Vascular Risk Factors in the Aetiology of

Idiopathic Sudden Sensorineural

Hearing Loss in Young Adults.

A thesis submitted to The University of Manchester for the degree of

Master of Philosophy (MPhil)
in the Faculty of Medical and Human Sciences.

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Abstract

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Master of Philosophy (MPhil).

Vascular Risk Factors in the Aetiology of Idiopathic Sudden Sensorineural Hearing Loss in Young adults.

Date: 22\textsuperscript{nd} October 2010

Objective:
To investigate the frequency of vascular risk factors in unilateral idiopathic sudden sensorineural hearing loss (ISSHL) in young adults.

Materials and Methods:
Twenty patients aged 18-50 years when suffering a unilateral ISSHL and 20 age and sex matched controls were examined with contrast-enhanced transcranial doppler to identify venous to arterial circulation shunts (v-aCS), flow mediated dilation of the brachial artery to identify endothelial dysfunction and blood tests for a thrombophilia screen, full blood count, fasting plasma lipids, p-selectin and soluble glycoprotein V.

Results:
There were no significant differences between cases and controls for any of the investigations performed. There was no difference at all between the larger (“significant” or “major”) v-aCS and ISSHL (25% vs 25%). There was a limited association for “small” v-aCS with ISSHL (35% vs 25%; p=0.77); between the presence of v-aCS and severity of hearing loss (no v-aCS 49.0+/− 26 dBHL vs v-aCS 73.4 +/- 34dBHL, p=0.11); for endothelial dysfunction with ISSHL (5.9 +/- 3.2% vs 7.2 +/- 3.2%; p=0.32); and for platelet hypereactivity with ISSHL (glycoprotein V: 67.0+/− 22.3ng/ml vs 64.2+/−21.5ng/ml, p=0.605; p-selectin: 81.7+/−40.2ng/ml vs 75.8+/−45.6ng/ml, p=0.590). Only 1 case had an inherited thrombophilia compared with 2 controls. No cases had positive antiphospholipid antibodies. High fibrinogen levels were more prevalent in cases: two cases and one control had hyperfibrinogenaeia with a further 5 cases and 4 controls in the upper quartile range. Hyperlipidaemia was more prevalent in controls.

Conclusions:
Paradoxical embolism and venous thrombosis are unlikely to be important causes of unilateral ISSHL in young adults. Mildly raised cardiovascular arterial risk factors such as endothelial dysfunction, hyperfibrinogenaeia and p-selectin suggest a possible vascular dysfunction in these patients that may warrant further study. The cause of ISSHL remains a mystery.
**Declaration**

I declare that no portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Ach</td>
<td>acetyl choline</td>
</tr>
<tr>
<td>aCL</td>
<td>anticardiolipin antibodies</td>
</tr>
<tr>
<td>AICA</td>
<td>anterior inferior cerebellar artery</td>
</tr>
<tr>
<td>AIED</td>
<td>autoimmune inner ear disease</td>
</tr>
<tr>
<td>APCR</td>
<td>activated protein C resistance</td>
</tr>
<tr>
<td>APTT</td>
<td>activated partial thromboplastin time</td>
</tr>
<tr>
<td>AT</td>
<td>antithrombin</td>
</tr>
<tr>
<td>CBS</td>
<td>cystathionine-β-synthase</td>
</tr>
<tr>
<td>CMV</td>
<td>cytomegalovirus</td>
</tr>
<tr>
<td>CoQ</td>
<td>coenzyme Q</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>DCM</td>
<td>degenerative cerebral microangiopathy</td>
</tr>
<tr>
<td>DRVVT</td>
<td>dilute Russell viper venom time</td>
</tr>
<tr>
<td>DVT</td>
<td>deep vein thrombosis</td>
</tr>
<tr>
<td>ECG</td>
<td>electrocardiogram</td>
</tr>
<tr>
<td>eNOS</td>
<td>endothelial nitric oxide synthase</td>
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<tr>
<td>ESS</td>
<td>endothelial shear stress</td>
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<tr>
<td>FMD</td>
<td>flow mediated dilation</td>
</tr>
<tr>
<td>fPS</td>
<td>free protein S</td>
</tr>
<tr>
<td>GP</td>
<td>glycoprotein receptors</td>
</tr>
<tr>
<td>HLA</td>
<td>human leukocyte antigens</td>
</tr>
<tr>
<td>HSP</td>
<td>heat shock protein</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>intercellular adhesion molecule</td>
</tr>
<tr>
<td>ISSHL</td>
<td>idiopathic sudden sensorineural hearing loss</td>
</tr>
<tr>
<td>KCT</td>
<td>kaolin clotting time</td>
</tr>
<tr>
<td>LA-PTT</td>
<td>lupus-anticoagulant-sensitive partial thromboplastin time</td>
</tr>
<tr>
<td>MCA</td>
<td>middle cerebral artery</td>
</tr>
<tr>
<td>MELAS</td>
<td>mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes</td>
</tr>
<tr>
<td>MES</td>
<td>microbubbles</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MTHFR</td>
<td>methylenetetrahydrofolate reductase</td>
</tr>
<tr>
<td>MTR</td>
<td>methionine synthase</td>
</tr>
</tbody>
</table>
NFkB nuclear factor-κ B
NO nitric oxide
PAI-1 plasminogen activator inhibitor
PC protein C
PFO patent foramen ovale
PICA posterior inferior cerebellar artery
PT prothrombin time
RCT randomised controlled trial
ROS reactive oxygen species
RPSHL rapidly progressive sensorineural hearing loss
SNHL sensorineural hearing loss
SSHL sudden sensorineural hearing loss
SVD small vessel disease
TCD transcranial doppler
TOE transoesophageal echocardiography
tPA tissue plasminogen activator
TT thrombin time
TTE transthoracic echocardiography
UHSM University Hospital of South Manchester
v-aCS venous-arterial circulation shunt
VBOD vertebrobasilar occlusive disease
VCAM-1 vascular cell adhesion molecule
VTE venous thromboembolism
vWF von Willebrand’s factor
WBV whole blood viscosity
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The Author
Simon Freeman graduated from the University of Manchester in 1993 with a Bachelor of Science in Experimental Immunology and Oncology and in 1995 with Bachelors of Medicine and Surgery. He has trained as a surgeon in the North West and in 2009 was appointed as a Consultant Otolaryngologist with special interests in Skull Base Surgery, Cochlear Implantation, Otology and Neurotology at Salford Royal and Central Manchester Foundation Trusts. He has presented both nationally and internationally and has over 20 publications.
1. INTRODUCTION

1.1. Definition of Idiopathic Sudden Sensorineural Hearing Loss (ISSHL).

The inner ear consists of two functionally separate parts: the cochlea for sound reception and the vestibular apparatus which senses both gravity and acceleration aiding the maintenance of balance. The cochlea is a coil shaped organ comprising of two and a half turns, making a basal, middle and apical turn. The vestibular apparatus consists of the vestibule, containing the saccule and utricle, and the lateral, posterior and superior semicircular canals. Sensorineural hearing loss (SNHL) occurs when there is damage to either the cochlea itself, the cochlear nerve that transmits sound from the cochlea to the brain or any part of the central auditory nervous system within the brain. Depending on the time frame over which it occurs, it has usually been defined as either sudden (occurring within 72 hours), rapidly progressive (occurring over weeks or months) (RPSHL) or gradually progressive. SSHL occurs due to a number of known causes but, despite detailed investigation, these are identified in only about 10% of cases and the term idiopathic sudden sensorineural hearing loss (ISSHL) is applied.

1.2. Epidemiology of ISSHL.

Case studies have estimated the incidence of ISSHL to be approximately 10 / 100 000 person-years, with an equal distribution between the sexes and between right and left sides (1-3). The majority of these patients suffered a single unilateral episode without further problems. However between 1 – 2% had further episodes, either ipsilaterally or contralaterally (bilateral sequential) (1). A further 1 – 2% of cases occurred bilaterally simultaneously.

When considering the age of onset, traditionally ISSHL was thought to be most frequent in middle age, with fewer cases reported in children and the elderly. Recent large population studies have found discordances, with one study showing an increased incidence with age, highest in the elderly (4). The reason for these discrepancies is not clear but may reflect either the geographical distribution and genetics of the different populations or the capabilities of differing societies for monitoring for these problems in sub-groups of their population (e.g. the elderly). Approximately 25% of cases presented under the age of 40 and a further 25% between 40 and 50 years (3, 5).
A great variation in severity and frequencies of hearing affected is also seen. There are several audiometric patterns recognised: low frequency, mid-frequency, high frequency, all frequencies (6). In general, studies have found that higher frequency losses and severe losses are less likely to recover. Another poor prognostic factor is the presence of vertigo with ISSHL which occurs in one third of patients. Although approximately 60% of patients undergo spontaneous full or partial recovery, a significant number are left with permanent deafness (3).

1.3. The Vascular Theory.
While the aetiology of ISSHL is by definition unknown, a number of theories exist. The vascular theory suggests that ISSHL predominantly occurs secondary to vascular disruption and subsequent ischaemia.

1.3.1. The vascular anatomy of the inner ear.
The arterial supply to the inner ear arises exclusively from the labyrinthine artery (also known as the internal auditory artery), which is usually a branch of the anterior inferior cerebellar artery (AICA) which in turn arises from the basilar artery (7, 8). There is no collateral circulation. The labyrinthine artery has two branches: the anterior vestibular artery supplies the utricle, superior part of the saccule, and the lateral and superior semicircular canals; the common cochlear artery divides into the spiral modiolar artery, which supplies the middle and apical turns of the cochlea and the vestibulo-cochlear artery. This latter vessel then divides into a cochlear branch which supplies the basal turn of the cochlea and a vestibular branch which supplies the inferior part of the saccule and the posterior semicircular canal. The two cochlear branches travel through the central bony part of the cochlea, the modiolus, and anastomose with each other at the junction of the basal and middle turns. The combination of the lack of collateral supply and the limited anastomoses within the inner ear potentially make it highly susceptible to arterial disease. In addition animal studies have found that these vessels may have a reduced autoregulation when compared to the brain (9) and the cochlea has a high oxygen requirement (10). Given the usual clinical scenario of unilateral rapid onset deafness, it would be very much in keeping with an arterial occlusive event akin to an ischaemic stroke.
The venous drainage from the inner ear follows a similar pathway to the arteries, the main exception being that there are three vessels draining the inner ear: one passing through the internal auditory meatus alongside the labyrinthine artery and the others exiting via the cochlear and vestibular aqueducts respectively. A similar pathophysiology might occur to the inner ear as occurs to the eye with retinal vein occlusion, a well-recognised cause of sudden blindness (11). Strokes can also occur secondary to venous sinus thrombosis within the cerebral circulation. Unfortunately with current diagnostic techniques there is no way to demonstrate acute occlusion of either the small arteries or veins supplying the inner ear following ISSHL.

1.3.2. Animal models of vascular disruption.

It is clear from animal models that arterial disruption leads to infarction of the cochlea (12-18). Temporary occlusion could cause cochlear damage with the vestibule largely spared, suggesting this latter structure is less susceptible to ischaemia. Cochlear electrical responses became depressed rapidly but permanent damage always followed if the blood supply was not restored within 30 minutes. The basal turn was more readily damaged than the middle and apical turns. Permanent occlusion close to or within AICA or the basilar artery damaged the cochlea but not consistently. Permanent occlusion of the labyrinthine artery caused more frequent and severe cochlear infarction with subsequent fibrosis and ossification (12-14). It has therefore been suggested that fibrosis and ossification are the diagnostic hallmarks of vascular damage (19). Animal experiments using microembolisation techniques to cause more localised damage demonstrated highly varied patterns of degeneration with fibrosis and ossification only where severe destruction had occurred (15-17). Small amounts of embolisation medium (barium sulphate) caused an initial decrease in cochlear blood flow followed by a reflex vasodilation with associated temporary hearing loss (17). Phenoxybenzamine (an α-adrenergic blocking agent) protected against the loss of cochlear potentials by reducing the decrease in blood flow. When larger amounts of barium were used, permanent blood flow reduction and cochlear damage ensued. Fibrosis was only seen in a small number of sacrificed animals, suggesting that this finding was related to the level of destruction.

Venous obstruction also causes cochlear damage in animal models (20, 21). When only part of the venous drainage was damaged, sensory cell atrophy did not occur, although there was localised haemorrhage. More extensive damage led to widespread destruction
with loss of cochlear potentials. As with arterial occlusion, the cochlea was more sensitive than the vestibule.

1.3.3. Potential mechanisms of vascular damage in ISSHL.
If vascular damage were to be the cause of ISSHL, then given the origin of its arterial supply, the pathophysiology would likely be the same as that for posterior circulation strokes. Mechanisms of stroke causation are well established (22, 23). The main ones are atherosclerosis of the large arteries with hypoperfusion and atherothrombosis with embolisation of the distal arteries, small vessel disease, emboli from a distant source (cardiac or paradoxical embolism) or other causes such as coagulopathy.

As many of these vascular disorders advance with age, it has been argued that the age distribution of ISSHL does not fit with a vascular cause in that western studies have shown a peak incidence of ISSHL at 50 – 60 year of age (24). However, as discussed above in section 1.2., Asian studies have reported a direct correlation of ISSHL with increasing age (4). The reason for this is unclear but may involve different reporting processes in different societies meaning ISSHL in elderly patients in western societies may be underdiagnosed. It has also been shown that the peak incidence of posterior strokes of any cause is 50-64 years with 25% under the age of 50 years (23), an age distribution which starts to resemble that of ISSHL.

1.3.4. Atherosclerosis and atherothrombosis.
Atherosclerosis is a complex pathological process whereby plaques develop in the vessel walls of medium to large arteries as a result of lipid accumulation and chronic inflammation (25-27). It has a predilection for areas of disturbed flow such as branch points and bifurcations and this is thought to be secondary to low endothelial shear stress (ESS) at these points which encourages lipid deposition (27). The process starts with large arteries and progresses to smaller ones with age (28). As the process advances, the plaque wall can rupture, activating the clotting cascade and gives rise to thrombus formation (atherothrombosis). This causes narrowing of the vessel and can result in ischaemia and infarction secondary to either reduced blood flow and hypoperfusion or release of the clot into the distal circulation with embolic occlusion.
Atherosclerosis affects the vertebral and basilar arteries leading to vertebrobasilar occlusive disease (VBOD) and can also affect smaller arteries such as AICA (29). The hypoperfusion that arises from VBOD can lead to SSHL, albeit in only about 10% of these patients (30-33). While this deafness may be due to damage to the cochlear nuclei in the brainstem secondary to stroke, audiological investigations demonstrate a cochlear dysfunction in about 50% (32, 34). The inconsistent rate of SSHL in VBOD is consistent with animal studies that proximal occlusion does not necessarily infarct the cochlea, presumably dependent on collateral circulation around the basilar artery and backflow from the anterior circulation. It has been argued that this is a rare cause of ISSHL as one study found only 4 of 333 cases of ISSHL were diagnosed with VBOD (35). However all four of these cases had bilateral disease and given others have found VBOD causes unilateral SSHL far more commonly than bilateral (32) it may be that the true rate was much higher.

Occlusion of AICA also causes SSHL, which again has been shown to be usually due to cochlear infarction rather than damage to the brainstem (36-38). This could be due to either hypoperfusion or embolisation. Interestingly a similar study found SSHL associated with strokes from occlusion of the posterior inferior cerebellar artery (PICA) (39). As this artery does not supply the labyrinthine artery, cochlear damage from hypoperfusion should not occur. Neither would these strokes be expected to damage the cochlear nuclei at the brainstem and indeed hearing tests again found a dysfunction of the inner ear. This would suggest that emboli causing the stroke, most likely arising from vertebro-basilar atherosclerosis, are multiple and travel to the cochlea at the same time.

Post-mortem findings have been reported following a stroke secondary to VBOD (40). This patient reported SSHL 17 days prior to death as part of his initial symptom complex. Atherosclerosis of the vertebral arteries and thrombosis of the basilar artery were found. Unfortunately the specimen did not include AICA. The distal labyrinthine artery was normal. Severe degeneration of the cochlea had occurred but no fibrosis or ossification had taken place. A further case of SSHL relating to subarachnoid haemorrhage occurring 2 months prior to death (41), also reported severe generalised degeneration within the inner ear without fibrosis or ossification. By 2 months, these findings would be expected to occur from animal studies although it may just be that the
process is slower in humans. It could also be further evidence that fibrosis and ossification are not always linked to vascular disruption. Post-mortem findings obtained from a 91 year old, 7 years after an episode of ISSHL, demonstrated atherosclerosis of vertebral and basilar arteries and AICA and PICA (42). Small arteries within the inner ear showed arteriosclerosis and there was degeneration of cochlear hair cells, most severe in the basal turn. No fibrosis or ossification had occurred. The authors suggested the findings of atherosclerosis meant the ISSHL must have been due to vascular occlusion however there was no direct evidence of this. It must be argued that, while this case demonstrates that atherosclerosis and arteriosclerosis occur within these arteries and that there is the potential for hypoperfusion, a 91 year old would be expected to have evidence of these diseases and so causation cannot be concluded.

There is no doubt that atherosclerosis can lead to SSHL. The question remains as to whether it is a common cause of ISSHL. There are many reports of temporal bone pathology in patients who have previously suffered ISSHL (19, 43-46) but these studies did not examine the vertebral and basilar arteries or AICA so any atherosclerosis remained undiagnosed. Radiographic studies have also tried to answer this question. Angiography of the vertebrobasilar system was carried out and suggested the vertebral arteries were smaller on the affected side of 7 of 10 ISSHL patients (47). However it was clear that the image was very different depending on which vertebral artery was catheterised and the controls only had one side done, resulting in an invalid comparison. Transcranial doppler (TCD) found 11 of 32 patients versus 4 of 30 controls had abnormal mean blood flow in either vertebral or basilar arteries and this was independent of age (48). Ultrasound demonstrated vertebral blood flow was slower in those with more severe hearing loss (49) and that the basilar artery resistance and pulsatility indices were higher following ISSHL (50). Magnetic resonance imaging (MRI) has found slow blood flow in the vertebrobasilar arteries in 12 of 57 patients with ISSHL, predominantly men over the age of 50 years (51). The inference from these studies is that the posterior circulation is diseased in approximately a quarter of ISSHL patients. Unfortunately there remains no direct evidence of atherosclerosis in the majority of cases.
1.3.5. Association of ISSHL with cardiovascular risk factors linked with atherosclerosis.

It is widely accepted that certain cardiovascular risk factors greatly increase the development of atherosclerosis. It follows that these risk factors should be associated with ISSHL patients as well if atherosclerosis were a common cause. There are a number of studies that have looked at this.

1.3.5.1. Hypertension, diabetes mellitus, hyperlipidaemia, smoking and a history of cardiovascular disease.

Hypertension, diabetes mellitus, hyperlipidaemia and smoking are widely recognised as leading cardiovascular risk factors (52, 53). Aimoni et al (54) compared 141 ISSHL patients to 271 controls with regard to cardiovascular risk factors. They found the risk of ISSHL increased with the number of risk factors. This was primarily due to significant associations with diabetes and hypercholesterolaemia. There was no difference between smoking or hypertension. A similar study (55) of 96 patients versus 179 controls found no significant difference for any of the traditional risk factors although they did find 12% of patients had a history of cardiovascular disease compared with only 3% of controls. Ballesteros et al (56) found higher rates of hypertension, diabetes and previous cardiovascular disease in ISSHL patients. A number of other case-control studies have included results for hyperlipidaemia and these vary between showing no difference (57-60) between ISSHL patients with controls to finding very clear differences (e.g. hypercholesterolaemia occurring in 79% of patients compared with 28% of controls) (61-68).

In addition to these relatively small studies, there have been 2 large population surveys, in Taiwan and Japan, which have addressed these factors (69, 70). Both of these found that hypertension and diabetes were increased in the patients with ISSHL. In particular, one of these studies showed an increased risk for subsequent stroke if there was a history of ISSHL (69). They also found a significant difference with regard to hyperlipidaemia but both rates were very low (0.8% in patients versus 0.3% among controls) (69).
1.3.5.2. Hyperhomocysteinaemia and folate.

Hyperhomocysteinaemia is another well-recognised risk factor for cardiovascular disease (71). It is toxic to many tissues but in particular affects endothelial cell function and the coagulation system resulting in a prothrombotic tendency and atherosclerosis. Folate directly lowers serum homocysteine although it also exerts an independent protective effect over endothelial function (72). Raised serum homocysteine has usually been found to be associated with ISSHL, particularly in larger studies, with abnormal levels found in up to 27% of patients (61, 63, 68, 73-76). Folate levels have been examined in 3 studies, all of which found reduced levels when compared to controls (68, 73, 74). Pathologically reduced levels were present in 4 and 73% respectively.

Genetic factors involving homocysteine metabolism have also been studied. Defects in the genes coding for methylenetetrahydrofolate reductase (MTHFR), cystathionine-β-synthase (CBS) and methionine synthase (MTR) are all associated with increased serum homocysteine (71). The commonest of these, MTHFR C677T, has been found by most studies to have a small association with ISSHL (62, 75-80). Another MTHFR gene, A1298C, has been found by 2 studies to also have a small association, particularly in combination with C677T (62, 75). MTR A2756G was found by one study to be increased in ISSHL (40% versus 23% abnormal). CBS was not found to have an association (75).

1.3.5.3. Hyperfibrinogenæmia.

There are a number of haemostatic factors that have been shown to have a strong association with cardiovascular risk factors and disease and fibrinogen is foremost among these (81). Fibrinogen is a large molecular weight protein and so is an important determinant of plasma and blood viscosity and therefore blood flow. It is converted to fibrin, an essential component of thrombus, during the process of coagulation. It is an acute phase protein that is increased in inflammatory states. Elevated fibrinogen has been demonstrated by meta-analysis to be a strong independent risk factor for cardiovascular disorders (82). However the relationship with ISSHL remains unclear. Four studies have shown an increased association of fibrinogen with ISSHL (60, 62, 64, 65) while 3 other have not (59, 77, 83). One of these studies also examined 2 genetic polymorphisms of the fibrinogen gene that are associated with increased cardiovascular risk but found no difference to controls (64).
Therapeutic plasmapheresis reduces the viscosity of blood by reducing certain plasma molecules, such as lipids and fibrinogen. It can improve endothelial dysfunction and is used as a treatment for atherosclerosis (84). A number of case series have been published suggesting it is an effective treatment for ISSHL. This was the subject of a single randomised controlled trial (RCT) which found improved hearing outcomes in a sub-group of patients with raised fibrinogen levels but not in the group as a whole (85), suggesting this treatment may benefit those patients who already have increased cardiovascular risk.

1.3.5.4. Plasminogen activator inhibitor (PAI-1).
Plasminogen activator inhibitor (PAI-1) is another acute phase protein that is involved in coagulation. PAI-1 is produced directly by endothelial cells. Plasminogen is converted to plasmin which lysed thrombus. This process is mediated by tissue plasminogen activator (tPA) or urokinase, both of which are inhibited by PAI-1. Elevated levels of PAI-1 are also well established as a cardiovascular risk factor (86). Three studies have examined PAI-1 levels in ISSHL patients and all found increased levels when compared to controls (63, 83, 87, 88). Elevated levels of PAI-1 are also associated with homozygosity of the 4G allele of a genetic polymorphism. However two studies examining this genetic factor found no difference with controls (78, 79).

1.3.5.5. Lipoprotein (a).
Lipoprotein(a) is a genetically determined variant which increases the risk of cardiovascular disease, likely as a result of its resemblance to and subsequent competition with plasminogen (89). Three studies examining this have all found higher proportions of abnormal levels in ISSHL patients (56, 63, 65).

1.3.5.6. Endothelial dysfunction and oxidative stress.
The vascular endothelium is composed of highly specialised cells essential to the regulation of the vascular milieu. It acts as both a barrier to noxious stimuli and a facilitator of interactions with the plasma and cellular components of blood. Endothelial dysfunction has long been recognised as part of the pathophysiology of atherosclerosis and is associated with both disease progression and future cardiovascular event rate (90, 91). It is a complex process that partly occurs because of oxidative stress, whereby there is local production of reactive oxygen species (ROS) secondary to systemic
inflammation that overwhelms the naturally occurring mechanisms to remove them (53, 92). ROS react with nitric oxide (NO), which is an essential vasodilator and inhibitor of platelet aggregation that is released by endothelial cells. This in turn reduces the bioavailability of NO resulting in localised inflammation and atherosclerosis as well as vasoconstriction and thrombus formation. ROS also have direct effects on cells themselves, causing oxidative modifications to cell components. NO has been shown to be present throughout the cochlea in animal models, particularly in the oxygen sensitive areas of the stria vascularis and organ of Corti, and to exert a tonic vasodilation (7). Many of the most important and well recognised cardiovascular risk factors, including hypertension, diabetes, smoking and hypercholesterolaemia all exert many of their deleterious effect by causing oxidative stress and are therefore associated with endothelial dysfunction (52).

Endothelial dysfunction can be measured using intracoronary infusion of acetyl choline (ACh), finger-pulse plethysmography, pulse curve analysis or flow mediated dilation of the brachial artery. Infusion of ACh has been considered as the gold standard but is invasive and requires the presence of an experienced interventionalist. There has also been some doubt cast over this method since ACh can lead to release of mediators of vasoconstriction produced outside the endothelium (93). Finger-pulse plethysmography and pulse curve analysis both have the potential to be effective methods of measuring endothelial dysfunction but at present are unproven (92). Currently the most accepted method is flow mediated dilation (FMD) of the brachial artery following 5 minutes of localised ischaemia secondary to inflation of a sphygmomanometer (92, 94, 95). The amount of dilatation reflects the vascular bioavailability of NO provoked by the ischaemic injury and is expressed as a percentage increase in the artery diameter following the stimulus. Different authors have found either <4.5% (96) or <8% (97) increase as associated with cardiovascular risk. In a small pilot study of 6 patients with ISSHL, 5 of them were found to have markedly reduced FMD (<5% increase) although no control data was presented (98).

1.3.5.7. Endothelial nitric oxide synthase.
Endothelial nitric oxide synthase (eNOS) is an endothelial cell membrane bound enzyme that plays a crucial role in the formation of nitric oxide (92). One study has examined three genetic polymorphisms of eNOS associated with reduced function
(T786C, G894T, 4a/4b) (99). They found that the G894T rare variant was independently associated with ISSHL and that the addition of the rare variants of the other 2 genes increased the odds ratio further.

1.3.5.8. Endothelial cell adhesion molecules.
Intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1) both undergo enhanced expression by endothelial dysfunction, increasing leukocyte adhesion and migration. The soluble forms can be measured in the serum. One study found significantly increased levels in ISSHL patients (59). However this was due to a small number of patients having very high levels (ICAM-1: 4 of 37 patients versus 0 of 47 controls; VCAM-1: 10 of 37 patients versus 4 of 47 controls). The level of the adhesion molecules did not correlate with the severity of hearing loss.

1.3.5.9. Platelet receptors.
Platelet glycoprotein receptors (GP) are involved in the activation of platelets. GP1a is one of the receptors that bind platelets to collagen when it is exposed following trauma to endothelial cells. A genetic polymorphism GP1a C807T has been found to have an association with cardiovascular disease although recent meta-analyses have cast some doubt (100-102). One study has examined this and found a strong association with both heterozygosity and homozygosity with ISSHL (64). GPIIb/IIIa complex is the essential component of the final pathway of platelet activation as it is essential for binding fibrin, leading to platelet aggregation. A genetic polymorphism of this receptor (GP IIIa PlA1/A2) has also been associated with cardiovascular disease (102). Two studies have examined this gene, one finding an association (62) and one finding no difference when compared to controls (78).

P-selectin is an adhesion receptor on both endothelial cells and platelets and is particularly a marker for platelet activation (103). It has been shown to have a role in leukocyte adhesion in the atherosclerotic process (104). Glycoprotein V (GPV) is part of the GP Ib-V-IX receptor complex that interacts with von Willebrand’s factor (vWF) to mediate adhesion of platelets to exposed collagen. GPV is also a marker for platelet hypereactivity (105, 106). At the present time neither has been examined with regard to ISSHL.
1.3.5.10. Coenzyme Q.
Coenzyme Q (CoQ) is an endogenous anti-oxidant that prevents oxidation of membrane-bound lipid peroxide free radicals, a process which contributes to atherosclerosis. Two studies, albeit by the same group, have examined CoQ levels and found considerably lower levels in ISSHL patients when compared to controls (61, 66).

1.3.5.11. The antiphospholipid syndrome.
The antiphospholipid syndrome is an autoimmune syndrome characterised by recurrent arterial and venous thromboses in association with antiphospholipid antibodies (107). The main antiphospholipid antibodies are anticardiolipin antibodies (aCL) IgG and IgM, lupus anticoagulant and anti-β2-glycoprotein-1. Emerging evidence supports the role of antiphospholipid antibodies accelerating atherosclerotic lesions, possibly by exacerbating the inflammatory response (108). Three studies examining these antibodies have all found increased rates of abnormal levels in patients with ISSHL when compared to controls (63, 109, 110).

1.3.5.12. Abnormal rheology and hyperviscosity.
Whole blood viscosity (WBV) is determined by plasma viscosity and the cellular components, principally haematocrit and erythrocyte deformability and aggregation. It is proportional to peripheral vascular resistance and hence affects blood pressure. Indeed hyperviscosity is associated with all cardiovascular risk factors and atherosclerosis itself (111).

Three studies have examined WBV and its relationship to ISSHL and all found a considerably increased association when compared to controls (65, 99, 112). Plasma viscosity has shown less of an association, with 2 studies finding higher levels (65, 112), one finding increased levels but not significantly (99) and one finding no difference (113).

Two studies have examined erythrocyte aggregation and both found it markedly increased in ISSHL patients (60, 65). Deformability was also increased in 3 out of 4 studies (65, 99, 113, 114), one of which found it correlated with eNOS genetic polymorphisms (99).
1.3.6. Small vessel disease.

Cerebral small vessel disease (SVD) describes a collection of disorders affecting the small penetrating arteries of the brain and their arterioles (115). These are functional end-arteries with little collateral circulation, much like the end arteries within the cochlea. SVD accounts for 20 – 30% of strokes and also has a role in neurodegenerative conditions such as Alzheimer’s disease (116, 117). A number of these conditions are rare but have clear links with sensorineural deafness, showing the possibility of SVD causing inner ear dysfunction. This includes the mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) syndrome and some of the retinocerebral vasculopathies such as Susac’s syndrome. These diseases are readily diagnosed and would not be responsible for ISSHL.

The commonest cause of SVD is degenerative cerebral microangiopathy (DCM) which is much like atherosclerosis but for the small arteries (115). It is associated with the usual vascular risk factors, in particular hypertension, diabetes mellitus and hyperhomocysteinaemia, and pathological findings include hyalinosis, fibrous proliferation and microatheroma with thrombosis. It is recognised that lifestyle changes such as smoking cessation that reduce atherosclerotic risk will also reduce DCM risk. While the typical presentation of DCM is slowly progressive deterioration over years, the significant association with strokes lend itself to the possibility of causing ISSHL. While the temporal bone studies carried out on people who had suffered ISSHL during life do not generally show the major arteries, they do contain the small vessels within the temporal bone. The case of the 91 year old unsurprisingly demonstrated arteriosclerosis, however the other 30 or so cases reported have found healthy arteries and arterioles. This would not be consistent with DCM as a common cause of ISSHL.

1.3.7. The Microcirculation.

The vessels of the microcirculation, including arterioles, capillaries and venules, are increasingly recognised as targets in their own right for cardiovascular risk factors and in particular the effects of endothelial dysfunction and oxidative stress (118). As defined by Poiseuille’s law, flow is proportional to both radius of the channel and viscosity of the fluid so hyperviscosity syndromes would be expected to especially affect the microcirculation. In addition, as blood vessel diameter reduces, the cellular component of blood becomes increasingly important to both viscosity and flow. Within capillaries
the erythrocyte aggregation and deformability seem to have the biggest effect (119) and, as discussed in section 1.3.5.12 above, these factors are associated with ISSHL. Given the high oxygen requirements of the cochlear, it could potentially be extremely vulnerable to these detrimental effects on the microcirculation.

Temporal bone studies have involved long time delays between the diagnosis of ISSHL and death, giving plenty of time for reparation of microcircular insults, so the lack of clear vascular pathology in these studies does not exclude this as a possible aetiology. There is only one case where death occurred nine days after the episode of ISSHL (45). There was no evidence of vascular occlusion in this case, the main pathology being severe swelling of the organ of Corti, where the sensory epithelium for hearing resides. There was also no evidence of the sort of leukocytic invasion that would be expected from a viral infection. Despite a preceding diagnosis of contralateral Meniere’s disease, there was no evidence of its pathologic correlate, endolymphatic hydrops, demonstrated at post-mortem in the ear with ISSHL. The authors proposed the “cellular stress response” theory based upon this. They hypothesized that distally produced circulating mediators caused activation of nuclear factor-κ B (NFκB), a cellular transcription factor which leads to localized inflammation. The only evidence they present to support this theory are unpublished experimental observations that lipopolysaccharide injected intraperitoneally into mice results in upregulation of NFκB in the mice cochleas. These changes were not associated with hearing loss, and indeed the authors have been unable to induce hearing loss in this situation. This raises a number of issues. The patient was admitted and anticoagulated following the episode of ISSHL. There is the possibility that a thrombus was cleared from the cochlear vessels prior to death. As cochlear infarction takes place after about 30 minutes, permanent damage would still be expected. The patient then suffered a femoral embolus followed by a coronary occlusion. Clearly this patient was an arteriopath at high risk of vascular events. There is a wealth of literature describing current theories on oxidative stress and endothelial dysfunction in patients with high cardiovascular risk and these make clear that many of the deleterious effects are mediated via NFκB (52, 53, 118). It seems more likely that the “cellular stress” resulted from a background of systemic inflammation secondary to cardiovascular risk factors and that the ISSHL occurred due to endothelial dysfunction causing microcircular localised inflammation, vasospasm or thrombosis. Given the subsequent arterial events, a proximal source of emboli is also a realistic possibility.
1.3.8. Emboli of cardiac origin.
Cardiac abnormalities leading to thromboembolism are another common cause of stroke (120). They are usually due to atrial fibrillation, valvular disease or atrial septal aneurysm (121). Only one study has compared echocardiograms of 86 ISSHL patients with 263 age-matched controls and found the incidence of mitral valve prolapse was 26% versus 2.7%, suggesting this aetiology may be important in ISSHL (122).

1.3.9. Venous thrombosis and thrombophilia.
The thrombophilias describe a group of disorders in which the balance between coagulation and anticoagulation is disrupted in favour of thrombosis, particularly in the venous system. Some factors can increase both venous and arterial risk, including hyperfibrinogenaemia, antiphospholipid antibodies and hyperhomocysteinaemia, which have been previously discussed. Five inherited factors are also well recognised. The commonest are 2 specific gene substitutions, factor V Leiden (G1691A) and Prothrombin G20210A. Between them they account for up to 60% of venous thromboembolism (VTE) and have a prevalence of approximately 5-10% and 2-4% in the general population respectively (123). Although they predominantly increase the risk of VTE, there is recent evidence that suggests they may have a role in arterial thrombosis as well (124). Currently any involvement in the causation of ISSHL is unclear. Factor V Leiden has been examined by 8 studies with 3 finding an association with ISSHL (62, 78, 125) but the majority finding no difference to controls (56, 63, 77, 79, 125). Even in the studies showing an association, factor V Leiden was only present in up to 16% of cases, which is only marginally higher than the prevalence in large population studies. Regarding the prothrombin gene mutation, one study (126) examined 368 patients with deep vein thrombosis (DVT) and found a high incidence of 18 with a history of ISSHL. This compared with only 10 patients out of 790 controls (half of whom had bleeding disorders), suggesting ISSHL was much more common in patients with DVT. All the inherited thrombophilias were significantly associated with the patients with DVT, however only the prothrombin gene deficiency was associated with ISSHL. Since then several studies have examined the prothrombin gene. One found a significant association with ISSHL (62) and a further study found it was only associated in patients under 40 years old (127). Again the majority have found no difference (56, 63, 77-79, 125).
The other inherited disorders are the deficiencies of antithrombin and proteins S and C. These are much rarer, having prevalences of approximately 1 in 1000 (123), although they also carry a greater relative risk of VTE. They are not thought to be involved in arterial disease. Only 2 studies have adequately examined these factors and both found no association with ISSHL (63, 76). Overall it appears that, while thrombophilias may increase the risk of ISSHL, venous thrombosis is probably not a common cause.

1.3.10. Paradoxical embolism.

“Paradoxical” embolism describes when a venous thrombus passes through a venous-arterial circulation shunt (v-aCS) to embolise the arterial circulation. As paradoxical embolism first requires a thrombus formation in the venous circulation, thrombophilia would theoretically increase this risk. Patent foramen ovale (PFO), a cardiac atrial septal defect, is the most frequent cause of v-aCS but ventricular septal defects and pulmonary arterio-venous fistulae also occur. The prevalence of PFO determined by autopsy was 27% overall but decreased from 34% in the first three decades of life to 20% in the 9th and 10th decade (128). The ease with which venous blood may shunt from the right to left atrium depends on the size of the PFO. Although it has been thought that high right atrial pressures are needed for shunting, it frequently occurs spontaneously and may be easily stimulated by coughing or a Valsalva manoeuvre.

There is growing interest in the role of paradoxical embolism in the causation of vascular events such as cryptogenic stroke in young people. This is an ischaemic stroke where no vascular pathology such as atherothrombosis has been identified. PFO has been found to occur in 50 - 54% of young patients with cryptogenic strokes compared to 15 - 44% of controls (129, 130). The YAMIS study, a major case-control study on the frequency of v-aCS in young adults surviving ischaemic stroke (IS) or myocardial infarction (MI), found an increased frequency of “major” v-aCS in young adults with IS compared with healthy age and sex matched controls (25% vs 13%) (131). A pilot study on the role of paradoxical embolism in sudden monocular blindness due to retinal artery occlusion found “significant” v-aCS in 70% of young adults suffering this disorder (132). Paradoxical embolism has also been found to be associated with Alzheimer’s disease (133). Another area of great interest is the role of paradoxical embolism in the pathogenesis of migraine with aura (134, 135). Indeed, not only are the two pathologies
associated, but even partial closure of v-aCS in these patients improved symptoms (136).

The onset of stroke immediately on waking or following exertion were both found to be independent predictive factors of the presence of a PFO (137). These are also the 2 commonest presentations of ISSHL with 65% of patients presenting in this manner (3). One study has examined the presence of PFO in patients with ISSHL (138). They used a transcranial doppler with microbubble contrast technique and found a significant association in 23 patients and 46 controls (48% vs 17%). They suggested a trend towards positivity in younger patients. No attempt was made to quantify the size of the PFO.

1.3.11. Other vascular mechanisms.
ISSHL has been reported in association with numerous vascular and haematological diseases such as migraine (139-141), sickle cell disease (142, 143), macroglobulinaemia (144), leukaemia (145) and Buerger’s disease (146) but adequate epidemiological studies have not been done. It is also well recognised after non-otologic surgery, particularly cardiac bypass surgery, for which vascular mechanisms such as hypotension and subsequent cochlear hypoperfusion have been proposed (147, 148).

1.3.12. Bilateral ISSHL.
While the nature of unilateral ISSHL lends itself to an embolic theory, bilateral ISSHL has usually been suggested to have an autoimmune cause. As it is so much rarer than its unilateral counterpart, bilateral disease has been infrequently studied (149-153). Two studies drew an association with increased cardiovascular disease over unilateral ISSHL patients but both could have been explained as the bilateral patients had a mean age 10 years older than the unilateral (149, 151). Another study found increased rates of autoimmune diseases and antibodies in bilateral disease, particularly where it occurred simultaneously (152).

An association that has been found is that with opiate medication, in particular hydrocodone, a synthetic analogue of codeine, and heroin (154-157). A recent study has shown this association with codeine users (158). This small study also found that all patients had a clinically raised mean cell volume, with no clear cause for the
macrocytosis identified. Macrocytosis has been shown to have an association with peripheral arterial disease (159) and is also associated with homocysteine (160) suggesting even bilateral ISSHL could be caused by a possible vascular mechanism.

1.4. Viral Infection.
Viral infection is a popular hypothesis and the literature is replete with uncontrolled case studies lending support to this theory. Viruses could potentially damage hearing by direct invasion of the cochlea or cochlear nerve during an acute infection or, in the case of the Herpesviridae family, by reactivation of dormant neurotropic virus (24).

ISSHL appears to be commoner at certain times of the year (4), but a positive association with the weather has not been seen (161, 162). It has been suggested that the change with seasonality was most likely due to increased viral load within the population.

Animal models have been used to determine what happens when the inner ear is inoculated with virus. These have usually shown variable patterns of destruction within the cochlea, sometimes resulting in fibrosis and ossification as described for vascular damage (24, 163, 164). Given that the most well recognised cause of cochlear ossification is bacterial meningitis (165), it would seem that this is a common end point of severe inflammatory destruction rather than a disease specific entity.

A report describing magnetic resonance imaging in two patients with clearly rising and falling viral titres in relation to their ISSHL (rubella and herpes varicella-zoster respectively) found clear evidence of contrast enhancement on T1 images (166). This sort of enhancement is however rarely documented in series of patients with ISSHL (167-171). A more recent study has used 3D-FLAIR to examine the cochlea and found 31 of 48 patients had enhancement, which is not seen in normal controls (169). With time this enhancement disappeared. The significance of this however remains unclear at this time and does not lend itself to a particular aetiology.

Viral causes gain little support from treatment studies. Two randomised controlled studies examining anti-viral treatments found that valacyclovir and acyclovir were ineffective (172, 173).
There is a good association of deafness with acute mumps infection (174). Similarly rubella and cytomegalovirus (CMV) are known to cause intrauterine infection and congenital deafness, among other things (175, 176). However despite the introduction of vaccination against mumps and rubella, ISSHL numbers are, if anything, increasing (70). Studies of serological viral data against many different viruses have usually found very low rates of positivity (177). Respiratory viruses have been implicated due to a history suggesting up to 35% of patients had concomitant infection. However these rates are similar to those of controls (3). Likewise a study examining ISSHL and interferon, a marker for systemic viral infection, found no evidence within the serum and gene expression did not differ from controls (178).

1.5. Intracochlear Membrane Rupture.
The bony cochlea is split into 3 compartments: the scala tympani and scala vestibuli contain perilymph, a sodium-rich fluid similar to extracellular fluid that freely communicates with the cerebrospinal fluid (CSF) via the cochlear aquaduct. The scala media contains endolymph, a potassium-rich fluid similar to intracellular fluid. Endolymph has been shown to be toxic to hair cells in vitro; therefore if there is a rupture of one of the membranes separating these compartments then damage to these cells could occur and this has been proposed as a potential mechanism for ISSHL (179). However, membrane breaks are rarely seen in most temporal bone studies (19, 43-46).

1.6. Autoimmune Inner Ear Disease.
Damage to the hair cells could be caused by hypersensitivity processes such as circulating antibodies cross-reacting to inner ear antigens or direct attack by activated T-cells (180). Autoimmune inner ear disease (AIED) is a well recognised disease entity that usually presents with bilateral RPSHL and can show good response to steroid and immunosuppressive treatment (181). Both sudden onset and unilaterality do occur but typically it progresses to bilateral disease. Antibodies from these patients show high reactivity for a 68kD protein identified by Western blotting (182). Initial experiments found these antibodies cross-reacted with heat shock protein (HSP) 70 which has a similar molecular weight (183, 184). HSP70 is found throughout the body including the cochlea. Although serum levels become elevated following ISSHL (185), levels of anti-HSP70 antibodies do not induce deafness (186, 187) and are not increased in ISSHL case-control studies (188, 189). However the target 68kD antigen now appears to be
choline transporter-like protein 2 (190, 191). No studies have examined this protein in relation to ISSHL.

Anti-endothelial cell antibodies are a heterogeneous group directed against various as yet unidentified antigens on endothelial cells. One study found their presence in 52% of ISSHL patients compared to 14% of controls (192). However the role of these antibodies is far from understood, with a probable role in normal physiology, so causation cannot be assumed (193).

Certain haplotypes of the human leukocyte antigens (HLA) are associated with systemic autoimmunity. A South Korean study found no difference between ISSHL patients and controls (194). A similar study in Spain found a marginally significant association with HLA DRB1*04 over controls but also found the presence of this gene predicted a poor prognosis (195).

1.7. Cellular Stress Response Pathway.

As discussed in section 1.3.7., the cellular stress response involves the stimulation of certain proteins and genes, one of which, NFkB, has been shown to be present within certain parts of the cochlea (45). It has been proposed that activation of this factor could set up a complex pathway leading to localised inflammation and damage but as yet there is little evidence to support this. It seems more likely this is a common pathogenesis to different aetiologies.

1.8. Endolymphatic Hydrops / Meniere’s Disease.

Endolymphatic hydrops is a condition whereby the fluid homeostasis within the inner ear is deranged, leading to an increase in volume within the endolymphatic compartment and can only be diagnosed histologically post-mortem. It is highly associated with (and thought to be the underlying cause of) Meniere’s disease, an idiopathic condition diagnosed clinically by recurrent vertigo and fluctuating SNHL, tinnitus and aural pressure. Patients with Meniere’s disease can sometimes suffer SSHL, particularly in the low frequencies, so differentiating this from ISSHL can prove difficult, particularly as Meniere’s disease is a highly variable condition clinically. It seems likely that some ISSHL may be due to endolymphatic hydrops. However one
study looking at disease progression found only 4% of ISSHL patients were subsequently diagnosed with Meniere’s disease (196).

1.9. Conclusions from Literature Evaluation.
The aetiology of ISSHL is still very much debated and it seems likely that it is multifactorial. However at the present time there is only limited evidence to support either viral or cochlear membrane breaks as common causes. Autoimmune conditions are more prevalent in bilateral ISSHL but evidence in unilateral disease is more related to antibodies that result in a prothrombotic state such as the anti-phospholipid syndrome. As understanding of the complex processes underpinning vascular dysfunction become clearer and studies start to examine the association of these conditions with ISSHL, a growing trend seems to be appearing. Traditional cardiovascular risk factors and atherosclerosis have some association and there is also an increasing picture of endothelial dysfunction and hyperviscosity at a microvascular level, possibly initiating cellular stress pathways. Given the exceptional nature of the cochlea in being both extremely sensitive to blood flow and oxygenation and having an end arterial supply, it seems likely that it is uniquely sensitive to these dysfunctions.

While atherosclerosis and cardiogenic emboli may be significant causes in the older population, these are much less likely to cause ISSHL in younger patients. Paradoxical embolism is increasingly recognised within this age group as a cause of cryptogenic stroke, a condition which clearly parallels ISSHL. Microemboli passing through a v-aCS in a susceptible individual could either directly obstruct the cochlear vasculature or lead to a localised inflammatory response causing intense reflex vasoconstriction and endothelial activation, particularly in a patient who already had increased cardiovascular risk.

1.10. Hypothesis.
This MPhil research study hypothesises that ISSHL in young adults is often caused by paradoxical embolism and that such patients may have increased cardiovascular risk.

1.11. Aims.
The MPhil will investigate the frequency of venous to arterial circulation shunts (v-aCS), thrombophilies, endothelial dysfunction and other cardiovascular risk factors in
20 young adult patients who have suffered unilateral ISSHL as a pilot study. The frequency of these risk factors will be compared with that in healthy controls matched for age and sex.

1.12. Experimental Approaches – Detection of v-aCS
The commonest methods for the diagnosis of v-aCS are transoesophageal echocardiography (TOE), transthoracic echocardiography (TTE) or transcranial doppler (TCD) in combination with a microbubble emulsion as an ultrasound contrast medium.

1.12.1. Transoesophageal echocardiography.
TOE involves placement of an ultrasound probe into the oesophagus which, due to proximity to the heart, provides excellent visualisation of the heart. It has been shown to be highly sensitive when compared to post-mortem examination (197). It is widely accepted as the reference technique in the detection of v-aCS but is semi-invasive, uncomfortable, involves sedation and has a recognised morbidity and mortality. As sedation and oesophageal intubation are required, provocation manoeuvres such as coughing or a standardised Valsalva are difficult.

1.12.2. Transthoracic echocardiography.
TTE involves ultrasonography across the chest wall. It is safe and minimally invasive and can be done with provocation manoeuvres but sensitivity for detecting PFO in comparison with TOE is less reliable because of the greater distance from the heart (198).

1.12.3. Transcranial Doppler.
TCD involves ultrasonography of the middle cerebral arteries (MCA) via a probe placed in the temporal region. The time between contrast injection and passage through the MCA indicates whether a v-aCS is present and the amount of contrast indicates its size. It is safe, minimally invasive and in comparison to TOE is both sensitive and specific for the detection of v-aCS (130, 199, 200). It also detects both intracardiac and pulmonary v-aCS. When compared to TOE in our Vascular Studies Unit, clear criteria for the detection of PFO could be identified enabling differentiation between small and large v-aCS (131, 200).
1.13. Relevance of the Research.

Identification of an association between ISSHL and v-aCS would help to explain the aetiology of this disease. Such patients may also be at increased risk of future vascular events such as stroke. Identification of factors that might make an individual susceptible to ISSHL, such as PFO, thrombophilia or endothelial dysfunction, may open new research opportunities for novel therapeutic options.
2. METHODS

2.1. Study Design.
This was a pilot prospective case-controlled study.

2.2. Ethical Approval.
Ethical approval was obtained from the local research ethics committee of the University Hospitals of South Manchester (UHSM).

2.3. Case and Control Identification and Recruitment.
The target group were young adults who were aged 18-50 years when suffering a first episode of unilateral ISSHL between 1996 and 2008 identified and referred by otolaryngologists working in hospitals within the North West of England including Mersey.

Controls were age and sex-matched individuals identified either through advertisement or as patients attending an otolaryngology department with a condition not associated with hearing loss, v-aCS or vascular disease (e.g. recurrent tonsillitis).

2.4. Inclusion Criteria.
i. Aged between 18 and 50 years inclusive when diagnosed with ISSHL and of either sex
ii. Consenting to study procedures
iii. No underlying disease that could be associated with SSHL as an aetiologic factor (listed under “exclusion criteria” below)
iv. For cases: A history of unilateral hearing loss with an onset over a period of less than 3 days and with audiometric confirmation in the affected ear of a masked bone conduction threshold of 30 dB or greater in at least 1 frequency.
v. For controls: Subjectively normal bilateral hearing.

2.5. Exclusion Criteria.
i. Evidence of fluctuating cochleovestibular dysfunction suggestive of endolymphatic hydrops (history of vertigo with either fluctuating hearing loss, aural pressure or episodic tinnitus) preceding the episode of ISSHL
ii. Evidence of pathology of the cerebello-pontine angle on magnetic resonance imaging (MRI)

iii. Patients with small-vessel diseases, including giant cell arteritis, Buerger’s disease and others

iv. The diagnosis of an autoimmune disorder by history with an abnormality on blood investigation to support this diagnosis

v. A history of otologic surgery, trauma to the head and neck or barotrauma in the 10 weeks prior to diagnosis of ISSNHL

vi. History or MRI findings of congenital cochlear malformations

vii. History of otitis media in the 10 weeks prior to diagnosis of ISSNHL

viii. Presence of neurologic disorders that may predispose to hearing loss

ix. Recent use of ototoxic medications

x. The diagnosis of a neoplasm within the previous two years

xi. Psychiatric illness or mental impairment that would impair informed consent

xii. History of other serious illness such as heart failure, severe lung disease, liver or renal dysfunction with supporting imaging or laboratory data

xiii. Unable to travel to UHSM

xiv. Controls with a history of abnormal hearing or an aetiologic factor associated with v-aCS (previous stroke, MI or sudden blindness)

2.6. Test Procedures.
Participants attended the Vascular Studies Unit at UHSM following overnight fasting and written informed consent was taken which included consent for a review of their medical notes to enable the diagnosis to be confirmed and data obtained at interview validated. The following procedures were then carried out.

2.6.1. The clinical interview.
A structured interview was completed detailing their symptoms at the time of the event (for cases), co-morbidity (including any history or family history of specific cardiovascular risk factors such as a hypertension, hypercholesterolaemia, ischaemic heart disease, stroke/transient ischaemic attack, smoking, obesity, diabetes, atrial fibrillation) and drug history.
2.6.2. Venous cannulation and phlebotomy for blood investigations and thrombophilia.

An 18g intravenous cannula was placed in the right antecubital vein for the v-aCS investigation. Venous blood was taken during cannulation for a thrombophilia screen, full blood count, fasting plasma lipids, fasting glucose, p-selectin and soluble glycoprotein V. The thrombophilia screen included the following tests: activated protein C resistance (APCR), free protein S (fPS), antithrombin (AT), protein C (PC), lupus anticoagulant (assessed by kaolin clotting time (KCT), lupus-anticoagulant-sensitive partial thromboplastin time (LA-PTT) and dilute Russell viper venom time (DRVVT)), derived fibrinogen, Clauss fibrinogen, thrombin time (TT), prothrombin time (PT), activated partial thromboplastin time (APTT) and anticardiolipin antibodies IgG and IgM. These investigations were carried out by the haematology and biochemistry laboratories at UHSM using commercially available kits.

2.6.3. Electrocardiogram.

A 12-lead electrocardiogram (ECG) was carried out.

2.6.4. Transcranial doppler investigation.

The participant then underwent contrast TCD investigation for v-aCS. This was performed using an established protocol developed in previous studies (131, 201-203). TCD was carried out with the participant in a semi-recumbent position. Insonation of the middle cerebral artery was carried out bilaterally through the temporal bone window using 2 MHz transcranial Doppler ultrasound probes to a depth of approximately 55mm. The subject was monitored for one hour at rest for spontaneous cerebral emboli.

Contrast was generated by mixing 8ml saline, 0.5ml blood and 1ml of air 12 times between two 10ml syringes through a three-way tap, generating an emulsion of agitated saline. The contrast was injected at rest and immediately preceding two provocation manoeuvres: a 10 second period of repeated coughing and a 5 second standardised Valsalva manoeuvre (the subject was asked to blow into a mouthpiece connected to a manometer maintaining a pressure of at least 40mmHg for 5 seconds). There was an interval of at least one minute between each injection. The time of contrast injection and the time to first microembolic signal in seconds and cardiac cycles was monitored and recorded by the research assistant, as was the maximum number of microembolic
signals. Throughout the test the TCD output was recorded onto a hard drive for validation.

2.6.5. Flow-mediated dilation of the brachial artery.
Part way through the study, equipment became available to allow participants to undergo testing for endothelial dysfunction using flow-mediated dilation of the brachial artery (FMD). This was added to the protocol and a sub-group of participants underwent this investigation according to a standardised protocol (95, 204).

The participant was placed in a recumbent, supine position with the right arm abducted to 90° and supported on a table covered with foam. A paediatric pneumatic tourniquet was placed around the forearm. The environment had a controlled temperature so it was the same for all individuals measured. The brachial artery was identified using ultrasound (Phillips HDI 5000 SonoCT) and measurements were carried out 2 to 15cm above the brachial bifurcation and in longitudinal section. The diameter of the artery was assessed in two dimensions. Ultrasound focus was at the near wall of the brachial artery to optimise the lumen/wall interface. Brachial artery blood flow velocity was assessed using velocity pulsed Doppler at a 70° angle to the vessel and range was at the centre of the artery. Once a good image was obtained the probe was secured in a clamp. A micrometer on the clamp allowed for minute changes in probe position to ensure the image remained optimised throughout the test.

Measurements were performed at rest for one minute to get baseline values. The tourniquet was then inflated to 200 mmHg of pressure for 4.5 minutes, following which it was promptly removed. A second scan was performed 30 seconds before and for 5 minutes after deflation of the cuff. Flow velocity was determined in the first 15 seconds after deflation. Results were recorded onto video tape. Image acquisition to hard drive and validation was carried out using hardware and software designed for purpose (Vascular Research Tools 5, Medical Imaging Applications, LLC). This software calculated the diameter of the vessel at any given time, providing an objective analysis. The baseline diameter was taken as the mean score over a 30 second period prior to cuff inflation. The maximal dilated diameter was found by taking the mean score over a 15 second period between 45 and 60 seconds post-cuff inflation. The percentage increase could then be calculated.
2.7. Outcome Measures.
The primary outcome measure was the presence and functional size of a v-aCS. The TCD protocol resulted in 3 non-parametric continuous variables (microbubbles found at rest and following coughing and a Valsalva manoeuvre) that were used to categorise the participants as having “no”, “small”, “significant” or “major” v-aCS (table 1). It has been shown in a previous study in our unit that only “significant” and “major” v-aCS correlate with PFO, as detected by TOE (201).

Table 1: Criteria for detection of v-aCS using TCD.

<table>
<thead>
<tr>
<th>Size of v-aCS</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>No microbubbles found following any injection.</td>
</tr>
<tr>
<td>“small”</td>
<td>Between 1-14 micro-embolic signals within 12 cardiac cycles of injection of contrast on any test.</td>
</tr>
<tr>
<td>“significant”</td>
<td>Greater than or equal to 15 micro-embolic signals within 12 cardiac cycles of injection of contrast without meeting criteria for “major”.</td>
</tr>
<tr>
<td>“major”</td>
<td>At least 50 embolic signals are detected at rest within 12 cardiac cycles, Or At least 10 microbubbles per 12 cardiac cycles are detected at rest and at least 80 microbubbles per 12 cardiac cycles detected during provocation tests.</td>
</tr>
</tbody>
</table>

Secondary outcome measures included: the presence of any spontaneous cerebral emboli, assessed by TCD monitoring at rest for one hour; blood levels of tests from the thrombophilia screen, full blood count, fasting plasma lipids, fasting glucose, p-selectin,
soluble glycoprotein V; and FMD of the brachial artery. A positive FMD result was considered to be where the arterial dilation was < 4.5% of the initial vessel diameter.

2.8. Data Handling.
Patient data was recorded onto a secure database in the Academic Surgery Unit, UHSM.

2.9. Data Validation.
Validation of the spontaneous cerebral emboli was undertaken by technicians working in the Vascular Studies Unit who were trained to do this from previous and on-going studies. Validation of the v-aCS and the FMD tests was performed by the principal author. The ECG computer analysis was validated by a cardiology research fellow.

2.10. Statistical Analysis.
2.10.1. Sample size.
PFO has an approximate prevalence of 25% and this correlates with “significant” or “major” v-aCS. Therefore, assuming a prevalence of “significant” or “major” v-aCS of 25% in the controls, then with 20 ISSHL cases and 20 matched controls, the study would have 80% power to detect a difference in prevalence of 45% or more (i.e. a prevalence of 70% in the cases vs 25% in the controls). This assumes a simple McNemar’s test is used to compare the difference in v-aCS proportions with an estimated discordant pair rate of 60% and a 5% level of significance.

2.10.2. Analysis.
McNemar’s test was used to carry out a comparison of prevalence between the ISSHL cases and their matched controls. Within the patient group, Spearman’s correlation coefficient was used to compare the association of v-aCS with severity of hearing loss and the Chi-square test was used to examine the association of v-aCS with other symptoms.

The most clinically relevant outcome for the blood tests is whether patients are within the well established laboratory reference ranges or not, as this determines whether there is an increased cardiovascular risk. For analysis therefore, dichotomous outcomes were created for these values by considering the number of participants within or without the laboratory reference range and the case and control rates were compared using
McNemar’s test. As the glycoprotein V test is less well established, the absolute values were also analysed using a paired samples t-test. No reference range was available for the p-selectin test so this was analysed using the paired samples t-test only.

FMD was analysed primarily by comparing the proportion of cases and controls with a value of <4.5%. As this is a less well validated test at our unit, the absolute values were also compared using the paired samples t-test.
3. RESULTS

3.1. Participants.
Twenty cases and 20 age and sex matched controls were successfully recruited to the study. All patients were confirmed to have suffered an episode of unilateral ISSHL conforming to the inclusion and exclusion criteria. The mean age at the time of ISSHL was 35 years (range 22 – 48 years). The mean time from date of ISSHL to testing was 29 months (range 2 – 120 months). Case and control ages were matched to within 28 months and the mean age difference between the groups was 3 months.

One patient had a history of hypertension and one patient had a history of migraine. Four patients and 4 controls were current smokers. There were no other known cardiovascular risk factors among either group. All participants had a normal ECG. One of the controls did not have thrombophilia testing as their bloods were misplaced during transit. The routine haematology and biochemistry testing for this participant were carried out. A sub-group of 12 cases and 12 age and sex matched controls underwent testing for endothelial dysfunction.

There were no adverse events.

3.2. Venous-Arterial Circulation Shunts.
These results are given in table 2. This has shown 8 (40%) cases and 10 controls (50%) had a normal examination, 7 (35%) cases and 5 controls (25%) had “small” shunts and 5 (25%) cases and 5 controls had “significant” or “major” shunts. Comparison of the presence or absence of v-aCS between patients and controls (60% vs 50%) using McNemar’s test found p = 0.77. If this trend persisted in a larger study, that study would require over 400 pairs of patients and controls to show significance with p=0.05 and 80% power.
Table 2: Results of v-aCS testing showing functional size as determined by number of microbubbles detected at rest and under provocation.

<table>
<thead>
<tr>
<th>ID</th>
<th>Case Rest</th>
<th>Control Rest</th>
<th>Case Cough</th>
<th>Control Cough</th>
<th>Case Valsalva</th>
<th>Control Valsalva</th>
<th>Case Size</th>
<th>Control Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>34</td>
<td>0</td>
<td>sig</td>
<td>sm</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>sm</td>
<td>sm</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>8</td>
<td>19</td>
<td>22</td>
<td>20</td>
<td>60</td>
<td>sig</td>
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<td>0</td>
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<td>sm</td>
<td>sm</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>26</td>
<td>4</td>
<td>99</td>
<td>sm</td>
<td>m</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>sm</td>
<td>sm</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<tr>
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<td>0</td>
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<td>0</td>
<td>sm</td>
</tr>
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<td>0</td>
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</tr>
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<td>0</td>
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<td>m</td>
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<td>sig</td>
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<td>0</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>37</td>
<td>0</td>
<td>62</td>
<td>0</td>
<td>41</td>
<td>0</td>
<td>sig</td>
<td></td>
</tr>
<tr>
<td>20</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

sm: small; s: significant; m: major.
Table 3 shows the patient details, including the hearing loss and recovery and other factors which are known to correlate with recovery. Hearing loss was calculated as the 4-frequency average using thresholds at 500Hz, 1kHz, 2kHz and 4kHz. These are categorised according to the v-aCS found.

**Table 3: ISSHL patient details compared with the size of any v-aCS.**

<table>
<thead>
<tr>
<th></th>
<th>Total (n=5)</th>
<th>Significant v-aCS (n-7)</th>
<th>Small v-aCS (n=8)</th>
<th>No v-aCS (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years (Mean ± STD)</strong></td>
<td>35 ± 8.7</td>
<td>34±12</td>
<td>36±7</td>
<td>36±9</td>
</tr>
<tr>
<td><strong>Sex (M:F)</strong></td>
<td>10:10</td>
<td>3:2</td>
<td>2:5</td>
<td>5:3</td>
</tr>
<tr>
<td><strong>Mean Hearing Loss, dBHL</strong></td>
<td>63±33</td>
<td>81±41</td>
<td>68±32</td>
<td>49±26</td>
</tr>
<tr>
<td><strong>Hearing recovery:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Partial</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>None</td>
<td>15</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Audiogram shape:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Flat</td>
<td>9</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>High</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><strong>Vertigo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tinnitus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aural pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sequential SNHL:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Contralateral</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
The size of v-aCS appears to be associated with severity of hearing loss and this relationship is displayed graphically in figure 1. It is most likely, given the lack of association of ISSHL with v-aCS, that this is a statistical quirk based upon the number of outcome measures examined. Never the less, analysis of the association of severity of hearing loss with size of v-aCS in the patient group found a Spearman correlation of \( r=0.34; \ p=0.14 \). To confirm this association with \( p=0.05 \) and 80% power, further studies would require over 60 patients in all. An alternative analysis using the t-test comparing mean hearing loss in those with and without v-aCS (no v-aCS: \( n=8 \), mean hearing loss = 49.0, sd=26; v-aCS: \( n=12 \), mean hearing loss = 73.4, sd=34) found \( p=0.11 \). To confirm this association with 80% power, a further study would require over 50 patients in all. Analysis of the size of v-aCS with other factors (hearing recovery, the presence of vertigo, tinnitus or aural pressure and whether the patient had a sequential hearing loss), performed using chi-square test, found no associations.

Figure 1: Boxplot showing the relationship between severity of hearing loss and size of v-aCS.
3.3. Spontaneous Cerebral Emboli.
No spontaneous cerebral emboli were identified.

3.4. Thrombophilia Testing.
Tables 4a and 4b provide details for the thrombophilia tests. As one control had not had these tests performed, the matched case was excluded from the McNemar analysis, however all results for this case were within the reference range.

Table 4a: Analysis of genetic thrombophilias and lupus anticoagulant tests.

<table>
<thead>
<tr>
<th>Test (Units)</th>
<th>Reference values</th>
<th>Mean+/− Standard deviation</th>
<th>Outside normal range (n)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated Protein C Resistance (iu/dl)</td>
<td>2.18 – 3.38</td>
<td>Case: 2.58+/−0.1</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 2.68+/−0.1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Free Protein S (iu/dl)</td>
<td>69.5 – 159.3</td>
<td>Case: 101.1+/−24.0</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 107.3+/−25.1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Antithrombin (iu/dl)</td>
<td>73 - 138</td>
<td>Case: 109.7+/−7.8</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 105.1+/−7.7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Protein C (iu/dl)</td>
<td>79 - 155</td>
<td>Case: 106.0+/−13.8</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 113.4+/−23.3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Kaolin Clot Time (secs)</td>
<td>&gt;168</td>
<td>Case: 135.68+/−8.12</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 138.01+/−9.94</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>LA-PTT (secs)</td>
<td>&gt;47.26</td>
<td>Case: 39.90+/−4.2</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 38.79+/−5.6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Dilute Russell Viper Venom Time (secs)</td>
<td>&gt;47</td>
<td>Case: 38.79+/−3.0</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 37.60+/−4.4</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

LA-PTT: Lupus anticoagulant-sensitive partial thromboplastin time;
NC: a 0 value means that a p-value is not calculable using McNemar’s test.
### Table 4b: Analysis of the fibrinogen, clotting and anticardiolipin antibody tests.

<table>
<thead>
<tr>
<th>Test (Units)</th>
<th>Reference values</th>
<th>Mean+/- Standard deviation</th>
<th>Outside normal range (n)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Derived Fibrinogen (g/L)</td>
<td>2.34 – 4.74</td>
<td>Case: 3.180 +/- 0.677</td>
<td>1</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 3.103 +/- 0.600</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Clauss Fibrinogen (g/L)</td>
<td>2 – 3.8</td>
<td>Case: 3.012 +/- 0.648</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 2.932 +/- 0.516</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Thrombin Time (secs)</td>
<td>7.9 – 10.8</td>
<td>Case: 8.79 +/- 0.73</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 9.17 +/- 0.75</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Prothrombin Time (secs)</td>
<td>11.6 – 14.34</td>
<td>Case: 13.25 +/- 0.82</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 13.03 +/- 1.06</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Activated Partial Thromboplastin Time (secs)</td>
<td>18.57 – 29.47</td>
<td>Case: 21.91 +/- 1.90</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 21.63 +/- 1.97</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Anticardiolipin IgG (GPL/ml)</td>
<td>&lt;10</td>
<td>Case: 2.7 +/- 2.0</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 3.5 +/- 2.3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Anticardiolipin IgM (MPL/ml)</td>
<td>&lt;7</td>
<td>Case: 1.4 +/- 0.7</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 2.2 +/- 1.4</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

NC: a 0 value means that a p-value is not calculable using McNemar’s test.

Analysis found no statistical difference between cases and controls for any test. Indeed very few participants had values outside of the reference range. One case (ID 4 from table 1) was found to have a free protein S deficiency and this case also had a small v-aCS. No participant had a raised thrombin time but 2 cases and 2 controls had a low thrombin time. One of these cases also had hyperfibrinogenaemia and a small v-aCS (ID 6 from table 1). No participant had hypofibrinogaemia. One further case (who had no other associated abnormal tests results) and one control had hyperfibrinogenaemia on the Clauss test only. Overall 7 cases and five controls were in the upper quartile range of Clauss fibrinogen (>3.2g/L). Two cases had marginally raised prothrombin times.
(14.4 and 15 seconds respectively). All cases had normal activated partial thromboplastin times and no evidence of lupus anticoagulant or anticardiolipin antibodies.

3.5. Platelet Tests.

Table 5 provides details of the platelet tests. There was no statistical difference between the two groups. One case had a mild thrombocytopaenia of 120 x 10^9/L. Approximately 50% of both groups had higher than normal values for glycoprotein V. Cases had mildly raised absolute values for both glycoprotein V and p-selectin (paired samples t-test p-value: 0.605 and 0.590 respectively).

Table 5: Analysis of platelet tests.

<table>
<thead>
<tr>
<th>Test (Units)</th>
<th>Reference values</th>
<th>Mean+/−/Standard deviation</th>
<th>Outside normal range (n)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets (x10^9/L)</td>
<td>150 – 400</td>
<td>Case 234+/−54 Control 246+/−45</td>
<td>1</td>
<td>NC</td>
</tr>
<tr>
<td>Glycoprotein V (ng/ml)</td>
<td>10 – 60</td>
<td>Case 67.0+/−22.3 Control 64.2+/−21.5</td>
<td>11</td>
<td>1.0</td>
</tr>
<tr>
<td>P-selectin (ng/ml)</td>
<td>Not available</td>
<td>Case 81.7+/−40.2 Control 75.8+/−45.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NC: a 0 value means that a p-value is not calculable using McNemar’s test.
Table 6 details the results from fasting lipids and glucose tests. Lipid abnormalities were more prevalent in controls but not significantly so. There were 7 cases with mild hypercholesterolaemia with all values <6 mmol/L although one of these cases had an associated triglyceride count of 3.0mmol/L. This compared with 9 controls with hypercholesterolaemia of whom 2 were more severe (6.3 and 7.1mmol/L respectively). LDL results were similar: 2 cases had mildly raised titres (3.3 and 3.7mmol/L) versus 6 controls, again with more severe raised levels (3.1, 3.5, 3.9, 3.9, 4.0 and 4.7mmol/L). One case, who was not known to be diabetic, had a fasting glucose of 7.1mmol/L.

**Table 6: Analysis of fasting lipids and glucose tests.**

<table>
<thead>
<tr>
<th>Test (Units)</th>
<th>Reference values</th>
<th>Mean+/- Standard deviation</th>
<th>Outside normal range (n)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>&lt;5.0</td>
<td>Case 4.55+/-0.61</td>
<td>7</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 4.73+/-1.12</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.3 – 1.7</td>
<td>Case 1.12+/-0.62</td>
<td>3</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 1.26+/-0.81</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>High Density Lipoprotein (mmol/L)</td>
<td>&gt;1.2</td>
<td>Case 1.82+/-0.51</td>
<td>3</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 1.68+/-0.53</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Low Density Lipoprotein (mmol/L)</td>
<td>&lt;3</td>
<td>Case 2.21+/-0.72</td>
<td>2</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 2.48+/-1.10</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>3.2 – 6.0</td>
<td>Case 4.67+/-0.70</td>
<td>1</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 4.66+/-0.52</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

NC: a 0 value means that a p-value is not calculable using McNemar’s test.
3.7. Erythrocyte and Leukocyte Tests.

Tables 7a and 7b provide details of the remaining haematology tests. There were no significant differences in any test. No participants had an abnormally high haematocrit that might be associated with hyperviscosity, although 5 had low values. Eleven cases and 7 controls had marginally reduced lymphocyte counts, none having abnormally high counts.

Table 7a: Analysis of erythrocyte tests.

<table>
<thead>
<tr>
<th>Test (Units)</th>
<th>Reference values</th>
<th>Mean+/− Standard deviation</th>
<th>Outside normal range (n)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/L)</td>
<td>Male: 130 – 175, Female: 115 - 165</td>
<td>Case: 139+/−15, Control: 137+/−14</td>
<td>1, 1</td>
<td>1.0</td>
</tr>
<tr>
<td>Red Blood Cells (x10^12/L)</td>
<td>Male: 4.5 – 6.5, Female: 3.8 – 5.8</td>
<td>Case: 4.60+/−0.45, Control: 4.66+/−0.45</td>
<td>2, 0</td>
<td>NC</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>Male: 0.4 – 0.54, Female: 0.37 – 0.49</td>
<td>Case: 0.41+/−0.04, Control: 0.41+/−0.04</td>
<td>3, 2</td>
<td>1.0</td>
</tr>
<tr>
<td>Mean Cell Volume (fl)</td>
<td>80 - 97</td>
<td>Case: 89.9+/−3.2, Control: 89.0+/−6.3</td>
<td>0, 2</td>
<td>NC</td>
</tr>
<tr>
<td>Mean Cell Haemoglobin (pg)</td>
<td>27 - 32</td>
<td>Case: 30.3+/−0.8, Control: 29.6+/−0.8</td>
<td>1, 3</td>
<td>0.63</td>
</tr>
<tr>
<td>Red Cell Distribution Width (%)</td>
<td>11.6 – 14.6</td>
<td>Case: 12.8+/−0.8, Control: 13.2+/−0.8</td>
<td>0, 1</td>
<td>NC</td>
</tr>
</tbody>
</table>

NC: a 0 value means that a p-value is not calculable using McNemar’s test.
Table 7b: Analysis of leukocyte tests.

<table>
<thead>
<tr>
<th>Test (Units)</th>
<th>Reference values</th>
<th>Mean +/- Standard deviation</th>
<th>Outside normal range (n)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Blood Cells (x10^9/L)</td>
<td>4.0 – 11.0</td>
<td>Case 5.78 +/- 1.87</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 6.47 +/- 1.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils (x10^9/L)</td>
<td>2.0 – 7.5</td>
<td>Case 3.49 +/- 1.61</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 3.82 +/- 1.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (x10^9/L)</td>
<td>1.5 – 4.0</td>
<td>Case 1.67 +/- 0.61</td>
<td>11</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 2.00 +/- 0.71</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Monocytes (x10^9/L)</td>
<td>0.2 – 0.8</td>
<td>Case 0.42 +/- 0.10</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 0.44 +/- 0.12</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Eosinophils (x10^9/L)</td>
<td>0.04 – 0.4</td>
<td>Case 0.16 +/- 0.11</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 0.18 +/- 0.11</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Basophils (x10^9/L)</td>
<td>0 – 0.1</td>
<td>Case 0.030 +/- 0.021</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 0.035 +/- 0.016</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

NC: a 0 value means that a p-value is not calculable using McNemar’s test.
These results are shown in figure 2. Four cases and 4 controls were found to have abnormal values (<4.5%) showing no difference for endothelial dysfunction. A small association was found with the absolute values and ISSHL which was not significant (5.9 +/- 3.2% vs 7.2 +/- 3.2%; p=0.32). A power calculation found that 90 pairs of cases and controls would be required to detect a significant difference at p=0.05 with 80% power.

Figure 2: Scatterplot showing the matched case-control results for flow mediated dilation of the brachial artery.
4. DISCUSSION

4.1 Synopsis of Findings.
This study found no significant differences between cases and controls for any of the investigations performed. There was no difference between the larger (“significant” or “major”) v-aCS and ISSHL. There was a limited association of ISSHL with small v-aCS, endothelial dysfunction, platelet hypereactivity and high fibrinogen levels. There was also an association between the size of v-aCS and severity of hearing loss. Only 1 case had an inherited thrombophilia compared with 2 controls and no cases had positive antiphospholipid antibodies. Hyperlipidaemia was more prevalent in controls.

4.2 Limitations and Strengths of the Study.
4.2.1. Recruitment.
The main limitation to this study was the difficulty encountered with recruitment. With a reported incidence of approximately 10 / 100,000, of which about 50% were under 50 years of age, then from a population in the North West of 6 million, there should be an expected rate of about 300 cases per year. In practice, there seem to be far fewer of these patients presenting acutely and hence the use of retrospective recruitment. Despite this, during a 3 year period only 42 patients were referred for the study and only 20 of these patients agreed to participate. This led to a large time gap between the ISSHL and the study date, potentially leading to bias. Fortunately v-aCS, the primary outcome measure for this study, show only slow variation over time. A large autopsy study (128) found that the prevalence of patent foramen ovale (PFO) decreased with age but that the mean size enlarged. This suggests that with age small shunts may close but larger shunts increase in size, presumably due to atrophy of cardiac muscles. However these changes occurred gradually over many decades so even a time gap of 10 years between ISSHL and study date seems unlikely to have much effect. Abnormalities in the majority of thrombophilic tests performed (APCR, PC, fPS, AT) are caused by genetic defects resulting in life-long deficiency. Although the antiphospholipid antibodies are an acquired thrombophilia, once developed they too remain life-long, so the time gap between ISSHL and study date should not have biased any of these blood results.

Other tests performed however are subject to variation with time and so conclusions drawn must take this into account. With regard to hyperlipidaemia, none of the
participants in this study had received treatment for this condition and as this disorder is highly associated with age, it seems unlikely that it would have been clinically relevant at the time of ISSHL given the negative findings at testing. Hyperfibrinogenaemia has a mixed aetiology. It varies with age, cardiovascular risk factors and other inflammatory states (81) so it is uncertain as to whether this would have been present at the time of ISSHL. However it can be used as a marker for current cardiovascular risk.

Endothelial dysfunction is also recognised to vary acutely dependent on a number of factors. Examples include fasting, recent exercise and smoking (95, 205, 206). Despite this it has been shown to be an accurate predictor of future cardiovascular disease and is thought to be a marker for mild, as yet undiagnosed vascular disease, perhaps even representing the sum of all on-going risk factors (207). It therefore seemed valid to perform in this study, even after some time since the ISSHL event. In essence this means we cannot conclude with any certainty whether or not endothelial dysfunction played a role in the acute event of ISSHL but we can say whether these patients may have an increased cardiovascular future risk.

The tests for platelet adhesiveness (GPV and p-selectin) are likely to suffer the same issues as described above, namely acute variation with the potential to be raised chronically in disease states. P-selectin particularly has been widely studied and clearly correlates with atherosclerotic risk factors as well as predicting future events in apparently healthy people (103, 208), so it seems reasonable to consider this as another marker for the development of cardiovascular disease. GPV is less well studied. It has been shown to be higher in those with chronic cardiovascular diseases (105, 106) but it is not known whether it might predict future disease.

4.2. Technical aspects of the TCD test.

The effectiveness of TCD for detecting v-aCS has been extensively studied previously. It has been found to be highly sensitive and specific albeit with some variability between studies. Although the way in which TCD is used to insonate most usually the middle cerebral artery is consistent in most studies, there is variation in type of contrast, position of patient and which provocation manoeuvres are used. The precise methodology used in our unit has been developed in order to try and maximise the sensitivity of the test. We have previously demonstrated that the addition of blood to the
saline/air mix improves sensitivity (209). Others have found that both coughing and the Valsalva manoeuvre are effective provocation manoeuvres and can be complementary (210) which is why both were used in our protocol. It has also been shown that a precise Valsalva manoeuvre to a minimum of 40cmH₂O also improves sensitivity (211).

The best evidence for the prevalence of intracardiac shunts comes from an autopsy study of 965 hearts which found probe-patent PFO was 27% overall but decreased from 34% in the first three decades through 25% between the 4th and 8th decades of life to 20% in the 9th and 10th decade (128). TCD would be expected to have a higher prevalence as it would not only pick up all cardiac shunts including minute ones that would be missed at autopsy but also those in the pulmonary circulation. The sensitivity of TCD in the literature in control patients seems to be highly variable. The YAMIS study (131), which was performed in our laboratory, found a surprisingly high positive rate of 70% of which 37% had “significant” or “major” shunts in 204 young adult controls. The findings in the current study are less than that (50% positive with 25% significant or major) but still higher than many other studies and this may be a reflection of the highly sensitive methodology used.

The categorisation used was developed by studying the relationship to TOE diagnosis of PFO in order to try and improve the specificity. It was shown that a cut-off rate for a “significant” v-aCS of 15 microbubbles (MES) following provocation made the test 100% sensitive and specific in 39 patients (200). One other study has compared the number of MES in TCD with those in TOE and they concluded that anything over 2 MES was significant. However they used TCD simultaneously with TOE which clearly led to a less sensitive methodology as their protocol failed to identify small shunts found by TOE (212). It has also been previously shown that simultaneous TCD produces fewer bubbles than TCD performed alone, probably both because of the left lateral position of the patient and the sedation necessary for TOE (200).

4.2.3. Technical aspects of FMD test.
FMD testing is well recognised to be a complex test requiring a high degree of training (94, 206) and expert recommendations have been published to help minimize variation (95). Although our unit had no previous experience with this technique, all FMD tests were carried out using the recommended methodology by a single investigator who had
undergone training elsewhere. Validation was carried out by computer software to ensure objectivity. Acute variations in endothelial dysfunction were minimized by carrying the test out after overnight fasting and asking the participants to refrain from smoking on the morning of the test.

4.2.4. Technical aspects of the thrombophilia test.
Thrombophilia testing was carried out using well established assays. Activated protein C resistance is widely used as an effective screen for factor V Leiden, with genetic screening reserved for positive cases. Due to financial constraints, we did not carry out the more expensive genetic tests which also meant we did not screen for the Prothrombin G20210A mutation. It is possible that cases of thrombophilia could have been missed because of this.

4.3. Comparison of TCD Data with the Literature.
In our study, there was no difference in the prevalence of “significant” and “major” v-aCS in either group but there was a small difference in the “small” v-aCS groups. This raises the question of whether these small shunts could be functionally relevant given the testing methodology used. This seems unlikely as it has been shown that both cryptogenic strokes and migraine are associated with large shunts to a much greater degree than small ones (130, 131, 134). Indeed migraine symptoms are markedly improved by even partial closure of a shunt (136), again suggesting that it is only the larger shunts that are functionally relevant.

The only other study to consider the association of v-aCS to sudden deafness found a significant association (138). They found v-aCS in 48% of 23 patients compared with 17% of 46 age and sex matched controls using a positive diagnosis on TCD of only 1 microbubble. This raises the question of whether the shunts they found were functionally relevant. The paper did not disclose full details of their TCD methodology but it can be noted that it is the same group that compared TCD and TOE described in section 4.2.2. (212) so one would expect their methodology to be less sensitive than our own. This may account for their low control rate. They also did not quantify the number of MES found although the first author relayed in a personal communication that “most had more than 30”, suggesting that they were functionally relevant v-aCS. Their findings are therefore in direct contrast to our own. There are several possible reasons
why this might be so. The small sample sizes used in both studies mean a statistical error may have occurred in either one. The different populations examined mean that paradoxical embolism may be a greater cause in the Japanese population over the British one. This seems unlikely as there seems to be little difference in the prevalence of PFO. Although Iguchi et al found a small trend towards younger patients having PFO, their population was still considerably older than our own and therefore they would potentially be more prone to cardiovascular complications. It may be that paradoxical embolism is a more prevalent cause of ISSHL in older populations.

A further point regarding our TCD data is that one of our patients developed ISSHL upon waking from a tonsillectomy operation. This patient also had a “significant” v-aCS. ISSHL occurring following non-otologic surgery is well recognised (147, 148). It is possible that paradoxical embolism may be the mechanism by which this occurs, secondary to microemboli developing as a result of stasis and the pro-inflammatory state of surgery.

4.4. Comparison of Blood Test Analysis with the Literature.

There was little evidence to suggest a role of thrombophilia within our patients. There was no evidence for Factor V Leiden, by far the commonest genetic thrombophilia, in any participant. This is consistent with the literature which, apart from isolated reports, does not support this as a common cause. The finding of one patient with a protein S deficiency is interesting in that the prevalence of this disorder is only 1 in 700. It is equally surprising that one control had this deficiency and a further control had a protein C deficiency which has a similar prevalence. Previous studies have demonstrated 1 patient with protein S deficiency out of 195 tested and, like us, no patients with protein C or antithrombin disorders (55, 76). The combination of protein S deficiency with a “small” v-aCS raises the possibility of paradoxical embolism in this patient. Equally, the combination of hyperfibrinogenemia and v-aCS could result in paradoxical embolism, as found in another patient. However, until such time as a definite diagnosis of vascular pathology can be made on investigations, this must remain conjecture.

Examination of the cardiovascular risk factors in our participants revealed little difference between the groups. The number of smokers was distributed evenly. Two cases had hypertension and one potentially had undiagnosed diabetes in comparison to
no controls. The mild increase in these risk factors in the ISSHL population is consistent with the literature but, given the small proportions involved, it seems unlikely to have a significant effect. Hyperlipidaemia was more prevalent in our control group, contrary to many other studies. Arguably the most important lipid measurement, LDL, demonstrated only 2 cases with mildly raised levels. This compared with 6 controls, 2 of whom had high levels of greater than 4mmol/L. The main relevance of this is that hyperlipidaemia is known to affect many of the other cardiovascular risk measurements, including endothelial dysfunction, fibrinogen and p-selectin. The fact that these other factors were still higher in the case group may mean that we have underestimated their significance. Unfortunately there were not enough participants to carry out a multiple regression analysis that might have tested this hypothesis.

Ridker et al demonstrated that P-selectin correlated with future cardiovascular events in healthy people (208). Their population was not restricted to young adults and so had a mean age of 55 years, in contrast to ours. Interestingly they found that those patients with plasma concentrations over 81ng/ml were twice as likely to suffer an event over the next 3 years, even after adjusting for other risk factors. Fifty percent of our cases had plasma concentrations above this level. They also found that their cases had higher levels of other risk factors whereas in our study, the opposite was true, as discussed above with regard to hyperlipidaemia. However it must be accepted that, while the marginally increased values might reflect a chronic state of platelet hypereactivity, it equally may have no clinical relevance given the limited difference between the cases and controls.

4.5. Comparison of Endothelial Dysfunction Testing with the Literature.

The literature on FMD is clear about how much this technique depends upon the operator and the international expert recommendations include carrying out regular validation to ensure quality is maintained (95). Since publication of this report, sophisticated computer software can now carry out the analysis, removing some of the variance, but the technique in acquiring the images is still challenging. It is unsurprising that different research groups have found varied levels of association with cardiovascular disease ranging from <4.5 to <8.1% (96, 97). We chose to use the lower end of this spectrum for analysis and found no difference between the two groups. We could equally have chosen the higher end and would have found that 10/12 cases were
abnormal compared with 7/12 controls. Although still not significant given the small sample size, a trend starts to become apparent. Given the varied results between research groups, it should probably be argued that the direct correlation between cases and controls is more important than trying to compare results with other centres. Again, while significance is not achieved, a trend is apparent in our study, suggesting these patients may be at increased risk of future cardiovascular events. The only other study to carry out FMD found 5 out of 6 ISSHL patients had evidence of endothelial dysfunction (98). While this study clearly suffers from a lack of control data for the reasons described above, the inference is the same as ours. This is certainly consistent with other studies that have all shown either an increased rate of previous cardiovascular disease (55, 56) or of future events (69).

4.6 Comparison of Aims with Extent of Achievement.
This study was designed as a pilot study to determine whether paradoxical embolism might be a common cause of unilateral ISSHL in young adults, and therefore to inform a larger study of what numbers would be required to answer this hypothesis. This was assessed by looking at the prevalence of what are considered to be functionally relevant “significant” or “major” v-aCS, in that it is these shunts that correlate with PFO. The fact that there was no difference suggests that paradoxical embolism is not a common cause and a larger study is unnecessary. This is also supported by the lack of evidence for thrombophilia. The findings that there was a slightly increased rate of “small” v-aCS and also that there was a slight association with severity of hearing loss with size of v-aCS are most likely to be statistical quirks due to the number of factors analysed. As an example, why would a “small” v-aCS be likely to be associated with ISSHL biologically when larger ones are not? This sounds implausible.

The findings that endothelial dysfunction, high fibrinogen levels and high p-selectin levels had a small association with cases are interesting and suggest there may be a role for studying these factors further in young adults. This would be better performed in the immediate aftermath of the ISSHL episode to determine whether they play a role in the acute event, as well as potentially predicting overall cardiovascular risk.
4.7. Conclusions.
Paradoxical embolism and venous thrombosis are unlikely to be important causes of unilateral ISSHL in young adults. Mildly raised cardiovascular arterial risk factors such as endothelial dysfunction, hyperfibrinogenaemia and p-selectin suggest a possible vascular dysfunction in these patients but they may all be non-specific measures of an inflammatory process. The causation of ISSHL remains a mystery as no single factor has yet been identified. It seems most likely that there are several different causes that may all be active.
5. REFERENCES

126. Mercier E, Quere I, Chabert R, Lallemant JG, Daures JP, Berlan J, et al. The 20210A allele of the prothrombin gene is an independent risk factor for perception


