheumatic heart disease (Wolf *et al.,* 1978). The beneficial effect of anticoagulation on this risk (Stroke Prevention in Atrial Fibrillation Study Group 1991; European Atrial Fibrillation Trial Study Group 1993), is further strong evidence of its importance as a cause of embolic stroke, though this evidence must also be viewed in the context that such anticoagulation could conceivably prevent artery to artery embolus and the formation of in-situ thrombosis, which are not related to the atrial fibrillation. This study found evidence of atrial fibrillation in 34% of our patients with ischaemic stroke, much higher than the 18 % reported in the Rochester study of ischaemic stroke (Whisnat *et al.*, 1996) although 24-hour "holter" monitoring was not used in that study to detect paroyxsmal atrial fibrillation. The use of this technique has been shown in the Lausanne study to have a positive 'pick-up' rate of at least 16% (Bogousslavsky *et al.*, 1988) which compares well with the 15% detection in this

population. Despite this however, Bogousslavsky *et al.*, reported a much lower overall incidence of atrial fibrillation of 18.3% among patients with ischaemic stroke though all strokes were first-events, and it is likely that the incidence would be higher in a population inclusive of recurrent stroke. The incidence of atrial fibrillation is also likely to be strongly influenced by age and incidence of ischaemic heart disease and our study population was older with a higher rate of heart disease (see later) than that reported by Bogousslavsky. Previous Irish studies have also found similar (33% -Fan *et al.*, 1998) and lower (27.3% - McDonnell *at al.*, 2000) incidences of atrial fibrillation in populations of ischaemic stroke, although again information on use or not of holter monitoring is not presented.

Another important cardioembolic source is that associated with valvular heart disease, not only in the setting of rheumatic atrial fibrillation but also increasingly with prosthetic valves. The association of mitral valve disease with stroke is well documented (Sandercock *et al.*, 1989), increasing the risk by a factor of two when all causes are considered (mitral stenosis and regurgitation). Mitral regurgitation was not documented as a major risk in this study given the difficulty in assessing clinically the degree of severity and the high prevalence of trivial regurgitation on echocardiography. Five patients (3.2%) had mitral stenosis comparable to 2.8% found by Bogousslavsky *et al.* in 281 consecutive strokes who underwent echocardiography. The risk of embolus from prosthetic heart valves is well documented and appears to be greater with mitral rather than aortic valve replacements (De Bono 1982). Embolism of thrombus formed on the valve, vegetation from infection of the valve or even material due to artificial valve disintegration may all result in ischaemic stroke. The estimated risk of embolism is 2% per annum for all valve types including patients

with artificial types on anticoagulation (Hammermeister *et al.*, 1993). 2.6% of this cohort had an artifical aortic valve, one of which had demonstrable vegetations on trans-thoracic echocardiogram. Bogousslavsky *et al.* reported 13 of 1,000 first ever stroke patients with a prosthetic valve, of which only one had demonstrable thrombus on echocardiography.

The association between coronary artery disease and stroke has been borne out in both post-mortem studies (Stemmerman *et al.*, 1984), case control studies (Wolf *et al.*, 1991.b) and has been shown to double the risk of ischaemic stroke (Whisnat *et al.*, 1996). The reasons for this are probably multiple; atheroma of the coronary circulation is likely to reflect a similar state in the cerebral arteries, mural thrombus leading to cardiac embolism may complicate myocardial infarction and both left ventricular failure from dilated cardiomyopathy and atrial fibrillation are frequent end-products of coronary artery disease. There was evidence of ischaemic heart disease in 41.8 % of our cohort with ischaemic stroke. This is comparable with other studies internationally, where up to 50% of patients with stroke have asymptomatic coronary artery disease with abnormal exercise ECG's or myocardial perfusion studies, two thirds of whom have serious underlying disease (Chimowitz *et al.*, 1997). The Lausanne registry reported an incidence of ischaemic heart disease of 29.3% among a population of 778 first-ever ischaemic strokes (Bogousslavsky *et al.*, 1988) although this was based on patient history alone and subsequently, a further 9.9% of patients without known cardiac disease were found to have ischaemic changes on their ECG analysis. The initial figure is similar to that reported in the Leigh Valley registry (25.3%) when myocardial infarction alone was taken as evidence of

ischaemic heart disease, and this relates well to previous Irish studies which quoted an incidence of 24% using the same criteria (Fan *et al.* 1998).

The presence of significant carotid artery stenosis ipsilateral to the symptomatic side is clearly a risk factor for ischaemic stroke given the beneficial results of surgical trials of endarterectomy in secondary prevention (North American Symptomatic Carotid Endarterectomy Trial Collaborators 1991; European Carotid Surgery Trialists' Collaborative Group 1998). Furthermore evidence that the treatment of asymptomatic men with carotid artery stenosis reduces the absolute risk of stroke by 1% (Asymptomatic Carotid Atherosclerosis Study [ACAS] Executive Committee 1995), also points to causation, although this remains controversial not least, because no such benefit was demonstrable in treated women, but also because such meagre benefit was only obtainable with extremely low rates of surgical complication. There was significant carotid artery stenosis ($\geq$ 70%) in 15% of our population, and when all stenotic disease $\geq$ 50% was included this rose to 34%. This is similar to the incidence of such disease prevalent in the Lausanne registry although a greater proportion of patients (25%) had $\geq$ 70% stenosis.

A history of previous stroke or TIA increases the relative risk of ischaemic stroke by a factor of ten. The Rochester epidemiological project reported an odds ratio of 5.6 (95% CI 3.7 –8.52) for a history of TIA, and found an incidence of 17.2 % among their cohort of ischaemic stroke patients (Whisnat *et al.*, 1996). This is higher than the 10.5% found among my population, although obviously the longer population follow-up over 25 years in the Rochester study is likely to increase the yield of TIA prior to stroke. A history of stroke/TIA in itself is not causative of stroke, but rather reflects

underlying disease mechanisms, risk factors and the state of the cerebral circulation in general. Attention to medical detail and addressing identified risk factors should modify this increased risk, but may be compromised by increased rates of haemorrhagic stroke when anticoagulants and antiplatelets are used in secondary prevention strategies. 20.3% of this cohort of patients with cerebral infarction had a prior history of stroke which is similar to the 24%, previously reported in an Irish stroke population (Fan *et al.*, 1998). This is also similar to the figures of 28% and 26% reported by the Stroke Data Bank (Hier *et al.*, 1991) and Swedish studies (Viitanen *et al.*, 1988).

Similarly family history of stroke has been identified as risk factor for ischaemic stroke (Kiely *et al.*, 1993; Jousilahti *et al.*, 1997), though not in all studies (Kubota *et al.*, 1997). While it is tempting to assume that any such relationship between family history and stroke reflects the strong association between family history and risks such as hypertension, diabetes and coronary artery disease, this does not appear to be the case. Indeed, when these and other factors such as smoking and socioeconomic group are controlled for, there is a consistent trend towards increased risk of stroke in all ethnic groups with a positive parental history of stroke (Liao *et al.*, 1997). Moreover this risk appears to be greater with a paternal history of stroke (Liao *et al.*, 1997; Jousilahti *et al.*, 1997). Also while some studies have demonstrated an association between parental history of coronary artery disease and cerebrovascular disease (Kiely *et al.*, 1993) this has not been a consistent finding. This suggests that some but not all of the genetic susceptibility to atherosclerosis in the coronary and cerebral circulation might be shared. More recently, parental history of stroke was associated with subclinical disease but not stroke (Morrison *et al.*, 2000), which may

suggest interaction between genetic susceptibilty and environment are necessary to produce clinical disease. Occasionally truly inherited disorders of collagen formation, thrombophilic states or neurological disorders which in themselves are causative of stroke, may account for isolated familial clusters, but these are not commonly encountered. 11.1 % of patients with ischaemic stroke in this study had a positive family history of stroke in a first-degree relative. This is much lower than the 36% reported in the Family Heart Study which included all stroke types (Liao *et al.*, 1997), or the 43% reported by Morrisson et al. in their study of ischaemic stroke, although the design of that study excluded, among others, people with unknown family histories and so is likely to be biased in favour of positive family history. However, figures in this study are similar to the 10.2 % observed in a Finnish population (Jousilahti *et al.*, 1997). No published Irish data is available for comparision.

Diabetes is an important risk factor for ischaemic stroke, increasing the relative risk by a factor of two independent of any concomitant hypertension (Burchfield *et al.*, 1994), and also resulting in higher mortality from stroke when compared to non-diabetic patients (Jorgensen *et al.*, 1994). It has also been demonstrated that diabetic patients have thicker carotid artery intimae compared to controls reflecting its strong association with atheroma (Folsom *et al.*, 1994). 18.3% of patients with ischaemic stroke in this study were diabetic, one third of whom were newly diagnosed. This is slightly higher than the 13.2% reported among cases of cerebral infarction in Rochester (Whisnat *et al.*, 1996) but lower than the 30.4 % reported incidence of diabetes in the Leigh Valley registry (Min Lai *et al.*, 1994) although both, different populations and diagnostic criteria are likely to account for such variation. Our

incidence of diabetes is similar to that reported in the Lausanne registry (16.7%) when all sub-types of infarction are considered (Bogousslavsky *et al.*, 1988).

The role of cholesterol as a risk factor for stroke is controversial. Large epidemiological studies have consistently shown cholesterol levels to have no bearing on risk of stroke (Prospective Studies Collaboration 1995), and even an absence of association with cardiovascular disease in the over 70-year age group (Krumholz *et al.*, 1994). However this is at variance with several large randomised control trials showing a risk reduction in stroke of 29%, when patients with ischaemic heart disease have their total cholesterol and LDL levels reduced by 3-hydroxyl-3methylglutaryl-Coenzyme-A (HMG-CoA) reductase inhibitors or "statins" (Blauw *et al.*, 1997; Bucher *et al.*, 1998). Furthermore some authorities feel that cholesterol as a vascular risk factor in the elderly becomes 'unmasked' when hypertension is controlled (Hachinski *et al.*, 1996). In addition treatment with statins have been shown to induce regression in carotid artery intimal and media thickness (MacMahon *et al.*, 1998), itself a recognised risk factor for stroke (O'Leary *et al.*, 1999), and statins may also reduce blood pressure which may contribute to their beneficial effect (Glorioso *et al.*, 1999).

In the light of such evidence it would seem prudent to treat hyperlipidaemia as a risk factor for stroke, at least in those with co-existant ischaemic heart or carotid artery disease. Among those with hyperlipidaemia in our study 16% had ischaemic heart disease and 7 % had definite carotid artery stenosis (1.3% with both conditions), so that elevated cholesterol could be considered a relevant risk factor in at least 22 % (33 patients) of the total patient population with ischaemic stroke. In truth this may be an underestimate given that many patients will have asymptomatic ischaemic heart

detectable only by provocative tests (Chimowitz *et al.*, 1997) and that conceivably patients with lower grades of carotid artery stenosis (i.e. < 70%) might also benefit from treatment with a statin.

More recent guidelines have suggested lower "ideal" total cholesterol and LDL levels of 5 and 3 mMol/L respectively, in at-risk patients (Recommendations of the Second Joint Task Force of the European and Other Societies on Coronary prevention, 1998). Using these lower levels as a criterion for hyperlipidaemia in this study had a negligible effect on overall numbers, where one additional patient could be considered for intervention with a statin.

In this study several differences were apparent between the first-ever ischaemic stroke group and the recurrent-stroke population. Patients with recurrent ischaemic stroke had a higher incidence of atrial fibrillation, valvular heart disease, ischaemic heart disease and carotid artery stenosis. There was no appreciable difference in the incidence of hypertension or diabetes between the two groups. Less patients were current smokers in the recurrent stroke group. This however, probably reflects a change in lifestyle after the first stroke, and when a history of previous tobacco use is considered, the overall incidence of smoking (ex- or current) is similar between the two groups – 65.3% of first-ever stroke patients and 62% of recurrent stroke patients having smoked tobacco regularly at some stage in their adult lives. Six patients with intracerebral haemorrhage also had a previous stroke (cerebral infarction in all cases), and in all but one case, patients were on either antiplatelet or anticoagulant agents. Incidence of other vascular risk factors among this group did not vary significantly as to affect the overall trend observed with cases of first and recurrent infarction.

The design and duration of this study does not allow for comparison of actual rates of recurrence with data published elsewhere, however it is interesting to compare risk factors with those known to influence recurrence. Atrial fibrillation is well recognised as both a risk for early (Moroney *et al.*, 1998) and later stroke recurrence (Min Lai *et al.*, 1994), with an estimated recurrence rate of 20% per year without anticoagulation (Sage & Van Uitert, 1983). This compares to our observation of a greater incidence of atrial fibrillation among the recurrent stroke group, though interestingly other studies have not identified it as a risk for recurrent stroke in their populations (Hankey *et al.*, 1998;). This may be the result of differences in medical management of atrial fibrillation in the secondary prevention of stroke, or a predominance of non-embolic recurrent strokes in the study group.

There did not appear to be any significant difference in rates of hypertension between the first and recurrent ischaemic stroke group, although several studies have implicated the importance of both diastolic and systolic hypertension as a risk for recurrent stroke. In a study of 621 ischaemic strokes with mean follow up of 24 months, Alter *et al.* reported significantly higher rates of recurrence in patients with poorly controlled diastolic hypertension (risk x 8), well controlled pressure (risk x 2) when compared to non-hypertensive patients (Alter *et al.*, 1994). However other studies have both failed to show this association (Burn *et al.*, 1994; Hankey *et al.*, 1998), or the impact of treatment of blood pressure on recurrence (Meissner *et al.*, 1988). Similarly there was no difference in rates of diabetes between the two groups although diabetes has been variously reported to increase (Hankey *et al.*, 1998; Elneihoum *et al.*, 1998; Alter et al. 1987) or was not associated with (Burn *et al.*, 1994) risk of stroke recurrence. Some of this disparity reflects variation in study

design, numbers of recurrent strokes analysed and models of variate analysis employed, but also the possible difference in impact of a given risk factor and its treatment on risk of recurrence in different populations.

4.3 Summary of Chapter Four

- A three-year audit of the stroke service is presented. There was a greater number of referrals of patients of a younger age from year to year. A non-significant trend towards reduced mortality, improved outcome and less institutional care was observed from year to year, without an increase in length of stay. This observation is likely to be multifactorial in nature, including better early treatment, organised multidisciplinary approach and a lower threshold of referral to the stroke service.

- A prospective audit of risk markers for acute stroke in an Irish population is presented. Data on patient demographics and stroke sub-type is similar to that published in international stroke data bases, though significantly higher rates of intracerbral haemorrhage were observed in years one and two. This may reflect the elderly catchment area of the stroke service in the first two years of its' operation, or a referral bias of more severe strokes to a newly-formed service.

- Hypertension was the most prevalent risk factor for stroke in my study population, although the majority of stroke patients had at least one other recognised risk factor. The incidence of major risk markers of stroke in an Irish population are consistent with data published elsewhere in international literature, although the incidence of atrial fibrillation and ischaemic heart disease was notably higher. Different ethnicity, diagnostic criteria and investigative techniques are likely to account for some of this difference. Data in this thesis is similar to that published from other Irish centres after this project had commenced.

Chapter Five

Results II

Chapter Five – Results II

Contents:

Section 5.1 Validation of SLC assay

5.1-1. Introduction

The method for sample preparation and assaying SLC has already been outlined in chapter three, section 3.2-2.of this thesis, and the calculation and kinetic analysis of the SLC discussed in section 3.2-3. The method employed remained similar for all experiments carried out. To test the reproducibility and reliability of the assay it was first necessary to check the inter-assay variation i.e. the variation in SLC value in a given individual when repeatedly assayed over time, under the same conditions and by the same operative (myself in all cases). It was also necessary to evaluate the intra-assay variation i.e. the variation in SLC value when the same sample is repeatedly assayed under the same conditions by the same operative (myself in all cases).

5.1-2 Inter-assay variation

To assess the inter-assay variation three healthy male subjects were recruited from staff within the Department of Pharmacology and Therapeutics, Trinity College Dublin, and a blood sample obtained under the same conditions. Male subjects were chosen to avoid any possible influence of the menstrual cycle on SLC over time in females, as has been reported elsewhere (Adebayo 1995). The method of sampling is discussed in section 3.2-2 and again, in brief, all sampling was done in the fasting state, between the hours of 08.00-10.00, by single venous puncture into sodium-heparin vacutainers (Becton & Dickson) until approximately 20 mls. of blood was obtained. All samples were processed in the same way by the same operative. The process was repeated on each of the volunteers on three separate occasions, over a six-week period. SLC, Km and Vmax. values were calculated and compared between

the different assays for each individual, by calculating the coefficient of variance (CV%), which is, put simply, the standard deviation of the sample expressed as a percentage of the mean. This gives an index of the inter-assay variation for each of the variables measured.

These results are outlined in table 5.1.

5.1-3 Intra-assay variation

To evaluate the intra-assay variation, three healthy volunteers were recruited, again from within the Dept. of Clinical Pharmacology and Therapeutics, Trinity College Dublin. A single sample of blood was obtained in the method outlined previously and the sample prepared and assayed in identical fashion by the same operative three times on the same day. SLC, Km and Vmax were calculated for each of the three assays and compared by calculation of the coefficient of variance (CV%), to give an index of intra-assay variation for each of the three variables.

The results are shown in table 5.2.

Table 5.1: Inter-Assay Variation.

Subject 1:

Weeks:	1	2	3	Mean	SD	%CV
SLC	0.1759	0.1623	0.1819	0.1745	0.0101	5.8%
Vmax	0.2433	0.22679	0.2542	0.24143	0.01379	5.7%
Km	78.93	92.67	102.58	91.3933	11.8803	11.9%

Subject 2:

Weeks:	1	2	3	Mean	SD	%CV
SLC	0.3413	0.3472	0.3275	0.3387	0.0101	3.0%
Vmax	0.5358	0.5573	0.5355	0.5429	0.01249	2.3%
Km	82.1	83.13	94.17	86.47	6.6911	7.7%

Subject 3:

Weeks:	1	2	3	Mean	SD	%CV
SLC	0.1850	0.1764	0.1763	0.1792	0.0049	2.8%
Vmax	0.2835	0.2935	0.2676	0.2815	0.0131	4.6%
Km	76.62	97.93	81.04	85.19	11.2467	13.2%

Table 5.2: Intra –Assay Variation with SLC.

Subject 1:

Assay no:	1	2	3	Mean	SD	%CV
SLC	0.2681	0.2806	0.2657	0.2714	.00803	2.9%
Vmax	0.4833	0.5411	0.512	0.5121	.02889	5.6%
Km	116.48	126.98	108.95	117.47	9.0557	7.71%

Subject 2:

Assay no:	1	2	3	Mean	SD	%CV
SLC	0.2293	0.2418	0.2550	0.2420	0.01284	5.3%
Vmax	0.3355	0.3675	0.3480	0.3503	0.01612	4.6%
Km	74.620	74.51	66.39	71.84	2.7244	3.8%

Subject 3

Assay no:	1	2	3	Mean	SD	%CV
SLC	0.2781	0.2646	0.2583	0.267	0.01011	3.7%
Vmax	0.3321	0.3195	0.3107	0.3207	0.02378	7.4%
Km	89.95	102.36	112.54	101.62	11.32	11.1%

5.1-4 Discussion

The inter-assay variation over a six-week period showed good reproducibility. The range of CV% from 2.8 –5.8% for SLC values was particularly encouraging and compared favourably to values of 1.80-9.56 % for work done previously by this method (Adebayo 1995). Similarly the intra-assay variation of 2.9-5.3% for SLC values was of similar magnitude to that reported previously by the same author, and indicates the robust nature of the assay even between observers.

The Vmax would appear to be an equally reproducible variable and the variation in value did not exceed that of SLC over time. This was important as it vindicates the mathematical model used for kinetic analysis in this regard. The Km value would seem a weaker variable in this respect although its reproducibility was also acceptable with an average variation in value of 10.9% over time and 7.5% on same-sample assay.

Overall inter-assay and intra-assay variation for SLC values were similar to those reported elsewhere, and were mirrored by the reproducibility of the Vmax. The Km would appear to be a less reproducible variable, although by no means to an unacceptable degree.

Section 5.2 Effect of smoking on SLC

5.2-1 Smoking and SLC

SLC is associated with essential hypertension and other vascular disease states. Because of the importance of smoking as a risk factor for vascular disease and the fact

that many of the groups studied with regard to SLC may also have a high prevalence of smoking, it is important to examine its effect on SLC. The effect of other environmental factors such as alcohol on the transporter has already been demonstrated (Adebayo *et al.*, 1994), but no study specifically addressing the effect of SLC on smoking has been published.

5.2-2 Study Design

In order to eliminate as many as possible confounding variables, 14 regular and otherwise, healthy smokers and 14 non-smokers were recruited from within the male medical staff of the Adelaide and Meath Hospital Dublin. Male staff were chosen only, in order to eliminate the possible confounding effect that, different phases of the menstrual cycle and exogenous oestrogen use among females, could have on SLC activity.

28 volunteers were recruited after informed consent was obtained. Subjects had their blood sampled for lipid analysis and SLC assay between 08.00-10.00 in a state of fasting and abstention from all alcohol and cigarettes, from midnight the previous evening. A family history of hypertension, cardiovascular disease, medication and smoking habits was obtained in all cases. Blood pressure and heart rate was measured in the sitting position prior to sampling, and the average of three readings taken as the final result. In addition weight and height were measured for determination of body mass index (BMI).

In order to examine the acute effect, if any, of smoking on SLC, smokers were then asked to smoke a cigarette containing 0.5mg nicotine and 5mg tar (Silk Cut mild –

Benson & Hedges), and a further blood sample taken to compare with the pre-smoking value.

5.2-3 Statistical Analysis

Statistical analysis employed the non-parametric model of Wilcoxon / Kruskal-Wallis and data is expressed as the median with 10^{th} to 90^{th} centiles in parantheses. P values used indicate presence or absence of statistical significance at the 5% level. Values for before and after smoking were also compared by a Wilcoxon signed rank test. All tests were performed using JMP software on an IMAC (© Apple MacKintosh) computer

5.2-4 Results

Baseline variables of BMI, systolic and diastolic blood pressure, and total cholesterol levels were similar for the smoking and non-smoking groups with no significant difference at the 5% level as tested for by Wilcoxon / Kruskal-Wallis.

Baseline variables for the two populations are summarised in **Table 5.3**.

Intracellular ion levels after the lithium loading procedure (described in section 3.2) were also similar for the two groups. Median intracellular lithium value for the smoking group was 6.45 (6.1-8.9) mMol Li^+ / Litre cells compared with 7.3 (6.15-9.25) for the non-smoking group (p=0.058). Median intracellular sodium levels were 2.61 (1.82-4.09) and 2.17 (1.61-3.84) mMol Na^+ /Litre cells respectively (p= 0.1781). Median intracellular potassium levels were 95.29 (73.55-120.28) and 90.78 (75.06-108) mMol K^+ / Litre cells respectively (p=0.6458). Neither was their any difference in cell volume observed with median corpuscular volumes (MVC) of 92.1 (86.7-96.5)

for the smoking and 91 (87.5-94.5) fL for the non-smoking group respectively (p=0.2966).

SLC:

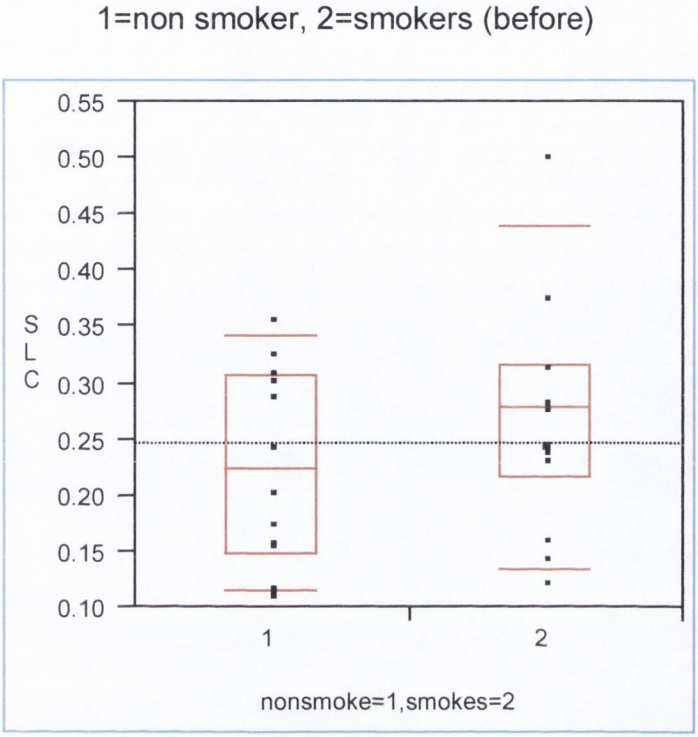
Median SLC for the smoking group was 0.2797 (0.1354-0.4409) mMol Li^+ / Litre Cells / Hour before smoking and 0.2841 (0.1557- 0.4782) after smoking versus an average SLC of 0.2251 (0.1152-0.3436) for the control group. Because of the relatively small sample size and consequent relatively large distributions, a non-parametric test, Wilcoxon / Kruskal-Wallis tests was chosen to compare the two populations. A Wilcoxon signed rank test was used to analyse the before and after effect of smoking among the smoking population.

There was no significant difference between smokers and non-smokers with regard to SLC values before smoking p =0.3012 (see **Fig. 5a**), or when values after smoking were used p = 0.2148 (see **Fig. 5b**). Baseline variables for body mass index (BMI), systolic and diastolic blood pressure were similar for the two groups and while there was a non-significant trend towards higher total cholesterol levels in the smoking group (see table 5.3), this was not reflected in increased SLC activity. Analysis of the smoking group before and after smoking however showed a significant effect of smoking on values, (p = 0.049). (see **Fig. 5c**)

Table 5.3 Baseline variables for smokers and non-smokers

Variable	Smokers n =14	Non-Smokers n=14	P value (Wilcoxon)
Age / years	26.64 ± 2.68	27.14 ± 2.44	
BMI / Kg m^{-2}	24.8 (19-27)	23.2 (19-26)	p= 0.1078
Family History of Hypertension	8	5	
Blood Pressure / mmHg			
Systolic	125 (115-132.5)	120 (115-130)	p = 0.1375
Diastolic	75 (70-90)	70 (65-80)	p= 0.1480
Total Cholesterol / mMol L^{-1}	5.87 (3.8-6.4)	4.55 (3.65-5.3)	p= 0.069

Fig 5a: Comparing Non Smokers With Smokers (Before) for SLC



Quantiles

Level	minimum	10.0%	25.0%	median	75.0%	90.0%	maximum
1	0.1123	0.11525	0.148075	0.2251	0.306725	0.3436	0.3597
2	0.1242	0.1354	0.21655	0.27965	0.315625	0.44085	0.504

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

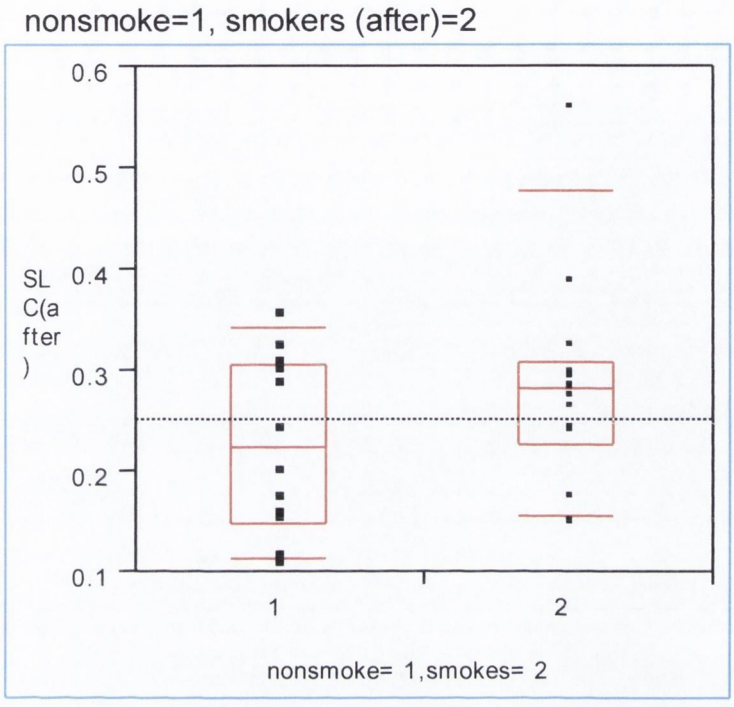
Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
1	14	180.5	12.8929	-1.011
2	14	225.5	16.1071	1.011

1-way Test, Chi-Square Approximation

ChiSquare	DF	Prob>ChiSq
1.0691	1	**0.3012**

Not significant at the 5% level.

Fig. 5b: Comparison of SLC values between smokers (after smoking) and non-smokers.



Quantiles

Level	minimum	10.0%	25.0%	median	75.0%	90.0%	maximum
1	0.1123	0.11525	0.148075	0.2251	0.306725	0.3436	0.3597
2	0.1547	0.15565	0.2288	0.2841	0.3096	0.4782	0.5642

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
1	14	176	12.5714	-1.218
2	14	230	16.4286	1.218

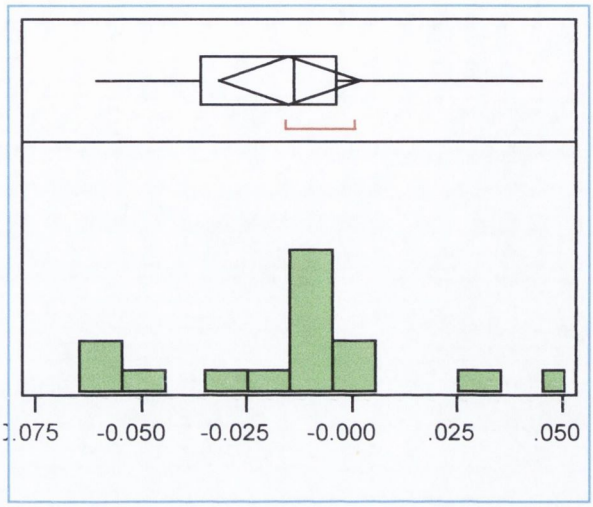
1-way Test, Chi-Square Approximation

ChiSquare	DF	Prob>ChiSq
1.5391	1	**0.2148**

Not significant at the 5% level.

Fig. 5c: Difference in SLC before and after smoking

difference SLC



Quantiles

maximum	100.0%	0.04500
	99.5%	0.04500
	97.5%	0.04500
	90.0%	0.03585
quartile	75.0%	-0.0033
median	50.0%	-0.0132
quartile	25.0%	-0.0356
	10.0%	-0.0587
	2.5%	-0.0602
	0.5%	-0.0602
minimum	0.0%	-0.0602

Test Mean=value

Hypothesized Value	0
Actual Estimate	-0.0145

Signed-Rank

Test Statistic	-31.500
Prob > |t|	0.049

Significant at the 5% level.

Vmax.

Kinetic analysis of the transporter was then carried out to determine Vmax. by the method described in section 3.2-3, and statistical comparisons between the groups as outlined above. Median value Vmax. for smokers (before smoking) was 0.4003 (0.2177-0.8023) versus 0.3408 (0.1508-0.7977) for the control group (p= 0.4347). Similarly, the median Vmax. value after smoking of 0.4802 was not significantly different from that of the control group (p= 0.2322), although when the smoking group were compared for the before and after effect of smoking the p value did shorten considerably (p= 0.068).

Km

Kinetic analysis of the transporter to determine its affinity for external sodium (Km) was carried out as outlined in section 3.2-3. Median value for the smoking population (before smoking), of 78.16 (33.78-163.46) did not differ significantly when compared to the non-smoking group value of 90.28 (34.76 – 196.29) (p= 0.3581). Again when the after smoking median Km value of 78.79 (38.14 – 197.55) was used in the statistical analysis there was no significant difference to the non-smoking group (p= 0.7132). Analysis of the before and after effect of smoking on the Km was likewise not significant (p= 0.542).

5.2-5 Discussion

As smoking is an important risk factor for vascular disease and because it is prevalent in people with such disease states, its importance as a possible confounder of studies looking at SLC in patient populations is important to assess. Previous work has shown alcohol to have an effect on SLC activity (Adebayo *et al.*, 1994) and as regular

ingestion of alcohol is associated with hypertension (Potter & Beevers 1984), such a finding is of particular relevance in the setting of SLC. This effect of alcohol on SLC is thought to be due to its interaction with fatty acid components of membrane phospholipids altering the lipid composition of the erythrocyte, resulting in increased membrane fluidity and expansion (Freund 1979; Winocour *et al.*, 1992). Although such a change is unlikely with smoking, smokers do tend to have higher LDL levels, and an equilibrium between plasma lipid levels and erythrocyte membrane make-up, is plausible.

There are several other good reasons to postulate that smoking may effect SLC. It may act as a co-confounder in that smokers may have higher LDL levels, have a greater alcohol consumption or take less exercise than a non-smoking population, all of which have been shown in themselves to effect SLC (Adragna *et al.*, 1985). It may also have a direct effect on the transporter. Erythrocytes in themselves do not have a nicotinic receptor to allow the active ingredient of tobacco affect its function, but smoking generates the production of a variety of soluble gases which are inhaled. Most notable among these is carbon monoxide (CO). Carbon monoxide binds strongly and irreversibly to haemoglobin, with an affinity a hundred times greater than that of oxygen, to form carboxyhaemoglobin, changing the structural configuration of the haem molecule and altering erythrocyte function. Smokers' erythrocytes are known to have a number of enzymatic differences, with lower carbonic anhydrase activity (Abel *et al.*, 1997) and increased susceptibility to peroxide-mediated free radical damage (Mehlhorn 2000) when compared to controls. In addition smoke inhalation is associated with decreased erythrocyte cholinesterase activity independent of carbon monoxide (Houeto *et al.*, 1999), suggesting that other soluble gases generated by smoke may effect erythrocyte function. Erythrocyte membranes from smokers have

also been shown to have lower essential fatty acid concentrations and are more prone to lipid per-oxidation (Brown *et al.*, 1998). Such disturbances in membrane composition are likely responsible for the increased aggregability (Weng *et al.*, 1998) and reduced deformability (Cicco *et al.*, 1999) observed in some smokers' erythrocytes. Clearly such effects could also impact significantly on membrane transport systems such as SLC.

My study was designed to examine smoking as specifically as possible by excluding as many extraneous variables as possible. This was achieved by recruiting volunteers from within a relatively healthy young male population of medical doctors, specifically according to smoking habits. While such an approach has its inherent biases, I felt it was necessary in order to examine the question of smoking having an effect on SLC, as specifically as possible. In this regard females were excluded as both pregnancy (Smith *et al.*, 1982.b) and the oral contraceptive pill (Stokes *at al.*, 1992) have both been shown to affect SLC. Both the control group and smoking group were not significantly different in terms of BMI, systolic and diastolic blood pressure and total cholesterol, although there was a trend towards higher cholesterol in the smoking group ($p=0.069$). Both groups were also of similar age range and had a similar prevalence of family history of hypertension. See **table 5.2** .

The wide distribution of SLC values obtained, in part, probably reflects a possible bias in selection and also the small sample population used. Two factors account for this limitation in sample size. Firstly and most notably, the time consuming, manual nature of this assay as it currently stands, limits the individual investigator as to what can be realistically accomplished. Secondly there are difficulties in finding a study

population within such parameters, and who are willing to participate in a "before and after" model of sampling.

Statistical analysis of such a study therefore requires the use of more rigorous non-parametric models, such as the Wilcoxon / Kruskal-Wallis test, used above. There was no demonstrable difference between smokers and non-smokers, in terms of SLC activity, Vmax. or Km, whether the pre- or post-smoking values were chosen, although median SLC values post cigarette were indeed higher. The decision to examine SLC activity before and after smoking is not without precedent. Previous findings indicated for example, that while acute ingestion of alcohol lowered SLC activity (Adebayo *et al.* 1994), chronic ingestion is associated with higher activity (Winocour *et al.*, 1992), and a subsequent period of abstinence lowered SLC (Ostrow *et al.*, 1986). Such a complex pattern of effect on SLC is difficult to interpret but suggests that the chronic effects of substances on SLC may be at odds with the observed acute effect.

When smokers were compared to themselves however, there was a significant increase in SLC after smoking a cigarette. There was no significant change in kinetic variables to account for this, although the pattern strongly suggests that it is a change in Vmax. ($p=0.068$ for 'before and after' analysis) which mediates this rather than any change in the affinity of the transporter for sodium (Km) [$p=0.542$ for 'before and after' analysis]. A number of theoretical possibilities may explain this observation.
To consider them one first needs to re-visit the kinetics of SLC. SLC obeys saturation kinetics as do most specific transport mechanisms and enzymes. The process depends initially on the binding of free substrate [S] to free catalyst [E], its transport across the membrane, in the case of SLC, and dissociation to form free catalyst capable of

binding again at the internal membrane surface. Thus it can be seen with SLC as with most membrane channel transporters that there are six possible rate constants (three steps forward and three back) (Stein 1986). Vmax. represents the theoretical maximum velocity of the transporter at an infinite concentration of substrate and in its simplest expression is a function of the total amount of catalyst and two rate constants – the rate of breakdown of the complex and return of the free complex to the binding site. In SLC this is a two-way process. Therefore in practical terms Vmax. is increased by increased catalyst concentration, rapid dissociation, or decrease in passage time of the catalyst across the membrane. The acute act of smoking is unlikely to affect catalyst concentration, but the generation of soluble gases acutely diffusing into the erythrocyte, may alter membrane fluidity or have effects on catalyst-substrate dissociation. Unfortunately a study of membrane fluidity changes with smoking was not possible in this study. The fact that there was no trend towards change in Km strongly suggests that whatever its exact effect, it is not through improved complexing with substrate. Part of the problem with kinetic analysis in SLC is, as yet, the exact catalyst remains unknown, though it can be inhibited by phloretin and N-ethylmaleimide (Sarkadi *et al.*, 1978; Duhm & Becker, 1979), a fact which suggests a protein is at least partly involved.

Thus smoking would appear to have an acute promotory effect on SLC. This in itself is important to know, as larger studies of patient populations should probably include some stipulation of abstinence from smoking as with food prior to testing. The fact that no demonstrable difference in SLC was evident between smokers and non-smokers, may be the result of too small a population sample, the fact that smokers had been in a state of abstinence for eight hours or most likely a combination of both

factors. Clearly the answer to this question would benefit from a larger study which includes measurement of erythrocyte membrane viscosity and carbon monoxide levels.

5.3 Summary

- SLC assay is robust over time and on same-sample analysis. Reproducibility of SLC values was similar to that published by other authors for both the inter-assay and intra-assay variation.

- There was no significant difference in SLC, Vmax. or Km values between smokers and non-smokers.

- The acute effect of smoking was to produce a significant increase in SLC value without significant change in kinetic values although, the pattern suggests that this is mediated through a change in Vmax. rather than Km.

Chapter Six

Results III

Chapter Six – Results III

Contents:

6.1 Effect of timing post-stroke on SLC

6.1-1 Introduction

Before conducting a study of SLC in patients with acute cerebral infarction, it was important to evaluate the possible effect of the timing of sampling post-stroke on SLC activity. There are many plausible reasons as to why such an effect might exist. Recovery from stroke is a dynamic process and often accompanied by neuro-medical complications (Collins & O'Neill 1998; Kalra *et al.*, 1995) such as dysphagia and aspiration pneumonia, urinary tract infections and uraemia, cardiac failure, decubitus ulcers or venous thrombo-embolism. Many of these complications can lead to various states of pyrexia, dehydration and electrolyte imbalance, activation of neuro-humoral systems such as the renin-angiotensin system or a reduction in plasma lipid levels, some of which have reported effects on SLC activity (Adebayo et al., 1996; Messner *et al.*, 1991). In addition many of drugs required to treat such complications may have an effect on SLC activity. This may have been previously reported as in the case of frusemide (Sarkadi *et al.*, 1978), though subsequently disputed (Beuckelmann & Erdmann 1986). This too has been the case with angiotensin converting enzyme (ACE) inhibitors (Niutta *et al.*, 1986; De la Sierra *et al.*, 1995), and the effect of other therapeutic agents may be as yet un-quantified by research. In any study of acute stroke patients such factors are difficult to control for and it would be important to assess the potential variation in SLC activity with time post-stroke, to see if such changes vary from the normal inter-assay variation.

6.1-2 Objectives

To determine the effect, if any, of sample time post-stroke on SLC activity.

To compare any such variation in SLC activity with the inter-assay variation for the assay reported in section 5.1.

6.1-3 Methodology

Five patients admitted, to the stroke-service at the Adelaide and Meath Hospital Dublin, with acute cerebral infarction confirmed by computerised tomography (CT), were recruited to participate in this study after written informed consent. A fasting blood sample was taken for lipid profile and SLC assay on days 1, 4-7 and 10 post-stroke. Blood pressure, heart rate, concurrent medications and medical complications of the stroke were also noted on the day of sampling. SLC assay and kinetic analysis was performed as outlined in sections 3.2-2 and 3.2-3. Analysis of variation was by calculation of the coefficient of variance (CV%) to allow for comparison with that previously found in the inter-assay reported in section 5.1. Values are expressed as mean + standard deviation, as values of a given assay over time in the same individual would expect to be normally distributed and obey the central limit theorem. This variation was also analysed by Wilcoxon rank sum, and significance taken at the 5% level.

6.1-4 Results

Five patients, two male and three female of mean age 80.4 years (range 64-87 years), were recruited for this study. Due to patient recumbancy it was not feasible to calculate body mass index, and therefore weight alone was used as a surrogate for body mass. Mean weight was 73.6 $\pm$ 13.4 Kgs. All patients had a clinical syndrome

consistent with either a total anterior circulation infarct (TACI) or partial anterior circulation infarct (PACI), and cerebral infarction was evident on CT in all patients. With regard to medical history three patients had a history of hypertension and the remaining two patients were hypertensive during admission, eventually requiring treatment. Two patients had atrial fibrillation and one had a prior history of ischaemic heart disease with angina. One patient had had a prior stroke and one was diabetic (non-insulin dependent). Only one patient was a current smoker. Accurate family history was available in three cases only, one of which was positive for hypertension and stroke and one for ischaemic heart disease. Baseline characteristics for the five patients are summarised in table 6.1.

A fasting blood sample was taken on days 1, 4-7 and 10 in all five patients and SLC and lipid profile analysed.

Cholesterol

Mean cholesterol on day 1 was 4.46 ± 1.16 but fell to 4.0 ± 0.7 by day 4-7 and again to 3.64 ± 0.59 on day 10. The cholesterol level fell for all but one patient between the first and fourth-seventh day sample, and fell again between this and the tenth day post-stroke in all patients. Magnitude of drop was greatest between days 1 and 4-7 in all patients except one where the drop was delayed. This is shown graphically for the five patients in **fig. 6a**.

Table 6.1 Baseline characteristics of patient population.

Patient	sex	Age (yrs.)	Weight (kg)	BP mmHg.	Cholest. mMol/L	Stroke type	Medical history	Family history
1	female	84	50	180/100	6.1	TACI (right)	Nil	Nil
2	male	80	68	160/100	4.1	TACI (left)	A-fib.	Nil
3	female	87	64	150/100	3.5	TACI (left)	A-fib ↑ BP NIDDM	Nil
4	male	64	95	180/110	5.2	PACI (right)	↑ BP IHD	++
5	female	87	63	190/90	3.4	PACI (left)	↑ BP stroke	Nil

BP = Blood pressure expressed as systolic / diastolic reading.
Cholest. = Fasting total cholesterol.
TACI = Total anterior circulation infarct [side of lesion indicated in '( )'].
PACI = Partial anterior circulation infarct [side of lesion indicated in '( )'].
A-fib = Atrial fibrillation.
↑ **BP** = Hypertension.
NIDDM = Non-insulin dependent diabetes mellitus.
IHD = Ischaemic heart disease.
++ = History of hypertension and stroke in a first degree relative.

Blood pressure

Mean systolic blood pressure on day one of 172 ± 16.4 mm Hg, decreased to 154 ± 16.7 mmHg on day 4-7 and fell again to 144 ± 8.9 mmHg by day 10. This was mirrored by a similar pattern of fall in diastolic blood pressure from 100 ± 7.1 mmHg to 87 ± 9.7 mmHg on day 4-7 with a further small drop to 84 ± 8.9 mm Hg on day 10. In three patients blood pressure readings fell over the three recorded days and in two patients it fell between days 1 and 4-7 but remained stable at day 10. This pattern is not surprising for two reasons. Firstly, it is common for blood pressure to rise acutely after a stroke as part of the stress reaction, and the higher readings on day one may reflect this tendency. Secondly, existing anti-hypertensive medication was restarted in

two patients between days 1 and 4-7, and existing anti-hypertensive medication increased in one other patient between these days. This would account for the subsequent decrease in systolic and diastolic pressure observed.

SLC

Mean SLC was 0.2118 ± 0.0349 mMol Li^+ / Litre Cells / Hour on the first day post-stroke for the group of patients. This had fallen slighty overall at 0.1980 ± 0.0532 by day 4-7, and again to 0.1749 ± 0.0392 by day 10 post-stroke. This however does not reflect the situation in individuals, but rather as a whole. In three patients SLC activity had fallen consistently between days 1 and 4-7 and 10 and by a magnitude greater than the inter-assay variation, reported in section 5.1. In one patient the SLC value fell between days 1 and 4-7 and had almost recovered to the original level by day 10. In one patient the level of activity rose slightly between the first two samples and dropped back to near the original value by day 10. This is shown in **fig. 6b.**

The inter-assay variation of SLC among healthy controls showed a coefficient of variance between 2.8 – 5.8 % (average 3.9%), as reported in section 5.1. When stroke patients were assayed over the first 10 days post-stroke this coefficient of variance increased in range to between 6.8 – 25.2 % (average 15.4%). Clearly this variation is well in excess of that reported both in this thesis and by others and as such, would not lead to an acceptable assay. Another way of analysing this variation is to compare the maximum differences between SLC values from the control group in the interassay experiment and stroke group and apply a Wilcoxon rank sum test. This was performed and showed a significant variation of SLC values from the stroke population over the control group 'Inter-assay' ($p=0.0253$).

Fig. 6a. Variation in total cholesterol post-stroke.

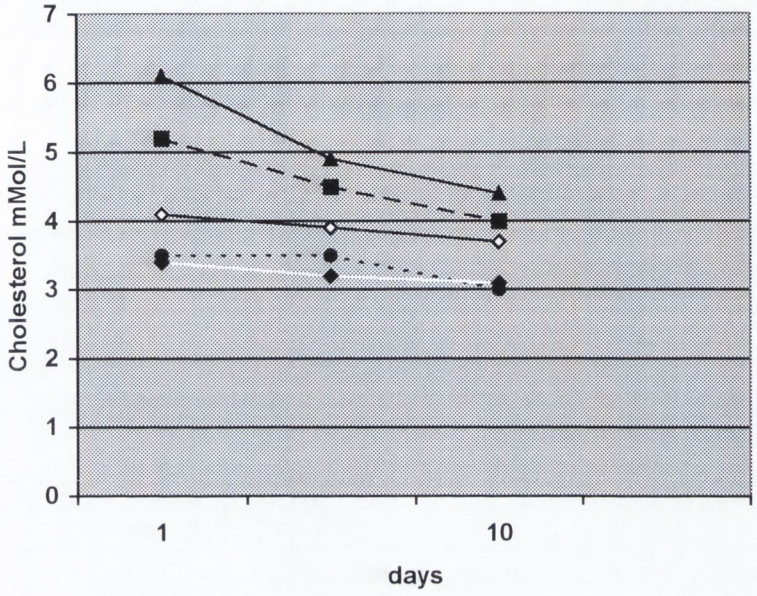
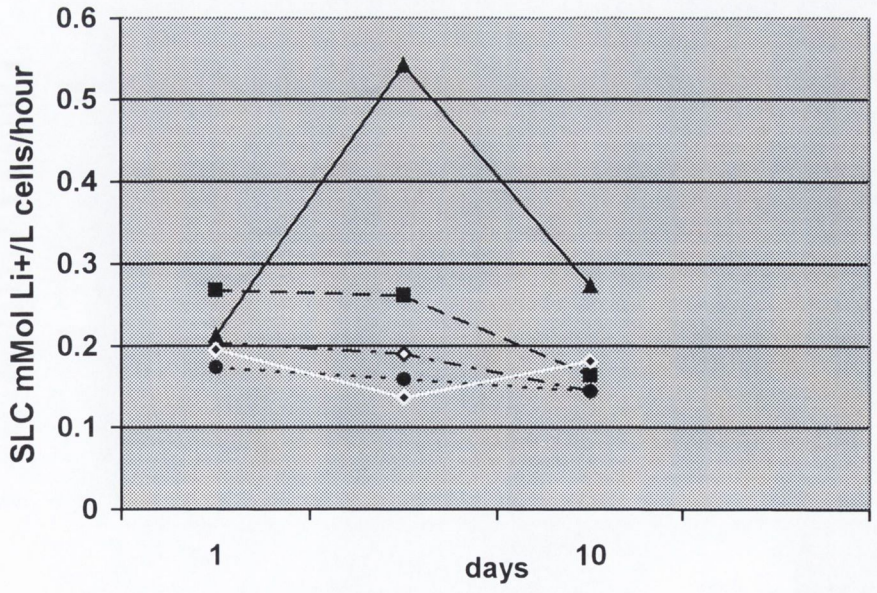


Fig. 6b. Variation in SLC values post-stroke.



Vmax.

This pattern of variation was also reflected by the Vmax. of the transporter in acute stroke patients. In general where SLC values fell this was mirrored by a fall in Vmax., and vice versa, so that the mean Vmax. value was 0.3126 ± 0.0676 on day one, 0.3129 ± 0.1518 on day four-seven and 0.2680 ± 0.0513 by day ten. Again these figures are a summation of the case as a whole and belie the individual situation where Vmax. fell over the three samples in two patients, fell between samples on day 1 and 4-7 but recovered towards the original value in a further two patients, and rose between the first two samples and fell back to the original value in another patient. I reported a coefficient of variance of between 2.3 –5.7 % (average 4.2%) in section 5.1, while this is far greater over the time period post-stroke with a range of 7.9 – 23.9% (average 20.2%). Again this mirrors the large change in the coefficient of variance observed with SLC. When Wilcoxon rank sum test is applied to the maximum differences in Vmax. values of individuals from the two groups, the result is again significant ($p = 0.025$).

6.1-5 Discussion

Clearly when coefficients of variance are compared there is a large difference observed between that reported in the inter-assay variation (section 5.1, chapter five) and that seen when stroke patients have their SLC measured over the first ten days post-stroke. The reasons for this are likely to be many, some of which have been previously reported including intercurrent illness, a fall in cholesterol levels (Corrocher *et al.*, 1985), changes in drug prescriptions and varying states of hydration and/or renal failure. In particular it is likely to correlate with cholesterol levels given the strong association between SLC and cholesterol. Previous work has shown total

cholesterol levels to fall from day one to a low point at day seven and return to a high at three months post cerebral infarction (Mendez *et al.,* 1997). Nevertheless while cholesterol levels fell in all five patients over the ten-day period and SLC also fell over this period, it did rise appreciably in one patient and other factors could clearly be at play. Additionally the two patients with lowest cholesterol levels at day 10 (3.1 and 3.3 mMol/L, respectively) did not have the lowest SLC values (0.2391 and 0.2878 mMol Li^+ /L cells/hour, respectively [mean value at day 10: 0.1749 ± 0.0392]) as one might expect if changes in cholesterol level alone were to account for the variation. Because such variation is impossible to control for given the dynamic process of recovery from stroke, it would be prudent to assay SLC when the patients' physiological state has stabilised. For this reason it was decided that the timing of SLC assay could vary significantly over the acute period post-stroke and therefore the timing of sampling would have to be standardised in all future experiments. A period of not less than two months from onset of stroke was chosen as most patients at this stage are relatively stable, have had fluid and electrolyte imbalances addressed and are on a relatively fixed regimen of medication.

There was also some indication from the above study that SLC tended to return towards day 1 values by the tenth day. For similar reasons I decided to exclude patients who were acutely unwell or had any evidence of recent clinical deterioration from further work. This model was also applied to patients recovering from myocardial infarction, although it was not possible to recruit enough acute patients to sequentially examine the effect of timing post MI on the SLC assay.

6.2 Comparison of SLC in hypertensive, stroke and myocardial infarction patients versus non-hypertensive controls.

6.2-1 Introduction

The positive association between SLC and hypertension and SLC and end-organ disease has been outlined in chapter 1, section 1.2. This association may be related to the genetic predisposition to hypertension, but SLC could also represent a broader marker of vascular risk and reflect membrane changes on the human erythrocyte which are repeated in other cell lines such as endothelial cells, renal tubule cells or neurones. Clearly when one talks of vascular disease, myocardial infarction and cerebral infarction are the two pathological entities with the greatest clinical significance in terms of patient morbidity and mortality. Little has been reported however about the possible association of SLC with these two disease processes.

6.2-2 Objectives

The aim of this study was to examine the possible association of SLC with stroke and myocardial infarction (MI) by comparison with known hypertensive patients without such disease and healthy non-hypertensive controls.

6.2-3 Methodology

A proposal was submitted to the Joint Research and Ethics Committee of the Federated Dublin Voluntary Hospitals outlining study objectives and design and approval was obtained prior to commencing work. Four study groups were recruited after informed consent was obtained. These were patients who had suffered a cerebral infarct not less than 2 months ago, patients who had suffered a myocardial infarction

not less than two months ago, known hypertensive patients attending a hypertension clinic and a group of healthy controls with no history of hypertension, stroke or MI. Groups were matched as closely for age and sex as possible.

All patients and controls had a full medical history and family history taken. Baseline blood pressure readings were recorded at five minute intervals in the sitting or recumbent position and the mean of three readings taken (Accuson mercury sphygmomanometer in the case of stroke, MI patients and the control group and by Omron 705a sphygmomanometer in the hypertensive group – as instrumentation available differed between the hospital ward and out-patient setting).
All patients had their weight recorded (Sega instruments) and a fasting blood sample drawn between 08.00 –10.00 for lipid analysis and SLC determination. The method of sample preparation and kinetic analysis has already been outlined in the methodology, section 3.2 of chapter three.

Statistical analysis was performed using a non-parametric model (Wilcoxon /Kruskal-Wallis) by JMP software on an IMAC computer (© Apple Macintosh). Values are expressed as the median with 10^{th} - 90^{th} percentiles in parantheses. Significance was at the 5% level (i.e. $P < 0.05$).

6.2-4 Results

10 people were recruited in the control group with no prior history of hypertension or cardiovascular disease and on no anti-hypertensive medication. One patient was found to be clearly hypertensive on baseline assessment and was referred for treatment. This patient was removed from subsequent analysis. Sixteen patients were recruited from

the hypertensive clinic. All patients had a history of hypertension, were on anti-hypertensive medication but had no prior history of stroke or myocardial infarction.

Sixteen patients with cerebral infarction were recruited, nine of whom were hypertensive (three with a prior history and six with a de novo diagnosis).

Twelve patients with acute myocardial infarction were recruited, two of whom had a prior history of hypertension. The baseline characteristics of the group are summarised in table 6.2. Statistical comparisons were made with reference to the control group in all cases.

Weight

Median weight did not differ significantly between the four groups – 76 Kgs (48 - 90.4 Kgs) for the control group; 81.3 Kgs (65.2 – 97.7 Kgs) for the hypertensive group (p=0.14); 71.3 Kgs (44.2-84.8 Kgs) for the stroke group (p=0.46); 72 Kgs (54.8 – 89 Kgs) for the MI group (p=0.62).

Blood Pressure

Median systolic blood pressure for the control group of 130 mmHg (110 – 140 mmHg) differed significantly from the hypertensive group with a median of 159 mmHg (130 – 179.8 mmHg) [p=0.002], but not from the stroke group with a median value of 125 mmHg (110 – 148 mmHg) [p=0.53] or the MI group, median value 120 mmHg (110 – 140 mmHg) [p=0.23]. This was similarly reflected in diastolic pressure readings, median 70 mmHg (65 – 85 mmHg) for the control group; 89.5 mmHg (70 – 105.3 mmHg) [p=0.001] for the hypertensive group; 80 mmHg (70 – 92 mmHg) [p=0.17] for the stroke group; 80 mmHg (60 – 90 mmHg) [p=0.55] for the MI group.

Cholesterol

Cholesterol levels showed some variation with median values of 5.5 mMol/L (4.4 – 7 mMol/L) for the control group significantly higher than that of the stroke group with a median value of 4.6 mMol/L (3.2 – 6.01 mMol/L) [p = 0.007], although no such difference was observed compared to the MI group, median value 5.05 mMol/L (4 – 6.57 mMol/L) [p= 0.19] or the hypertensive group, median value 5.2 mMol/L (4.2 – 5.97 mMol/L) [p= 0.12].

Intracellular ions

The levels of intracellular ions after the lithium loading procedure were also compared. Median lithium levels were 7.24 mMol/L (6.18 – 8.44) for the control group; 6.81 mMol/L (6.14 – 8.35) for the hypertensive group [p=0.21]; 7.9 mMol/l 9 6.23 – 11.79) for the stroke group [p=0.09]; 7.93 mMol/L (6.3 – 12.77) for the MI group [p=0.29].

Similarly potassium levels did not differ between the control or hypertension groups with median values of 112.9 mMol/L (101.6 – 119.92) and 107.28 (93.91 – 126.3), respectively [p=0.82]. Nor was there a difference between the controls and stroke group, median 107 .9 mMol/L (52.06 – 148.2) [p= 0.57], or the MI group 132.7 mMol/L (87.52 – 174.7) [p=0.06].

Sodium levels were also similar between control and hypertensive groups, median values 3.19 mMol/L (1.59 – 4.87) and 3.41 (1.85 – 4.66) respectively [P= 0.61]. Neither was a difference observed compared to the stroke group, median value 3.03 mMol/L (0.4 – 5.6) [p= 0.78], though some difference was apparent with the MI group, median value 5.4 mMol/L (0.74 – 7.45) [p=0.03].

Table 6.2 Baseline characteristics of study groups

Group	sex m/f	age (yrs.)	blood pressure (mmHg)	Weight (Kgs)	Cholesterol (mMol/L)
Controls n=9	4 /5	69 (61-82)	Sys. 130 (110-140) Dias. 70 (65-85)	76 (48– 90.4)	5.4 (4.4-7)
Hypertension n=16	7/9	66 (57-77)	Sys. 159 (130-179) ***p=0.002** Dias. 89.5 (76.3-105.3) ***p= 0.001**	81.3 (65.2-97.7) p=0.14	5.2 (4.2-5.97) P =0.12
Stroke n=16	8/8	72 (48-87)	Sys. 125 (116-148) p=0.53 Dias. 80 (70-92) p= 0.17	71.3 (44.2-84.8) p=0.46	4.6 (3.27-6.01) ***p=0.007**
MI n=12	5/7	67 (55-78)	Sys. 120 (110-140) p=0.26 Dias. 80 (60-90) p= 0.55	72 (54.8-89) p=0.62	5.05 (4.15-6.57) p=0.19

* significant at the 5% level (Wilcoxon / Kruskal-Wallis)

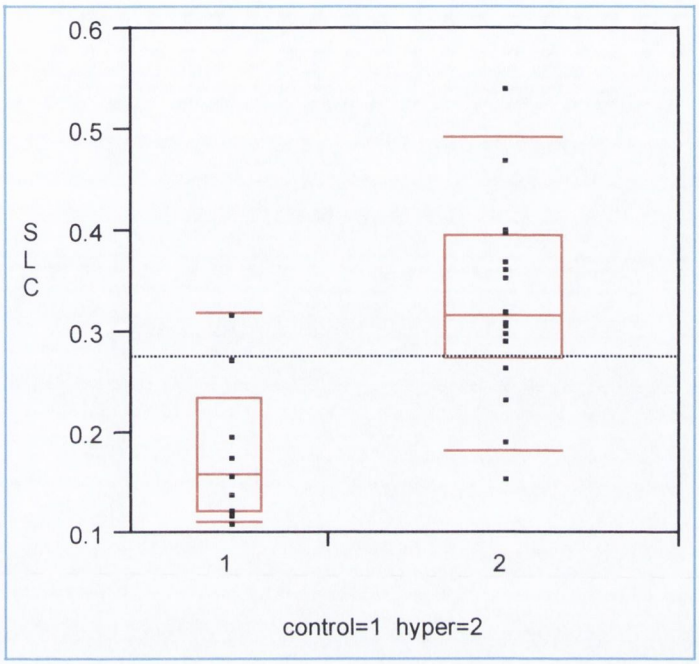
SLC

SLC activity differed significantly between the control and hypertension groups with activity being higher in the latter. Median control group value of 0.1601 mMol Li^+ / L cells / Hour (0.1121 – 0.3203) versus 0.3171 mMol Li^+ / L cells / Hour (0.1819 – 0.4947) for the hypertensive group [p= 0.002]. This to some extent confirms what was already known, the positive association between SLC and essential hypertension (**Fig. 6c**). This difference was mainly mediated by transport activity with Vmax values for the hypertensive group of 0.5105 (0.288 – 0.8312) being much higher than the control group, 0.2462 (0.1514 – 0.3973) [p= 0.0004]. No difference was observed in affinity of the transporter for external sodium (Km), median values of 63.59 (29.12 – 91.03) for the control group and 83.42 (50.84 – 105.03) for the hypertensive group [p= 0.06].

SLC activity was also significantly higher in the stroke group when compared to controls, median value 0.2199 mMol Li^+ / L cells / Hour (0.1572 – 0.4527) [p= 0.03]. **Fig. 6d**. Again this appeared to be mediated by increased turnover, (Vmax) with median values of 0.3364 (0.2536 – 0.6979) for the stroke group versus 0.2462 (0.1514 – 0.3973) for the control group [p= 0.03]. No difference was observed between transporter affinity for external sodium (Km) with values of 63.59 (29.12 –91.03) for the controls and 74.09 (46.26 – 128.04) for the stroke patient group [p= 0.09]. When patients with a history of, and newly diagnosed hypertension were excluded and analysis repeated this effect disappeared. Rank sum Wilcoxon test for non-hypertensive stroke patients versus controls revealed a non-significant difference for SLC (p=0.1858) and Vmax. (p=0.2235).

Fig. 6c: Comparison of SLC values between hypertensive patients and controls.

SLC by control=1, hypertensive =2.



Quantiles

Level	minimum	10.0%	25.0%	median	75.0%	90.0%	maximum
1	0.1121	0.1121	0.1223	0.1601	0.23655	0.3203	0.3203
2	0.1579	0.18191	0.27385	0.3171	0.3953	0.49473	0.5438

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

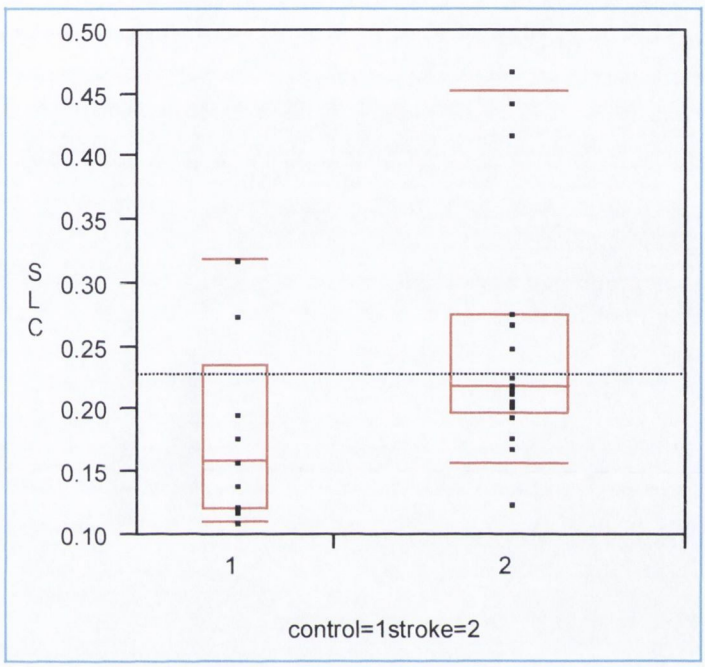
Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
1	9	61	6.7778	-3.142
2	16	264	16.5000	3.142

1-way Test, Chi-Square Approximation

ChiSquare	DF	Prob>ChiSq
10.0513	1	0.0015

Significant at the 5% level

Fig 6d: Comparison of SLC values between stroke patients and controls.



Quantiles

Level	minimum	10.0%	25.0%	median	75.0%	90.0%	maximum
1	0.1121	0.1121	0.1223	0.1601	0.23655	0.3203	0.3203
2	0.1253	0.15715	0.1979	0.21985	0.275925	0.45271	0.47

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
1	9	78	8.6667	-2.180
2	16	247	15.4375	2.180

2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
78	-2.17963	0.0293

1-way Test, Chi-Square Approximation

ChiSquare	DFProb>	ChiSq
4.8750	1	0.0272

Significant at the 5% level.

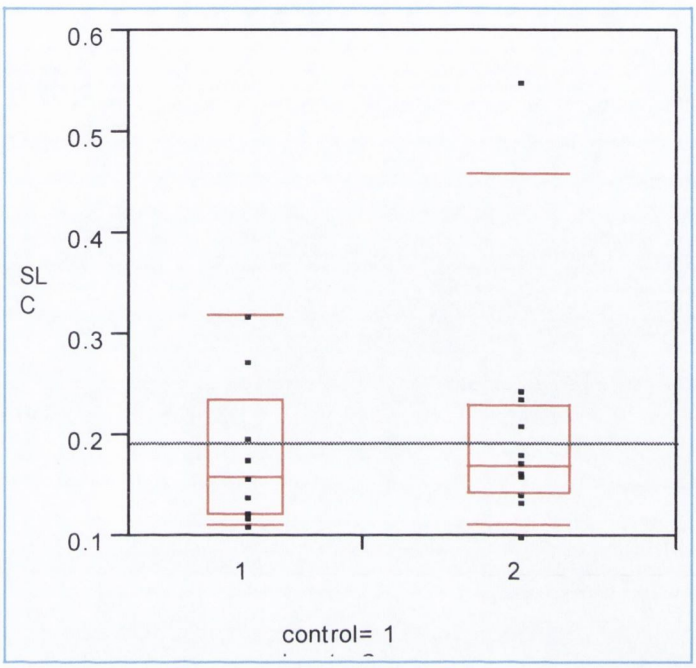
SLC activity did not differ between the MI and control groups, with median values for the MI group 0.1695 mMol Li^+ / L cells / Hour (0.1120 – 0.4600) [p= 0.57]. **Fig. 6e.** Vmax values of 0.2959 (0.2096 – 0.8968) likewise did not differ [p= 0.23] although transporter affinity appeared to somewhat lower (i.e higher Km) with a median value of 98.605 (70.9 – 149.03) [p=0.008].

6.2-5 Discussion

My study explored the possible role of SLC as a broad marker of cardiovascular disease, by examining the rates of SLC activity in four groups of patients – patients who had suffered a cerebral or myocardial infarct, patients with essential hypertension but no previous vascular event or renal failure (i.e. no evidence of end-organ damage), and a group of healthy normotensive controls. All groups were matched as closely as possible for sex and age to limit possible co-founders though, obviously it was not feasible to screen large numbers of patients with regard to variables such as fasting lipid levels or body mass index. Nevertheless the baseline variables were remarkably similar for all four groups, with similar distribution of males to females, similar age profile, and no significant difference in weight (see **table 6.2**) or fasting cholesterol levels, except the noted lower levels in the stroke group compared to controls.

This lower cholesterol in stroke patients is probably due to a combination of factors including a natural fall in levels post-stroke, variable periods of fasting due to dysphagia initially after the stroke and the treatment of elevated cholesterol in the secondary prevention of stroke. Blood pressure measurement did not vary significantly between stroke and MI patients and controls, though highly significant

Fig. 6e: Comparison of SLC values between MI patients and controls.



Quantiles

Level	minimum	10.0%	25.0%	median	75.0%	90.0%	maximum
1	0.1121	0.1121	0.1223	0.1601	0.23655	0.3203	0.3203
2	0.102	0.11196	0.143075	0.16945	0.2311	0.46003	0.5518

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
1	9	91	10.1111	-0.533
2	12	140	11.6667	0.533

2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
91	-0.53300	0.5940

1-way Test, Chi-Square Approximation

ChiSquare	DF	Prob>ChiSq
0.3232	1	0.5697

Not significant at 5% level

differences were observed in the hypertension group. This would be expected amongst a cohort of patients regularly attending a hypertension clinic.

The result that SLC activity is significantly raised in hypertensive patients compared to controls confirms what is all ready known from the literature and outlined in section 1.2-3. These patients were chosen from a hypertension clinic as such specialist clinics are likely to have a population of patients with essential hypertension from initial diagnosis, making it easier to find patients without end-organ events. There was considerable overlap however in values between the two groups **(fig 6.c)** and this has been a consistent finding in previous work (Brugnara *et al.*, 1983; Beuckelmann & Erdmann 1984). Vmax. for the transporter was significantly higher in the hypertensive group ($p=0.004$) suggesting that it is increased catalyst concentration, altered membrane structure resulting in decreased transit time or rapid dissociation to create free catalyst again, which is responsible for this increased rate. Of these theoretical possibilities it is altered membrane structure which has gained most credence with the observation that patient groups with elevated SLC are not infrequently found to have other ion flux anomalies in their erythrocytes (Lijnen *et al.*, 1993; Rutherford *et al.*, 1992). Patients with elevated SLC could also have increased carrier molecules on the erythrocyte membrane, but this is difficult to substantiate when the carrier molecule for SLC remains unknown.

SLC activity was also significantly higher in stroke patients when compared to controls ($p=0.03$), but again there was considerable overlap in the range of values obtained **(fig. 6d.)**. When patients with known hypertension ($n=3$) and those with a de novo diagnosis ($n=6$) were excluded however, no apparent difference was evident ($p=$

0.1858), although the resultant small numbers have restrictive power and are less amenable to statistical analysis. It is interesting that the stroke group had similar blood pressure readings to the controls, in contrast to the hypertensive group. This is likely to reflect the smaller numbers with a true history of hypertension in the stroke group, the possibility that many patients with a new diagnosis had merely reactive hypertension acutely post-stroke and perhaps more rigorous control of blood pressure in the hospital in-patient setting. When the possibility that the majority of these stroke patients did not have primary hypertension, and the fact that their total cholesterol levels were significantly lower than the control group (which would be expected to be associated with lower SLC activity) are considered, the possibility of genuinely increased SLC in patients with cerebral infarction independent of hypertension arises. Again the kinetic pattern observed is remarkably similar to that seen in the hypertensive group, with significantly higher Vmax. than controls ($p=0.03$) with no significant difference in Km. However, there was no evidence of increase or indeed decrease in SLC among patients with myocardial infarction, and the significantly higher Km is difficult to explain in this light.

While the overall result might suggest that SLC could be raised in stroke patients, this study has a number of limitations that require comment. Most importantly the numbers examined were small, an unfortunate practical necessity of the manual nature of the assay, the difficulty in obtaining suitable patients who are not acutely unwell in the hospital setting and the difficulty in identifying suitable age-matched, and hence elderly controls, with no history of hypertension or vascular disease.

The second factor relates to the inability to control for extraneous factors such as medications. Many of the patient groups would be expected to be on different combinations of diuretics, angiotensin converting enzyme inhibitors or perhaps insulin all with reported effects on SLC (Chi *et al.*, 1996; De la Sierra *et al.*,1995; Sarkadi *et al.*, 1978) though all have been reported to be inhibitory and other work suggests an absence of effect (Beuckelmann & Erdmann 1986; Niutta *et al.*, 1986; Rutherford *et al.*, 1993). Another variable in this equation is the influence of genetic predisposition. Family history of hypertension and/or cardiovascular events has been associated with higher SLC values in hypertensive patients when compared to patients without such a history (Morgan *et al.*, 1986; Carr *et al.*, 1989), and it could be argued the cohort of stroke patients studied here had very strong familial histories of cardiovascular disease (or conversely that the MI patients did not). Many of the stroke patients were elderly however, and did not know their parental history or cause of death. In addition due to dysphasia/cognitive impairment post-stroke, many were unable to communicate this information. The result therefore of five positive family histories in the stroke population could be misleading.

The third factor relates to the assay itself. The criteria for intracellular Li^+ loading prior to kinetic study require that the Km for internal Li^+ be exceeded 10-fold in order to ensure maximum saturation of the transporter in the face of passive leak of Li^+ ions. A value of intracellular Li^+ of 6-10 mMol/L has been suggested as adequate to ensure 80-90% saturation (Canessa, 1989), although higher ranges of 8-12 mMol/L have been used by others (Duhm & Becker 1977) and shown to improve precision of the assay. In addition there is considerable variation in the Km for internal Li^+ and although generally quoted at 0.5 mMol/L (Sarkadi *et al.*, 1978) it can range from 0.5-

2.0 mMol/L (Hannaert & Garay, 1986). Strictly speaking the intracellular levels achieved in this experiment met the criteria for adequate saturation of the carrier in all assays in all groups, but the median levels achieved were generally in the 6.8-7.9 range, the lower range of acceptable Li^+ loading. The consequence of inadequate loading, were it to occur, are two-fold. Firstly there could theoretically be a slower passive diffusion of ions into both Na^+-free and Na^+-rich media, and as SLC is the subtraction of passive (Na^+ -free medium) from total efflux (Na^+ rich medium), the resultant value obtained could be an overestimation. However this is likely to be compensated as carrier activity itself is likely to be reduced, and other work has shown inadequate intracellular levels of Li^+ to result in underestimation of Vmax (Hannaert & Garay, 1986). The fact that the quoted range of intracellular Li^+ for an accurate assay was met and that there was no statistical difference in intracellular Li^+ levels between all four groups prior to kinetic study, however was reassuring. In addition the range of values obtained with considerable overlap between the four patient groups is consistent with most published data.

It is clear from the literature that SLC is associated with essential hypertension (Canessa *et al.*, 1980), predisposition to hypertension and nephropathy in patients with both insulin and non-insulin dependent diabetes (Fujita *et al.*, 1994; Viberti & Earle, 1992) and the development of left ventricular hypertrophy in hypertensive patients (Yap *et al.*, 1992). What the exact role of SLC is in these pathological processes is unknown. It is unlikely that SLC is in itself pathological, its' observed rates of normal activity of 0.1-0.39 mMol/L cells per hour is dwarfed by other transport systems for sodium, (take the sodium-hydrogen exchanger (NHE) for example with reported rates of 25-60 mMol/l cells /hour when 95% activated (Corry

et al., 1993). However it may be that SLC represents a membrane change on the erythrocyte that is mirrored in other cell lines, which in themselves could be causative or the result of vascular disease. If this could be substantiated then SLC could indeed serve as a useful marker of vascular disease.

The fact of a reported familial link to SLC activity (Wilson & Meyer, 1981) is supportive of this theory of a genetically determined cell-membrane structural change. A level of activity for SLC of 0.4 mMol/L cells /hour has been suggested as discriminatory between hypertensive and normotensive populations (Carr *et al.*, 1989) from Canessa's original paper (Canessa *et al.*, 1980). Studies have shown considerable overlap in values between the two groups and while family history of either hypertension or cardiovascular disease is more likely to result in values > 0.4 mMol/L cells / hour (Morgan *et al.*, 1986), it has also been shown in one study that some 12 % of normotensive adults have this level of activity (Williams *et al.*, 1987). Other work has also indicated that even amongst hypertensive patients with a positive family history, few will achieve this level of SLC (Weder *et al.*, 1984). This is not helpful in teasing out the relationship of SLC to vascular disease, but has led to the suggestion of two groups of hypertensive patients, with those with higher SLC activity perhaps more at risk of cardiovascular disease. This study, despite its small numbers, bears out the degree of overlap between hypertensive patients and controls, with few hypertensive patients exceeding 0.4 mMol/L cells /hour, a pattern which was even more pronounced with stroke patients.

Another difficulty in assessing the relationship of SLC to vascular disease, is that the catalyst of SLC is unknown. Clearly in normal physiological, in vivo setting there is

no lithium to act as the countertransport ion, so what could be the association of iso-electric Na^+-Na^+ countertransport with hypertension ? The most frequent model proposed to that question is that SLC in itself may be catalysed by the Na^+-H^+ exchanger (NHE) (Aronson, 1982) and Na^+-H^+ exchange on the human erythrocyte has also been reported to be raised in hypertensive individuals (Semplicini *et al.*, 1995) The Na^+-H^+ exchanger is present in all mammalian cell lines and is thought to be important in pH and cell volume regulation, and in addition will accept Li^+ ions in a bi-directional fashion similar to SLC. Different isomers of the transporter have been identified through cloning of messenger RNA (Sardet *et al.*, 1989; Tse *et al.*, 1993) and the identification of an amiloride-resistant isomer in the kidney (NHE-3) responsible for Na^+ reabsorption in the proximal tubule of animals (Montrose & Murer, 1990), could provide a simple aetiological link between SLC and hypertension. However a number of difficulties exist with this assertion, not least that SLC has only been demonstrated in human erythrocytes (and rat glial cells), amiloride inhibits the NHE-1 isomer on erythrocytes but not SLC, uraemia appears to up-regulate and increase NHE-1 expression on erythrocytes but SLC is not raised (Corry *et al.*, 1993), and perhaps most convincingly the demonstration that no common genetic locus is shared for NHE-1 and SLC (Lifton *et al.*, 1991).

Other possible models of association of SLC with hypertension and vascular disease have been postulated (West *et al.*, 1998). SLC could serve as a marker of change in cell membrane viscosity resulting in other ion flux changes, which in itself is the result of alterations in blood cholesterol and membrane phospholipid levels, or in themselves result in significant disease. While enriching the erythrocyte membrane with cholesterol and thereby increasing its viscosity has been shown experimentally to

alter the rate of several ion-transport mechanisms including Na^+-K^+ ATPase (Giuraud *et al.*, 1976) and SLC, the relationship is not straightfoward. No correlation between SLC and membrane viscosity was detected in one study of normolipaemic hypertensive patients although when a positive family history was considered as a sub-group significant increases in membrane viscosity were evident (Carr *et al.*, 1995). In addition while cholesterol enriching of the membrane is generally associated with reduced SLC (Lijnen & Petrov, 1995), this does not explain the observed increase in SLC in type 1 diabetic patients with raised membrane cholesterol (Lijnen *et al.*, 1993).

An alternative model of association comes from this observed increase in SLC among diabetic patients. Whether SLC could directly be stimulated by insulin, and insulin in itself play a role in primary hypertension has been postulated (Grunfield *et al.*, 1994). Incubation with insulin has been shown to affect the kinetics of SLC (Zerbini *et al.*, 1995), and insulin has been shown to affect other cation transporters on the erythrocyte (Pontremoli *et al.*, 1994). Much more work however, remains in this puzzle.

It is likely that the final answer may lie in a combination of factors affecting erythrocyte membrane viscosity, SLC carrier expression and affinity for substrate, and the influence of extraneous agents on both. The fact that SLC could serve as a marker of cardiovascular risk is of interest in itself, as it could help identify individuals at risk and intensify primary preventative strategies in such people. More fundamentally however, through a better understanding of its association with disease states, the underlying cellular mechanisms of pathology may be better understood.

6.3 Summary of chapter six.

- SLC is associated with essential hypertension, diabetes, the development of hypertension and nephropathy in diabetic patients and of left ventricular hypertrophy in hypertensive patients. It may represent a broad risk marker of cardiovascular disease. Its possible association with stroke and myocardial infarction is unknown.

- An initial study examined the effect of sample timing post-stroke on SLC and found that in the majority of patients SLC fell over a ten- day period post-stroke. This was accompanied by a corresponding fall in serum total cholesterol.

- Four groups of patients were recruited and matched for age and sex; a control group, hypertensive patients with no other vascular pathology, patients with cerebral infarction and patients with myocardial infarction. Baseline variables were similar for the four groups except significantly higher systolic and diastolic blood pressure was evident in the hypertensive group, and significant lower total cholesterol in the stroke group. SLC was measured under standard conditions in all four groups. Both the hypertensive group and stroke group were found to have higher SLC and Vmax when compared to controls. There was no significant difference found in the myocardial infarct group.

Chapter Seven

Conclusion

When work on this thesis commenced there was no organised acute stroke care in an Irish teaching hospital and no published data on risk factors for stroke in an Irish population. The initial objective of this thesis was to describe the setting-up of an acute stroke service, audit its' initial results and describe the risk factors for acute stroke in this population. The second part of my thesis was laboratory based and sought to examine the role of SLC as a potential risk marker in stroke, and the effect of smoking (a relevant environmental factor) on SLC activity. Much work had already described important associations of SLC with hypertension, nephropathy and diabetes, and the effect of alcohol and steroid hormones on its' activity. I sought to add to our knowledge by examining unexplored subjects relevant to its' role as a potential broader risk marker of vascular disease.

The audit of the stroke service showed a pattern of increased referrals of younger stroke patients from year to year as our stroke service developed. The initial two years of operation show a marked increase in rates of intracerbral haemorrhage (19 & 27% respectively) compared to international trends, but is probably explained by our very elderly catchment area and a bias of referral of more severe stroke patients to a newly-formed specialist service. When our catchment area changed with a move of hospital site in year three, more 'traditionally' accepted rates of haemorrhage (11%) were evident. Although it did not reach statistical significance, there was an encouraging trend towards reduced mortality and less need for intitutional care at outcome from year to year, without increasing length hospital stay. Numbers in this audit compared to published stroke-unit trials were small, particularly in year one as the service was setting-up, and that the trend towards improved outcome was significant at the 10% level is supportive of an organised approach to stroke care.

Risk markers for stroke were audited over a three-year period using accepted diagnostic criteria. In line with many other international studies, hypertension would appear to be the most prevalent risk factor in our stroke population, occuring in 65% of those with cerebral infarcts and in 58% of cases of intracerebral haemorrhage. More obvious a feature was however, the prevalence of multiple risk factors in those with cerebral infarction and that hypertension never occurred in isolation. This lends support to the 'additive effect' of hypertension with other risk factors in the aetiology of stroke reported by many others. When patients with 'first ever' ischaemic stroke were compared to those with recurrent events, the incidence of hypertension was similar in the two groups. The prevalence of valvular heart disease, atrial fibrillation and carotid artery disease was notably higher however in the recurrent group, as one might anticipate in view of the increased risk of embolic phenomena.

The prevalence of major risks markers for stroke in my patient population would appear similar to those quoted in large international stroke data-bases, with the exception of atrial fibrillation (34%) and perhaps ischaemic heart disease (42%). Differences in ethnicity, diagnostic and selection criteria for inclusion into studies probably account for some of this observation, and data published from similar Irish centres after this work had commenced concurs with my findings in terms of the high incidence of atrial fibrillation.

SLC activity has important associations with hypertension and the development of certain forms of end-organ damage in both hypertensive and diabetic patients as outlined in chapter one. It is plausible that elevated SLC activity may represent a broader marker of cardiovascular risk. In chapters five and six of this thesis I tried to

delineate further the effect of important environmental factors (namely smoking) on SLC activity and examine further the association of SLC with vascular disease.

The inter- and intra-assay variation of the kinetic assay used and reported in section 5.1, ranged from 2.8-5.8% and 2.9-5.3% respectively for SLC values. This reflected good reproducibility and was similar in magnitude to that reported in the work of others. To examine the possible effect of smoking on SLC, a group of otherwise healthy male doctors with similar baseline characteristics but differentiated by smoking habits were compared for SLC values. While no difference in SLC activity was observed between the two groups ($p=0.3012$), the acute effect of smoking was to produce a higher activity amongst smokers compared to baseline levels ($p=0.049$). This suggests that smoking may have an important effect on SLC activity and may be a relevant enviromental factor to consider in future studies of SLC. The study is limited by sample size however, and the effect of smoking on SLC activity in non-smokers remains unknown. Nevertheless there is no published literature on smoking and SLC and my results support the need for further study of this issue.

In specifically studying SLC activity in patients with hypertension, stroke and myocardial infarction versus controls, significantly higher activity was observed in both the hypertensive and stroke groups ($p = 0.002$ and 0.03 respectively). These differences in SLC activity appeared to relate to transporter activity (Vmax) rather than any alteration in affinity of the transporter for substrate (Km). No such difference was observed in the myocardial infarction group ($p = 0.57$). This observation is not easily explained and while it seems logical to assume that underlying hypertension in the stroke group would readily explain the higher SLC activity compared to controls,

no such difference was observed in the myocardial infarction group, although baseline characteristics were similar in terms of blood pressure and history of hypertension in both groups. A higher proportion of stroke patients did have a *de novo* diagnosis of hypertension, but this may reflect a 'reactive' phenomenon post-stroke. It is also interesting that the ischaemic stroke group had lower mean cholesterol levels than controls, a factor which would be expected to be associated with lower SLC activity.

While this study is limited by sample size the trend observed is intersesting and SLC activity may be a risk marker for ischaemic stroke. There are several theoretical associations of SLC activity with erythrocyte micro- and macroviscosity, and membrane transport characteristics that would explain a common role in the development of hypertension and ischaemic stroke. There are no published reports of elevated (or otherwise) SLC in patients with stroke or myocardial infarction and this study represents an initial exploration of the subject. Were a further larger study to confirm the association observed in this thesis it would represent another important advance in our understanding of the relationship of SLC activity to the develoment of 'end-organ' damage.

References

Abel P, Wussow S, Blucher H, Gros G, Rettig R, Honig A. (1997).
Erythrocyte carbonic anhydrase activity in smokers and in diabetic patients.
Experimental & Clinical Endocrinology & Diabetes;105 (suppl 2):17-19.

Abercrombie J. (1836).
Pathological and Practical Researches on Diseases of the Brain and Spinal Cord.
2nd edn. (from 3rd British edn.). Philadelphia: Carey, Lea & Blanchard.

Adebayo GI., Feely J. (1992).
Calculation of sodium-lithium countertransport (SLC): A comparison of two methods.
Ir J. Med. Sci; 161:469.

Adebayo GI, Gaffney P, Buggy D, Feely J. (1994).
Acute inhibitory effect of alcohol on sodium-lithium countertransport.
Alcohol; 11(5):367-370.

Adebayo GI. (1995).
Erythrocyte sodium-lithium countertransport in health and disease states.
Ph.D. Thesis, Trinity College Dublin chapter 2.1.4. p30.

Adebayo GI, Gaffney P, Sinnott M, Feely J. (1996).
Assay of human erythrocyte sodium-dependent lithium efflux. The importance of timing of blood sample.
Eur. J. Clin. Invest; 26(2):131-135.

Adragna MC, Chang JL, Morey MC, Williams RS. (1985).
Effect of exercise on cation transport in human red cells.
Hypertension; 7:132-139.

Alfthan G, Pekkanen J, Jauhianen M, Pitkaniemi J, Karvonen M, Tuomilehto J *et al.* (1994).
Relation of serum homocysteine and lipoprotein(a) concentrations to atherosclerotic disease in a prospective Finnish population based study.
Atherosclerosis; 106:9-19.

Alter M, Sobel E, McCoy R, Francis ME, Davanipour Z, Shofer F, et al. (1987).
Stroke in the Leigh Valley: risk factors for recurrent stroke.
Neurology; 37:503-507.

Alter M, Friday G, Lai S, O'Connell J, Sobel E. (1994).
Hypertension and risk of stroke recurrence.
Stroke; 25:1605-1610.

Alter M, Lai SM, Friday G, Singh V, Kumar Vm, Sobel E. (1997).
Stroke Recurrence in Diabetics. Does Control of Blood Glucose Reduce Risk?
Stroke; 28:1153-1157.

Amarenco P, Cohen A, Tzourio C, Bertrand B, Hommel M, Besson G et al. (1994).
Atherosclerotic disease of the aortic arch and the risk of ischaemic stroke.
N. Engl. J. Med; 331:1474-9.

American Diabetes Association. (1997).
Report of the expert committees on the diagnosis and classification of diabetes mellitus.
Diabetes Care; 20:1183-97.

Anand SS, Yusuf S, Vukshan V, Devanesen S, Teo KK, Montague P, et al. (for the SHARE Investigators). (2000).
Differences in risk factors, atherosclerosis and cardiovascular disease between ethnic groups in Canada: the Study of Health Assessment and Risk in Ethnic groups (SHARE).
Lancet; 356:279-284.

ARIC Investigators. (1989).
The Atherosclerosis Risk in Communities (ARIC) study: design and objectives.
Am J Epidemiol; 129:687-702.

Aronson PS. (1982).
Red-cell sodium-lithium countertransport and essential hypertension (letter).
N. Engl. J. Med; 307:317

Asymptomatic Carotid Atherosclerosis Study Executive Committee. (1995)
Endarterectomy for Asymptomatic Carotid Artery Stenosis.
JAMA; 273:1421-28.

Bamford J, Sandercock PAG, Warlow CP, Slattery J. (1989).
Interobserver agreement for the assessment of handicap in stroke patients.
Stroke; 20:828.

Bamford J, Sandercock P, Dennis M, Burn J, Warlow C. (1991).
Classification and natural history of clinically identifiable subtypes of cerebral infarction.
Lancet; 337:1521-6.

Barnett HJ, Taylor DW, Eliasziw M, Fox AJ, Ferguson GG, Haynes RB *et* al. (1998).
Benefit of Carotid Endarterectomy in patients with symptomatic moderate or severe stenosis.
N. Engl. J. Med; 339:1415-1425.

Barrett-Connor E, Khaw ET.(1988).
Diabetes Mellitus: an independent risk factor for stroke?
Am J Epidemiol; 128:116-123.

Beuckelmann D, Erdmann E. (1984).
Exogenous factors influencing the human erythrocyte sodium-lithium countertransport system.
Eur. J. Clin. Invest; 14:392-397.

Beuckelmann D, Erdmann E. (1986).
Na+-Li+ countertransport and electrolyte composition in erythrocytes of patients with essential hypertension before and after antihypertensive treatment.
Klin.Wochenschr; 64:1101-1105.

Blauw GJ, Lagaay AM, Smelt AHM. (1997).
Stroke, statins and cholesterol.
Stroke; 28:946-950.

Bogousslavsky J, Van Melle G, Regli F. (1988).
The lausanne stroke registry: Analysis of 1,000 consecutive patients with first stroke.
Stroke; 19:1083-1092.

Bonita R. (1992).
Epidemiology of stroke.
Lancet; 339:342-4

Bonita R, Duncan J, Truelsen T, Jackson RT, Beaglehole R. (1999).
Passive smoking as well as active smoking increases the risk of acute stroke.
Tobacco Control; 8(2):156-160.

Bonora E, Willeit J, Kiechl S, Oberhollenzer F, Egger G, Bonadonna R *et al.*.(1997).
Relationship between Insulin and Carotid Atherosclerosis in the general population. The Bruneck Study.
Stroke; 28:1147-1152.

Bousser MG, Chiras J, Bories J, Castaigne P. (1985).
Cerebral venous thrombosis – a review of 38 cases.
Stroke; 16:199-213.

Brand SC., Whittam R. (1984).
The effect of furosemide on sodium movements in human red cells.
J. Physiol; 348:301-306.

Brown KM, Morrice PC, Duthie GG. (1998).
Erythrocyte membrane fatty acid composition of smokers and non-smokers: effects of vitamin E supplementation.
European Journal of Clinical Nutrition; 52(2): 145-150..

Brugnara C, Corrocher R, Foroni L, Steinmayr M, Bonfanti F, De Sandre G. (1983).
Lithium-sodium countertransport in erythrocytes of normal and hypertensive subjects. Relationship with age and plasma renin activity.
Hypertension; 5:529-534.

Bucher HC, Griffith LE, Guyatt GH. (1998).
Effect of HMG CoA reductase inhibitors on stroke: a meta-analysis of randomised, controlled trials.
JAMA; 128:89-95.

Burchfield CM, Curb JD, Rodriguez BL, Abbot RD, Chiu D, Yano K. (1994).
Glucose intolerance and 22 year stroke incidence : the Honolulu Heart Program.
Stroke; 25:951-7.

Burn J, Dennis M, Bamfotrd J, Sandercock P, Wade D, Warlow C. (1994).
Long-term risk of recurrent stroke after a first-ever stroke. The Oxfordshire community stroke project.
Stroke; 25:333-337.

Bushnell CD, Goldstein LB. (2000)
Diagnostic testing for coagulopathies in patients with ischemic stroke.
Stroke; 31:3067-3078.

Cabantchik I., Knauf PA., Rothstein A. (1978).
The anion transport system of the red blood cell. The role of membrane protein evaluated by the use of 'probes'.
Biochem. Biophys. Acta; 515:239-302.

Canali M, Borghi I, Sani E. (1987).
Increased red blood cell sodium-lithium countertransport in essential hypertension. Its' relationship to family history of hypertension.
Clin. Sci; 61:135-155.

Candelise L, Pinardi G, Aritzu E, Mussico M. (1994).
Telephone interview for stroke outcome assessment.
Cerebrovasc Dis; 4:341-343.

Canessa M, Tosteson D. (1979).
Determination of the sodium-lithium countertransport system of human erythrocytes.
In: Lithium, Controversies and Unresolved Issues: Proceedings International Lithium Conference.
Excerpta Medica: Amsterdam: 978-982.

Canessa M, Adragna N, Solomon H, Connolly T, Tosteson D. (1980).
Increased sodium-lithium countertransport in red cells of patients with essential hypertension.
N. Eng. J. Med; 302:722-726.

Canessa M., Morgan K., Semplicini A., (1988).
Genetic difference in lithium-sodium exchange and regulation of the sodium-hydrogen exchanger in essential hypertension.
J.Cardiovasc.Pharmacol; 12(suppl.3):S92-S98.

Canessa M. (1989)
Kinetic properties of Na+/H+ exchange and Li+/Na+, Na+/Na+, and Na+/Li+ exchanges of human red cells.
In: Methods in Enzymology, (Fleischer S., Fleischer B., Eds.), 173:pp.176-191.
Academic Press: New York.

Canessa M, Zerbini G, Leffel LMB. (1992).
Sodium activation kinetics of red blood cell Na+-Li+ countertransport in diabetes. Methodology and controversy.
J.Am.Soc.Nephrol; 3:S41-S49.

CAPRIE Steering Committee (1996).
A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE).
Lancet; 348:1329-39.

Carr SJ, Thomas TH, Wilkinson R. (1989).
Erythrocyte sodium-lithium countertransport in primary and renal hypertension : relation to family history
Eur J Clin Invest; 19:101-106.

Carr SJ, Thomas TH, Laker MF, Wilkinson R. (1990).
Elevated sodium-lithium countertransport: a familial marker of hyperlipidaemia and hypertension?
J Hypertension; 8:139-146.

Carr SJ, Thomas TH, Laker MF, Wilkinson R. (1991).
Lipid lowering therapy leads to a reduction in sodium-lithium countertransport activity.
Arteriosclerosis; 87:103-108.

Carr SJ, Sikand K, Moore D, Norman RI. (1995).
Altered membrane microviscosity in essential hypertension: relationship with family history of hypertension and sodium-lithium countertransport activity.
J.Hypertens; 13:139-146.

CAST (Chinese Acute Stroke Trial) Collaborative Group (1997).
CAST: randomised placebo-controlled trial of early aspirin use in 20,000 patients with acute ischaemic stroke.
Lancet; 349:1641-1649.

Central statistics Office (Ireland). (1999).
Vital statistics, fourth quarter and yearly summary 1998.
CSO stationary office, Dublin.

Charcot JM, Bouchard C. (1868).
Nouvelles recherches sur la pathogénie de l' hémorrhagie cérébbrale.
Arch. Physiol. Norm. Pathol; 1: 110-27, 643-65, 725-34.

Chiari H. (1905).
Uber das Verhalten des Teilungswinkels des Carotis Communis bei der Endarteritis chronica deformans.
Verh. Ddtsch. Path. Ges; 9:326-330.

Chi Y, Mota de Freitas D, Sikora M, Bansal VK. (1996).
Correlation of Na+-Li+ exchange activity with Na+-Li+ binding and phospholipid composition in erythrocyte membranes of white hypertensive and normotensive individuals.
Hypertension; 27: 456-464.

Chimowitz MI, Poole RM, Starling MR, Schwaiger M, Gross MD. (1997).
Frequency and severity of asymptomatic coronary disease in patients with different causes of stroke.
Stroke; 28(5):941-945.

Chiu D, Schwartz R, Yee M, Lubin B. (1983).
Calcium-induced abnormal membrane phospholipid organisation in human erythrocytes.
Clin. Exp.Hypertens; A6:2279-2296.

Cicco G, Dolce E, Vicenti P, Stingi GD, Tarallo MS, Pirrelli A. (1999).
Hemorheological aspects in hypertensive menopausal smokers treated with female hormones.
Clinical Hemorheology & Microcirculation; 21(3-4):343-347.

Cole FM, Yates PO. (1967).
The occurrence and significance of intracerebral microaneurysms.
J. Pathol Bacteriol; 93:393-411.

Collin C, Wade DT (1988).
The Barthel ADL Index: A reliability study.
Int. Disabil. Stud; 10:61-63.

Collins R, MacMahon S. (1994).
Blood pressure, antihypertensive drug treatment and the risk of stroke and coronary heart disease.
Br. Med. Bull; 50:272-98.

Collins DR, O'Neill D. (1998).
Stroke: non-motor sequelae, medical co-morbidity and patterns of intervention after referral to a special interest service.
Ir. J. Med. Sci; 1:33-34.

Cooper RA.(1977).
Abnormalities of cell membrane fluidity in the pathogenesis of disease.
N.Eng.J.Med; 297:371-377.

Corrocher R, Steinmayr M, Ruzzenente O, Brugnara C, Bertinato L, Mazzi M. et al. (1985).
Elevation of red cell sodium-lithium countertransport in hyperlipidaemias.
Life Sci; 36:649-655.

Corry DB, Tuck ML, Nicholas S, Weinman EJ. (1993).
Increased Na +/ H+ antiport activity and abundance in uraemic red blood cells.
Kidney Int; 44:574-578.

Crompton MR. (1964).
The pathogenesis of cerebral infarction, following the rupture of cerebral berry aneurysms.
Brain; 87:491-510.

Crompton MR. (1966).
The pathogenesis of cerebral aneurysms.
Brain; 89:797-814.

Currie CJ, Morgan CL, Gill L, Stott NCH, Peters JR. (1997).
Epidemiology and costs of acute hospital care for cerebrovascular disease in diabetic and non-diabetic populations.
Stroke; 28:1142-1146.

De Bono DP. (1982).
Cardiac causes of of transient neurological disturbances.
In: Transient Ischaemic Attacks. New York: Dekker: 99-124.

De la Sierra A, Insa R, Compte M, Martinez- Amenos A, Sierra C, Hernandez Herrero et al. (1995).
Effect of longterm antihypertensive therapy with angiotensin II converting enzyme inhibitors on red blood cell Na+ transport.
Am. J. Hypertension; 8:622-625.

Diabetic Control and Complications Trial Research group (1993).
The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin dependent diabetes mellitus.
N. Engl. J. Med; 329:977-986.

Diez J, Arrazola A. (1991).
Is the erythrocyte sodium-lithium countertransport a molecular marker of cardiac risk in hypertension.
Cardioscience; 2:87-92.

Donnan GA, You R, Thrift A, McNeill JJ. (1993).
Smoking as a risk factor for stroke.
Cerebrovasc Dis; 3:129-38.

Donnan GA, Davis SM, Chambers BR, Gates PC, Hankey GJ, McNeill JJ *et al.* (1995).
Trials of streptokinase in severe acute ischaemic stroke.
Lancet; 345:578-579.

Duhm J, Eisenreid F, Becker BF, Griel W. (1976)
Studies on the lithium transport across the red cell membrane. Li+ uphill transport by the Na-dependent Li+ countertransport system of human erythrocytes.
Pflugers Arch; 364:147-155.

Duhm J, Becker BF. (1977).
Studies on the lithium transport across the red cell membrane. IV. Inter-individual variations in the Na+-dependent Li+ countertransport systems of human erythrocytes.
Pflugers Arch; 370: 211-219.

Duhm J, Becker BF. (1979)
Studies on lithium transport across the red cell membrane. On the nature of the Na-dependent Li countertransport system of mammalian erythrocytes.
J. Membr.Biol; 51:263-286.

Duhm J, Behr J. (1986).
Role of exogenous factors in alterations of red cell Na+-Li+ exchange and Na+-K+ cotransport in essential hypertension, primary hyperaldonsteronism and hypokalaemia.
Scand.J.Clin.Lab.Invest; 180:82-95.

Early Supported Discharge Trialists. (2000).
Services for reducing duration of hospital care for acute stroke patients (Cochrane review). In: Cochrane Library, issue 2 [database online]. Oxford, UK: Cochrane Library;2000.

Elneihoum AM, Goransson M, Falke P, Janzon L. (1998).
Three-year survival and recurrence after stroke in Malmo, Sweden.
Stroke; 29:2114-2117.

Englemann B, Duhm J. (1991).
Effect of cholesterol and dipalmitoyl poosphatidylcholine enrichment on the kinetics of Na+-Li+ exchange of human erythrocytes.
J.Membr.Biol; 122:231-238.

Englemann B, Duhm J, Schonthier UM, Streich S. (1993).
Relations of sodium-lithium countertransport kinetics to plasma and red cell membrane phospholipids in hyperlipidaemia.
Athersclerosis; 99:151-163.

EPSP 2 Group (1997).
European stroke prevention study.2. Efficacy and safety data.
J Neurol Sci; 151: (suppl) S1-77.

Escobales N, Canessa M. (1986).
Amiloride sensitive Na+ transport in human red cells. Evidence for a Na+/H+ exchange system.
J Membr Biol; 90:21-28.

European Atrial Fibrillation Trial Study Group. (1993)
Secondary prevention in non-rheumatic atrial fibrillation after transient ischaemic attack or minor stroke.
Lancet; 342:1255-1262.

European Carotid Surgery Trialists Collaborative Group (1998).
Randomised trial of enderarterectomy for recently symptomatic carotid stenosis: final report of the MRC European Carotid Surgery Trial.
Lancet; 351:1379-1387.

European Cooperative Acute Stroke Study Group II. (1998).
Randomised double-blind, placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II).
Lancet; 352:1245-51.

Evans RW, Shaten J, Hempel JD, Cutler JA, Kuller LH. (1997).
Homocyst(e)ine and risk of cardiovascular disease in the Multiple Risk Factor Intervention Trial.
Arterioscler Thromb Vasc Biol; 17:1947-1953.

Evers SMAA, Engel GL, Ament AJHA. (1997).
Cost of stroke in the Netherlands from a societal perspective.
Stroke; 28:1375-81.

Fan CW, McDonnell B, Crowe M, Johnson Z. (1998).
Prospective analysis of risk factors in 100 acute stroke admissions to a Dublin hospital.
Ir. J. Med. Sci; 167 (suppl. 9): 19.

Fan CW, McDonnell R, Johnson Z, O'Keefe S, Crowe MJ. (2000).
Hospital-based stroke care in Ireland: results from one regional register.
Irish Medical Journal; 169(1):30-33.

Folsom AR, Eckfeldt JH, Weitzman S, Ma J, Chambless LE, Barnes RW *et al.* (1994)
Relation of carotid artery wall thickness to diabetes mellitus, fasting glucose and insulin, body size and physical activity.
Stroke; 25:66-73.

Fortes-Meyer KD, Starkey BJ. (1977).
Simple flame photometric determination of erythrocyte sodium and potassium: the reference range for apparently healthy adults.
Clin. Chem; 23:275-278.

Foulkes MA, Wolf PA, Price TR, Mohr JP, Hier DB. (1988).
The Stroke Data Bank: design, methods and baseline characteristics.
Stroke; 19:547-554.

Freund G. (1979).
Possible relationship of alcohol in membranes to cancer.
Cancer Res; 39:2899-2901.

Friday G, Lai SM, Alter M, Sobel E, La Rue L, McCoy RL *et al.* (1989)
Stroke in the Lehigh Valley: racial/etnic differences.
Neurology; 39:1165-1168.

Fujita J, Tsuda K, Seno M, Obayashi H, Seino Y. (1994).
Erythrocyte sodium-lithium countertransport activity as a marker of predisposition to hypertension and diabetic nephropathy in NIDDM.
Diabetes Care; 17:977-982.

Funder J, Wieth JO (1974).
Human red cell sodium and potassium in metabolic alkalosis.
Scand J Clin Invest: 34:49-59.

Fung MM, Barrett-Connor E, Bettencourt RR. (1999).
Hormone replacement therapy and stroke risk in older women.
Journal of Womens' Health; 8(3):359-364.

Gillum LA, Mamidipudi SK, Johnston SC. (2000).
Ischemic stroke risk with oral contraceptives: A meta-analysis.
JAMA; 284(1): 72-78.

Giraud F, Claret M, Garay R. (1976).
Interactions of cholesterol with the Na pump in red blood cells.
Nature; 264:646-648.

GISSI-1 (1986).
Effectiveness of intravenous thrombolytic treatment in acute myocardial infarction.
Lancet; i:397-401.

Glorioso N, Troffa C, Filigheddu F *et al.* (1999).
Effect of the HMG-CoA reductase inhibitors on blood pressure in patients with essential hypertension and primary hypercholesterolaemia.
Hypertension;34:1281-1286..

Glynn IM, Karlish SJ.(1975).
The Sodium Pump.
Ann.Rev.Physiol; 37:13-55.

Grunfield B, Balzerti M, Romo M, Gimenez M, Gutman R. (1994).
Hyperinsulinaemia in normotensive offspring of hypertensive parents.
Hypertension; 23:112-115.

Haas M, Scooler J, Tosteson DC. (1975).
Coupling of lithium to sodium transport in human red cells.
Nature; 258:424-427.

Hachinski V, Graffagnino C, Beaudry M, Bernier G, Buck C, Donner A *et al.* (1996).
Lipids and stroke: a paradox resolved.
Arch. Neurol; 53:303-08.

Hammermeister KE, Sethi GK, Henderson WG, Oprian C, Kim T, Rahimtoola S. (1993)
A comparison of outcomes in men 11 years after heart-valve replacement with a mechanical valve or bioprosthesis.
N. Eng. J. Med; 328:1289-96.

Hankey GJ, Jamrozik K, Broadhurst RJ, Forbes S, Burvill PW, Anderson CS *et al.* (1998).
Long-term risk of first recurrent stroke in the Perth Community Stroke Study.
Stroke; 29: 2491-2500.

Hankey GJ, Eikelboom JW. (1999).
Homocysteine and vascular disease.
Lancet; 345:407-413.

Hannaert PA, Garay RP. (1986).
A kinetic analysis of Na-Li countertransport in human red blood cells.
J. Gen. Physiol; 87: 253-368.

Hannaford PC, Croft PR, Kay CR. (1994).
Oral contraception and stroke. Evidence from the Royal College of General Practitioners' Oral Contraception Study.
Stroke; 25:935-942.

Hansagi H, Romelsjo A, Gerhardsson de Verdier M, Andreasson S, Leifman A. (1995).
Alcohol consumption and stroke mortality. 20 year follow-up of 15.077 men and women.
Stroke; 26(10):1768-1773.

Hansson L, Zanchetti A, Carruthers SG, Dahlof B, Elmfeldt D, Julius S *et al.,* (1998).
Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. HOT study group.
Lancet; 351:1755-1762.

Hardmann TC, Dubrey SW, Leslie DG, Hafiz M, Noble MI, Lant AF. (1992).
Erythrocyte sodium-lithium countertransport and blood pressure in identical twin pairs discordant for insulin-dependent diabetes.
Br.Med.J; 305:215-219.

Hart RG, Halperin JL. (1994)
Atrial fibrillation and stroke: revisiting the dilemmas.
Stroke; 25: 1337-41.

Hart RG, Pearce La, McBride R, Rothbart RM, Asinger RW. (1999).
Factors associated with ischemic stroke during aspirin therapy in atrial fibrillation: analysis of 2012 participants in the SPAF I-III clinical trials. The Stroke Prevention in Atrial Fibrillation (SPAF) Investigators.
Stroke; 30(6):1223-9.

Hasstedt SJ, Hunt SC, Wu L, Williams RR.(1994).
Evidence for multiple genes determining sodium transport.
Genet.Epidemiol; 11:553-568.

Hatano S. (1976).
Experience from a multicentre stroke register: a preliminary report.
Bull. WHO; 54:541-53.

Herbert PR, Gaziano JM, Chan KS, Hennekens CH. (1997).
Cholesterol lowering with statin drug, risk of stroke, and total mortality.
JAMA; 278:313-21.

Hier DB, Foulkes Ma, Swiontoniowski M, Sacco RL, Gorelick PB, Mohr JP, Price TR, Wolf PA. (1991).
Stroke recurrence within 2 years after ischaemic stroke.
Stroke; 22: 15-161.

Homer D, Ignall TJ, Baker HL, O'Fallon WM, Kottke BA, Whisnat JP. (1991)
Serum lipids and lipoproteins are less powerful predictors of extracranial carotid artery atherosclerosis than are cigarette smoking and hypertension.
Mayo Clin. Proc; 66:259-67.

Homocysteine Lowering Trialists' Collaboration. (1998).
Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials.
BMJ; 316:894-898.

Houeto P, Borron SW, Baud FJ, Muszynski J, Buisine A, Gourlain H *et al.* (1999).
Assessment of erythrocyte cholinesterase activity in victims of smoke inhalation.
Journal of Toxicology; 37)3):321-326.

Houtman PN, Shah V, Dillon MJ. (1993).
Sodium-lithium countertransport and family history of hypertension in childhood.
Acta.Paediatr; 82:1057-1060.

Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E. (1998).
Randomised trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart Estrogen/progestin Replacement Study (HERS) Research group.
JAMA; 280(7):605-613.

Hunt SC, Williams RR, Ash KO. (1990).
Changes in sodium-lithium countertransport correlate with changes in triglyceride levels and body mass index over two and a half years follow-up in Utah.
Cardiovasc.Drugs Ther; 4(supp.2):357-362.

Hsiang JN, Zhu XL, Wong LK, Kay R, Poon WS. (1996).
Putaminal and thalamic hemorrhage in ethnic Chinese living in Hong Kong.
Surg. Neurol; 46:441-445.

Ibsen K., Jensen H., Wieth J., Funder J. (1982)
Essential hypertension: sodium-lithium countertransport in erythrocytes from patients and from children having one hypertensive parent.
Hypertension; 4:703-709.

Indredavik B, Fjeartoft H, Ekeberg G, Loge AD, Morch B. (2000).
Benefit of an extended stroke unit service with early supported discharge. A randomised controlled trial.
Stroke; 31:2989-2994.

International Stroke Trial Collaborative Group. (1997).
International Stroke Trial (IST): a randomised trial of aspirin, subcutaneous heparin, both, or neither among 19,435 patients with acute ischaemic stroke.
Lancet; 349:1569-1581.

Isard PA, Forbes JF. (1992).
The cost of stroke to the National Health Service in Scotland.
Cerebrovasc Dis; 2:47-50.

ISIS-2 (1988).
Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction.
Lancet; ii:349-60.

Jensen JS, Mathiesen ER, Norgaard K, Hommel E, Borch-Johnsen K, Funder J *et al.* (1990)
Increased blood pressure and erythrocyte sodium-lithium countertransport activity are not inherited in diabetic nephropathy.
Diabetologia; 33:619-624.

Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS. (1994).
Stroke in patients with diabetes: the Copenhagen Stroke Study.
Stroke; 25:1977-84.

Jousilahti P, Rastenyte D, Tuomilehto J, Sarti C, Vartianen E. (1997).
Parental history of cardiovascular disease and risk of stroke.
Stroke; 28(1361-1366).

Juvela S, Hillbom M, Palomaki H. (1995).
Risk factors for spontaneous intracerebral hemorrhage.
Stroke; 26:1558-1564.

Kalra L, Uyu J, Wilson K, Ruths P. (1995).
Medical complications during stroke rehabilitation.
Stroke; 26(6):990-3.

Kalra L, Perez I, Melbourn A. (1998).
Stroke risk management – changes in mainstream practice.
Stroke; 29:53-57.

Kannel WB, McGee DL. (1979).
Diabetes and cardiovascular disease: the Framingham Study.
JAMA; 241:2035-2038.

Kannel WB, Wolf PA, Verter J. (1983).
Manifestations of coronary disease predisposing to stroke: the Framingham study.
JAMA; 250:2942-2946.

Kargman DE, Tuck C, Berglund L, Lin IF, Mukherjee RS, Thompson EV *et al.* (1998).
Lipid and lippoprotein levels remain stable in acute ischaemic stroke: the Northern Manhattan Stroke Study.
Atherscleros is; 139:391-399.

Kaste M, Palomaki H, Sarna S. (1995).
Where and how should elderly stroke patients be treated? A randomised trial.
Stroke; 26:249-53.

Kiely DK, Wolf PA, Cupples LA, Beiser AS, Myers RH. (1993)
Familial aggregation of stroke: the Framingham Study.
Stroke; 24:1366-71.

Kirkes WS. (1855).
On apoplexy in relation to chronic renal disease.
Med Times Gazette; 11:515-516.

Kittner SJ, Bousser MG. (2000).
Post-menopausal hormone replacement therapy and stroke risk.
Cephalgia; 20(3):208-213.

Klungel OH, Stricker BHC, Paes AH, Seidell JC, Bakker A, Voko Z *et al.* (1999).
Excess stroke among hypertensive men and women attributable to under treatment of hypertension.
Stroke; 30:1312-1318.

Krumholz HM, Seeman TE, Merrill SS, Mendes de Leon CF, Vaccarino V, Silverman DI. (1994).
Lack of association between cholesterol and coronary heart disease mortality and morbidity and all-cause mortality in persons over 70 years.
JAMA; 272:1335-40.

Laboratory Standardization Panel of the National Cholesterol Education Program. (1998).
Current status of blood cholesterol measurement in clinical laboratories in the United States: A report from the Laboratory Standardization Panel of the National Cholesterol Education Program.
Clin Chem; 34:193-201.

Langhorne P, Williams BO, Gilchrist W, Howie H. (1993).
Do Stroke units save lives?
Lancet; 342:395-398.

Langhorne P, Dennis M. (1998).
Stroke Units: an evidence based approach.
British Medical Journal books.

Liao D, Myers R, Hunt S, Shahar E, Paton C, Burke G, Province M, Heiss G. (1997).
Familial history of stroke and stroke risk.
Stroke; 28:1908-1912.

Lifton RP, Hunt SC, Williams RR, Pouyssegur J, Lalouel JM. (1991)
Exclusion of the Na+ -H+ antiporter as a candidate gene in human essential hypertension.
Hypertension; 17: 8-14.

Lijnen P, Fagard R, Staessen J, Thijs L, Amery A. (1992).
Erythrocyte membrane lipids and cationic transport systems in men.
J.Hypertension; 10:1205-1211.

Lijnen P, Fenyvesi A, Bex M, Bouillon R, Amery A. (1993).
Erythrocyte cation transport systems and membrane lipids in insulin dependent diabetes.
Am. J. Hypertens; 6:763-770.

Lijnen P, Petrov V. (1995).
Cholesterol modulation of transmembrane cation transport systems in human erythrocytes.
Biochem. Mol. Med; 56: 52-56.

Long-term intervention with pravastatin in Ischaemic Disease (LIPID) Study Group (1998).
Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels.
N. Engl. J. Med; 333:1349-57.

Mahnensmith RL, Aronson PS. (1985).
The plasma membrane sodium/hydrogen exchange and its role in physiological and pathophysiological processes.
Circulation Res; 56:773-788.

Makino Y, Kawano Y, Minami J, Yamaguchi T, Takishita S. (2000).
Risk of stroke in relation to level of blood pressure and other risk factors in treated hypertensive patients.
Stroke; 31:48-52.

Mangili R, Bending JJ, Scott G, Li LK, Gupta A, Viberti G. (1988).
Increased sodium-lithium countertransport activity in red cells of patients with insulin-dependent diabetes and nephropathy.
N. Eng. J. Med; 318:146-150.

Mangili R, Zerbini G, Barlassina C, Cusi D, Pozza D. (1993).
Sodium-lithium countertransport and triglycerides in diabetic nephropathy.
Kidney Int; 44:127-133.

McDonnellR, Fan CW, Johnson Z, Crowe M. (2000).
Prevalence of risk factors for ischaemic stroke and their treatment among a cohort of stroke patients in Dublin.
Ir J Med Sci; 169(4): 253-257.

McMahon S, Sharpe N, Gamble G, Hart H, Scott J, Simes J *et al.* (1998).
Effects of lowering average or below-average cholesterol levels on the progression of carotid athersclerosis.
Circulation; 97:1784-90.

Mehlhorn RJ. (2000).
Increased vulnerability of human erythrocytes to hydroperoxide damage after exposure to cigarette smoke or 1-chloro-2,4-dinitrobenzene in vitro.
Nicotine Tob. Res; 2(2):141-148.

Meissner I, Whisnat JP, Garraway M. (1988).
Hypertension management and stroke recurrence in a community (Rochester, Minnesota, 1950-1979).
Stroke; 19:459-463.

Mendels J, Frazier A. (1973).
Intracellular lithium concentration and clinical response. Towards a membrane theory of depression.
J. Psychiatr. Res; 10:9-18.

Mendez I, Hachinski V, Wolfe B. (1987).
Serum lipids after stroke.
Neurology; 37:507-511.

Messner H, Kleophas W, Hein D, Gries FA, Kobberling J. (1991)
Sodium-lithium countertransport is acutely influenced by heparin induced extracorporal LDL precipitation.
Eur. J. Clin. Invest; 21:215-218.

Min Lai S, Alter M, Friday G, Sobel E. (1994).
A multifactorial analysis of risk factors for recurrence of ischaemic stroke.
Stroke; 25:958-962.

Monforte R, Estruch R, Graus F, Nicolas JM, Urbano-Marquez A. (1990).
High ethanol consumption as a risk factor for intracerebral hemorrhage in young and middle-aged people.
Stroke; 21: 1529-1532.

Montrose MH, Murer H. (1990).
Polarity and kinetics of Na+-H+ exchange in cultured opossum kidney cells.
Am. J. Physiol; 259:C121-C133.

Morgagni GB. (1761).
De sedibus et causis morborum per anatomen indigatis libri quinque.
Vienna: ex typographia Remondiana.

Morgan DB, Steward AD, Davidson C. (1986).
Relations between erythrocyte lithium efflux, blood pressure and family history of hypertension and cardiovascular disease. Studies in a factory workforce and hypertension clinic.
J.Hypertension; 4:609-615.

Moroney JT, Bagiella E, Paik MC, Sacco RL, Desmond DW. (1998).
Risk factors for early recurrence after ischaemic stroke.
Stroke; 29:2118-2124.

Morrison AC, Fornage M, Liao D, Boerwinkle E (2000).
Parental history of stroke predicts subclinical but not clinical stroke.
Stroke; 31:2098-2102.

Multicentre Acute Stroke Trial-Italy (MAST-I) Group (1995).
Randomised controlled trial of streptokinase, aspirin, and combination of both in treatment of acute ischaemic stroke.
Lancet; 346:1509-1514.

Murray CJL, Lopez AD. (1997).
Mortality by cause for eight regions of the world: global burden of disease study.
Lancet; 349:1269.

National Institute of Neurological Disorders and Stroke (NINDS) rt-PA Stroke Study Group. (1995).
Tissue plasminogen activator for acute ischaemic stroke.
N. Engl. J. Med; 333:1581-1587.

Niutta E, Tripodi MG, Cusi D, Pati C, Dossi F, Elli A, Bianchi G. (1986).
Effects of captopril and of other antihypertensive drugs on cell membrane ion transport – a preliminary report.
Postgrad. Med. J; 62(suppl.1):13-15.

North American Symptomatic Carotid Endarterectomy Trial Collaborators (1991).
Beneficial effect of carotid endarterectomy in symptomatic patients with high grade stenosis.
N. Engl. J. Med; 325:1191-1200.

O'Brien E, Staessen JA. (2000).
Critical appraisal of the JNC VI, WHO/ISH and BHS guidelines for essential hypertension.
Expert Opinion on Pharmacotherapy; 1(4): 675-682.

O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SKJ. (1999).
Carotid-artery intima and media thickness as a risk factor for myocardial infarction and strokes in older adults.
N. Engl. J. Med; 340:14-22.

Ostrow DG, Dorus W, Okonek A, Desai P, Bauer J, Bresolin LB, Davis JM. (1986).
The effect of alcoholism on membrane lithium transport.
J.Clin.Psychiat; 47:350-353.

Paganini-Hill A. (1995).
Estrogen replacement therapy and stroke.
Progress in Cardiovascular Diseases; 38(3):223-242.

Pandey GN, Sarkadi B, Haas M, Gunn RB, Davis JM, Tosteson DC. (1978).
Lithium transport pathways in human red blood cells.
J. Gen. Physiol; 72:233-246.

Pandey GN, Dorus E, Davies JM, Tosteson DC. (1979).
Lithium transport in human red cells: Genetic and clinical aspects.
In: Lithium: Controversies and unresolved issues (International Lithium Conference) (Cooper TB, Gersohn S, Kline NS, Shou M. editors), pp. 736-757. Excerpta Medica, Amsterdam.

Parker JC. (1986).
Interaction of lithium and protons with the sodium proton exchanger of dog red blood cells.
J. Gen. Physiol; 87:189-200.

Pery IJ, Refsum H, Bonaa KJ, Ueland PM, Forde OH, Nordrehaug JE. (1995).
Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men.
Lancet; 346:1395-1398.

Peterson JC, Spence JD. (1998).
Vitamins and progression of atherosclerosis in hyperhomocyst(e)inaemia.
Lancet; 351: 263.

Petrov VV, Arabidze CG, Levitsky DO, Eliseen AO, Lijnen PJ. (1994).
Red blood cell sodium-lithium countertransport in patients with essential and renal hypertension.
Methods Find.Exp.Clin.Pharmacol;16:153-157.

Pontremoli R, Zerbini G, Rivera A, Canessa M. (1994).
Insulin activation of red blood cell Na+/H+ exchange decreases the affinity of sodium sites.
Kidney Int; 46:365-375.

Porzig H. (1983).
Red cell cation transport systems – clues for the 'missing link' in a unifying theory of essential hypertension.
Trends Pharmacol.Sci; 4:410-411.

Potter JF, Beevers DG. (1984).
Pressor effect of alcohol in hypertension.
Lancet; 1:119-122.

Prineas RJ, Crow RS, Blackburn H. (1982).
The Minnesota Code Manual of electrocardiographic findings: Standards and procedures for measurement and classification.
Littleton, Mass. John Wright-PSG Inc.

Prospective Studies Collaboration (1995).
Cholesterol, diastolic blood pressure and stroke: 13,000 strokes in 450,000 people in 45 prospective cohorts.
Lancet; 346:1647-53.

Rankin J. (1957).
Cerebral vascular accidents in people over the age of 60. II prognosis.
Scottish Medical Journal;2:200-215.

Recommendations of the Second Joint Task Force of the European and Other Societies on Coronary prevention (1998).
Prevention of coronary heart disease in clinical practice.
Eur Heart J; 19:1434-1503.

Ross Russell RW. (1963).
Observations on intracerebral aneurysms.
Brain; 86:425-42.

Rutherford PA, Thomas TH, Wilkinson R. (1992).
Plasma lipids affect maximum velocity not sodium affinity of human erythrocyte sodium-lithium countertransport: distinction from hypertension.
Eur.J.Clin.Invest; 22:719-724.

Rutherford PA, Thomas TH, Hardman T, Lant Af, Wilkinson R. (1993).
Sodium-lithium countertransport activity is not affected by short-term insulin exposure in vivo or in a physiological medium in vitro.
Metabolism; 42:1087-1089.

Sacco Rl, Wolf Pa, Kannel WB, McNamara PM. (1982).
Survival and recurrence following stroke: the Framingham study.
Stroke; 13:290-295.

Sacco Rl. (1995).
Risk factors and outcomes for ischemic stroke.
Neurology; 45(suppl 1):S10-S14.

Sacco RL, Elkind M, Boden-Albala B, Lin IF, Kargman DE, Hauser WA *et al.* (1999)
The Protective effect of moderate alcohol consumption on ischemic stroke.
JAMA; 281(1):53-60.

Sage JI, Van Uitert RL. (1983).
Risk of recurrent stroke in patients with atrial fibrillation and non-valvular heart disease.
Stroke; 14:537-540.

Sardet C, Franchi A, Pouyassegur J. (1989).
Molecular cloning, primary structure and expression of the human growth factor activatable Na+/H+ antiporter.
Cell; 56:271-280.

Sarkadi B., Alifimoff JK., Gunn RB., Tosteson DC. (1978)
Kinetics and stoichiometry of Na-dependent Li+ transport in human red cells.
J. Gen.Physiol; 72:249-265.

Scandinavian Simvastatin Survival Group (1994).
Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S).
Lancet; 344:1383-9.

Scandinavian Stroke Study Group. (1985).
Multicenter trial of hemodilution in ischaemic stroke: background and study protocol.
Stroke; 16:885-90.

Schatzman HJ. (1983).
The red cell calcium pump.
Ann.Rev. Physiol; 45:303-312.

Schwartz SM, Petitti DB, Siscovick DS, Longstreth WT Jr, Sidney S, Ragunathan TE *et al.* (1998).
Stroke and use of low-dose oral contraceptives in young women; a pooled analysis of two US studies.
Stroke; 29(11):2277- 2284.

Seifter JL., Aronson PS. (1986).
Properties and physiologic roles of the plasma membrane sodium-hydrogen exchanger.
J.Clin.Invest; 78:859-864.

Seldinger SI. (1953).
Catheter replacement of the needle in percutaneous arteriography.
Acta Radiol; 39:368-78.

Semplicini A, Ceoletta G, Felice M, Reato S, Valle R, Gennin A *et al.* (1995).
Kinetic properties of erythrocyte Na+-Li+ and Na+-H+ exchange in hypertensive patients.
J. Hypertens; 13:1566-1569.

SHEP Cooperative Research Group (1991).
Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension.
JAMA; 265:3255-3264.

Sherif K, Barrett M, Kushner H, Falkner B. (2000).
The association of RBC sodium lithium countertransport (Vmax) with left ventricular mass in African American women.
Journal of Human Hypertension; 14(3):213-9.

Siebens A, Kregenow FM. (1978).
Volume regulatory responses of salamander red cells incubated anisosmotic media: effect of amiloride.
Physiologist: 21:110.

Simons LA, McCallum J, Friedlander Y, Simons J. (1998).
Risk factors for ischaemic stroke. Dubbo study of the elderly.
Stroke; 29:1341-1346.

Smith JB, Price AL, Williams RR, Hentschel WM, Sprowell W, Hunt SC *et al.* (1982).
A reproducible sodium-lithium countertransport assay: the outcome of changing key laboratory parameters.
Clin. Chem. Acta; 122:327-335.

Smith JB, Ash KO, Hentschel WM, Worley RJ, Astle CD, Williams RR. (1982.b).
Sodium-lithium countertransport in erythrocytes of pregnant women.
N. Engl. J. Med; 307:1645-1646.

Soloway HB, Aronson SM. (1964).
Atheromatous emboli to central nervous system.
Arch Neurol; 11:657-667.

Stein WD. (1986).
Transport and diffusion across cell membranes.
San Diego Academic Press.

Stemmerman GN, Hayashi T, Resch JA, Chung CS, Reed DM, Rhodes GG. (1984)
Risk factors related to ischaemic and haemorrhagic cerebrovascular disease at autopsy: the Honolulu Heart Study.
Stroke; 15:23-8.

Stampfer MJ, Malinow R, Willett WC, Newcomer LM, Upson B, Ullman D *et al.* (1992).
A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians.
JAMA; 268:877-881.

Stevens RS, Ambler NR, Warren MD (1984).
A randomised controlled trial of a stroke rehabilitation ward.
Age & Ageing; 13:65-75.

Stokes GS, Monaghan JC, Flemming CL, Johnston H, Jones M, Pinkerton G *et al.* (1992)
Effects of oral contraceptives containing oestrogen combined with norethisterone or levonorgestrol on erythrocyte cation transport in normal women.
Clin. Sci; 82:505-512.

Stone SP (1987).
The Mount Vernon stroke service: a feasibility study to determine whether it is possible to apply the principles of stroke management to patients and their families on general medical wards.
Age & Ageing; 16:81-8.

Strazzullo P, Galletti F, Cirillo M, Siani A, Nunziata V, Giannattasio R *et al.* (1986).
Altered extracellular calcium homeostasis in essential hypertension: a consequence of abnormal cell calcium handling.
Clin.Sci; 71:239-244.

Stroke Prevention in Atrial Fibrillation Study Group. (1991).
The stroke prevention in atrial fibrillation study: final results.
Circulation; 84:527-539.

Stroke Unit Trialists Collaboration (1997).
Collaborative systematic review of the randomised trials of organised inpatient (stroke unit) care after stroke.
Br. Med. J; 314:1151-59.

Sudlow CLM, Warlow CP. (1996).
Comparing stroke incidence worldwide: what makes studies comparable?
Stroke; 27:550-8.

Sutton-Tyrell K, Alcorn Hg, Wolfson SK, Kelsey SF, Kuller LH. (1993).
Predictors of carotid stenosis in older adults with and without isolated systolic hypertension.
Stroke; 24:355-61.

Sutton-Tyrell K, Wolfson SK, Kuller LH. (1994).
Blood pressure treatment slows progression of carotid stenosis in patients with isolated systolic hypertension.
Stroke; 25:44-50.

Swedish Aspirin Low-dose Trial Collaborative Group. (1991).
Swedish aspirin low-dose trial (SALT) of 75 mg aspirin as secondary prophylaxis after cerebrovascular ischaemic events.
Lancet; 338:1345-1349.

Szaz I., Sarkadi B., Schubert A., Gardos G. (1978).
Effects of lanthanum on calcium-dependent phenomena in human red cells.
Biochem.Biophys.Acta; 512:331-340.

Takebayashi S, Kaneko M. (1983).
Electron microscopic studies of ruptured arteries in hypertensive intracerebral haemorrhage.
Stroke; 14:28-36.

Tavani A, La Vecchia C. (1999).
The adverse effects of hormone replacement therapy.
Drugs & Aging; 14(5):347-357.

Trevisan M, Laurenzi M. (1991).
Correlation of sodium-lithium countertransport. Findings from Gubbio epidemiological study.
Circulation; 84:2011-2019.

Tse M, Levine S, Yun C, Brant S, Counillon LT, Pouyssegur *et al.* (1993)
Structure/function studies of the epithelial isoforms of the mammalian Na+/H+ exchanger gene family.
J. Membr. Biol; 135:93-108.

Turner ST, Boerwinkle E, Johnson M, Richelson E, Sing CF. (1987).
Sodium-lithium countertransport in ambulatory hypertensive and normotensive patients.
Hypertension; 9:24-34.

Turner ST, Michels VV .(1991).
Sodium-lithium countertransport and hypertension in Rochester, Minnesota.
Hypertension; 18:183-190.

UK Prospective Diabetes Study (UKPDS) Group. (1998).
Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33).
Lancet; 352:837-853.

UK Prospective Diabetes Study (UKPDS) Group. (1998.b).
Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34).
Lancet; 352:854-865.

Vaughan CJ, Delanty N. (1999).
Neuroprotective properties of statins in cerebral ischaemia and stroke.
Stroke; 30:1969-1973.

Viberti GC, Earle K. (1992).
Predisposition to essential hypertension and the development of diabetic nephropathy [review].
J AM Soc Nephrol; 3:S27-33.

Viitanen M, Eriksson S, Asplund K. (1988).
Risk of recurrent stroke, myocardial infarction and epilepsy during long-term follow-up after stroke.
Acta Neurol; 28:227-231.

Villar J, Montilla C, Muniz-Grijalvo O, Muriana GJ, Stiefel P, Ruiz-Gutierrez V *et al.* (1996).
Erythrocyte Na+-Li+ countertransport in essential hypertension: correlation with membrane lipids levels.
J.Hypertens; 14:969-973.

Vinters HV. (1987)
Cerebral amyloid angiopathy – a critical review.
Stroke; 18:311-24.

Virchow RLK. (1847).
Ueber die akute Entzundung der Arterien.
Archiv. Pathol. Anat; 1:272-378.

Virchow RLK. (1856).
Thrombose und Embolie: GefassentzUndung und septische Infektion.
In Virchow RLK,ed. Gesammelte abhandlungen zur wissenschaftlichen Medizin.
Frankfurt: Medinger, 219-735.

Wade DT, Collin C. (1988).
The Barthel ADL Index: A standard measure of physical disability?
Int. Disabil. Stud; 10:64-67.

Wagenknecht LE, d'Agostino R, Savage P, O'Leary DH, Mohammed FS, Haffner SM.(1997).
Duration of Diabetes and Carotid Wall Thickness. The Insulin Resistence Atherosclerosis Study (IRAS).
Stroke; 28:999-1005.

Walker JD., Tariq T., Viberti G. (1990).
Sodium-lithium countrtransport activity in red cells of patients with insulin dependent diabetes and nephropathy and their parents.
Br.Med. J; 301:635-638.

Wannamethee SG, Shaper G, Ebrahim S. (2000)
HDL-Cholesterol, total cholesterol and the risk of stroke in middle-aged British men.
Stroke;31:1882-1888.

Warlow CP, Dennis MP, van Gijn J, Hankey GJ, Sandercock PAG, Bamford JM, Wardlaw J. (1996a).
Stroke *A Practical Guide to management.*
Blackwell Science: Chpt. 6. pp.190-197.

Warlow CP, Dennis MP, van Gijn J, Hankey GJ, Sandercock PAG, Bamford JM, Wardlaw J. (1996b).
Stroke *A Practical Guide to management.*
Blackwell Science: Chpt. 5. pp. 150-159.

Weder AB, Toretti BA, Julius S. (1984).
Racial differences in erythrocyte cation transport.
Hypertension; 6:115-123.

Welin L, Svardsudd K, Wilhelmsen L, Larsson B, Tibblin G. (1987).
Analysis of risk factors for stroke in a cohort of men born in 1913.
N. Engl. J. Med.; 317:521-526.

Weng X, Roederer GO, Beaulieu R, Cloutier G. (1998).
Contribution of acute phase proteins and cardiovascular risk factors to erythrocyte aggregation in normolipiaemic and hyperlipiaemic individuals.
Thrombosis & Haemostasis; 80(6):903-908.

Wentworth DA, Atkinson RP. (1996).
Implementation of an acute stroke program decreases hospitalisation costs and length of stay.
Stroke; 27(6): 1040-1043.

Wepfer JJ (1658).
Observationes anatomicae, ex cadaveribus eorum, quos sustulit apoplexia, cum exercitationale de ejus loco affecto.
Schaffhausen: JC Suteri.

West IC, Rutherford PA, Thomas TH. (1998).
Sodium-lithium countertransport: physiology and function.
J Hypertens; 16:3-13.

Whisnat JP, Wiebers DO, O'Fallon WM, Sicks JD, Frye RL. (1996).
A population-based model of risk factors for ischaemic stroke: Rochester, Minnesota.
Neurology; 47:1420-1428.

Whisnant, J.P. (1997).
Modelling of Risk Factors for Ischaemic Stroke.
Stroke; 28:1839-1843.

WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. (1996).
Haemorrhagic stroke, overall stroke risk and combined oral contraceptives: results of an international, multicentre, case-control study.
Lancet; 348(9026): 505-510.

WHO – International Society of Hypertension. (1999).
1999 World Health Organisation – International Society of Hypertension guidelines for the management of hypertension.
Journal of Human Hypertension; 17(2):151-183.

WHO MONICA project (prepared by Stegmayr B. et al.) (1997).
Stroke Incidence and Mortality Correlated to Stroke Risk Factors in the WHO MONICA project.
Stroke; 28:1367-1374.

WHO. (1980)
WHO expert committee on Diabetes Mellitus: a second report.
WHO Technical Report Series 646. Geneva: WHO.

Wieth JO. (1970).
Effects of monovalent cations on sodium permeability of human red cells.
Acta.Physiol.Scand; 79:76-87.

Wiley JS, Cooper RA. (1974).
A furosemide-sensitive cotransport of sodium plus potassium in the human red cell.
J.Clin.Invest; 59: 1113-1119.

Williams RR, Hasstedt SJ, Hunt SC, Wu LL, Ash KO.(1987).
Genetic studies of cation tests and hypertension.
Hypertension; 10:137-141

Wilson NV, Meyer BM.(1981).
Early prediction of hypertension using exercise blood pressure.
Prev. Med; 10:62-68.

Winocour PH, Thomas TH, Brown L, Laker MF, Wilkinson R, Alberti KGMM. (1992).
Serum triglyceride and insulin levels are associated with erythrocyte sodium-lithium countertransport activity in normoglycaemic individuals.
Clin.Chim.Acta; 208:193-203.

Wolf PA, Dauber TR, Thomas E, Kannel WB. (1978)
Epidemiological assessment of chronic atrial fibrillation and risk of stroke: the Framingham Study.
Neurology; 28:973-7.

Wolf PA, Abbot RD, Kannel WB. (1991)
Atrial fibrillation as an independent risk factor for stroke: the Framingham Study.
Stroke; 22: 983-8.

Wolf PA, d'Agostini RB, Belanger AJ, Kannel WB. (1991.b).
Probability of stroke: a risk profile from the Framingham Study.
Stroke; 22:312-318.

Woods JW, Falk RJ, Pittman AW, Klemmer PJ, Watson BS, Namboodiri K. (1982).
Increased red cell sodium-lithium countertransport in normotensive sons of hypertensive parents.
N. Eng. J. Med; 306:593-595.

Woods-Dauphinee S, Shapiro S, Bass E. (1984).
A randomised trial of team care following stroke.
Stroke; 5:864-72.

Yamamoto H., Bogousslavsky J. (1997).
Pathophysiological patterns of stroke recurrence.
Cerebrovasc Dis; 7(suppl 1):5-9.

Yano K, Reed DM, MacLean CJ. (1989).
Serum cholesterol and hemorrhagic stroke in the Honolulu Heart Program.
Stroke; 20:1460-1465.

Yap I, Arrazola A, Soria F, Diez J. (1989).
Is there increased cardiovascular risk in essential hypertensive patients with abnormal kinetics of red cell sodium-lithium countertransport ?
J.Hypertens; 7:667-673.

You RX, McNeil JJ, Hurley SF, Farish SJ, O'Malley HM, Quang L *et al.* (1993).
Smoking as a risk factor for cortical ischaemia presumably due to carotid occlusive disease.
Neuroepidemiology; 12(3):141-147.

You RX, Thrift AG, McNeill JJ, Davis SM, Donnan GA. (1999).
Ischemic stroke risk and passive exposure to spouses' cigarette smoking. Melbourne Stroke Risk Factor Study (MEFRS) Group.
American Journal of Public Health; 89(4):572-575.

Zerbini G, Ceolotto G, Gaboury C, Mos L, Pessina AC, Canessa M. (1995)
Sodium-lithium countertransport has low affinity for sodium in hyperinsulinaemic hypertensive subjects.
Hypertension; 25:986-993.

Zidovetzki R, Chen P, Fisher M, Hofman FM, Faraci FM. (1999).
Nicotine increases plasminogen activator inhibitor-1 production by human brain endothelial cells via protein kinase C-associated pathway.
Stroke; 30(3):651-655.

APPENDICES

Appendix 1. Stroke Data Sheet

General Information:

Name:
Hospital no.:
Age:
Sex:

Time of onset:
Date of Onset:
Date of Admission:
Date of transfer:

Marital status:
Lives alone: Y / N
House Type:

Education Level:

Main Carers:

Stroke:

Side of deficit: R / L
Power upper limb: Prox. /5
Dist . /5
Power lower limb: Prox. /5
Dist. /5

Sensory deficit: Y / N
Visual field defect: Y / N
Facial Weakness: Y / N
Cranial nerve palsy: Y / N
Admission SSNS score: /58
(consciousness /6)

Clinical Stroke type:

CT Findings:

Speech & language:

Dysphasia: Express. Y / N
Recept: Y / N
Dysphagia: Y / N
Alternative feeding: Y / N
NG
PEG
Dysarthria: Y / N

Dyspraxia: Y / N

Videofluroscopy: Y/N

Likely Aetiology: Cardioembolic
(if infarct) Large vessel
Intracranial

Cardiovascular:
Risk factors:
Hx. MI / Angina: Y / N
Hx Valve Disease. Y/N
Hx A-Fib: Y / N
Hx. Hypertension: Y / N
Hx CVA : Y / N
Hx TIA: Y / N
Hx Hyperlipidaemia: Y / N
Hx Diabetes: Y / N
Smoking habits: /day. (ex = x yrs.)
OCP use: x yrs.
Alcohol: units/week
Other:

Fasting - Chol: mMol/l
- Trig: mMol/l
- HDL: mMol/L
- LDL: mMol/L
- Glucose: mMol/L
- HbA1C: %
- Urea: mMol/L
- Creat: mMol/L
- Albumin: g/dL

Admission ECG: **Admission BP:**

HR = Rhythm;
Qwaves: Y / N TWI: Y / N
ST depression: Y / N ST elevation: Y /N
LBBB. Y / N Other conduction: Y/ N

Investigations

24 Hr. Holter: Y /N Result Other:

Carotid Dopplers: Y / N Result

Echocardiogram: Y / N Result

MRI: Y / N Result:

Thrombophilia Screen: Y/N Result:

LP: Y / N Result:

Chest radiograph:

Cardiomegaly: Y/N Pulm oedema/ULD: Y/N
Pneumonia: Y/N Tumour: Y/N
Other:

Medical Co-Morbidity:

Premorbid:
1.
2.
3.
4.
5.

New onset since Stroke:
1.
2.
3.
4.
5.

Nutrition:

Body Weight: (actual/estimate). BMI (est):
(ideal)
(upon discharge)
Caloric Requirement: Kcal/day.
Special dietary needs: Y/N
Dietary supplements: Y/N
Weight loss (If occurred) (Kgs)
Reduction in albumin: g/dl

Occupational Therapy;

Pre-Morbid: Barthel Index : MMSE: Other:

Week 2: " " " "

Week 6: " " " "

Cognitive impairment: Y/N Seat/Pressure relief. Y/N

Perceptual problems/ Visual: Y/N
Inattention Y/N
Apraxia? Y/N (activity;)

Functional Deficits: Y/N
Where?

Hand Splinting : Y/N
Adaptation/aids: Y/N
Home Visit: Y/N

Home suitable (as exists): Y/N.
Home requires adaptation: Y/N.
Not suitable to return home: Y/N.

Physiotherapy:

Week 2: **Tone:** Prox. UL Power: /5 Shoulder Sublux. Y/N
Dist UL /5 Shoulder-Hand Y/N
Prox LL /5
Dist LL /5

Balance: Sitting Y/N **Mobility:** Transfers: Ind. +1 +2 N/A
Standing Y/N Walking: Ind. +1 +2 N/A
+1
+2

Sensory Deficit: Y/N (site:)
Proprioceptive deficit : Y/N (Site:)

Week 6: **Tone:** Prox UL /5 Shoulder Sublux. Y/N
(or D/C) Dist UL /5 Shoulder-Hand Y/N
Prox LL /5
Dist LL /5

Balance: Sitting: Y/N **Mobility:** Transfers: Ind. +1 +2 N/A
Standing: Y/N Walking: Ind. +1 +2 N/A
+1 Stairs: Ind. +1 +2 N/A
+2

Sensory Deficit: Y/N (site:)
Proprioceptive deficit : Y/N (Site:)

Discharge: Date: Destination :

Length of stay: SSNS score: = /58

Discharge Grade: (mod. Rankin=).

Date of listing LTC:

Delay from date of planned d/c:

Why delay Occurred:

Medications on Admission:	Discharge:
1.	
2.	2.
3.	3.
4.	4.
5.	5.
6.	6.
7.	7.
8.	8.

Liasion Consultations ?: (tick as appropriate)

Urology:	Cardiology
General Surgery:	Respiratory:
Vascular Surgery	Neurology
Orthopaedics:	Diabetes
Gastroenterology	

Follow-Up Rehab: (circle relevant))

Baggot st.

DCU

Day Hosp.

OPD

Social Supports:

Family Member at Home	Y/N
Family Member Visits	Y/N
Home Help	Y/N
Neighbour	Y/N
Care Assistant:	Y/N

Appendix 2: Bamford Classification of Sub-types of cerebral Infarction.

Total Anterior circulation Infarct (TACI):

Acute onset of hemiparesis (with or without sensory loss) affecting at least two areas of ; the contralateral face, upper or lower limb + contralateral homonymous hemianopia + evidence of new cortical dysfunction (eg dysphasia, inattention, apraxia).

Partial Anterior circulation Infarct (PACI):

Acute onset of two of the three components of TACI, or isolated cortical dysfunction (e.g. isolated dysphasia), or with a motor/sensory deficit more restricted than in TACI e.g restricted to one body area or part thereof e.g. one hand.

Lacunar Infarction (LACI):

Acute onset of pure motor, sensory or sensorimotor deficit affecting the contralateral limbs and face, or isolated ataxia.

Posterior Circulation Infarction (POCI):

Acute onset of isolated homonymous hemianopia, cerebellar signs (other than isolated ataxia), cranial nerve palsy, or bilateral sensory, motor or sensorimotor signs.

(taken from **Bamford J. (1992) Clinical examination in diagnosis and subclassification of stroke. Lancet 339:400-2**).

Appendix 3: Barthel Index of Activities of Daily Living

Activity	category	score
Bowels	Incontinent	0
	Occasional incontinence (< once per week)	1
	Continent	2
Bladder	Incontinent/catheterised unable to manage	0
	Occasional accident (< 1 per day)	1
	Continent	2
Grooming	Needs help with washing, hair, shaving, teeth.	0
	Independent	1
Toilet use	Dependent	0
	Need some help	1
	Independent, on, off, dressing and cleaning.	2
Feeding	Dependent	0
	Need some help (cutting, spreading)	1
	Independent	2
Transfer	Unable, no sitting balance	0
	Major help (2 people)	1
	Minor help (I person)	2
	Independent	3
Mobility	Unable	0
	Wheelchair independent	1
	Walks with help or supervision	2
	Independent (can use aid)	3

Dressing	Dependent	0
	Needs some help	1
	Independent (incl. Zips, buttons etc.)	2
Stairs	Unable	0
	Need some help or supervision	1
	Independent (up & down)	2
Bathing	Dependent	0
	Independent in bath or shower	1

Total Score **/ 20**