

**Vascular investigation and management of ischaemic  
stroke**

A thesis by

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## Summary

A variety of aetiological factors serve as targets for both treatment and prevention in ischaemic stroke. Research has shifted towards acute therapy and improving the risk / benefit ratio in secondary prevention.

Current evidence based practice in ischaemic stroke is discussed in **chapter one**, and the background to subsequent chapters is introduced. The thesis is then divided into two broad areas that examine mechanisms by which vascular disease may cause ischaemic stroke, and the manner in which vascular risk may be modulated. Chapters two to four highlight controversies in investigation and management. **Chapter two** addresses a clinical dilemma - essentially whether a carotid lesion ipsilateral to a lacunar stroke should be considered symptomatic. Observational data comparing incidence of lacunar disease contralateral (group 1) or ipsilateral (group 2) to carotid artery disease, and stroke recurrence in patients in whom co-existent lacunar and carotid disease had been identified, were analysed. 32 patients had carotid disease contralateral to their lacunar stroke, compared to 61 patients with ipsilateral lacunar disease. Chi-squared testing indicated a positive association between unilateral lacunar stroke and ipsilateral carotid disease ( $p=0.003$ ), and a just significant trend towards more severe carotid disease in group 2 ( $p=0.049$ ). Recurrent ischaemic stroke occurred more commonly in group 2 than group 1, although this difference just failed to reach statistical significance ( $p=0.059$ ). A positive association therefore exists between lacunar stroke and ipsilateral carotid disease that confers a poor prognosis.

Cryptogenic stroke accounts for approximately 30% of ischaemic stroke. Inter-atrial septal abnormalities (patent foramen ovale (PFO) and atrial septal aneurysm) have been proposed as a cause of stroke. **Chapter three** details a meta-analysis of data examining the relationship between inter-atrial septal abnormalities and stroke. Comparing ischaemic stroke to controls, the odds



ratio (OR) associated with PFO for all ages was 1.83 (95% C.I.=1.25-2.66). For atrial septal aneurysm it was 2.35 (1.46-3.77), and for both lesions in conjunction it was 4.96 (2.37-10.39). Homogeneous results were found within the group  $\leq 55$  years (3.10 (2.29-4.21), 6.14 (2.47-15.22) and 15.59 (2.83-85.87) respectively), and  $\geq 55$  years (1.27 (0.80-2.01), 3.43 (1.89-6.22) and 5.09 (1.25-20.74) respectively). Comparing cryptogenic stroke patients to patients with known stroke cause, heterogeneous results derived from total group examination (PFO 3.16 (2.30-4.35), atrial septal aneurysm 3.65 (1.34-9.97), PFO and atrial septal aneurysm 23.26 (5.24-103.20)). In those  $\leq 55$ , for PFO the OR was 6.00 (3.72-9.68) with only one study examining atrial septal aneurysm or combined lesions. In those  $\geq 55$ , 3 studies produced heterogeneous results for PFO (2.26 (0.96-5.31)), while no data were available on atrial septal aneurysm prevalence. Meta-analysis therefore demonstrates significant association between both PFO and atrial septal aneurysm and ischaemic stroke in patients  $\leq 55$  years. Further studies are needed to firmly establish whether an association exists between PFO and ischaemic stroke in those  $\geq 55$  years.

The climate of uncertainty that surrounds the subject of 'PFO-associated stroke' has led to a wide spectrum of practice amongst specialists, which is examined in **chapter four**, using a questionnaire. 17% of respondents would investigate for PFO in all cryptogenic stroke patients, while 60% investigate only in those  $< 55$  years. 23% would not investigate for PFO at all. Antiplatelet therapy alone was chosen as an initial strategy by 47% of respondents for those  $> 55$  years, and by 33% of respondents for those  $< 55$  years ( $p < 0.01$  for comparison of proportions). In a patient of any age with recurrent events, less than 5% of respondents would continue to use antiplatelet therapy alone. 45% would use warfarin, and 42% would refer the patient for a corrective procedure. For a patient with a large PFO, 57% ( $< 55$  years) vs 45% ( $> 55$  years) would refer ( $p = 0.01$ ), and for a patient with concomitant atrial septal aneurysm, 62% ( $< 55$  years) vs 44% ( $> 55$  years) would refer ( $p < 0.01$ ). A large

PFO was felt to be the most important factor in decisions regarding lesion correction. Investigation practice varied considerably amongst specialists. Randomised trial design should reflect management practice in this area.

The second part of the thesis reports clinical trials designed to examine the manner in which agents aimed at the modification of vascular risk may act in the acute and subacute phase of ischaemic stroke. The cholesterol-independent effects of statins may explain their efficacy in patients at high vascular risk. **Chapter five** reports a small randomised trial of pravastatin versus placebo in ischaemic stroke. No difference in whole cerebral perfusion was seen after 2 months between the treatment and control arms ( $p=0.88$ ). Similarly, no difference in common ( $p=0.41$ ) or internal ( $p=0.84$ ) carotid artery flow was evident. No effect on mean arterial blood pressure was detected in the pravastatin treated group ( $p=0.44$ ). Early total and LDL cholesterol reduction in the pravastatin group was accompanied by trends towards reduction in factor VII ( $p=0.067$ ) and haematocrit levels ( $p=0.101$ ), but no effect or trend to effect was seen in fibrinogen, C-reactive protein, plasma viscosity, von-Willebrand factor or other haemorrhological markers.

*Chlamydia pneumoniae* infection and raised levels of fibrinogen are risk factors for ischaemic stroke in observational studies. Seropositivity to *chlamydia pneumoniae* is independently associated with raised fibrinogen, and *chlamydia pneumoniae* infection may increase vascular risk by increasing fibrinogen. The pilot study reported in **chapter six** was a randomised, double-blind comparison of azithromycin versus placebo after ischaemic stroke. Thirty patients were randomised irrespective of *chlamydia pneumoniae* status. No difference in fibrinogen reduction after ischaemic stroke was detected between azithromycin and placebo treated patients ( $p=0.91$ ), or between *chlamydia pneumoniae* positive and negative patients ( $p=0.65$ ). No differential effect of treatment was detected in the seropositive group ( $p=0.77$ ). No effect of *chlamydia pneumoniae* seropositivity, azithromycin

treatment or their interaction was detected on plasma viscosity, C-reactive protein or von-Willebrand factor. This pilot phase excludes effects of macrolides on fibrinogen levels in unselected patients after ischaemic stroke of more than 0.36g/l. A project to investigate antibiotic effects as small as 0.2g/l will require over 200 patients, and is planned.

The heterogeneity of stroke militates against the view that 'stroke medicine' is merely an extension of cardiovascular medicine. While some therapies are suitable for a broad spectrum of patients, a variety of different causes and risk factors may be at play in the individual, and secondary prevention should be tailored to that individual. Further work should aim to define the importance of PFOs in the old, the correct management strategy in 'PFO associated stroke', the indications for and actions of statins in stroke, and the possible effects of anti-chlamydial therapy.

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Total group: *recurrent events*

Total group: *large PFO or concomitant ASA*

Total group: *concurrent DVT*

YY investigators: *uncomplicated scenario*

YY investigators: *recurrent events*

YY investigators: *large PFO or concomitant ASA*

YY investigators: *concurrent DVT*

YN investigators: *uncomplicated scenario*

YN investigators: *recurrent events*

YN investigators: *large PFO or concomitant ASA*

YN investigators: *concurrent DVT*

NN investigators: *uncomplicated scenario*

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## Introduction



## 1.1 Stroke: a vascular brain disease

The pathogenesis of stroke involves *vascular* injury to *neural* tissue. Stroke *risk factors* and *causes* exert their effects on cerebral vasculature, and form the basis for secondary prevention strategies, while acute management of stroke may be directed at the primary circulatory insult, or its secondary neural effects. Considerable research efforts have been directed at each of these putative strategies. Large scale randomised trials of intravenous thrombolysis have established acute therapy as both feasible and beneficial for selected patients <sup>1</sup>, while neuroprotective agents, despite considerable research interest <sup>2</sup>, have failed to show benefit in clinical trials to date.

Secondary prevention of stroke has focused chiefly on the modification of established vascular risk factors (for example smoking and hypertension), and on the treatment of vascular stroke *causes* (for example carotid endarterectomy in severe carotid artery stenosis). While certain questions have been answered, an example being the use of low to medium dose aspirin in ischaemic stroke <sup>3</sup>, controversies remain with regard to vascular risk factors, aetiology and management, a number of which the following chapters seek to address. Each chapter addresses aspects of 'what to do second', once the acute phase of ischaemic stroke has passed. A common theme is the detailed consideration of the *mechanism* of stroke recurrence, and how established and novel approaches to secondary prevention may affect such mechanisms.

To better explain the setting and nature of each controversy, current evidence based practice for an individual stroke patient will be described. The difficult decisions regarding his / her investigation and management will be highlighted, and the research topics will be introduced in turn. While rehabilitation and patient-centred occupational and physiotherapy represent vitally important factors in the care of stroke patients, these will not be

addressed as they are not within the remit of this thesis. The specifics of each research subject will be dealt with in the succeeding chapters.

## **1.2 Evidence based management of the patient with ischaemic stroke**

### ***Acute investigation and therapy***

After symptom onset, rapid evaluation of the stroke patient is recommended. Such a policy allows prompt investigation, safe nursing, and specific therapy if appropriate. After clinical evaluation by emergency medicine staff and a specialist, urgent imaging (Computed Tomography (CT) and/or Magnetic Resonance Imaging (MRI)) should be performed. Such investigations may be delayed, particularly if thrombolytic therapy is not considered worthwhile. The Scottish Intercollegiate Guidelines Network (SIGN) state that CT scanning should occur '*as soon as possible – preferably within 48 hours – and no later than 7 days*'. The proportion of stroke admissions fulfilling these criteria is one measure of the quality of stroke care, and adherence to these guidelines has proved difficult to achieve within many Scottish hospital trusts. In contrast, delays of up to 7 days would be considered unacceptable in other European industrialised nations.

Modern MRI techniques are both sensitive and specific in the detection of acute haemorrhage, and allow semi-functional assessment of ischaemic damage by diffusion/perfusion weighted imaging (DWI/PWI). Such techniques may prove useful in choosing individuals who are suitable for thrombolysis outside the conventional 3 hour time window <sup>4</sup>. Other investigations will depend on the individual presentation, but an electrocardiogram (ECG), to detect atrial fibrillation (AF), left ventricular hypertrophy (LVH) or evidence of cardiac ischaemia / infarction, chest x ray (CXR) for assessment of heart size or concurrent infection, and basic haematology and biochemistry would be included in the standard assessment in the casualty department.

Acute management, as alluded to previously, should consist of thrombolytic therapy for selected individuals. The disadvantages of intravenous thrombolysis with tissue plasminogen activator (IV tPA), namely increased early death and symptomatic haemorrhage, are offset by a reduction in disability in survivors, so that overall there is a significant reduction in the proportion of patients dead or dependent in activities of daily living at follow up <sup>1</sup>. Current evidence thus favours the use of thrombolysis within 3 hours. Data from recent trials have suggested that in patients who have middle cerebral artery (MCA) occlusion, the time window may be extended to 6 hours with the use of intra-arterial thrombolysis <sup>5</sup>.

One of the difficulties with stroke care in the 'thrombolytic era' has been the assessment of patients within the time window that thrombolytic therapy has been proven to be beneficial – its use is by no means common, even in those countries in which a licence has been granted. Recently an observational study in the Cleveland area reported that only 1.8% of acute stroke patients received intravenous tissue plasminogen activator (IV tPA), despite numerous protocol violations <sup>6</sup>. Debate also continues about clinical and radiological indications, and the correct setting for the use of IV tPA <sup>7</sup>. In the UK and Europe, thrombolytic therapy is limited to a few specialist centres, and ischaemic stroke remains an unlicensed indication. This situation is likely to change in the near future: numerous centres have now reported outcome results similar to those in the National Institute of Neurological Diseases (NINDS) trial <sup>8</sup>, and it has become clear that if used by specialists in the correct setting, IV tPA is a useful therapy for a minority of cases <sup>9</sup>. It may be that selected patients will benefit from intravenous treatment beyond 3 hours, a contention which is currently being tested in the third international stroke trial (IST III).

Trials of neuro-protective agents have until now proved negative, which in some instances may reflect poor trial design rather than absence of effect. There is little pathophysiological basis for neuroprotection in stroke that is confined to white matter. Despite this, patients with white matter stroke have been included in clinical trials of neuroprotective agents, in the same way as individuals with cortical disease. Clinical trials restricted to patients with large MCA stroke accompanied by radiological evidence of an ischaemic penumbra (using diffusion/perfusion MRI) may be more likely to detect drug effect <sup>10</sup>. It is hoped that slower recruitment may be offset by extended time windows and requirements for fewer patients. Surrogate evidence of biological effect in man may therefore be defined prior to embarking on further research into clinical endpoints, so that ineffective agents are identified quickly and development is discontinued. Natural history data from existing trials may enable better definition of endpoints relevant to the individual stroke patient, another means by which agent efficacy may be more efficiently assessed.

Placement of the patient presenting with stroke is at least in part dependent on the clinical presentation. Patients with haemorrhagic stroke requiring surgery should be transferred to a specialist unit for urgent evaluation and management. If symptoms have resolved within 24 hours (that is, if the diagnosis is of transient ischaemic attack (TIA) rather than stroke), the clinician may elect not to manage the patient in hospital. If such a policy is employed, it is imperative that investigations and clinic review occur shortly after presentation so that secondary measures may be instituted quickly. A short admission may be more practicable. In-patient care should be based in a specialist unit <sup>11</sup>, and acceptance of such a policy has led to a considerable expansion in stroke units in recent years. The situation remains, however, unbalanced across the United Kingdom, with the consistent recommendation of working groups that stroke care should take place within a specialist unit being adhered to variably by different hospital trusts <sup>12</sup>.

Another argument supporting the development of specialist units is that control of physiological parameters in the acute stages of stroke may improve prognosis. Certainly pyrexia <sup>13</sup>, hypoxia and hyperglycaemia <sup>14</sup> are associated with poor outcome, but policy on treating such abnormalities differs widely. Abnormal physiological measurements may represent co-existent conditions, the effect of stroke, or in some instances the cause of stroke, and the effect of corrective treatment is likely to depend on which of these associations is relevant in the individual. Admission to a unit with a stated policy on the correction of such abnormalities makes both detection and correction more likely. Evidence that such measures will result in improved outcome has recently been published by workers in Scandinavia. *Ronning et al* noted that more aggressive rehabilitation and use of parenteral fluid, aspirin, antipyretics and antibiotics was more common in their stroke unit than in the general medical ward <sup>15</sup>. *Indredavik et al* assessed which aspects of stroke unit care resulted in improved outcomes <sup>16</sup>. Stroke units mobilised patients more quickly, and used intravenous fluid, heparin, antipyretics and oxygen more readily, resulting in less variable blood pressure, avoidance of low diastolic blood pressure, and lower glucose levels and temperature. Each of these factors was significantly associated with discharge to home within 6 weeks. In a multivariate model, shorter time to mobilisation and stabilised diastolic blood pressure were both independent factors significantly associated with discharge to home within 6 weeks. Whether specific therapy for hyperglycaemia will improve outcome is currently being investigated in a large trial, which recently reported pilot data <sup>17</sup>.

Adequate nutrition is also important in the acute and subacute phases. Current practice in this area varies considerably, and randomised evidence is scarce <sup>18</sup>. What little evidence is available suggests that percutaneous gastroenterostomy (PEG) may reduce fatalities and improve nutritional status <sup>18</sup>. The best use of nasogastric (NG) or PEG tubes, and the timing of feeding

interventions, is the subject of the ongoing 'Feed or ordinary diet' (FOOD) trial.

Control of blood pressure is a contentious area of ischaemic stroke management. Because the autoregulatory mechanisms governing cerebral perfusion across a range of blood pressures are disturbed after stroke<sup>19</sup>, falls in blood pressure may be directly transferred to the infarcted area, resulting in worse neurologic outcome<sup>20</sup>. Conversely, high blood pressures increase the theoretical likelihood of haemorrhagic transformation and persistent or worsening cerebral oedema, with the consequent possibility of clinical deterioration. Clinical data support an increased risk of haemorrhagic transformation at diastolic blood pressures above 100mmHg<sup>21</sup>. Thus a therapeutic quandary exists: when to treat, with what and for how long. Published small trials on this subject have been summarised in a recent Cochrane Review<sup>22</sup>, but good randomised controlled data are scarce, and larger scale trials must assess the efficacy of pharmacological blood pressure control both within and without the context of thrombolysis. The rationale behind the numerous ongoing studies in this area is explained in **table 1.1** (uses references<sup>23</sup> and<sup>24</sup>).

Aspirin therapy should begin in the acute stroke phase, as soon as haemorrhage has been excluded by CT or MR imaging. Large scale randomised trials were required to show a beneficial effect of antiplatelet therapy in the acute prophylaxis of further vascular events. In a recent Cochrane review antiplatelet therapy was associated with a small but definite excess of 2 symptomatic intracranial haemorrhages for every 1000 patients treated, but this was more than offset by a reduction of 7 recurrent ischaemic strokes for every 1000 patients treated<sup>3</sup>. Only doses higher than 160mg have been tested in this acute setting.

### ***Modification of vascular risk and secondary prevention***

The cornerstone of secondary prevention for both the primary and secondary care physician is the modification of vascular risk factors. Patients are advised not to smoke (since cigarette use is strongly associated with increased stroke risk <sup>25</sup>), and considerable evidence now exists for the use of pharmacological strategies in achieving this goal <sup>26</sup>. While good diabetic control has been demonstrated to reduce the incidence of microvascular complications <sup>27</sup>, secondary prevention of macrovascular disease is assumed rather than proven.

Meta-analysis of randomised trials examining the long term effects of antiplatelet agents in 1994 by the Antiplatelet Trialists Collaboration confirmed modest effects in the secondary prevention of vascular events <sup>28</sup>. The findings have been recently updated <sup>29</sup>, and the relative risk reduction (RRR) afforded by antiplatelet therapy in all high risk vascular patients remained approximately 25%. In those with previous ischaemic stroke or TIA, allocation to a mean duration of 29 months of antiplatelet therapy resulted in 36 fewer serious vascular events per 1000 patients treated <sup>29</sup>. Most of this evidence relates to aspirin used as a single agent, and there does not seem to be an aspirin dose effect <sup>29 30</sup>.

Two major stroke secondary prevention trials have been published since the 1994 meta-analysis <sup>28</sup>, both of which examined ways in which secondary prevention afforded by aspirin could be improved. Criticism was aimed at the second European Stroke Prevention Study (ESPS-2), which was designed to investigate the contention that addition of dipyridamole to aspirin would improve rates of recurrent stroke and death in stroke patients. This criticism stemmed from the choice of aspirin dose (50mg) (which was deemed too low), from the inclusion of a placebo group (which with the benefit of data that emerged during recruitment was deemed inappropriate) and from one centre falsifying results, which were discounted from the final analysis. In fact, the

results for the aspirin treated group were entirely in line with those of other trials that have used much higher doses of aspirin in the secondary prevention of stroke <sup>31</sup>. The finding that the combination of aspirin and dipyridamole afforded a 37% RRR in recurrent stroke over placebo, twice that of either aspirin (18%) or dipyridamole (16%) alone <sup>32</sup>, has not resulted in routine use of this 'combination' strategy. Current Scottish guidelines (SIGN guideline number 36: Antithrombotic therapy) recommend the addition of dipyridamole to aspirin for patients with recurrent events on aspirin. This suggests that its authors accept that the addition of dipyridamole confers improved efficacy in secondary prevention. If this is the case, and one accepts the low bleeding rates demonstrated in ESPS-2 <sup>32</sup>, the only barrier to the routine use of combination aspirin and dipyridamole therapy is cost. Even using conservative estimates of the cost of stroke, the cost of additional dipyridamole therapy is outweighed by the cost of recurrent events <sup>33</sup>.

The CAPRIE trial <sup>34</sup> compared 325mg aspirin and 75mg clopidogrel, a new thienopyridine derivative which inhibits platelet aggregation induced by adenosine diphosphate. In the overall trial cohort, which included patients with ischaemic heart disease (IHD), cerebrovascular disease (CVD) and peripheral vascular disease (PVD), a significant benefit in the reduction of a combined endpoint (ischaemic stroke, myocardial infarction (MI) or vascular death) was detected. The benefit of clopidogrel over aspirin was small, and was largely evident in the subgroup of patients with PVD. Chi-squared test for heterogeneity of effects in the different subgroups was significant. To prevent a single vascular event, 200 patients would have to be treated with clopidogrel rather than aspirin. Given the cost of the new agent (£370 per year), current guidelines recommend its use in those who, for whatever reason, cannot take aspirin.

Current research interest in this area centres on alternative 'combination therapies', and in the use of oral anticoagulation. The recent publication of the



Warfarin-Aspirin Recurrent Stroke Study (WARSS) <sup>35</sup> confirmed previous meta-analyses <sup>36</sup> that failed to demonstrate superior efficacy of warfarin over aspirin for patients in sinus rhythm. The Management of ATherothrombosis with Clopidogrel in High-risk patients with recent TIA or ischaemic stroke (MATCH) trial is comparing the combination of clopidogrel and aspirin with clopidogrel alone for the prevention of new ischaemic events (cerebrovascular or cardiovascular). Patients will be randomised within three months of an ischaemic stroke or TIA, and followed up for 18 months, with an anticipated sample size of 7600 patients. The European and Australian Stroke Prevention in Reversible Ischaemia (ESPRIT) trial is randomising patients with cerebral ischaemia of arterial origin to oral anticoagulation, aspirin and dipyridamole or aspirin alone (anticipated sample size 4500).

In patients with AF and recent cerebral ischaemia, meta-analysis of randomised evidence suggests that anticoagulants are beneficial, without serious adverse effects, for people with nonrheumatic atrial fibrillation and recent cerebral ischaemia <sup>37</sup>. Anticoagulants reduced the risk of recurrent stroke by two-thirds (odds ratio 0.36, 95% confidence interval 0.22 to 0.58). The risk of all vascular events was shown to be almost halved by treatment (odds ratio 0.55, 95% confidence interval 0.37 to 0.82).

Hypertension increases ischaemic stroke risk, and its treatment (as primary prevention) reduces stroke risk <sup>38</sup>. Management of blood pressure as a secondary prevention measure after stroke was investigated by the recently reported Perindopril Protection Against Recurrent Stroke Study (PROGRESS), which randomised 6105 patients <sup>39</sup>. It was designed to determine the effects of blood pressure lowering in both 'hypertensive' (systolic >160 mmHg or diastolic >90 mmHg) and 'non-hypertensive' patients with a history of stroke or TIA. Active treatment comprised a flexible regimen based on the angiotensin converting enzyme (ACE) inhibitor perindopril, with the addition of the thiazide diuretic indapamide at the physician's discretion.

Active therapy reduced blood pressure by 9/4 mmHg, and the risk of recurrent stroke by 28% (95% CI 17-38), with a similar highly significant reduction in total vascular events. The effects were seen in both hypertensive and non-hypertensive subjects, and after all types of initial event. They reproduce (and extend to the normotensive population) the findings of an overview which summarised antihypertensive secondary prevention studies prior to the publication of PROGRESS <sup>40</sup>.

The PROGRESS trial, while supporting an intensified approach to blood pressure control in all stroke patients, also generates a number of questions. Firstly, should all patients be started on a regime of perindopril and indapamide? Single agent perindopril therapy (which reduced blood pressure by 5/3 mmHg) had no discernable effect on stroke risk: the effects were only seen in the combination therapy (perindopril + indapamide) group, in which blood pressure was lowered further (12/5 mmHg). It is unclear whether the effects seen can be generalised to the use of all antihypertensive agents - in other words the reduction in stroke risk may have merely reflected reduction in blood pressure, rather than an effect of the specific agents chosen. If blood pressure is the clinical target (rather than the prescription of perindopril and indapamide), should we be aiming for a target blood pressure, or a target pressure reduction? It also remains unclear whether patients with occlusive or stenotic disease of major cerebral arteries can safely be started on the PROGRESS regime, although small trial data would support the view that such an approach will not result in dangerous falls in cerebral perfusion or flow <sup>41</sup>.

### **1.3 Carotid endarterectomy and subcortical stroke**

Carotid endarterectomy for the secondary prevention of stroke in patients with varying degrees of carotid stenosis has been tested in randomised controlled trials. Large scale studies have been reported from both America <sup>42 43</sup> and

Europe <sup>44</sup>. These trials included patients with *symptomatic* carotid disease, defined as symptoms referable to the hemisphere supplied by the relevant carotid artery, or amaurosis fugax in the ipsilateral eye. They showed clear benefit for surgery over best medical treatment for patients with more than 50% stenosis (measured by the NASCET method) or 70% (measured by the ECST method) <sup>45</sup>. Given the less impressive results of trials in asymptomatic carotid artery stenosis <sup>46</sup>, despite extremely low complication rates in the surgically treated group, the classification of a lesion as *symptomatic* is central to the choice of correct therapy for patients with carotid artery stenosis.

The effect of surgery in symptomatic individuals with carotid artery stenosis is not uniform, and subgroups that respond differently to surgical intervention can be identified both clinically and angiographically. It appears that as age and level of stenosis increase, so does the risk of stroke with medical therapy, and the benefit to be gained from surgery <sup>45</sup>. Clinical characteristics that are associated with an increased risk of stroke or death with surgery include contralateral carotid occlusion, left-sided carotid disease, the absence of a history of MI or angina, a history of diabetes mellitus and a diastolic blood pressure higher than 90mmHg <sup>43</sup>. It seems that the long term benefit of surgery (over medical treatment) is higher for men than for women, for patients who have had a stroke than for those who have had a TIA, and for patients with hemispheric symptoms than for those with retinal symptoms <sup>43</sup>. Simple classification systems have been developed using ECST data that incorporate both clinical and angiographic data <sup>47</sup> to enable surgical therapy to be offered only to those patients most likely to benefit. These are likely to improve with further analysis of individual patient data from trials already conducted and from randomised trials that are ongoing.

Lacunar syndromes comprise four different well defined clinical presentations: pure motor stroke, pure sensory stroke, sensorimotor stroke and ataxic

hemiparesis (including dysarthria clumsy hand syndrome and homolateral ataxia and crural paresis). These presentations correlate well with small infarctions in the subcortical areas of the brain, and it is likely that most are caused by a specific vascular pathology affecting the small perforating arteries <sup>48</sup>. These comprise the lenticulostriate perforating branches of the MCA, the thalamoperforating branches of the proximal posterior cerebral artery (PCA) and the perforating branches of the basilar artery (BA). Although the reported associated risk factors differ between datasets, at least in part because of the population that they have studied, hypertension, diabetes and hyperlipidaemia are the most consistent correlates of lacunar stroke.

There are substantial pathological and experimental data to support the view that lacunar stroke is caused by small vessel pathology, rather than by embolism (from carotid arteries or the heart) or haemodynamic mechanisms <sup>48</sup>. Epidemiological and observational studies have found low rates of embolic sources in lacunar stroke populations <sup>49</sup>. It seems clear that in the *population*, lacunar stroke is rarely associated with carotid stenosis, or other traditional embolic sources <sup>50</sup>. Other strokes in the subcortical territories include centrum ovale infarction, occurring in the territory of the superficial (pial) branches of the MCA, and internal borderzone infarcts, occurring between the deep and superficial perforating arterial territories. Both of these types of infarction have been associated, in literature which stems from analysis of data from the major carotid endarterectomy trials, with large vessel carotid disease <sup>51 52</sup>.

Thus, data from epidemiological literature provide useful pointers to the clinician in deciding which patients should be investigated for the presence of a proximal (carotid or cardiac) source. Patients with lacunar stroke, in whom such lesions are uncommon, are less likely to have positive carotid Doppler or echocardiographic investigations. Such considerations are less helpful in managing *the individual who has been investigated* however. Most stroke services employ a routine series of investigations in their patients. While small

subcortical strokes are not *usually* caused by embolism or haemodynamic mechanisms, the detection of a carotid stenotic lesion or cardiac embolic source in a patient with a lacunar stroke leaves the practising physician with the uncertainty of whether the lacunar syndrome is incidental, or whether it has been caused by the embolic source. Practising physicians and surgeons need advice on whether patients with severe stenosis and small subcortical strokes should be referred for endarterectomy.

Observational information on both association and prognosis, using the sort of clinical data that are likely to be available to the practising clinician, may provide useful guidance in this situation. The two published subgroup analyses from the major trials of carotid stenosis failed to show a significant benefit of surgical over medical therapy in lacunar disease patients<sup>53 54</sup>. In the NASCET paper, a trend to benefit was observed, but was much less marked than in the larger cohort of patients with cortical stroke, with the confidence interval of the RRR crossing the line of no effect. More recent individual patient data from randomised trials of carotid endarterectomy, currently reported only in abstract form, suggested benefit was evident in the lacunar stroke subgroup for those patients with high degrees of stenosis (80-99%)<sup>55</sup>

**Chapter 2** examines information from the Glasgow Western Infirmary database, aiming to provide an observational aid in what is a difficult management decision. With simple clinical data, and information from routine investigations, the study provides useful data that help guide the clinician. The literature pertaining to this subject to date is discussed, as are the weaknesses and strengths of the analysis presented.

## **1.4 Echocardiography: investigation for major and minor embolic sources**

Transthoracic echocardiography (TTE), transoesophageal echocardiography (TOE), and more specialised transcranial Doppler (TCD) techniques to assess the presence of cardiac right-to-left shunt, are investigations that are conducted in a smaller proportion of stroke patients than brain imaging and carotid Doppler. Generally these are conducted to determine *cause* of stroke, although the information they provide may be useful in stratifying cardiac risk or in investigating abnormalities that are felt not to have played a role in stroke pathogenesis. Features that may favour a request for cardiac ultrasound are patients with lesions considered to be embolic, in the absence of more common embolic sources (e.g. AF and carotid stenosis), multiple cerebral lesions in different sites, the presence of a cardiac murmur or abnormal ECG, and absence of conventional risk factors, including age, in the individual stroke patient. Diagnostic yield depends on the stroke population being studied, and may not lead to a change in immediate management.

It is of note that in up to 40% of strokes, no aetiology is found<sup>56</sup>. These are known as cryptogenic strokes. Various classification systems in different stroke data banks produce different rates of cryptogenic stroke. This depends on the population being studied, and on the extent of investigations that have been conducted to determine stroke cause. Moreover, it hinges on which positive investigation results are regarded as causal and which are felt to be incidental.

Certain cardiac characteristics felt to represent high cardioembolic risk (e.g. left ventricular thrombus, atrial myxoma) will alter management strategy in the short term. Other lesions may be of importance in themselves, while probably not implicated in the pathogenesis of stroke (for example mitral regurgitation and aortic stenosis<sup>57</sup>). There remains, however, a group of lesions that may be diagnosed by cardiac echocardiography that have an as yet *undefined* pathogenic role. Such lesions have been found in case control literature to be

associated with stroke (that is they are more common in a stroke population than a control population), but are not regarded as definite causes of stroke. Causation is difficult to prove, and in practical terms is overshadowed by a more pertinent clinical question: does therapy aimed at treatment of the lesion, or of its vascular effects, improve the patient's prognosis?

### ***Minor cardiac embolic sources***

Examples of such abnormalities are mitral valve prolapse (**MVP**), mitral annular calcification (**MAC**), aortic valve calcification (**AVC**), spontaneous echo contrast (**SEC**), aortic arch atherosclerosis (**AAA**), atrial septal aneurysm (**ASA**) and patent foramen ovale (**PFO**). The extent to which different physicians will investigate individual stroke patients for each of these abnormalities, and the prescribed therapy on detection, is extremely variable. Each lesion may merely be a concomitant abnormality, and so any data suggesting causation rather than merely association are of vital importance.

The reported association of stroke with **MVP** has been drawn into question by recent revision of diagnostic criteria: the prevalence of MVP is substantially higher, and considerably more operator dependent, when single dimensional (M-mode) techniques are used. Specificity (for thickened, myxomatous or regurgitant valves) is improved without decreasing sensitivity by the adoption of 3-dimensional criteria<sup>58</sup>. A recent case control study, using the new criteria, failed to find an association between MVP and stroke<sup>59</sup>. Since the rate of diagnosis with these new criteria is much lower (~2%<sup>59</sup>) than previously reported, exclusion of an association would necessitate a study involving extremely large numbers of young stroke patients. It seems clear, however, that true MVP is a rare and usually benign cardiac abnormality<sup>60</sup>.

Autopsy studies have established a higher prevalence of **AAA** in stroke patients as compared to patients with non-vascular neurological disease. Aortic arch atheroma was associated with an odds ratio for ischaemic stroke

of 4.0 (95%CI= 2.1 to 7.8) in a pathological study by *Amarenco et al*<sup>61</sup>. The association became stronger when just individuals with cryptogenic stroke were examined (OR 5.7; 95%CI= 2.4 to 13.6). As might be expected, the incidence of AAA increased with age. The same group proved the same association in life, using TOE in 250 stroke patients<sup>62</sup>. While plaque thickness >1mm and <3.9 mm could not be established as an independent risk factor, due to the frequent co-existence of carotid stenosis, plaque thickness >4mm was associated with an odds ratio for ischaemic stroke of 13.8 (95% CI= 5.2 to 36.1). The association remained significant after accounting for other risk factors. Further case-control work has confirmed these findings, and suggested that mobile plaques are particularly strongly associated with ischaemic stroke<sup>63 64</sup>. Furthermore, recurrent vascular events are more common in patients with larger degrees of aortic arch wall thickness – the French Study of Aortic Plaques in Stroke group study found the annual risk of stroke, myocardial infarction, systemic embolisation or death was 25% in individuals with aortic atheroma >4mm, compared to 5.9% in individuals with aortic atheroma <1mm<sup>65</sup>. Plaque morphology (lack of calcification and presence of ulceration) has also been found to be predictive of recurrence<sup>66</sup>.

Whether such clear evidence of association merely identifies co-existent vascular disease in high-risk individuals likely to develop recurrent stroke, or whether AAA is an *embolic source* is unclear. Cerebrovascular disease is likely to worsen in line with aortic arch disease (both resulting from the same vascular risk factors). Thus suggesting that the association between AAA and stroke is a causal one on the basis of a dose response relationship is problematic. Furthermore, putative treatments (antiplatelet therapy, anticoagulants or surgical therapy (atherectomy)) have never been subjected to randomised trials. If cardio-embolism is postulated and preventative therapy planned, a crucial distinction in therapy must be made between prophylaxis against thromboembolism and prophylaxis against cholesterol embolism. While the former mechanism favours the use of warfarin as a



prophylactic therapy, a strategy supported by data from the non-randomised series published by *Dressler et al*<sup>67</sup>, anticoagulants can increase the likelihood of cholesterol emboli<sup>68 69</sup>.

**MAC** is more likely to be a marker of vascular calcification and embolic risk than a true embolic source itself. Again case control literature<sup>70</sup> supports the contention that MAC is more common in stroke populations than amongst control subjects. A placebo treated cohort of patients with MAC from the second Stroke Prevention in Atrial Fibrillation (SPAF II) study<sup>71</sup> failed to confirm the suggestion that MAC conferred higher embolic risk in patients with AF<sup>72</sup>. Prospective data from the Framingham cohort have suggested that MAC is associated with a relative risk of 2 for stroke after adjustment for conventional risk factors, excluding carotid disease<sup>73</sup>. It seems unlikely that calcific embolus commonly causes stroke, although the presence of MAC in conjunction with other embolic factors may provide a clinical indication that prophylactic therapy should be more aggressive.

Although case reports and autopsy series<sup>74</sup> have suggested spontaneous embolization of calcific material from **AVC** as a cause of stroke, the small size of the calcific emboli makes asymptomatic infarction a more likely manifestation. A prospective case control study comparing patients with AVC (either with or without aortic stenosis) to controls found no difference in stroke rate<sup>57</sup>. Surgical treatment should be restricted to those with valvular stenosis of sufficient degree. Whether medical therapy for secondary stroke prevention should be altered is unclear.

**SEC** is also postulated as a marker of increased embolic risk. It is an echocardiographic finding, taking the appearance of 'smoke' in the left atrium, and is felt to represent stasis of blood and microaggregates of blood constituents. It is much more common in patients with atrial fibrillation and mitral valve disease than the general population (19% vs up to 74%)<sup>75</sup>. In a

prospective follow up study of 272 patients with non-valvular atrial fibrillation undergoing TEE, the stroke / embolic event rate was 12% per year in patients with SEC compared to 3% per year in those without SEC, despite higher levels of anticoagulation in the SEC group <sup>76</sup>. In the third Stroke Prevention in Atrial Fibrillation (SPAFIII) trial, a cross-sectional analysis found SEC to be independently associated with an increased risk of thromboembolic events (RR 3.7;  $p < 0.001$ ) <sup>77</sup>. Although other published reports challenge such findings <sup>78</sup>, the weight of evidence appears to be in favour of SEC being a marker of higher embolic risk in patients with AF. Such a relationship also makes pathophysiological sense, and implies that more aggressive management policies should be employed in those patients with AF who have SEC on TEE. Similarly, mitral valvular strands are associated with stroke risk in some studies <sup>79 80</sup>.

It is clear from literature review that such 'new' or 'minor' cardiac sources of embolic stroke may be of great importance in the individual clinical situation, while their importance becomes both more questionable and difficult to quantify in populations. There is literature supporting and refuting association for each. Much of the discrepancy is a function of different types and ages of patients being enrolled in studies of different design with varying levels of stroke definition. Diagnostic criteria and rates of follow up vary, and publication bias is likely to play a role.

### ***Patent Foramen Ovale and Atrial Septal Aneurysm***

The foramen ovale – a natural inter-atrial channel allowing a functional right-to-left shunt of oxygenated blood from the maternal placenta to the fetus – normally closes after birth as pressure in the left atrium exceeds that in the right atrium. Permanent closure of the septum is achieved by fibrous adhesions between the septum primum and secundum, normally within the first 3 months of life. The foramen ovale remains patent in approximately one third of the population; largely a small communication (2 to 5mm) is present,

but in 6% of autopsies a larger PFO is detected (6 to 10mm)<sup>81</sup>. Closure of patent foramen ovale (PFO) continues to occur throughout life, since its incidence declines with increasing age, while average size of lesions tends to increase<sup>81</sup>, presumably reflecting increased right atrial pressures in the older population (see **figure 1.1**<sup>81</sup>).

Normally, mean right atrial pressure is lower than left atrial pressure. However, a number of pathological states (for example tricuspid regurgitation), and physiological states (for example during coughing or Valsalva manoeuvre) can lead to a higher right atrial pressure, allowing shunting through a patent channel. Also, even though mean pressure in the right atrium is lower than that in the left atrium, the pressure difference may be reversed transiently at end-systole, potentially leading to right-to-left shunting in the presence of otherwise normal intra-cardiac pressures<sup>82</sup>. Thus, in the presence of thrombosis within the venous system, a persistent foramen ovale may allow shunting of embolic material to the arterial system and brain. Alternatively, turbulence at inter-atrial level may predispose to the formation of thrombotic material, or the defect may predispose to atrial arrhythmias, with subsequent embolic potential<sup>83</sup>.

An atrial septal aneurysm (ASA) is an outpouching of the inter-atrial septum into either the left or the right atrium (see **figure 1.2a**). Echocardiographic criteria for its diagnosis are based on the size and excursion of the aneurysm (see **figure 1.2b**). ASA is proposed to cause stroke by acting as a thrombotic surface and embolic source. It is much less prevalent than PFO (1-5% of individuals), but PFO is often found in conjunction with it. Stroke mechanism in this instance has been hypothesised to be due to thrombus formation within the right side of the ASA, with shunting to the arterial side through the PFO.

The literature on the association of **PFO** and **ASA** ('inter-atrial septal abnormalities') with stroke is similar to that on the other 'minor' embolic sources detailed above. It is characterised by numerous small studies, which published reviews have approached in a manner that is neither systematic nor complete. In a climate in which randomised trials of secondary prevention in patients that have stroke and an inter-atrial septal abnormality are planned, *definition of the presence and quantification of the level* of any association assumes great importance. **Chapter 3** describes a systematic review and meta-analysis of published case-control literature on inter-atrial septal abnormalities and stroke. Firm conclusions are drawn, with recommendations for future work. **Appendix 1** describes a planned study which will aim to determine the presence of association in the elderly stroke population.

## **1.5 Current status of investigation and management in PFO associated cryptogenic stroke**

Interpretation of observational data, and the translation of the information it provides into decisions that truly influence patient management, is fraught with difficulty. Although certain characteristics of 'association' suggest that the relationship detected is more likely to be a causal one, for example the presence of a 'dose-response' relationship, it does not necessarily follow that treatment of a putative 'cause' will influence the incidence of its proposed effect. A randomised controlled trial is the only proper way to test such a hypothesis and the information provided by such studies forms the basis of the practice of 'evidence based medicine'. Designing randomised studies, however, requires great skill and attention, especially in the context of rare or uncommon diseases or low rates of outcome events. Moreover, observational data tend to become available to practising clinicians a number of years before proper randomised trials are designed or completed. Clinicians are left to practise in an environment where there is no 'right answer'.

'PFO-associated stroke' illustrates this situation well. Association has been demonstrated in numerous studies between PFO and stroke, and because of the 'biologically plausible' explanation that venous thrombosis and paradoxical embolism result in stroke, attention has shifted to treatment. This is despite numerous studies that have failed to demonstrate a relationship, especially in the older patients that make up the majority of stroke patients, and the consistent finding both in clinical practice and published series that venous thrombosis is extremely difficult to detect<sup>84</sup>. It would be pragmatic to argue that such patients should not currently be treated with potentially deleterious anti-thrombotic agents, except in the context of randomised controlled trials. However, physicians faced with such patients, in whom the consequences of stroke recurrence could be devastating, understandably find it difficult to await the results of randomised trials.

How physicians react in such a situation is dependent on both training and local service availability. It will also depend on the nature of the individual physician, and the individual patient. There will always be those who translate the information provided by observational literature more readily, just as there will always be physicians who treat patients 'aggressively', in the belief that such an approach will reap long-term benefits. Since no therapy is proven, more conservative doctors will favour the secondary preventative measure that is likely to carry the least chance of side effects. Others will approach the problem more mechanistically: if the root of ischaemic stroke in this situation is embolism, would the best preventative measure not be systemic anticoagulation?

The two 'medical' approaches available were recently examined in a substudy of the Warfarin and Aspirin in Recurrent Stroke Study (WARSS)<sup>35</sup>. In WARSS 2206 stroke patients were prospectively randomised to prophylactic therapy with either warfarin (to maintain the International Normalized Ratio, or INR, in the range of 1.4 to 2.8) or aspirin (325 mg daily),

and followed for recurrent events during a 2-year period. For the whole study group no difference was detected between aspirin and warfarin in the prevention of recurrent ischaemic stroke or death or in the rate of major haemorrhage.

The PICSS study (Patent foramen ovale In Cryptogenic Stroke Study), a substudy of the larger WARSS cohort, has not yet been published. Preliminary data have been presented at a recent meeting (27th International Stroke Conference, San Antonio, Texas, February 7-9 2002). PICSS aimed to determine the two-year rate of stroke recurrence or systemic embolisation in medically treated (warfarin or aspirin) cryptogenic stroke patients with a patent foramen ovale (PFO), and to compare it to the two-year rate of stroke recurrence or systemic embolisation in medically treated cryptogenic stroke patients without a PFO. It was powered on the basis that the presence of PFO will double the two-year rate of stroke recurrence or systemic embolization in cryptogenic stroke patients. The study was *not* powered to detect a difference between aspirin and warfarin. The total number of patients randomised was 630 with a mean age at randomisation of 59.7 years. There were a number of secondary aims:

- To determine the two-year rate of stroke recurrence or systemic embolization in warfarin treated cryptogenic stroke patients with a PFO, and to compare it to the two-year rate of stroke recurrence or systemic embolization in warfarin treated cryptogenic stroke patients without a PFO.
- To determine the two-year rate of stroke recurrence or systemic embolization in aspirin treated cryptogenic stroke patients with a PFO, and to compare it to the two-year rate of stroke recurrence or systemic embolization in aspirin treated cryptogenic stroke patients without a PFO.
- To obtain pilot data on the natural history of medically treated cryptogenic stroke patients with varying sizes of PFO.

- To obtain pilot data on the natural history of medically treated cryptogenic stroke patients with transoesophageal echocardiographically detected potential cardiac embolic sources defined as aortic arch mass, left atrial spontaneous contrast, or atrial septal aneurysm.

The probability of an event at 2 years in the PFO positive group was 14.8% compared to 15.3% in the PFO negative group. No difference in recurrence between PFO positive and PFO negative patients was detected in the warfarin or aspirin treated cohort. Full data from PICSS will help to delineate those features that are associated with the highest risk of recurrence, but to answer central management questions, trials concentrating on a younger and more stringently selected group will be required.

It is becoming increasingly common, especially in mainland Europe and America, to correct PFO lesions surgically. Numerous surgical series have been reported, some with flawless results<sup>85</sup>. To avoid the need for an open procedure, and a period spent on cardiopulmonary bypass, many cardiologists are using percutaneous closure devices to correct inter-atrial septal defects. Recently, large series of patients treated with percutaneous closure have reported a crucial finding: that persistent patency of the PFO after attempted closure is associated with stroke recurrence<sup>86 87</sup>. Any clinical trial to compare medical versus surgical therapy (percutaneous or open closure) for PFO associated stroke will require a large number of patients to discern what is likely to be a small difference in overall outcome, since reported recurrence rates are low in both retrospective series<sup>88 89</sup> and more recently in a large prospective cohort<sup>90</sup> (*see chapter 4 for detailed discussion of these data*).

It is in the climate of uncertainty described that the survey reported in **chapter 4** was conducted. A questionnaire was distributed to assess whether United Kingdom experts felt that patients should be investigated for PFO, and how

they would act if such a lesion were found. Randomised studies are underway in both America (percutaneous closure vs aspirin vs warfarin) and Germany (percutaneous closure vs warfarin), and a proper assessment of current management practice is a useful precursor to the design of a UK based study.

## **1.6 Stroke, statins and cholesterol**

For stroke, in contrast to coronary heart disease (CHD), the relation between an increased risk of stroke and increased plasma total cholesterol or low density lipoprotein (LDL) cholesterol is in dispute. The largest observational analysis, a review of 45 prospective observational cohorts involving 450,000 individuals and a mean follow up of 16 years, found no association between blood cholesterol and total stroke<sup>91</sup>. However, many trials reported merely fatal strokes (allowing a possible association with non-fatal stroke to be missed), and any association with ischaemic stroke may have been diluted by an inverse association of serum cholesterol and haemorrhagic stroke, which has been reported in other series<sup>92 93</sup>. Both these series also suggested positive correlations between serum cholesterol and non-haemorrhagic stroke risk.

There are several reasons why the epidemiological relationship between cholesterol and stroke risk may be weak or absent. First, stroke is a heterogeneous condition, with less than half of all strokes being caused by large vessel atheroma. This contrasts with the situation in coronary artery disease in which nearly all presentations are due to coronary artery atheroma. One would not expect cholesterol levels to influence the incidence of cardioembolic stroke, or small vessel arteriosclerosis. Second, since coronary deaths generally occur in a younger age group, the population with cervical or cerebral large vessel atheroma secondary to raised plasma lipids



is lower by the time that most strokes occur. Total and LDL cholesterol decrease with advancing age, as does their relation to CHD.

In acute stroke, poor outcome has been found to correlate with lower total cholesterol concentrations by two major stroke registries<sup>94 95</sup>. Both these groups reported the association in both ischaemic and haemorrhagic subtypes. These findings draw into question the use of cholesterol lowering agents in the acute phase of stroke: while the Swiss group reported poor functional outcome at one month, higher serum cholesterol levels were associated with reduced long term mortality in the Glasgow study<sup>94</sup>.

Stroke has been studied as a secondary endpoint in cholesterol lowering trials which have examined HMG-CoA reductase inhibitors (statins) both as a primary prevention measure and after MI. These data do suggest a beneficial effect on incidence of cerebrovascular events. Four published meta-analyses, three of which examined specifically the effect of statins<sup>96 97 98</sup> and one of which examined all lipid lowering drugs<sup>99</sup>, have reached broadly similar conclusions. They indicate that statins reduce the risk of stroke by approximately 30% in patients with CHD. These analyses are dominated by data from three large studies: the West Of Scotland COronary Prevention Study (WOSCOPS) (primary prevention in high risk hypercholesterolaemic individuals), the Scandinavian Simvastatin Survival Study (4S) (secondary prevention in hypercholesterolaemic individuals) and the Cholesterol and Recurrent Events (CARE) study (secondary prevention in patients with 'normal' (<6.2 mmol/l) cholesterol). The more recently published LIPID trial (again a secondary prevention trial in patients with CHD) showed consistent results.

Some of the uncertainty in the cerebrovascular population *without CHD* will be addressed by the publication of the recently completed Heart Protection Study (HPS)<sup>100</sup>. This enrolled 20,000 high risk patients, and a preliminary

subgroup analysis has been posted on the internet ([www.ctsu.ox.ac.uk](http://www.ctsu.ox.ac.uk)). Although full publication and adjudication of all events is awaited, significant benefit (~24% RRR) in the reduction of all vascular events was seen in patients with a history of ischaemic stroke (and no history of CHD). No heterogeneity of benefit was seen between groups with different types of vascular event or risk at baseline.

Two major multicentre trials are awaited. The Risk Evaluation and Stroke Prevention in the Elderly- Cerivastatin Trial (RESPECT) trial is a primary prevention study with cerivastatin, ongoing in Northern Europe, which has stroke incidence as its primary outcome measure <sup>101</sup>. The Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) study examines the role of the statin which has the most potent effect on serum cholesterol (atorvastatin) in the secondary prevention of stroke and other major vascular events after an initial ischaemic or haemorrhagic cerebral event. Patients with CHD will be excluded from these studies.

The mechanism by which statin therapy produces effects on CHD mortality may be particularly pertinent to stroke prevention. It is clear that the profound reduction in cholesterol levels attributable to statin therapy produces regression in both coronary and carotid atheromatous lesions <sup>102 103</sup>. This improvement occurs over 4 years, and was not evident at 2 years in the Multicentre Anti-Atheroma Study (MAAS) <sup>102</sup>. However, the reduction in vascular events observed in both 4S and WOSCOPS occurred within 2 years: much sooner than would be expected as a result of reduced atheroma progression or regression. Moreover, no relationship between risk reduction of major coronary events by statin and baseline LDL concentration was found <sup>104 105</sup>. In WOSCOPS the effect of therapy was highest in those patients in the middle quintile of LDL at baseline: it appeared that treatment effect was independent of LDL concentration <sup>105</sup>. Similarly, preliminary data from HPS

show similar benefit in vascular event reduction in all three tertiles of baseline LDL and total cholesterol.

### ***Statins do more than just lower cholesterol***

For the reasons above alternative mechanisms have been proposed to explain the action of statins. Numerous authors have investigated the possible effects of statins on vascular endothelial reactivity, haemorrhological markers and vasomotor tone in an attempt to explain their effects<sup>106 107</sup>. Statins appear to stabilise plaque by a number of mechanisms, and have antiproliferative effects due to reduced levels of oxidized LDL and intermediates in cholesterol metabolism, which have been implicated in the control of cell proliferation. Improvements in endothelial function and vasomotor tone have been demonstrated in both coronary and forearm vasculature. Cardiovascular reactivity (as measured by blood pressure response to incremental infusions of angiotensin II and noradrenaline) is improved by pravastatin<sup>108</sup>. Conclusive evidence from a recent controlled trial demonstrated that pravastatin lowered systolic, diastolic and pulse pressures in hypercholesterolaemic patients with moderate hypertension<sup>109</sup>.

Improved myocardial perfusion (measured by thallium-201 SPECT scanning) has been demonstrated after only 12 weeks of fluvastatin therapy<sup>110</sup>. Such effects of statins have not been studied in cerebral vasculature in humans. However, acute LDL and fibrinogen reduction has been shown to have beneficial effects on cerebral blood flow<sup>111</sup>. An alternative mechanism by which statins may improve cerebral blood flow has been elegantly demonstrated in a murine model: improved perfusion, reduced infarct size and improved neurological function occurred by selective up-regulation of endothelial nitric oxide synthase (*eNOS*)<sup>112</sup>. The blood flow and neuroprotective effects of statins were absent in *eNOS* knockout (deficient) mice. Both up-regulation of *eNOS* and down regulation of inducible nitric oxide synthase (*iNOS*) are potentially neuroprotective<sup>113</sup>.

### ***Effects on intravascular thrombosis***

The effects of statins on endothelial function, smooth muscle cell proliferation and plaque composition may only partially explain their effects on myocardial and cerebral perfusion and early effects on vascular event rate. Numerous studies have documented the effects of statins on blood constituents <sup>114</sup>, including **fibrinogen, plasma viscosity, endogenous tissue plasminogen activator (tPA), plasminogen activator inhibitor 1 (PAI-1), von-Willebrand factor, d-dimers and factor VII**. Each of these 'haemorrhheological' markers has been proposed as a marker of vascular risk, and as a potential target for pharmacologic therapy in cerebrovascular disease. The evidence linking each of these markers of blood 'thickness' with stroke is discussed below.

The Edinburgh Artery Study reported the relationship between **blood viscosity** and incident vascular events, and examined the complex inter-relationships between plasma and whole blood viscosity, haematocrit and fibrinogen <sup>115</sup>. The results suggested that blood viscosity was as strong a predictor of cardiovascular events in older individuals as LDL cholesterol or diastolic blood pressure. Furthermore, the component of viscosity due to plasma (which is largely determined by fibrinogen level) seemed to be at least as important as that due to haematocrit. In addition to thrombogenic effects, fibrinogen and viscosity may have a direct effect on cerebral blood flow – an inverse relation has been shown to exist in the elderly between fibrinogen and viscosity levels and middle cerebral artery velocity measured by transcranial Doppler <sup>116</sup>.

There is a large amount of epidemiological evidence suggesting that plasma **fibrinogen** levels are significantly associated with cerebrovascular events, and that the association may explain to some extent the association between smoking and stroke. Data from the Gothenberg and Framingham studies have shown that plasma fibrinogen is an independent prognostic risk factor

for stroke <sup>117 118</sup>. Similarly, the Oxfordshire Community Stroke Project (OCSP) demonstrated that an elevated plasma fibrinogen level (in addition to generalised hypercholesterolaemia and elevated low-density lipoproteins) was a risk factor for prevalent cerebrovascular events <sup>119</sup>.

**Fibrinogen** is an acute phase protein which commonly rises during the acute phase of stroke and is a potent predictor of vascular risk in stroke survivors <sup>120</sup>. Randomised clinical trials with ancrod, a serine protease derived from the venom of the Malayan pit viper and which acts as a defibrinogenating agent, have been recently reported. The Stroke Treatment with Anicrod Trial (STAT) <sup>121</sup> showed efficacy similar to that of tPA, with a 23% increase in patients with a favourable functional status (Barthel score  $\geq 95$ ) in comparison to placebo. The study was, however, dogged with the methodological problems of administering anicrod as a 74 hour infusion requiring plasma fibrinogen monitoring, and sullied by the almost simultaneous termination of the concurrently running European Trial <sup>122</sup>. Patients receiving anicrod in Europe had a higher 90 day mortality than placebo treated patients: it is not yet apparent why.

Endogenous **tPA** is the primary mediator of intravascular fibrinolysis: in a paper based on data from the Physicians Health Study the age-adjusted relative risk for total stroke among men with baseline tPA concentrations above the 95<sup>th</sup> percentile of the control distribution was 3.51 (95% CI=1.72-7.17) <sup>123</sup>. This finding was confirmed by the Edinburgh Artery Study <sup>124</sup>. **PAI-1**, which is also produced by endothelial cells, inhibits the activity of tPA and thereby the fibrinolytic process (see **figure 1.3** <sup>125</sup>). Levels have been shown to be independently associated with prevalent IHD and increased incident IHD in persons with established arterial disease <sup>126</sup>. In a study of 135 acute stroke patients and 77 controls, increased levels of both tPA (reflecting fibrinolytic activation) and PAI-1 (reflecting increased fibrinolytic inhibition) were seen in the acute phase of stroke <sup>127</sup>. In the acute stroke group, the

concentrations of tPA and PAI-1 antigen appeared to differ little between the acute and convalescent phases (they remained raised), arguing against those authors who have suggested their presence to reflect an acute phase reaction. However, only 23% of stroke subjects were re-examined in the convalescent phase. More recently, assays that measure tPA complexed with PAI-1, have demonstrated that tPA/PAI-1 complex is independently associated with the development of first ever stroke <sup>128</sup>. The interplay between these factors in the activation and inhibition of the fibrinolytic pathway is shown in **figure 1.4** <sup>125</sup>.

**D-dimer** levels reflect fibrin turnover and thrombogenesis, and may be a useful marker of thrombogenesis. Their use in the clinical diagnosis of venous thromboembolism has received increasing interest in recent years, but they have also been associated with prevalent and incident IHD in longitudinal studies <sup>129</sup>. In the Physicians Health Study, d-dimer levels were significantly predictive of incident arterial, but not venous, vascular events in a population free of vascular disease at outset <sup>130</sup>.

**Von Willebrand factor (vWF)** is synthesised by and stored in endothelial cells. When released, vWF seems to mediate platelet aggregation and adhesion to the vascular endothelium, and has been proposed as an indicator of endothelial disturbance or dysfunction <sup>131</sup>. Levels have been shown to correlate with the presence of left ventricular dysfunction, atrial fibrillation and peripheral vascular disease <sup>132</sup>. High concentrations of vWF are associated with ischaemic cerebrovascular disease, although the association has only been detected in small studies. It may reflect either endothelial dysfunction associated with cerebral thrombosis or its risk factors, or ischaemia related release of vWF from infarcted tissue.

The relationship between **factor VII** and vascular disease appears less strong than those described for fibrinogen, tPA and PAI-1. The Northwick Park Heart

Study reported a strong relationship between factor VII activity and recurrent vascular events <sup>133</sup>. This has not, however, been confirmed by subsequent data: the PRIME study confirmed relationships reported for other haemostatic factors but showed evidence of an acute phase or consumptive fall in factor VIIc levels after myocardial infarction, with levels approaching those of healthy matched controls at 3 months <sup>134</sup>.

Studies that have examined statin effects on such vascular markers are small. Different effects have been reported for each of the different naturally occurring (lovastatin and pravastatin), semi-synthetic (simvastatin) and synthetic compounds (fluvastatin, cerivastatin and atorvastatin). Some of the reported effects have been deleterious <sup>135</sup>. The most promising in terms of atherothrombotic effects appears to be pravastatin <sup>114</sup>. In a randomised trial pravastatin had significant (and clinically important) effects on plasma and whole blood viscosity and fibrinogen over a 10 week course, while simvastatin had no such effects <sup>136</sup>. The effect of statins on haemostatic markers in the acute phase of ischaemic stroke has not been reported.

An acute phase response during and immediately after acute vascular events is well documented. Patients with high levels of markers of acute phase response (for example CRP) have a poorer prognosis after both acute stroke and acute MI than patients without such findings <sup>137 138</sup>. As well as marking poor prognosis after acute events, it seems that a chronic low grade inflammatory response is associated with incident vascular events over long periods of follow-up. A recent study by *Danesh et al* confirmed previous findings that a twofold increase in the risk of future cardiovascular events is associated with even mildly raised concentrations of CRP <sup>139</sup>. The accompanying meta-analysis revealed an odds ratio for CHD of 1.9 (95% C I 1.5 to 2.3) when comparing individuals in the highest and lowest CRP tertiles. A weaker association was discovered for serum amyloid A protein.

It has been proposed that in addition to effects on rheological markers, many of which demonstrate an 'acute phase response' as alluded to above, statin therapy may have a more generalised 'anti-inflammatory' action, which may benefit patients with vascular disease. Such theories are supported by data from the CARE study, which examined those patients with evidence of 'acute inflammation' (high CRP or serum amyloid A levels) who were randomised to pravastatin or placebo. In stratified analyses the association between inflammation and risk was significant among those randomised to placebo (RR=2.11, p=0.048), but was attenuated and non-significant among those randomised to pravastatin (RR=1.29, p=0.5), suggesting an anti-inflammatory effect of statin therapy which modulates cardiovascular risk. Again, statin effects on such markers after stroke have not been reported in a randomised trial.

**Chapter 5** reports a clinical trial designed to investigate the acute effect of statins in stroke. As endothelial, anti-proliferative, anti-oxidant, anti-inflammatory and rheological effects of statins are becoming increasingly recognised in the field of cardiology, their use in the acute phase of coronary syndromes is becoming more widespread. In stroke, statins may have a role in maintaining cerebral perfusion, neuroprotection, reduction of final infarct size and in the acute prophylaxis of further vascular events. If beneficial effects on important surrogate markers can be demonstrated in the acute phase, larger studies with hard clinical endpoints may be designed to examine the effects of acute statin therapy in the wider stroke population.

## **1.7 Alternative vascular risk factors: can infections cause stroke?**

While identified risk factors (for example smoking and blood pressure) play a large part in the genesis of ischaemic stroke, numerous other factors have become evident over recent years. The association between elevated levels



of homocysteine and both coronary and cerebral vascular disease is becoming increasingly clear <sup>140</sup>, and two large trials (Vitamin Intervention in Stroke Project (VISP) and VITamins TO Prevent Stroke (VITATOPS)) are currently following-up randomised cohorts with the aim of establishing whether homocysteine lowering with B vitamins can influence vascular risk. Another active research area concerns the possibility that chronic infections may cause stroke.

Both pyrexia <sup>13</sup> and high levels of systemic inflammatory markers <sup>137</sup> confer a poor outcome in stroke, but may reflect the effects of the stroke insult itself rather than marking an inflammatory *cause*. Associations have been reported between stroke and recent bacterial infection <sup>141</sup>, viral infection <sup>141</sup>, and with clinical correlates of chronic dental infection (for example severe periodontal disease and missing teeth) <sup>142</sup>. Other studies have reported histological evidence of bacteria or viruses in atheromatous or non-atheromatous blood vessels (both cerebral and coronary), in addition to sero-epidemiological studies reporting association with antibody measurements. These studies, pertaining largely to the CHD population, are well summarised elsewhere <sup>143</sup>. Many of these studies can be criticised with regard to their selection of control patients, and the choice of antibody titre and type chosen empirically to indicate 'seropositivity' or 'active infection'.

Numerous mechanisms by which infections may increase stroke risk have been proposed. These are outlined in **figure 1.5** <sup>143</sup>. The infectious agent may have effects on the arterial wall, causing endothelial injury or dysfunction, smooth muscle proliferation or local inflammation. The most convincing experimental evidence supports the effects of chronic or acute infection in the circulation, through systemic inflammation, cross reactive antibodies, or effects on cardiovascular risk factors, such as LDL, homocysteine or fibrinogen <sup>143</sup>.

### ***Chlamydia Pneumoniae and its association with stroke***

*Chlamydia pneumoniae* frequently causes community acquired respiratory infections such as sinusitis, pharyngitis, and pneumonia. There are epidemics due to *C. pneumoniae* infection every 5 to 7 years in Northwestern countries, with a 50% to 70% prevalence of seropositivity in middle aged adults. Most adults are infected 2 to 3 times during their lifetime. It is an obligate intracellular gram-negative bacterium, and after ingestion by circulating macrophages may trigger or perpetuate inflammatory changes that contribute to the development of atherosclerosis, either directly or indirectly (see **figure 1.6**<sup>144</sup>). Both chronic and acute chlamydial infection appear to be associated with a pro-coagulant state.

The specific association of chlamydial infection and stroke has been reported by numerous workers. *Wimmer et al* demonstrated (with microimmunofluorescence antibody techniques) that chlamydia IgA and specific IgG levels in circulating immune complexes were significantly higher in stroke patients than controls<sup>145</sup>. An even stronger effect was demonstrated by *Cook et al*, with acute chlamydial infection being associated with an odds ratio of 4.2 (95% CI 2.5 to 7.1) for stroke/TIA, and chronic chlamydial infection being associated with an odds ratio of 4.4 (95% CI 3.0 to 6.5)<sup>146</sup>. Reported associations with prevalent cerebrovascular disease are strengthened by recent work on incident stroke: *Fagerberg et al* reported that high titres to chlamydia pneumoniae were associated with a relative risk of 8.58 for future stroke in a high risk population, albeit with a wide confidence limit (1.07 to 68.82)<sup>147</sup>. Other workers, however, have failed to confirm these findings<sup>148</sup>.

Hyperfibrinogenaemia has been found to be consistently correlated with chlamydial infection. The association was originally described in 1994<sup>149</sup>, when seropositivity to both *C. Pneumoniae* and *Helicobacter Pylori* was independently associated with raised fibrinogen in a small general practice based case-control study. Subjects had no history of vascular disease. A

larger study by the same authors found both *H.pylori* and *C.pneumoniae* to be associated with CHD, even after extensive adjustment for confounders, and confirmed the association of infection with leucocyte count and with fibrinogen <sup>150</sup>. The same authors found that *C. pneumoniae* was associated with higher concentrations of factor VII antigen <sup>150</sup>. In a study of patients with unstable angina, the association between persistent *C.pneumoniae* infection (assessed by IgA titres) and increased fibrinogen levels was independent of other risk factors evaluated in multivariate analysis <sup>151</sup>.

Treatment trials that have used antibiotics in CHD have already been published, and represent a promising avenue for modern secondary prevention. No treatment trials have been conducted in cerebrovascular patients. In a randomised, placebo controlled study macrolide antibiotics (azithromycin) were given to a series of male survivors of MI with raised serum anti-*C.pneumoniae* titres <sup>152</sup>. The study found a four-fold increase of adverse cardiovascular events among the group with elevated *C.pneumoniae* titres who received no antibiotic therapy compared with the group that had negative serology (odds ratio 4.2; 95% CI 1.2 to 15.5). In contrast, seropositive treated patients treated with azithromycin had a similar outcome to seronegative patients (OR 0.9; 95%CI 0.2 to 4.6). Subjects receiving azithromycin had a significant fall in certain levels of serum and monocyte activation markers, including fibrinogen (see below). Unfortunately the results of this study do not help to distinguish between a chlamydia-specific effect of azithromycin and a more generalised anti-inflammatory effect, because only seropositive patients received therapy.

The ROXIS (roxithromycin in ischaemic syndromes) study found a significant effect of antibiotic therapy on recurrent ischaemic event rate at 30 days after non-Q wave coronary syndromes <sup>153</sup>. However, 6 months after the study treatment termination, there was no longer any significant difference between the treatment and placebo groups <sup>154</sup>. About half the population studied were

seropositive (IgG>1:64) at inclusion, and event rates in this subgroup did not differ significantly between those receiving placebo and those receiving roxithromycin. Other trials in the CHD population are planned, and are well summarised elsewhere <sup>155</sup>. These are hoped to provide worthwhile information on hard endpoints, while also adding to knowledge about *how* chlamydia may lead to vascular events. The proper elucidation of this mechanism is vitally important in the delivery of therapy to those most likely to benefit.

If chlamydial infection leads to hyperfibrinogenaemia, and therefore a hypercoagulable state predisposing to vascular events, then treatment of chlamydial infection may reduce fibrinogen. Demonstrating this would improve arguments that chlamydia is a *cause* of vascular disease, rather than an incidental association, since its treatment reduces a potent factor in determining vascular risk. Recently *Torgano et al* published a study of 84 'chronic' CHD patients who were seropositive for both *C.pneumoniae* and *H.pylori* antibodies, and who had normal acute phase reactants <sup>156</sup>. They assessed the effect of treatment (omeprazole, clarithromycin and tinidazole for *H.pylori* positive patients, and clarithromycin for *C.pneumoniae* positive patients) on fibrinogen levels at 6 months. Treatment significantly reduced fibrinogen at 6 months in the overall study population and in the groups of patients divided according to *H.pylori* or *C.pneumoniae* positivity, the largest decrease being observed in patients with both infections. Unfortunately, the study was not placebo-controlled, and gave no information about antibiotic effect on fibrinogen in patients without *H.pylori* or *C.pneumoniae* infections.

Other markers of vascular risk or inflammation have been studied. As mentioned previously, *Gupta et al* demonstrated significant effects on monocyte/macrophage tissue factor and the surface adhesion molecule CD11b <sup>152</sup>. The ACADEMIC (Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with Chlamydia) study, a placebo

controlled study of azithromycin vs placebo in 302 *C pneumoniae* positive CHD patients, found antibiotic effects on CRP and interleukin-6 (IL-6) levels after 6 months. Fibrinogen was not measured, and no effect on cardiovascular endpoints was detected. What characterises all of these studies is the lack of distinction between a 'generalised' anti-inflammatory effect of macrolide antibiotics, and a chlamydia-specific effect, which should play a part only in those individuals who are seropositive for the infection.

**Chapter 6** describes the pilot phase of a small randomised placebo controlled trial in acute ischaemic stroke patients designed to answer some of the mechanistic questions that have stemmed from the work summarised above. Both patients with and without serological evidence of chlamydial infection will be treated with azithromycin or placebo, and the effect on plasma fibrinogen followed. If an effect on plasma fibrinogen is detected, the study will be able to establish whether all patients benefit in terms of fibrinogen reduction, or just those seropositive for chlamydia. Effects on other markers (CRP, plasma viscosity and vWf) will be explored.

## **1.8 Concluding comments**

If ongoing research into 'novel' vascular markers proves positive, the secondary prevention of stroke will change considerably. Patients of the future may be provided not just with antithrombotic and anti-hypertensive therapy, but with a statin, an antibiotic, B-vitamins and other supplements. The interaction of the different agents in the reduction of future risk, the cost-effectiveness of different therapies and drug compliance in a functionally impaired population are some of the issues that will require clarification if randomised trials prove positive.

Epidemiological data suggest that the causes and risk factors for stroke may not necessarily be synonymous with those for CHD. Interest in novel 'stroke'

risk factors has largely followed preliminary work in the CHD population, which is larger, easier to study and younger. However, vascular 'brain' disease is a more complex and heterogeneous condition than vascular heart disease. The three main mechanisms for stroke, namely small vessel arteriosclerosis and thrombosis, embolism from a proximal source, and large vessel atherothrombosis, contrast with the one mechanism for MI: coronary atheroma. Additionally, stroke can affect *grey* or *white* matter, the neuroprotective targets for each being different. It is this complexity which has militated against positive trial results in stroke. Such considerations suggest that inclusion and classification of stroke in both trials and clinical practice should become more detailed, acknowledging the complexity that exists. Furthermore, experimental and 'pre-clinical' research should concentrate on 'stroke-specific' risk factors and causes, rather than merely transcribing work from the CHD population.

<b>Uncertainties concerning treatment of hypertension in acute stroke: rationale for a clinical trial</b>	
<b>Antihypertensive treatment is hazardous</b>	<b>Antihypertensive treatment is beneficial</b>
Hypertension typical in acute stroke, with spontaneous decline over days	Focal oedema shown with acute hypertension in animal models
Hypertension not linked to worse prognosis	Hypertension may predict worse prognosis
Reduced BP could worsen ischaemic penumbra due to paralysed autoregulation	Cerebral perfusion may not be worsened with newer antihypertensive drugs
Pressor therapy could be effective in some patients	Elevated BP increases the risk of haemorrhage with thrombolysis
Moderate hypertension useful in vasospasm / ischaemia after subarachnoid haemorrhage	Controlling hypertension may reduce the risk of haemorrhagic transformation
Acute hypotension likely cause of adverse effects of IV nimodipine ( <i>Ahmed et al, 2000</i> )	Lowered BP at day 2-3 predicted better outcome in a clinical study ( <i>Chamorro et al, 1998</i> )

**Table 1.1: The rationale for a clinical trial of blood pressure reduction in acute stroke**

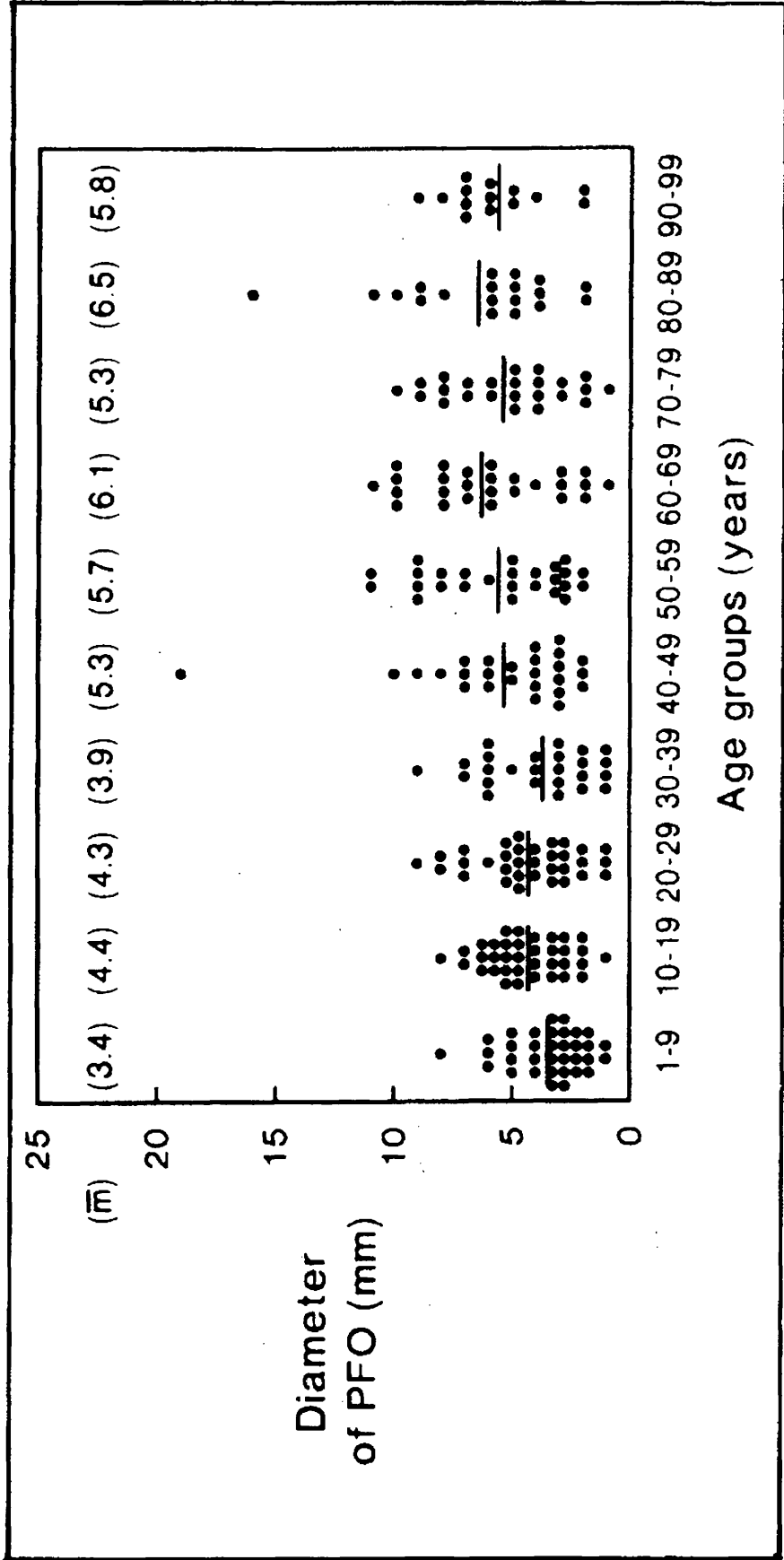
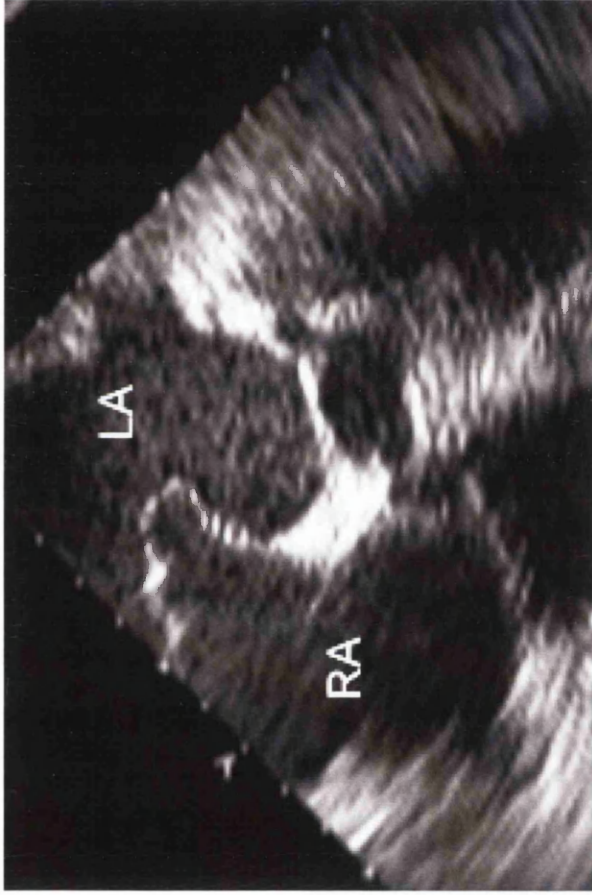
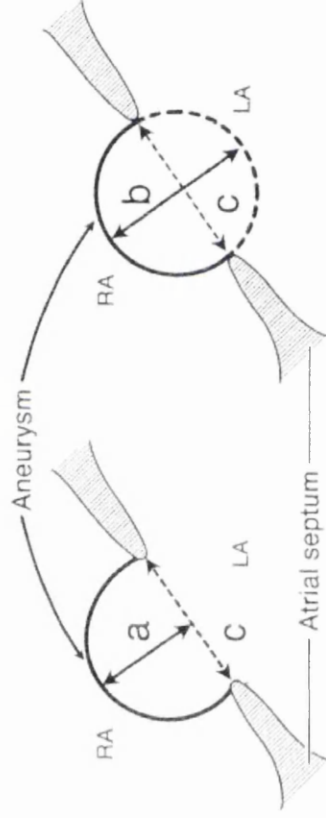


Figure 1.1: Size distribution of Patent Foramen Ovale amongst patients of different ages (from Hagen et al, 1984).



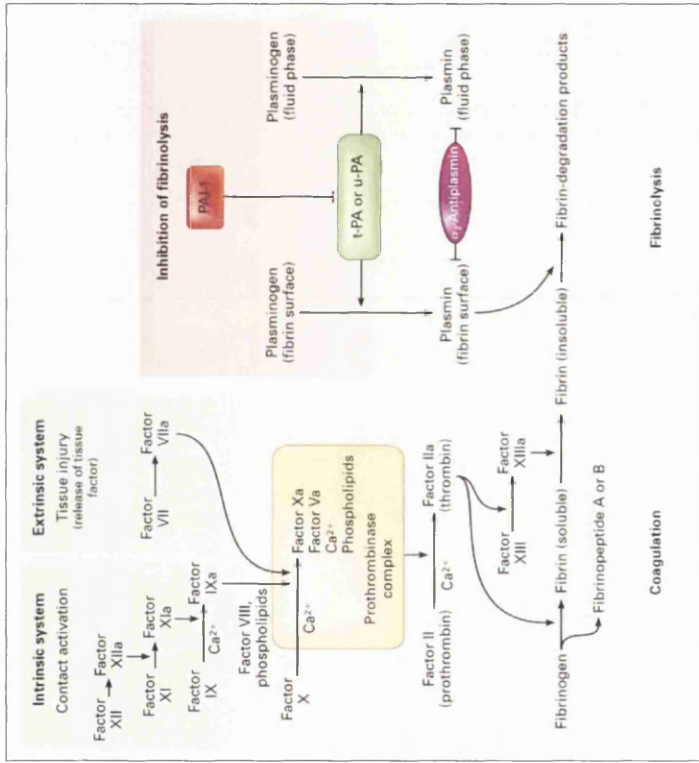


a)



b)

**Figure 1.2 a) Transoesophageal echocardiographic image of atrial septal aneurysm; LA=left atrium, RA=right atrium.**  
**b) Echocardiographic criteria for the diagnosis of atrial septal aneurysm. Distance (a) or (b) (aneurysm excusion) must equal or exceed 15mm. Distance (c) must equal or exceed 10mm.**



**Figure 1.3: The Coagulation and Fibrinolytic Pathways.** The main coagulation reactions are divided into the intrinsic and extrinsic systems. Activation of factor XII on contact with a negatively charged surface initiates the intrinsic coagulation system. (The activated form of the factor is indicated by "a.") The extrinsic coagulation system induces the formation of a complex composed of factor VII and tissue factor, which is released after tissue injury. Thrombin is formed by an enzyme complex called prothrombinase, composed of factor X, factor V, negatively charged phospholipids, and calcium ions. Intrinsic and extrinsic activation of the coagulation cascade leads to the generation of thrombin, the activation of fibrinogen, the release of fibrinopeptides, the formation of soluble fibrin, and finally, the formation of factor XIII-mediated, cross-linked, insoluble fibrin. The main fibrinolytic reactions involve the inhibition of fibrinolysis by plasminogen-activator inhibitor type 1 (PAI-1) and (alpha)<sub>2</sub>-antiplasmin. Fibrinolysis is initiated by tissue plasminogen activator (t-PA), urinary-type plasminogen activator (u-PA), and plasmin. Plasmin bound to the surface of fibrin initiates the lysis of insoluble, cross-linked fibrin, with the subsequent generation of fibrin-degradation products. Plasmin bound to the surface of fibrin is better protected from inhibition by (alpha)<sub>2</sub>-antiplasmin than is plasmin generated in the fluid phase (from Kohler and Grant, 2000).



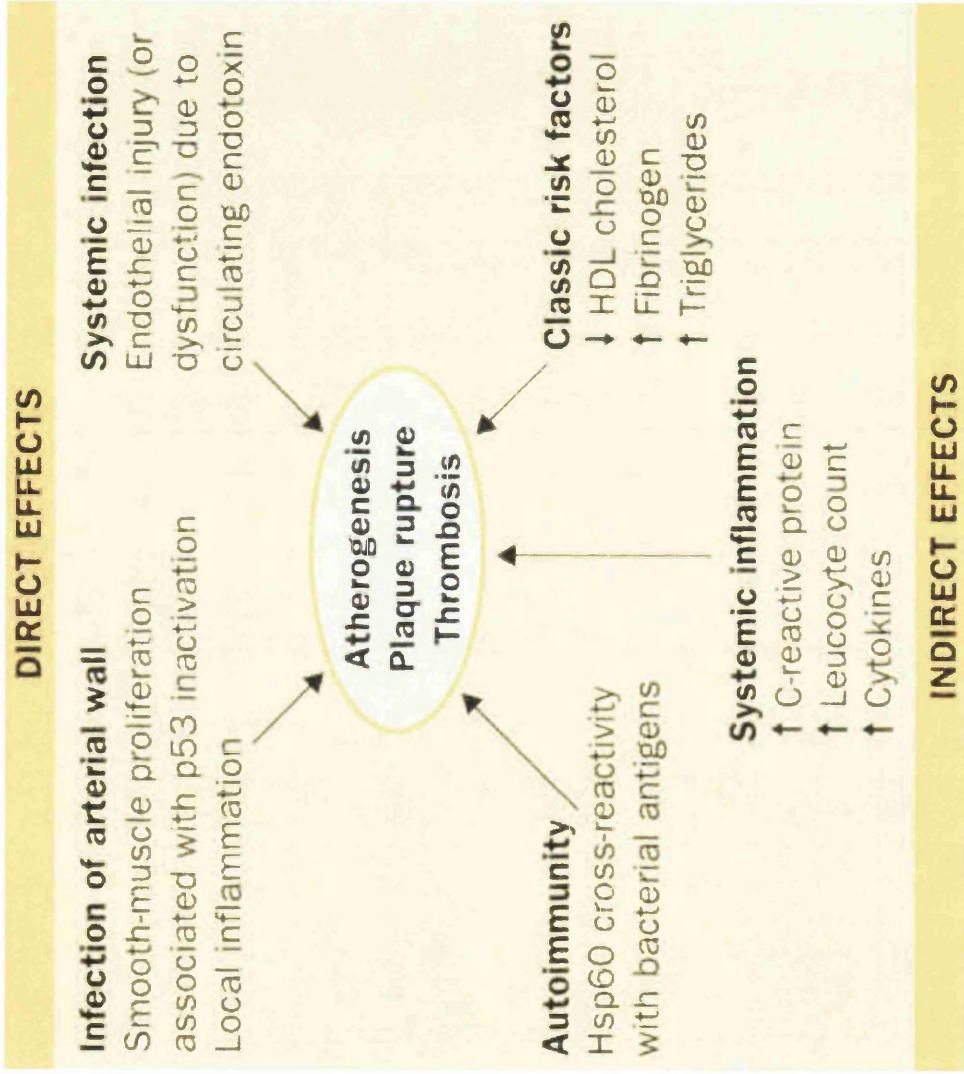


Figure 1.5: Postulated mechanisms to link infections and vascular disease Hsp=heat-shock protein; HDL=high-density lipoprotein (From Danesh et al, 1997)



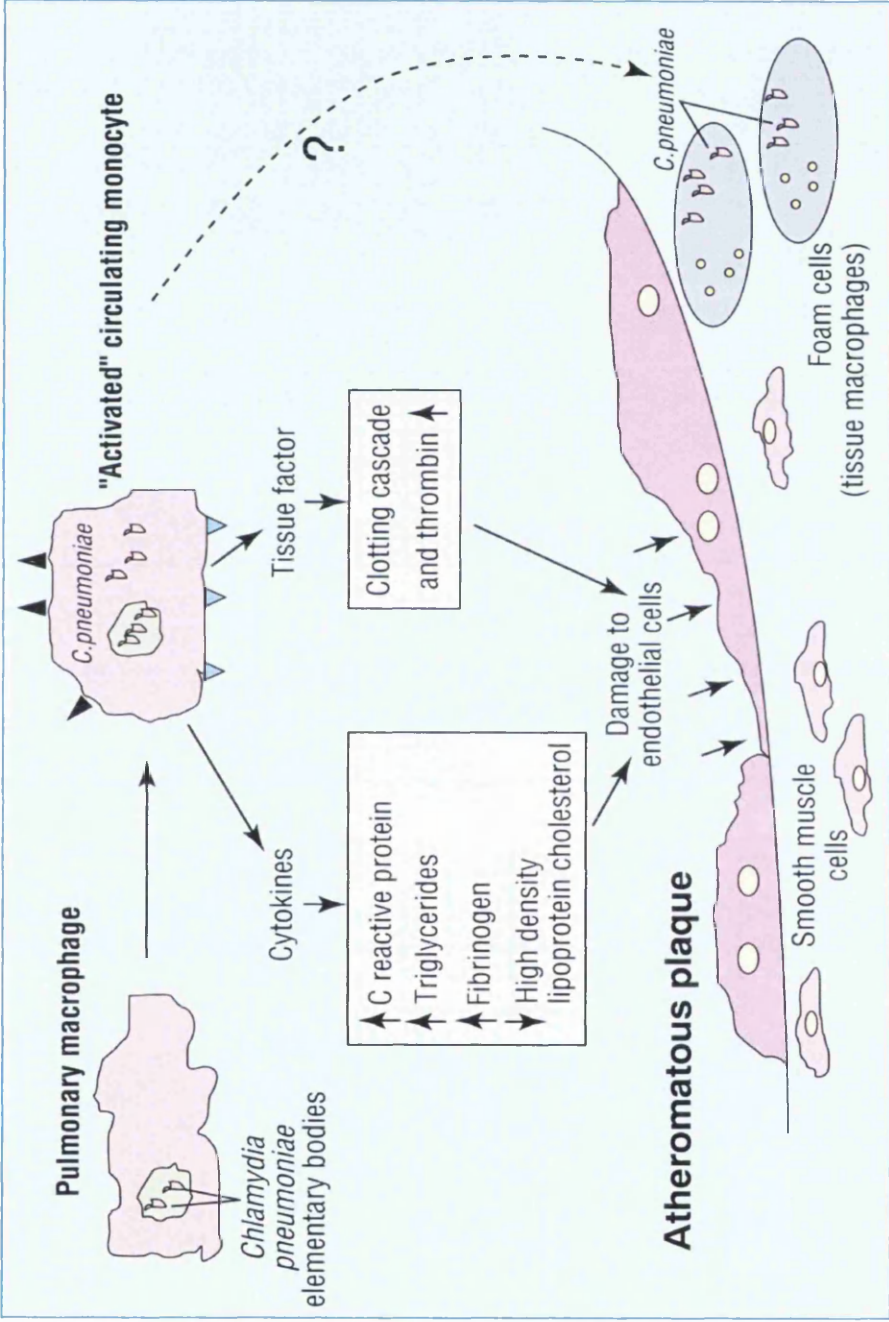


Figure 1.6: Possible mechanisms for the involvement of Chlamydia Pneumoniae in atherogenesis (from Gupta and Camm, 1997)

## **Chapter 2: Association between carotid disease and ipsilateral lacunar stroke**

## 2.1 Introduction

Major surgical intervention trials have established the role of carotid endarterectomy in the management of patients with carotid stenosis <sup>44 157 42</sup>. Increasingly, consideration of the benefit that is likely to be derived by patients with specific clinical characteristics <sup>47</sup> and the costs that might be incurred by surgical intervention <sup>158</sup>, has resulted in calls for a more selective policy of referral for vascular surgical attention. Central to evidence-based decision making in this area is the assessment of whether a carotid lesion is symptomatic: patients with symptomatic stenoses are at much higher stroke risk than those with asymptomatic lesions <sup>159</sup>, at least in the first 3 years <sup>160</sup>, and derive greater benefit from intervention than those with asymptomatic lesions <sup>42 46</sup>. Moreover, recent data on 'surgical skill' have shown that peri-operative stroke rates in clinical practice are likely to be considerably higher than those achieved in carotid endarterectomy trials <sup>161</sup>.

Published guidelines currently recommend consideration of endarterectomy in patients with *symptomatic severe stenosis* <sup>162</sup>. While evidence of cortical stroke or transient ischaemic attack (TIA) ipsilateral to a severely stenotic carotid lesion strongly suggests that the stenosis caused the cerebral lesion, lacunar or subcortical stroke in combination with ipsilateral carotid stenosis presents a more challenging clinical picture. Because most lacunar strokes and subcortical lesions are caused by intrinsic small vessel disease (often secondary to hypertension or diabetes) <sup>48</sup>, stenosis in these patients may be incidental, and surgical correction may expose the patient to the risks of surgery with no more benefit than would be derived from a 'blanket' policy of surgical correction in all detected stenoses. Published literature supports the view that large vessel stenosis is rare in lacunar stroke and TIA in comparison to cortical disease <sup>163 164 165 166</sup>, but more common than in the general population <sup>167 168</sup>. Studies have not been of sufficient size to detect a clear association between 'lacunar' presentation and extracranial vascular disease

<sup>165</sup> <sup>169</sup> <sup>170</sup>. The two major trials of 'symptomatic' carotid stenosis included patients with lacunar syndromes and subcortical lesions on CT scanning, but subgroup analysis was unable to detect significant benefit from surgery in either the ECST <sup>53</sup> or NASCET <sup>54</sup> study. Sample sizes were small however: in ECST only 43 patients with severe stenosis had CT evidence of lacunar infarction at randomisation, and only 5 ischaemic strokes occurred in this group during the follow-up period <sup>53</sup>.

The aim of this analysis was to investigate any association between carotid lesions and subcortical syndromes, by comparing the prevalence of clinical lacunar syndromes and subcortical strokes ipsilateral and contralateral to ultrasonically detected carotid disease. Furthermore the risk of recurrent stroke was determined by record-linkage analysis in the two groups.

## **2.2 Methods**

The Western Infirmary Acute Stroke Unit serves a catchment population of 200,000. All patients who present within 72 hours of onset of acute neurological deficit likely to be of vascular origin are admitted irrespective of age or severity of presentation. Approximately 800 patients are admitted each year. Patients who present less acutely, or who may be better assessed in a clinic environment, are seen at a rapid referral clinic that occurs twice a week. All patients given a stroke diagnosis are classified using the Oxford Community Stroke Project <sup>171</sup> method. Patients with a clinical diagnosis of TIA are classified in a similar manner <sup>166</sup>. Classification is performed by the admitting Senior House Officer, and verified by a consultant physician within 24 hours. Brain imaging using either CT or MRI is performed within 72 hours of admission (or within 1 week if seen in clinic) on all patients with a clinical diagnosis of stroke, and almost all patients with TIA. A carotid Doppler examination is routinely performed in all patients. Clinical and radiological data from each patient are reviewed and verified during a weekly meeting



attended by a neurologist, a radiologist and a team of specialist stroke physicians (one of whom will have been involved in the original clinical classification) before being recorded prospectively on a database. Carotid ultrasound findings are not used in clinical or radiological classification of stroke subtypes, and are recorded on a separate database. Clinical and radiological data for each patient have been linked to ultrasound data for the purposes of this study.

We analysed the database records for those patients admitted between April 1994 and 1998. The database for this period contained information for 3721 patients in total, 2445 (66%) of whom had linked data on Doppler results, and 3213 (86%) of whom had CT or MRI results recorded. We extracted data for those patients who had concurrent evidence of unilateral lacunar disease (LD) (as defined by a clinical lacunar syndrome with normal imaging or imaging evidence of unilateral subcortical infarction) and unilateral carotid disease (CD) (as defined by a moderate (50-70%), severe (>70%) or occluded single carotid vessel on ultrasound). Of 1093 LD patients on the database, 835 had linked Doppler data (since CT or MRI formed part of the definition of LD, all LD patients had imaging data). Individuals with bilateral LD or bilateral CD were *not* included in the analysis. These amounted to 4 patients with bilateral CD and bilateral LD, 16 patients with bilateral CD and right LD, 14 patients with bilateral CD and left LD, 7 patients with right CD and bilateral LD and 6 patients with left CD and bilateral LD. Patients with striato-capsular infarction or large subcortical infarction extending into cortical territories on CT or MRI were classified on the database as cortical/subcortical and were excluded, while those with a *separately classified* cortical lesion or event were included. The incidence of contralateral LD and CD (group 1) was compared to the incidence of ipsilateral LD and CD (group 2). Risk factors (smoking, hypertension, atrial fibrillation, hyperlipidaemia and diabetes (types I and II)) in the two groups were assessed. Proportions with moderate, severe and occluded vessels in the two

groups were calculated. Age and first recorded ward blood pressure were also analyzed and compared.

Using follow-up data we assessed the recurrence rate of ischaemic stroke in the two groups. Follow up was by record-linkage <sup>172</sup> to both hospital discharge records (to obtain information on recurrent ischaemic stroke) <sup>173</sup> and death records from the Registrar General of Scotland. This technique has been validated in an epidemiological study of hypertension <sup>174</sup> and has been used for monitoring end points in a large clinical trial <sup>175</sup>. The method of record-linkage is reliable, but admissions to private hospitals or institutions outside Scotland are not recorded. Patients in groups 1 and 2 with separate, additional cortical lesions or cortical events ipsilateral to the side of their CD, who thereby fulfilled conventional descriptions of 'symptomatic carotid disease' (4 in group 1, 8 in group 2), were excluded from the follow-up analysis. Data for those patients whose first recorded stroke diagnosis was during their admission to our unit, and for whom follow-up data were available (18 in group 1, 37 in group 2), were used to calculate the cumulative proportion in each group remaining stroke-free. The comparison of groups 1 and 2 formed the primary follow-up analysis. In addition, stroke-recurrence data for all patients on the database whose first recorded stroke diagnosis was during their admission to our unit, and who presented with LD but had no CD (n=435), or who had CD but no evidence of LD (n=126), were analyzed as supplementary information.

A Chi-squared test of association was performed to determine whether there was an association between unilateral CD and unilateral LD. A significance level of 5% was used (without Yates' correction). Treating moderate, severe and occluded carotid vessels as ordered and equally spaced points on a scale, a chi-squared test for trend was conducted to test for linear trend in severity of CD in groups 1 (contralateral) and 2 (ipsilateral). The proportion of patients with specific risk factors was assessed in both groups and compared.

With contralateral / ipsilateral disease as the response variable, a multivariate analysis by forward stepwise logistic regression was conducted using *BMDP* software. A significance level of 5% was used for inclusion in the model. Mean age and first recorded ward blood pressure in the two groups were normally distributed, and were compared by t-testing. Kaplan-Meier analysis, using *Statistica* software (version 5.1 StatSoft, Inc. (1997)) was performed in both groups, and the 'ipsilateral' and 'contralateral' groups were compared using a Log-rank test. Data for patients on the database within the same period with just LD (no CD) (group 3) and just CD (no LD) (group 4) are also presented, and were compared to the initial two groups, by an extended (multiple sample) Log-rank test. No specific adjustments were made when directly comparing groups 3 and 4 to the original two groups, but data on these comparisons were viewed in the context of multiple tests having been conducted.

## 2.3 Results

The incidence of right and left LD in those with right and left CD is shown in **table 2.1**. The chi-squared test of association was strongly positive ( $p=0.003$ ), and it can be seen from the table that lacunar strokes were more common on the side of carotid lesions. Mean age in (contralateral) group 1 was 73.4 years compared to 70.1 years in (ipsilateral) group 2 ( $p=0.12$ ). Mean (first ward reading) systolic blood pressures were 154 (group 1) and 165 (group 2) ( $p=0.06$ ), while diastolic blood pressures were 91 and 89 respectively ( $p=0.31$ ). **Table 2.2** shows a chi-squared test for linear trend across the carotid disease groups (moderate stenosis, severe stenosis and occluded). The chi-squared test proved narrowly positive ( $p=0.049$ ), with a trend towards higher severity of CD in group 2 (ipsilateral). The proportions of patients with each stroke risk factor are presented in **table 2.3**. No significant differences were found between the two groups. In the logistic regression model, severity of carotid disease was significantly predictive ( $p=0.048$ ) of the presence of

ipsilateral LD. No other risk factor was significant after carotid disease was added to the model (smoking,  $p=0.10$ ; hypertension,  $p=0.97$ ; AF,  $p=0.72$ ; hyperlipidaemia,  $p=0.43$ ; type II diabetes mellitus,  $p=0.93$ ; type I diabetes mellitus,  $p=0.45$ ).

The cumulative proportion of patients remaining stroke-free for groups 1 and 2 is shown in **figure 2.1**. No stroke / TIA outcome events were recorded in group 1. Clear divergence can be seen between the two groups in the figure, but this trend did not reach conventional statistical significance ( $p=0.059$ ). Eight patients were treated with carotid endarterectomy during follow up, one in group 1, and seven in group 2. Only one of these patients (from group 2) sustained a recurrent ischaemic stroke, 570 days after endarterectomy. **Figure 2.2** presents data for LD (without CD) (group 3,  $n=435$ ) and CD (without LD) (group 4,  $n=126$ ), as well as groups 1 and 2. Multiple-sample testing showed survival to be significantly different in the 4 groups ( $p=0.003$ ) shown in **figure 2.2**. Comparing the groups individually, there was no significant difference between group 1 and group 3 ( $p=0.320$ ), or between group 1 and 4 ( $p=0.196$ ). Neither groups 3 and 4 ( $p=0.248$ ) nor groups 2 and 4 ( $p=0.073$ ) differed significantly, but comparison of groups 2 and 3 indicated that the presence of ipsilateral (*but not contralateral*) large vessel carotid disease confers a markedly poorer prognosis to those patients presenting with lacunar stroke or TIA ( $p=0.002$ ). Stroke-free survival (with both ischaemic cerebrovascular events and death from all causes counted as a complete response) was also examined. Eight deaths not due to recurrent ischaemic stroke occurred in group 1 (3 vascular, 3 oncological and 2 respiratory), while 6 such deaths occurred in group 2 (4 vascular, 1 oncological and 1 unknown). The trend to better outcome in group 2 (compared to group 1) disappeared ( $p=0.688$  for comparison).

## 2.4 Discussion

The impetus for this analysis stemmed from a genuine uncertainty as to the correct management course in what is a challenging clinical situation. We have found a strong association between unilateral carotid disease and subcortical syndromes. A trend towards increased severity of carotid disease in patients with ipsilateral lacunar stroke suggests a 'dose-response' relationship, which supports an aetiological hypothesis as the *explanation* for that association. In the logistic regression model, a single step increase in carotid disease severity (from moderate to severe, or from severe to occluded) was associated with an odds ratio for ipsilateral lacunar disease of 1.93, with a wide and marginally significant 95% confidence interval of 1.01 to 3.67.

If causation is discounted, it is difficult to explain the association detected. The implication is that, either by embolism or haemodynamic ('low flow') mechanisms, lacunar syndromes have resulted from ipsilateral large vessel disease. One might feel more able to accept that an ipsilateral carotid stenosis has caused a lacunar stroke if, in the individual patient, risk factors for small vessel disease are absent. However, when data for just those patients who have risk factors for small vessel disease (defined as a history of hypertension (treated or untreated) or a history of diabetes (type I or II)) are examined, chi-squared analysis continues to reveal a positive association between unilateral LD and ipsilateral carotid lesions ( $p=0.042$ ). Thus, even in those patients with subcortical presentation or pathology in whom one might reasonably assume an ipsilateral carotid lesion to be incidental, there remains evidence of association between the two.

Calculation of the cumulative proportion of patients remaining 'stroke-free' adds some weight to the assertion that carotid disease is instrumental in the pathogenesis of ipsilateral subcortical infarction. A trend towards higher rates of cerebrovascular events in group 2 (with ipsilateral LD and CD) compared to group 1 failed to reach conventional significance ( $p=0.059$ ), probably due to

the low patient numbers for whom data were available and the absence of outcome events in (contralateral) group 1. The divergent findings shown in figure 2 reflect natural history rather than surgical morbidity. Patients in both groups received best medical therapy, including antiplatelet treatment and modification of risk factors. While those with lacunar and carotid disease on opposite sides had a similar prognosis to those with just lacunar stroke / TIA (and no carotid lesion), the prognosis of patients with carotid and lacunar disease on the same side was substantially (and significantly) worse.

Doppler data were not available for 24% of the LD patients on the database. While Dopplers were conducted in these patients, the information is not linked to the main database containing clinical and radiological data. This is an important source of bias within our sample, because the LD patients lacking ultrasound data may have had a different incidence of CD. Carotid Doppler, however, has been routinely conducted on all patients in our stroke unit since 1994 and is not requested on the basis of clinical syndrome or scan appearance. While every effort is made in the weekly clinical meetings to ensure validity of the data entered into the database, it remains possible that incorrect clinical classification of lacunar strokes may also have biased our results.

The follow-up analysis was performed on 56% of group 1 (18 of 32) and 61% of group 2 (37 of 61). Patients were excluded from follow-up if the stroke that necessitated admission to our unit was not their first. Exclusion was necessary since their first stroke may have been a cortical event ipsilateral to their CD, thus denoting a 'conventional' symptomatic stenosis. Twelve patients (4 in group 1 and 8 in group 2) had a separately classified cortical stroke ipsilateral to their CD (on our database), and so were removed from follow-up analysis. However, record-linkage data were *unavailable* at the time of the study for 10 patients in group 1, and 20 patients in group 2. These

missing data, representing a third of the total dataset, represent another important potential source of bias.

It is unfortunate that the record-linkage data used in this study are not side-specific, and also do not enable us to distinguish between recurrent cortical and subcortical ischaemic events. It has been previously reported that recurrent large vessel strokes are more common in patients who present with small vessel disease than are small vessel recurrent events in patients with large vessel presentations<sup>50 176</sup>. The most likely explanation for such a finding was felt to be the co-existence of large and small vessel disease in the patients that presented with a small vessel syndrome (routine screening of the carotid arteries was not performed in these studies). A possible explanation in the light of the data presented here is that some of the presenting small vessel syndromes represented symptomatic manifestations of large vessel disease in the same way as the recurrent cortical strokes.

There is both pathological<sup>177</sup> and clinical trial<sup>52</sup> evidence that infarcts of the centrum ovale (in the territory of the superficial rather than deep perforating arteries) are associated with large vessel disease, though observational data are less conclusive<sup>178 179</sup>. Internal watershed (internal borderzone) infarction, between the superficial and deep territories, is also regarded to result from haemodynamic effects distal to diseased large vessels<sup>180 51</sup>. These infarct types, along with large (>15mm) subcortical infarctions, may have been included in the 'lacunar disease' group, since the level of vascular localisation required to confidently diagnose such lesions is not documented on the database. Such infarct types represent a problem with the analysis presented, because there is literature to suggest that they are associated with large vessel disease, and so their presence may denote a 'symptomatic' carotid lesion. Our patients had no clinical cortical features, however, and our assessment is a pragmatic one: many clinicians dealing with stroke have seen patients present with clinical features suggesting a subcortical locality

and either a normal scan or a scan demonstrating a subcortical stroke. Our results indicate that in such patients ipsilateral carotid disease is more common and more severe than contralateral carotid disease, and that its presence is associated with a poor prognosis. Such findings support investigation for carotid disease and also consideration of surgical referral in this group.

A related problem in the assignment of whether a carotid lesion is 'symptomatic' is those patients who present with AF and are found to have carotid stenosis. Should one blame the ischaemic stroke on the cardiac or the carotid abnormality? Such individuals were not included in either major endarterectomy trial<sup>45</sup>. Recently, work examining stroke recurrence published from the NASCET trial has advised caution in referral: it was estimated that approximately 20% of strokes in the territory of symptomatic carotid arteries with 70-99% stenosis were 'unrelated to the carotid lesion' (that is, they were classified as lacunar or cardioembolic)<sup>181</sup>. In patients with less than 70% stenosis, 'approximately 35% of strokes were due to causes other than the large artery lesion'<sup>181</sup>.

Another recently published analysis from NASCET detailed those patients included in the trial with 'probable' and 'possible' lacunar stroke<sup>54</sup>. Patients with high grade carotid stenosis had a greater risk of lacunar infarction than patients with lesser degrees of stenosis. A trend towards benefit of surgery for patients with 50-99% stenosis was observed in those with 'probable' (relative risk reduction (RRR) 35%, 95% CI= -100% to 81%) and 'possible' (RRR 53%, 95% CI= -62% to 88%) lacunar stroke. Benefit was most marked in the group presenting with non-lacunar stroke, who gained highly significant benefits from surgery (RRR 61%, 95% CI= 34% to 82%). There were, however, three times as many patients in this group (665 vs 210 in the probable lacunar group).



The trend to benefit observed in the lacunar subset of the NASCET trial<sup>54</sup> does not necessarily imply that a large vessel lesion *caused* the 'possible' or 'probable' lacunar lesion – benefits are evident in asymptomatic individuals – but it is reassuring that trend is to benefit rather than to harm. The results suggest that a larger sample size would confirm benefit in individuals with lacunar stroke and carotid stenosis. Such an analysis has now been undertaken with individual patient data from the NASCET, ECST and Veterans Affairs trials<sup>55</sup>, after completion and presentation of the analysis presented here, and is currently published in abstract form. It showed that in patients with high degrees of stenosis (70-99%), endarterectomy was beneficial in patients presenting with lacunar events. Such patients benefit less than those with cortical stroke and the same degree of stenosis. Patients presenting with cortical stroke also benefit from endarterectomy for lesser degrees (50-69%) of stenosis<sup>55</sup>. Incorporation of stroke type into a simple clinical scoring system, like that already proposed by *Rothwell et al*<sup>47</sup>, could greatly improve the efficiency by which referrals are made.

To discount the possibility that lacunar and subcortical syndromes may relate to either embolism or low flow from a proximal carotid source could therefore deny worthwhile preventative therapy to an important subset of patients. Published literature increasingly suggests that lacunar stroke is a complex and heterogeneous entity. Higher rates of embolic sources have been observed in lacunar stroke than comparative control groups<sup>182 183</sup>, and diffusion weighted imaging has called into question the theory that all lacunar lesions are due to disease of a single perforator<sup>184</sup>.

Other related analyses are reported in the literature. In a paper based on data from the NASCET trial, no association was reported between leukoaraiosis (white matter change) and angiographically detected carotid stenosis<sup>185</sup>. While leukoaraiosis may confer a poor prognosis<sup>186</sup>, it is unlikely to arise from artery-to-artery embolism, and represents a different form of white matter

pathology to that studied here. Increasing severity of carotid stenosis has been linked to higher incidence of radiographic central infarction <sup>187</sup>. Another study <sup>170</sup> reported no significant difference in severity between ipsilateral and contralateral internal carotid artery disease in lacunar infarction. Our study differed in that we examined the incidence of *unilateral* LD and *unilateral* CD, and compared patients with contralateral and ipsilateral findings. This is likely to increase the possibility of detecting an association (should one exist), since patients with generalized vascular disease manifesting as concurrent (and often bilateral) carotid and small vessel disease are less likely to be included. The methodology presented here concentrates on the possibility of an embolic or haemodynamic mechanism ('low flow') linking the two, and requires far higher patient numbers than have been previously reported. The finding of bilateral carotid disease in the individual patient with a lacunar syndrome should not deter the clinician from considering the possibility that the ipsilateral lesion may be responsible for the clinical presentation.

Endarterectomy, performed by centres with very low peri-operative stroke and death rates, has been proven to be effective in carefully selected asymptomatic patients with severe carotid stenosis <sup>46 188</sup>. Debate continues about the wisdom of adopting such policies in clinical practice <sup>189 190</sup>, and whether the impressive surgical results achieved in clinical trials are possible in general hospital settings <sup>191 161</sup>. Even if a policy of endarterectomy in asymptomatic severe stenosis is favoured, priority must be given to those lesions considered symptomatic, since yearly risk of stroke on medical treatment is so much higher for symptomatic lesions, and absolute risk reduction achieved by surgical intervention is so much greater. After two years the number needed to treat to prevent one stroke in 'symptomatic' severe stenosis is 6 <sup>42</sup>, compared to 67 in asymptomatic lesions <sup>46</sup>. Many other factors will play a part in the decision to recommend endarterectomy in the individual clinical setting <sup>47</sup>, but data supporting the view that patients with lacunar stroke and high degrees of stenosis will benefit from intervention are

emerging <sup>54</sup> <sup>55</sup>. The simple acknowledgement that an ipsilateral lacunar syndrome can denote a *symptomatic* stenosis is important in both risk stratification and in the planning of investigation and management.

	Left CD	Right CD	TOTAL
Left LD	34	16	50
Right LD	16	27	43
TOTAL	50	43	93

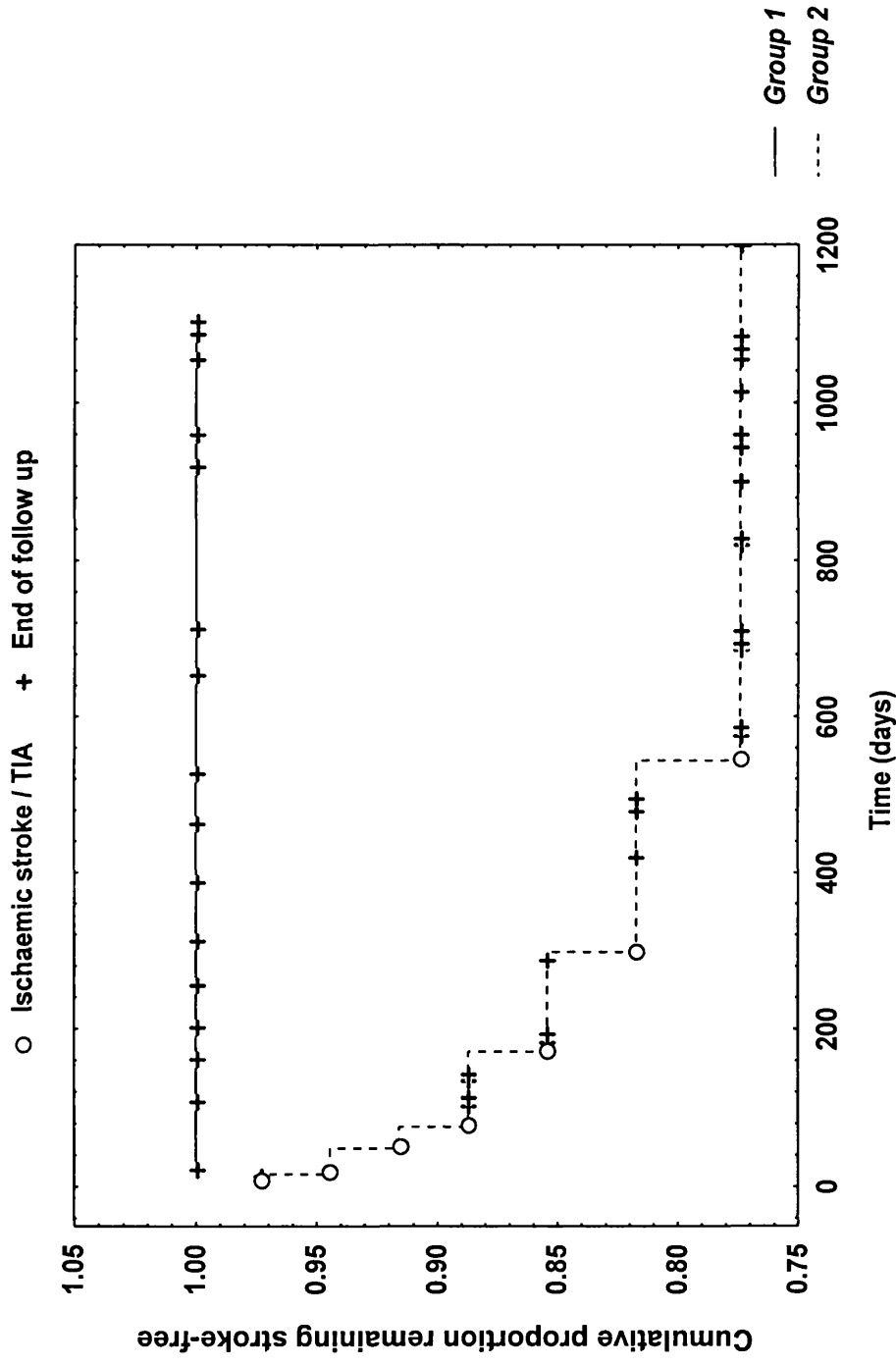
**Table 2.1: Chi squared Table** showing the numbers of patients with unilateral right and left lacunar disease (LD) who also had unilateral right or left carotid disease (CD). Chi-squared test of association gives  $X^2= 8.82$ , d.f. = 1,  $p=0.003$ .

	Moderate	Severe	Occluded
<b>Group 1</b>	7 (50%)	17 (39%)	8 (23%)
<b>Group 2</b>	7 (50%)	27 (61%)	27 (77%)

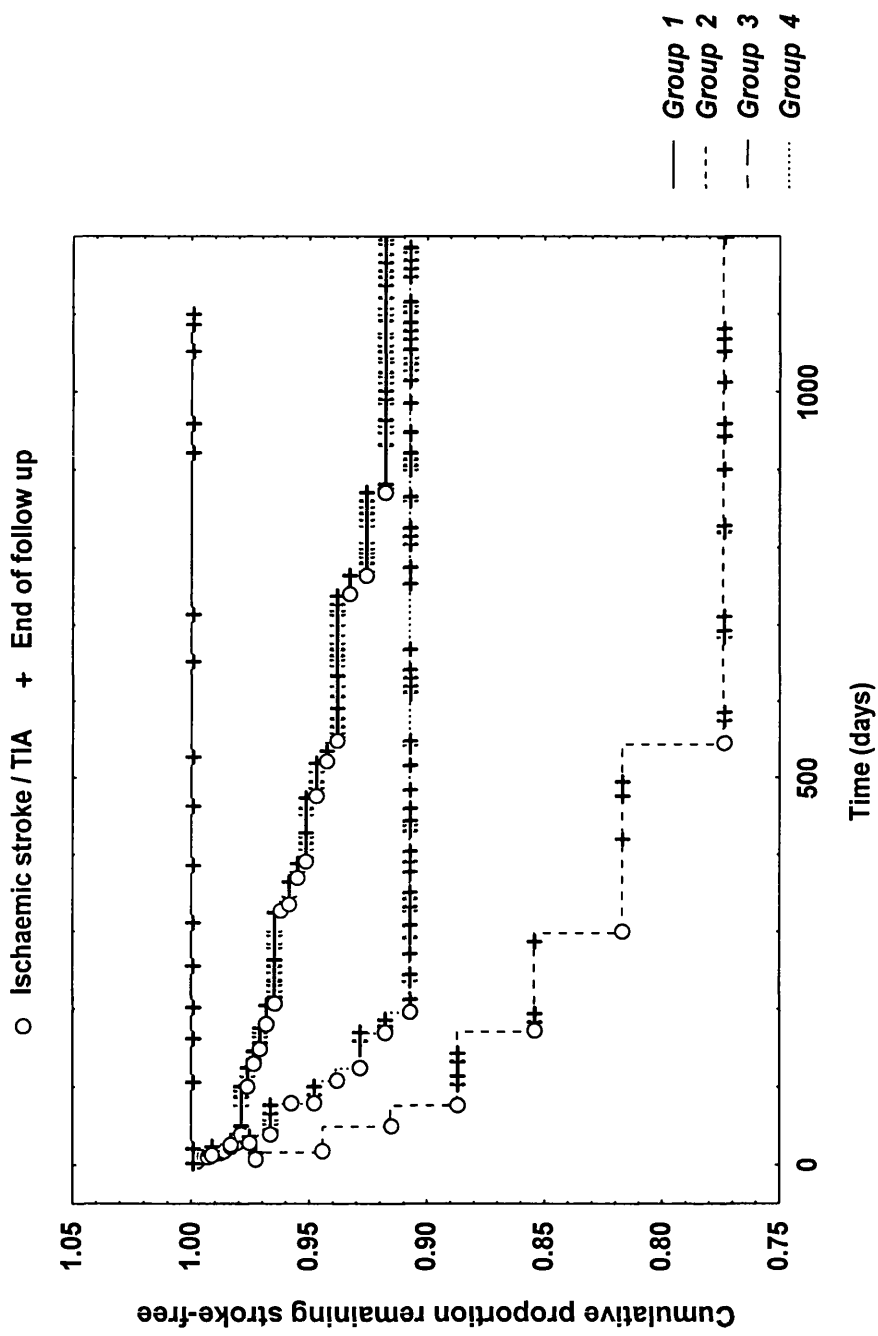
**Table 2.2: Chi-squared test for trend** showing the numbers of patients in groups 1 and 2 moderate stenosis, severe stenosis or occluded carotid vessels on carotid ultrasound examination. Percentage of the total in each carotid disease category are shown in brackets. Chi-squared test for linear trend gives  $X^2 = 3.88$ , d.f. = 1,  $p = 0.049$ .

<b>Risk Factor</b>	<b>Proportion (as percentage) in group 1 (contralateral)</b>	<b>Proportion (as percentage) in group 2 (ipsilateral)</b>	<b>p-value for comparison of proportions in the two groups</b>
<b>Smoking</b>	68%	55%	0.21
<b>History of hypertension</b>	59%	55%	0.69
<b>Atrial fibrillation</b>	15%	11%	0.59
<b>History of hyperlipidaemia</b>	3%	9%	0.24
<b>History of type II diabetes</b>	6%	6%	0.94
<b>History of type I diabetes</b>	0%	2%	0.46

**Table 2.3: The proportion of patients in group 1 (contralateral) and group 2 (ipsilateral) with each stroke risk factor. In the third column, a (two-sided) p-value for the comparison of the 2 proportions is given, none of which reached the conventional level of significance (5%).**



**Figure 2.1:** Kaplan-Meier analysis for patients in groups 1 (n=18) and 2 (n=37). The patients are classified as having a complete response (O) if they have a recurrent ischaemic stroke (fatal or non-fatal) or TIA and are censored (+) at the end of the period for which follow-up data were available. Death from causes other than stroke is censored (+). The cumulative proportions remaining stroke-free are shown over time in the 2 groups. Follow-up data are restricted to 1200 days.



**Figure 2.2:** Kaplan-Meier analysis of patients in group 1 (n=18), group 2 (n=37), group 3 (LD without CD, n=435) and group 4 (CD without LD, n=126). The patients are classified as having a complete response (O) if they have a recurrent ischaemic stroke (fatal or non-fatal) or TIA and are censored (+) at the end of the period for which follow-up data were available. Death from causes other than stroke is censored (+). The cumulative proportions remaining stroke-free are shown over time in the 4 groups. Follow-up data are restricted to 1200 days.



## **Chapter 3: Inter-atrial septal abnormalities and stroke: a meta-analysis of case control studies**

### 3.1 Introduction

Individual case studies and series in the 1980s postulated a causal role for inter-atrial septal abnormalities (patent foramen ovale (PFO) and atrial septal aneurysm (ASA)) in the etiology of embolic stroke<sup>192 193</sup>. Numerous case control studies have been published since examining the frequency of inter-atrial septal abnormalities in patients with stroke or transient ischaemic attack (TIA) in comparison to controls. Similarly designed studies aiming to establish whether such abnormalities were more likely to be found in individuals with 'cryptogenic' stroke (stroke without other clear cause) followed. Ambiguous findings have left clinicians uncertain as to whether they should investigate for such abnormalities, and whether positive results should be regarded as causal or incidental. Published reviews have provided an interpretation of the literature without systematically evaluating available evidence<sup>194</sup>. Studies differ in the age groups examined and in the control groups chosen, but similarities can be found in design and methodology, such that they may be grouped together using simple, clinically intuitive criteria. Whilst the results of such an exercise cannot address the appropriate management of such patients, a systematic review and meta-analysis has implications for current investigational and therapeutic strategies, and for further research.

### 3.2 Methods

A systematic search was made using both Medline and BIDS (Bath Information and Data Services) bibliographic databases for the keywords 'patent foramen ovale', 'atrial septal aneurysm' and 'right-to-left shunt'. This resulted in a total of 2738 references, which were individually assessed. All studies that examined the prevalence of PFO, ASA or a combination of both in a stroke population were examined in full. All those that met the criteria defined below were included in the meta-analysis. The bibliographies of all included studies, many excluded studies, and any review articles were

searched for additional suitable studies. Both English and foreign language journals were examined. Unpublished data were not sought. Inclusion criteria were:

1. Case control studies that compared prevalence of *PFO* or *ASA* in *ischaemic stroke or TIA* patients to *non-stroke control* patients using a validated *diagnostic technique*.
2. Case control studies that compared prevalence of *PFO* or *ASA* in patients with *cryptogenic stroke* to patients with *known stroke cause* using a validated *diagnostic technique*.
3. Case control studies that compared prevalence of *PFO* or *ASA* in patients with *cryptogenic stroke* to *non-stroke control* patients using a validated *diagnostic technique*.

Clear definitions of the terms in *italic* are given in the **definitions section** (see below). The three broad comparisons above were each divided into 'total' (patients of all ages), 'young' ( $\leq 55$  years) and 'old' ( $\geq 55$  years). Differing inclusion criteria dictate that patients aged 55 may be included in either category. If *planned* analysis of a different age group above or below 55 years was stated in the objectives / methods section of the paper, results are included within the relevant comparison. If such an age subgroup was studied as a 'post-hoc' analysis within a larger study (i.e. it may have been *defined and analyzed* after knowledge of results) these data are excluded. Two studies divided patients into groups older or younger than 50<sup>195 196</sup>, and one used an age cut-off of 60 years<sup>197</sup>. These are included in the separate age analyses as if '55 years' was the division used.

Both *fixed effects* (Mantel-Haenszel) and *random effects* (Der-Simonian Laird) methods of meta-analysis were used<sup>198</sup>, and the combined odds ratio (OR) from each method was tabulated. Using a significance level of 10%, *homogeneity* of trials was assessed for each comparison. If significant

*heterogeneity* was not detected, results were presented graphically (*forest plot*) using the *fixed effects* method. If significant *heterogeneity* was present, reasons for this were investigated, and the comparison was presented graphically using the *random effects* method. Sensitivity analysis was performed for each group of trials, and possible sources of bias were examined. *Funnel plots* were constructed to investigate publication and related bias <sup>199</sup>. Explanations of the terms in italics are given in **appendix 2**.

### **Definitions**

#### ATRIAL SEPTAL ABNORMALITIES

##### *Patent foramen ovale (PFO)*

Presence of right-to-left shunt (R-to-L shunt) at inter-atrial level. One study included patients with atrial septal defect (ASD) in the published data <sup>195</sup>. This was defined as a defect in the septum primum or secundum, or a defect >0.5cm wide in the region of the fossa ovalis, with left-to-right flow. The investigators reported that the relative frequencies in the case and control groups were not substantially altered if analysis was restricted to patients with PFO (i.e. just those with R-to-L shunt). All other trials excluded patients with ASD.

##### *Atrial Septal Aneurysm (ASA)*

Base width 1.5cm or greater and with at least 1.1cm excursion into either the left or the right atrium or a sum of the total excursion into the left or right atrium of 1.1cm or greater <sup>200</sup>. Some studies used stricter criteria <sup>201</sup>. All studies used transesophageal echocardiography. Base width not mentioned in one paper <sup>202</sup>.

DIAGNOSTIC TECHNIQUE (*When more than one technique was used in a study, the most sensitive has been chosen for analysis*)

##### *Transcranial Doppler (TCD)*

(PFO)-Gelatin or saline contrast. Timing of microbubble spike(s) ranged between 4 and 20 seconds after injection. Studies performed at rest, coughing and with Valsalva maneuver in most studies.

#### *Transthoracic Echocardiography (TTE)*

(PFO)-Gelatin or saline contrast. Appearance of at least one microbubble of contrast in left atrium (LA) within 4 cardiac cycles of opacification of the right atrium (RA).

#### *Transesophageal echocardiography (TEE)*

(PFO)-Gelatin or saline contrast. Contrast opacification (at least 1 microbubble) of LA within 3 seconds or 4 cardiac cycles of opacification of RA. All studies examining ASA used TEE, and the echocardiographic definition is given above.

### STROKE SUBCLASSIFICATION

#### *Non-stroke control patients*

Normal volunteers, hospitalized or echocardiographic (patients receiving an echocardiographic examination for another reason) control patients who are compared to patients with stroke / TIA.

#### *Ischaemic stroke / transient ischaemic attack (TIA)*

Sudden clinical focal neurological deficit consistent with the diagnosis of stroke (confirmed as ischaemic by computed tomography (CT) or magnetic resonance imaging (MRI)), or TIA (lasting less than 24 hours). One study did not mention cranial imaging <sup>203</sup>. One study included patients with peripheral (arterial) embolus <sup>204</sup>; three others examined such patients, but they were removed from the analysis where possible <sup>205 206 197</sup>.

*Known stroke / TIA cause (non-cryptogenic stroke)*

Levels of clarification of cause differ considerably between studies. Minimum requirements are assessment of cardiac rhythm (electrocardiogram (ECG)), assessment of presence of carotid stenosis (by Doppler, with one exception that used a clinical bruit <sup>203</sup>) and assessment of alternative cardioembolic source (by TTE). Many earlier papers used angiography in their assessment <sup>207 208 84</sup>. Assessment of pro-coagulant markers differs, and if conducted these have been used for classification <sup>209 202 84 210 211</sup>. Many studies used accepted stroke data bank criteria in their definition of cryptogenic stroke, namely those of the NINDS (National Institute of Neurological Diseases) stroke data bank <sup>212 213 214 211</sup>, and the BADISEN (Banco de datos de Ictus de la Sociedad Espanola de Neurologia) system <sup>215</sup>. These classify strokes into those of determined cause (cardioembolic, lacunar (small vessel), large artery atherosclerotic or of unusual but determined cause) or cryptogenic. All other studies used a similar system, and for this analysis non-cryptogenic stroke includes patients with the following causes of stroke: arterial dissection, carotid stenosis > 50% (one study used stenosis  $\geq$  31% <sup>196</sup>), intra-cranial atherosclerosis with stenosis > 50% of the corresponding vessel, angiitis, migrainous infarction, coagulopathies, systemic disorders (e.g. lupus / Hughes syndrome), atrial fibrillation (AF) (chronic or paroxysmal on ECG), recent (within 6 weeks) myocardial infarction, dilated cardiomyopathy, rheumatic mitral stenosis, mitral / aortic vegetation or prosthesis, left atrial or left ventricular tumor / thrombus, spontaneous left atrial echo contrast and complex atheroma between the aortic valve and the left subclavian artery origin. Mitral valve prolapse (MVP) is regarded as a cause of stroke in some trials <sup>212 213 214 211 216 204 215 217</sup>, but generally as a risk factor (see below). Two studies <sup>183 218</sup> assessed as 'undetermined' those individuals with more than one cause of stroke. These patients are defined as having non-cryptogenic strokes for this analysis.

### *Cryptogenic stroke / TIA*

Those patients without predetermined cause for stroke, as described above. One study included those with AF and spontaneous echo contrast in its cryptogenic stroke group<sup>219</sup>. Another classified those with AF on TEE / Holter monitoring as cryptogenic<sup>220</sup>. One study excluded (i.e. defined as non-cryptogenic) patients with hypertension (> / = 170/95)<sup>203</sup>. In all other studies hypertension is regarded as a risk factor.

### RISK FACTORS

Increasing the risk of stroke, but not necessarily the cause of stroke (e.g. hypertension, diabetes, hypercholesterolaemia, smoking, alcohol use, oral contraceptive pill, migraine, and generally MVP (see above)). ASA is regarded as a risk factor in those studies examining PFO, and PFO is regarded as a risk factor in those studies examining ASA.

## 3.3 Results

Details of the comparisons performed in the total category (all ages) (**table 3.1**), in the young (<55 years) (**table 3.2**) and in the old (>55 years) (**table 3.3**) will be considered in turn:

### ***Patent Foramen Ovale: stroke vs non-stroke controls***

The 'total' comparison, comparing ischaemic stroke patients to non-stroke controls yielded 15 studies for PFO<sup>202 221 222 223 205 216 195 208 224 206 225 215 207 197 204</sup> (**see figure 3.1**). Significant heterogeneity was detected, which appeared largely to result from the different ages of participants. If the trials are divided into two separate groups, of positive studies<sup>202 221 222 208 206 207 197 204</sup> and neutral/negative studies<sup>223 205 216 195 224 225 215</sup>, and the ages of included patients are compared, a difference exists between the mean age of patients in the positive (44.8 years) and neutral/negative (61.1 years) trials (p=0.022). No significant trends were observed in the diagnostic technique

employed, attempts to blind observers to the clinical diagnosis, choice of controls, or retrospective vs prospective design. A funnel plot did not suggest publication bias.

Homogeneous results were obtained in the younger age group (**figure 3.2**). This comparison included nine trials<sup>202 221 222 216 195 208 207 197 204</sup>, which produced a common effect measure of 3.10 (2.29-4.21). A funnel plot was asymmetric, suggesting possible bias towards the publication of positive results. In the older age group (**figure 3.3**), more heterogeneous results were evident<sup>195 197 204</sup>. The odds ratio from both fixed and random effects analysis crossed the line of no effect. The largest study showed the least association, but examination of individual trials provided no explanation for the difference in effect.

#### ***Patent foramen ovale: cryptogenic stroke vs known stroke cause***

This comparison was characterized by numerous small studies estimating a reasonably consistent positive effect. Twenty-two studies were included<sup>183 202 211 212 205 213 217 216 195 226 210 208 214 84 225 209 215 227 207 196 197 204</sup> (**see figure 3.4**). Chi-squared test for heterogeneity was significant, but the studies that were the source of the heterogeneity detected within the group<sup>183 211 205</sup> were well conducted, with clear definitions of cryptogenic stroke. Funnel plot was symmetrical.

When considering purely the younger age group, more consistent results were obtained (**figure 3.5**). Effects were homogeneous ( $p=0.29$ ), and the nine eligible studies<sup>202 211 217 216 195 208 84 207 196</sup> gave a symmetric funnel plot. Data were scarce in the older age group with each study employing a different diagnostic technique (TTE<sup>211</sup>, TEE<sup>195</sup> and TCD<sup>196</sup>), and the two most recent studies estimating a consistent non-significant effect (**figure 3.6**).

#### ***Patent foramen ovale: cryptogenic stroke vs non-stroke controls***



The heterogeneity evident in this group (see figure 3.7) enabled trials to be divided into those with a point OR of 1-2<sup>205 195 225</sup>, those with a point OR 2-4<sup>216 219 215 220 203 197</sup> and those with a point OR >4<sup>202 208 207 204</sup>. Analysis of these three groups showed a trend towards greater patient age in trials with a lower OR (p=0.08, by ANOVA). The groups did not differ in diagnostic technique, the presence of blinding, in their choice of controls or in design. The group with OR 2-4 tended to have less detailed criteria for the diagnosis of cryptogenic stroke, and one trial in each group included lacunar stroke within their 'cryptogenic' classification. A funnel plot did not suggest bias. Again, subdivision into age bands revealed much more homogeneous results, both in the young<sup>202 216 195 208 207</sup> (figure 3.8), and in the old<sup>195 203</sup> (figure 3.9).

#### ***Atrial septal aneurysm: stroke vs non-stroke controls***

For the total comparison (figure 3.10), negative studies<sup>223 224 225</sup> recruited older patients than the positive studies<sup>228 206 200 197 229 202</sup> (p=0.014). Once more, funnel plot appearance did not suggest publication bias. Comparisons in the young<sup>228 202 206 229</sup> (figure 3.11) and old<sup>228 206</sup> (figure 3.12) were homogeneous, and estimated a clear increased prevalence of ASA in stroke patients compared to controls.

#### ***Atrial septal aneurysm: cryptogenic stroke vs known stroke cause***

The heterogeneity evident in the total comparison (figure 3.13) was due to the presence of one negative/neutral study<sup>183</sup>. This study was well conducted (although blinding of echocardiographers was not mentioned) with clear definitions of cryptogenic stroke. It found an increased prevalence of ASA in lacunar stroke. The four remaining studies<sup>202 226 218 225</sup> estimate a combined odds ratio of 5.30 (2.70-10.42). Only one study defined and studied a younger age group<sup>202</sup>. No studies were available for analysis in those over 55 years.

#### ***Atrial septal aneurysm: cryptogenic stroke vs non-stroke controls***

Data are scarce for this comparison, but homogeneous results were obtained in the 'total' group<sup>202 219 225</sup> (**figure 3.14**), estimating a significant effect. Only one study has been conducted in the young<sup>202</sup>, and none in the old.

***Combination of patent foramen ovale and atrial septal aneurysm: stroke vs non stroke controls***

Homogeneous results are obtained for total patients<sup>202 223 206 200</sup> (**figure 3.15**) and in the younger group<sup>202 206</sup> (**figure 3.16**), albeit with extremely wide confidence limits. Only one study was found in the old<sup>206</sup>.

***Combination of patent foramen ovale and atrial septal aneurysm: cryptogenic stroke vs known stroke cause***

Assessment yields a significant odds ratio, with a very wide confidence interval, in the total group<sup>202 226</sup> (**figure 3.17**), and only one study in the younger group<sup>202</sup>.

Data on the final comparison for the combination of both lesions (cryptogenic stroke vs non-stroke controls) comes from a single study<sup>202</sup>.

### **3.4 Discussion**

The literature on prevalence of inter-atrial septal abnormalities in stroke is both extensive and confusing. Erroneous conclusions can be drawn from observational data on a small number of subjects viewed in isolation. A number of studies have failed to show a significant association of inter-atrial septal abnormalities with stroke in the young<sup>216 195 204 228 229</sup>. Consideration of the greater body of literature supporting an association is biased, and fails to provide proper assessment of the level of association. The data presented in this analysis allow a number of firm conclusions to be reached, in addition to suggesting areas requiring further research.

Meta-analysis of observational studies has been criticized since it can generate 'spurious precision' from disparate data sets. It may, however, clarify an extensive and confusing literature by organization and collation of the available information. Pre-defined inclusion criteria, systematic examination of trials to detect sources of heterogeneity and bias, and the consistent results found in separate age bands support the methods presented here. We did not seek unpublished data when conducting this analysis. Publication and related bias was examined by visual inspection of funnel plots, and asymmetry was not often evident. Funnel plots, however, remain an insensitive measure, open to different interpretation between observers<sup>198</sup>. Referral bias presents a major source of difficulty, particularly in those studies employing retrospective design or TEE. Some studies included only those referred for TEE, introducing bias<sup>227 212 213 223 214 200 206</sup>. Such populations are likely to be similar to those with 'embolic' stroke<sup>228</sup>. Numerous studies only investigated for, or reported, either ASA or PFO, so the associations demonstrated may partly be with both lesions in conjunction rather than with either alone. Blinding of observers was often ignored or poorly defined, which is concerning given the amount of inter-observer disagreement in diagnosis<sup>230</sup>. Such disagreement may result from different diagnostic criteria, or from methodological inconsistencies<sup>231</sup>, and may partly explain the widely different detection rates in the studies included. These ranged (for PFO) from 10%-44% for stroke, 31%-77% for cryptogenic stroke, 4%-25% for known stroke cause and 3%-22% for controls, and (for ASA) from 2-17% for stroke, 4-25% for cryptogenic stroke, 0.2-22% for known stroke cause and 0-15% for controls. Prevalence of PFO declines with advancing age, whilst average lesion size increases<sup>81</sup>. Variation in prevalence also stems from the different diagnostic techniques employed. Although the specificity and sensitivity of TCD in comparison to TEE are well defined<sup>232</sup>, TTE underestimates the presence of PFO and ASA<sup>233</sup>. The different extents to which cryptogenic stroke was investigated for and defined, and the details of that definition, did not exert any clear effect on study conclusion.

Recruitment rates were lower in TEE studies (65%-76%), with investigators experiencing difficulties with patients' consent, tolerance of the procedure, and in obtaining satisfactory images.

When classified on the basis of age and abnormality detected, published studies have been shown to demonstrate a fairly homogeneous effect, despite the differences in their design. The unequivocal finding that both PFO and ASA are associated with ischaemic stroke in the young is important. The implication for planned investigation is that PFO should be sought in the young ( $\leq 55$  years), and if found should not be regarded as incidental. Although less frequently detected, ASA is more strongly associated with ischaemic and cryptogenic stroke than PFO. Since reliance on TCD and TTE will not provide the clinician with accurate information on this and other potentially important cardiac embolic sources <sup>194</sup>, this meta-analysis supports wider use of TEE in young stroke patients.

Less work has been conducted in the older age group ( $\geq 55$  years), and the results presented highlight the areas in which data are lacking. The finding that heterogeneity within 'total' comparisons is eliminated by grouping into age bands, and that negative trials are more likely to stem from the inclusion of older patients, suggests that age exerts an effect on the relationship between inter-atrial septal abnormalities and stroke which is clinically relevant. Other stroke causes and risk factors are more likely to play their part in the old, and the association of PFO with both total and cryptogenic ischaemic stroke in those  $\geq 55$  years remains unconfirmed. The odds ratio for cryptogenic stroke compared to known stroke cause in those  $\geq 55$  years nearly reached significance, while results in the stroke vs control comparison were much more equivocal. These findings are consistent with the hypothesis that a true effect exists which is easier to detect once patients with more common causative factors have been excluded.

Does the association demonstrated in the young imply causation? Both abnormalities in conjunction are consistently more strongly associated with ischaemic stroke than either alone, and are associated with higher rates of recurrent stroke<sup>88 90</sup>. This, coupled with the reported finding that larger PFOs<sup>213 227 216</sup> and ASAs<sup>202</sup> are more strongly associated with cryptogenic stroke than smaller abnormalities, and are more likely to lead to recurrence<sup>234</sup>, suggests a 'dose-response' relationship supporting causality. In addition the relationship is biologically plausible, cerebral ischaemia or infarction resulting either by paradoxical embolism from a venous source or by in situ thrombosis at an atrial level. Despite such arguments, documentation of venous thrombosis in patients whose stroke mechanism is felt to be paradoxical embolism has proved unrewarding<sup>84</sup>.

Secondary prevention for stroke patients with PFO is a subject of considerable debate, and is addressed further in **chapter 4**. Neither open heart surgery<sup>235</sup>, nor transcatheter closure<sup>87</sup> guarantee freedom from recurrent events. A surgical series (with flawless clinical outcome) has outlined proposed criteria for surgical correction which have been reached after many years of both clinical practice and research in this area<sup>85</sup>. A large prospective series recently reported the risk of recurrent cerebrovascular events in 581 patients < 55 years with unknown stroke cause who were treated with aspirin<sup>90</sup>. After 4 years, the risk of recurrent stroke was 2.3% (95% CI 0.3 to 4.3%) among patients with PFO alone, 15.2% (95% CI 1.8 to 28.6%) among patients with PFO and ASA, and 4.2% (95% CI 1.8 to 6.6%) among patients with neither abnormality. There were no recurrences in the small (n=10) group with ASA alone. The currently unpublished Patent Foramen Ovale in Cryptogenic Stroke (PICSS) substudy of the Warfarin Aspirin Recurrent Stroke Study (WARSS) (see **chapter 1**) provides no information regarding the role of corrective techniques and does not have the statistical power to determine best medical therapy. In addition to ongoing randomised trials of closure versus antithrombotic therapy, active

investigation for atrial septal abnormalities, and large scale prospective data collection, as proposed by Kasner et al <sup>236</sup>, may improve estimation of the risks and benefits of the different therapeutic strategies available, and provide useful evidence on which to base therapeutic decisions.

Patient group	Comparison	Abnormality	Number of included studies	Experimental patients (n)	Control patients (n)	p value (Chi-squared test for heterogeneity)	Fixed effects odds ratio (95% C.I.)	Random effects odds ratio (95% C.I.)
Total (all ages)	Stroke vs non-stroke controls	▲PFO	15	2014	2020	0.00	1.57 (1.32-1.87)	1.83 (1.25-2.66)
		▲PFO + ASA	4	770	1284	0.24	5.25 (2.91-9.45)	4.96 (2.37-10.39)
		▲ASA	9	1640	1884	0.02	2.24 (1.71-2.94)	2.35 (1.46-3.77)
	Cryptogenic stroke vs known stroke cause controls	▲PFO	22	1163	1679	0.00	3.03 (2.51-3.66)	3.16 (2.30-4.35)
		▲PFO + ASA	2	135	469	0.65	20.74 (4.14-103.90)	23.26 (5.24-103.20)
		▲ASA	5	275	749	0.03	3.06 (1.82-5.15)	3.65 (1.34-9.97)

Patient group	Comparison	Abnormality	Number of included studies	Experimental patients (n)	Control patients (n)	p value (Chi-squared test for heterogeneity)	Fixed effects odds ratio (95% C.I.)	Random effects odds ratio (95% C.I.)
	<i>Cryptogenic stroke vs non stroke controls</i>	▲PFO	13	926	1747	0.00	2.76 (2.22-3.42)	2.95 (2.01-4.33)
		PFO + ASA	1	134	125	-	23.93 (3.09-185.42)	-
		▲ASA	3	401	890	0.14	4.12 (2.72-6.26)	3.98 (1.85-8.55)

**Table 3.1: Total patients** the three major comparisons are subdivided into prevalence of patent foramen ovale (PFO), prevalence of atrial septal aneurysm (ASA) and the prevalence of a combination of both abnormalities (PFO + ASA). The number of included studies in each comparative group, and the number of patients in the control and experimental groups within each comparison, are shown. A chi-squared test of heterogeneity of effects is given. Combined odds-ratios from both fixed and random effects meta-analysis are provided. If the chi-squared test of heterogeneity is positive (at a significance level of 0.1 (10%)), then the random-effects odds ratio is shown in **bold**. If it is negative, the fixed-effects odds ratio is shown in **bold**. When no data are available, the cell is marked by (-). ▲ denotes those comparisons which are illustrated by a figure.



Patient group	Comparison Abnormality Number of included studies Experimental patients (n) Control patients (n) p value (Chi-squared test for heterogeneity) Fixed effects odds ratio (95% C.I.) Random effects odds ratio (95% C.I.)
<b>Young (<math>\leq 55</math> years)</b>	Stroke vs non-stroke controls ▲PFO 9 566 456 0.31 3.10 (2.29-4.21) 3.16 (2.24-4.45)
	▲PFO + ASA 2 134 125 0.79 15.59 (2.83-85.87) 16.12 (3.02-86.13)
	▲ASA 4 277 258 0.75 6.14 (2.47-15.22) 5.56 (2.25-13.75)
	Cryptogenic stroke vs known stroke cause controls ▲PFO 9 307 164 0.29 6.00 (3.72-9.68) 5.51 (3.06-9.91)
	PFO + ASA 1 64 36 - 17.09 (2.19-133.46) -
	ASA 1 64 36 - 6.65 (1.45-30.62) -

Patient group	Comparison	Abnormality	Number of included studies	Experimental patients (n)	Control patients (n)	p value (Chi-squared test for heterogeneity)	Fixed effects odds ratio (95% C.I.)	Random effects odds ratio (95% C.I.)
	<i>Cryptogenic stroke vs non stroke controls</i>	▲PFO	5	194	272	0.33	5.01 (3.24-7.75)	5.10 (3.15-8.25)
		PFO + ASA	1	64	50	-	23.93 (3.09-185.42)	-
		ASA	1	64	50	-	19.17 (2.46-149.47)	-

**Table 3.2: Young (≤55 years) patients** the three major comparisons are subdivided into prevalence of patent foramen ovale (PFO), prevalence of atrial septal aneurysm (ASA) and the prevalence of a combination of both abnormalities (PFO + ASA). The number of included studies in each comparative group, and the number of patients in the control and experimental groups within each comparison, are shown. A chi-squared test of heterogeneity of effects is given. Combined odds-ratios from both fixed and random effects meta-analysis are provided. If the chi-squared test of heterogeneity is positive (at a significance level of 0.1 (10%)), then the random-effects odds ratio is shown in **bold**. If it is negative, the fixed-effects odds ratio is shown in **bold**. When no data are available, the cell is marked by (-). ▲ denotes those comparisons which are illustrated by a figure.

Patient group	Comparison	Abnormality	Number of included studies	Experimental patients (n)	Control patients (n)	p value (Chi-squared test for heterogeneity)	Fixed effects odds ratio (95% C.I.)	Random effects odds ratio (95% C.I.)
Old (≥55 years)	Stroke vs non-stroke controls	▲PFO	3	326	265	0.08	1.27 (0.80-2.01)	1.60 (0.63-4.06)
		PFO + ASA	1	112	273	-	5.09 (1.25-20.74)	-
		▲ASA	2	364	532	0.73	3.43 (1.89-6.22)	3.38 (1.86-6.13)
	Cryptogenic stroke vs known stroke cause controls	▲PFO	3	170	293	0.06	1.95 (1.20-3.15)	2.26 (0.96-5.31)
		PFO + ASA	-	-	-	-	-	-
		ASA	-	-	-	-	-	-

Patient group	Comparison	Abnormality	Number of included studies	Experimental patients (n)	Control patients (n)	p value (Chi-squared test for heterogeneity)	Fixed effects odds ratio (95% C.I.)	Random effects odds ratio (95% C.I.)
	<i>Cryptogenic stroke vs non stroke controls</i>	▲PFO	2	95	216	0.61	1.20 <b>(0.56-2.56)</b>	1.19 <b>(0.55-2.55)</b>
		PFO + ASA	-	-	-	-	-	-
		ASA	-	-	-	-	-	-

**Table 3.3:** Old ( $\geq 55$  years) patients the three major comparisons are subdivided into prevalence of patent foramen ovale (PFO), prevalence of atrial septal aneurysm (ASA) and the prevalence of *both* abnormalities (PFO + ASA). The number of included studies in each comparative group, and the number of patients in the control and experimental groups within each comparison, are shown. A chi-squared test of heterogeneity of effects is given. Combined odds-ratios from both fixed and random effects meta-analysis are provided. If the chi-squared test of heterogeneity is positive (at a significance level of 0.1 (10%)), then the random-effects odds ratio is shown in bold. If it is negative, the fixed-effects odds ratio is shown in bold. When no data are available, the cell is marked by (-). ▲ denotes those comparisons which are illustrated by a figure.

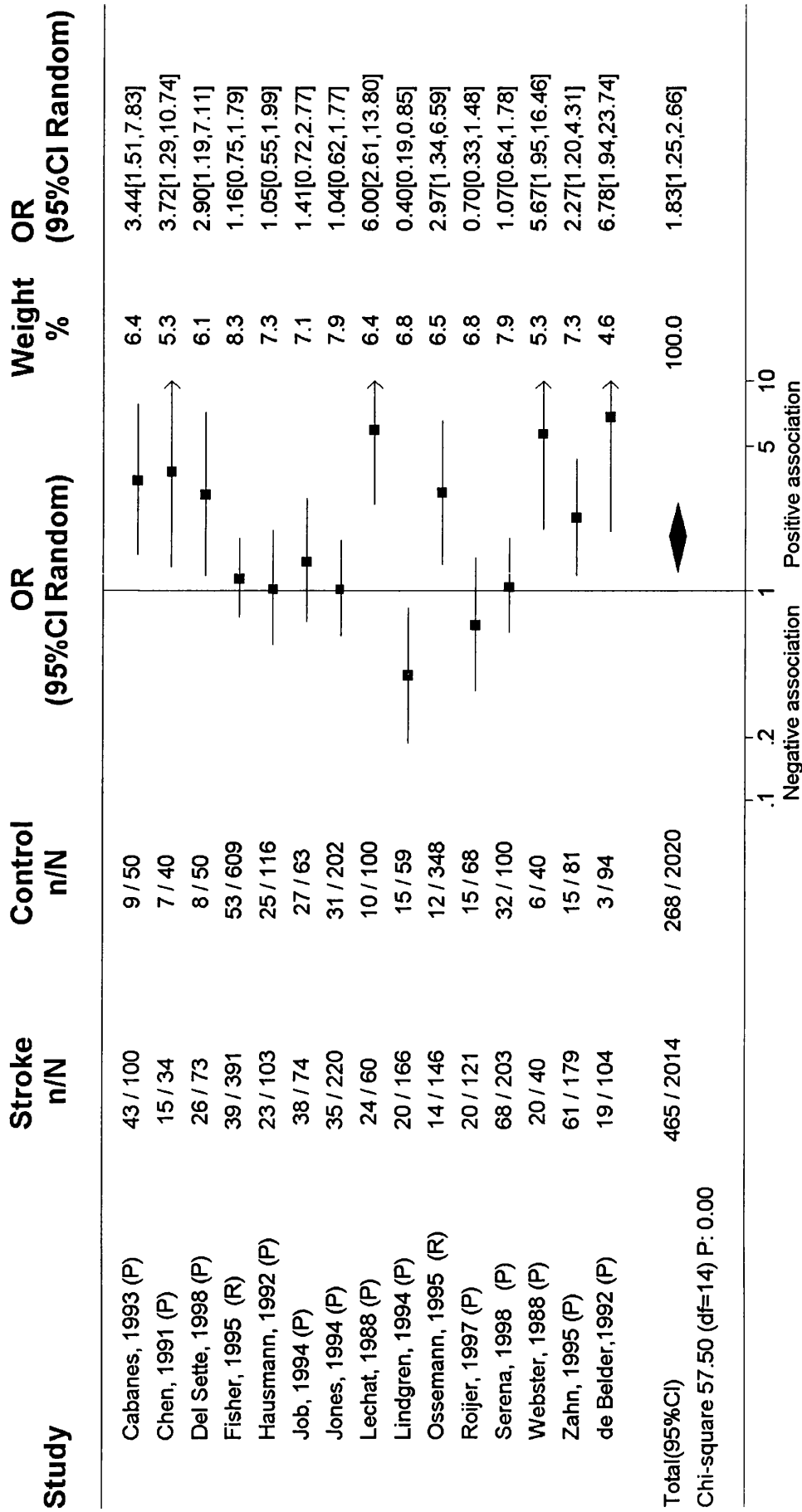


Figure 3.1: Total patients (all ages), comparing prevalence of patent foramen ovale (PFO) in ischaemic stroke (IS) patients to that in non-stroke controls (C).

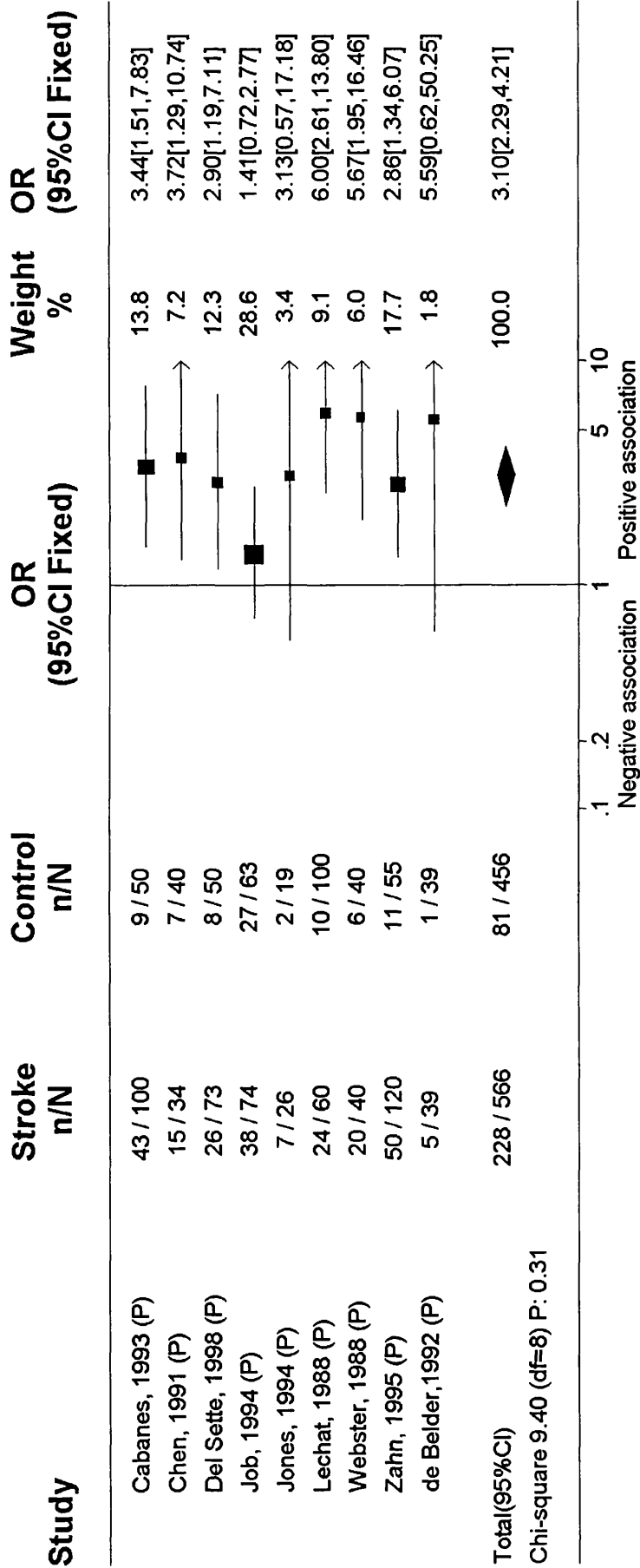


Figure 3.2: Young patients ( ≤55 years), comparing prevalence of patent foramen ovale (PFO) in ischaemic stroke (IS) patients to that in non-stroke controls (C).

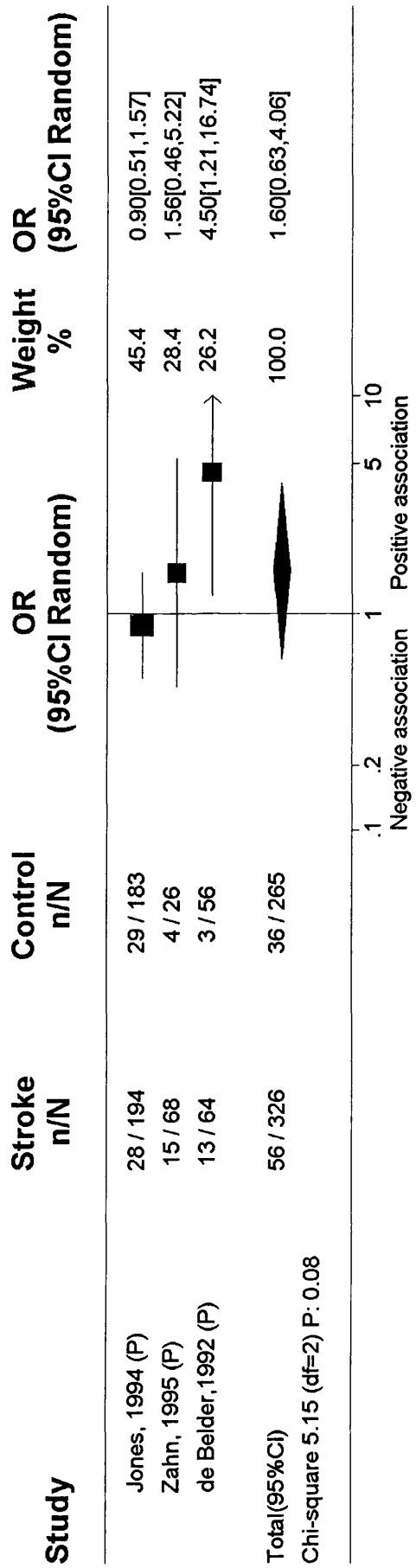
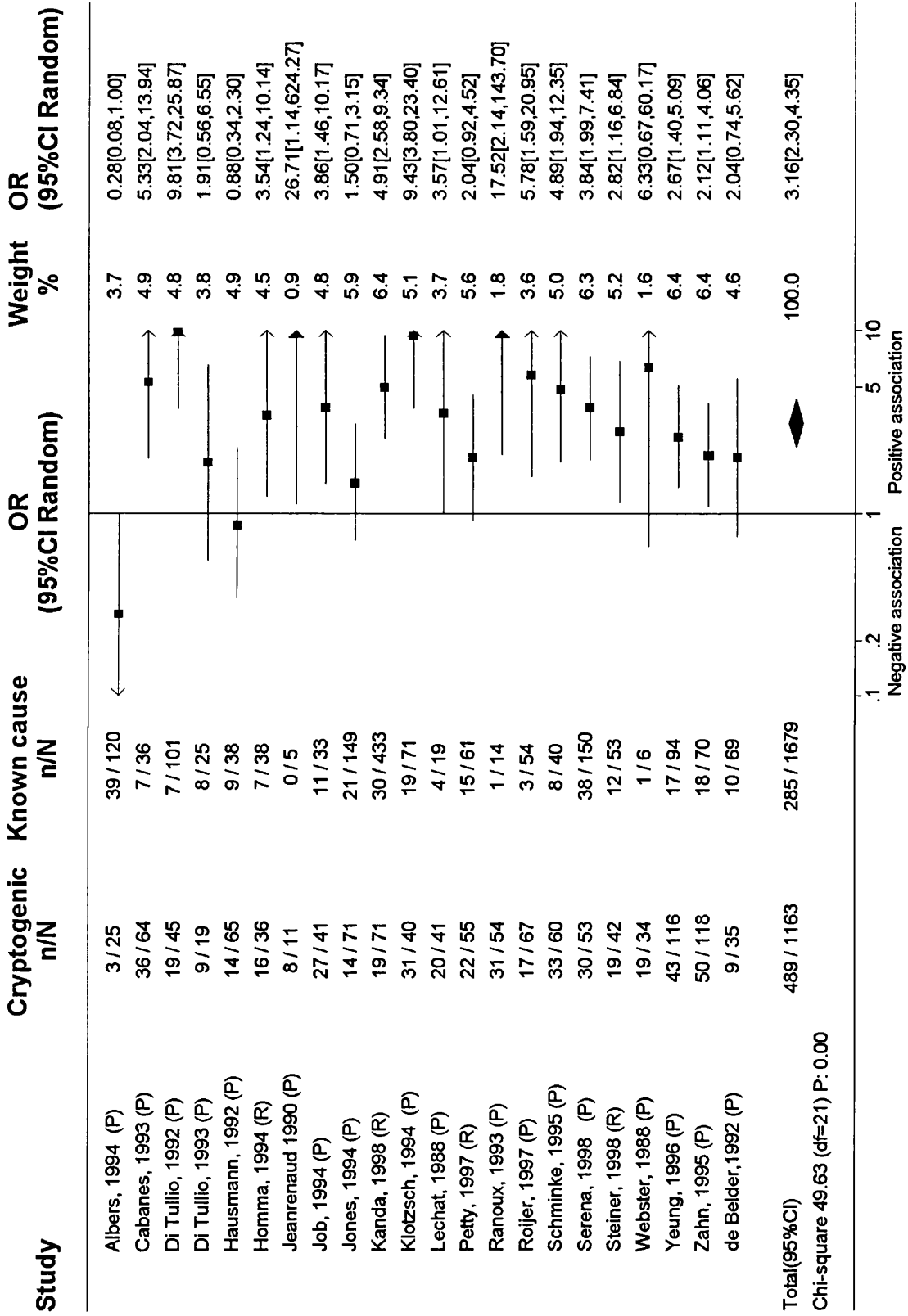
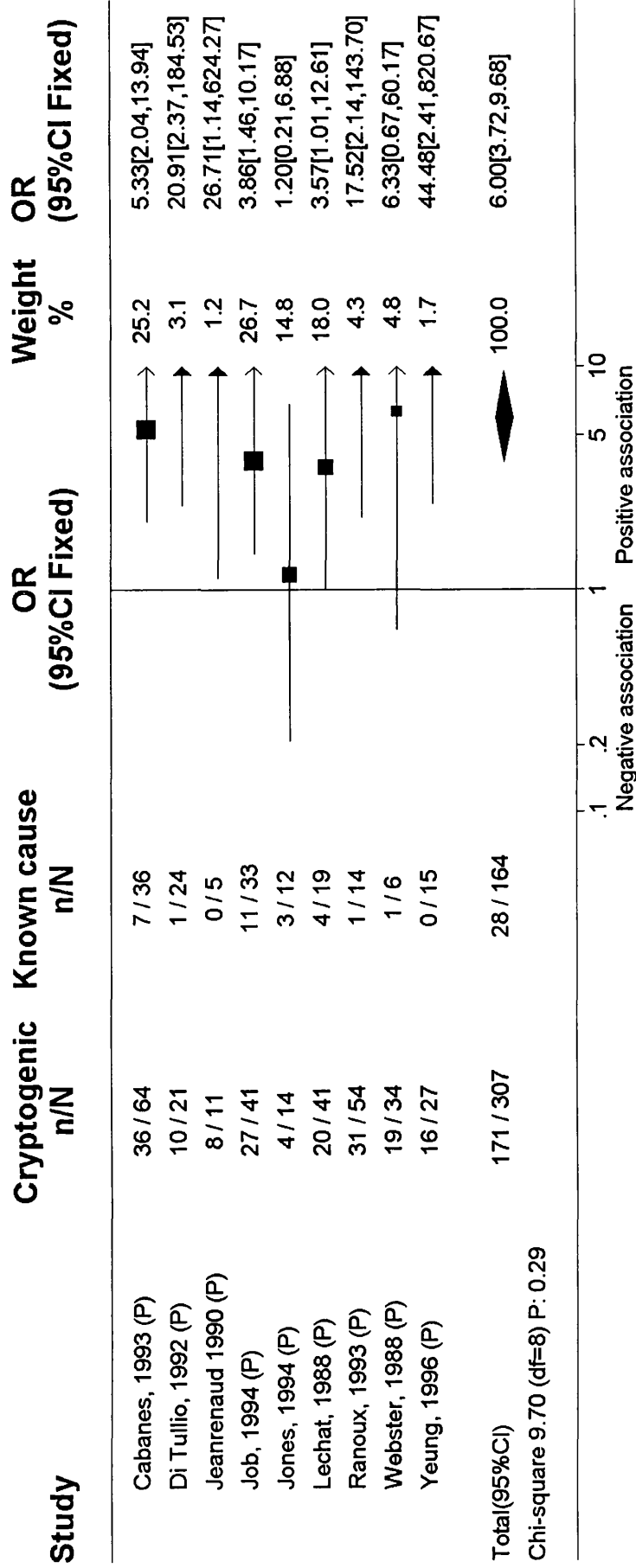


Figure 3.3: Old Patients ( $\geq 55$  years), comparing prevalence of patent foramen ovale (PFO) in ischaemic stroke (IS) patients to that in non-stroke controls (C).

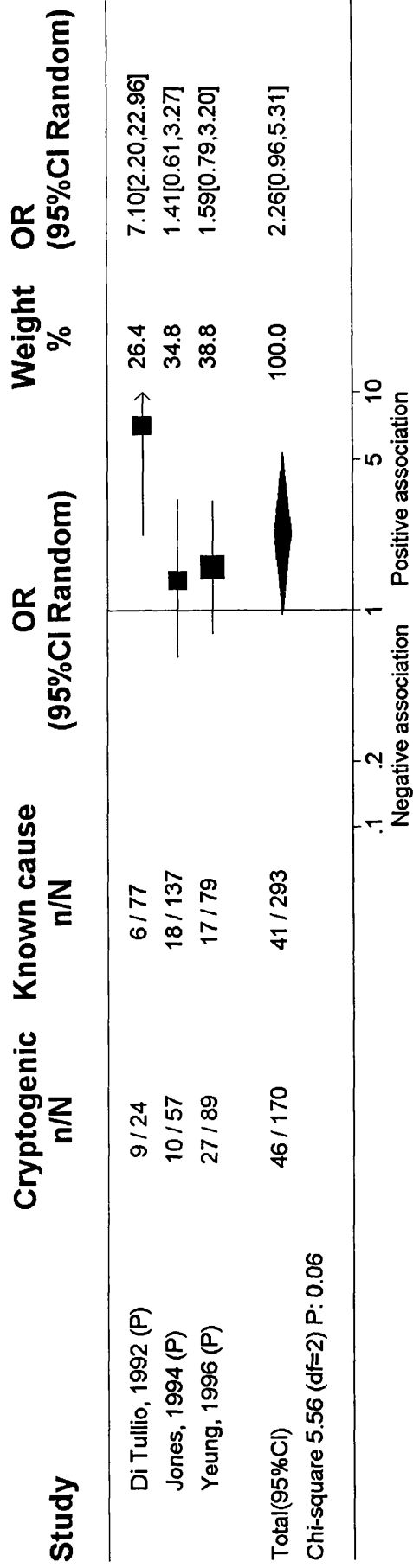


**Figure 3.4:** Total patients (all ages), comparing prevalence of patent foramen ovale (PFO) in cryptogenic stroke (CS) patients to that in patients with known stroke cause (KSC).

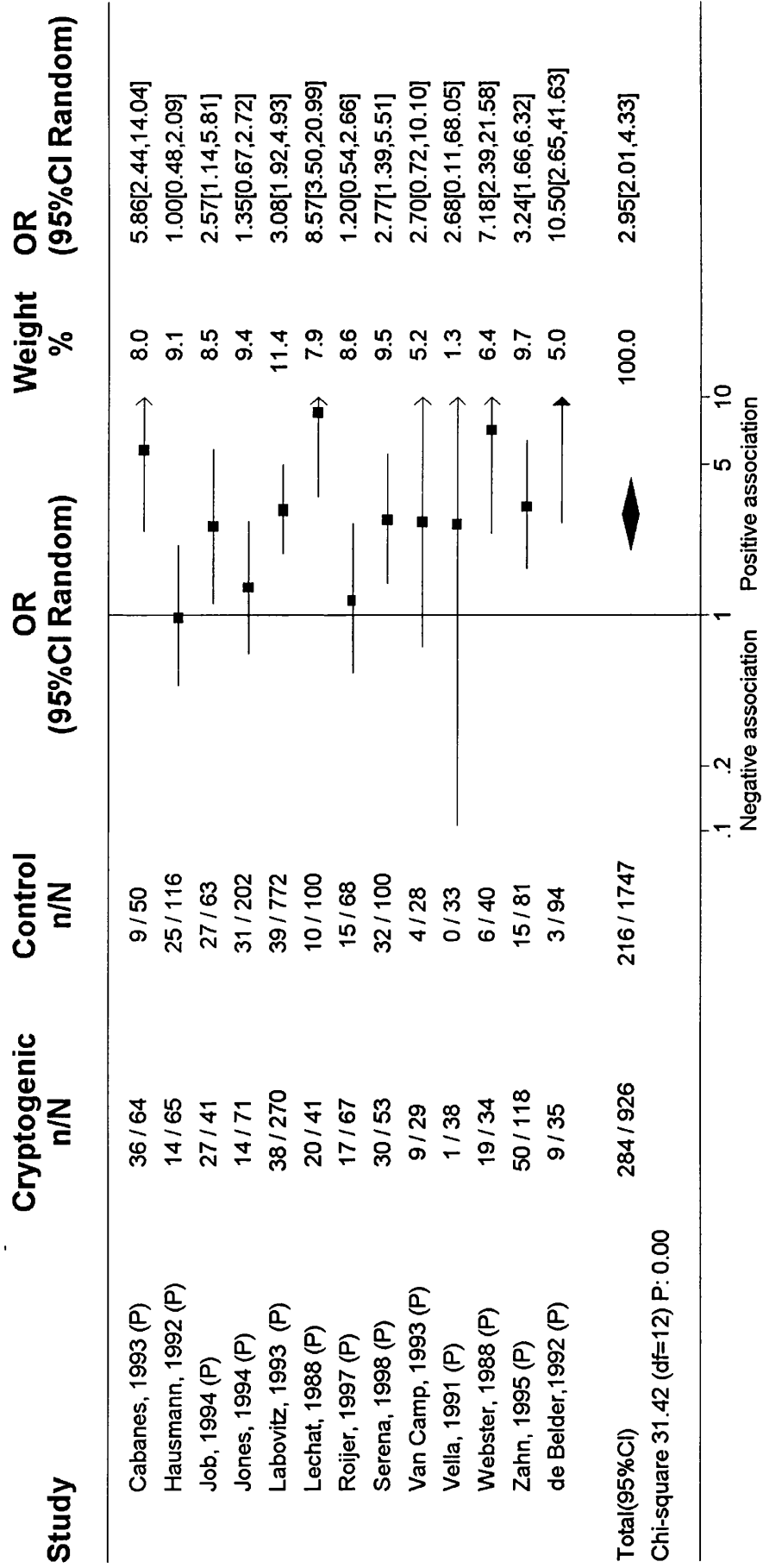




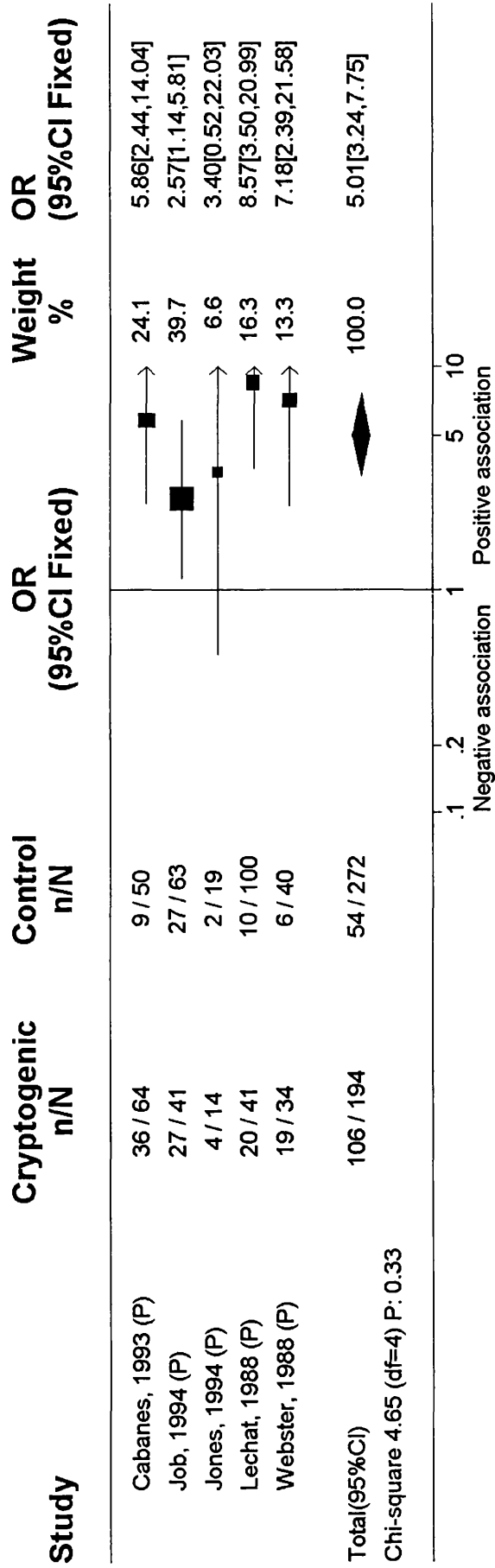
**Figure 3.5:** Young patients ( $\leq 55$  years), comparing prevalence of patent foramen ovale (PFO) in cryptogenic stroke (CS) patients to that in patients with known stroke cause (KSC).



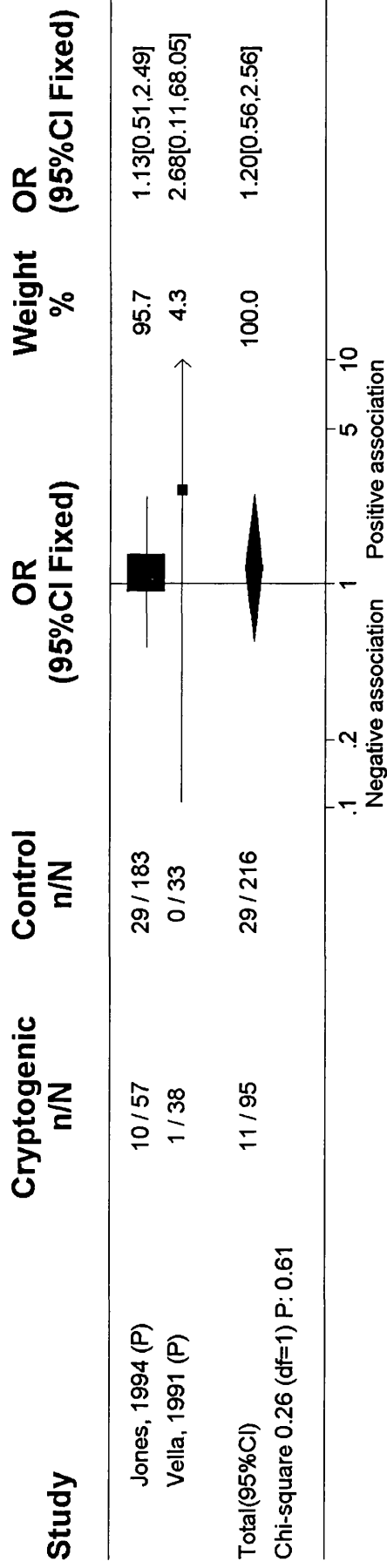
**Figure 3.6:** Old patients ( $\geq 55$  years), comparing prevalence of patent foramen ovale (PFO) in cryptogenic stroke (CS) patients to that in patients with known stroke cause (KSC).



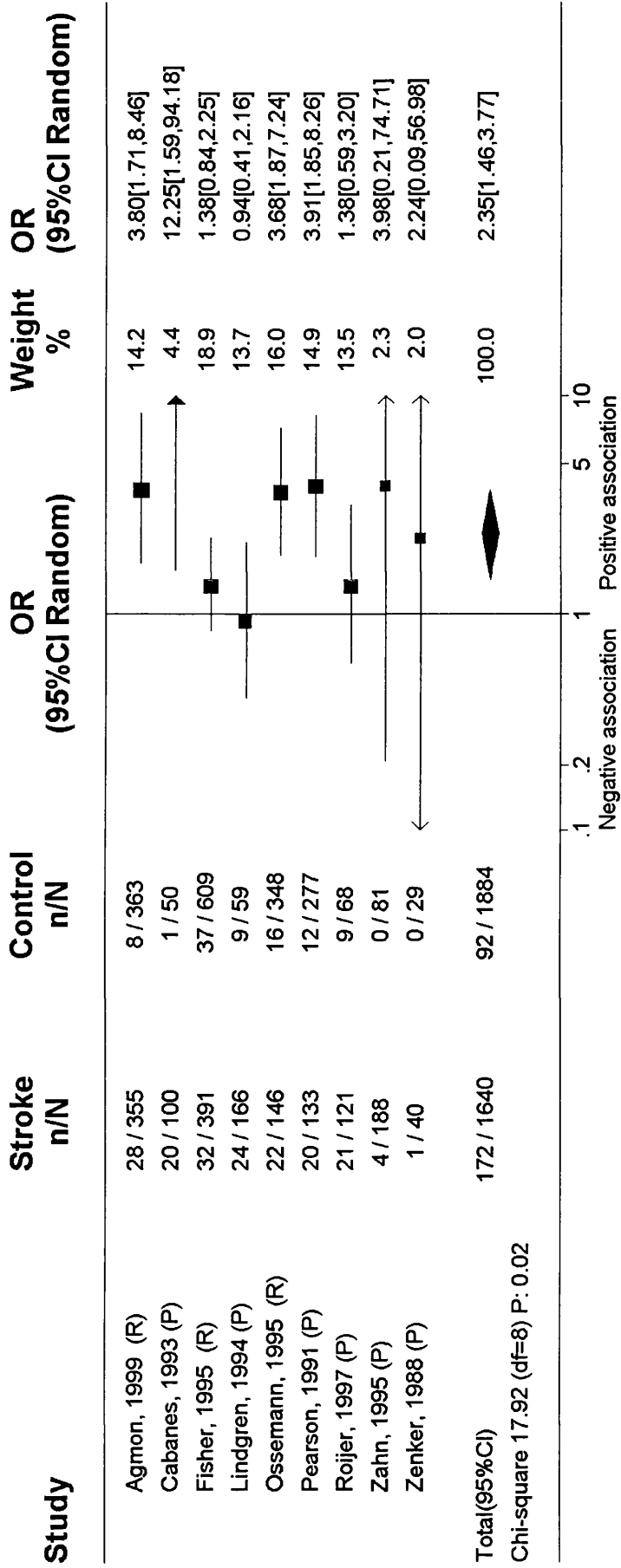
**Figure 3.7:** Total patients (all ages), comparing prevalence of patent foramen ovale (PFO) in cryptogenic stroke (CS) patients to that in non-stroke controls (C).



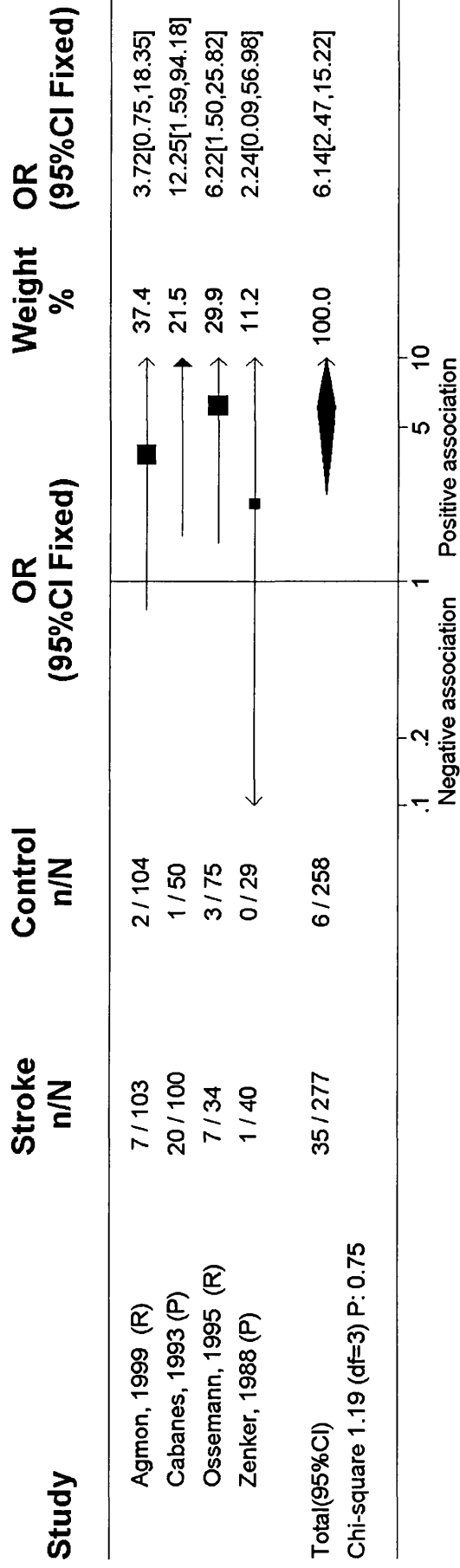
**Figure 3.8:** Young patients ( $\leq 55$  years), comparing prevalence of patent foramen ovale (PFO) in cryptogenic stroke (CS) patients to that in non-stroke controls (C).



**Figure 3.9:** Old patients ( $\geq 55$  years), comparing prevalence of patent foramen ovale (PFO) in cryptogenic stroke (CS) patients to that in non-stroke controls (C).



**Figure 3.10:** Total patients (all ages), comparing prevalence of atrial septal aneurysm (ASA) in ischaemic stroke (IS) patients to that in non-stroke controls (C).



**Figure 3.11:** Young patients ( $\leq 55$  years), comparing prevalence of atrial septal aneurysm (ASA) in ischaemic stroke (IS) patients to that in non-stroke controls (C).

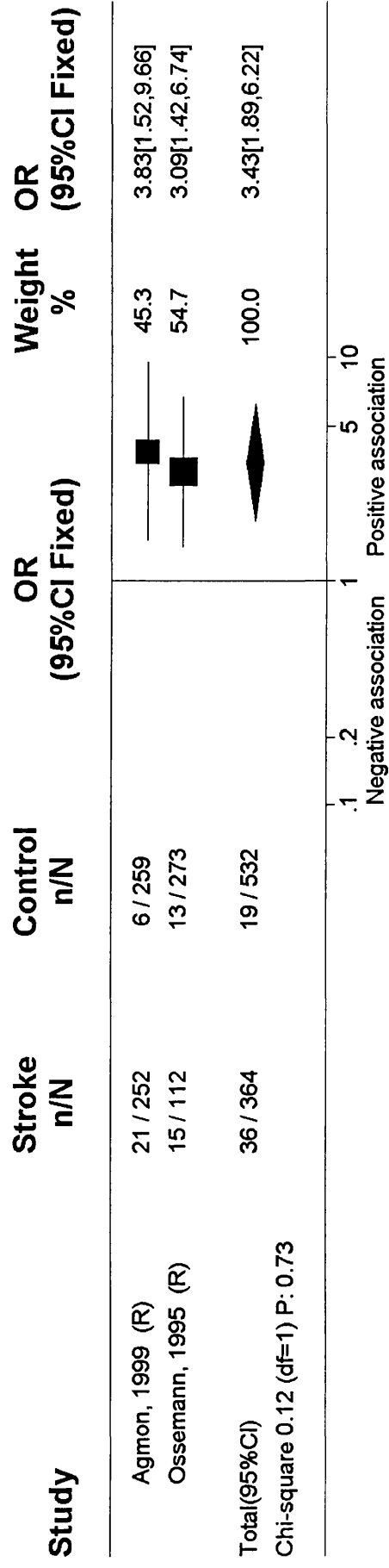
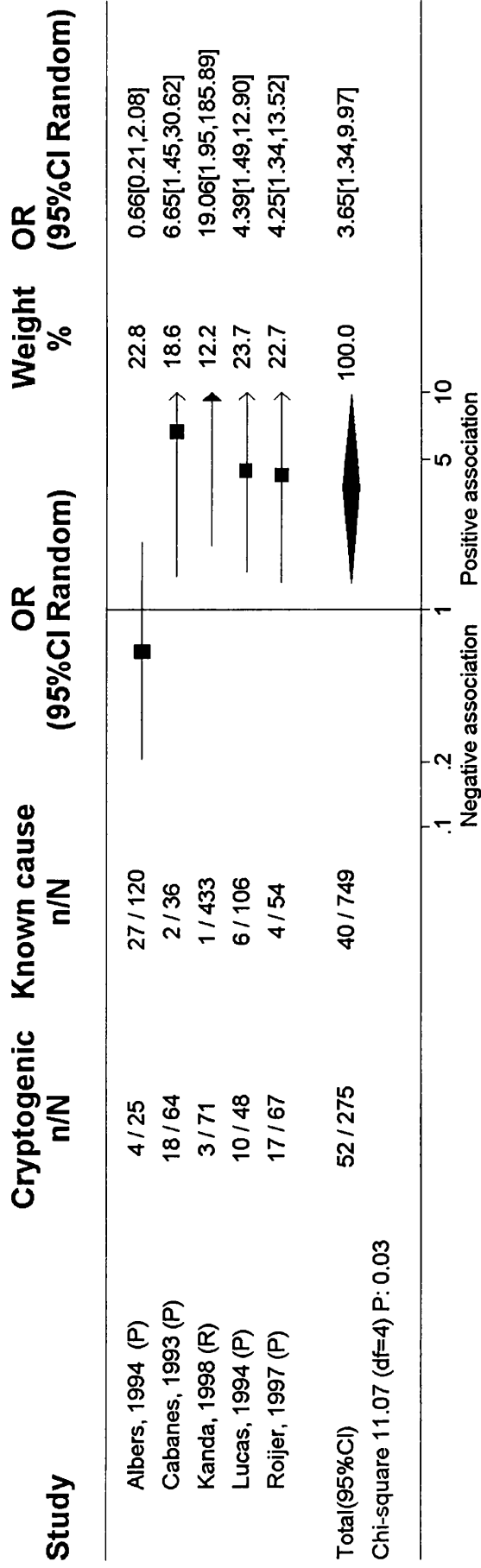
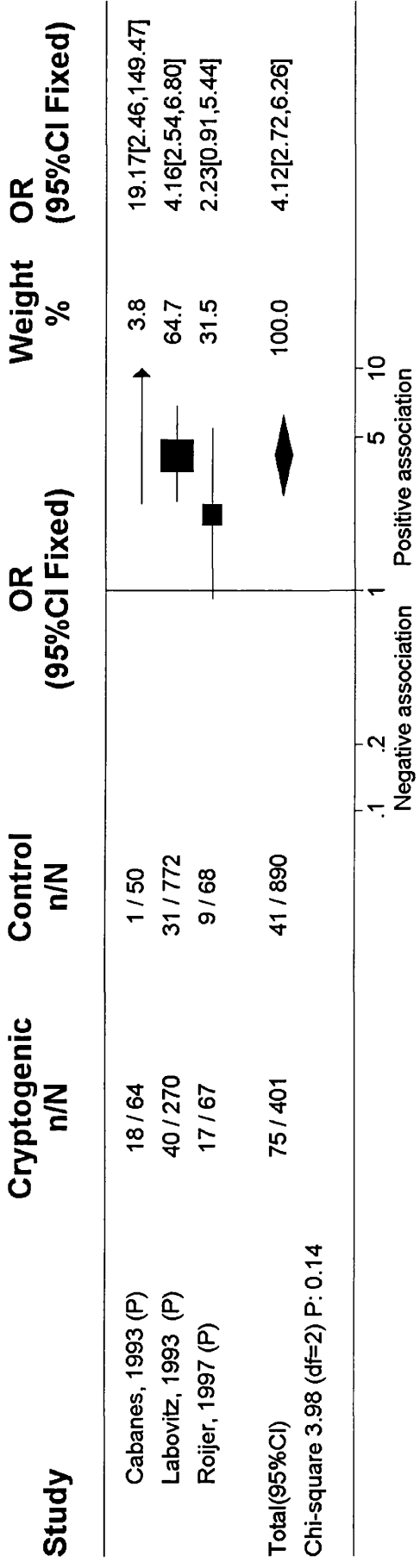


Figure 3.12: Old patients ( $\geq 55$  years), comparing prevalence of atrial septal aneurysm (ASA) in ischaemic stroke (IS) patients to that in non-stroke controls (C).

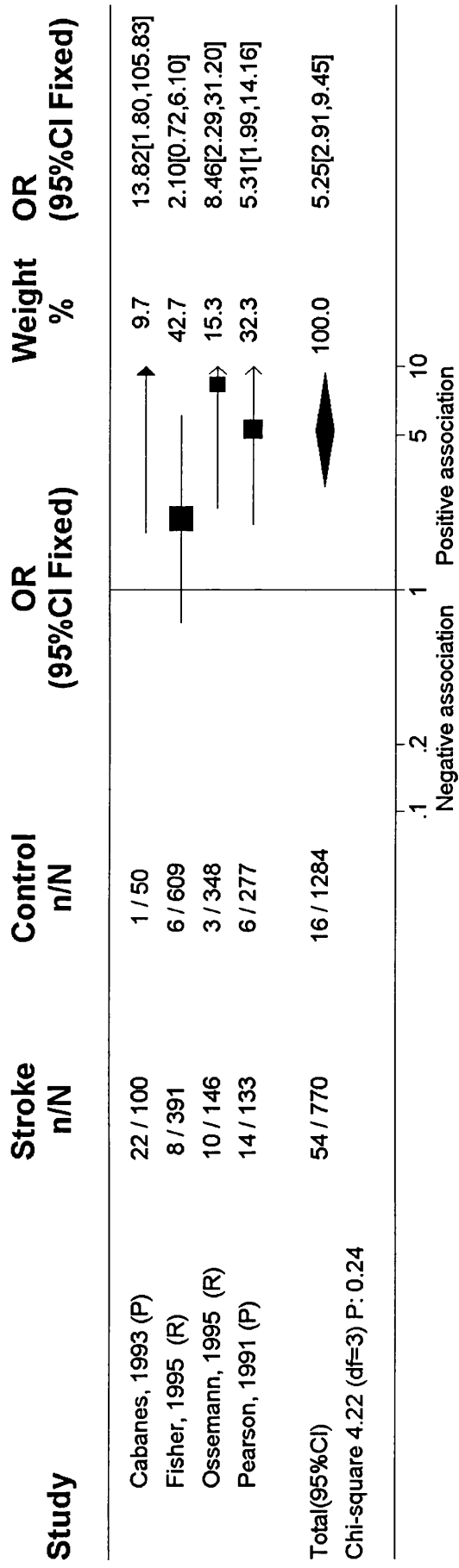




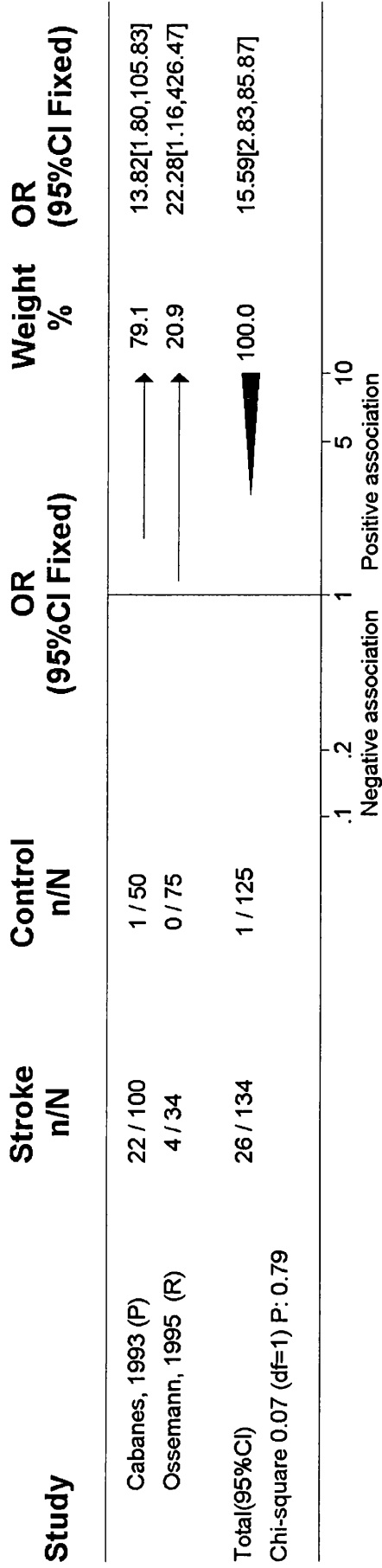
**Figure 3.13:** Total patients (all ages), comparing prevalence of atrial septal aneurysm (ASA) in cryptogenic stroke (CS) patients to that in patients with known stroke cause (KSC).



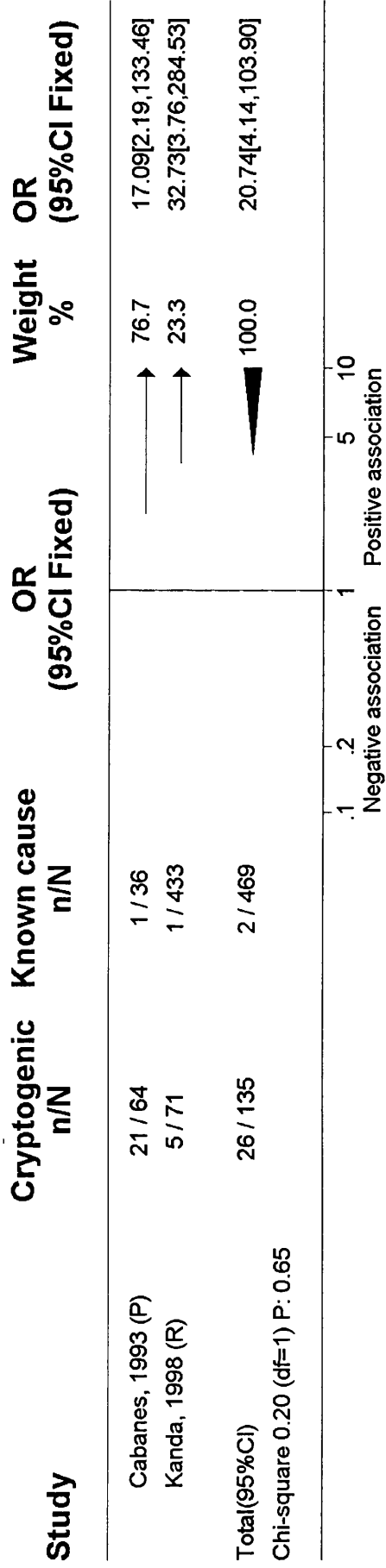
**Figure 3.14:** Total patients (all ages), comparing prevalence of atrial septal aneurysm (ASA) in cryptogenic stroke (CS) patients to that in non-stroke controls (C).



**Figure 3.15:** Total patients (all ages), comparing prevalence of the combination of both patent foramen ovale (PFO) and atrial septal aneurysm (ASA) in ischaemic stroke (IS) patients to that in non-stroke controls (C).



**Figure 3.16:** Young patients ( $\leq 55$  years), comparing prevalence of the combination of both patent foramen ovale (PFO) and atrial septal aneurysm (ASA) in ischaemic stroke (IS) patients to that in non-stroke controls (C).



**Figure 3.17:** Total patients (all ages), comparing prevalence of the combination of both patent foramen ovale (PFO) and atrial septal aneurysm (ASA) in cryptogenic stroke (CS) patients to that in patients with known stroke cause (KSC).

### Legend for Figures 3.1 to 3.17

Individual studies are listed on the left, with (P) denoting prospective studies and (R) denoting retrospective studies. Total patient numbers (N), and numbers with an atrial septal abnormality (n) are shown in the experimental and control groups for each study, and total numbers are provided at the foot of the figure. Odds ratios for individual studies are represented by black boxes (■), the size of which corresponds to the weight attached to each, and are presented with their 95% confidence intervals (thin black line). Results to the right of the line of no effect (OR=1) denote a positive association of PFO (figures 3.1 to 3.5), ASA (figures 3.6 to 3.10) or both (figures 3.11 to 3.13) with IS (figures 3.1, 3.6, 3.9, 3.10, 3.11 and 3.13) or CS (figures 3.2, 3.3, 3.4, 3.5, 3.7, 3.8 and 3.12). The combined odds ratio is presented with 95% confidence intervals at the bottom right (◀▶). A chi-square test (for heterogeneity) is shown at the bottom left in each figure.

## **Chapter 4: Investigation and management of stroke associated with patent foramen ovale**

## 4.1 Introduction

The association of patent foramen ovale (PFO) with ischaemic stroke is confirmed in patients  $\leq 55$  years, but remains uncertain in the older age group<sup>237</sup>. Atrial septal aneurysm (ASA), a less common abnormality characterised by 'bulging' of the inter-atrial septum, has been shown to be even more strongly associated with ischaemic stroke. Such associations are evident when stroke patients are compared with normal controls, and are more clearly apparent when patients with cryptogenic stroke (stroke that has no known cause) are compared to patients with a 'known' cause of stroke<sup>237</sup>. Association does not necessarily imply causation, but such a relationship is both biologically plausible (either by paradoxical embolism from a venous source or by in situ thrombosis at atrial level) and supported by observational data. Larger PFOs are more strongly associated with cryptogenic stroke than smaller lesions<sup>213 227 216</sup>. ASA size also determines the strength of its association with cryptogenic stroke<sup>202</sup>. Additionally, the combination of PFO and ASA is more strongly associated with stroke than either alone<sup>237</sup>, and more likely to lead to recurrence than either alone<sup>88 90</sup>.

At present it is unclear whether patients should be investigated for PFO – only association is proven. With no randomised data to inform a therapeutic approach, management decisions similarly lack a firm basis. Stroke is often attributed to the presence of a PFO after other causes of stroke (for example atrial fibrillation (AF)) are discounted, but whether suspicion of a pathogenic role should dictate changes in therapy is extremely difficult to answer at present. The natural history of patients with 'PFO-associated stroke' seems benign: published retrospective literature suggests a yearly recurrence rate of stroke or TIA of approximately 3% per year<sup>88 89</sup>. A recent large prospective series found a stroke recurrence rate of 2.3% (95% CI 0.3 to 4.3%) in aspirin treated patients < 55 years with isolated PFO after a follow-up period of 4 years (5.6% for stroke or TIA)<sup>90</sup>. In contrast to single lesions (which seemed



to have no impact on outcome), the presence of both ASA and PFO was predictive of stroke recurrence in this study; patients with both lesions had a recurrence rate of 15.2% (95% CI 1.8 to 28.6%) after 4 years. These data concur with the same authors' retrospective series<sup>88</sup>. Clinical context, recurrent events and large echocardiographic lesions may also characterise those patients with a poor prognosis.

Series of patients treated with medical therapy (aspirin or anticoagulants)<sup>88 89 90</sup>, surgery<sup>85</sup> and percutaneous closure<sup>86 87</sup> have provided useful information, without defining the correct approach in those patients who are investigated. The Patent foramen ovale In Cryptogenic Stroke Study (PICSS) (see chapter 1), will improve data on the natural history of medically treated patients with PFO when published in full. PICSS was not, however, powered to address the central management question of whether antiplatelet or anticoagulant therapy is more appropriate in the individual, nor did it assess the role of corrective techniques. The mean age of the randomised population in PICSS (59.7 years) was higher than that of the group for whom association has been firmly established<sup>237</sup>, and this is a significant factor in the interpretation of its findings. Mechanisms of index stroke and recurrence other than embolism from PFO occur more commonly in such older cohorts, because other stroke causes and risk factors are more prevalent. This is borne out by the discrepancy between recurrence rates in PICSS (~15% over 2 years) and the large prospective series of younger patients treated with aspirin discussed above (~4% over 2 years)<sup>90</sup>.

In the absence of randomised evidence, decision making in 'PFO associated cryptogenic stroke' is likely to depend highly on individual experience and local expertise. We wished to assess the clinical practice of physicians with specialist experience in stroke and neurology. Such an analysis may provide useful information that will aid the design of pragmatic clinical trials.

## 4.2 Methods

A clinical questionnaire was distributed to members of the British Association of Stroke Physicians (BASP), and to members of the Association of British Neurologists (ABN). A total of 860 questionnaires were posted, 136 to the 111 full and 25 associate members of the BASP and 724 to the 474 ordinary (consultants) and 250 associate (trainees in neurology) members of the ABN. Questionnaires were posted between December 1999 and February 2000, *before* the publication of the prospective stroke recurrence data detailed above <sup>90</sup>, and the presentation of the PICSS results summarised in **chapter 1**. The questionnaire is provided in the information box below.

Investigation for inter-atrial septal abnormalities may be a standard part of a policy of programmed investigation for some physicians. We wished to assess whether specialists felt that information about the presence of PFO was useful when other causes of stroke had been ruled out, both in patients older and younger than 55 years.

The management decision 'tree' is based on the criteria proposed by Devuyst and Bogousslavsky <sup>85</sup>. They postulate that surgical therapy should be reserved for patients < 60 years of age with PFO and stroke who satisfy two of the following criteria: 1) recurrent events on initial therapy or multiple ischaemic lesions on magnetic resonance imaging, 2) Valsalva manoeuvre or cough preceding stroke, 3) large (>50 microbubbles in the left atrium on transoesophageal echocardiography (TOE)) PFO and 4) associated inter-atrial septal aneurysm (ASA) <sup>85</sup>. Initially, a patient displaying none of these characteristics was considered. Subsequently, a patient displaying each of these features in isolation was presented, and alterations in management were assessed. We used 'recurrent events' rather than 'recurrent events or multiple ischaemic lesions' as a clinical scenario, and a 'large PFO' was defined as > 25 microbubbles of right-to-left shunting on TOE or transcranial

Doppler (TCD) <sup>213 234</sup>, or > 4mm diameter on TEE <sup>227</sup>. A further finding (documented venous thrombosis at the time of stroke) was felt clinically relevant in the context of 'presumed' paradoxical embolus <sup>238 84</sup>, and was included as a fifth clinical feature.

Finally, the clinician was then asked whether they would refer the patient to a cardiologist for consideration of a corrective procedure (percutaneous or surgical closure) if two or three of these factors were present. If more than one combination of clinical findings would lead to referral, the respondent was asked to state the combination they felt would confer the highest risk of stroke recurrence. The entire series of management questions was presented in the context of a patient both younger and older than 55 years.

Investigational data were tabulated, allowing division of respondents into four groups: those who would investigate all patients (YY), those who would investigate only the young (YN), those who would investigate only the old (NY), and those who would not investigate a patient in either age group (NN). Management data were tabulated for the entire group of respondents, and for each of these 'investigator' subgroups. Relevant proportions were compared using chi-squared tests (significance level 5%), and were predefined.



Would you consider referral for corrective procedure (option D) if the patient had

- 6 a combination of two of factors 1 to 5? Y (factors .... and ....) N
- 7 a combination of three of factors 1 to 5? Y (factors .... , .... and ....) N

*(if more than one is appropriate, answer with the combination which you feel confers the greatest risk of recurrent events)*

**Management:** In a older patient (> 55 years) with a small PFO (< 25 micro-bubbles of right-to-left shunt; < 4mm on TOE), no contra-indication to anticoagulation or surgery, and negative investigations for embolic stroke cause (*cryptogenic stroke*), do you:

- A prescribe an antiplatelet agent (e.g. aspirin) / combination (e.g. aspirin / dipyridamole)
- B prescribe warfarin
- C insert *vena cava* filter
- D refer for cardiological opinion regarding a corrective surgical procedure

Which course of action would you take in the following situations?

- |   |  |   |   |   |   |
|---|--|---|---|---|---|
| 1 | recurrent events on initial therapy                | A | B | C | D |
| 2 | Valsalva manoeuvre or cough preceding stroke       | A | B | C | D |
| 3 | large (>25 microbubbles; >4 mm on TOE) PFO         | A | B | C | D |
| 4 | associated atrial septal aneurysm (ASA)            | A | B | C | D |
| 5 | documented venous thrombosis at the time of stroke | A | B | C | D |

Would you consider referral for corrective procedure (option D) if the patient had

- 6 a combination of two of factors 1 to 5? Y (factors .... and ....) N
- 7 a combination of three of factors 1 to 5? Y (factors .... , .... and ....) N

*(if more than one is appropriate, answer with the combination which you feel confers the greatest risk of recurrent events)*

### 4.3 Results

A total of 261 questionnaires were returned, representing a 30% response rate. Investigation policy is summarised in **table 4.1**. The majority of respondents (60%) reported that their normal practice was to investigate 'young' patients with cryptogenic stroke, but not to investigate the old (YN investigators). Importantly, nearly a quarter of the physicians who replied (23%) do not investigate for PFO (NN investigators) at all, even in a young patient whose stroke cause is undefined.

The second portion of the analysis concerned patient management. Considering the young (<55 years) initially, the response to the first question (examining practice outwith the context of putative markers that might persuade the clinician to adopt a more aggressive management plan) is tabulated in **table 4.2a**. Responses for each of the five situations that are thought to support a paradoxical embolic mechanism or to mark a worse prognosis are given in **tables 4.2b** (recurrent events on initial therapy), **4.2c** (Valsalva manoeuvre or cough preceding stroke), **4.2d** (large PFO), **4.2e** (associated ASA) and **4.2f** (documented concurrent DVT). Questions 6 and 7 assess whether combinations of these five situations may persuade the clinician to opt for a corrective procedure, and indicate which factors are felt to confer the highest risk: they are presented in the form of a pie chart (**figure 4.1 a-d**).

Patient management in the old (> 55 years) is presented in **tables 4.3a** (uncomplicated patient), **4.3b** (recurrent events on initial therapy), **4.3c** (Valsalva manoeuvre or cough preceding stroke), **4.3d** (large PFO), **4.3e** (associated ASA) and **4.3f** (documented concurrent DVT). **Figures 4.2 a-d** illustrate the effect of multiple factors on referral decisions in the old. About 40% of respondents did not answer questions 6 and 7 in either age group.

### ***General considerations***

The occurrence of recurrent events ('clinical worsening') tended to lead to more aggressive medical management, rather than referral for lesion correction. This effect was evident across investigator groups, including those who would not investigate for PFO. 'Structural worsening' (co-existence of ASA or a large PFO) prompted referral for correction, a policy used on its own by 'non-investigators' (NN) and in combination with warfarin therapy by 'investigators' (YY). Rates of non-response were higher for questions regarding management in the old and for the question regarding a history of Valsalva manoeuvre or cough preceding stroke. A history of Valsalva manoeuvre or cough at stroke onset did not significantly alter rates of warfarin use or rates of referral for correction in any investigator group in the young. Consistent with this are the responses to questions 6 and 7, which indicate that the presence of Valsalva manoeuvre or cough at stroke onset is the least important variable when clinicians determine suitability for a corrective procedure. Use of inferior vena cava filter was low in all groups and for all clinical scenarios.

### ***Total group: uncomplicated scenario***

Antiplatelet therapy alone was chosen as an initial strategy by 47% of respondents in the older age group (>55 years), and by 33% of respondents in the younger age group (<55 years) ( $p < 0.01$  for comparison of proportions). However, antiplatelet therapy remained more popular than warfarin for initial *medical* management in young uncomplicated patients (51% vs 17%,  $p < 0.01$ ). A higher proportion of all respondents refer for an opinion regarding correction first-line in the young than the old (42% vs 18%,  $p < 0.01$ ). Warfarin usage did not differ significantly between young and old (18% vs 13%,  $p = 0.12$ ).

**Total group: recurrent events**

If cerebrovascular events recur, less than 5% of respondents would leave a patient of any age on antiplatelet therapy alone. There is a trend to higher use of a combination of both warfarin and correction in the young compared to the old ( $p=0.05$ ). Referral for correction, alone or in combination with antiplatelet therapy, is more common in younger patients ( $p<0.01$ ), while there was a trend to higher use of warfarin alone in older patients ( $p=0.06$ , comparing young and old).

**Total group: large PFO or concomitant ASA**

In both age groups, warfarin was used by ~17% of respondents for individuals with large PFOs or with co-existent ASAs. It was used alone in only ~10%, representing no change from use in the uncomplicated patient, and in combination with referral for correction by ~7%. In general, such 'structural findings' prompted referral for correction: for a patient with a large PFO, 57% (young) vs 45% (old) would refer ( $p=0.01$  for comparison), and for a patient with concomitant ASA, 62% (young) vs 44% (old) would refer ( $p<0.01$  for comparison).

**Total group: concurrent DVT**

Concurrent DVT commonly led to treatment with warfarin both for patients <55 years (62%) and >55 years (53%). Inferior vena cava filter insertion, while remaining rare, was most common for this scenario (7% in both age groups). In those <55 years referral for correction was less common than in uncomplicated patients (24% vs 43%,  $p<0.01$ ), rates in the old remaining unchanged (19% vs 18%,  $p=0.77$ ).

**YY investigators: uncomplicated scenario**

Aspirin was chosen as the most appropriate medical management in both young and old age groups, although YY investigators were the group least likely to use aspirin monotherapy as first line. Referral for correction was



chosen by 54% of respondents for patients <55 years and by 37% of respondents for patients >55 years (a significant increase when compared to the total group,  $p<0.01$ ). Warfarin was used as an initial therapy in ~20% of patients (young or old).

***YY investigators: recurrent events***

More than half of the YY group would now prescribe warfarin for a patient of any age. 51% of YY investigators would refer for correction in the young, though are a little more reluctant to do so in the old (39%).

***YY investigators: large PFO or concomitant ASA***

In the young, 63% would refer for a corrective procedure with a large PFO, and 63% would refer for correction with a concomitant ASA. These rates are not significantly altered by patient age. Warfarin is more likely to be used in combination with surgery than on its own in both age groups.

***YY investigators: concurrent DVT***

73% would use warfarin in the young, and 63% in the old. This is used in combination with surgery by only a minority (15% and 10% respectively).

***YN investigators: uncomplicated scenario***

Aspirin monotherapy was significantly more common in the older age group (54% vs 35%,  $p<0.01$ ), and referral for correction more common in the young (43% vs 16%,  $p<0.01$ ).

***YN investigators: recurrent events***

Policy in the old now switches to warfarin in the majority (54%), and cardiological referral in a quarter. This represents a lower referral rate than YY investigators (YN=25%; YY=41%;  $p<0.05$ ), but a shift towards active management strategies by doctors who would not routinely investigate this

age group. In the young, referral rates did not differ from those seen in the YY group (YN=44%; YY=51%;  $p=0.43$ ).

***YN investigators: Large PFO or concomitant ASA***

Rates of surgical referral were higher for the young than the old (for large PFO, 58% young, 40% old; for ASA 65% young, 51% old). However, referral was significantly more common in the older age group than for the uncomplicated patient aged over 55 years ( $p < 0.01$  for either abnormality).

***YN investigators: associated DVT***

Warfarin monotherapy was again the most popular choice, with referral for correction being recommended by 27% in the young and 20% in the old.

***NN investigators: uncomplicated scenario***

NN investigators showed the highest rate of non-response (30%). Nearly a quarter of respondents in this group felt referral to be appropriate first-line in the young.

***NN investigators: recurrent events***

High warfarin usage (~40%) was observed in both the young and old. Referral for correction was common in the young (41% of respondents). Referral for correction was also common in the old, where significantly more patients were referred than in the YN group (33% vs 25%,  $p=0.01$ ).

***NN investigators: Large PFO or concomitant ASA***

Rates of referral in the NN group were not significantly different from those in the YY group. In the young, 63% of YY investigators and 63% of NN investigators would refer for a corrective procedure with a large PFO. 63% of YY and 65% of NN investigators would refer for correction with a concomitant ASA. The age of the patient had no appreciable effect on decision making.

### ***NN investigators: associated DVT***

NN investigators differed from other subgroups in their rate of cardiological referral for patients with associated DVT. 34% of YY investigators regard referral as part of their management plan in this situation compared to 13% of NN investigators ( $p=0.0164$ ). Warfarin is used by about half of the respondents in both age groups, and 7% would recommend insertion of an inferior vena cava filter.

### ***Questions 6 and 7***

For a patient <55 years 49% of clinicians answered 'yes' to question 6. The majority of these responders had recommended referral on the basis of one abnormality in the previous series of questions (121/130, 93%). Moreover, the majority of *non-responders* to question 6 had also recommended referral on the basis of a single 'poor prognostic marker' (69/105, 66%). Thus, 72% would refer with one clinical indicator of likely recurrence, and 76% would refer on the basis of two abnormalities or fewer. Using data from question 7, 78% would recommend referral with three abnormalities or fewer. The older age group are thought to merit referral less, although response rates were similar: 64% with two abnormalities or fewer, and 67% with three abnormalities or fewer. There remains a substantial minority who do not feel that multiple clinical or structural characteristics would persuade them to seek a cardiological opinion: even with 3 factors present, 17% would not refer a patient >55 years.

The factors that are felt by responders to questions 6 and 7 to represent the greatest risk of recurrent events are illustrated in **figure 4.1b**, **figure 4.1d**, **figure 4.2 b** and **figure 4.2d**. No weighting is given to the order in which the responses were made. A large PFO was consistently felt to be the most important factor in decisions regarding lesion correction in the complicated patient, followed closely by the presence of recurrent events and then by a concurrent ASA. The presence of a large PFO combined with an ASA was felt

to be the combination of two factors that most merited referral for correction (19 respondents for patients < 55 years, 15 respondents for patients > 55 years), followed by the combination of recurrent events and a large PFO.

#### 4.4 Discussion

Questionnaires examine practice only in those who respond to them and can never properly illustrate clinical management situations: each patient is different, and in real life we are not restricted to a finite set of responses. However, each physician has his or her own concept of a 'reasonable' investigation and management plan in clinically defined groups of individuals, based on their experience, their teaching, and their assessment of pertinent literature. There are many controversial areas of medicine where the collective opinion of those involved in making 'difficult' decisions can help to guide those with less experience. Furthermore, a proper assessment of practice in an area in which no consensus has been reached informs the design and planning of pragmatic clinical trials.

Observational studies and indirect analyses have failed to provide clarity for clinicians in PFO associated unexplained stroke, and randomised evidence is badly needed. Decision-analysis models have been used to simulate the decision-making process<sup>239</sup>. As is necessary in such exercises numerous assumptions were made, including that paradoxical embolism *caused* stroke in the hypothetical cohort of patients, that the risk of stroke recurrence remained constant over time, and that the efficacy of anticoagulation (compared with aspirin) would equal that seen in the major trials of patients with AF. The authors concluded that if the estimated risk of paradoxical stroke recurrence exceeded 0.8% per year, anticoagulation or surgical closure represented the preferred strategies. The annual stroke or TIA recurrence rate does not differ between the published series of medical therapy (aspirin or warfarin) (3.4%<sup>88</sup> and 3.8%<sup>89</sup>) and percutaneous closure (3.4%<sup>86</sup>), but is

clearly above the level at which decision analysis would favour 'therapeutic abstention'.

The most common investigation group in this study (YN investigators) reflect the balance of observational evidence <sup>237</sup>. A trend to association is, however, evident in the older age group, particularly (as in this questionnaire) when a cryptogenic group is defined <sup>237</sup>, and investigation should not be avoided on the basis of age alone. Management decisions seem less dependant on age: for the complicated patient detailed in questions 6 and 7, responses for those patients older and younger than 55 are strikingly similar.

The questionnaire was worded with the aim of reflecting the practical decisions presented by clinical practice. Respondents were asked whether they would 'refer to a cardiologist for consideration of a corrective procedure', rather than whether they would *recommend correction*. This may explain the high level of referral in uncomplicated patients <55 years with a small PFO (42%). Antiplatelet therapy was the preferred initial strategy in those over 55 years. 'Structural worsening' (a large PFO or a concomitant ASA) precipitated referral, whereas 'clinical worsening' (recurrent events) led to the prescription of warfarin.

Non-responders were more common for questions regarding management in the older age group, probably reflecting either uncertainty about management in this group, or respondents tiring during completion of the questionnaire. Fatigue may also have contributed to the similarity in response to questions 6 and 7 in the old. Alternatively, age may be discounted (or relatively less important) when a patient is regarded at high risk of recurrence. Response to the question about Valsalva manoeuvre was also low, perhaps indicating that respondents interpret this aspect of the history as less 'concrete' than the others addressed. It is unfortunate that individuals responding to the questionnaire were not classified by their background (ABN or BASP) or their

level of experience (consultant or trainee), since it would have been interesting to assess whether any significant differences were found between such groups.

Often before requesting an investigation we are asked 'will it change your management?' This is a reasonable question: it guards against excessive use of investigations, limits costs and saves patients from unnecessary distress. A striking finding in this analysis is that those individuals who would not investigate a patient for PFO (NN investigators) reported similar rates of warfarin use and referral for closure to YY investigators. For these individuals, knowledge about PFO clearly *would* change management plan, and avoidance of investigation is not serving the best interests of the patients under their care. A change in management is not the only consideration in deciding whether to investigate patients for PFO after stroke: advice about natural history can be given from observational data and the documented presence of PFO may be helpful if a patient returns with further symptoms suggestive of stroke disease.

Because management *does* change even in non-investigators, the results of this analysis support more thorough investigation of stroke patients. The nature of the stroke lesion and the presence of co-existent risks and causes should influence investigation strategy. In cryptogenic (unexplained) stroke, PFO should be sought, certainly in the young and possibly in the old, and while TOE is the 'gold standard' investigation, a useful screening test for clinicians with limited access to it is TCD with microbubble injection. If PFO is found, a TOE should be requested, so that lesion size can be more closely defined and the presence of ASA determined. Clinical evidence of DVT should be sought and investigated if detected. Ultrasound or venographic investigation of patients without symptoms or signs of DVT is likely to be unrewarding<sup>84 240</sup>. Positive investigations for thrombophilia may lead to the prescription of warfarin in patients known to have a PFO<sup>241</sup>, but such

decisions (without concurrent evidence of DVT) lack any evidence base. It would have been interesting to document current practice in this area, but thrombophilias are a heterogeneous group of disorders, making such an assessment extremely difficult.

The design of therapeutic trials must reflect the management practices detailed here. Stratified randomisation techniques, informed by data from this analysis and from recent prospective observational studies<sup>90</sup>, may permit a more inclusive approach that takes patient risk into account before randomisation. A consensus for patients with cryptogenic stroke and PFO does emerge from analysis of expert opinion, which could be used to inform the design of hospital protocols and local guidelines. Initial management should consist of antiplatelet therapy, commonly aspirin. There is some evidence that dipyridamole is effective in the prophylaxis of thromboembolic events<sup>242</sup>, and in combination with aspirin was superior to aspirin monotherapy in the ESPS-2 trial<sup>32</sup>. A case could therefore be made for the first-line use of aspirin and dipyridamole in patients with inter-atrial septal abnormalities. The recent prospective recurrence data<sup>90</sup>, published *after* circulation of this questionnaire, concur with a conservative approach - isolated PFO lesions appeared to have no effect on stroke recurrence.

If investigations document large PFO or concomitant ASA, referral for lesion correction should be considered, particularly if multiple events have been documented. PFO and ASA in conjunction are significantly predictive of recurrence in both retrospective<sup>88</sup> and prospective<sup>90</sup> series. The recently published prospective study<sup>90</sup> failed to confirm previous reports<sup>234</sup> that the degree of shunting was a significant predictor of recurrence. The optimal technique for correction is not yet defined. Recent series of patients treated with percutaneous closure have showed that persistent shunt is predictive of recurrence<sup>86 87</sup>. Reported rates of persistent shunt after open heart surgery are lower than those after percutaneous closure<sup>86 85</sup>, but surgery involves the

attendant risks of cardiopulmonary bypass.

If clinical events recur the first consideration should be a switch to warfarin: the aetiology of recurrence may not be the PFO, an abnormality which is found in approximately 25% of the population<sup>81</sup>, and warfarin therapy is appropriate prophylaxis for all sources of embolism. The presence of DVT should also lead to the use of warfarin. Therapy may be deferred if haemorrhagic transformation is a concern in the acute phase of stroke. The trend to longer periods of anticoagulation for DVT, on the basis of randomised data<sup>243</sup>, seems even more prudent when there is a risk of recurrent stroke as well as venous thromboembolism. Whether and when therapy should stop remains unclear, and should be influenced by clinical course. The use of corrective techniques in combination with warfarin in a patient known to have a DVT was uncommon in respondents to the questionnaire. Such reluctance may reflect the fact that the period during which the patient is most at risk of stroke recurrence will be covered by warfarin therapy for venous thrombosis, making correction less of a priority. Also, although it provides support for a paradoxical embolic mechanism, DVT may occur as a consequence of stroke.

Many respondents who returned but did not fully complete the questionnaire wrote that they would leave all the decisions which were addressed in the questionnaire to a cardiologist, from whom they would seek advice as a matter of course. Such a policy means that the secondary prevention of stroke is planned by cardiologists, and seems incongruent with other areas of neurological practice; we do not leave decisions regarding appropriate use of carotid endarterectomy to vascular surgeons. The secondary prevention of cerebral infarction should be the realm of neurologists and stroke physicians, in consultation with cardiologists and cardiothoracic surgeons. Moreover, clinical trials in this area should be initiated, designed and conducted by stroke specialists, so that some of the real uncertainty illustrated here may be addressed.



Response Group	Investigate Young?	Investigate Old?	Number (% of complete investigation responders)
Group 1	Y	Y	41 (17%)
Group 2	Y	N	145 (60%)
Group 3	N	Y	0 (0%)
Group 4	N	N	54 (23%)

**Table 4.1: Investigation policy** Respondents are grouped by their differential response to a question asking whether they would investigate a cryptogenic ischaemic stroke patient < or > 55 years (see information box for full question). 21 respondents did not completely answer the investigation question.



group	no resp -onse	single response				double response				treble response				A				
		A	B	C	D	A	B	C	D	A	B	C	D		A	B	C	D
<b>Total</b>	<b>45</b> (17%)	9 (3%)	91 (35%)	6 (2%)	72 (28%)	5 (2%)	26 (10%)	3 (1%)	0	0	0	0	1 (0%)	3 (1%)	0	0	1 (0%)	
<b>(1)</b>	<b>5</b> (12%)	1 (2%)	14 (34%)	0	12 (29%)	0	7 (17%)	1 (2%)	0	1 (2%)	0	0	1 (2%)	0	0	0	0	
<b>YY</b>																		
<b>(2)</b>	<b>14</b> (10%)	7 (5%)	56 (39%)	4 (3%)	45 (31%)	3 (2%)	12 (8%)	1 (1%)	0	0	0	0	0	3 (2%)	0	0	0	
<b>YN</b>																		
<b>(3)</b>	<b>-</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<b>NY</b>																		
<b>(4)</b>	<b>13</b> (24%)	1 (2%)	17 (31%)	1 (2%)	13 (24%)	2 (4%)	6 (11%)	1 (2%)	0	0	0	0	0	0	0	0	0	
<b>NN</b>																		

**Table 4.2b: Management policy (< 55 years), recurrent events on initial therapy.** Responses are documented for all respondents and then for each investigation subgroup (YY, YN, NY and NN). Responses are categorised using the key from the questionnaire provided in appendix 3. Thus, A = antiplatelet agent, B = warfarin, C = insert vena cava filter and D = refer for cardiological opinion. Respondents who circled more than one management option are characterised by the 'double', 'treble' and 'quadruple' response columns. Percentages of the total number, or subgroup number for the final four rows, are also provided.

group	no resp -onse	single response				double response				treble response				A				
		A	B	C	D	A	B	C	D	A	B	C	D		A	B	C	D
<b>Total</b>	<b>63</b> (24%)	44 (17%)	43 (16%)	7 (3%)	78 (30%)	11 (4%)	9 (3%)	1 (0%)	0	0	0	2 (1%)	0	0	1 (0%)	1 (0%)	1 (0%)	1
<b>(1)</b> <b>YY</b>	<b>8</b> (20%)	5 (12%)	7 (17%)	1 (2%)	16 (40%)	0	2 (5%)	0	0	0	0	0	0	0	1 (2%)	0	0	1 (2%)
<b>(2)</b> <b>YN</b>	<b>22</b> (15%)	30 (21%)	30 (21%)	3 (2%)	44 (30%)	7 (5%)	6 (4%)	1 (1%)	0	0	0	1 (1%)	0	0	0	1 (1%)	0	0
<b>(3)</b> <b>NY</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>(4)</b> <b>NN</b>	<b>18</b> (33%)	8 (15%)	4 (7%)	3 (6%)	17 (31%)	3 (6%)	1 (2%)	0	0	0	0	0	0	0	0	0	0	0

**Table 4.2c: Management policy (< 55 years), Valsalva manoeuvre or cough preceding stroke.** Responses are documented for all respondents and then for each investigation subgroup (YY, YN, NY and NN). Responses are categorised using the key from the questionnaire provided in appendix 3. Thus, A = antiplatelet agent, B = warfarin, C = insert vena cava filter and D = refer for cardiologist opinion. Respondents who circled more than one management option are characterised by the 'double', 'treble' and 'quadruple' response columns. Percentages of the total number, or subgroup number for the final four rows, are also provided.

group	no resp -onse	single response				double response				treble response				A				
		A	B	C	D	A	B	C	A	A	B	C	D		A	B	C	D
<b>Total</b>	<b>56</b> (21%)	26 (10%)	26 (10%)	3 (1%)	117 (45%)	11 (4%)	18 (7%)	3 (1%)	0	0	0	0	0	1	0	0	0	0
<b>(1)</b> <b>YY</b>	<b>5</b> (12%)	5 (12%)	5 (12%)	0	18 (44%)	0	6 (15%)	1 (2%)	0	0	0	0	0	1	0	0	0	0
<b>(2)</b> <b>YN</b>	<b>23</b> (16%)	16 (11%)	18 (12%)	3 (2%)	66 (46%)	9 (6%)	10 (7%)	0	0	0	0	0	0	0	0	0	0	0
<b>(3)</b> <b>NY</b>	<b>-</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>(4)</b> <b>NN</b>	<b>15</b> (28%)	3 (6%)	2 (4%)	0	30 (56%)	1 (2%)	2 (4%)	1 (2%)	0	0	0	0	0	0	0	0	0	0

**Table 4.2d: Management policy (< 55 years), large (>25 microbubbles; >4mm on TEE) PFO.** Responses are documented for all respondents and then for each investigation subgroup (YY, YN, NY and NN). Responses are categorised using the key from the questionnaire provided in appendix 3. Thus, A = antiplatelet agent, B = warfarin, C = insert vena cava filter and D = refer for cardiological opinion. Respondents who circled more than one management option are characterised by the 'double', 'treble' and 'quadruple' response columns. Percentages of the total number, or subgroup number for the final four rows, are also provided.

group	no resp -onse	single response				double response				treble response				A				
		A	B	C	D	A	B	C	A	A	B	C	D		A	B	C	D
<b>Total</b>	<b>51</b> (20%)	21 (8%)	27 (10%)	1 (0%)	135 (52%)	7 (3%)	18 (7%)	0	0	0	0	0	0	0	1 (0%)	0	0	0
<b>(1)</b> <b>YY</b>	<b>5</b> (12%)	6 (15%)	4 (10%)	0	20 (49%)	1 (2%)	4 (10%)	0	0	0	0	0	0	0	1 (2%)	0	0	0
<b>(2)</b> <b>YN</b>	<b>18</b> (12%)	12 (8%)	19 (13%)	1 (1%)	80 (55%)	5 (3%)	10 (7%)	0	0	0	0	0	0	0	0	0	0	0
<b>(3)</b> <b>NY</b>	<b>-</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>(4)</b> <b>NN</b>	<b>15</b> (28%)	2 (4%)	2 (4%)	0	32 (59%)	1 (2%)	2 (4%)	0	0	0	0	0	0	0	0	0	0	0

**Table 4.2e: Management policy (< 55 years), associated ASA.** Responses are documented for all respondents and then for each investigation subgroup (YY, YN, NY and NN). Responses are categorised using the key from the questionnaire provided in appendix 3. Thus, A = antiplatelet agent, B = warfarin, C = insert vena cava filter and D = refer for cardiologist opinion. Respondents who circled more than one management option are characterised by the 'double', 'treble' and 'quadruple' response columns. Percentages of the total number, or subgroup number for the final four rows, are also provided.

group	no resp -onse	single response				double response				treble response				A				
		A	B	C	D	A	B	C	D	A	B	C	D		A	B	C	D
<b>Total</b>	<b>48</b> (18%)	9 (3%)	128 (49%)	8 (3%)	32 (12%)	1 (0%)	24 (9%)	0	1 (0%)	0	5 (2%)	0	1 (0%)	4 (2%)	0	0	0	
<b>(1)</b>	<b>4</b> (10%)	1 (2%)	21 (51%)	0	6 (15%)	0	6 (15%)	0	0	0	1 (2%)	0	1 (2%)	1	0	0	0	
<b>(2)</b>	<b>17</b> (12%)	7 (5%)	77 (53%)	3 (2%)	20 (14%)	1 (1%)	15 (10%)	0	1 (1%)	0	1 (1%)	0	0	3 (2%)	0	0	0	
<b>(3)</b>	<b>-</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<b>NY</b>																		
<b>(4)</b>	<b>14</b> (26%)	1 (2%)	26 (48%)	4 (7%)	5 (9%)	0	2 (4%)	0	0	0	2 (4%)	0	0	0	0	0	0	
<b>NN</b>																		

**Table 4.2f: Management policy (< 55 years), documented DVT at the time of stroke.** Responses are documented for all respondents and then for each investigation subgroup (YY, YN, NY and NN). Responses are categorised using the key from the questionnaire provided in appendix 3. Thus, A = antiplatelet agent, B = warfarin, C = insert vena cava filter and D = refer for cardiologist opinion. Respondents who circled more than one management option are characterised by the 'double', 'treble' and 'quadruple' response columns. Percentages of the total number, or subgroup number for the final four rows, are also provided.

group	no resp -onse	single response				double response				treble response				A				
		A	B	C	D	A	B	C	D	A	B	C	D		A	B	C	D
<b>Total</b>	<b>67</b> (26%)	123 (47%)	22 (8%)	0	17 (7%)	19 (7%)	7 (3%)	0	0	3 (1%)	0	0	0	2 (1%)	0	0	0	1 (0%)
<b>(1)</b> <b>YY</b>	<b>10</b> (24%)	14 (34%)	3 (7%)	0	6 (15%)	4 (10%)	2 (5%)	0	0	1 (2%)	0	0	0	2 (5%)	0	0	0	1 (2%)
<b>(2)</b> <b>YN</b>	<b>28</b> (19%)	78 (54%)	14 (10%)	0	8 (6%)	11 (8%)	4 (3%)	0	0	2 (1%)	0	0	0	0	0	0	0	0
<b>(3)</b> <b>NY</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>(4)</b> <b>NN</b>	<b>16</b> (30%)	25 (46%)	2 (7%)	0	3 (6%)	4 (7%)	2 (4%)	0	0	0	0	0	0	0	0	0	0	0

**Table 4.3a: Management policy (> 55 years), uncomplicated patient with cryptogenic stroke and a small PFO.** Responses are documented for all respondents and then for each investigation subgroup (YY, YN, NY and NN). Responses are categorised using the key from the questionnaire provided in appendix 3. Thus, A = antiplatelet agent, B = warfarin, C = insert vena cava filter and D = refer for cardiological opinion. Respondents who circled more than one management option are characterised by the 'double', 'treble' and 'quadruple' response columns. Percentages of the total number, or subgroup number for the final four rows, are also provided.



group	no resp -onse	single response				double response				treble response				A				
		A	B	C	D	A	B	C	D	A	B	C	D		A	B	C	D
<b>Total</b>	<b>60</b> (23%)	11 (4%)	112 (43%)	5 (2%)	46 (18%)	6 (2%)	15 (6%)	2 (1%)	0	1 (0%)	0	1 (0%)	0	0	1 (0%)	2 (1%)	0	
<b>(1)</b> <b>YY</b>	<b>7</b> (17%)	1 (2%)	16 (39%)	0	9 (22%)	1 (2%)	5 (12%)	1 (2%)	0	0	0	0	0	0	1 (2%)	0	0	
<b>(2)</b> <b>YN</b>	<b>27</b> (19%)	8 (6%)	70 (48%)	3 (2%)	25 (17%)	4 (3%)	5 (3%)	0	0	1 (1%)	0	0	0	0	0	2 (1%)	0	
<b>(3)</b> <b>NY</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<b>(4)</b> <b>NN</b>	<b>13</b> (24%)	2 (4%)	20 (37%)	1 (2%)	12 (22%)	1 (2%)	4 (7%)	1 (2%)	0	0	0	0	0	0	0	0	0	

**Table 4.3b: Management policy (> 55 years), recurrent events on initial therapy.** Responses are documented for all respondents and then for each investigation subgroup (YY, YN, NY and NN). Responses are categorised using the key from the questionnaire provided in appendix 3. Thus, A = antiplatelet agent, B = warfarin, C = insert vena cava filter and D = refer for cardiologist opinion. Respondents who circled more than one management option are characterised by the 'double', 'treble' and 'quadruple' response columns. Percentages of the total number, or subgroup number for the final four rows, are also provided.

group	no resp -onse	single response				double response				treble response				A					
		A	B	C	D	A	B	C	A	A	B	C	D		A	B	C	D	
<b>Total</b>	<b>75</b> (29%)	53 (20%)	48 (18%)	10 (4%)	59 (23%)	6 (2%)	6 (2%)	1 (0%)	0 (0%)	1 (0%)	0 (0%)	0 (0%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)	0 (0%)	
<b>(1)</b> <b>YY</b>	<b>8</b> (20%)	6 (15%)	8 (20%)	2 (5%)	13 (32%)	0 (0%)	2 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)
<b>(2)</b> <b>YN</b>	<b>34</b> (23%)	36 (25%)	30 (21%)	5 (3%)	31 (21%)	3 (2%)	4 (3%)	0 (1%)	1 (1%)	1 (1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>(3)</b> <b>NY</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>(4)</b> <b>NN</b>	<b>18</b> (33%)	10 (19%)	6 (11%)	3 (6%)	15 (28%)	2 (4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

**Table 4.3c: Management policy (> 55 years), Valsalva manoeuvre or cough preceding stroke.** Responses are documented for all respondents and then for each investigation subgroup (YY, YN, NY and NN). Responses are categorised using the key from the questionnaire provided in appendix 3. Thus, A = antiplatelet agent, B = warfarin, C = insert vena cava filter and D = refer for cardiological opinion. Respondents who circled more than one management option are characterised by the 'double', 'treble' and 'quadruple' response columns. Percentages of the total number, or subgroup number for the final four rows, are also provided.

group	no resp -onse	single response				double response				treble response				A				
		A	B	C	D	A	B	C	A	A	B	A	B		A	B	C	D
<b>Total</b>	<b>74</b> <b>(28%)</b>	37 (14%)	30 (11%)	2 (1%)	93 (36%)	6 (2%)	16 (6%)	1 (0%)	0 (0%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0
<b>(1)</b>	<b>7</b> <b>(17%)</b>	4 (10%)	5 (12%)	0	16 (39%)	0	7 (17%)	0	1 (2%)	0	0	0	0	1	0	0	0	0
<b>YY</b>																		
<b>(2)</b>	<b>38</b> <b>(26%)</b>	28 (19%)	19 (13%)	1 (1%)	48 (33%)	4 (3%)	6 (4%)	0 (1%)	0	1	0	0	0	0	0	0	0	0
<b>YN</b>																		
<b>(3)</b>	<b>-</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>NY</b>																		
<b>(4)</b>	<b>15</b> <b>(28%)</b>	3 (6%)	5 (9%)	0	27 (50%)	1 (2%)	3 (6%)	0	0	0	0	0	0	0	0	0	0	0
<b>NN</b>																		

**Table 4.3d: Management policy (> 55 years), large (>25 microbubbles; >4mm on TEE) PFO.** Responses are documented for all respondents and then for each investigation subgroup (YY, YN, NY and NN). Responses are categorised using the key from the questionnaire provided in appendix 3. Thus, A = antiplatelet agent, B = warfarin, C = insert vena cava filter and D = refer for cardiologist opinion. Respondents who circled more than one management option are characterised by the 'double', 'treble' and 'quadruple' response columns. Percentages of the total number, or subgroup number for the final four rows, are also provided.

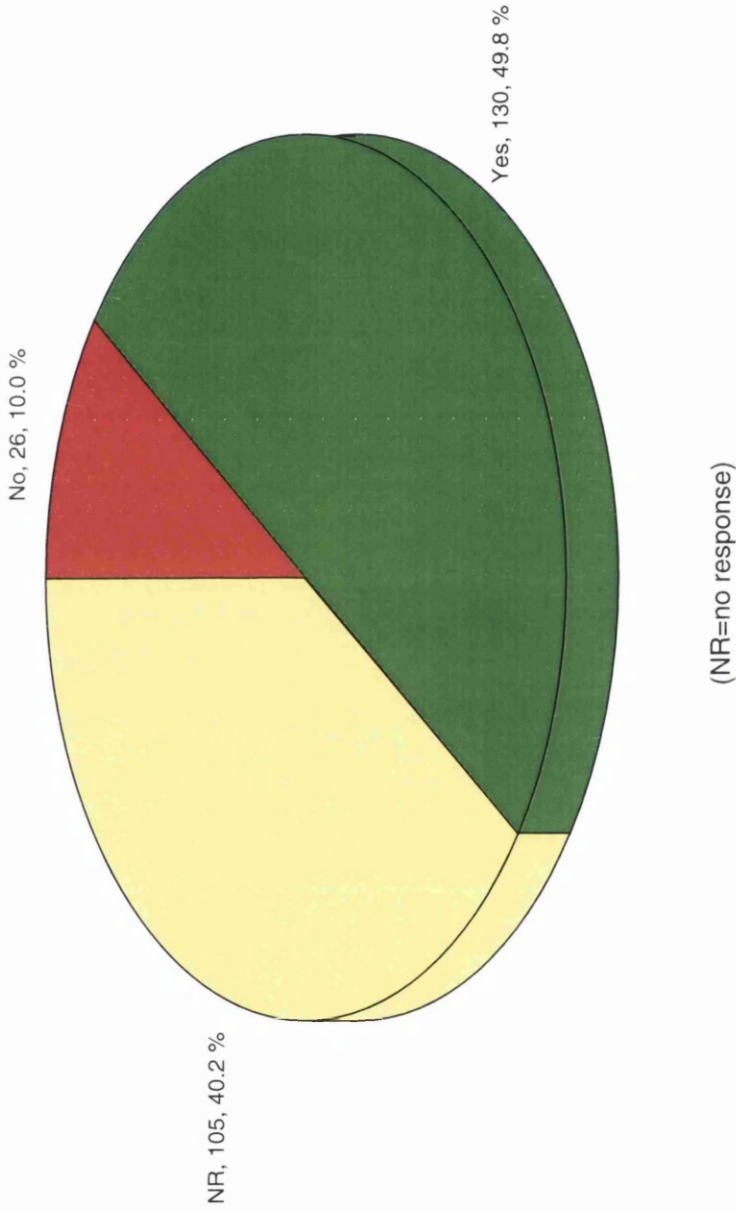
group	no resp -onse	single response				double response				treble response				A			
		A	B	C	D	A	B	C	A	A	B	A	A		B	C	D
Total	69 (26%)	25 (10%)	29 (11%)	0	115 (44%)	5 (2%)	16 (6%)	0	0	1 (0%)	0	0	0	1 (0%)	0	0	0
(1) YY	8 (20%)	5 (12%)	4 (10%)	0	19 (46%)	0	4 (10%)	0	0	0	0	0	0	1 (2%)	0	0	0
(2) YN	33 (23%)	15 (10%)	22 (15%)	0	62 (43%)	4 (3%)	8 (6%)	0	0	1 (1%)	0	0	0	0	0	0	0
(3) NY	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(4) NN	15 (28%)	3 (6%)	2 (4%)	0	31 (57%)	1 (2%)	2 (4%)	0	0	0	0	0	0	0	0	0	0

**Table 4.3e: Management policy (> 55 years), associated ASA.** Responses are documented for all respondents and then for each investigation subgroup (YY, YN, NY and NN). Responses are categorised using the key from the questionnaire provided in appendix 3. Thus, A = antiplatelet agent, B = warfarin, C = insert vena cava filter and D = refer for cardiological opinion. Respondents who circled more than one management option are characterised by the 'double', 'treble' and 'quadruple' response columns. Percentages of the total number, or subgroup number for the final four rows, are also provided.

group	no resp -onse	single response				double response				treble response				A				
		A	B	C	D	A	B	C	A	A	B	A	B		A	B	C	D
<b>Total</b>	<b>64</b> (25%)	12 (5%)	118 (45%)	10 (4%)	32 (12%)	1	14 (5%)	1	1	1	4 (2%)	0	1 (0%)	0	1 (0%)	2 (1%)	0	0
<b>(1)</b> <b>YY</b>	<b>7</b> (17%)	0	19 (46%)	0	8 (20%)	0	4 (10%)	0	0	0	2 (5%)	0	0	0	1 (2%)	0	0	0
<b>(2)</b> <b>YN</b>	<b>30</b> (21%)	10 (7%)	68 (47%)	6 (4%)	19 (13%)	1	7 (5%)	0	1	1	0	1 (1%)	0	0	0	2 (1%)	0	0
<b>(3)</b> <b>NY</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>(4)</b> <b>NN</b>	<b>14</b> (26%)	1 (2%)	28 (52%)	4 (7%)	4 (7%)	0	2 (4%)	0	0	0	1 (2%)	0	0	0	0	0	0	0

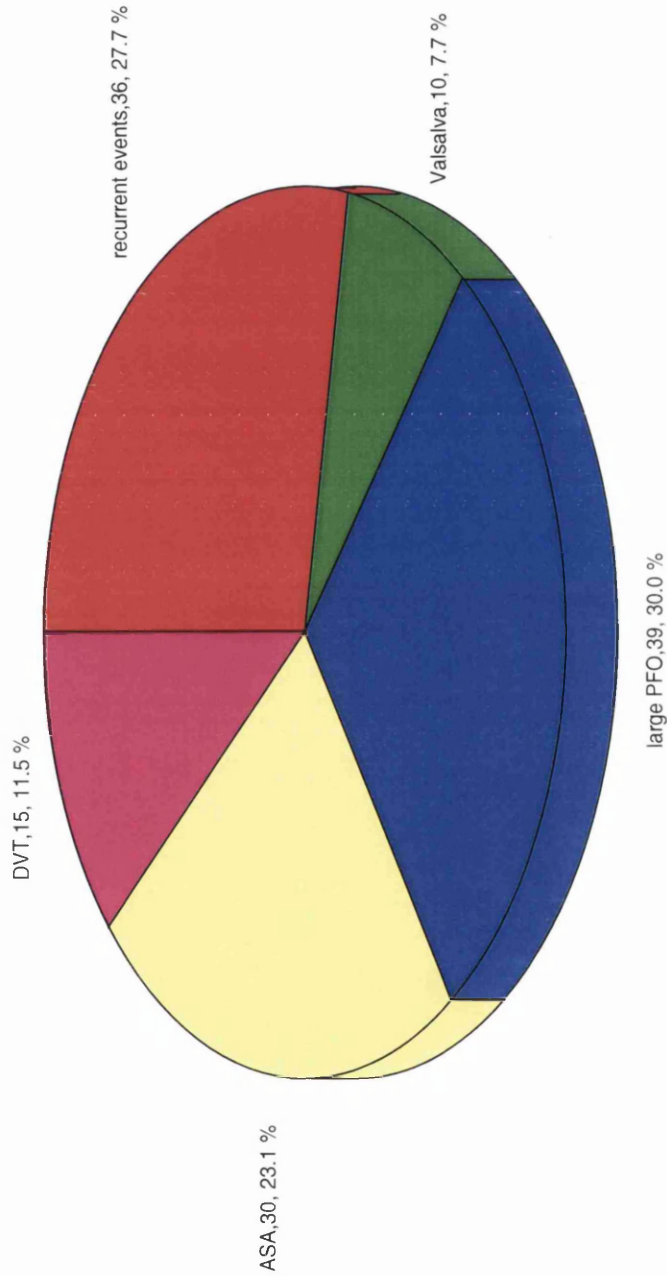
**Table 4.3f: Management policy (> 55 years), documented DVT at the time of stroke.** Responses are documented for all respondents and then for each investigation subgroup (YY, YN, NY and NN). Responses are categorised using the key from the questionnaire provided in appendix 3. Thus, A = antiplatelet agent, B = warfarin, C = insert vena cava filter and D = refer for cardiologist opinion. Respondents who circled more than one management option are characterised by the 'double', 'treble' and 'quadruple' response columns. Percentages of the total number, or subgroup number for the final four rows, are also provided.

< 55 years: referral with a combination of two of factors 1 to 5?



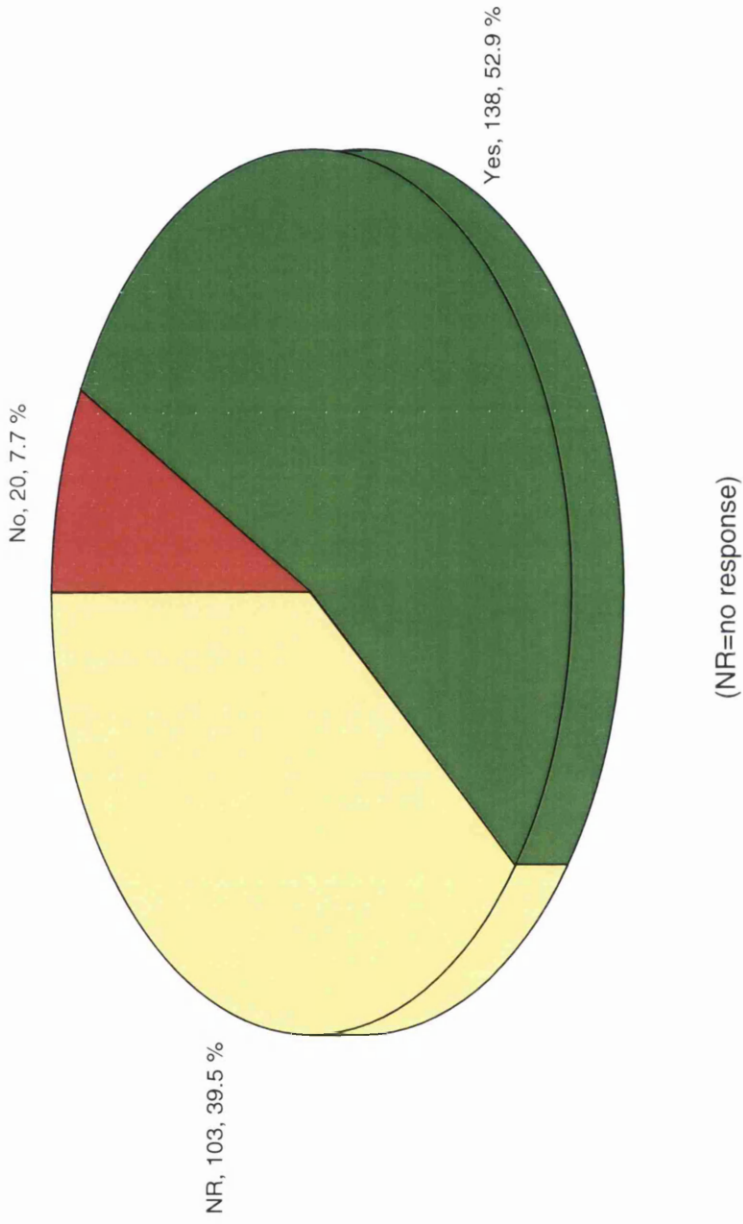
**Figure 4.1a < 55 years: referral with a combination of two of factors 1 to 5?** Illustrates the initial response to question 6 in the age group < 55 years. Respondents were requested to answer 'yes' if they would consider referral if two of the five clinical factors that may justify more aggressive management were present.

< 55 years: which factor(s) merit referral, or confer the greatest risk (maximum of two responses)?



**Figure 4.1b < 55 years: which factor(s) merit referral, or confer the greatest risk (maximum of two responses)?** Respondents answering 'yes' to question 6 were asked which combination of two clinical factors would make them consider referral for a corrective procedure, or, if more than one combination was appropriate, conferred the highest risk of recurrence. The frequency of each of the five clinical factors in these responses is illustrated. No weighting is given to the order in which the responses were documented, or to the way in which they were combined, and partial responses are included. Non-responders are not documented in this figure.

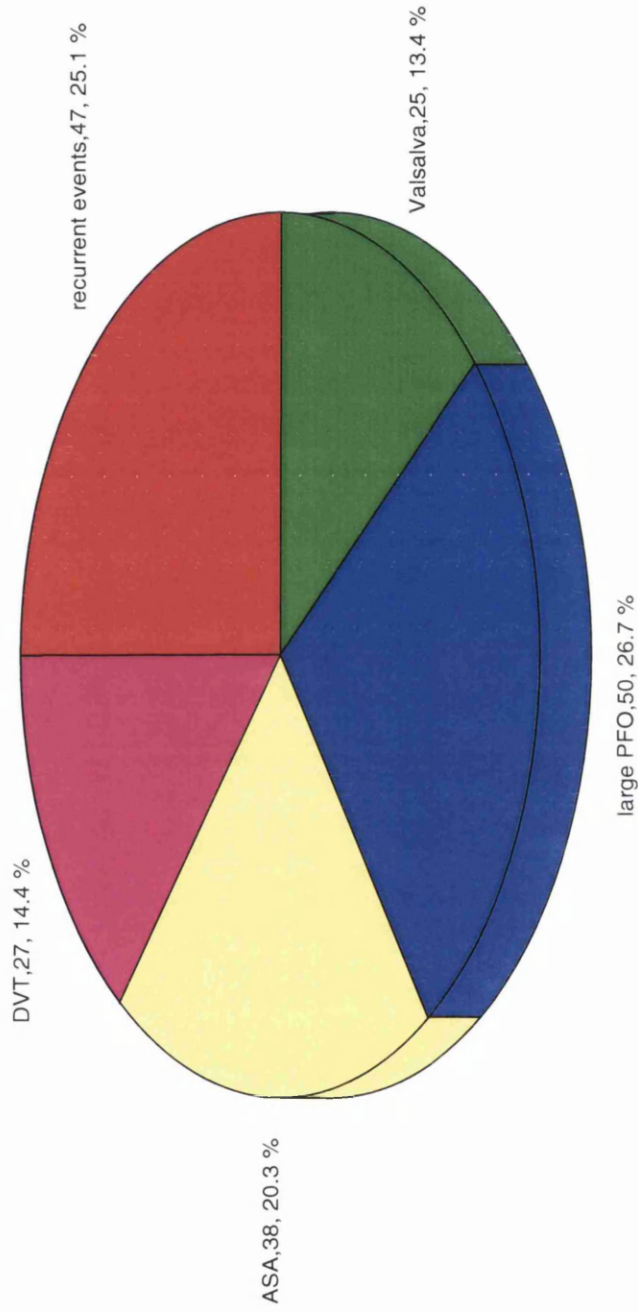
< 55 years: referral with a combination of three of factors 1 to 5?



**Figure 4.1c < 55 years: referral with a combination of three of factors 1 to 5?** Illustrates the initial response to question 7 in the age group < 55 years. Respondents were requested to answer 'yes' if they would consider referral if three of the five clinical factors that may justify more aggressive management were present.

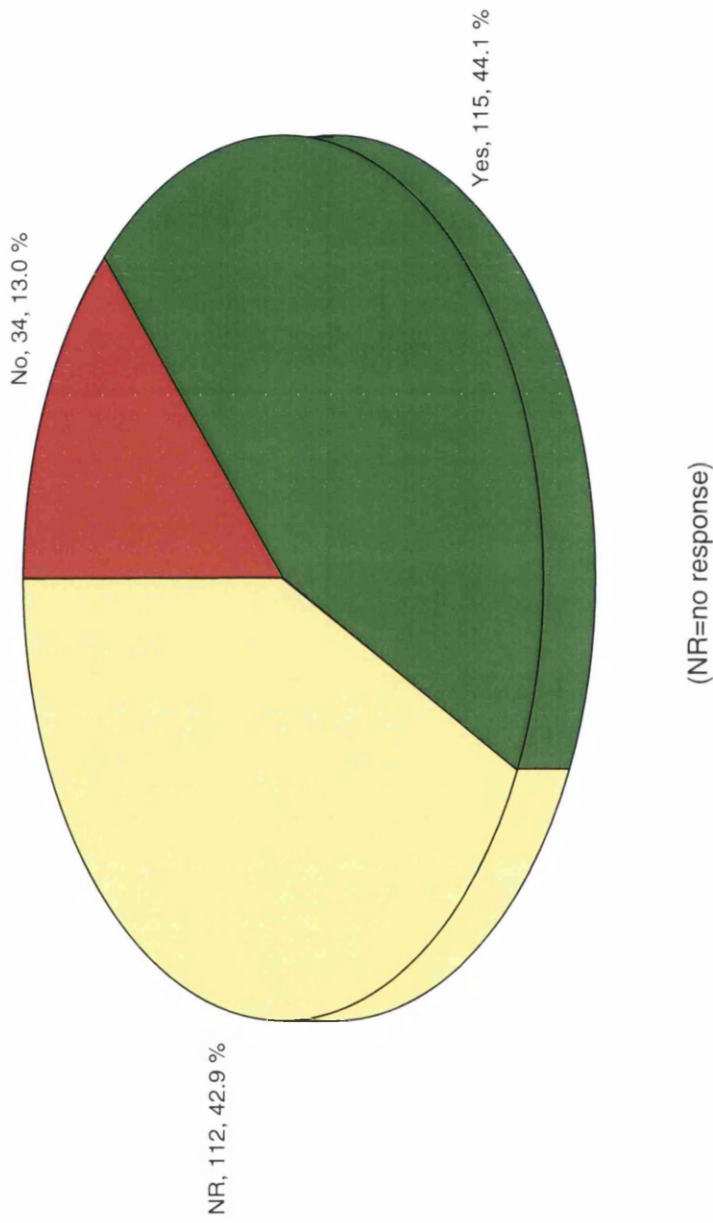


< 55 years: which factor(s) merit referral, or confer the greatest risk (maximum of three responses)?



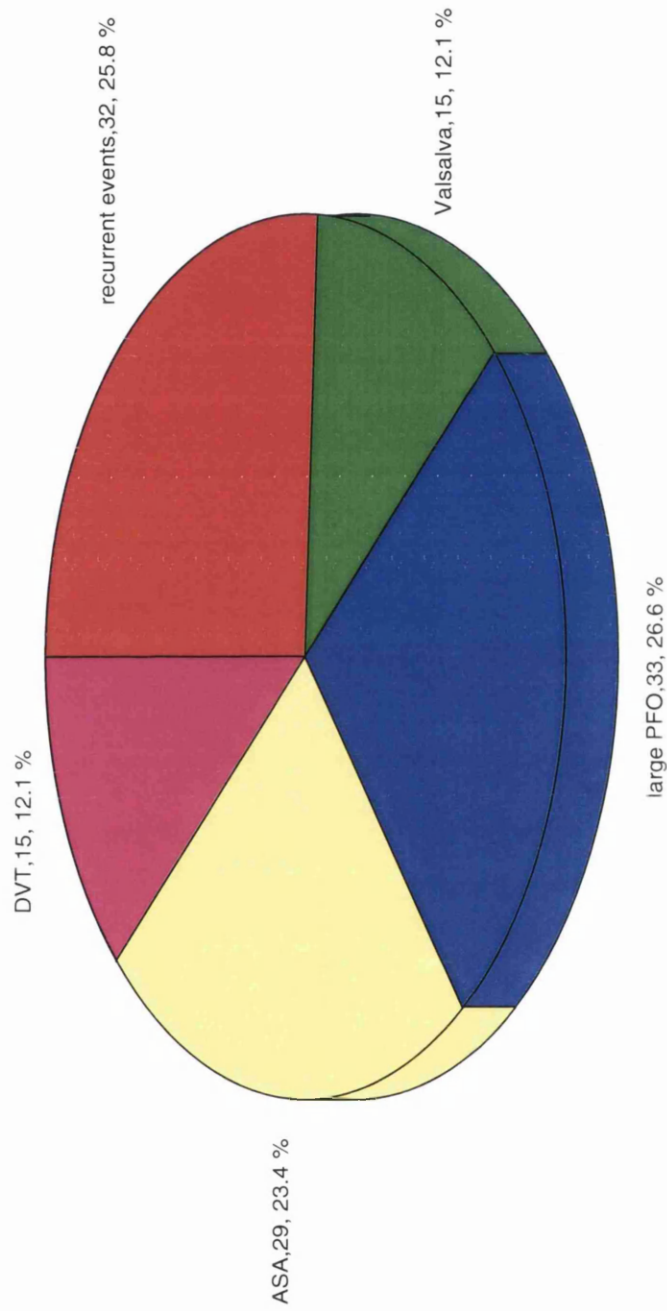
**Figure 4.1d < 55 years: which factor(s) merit referral, or confer the greatest risk (maximum of two responses)?** Respondents answering 'yes' to question 7 were asked which combination of three clinical factors would make them consider referral for a corrective procedure, or, if more than one combination was appropriate, conferred the highest risk of recurrence. The frequency of each of the five clinical factors in these responses is illustrated. No weighting is given to the order in which the responses were documented, or to the way in which they were combined, and partial responses are included. Non-responders are not documented in this figure.

> 55 years: referral with a combination of two of factors 1 to 5?



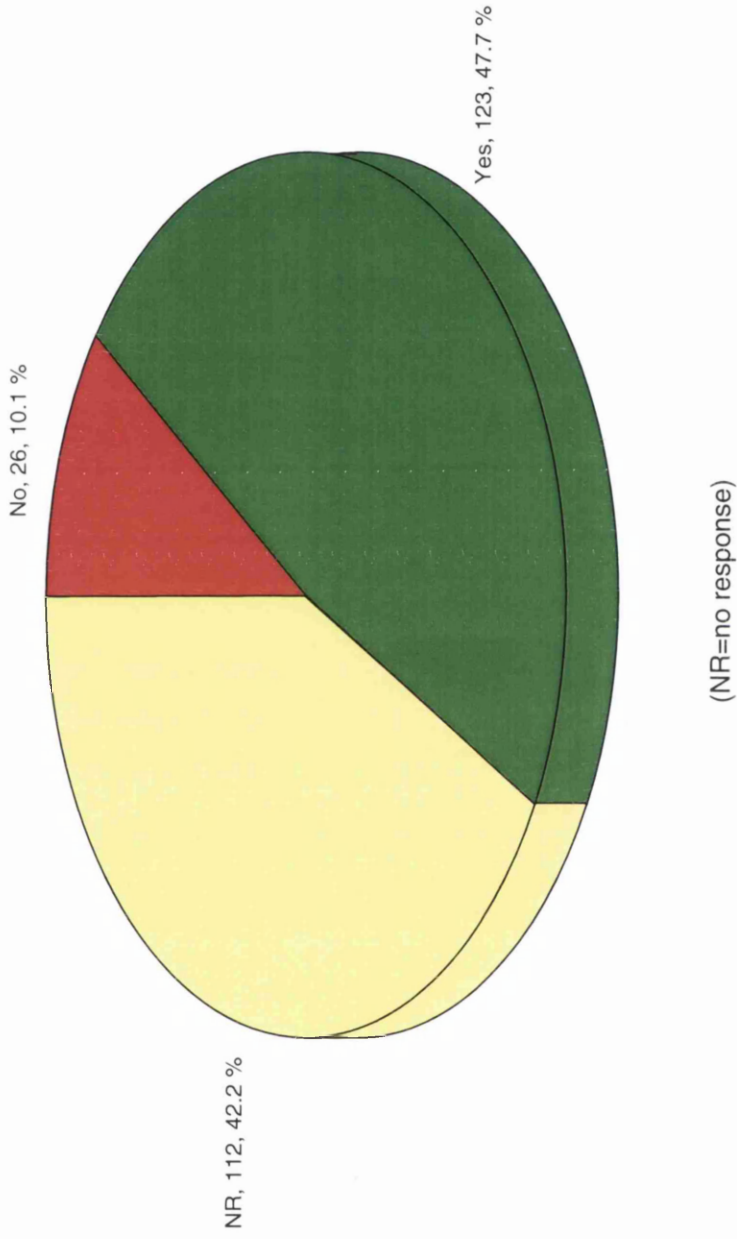
**Figure 4.2a > 55 years: referral with a combination of two of factors 1 to 5?** Illustrates the initial response to question 6 in the age group > 55 years. Respondents were requested to answer 'yes' if they would consider referral if two of the five clinical factors that may justify more aggressive management were present.

> 55 years: which factor(s) merit referral, or confer the greatest risk (maximum of two responses)?



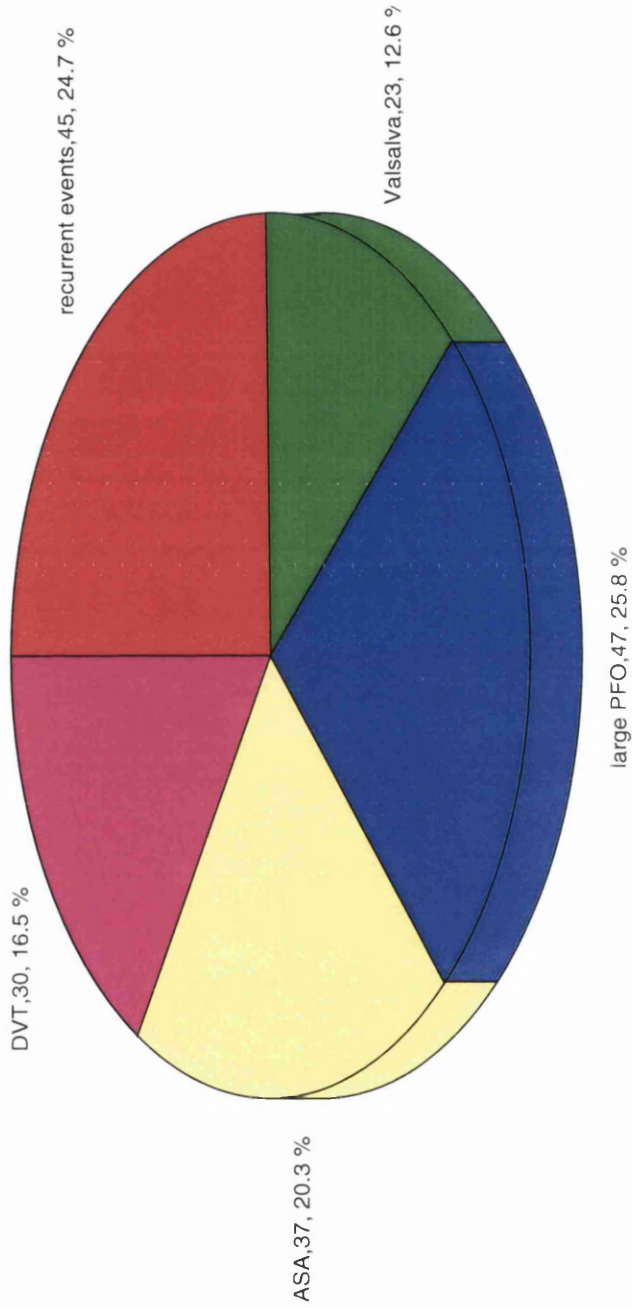
**Figure 4.2b > 55 years: which factor(s) merit referral, or confer the greatest risk (maximum of two responses)?** Respondents answering 'yes' to question 6 were asked which combination of two clinical factors would make them consider referral for a corrective procedure, or, if more than one combination was appropriate, conferred the highest risk of recurrence. The frequency of each of the five clinical factors in these responses is illustrated. No weighting is given to the order in which the responses were documented, or to the way in which they were combined, and partial responses are included. Non-responders are not documented in this figure.

> 55 years: referral with a combination of three of factors 1 to 5?



**Figure 4.2c > 55 years: referral with a combination of three of factors 1 to 5?** Illustrates the initial response to question 7 in the age group > 55 years. Respondents were requested to answer 'yes' if they would consider referral if three of the five clinical factors that may justify more aggressive management were present.

> 55 years: which factor(s) merit referral, or confer the greatest risk (maximum of three responses)?



**Figure 4.2d > 55 years: which factor(s) merit referral, or confer the greatest risk (maximum of two responses)?** Respondents answering 'yes' to question 7 were asked which combination of three clinical factors would make them consider referral for a corrective procedure, or, if more than one combination was appropriate, conferred the highest risk of recurrence. The frequency of each of the five clinical factors in these responses is illustrated. No weighting is given to the order in which the responses were documented, or to the way in which they were combined, and partial responses are included. Non-responders are not documented in this figure.

## **Chapter 5: The Statins in Acute Stroke (SAS) Trial**

## 5.1 Introduction

Inhibitors of the enzyme HMG Co-A reductase, often called 'statins', are well tolerated and potent drugs used to lower cholesterol. In large scale clinical trials, HMG Co-A reductase inhibitors have been shown to reduce the long term incidence of myocardial infarction and death, in both primary<sup>244</sup> and secondary<sup>245</sup> prevention. Published meta-analyses have demonstrated a 30% reduction in the incidence of stroke in individuals with coronary heart disease (CHD)<sup>246</sup>. The secondary prevention of stroke with statins is currently being investigated by randomised trials, but subgroup analysis of the large (but unpublished) Heart Protection Study<sup>100</sup> has suggested benefits in the secondary prevention of stroke similar to those demonstrated in CHD.

There appear to be both short and long-term benefits in prescribing statins to individuals with coronary artery disease. The effect cannot be solely attributed to cholesterol lowering in individuals with hyperlipidaemia, because reductions in vascular events seem to occur earlier than could be explained by plaque regression, and appear to occur in individuals with both low and high initial cholesterol levels<sup>247 104</sup>. The effect of statins on endothelium, blood rheology and vasomotor tone may explain their efficacy in patients at high vascular risk<sup>106</sup>.

Various rheological markers (fibrinogen, viscosity, tissue plasminogen activator (tPA), plasminogen activator inhibitor (PAI-1) and D-dimer) have been shown to be independent predictors of stroke risk<sup>120 127 124 115</sup> (see **chapter 1**). It is thought that treatment to lower such markers may reduce the incidence of further stroke and myocardial infarction, and there is limited trial evidence that statins may be beneficial in this regard<sup>114</sup>. Other putative effects of statins have been discussed in **chapter 1**; effects on blood pressure and cerebral blood flow and perfusion have been suggested,

either directly or indirectly by effects on rheological markers. Anti-inflammatory effects of statins have generated considerable interest in the cardiovascular research community<sup>138</sup>, and have also been suggested in the cerebrovascular population by observational data<sup>248</sup>. The use of statins in the acute phase of stroke has not been studied. Knowledge of their effects on rheological and inflammatory markers, blood pressure, cerebral blood flow and perfusion in the recovery phase of stroke will provide important data that should complement the large-scale primary and secondary stroke prevention trials due to report in the next few years.

## **5.2 Methods**

### ***Study design and objectives***

The study was designed as a randomised, double blind, placebo-controlled trial of pravastatin versus placebo. We hypothesised that statin therapy would improve cerebral perfusion and cerebral blood flow after ischaemic stroke or transient ischaemic attack (TIA). The effect of statin therapy on blood pressure and markers of blood rheology was also examined. It was hoped that these data would be helpful in generating hypotheses for further investigations. Approval was obtained from the West Ethics Committee, and patients gave written informed consent to participate.

### ***Patient qualification, enrolment, randomisation and dosing***

The following inclusion and exclusion criteria applied to the study population:

#### ***Inclusion criteria***

- Over 40 years of age
- Stroke 36 to 168 hours before randomisation
- Informed consent of patient
- Haemorrhage excluded on CT scanning



### *Exclusion criteria*

- Treatment with statin or alternative lipid lowering therapy within the previous 4 weeks
- History of intolerance to statin therapy
- Concomitant cardiovascular drug therapy maintained or initiated since stroke onset, excluding aspirin and digoxin, but including antihypertensives, warfarin and heparin.
- Unstable clinical state
- Women in whom possibility of pregnancy cannot be excluded
- Clinically significant liver disease or myositis

After full explanation of the procedures and risks, patients who gave informed consent underwent baseline investigations (day 0). These included  $^{99m}\text{Tc}$  hexamethyl propylene amine oxide single photon emission computed tomography (HMPAO SPECT) brain scanning, common and internal carotid artery Doppler examination, fasting blood samples for haemorrhology, total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides, liver function tests, creatinine kinase (CK), inflammatory markers and 24 hour blood pressure monitoring. These techniques, and their context in the study, are described further below.

Once baseline investigations were completed, all patients were randomised to receive either pravastatin 40mg per day for 56 days or matching placebo, in a ratio of 1:1. Randomisation was conducted by an unblinded independent research pharmacist who was uninvolved in clinical management except for the provision of blinded drug supplies. The day on which the first dose of drug was received was labelled day 1. Randomisation took place between October 1999 and August 2001.

***Study flow (illustrated in figure 5.1)***

### *Day 3*

24 hr blood pressure monitoring and carotid artery Doppler measurements were taken during the course of the day. Standard post-stroke management was employed, either in the acute stroke unit or in the rehabilitation setting.

### *Day 28*

Patients returned for repeat measurements of blood rheology, inflammatory markers, lipid concentrations, CK, liver function tests, carotid artery Doppler, and 24hr blood pressure monitoring.

### *Day 56*

The measurements made on day 28 were repeated, and SPECT scanning was repeated at the same time of day as the baseline measurement.

Measurements of liver function and plasma CK were made at baseline and on day 28, to exclude the possibility of drug-induced hepatitis or myositis. If substantial rises in either of these markers were detected, the patient was withdrawn from the study.

## ***Explanation of investigations***

### *HMPAO SPECT scanning*

SPECT was undertaken at baseline and on day 56 in the Department of Nuclear Medicine of the Western Infirmary using a Picker Prism 2000XP large field of view double-headed gamma camera. On each occasion 500 MBq  $^{99m}\text{Tc}$  HMPAO dissolved in 5 millilitres 0.9% saline was injected as a bolus over one second through an 18 gauge intravenous catheter, followed by a further bolus of 10 millilitres of saline. An anterior image of 200 x 1s frames was then acquired. The patient's head and as much as possible of their heart was included in the field of view. SPECT imaging was undertaken 15 minutes later using a circular orbit and acquiring 60 angles around 360 degrees with 30s per angle. Analysis of the SPECT data was undertaken using a combination of Picker supplied software and some specialised software written in house. The data were reconstructed using a

back projection algorithm with a Butterworth filter of order 3.14. Attenuation correction was undertaken using the Chang algorithm. A brain perfusion index (BPI) was calculated from the first-pass data as described by *Matsuda et al*<sup>249 250</sup>, using the unaffected hemisphere as the reference region. The reconstructed data were reorientated to transaxial oblique slices (8mm thick) parallel to the orbito-meatal line. Image registration was undertaken, registering the two sets of SPECT data for each of the patients (from day 0 and day 56).

An elliptical region of interest was manually fitted to the outer edge of each transaxial oblique slice for each of the sets of data and a set of templates constructed<sup>251</sup>. Regional cerebral blood flow (rCBF) in ml/100g/min was then calculated in each of the segments of the template using the previously obtained BPI. Segments were classified according to whether they were from right or left hemisphere, or from a cortical or subcortical locality. For each analysis (whole brain perfusion, cortical perfusion, symptomatic hemisphere perfusion and symptomatic cortex perfusion) the difference (day 56 - day 0) in rCBF was calculated for each of the relevant segments, and expressed as a percentage of the baseline (day 0) value. The SPECT method is analogous to that employed in previous studies of the cerebral perfusion effects of antihypertensives reported from our unit<sup>41</sup>.

#### *Carotid Doppler examination*

Doppler measurements (common carotid artery (CCA) and internal carotid artery (ICA)) were conducted at 08.45 hours at baseline, and on days 3, 28, and 56. Doppler studies were conducted using an Acuson Aspen duplex ultrasound machine and a 5MHz probe. The product of peak systolic area and mean time averaged velocity gave a measure of total CCA flow (millilitres / second). Total ICA blood flow and pulsatility index (a measure of peripheral vascular resistance, equalling (systolic velocity - diastolic velocity

/ mean velocity)<sup>252 253</sup>) were calculated from bilateral ICA insonation. Arterial flow was calculated (in ml/second) as:

$$[\pi \times (\text{peak systolic diameter})^2 \times \text{mean time averaged velocity}] / 4$$

Details of the Doppler methods have been published previously<sup>254</sup>. Each Doppler measurement at each time point was conducted in triplicate, and averages were calculated. Differences between follow-up measurements and the baseline value were expressed as a percentage change from baseline (day 0). No significant difference was detected between right and left percentage flow changes at each timepoint in the whole group, and so for each individual percentage flow change on right and left were averaged to provide a summary measure of change in CCA or ICA flow in that individual. This value was used for statistical comparisons.

#### *24 hour blood pressure monitoring*

Blood pressure monitoring (day 0, day 3, day 28 and day 56) was performed using standard ambulatory monitors (Spacelabs Inc, model 90207). Monitoring commenced at 0900 and was performed for 24 hours. Recordings were taken at 30 minute intervals during the day, and hourly during the night, and were preceded by a warning bleep. Patients were provided with an information sheet detailing how often their blood pressure would be recorded, and asking them to keep their arm stationary during recordings.

#### *Rheological / Inflammatory marker assays*

Samples were stored at -70 degrees Celsius at the Western Infirmary, and transferred at the end of the study to the haemorrhology laboratories at the Glasgow Royal Infirmary for analysis. Plasma levels of **tPA** were measured with a commercially available enzyme linked immunosorbent assay (ELISA) from Biopool AB, Umea, Sweden. Plasma von Willebrand factor (**vWf**)

antigen levels were measured using an in-house ELISA, employing rabbit anti-human polyclonal antibodies obtained from DAKO plc, High Wycombe, UK. The measurement of plasma fibrin **D-dimer**, which is present in several cross-linked fibrin degradation products, was carried out using the ELISA kit from Biopool AB, Umea, Sweden. Clottable **fibrinogen** was measured by the dilute thrombin clotting time on an automated coagulometer (MDA 180, Organon Teknika, Cambridge UK), using calibrants and reagents provided by the manufacturer. **Factor VII** was measured by standard clotting assays on an automated coagulometer (MDA 180, Organon Teknika, Cambridge UK), using calibrants and reagents provided by the manufacturer. C-reactive protein (**CRP**) was measured on a Prospec Nephelometer (Dade Behring, Marburg, Germany) using a high sensitivity method with the manufacturer's calibrant and reagent. **Plasma viscosity** was measured using a semi-automated capillary viscometer (Beckman Coulter, High Wycombe, UK).

#### ***Statistical considerations and justification for sample size***

This was based on repeated measurements of extracranial arterial flow rates, assessed at the Doppler examinations described. From a previous study <sup>255</sup>, the average difference between two ICA flow measurements within a single patient was 0.07 with a standard deviation of 1.75 ml/sec. An analysis based on a simple t-test comparing the mean difference between flow measurements at baseline and at day 56 within individual patients was calculated to have 77% power to detect a difference between the treated and control groups of 1.4 ml/sec (~16% difference) in change over time with 12 patients per group, at a significance level of 5%. Thus, a sample size of 24 patients was our recruitment target.

In the SAS trial, each recording was conducted in triplicate by a single observer which we estimated would reduce variability by 30% to 1.225 ml/sec. Using this estimate of sample variability, power levels in excess of 95% could be obtained with 12 patients per group, and a satisfactory power

of 90% was achievable with 18 patients (9 per group). SPECT data from similar post-stroke studies became available during the course of the trial, and indicated that a sample size of 18 would provide sufficient power to detect a cerebral perfusion difference between groups of 7ml/100g/min with 80% power <sup>41</sup>. These considerations permitted an alteration in our initial assumptions about data variability and sample size, and enabled us to absorb dropouts (see below) without compromising the trial.

For all variables measured, changes between the day 56 and baseline value within individuals were collated for both the pravastatin and placebo groups, using *Microsoft Excel 97*. For each parameter this difference (day 56 - day 0), or the percentage difference from baseline calculated using this value ((day 56 - day 0) / day 0), was regarded as the primary endpoint. More acute effects at day 28 or day 3 ((day 28 - day 0) or (day 3 - day 0)) were regarded as secondary for the purposes of statistical analysis. The series of differences for each variable was checked for normal (parametric) data distribution by visual inspection of histograms, and groups were compared by standard t-tests for independent samples. All calculations and figures were generated using *Statistica for windows* (Statsoft Inc., version 5.1, 1997).

### 5.3 Results

24 patients were recruited in total, but a number of dropouts occurred during the course of the trial. Patient 5 (placebo) was too obese to undergo SPECT examination, and so did not continue in the trial. Patient 9 (placebo) was discovered by the clinical team to have worsening liver function tests a week after randomisation, and was therefore withdrawn. On review, these continued to rise after discontinuation of trial medication, suggesting an alternative cause. Patient 19 (pravastatin) was also discovered to have an increase in alkaline phosphatase to 461 u/l at the day 28 assessment, and

trial medication was discontinued at that point. Patient 14 (placebo) withdrew from the trial after day 3, because the rehabilitation staff caring for her felt that further trial visits were too strenuous. Patient 17 (placebo) did not attend for final SPECT assessment. Concomitant antihypertensives were prescribed to patient 23 (placebo) on the third day after randomisation, which may have interfered with our assessment of statin effect on blood pressure and cerebral perfusion, and so led to withdrawal. Patient 24 did not attend for final follow-up, and so data were only available for the first 3 visits.

As a result of these numerous dropouts, 18 (10 pravastatin, 8 placebo) patients had a full set of Doppler and haemorrhheological results, with two additional patients (19 and 24) having data for day 0, 3 and 28 comparisons. Full SPECT data were available for 17 patients (10 pravastatin, 7 placebo). Problems with compliance and equipment failure led to only 13 patients having full blood pressure data. Two further patients had day 0 and 3 blood pressure assessments (patient 9 and 14), and three had day 0, 3 and 28 assessments (patients 13, 21 and 24). Patients 12, 17 and 18 had no baseline blood pressure measurement with which to make comparisons and patients 19 and 24 had only a baseline measurement.

Clinical details and treatment allocation are shown in **table 5.1**. Mean patient age was 66 years (standard deviation (SD) 14). No significant difference in mean age was detected between pravastatin and placebo groups ( $p=0.34$ ). Mean entry NIH score was 2.5 (SD 3.2) for all randomised patients, 1.5 for the pravastatin group and 3.5 for the placebo group ( $p=0.13$  for comparison). At inclusion, 3 patients had suffered posterior circulation events, 9 had lacunar events and 12 patients had cortical anterior circulation events. No patient had further stroke events during follow up, but patient 12 was admitted with DVT (to L external iliac) shortly before his day

56 visit. He was found during admission to have multiple pulmonary emboli and metastatic cancer in his liver.

**Table 5.2** details changes in lipid and safety parameters in the pravastatin and placebo groups. Mean total cholesterol at baseline was 5.44 mmol/l (SD 0.66 mmol/l) in the pravastatin group and 5.49 mmol/l (SD 0.74 mmol/l) in the placebo group. Compared with minor increases in the placebo group, significant falls were seen in total ( $p<0.001$ ) and LDL ( $p<0.001$ ) cholesterol in pravastatin treated patients at 56 days. No significant differences between pravastatin and placebo were seen when comparing changes in triglyceride concentration or HDL level. Although patients 9 and 19 withdrew from the trial because of worsening liver function, neither case on review could be attributed to statin therapy. Comparisons of change in transaminase and CK levels between pravastatin and placebo groups revealed no significant difference.

SPECT cerebral perfusion results are illustrated in **figure 5.2**. Insignificant trends towards improvement in cerebral perfusion over the 2 month trial time course were seen in both groups, but there was no difference between pravastatin and placebo treated patients when comparing whole ( $p=0.88$ ), cortical ( $p=0.94$ ), symptomatic hemispheric ( $p=0.78$ ) or symptomatic cortical ( $p=0.99$ ) perfusion.

**Figures 5.3 and 5.4** demonstrate the change from baseline in common (**figure 5.3**) and internal (**figure 5.4**) carotid artery flow over time for both the pravastatin and placebo groups. No significant benefit of treatment was detected when comparing common carotid ( $p=0.41$ ) or internal carotid ( $p=0.84$ ) arterial flow changes in the pravastatin and placebo groups between day 56 and baseline. Pulsatility index change over the course of the trial was not altered by treatment allocation ( $p=0.58$ ).



**Figure 5.5** illustrates changes from baseline in mean arterial blood pressure (MAP) at the 3 follow-up examinations. No significant difference in MAP change over time was detected between the pravastatin and placebo treated patients ( $p=0.44$  for (day 56 - day 0) comparison). Similarly, no significant effects of treatment were apparent when comparing systolic ( $p=0.42$ ) or diastolic ( $p=0.20$ ) pressures.

**Figures 5.6 and 5.7** illustrate the mean difference between the day 56 and baseline values of each of the haemorrhological variables for the pravastatin and placebo groups. These differences are tabulated, along with the differences between day 28 and baseline values, in **table 5.3**. Insignificant trends towards reductions in haematocrit and factor VII were detected in the pravastatin group. No effect was seen on plasma fibrinogen, or plasma viscosity at 56 days. Plasma CRP appeared to rise in the treatment group, attributable to an increase in CRP to 136 mg/l in patient 12, concurrent with the diagnosis of metastatic cancer.

## 5.4 Discussion

Cardiovascular research interest into the cholesterol independent<sup>256</sup> and acute<sup>257</sup> effects of statins continues to expand, fuelling new approaches to their use in the clinical arena. Statin use as a secondary preventative measure in cerebrovascular disease is also becoming increasingly common. The effects of statins on endothelial function, arterial tone, inflammatory processes and haemorrhology are an important and to date poorly examined area, both in the cerebrovascular population and in the acute phase of stroke itself. Pravastatin was well tolerated and safe in the acute phase of cerebrovascular events, and a marked early effect on total and LDL cholesterol was observed. Previous studies have shown improved blood pressure control with statin therapy in both untreated and treated hypertensive patients<sup>258</sup>, but no effect on mean arterial blood pressure was

observed in our cohort, possibly reflecting the small number of patients with full data. No effect or trend to effect was seen in common carotid or ICA flow, or cerebral perfusion as measured by SPECT.

There are trial design factors that serve as possible explanations for our failure to detect an effect of pravastatin on the primary outcome measures of SPECT perfusion and ICA flow. The numbers recruited were small, and this was compounded by missing data due to dropouts. It was felt that these dropouts could be absorbed because of new data on SPECT variability that became available during the trial, and improved Doppler accuracy from the use of a single observer and triplicate recordings. In fact, variability of SPECT differences in whole perfusion was lower in the current study than in that which informed our decision <sup>41</sup>, probably an effect of the same observer examining baseline and follow-up scans, and increased familiarity of nuclear medicine staff with the quantitative technique employed <sup>249 250</sup>. Even so, we were unable to detect even a trend towards improvement in SPECT perfusion and have ruled out differences in whole cerebral perfusion at two months between pravastatin and placebo treated ischaemic stroke patients of more than 7 ml/100g/min. While it remains possible that small improvements in perfusion may be conferred by statin therapy over longer periods, these are unlikely to be clinically significant after two months of therapy. Similarly sized studies in hyperlipidaemic patients with CHD have demonstrated improvements in Positron Emission Tomography (PET) coronary flow reserve under dipyridamole stress after 6 months <sup>259</sup> and thallium SPECT perfusion after 12 weeks <sup>110</sup>.

The standard deviation of differences in ICA flow recordings between follow-up and baseline scans was higher than those obtained during previous studies in our unit: 4.2 ml/sec for day 56 - day 0 difference, 4.08 ml/sec for day 28 - day 0 difference and 3.99 ml/sec for day 3 - day 0 difference. This reduced level of accuracy in the recordings had a major impact on the ability

of the trial to detect a treatment effect on ICA flow: only differences between placebo and pravastatin of approximately 3.2 ml/sec were detectable with the numbers recruited. The technique used to assess ICA flow assumes that the lumen of the vessel being studied is cylindrical (in other words, the cross-sectional area of the vessel can be calculated from its diameter). Some of the patients recruited into the trial had haemodynamically significant internal carotid disease (patient 6: left carotid occlusion; patient 13: bilateral moderate stenosis; patient 14 moderate right stenosis; patient 18: bilateral severe stenosis; patient 19: bilateral occlusion) and if these lesions caused a non-concentric reduction in ICA flow, errors may have been introduced into its calculation. Such lesions are also likely to increase variability in the assessment of flow. However, no significant change over time in arterial diameter was seen within groups (mean change pravastatin=0.24mm (95% CI -0.38 to 0.85), mean change placebo=0.25mm (-0.31 to 0.80)) or between groups ( $p=0.98$ ), and so the comparison of the change in flow between groups remains valid.

The trial was not powered to detect clinically significant changes in haemorrhological variables, but some observations can be made from the data presented. Trends towards reduction in factor VII, which plays an important role in fibrin generation and thrombus formation, concur with previous reports that atorvastatin significantly reduces factor VII levels in chronically hyperlipidaemic patients<sup>260 261</sup>. Haematocrit (the volume fraction of red blood cells) is a major determinant of blood viscosity, and after 56 days showed a trend towards reduction in the treated group. Higher numbers of patients recruited to the trial may have led to significant results. Plasma viscosity itself appeared to reduce in the treated group at 28 days ( $p=0.04$ ), but comparison with the placebo group was insignificant at 56 days. Our failure to demonstrate an effect on fibrinogen and plasma viscosity (which have shown significant reductions with pravastatin therapy in studies of similar size<sup>114</sup>) may be a function either of assessing

fibrinogen change in the immediate aftermath of acute cerebral ischaemic events, or of the short length of therapy. TPA antigen, D-dimer and vWf were unaffected by therapy. Whether pharmacological intervention will affect these variables, and what significance in terms of vascular event prevention this may have, is unclear.

Numerous investigators have reported improvements in CRP level in hyperlipidaemic and cardiovascular populations with pravastatin therapy<sup>262</sup><sup>263</sup>. A recent retrospective analysis of data from the Air Force/Texas Coronary Atherosclerosis Prevention Study has suggested that CRP could be used as a means for targeting statin therapy in patients with relatively low lipid levels<sup>264</sup>. While CRP levels predict poor outcome after stroke<sup>137</sup>, the effect of statins on CRP in acute ischaemic stroke has not been previously studied in a randomised trial. Because of small numbers, the results obtained (using a high sensitivity method of CRP detection) were strongly influenced by a single individual in the treatment group (patient 12), whose CRP rose from 14mg/l at 28 days to 136mg/l at 56 days. The same individual similarly affected measurements of differences in other haemorrhological parameters that rise as part of the acute phase response (fibrinogen, vWF and d dimers). Whether CRP will be useful in assessing vascular risk and guiding therapy in ischaemic stroke patients is currently unclear. In common with fibrinogen, it may be a practical surrogate marker for testing the effectiveness of therapies such as statins and antibiotics in targeting the acute phase response element of atherothrombosis<sup>256</sup>.

A trend towards improved stroke outcome in patients receiving statins at the time of their stroke has been reported in a retrospective case-referent study<sup>265</sup>. While the statistical power and methods of outcome assessment were weak, in a logistic regression model the use of statins was a moderately strong but statistically non-significant predictor of discharge home (multiple adjusted odds ratio 1.42, 95% CI 0.90 to 2.22). As discussed in **chapter 1**,

possible explanations for such an effect include statin-mediated preservation of endothelial nitric oxide synthase activity in cerebral vasculature, modulation of endothelial function with preservation of blood flow to ischaemic regions or the putative anti-inflammatory and antioxidant properties of statins <sup>113</sup>. The authors <sup>265</sup> indicate that the design of a trial examining the acute effects of statins on *patient outcome* in stroke should be based on an estimated 20 to 40% reduction in the risk for poor outcome.

Randomised data from cardiovascular trials have confirmed that statins confer a reduced risk of stroke in individuals with CHD across a range of cholesterol levels (see **chapter 1**). Preliminary data from the Heart Protection Study <sup>100</sup>, which currently remains unpublished, have suggested a significant effect on vascular events in individuals with no prior CHD, a finding which is being investigated in 'pure' stroke populations by ongoing randomised trials. No significant effect on arterial flow, cerebral perfusion or haemorrhological markers was demonstrated in this trial, but the early use of statin therapy may have wider implications than merely early reduction of cholesterol. In common with early ACE inhibitor use after stroke following the publication of the PROGRESS trial <sup>39</sup>, there may be effects on both recovery from the index stroke and vascular risk reduction in the early subacute phase, and these must be examined in larger studies with clinical endpoints. Such studies must also examine the interaction between statins and ACE inhibitors, particularly on blood pressure control, whether different statins have different effects, and whether effects are seen only in subgroups of the heterogeneous stroke population.

Patient number	Initials	Age	Date of qualifying event	Event type	MRI / CT	Comorbid history	Treatment allocation	Other medication	Entry NIH score	Final NIH score
1	PS	71	07-Oct-99	R POCI	PVWMI	Nil	pravastatin	aspirin	3	1
2	JM	68	18-Oct-99	R POCI	R pons infarct SVD	Nil	pravastatin	aspirin omeprazole	0	0
3	JP	58	28-Oct-99	L PACI	L subcortical infarct	Dukes B adenocarcinoma: Recent sigmoid colectomy	placebo	aspirin ralitrexed	1	0
4	BM	59	22-Nov-99	L PACI	Normal	Nil	pravastatin	aspirin solpadol	0	0
5	MF	71	25-Nov-99	L PACI	L cortical infarct	Type II diabetes Obesity	placebo	gliazide lansoprazole	3	N/A
6	JON	64	11-Jan-00	L LACI	L subcortical infarct	Type I diabetes Squamous jaw tumour	placebo	insulin aspirin	4	4
7	WS	35	04-Feb-00	R LACI	Normal	Hypertension (untreated)	placebo	aspirin	1	0
8	AJ	64	18-Feb-00	R LACI	Normal	Breast carcinoma	pravastatin	aspirin	3	2
9	AT	52	06-Apr-00	L LACI	Old subcortical infarcts	Crohns Disease Osteoporosis Wernicke syndrome 1995	placebo	azathioprine prednisolone Aspirin IV rPa (stroke)	9	N/A
10	DM	70	10-May-00	R LACI	PVWMI Atrophy	Nil	pravastatin	aspirin	0	0
11	CR	61	16-Jun-00	L PACI	Large L subcortical infarct	Nil	placebo	aspirin	12	6
12	WK	75	07-Aug-00	L PACI	Normal	Osteoarthritis Asbestosis Gastro-oesophageal reflux	pravastatin	Losec Co-danthramer	1	0
13	JS	62	19-Oct-01	R PACI	Atrophy	Osteoarthritis Angina	pravastatin	omeprazole aspirin	1	1

Patient number	Initials	Age	Date of qualifying event	Event type	MRI / CT	Comorbid history	Treatment allocation	Other medication	Entry NIH score	Final NIH score
14	EM	90	07-Nov-00	R PACI	R subcortical infarct	Osteoarthritis Chronic renal failure Iron deficiency anaemia	placebo	iron sulphate aspirin	7	N/A
15	MM	75	09-Nov-00	R PACI	Normal	Hysterectomy	pravastatin	aspirin dipyridamole brufen	1	0
16	JW	77	15-Nov-00	R LACI	R subcortical infarct	Hypertension (treated) OA	placebo	aspirin dipyridamole (bendrofluazide stopped)	1	0
17	GH	75	09-Feb-01	L POCI	PVWMI Old subcortical infarcts	Nil	placebo	aspirin	0	0
18	MB	79	01-Mar-01	L PACI	PVWMI Atrophy	Aortic stenosis	pravastatin	aspirin	1	0
19	EA	58	27-Mar-01	R PACI	R cortical (MCA) infarct	Type II diabetes Alcoholic liver disease Previous stroke	pravastatin	aspirin	1	N/A
20	JG	41	19-Jun-01	R LACI	Normal	Hypertension (treated)	placebo	aspirin (amlodipine stopped)	0	0
21	MC	55	19-Jun-01	R PACI	Normal	Nil	placebo	aspirin	1	0
22	HOB	51	08-Aug-01	R LACI	Normal	Previous stroke 1998	pravastatin	aspirin dipyridamole	7	1
23	DG	81	26-Aug-01	R LACI	R subcortical infarct	Hypertension (untreated)	placebo	clopidogrel	3	N/A
24	DF	95	30-Aug-01	L PACI	Moderate atrophy	Nil	pravastatin	aspirin	0	N/A

**Table 5.1: Characteristics of patients.** Entry event (stroke or TIA) date, and event type are tabulated for each patient. Prefix L or R denotes the side of the ischaemic lesion. PACI = partial anterior circulation stroke, LACI = lacunar infarction, POCI = posterior circulation stroke. CT or MRI findings are summarised in the sixth column. PVWMI = periventricular white matter ischaemia. Significant medical history for each patient at entry is shown, along with treatment allocation and concurrent medication at entry. NIH stroke scale score (see **appendix 3**) is tabulated for each patient on days 0 and 56.

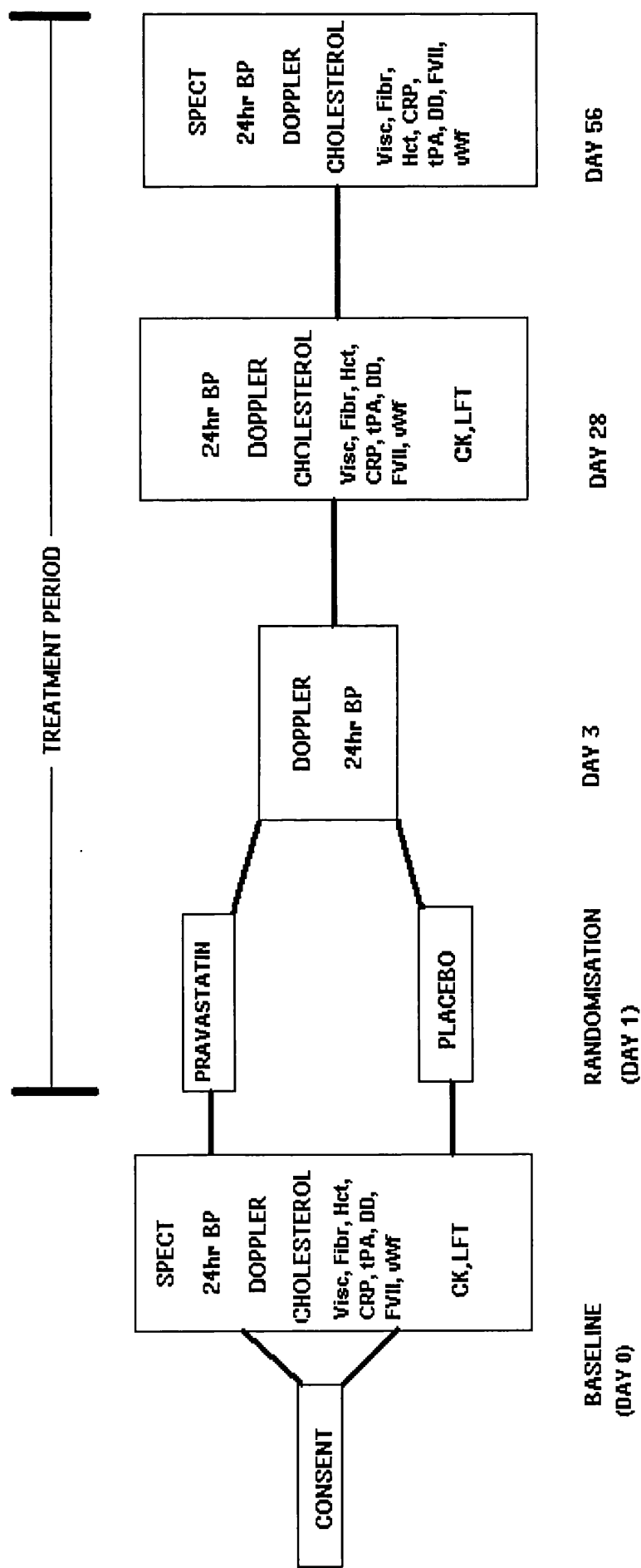
	Day 56 - baseline			Day 28 - baseline		
	Placebo mean change (95% CI)	Pravastatin mean change (95% CI)	<i>p</i> -value for comparison	Placebo mean change (95% CI)	Pravastatin mean change (95% CI)	<i>p</i> -value for comparison
Total cholesterol (mmol/l)	0.23 (-0.48 to 0.93)	-1.30 (-1.75 to -0.84)	<0.001	0.15 (-0.45 to 0.74)	-0.96 (-1.45 to -0.48)	0.004
LDL cholesterol (mmol/l)	0.25 (-0.31 to 0.81)	-1.39 (-1.77 to -1.01)	<0.001	0.05 (-0.50 to 0.61)	-1.05 (-1.90 to -0.20)	0.049
HDL cholesterol (mmol/l)	0.04 (-0.06 to 0.15)	0.06 (-0.17 to 0.28)	0.890	0.09 (-0.03 to 0.21)	0.06 (-0.12 to 0.24)	0.778
Triglycerides (mmol/l)	-0.24 (-1.06 to 0.58)	-0.14 (-0.91 to 0.62)	0.851	0.04 (-0.70 to 0.79)	-0.03 (-0.64 to 0.57)	0.851
Creatinine kinase (CK) (u/l)	-	-	-	22.75 (-16.45 to 61.95)	7.73 (-23.96 to 39.42)	0.501
Alanine aminotransferase (ALT) (u/l)	-	-	-	-9.13 (-30.05 to 11.80)	-0.17 (-10.01 to 9.68)	0.334
Aspartate aminotransferase (AST) (u/l)	-	-	-	-2.25 (-11.08 to 6.58)	2.83 (-4.24 to 9.90)	0.321

**Table 5.2: Change in lipid and muscle and liver enzyme values over time**

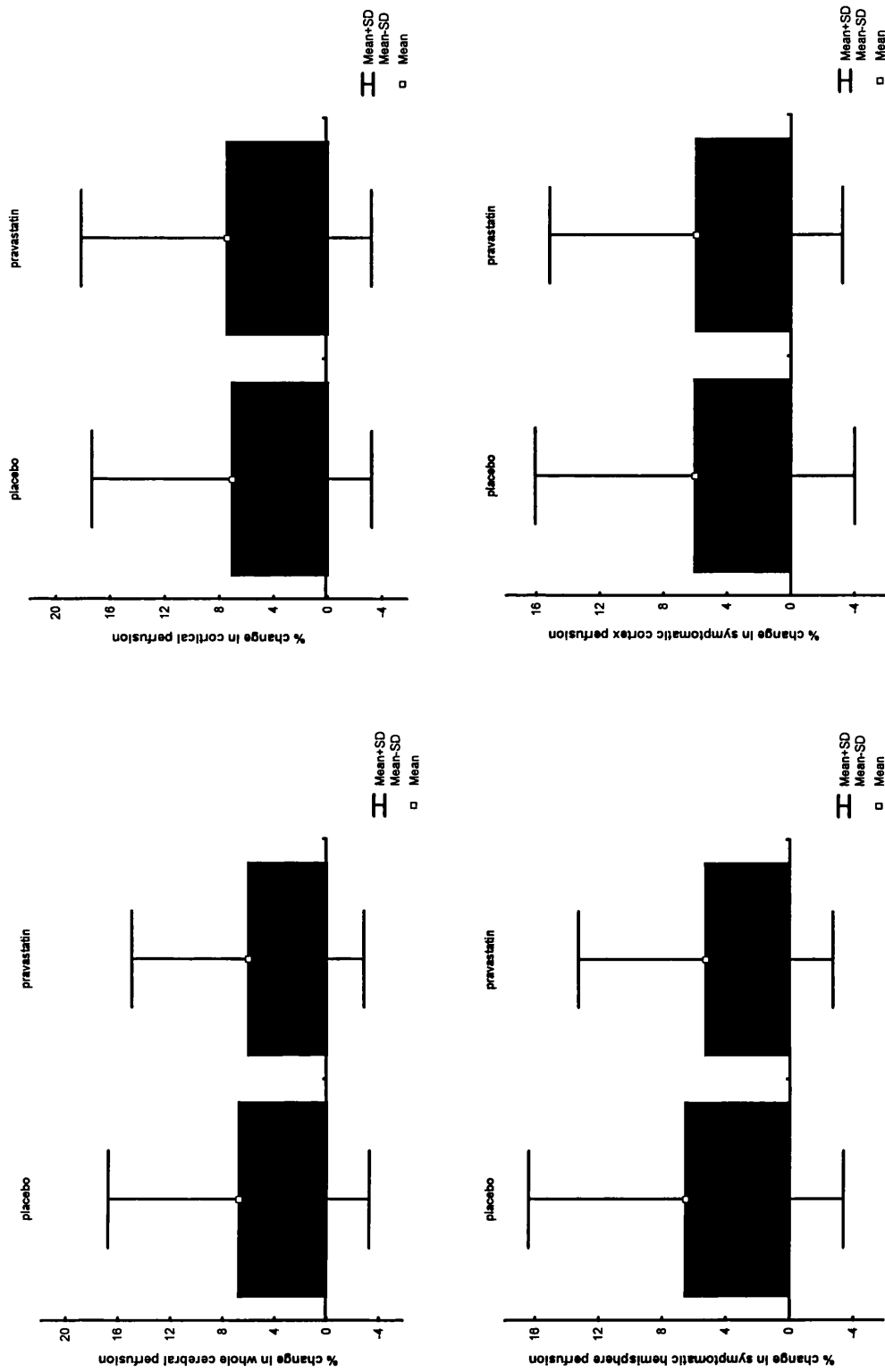


	Day 56 - baseline		Day 28 - baseline			
	Placebo mean change (95% CI)	Pravastatin mean change (95% CI)	p-value for comparison	Placebo mean change (95% CI)	Pravastatin mean change (95% CI)	p-value for comparison
<b>Plasma viscosity (mPa.sec)</b>	0.017 (-0.045 to 0.081)	-0.007 (-0.062 to 0.049)	0.509	0.039 (0.007 to 0.070)	-0.024 (-0.072 to 0.025)	0.040
<b>Fibrinogen (g/l)</b>	-0.249 (-1.327 to 0.830)	-0.090 (-0.882 to 0.702)	0.782	-0.414 (-1.468 to 0.641)	-0.603 (-1.537 to 0.332)	0.770
<b>Haematocrit</b>	0.006 (-0.012 to 0.023)	-0.011 (-0.025 to 0.003)	0.101	0.007 (-0.011 to 0.0242)	0.0003 (-0.011 to 0.011)	0.462
<b>Factor VII (iu/dl)</b>	16.63 (3.64 to 29.62)	-3.40 (-21.42 to 14.62)	0.067	12.50 (-8.60 to 33.60)	5.00 (-8.91 to 18.91)	0.489
<b>C reactive protein (mg/l)</b>	-5.77 (-18.22 to 6.68)	7.46 (-20.01 to 34.94)	0.373	-4.35 (-16.83 to 8.14)	-6.80 (-15.99 to 2.39)	0.718
<b>Tissue plasminogen activator (ng/ml)</b>	0.775 (-0.573 to 2.123)	0.630 (-3.380 to 4.63998)	0.945	0.925 (0.278 to 1.572)	1.833 (-1.851 to 5.517)	0.668
<b>D dimer (ng/ml)</b>	-117.38 (-304.35 to 69.60)	-52.30 (-122.99 to 18.38)	0.419	-108.50 (-266.10 to 49.10)	-2.83 (-37.33 to 31.66)	0.080
<b>von-Willebrand factor (iu/dl)</b>	-11.63 (-47.56 to 24.31)	6.00 (-24.16 to 36.16)	0.395	-22.50 (-62.81 to 17.81)	-13.75 (-31.306 to 3.81)	0.611

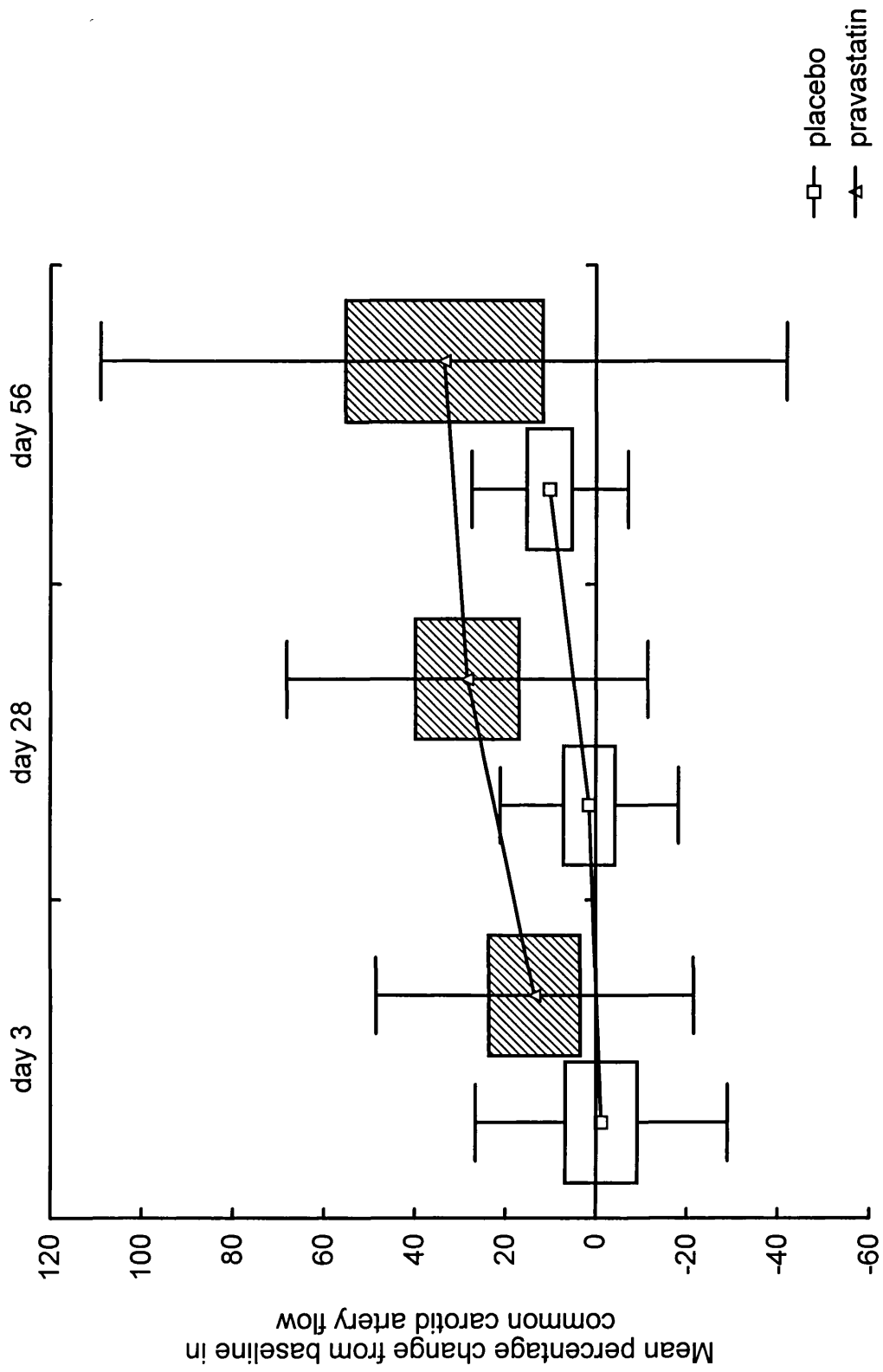
**Table 5.3: Change in haemorrhological variables over time**



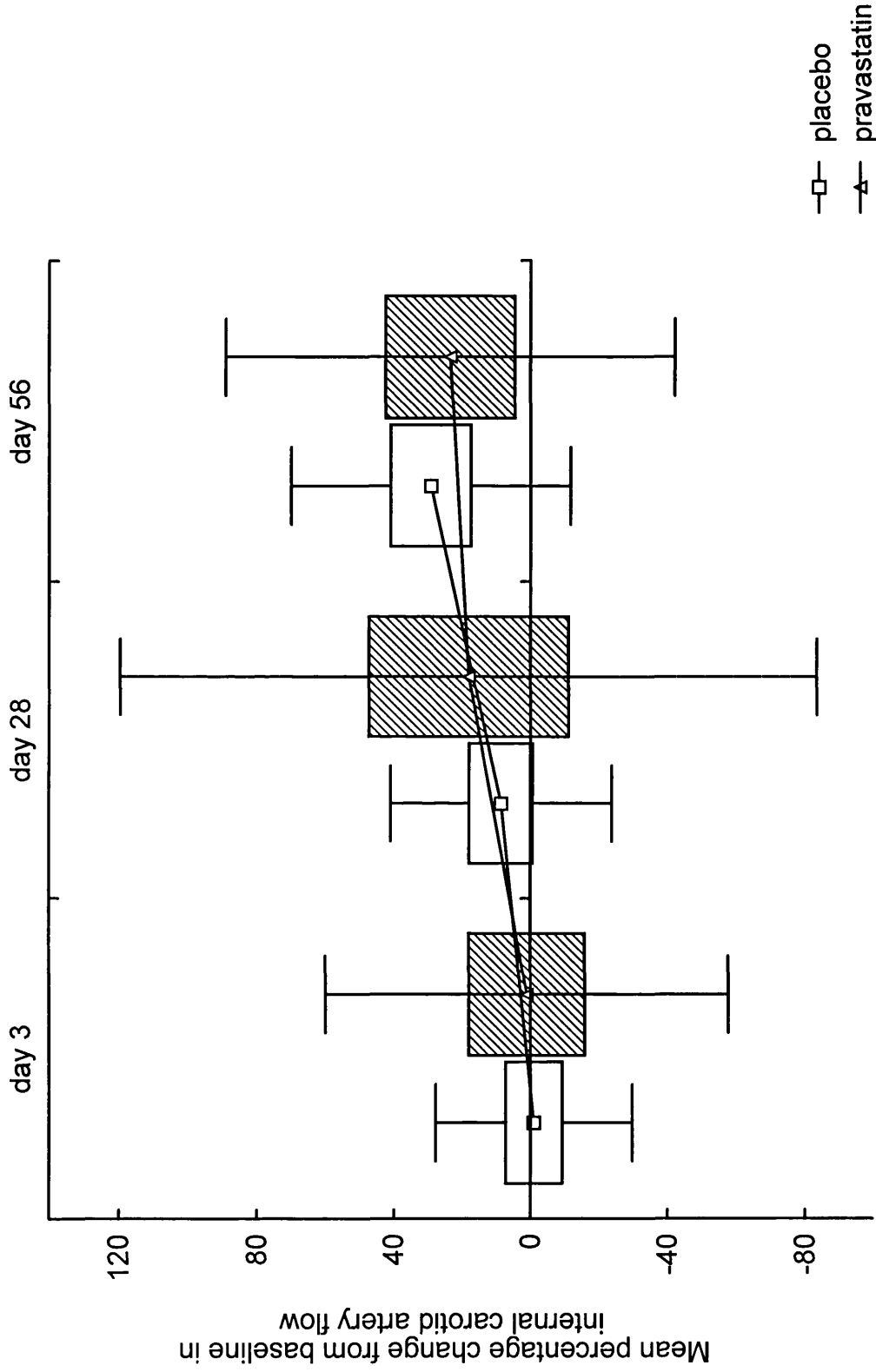
**Figure 5.1: Design of the Statins in Acute Stroke Trial.** SPECT= Single photon emission computed tomography, CK= creatinine kinase, LFT= liver function tests, Visc= plasma viscosity, Fibr= fibrinogen, Hct= haematocrit, CRP= C reactive protein, tPA= tissue plasminogen activator, DD= fibrin d-dimer, FVII= factor VII and vWF= von-Willebrand factor.



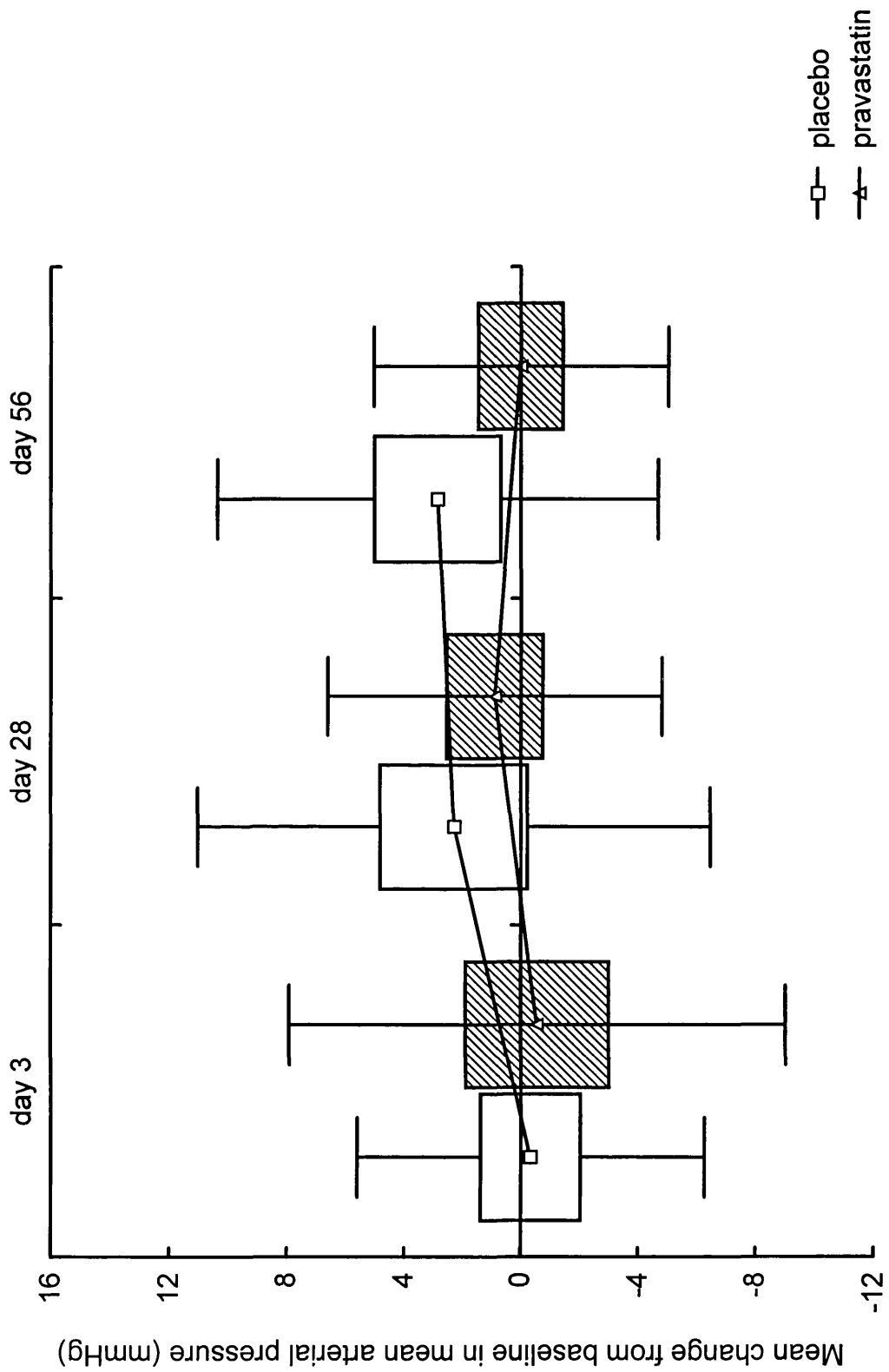
**Figure 5.2: SPECT comparison of percentage change in cerebral perfusion (56 day scan - baseline scan) in pravastatin treated and placebo groups.** Comparison is made between the two groups using four different measures of cerebral perfusion, namely whole brain perfusion, whole cortical perfusion, symptomatic hemispheric perfusion and symptomatic cortex perfusion. The mean value for each group is represented by a bar, and the standard deviation of each mean value is illustrated by brackets.



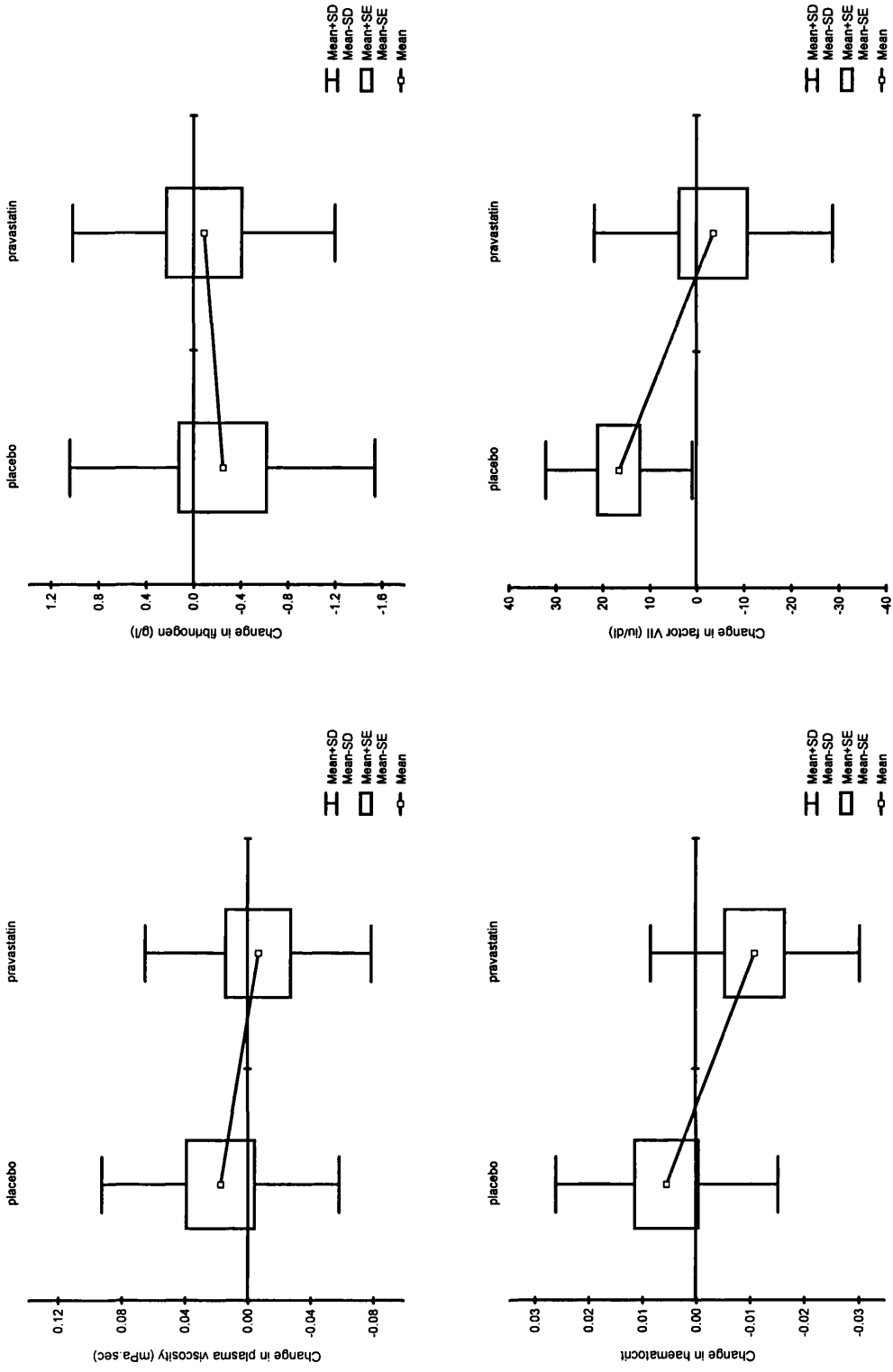
**Figure 5.3: Common carotid artery flow in pravastatin and placebo groups.** For each follow-up timepoint change from the baseline flow value was calculated for each individual and expressed as a percentage of that value. The mean percentage change for the placebo and pravastatin groups at each timepoint is represented by the point value shown. The standard error is shown by the box, and the standard deviation by brackets.



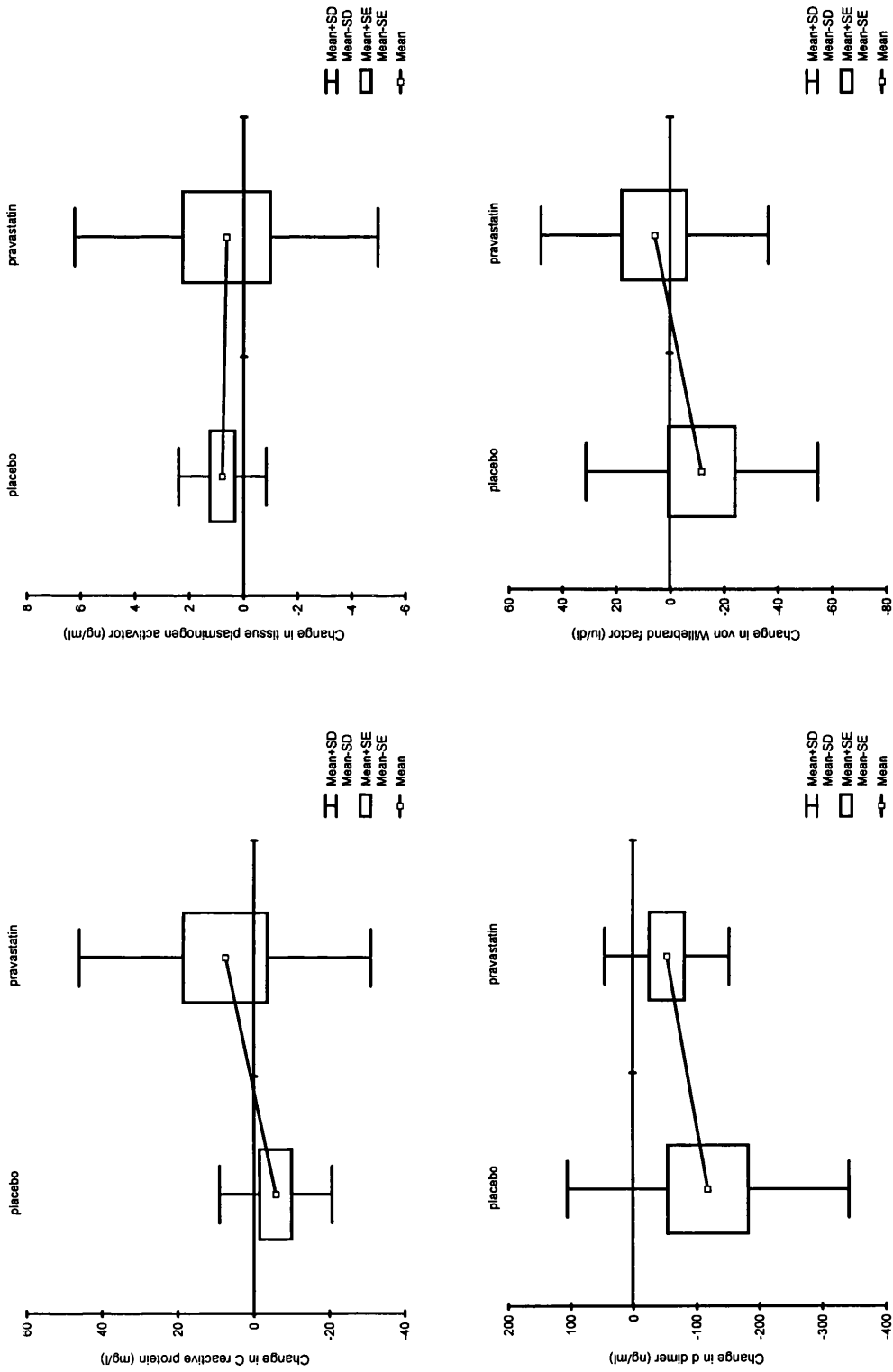
**Figure 5.4: Internal carotid artery flow in pravastatin and placebo groups.** For each follow-up timepoint change from the baseline flow value was calculated for each individual and expressed as a percentage of that value. The mean percentage change for the placebo and pravastatin groups at each timepoint is represented by the point value shown. The standard error is shown by the box, and the standard deviation by brackets.



**Figure 5.5: Mean arterial blood pressure in the pravastatin and placebo groups.** A change from their baseline reading was calculated for each individual at each timepoint. The mean change for placebo and pravastatin treated patients at each timepoint is represented by the point value shown. The standard error is shown by the box, and the standard deviation by brackets.



**Figure 5.6: Change in plasma viscosity, fibrinogen, haematocrit and factor VII.** The difference between the value at day 56 and baseline (day 56 - day 0) was calculated for each randomised individual. The mean, standard error and standard deviation for these differences are shown for the placebo and pravastatin groups. A line joins the mean values for the placebo and pravastatin groups.



**Figure 5.7: Change in C-reactive protein, tissue plasminogen activator, d dimer and von-Willebrand factor.** The difference between the value at day 56 and baseline (day 56 - day 0) was calculated for each randomised individual. The mean, standard error and standard deviation for these differences are shown for the placebo and pravastatin groups. A line joins the mean values for the placebo and pravastatin groups.



**Chapter 6: The effect of anti-chlamydial antibiotics on fibrinogen levels after stroke: the Fibrinogen And Chlamydia Experiment (FACET)**

## 6.1 Introduction

Numerous observational studies have suggested a link between chronic infection and vascular disease. Amongst the putative chronic or acute infections that have been studied, *chlamydia pneumoniae* infection has been closely correlated with both coronary heart disease and cerebrovascular disease<sup>143 146</sup>. Treatment trials with anti-chlamydial antibiotics, namely macrolides, have been performed in patients with unstable angina and non-Q wave myocardial infarction, and have yielded mixed results<sup>152 154 266</sup>. No treatment trial in the stroke population has yet been published.

Fibrinogen is a well established risk factor for vascular disease in general, and more specifically for cerebrovascular disease<sup>117 118</sup>. It also appears to be associated with chlamydial infection in observational studies<sup>149 150 151</sup>. Reduction in fibrinogen is a pharmacological goal that may be achieved with anacard in the acute phase<sup>121</sup> or fibrates in the longer term, but whether fibrinogen can be reduced in a cerebrovascular population by treating chlamydial infection is not known. It is also unknown whether treatment of stroke patients with macrolide antibiotics will reduce their risk of further cardiac and cerebral events or death.

Large scale intervention studies are needed in this area. The study reported here represents the pilot phase of a project designed to establish whether anti-chlamydial macrolide antibiotics represent a mechanism by which fibrinogen can be reduced in the post-stroke phase. A further aim was to examine whether this might occur just in those patients seropositive for chlamydia or whether macrolides may exert their effects via a more generalised anti-inflammatory response, irrespective of chlamydia status.

## 6.2 Methods

### ***Study Design and objectives***

The study was designed as a randomised, double-blind, placebo-controlled trial of azithromycin versus placebo in patients with acute ischaemic stroke, and was approved by our local ethics committee. The hypothesis was that the difference between baseline acute fibrinogen levels and fibrinogen levels at 1 and 3 months after stroke would be greater in those treated with macrolide antibiotics than in those who received placebo. We wished to assess whether such a difference was present only in the group seropositive for chlamydia, and whether amongst those patients who received azithromycin larger falls in fibrinogen would be seen in seropositive than in seronegative patients, thus suggesting a link between chlamydial seropositivity and hyperfibrinogenaemia. The effect of azithromycin on other markers of blood rheology and inflammation was also examined, to provide exploratory data and to generate further hypotheses. Due to a lack of suitable data on the variability of differences between fibrinogen levels acutely and at 1 month a pilot phase of 30 patients was planned, which is reported in this thesis. Sample size calculations using this dataset were conducted in order to plan a definitive study. Ethical approval was obtained from the West Ethics Committee, and patients gave written informed consent to participate.

### ***Patient qualification, enrolment, randomisation and dosing***

The following inclusion and exclusion criteria applied to the study:

#### ***Inclusion criteria***

- Over 40 years of age
- 4 days to 4 weeks post stroke or TIA
- Written informed consent of patient
- Haemorrhage excluded on CT or MRI scanning

#### ***Exclusion criteria***

- Chronic Bronchitis

- Recent (previous month) or current prescription of macrolide antibiotics, or prescription of alternative broad spectrum antibiotics during course of trial
- Documented bacterial infection
- Swallowing difficulty, or anticipated high risk of aspiration pneumonia
- QT prolongation on ECG, or concomitant antihistamine preparation
- Prescription of warfarin or heparin before enrolment
- Hepatic impairment
- Pregnancy or breast feeding

Co-prescription of other agents known to interact with macrolides (for example digoxin) was monitored, but did not exclude the patient from the study.

After explanation of the procedures and gaining of informed consent, eligible patients underwent baseline investigations, consisting of fibrinogen level, plasma viscosity (PV), von-Willebrand factor (vWF) and C-reactive protein (CRP), as well as anti-chlamydial immunoglobulins (IgG and IgA). These investigations were carried out in the haemorrhology laboratories of Glasgow Royal Infirmary and the West of Scotland Regional Virus Laboratory respectively. The qualifying event was classified using Oxfordshire Community Stroke Project (OCSP) definitions<sup>171</sup>, and stroke severity was classified using the National Institutes of Health (NIH) stroke scale (see **appendix 3**).

Once baseline investigations were completed all patients, irrespective of chlamydia status which was at that point unknown, were randomised to receive either azithromycin capsules 500mg per day for 6 days or matching placebo, in a ratio of 1:1. The dosage chosen is double the standard azithromycin dose quoted in the *British National Formulary*, and at the time the trial was designed these smaller dosages of 500mg for 3 days had been demonstrated to have an effect on event rate in patients with ischaemic

heart disease <sup>152</sup>. Randomisation was conducted by an unblinded independent research pharmacist who was uninvolved in clinical management except for the provision of blinded drug supplies. Randomisation took place between November 1999 and September 2000.

### ***Study flow***

The drug course was for 6 days, and fibrinogen levels were measured at the end of the course of tablets, along with PV, vWf and CRP. NIH score was reassessed. Standard post-stroke management was employed, either in the acute stroke unit or the rehabilitation setting. Concurrent antibiotic therapy led to withdrawal from the study, and the decision to initiate antibiotic therapy outwith the study was that of the clinical team.

Patients returned in 1 month for repeat measurements of chlamydial serology and fibrinogen, PV, vWf, and CRP. Fibrinogen, PV, vWf and CRP were repeated at 3 months to confirm that any detected effect persisted. NIH score was assessed at each visit. An overview of the study design is provided in **figure 6.1**.

### ***Laboratory testing***

Anti-chlamydia immunoglobulins were measured using enzyme linked immunosorbent assay (ELISA). The use by previous investigators of microimmunofluorescence (MIF) and complement fixation (CFT) tests in their assessment of 'chlamydia seropositivity' has been criticised, with the 'cut-offs' denoting seropositivity and seronegativity varying widely <sup>143</sup>. Defining boundaries that provide adequate sensitivity and specificity has proven problematic <sup>267</sup>. After comparing assays using 4 tests (MIF, ELISA IgG, ELISA IgA and CFT) on 188 local cases (with proven respiratory infection) and 88 healthy controls, the West of Scotland Regional Virus Laboratory concluded that switching to an ELISA based diagnostic series with higher cut-offs than those recommended by the manufacturers provided adequate diagnostic

accuracy without unacceptable false positive results (personal communication, Dr W Carman). Consequently, both IgG and IgA levels were measured in the trial. Seropositive patients were defined as having probable (IgG index  $>1$ ) or definite (IgG index  $>2$ ) previous infection, or evidence of infection in the recent past (IgA index  $> 2.5$ ).

Samples were stored at  $-70$  degrees Celsius at the Western Infirmary, and transferred at the end of the study to the haemorrhology laboratories at the Glasgow Royal Infirmary for analysis. Clottable fibrinogen was measured by the dilute thrombin clotting time on an automated coagulometer (MDA 180, Organon Teknika, Cambridge UK), using calibrants and reagents provided by the manufacturer. CRP was measured on a Prospec Nephelometer (Dade Behring, Marburg, Germany) using a high sensitivity method with the manufacturer's calibrant and reagent. PV was measured using a semi-automated capillary Viscometer (Beckman Coulter, High Wycombe, UK). Plasma vWF antigen levels were measured using an in-house ELISA, employing rabbit anti-human polyclonal antibodies obtained from DAKO plc, High Wycombe, UK.

### ***Statistical considerations and justification for sample size***

The pilot phase (the initial 30 fully evaluable patients) is reported here, with the intention being to estimate the variability in fibrinogen in our population and likely treatment effect of azithromycin. We expect these data to inform both the sizing and the design of a definitive project.

Descriptive statistics and significance calculations were prepared using Statistica for Windows (Version 5.1, Statsoft Inc.). Values of rheological variables at each timepoint, and calculated differences within individuals between values at different timepoints, were normally distributed. After calculation of individual differences the data were tested for the effect of seropositivity, the effect of treatment, or for an interaction between these

factors, using two-way factorial analysis of variance (ANOVA). These effects were quantified and confidence limits for the effects were generated using Minitab for windows (Version 10.1). Sample size calculations were conducted with PS sample size calculation software (Version 1.0.13, Dupont and Plummer), using variability data detected within the pilot trial sample.

### 6.3 Results

A total of 34 patients was randomised. Three patients withdrew from the study before day 6 (patient numbers 4, 19 and 29). One patient died between day 6 and day 30 (patient number 14). Thus 30 patients were available for primary end point analysis, with 16 randomised to azithromycin and 14 to matching placebo. No patient received heparin during the acute stroke phase, nor alternative broad spectrum antibiotics during the course of the trial. Two patients were started on warfarin during the course of the study (patient number 7 and patient number 22, both for deep venous thrombosis). One patient was randomised while taking warfarin (patient number 33 who was taking warfarin for atrial fibrillation), which represented a protocol violation. These three patients were included in the final analysis because the prescription of warfarin does not appear to affect fibrinogen levels<sup>268 269</sup>.

**Table 6.1** shows the characteristics of the 34 randomised patients. Patients completing the trial and whose results are reported subsequently are in bold type in the table. Mean age was 67 (+/- standard deviation (SD) 14). Mean NIH score for all patients at entry was 2.6 (+/- SD 3.09); mean NIH score for evaluated patients was 2.0 (+/- SD 2.0). Amongst evaluated patients there was no significant difference in NIH score between azithromycin and placebo treated groups ( $p=0.78$ ), or between seropositive and seronegative groups ( $p=0.94$ ). Twelve evaluated patients suffered lacunar events, 3 posterior circulation events and 15 cortical anterior circulation events. Three patients had further stroke events during follow-up: patients 5 (seronegative-placebo)

and 33 (seropositive-placebo) had a TIA between day 6 and 30 and patient 9 (seropositive azithromycin) suffered a repeat cortical stroke between day 30 and day 90 causing an increase in NIH score to 11 at day 90.

**Figure 6.2** shows the mean and spread of values for the total patient group for each haemorheological variable at each timepoint. It can be seen from the figures that both CRP and fibrinogen tended to rise at day 6 and fall subsequently, consistent with an acute phase reaction <sup>270</sup>. **Table 6.2** and **table 6.3** detail the mean levels of haemorrheological parameters at each timepoint for each subgroup, categorising the patients according to treatment (azithromycin or placebo) and chlamydia status.

To eliminate the effect of inter-subject variability, the difference between fibrinogen at day 1 and day 90 (day 1-day 90, since it was anticipated that fibrinogen would fall after the acute stroke event), and between fibrinogen at day 1 and day 30 (day 1 - day 30), was calculated for each patient. This represented a summary measure of the change in fibrinogen over time in each individual. Comparison was then made between the seropositive and seronegative groups and between the azithromycin treated and placebo groups. The interaction between these factors was also assessed. Analogous calculations were conducted for CRP, PV and vWf. The effects of the 3 different factors (treatment, seropositivity and the interaction between them) were quantified and are presented in **table 6.4**. Neither azithromycin therapy nor chlamydia status had any effect on fibrinogen, CRP, PV or vWf change over time. No differential effect of treatment was detected in the seropositive group on any of the parameters tested.

Sample size calculations were planned using variability (standard deviation) data on the differences within individuals between fibrinogen levels at day 1 and day 30. With a ratio of experimental to control subjects of 1 (comparing azithromycin to control), and a type 1 error probability of 0.05 for a 2 sided



test, a planned study would have 90% power to detect a difference of 0.2g/l between treated and control groups with an experimental sample size of 202 patients. The spread of values for alternative detectable mean differences, and varying levels of power, are depicted in **figure 6.3**. Two-thirds of the patients we included were seropositive; to compare treatment and control only in these patients, we would need to screen approximately 300 patients. To assess whether falls in fibrinogen are higher in treated seropositives than in treated seronegatives, the ratio of control to experimental patients falls to 0.5, and 304 unselected patients would need to be assigned azithromycin treatment to detect a difference of 0.2g/l at 90% power.

## 6.4 Discussion

The data from this pilot phase have allowed us to size the next stage of the project, and have excluded effects of macrolide antibiotics on fibrinogen levels in unselected patients after ischaemic stroke of more than 0.36g/l (the upper end of the confidence interval for the difference between treated and placebo - see **table 6.4**). Data from epidemiological studies suggest that clinically important differences in event rate may be seen between groups whose fibrinogen differs by as little as 0.2g/l <sup>117 118</sup>, and the adjusted difference between seropositive and seronegative patients in the largest epidemiological series in which the association between fibrinogen and chlamydia status has been described was 0.18g/l (95% C.I.= 0.015 to 0.345) <sup>150</sup>. For this reason a larger study of the size stated above is required to evaluate the primary and secondary hypotheses formulated at the planning stage.

Trials in the coronary population have used 'hard' endpoints rather than surrogate markers, and treatment trials like these will be needed to assess whether antichlamydial antibiotics have any practical role in cerebrovascular disease. Longer courses of azithromycin (between 3 and 12 months of

therapy) have been incorporated into the design of recent trials<sup>271</sup>. Alteration of the FACET trial protocol to include a longer course of therapy is likely to maximise any effect on chronic chlamydial infection and its sequelae. There is, as yet, little evidence that lowering fibrinogen reduces rates of vascular events. Investigations into treatment effects on surrogate markers such as fibrinogen can, however, be useful in confirming hypotheses without the considerable expense and planning required for a large scale project. No effects on the other haemorrhological parameters tested were detected; trends or effects on these parameters may become more evident when larger patient numbers are recruited.

As alluded to previously, what constitutes 'seropositivity' is disputed and uncertain. Previous studies in the cerebrovascular population have generally used MIF techniques, although their 'cut-off' levels for seropositive titres have varied considerably. When strict or complex criteria involving repeated sampling are used, as in the study by *Cook et al*<sup>146</sup>, detected levels of 'acute' (13.6%) and 'previous' (32.4%) infection are low. In other published case control studies IgA levels  $\geq 1:16$  have been reported to be present in 46.1%<sup>272</sup>, 46.6%<sup>145</sup> and 45%<sup>148</sup> of ischaemic stroke patients. 'Seropositive' IgG levels have been reported in much higher proportions of ischaemic stroke patients with the MIF technique (80.9%<sup>272</sup> and 86%<sup>148</sup>  $\geq 1:16$ , 74.1%<sup>145</sup> and 79%<sup>148</sup>  $\geq 1:32$ ). In this study, two-thirds of ischaemic stroke patients were classified as seropositive, 43% having IgA index  $>2.5$ , and 50% having an IgG index  $>1$ . Such findings were expected, and reflect the improved specificity of the ELISA tests and modified cut-off points employed.

Patients included in this study generally had mild strokes (mean NIH score = 2.6), a reflection in part of the environment in which the study was conducted. The Western Infirmary Acute Stroke Unit is a very active research facility, with a number of acute and secondary prevention studies running concurrently. Patients with more severe strokes will often have been included in one of the

acute studies, precluding their involvement in FACET. Patients with swallowing difficulties were not included as a result of concerns about aspiration risk and likely need for broad spectrum antibiotics. Explaining trial plans, and making arrangements to review patients at follow-up visits, requires a level of understanding and mobility which renders severely affected patients difficult subjects in trials that include repeated assessments. This is undoubtedly a difficult issue in stroke research, but trials should be conducted in a representative population, to widen the applicability of any results, and to broaden the possibilities of subgroup analysis. Attempts will be made to recruit a more varied cohort when the definitive project begins.

Elevation of fibrinogen and CRP levels in acute stroke has been amply documented, and large prospective studies have demonstrated that each is an independent risk factor for both stroke and other vascular events in the longer term<sup>120 139</sup>. It seems that the 'acute phase response' is superimposed upon what appears to be a chronic elevation of fibrinogen and CRP levels in patients with cerebrovascular disease. Antibiotic use and recent clinical infection preceding ischaemic stroke have correlated strongly with levels of CRP but not fibrinogen in observational studies<sup>273 274</sup>, and so differential effects on CRP and fibrinogen may be useful in separating a 'generalised' anti-inflammatory effect from a specific 'anti-chlamydial effect'. The planned study randomising 202 patients would be able to detect a difference between groups of 7.3mg/l in difference in CRP level between day 1 and day 90 with 90% power. In a longitudinal analysis of acute phase markers after acute ischaemic stroke, *Beamer et al* found that while CRP, interleukin-6 and interleukin-1 receptor antagonist levels returned to those of a 'vascular risk' group without a history of recent stroke, fibrinogen levels remained elevated, even after a follow-up period of a year ( $p < 0.001$ )<sup>273</sup>. Fibrinogen levels predicted new vascular events in this cohort. Other workers have reported similar findings<sup>275</sup>.

Multiple stimuli may be responsible for upregulating fibrinogen synthesis: smoking, body mass index and serum lipid levels over longer periods, and tissue injury and necrosis in the acute phase of vascular events. Chlamydial infection may play a role both in the peri-stroke period, contributing to the 'acute phase response', and in the longer term, as suggested by epidemiological data <sup>149</sup>. In this trial baseline fibrinogen was assessed at a time when the acute phase response may have been an important determinant of its level: a mean of 3 days with a maximum of 6 days and a minimum of 1 day. If fibrinogen had been measured earlier (<12 hours after the acute event) it is less likely that an acute phase reaction, with infarction *itself* being a confounding factor, would interfere with the results. There was, however, no significant difference between treatment and placebo groups in the time from the stroke event that the baseline samples were taken or in stroke severity (as measured by NIH scores), which is an important determinant of the level of the acute phase response <sup>276</sup>.

Peri-stroke infection (chlamydial or other) may be an important factor in the acute rise in fibrinogen levels which occurs, and may be modifiable by antibiotic therapy. This in turn may affect the fibrinogen level which is attained in the year post-stroke, and which is clearly associated with recurrent vascular events <sup>273</sup>. The use of day 6 fibrinogen levels (which showed a trend upwards from baseline as a result of acute phase response), or of 'area under the curve' of fibrinogen levels plotted against time, in outcome summary measures would assess such an acute effect more closely, but these are less clinically relevant than the outcome measure chosen. The aim was to determine whether fibrinogen could be lowered in the recovery phase, with the assumption that this would carry a reduced risk of recurrent vascular events, not to assess any effect on the acute rise in fibrinogen itself. Acute rises in fibrinogen are much more effectively treated with defibrinogenating agents such as ancrod <sup>121</sup>. It is important that the planned trial continues to assess fibrinogen at 90 days, to ensure that any detected effect persists.

Recent prospective data have raised questions about the association between chlamydial infection and CHD. In comparison with retrospective studies, prospective studies should reduce selection biases, minimise any influence of disease itself on the factor being investigated, and generally include better adjustment for potential confounding factors. However, adjustment for potential confounding factors, when we know little of the mechanism by which such factors may affect the disease under study, may lead to 'over-adjustment', thus diluting any true effect. *Danesh et al* reported a large epidemiological survey of 5661 men who provided blood samples during 1978-80<sup>271</sup>. 40% of the 496 men with CHD were in the top third of *C pneumoniae* titres compared with 33% of the 989 controls. The corresponding odds ratio for CHD was 1.66 (95% CI = 1.25 to 2.21). This remained highly significant when adjusted for age, town, smoking and social class, but reduced to 1.22 (95% CI = 0.82 to 1.82) after adjustment for childhood social class. There is poor understanding of the mechanism by which childhood social class influences adult CHD, but chlamydial infection may play a part, therefore possibly rendering such adjustment 'over-adjustment'<sup>277</sup>.

It was the fully adjusted estimate that was included in *Danesh et al's* accompanying meta-analysis of the 15 published prospective studies of *C. Pneumoniae* and CHD, which yielded a combined odds ratio of 1.15 (95% CI = 0.97 to 1.36). They concluded that the meta-analysis reliably excluded any strong association between *C pneumoniae* IgG titres (or IgA titres) and CHD, and that planned randomised trials were unlikely to be large enough to confirm or refute the small effect on coronary risk that antibiotics such as azithromycin may confer. In a study that minimised the possible detrimental effects of over-adjustment, *Wald et al*<sup>278</sup> reported no association between infection with *C. pneumoniae* and mortality from IHD in a socially homogeneous population of middle aged professional men.

Thus recent data in the cardiovascular population have raised questions about whether an association between chlamydia seropositivity and vascular disease exists at all, and have strongly suggested that if an association is present, it is likely to be only small to moderate. Recent data in the cerebrovascular population, however, have continued to find moderate associations between chlamydial seropositivity and stroke. *Elkind et al*<sup>272</sup> recently reported case-control data from the Northern Manhattan Stroke Study that correlates well with previous case control literature<sup>145 146</sup>. Elevated *C. pneumoniae* IgA titres were significantly associated with risk of ischaemic stroke after adjusting for other stroke risk factors (adjusted OR 4.51, 95% CI 1.44 to 14.06). IgG titres were not significantly associated with stroke risk (adjusted OR 2.59, 95% CI 0.87 to 7.75), suggesting that it is persistent infection, rather than remote, completed infection which confers risk. *Schmidt et al*<sup>279</sup> demonstrated that seropositivity for *C. pneumoniae* is associated with an increased intima-media thickness (an early sign of atherosclerosis) in the common carotid artery in hypertensive men at high risk for cardiovascular disease.

Recently it has been stated in the stroke literature that the best means of ascertaining a link between *C. pneumoniae* infection and vascular disease, in the context of data suggesting association but not establishing causation, is by conducting interventional trials aiming to look at the effect of antibiotics that eradicate *C. pneumoniae*<sup>280</sup>. The study presented here is the first of these to report pilot data.

Patient number	Initials	Age	Date of qualifying event	Event type	Chlamydia status	Treatment allocation	Day 1		Day 6		Day 30		Day 90	
							NIH	NIH	NIH	NIH	NIH	NIH	NIH	NIH
1	JS	85	19-Nov-99	L LACI	positive	Azithromycin	2	1	1	1	1	1	1	1
2	MC	67	28-Nov-99	R PACI	positive	Placebo	2	2	2	2	2	2	2	1
3	JH	56	28-Nov-99	POCI	negative	Azithromycin	2	1	1	1	1	1	1	1
4	GB	86	30-Nov-99	L PACI	positive	Azithromycin	4	N/A	N/A	N/A	N/A	N/A	N/A	N/A
5	EE	65	12-Dec-99	POCI	negative	Placebo	0	0	0	0	0	0	0	0
6	AM	50	11-Dec-99	R LACI	positive	Azithromycin	0	0	0	0	1	1	0	0
7	CM	87	06-Jan-00	L PACI	negative	Placebo	3	3	3	2	2	4	4	4
8	AM	55	02-Jan-00	R LACI	negative	Placebo	4	3	3	4	4	2	2	2
9	RC	65	24-Jan-00	L PACI	positive	Azithromycin	3	3	3	3	1	11	11	11
10	HR	48	27-Jan-00	L LACI	positive	Placebo	0	0	0	0	0	0	0	0
11	CC	73	18-Feb-00	L PACI	positive	Placebo	1	0	0	0	0	0	0	0
12	HC	83	05-Mar-00	L LACI	negative	Placebo	2	3	3	1	1	1	1	1
13	GF	80	06-Mar-00	L PACI	positive	Azithromycin	0	0	0	0	0	0	0	0
14	JD	74	25-Mar-00	L TACI	positive	Placebo	16	13	13	N/A	N/A	N/A	N/A	N/A

Patient number	Initials	Age	Date of qualifying event	Event type	Chlamydia status	Treatment allocation	Day 1	Day 6	Day 30	Day 90
							NIH	NIH	NIH	NIH
15	JM	69	30-Mar-00	L LACI	positive	Placebo	1	1	1	1
16	WH	61	10-Apr-00	R PACI	positive	Azithromycin	2	1	1	1
17	SW	74	25-Apr-00	POCI	negative	Azithromycin	0	1	1	1
18	SM	69	28-Apr-00	R LACI	negative	Azithromycin	4	0	0	0
19	JS	79	07-May-00	L PACI	negative	Placebo	4	N/A	N/A	N/A
20	MW	79	20-May-00	L LACI	positive	Azithromycin	5	3	3	3
21	AD	40	21-May-00	R PACI	positive	Azithromycin	2	2	0	1
22	DM	59	24-May-00	R LACI	positive	Azithromycin	3	5	4	3
23	MM	68	06-Jun-00	L PACI	positive	Placebo	4	3	1	1
24	WB	62	29-Jun-00	R PACI	negative	Azithromycin	2	1	0	0
25	MM	75	10-Jul-00	R LACI	positive	Placebo	0	1	1	1
26	JR	82	30-Jul-00	L PACI	positive	Azithromycin	1	1	0	0
27	EF	78	07-Aug-00	R PACI	positive	Placebo	0	1	1	0
28	AC	62	15-Aug-00	L PACI	positive	Placebo	4	6	5	4



Patient number	Initials	Age	Date of qualifying event	Event type	Chlamydia status	Treatment allocation	Day 1 NIH	Day 6 NIH	Day 30 NIH	Day 90 NIH
29	FM	68	22-Aug-00	L LACI	positive	Azithromycin	4	N/A	N/A	N/A
30	MW	73	31-Aug-00	R LACI	positive	Placebo	9	9	7	5
31	DT	30	03-Sep-00	POCI	negative	Azithromycin	0	0	0	0
32	EF	79	05-Sep-00	L PACI	negative	Azithromycin	4	2	0	0
33	AE	60	12-Sep-00	L PACI	positive	Placebo	0	0	0	0
34	AS	49	18-Sep-00	R LACI	negative	Azithromycin	1	1	0	0

**Table 6.1: Patient characterisation and chlamydia status.** Entry event (stroke or TIA) date, and event type are tabulated for each patient. Prefix L or R denotes the side of the ischaemic lesion. TACI = total anterior circulation stroke, PACI = partial anterior circulation stroke, LACI = lacunar infarction, POCI = posterior circulation stroke. Chlamydia status is tabulated for each patient at entry (for definition of 'positive' and 'negative' see text). NIH stroke scale score (see **appendix 3**) is tabulated for each patient on days 1, 6, 30 and 90. Patients completing the trial, and whose results were included in the analysis, are in **bold type**. Patients recruited but not completing the trial are in *italic type* (see text for details).

Rheological marker	Chlamydia seropositive (n=19)	Chlamydia seronegative (n=11)	Azithromycin treated (n=16)	Placebo treated (n=14)	Seropositive azithromycin treated (n=9)	Seronegative azithromycin treated (n=7)	Seropositive placebo treated (n=10)	Seronegative placebo treated (n=4)
Fibrinogen (g/l) (baseline)	3.86 (+/- 0.86)	3.48 (+/- 0.95)	3.57 (+/- 0.82)	3.89 (+/- 0.98)	3.63 (+/- 0.81)	3.48 (+/- 0.89)	4.06 (+/- 0.89)	3.46 (+/- 1.20)
Fibrinogen (g/l) (6 days)	4.08 (+/- 0.93)	3.36 (+/- 0.87)	3.80 (+/- 0.99)	3.82 (+/- 0.96)	4.15 (+/- 0.95)	3.35 (+/- 0.91)	4.01 (+/- 0.96)	3.36 (+/- 0.94)
Fibrinogen (g/l) (30 days)	3.61 (+/- 0.83)	3.29 (+/- 0.99)	3.26 (+/- 0.76)	3.76 (+/- 0.97)	3.31 (+/- 0.65)	3.18 (+/- 0.92)	3.88 (+/- 0.90)	3.47 (+/- 1.21)
Fibrinogen (g/l) (90 days)	3.49 (+/- 0.74)	3.23 (+/- 0.75)	3.28 (+/- 0.56)	3.53 (+/- 0.91)	3.32 (+/- 0.53)	3.23 (+/- 0.64)	3.64 (+/- 0.89)	3.25 (+/- 1.03)
CRP (mg/l) (baseline)	11.06 (+/- 27.45)	16.92 (+/- 28.32)	10.99 (+/- 23.49)	15.74 (+/- 32.08)	5.48 (+/- 7.44)	18.07 (+/- 34.67)	16.08 (+/- 37.40)	14.89 (+/- 16.16)
CRP (mg/l) (6 days)	17.44 (+/- 30.33)	9.28 (+/- 11.98)	12.07 (+/- 16.80)	17.16 (+/- 32.85)	16.23 (+/- 20.40)	6.72 (+/- 9.58)	18.53 (+/- 38.30)	13.75 (+/- 15.91)
CRP (mg/l) (30 days)	8.66 (+/- 22.35)	7.10 (+/- 8.78)	4.36 (+/- 4.79)	12.35 (+/- 26.25)	2.95 (+/- 9.48)	6.18 (+/- 6.40)	13.80 (+/- 30.51)	8.71 (+/- 13.01)
CRP (mg/l) (90 days)	5.14 (+/- 8.04)	5.21 (+/- 5.30)	3.58 (+/- 2.36)	6.97 (+/- 9.90)	3.60 (+/- 2.84)	3.56 (+/- 1.79)	6.53 (+/- 10.85)	8.10 (+/- 8.34)

Table 6.2: Mean values (+/- standard deviation) of fibrinogen and C-reactive protein (CRP) at each timepoint for each subgroup.

Rheological marker	Chlamydia seropositive (n=19)	Chlamydia seronegative (n=11)	Azithromycin treated (n=16)	Placebo treated (n=14)	Seropositive azithromycin treated (n=9)	Seronegative azithromycin treated (n=7)	Seropositive placebo treated (n=10)	Seronegative placebo treated (n=4)
PV (mPa.sec) (baseline)	1.29 (+/- 0.05)	1.25 (+/- 0.08)	1.28 (+/- 0.07)	1.27 (+/- 0.06)	1.30 (+/- 0.05)	1.27 (+/- 0.09)	1.29 (+/- 0.05)	1.22 (+/- 0.07)
PV (mPa.sec) (6 days)	1.31 (+/- 0.07)	1.24 (+/- 0.08)	1.30 (+/- 0.09)	1.28 (+/- 0.07)	1.32 (+/- 0.08)	1.26 (+/- 0.09)	1.30 (+/- 0.06)	1.22 (+/- 0.07)
PV (mPa.sec) (30 days)	1.31 (+/- 0.07)	1.25 (+/- 0.08)	1.27 (+/- 0.06)	1.30 (+/- 0.09)	1.28 (+/- 0.04)	1.26 (+/- 0.08)	1.34 (+/- 0.08)	1.22 (+/- 0.06)
PV (mPa.sec) (90 days)	1.30 (+/- 0.06)	1.26 (+/- 0.07)	1.29 (+/- 0.06)	1.28 (+/- 0.07)	1.29 (+/- 0.05)	1.29 (+/- 0.07)	1.31 (+/- 0.07)	1.22 (+/- 0.05)
vWf (i.u./dl) (baseline)	164 (+/- 37)	168 (+/- 46)	156 (+/- 38)	176 (+/- 41)	156 (+/- 35)	157 (+/- 44)	172 (+/- 39)	187 (+/- 50)
vWf (i.u./dl) (6 days)	161 (+/- 38)	148 (+/- 43)	142 (+/- 39)	173 (+/- 34)	151 (+/- 38)	129 (+/- 40)	170 (+/- 38)	182 (+/- 25)
vWf (i.u./dl) (30 days)	155 (+/- 44)	144 (+/- 44)	140 (+/- 34)	164 (+/- 51)	146 (+/- 29)	132 (+/- 39)	164 (+/- 54)	165 (+/- 48)
vWf (i.u./dl) (90 days)	156 (+/- 41)	139 (+/- 35)	142 (+/- 34)	159 (+/- 43)	151 (+/- 39)	130 (+/- 26)	161 (+/- 43)	153 (+/- 47)

Table 6.3: Mean values (+/- standard deviation) of plasma viscosity (PV) and Von Willebrand factor (vWf) at each timepoint for each subgroup.

Difference in rheological marker	Effect of seropositivity Mean seropositive - mean seronegative ( <i>p</i> -value for comparison)	Effect of azithromycin (mean treated - mean placebo) ( <i>p</i> -value for comparison)	Effect of treatment in seropositives (mean treated seropositive - mean other groups) ( <i>p</i> -value for comparison)
Day 1 fibrinogen - day 90 fibrinogen (g/l)	0.06 (-0.22 to 0.34) <i>p</i> =0.65	-0.02 (-0.30 to 0.27) <i>p</i> =0.91	0.04 (-0.24 to 0.32) <i>p</i> =0.77
Day 1 fibrinogen - day 30 fibrinogen (g/l)	0.05 (-0.20 to 0.30) <i>p</i> =0.67	0.11 (-0.14 to 0.36) <i>p</i> =0.37	0.04 (-0.21 to 0.29) <i>p</i> =0.74
Day 1 CRP - Day 90 CRP (mg/l)	-2.47 (-11.5 to 6.57) <i>p</i> =0.59	0.01 (-9.03 to 9.05) <i>p</i> =1.0	3.84 (-5.19 to 12.88) <i>p</i> =0.40
Day 1 CRP - day 30 CRP (mg/l)	-3.32 (-10.81 to 4.17) <i>p</i> =0.38	1.49 (-6.00 to 8.98) <i>p</i> =0.69	1.36 (-6.13 to 8.85) <i>p</i> =0.72
Day 1 PV - day 90 PV (mPa.sec)	-0.0003 (-0.03 to 0.03) <i>p</i> =0.98	-0.0008 (-0.03 to 0.03) <i>p</i> =0.96	-0.0092 (-0.04 to 0.02) <i>p</i> =0.55
Day 1 PV - day 30 PV (mPa.sec)	-0.01 (-0.03 to 0.02) <i>p</i> =0.50	0.02 (-0.01 to 0.04) <i>p</i> =0.15	-0.02 (-0.04 to 0.01) <i>p</i> =0.16
Day 1 vWF - Day 90 vWf (i.u./dl)	-11.05 (-22.43 to 0.34) <i>p</i> =0.06	-3.05 (-14.44 to 8.33) <i>p</i> =0.60	-0.68 (-12.07 to 10.71) <i>p</i> =0.91
Day 1 vWF - day 30 vWf (i.u./dl)	-6.99 (-18.20 to 4.23) <i>p</i> =0.22	1.06 (-10.16 to 12.27) <i>p</i> =0.85	0.21 (-11.00 to 11.43) <i>p</i> =0.97

**Table 6.4: The effect of seropositivity and azithromycin treatment on the differences between haemorrhological values within individuals between day 1 and day 90 and between day 1 and day 30.** CRP=C reactive protein, vWf=von Willebrand factor and PV=plasma viscosity. See text for statistical method. The differences that were calculated for each individual are tabulated on the far left. The mean difference between seropositive and seronegative patients for each variable ('the effect of seropositivity') is shown in the first column. The mean difference between azithromycin treated and placebo patients for each variable ('the effect of treatment') is shown in the second column. The interaction between these factors is shown in the third column, which compares azithromycin treated seropositive patients to placebo treated and seronegative patients ('the effect of treatment in seropositives'). Each value is stated with its confidence limit, and a *p*-value for the comparison is given.

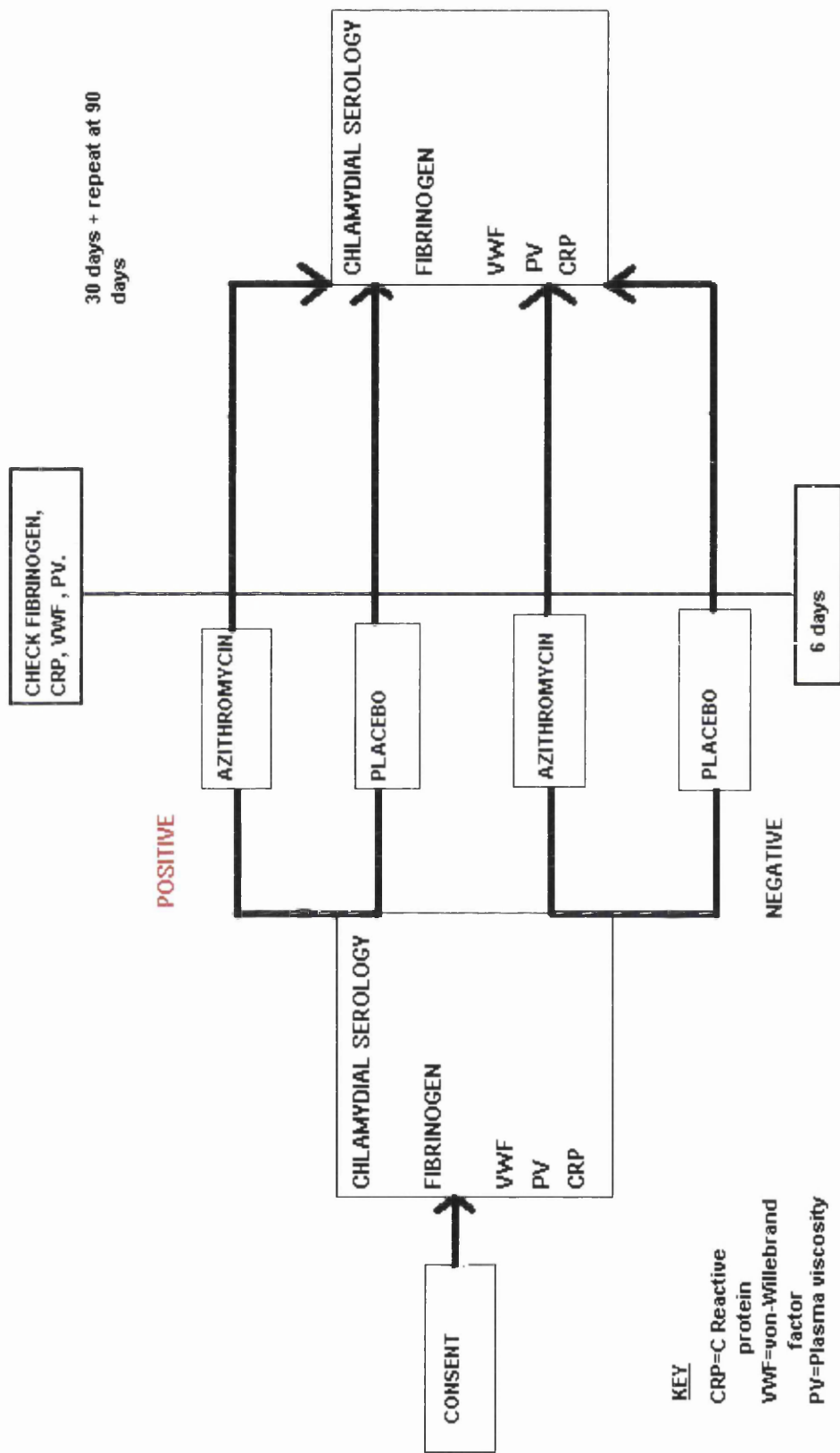
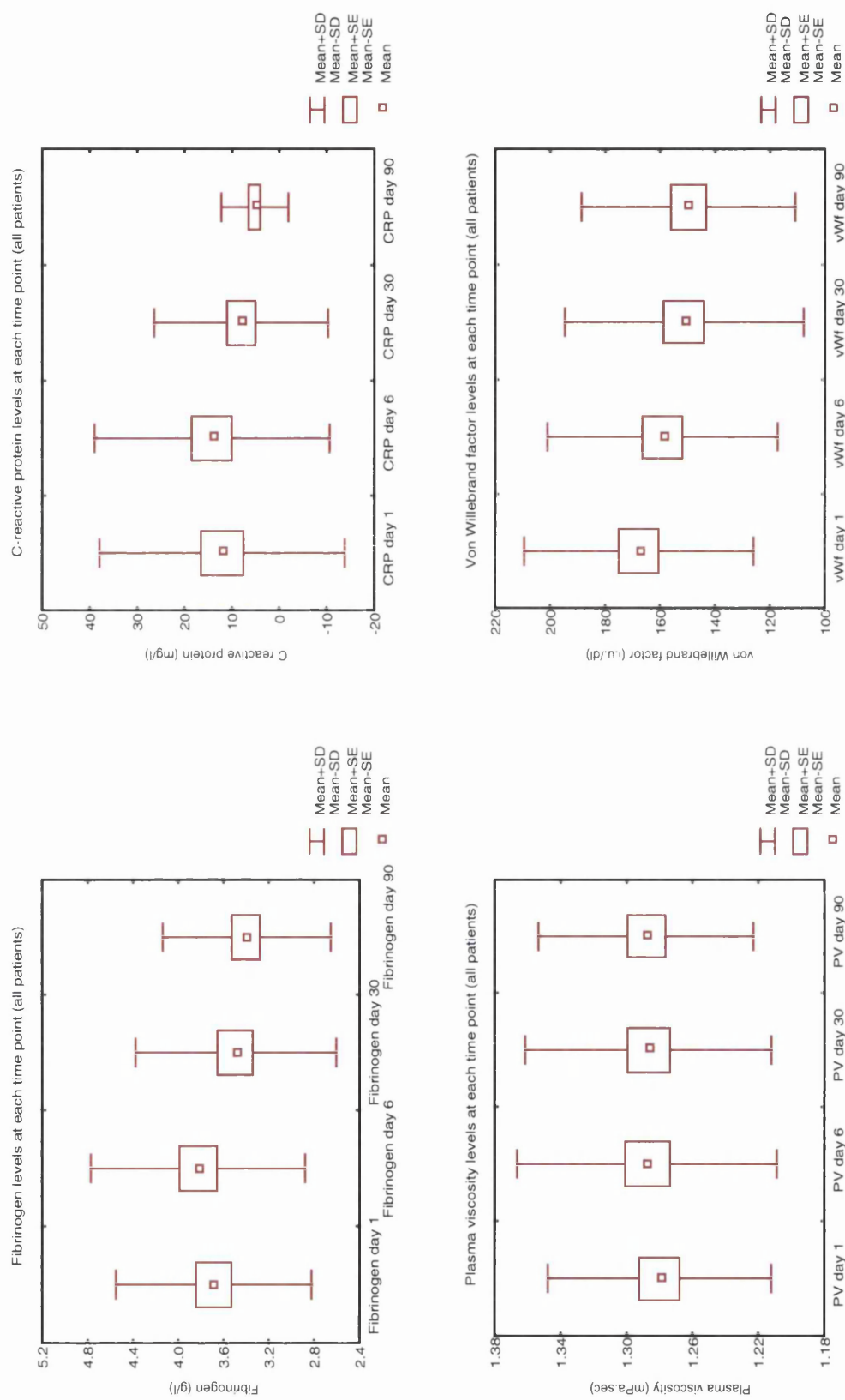
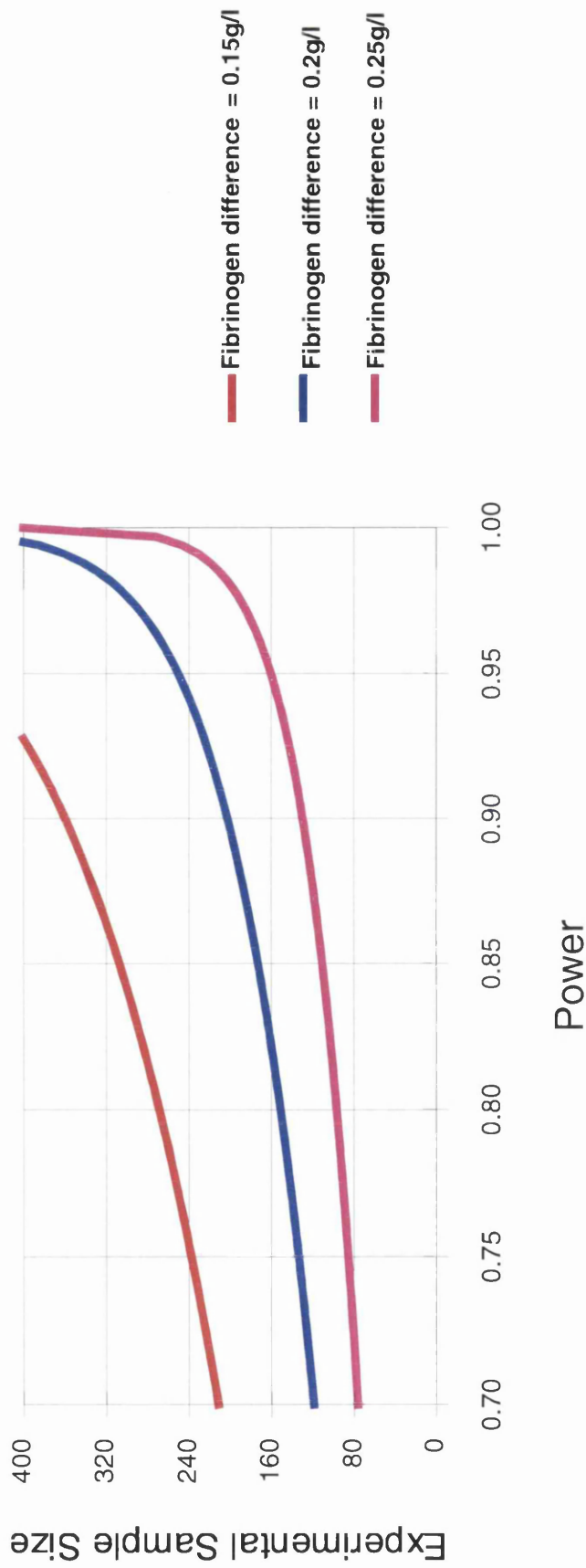


Figure 6.1: Design of the FACET trial



**Figure 6.2** : The mean levels of each rheological variable on days 1, 6, 30 and 90 for the total patient group. CRP= C reactive protein, PV=Plasma viscosity and vWf= von Willebrand factor. The mean, standard error (SE) and standard deviation (SD) are shown for each value.



**Figure 6.3: Sample size calculation graph**, based on variability data derived from the spread of detected differences between fibrinogen levels at day 1 and day 30. Experimental sample size (total patient number) is plotted on the y-axis, against study power on the x-axis. The difference in population means that can be detected between experimental and treated groups is set at 3 different values (0.15 g/l, 0.2 g/l and 0.25 g/l), with the combination of power and sample size values being shown for each.

## **Conclusions, discussion and future directions**



The preceding chapters have detailed clinically based investigations addressing some of the controversies in ischaemic stroke. The work described contributes to a number of areas of expanding research interest. The setting, merits and problems with each investigation have been discussed in each chapter; further debate and consideration of research direction in the future is provided here.

Recent reports from the NASCET trial have improved the accuracy of data available to clinicians on the effect of endarterectomy, and the natural history of patients with carotid stenosis, but have not fully answered the question addressed in **chapter 2**. In an analysis of stroke patterns in patients with asymptomatic internal carotid artery stenosis, *Inzitari et al*<sup>281</sup> recommend that only patients with symptomatic carotid artery stenosis should undergo carotid endarterectomy, since 45% of strokes in patients with asymptomatic stenosis of 60-99% are attributable to lacunes or cardioembolism, and therefore have no relation to their carotid lesion.

This argument, however, seems incongruous with the same authors' other recent publication<sup>54</sup>, which was discussed in **chapter 2**. In those patients with lacunar stroke who entered NASCET, a trend to benefit was observed in the surgical group, and the authors recommended that such patients be considered for endarterectomy. Therefore lacunar stroke denotes a patient with sufficient risk of subsequent events to justify surgical correction, yet the same authors do not regard lacunar stroke as a relevant endpoint in endarterectomy trials. This is despite observations that higher degrees of stenosis were associated with a hazard ratio of 3.6 for lacunar stroke<sup>281</sup>, and that five year risk of ipsilateral lacunar stroke was halved from 5.7% to 2.8% by surgery in NASCET<sup>181</sup>.

We have shown that an association exists between lacunar stroke and carotid disease that confers a poor prognosis. In conjunction with the observations

from NASCET above and individual patient data from the major randomised trials which confirmed the benefit of surgery in lacunar stroke patients with higher degrees of stenosis <sup>55</sup>, these data suggest that patients with ipsilateral lacunar stroke should be considered for endarterectomy. They also indicate that it is prescriptive to disregard recurrent lacunar stroke from follow-up analysis.

Vascular localisation remains a concern with the analysis presented in **chapter 2**. Distinct subcortical infarct patterns are becoming increasingly recognised, and different associations described. A recent report described very eloquently the different degrees of stenosis found in the NASCET dataset between perforating artery infarction (PAI) and internal border zone infarction (IBI) <sup>51</sup>. Sixty-three percent of the patients with IBI had severe (70-99%) stenosis compared with 42% of patients with PAI; 18% of the IBI patients had stenosis of 90% or more compared with 8% of the patients with PAI. Similar data support a 'large artery hypothesis' for centrum ovale infarction <sup>52</sup> and anterior choroidal territory infarction <sup>179</sup>. Such detailed vascular localisation, requiring templates for the identification of vascular territories, was not conducted on the data presented in **chapter 2**. Coupled with blinded neuroradiological review it would strengthen the analysis presented. Furthermore, improved (and updated) record-linkage data, and re-analysis of the same database to include patients admitted after 1998, would improve the power of the study to detect a significant difference between those patients with ipsilateral and contralateral lacunar and carotid disease. Both such approaches have been discussed as means of improving information about this important clinical issue. It is unlikely that a randomised trial will answer directly whether patients with lacunar stroke and ipsilateral carotid stenosis should be offered endarterectomy. However, continued analysis of individual patient data from the randomised trials already conducted is likely to be able to improve our ability to predict whether an

individual with a particular set of clinical characteristics will benefit from endarterectomy.

**Chapter 3** summarises data reported over the course of 15 years that has examined association between patent foramen ovale (PFO) and atrial septal aneurysm (ASA) and stroke. It provides considerable clarity in a confusing area by collating and grouping similar studies together. Moreover, careful analysis of the data reported in **chapter 3** suggests clear directions for future work. Firstly, association studies examining the frequency of PFO, ASA or both in stroke patients  $\leq 55$  years are no longer necessary. Association between both lesions and ischaemic or cryptogenic stroke is proven and indisputable. Association is stronger for ASA than PFO, and is strongest when both abnormalities co-exist. Recurrence data have improved since the meta-analysis reported in **chapter 3** was published <sup>237</sup> - *Mas et al* have reported a large prospective observational study which is discussed in **chapters 3** and **4**. This confirms the importance of the two lesions being present in conjunction - only combined lesions were significantly predictive of recurrent stroke. Further data on the clinico-radiologic characteristics of the patients included in this trial are in press. Useful data on natural history and how prognosis may be better defined with echocardiographic information will be provided by the full publication of the Patent foramen ovale in Cryptogenic Stroke Study (PICSS).

**Chapter 3** also confirms that association is *not* proven in patients older than 55 years. There are fewer studies that have examined the older age group, and they have reached heterogeneous conclusions. In older patients other stroke causes are more frequent, and so isolating any effect of PFO is more difficult. In the meta-analysis reported heterogeneity within total comparisons was eliminated by grouping into age bands, and negative trials were more likely to stem from the inclusion of older patients. These findings suggest that age exerts an effect on the relationship between interatrial septal

abnormalities and stroke that is clinically relevant. The presence of PFO showed a strong inverse correlation with age in the largest pathological study in the literature<sup>81</sup>. To address the important issue of whether association exists between PFO and stroke in older patients ( $\geq 55$  years), **appendix 1** describes a planned age matched case control study in patients over 55 years.

Neither further studies of association, nor improved data on natural history will answer the central management questions: 'aspirin or warfarin?' 'warfarin or lesion correction?' and 'surgical or percutaneous closure?'. **Chapter 4** examines current practice and opinion in a sample of British neurologists and stroke physicians. Generally, recurrent events led to warfarin prescription while large lesions or concurrent ASA led to consideration of lesion correction. However, considerable uncertainty and disagreement was evident, in both investigation and management strategies. Experts who regarded investigation for PFO as *unnecessary* were very clearly influenced both by the results of investigations and by clinical course in those with positive investigations. While 'consensus' can be reported from analysis of these responses (discussed in the conclusion to **chapter 4**), both the sample of respondents and the basis upon which their responses were made are open to bias. The analysis, however, does generate useful background data for the design of a pragmatic clinical trial.

A randomised trial in PFO-associated stroke is the only way in which the uncertainty evident in the survey of specialist opinion can be addressed. The current climate of uncertainty is in many ways a perfect backdrop for such an investigation. However, the low recurrence rate in patients with cryptogenic stroke and PFO presents difficulties in trial design. In neither PICSS nor the large prospective series from *Mas et al*<sup>90</sup> did the presence of isolated PFO have any effect on stroke recurrence. This suggests that aspirin alone is sufficient for patients with an isolated PFO and a single otherwise

unexplained stroke. A benign natural history means that large randomised trials will be required to investigate any significant benefit of interventional techniques, or to study the relative merits of warfarin and aspirin. It militates against positive findings in the ongoing randomised investigations of closure versus anticoagulants. A trial concentrating on those with combined lesions (PFO + ASA) would need a smaller sample size because recurrence risk is higher, but these abnormalities are much rarer in clinical practice. Including patients with large isolated PFOs in such a 'high risk' trial is supported by observational data<sup>213 227 216</sup>, but not by the prospective recurrence trial which found degree of shunt to be an insignificant factor in predicting recurrence<sup>90</sup>. Adaptive randomisation techniques may enable both patients with small lesions and transient isolated symptoms, and patients with combined lesions and recurrent stroke, to be randomised to appropriate comparative therapies. Alternatively, use of the uncertainty principle may allow the disparate views of randomising physicians to be catered for, while still producing a varied study cohort.

Statins are becoming increasingly used in secondary stroke prevention, a trend likely to continue after full publication of the Heart Protection Study (HPS). The HPS study concurs with data from previous large statin trials in the cardiovascular population that have demonstrated benefit in patients with a wide range of initial cholesterol levels. The discrepancy between the cerebrovascular observational data, which have shown better outcome in individuals with high cholesterol<sup>95</sup> and only very weak relations between serum cholesterol and stroke risk<sup>91</sup>, and the increasing proof of beneficial statin effects in intervention trials, is perhaps best explained by the effects of statins that are independent of their effect on plasma cholesterol. These effects on endothelium, rheology and vasomotor tone may be important both in secondary prevention and in the acute phase, and the small trial reported in **chapter 5** is the first to specifically examine these effects in acute ischaemic stroke. The study had sufficient power to exclude a possible effect

of statins on subacute whole cerebral perfusion of more than 7ml/100g/min. Putative effects on arterial flow, blood pressure, haemorrhological and inflammatory markers were not detected, but the sample size was small and the analysis cannot exclude clinically significant effects on these markers. A number of markers (such as interleukin-6 and plasminogen activator inhibitor-1) were not examined. If large statin trials in stroke prevention confirm the effects seen in the stroke subgroup of the HPS trial, studies examining acute statin effects on clinical endpoints (early recurrence and outcome) will follow. In the interim, further investigations properly powered to detect minor to moderate effects on surrogate endpoints should be considered, both in acute stroke and in the more chronic cerebrovascular population, whose response to statins may be at least in part independent of effects on plasma cholesterol.

**Chapter 6** reports the pilot phase of a project designed to examine macrolide antibiotic effects on fibrinogen levels in acute stroke. Variability data have allowed us to size the next stage of the project. Fibrinogen remains persistently raised after ischaemic stroke<sup>273</sup> and predicts new events<sup>273 120</sup>, and so it remains a reasonable surrogate marker to serve as a target for macrolides. Whether impact on fibrinogen levels will alter long term risk of clinical events is uncertain. A similarly designed trial randomising patients 6 months or more after stroke would eliminate the 'acute phase response' element of fibrinogen elevation. The most recent studies in the CHD population are randomising patients to longer courses of antibiotics (up to 18 months) but have continued to use microimmunofluorescence techniques to define seropositivity (generally a titre > 1:16)<sup>271</sup>. Most, like the study we plan, are randomising both seropositive and seronegative patients.

Recent epidemiological data have called into question the association of chlamydial infection and vascular disease<sup>271</sup>. Observational trials examining the association of stroke with chlamydial infection are greatly outnumbered by

those examining associations with CHD. Given the heterogeneity of stroke, and the fact that retrospective observational data pointed to a far stronger association in CHD than was eventually evident after meta-analysis of the prospective data <sup>271</sup>, a properly planned large prospective study and systematic review of the evidence linking chlamydia and ischaemic stroke should be conducted before large scale intervention trials are planned. Any plans for definitive trials in the cerebrovascular population are also likely to be informed by the results of trials ongoing in the CHD population (summarised in *Danesh et al* <sup>271</sup>), but these are only large enough to detect reductions in coronary events with antibiotic therapy of ~25%. If effects are smaller than this, as seems likely from the strength of the epidemiological association, they may be missed by the current group of trials.

This thesis has explored a number of aspects of stroke secondary prevention that may become increasingly important in the future. Post-stroke management is likely to involve intensified control of traditional risk factors, such as hypertension. Research must concentrate on refining the indications for more established interventions and on establishing the importance of novel causes and risk factors. The work presented here contributes to that goal. Randomised trials will be required before therapies designed to combat 'newer' causes and risk factors are routinely employed.

**Appendix 1: Is patent foramen ovale associated with stroke in patients over 55 years?**



The systematic review examining studies that have quantified the relationship between patent foramen ovale (PFO) and ischaemic stroke described in **chapter 3** reported clear evidence of association in patients  $\leq 55$  years <sup>237</sup>. Randomised trials must define the best medical therapy for such patients, and the relative merits of medical therapy, surgical treatment and percutaneous closure techniques.

In patients  $\geq 55$  years, association is not yet established <sup>237</sup>. Other causes and stroke risk factors such as atrial fibrillation and hypertension are more likely to lead to stroke in older individuals, because they are more common. The largest study which compared the prevalence of PFO in stroke patients with that in controls contained 194 patients in the 'stroke' group, and 183 patients in the 'control group' <sup>195</sup>. Random effects meta-analysis combining this and the two other studies that have examined the same question <sup>197 204</sup> yielded a combined odds ratio of 1.60 (95% CI=0.63 to 4.06), thus excluding an odds ratio greater than 4.06 or less than 0.63 <sup>237</sup>. Trend to association was more evident in patients  $\geq 55$  years when comparing cryptogenic stroke to known stroke cause. This resulted in an odds ratio of 2.26 (95% CI=0.96 to 5.31).

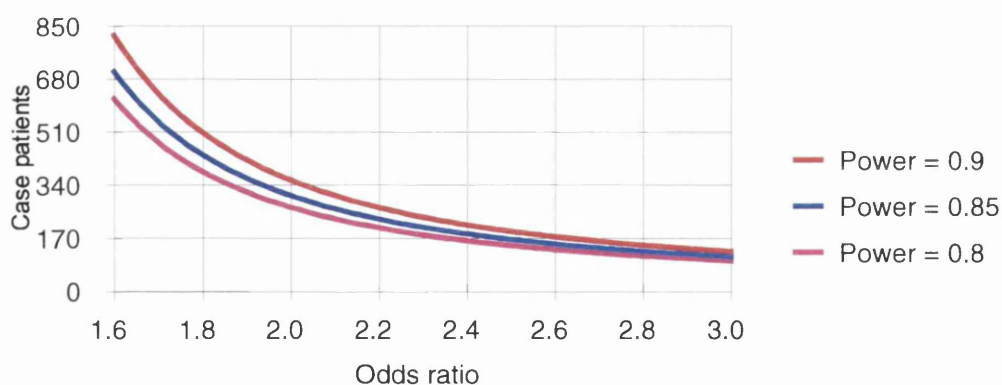
A prospective study in this older population is planned. Eligible patients will be aged 55 or greater, and have a history of ischaemic stroke (confirmed by CT scanning) or TIA within the preceding 2 months. Stroke type will be classified clinically using the Oxfordshire Community Stroke Project (OCSP) method <sup>171</sup>, but final classification into atherosclerotic stroke, cardioembolic stroke, lacunar stroke, stroke of unusual but determined cause or cryptogenic stroke will be made using the criteria developed by the National Institute of Neurological Disorders and Stroke (NINDS) <sup>282</sup>. Stroke classification will be made by a neurologist or physician blinded to the presence or absence of PFO (see below). Patients with all ischaemic stroke subtypes will be included in the study, but pre-defined subgroups will be analysed as stated below.

After informed consent, PFO will be detected using transcranial Doppler (TCD). The main stem of the middle cerebral artery (MCA) will be insonated trans-temporally with a 2MHz transducer. Patients in whom MCA insonation fails will be withdrawn from the study. Patients will receive a 10ml bolus injection of agitated saline into a 16 gauge venflon placed in an antecubital vein. Agitated saline will be prepared by mixing 8ml of saline with 2 ml of air, using 2 syringes mounted on a 3-way stopcock. PFO will be defined as the detection of 1 or more spike-like interruptions of the normal pulsatile arterial signal detected by the MCA transducer, 4 to 20 seconds after saline injection. Examinations will be performed at rest, with coughing and with Valsalva manoeuvre. Valsalva manoeuvre will be standardised by having the subjects blow into a manometer until 50 to 60 mmHg of pressure is reached and asking them to maintain this for a period of 5 to 7 seconds immediately prior to injection. Patients unable to perform the Valsalva manoeuvre will continue in the study.

PFO size will be semi-quantified by dividing patients with right-to-left shunt into those with a small (1-5 microbubbles), moderate (6 to 15 microbubbles) or large (16 or more microbubbles) degree of shunt detected on middle cerebral artery screening. The highest degree of shunt detected during the 3 phases of the TCD test (rest, Valsalva and cough) will define PFO size. All examinations will be video-taped and classified by a second observer, blinded to patient details. The classification of this second observer will be used as the study data. Control patients will be recruited from the inpatient medical population at participating hospitals. They will have no history of cerebrovascular disease, nor of valvular heart disease. PFO presence has been shown to be highly dependant on age<sup>81</sup>, and so cases and controls will be matched for age and sex.

The primary endpoint of the study will be the relative frequency of PFO in stroke patients compared to control patients. Since the trial subjects will be matched for age and sex, McNemar's version of the chi-squared test with Yates's continuity correction will be used to test for association between PFO and stroke. Statistical significance will be set at the level of  $p < 0.05$ . Odds ratios will be calculated with the Mantel-Haenszel test. Other (secondary) comparisons will include PFO frequency in cryptogenic stroke patients compared to known stroke cause and in cryptogenic stroke patients compared to control subjects. To determine whether there is a trend towards larger degrees of right-to-left shunt in stroke patients (in comparison to controls) and cryptogenic stroke patients (in comparison to patients with known stroke cause), a chi-squared test for trend across the PFO size categories will be conducted.

The graph shown below details the results of calculations performed to determine the correct sample size for the study. The calculations were performed using PS power and sample size calculation software (1997).



alpha=0.05 phi=0.2 p\_0=0.15 m=1, Matched or paired, Case control design

*Key: alpha: type 1 error probability for a two sided test*

*phi: correlation co-efficient for PFO presence between matched cases and controls*

*p: prevalence of PFO amongst controls*

The table below gives the numbers of patients required in each group (stroke and control) to detect different odds ratios, with varying degrees of power, derived from the same calculations that produced the graph above.

<b>Odds ratio</b>	<b>90% power</b>	<b>85% power</b>	<b>80% power</b>
<b>1.6</b>	821	703	616
<b>1.698</b>	637	546	479
<b>1.81</b>	499	428	375
<b>1.908</b>	415	356	313
<b>2.006</b>	353	303	267
<b>2.51</b>	191	165	146
<b>3</b>	129	112	99

In the absence of *age matched* case-control data from previous studies, the correlation co-efficient ( $\phi$ ) has been estimated at 0.2, as recommended in the methodological papers used to design the sample size software that was used<sup>283</sup>. The prevalence of PFO ( $p$ ) has been estimated at 15% in control subjects over 55 years. Prevalence rates in the studies included in the systematic review varied enormously, but for the studies that examined the comparison of PFO presence in stroke vs control patients over 55 years<sup>195</sup><sup>204</sup><sup>197</sup>, the control frequencies for PFO were 16%, 15% and 6%. A study containing 353 patients in each group would have 90% power to detect an odds ratio of 2 or more, should one exist. If such a study were to be included

in the meta-analysis already reported, and found an odds ratio of 2 (the lowest odds ratio that it would be powered to detect), the resulting combined odds ratio from 4 studies would be 1.65 (95% CI=1.22 to 2.23) by fixed effects, and 1.67 (0.92 to 3.04) by random effects. If the study were to detect a lower odds ratio, a value of 1.47 would still render the combined odds ratio of the four studies significant.

Funding is currently being sought for this study, and it is hoped that it will run in 3 centres in the Glasgow area, though will be dependant on the provision of equipment (for TCD) and staff training in MCA insonation that can be provided.

## Appendix 2: Definitions of meta-analytical terms<sup>198 199</sup>

**Heterogeneity** The magnitude of statistical diversity that exists between the results of different sets of data. The *chi-squared test for heterogeneity* assesses whether the differences between the effect estimated by each data set can be assumed to be a consequence of random sampling variation. If this test is not significant, outcomes in the individual studies are said to be *homogeneous*, in that they estimate a single underlying effect.

**Fixed effects meta-analysis** considers that the difference between the effect found in each study is exclusively due to random variation. Thus, if all the studies were infinitely large they would give identical results.

**Random effects meta-analysis** assumes a different underlying effect for each study and takes this into consideration as an additional source of variation.

**Funnel plot** A plot of effect estimate against sample size which is used to assess validity and to detect bias in meta-analyses. These are skewed and asymmetrical in the presence of publication and other bias.

**Forest plot** A figure comprising the estimate and 95% confidence interval for each trial, together with an overall estimate and confidence interval.

## Appendix 3: NIH Stroke Scale

Administer stroke scale items in the order listed. Record performance in each category after each subscale exam. Do not go back and change scores. Follow directions provided for each exam technique. Scores should reflect what the patient does, not what the clinician thinks the patient can do. The clinician should record answers while administering the exam and work quickly. Except where indicated, the patient should not be coached (i.e., repeated requests to patient to make a special effort).

**IF ANY ITEM IS LEFT UNTESTED, A DETAILED EXPLANATION MUST BE CLEARLY WRITTEN ON THE FORM. ALL UNTESTED ITEMS WILL BE REVIEWED BY THE MEDICAL MONITOR, AND DISCUSSED WITH THE EXAMINER BY TELEPHONE.**

Instructions	Scale Definition	Score
<p><b>1a. Level of Consciousness:</b> The investigator must choose a response, even if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation.</p>	<p>0 = Alert; keenly responsive.            1 = Not alert, but arousable by minor stimulation to obey, answer, or respond.            2 = Not alert, requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped).            3 = Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, areflexic.</p>	<p>_____</p> <p>-</p>
<p><b>1b. LOC Questions:</b> The patient is asked the month and his/her age. The answer must be correct - there is no partial credit for being close. Aphasic and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues.</p>	<p>0 = Answers both questions correctly.            1 = Answers one question correctly.            2 = Answers neither question correctly.</p>	<p>_____</p> <p>-</p>
<p><b>1c. LOC Commands:</b> The patient is asked to open and close the eyes and then to grip and release the non-paretic hand. Substitute another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to them (pantomime) and score the result (i.e., follows none, one or two commands). Patients with trauma, amputation, or other physical impediments should be given suitable one-step commands. Only the first attempt is scored.</p>	<p>0 = Performs both tasks correctly            1 = Performs one task correctly            2 = Performs neither task correctly</p>	<p>_____</p> <p>-</p>
<p><b>2. Best Gaze:</b> Only horizontal eye movements will be tested. Voluntary or reflexive (oculocephalic) eye movements will be scored but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an isolated peripheral nerve paresis (CN III, IV or VI) score a 1. Gaze is testable in all aphasic patients. Patients with ocular trauma, bandages, pre-existing blindness or other disorder of visual</p>	<p>0 = Normal            1 = Partial gaze palsy. This score is given when gaze is abnormal in one or both eyes, but where forced deviation or total gaze paresis are not present.            2 = Forced deviation, or total gaze paresis not overcome by the oculocephalic maneuver.</p>	<p>_____</p> <p>-</p>

<p>acuity or fields should be tested with reflexive movements and a choice made by the investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence of a partial gaze palsy.</p>		
<p><b>3. Visual:</b> Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat as appropriate. Patient must be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia is found. If patient is blind from any cause score 3. Double simultaneous stimulation is performed at this point. If there is extinction patient receives a 1 and the results are used to answer question 11.</p>	<p>0 = No visual loss  1 = Partial hemianopia  2 = Complete hemianopia  3 = Bilateral hemianopia (blind including cortical blindness)</p>	<p>_____  -  _____</p>
<p><b>4. Facial Palsy:</b> Ask, or use pantomime to encourage the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barrier obscures the face, these should be removed to the extent possible.</p>	<p>0 = Normal symmetrical movement  1 = Minor paralysis (flattened nasolabial fold, asymmetry on smiling)  2 = Partial paralysis (total or near total paralysis of lower face)  3 = Complete paralysis of one or both sides (absence of facial movement in the upper and lower face)</p>	<p>_____  -  _____</p>
<p><b>5 &amp; 6. Motor Arm and Leg:</b> The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine) and the leg 30 degrees (always tested supine). Drift is scored if the arm falls before 10 seconds or the leg before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in the case of amputation or joint fusion at the shoulder or hip may the score be "9" and the examiner must clearly write the explanation for scoring as a "9".</p>	<p>0 = No drift, limb holds 90 (or 45) degrees for full 10 seconds.  1 = Drift, Limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support.  2 = Some effort against gravity, limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity.  3 = No effort against gravity, limb falls.  4 = No movement  9 = Amputation, joint fusion explain: _____</p> <p><b>5a. Left Arm</b>  _____</p> <p><b>5b. Right Arm</b>  _____</p>	<p>_____  -  _____</p>
	<p>0 = No drift, leg holds 30 degrees position for full 5 seconds.  1 = Drift, leg falls by the end of the 5 second period but does not hit bed.  2 = Some effort against gravity; leg falls to bed by 5 seconds, but has some effort against gravity.  3 = No effort against gravity, leg falls to bed immediately.  4 = No movement  9 = Amputation, joint fusion explain: _____</p> <p><b>6a. Left Leg</b>  _____</p> <p><b>6b. Right Leg</b>  _____</p>	<p>_____  -  _____</p>



<p><b>7. Limb Ataxia:</b> This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual defect, insure testing is done in intact visual field. The finger-nose-finger and heel-shin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion may the item be scored "9", and the examiner must clearly write the explanation for not scoring. In case of blindness test by touching nose from extended arm position.</p>	<p>0 = Absent  1 = Present in one limb  2 = Present in two limbs</p> <p>If present, is ataxia in  Right arm 1 = Yes 2 = No  9 = amputation or joint fusion, explain</p> <hr/> <p>Left arm 1 = Yes 2 = No  9 = amputation or joint fusion, explain</p> <hr/> <p>Right leg 1 = Yes 2 = No  9 = amputation or joint fusion, explain</p> <hr/> <p>Left leg 1 = Yes 2 = No  9 = amputation or joint fusion, explain</p> <hr/>	<p>_____</p> <p>-</p> <p>_____</p> <p>-</p> <p>_____</p> <p>-</p> <p>_____</p> <p>-</p> <p>_____</p> <p>-</p>
<p><b>8. Sensory:</b> Sensation or grimace to pin prick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas [arms (not hands), legs, trunk, face] as needed to accurately check for hemisensory loss. A score of 2, "severe or total," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will therefore probably score 1 or 0. The patient with brain stem stroke who has bilateral loss of sensation is scored 2. If the patient does not respond and is quadriplegic score 2. Patients in coma (item 1a=3) are arbitrarily given a 2 on this item.</p>	<p>0 = Normal; no sensory loss.</p> <p>1 = Mild to moderate sensory loss; patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick but patient is aware he/she is being touched.</p> <p>2 = Severe to total sensory loss; patient is not aware of being touched in the face, arm, and leg.</p>	<p>_____</p> <p>-</p>
<p><b>9. Best Language:</b> A great deal of information about comprehension will be obtained during the preceding sections of the examination. The patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet, and to read from the attached list of sentences. Comprehension is judged from responses here as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to identify objects placed in the hand, repeat, and produce speech. The intubated patient should be asked to write. The patient in coma (question 1a=3) will arbitrarily score 3 on this item. The examiner must choose a score in the patient with stupor or limited cooperation but a score of 3 should be used only if the patient is mute and follows no one step commands.</p>	<p>0 = No aphasia, normal</p> <p>1 = Mild to moderate aphasia; some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided material difficult or impossible. For example in conversation about provided materials examiner can identify picture or naming card from patient's response.</p> <p>2 = Severe aphasia; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response.</p> <p>3 = Mute, global aphasia; no usable speech or auditory comprehension.</p>	<p>_____</p> <p>-</p>
<p><b>10. Dysarthria:</b> If patient is thought to be normal an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other</p>	<p>0 = Normal  1 = Mild to moderate; patient slurs at least some words and, at worst, can be understood with some difficulty.  2 = Severe; patient's speech is so slurred as to be unintelligible in the absence</p>	<p>_____</p> <p>-</p>

<p>physical barrier to producing speech, may the item be scored "9", and the examiner must clearly write an explanation for not scoring. Do not tell the patient why he/she is being tested.</p>	<p>of or out of proportion to any dysphasia, or is mute/anarthric. 9 = Intubated or other physical barrier</p>	
<p><b>11. Extinction and Inattention (formerly Neglect):</b> Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosagnosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable.</p>	<p>0 = No abnormality.  1 = Visual, tactile, auditory, spatial, or personal inattention or extinction to bilateral simultaneous stimulation in one of the sensory modalities.  2 = Profound hemi-inattention or hemi-inattention to more than one modality. Does not recognize own hand or orients to only one side of space.</p>	<p>_____</p> <p>-</p>

Additional item, not a part of the NIH Stroke Scale score.

<p><b>A. Distal Motor Function:</b> The patient's hand is held up at the forearm by the examiner and patient is asked to extend his/her fingers as much as possible. If the patient can't or doesn't extend the fingers the examiner places the fingers in full extension and observes for any flexion movement for 5 seconds. The patient's first attempts only are graded. Repetition of the instructions or of the testing is prohibited.</p>	<p>0 = Normal (No flexion after 5 seconds)  1 = At least some extension after 5 seconds, but not fully extended. Any movement of the fingers which is not command is not scored.  2 = No voluntary extension after 5 seconds. Movements of the fingers at another time are not scored.</p> <p><b>a. Left Arm</b></p> <p><b>b. Right Arm</b></p>	<p>_____</p> <p>-</p> <p>_____</p> <p>-</p>
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## **Appendix 4: Publication List**

## Chapter 1

Overell J, Lees K. Cardiac causes of stroke. **Trends in Cardiology and Vascular Disease** 1999; **1**: 13-14

Overell J, Weir C, Walker A, Lees K. The cost effectiveness of clopidogrel and the combination of aspirin and dipyridamole in stroke prevention. **Cerebrovascular Diseases** 1999; **9 (S1)**: 66

Overell J, Weir C, Walker A, Lees K. Treatment and secondary prevention of stroke. **Lancet** 2000; **355**: 319-320

Nazir F, Overell J, Bolster A, Hilditch T, Lees K. The effect of perindopril on cerebral and renal perfusion in early ischaemic stroke. **Cerebrovascular Diseases** 2002 (abstract in press).

## Chapter 2

Overell J, Sim I, Lees K. Association between carotid disease and ipsilateral lacunar stroke. **Stroke** 2000; **31**: 334

Overell J, Sim I, Lees K. Association between carotid disease and ipsilateral lacunar stroke. **Cerebrovascular Diseases** 2000; **10**: 42

## Chapter 3

Overell J, Bone I, Lees K. The association of inter-atrial septal abnormalities and stroke: a meta-analysis of case control studies. **Neurology** 2000; **54**: A466

Overell J, Bone I, Lees K. The association of inter-atrial septal abnormalities and stroke: a meta-analysis of case control studies. **Cerebrovascular Diseases** 2000; **10**: 32

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Overell JR, Bone I, Lees KR. Percutaneous closure of patent foramen ovale in patients with paradoxical embolism. **Circulation** 2001; **103**: e56

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#### **Chapter 6**

Overell JR, Lees KR, Lowe GDO. The effect of anti-chlamydial antibiotics on fibrinogen levels after ischaemic stroke. **Cerebrovascular Diseases** 2002 (abstract in press).

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