THE UNIVERSITY
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Imaging Intracranial Arterial Patency and Intravenous Thrombolysis in Acute Ischaemic Stroke

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Summary

Among patients presenting acutely with ischaemic stroke who are being considered for intravenous thrombolysis, prompt brain imaging is used to exclude contra-indications to treatment (chiefly haemorrhagic stroke or other conditions mimicking stroke) rather than to identify which patients are more or less likely to benefit from thrombolysis. For example, it is unclear whether the presence or absence of arterial obstruction on imaging should be used to guide thrombolysis treatment decisions.

In this thesis I explore methods of imaging arterial patency among patients presenting acutely with ischaemic stroke and look for associations between these early imaging findings, response to intravenous thrombolysis and functional outcome six-months after stroke onset. I primarily use data from the Third International Stroke Trial (IST-3), the largest ever randomised-controlled trial testing the use of intravenous alteplase for the acute treatment of ischaemic stroke.

I begin by summarising the main features of stroke, covering techniques for imaging the brain and for imaging arterial patency, and post-stroke outcomes. Next I describe two literature reviews which I compiled to increase my understanding of the topic with particular reference to imaging arterial patency. This is followed by a summary of IST-3. Then I describe the general methods I used to address my thesis aims exploring relationships between imaging characteristics of arterial patency, treatment with intravenous alteplase and functional outcome after ischaemic stroke. Specifically, I investigated the following imaging features:

- The hyperattenuating artery sign (HAS), which is a non-contrast enhanced CT finding thought to be indicative of acute arterial obstruction by thrombus or embolus
- Arterial patency or obstruction as demonstrated using contrast enhanced CT and MR angiographic imaging.

In addition to providing better characterisation of the HAS and a better understanding of how angiography helps to assess ischaemic stroke patients, I found that arterial obstruction (however this is identified on imaging) is associated with

more severe stroke at baseline and worse functional outcome six months after stroke. I also prove that intravenous alteplase is effective in the presence of arterial obstruction, counter to a widely held concern that it may not be effective in this context. Most of my work has been published in peer reviewed journals.

My work should give front line clinicians greater confidence to use intravenous alteplase for the treatment of ischaemic stroke associated with arterial obstruction on imaging, but more work is needed to better understand the implications of apparently normal arterial patency on imaging among patients with ischaemic stroke.

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Preface and Declaration

I submit this thesis as evidence of the work I have undertaken in completion of my MD research project at the *University of Edinburgh*. I have been a registered as a part time MD student with the *University of Edinburgh* since September 2012.

During that time I also completed my higher clinical training in radiology, was awarded my Certificate of Completion of Training (CCT) by the General Medical Council and entered onto the Specialist Register for Radiology, completed a one year diagnostic neuroradiology fellowship in Perth, Australia and returned to Edinburgh to take up my current role as a senior clinical fellow and honorary consultant in neuroradiology.

I make the following declaration:

- This thesis was composed entirely by me although I received editing support from my research supervisors
- Most of the work described herein, was undertaken as part of the Third International Stroke Trial Collaborative Group. However, I was the major contributor for all collaborative work contained within
- This work has not been submitted for any other degree or professional qualification
- All publications included in the appendices are my own work

Signed _____ Date __12th September 2017__

Dr Grant Mair.

Acknowledgements

Very little of the work in this thesis would be possible without the Third International Stroke Trial (IST-3).

Foremost, I wish to thank all patients who enrolled in the trial, their families and their carers.

I also wish to thank the many many contributors and collaborators (IST-3 Collaborative Group, see Appendix 1, Chapter 11.1), generous supporters and funders (see Appendix 2, Chapter 11.2) that made IST-3 both possible and ultimately, successful.

I am deeply grateful to my MD project supervisors Prof Joanna M Wardlaw and Prof Peter AG Sandercock. From granting me privileged access to the unrivalled IST-3 dataset, to providing guidance and initial ideas for its analysis and for continually supporting me as I gradually worked through, developed and expanded those ideas as my own.

I need to thank my 13 radiology colleagues who used some of their own time to assess CT and CT angiography enabling me to complete the reliability analyses (see Chapter 8).

Finally I would like to thank my family for supporting me on this substantial endeavour; thank you Suzanne for your constant patience and support, thank you Mila and Sadie for coping with my Sundays in the ‘work study’.

Thesis Aims

The main aims and objectives of the work contained within this thesis are to:

1) Quantify the prevalence and observer reliability of the Hyperattenuating Artery Sign (HAS) on CT performed for the initial imaging assessment of patients with suspected ischaemic stroke
2) Determine how intravenous alteplase affects HAS and whether the presence or absence of HAS influences the response to intravenous alteplase and outcome and whether the characteristics of HAS such as attenuation, location and extent are important
3) Calculate the sensitivity and specificity of HAS for arterial obstruction
4) Quantify observer reliability for the assessment of CT Angiography (CTA) performed routinely as part of the initial CT imaging assessment of patients presenting with ischaemic stroke
5) Determine if the addition of CTA to standard plain CT improves ischaemic stroke detection, and prognosis prediction, by CT when performed as part of the initial imaging assessment and determine how much extra time is needed to perform CTA in this context
6) Determine whether the presence or absence of arterial obstruction on angiography performed acutely after ischaemic stroke influences the response to intravenous alteplase and outcome and whether characteristics of obstruction such as location, completeness and extent are important

Chapter 1 Introduction and Background

1.1 Aetiology of Stroke

Stroke is a medical emergency which is caused by disruption of blood supply to the brain and leads to a focal neurological deficit. There are two major causes of blood supply disruption. The majority of strokes are ischaemic (approximately 80%) and most of these relate to interruption of arterial blood flow to the brain; ischaemic stroke secondary to venous insufficiency is much less common. Haemorrhagic stroke accounts for the remaining 20% and denotes bleeding into the brain (to be differentiated from bleeding around the brain, i.e. subarachnoid haemorrhage).

The aetiologies of ischaemic and haemorrhagic stroke are usually quite different, but ischaemic strokes are often complicated by secondary haemorrhagic transformation. In general, large artery ischaemic stroke occurs in the presence of an intra-arterial thrombus or embolus. Occlusive atherosclerotic vascular disease represents the major underlying pathology. This can be further divided into cardioembolic (for example, cardiac emboli arising secondary to atrial fibrillation or valvular heart disease) and atherothromboembolic causes (thrombus or embolus developing from atheromatous plaques of the extra and/or intracranial arteries). Hypertension, hypercholesterolaemia, diabetes and smoking act as major risk factors for the development of atherosclerotic disease. Haemorrhagic stroke can occur secondary to an underlying vascular abnormality such as aneurysm, amyloid angiopathy or venous sinus thrombosis or in the presence of medicinal anticoagulation (e.g. warfarin); hypertension is also a major risk factor for haemorrhagic stroke. Less commonly, both ischaemic and haemorrhagic stroke can occur secondary to a variety of neurological diseases including vasculitis, CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy), Moyamoya syndrome, Sneddon's syndrome, MELAS (Mitochondrial Encephalomyopathy Lactic Acidosis and Stroke-like episodes).

1.2 Stroke Prevalence

Stroke is a major health concern worldwide but it is a particular problem in less developed countries where there are increased risk factors (including vascular risk factors and exposure to pollution[1;2]). But even in developed nations stroke remains a significant concern among our increasingly aged populations. Recent figures from the World Health Organization show that in the UK, cerebrovascular disease (predominantly stroke but includes subarachnoid haemorrhage from aneurysmal rupture) causes approximately 10.6% of all deaths and has a death rate of 45.6 per 100,000 of population. Table 1-1 compares world rankings for death rate (lower death rates at the top) from cerebrovascular disease for selected countries, the UK is placed 34th; the worldwide median death rate is 101.8 per 100,000 of population.[3]

Table 1-1 *Comparison of global death rates from cerebrovascular disease (predominantly stroke) for selected countries*

Rank	Country	Death Rate per 100,000 Population
1	Seychelles	25.0
2	Canada	25.7
3	Switzerland	26.5
4	Israel	26.6
5	Monaco	27.8
6	France	27.9
7	United States	30.4
8	Bahrain	30.8
9	Austria	31.3
10	Australia	32.6
18	Ireland	36.0
22	Germany	37.5
26	Italy	41.4

28	Japan	42.2
34	United Kingdom	45.6
101	India	108.0
168	China	156.5
190	Russian Federation	228.0

1.3 Clinical Presentation of Stroke

Disruption of intracranial blood supply in stroke reduces delivery of oxygen and other essential nutrients, particularly glucose, to brain cells. Deprived of oxygen and glucose, neural tissue ceases to function almost immediately leading to loss of normal body functionality. Due to the standardised routes of blood supply to the human brain and the commonality of certain aetiologies (especially acute middle cerebral artery occlusion), the loss of neural function in stroke often results in facial and/or limb paresis and sensory loss, deficits in cognition and problems understanding and producing speech, but clinical presentation is variable (see Table 1-2). Cell death follows shortly thereafter, potentially within minutes of the disruption to blood supply; cell death can be postponed or avoided if there are alternative routes for blood delivery - i.e. as collateral supply - but such vascular redundancy may only offer a temporary reprieve. Treatments for stroke therefore, aim to restore the primary blood supply before neuronal cells die and deficits in body functionality become permanent.

Table 1-2 *Clinical presentation of stroke*

Ischaemic Stroke	
Middle Cerebral Artery (MCA) occlusion	Total occlusion: • Hemiparesis (arm > leg) • Hemisensory loss • Aphasia (if dominant hemisphere) • Conjugate eye deviation • Hemianopia Partial (branch) occlusion: • Upper branch occlusion – hemiparesis, hemisensory loss, expressive dysphasia • Lower branch occlusion – receptive dysphasia • Small cortical branch occlusion – isolated cortical signs • Striatocapsular – motor and sensory loss
Anterior Cerebral Artery (ACA) occlusion	• Hemiparesis (leg > arm) • Executive dysfunction • Disinhibition • Lack of insight
Posterior Cerebral Artery (PCA) occlusion	• Hemianopia (cortical blindness if bilateral) • Memory impairment
Large vessel cortical infarct	Following occlusion of large cerebral artery branches (i.e. MCA, ACA, PCA), the following cortical symptoms & signs are common: • Confusion/ cognitive impairment • Dysphasia • Neglect • Apraxia
Vertebro-basilar artery occlusion	• Lateral medullary syndrome • Bulbar/ pseudobulbar palsy • Ophthalmoplegia • Quadriplegia • Coma
Lacunar infarct	Absence of cortical signs with unilateral weakness or sensory loss of face and/or limbs, the following are common: • Pure motor hemiparesis • Sensorimotor hemiparesis • Pure sensory stroke • Ataxic hemiparesis
Borderzone ischaemia	Following a global fall in perfusion pressure (e.g. during

	cardiac arrest), the regions between major arterial territories can be ischaemic. Symptoms & signs include: • Behavioural abnormality • Memory impairment/ amnesia • Complex visual abnormalities
Transient Ischaemic Attack (TIA)	Can present like and precede any of the other ischaemic stroke syndromes listed above but common TIA symptoms include: • Monocular visual loss • Hemiparesis • Dysphasia • Dysarthria • Facial or tongue numbness • Diplopia
Haemorrhagic Stroke	
Cerebral haemorrhage	Often described as deep (basal ganglia and thalamus) or lobar. Deep: • Hemiparesis • Hemisensory loss • Conjugate eye deviation Lobar: • Hemiparesis • Hemisensory loss • Dysphasia • Neglect
Posterior fossa haemorrhage	Brainstem: • Ophthalmoplegia • Quadriplegia • Coma Cerebellum: • Headache • Vomiting • Unilateral ataxia

Adapted from Clinical Neurology – 4th Ed. JW Scadding & NA Losseff. Hodder Arnold 2012

1.4 Brain Imaging

Need for early imaging after stroke onset

To allow for the prompt delivery of appropriate treatment, it is important to make a rapid and accurate diagnosis of stroke type as soon as possible following symptom onset - imaging is necessary to differentiate ischaemia from haemorrhage. Although it is perhaps more accurate to state that CT imaging in early stroke is primarily used to exclude haemorrhage and non-vascular causes of stroke (i.e. structural stroke mimics such as encephalitis, acute demyelination, meningitis, abscess, subdural haematoma or other space occupying lesion) rather than confirm the presence of infarction. Haemorrhage (or a structural lesion) is a contra-indication to intravenous thrombolysis but the absence of a visible infarct is not.

CT versus MRI

CT scanning without contrast enhancement (hereafter referred to as non-contrast CT) has become the primary imaging modality (rather than MRI) in the initial assessment of acute stroke for several reasons:

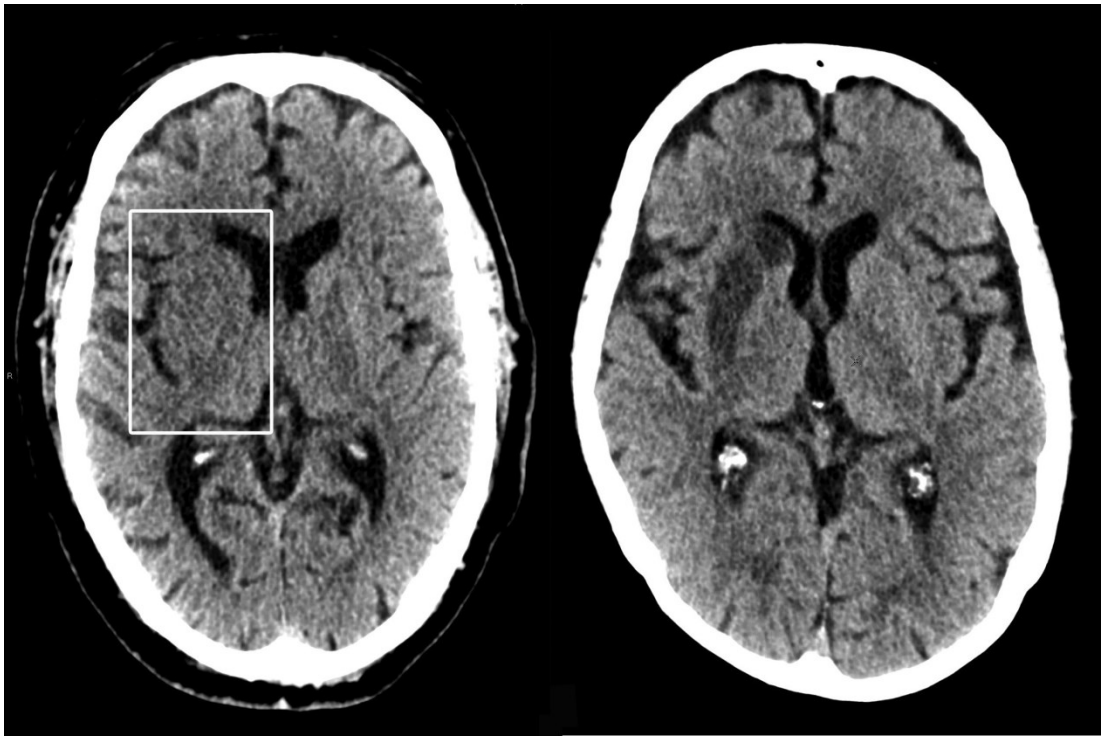
1. CT is widely available whereas MRI is not, especially out-of-hours[4]
2. On modern equipment, a non-contrast brain CT can be performed in seconds while even a few basic MRI sequences can take several minutes
3. Due to the inherent differences in machine structure and function, CT makes it easier to manage an unstable patient during scanning and CT is better tolerated by those who suffer claustrophobia
4. A proportion of older patients have cardiac pacemakers or other contra-indications to MR imaging
5. As a means to answer the basic imaging question, of whether a stroke is ischaemic, haemorrhagic or due to a non-vascular cause, CT is very accurate for identifying haemorrhage and non-vascular causes.[5;6]

Other benefits of CT

In addition to differentiating ischaemic from haemorrhagic stroke, non-contrast CT also provides some information on the presence of arterial obstruction (intra-arterial hyperattenuation, see 1.6.1) and on the extent of ischaemia (loss of grey-white matter

differentiation, hypoattenuation of brain tissue, evidence of swelling), Figure 1-1, especially when medium to large areas are affected; small volume ischaemia in the early stages of stroke can however be more difficult to detect particularly for those with less experience of doing so and especially if insufficient time is allowed for assessment.[7;8] Finally, non-contrast CT provides valuable information on the remainder of the imaged brain. For example, the identification of background leukoaraiosis has been shown in meta-analysis of case series and observational studies (there is limited randomised-controlled trial data), to increase the risk of haemorrhage following treatment with thrombolysis[9] while an unexpected subacute infarct, i.e. a silent infarct that occurred several days prior to the presenting clinical deterioration,[10] would represent a contra-indication to the use of intravenous thrombolysis.

Figure 1-1 *Brain tissue changes of early ischaemic stroke identifiable on non-contrast CT*



Footnote: Left image was obtained within a few hours of stroke symptom onset (hyperacute). Note that the right basal ganglia and insular cortex are difficult to identify (boxed area).

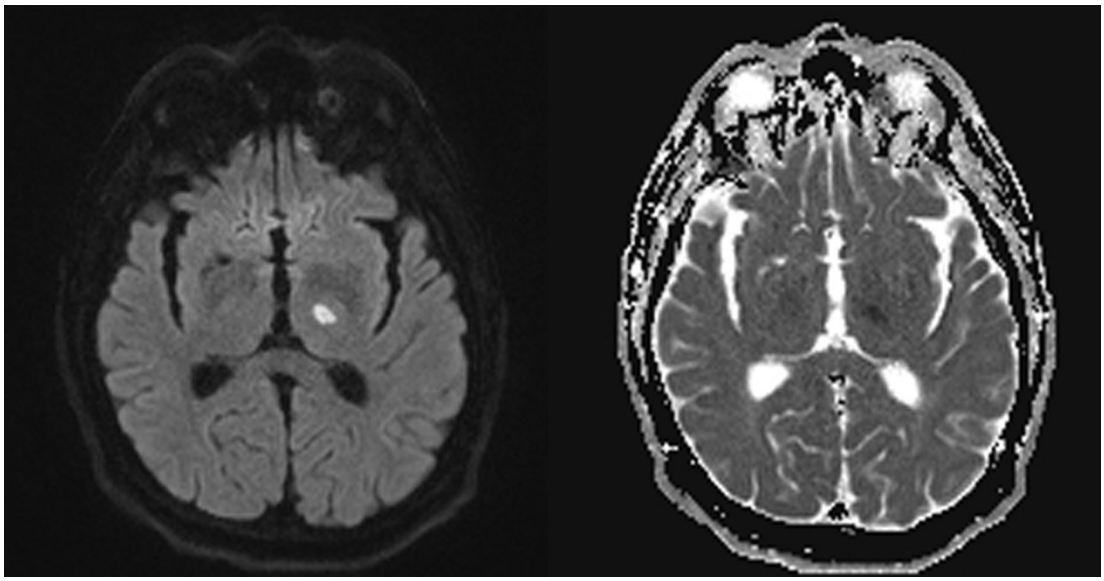
Right image was obtained at 24 hours after stroke symptom onset (subacute) in a different patient with a similar distribution of ischaemic lesion.

Benefits of MRI

MRI is used for assessing acute stroke in many centres. Ideally, MRI in stroke includes at least four sequences, namely T2 weighted, diffusion-weighted imaging (DWI), T2* (or an alternative haem sensitive sequence) and FLAIR. In this context, MRI is usually most appropriate in patients with mild stroke since these minimum four sequences will require approximately 15 minutes of in-scanner time for the patient using standard modern equipment, and this will increase if other sequences, e.g. T1 weighted, time-of-flight angiography, are applied. In some series, patients with moderate or severe stroke were unable to complete imaging in up to 45% of examinations.[11] Depending on availability, MRI may be used instead of non-contrast CT but only if blood-sensitive sequences like T2*, SWI, SWAN are used – MRI without these is not able to differentiate acute haemorrhage reliably; both CT and MRI are excellent at differentiating acute ischaemia from haemorrhage (and for excluding other structural abnormalities that may present as stroke) in the first five days after stroke if suitable MRI sequences are used. Allowing for the aforementioned limitations of MRI, it also has certain advantages over non-contrast CT. The main advantage of MRI in acute stroke relates specifically to the use of diffusion-weighted imaging which can provide very clear evidence of the extent of parenchymal ischaemia and/or infarction within minutes of stroke onset and can do so with greater sensitively than non-contrast CT.[12-14] Early parenchymal changes are appreciated as increased restriction in the normal diffusivity of intracellular water (i.e. restricted diffusion – high signal on DWI sequences and correspondingly low on ADC maps), Figure 1-2. It should be noted that DWI provides most benefit in patients with small volume infarct (like the example provided) since these infarcts are more likely to be overlooked using non-contrast CT; large volume infarcts are usually readily apparent on both modalities. Unfortunately, is has been demonstrated in an observational study of over 200 patients that, for various reasons,[15] DWI changes may not be apparent in up to 33% of those with minor stroke.[16] Changes on the other standard MRI sequences (i.e. increased T2 signal within affected tissues, best seen on FLAIR) occur later with the development of parenchymal oedema and are therefore seen in a similar time course (and with no greater sensitivity) than the changes described above for non-contrast CT. This time difference between DWI

and other MRI sequences has been used to estimate the time of ischaemic stroke onset by assessing for a DWI-FLAIR mismatch. It has been shown that while DWI changes can be identified within minutes of stroke onset, in a large proportion of patients, FLAIR changes may not be apparent until several hours from onset. Patients with large areas of mismatch are therefore deemed to have had a recent onset time of stroke and could potentially be eligible for intravenous thrombolysis even if an absolute stroke onset time is not available.[17;18] This concept is currently being tested in the WAKE-UP randomised-controlled trial.[19]

Figure 1-2 *Diffusion weighted imaging (DWI) showing early infarct on MRI*



Footnote: DWI (left) and corresponding ADC map (right) confirming small focus of abnormal diffusion restriction within the left thalamus.

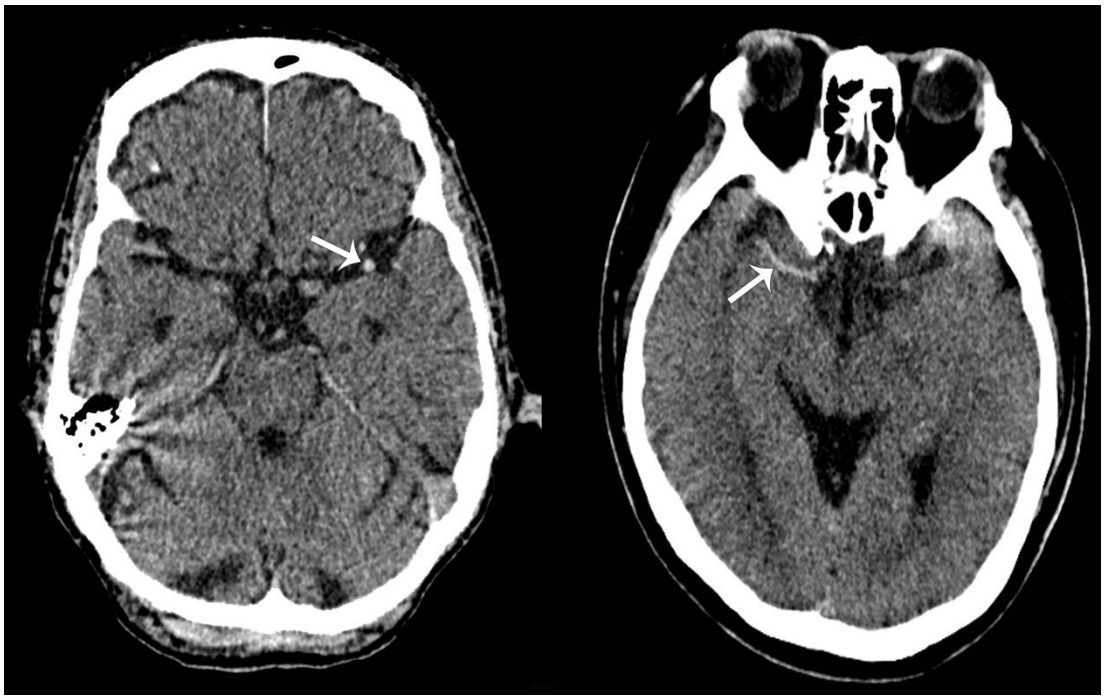
1.5 Imaging Intracranial Arterial Patency

1.5.1 Hyperattenuating artery sign (HAS) on non-contrast CT

In 1983, an article by Gács and colleagues documented several cases of vascular hyperattenuation identified on non-contrast CT performed acutely after stroke. The authors correctly postulated that this appearance represented acute thrombus or embolus within intracranial arteries.[20] The hyperattenuating artery sign (HAS) provides indirect evidence of arterial obstruction.

As discussed above, non-contrast CT is usually performed as first line imaging immediately after stroke onset predominantly to exclude haemorrhage or a structural stroke mimic. While evidence of parenchymal ischaemia can be difficult to identify in these very early stages on CT, HAS are usually recognised more readily, see Figure 1-3.[7;8]

Figure 1-3 *HAS visible on two different non-contrast CT scans (arrows)*



1.5.2 Susceptibility sign and flow voids on MRI

Indirect evidence of arterial obstruction can also be observed in standard MRI sequences of the brain.

MRI works by transmitting radio frequency energy into magnetised tissues then detecting returned radiofrequency signals derived as the tissue returns to its pre-excitation state; the frequency of returned energy varies by tissue type and position within the scanner allowing for an accurate image representation of the body to be created. Inherent in this model is the requirement that tissue remains in the same location during excitation and relaxation. Flowing blood therefore does not return sufficient signal on standard MRI sequences (compare with time of flight

angiography, see 1.6.3) since many of the excited molecules in blood have left the image plane before relaxation begins. Thus 'flow voids' are demonstrated within normal arteries, and to a lesser extent within veins (slower flowing venous blood may well remain within the image plane for long enough). Comparatively if there is an arterial obstruction, this static tissue will return an MRI signal and the normal flow void will be absent.

In addition, if acute arterial obstruction is caused by blood clot (thrombus) rather than embolus (which may not consist of blood products) some unique MRI features of clotting blood can be appreciated. First, at certain stages in the development and subsequent dissolution of blood clot, it becomes paramagnetic and causes an artefact on the MR images by disrupting the homogeneity of the local magnetic field. Second, blood clot returns a high signal on T1 imaging during certain stages in its development (see Figure 1-4), and very few other things can return a high signal on standard T1 weighted spin echo acquisitions (e.g. fat, proteinaceous material, gadolinium). In the correct clinical context then, if either susceptibility artefact or T1 hyperintensity are identified within a relevant artery these could be convincing indicators of recent thrombus.

Figure 1-4 *Sequence of changing imaging appearances for aging blood on MRI*

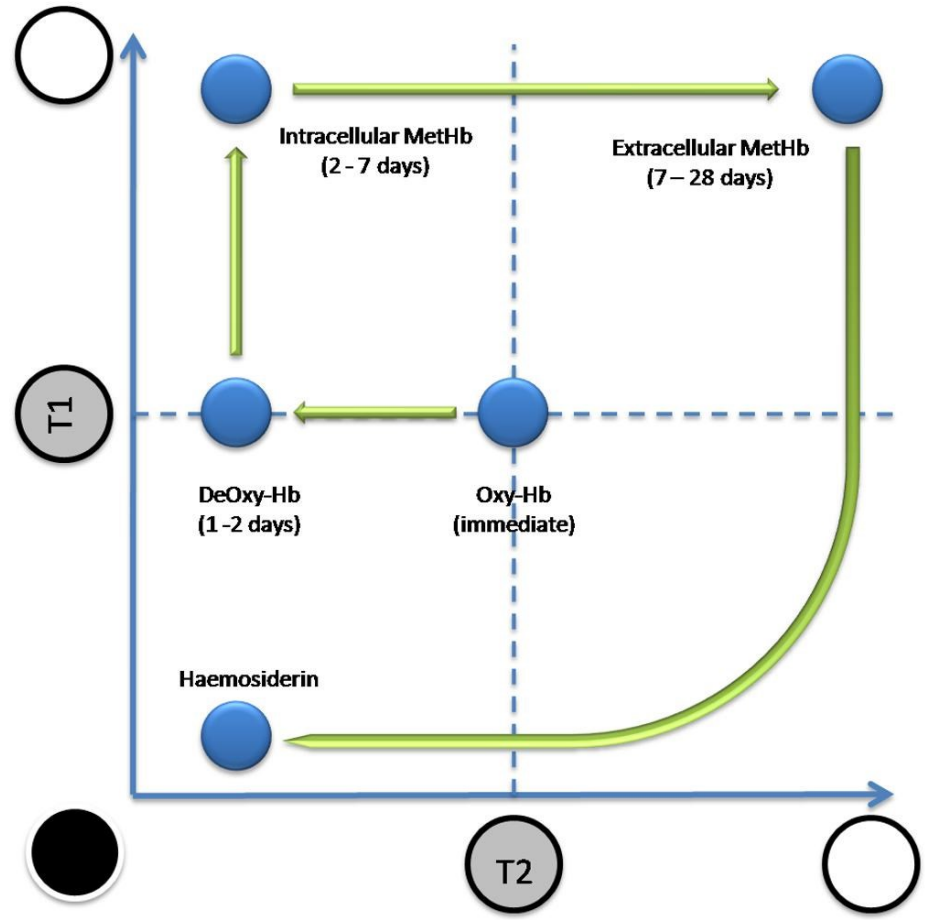


Image used with permission from Radiopaedia.org[21]

1.5.3 Angiography

Catheter angiography

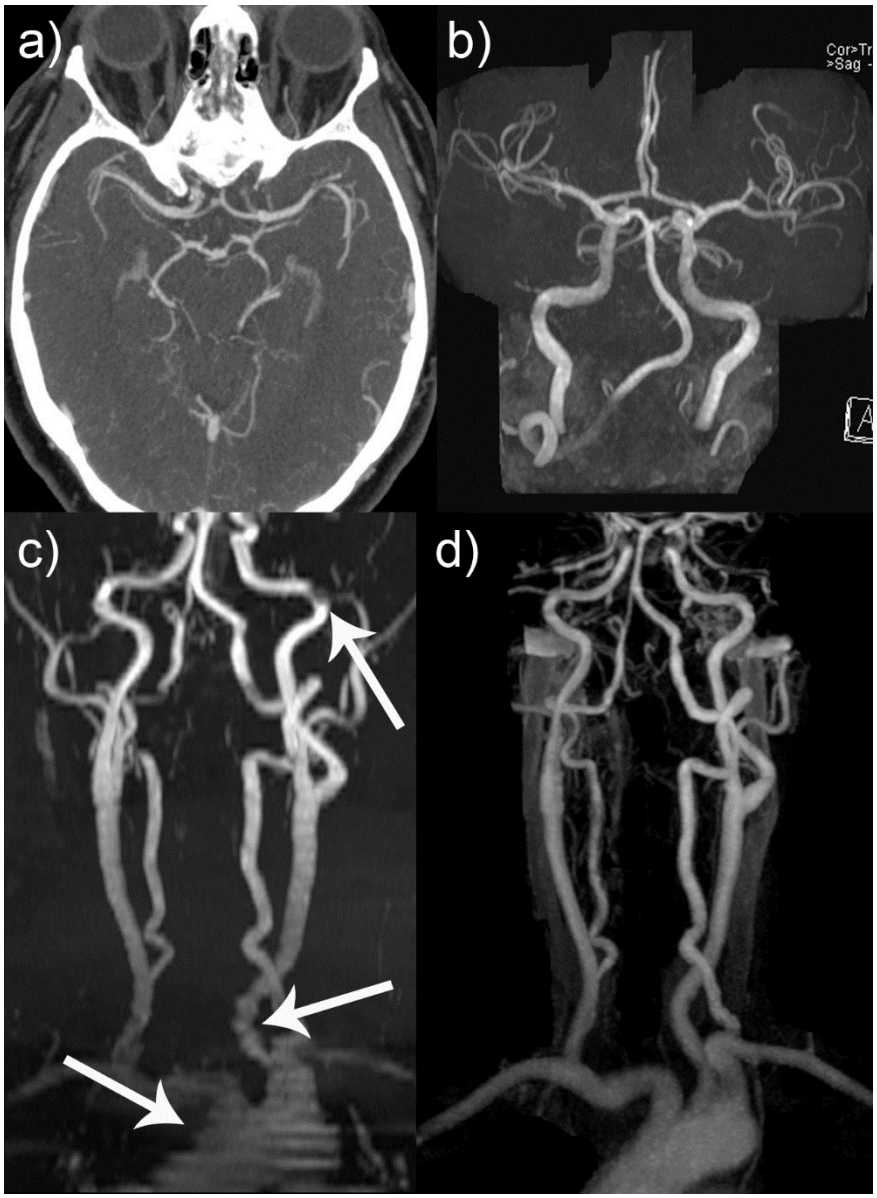
By placing a catheter into proximal intracranial arteries and directly infusing a radio-opaque contrast medium, high resolution, three-dimensional radiographic vascular imaging can be obtained. This remains the gold-standard for assessing arterial (and venous) flow in real time. It provides excellent information on vascular anatomy, vessel patency and extent of collateral supply (including the length of time that it takes and the direction travelled by contrast if it bypasses an occlusion). Catheter angiography is however an invasive procedure that comes with its own risks.

Neurologic complications of any type occur in up to 3% of stroke cases while permanent disability or death is reported in less than 1%.[22-24]

CT and MR angiography (CTA & MRA)

CT and MRI offer a means of obtaining minimally invasive and non-invasive angiography. Minimally invasive indicates that the patient will require only an intravenous injection of a contrast agent (CT or MRI), usually via an arm vein. Non-invasive implies that no contrast is given to the patient; angiographic images are obtained by harnessing features of moving blood (MRI only). Angiography performed with contrast generally provides more robust imaging than angiography without contrast since the former is less likely to be affected by imaging artefacts. In an acute setting however, it can be difficult to obtain the baseline renal status or allergy profile of a stroke patient. In addition, many stroke patients will be dehydrated at presentation which can further exacerbate the problem. In these scenarios, intravenous contrast may be contraindicated. Non-contrast time-of-flight MR imaging (saturates or removes signal from stationary tissue to isolate the small signal returned from flowing blood entering the imaging plane[25]) provides an alternative option, but as alluded to, this approach is potentially limited by imaging artefact. Figure 1-5 displays contrast enhanced CT angiography and compares MR time of flight angiography with contrast enhanced MR angiography.

Figure 1-5 *Comparison of CT and MR angiography techniques performed in the same patient*



Footnote: Upper panel compares contrast enhanced CT (a) and contrast enhanced MR (b) angiography of the circle of Willis.

Lower panel compares time-of-flight (c) and contrast enhanced (d) MR angiography of the major arteries in the neck. Note multiple imaging artefacts within the time-of-flight sequence (arrows) which are not visible on the contrast enhanced images.

1.6 Treatment of Stroke

Development of stroke treatments

Advances in care over the last 20 years have greatly improved the outcome for stroke patients with less mortality and a greater number of patients ultimately achieving independence. Amongst several possible changes, three factors are largely responsible for this improvement. First was the emergence of dedicated stroke units which offer both acute and rehabilitative care for the various and variable needs of stroke patients;[26] prior to this, stroke patients were cared for on general medical wards. Second was the introduction of intravenous thrombolysis which represented the first effective treatment for acute ischaemic stroke[27] (aspirin has a very modest effect).[28] Third is a better understanding among the general public at large, thanks in part to the FAST (Face, Arm, Speech, Time) mass media campaign[29] that stroke represents a potentially treatable medical emergency[30]; a 'brain attack!' Despite these advances however, many stroke survivors remain disabled; in general around one third of stroke patients die, one third remain disabled long term and one third regain or retain their independence. Stroke related disability has enormous implications and costs for both the patient and for society as a whole. It is estimated that stroke costs the UK economy around £9 billion per year. Around 49% of this is billed directly to the NHS (health and social care), 27% is for the cost of informal care, loss of productivity is estimated to account for 15%, while benefits account for the remaining 9%.[31]

Intravenous thrombolysis

Intravenous thrombolysis is now established as an effective treatment for acute ischaemic stroke and acts by disrupting and dissolving occlusive thrombus or embolus and thereby restoring arterial blood flow to the brain.[32-44] A recently updated systematic review and meta-analysis incorporated data from the 12 major randomised-controlled trials performed to date that tested intravenous alteplase (recombinant tissue plasminogen activator, rt-PA). In 7012 patients randomised over a 20 year period, this meta-analysis showed that those treated with alteplase within six hours of stroke were significantly more likely to be alive and independent at follow up (46.3% versus 42.1%, respectively, p=0.001). This is despite evidence that

there is an increase in early death rate secondary to haemorrhage for those treated with alteplase (8.9% versus 6.4%, respectively, p=0.0003). Furthermore, the power of this thrombolytic effect declines with time and greatest benefit is achieved if treatment begins within the first 3 hours from ischaemic stroke onset (among treated patients, 40.7% alive and independent <3 hours versus 31.7% >3 hours, p<0.0001).[45] The steep time dependency of thrombolytic effect on good outcome was similarly identified in a subsequent patient level data meta-analysis including nine of the major trials (n=6756) which showed greater proportional benefits for those treated earlier.[46] Importantly, approximately 10 years of observational data from the SITS register (Safe Implementation of Thrombolysis in Stroke) which monitors alteplase use in routine clinical practice across Europe has shown, in nearly 30,000 patients that intravenous alteplase can be used safely in this context. SITS data show comparable rates of haemorrhage, death and good outcome among stroke patients treated with intravenous alteplase within the first 3 hours (n=25,279) and those treated between 3 to 4.5 hours (n=4056); even those treated 'off-licence', i.e. between 4.5 and 6 hours from stroke onset (n=283) did not show a significant increase in the rates of poor outcome, though the authors acknowledge the limitations of this latter comparison.[47-50] Presently, there is no evidence to support the use of intravenous thrombolysis therapy beyond 6 hours of ischaemic stroke onset and the license in most countries only extends to 4.5 hours after stroke (3 hours in the USA). Trials are ongoing to find out if the time window can be extended and in which patients.

Mechanical thrombectomy

Endovascular retrieval of intra-arterial clot (mechanical thrombectomy or embolectomy) offers an effective means to restore blood flow to the brain after ischaemic stroke secondary to proximal, large vessel arterial occlusion. A recent series of clinical trials compared mechanical thrombectomy plus intravenous thrombolysis to intravenous thrombolysis alone for the early treatment of ischaemic stroke. At the time of writing, eleven randomised-controlled trials have been completed and six of the eight trials published since 2015 have each independently demonstrated (on primary end point analysis) that endovascular thrombectomy in addition to intravenous thrombolysis improves functional outcome compared with intravenous thrombolysis alone.[51-61] In meta-analysis of ten of these trials including

2925 patients, those treated with endovascular thrombectomy had a significantly higher chance of achieving a good outcome (modified Rankin score ≤2, see 1.7.2) compared to patients treated with intravenous thrombolysis alone: risk ratio 1.37 (95% confidence interval 1.14-1.64). This means that for every 1000 patients treated with mechanical thrombectomy rather than intravenous thrombolysis alone, 123 extra patients achieved a good outcome.[62]

Other medical treatments

The treatment for haemorrhagic stroke is currently primarily supportive unless there is a specific underlying cause such as an aneurysm.

A large number of trials for agents that might improve medicinal thrombolysis or offer neuroprotection in both ischaemic and haemorrhagic stroke have been and continue to be tested. As yet, no such agents have been convincingly identified.

1.7 Outcome after Stroke

1.7.1 Short-term outcomes

As noted above, the clinical stroke syndrome with which a patient presents to hospital can be partially, or even completely reversible. This will occur if blood supply to brain is restored, either spontaneously or following treatment, before ischaemia becomes infarction (that is before brain cells die). Reversible ischaemia causes stunning rather than death of neurons.

Also, it has been demonstrated that early and sustained arterial recanalisation is related to better early and late outcomes in stroke[63;64] presumably due to return of normal arterial perfusion leading to greater neuronal recovery, less cell death and less secondary haemorrhage.

Transient ischaemic attack

If a presenting neurological deficit spontaneously resolves within 24 hours of onset, by definition this is a transient ischaemic attack, or TIA, rather than a stroke. TIAs

are often a precursor to an ischaemic stroke and their appearance usually prompts a thorough assessment for and treatment of vascular risk factors in the affected patient. For example:

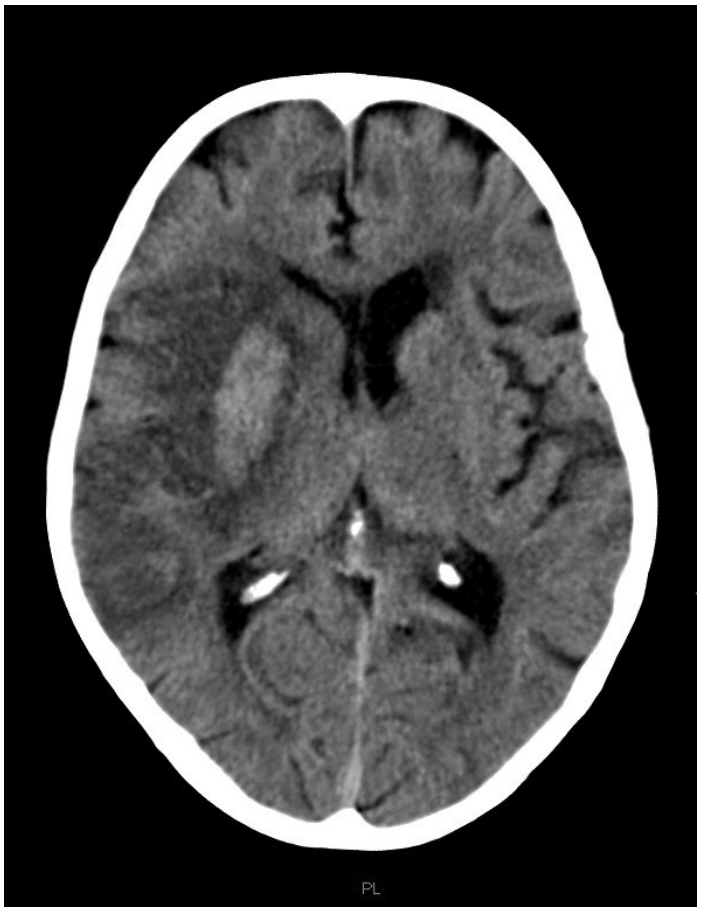
- Encouragement to stop smoking
- Control of blood pressure, diabetes and cholesterol levels
- Oral anti-platelet therapy
- Treatment for atrial fibrillation
- Treatment for right-to-left cardiac shunt
- Carotid endarterectomy surgery

Haemorrhagic transformation of infarct

Following infarction there is likely to be disruption to the blood-brain barrier and damage to the capillary bed supplying the region. If there is some residual arterial blood supply, or more likely blood supply has been restored following treatment, haemorrhage into the area of infarct can occur.

If this haemorrhage is of small volume, does not extend beyond the margins of infarct and does not worsen local swelling or change the severity of stroke, it is termed petechial haemorrhage and the clinical implications of this change are likely to be minimal (see Figure 1-6). Ongoing post-stroke care may not change in the face of petechial haemorrhage, e.g. even antiplatelet therapy may continue.

Figure 1-6 *Petechial haemorrhage (hyperattenuating) into an area of recent infarct (hypoattenuating)*

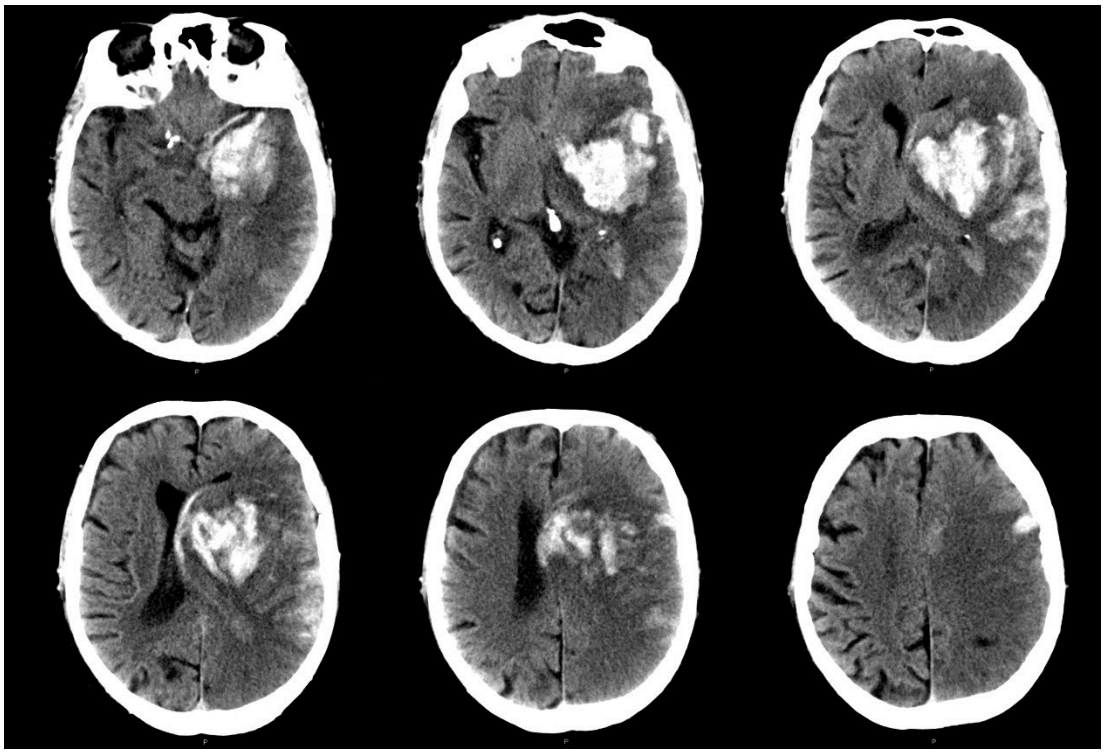


If however, haemorrhage into an infarct is more significant, extends beyond the margins of the original injury and the mass effect from this haemorrhage causes new shifts in brain position and thereby worsening symptoms, it is termed symptomatic intracranial haemorrhage (SICH – sometimes the term symptomatic intracerebral haemorrhage is used synonymously but the former term is preferred and more often correct since it also encapsulates the brainstem and cerebellum. Note however that SICH is sometimes also used to denote *spontaneous* intracranial haemorrhage), see Fig 1-7. SICH is likely to significantly change post-stroke care for that patient, e.g. cessation of antiplatelet therapy, possibly the need for surgery. In addition, SICH is associated with worse long and short term outcomes.[65]

Hydrocephalus after haemorrhagic stroke

Extension of haemorrhage from brain parenchyma into the CSF-filled ventricular system can occur after primary haemorrhagic stroke or after significant haemorrhagic transformation of infarct. The end result is the same; haemorrhage obstructs normal CSF flow and drainage through the arachnoid villi resulting in accumulation of CSF, termed hydrocephalus (see Figure 1-7). This causes an increase in intracranial pressure and worsening of the patient's clinical status, often reducing conscious level. In severe cases if left untreated, hydrocephalus can be fatal.

Figure 1-7 *Large volume haemorrhage into an area of recent infarct and adjacent lateral ventricle associated with hydrocephalus and worsening of the patient's symptoms*

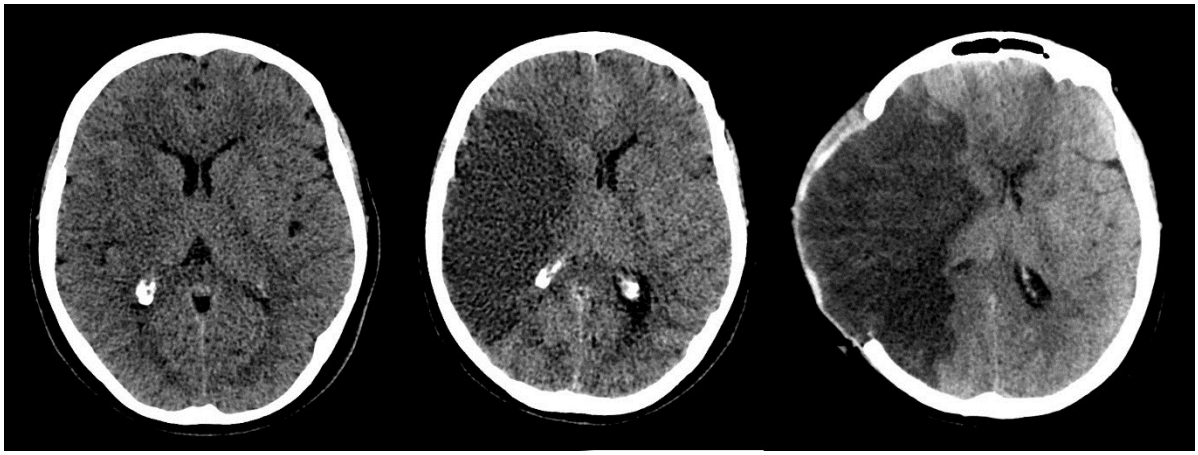


Malignant middle cerebral artery syndrome

When a large volume of brain is infarcted (commonly, but not exclusively the entire middle cerebral artery – MCA – territory) this leads to a correspondingly greater volume of post-infarct swelling secondary to oedema. Swollen brain can then displace and compress adjacent normal brain, worsening the patient's status. This is a particular problem for younger patients since there is minimal redundant space

around the brain to allow for expansion and the skull is a solid, fixed volume. Older patients are less at risk of malignant swelling syndromes if previous, age-related global volume loss of brain has occurred, the extra intracranial space created is protective. Figure 1-8 demonstrates a malignant MCA syndrome in a young patient.

Figure 1-8 *Malignant MCA syndrome in a young patient*



Footnote: Sequential non-contrast CT images taken at baseline, 1 and 6 days after ischaemic stroke onset. Craniectomy was performed between the latter two images to relieve raised intracranial pressure following entire right MCA territory infarct. In the final image infarcted and swollen brain herniates through the skull defect.

Randomised trials of surgical intervention by hemicraniectomy for patients at risk of significant swelling after ischaemic stroke have shown mixed results; while there is a significant reduction in mortality following hemicraniectomy, there is also an increase in the proportion of patients surviving but with severe disability.[66]

1.7.2 Long-term outcomes

For those patients that retain a neurological deficit more than 24 hours after stroke onset, post-stroke rehabilitative therapy can help to minimise long term disability by encouraging the patient to relearn particular activities. For example, how to articulate speech or to recover some mobility. Younger patients with greater potential for neuroplasticity may have the most to gain from neuro-rehabilitation.[67-69]

Stroke trials investigating the impact of intravenous thrombolysis or more recently, endovascular thrombectomy have usually assessed the patient's functional status at three or six months after stroke onset to allow for a 'final' stable post-stroke outcome to become evident.

The modified Rankin Scale (mRS) has been most commonly used to assess 3-6 month outcome in stroke trials.[70-72] mRS is graded 0-6:

0. No symptoms.
1. No significant disability. Able to carry out all usual activities despite some symptoms.
2. Slight disability. Able to look after own affairs without assistance, but unable to carry out all previous activities.
3. Moderate disability. Requires some help, but able to walk unassisted.
4. Moderately severe disability. Unable to attend to own bodily needs without assistance, and unable to walk unassisted.
5. Severe disability. Requires constant nursing care and attention, bedridden, incontinent.
6. Dead.

Traditionally, trial patients said to have derived benefit from treatment were those without or with only minimal disability (mRS 0-1 or 0-2) who retained their independence. Thus analysis of results was based on converting mRS into a simple dichotomy comparing patients with 'good' versus 'bad' outcomes. Straightforward statistical testing is required in this type of analysis, e.g. a two by two table and chi-squared (χ^2) statistics; see Table 1-3 for an example using imaginary stroke trial data.

Table 1-3 *Example data from imaginary stroke trial comparing treatment allocation with outcome as a dichotomy*

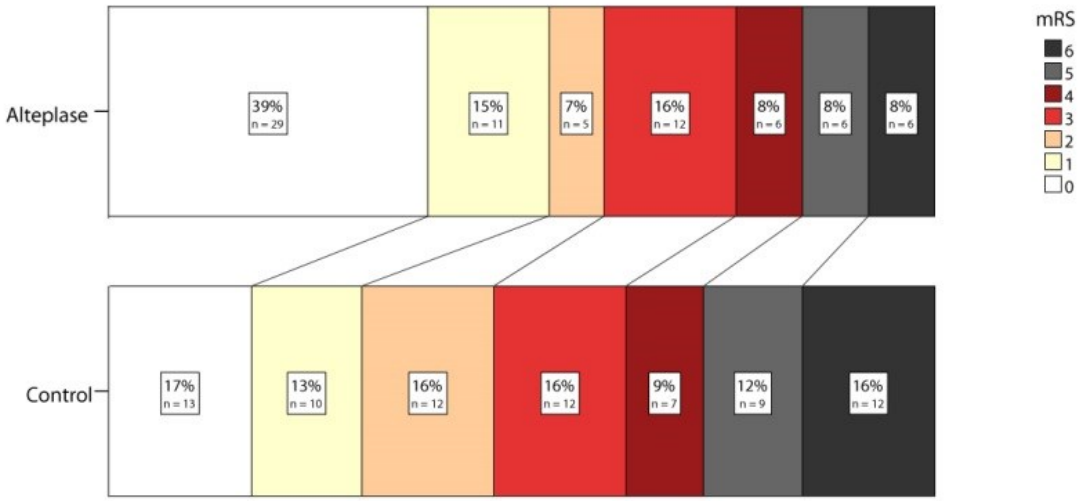
		Treatment Allocation	
		IV Alteplase	Control
3-Month Outcome	mRS 0-2	45	35
	mRS 3-6	30	40

Footnote: mRS = modified Rankin Scale. n=150

$\chi^2 = 2.7$, $p = 0.102$, i.e. despite an extra 10 patients achieving a good outcome following treatment with intravenous thrombolysis (in treatment allocation groups which are matched for size), the treatment effect on outcome in this analysis is non-significant.

However, this approach fails to take account of trial patients who moved along the mRS scale but did not cross the line of dichotomy, i.e. it has the effect of smoothing data since seven mRS groups become two. Conceivably many patients enrolled in trials who did not cross the line would nevertheless demonstrate improved or worsened outcomes (depending on direction of travel) as a consequence of their treatment allocation in the trial. Figure 1-9 uses the same imaginary stroke trial data but displays all mRS levels as stacked horizontal bar graphs. Figures of this type are commonly used to report stroke trial results (sometimes termed Grotta bars)[73] as they highlight that multilevel scalar shifts occur in such datasets. Unfortunately, more complex statistics are required to assess stepwise shifts over multiple levels, e.g. ordinal regression analysis.[74] The benefit of such analyses is that with a greater number of data points per treatment allocation group (7 rather than 2), the results are more powerful for the same number of included patients.[75] Note that from Table 1-3 to Figure 1-9, in my imaginary dataset, the result of the latter analysis is significantly in favour of treatment when data are analysed using the more statistically powerful regression technique incorporating all data points (i.e. non-smoothed data).

Figure 1-9 *Example data from imaginary stroke trial comparing treatment allocation with outcome*



Footnote: Result of ordinal regression analysis with mRS (modified Rankin Scale) as the dependent variable, n=150: Odds ratio for improved outcome with alteplase versus control (shift toward the left) = 2.25 (95% confidence interval = 1.27-4.00), p=0.006.

1.8 References for Chapter 1

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Chapter 2 Literature Reviews

In the early stages of my MD research project, I undertook two reviews to investigate the major topics for consideration in my thesis:

I. Systematic review of the hyperattenuated artery sign (HAS) in ischaemic stroke with meta-analysis of HAS prevalence data
II. Narrative review of CT and MR angiography (CTA and MRA, respectively) performed acutely in the assessment of ischaemic stroke

These reviews sought to refine the aims of my thesis and to direct and inform subsequent analyses designed to address those aims.

2.1 Systematic Review of Prior HAS Research

This systematic review was designed as a comprehensive review of published HAS research.

2.1.1 Methods

Search strategy

I performed concurrent searches on the Embase and Medline databases using the strategy outlined in Figure 2-1. Keywords pertaining to the three topic areas of CT, ischaemic stroke and hyperattenuating arteries (in any intracranial location) were combined within each topic area using the Boolean operator OR. I combined results from these three topic area searches using the Boolean operator AND. Since hyperattenuating arteries in stroke were first described in the early 1980s,[1;2] I included all reports from 1980 to the date at the time of search (October 2012). I hand searched and cross checked the reference lists from returned citations

(especially review articles) to identify any further relevant work that may have been overlooked by the searches.

Figure 2-1 *Search strategy for systematic review of prior HAS research*

```
1. exp computer assisted tomography/
2. exp Tomography, X-Ray Computed/
3. ct.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui]
4. cat.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui]
5. 1 or 2 or 3 or 4
6. exp brain artery/ or exp carotid artery/ or exp temporal artery/
       or exp vertebral artery/
7. exp basilar artery/ or exp carotid arteries/ or exp cerebral arteries/
       or exp meningeal arteries/ or exp vertebral artery/
8. arter*.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui]
9. 6 or 7 or 8
10. exp density/
11. dens*.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui]
12. 10 or 11
13. 9 and 12
14. hyperdens*.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui]
15. hyperatten*.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui]
16. hyper-dens*.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui]
17. hyper-atten*.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui]
18. hmcas.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui]
19. 13 or 14 or 15 or 16 or 17 or 18
20. exp cerebrovascular disease/ or exp brain infarction/ or exp brain ischemia/
       or exp carotid artery disease/ or exp cerebral artery disease/
       or exp cerebrovascular accident/ or exp occlusive cerebrovascular
       disease/ or exp stroke/ or exp vertebrobasilar insufficiency/
21. exp stroke patient/
22. 20 or 21
23. cerebrovascular disorders/ or exp basal ganglia cerebrovascular disease/
       or exp brain ischemia/ or exp carotid artery diseases/
       or exp cerebral small vessel diseases/ or exp intracranial arterial
       diseases/ or exp "intracranial embolism and thrombosis"/ or exp stroke/
       or exp vertebral artery dissection/
24. exp Cerebrovascular Circulation/
25. 22 or 23 or 24
26. 5 and 19 and 25
```

Inclusion and exclusion criteria

First I screened titles and abstracts. For efficiency, I sought only peer reviewed original research articles published in English. Results published only in abstract, review articles, editorials and case studies were not included. Articles had to contain original data on intracranial hyperattenuated arteries identified on CT in the course of investigating stroke.

To be widely inclusive, I imposed no further exclusion criteria (i.e. I did not use quality criteria to determine eligibility) and I included all types of HAS research. I then subclassified articles into four topics based on their commonality in the search output:

- Prevalence/ identification of HAS

- Pathological composition of HAS
- Correlation with angiography
- Clinical implications of HAS (on stroke severity and response to thrombolysis)

Meta-analysis of HAS prevalence

To derive an overall prevalence for HAS in ischaemic stroke, I included only absolute numbers for HAS presence within individual study populations. However, I did not examine differences between these study populations. I collected data in a spreadsheet and calculated sum totals for the numerator and denominator for each study prior to calculation of the overall prevalence.

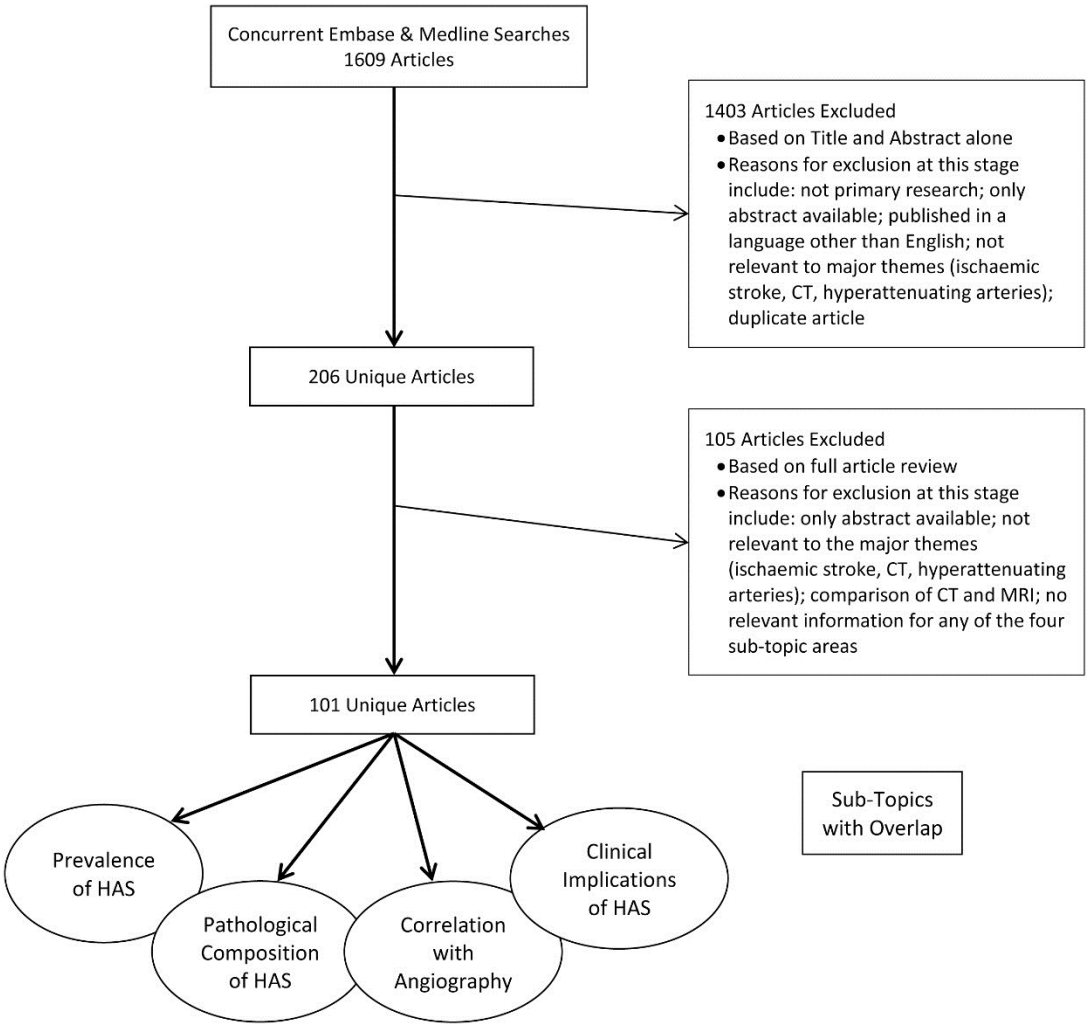
Literature review

I did not otherwise meta-analyse articles identified in my systematic review but all relevant citations are included below in a narrative literature review exploring the status of published HAS research at the outset of my MD project.

2.1.2 Results of search

My searches identified 1609 unique citations from which 206 were selected after abstract screening for further reading and 101 were ultimately included in the review, see Fig 2-2. The main reasons for exclusion from the review were: studies comparing MRI findings to CT hyperattenuation; review articles (including pictorial), editorials or letters adding no new data to the field; case studies describing unusual findings that were not relevant to the declared HAS topics for review. Most of the articles represented observational studies (86/101 = 85%), there was very little data from randomised-controlled stroke trials (7 articles derived from 4 trials).[3-9] Most of the observational studies were conducted retrospectively in single centres and included fewer than 100 patients. I identified only 2 large multicentre observational datasets[10;11] and only 1 systematic review which investigated early CT findings among ischaemic stroke patients.[12]

Figure 2-2 *Flow chart for systematic review showing numbers of articles identified and selection of reports for inclusion*



The 101 citations identified after systematic review contributed to the predetermined HAS topics as follows in Table 2-1. Note that many articles contributed to more than one topic.

Table 2-1 *Contribution of articles to each of the HAS research topic areas*

HAS Research Topic	**n (% of 101 identified)**
Prevalence/ identification of HAS	54 (53.5)
Pathological composition of HAS	7 (6.9)
Correlation with angiography	21 (20.8)
Clinical implications of HAS – Stroke severity & outcome	52 (51.5)
Clinical implications of HAS – Response to thrombolysis	27 (26.7)

Meta-analysis of HAS prevalence in the published literature

Among 54 articles providing data on HAS prevalence, I identified a wide range of values (7-88%) which may reflect variability in the included study populations; the overall prevalence from over 20,000 scans was 21.2%, Table 2-2. Figure 2-3 demonstrates that there was greater variability in published prevalence for studies with fewer than 100 patients.

Table 2-2 *Published prevalence of HAS*

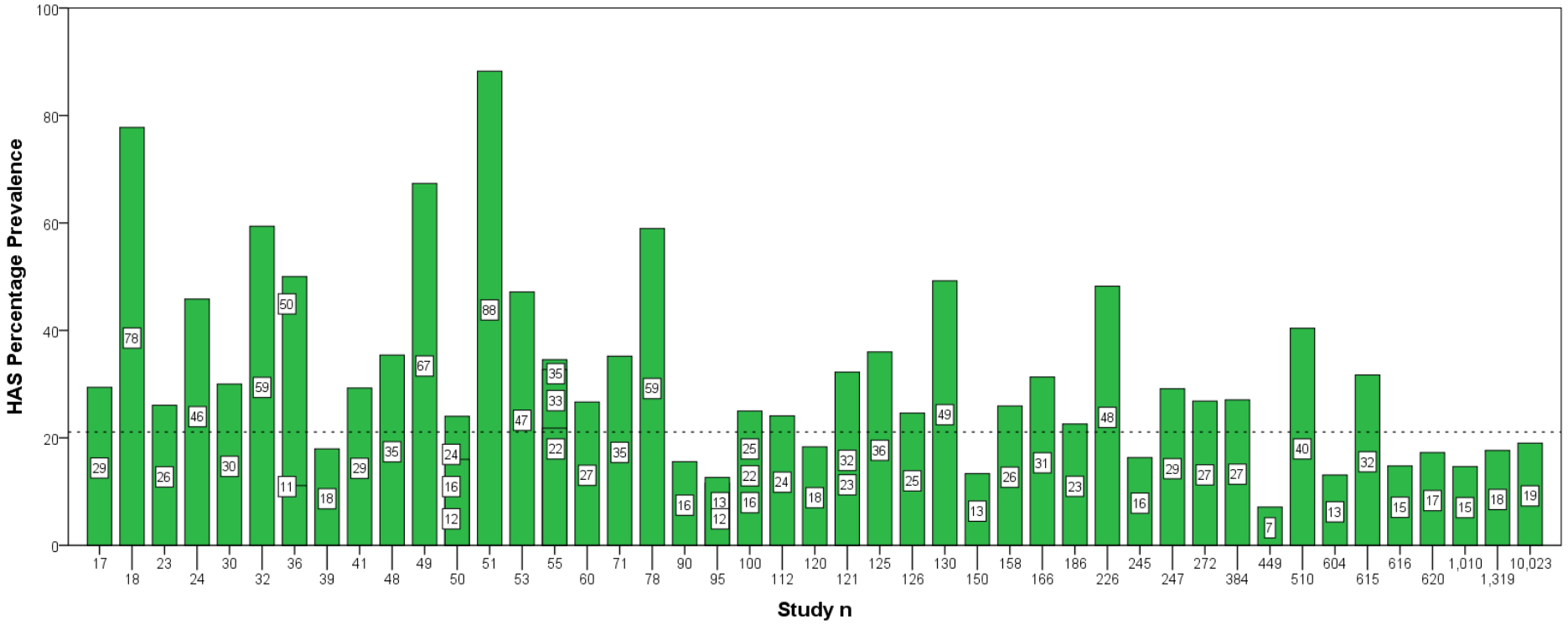
First Author of Publication (Year)	**Prevalence, n (%)**
Tomsick (1989)[13]	6/50 (12.0)
Schuknecht (1990)[14]	28/121 (23.1)
Bastianello (1991)[15]	18/36 (50.0)
Ricci* (1991)[16]	14/90 (15.6)
Leys (1992)[17]	73/272 (26.8)
Tomsick (1992)[18]	19/55 (34.5)
Wolpert (1993)[19]	16/60 (26.7)
von Kummer (1994)[20]	25/53 (47.0)
Moulin (1996)[21]	22/100 (22.0)
Tomsick (1996)[22]	18/55 (33.0)

Büttner (1997)[23]	11/95 (11.6)
Barber (1999)[24]	5/17 (29.0)
Dávalos (1999)[4]	195/615 (31.7)
Manelfe (1999)[5]	107/620 (17.7)
Scott (1999)[25]	7/39 (18.0)
Fiorelli (2000)[26]	41/158 (26.0)
Flacke (2000)[27]	6/23 (26.0)
Barber (2001)[28]	16/100 (16.0)
Berge (2001)[29]	32/449 (7.0)
Mendizabal (2001)[30]	4/36 (11.0)
Gadda* (2002)[31]	19/32 (59.3)
Somford (2002)[32]	42/186 (22.6)
Koga (2003)[33]	27/112 (26.0)
Leary* (2003)[34]	12/50 (24.0)
Aronovich (2004)[35]	20/150 (13.3)
Barber (2004)[36]	25/100 (25.0)
Gadda (2005)[37]	33/49 (67.3)
Kim* (2005)[38]	45/51 (88.0)
Mandava (2005)[39]	14/18 (78.0)
Krings (2006)[40]	17/48 (35.4)
Qureshi (2006)[8]	91/616 (15.0)
Tei (2006)[41]	52/166 (31.3)
Poniatowska (2007)[42]	45/125 (35.7)
Kim (2008)[43]	46/78 (59.0)
Nichols (2008)[44]	79/604 (13.0)
Ozdemir* (2008)[45]	25/71 (35.0)

Tartaglia (2008)[46]	64/130 (49.0)
Abul-Kasim* (2009)[47]	39/121 (32.0)
Aries (2009)[48]	104/384 (27.0)
Goldmakher (2009)[49]	12/95 (12.6)
Kharitonova (2009)[11]	1905/10023 (19.0)
Kobayashi (2009)[9]	206/510 (40.0)
Jensen (2010)[50]	11/24 (46.0)
Nedeltchev* (2010)[51]	40/245 (16.3)
van Overbeek (2010)[52]	72/247 (29.1)
Tan (2010)[53]	31/126 (24.6)
Ambrosius (2011)[54]	12/55 (21.8)
Fitzsimmons* (2011)[55]	22/120 (18.3)
Paciaroni (2011)[56]	148/1010 (14.7)
Guo (2012)[57]	9/30 (30.0)
Lingegowda (2012)[58]	12/41 (29.3)
Paliwal (2012)[59]	109/226 (48.2)
Sakamoto** (2012)[60]	8/50 (16.0)
Strbian* (2012)[61]	233/1319 (17.7)
Sum Totals (Overall Prevalence)	**4292/20256 (21.2)**

Footnote: *Several results were presented, not all are listed here. ** Excluded 17 patients with primarily haemorrhagic stroke.

Figure 2-3 *Bar chart ordering the 54 studies included in the HAS prevalence analysis by sample size*



Footnote: HAS = Hyperattenuated Artery Sign. Studies with matching sample size are grouped. Dotted line represents overall prevalence of HAS, 21.2%.

2.1.3 Narrative literature review of HAS

In the following sections I provide an overview of the HAS literature summarising the remainder of the articles identified in my systematic review and grouped by the subcategories described above in Table 2-1. Note that I did not undertake any further meta-analyses with the data from these articles.

Variability in the identification of HAS

The location of HAS may determine how often it is detected. Most of the early case studies describing HAS related to hyperattenuation within one of the middle cerebral arteries (MCA),[2;62-64] which is arguably one of the easier locations to identify HAS given that the main MCA trunk lies roughly in parallel with the standard axial plane for head CT. Less commonly, HAS has also been described in all of the other named intracranial arteries.[14;40;45;49;50;65-67]

Several papers describe HAS as a transient phenomenon[5;15;17;21;52;62;68;69] more frequently found early in the process of acute stroke.[29;50] However one article noted that HAS prevalence increased from 3 to 6 to 9 to 12 hours after stroke onset.[42] These results imply that there is a peak time soon after stroke onset when HAS is most likely to be identified. This may relate to the normal temporal development and subsequent breakdown (natural or treatment related) of the thrombus or embolus that gives rise to the appearance of HAS.

Several other factors might also account for the described variance in HAS prevalence. Significantly improved results have been reported where thin slice CT data were used. Kim et. al. compared 5 mm slice data with that from 1.25 mm or 1 mm slices (defined as thin slice) for detecting HAS. The prevalence of HAS with thin slice CT was 88% versus 32% for images based on 5 mm slices.[38] Other authors have also shown that intravascular thrombus can be identified more readily with thin slice CT.[31;37;70;71] Similarly, prevalence is increased if larger, more proximal vessels are examined, i.e. likely to represent a greater volume of thrombus in positive cases.[33] Instinctively it makes sense that a greater clot load is more likely to be identified on CT.[72] Notwithstanding, the basilar artery is uniquely recognised as a

difficult vessel to assess on non-contrast CT due to bony artefact within the posterior fossa and the lack of a paired vessel for comparison.[67]

False positive HAS can be produced by non-thrombotic or embolic causes. Vessel wall calcification, raised haematocrit, arterial dissection, circulating intravascular contrast agents (residual iodinated contrast in patient with renal failure, bromine as a treatment for epilepsy, cocaine) and reduced density within the surrounding brain parenchyma have all been reported.[73-78]

Several of these reported problems relate to subjective assessment of vessel hyperattenuation, i.e. the vessel appears bright to the human eye. In addition, they make no allowance for whether the finding is generalised or isolated to a single vessel. Koo and colleagues attempted to ascertain criteria for validating an apparently dense intracranial vessel. They found that an absolute attenuation greater than 43 HU or a ratio (with matched contralateral vessel – more dense:less dense) greater than 1.2 might be used to define 'true' hyperattenuation as defined by an expert reader. The ratio of affected vessel to adjacent brain parenchyma was not found to be useful as a discriminator of true intravascular hyperattenuation.[79] An intravascular attenuation >40 HU has also been independently identified as an optimal cut-off for defining intra-arterial hyperattenaution.[54;67] It is important to correct attenuation measures for background intravascular attenuation.[47;80] An added benefit of taking into account attenuation within the contralateral vessel is that it also controls for decreases in background intravascular attenuation (e.g. in states of anaemia); this may allow for more subtle hyperattenuation to be appreciated.

Finally, it is worth noting that bilateral MCA hyperattenuation related to bihemispheric stroke has been reported.[81] While this rarity would clearly obviate use of the contralateral vessel as a measure of normality, this case nevertheless demonstrates the need for correlation between radiological and clinical findings.

Pathological composition of HAS

Several research articles sought to determine the pathological composition of thrombus or embolus in stroke and to relate this to differences in attenuation that might be appreciated on CT.

A linear relationship between the attenuation of blood and haematocrit has been described.[82] Ischaemic strokes can be caused by either erythrocyte rich or platelet rich thrombus, termed red and white clots, respectively.[83] In addition, histochemical differences are appreciated between thrombi that form locally and those that proceed to embolisation.[84] Clots retrieved from patients following acute ischaemic stroke demonstrate a variable mixture of red cells, platelets and fibrin.[85] Mixed or fibrin rich clots are most common while pure red clots are rare and it has been postulated that these may only occur in stagnant blood following primary occlusion by a mixed or fibrin rich clot.[86;87] Phantom studies have demonstrated that clot composition directly affects attenuation. As with circulating whole blood, higher erythrocyte levels lead to a higher CT measurable attenuation. In one study using phantoms, over a time range of 6-144 hours, white thrombi demonstrated HU of 16-24, mixed measured 22-52 HU, while red thrombi measured 69-76 HU.[88]

Koo et. al. and other authors published mean attenuation values within affected vessels in the range 46 to 89 HU[14;55;79] while another study comparing CT and MR findings to the pathology of retrieved clot found a mean attenuation of 61 HU in cases where HAS was present; hyperattenuated clots were more likely to be red cell dominant or mixed and to have a higher red cell content compared with clots that did not demonstrate a HAS (47% versus 22%).[87] Similarly, Sakamoto and colleagues found that HAS on CT and susceptibility artefact on MRI were highly consistently identified in the same individuals, i.e. both signs were usually synchronously either present or absent. Given that susceptibility artefact is an indicator of haem, this finding also implies HAS are red cell dominant.[60] Finally, some authors have provided data suggesting that HAS is more likely after stroke secondary to cardioembolism rather than large artery atherosclerosis which may have implications for the differences in underlying clot composition but these data are subjective due to the nature of estimating stroke aetiology.[56;89]

That only a proportion of thrombi contain enough red cells to be registered as high attenuation on CT might also help to account for some of the variability of published prevalence results for HAS described above.

Correlation with angiography

Catheter angiography remains the gold standard for assessment of intracranial vascular anatomy and arterial patency. Early studies validated the HAS using catheter angiography and confirmed the presence of cerebral artery occlusion in arterial segments with intravascular hyperattenuation. In these studies correlation was often 100%, i.e. a hyperattenuating vessel almost always correlated with an angiographically detected occlusion but conversely, an occlusion on catheter angiography was not always related to the finding of a hyperattenuated vessel on CT.[14;15;18;34] Hyperattenuating vessels have also been shown to signify vascular occlusion as determined with CTA.[43;90-92]

To establish the potential value of HAS in routine practice, it is important to quantify the sensitivity and specificity of HAS for the identification of true arterial occlusion. Reported numbers for sensitivity, specificity, positive predictive (PPV) and negative predictive values (NPV) varied across 16 studies where these figures were reported and they did not always use the same angiography gold standard.[16;17;19;20;27;34;36;38;40;45;47;49;58;60;67;93] Sensitivity ranged from 26% to 100% (median 44.5%), i.e. fewer than half of acute stroke patients with intravascular thrombus had an identifiable HAS. Specificity was often reported as 100% but estimates ranged from 51% to 100% (median 100%), i.e. an identifiable HAS was highly likely to relate to an acute stroke. PPV was estimated within the range 32 to 100% (median 100%) while NPV was reported from 15 to 100% (median 63.1%), i.e. similar to sensitivity and specificity findings, the presence of the sign was likely to relate to stroke but its absence did not exclude stroke. The use of thin-slice CT has been shown to improve rates of sensitivity, specificity, PPV and NPV; two studies reported values of 100% for all measures when thin-slice CT was used.[38;47] It is also worth noting that in several of the studies reporting accuracy data for HAS, there was apparently no direct assessment of vascular patency, i.e. neither catheter or CT angiography was used to confirm arterial occlusion in the presence of an apparent HAS. Instead, several authors used clinical outcomes (confirmed stroke or death) to imply that an apparent HAS was a genuine finding which calls the validity of these results into question.[16;17;20;40;47] For completeness, these studies have been included in the summary ranges quoted above.

Clinical implications

Stroke severity and outcome: A large body of early work demonstrated on univariate analyses an association between the presence of HAS and increased stroke severity[50] and that both short and longer term poor outcome including an increased rate of death,[20] were more frequent in patients with HAS.[12;16;22;23;32;41;45;94-98] HAS is related to larger volume infarcts[17-19;22;23;29;40;54;56;98] that demonstrate earlier - CT identifiable - changes in brain parenchyma,[15;21] particularly if the HAS resides within a proximal vessel.[28;32;37;55] van Overbeek et. al. reported that striatocapsular infarcts due to MCA occlusion (which may present with a clinical syndrome indistinguishable from a lacunar stroke) are more likely if HAS within the middle cerebral artery has resolved by 24 hours. In their study, patients for whom HAS persisted were more likely to suffer additional cortical infarction.[52] Comparatively, reversal of the sign has been related to better clinical outcomes.[39;59;99] At the time of writing, in the largest published cohort of patients with HAS (1905 from the Safe Implementation of Thrombolysis in Stroke, SITS, register), Kharitonova and colleagues found that HAS patients had a more severe stroke both at presentation and up to three months later and were less likely to regain independence.[11] Very similar results are also reported in other large cohorts such as the European Cooperative Acute Stroke Study 1 – ECASS1, a double blind, randomised, multi-centre trial with 620 patients.[5]

Several authors have demonstrated, using regression analyses, that associations between HAS and poor clinical outcomes occur independently of other clinical and demographic factors such as patient age and sex, stroke severity at baseline.[48;49;53;54;56;61;100] This includes data from the National Institute of Neurological Disorders and Stroke – NINDS intravenous thrombolysis stroke trial (placebo controlled, randomised, double-blind, multicentre) which showed that intravascular thrombus identified on CT was an independent predictor of both clinical deterioration within 24 hours of stroke onset and also poor 3-month outcome.[3;6;8] Conversely, Mustanoja et. al. demonstrated in their large cohort of 957 patients that absence of HAS at baseline predicted a good three month outcome[101] and Paliwal et. al. showed that persistence of HAS on follow-up CT (22-36 hours after the first) was independently associated with a poor three month outcome.[59] Meanwhile, Dávalos

and colleagues found that HAS independently predicted an early progression of stroke severity – defined as deterioration within 24 hours[4] while Berge et. al. found independent associations between HAS and parenchymal haemorrhage and death.[29] This latter association with mortality was also demonstrated by both Zorzon and Kharitonova.[11;102] One large study recently demonstrated an independent association between HAS and increasing severity of cerebral oedema.[103] However, despite significant correlations on univariate analysis in most cases, not all studies that performed regression testing of their data found HAS to be independently associated with prognosis.[5;17;26;27;30;41;46;51;96;104] Some of these results were summarised in a systematic review that nevertheless found clinical outcomes in stroke to be significantly worse in the presence of HAS (cumulative odds ratio 2.09, 95%CI 1.47-2.96).[12]

In summary, these results are consistent with the observation that increased arterial clot burden is associated with poorer outcomes after ischaemic stroke and imply that visible clot is likely to be of a higher volume than one which is not visible on CT.[43] Larger volume clots are probably more likely to occlude the primary vessel and more likely to extend to distal branches and collaterals. Larger volume clots might take longer to dissipate - whether occurring naturally or secondary to treatment[99;105] - thereby increasing the duration of any distal ischaemia and reducing the chance of a full recovery.

Response to thrombolysis: Patients with ischaemic stroke secondary to small, distally sited clots may respond best to intravenous thrombolysis.[106-109] Wolpert et. al. found that while recanalisation following intravenous thrombolysis was more likely in distal occlusions rather than proximal, recanalisation was not related to the presence, or absence, of a HAS.[19] Several retrospective studies have demonstrated that HAS is not significantly associated with outcomes following intravenous thrombolysis.[30;46] Secondary analysis of the NINDS data showed that while HAS was independently associated with outcomes, the sign was not itself a predictor of response to intravenous thrombolysis.[3] Patients with HAS treated with intravenous thrombolysis had increased frequency of recanalisation and reduced infarct volumes at 24 hours.[8;44] Data from ECASS1 also demonstrated that patients with HAS

receiving intravenous thrombolysis had significantly better clinical outcomes than those given placebo.[5] Kharitonova and colleagues have shown that following thrombolysis, 48% of patients in their large SITS cohort demonstrated clearance of HAS within 36 hours. Patients within their cohort for whom the sign had disappeared following thrombolysis suffered a less severe stroke and had better long-term outcomes.[110] Interestingly, two groups found that clots with lower attenuation on CT were more resistant to intravenous thrombolysis[80;89] and postulated that this relates to the underlying pathological composition where erythrocyte rich clots, which are known to be more sensitive to thrombolytics,[111;112] are of higher attenuation. While this correlates well with the pathological evidence presented above, it is somewhat at odds with the cumulative evidence presented here; HAS is associated with more severe strokes but the effect on post-thrombolysis outcomes is not directly related to the presence or absence of the sign despite pathological evidence that HAS should be more sensitive to intravenous thrombolysis. This apparent contradiction might be explained by consideration of the CT data that is utilised in most of these studies. As noted above, CT slice thickness is associated with HAS detection, with thin slice data providing better results. The true prevalence and sensitivity rates of HAS therefore are likely to be much higher than is currently widely described, i.e. nearer 100%.[38] It may be that the increased effectiveness of intravenous thrombolysis on hyperattenuating clots is masked by the limited detection rate of HAS in those studies where only large, central HAS (the type that reportedly do not respond well to intravenous thrombolysis[99]) are included. The repeated observation that patients with HAS do, nevertheless, respond to intravenous thrombolysis would seem to solidify this supposition.[5;8;110;113]

A number of non-randomised prospective observational studies have investigated the effectiveness of thrombolytics delivered intra-arterially. Two of these studies described more favourable outcomes for HAS patients following intra-arterial delivery of thrombolytics when compared with the same delivered intravenously.[114;115] In contrast, Ozdemir et. al. found no difference in the long term outcomes for HAS patients treated with intravenous compared to intra-arterial thrombolysis.[45] Similarly, Arnold et. al. did not find HAS to be an independent predictor of 3-month functional outcome but did identify HAS as an independent

predictor of recanalisation among patients with proven basilar artery occlusion treated with intra-arterial thrombolysis.[116] Data from the PROACT study (a randomised trial investigating the efficacy of intra-arterial thrombolysis), found that HAS patients demonstrated less of a response to intra-arterial therapy compared to those without the sign but this finding was not significant; the trial was likely underpowered to detect an interaction between intra-arterial thrombolysis and HAS.[7] Subsequent work by Barreto and colleagues, found intra-arterial therapy to be less effective with larger volume clots.[117] Given my previous suggestion that clot size may relate to whether or not HAS is visible, there is a risk of bias in these HAS versus intra-arterial thrombolysis data.

An increased rate of haemorrhage after thrombolysis might be anticipated in patients with HAS given the associations between HAS, a higher likelihood of proximally sited larger volume thrombus, worse stroke severity and outcome. Indeed, some small studies have found HAS to be associated with an increased risk of intracranial haemorrhage following treatment with intravenous thrombolysis.[33;118] Similarly, in a large multicentre review including data from 1205 patients, Tanne et. al. found more asymptomatic haemorrhage in those with HAS but no significant difference in the risk of symptomatic haemorrhage.[10] However, analysis of RCT data from the NINDS showed no significant relationship between the use of intravenous thrombolysis and intracranial haemorrhage,[8] a finding that has also been demonstrated elsewhere.[48;55;113] Similarly, neither Kharitonova et. al. nor Marti-Fabregas et. al. found HAS to be an independent predictor of symptomatic intracranial haemorrhage in their large datasets following treatment with intravenous thrombolysis when defined using the SITS classification: parenchymal haemorrhage within 36 hours of treatment accompanied by a decrease in the baseline NIHSS score of ≥4 points or death within 24 hours of thrombolysis.[11;119] Conversely, HAS was independently predictive of symptomatic haemorrhage in Kharitonova's cohort if a broader definition (as described in previous randomised-controlled trials) was used: any haemorrhage on follow up imaging combined with an NIHSS decrease of one point or death before day seven. The authors postulated that this difference was related to the increased severity of strokes seen with HAS however, given that baseline NIHSS was also included in their regression model (i.e. the results were

adjusted for stroke severity), this explanation does not adequately rebuff the importance of their former finding.[11] Subsequent work by Strbian and colleagues concluded that this broader definition of symptomatic haemorrhage does drive a larger contribution to worse outcomes when compared to the SITS definition.[120] Notwithstanding, Strbian and colleagues recently developed the SEDAN score for predicting symptomatic intracranial haemorrhage after intravenous thrombolysis and they identified intra-arterial hyperattenuation as one of the predictor variables for SEDAN.[121] Given these mixed results, it is not clear if haemorrhage is more likely among all HAS patients following intravenous thrombolysis. Whether haemorrhage subsequently affects outcomes in this cohort has been inadequately defined.

2.1.4 What remained unclear after HAS systematic review

Published evidence demonstrates a wide variability in the prevalence of HAS among patients with CT brain imaging performed soon after stroke onset, but it is probably more common than at first supposed, particularly within the early phase of stroke evolution. In addition, the sign is naturally transient. More frequent use of thin slice CT in the hyperacute assessment of stroke is likely to reveal HAS more reliably and in a greater number of patients, especially if HAS involves smaller vessels more distally sited. It will be important to assess reader reliability for the identification of HAS. Measurement of intravascular attenuation of clot assessed objectively may improve the identification of HAS and give insights into the pathological composition and potentially, the aetiological origin of HAS.

Comparative angiography data suggest that the presence of HAS is highly specific for arterial occlusion but is less sensitive, perhaps in part due to the natural variability in clot composition and the technical variability in HAS identification. However, the estimates of sensitivity and specificity of HAS for detecting arterial obstruction are highly variable and several studies did not use angiography to confirm arterial occlusion.

Much of the published data on HAS indicates that it is associated with worse stroke severity at baseline and poorer post-stroke outcomes both in the short and longer

term. Whether these relationships are independent of other stroke severity and outcome variables remains unclear. Some evidence suggests that reversal or persistence of HAS is important for outcome but this has not been tested rigorously.

It is likely that HAS represents that proportion of thrombus, rich in erythrocytes, that is most sensitive to thrombolysis. It appears that patients with HAS do gain benefit from intravenous thrombolysis but with a greater underlying clot volume than patients without the sign, it is perhaps erroneous to compare the two cohorts directly, at least without the benefit of thin-slice CT data. There is limited randomised-controlled trial data to assess whether the presence versus absence of HAS, its location and extent and its measurable attenuation value are associated with or predictive of response to intravenous thrombolysis. The contingent risk of post-thrombolytic haemorrhage in patients with versus without HAS remains controversial.

Limitations of this analysis

I had intended to perform a comprehensive systematic review of the HAS literature and to undertake a full meta-analysis. However, initial searches rapidly established that the broad scope of the topic and the amount of literature to be reviewed meant that this aspect of the project was not feasible. In order to complete the more rigorous original research sections of my work described in the chapters that follow, I restricted my investigation of prior HAS research to a largely narrative review.

2.2 Review of CT and MR Angiography use in Stroke

This review was commissioned by the British Journal of Radiology (BJR) in July 2013. The remit was to cover 'imaging of acute stroke prior to treatment', including 'current practice and evolving techniques' (advanced imaging). A large part of the review therefore discussed CTA and MRA in this context and is included below in a slightly adapted format. This narrative review was not based on a systematic literature search.

The final peer-reviewed published article, which also includes a discussion of non-contrast CT and CT/MR perfusion studies, is included as Appendix 3 (see Chapter 11.3.1):

> Mair G and Wardlaw JM. Imaging of acute stroke prior to treatment: Current practice and evolving techniques. *British Journal of Radiology* 2014;87:20140216

2.2.1 CTA & MRA for acute assessment of ischaemic stroke

CTA and/or MRA are used increasingly to assess arterial patency in patients with ischaemic stroke in addition to plain CT or MR brain tissue imaging. Two US based studies examined a nationwide dataset that captures 15% of all US hospitalisations from over 600 hospitals. From 2006 to 2011, both studies showed an increase in the use of CTA for assessing ischaemic stroke acutely. This was true for all ischaemic stroke patients (3.8% had CTA in 2006 versus 9.1% in 2010)[122] and for ischaemic stroke patients treated with intravenous thrombolysis (18% had CTA in 2008 versus 20% in 2011).[123] This trend for CTA use is likely to have increased further recently following a new recommendation from the American Heart and American Stroke Associations on endovascular intervention following ischaemic stroke[124] which are based on the recent highly positive results from several trials of endovascular therapy (see 1.4).[125] The new guideline states that:

> "Patients should receive endovascular therapy with a stent retriever if they meet all the following criteria [a.. b..] c. Causative occlusion of the ICA or proximal MCA [d.. e..]."

However, it is not explicitly stated how the presence of this occlusion should be determined.

CTA/MRA versus catheter angiography

Angiography performed with either CT or MRI is known to provide very accurate information of arterial flow when compared with the gold standard, catheter angiography.[90;91;126-129] The major limitation is that a standard CTA or MRA obtains imaging over a short time period (in the case of CT, this is only a few seconds) and therefore provides no information on the speed or direction of blood flow (information that can be especially important if collateral supply is being assessed). To incorporate this, four-dimensional CTA and MRA have been developed. These techniques reimage the field of interest at several time points following the injection of contrast or initiation of the stimulatory radio-frequency pulse. The resultant dynamic imaging provides flow information very similar to that obtained by catheter angiography.[130-132]

Grading CTA or MRA

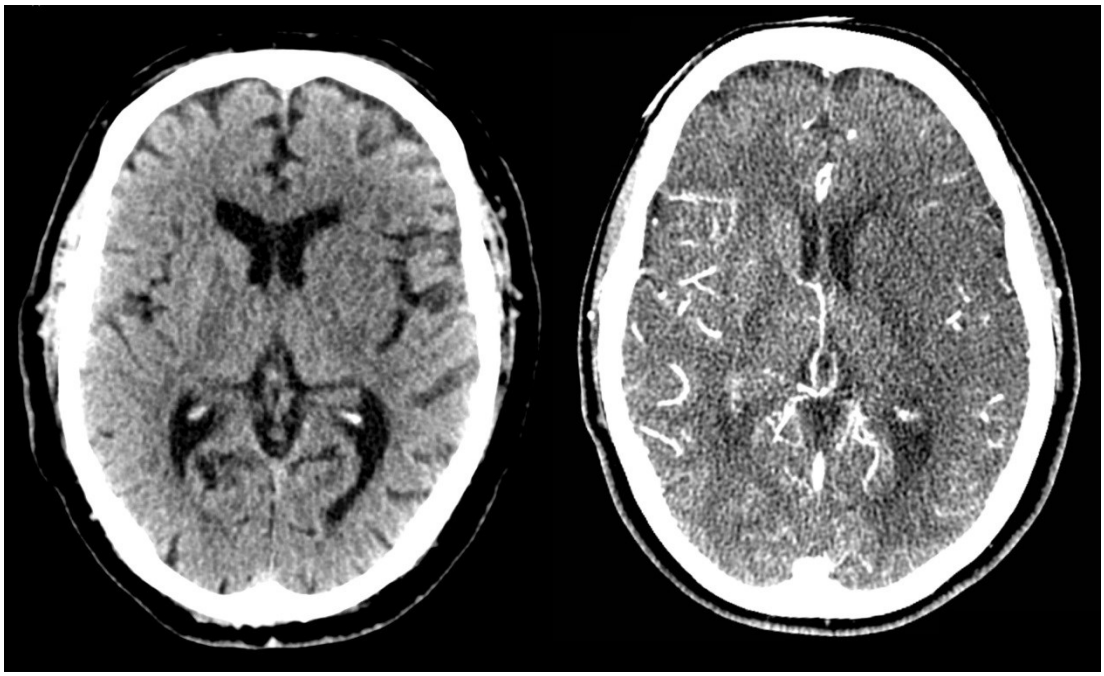
CT and MR angiography in stroke are commonly assessed using either the Thrombolysis in Cerebral Infarction (TICI) score or the Mori scale.[133;134] Both scales were however originally developed for the assessment of catheter angiography. When properly applied, these scales assess distal tissue perfusion in addition to focal arterial luminal narrowing and were developed to be used both in the initial assessment of an arterial occlusion and also to measure the extent of recanalisation following treatment, i.e. several different components of tissue blood supply are being conflated into one scoring system. Comparatively, standard CTA and MRA only allow interpretation of luminal narrowing or focal occlusion (unless they are performed in four-dimensions as described above) and therefore the standard catheter angiography scores are not wholly appropriate in this context.[135] Many of the studies that have used these scales for assessing CTA or MRA have therefore used modified (and non-standardised) versions.[136] Consequently, it is difficult to assess the wider application of angiography rating scales from the available literature; as recommended in a recent consensus group statement, greater standardisation is required.[137] The Acute Stroke Imaging Research Roadmap II aims

to do exactly that and has suggested use of their modified TICI scale for studies assessing CT and MR angiography.[138]

Additional data with CTA or MRA

A major benefit of performing angiography with CT or MRI rather than via an intra-arterial catheter is the additional brain parenchymal imaging that can simultaneously obtained. For a few extra minutes in the scanner, CTA may be preceded by a non-contrast brain CT while MRA may be enhanced with a few basic stroke sequences (e.g. FLAIR, DWI, T2*). In fact, even if angiography sequences were acquired in isolation, additional parenchymal information will still be available. For example, when the window settings of CT angiography images are reset to those of a standard non-contrast brain, a simple 'perfusion' study is achieved. Such use of the CTA source images (CTA-SI) has been shown to enhance the interpretation of non-contrast CT and CTA, providing results that are more equivalent to MR imaging in stroke by improving the sensitivity of CT for detecting infarct and predicting outcome, particularly among less experienced observers, Figure 2-4.[139-142]

Figure 2-4 *Comparison of visible acute ischaemic changes on standard non-contrast CT and CTA-source images*



Footnote: CT brain scans performed soon after the onset of severe left MCA territory ischaemia in different patients.

The image on the left is from a standard non-contrast CT; early ischaemic changes in the left MCA territory (right side of left image) are very subtle with only loss of normal grey-white matter contrast affecting the insular cortex and basal ganglia.

The image on the right is from a CT angiographic study (note hyperattenuating arterial vessels throughout) windowed to best display brain tissue; here, early ischaemic changes in the left MCA territory (right side of right image) are comparatively much easier to identify. In addition, reduced arterial perfusion can be appreciated on the affected side.

2.2.2 Practicalities of imaging arterial patency in stroke

It is estimated that during an acute stroke, approximately two million neurons, 14 billion synapses and 12 km of myelinated fibres are destroyed per minute, i.e. *time is brain*.[143] Therefore the time required for every intervention or investigation that occurs between the acute presentation of a stroke patient and the initiation of definitive reperfusion therapy needs to be justified and proven to provide benefit by improving outcome, while not causing undue harm. Inevitably, performing more scanning takes more time, increases radiation dose in CT or the risk of aspiration while supine in MRI, and reduces oxygen saturation.[144] Prior to the widespread

utilisation of advanced imaging in acute stroke therefore, several factors need to be considered.

Reader reliability

The first consideration is whether the information from imaging can be consistently and repeatedly extracted. There is variability in the interpretation of non-contrast CT for ischaemic stroke, especially in the identification of subtle parenchymal changes and potentially among less experienced observers. A systematic review of inter-observer agreement for the detection of stroke on non-contrast CT demonstrated kappa statistics in the range 0.14-0.78 for the identification of any relevant CT finding but the experience level of observers or their number was not clearly stated in all cases.[12] Two more recent observer reliability studies, including large numbers of observers (>200) with varying levels of experience and expertise, showed that while expert neuroradiologists were more likely than non-neuroradiologists to identify subtle stroke signs, the number of years of experience was less important.[145;146] Notwithstanding, such reader variability is the baseline upon which more advanced imaging techniques will build, i.e. advanced techniques should not significantly increase reader variability as this might diminish the value of performing those additional techniques in the first place.

In contrast, there is very little information on the observer reliability of angiographic imaging in stroke. One small study of 17 patients and four observers reported inter-observer agreement for the identification of arterial occlusion in acute stroke using MRA as kappa=0.78.[147] Another small study assessed the degree of chronic arterial stenosis in 25 patients using two observers and found better agreement using MRA (kappa=0.78) than CTA (kappa=0.51).[148]

Technical factors and delay

Other issues to consider relate to the technical aspects in implementing an advanced imaging technique. These include, a reasonable estimate of the average time each additional imaging study is likely to take, a measure of how consistently complex imaging is acquired by radiographic staff, the expected increase in radiation exposure for CT based modalities, and whether additional hardware or software are required for either the creation or interpretation of imaging (i.e. departmental extra costs).

Very little data exist to accurately quantify the extra time that is required for additional angiographic imaging following non-contrast CT in acute stroke. In general, advocates for these techniques suggest and imply that these extra sequences add only a few minutes to the basic imaging requirement, but rarely publish actual results. In reality, staff working in busy imaging centres are likely to have variable levels of experience and the efficiency of performing complex imaging is also likely to drop when performed out-of-hours. Additionally, angiographic imaging requires more time for image processing and for interpretation. It has been reported that CT angiography requires approximately 15-20 minutes for acquisition and interpretation, while a full stroke MR protocol can take more than 20 minutes.[149-152]

Compared with non-contrast scanning, the acquisition of more complex imaging requires greater planning and therefore more opportunity to introduce human error. For example, good contrast enhanced angiography requires images to be obtained during the peak of the arterial contrast bolus.

CT radiation dose

While radiation dose is rarely a concern when assessing acutely unwell patients with life threatening pathology, not all of those suffering from an acute stroke can be described as such. The radiosensitive corneas are especially at risk from too much radiation as these often lie directly within the x-ray beam when head CT is performed and can develop radiation induced cataracts in the medium term. Moreover, many stroke patients are of an age where the long term effects of too much radiation, i.e. development of a radiation induced malignancy, remains a concern. It should therefore be noted that a non-contrast CT brain requires a median total dose (including planning sequences) of approximately 2mSv (inter-quartile range 2-3 mSv). In comparison, a full stroke protocol CT (non-contrast CT, CTA and CT perfusion) requires a median dose of 14 mSv (inter-quartile range 9-20 mSv), i.e. equivalent to an additional five years of normal background radiation over non-contrast CT alone. At these doses, it is estimated that approximately one in every 2000 patients aged 60 years undergoing a stroke protocol CT will develop a radiation induced cancer; the estimated frequency is 2-3 times higher for younger patients.[153]

2.2.3 Association between CTA/MRA and acute stroke treatment and outcome

Compared with standard non-contrast imaging, angiography provides extra information on the arterial supply to the brain (such as whether there is arterial obstruction or occlusion, the location and extent of arterial obstruction/occlusion and if there is a collateral supply) but it is less clear whether this information should influence management decisions in acute stroke. It is worth considering how the results of CT or MR angiography might alter diagnosis, treatment decisions and ultimately, affect clinical outcome.

Observational studies

According to a number of non-randomised, single-centre retrospective studies, angiographic imaging might improve selection of patients with ischaemic stroke for intravenous thrombolysis but some of these results are conflicting and the overall picture is non-conclusive. A selection of these results is provided here. In one non-randomised study of 188 patients with occlusion on CTA, those given intravenous thrombolysis were more likely to be independent at six months (35% treated versus 17% not given thrombolysis, p=0.031).[154] In two studies comparing proximal versus distal MCA occlusions (n=71 and n=225), both studies found proximal occlusion to be independently predictive of poor functional outcome at three months.[155;156] One small cohort (n=47) found absence versus presence of occlusion to be an independent predictor of independence at day seven (odds ratio, OR=6.8, 95% confidence interval, 95%CI=1.3-34.6, p=0.02).[106] Another analysis comparing those with and those without occlusion on CTA (n=168 and n=119, respectively) found no difference in three month outcome whether intravenous thrombolysis was given or not (p=0.94).[157] Two non-randomised studies assessed only patients without arterial occlusion on imaging. One found better outcomes (OR=3.79, 95%CI=2.04-7.02, p<0.01) for those given intravenous thrombolysis (n=103) versus those not treated with thrombolysis (n=153)[158] while the other (n=99) found no association between use of intravenous thrombolysis and outcome.[159] A large (n=654) retrospective observational stroke registry meanwhile observed a non-significant trend for more favourable clinical outcome after intravenous thrombolysis in patients with intracranial arterial occlusion versus those with no/minimal obstruction in whom it

was unclear if intravenous thrombolysis was beneficial or not.[160] Less extensive arterial occlusion/obstruction has been shown to independently predict reperfusion following intravenous thrombolysis (OR=1.35, 95%CI=1.14-1.58, p=0.001, n=178)[161] and presumably as a consequence of successful reperfusion, a favourable clinical outcome at 90 days (OR=1.56, 95%CI=1.12-2.18, p=0.009, n=85).[162] While in the presence of distal ICA (internal carotid artery) or proximal MCA occlusions, poor arterial collateral supply has been shown to predict poor outcome in moderately sized (n>200) cohorts and one study showed that multiple scoring methods were equally effective and provided comparable results.[163;164]

Randomised trials

Very few randomised-controlled trials of intravenous thrombolysis have included CTA or MRA to assess whether angiographic findings modify the treatment response. A pooled analysis of trials testing desmoteplase (a fibrin specific thrombolytic agent derived from vampire bat saliva) - DIAS (Desmoteplase In Acute Ischemic Stroke), DIAS-2 and DEDAS (Dose Escalation of Desmoteplase for Acute Ischemic Stroke) demonstrated that patients with complete arterial occlusion or severe obstruction had better outcomes after desmoteplase rather than placebo, but in patients with minimal obstruction or normal arteries, there was no significant difference between desmoteplase versus placebo and no significant difference between these two groups based on arterial patency (i.e. no interaction between arterial patency and thrombolysis).[165] A post hoc analysis of EPITHET (Echoplanar Imaging Thrombolytic Evaluation Trial) demonstrated better outcome amongst patients with MCA than ICA occlusion following intravenous alteplase, but they did not test for any alteplase-arterial patency interaction.[166]

2.2.4 What remained unclear after CT/MR angiography review

CT and MR angiography are being used more commonly in general clinical practice for the assessment of ischaemic stroke. While these techniques can provide an accurate representation of arterial flow to the brain, it is not clear if this knowledge improves diagnostic accuracy or how it should influence decisions on the use of intravenous thrombolysis for acute ischaemic stroke.

Unfortunately, CT and MRI angiography are limited by a lack of standardisation. Questions remain over how, when and in whom we should best acquire such imaging and how to grade and assess the myriad appearances. We need better data on how accurately angiography is scored by readers. It is important to understand more clearly the likely delays angiographic techniques will cost the average patient in a non-specialist imaging department. Without standardisation it is not possible to understand the impact of these technologies on stroke treatment. Specifically, we need to know whether angiographic occlusion or its absence should alter the decision to treat with intravenous thrombolysis. Similarly, it is unclear if the location, completeness and longitudinal extent of arterial occlusion need to be considered and whether we should also assess collateral supply. Much of this vital information is not currently available in a robust form, i.e. there is very little relevant randomised-controlled trial data to test for treatment interaction between angiographic findings and response to thrombolysis.

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Chapter 3 Third International Stroke Trial (IST-3)

The following details of IST-3 are provided only for context. Most of this work was completed before I joined IST-3 as a member of the angiography expert panel.

3.1 Background and Aims of IST-3

The Third International Stroke Trial (IST-3) was an international multicentre prospective randomised open-label blinded endpoint (PROBE) trial that tested intravenous thrombolysis (alteplase) versus control for ischaemic stroke presenting within 6 hours of symptom onset. IST-3 was devised to become the largest single trial of intravenous alteplase for ischaemic stroke.

The primary measure of outcome was the proportion of patients alive and independent at 6 months from randomisation in the alteplase versus control groups.[1]

At the time of trial planning, there was uncertainty over several specific treatment issues which IST-3 aimed to address:[2]

- Is alteplase of benefit to a wider variety of patients than that defined by current approvals, including:
    - Is alteplase effective between 3 and 6 hours from symptom onset?
    - Can alteplase be used safely and effectively in patients older than 80 years of age?
- Do early CT imaging features (both those associated with the acute ischaemic insult and background chronic changes) predict response to treatment with alteplase?
- Does alteplase affect stroke mortality?

Various subgroup analyses were therefore also pre-planned in the trial protocol[1] to address these (and other) specific uncertainties, see Table 3-1.

Table 3-1 *Pre-planned subgroup analyses for IST-3*

Analyses to be performed on the effect of treatment at six months among all those allocated alteplase versus control, subdivided by:
Time from symptom onset (0-3 versus 3-6 hours)
Age
Sex
Clinical stroke syndrome (OCSP classification, see 3.3)
Presence or absence of atrial fibrillation
Pre-randomisation brain imaging appearances (extent and severity of visible infarct, visible infarct versus not, presence of the dense artery sign [HAS], leukoaraiosis, atrophy)
Use of antiplatelet drugs at time of randomisation
Stroke severity (NIHSS, see 3.3)
Blood pressure at randomisation
Randomisation in a centre with prior experience of using intravenous thrombolysis
Randomisation during the double-blind start-up phase versus randomisation during the main (open label) phase

Footnote: Adapted from the IST-3 main trial protocol.[1] OCSP = Oxfordshire Community Stroke Project. NIHSS = National Institutes of Health Stroke Scale.

In addition, in IST-3 recruitment centres where standard practice included the acquisition of 'advanced' imaging (i.e. CT or MR angiography and/or perfusion imaging) for all patients presenting acutely with stroke, the trial allowed for these extra imaging datasets to also be submitted. Advanced imaging would not however be used within the main trial analysis. Instead, a pre-specified perfusion and angiography imaging sub-study was developed with the explicit aim of determining whether CT and MR perfusion or angiographic imaging can be used acutely to identify patients with ischaemic stroke more or less likely to benefit from intravenous alteplase.[3]

3.2 Ethics, Consent, Trial Registration and Funding

Ethical approval for IST-3 was granted by the Scotland A Multicentre Research Ethics Committee (MREC/99/0/78) and by all national and local ethical committees.

Written informed consent was obtained for all patients. In the event of patient debility too severe to allow an informed discussion, consent was obtained by proxy from family members either in person or over the phone, as appropriate.

IST-3 was registered with ISRCTN, an open access primary clinical trial registry: http://www.isrctn.com/ISRCTN25765518.

IST-3 was funded from a large number of sources; these are fully documented in Appendix 2 (see Chapter 11.2). The IST-3 main trial was primarily funded by the UK Medical Research Council (MRC, grant numbers G0400069 and EME 09-800-15). The IST-3 angiography and perfusion substudy was funded by the National Institute for Health Research (NIHR) Efficacy and Mechanisms Evaluation Panel (EME 08-43-52). Development of the Systematic Image Review System 2 (SIRS-2, an image analysis platform, see 3.4.2) was funded by the Edinburgh MRC Hub for Trials Methodology Research (G0800803).

3.3 Recruitment, Randomisation and Data Collection

IST-3 recruited patients from 156 hospitals in 12 countries. Recruitment for IST-3 ran from May 2000 to July 2011. Adult patients (18 years or older; importantly, there was no upper age limit) with acute stroke of any severity were eligible for recruitment into IST-3 when:

- There was no clear indication for treatment, i.e. they did not meet the prevailing license criteria for treatment with intravenous alteplase (first licensed for use in the European Union in 2002; administration was initially restricted to within 3 hours of stroke onset but this was increased to the current 4.5 hours in 2012)

- There was no clear contra-indication to treatment
- Both the treating doctor and patient were uncertain whether or not to give treatment to that patient (i.e. the uncertainty principle)
- Treatment could be started within 6 hours of known stroke onset
- CT or MR imaging had reliably excluded intracranial haemorrhage or any intracranial structural stroke mimic.

Demographic and baseline clinical data were collected and included: patient age and sex, witnessed time and date of stroke onset; time from stroke onset to baseline CT or MRI scan; time from stroke onset to randomisation; clinical stroke syndrome as defined using the Oxfordshire Community Stroke Project (OCSP) classification, i.e. one of - Total Anterior Circulation Infarct (TACI), Partial Anterior Circulation Infarct (PACI), Lacunar Circulation Infarct (LACI), or Posterior Circulation Infarct (POCI).[4]

The National Institutes of Health Stroke Scale (NIHSS) was used to assess stroke severity at onset: http://www.ninds.nih.gov/doctors/NIH_Stroke_Scale.pdf. NIHSS ranges from 0-42 where:

- 0 = No stroke symptoms
- 1-4 = Minor stroke
- 5-15 = Moderate stroke
- 16-20 = Moderate to severe stroke
- 21-42 = Severe stroke

Patients were randomly allocated to intravenous alteplase (rt-PA, recombinant tissue plasminogen activator, 0.9 mg/Kg) or open control. Treating clinicians used an automated telephone or secure online system to enter baseline data and obtain a randomised treatment allocation. Excepting the first 276 patients (initial double-blinded start-up phase), treatment was given open label, i.e. neither treating clinicians nor patients were blinded to treatment allocation. Trial design was changed from double-blinded to open label due to cessation in the supply of placebo.

Follow-up clinical data were primarily collected at 7 days and 6 months for assessment of the primary trial outcome. When patients were uncontactable at any time point, efforts were made to determine if they had died. Longer term follow-up was carried out at 18 months for all survivors and at 3 years for patients from the UK and Scandinavia (Sweden and Norway). Data collected at 7 days included: symptomatic intracranial haemorrhage (SICH); recurrent ischaemic stroke; neurological deterioration due to brain swelling post infarct; extra-cranial haemorrhage and death. At 6 months functional outcome was assessed by the Oxford Handicap Scale (OHS) administered by postal questionnaire or telephone interview; those conducting telephone interviews were blinded to treatment allocation.[5] OHS is similar to the modified Rankin Scale (mRS, see 1.7.2)[6] and ranges from 0-6 where:

- 0 = Normal
- 1 = Minor symptoms not restricting lifestyle
- 2 = Minor handicap with some restrictions but independent
- 3 = Moderate handicap, unable to lead a totally independent existence
- 4 = Moderate-severe handicap, unable to live independently but does not require constant attention
- 5 = Severe handicap requiring constant attention day and night
- 6 = Death.

At 18 months from randomisation, OHS was repeated and quality of life was measured for survivors using the EuroQol instrument.[7;8] At 3 years from randomisation, survival was assessed in the UK, Norway and Sweden.

3.4 Imaging

3.4.1 Imaging protocol

Prior to recruiting patients into IST-3, receiving centres had to submit test scans to ensure their routine image quality was adequate, that the trial imaging protocol standards could be met and to test file transfer and image handling processes. All imaging was sent to the central trials office for quality control and validation.

Minimum standards were provided for all imaging acquisition to ensure scans were of sufficient quality but without being too restrictive so that most departments did not need to change their imaging protocols.

The IST-3 protocol required that CT scans should cover the entire brain from the foramen magnum to the vertex, with maximum slice thickness 4-5mm through the posterior fossa and 8-10mm for the cerebral hemispheres, with no slice gap. Thinner slices and spiral scans were also acceptable; given that trial recruitment lasted for11 years, scanning technology changed considerably during this time and thin-slice CT became more common as the trial progressed.

MRI scans had to include, as a minimum, a gradient echo sequence (T2*) to exclude haemorrhage, Diffusion Weighted Imaging (DWI) to identify recent infarct and T1 & T2/FLAIR to assess for structural abnormality, white matter lesions or old infarcts.

More specific advisory minimum standards were provided for centres proposing to include CT or MR angiography,[3] Table 3-2.

Table 3-2 *Advisory minimum standards for a) CT and b) MR angiography acquisition*

a) CT Angiography	
Kvp	100
mAs	120
Contrast (volume/type/rate)	50 ml Omnipaque 300 at 4 ml/sec
Flush (volume/type/rate)	40 ml saline at 4 ml/sec
Delay	15 secs
Coverage	Circle of Willis (upwards)
Slice collimation	0.75 mm
Pitch	1.25

b) MR Angiography	
Sequence	3D TOF 2 slab HR
TR (ms)	23
TE (ms)	2.7
Flip angle	20°
Locs / slab	32
Slice thickness	1.6
Slice gap	0
Matrix	320 x 224
Φ FOV	1
FOV	16 cm
Slice orientation	Straight axial
Tscan	5:46

Footnote: TOF = Time of flight

Follow-up brain imaging was obtained for all patients where possible. It was expected that follow-up imaging, either CT or MRI, would be obtained at 24-48 hours from stroke onset to the same protocol as for baseline imaging. However scans performed earlier due to clinical deterioration were also accepted. All brain imaging for each patient performed within 7 days of stroke onset was to be sent to the IST-3 central office. Follow-up imaging was not performed if patients were deemed too unwell at the proposed time of scanning.

3.4.2 Image analysis

All imaging in IST-3 was collected centrally as DICOM (digital imaging and communications in medicine) where possible; cut film was collected if digital imaging was not available for transfer. Cut film was later redigitised. All imaging was then assessed centrally by experts at reviewing brain imaging in stroke. Expert assessment was performed masked to patient demographics, clinical data and any other imaging, i.e. those assessing baseline imaging were not given access to follow-up imaging and vice versa, and for the main IST-3 trial, expert readers were not given access to concurrently acquired angiography (or perfusion) data. For assessment of imaging data in the IST-3 angiography substudy, expert readers were given simultaneous access to both plain and angiographic imaging when it was acquired concurrently.

Two centralised expert imaging assessment panels, each of ten readers were constructed; one for the main IST-3 trial and one for the angiography substudy (see Appendix 1, Chapter 11.1.1 and 11.1.2, respectively). Expert panel assessments of imaging for the main trial and for the angiography substudy were performed independently. Imaging assessment for the main trial occurred in line with recruitment from the outset of IST-3 until the 27th March 2012. Expert panel assessments of imaging for the angiography substudy occurred between 25th April 2012 and 8th July 2013, i.e. there was no overlap, but angiography subgroup plain scans were read by both expert panels.

All image analysis for IST-3 was performed using an in-house image viewing platform compatible with both DICOM and all redigitised imaging, entitled the Systematic Image Review System, SIRS. SIRS was developed for IST-3 and tested in a prior analysis of CT reader reliability in stroke comprising many readers from different international locations.[9;10] The system was later developed further; SIRS-2 also allowed for the assessment of angiographic and perfusion imaging in addition to standard CT and MR imaging, see: http://sirs2.neuroimage.co.uk/sirs2/. SIRS-2 allowed readers to review scans online via a standard web browser. Readers could only access those scans which had been allocated to them, i.e. appropriate blinding was assured. SIRS-2 allowed users to display two allocated scans side by side and to perform all standard image manipulations (e.g. zoom, pan, scroll), but users could not create multiplanar reformats. Plain CT scans were presented with the following default settings but these could be changed to suit user preferences: windowed on 80 Hounsfield Units (HU) width and centre level of 35-40 HU.

Questionnaires were developed and validated for the expert assessment of CT and MR imaging in IST-3. These questionnaires included scoring for both acute (imaging features representing ischaemia; secondary brain swelling; hyperattenuated arteries – HAS; presence, completeness and extent of angiographic obstruction or occlusion; arterial collateral status on angiography; brain tissue 'perfusion' on CTA source images – CTA-SI; haemorrhage) and chronic brain changes (atrophy, leukoaraiosis, old stroke lesions, non-stroke lesions); see Appendices 9 and 10 for all available CT and MRI scoring options; Chapters 11.4.1 and 11.4.2, respectively. Table 3-3 summarises the questions and available selection options relevant to assessment of HAS and also CT and MR angiography as the main points of interest in this thesis. The SIRS-2 platform displayed imaging and the appropriate questionnaire on the same screen; readers could view imaging and submit their interpretation of imaging findings simultaneously (Figure 3-1). Reported image findings were then immediately and securely stored in a central database.

Table 3-3 *Questions presented to SIRS-2 users for assessing the hyperattenuating artery sign on plain CT and for assessing CT/MR angiography*

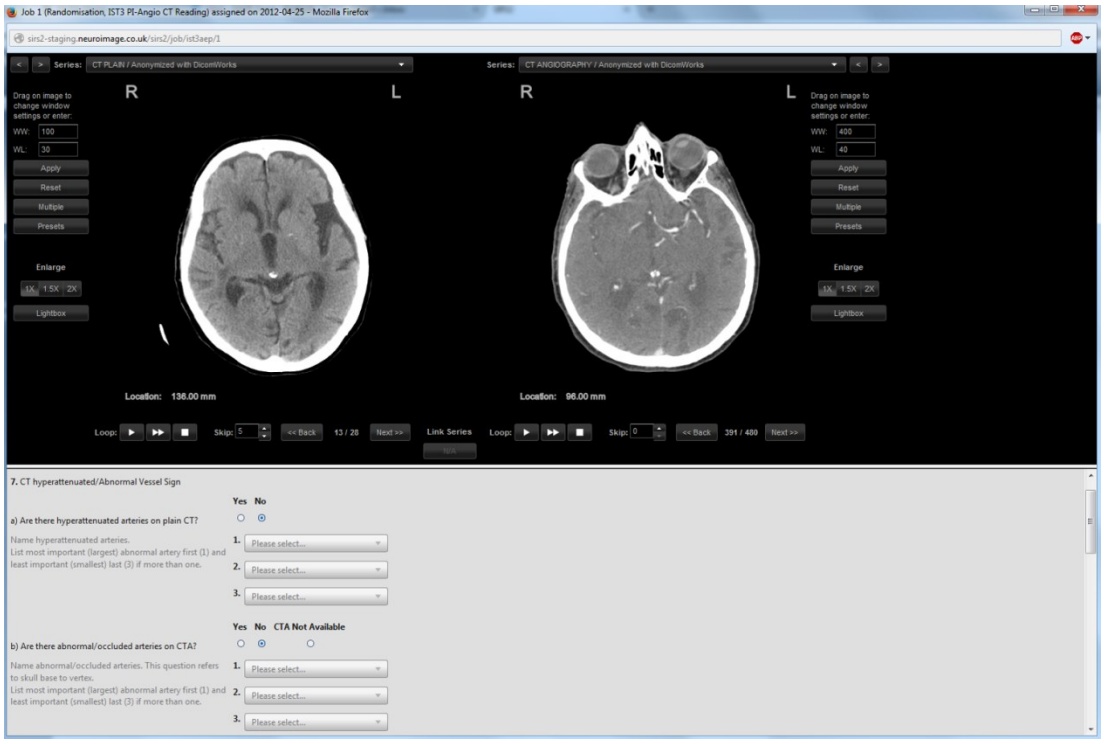
Question	**Options**
Is there a hyperattenuated artery on plain CT?	Y or N
Is there an abnormal/occluded artery on CTA/MRA?	Y or N
Name abnormal artery if 'Y' to either of above, indicate which artery(ies). List most important (largest) abnormal artery first (1) and least important (smallest) last (3) if more than one.	1) ICA 2) MCA stem 3) MCA Sylvian branch 4) PCA 5) ACA 6) BA 7) VA 8) Other
Thrombolysis in Cerebral Infarction (TICI) score[11]	0) No flow/patency 1) Minimal flow/patency 2a) Partial flow/patency <50% of expected territory 2b) Partial flow/patency >50% of expected territory 3) Complete flow/patency
IST-3 Angiography Score[3]	0) No patency 1) Minimal patency – some contrast penetrates obstruction but no/minimal enters distal artery 2) Patency of <50% of the lumen and some filling of branches of the distal artery 3) Patency of >50% of the lumen and filling of most branches of the distal artery 4) Complete patency – normal artery
Clot Burden Score[12]	From a total score for normal arteries of 10, two points are subtracted for thrombus found on angiography in the supraclinoid ICA and each of the proximal and distal halves of the MCA trunk. One point is subtracted for

	thrombus found in the infraclinoid ICA and A1 segment and for each affected M2 branch.
In occluded ICA/MCA only: Score for collateral status[13]	A 'Good' = entire MCA distal to the occluded segment reconstituted with contrast B 'Moderate' = if some of the MCA branches reconstituted within the Sylvian fissure C 'Poor' = if only the distal superficial MCA branches reconstituted with contrast
Is there are area on CTA-SI of brain tissue contrast enhancement deficit?	Y or N

Footnote: This is an abridged version of the IST-3 image scoring proforma; see Appendices 9 and 10 for the full CT and MRI questionnaires, respectively.

ICA = Internal Carotid Artery, MCA = Middle Cerebral Artery, PCA = Posterior Cerebral Artery, ACA = Anterior Cerebral Artery, BA = Basilar Artery, VA = Vertebral Artery. CTA-SI = CTA source images.

Figure 3-1 *Screenshot from SIRS-2 displaying simultaneous image viewing and image rating options for expert readers*



3.5 Storage and Management of Data

All clinical and imaging datasets in IST-3 were checked for quality and consistency, catalogued and de-identified prior to being entered into final databases for analysis and storage.

De-identification included removal of DICOM headers as appropriate. For each patient, identifiable data was replaced with a unique IST-3 participant number allocated chronologically from IST30001 as patients were enrolled. The de-identification key was stored in a separate database from the main trial data and only a few individuals had access to this key.

All data were stored centrally within University of Edinburgh servers with strict user access control and in full compliance with the University of Edinburgh Research Data Management Policy: http://www.ed.ac.uk/information-services/about/policies-and-regulations/research-data-policy.

In accordance with the UK MRC recommendations for sharing of individual patient level data from publically funded clinical trials,[14] the IST-3 collaborative group has lodged an anonymised version of the clinical dataset (this does not currently include DICOM or redigitised imaging but does include expert panel assessments of imaging) with Edinburgh DataShare: http://datashare.is.ed.ac.uk/handle/10283/1931. This will enable interested bona fide researchers to apply for direct access to IST-3 data.[15]

3.6 Statistical Analysis Plan

In order to be completely transparent over data analysis, the IST-3 Collaboration published a statistical analysis plan for the trial shortly before the final data set was locked, before data analysis commenced and therefore before the IST-3 investigators were un-blinded to the results.[16] By this means there was no risk of introducing analysis bias where researchers might, even inadvertently, manipulate the analysis strategy to produce the most favourable results.

Analyses were performed by intention to treat. That is, patients were included in the treatment group they were randomised to for all analyses, even if their treatment subsequently changed for clinical or technical reasons (for example, if a control patient eventually received intravenous alteplase outside of the trial or if there was failure to administer intravenous alteplase to a patient allocated to the treatment arm of the trial).

For assessment of the primary measure of outcome (proportion alive and independent six months after randomisation), a simple two-by-two analysis (crossing outcome dichotomy with treatment allocation) was planned in keeping with previous randomised-controlled trials of intravenous alteplase. This analysis was performed both with and without adjustment for: patient age; NIHSS; time from stroke onset to randomisation; and presence (versus absence) of signs of acute ischaemia on the baseline scan. In addition, a further analysis was planned using ordinal logistical regression analysis with OHS as the dependent variable.[16]

IST-3 was powered to deliver definitive results on the efficacy of alteplase in this context. The initial recruitment target was 6000; at this level there was 80% power to detect a 3% difference. However the recruitment target was reduced to 3100 in 2007, due to lower recruitment rates than expected; at this level there was 80% power to detect a 4.7% difference.[1]

3.7 Major IST-3 Results and Impact

In total, IST-3 recruited 3035 patients. The main IST-3 results were published in 2012. For the primary measure of outcome, a greater proportion of patients were alive and independent at six-months in the treatment versus control groups (554/1515 = 37% versus 534/1520 = 35%, respectively), but this difference was not significant (p=0.181). However, when OHS was analysed as a scalar variable rather than dichotomously, i.e. using ordinal regression analysis, patients in the treatment group had significantly better outcomes; adjusted odds ratio, OR = 1.27 (95% confidence interval, 95%CI = 1.10-1.47, p=0.001).[17]

Secondary analyses showed that while more deaths occurred in the first 7 days among patients treated with alteplase compared to controls, there were correspondingly fewer deaths in the treatment group over the following 6 months such that in total, death rates were the same in both groups (27%). IST-3 demonstrated better outcomes for patients treated within 3 hours (OR = 1.64, 95%CI = 1.03-2.62) but was underpowered to detect a significant difference in treatment effect between patients treated <3 hours versus >3 hours (p value for interaction = 0.613). Compared with prior trials, IST-3 has a large proportion of patients >80 years old (1617/3035 = 53%). Contrary to conventional wisdom at the time, IST-3 showed a greater benefit for alteplase among patients >80 years old compared to those <80 years old (p=0.029).[17]

After publication, the main IST-3 results were included in two systematic reviews of tabular randomised-controlled trial data of thrombolytics for the treatment of ischaemic stroke. The first comprised 12 trials testing intravenous alteplase and included 7012 patients (i.e. IST-3 contributed 43% new data).[18] The second was an

update of the Cochrane Collaboration systematic review and comprised 27 trials and included 10187 patients (IST-3 contributed 30% new data) testing one of five different thrombolytics (urokinase, streptokinase, alteplase, recombinant pro-urokinase or desmoteplase) delivered either intravenously or intra-arterially.[19] Both reviews reached the same main conclusion: that despite the early risk of haemorrhage, thrombolytics were of significant net benefit for the treatment of ischaemic stroke and 1) increased the odds of being alive and independent at final follow-up (OR = 1.17, 95%CI = 1.06-1.29, p=0.001)[18] or 2) reduced the odds of being dead or dependent 3 to 6 months after stroke (OR = 0.85, 95%CI = 0.78-0.93, p=0.0002).[19]

Longer follow-up of IST-3 patients at both 18 months and 3 years from randomisation showed continued benefit for treatment with alteplase. At 18 months, the favourable shift in OHS for the treatment group remained significant, i.e. treated patients were still more likely to have better functional outcome on ordinal regression analysis (adjusted OR = 1.30, 95%CI = 1.10-1.55, p=0.002). In addition and perhaps as a consequence, treated patients self-reported a significantly better quality of life at 18 months using the EuroQol instrument (adjusted mean difference = 0.06, p=0.019).[20] At 3 years, patients treated with alteplase were less likely to have died (453/967 = 47%) than controls (494/979 = 50%), risk difference 3.6%, 95%CI = -0.8-8.1.[21]

A secondary analysis of the expert panel assessment of IST-3 imaging tested whether evidence of early ischaemia or pre-existing structural changes on baseline brain imaging affected the response to alteplase and whether there were any associations between baseline brain imaging and 6-month outcome.[22] Although several imaging characteristics (tissue hypoattenuation on CT, a large lesion, swelling, a hyperattenuated artery, old infarct, atrophy and leukoaraiosis) independently predicted symptomatic intracranial haemorrhage and/or poorer outcome in IST-3 (adjusted for age, NIHSS, time to randomisation and treatment allocation), none of the tested imaging characteristics (individually or combined) modified the effect of alteplase on symptomatic intracranial haemorrhage risk (p-value 0.082 to 0.568) or likelihood of achieving a good functional outcome (p-value 0.237 to 0.960). In other

words, the treatment and control groups in IST-3 did not differ significantly in their risk of symptomatic haemorrhage or likelihood of a better functional outcome for patients with and without any of the specific imaging characteristics assessed.[22]

3.8 IST-3 Data Used Within this Thesis

Much of the work contained within this thesis is based on analysis of IST-3 patient data. In addition to the complete IST-3 trial dataset (n=3035) I also used two overlapping subsets:

1. All IST-3 patients with non-contrast CT performed at baseline (pre-randomisation)
2. All IST-3 patients with CT or MR angiography performed at baseline.

For a small number of IST-3 scans (18 baseline and 55 follow-up), imaging was not received centrally and could therefore not be included in any analyses based on central expert reads (in these cases, the main trial analysis was based on image interpretation provided by local experts). In addition, 50 patients were too unwell (13) or had died (37) at the proposed time of follow-up scanning and so never had a repeat scan.

Most centrally reviewed brain imaging in IST-3 was non-contrast CT: at baseline 2961/3035 (97.6%) scans were non-contrast CT and 56/3035 (1.8%) were MRI; at 24-48 hour follow-up 2779/2985 (93.1%) scans were non-contrast CT and 151/2985 (5.1%) were MRI. In total, 2731 (90.0% of 3035) patients had centrally reviewed non-contrast CT performed both at baseline and follow-up.

IST-3 recruited 300 patients with baseline angiography: most were performed using CT (271/300, 90.3%) rather than MRI (29/300, 9.7%).

Table 3-4 compares demographic and clinical data for the entire IST-3 group (n=3035) with the baseline non-contrast CT (n=2961) and angiography (n=300) subgroups, respectively.

Table 3-4 *Demographic and clinical data for imaging subgroups in IST-3*

	Full IST-3 Group (n=3035)	**NCCT at Baseline (n=2961)**	**p-value for comparison full IST-3 & baseline CT groups**	**CT or MR Angiography at baseline (n=300)**	**p-value for comparison full IST-3 & angiography groups**
Age, years	81 (72-86)	81 (72-86)	0.736	80 (71-85)	0.510
Male Sex	1465 (48.3%)	1426 (48.2%)	0.952	132 (44.0%)	0.119
Mean Time Stroke Onset to Baseline Scan, minutes (SD)	164 (73)	163 (73)	0.707	180 (80)	<0.0001
Mean Time Stroke Onset to Randomisation, minutes (SD)	231(73)	231(73)	0.844	241 (80)	0.019
Time to Follow-up Scan, hours	26 hours (24-36)	26 hours (24-36)	0.869	25 hours (24-29)	0.006
NIHSS	11 (6-17)	11 (6-18)	0.821	9 (5-17)	0.003
OHS	4 (2-6)	4 (2-6)	0.850	3 (1-5)	0.001
Independent at 6 Months (OHS 0-2)	1088 (35.8%)	1053 (35.6%)	0.817	134 (44.7%)	0.001
Dead by 6 Months	815 (26.9%)	797 (26.9%)	0.956	67 (22.3%)	0.063
Treated with rt-PA	1515 (49.9%)	1484 (50.1%)	0.877	147 (49.0%)	0.738

Footnote: Results are n (%) or median (interquartile range), unless stated otherwise. NCCT = Non-contrast CT. SD = Standard Deviation. NIHSS = National Institutes of Health Stroke Scale (baseline). OHS = Oxford Handicap Scale (at 6 months).

There were no significant differences between IST-3 patients with non-contrast CT at baseline and the full IST-3 group (i.e. including those without centrally assessed imaging or MRI at baseline). Patients with non-contrast CT at baseline were well

balanced for age, sex, NIHSS and time from stroke onset to scan/randomisation between alteplase and control, with no significant differences between groups (Table 3-5a).

Patients with angiography had less severe strokes at baseline than those in the full IST-3 trial (median NIHSS 9 versus 11 respectively, p=0.003), better six-month outcomes (median OHS 3 versus 4 respectively, p=0.001), had baseline scanning later (mean 180 minutes versus 164 minutes respectively, p<0.0001) and were randomised later after stroke onset (mean 241 minutes versus 231 minutes for the full IST-3 trial, p=0.019). There was no significant difference between patients with angiograms versus the full IST-3 trial for patient age, sex or treatment allocation. Patients with angiograms were well balanced for age, sex, NIHSS and time from stroke onset to scan/randomisation between alteplase and control, with no significant differences between groups (Table 3-5b).

Table 3-5 *Comparison of alteplase and control groups:*

a) IST-3 patients with non-contrast CT at baseline (n=2961)

	Alteplase (n=1484)	**Control (n=1477)**	**p-value for Comparison**
Age, years	81 (72-86)	81 (72-86)	0.639
Male Sex	718 (48.4%)	709 (48.0%)	0.836
Mean Time Stroke Onset to Baseline Scan, minutes (SD)	163 (72)	163 (72)	0.773
Mean Time Stroke Onset to Randomisation, minutes (SD)	231 (73)	231 (72)	0.902
NIHSS	11 (6-18)	11 (6-17)	0.995
OHS	3 (2-6)	4 (2-6)	0.072

b) IST-3 patients with CT or MR angiography at baseline (n=300)

	Alteplase (n=147)	**Control (n=153)**	**p-value for Comparison**
Age, years	81 (73-86)	80 (69-85)	0.274
Male Sex	63 (42.9%)	69 (45.1%)	0.697
Mean Time Stroke Onset to Baseline Scan, minutes (SD)	184 (82)	176 (78)	0.344
Mean Time Stroke Onset to Randomisation, minutes (SD)	243 (82)	238 (79)	0.584
NIHSS	10 (5-17)	9 (5-16)	0.603
OHS	3 (1-5)	3 (1-5)	0.726

Footnote: Results are n (%) or median (interquartile range), unless stated otherwise. SD = Standard Deviation. NIHSS = National Institutes of Health Stroke Scale (baseline). OHS = Oxford Handicap Scale (at 6 months).

3.9 References for Chapter 3

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Chapter 4 General Methods

4.1 Overview of Analyses Undertaken in Pursuit of Thesis Aims

Most of the work in this thesis focusses on imaging arterial patency using CT rather than MRI since CT is far more frequently used in routine practice for the hyperacute initial imaging assessment of the brain in stroke and consequently was the predominant imaging modality collected in IST-3.

Work undertaken to address thesis aims was based on both newly collected data and assessment of existing data as follows:

Collection and assessment of new data

- In IST-3 patients with baseline angiography, I reviewed imaging to identify, classify and measure the attenuation of arterial obstructions on plain CT and correlated the location and extent of plain CT findings with angiographic appearances
- I derived sensitivity and specificity data of HAS for the identification of arterial obstruction from my assessment of IST-3 imaging and compiled these results in a systematic review and meta-analysis
- I arranged a multi-reader CTA observer reliability analysis using representative cases from IST-3

Assessment of existing data

- I analysed data from the IST-3 expert scan reading panel's assessment of baseline and follow-up CT imaging (see 3.4.2) to investigate the importance of HAS in relation to stroke severity, response to intravenous thrombolysis and outcome
- I analysed data from the IST-3 expert scan reading panel's assessment of baseline CT and MR angiography (see 3.4.2) to investigate the importance of arterial obstruction and other angiographic appearances in relation to stroke severity, response to intravenous thrombolysis and outcome

4.1.1 Datasets used

As described in the previous Chapter (3.8), the following datasets have been used throughout in the production of this thesis and are defined here with reference to appropriate subchapters.

Main IST-3 trial data

This dataset represents all 3035 patients recruited into IST-3 and was used for establishing the representation of subgroups (Chapter 3.8).

Main IST-3 trial non-contrast CT data

This dataset represents the 2961 patients recruited into IST-3 with non-contrast CT at baseline and is used for analyses of HAS (Chapter 5).

IST-3 angiography subgroup

This dataset contains 300 patients who had CT or MR angiography routinely performed at baseline and is used for the analyses: quantifying the attenuation of HAS and other non-hyperattenuating arterial obstructions (Chapter 6); identifying the sensitivity and specificity of HAS as a marker of arterial obstruction (Chapter 7); and for all analyses of CT and MR angiography (Chapters 8 and 9).

Full details of these datasets and comparisons between the full trial and subgroups are presented in Chapter 3.8.

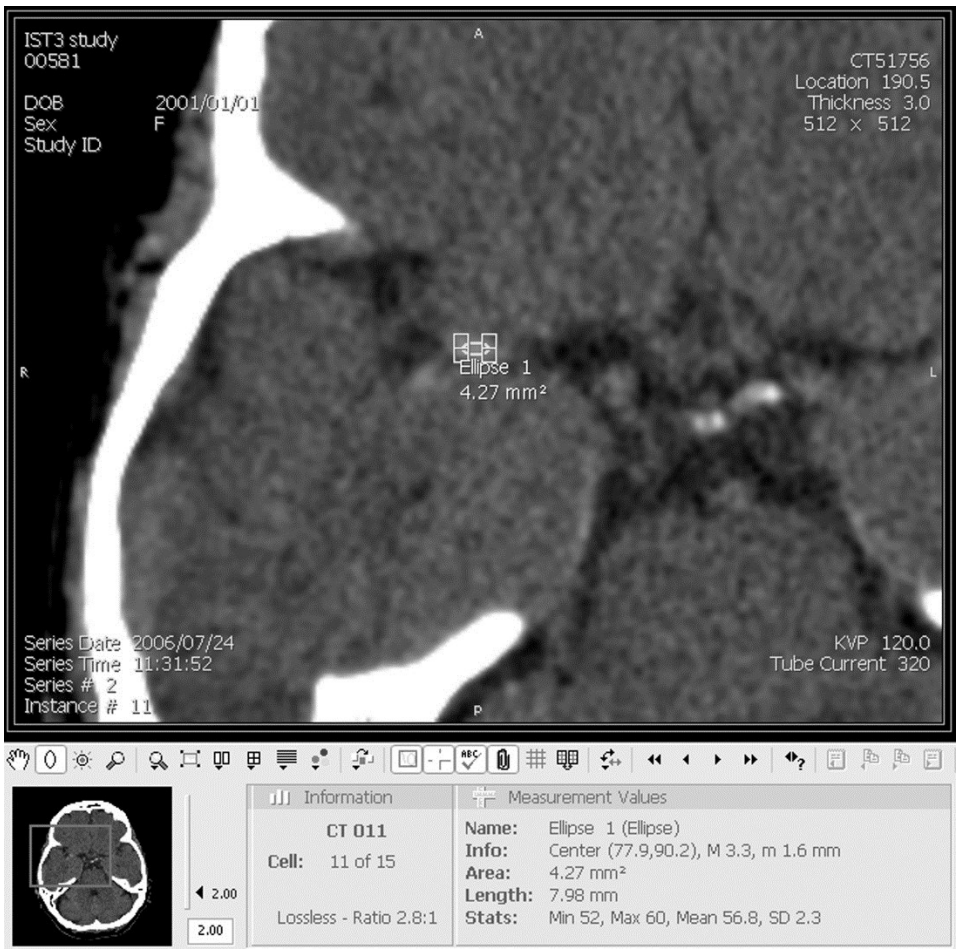
4.2 New Analysis of IST-3 Imaging

Image analyses discussed in this section are listed in the chronological order in which they were performed by me. Note that this order does not necessarily match the order of the thesis aims or therefore the sequence of the major research chapters of the thesis. This choronology was dictated so that at all times, IST-3 data were made available to me incrementally as needed to maintain reader blinding (especially for treatment allocation of individual patients) and to minimise the chance of introducing bias. The IST-3 data management team handled the provision of data.

First, I identified all patients in IST-3 for whom a baseline angiographic study was available (ideally performed within 30 minutes of the initial pre-randomisation CT scan). Both CT and MR angiography were deemed acceptable but since HAS is a CT sign, pre-randomisation plain imaging needed to include a CT.

For my analyses, I reviewed all imaging using *Digital Jacket* (V5.0, DesAcc Inc, Terre Haute, IN, USA) DICOM viewing software. Digital Jacket has a standard viewing window upon which basic measurement annotations can be applied (see Figure 4-1).

Figure 4-1 *Screenshot from Digital Jacket software demonstrating application of intra-arterial attenuation measurement (Ellipse 1 placed within the main branch of right middle cerebral artery) on plain CT*



I performed my image analysis in a similar manner to that undertaken by the IST-3 expert panels as described in the previous chapter (3.4.2) and I used the same image assessment questionnaires developed for IST-3 (see Appendices 9 and 10, Chapters 11.4.1 and 11.4.2, respectively) although I selected only those questions relevant to my work. All data were stored in a spreadsheet (Appendix 11, Chapter 11.4.3).

Non-contrast CT

I reviewed the pre-randomisation non-contrast CT first in each case. Using the validated IST-3 CT assessment proforma (Appendix 9, Chapter 11.4.1) for standardisation, I assessed scans for: the location and extent of any acute ischaemia; the location and extent of any HAS; the extent of secondary oedema; the presence of other infarcts (old or new); and for the presence and extent of chronic brain changes (leukoaraiosis and atrophy). For each scan, I noted the DICOM stored date and time and assessed image quality (good, moderate or poor) based on patient position and the presence of any imaging artefacts (e.g. movement or beam hardening artefact).

I assessed extent of ischaemic change with the IST-3 ischaemia score[1], ASPECTS (Alberta Program Early CT Score),[2] and the 1/3 MCA concept (that is, to make a visual assessment of whether more than one third of the MCA territory is affected by ischaemia, this idea was first popularised following a post-hoc analysis of ECASS [European Cooperative Acute Stroke Study][3]); the IST-3 score covers all brain regions and ranges from 0 (normal scan) to 4 (very large volume of brain affected), see Table 4-1 . In addition, the IST-3 score contains coding for the location of affected brain. ASPECTS assesses only the middle cerebral artery (MCA) territory and normally ranges from 10 (normal) to 0 (entire territory affected); the anterior and posterior cerebral artery territories can be added to the standard ASPECTS giving a total normal score of 12 covering the entire cerebral hemisphere. If present, old infarcts were also assessed using the IST-3 ischaemia score.

Table 4-1 *The IST-3 Ischaemia Score*

Site	Subsite	IST-3 Ischaemia Score
Middle Cerebral Artery (MCA) Territory	Small cortical	1
	Basal ganglia (>2 x 2 x 2 cm)	2
	White matter adjacent to lateral ventricle (>2 x 2 x 2 cm)	2
	Anterior or posterior half of peripheral MCA territory not involving basal ganglia	2
	Anterior or posterior half of peripheral MCA territory involving part of the basal ganglia	3
	Whole of the peripheral MCA territory	3
	Whole of the peripheral MCA territory and part of the basal ganglia	4
	Whole of the MCA territory	4
Anterior Cerebral Artery (ACA) Territory	Up to half of ACA territory	1
	More than half of ACA territory	2
Posterior Cerebral Artery (PCA) Territory	Up to half of PCA territory	1
	More than half of PCA territory	3
MCA territory & up to half of ACA or PCA territories		3/4*
MCA territory & more than half of ACA or PCA territories		3/4*
Whole of MCA, ACA and PCA territories		4
Lacune in basal ganglia, internal capsule, internal borderzone, centrum semiovale, thalamus or brainstem		1
Anterior or posterior borderzone		1
Cerebellum	Small cortical	1
	Less than half of a hemisphere	2
	More than half a hemisphere	3

Brainstem	Small (e.g. less than half of pons)	1
	Extensive (e.g. pons & medulla)	2

Footnote: MCA = middle cerebral artery. ACA = anterior cerebral artery. PCA = posterior cerebral artery. This classification system was also used by the expert panel readers in IST-3.

* 3 if MCA component 1 or 2, 4 if MCA component 3 or 4.

I identified hyperattenuated arteries by applying the following criteria. HAS was considered to be present when:

- An intracranial artery was affected
- Intraluminal attenuation (to differentiate from vessel wall calcification, a common finding in the elderly) was clearly greater (i.e. brighter on imaging) than equivalent vessels or adjacent brain[4]

I classified HAS location by selecting the three largest vessels affected from a pre-defined list:

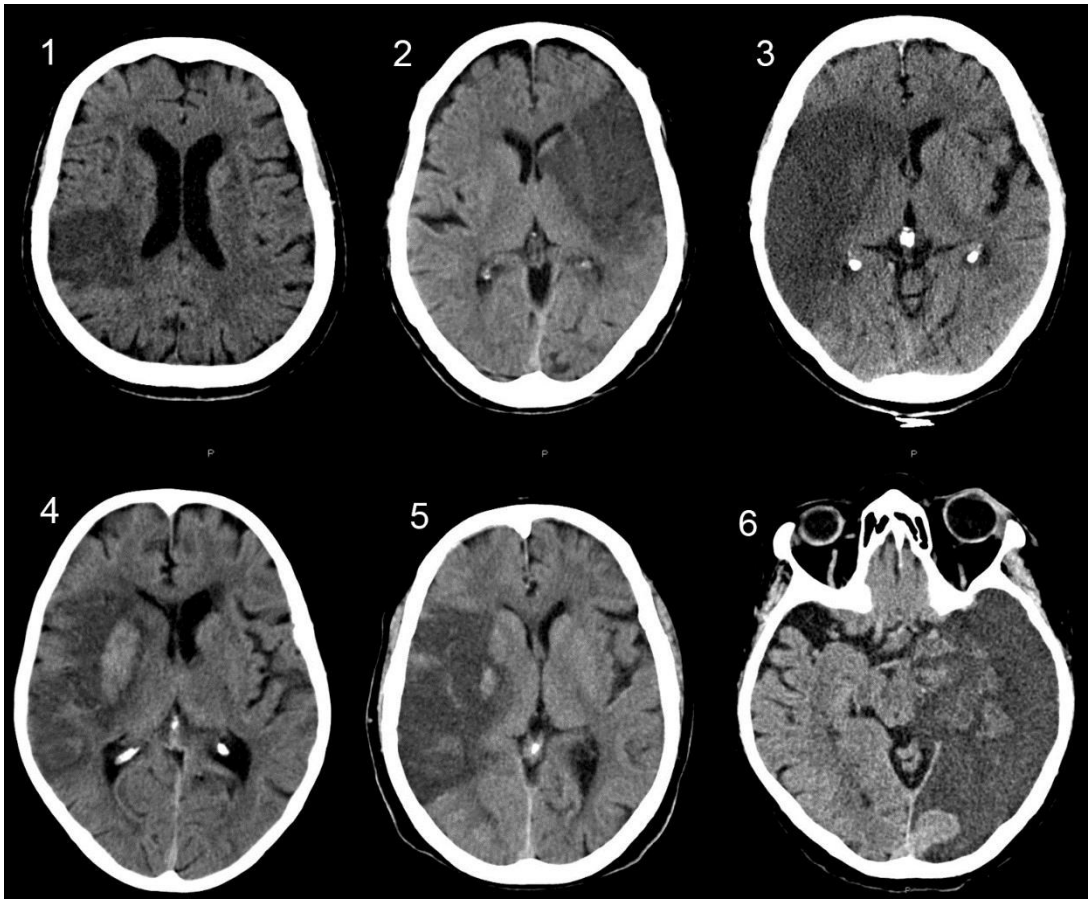
- Internal carotid artery (ICA)
- MCA main stem (horizontal segment on axial imaging, sometimes referred to as M1)
- MCA Sylvian or cortical branches (vertical branches on axial imaging, M2-M4)
- Vertebral artery (VA)
- Basilar artery (BA)
- Anterior cerebral artery (ACA)
- Posterior cerebral artery (PCA)

I assessed brain swelling using a validated scale that ranges from 0 to 6[1], Figure 4-2:

- 0 = no swelling
- 1 = effacement of the sulci overlying the infarct
- 2 = 1 + minor effacement of adjacent lateral ventricle

- 3 = 1 + complete effacement of lateral ventricle
- 4 = 1 + effacement of the lateral and third ventricle
- 5 = 4 + shift of the midline away from the side of the ventricle
- 6 = 5 + effacement of the basal cisterns

Figure 4-2 *Examples of the different grades of brain swelling as demonstrated on plain CT following infarct*



Footnote: Based on pre-defined brain swelling classification system also used by expert panel readers in IST-3.[1]

I then proceeded to take intra-arterial attenuation measurements from within all HAS and in up to three standard locations for each case: a proximal section of both the left and right MCAs and within the basilar artery. Where HAS was identified in one of the standard locations, only HAS measurements were recorded for that location. In

each location, using an attenuation measurement tool, three ellipses were drawn taking care to ensure that each ellipse was contained entirely within arterial lumen, i.e. to minimise the effect of arterial wall calcification on attenuation measures (Figure 4-1). I did not standardise ellipse dimensions but adapted these for each case to ensure best intraluminal fit. Where possible, overlap of the ellipses was avoided. Later, I calculated a mean intraluminal attenuation value from the three elliptical measurement values for each standard arterial location and for each HAS, if present.

CT or MR angiography

I only reviewed angiographic imaging after the corresponding non-contrast CT had been completely assessed; i.e. my assessments of HAS were performed blind to angiography appearances. In addition, I performed baseline imaging assessment blind to follow-up imaging. Several angiography measurement scales were used as detailed in the validated IST-3 angiography assessment proforma (Appendix 9 for CTA, Chapter 11.4.1 and Appendix 10 for MRA, Chapter 11.4.2) and listed in Table 3-3, including the IST-3 angiography score, the Thrombolysis in Cerebral Infarction (TICI) score, the Clot Burden Score and a collateral status score.[5-8] CTA source image review was also performed as previously described. For each CT angiographic scan, I noted the DICOM stored date and time and objectively assessed image quality (good, moderate or poor) based on timing of arterial contrast bolus (i.e. not too early or too late) and scan coverage (ideally centred on the circle of Willis and of significant imaging volume to assess all major intracranial arteries). Later, I subtracted the date and time of non-contrast CT from the date and time of CT angiography to calculate the extra time required for CT angiography in IST-3.

If I identified an angiographic obstruction in the absence of HAS, intra-arterial attenuation measures were then repeated retrospectively in an identical location on non-contrast CT using the technique as described above. Arterial locations were matched by viewing non-contrast CT and angiographic imaging side by side on the same screen at equivalent slice locations.

Follow-up imaging

I assessed follow-up non-contrast CT or MR imaging in the same manner as for baseline imaging but with knowledge of baseline imaging findings, i.e. non-blinded.

First, I noted the DICOM stored date and time so that an accurate time from baseline to follow-up scan could be calculated.

In all cases, I noted and scored the presence of subacute ischaemia or new infarct using both the IST-3 ischaemia score and ASPECTS. If haemorrhage was present, this was measured and classified (e.g. minor petechial or extensive haemorrhagic transformation of infarct, haemorrhage remote from infarct, etc.) as per the validated IST-3 scoring options for CT and MRI; Appendix 9 for CT, Chapter 11.4.1 and Appendix 10 for MRI, Chapter 11.4.2.

I noted whether HAS had completely resolved or persisted between baseline and follow-up non-contrast CT brain imaging. For patients with persistent HAS on follow-up imaging, I repeated intra-arterial attenuation measures as described above.

4.3 References for Chapter 4

(1) Wardlaw JM, Sellar RJ. A simple practical classification of cerebral infarcts on CT and its interobserver reliability. *AJNR Am J Neuroradiol* 1994; 15:1933-1939.

(2) Barber PA, Demchuk AM, Zhang J, Buchan AM. Validity and reliability of a quantitative computed tomography score in predicting outcome of hyperacute stroke before thrombolytic therapy. ASPECTS Study Group. Alberta Stroke Programme Early CT Score. *Lancet* 2000; 355(9216):1670-1674.

(3) von Kummer R, Allen KL, Holle R, Bozzao L, Bastianello S, Manelfe C et al. Acute stroke: usefulness of early CT findings before thrombolytic therapy. *Radiology* 1997; 205(2):327-333.

(4) Koo CK, Teasdale E, Muir KW. What constitutes a true hyperdense middle cerebral artery sign? *Cerebrovasc Dis* 2000; 10(6):419-423.

(5) Wardlaw JM, von Kummer R, Carpenter T, Parsons M, Lindley RI, Cohen G et al. Protocol for the perfusion and angiography imaging sub-study of the Third International Stroke Trial (IST-3) of alteplase treatment within six-hours of acute ischemic stroke. *Int J Stroke* 2015; 10(6):956-968.

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(8) Miteff F, Levi CR, Bateman GA, Spratt N, McElduff P, Parsons MW. The independent predictive utility of computed tomography angiographic collateral status in acute ischaemic stroke. *Brain* 2009; 132(8):2231-2238.

Chapter 5 Hyperattenuating Artery Sign

In this chapter, I use existing IST-3 data (expert panel assessment of non-contrast CT imaging, n=2961) to identify the prevalence of HAS in IST-3 and to quantify HAS location and extent. I also use these data to investigate relationships between HAS presence, stroke severity at baseline, response to intravenous alteplase and functional outcome at six months.

This chapter addresses **Thesis Aims 1) and 2).**

This work is also published in a peer reviewed journal, the final draft of which is included as Appendix 4, see Chapter 11.3.2:

> Mair G, von Kummer R, Morris Z, von Heijne A, Bradey N, Cala L, Peeters A, Farrall AJ, Adami A, Potter G, Cohen G, Sandercock PAG, Lindley RI and Wardlaw JM for the IST-3 Collaborative Group. Effect of alteplase on the CT hyperdense artery sign and outcome after ischemic stroke. *Neurology* 2016;86:1-8

5.1 Methods for Analysis of IST-3 Expert Panel Image Assessment Data

HAS prevalence was calculated by identifying all patients with HAS in any intracranial location visible on baseline imaging. Using the largest vessel affected, HAS location was classified as proximal (ICA, main stem MCA, VA, BA) or distal (Sylvian branch of MCA, ACA, PCA). HAS extent was classified by the number of contiguous named vessels involved (0, 1, 2 or 3 as per the pre-defined options listed previously in Table 3-3).

Baseline and follow-up scans were compared in those who had non-contrast CT performed in both instances. The absolute change in HAS segment number from -3 to +3 was calculated (negative numbers indicate shrinkage and positive numbers indicate growth of HAS). This new variable was then used to define post-hoc subgroups for patients with HAS as follows: (a) Persisting HAS, i.e. unchanged, (b)

Disappearing HAS, i.e. completely disappeared, (c) New HAS, (d) Growing HAS, and (e) Shrinking HAS.

Patients with any evidence of HAS on baseline scan were compared with those without HAS at baseline. Associations were sought between the presence, location, extent and persistence of HAS, stroke severity at onset (NIHSS), six-month functional outcome (OHS) and treatment with alteplase (alteplase versus control).

5.1.1 Statistical testing

Univariate analyses

Simple t-tests were employed to compare ratios and to compare the means of normally distributed continuous data. The medians of non-parametric continuous data were compared using a Mann-Whitney U test. Odds ratios, OR (with 95% confidence intervals, 95%CI) and chi-square statistics (χ^2) were used to compare nominal and dichotomous data.

Multivariable analyses

Ordinal regression methods[1] were used to calculate common ORs for dependent variables: OHS at six months and *change in HAS segment number*.

To stabilise ordinal regression estimates, I used the same approach as the original IST-3 analysis of functional outcome, where the more severe grades of OHS (4-6) were grouped as one, leaving 5 groups for ordinal analysis (0, 1, 2, 3 and 4-6).[2] Similarly, several other variables were stratified: *time from stroke onset to baseline scan*, was grouped into 6 one-hour windows (0-60, 61-120, 121-180, 181-240, 241-300 and 301-360 minutes); the variable *time from baseline scan to follow-up scan* was grouped into 5 twelve-hour windows (<=12, 13-24, 25-36, 37-48 and >48 hours); and *change in HAS segment number* was grouped into three outcomes (fewer segments = shrinkage, no change, more segments = growth).

Statistical software

Comprehensive Meta-Analysis software (Biostat, Englewood, NJ, USA) was used to compute summary ORs and to perform tests of interaction between treatment groups.

All other analyses were performed using IBM SPSS Statistics software, version 20.0 (IBM Corporation, Armonk, NY, USA).

A p-value <0.05 was considered significant.

5.2 Prevalence, Location and Extent of HAS in IST-3

Prevalence of HAS

Among 2961 patients in IST-3 with NCCT at baseline, HAS was present in 716 (24.2%) and absent in 2245 (75.8%) according to the expert panel assessment of imaging, Table 5-1.

Similarly, expert readers independently identified HAS on 531 of 2779 (19.1%) NCCT at follow-up (HAS data were not available for 1 follow-up NCCT scan).

The total number of IST-3 patients with NCCT performed at baseline and follow-up was 2731. Of these, 870 patients had HAS on at least one of their scans.

Location and extent of HAS

A proximal location for HAS (ICA, horizontal segment of MCA, VA or BA) was most common, found in 534 IST-3 patients (74.6% of those with the sign) at baseline. HAS exclusively affected a distal vessel in 182 patients (25.4% of those with the sign) at baseline.

Most HAS affected only a single vessel segment at baseline (589, 82.3% of those with the sign); having 2 or 3 affected vessel segments was progressively less common.

Table 5-1 *Prevalence, location and extent of HAS in IST-3 on baseline CT*

Hyperattenuating Artery Sign Characteristics		**Baseline CT n=2961**
Prevalence		716 (24.2)
Location	Proximal Vessels Only	430/716 (60.1)
	Distal Vessels Only	182/716 (25.4)
	Proximal and Distal Vessels	104/716 (14.5)
Extent	1 Segment	589/716 (82.3)
	2 Segments	115/716 (16.1)
	3 Segments	12/716 (1.7)

Footnote: Results are n (%).

5.3 Effect of HAS on Stroke Severity, Six-Month Outcome and Response to Intravenous Alteplase in IST-3

Comparison of those with and without HAS at baseline

Patients in IST-3 with HAS on baseline CT were slightly younger than those without HAS (median 80 versus 81 years, p=0.003). Patients with HAS had a more severe stroke at baseline (median NIHSS 16 versus 9, p<0.001) and worse six-month outcomes (median OHS 5 versus 3, p<0.001; 37% versus 24% dead, p<0.001), Table 5-2.

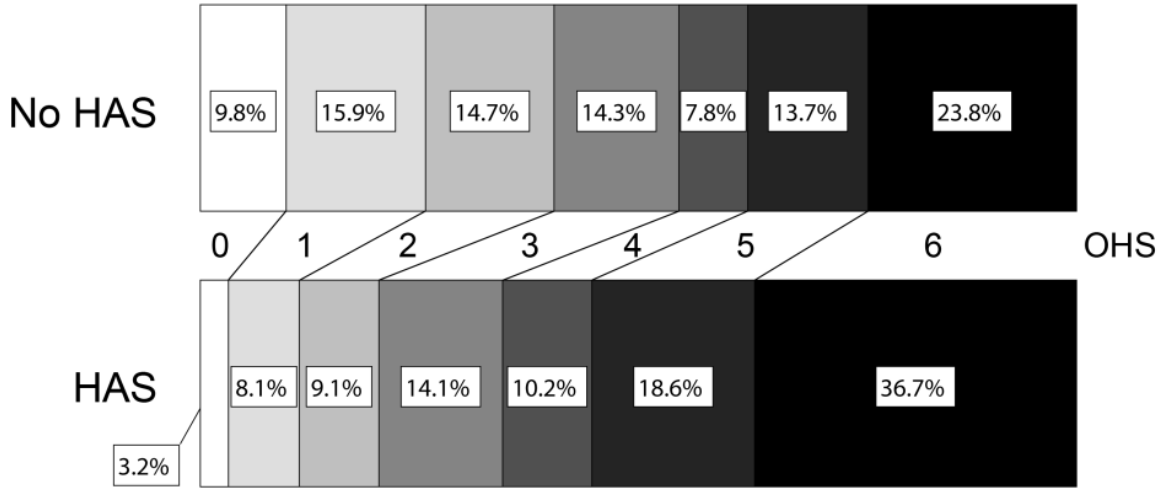
Table 5-2 *Demographic and clinical data of patients with and without a HAS on baseline CT*

	HAS n = 716	**No HAS n = 2245**	**p-value for difference**
Age (median, IQR)	80 years (70-85)	81 years (72-86)	0.003
Male sex	356 (49.7%)	1071 (47.7%)	0.348
Stroke onset to baseline Scan (mean, SD)	159 minutes (71)	164 minutes (73)	0.082
Time to imaging follow-up (median, IQR)	26 hours (24-38)	26 hours (24-35)	0.801
Baseline NIHSS (median, IQR)	16 (10-20)	9 (6-16)	<0.001
OHS at six months (median, IQR)	5 (3-6)	3 (1-5)	<0.001
Death by six months	263 (36.7%)	534 (23.8%)	<0.001
Treated with alteplase	368 (51.4%)	1116 (49.7%)	0.432

Footnote: Results represent n (%) unless otherwise stated. NIHSS = National Institutes of Health Stroke Scale. OHS = Oxford Handicap Scale. IQR = Interquartile range. SD = standard deviation.

Figure 5-1 compares OHS distribution between the groups with and without HAS at baseline. There were no significant differences between those with and without HAS at baseline for sex distribution (49.7% versus 47.7% male, p=0.348), stroke onset to scan time (mean 159 versus 164 minutes, p=0.082), time to follow-up (both 26 hours, p=0.801), or treatment allocation (51.4% versus 49.7% treated with alteplase, p=0.432).

Figure 5-1 *Comparison of six-month functional outcome distribution (OHS) for patients with and without HAS at baseline*



Footnote: OHS = Oxford Handicap Scale

Stroke severity and 6-month outcome were significantly worse if HAS was found only in proximal rather than only in distal vessels: median NIHSS 17 (interquartile range 11-21) versus 12 (7-17), p<0.001; median OHS 5 (3-6) versus 4 (2-6), p<0.001). Similarly, stroke severity and 6-month outcome were significantly worse if HAS was more versus less extensive: median NIHSS 17 (13-21) versus 15 (10-20), p=0.001; median OHS 5 (4-6) versus 5 (3-6), p=0.008.

Table 5-3 compares the effect of baseline HAS presence, extent and location on outcome in separate multivariable ordinal regression analyses (OHS as the dependent variable in each). The presence of HAS was an independent predictor of poor outcome; OR=0.66, 95%CI=0.55-0.80, p<0.001. More extensive HAS (1, 2 or 3 segments) also independently predicted a worse outcome among patients with the sign (n=716); OR=0.61, 95%CI=0.39-0.94, p=0.027. In patients with HAS in only 1 named arterial segment (n=589), proximal versus distal location was not independently associated with outcome, OR=1.17, 95%CI=0.80-1.71, p=0.420. These regression analyses are adjusted for the effects of age, NIHSS, time from stroke onset to scan and treatment allocation.

Table 5-3 *Multivariable analyses testing the importance of HAS presence, extent and location on six-month outcome*

a) Presence of HAS	**OR**	**95% CI**	**p-value**
Age (years)	0.96	0.96-0.97	<0.001
NIHSS	0.84	0.83-0.85	<0.001
Stroke onset to baseline scan (hours)	1.07	1.01-1.14	0.018
HAS present	**0.66**	**0.55-0.80**	**<0.001**
Alteplase treatment allocation	1.29	1.12-1.50	0.001
b) Extent of HAS			
Age (years)	0.95	0.94-0.96	<0.001
NIHSS	0.84	0.82-0.87	<0.001
Stroke onset to baseline scan (hours)	1.06	0.92-1.22	0.412
HAS extent (1-3 named segments)	**0.61**	**0.39-0.94**	**0.027**
Alteplase treatment allocation	1.06	0.76-1.48	0.714
c) Location of HAS			
Age (years)	0.95	0.94-0.97	<0.001
NIHSS	0.84	0.81-0.87	<0.001
Stroke onset to baseline scan (hours)	1.03	0.89-1.20	0.681
HAS location (proximal or distal)	**1.17**	**0.80-1.71**	**0.420**
Alteplase treatment allocation	1.15	0.81-1.64	0.442

Footnote: Ordinal regression analyses with Oxford Handicap Scale (OHS) as the dependent variable for each:

a) All IST-3 patients with CT at baseline (n=2961)

b) Includes only patients with HAS on baseline CT (n=716)

c) Includes only patients with HAS in a single arterial segment on baseline CT (n=589)

OR = Odds ratio (<1 indicates worse outcome). NIHSS = National Institutes of Health Stroke Scale. CI = Confidence Interval.

Change in HAS between baseline and follow-up CT

Among 2731 patients with NCCT at baseline and follow-up, 674 had HAS at baseline and 2057 did not. Shrinkage of HAS (fewer affected segments on follow-up compared with baseline CT) was more common than HAS growth (including development of new HAS) between baseline and follow-up scans in IST-3. Among patients with HAS on at least one scan, shrinkage occurred in 388/870 (44.6%), and completely disappeared in 349. HAS growth occurred in 237/870 (27.2%) patients which includes development of a new HAS in 196 patients at follow-up, i.e. no baseline HAS was identified in these patients. There was no change in HAS extent between baseline and follow-up NCCT for 245/870 (28.2%) patients, Table 5-4.

Table 5-4 *Univariate associations between imaging characteristics of HAS on baseline and follow-up CT, stroke severity at baseline and six-month outcome*

Change in HAS from Baseline to Follow-up CT		**n**	**NIHSS**	**Median**
Extent change from onset to follow-up	Growth	237	17 (10-21)	5 (3-6)
	Shrinkage	388	15 (9-20)**	4 (2-6)*
Persistence to follow-up	Persisted	324	17 (13-21)	5 (3-6)
	Disappeared	349	14 (9-19)*	4 (2-6)*
New HAS at follow-up	Yes	196	16 (10-20)	5 (3-6)
	No	1861	9 (5-15)*	3 (1-5)*

Footnote: Total numbers in each analysis vary because growth/shrinkage data does not include patients where HAS extent remained unchanged between scans. Total n=2731.

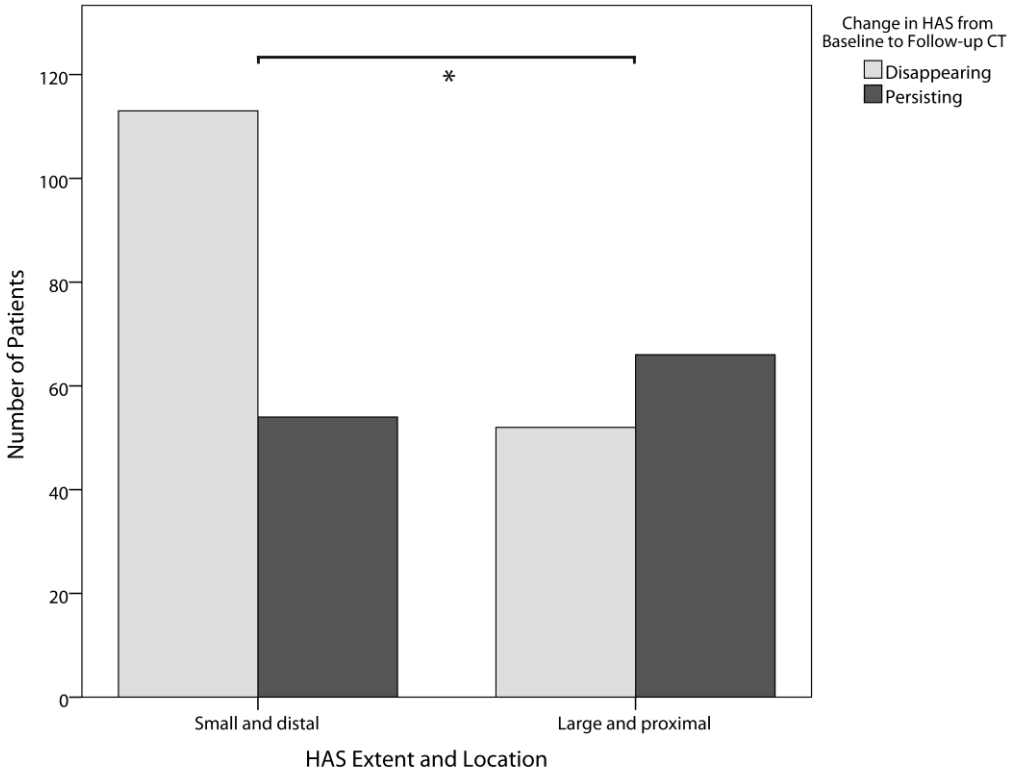
NIHSS = National Institutes of Health Stroke Scale. OHS = Oxford Handicap Scale.

Follow-up CT was only available in 674 of those with and in 2057 of those without HAS at baseline. HAS data were not available for one follow-up CT.

Results are median (inter-quartile range). * p-value for difference <0.001, ** p=0.010.

Location and extent of baseline HAS were associated with persistence or disappearance of the sign at follow-up on univariate analysis. Smaller HAS (fewer arterial segments affected) were more likely to disappear (297/553 = 53.7%) between baseline and follow-up than larger HAS (52/120 = 43.3%), $\chi^2 = 4.3$, p=0.039. Similarly, HAS sited in distal vessels were more likely to disappear (112/166 = 67.5%) between baseline and follow-up than HAS sited in proximal vessels (185/387 = 47.8%), $\chi^2 = 18.1$, p<0.001. These results are grouped and summarised in Figure 5-2. Note that while clearance was less common in the presence of a large and proximal HAS, it still occurred in 44% (52/118) of these patients. Small and distal HAS cleared in 67% (112/166) of cases.

Figure 5-2 *Effect of HAS location and extent on HAS persistence to follow-up*



Footnote: Small HAS = single arterial segment. Large HAS = more than one arterial segment. Proximal HAS = internal carotid, middle cerebral artery main stem, vertebral and basilar arteries. Distal HAS = anterior, middle (sylvian branch) and posterior cerebral arteries. * $\chi^2 = 15.5$, p<0.001.

Stroke severity and 6-month outcome were significantly worse if HAS showed growth rather than shrinkage between scans (median NIHSS 17 versus 15, p=0.010, median OHS 5 versus 4, p<0.001), if HAS persisted to follow-up rather than disappeared (median NIHSS 17 versus 14, p<0.001, median OHS 5 versus 4, p<0.001) or if a new HAS developed between scans (median NIHSS 16 versus 9, p<0.001, median OHS 5 versus 3, p<0.001), Table 5-4.

Effect of alteplase on HAS

The following analyses demonstrate the influence of alteplase on both the persistence and any change in the extent of HAS at follow-up and on the development of new HAS in the interim between pre-randomisation and follow-up CT.

On univariate analysis, there were significant associations between alteplase treatment and an increased likelihood of both HAS shrinkage (222/440 = 50.5% treated with alteplase versus 166/429 = 38.7% controls shrank, χ^2 = 12.6, p=0.002) and HAS disappearance (198/350 = 56.6% treated with alteplase versus 151/323 = 46.7% of controls disappeared, χ^2 = 6.5, p=0.011). In patients treated with alteplase, distal versus proximal HAS (65/86 = 75.6% versus 105/198 = 53.0%, respectively, χ^2 = 12.7, p=0.001) and single segment versus multi-segment HAS (170/284 = 59.9% versus 28/66 = 42.4%, respectively, χ^2 = 6.6, p=0.010) were more likely to have disappeared at follow-up. Among patients without a baseline HAS, fewer who received alteplase had developed a new HAS in the interim between baseline and follow-up CT, although this difference was not significant (90/1047 = 8.6% versus 106/1010 = 10.5%, χ^2 = 2.2, p=0.143), see Table 5-5.

Table 5-5 *Combined 2x2 tables comparing alteplase treatment allocation with changes in the presence of HAS between baseline and follow-up CT*

Hyperattenuating Artery Characteristics		**Treated with Alteplase**		**Total**
		Yes	No	
Extent change from baseline to follow-up	Growth	111	126	237
	Shrinkage	222	166	388
	No Change	107	137	244
Total		440	429	869
χ^2 = 12.6, p=0.002, OR for HAS shrinkage with treatment = 1.52 (95%CI=1.1-2.1)*				
Persistence to Follow-up	Persisted	152	172	324
	Disappeared	198	151	349
Total		350	323	673
χ^2 = 6.5, p=0.011, OR for HAS disappearance with treatment = 1.48 (95%CI=1.1-2.0)				
New HAS at Follow-up	Yes	90	106	196
	No	957	904	1861
Total		1047	1010	2057
χ^2 = 2.2, p=0.143, OR for developing new HAS on treatment = 0.80 (95%CI=0.6-1.1)				

Footnote: OR = Odds Ratio. 95%CI = 95% Confidence Interval. HAS data were not available for one follow-up CT. *This OR is calculated only for the groups with either growth or shrinkage, i.e. those with no change have been excluded.

For the multivariable analysis testing factors associated with *change in HAS segment number* (dependent variable), alteplase treatment was an independent predictor of HAS shrinkage at follow-up (OR 0.77, 95%CI=0.65-0.93, p=0.006) while HAS growth was more likely in older patients (OR 1.01, 1.00-1.02, p=0.013). This result is adjusted for the effects of time (between baseline and follow-up scans) and stroke severity. Increased time between scans showed a non-significant trend for HAS shrinkage (OR 0.91, p=0.059); Table 5-6.

Table 5-6 *Ordinal regression analysis for factors associated with change in HAS extent (growth or shrinkage) between baseline and follow-up CT scans (n=2730)*

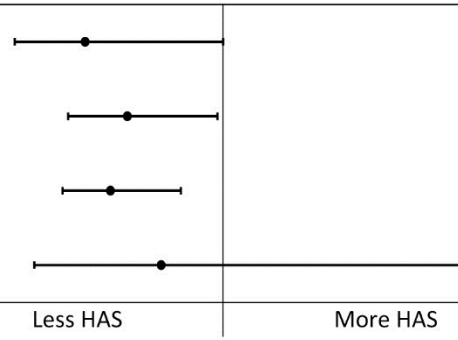
	Raw Data	**Odds Ratio**	**95% Confidence Interval**	**p-value**
Age	81 (72-86) years	1.01	1.00-1.02	0.013
NIHSS	11 (6-18)	1.00	0.98-1.01	0.475
Time from baseline to follow-up scan	26 (24-36) hours	0.91	0.82-1.00	0.059
Treated with alteplase	n = 1397	0.77	0.65-0.93	0.006

Footnote: NIHSS = National Institutes of Health Stroke Scale. Raw data provided as median (inter-quartile range) unless otherwise stated. An odds ratio <1 indicates HAS shrinkage, >1 indicates HAS growth. n=2730, HAS data were not available for one follow-up CT.

Both proximal and distal HAS were equally likely to shrink following alteplase treatment (OR 0.66, 95% CI 0.45–0.98, and OR 0.51, 95% CI 0.26–1.00, respectively), with no evidence of an interaction between HAS location and alteplase effect (p=0.516), Figure 5-3. HAS affecting a single arterial segment at baseline was more likely to shrink following alteplase treatment (OR 0.60, 95% CI = 0.43-0.85), but alteplase was not an independent predictor of HAS shrinkage if more than one arterial segment was affected at baseline (OR 0.78, 95% CI 0.33–1.86). Nevertheless, there was no evidence of an interaction between HAS extent and alteplase effect (p=0.580), Figure 5-3. These results are adjusted for patient age, time (between baseline and follow-up scans), and stroke severity.

Figure 5-3 *Ordinal regression analyses for the effect of alteplase treatment on change in HAS extent from baseline to follow-up scan*

Hyperdense Artery Sign (HAS) Pre-Randomization		Change in HAS from Pre-Randomization to Follow-up			Treated with Alteplase	Adjusted Odds Ratio for Effect of Alteplase on HAS Extent (95% Confidence Interval)	Test for Interaction
		Shrank	Stable	Grew			
Location*	Distal (n=166)	65	18	3	Yes (86)	0.51 (0.26-1.00)	p = 0.516
		47	32	1	No (80)		
	Proximal (n=409)	114	76	18	Yes (208)	0.66 (0.45-0.98)	
		90	92	19	No (201)		
Extent	1 Segment (n=553)	170	93	21	Yes (284)	0.60 (0.43-0.85)	p = 0.580
		127	122	20	No (269)		
	> 1 Segment (n=120)	52	14	0	Yes (66)	0.78 (0.33-1.86)	
		39	15	0	No (54)		



Footnote: Ordinal regression analyses with change in HAS segment number (shrinkage, no change, growth) as the dependent variable assessing the importance of baseline (pre-randomisation) HAS location (proximal = internal carotid artery, middle cerebral artery mainstem, vertebral or basilar arteries; distal = anterior or posterior cerebral arteries or sylvian branches of the middle cerebral artery) and HAS extent on the effect of alteplase.

*Location analysis does not include patients with HAS in both proximal and distal arteries.

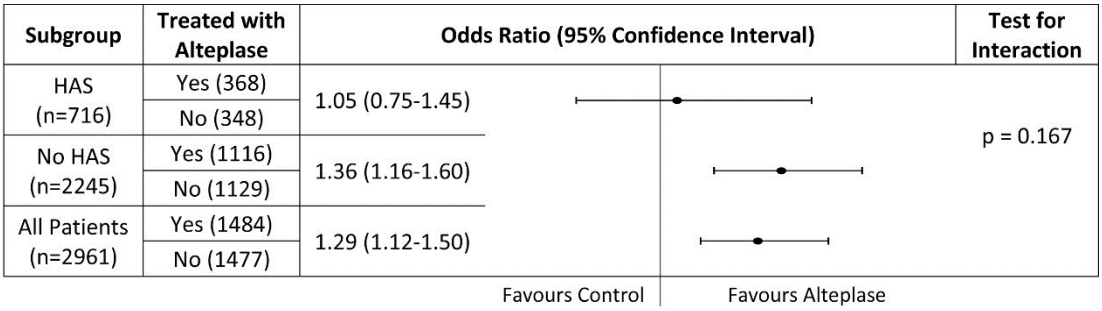
Odds ratio <1 (left of line) indicates HAS shrinkage, >1 (right of line) indicates HAS growth.

Effect of alteplase on outcome in those with and without HAS

The result of ordinal regression analysis for alteplase effect on outcome (OHS as dependent variable) is shown in Figure 5-4 for the entire group (n=2961) and for the subgroups with and without HAS on baseline CT. These results are adjusted for patient age, time (between baseline and follow-up scans), stroke severity and (for the full group only) presence versus absence of HAS.

Overall, alteplase increased the odds of better functional outcome (OR=1.29, 95%CI=1.12-1.50, p=0.001), as previously described in the complete IST-3 dataset, see Chapter 3.7, but there was no evidence of an interaction with the presence versus absence of HAS (p=0.167).

Figure 5-4 *Ordinal regression analyses showing odds ratios for the effect of alteplase treatment on outcome in the full group and in the subgroups with and without HAS on baseline CT.*



Footnote: Odds ratio >1 (right of line) indicates better outcome (lower six month OHS). Results are adjusted for the effect of age, time from stroke onset to scan (hours) and National Institutes of Health Stroke Scale (NIHSS).

5.4 Discussion of HAS Results

In this analysis of IST-3 data, I have shown that HAS is present in approximately 25% of patients undergoing non-contrast CT within six hours of acute ischaemic stroke. This is consistent with other published large series using similar scanning protocols[3;4] however prevalence rates in the range 47-59% have been demonstrated in smaller studies.[5;6] I confirmed that patients with HAS have more severe strokes and worse six-month outcomes than patients without HAS and that six-month outcome is worst when HAS is more extensive and proximally located. The effect of HAS presence on outcome is independent of age, time from stroke onset, stroke severity and treatment with intravenous alteplase, i.e. patients with and without HAS both benefit similarly from alteplase. Other authors have demonstrated that HAS independently predicts outcome[7-9] but my work further demonstrates that more extensive HAS independently predicts a worse outcome among patients with the sign. I did not demonstrate an independent association between HAS location and outcome despite highly significant univariate results as demonstrated elsewhere;[10] however, my multivariable analysis of outcome including HAS location is limited by the exclusion of those HAS affecting more than one arterial segment (n=127) and is therefore likely to be underpowered.

I provide evidence that intravenous alteplase both reduces the persistence (more likely to shrink, or disappear) and limits the formation (less likely to grow) of HAS among patients with acute ischaemic stroke compared to patients allocated control. I found that alteplase increases HAS shrinkage/disappearance independent of age, baseline stroke severity and time between baseline and follow-up scans. Moreover, these changes occur in both large volume proximal HAS and in smaller, more distally sited HAS although clearance is more common in the latter. These findings are important since I, and others,[9;11] have shown improved outcome for patients where HAS shrinks or disappears in the presence of alteplase. I believe my work is the first to demonstrate an association between intravenous alteplase and measurable growth/shrinkage of HAS using randomised controlled trial data. I found no evidence that presence or absence of HAS materially altered the benefit of alteplase (the favourable shift in six-month OHS) which was seen in patients with and without HAS. The appropriate interpretation is that there is no evidence of a difference in

treatment effect between those with and without HAS at baseline and not that alteplase has no effect in those with HAS. The multivariable analyses support this interpretation since HAS predicts poor outcome in ischaemic stroke, independent of alteplase use. This post-hoc subgroup analysis may be underpowered (95% confidence intervals overlap substantially) and must therefore be interpreted with caution. Large patient numbers are likely to be required to detect/exclude a true interaction which if present, is likely to be very small. To understand the apparent benefit of alteplase in patients without HAS, the presence of undetected thrombosis may be an answer; a recent report of case series demonstrated apparent benefit from thrombolysis for patients without arterial obstruction on angiography.[12] I will explore this further in Chapter 9.

Strengths and limitations

Firstly, most of the CT scans were not thin-section. Previous work has demonstrated that thrombus detection on CT is affected by slice thickness and that prevalence rates for HAS are larger with thin-slice protocols.[13] It is likely therefore that the baseline prevalence of HAS is an underestimate. Variability in the detection of HAS could also have led to misclassification of *new* or *disappearing* HAS when baseline and follow-up scans were compared. I could have verified the changing HAS appearances in a subset of these cases. Secondly, HAS was not defined by a specified level of attenuation measured by Hounsfield units. Though image analysis was performed centrally by an expert panel of readers, (inter-observer reliability for the identification of HAS is dealt with in Chapter 8) these assessments were qualitative rather than quantitative to reflect how the scan would be used in routine acute stroke care. A recent small cohort study demonstrated that following intravenous alteplase, ischaemic stroke patients with persistent arterial occlusion had significantly lower mean attenuation of thrombus compared to those who achieved recanalisation.[14] I will explore the effect of thrombus attenuation on outcome in IST-3 in Chapter 6. Thirdly, estimating HAS extent from the number of arterial segments affected is relatively crude and could have over or underestimated the true extent of HAS especially where HAS crossed segment boundaries. A volumetric measurement would have provided numerically continuous data and possibly a more accurate assessment of changes in HAS volume. However, it remains unclear if HAS volume

can be measured reliably. The IST-3 method of assessing thrombus extent is feasible and therefore relevant to daily practice. Finally, IST-3 has insufficient power to reliably explore interactions between HAS and the effect of alteplase but I found little evidence that patients with HAS responded differently to alteplase than those without HAS.

Conclusion

IST-3 data confirmed significant associations between the presence and extent of HAS, increased baseline stroke severity and poor six-month outcomes. I show clearly that alteplase accelerates the disappearance of the HAS. Furthermore, I found no evidence that the improvement in functional outcome following alteplase was materially different in the presence or absence of HAS. Therefore, while the presence of HAS is useful to support a diagnosis of acute ischaemic stroke, these data suggest that the presence or absence of the sign should not influence decisions on whether or not to use intravenous alteplase.

5.5 References for Chapter 5

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Chapter 6 CT Attenuation of Arterial Obstruction in Ischaemic Stroke

In this chapter, I use data from my own assessment of IST-3 imaging of patients with non-contrast CT and CT or MR angiography at baseline to calculate the CT attenuation within acute arterial obstructions (i.e. thrombus or embolus). Using the IST-3 angiography subgroup for this analysis allowed me to calculate the attenuation of obstructions that were both visible (i.e. HAS) and not visible on non-contrast CT. I use the derived data to investigate relationships between the CT attenuation of acute arterial obstructions, response to intravenous alteplase and functional outcome at six months.

This chapter addresses **Thesis Aim 2).**

This work is also published in a peer reviewed journal, the complete draft of which is included as Appendix 5, see Chapter 11.3.3:

> Mair G, von Kummer R, Lindley RI, Sandercock PAG, Wardlaw JM. Effect of X-ray attenuation of arterial obstructions on intravenous thrombolysis and outcome after ischemic stroke. *PLOS ONE* 2015;10:e0145683.

6.1 Methods for Assessment of CT Attenuation of Arterial Obstructions

As discussed in Chapter 4.3, I had determined the intra-arterial CT attenuation for all arterial obstructions (i.e. *obstruction attenuation*) at baseline and, if still present, at follow-up among patients with CT or MR angiography at baseline in IST-3.

In addition to analysing the obstruction, I also obtained attenuation measures within any equivalent contralateral normal artery (for example, the opposite MCA at an equivalent location) and also within the basilar artery. I therefore measured intra-arterial density at three intra-cranial locations for most patients. At each location, three separate elliptical regions of interest were applied by hand, taking care not to include the arterial wall or any surrounding tissue; a mean attenuation value of the

three readings was later calculated. Attenuation measurements were performed following x2 magnification of images (Figure 4-1). The subjective appearance of obstruction on non-contrast CT was also noted as either iso-attenuating to normal vessels or hyperattenuating (if obstruction appeared more attenuating than adjacent or equivalent contralateral arteries but non-calcified). I calculated attenuation ratios for each patient to assess obstruction attenuation in relation to normal background intra-arterial attenuation as:

> mean obstruction attenuation ÷ mean normal intra-arterial vessel attenuation.

I repeated attenuation measurements, in the same location, on follow-up non-contrast CT. I calculated change in obstruction attenuation as:

> mean follow-up attenuation – mean baseline attenuation

i.e. a negative difference in obstruction attenuation represented a reduction in obstruction attenuation between the two scans.

As for all patients within the IST-3 angiography subgroup, an IST-3 angiography score was also calculated to determine the completeness of arterial obstruction.

For the present analysis, I identified how many patients within the angiography subgroup were included, i.e. angiography patients with measurable obstruction on non-contrast CT. Patients were excluded from this analysis if the angiographic abnormality was insufficient to allow for attenuation measures to be performed (i.e. minor stenosis without a discrete measurable filling defect). Patients identified for this present subgroup analysis (obstruction attenuation subgroup) were compared with the full IST-3 group.

I tested whether change in the attenuation of obstructions from baseline to follow up was different for the treatment versus control groups. Whether baseline obstruction attenuation affected functional outcome at six months and assessed for interaction between obstruction attenuation and treatment with alteplase.

6.1.1 Statistical testing

Univariate analyses

Simple t-tests were employed to compare ratios and to compare the means of normally distributed continuous data. The medians of non-parametric continuous data were compared using a Mann-Whitney U test. I used Pearson's correlation coefficient, analysis of variance and t-tests to look for associations between baseline obstruction HU and clinical characteristics, other imaging appearances and outcome in separate univariate analyses.

Multivariable analyses

Ordinal regression methods[1] were used to calculate common odds ratios (ORs) for OHS at six months as the dependent variable.

To stabilise ordinal regression estimates, I again used the same approach as the original IST-3 analysis of functional outcome, where the more severe grades of OHS (4-6) were grouped as one, leaving 5 groups for ordinal analysis (0, 1, 2, 3 and 4-6).[2] Similarly, *time from stroke onset to baseline scan*, was grouped into 6 one-hour windows (0-60, 61-120, 121-180, 181-240, 241-300 and 301-360 minutes).

Statistical software

Comprehensive Meta-Analysis software (Biostat, Englewood, NJ, USA) was used to compute summary ORs and to perform a test of interaction between treatment and control groups.

All other analyses were performed using IBM SPSS Statistics software, version 20.0 (IBM Corporation, Armonk, NY, USA).

A p-value <0.05 was considered significant.

6.2 Results for the Obstruction Attenuation Subgroup

From the 300 patients in IST-3 with angiography performed at baseline, I identified 109 who had both a concurrent non-contrast CT and a measurable angiographic abnormality; this subgroup is termed the obstruction attenuation subgroup.

Compared with the remainder of the full IST-3 group, patients within the obstruction attenuation subgroup had significantly more severe strokes at baseline (NIHSS 17 versus 11 for the full group, $p<0.001$), with more extensive early brain parenchymal changes (ASPECTS 9 versus 10 for the full group, $p=0.001$) and worse six-month functional outcome (OHS 5 versus 4 for the full group, $p<0.001$) including an increased death rate (39% versus 27% for the full group). Subgroup patients were more likely to have a total anterior circulation infarct (TACI 62% versus 42% with correspondingly fewer partial anterior, posterior or lacunar type infarcts, $p<0.001$). In addition, there were significantly fewer men in the obstruction attenuation subgroup (35% versus 48% in the full group, $p=0.004$). There were no significant differences in patient age, rate of atrial fibrillation, time from stroke onset to scan, time to randomisation or time to imaging follow-up between the obstruction attenuation subgroup and the full IST-3 group, Table 6-1.

Table 6-1 *Clinical characteristics of the IST-3 obstruction attenuation subgroup and the full IST-3 group*

	Obstruction Attenuation Subgroup (n=109)	**Remainder of the Full IST-3 Group (n=2926)**	**p-value for Difference**
Age (median, IQR)	82 (75-86)	81 (72-86)	0.192
Male Sex (n, %)	38 (34.9)	1465 (48.3)	0.004
Clinical Stroke Syndrome (n, %)			<0.001
TACI	68 (62.4)	1238 (42.3)	
PACI	32 (29.4)	1114 (38.1)	
POCI	5 (4.6)	241 (8.2)	
LACI	4 (3.7)	328 (11.2)	
Other	0	5 (0.2)	
Atrial fibrillation at baseline (n, %)	35 (32.1)	879 (30)	0.644
Time (mins) from stroke onset to baseline CT (mean, SD)	171 (79)	164 (73)	0.282
Time (mins) from stroke onset to randomisation (mean, SD)	230 (79)	231 (73)	0.891
Baseline NIHSS (median, IQR)	17 (11-21)	11 (6-17)	<0.001
Baseline ASPECTS (median, IQR)	9 (6-10)	10 (8-10)	0.001
Treated with rt-PA (%)	51 (46.8)	1515 (49.9)	0.506
Time (hours) from baseline to follow-up CT (median, IQR)	25 (24-29)	26 (24-36)	0.110
Six-month OHS (median, IQR)	5 (3-6)	4 (2-6)	<0.001
Independent at six-months (OHS 0-2)	26 (23.9)	1088 (35.8)	0.008
Dead by six-months (%)	43 (39.4)	815 (26.9)	0.003

Footnote: TACI = Total Anterior Circulation Infarct. PACI = Partial Anterior Circulation Infarct. POCI = Posterior Circulation Infarct. LACI = Lacunar Infarct. NIHSS = National Institutes of Health Stroke Scale. ASPECTS = Alberta Stroke Program Early CT Score. OHS = Oxford Handicap Scale. IQR = Interquartile Range. SD = Standard Deviation.

Imaging findings

Table 6-2 demonstrates the results of imaging assessment for the 109 patients in the obstruction attenuation subgroup. I identified HAS in 64 patients (59%). The mean CT attenuation within these hyperattenuating obstructions was 51.0 Hounsfield Units (HU). This compares with a mean attenuation of 37.9 HU in iso-attenuating obstructions, $p<0.001$. Similarly, the mean attenuation within normal vessels (i.e. normally flowing blood) was 38.1 HU. The mean HU ratio of hyperattenuating obstructions was 1.38 compared with a mean HU ratio of 0.97 for iso-attenuating obstruction, $p<0.001$. The majority of patients had obstruction within an ICA (20%) or an MCA branch (75%). In most patients, obstruction was isolated to a single named arterial segment (56%); fewer patients had obstruction affecting 2 or 3 arterial segments (37% and 7%, respectively). The mean length of hyperattenuating obstructions on non-contrast CT was 19 mm (standard deviation 10 mm). By IST-3 angiography score, 84% of patients had >50% luminal stenosis (IST-3 angiography score 0-2b) and in 44%, there was either complete occlusion or severe luminal stenosis (IST-3 angiography score 0-1).

Table 6-2 *Imaging characteristics of the IST-3 obstruction attenuation subgroup*

Non-contrast CT		
Hyperattenuating artery sign (%)		64 (58.7)
Intra-arterial attenuation (mean, SD)	Hyperattenuating obstruction	51.0 (8.3) HU
	Iso-attenuating obstruction	37.9 (10.7) HU
	Normal vessel	38.1 (5.2) HU
	Follow-up hyperattenuating obstruction	45.1 (9.2) HU
	Follow-up iso-attenuating obstruction	38.2 (8.2) HU
Obstructed: normal vessel attenuation ratio (mean, SD)	Hyperattenuating obstruction	1.38 (0.23)
	Iso-attenuating obstruction	0.97 (0.21)
CT or MR angiography		
Location of arterial obstruction (%)	ICA	22 (20.2)
	MCA mainstem	61 (55.9)
	MCA sylvian branch	21 (19.3)
	ACA	0
	PCA	0
	Vertebral artery	2 (1.8)
	Basilar artery	3 (2.8)
Number of obstructed arterial segments (%)	1	61 (56.0)
	2	40 (36.7)
	3	8 (7.3)
IST-3 angiography score (%)	0	11 (10.1)
	1	37 (33.9)
	2a	17 (15.6)
	2b	27 (24.8)
	3	17 (15.6)
	4	0

Footnote: n=109. SD = Standard Deviation. HU = Hounsfield Units. ICA = Internal Carotid Artery. MCA = Middle Cerebral Artery. ACA = Anterior Cerebral Artery. PCA = Posterior Cerebral Artery.

IST-3 angiography scoring: 0 = occluded, 1 = minimal patency (some contrast penetrates obstruction but none or only a minimal amount enters distal artery), 2 = patency <50% of the lumen at the point of obstruction and a) only partly filling (<half) or b) incomplete filling (but >half) of the major branches of the affected artery, 3 = patency >50% of the lumen and filling of most of the branches of the affected artery, 4 = normal.

Associations with obstruction attenuation at baseline

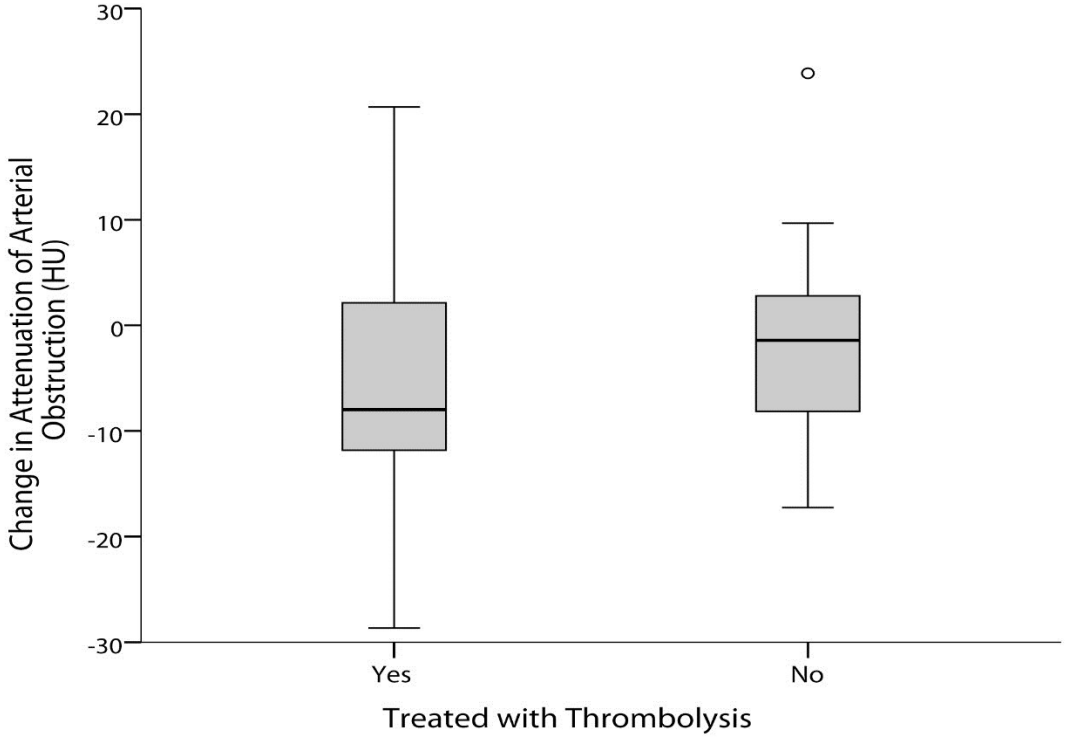
On univariate analysis, there was no significant difference in the mean attenuation of baseline arterial obstruction when patients with versus without atrial fibrillation were compared (45.9 HU versus 45.5 HU, respectively, p=0.859) or among the different clinical stroke subtypes (F = 1.5, p=0.220). Similar non-significant results were obtained in all cases if those with hyper- versus iso-attenuating arterial obstructions were compared. There was no correlation between the length of the hyperattenuating arterial segment and the attenuation of that obstruction (r = 0.03, p=0.825). Similarly, the mean attenuation of arterial obstruction at baseline did not differ between those with 1 versus those with 2 or 3 obstructed arterial segments (44.8 HU versus 46.6 HU, respectively, p=0.418). Finally, there was no correlation between the time from stroke onset to baseline scan and the attenuation of arterial obstruction on the baseline scan (r = 0.13, p=0.203).

Effect of alteplase on obstruction attenuation

For all attenuation subgroup patients, the median time between baseline and follow-up non-contrast CT was 25 hours. At follow-up, the attenuation of hyperattenuating obstruction had fallen to mean 45.1 HU while the attenuation of iso-attenuating obstruction was essentially unchanged at mean 38.2 HU. There was a non-significant (p=0.117) greater reduction of obstruction attenuation between baseline and follow-up non-contrast CT for patients treated with alteplase (median reduction of 8.0 HU) compared to controls (median reduction of 1.4 HU), Figure 6-1. Similar results were obtained if only patients with hyperattenuating obstructions at baseline

were included; median reduction of 8.4 HU in those treated with alteplase versus a median reduction of 0.5 HU in those allocated control, p = 0.094.

Figure 6-1 *Effect of alteplase on change in attenuation of arterial obstruction between baseline and follow-up CT*



Footnote: A negative change in arterial obstruction HU (Hounsfield Units) between baseline and follow-up CT indicates a reduction in attenuation of the obstruction.

This analysis includes patients with both iso- and hyperattenuating obstructions at baseline.

There was no significant difference between the groups treated with alteplase (median -8.0 HU, IQR -12.4 to 2.5) and control (median -1.4 HU, IQR -8.7 to 2.9), p=0.117.

Obstruction attenuation and six-month outcome

There were no significant associations on univariate analysis between the attenuation of arterial obstruction at baseline and six-month functional outcome in either the alteplase or control groups. For correlation between obstruction attenuation and six-month OHS the results were: r =0.03, p=0.851 for those treated with alteplase (n=51) and r=0.13, p=0.342 in the control group (n=58). Comparing baseline arterial obstruction attenuation between the patients reaching independence (OHS 0-2) and those patients dependent or dead at six months (OHS 3-6), the mean attenuation difference was: 0.2 HU, p=0.944 for those treated with alteplase and 1.5 HU, p=0.673 in the control group. In both cases, baseline HU values were non-significantly higher among patients with poor six months outcome (OHS 3-6).

Test for interaction between alteplase and obstruction attenuation

Baseline arterial obstruction attenuation was not an independent predictor of six-month functional outcome on adjusted multivariable ordinal regression analysis in either the alteplase (OR=1.00, 95%CI=0.94-1.07, p=0.911) or control groups (OR=0.99, 95%CI=0.93-1.05, p=0.680) and there was no significant difference between these results, i.e. no treatment interaction between alteplase and obstruction attenuation (p=0.824), Table 6-3.

Similarly, there was no evidence that baseline arterial obstruction attenuation was an independent predictor of outcome in the whole group (n=109) using either the quantitative (absolute HU of obstruction, OR=0.99, 95%CI=0.94-1.03, p=0.516) or qualitative analyses (hyper- versus iso-attenuating obstruction, OR=0.53, 95%CI=0.20-1.41, p=0.203) in a model adjusting for treatment allocation, Table 6-4. There was a non-significant trend towards poorer outcome among patients with hyperattenuating rather than iso-attenuating obstructions.

For all analyses, replacing absolute arterial obstruction attenuation values with obstruction attenuation ratios did not alter the results (data not shown).

Table 6-3 *Ordinal regression analysis with six-month OHS as the dependent variable for a) patients treated with alteplase and b) controls*

a) Treated with alteplase (n = 51)	**Odds Ratio**	**95%CI**	**p-value**
Age (years)	0.93	0.89-0.98	0.003
Time from stroke onset to baseline scan (hours)	0.66	0.38-1.15	0.139
NIHSS	0.87	0.79-0.97	0.009
Obstruction attenuation at baseline (HU)	1.00	0.94-1.07	0.911
Location of arterial obstruction	1.45	0.67-3.15	0.349
Number of obstructed arterial segments	0.79	0.24-2.61	0.696
IST-3 angiography score	1.41	0.78-2.52	0.253

b) Control group (n=58)	**Odds Ratio**	**95%CI**	**p-value**
Age (years)	0.97	0.92-1.02	0.254
Time from stroke onset to baseline scan (hours)	1.84	1.00-3.37	0.050
NIHSS	0.79	0.67-0.92	0.002
Obstruction attenuation at baseline (HU)	0.99	0.93-1.05	0.680
Location of arterial obstruction	1.06	0.60-1.90	0.834
Number of obstructed arterial segments	1.73	0.37-8.05	0.485
IST-3 angiography score	0.81	0.43-1.55	0.533

Footnote: 95%CI = 95% Confidence Interval. NIHSS = National Institutes of Health Stroke Scale. HU = Hounsfield Units. Odds ratio <1 indicates a worse outcome.

Table 6-4 *Ordinal regression analysis with six-month OHS as the dependant variable for all patients in the obstruction attenuation subgroup; obstruction attenuation is included as a) a quantitative continuous variable and b) a qualitative dichotomous variable.*

a) Obstruction attenuation continuous variable	**Odds Ratio**	**95%CI**	**p-value**
Age (years)	0.95	0.92-0.98	0.001
Time from stroke onset to baseline scan (hours)	1.12	0.78-1.62	0.539
NIHSS	0.85	0.79-0.92	<0.0001
Obstruction attenuation at baseline (HU)	0.99	0.94-1.03	0.516
Location of arterial obstruction	1.10	0.73-1.68	0.645
Number of obstructed arterial segments (1-3)	0.70	0.30-1.62	0.407
IST-3 angiography score (0-3)	1.03	0.71-1.51	0.865
Treated with alteplase (versus control)	2.17	0.80-5.91	0.131

b) Obstruction attenuation dichotomous variable	**Odds Ratio**	**95%CI**	**p-value**
Age (years)	0.95	0.92-0.98	0.001
Time from stroke onset to baseline scan (hours)	1.13	0.79-1.63	0.508
NIHSS	0.86	0.79-0.92	<0.0001
Hyperattenuating obstruction at baseline (versus isoattenuating obstruction)	0.53	0.20-1.41	0.203
Location of arterial obstruction	1.12	0.75-1.67	0.589
Number of obstructed arterial segments (1-3)	0.70	0.30-1.60	0.396
IST-3 angiography score (0-3)	1.02	0.70-1.49	0.902
Treated with alteplase (versus control)	2.29	0.85-6.17	0.102

Footnote: n=109. 95%CI = 95% Confidence Interval. NIHSS = National Institutes of Health Stroke Scale. HU = Hounsfield Units. Odds ratio < 1 indicates a worse outcome.

6.3 Discussion of Obstruction Attenuation Results

These IST-3 subgroup data do not support the hypothesis that baseline x-ray attenuation of angiographically-proven intra-arterial obstruction measured on non-contrast CT is an independent predictor of functional outcome at 6 months. Age, baseline stroke severity and time to treatment are such powerful predictors of outcome after ischaemic stroke and response to intravenous alteplase that the addition of measured obstruction attenuation does not appear to have any value in guiding decisions about treatment with intravenous alteplase.

The value of measuring obstruction attenuation on CT to select patients for treatment has been debated. Two recent non-randomised studies found no association in samples of about 100 patients.[3;4] Three recent cohort studies have however demonstrated with highly consistent results that intravenous thrombolysis more often resulted in successful arterial recanalisation in patients with highly attenuating arterial obstruction at baseline. Puig et. al. (n=45) demonstrated a mean relative HU (equivalent to mean HU ratio in my analysis) of 1.57 for patients achieving recanalisation versus 1.11 for those without recanalisation ($p<0.001$).[5] Similarly, Moftakhar et. al. (n=90) demonstrated a mean relative HU of 1.58 in patients that recanalised versus 1.39 in those that did not ($p=0.01$).[6] Finally, Niesten et. al. (n=88) demonstrated a mean relative HU of 1.54 in patients that recanalised versus 1.29 in those that did not ($p<0.001$).[7] With IST-3 data, I was not able to assess recanalisation following treatment with intravenous alteplase as follow-up angiography was not mandatory in IST-3. The apparent discrepancy between my results and these three cohort studies may indicate that success of recanalisation therapy is not the only predictor of outcome. Indeed the literature assessing relationships between obstruction attenuation and success of endovascular thrombectomy, has demonstrated that while recanalisation is more likely with hyperattenuating obstructions, where reported, this does not translate to a proportionally improved functional outcome.[8-12] In addition, some authors have identified significant differences in obstruction attenuation when stroke cohorts are stratified by aetiological stroke subtype. For example, cardioembolic thrombus may be more attenuating than atherothrombotic thrombus,[5;13] but such results have not always been replicated[4] and a large retrospective analysis of over 8000 patients failed

to demonstrate an independent association between hyperattenuating obstruction and stroke aetiology.[14] I similarly did not find any association in IST-3 between clinical stroke subtype (which may vary with differing underlying aetiologies[15]) or the presence of atrial fibrillation and the attenuation of arterial obstruction.

My analysis assessing the change in obstruction attenuation from baseline to short-term follow-up CT suggested that the HU of obstruction may reduce more rapidly in patients given intravenous alteplase than controls; however, the difference in obstruction attenuation between treatment and control groups was non-significant. This result is nevertheless consistent with my analysis of HAS in the whole IST-3 dataset (Chapter 5) which demonstrated that intravenous alteplase accelerates the removal of HAS, a known surrogate for intra-arterial thrombus. I assume a similar process of removal is occurring in the obstruction attenuation subgroup but that smaller patient numbers in the present analysis have limited the power of this assessment.

Strengths and limitations

In a large pragmatic multicentre trial like IST-3 there is inevitably some variability in scan parameters and protocols but, on the other hand, the study design closely represents normal working practice. Nevertheless, I cannot exclude the possibility that x-ray attenuation of thrombus was under-estimated due to partial volume effects of thick CT slices for some patients. Similarly, attenuation ratios may have varied with the hydration status of patients. We might expect patients to be dehydrated at baseline and for this to improve following admission to hospital. Therefore circulating blood could be relatively hyperattenuating on baseline versus follow-up scans due to differences in haematocrit. However, I did not find that use of attenuation ratios rather actual attenuation values materially altered results. IST-3 angiography was performed only in some centres and may therefore be biased toward patients for whom it could convey the greatest perceived benefit. Though the total patient numbers for this assessment are similar to previous work, my analyses are underpowered. Nevertheless, as a randomised-controlled trial, IST-3 provides an unbiased assessment of the relationships between the use of intravenous alteplase and functional outcome. I used manually applied regions of interest to measure the

attenuation of obstruction. Other centres have developed automated segmentation programs for this task but it remains to be seen whether such an approach improves the accuracy of the data collected. I used standardised and validated methods of accessing and scoring imaging with absolute blinding for robust and repeatable data collection.

Conclusion

These data from a prospective analysis of a large randomised trial do not provide evidence that measurement of arterial obstruction x-ray attenuation on CT adds useful additional information on prognosis or response to intravenous alteplase in patients with ischaemic stroke.

6.4 References for Chapter 6

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Chapter 7 Sensitivity and Specificity of HAS for Arterial Obstruction

In this chapter, I use data from my own assessment of IST-3 imaging of patients with non-contrast CT and CT or MR angiography at baseline to calculate the sensitivity and specificity of HAS for identifying true arterial obstruction or occlusion in ischaemic stroke. To broaden the applicability of the results of this analysis, IST-3 sensitivity and specificity data were then included in a systematic review and meta-analysis.

This chapter addresses **Thesis Aim 3).**

This work is also published in a peer reviewed journal, the complete draft of which is included as Appendix 6, see Chapter 11.3.4:

> Mair G, Boyd EV, Chappell FM, von Kummer R, Lindley RI, Sandercock P, Wardlaw JM, The IST-3 Collaborative Group. Sensitivity and specificity of the hyperdense artery sign for arterial obstruction in acute ischemic stroke. *Stroke* 2015;46:102-7.

7.1 Sensitivity and Specificity of HAS in IST-3

7.1.1 Methods

As discussed in Chapter 4.3, among IST-3 patients with CT or MR angiography at baseline, I had identified both HAS on non-contrast CT (where available) and angiographic obstruction on the concurrent CTA or MRA scan. First I assessed non-contrast CT scans for the presence or absence of HAS. Only thereafter did I assess angiography for the presence or absence of arterial obstruction, graded using TICI (see Table 3-3); i.e. I identified HAS without knowledge of angiography appearances. I was then able to determine when angiographic obstruction correlated with HAS, when it did not and vice versa.

The following were then calculated:

- True positive (TP) – When the presence of HAS on non-contrast CT correlated with an arterial obstruction on angiography in the same anatomical location
- False positive (FP) – When the presence of an apparent HAS on non-contrast CT did not correlate with an arterial obstruction on angiography
- True negative (TN) – When neither HAS nor arterial obstruction were identified
- False negative (FN) – When arterial obstruction was observed in the absence of HAS

Next, I derived sensitivity and specificity data for this IST-3 subgroup:

- Sensitivity (%) was calculated as TP / (TP + FN) x 100
- Specificity (%) was calculated as TN / (TN + FP) x 100

For completeness, the positive predictive value was calculated as the number of true positives divided by the number of positive calls (true positives plus false positives). The negative predictive value was calculated as the number of true negatives divided by the number of negative calls (true negatives plus false negatives).

Statistical testing

Simple t-tests were employed to compare percentages. Chi-square statistics (χ^2) were used to compare categorical data. Pearson and Spearman's Rank correlation coefficients were used to test for relationships between normally distributed and non-parametric continuous data, respectively.

All analyses were performed using IBM SPSS Statistics software, version 20.0 (IBM Corporation, Armonk, NY, USA).

A p-value <0.05 was considered significant.

7.1.2 Results

From the 300 IST-3 patients with angiography performed at baseline, I included 273 in this analysis to determine the sensitivity and specificity of HAS for true arterial obstruction following comparison of non-contrast CT imaging and concurrent CTA (n=269) or MRA (n=4). From 29 IST-3 patients with MRI performed at baseline, 25 had no correlative non-contrast CT (<30 minutes of the MRI) with which to assess for HAS. In addition, at the time of performing this research, the IST-3 angiography subgroup data was still being compiled and ratified; 2 patients with CTA were added to the subgroup with baseline angiography subsequent to my present analyses. For completeness, Table 7-1 compares the n=273 subgroup with the entire IST-3 group. These results are not materially different to the previously reported comparison between the n=300 angiography subgroup and the entire IST-3 group, Table 3-4.

Table 7-1 *Baseline clinical and imaging characteristics and six-month outcome for IST-3 patients included in the sensitivity and specificity analysis*

	IST-3 Patients with Baseline CT or MR Angiography, n = 273	**Entire IST-3 Group, n=3035**	**p-value for Difference**
Age (median, IQR)	81 years (71-86)	81 years (72-86)	0.815
Male Sex	120 (44.0%)	1465 (48.3%)	0.135
NIHSS (median, IQR)	10 (5-17)	11 (6-17)	0.020
Hyperdense Artery	69 (25.3%)	716/2961 (24.2%)*	0.687
OHS (median, IQR)	3 (1-5)	4 (2-6)	0.002
Independent at 6 Months (OHS 0-2)	120 (44.0%)	1088 (35.8%)	0.003
Dead by 6 Months	61 (22.3%)	815 (26.9%)	0.078
Treated with rt-PA	138 (50.5%)	1515 (49.9%)	0.827

Footnote: Results represent n (%) unless otherwise stated. NIHSS = National Institutes of Health Stroke Scale. OHS = Oxford Handicap Scale (six-month follow up). IQR = Inter-Quartile Range.

* From the entire IST-3 group 2961 had non-contrast CT at baseline, the remainder had MRI.

From these 273 patients, I identified an abnormal angiogram in 114 patients (42%) i.e. at least some luminal stenosis or obstruction (TICI < 3). I identified HAS in 69 patients (25%).

Table 7-2 compares the sensitivity and specificity rates of HAS as an indicator of true arterial obstruction using different dichotomies of the TICI angiography scale. In general, higher grades of TICI (increasing vessel patency towards normal) were associated with greater sensitivity and specificity rates. At the cut-off level used to define an abnormal angiogram (TICI < 3), HAS correctly identified arterial obstruction in 62/114 (54%), was falsely positive for angiographic abnormality in 7 of 69 cases (10%) and falsely negative for angiographic abnormality in 52 of 114 cases (46%). As a measure of angiographic abnormality in IST-3 therefore, the hyperattenuating artery sign has a sensitivity of 54% (95% Confidence Interval = 45-64%), a specificity of 96% (92-99%), a positive predictive value of 90% (83-97%) and a negative predictive value of 75% (68-81%). Note that in 90% (56/62) of true positive cases, HAS was present in patients with arterial obstruction on angiography rather than occlusion (i.e. TICI 1, 2a or 2b).

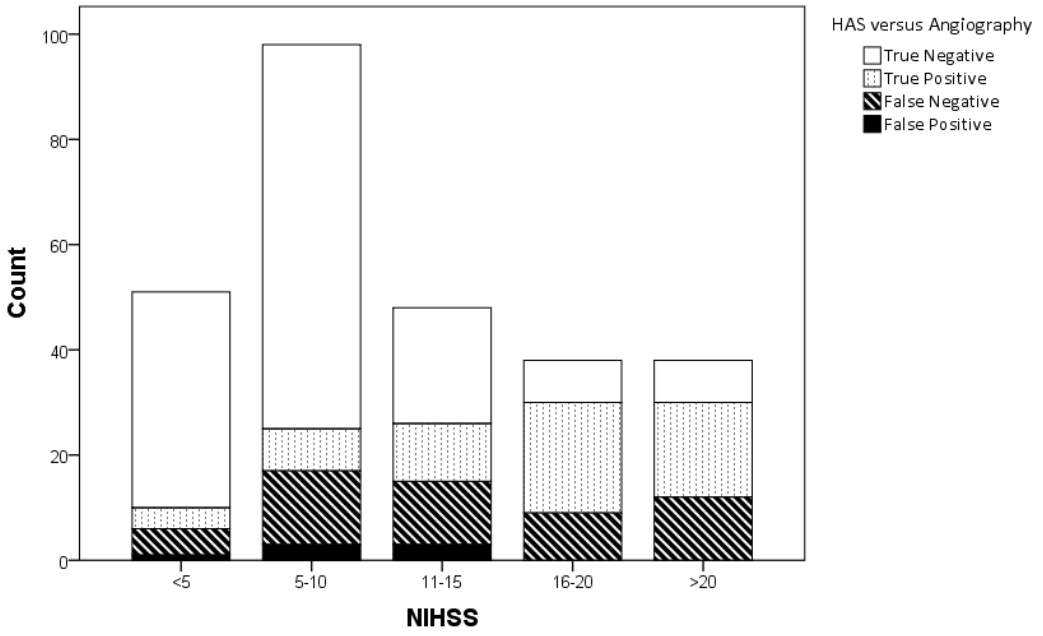
Table 7-2 *Sensitivity and specificity of HAS for identifying arterial obstruction on angiography as defined using different angiography grades in IST-3*

Definition of Angiographic Abnormality	**Hyperattenuating Artery**		**Sensitivity**	**Specificity**
	Yes	No		
TICI = 0	6	6	50%	76%
TICI <= 1	27	18	58%	83%
TICI <= 2a	12	9	58%	88%
TICI <= 2b	17	19	54%	96%
TICI = 3	7	152	-	-

Footnote: TICI = Thrombolysis in Cerebral Infarction: 0 = No flow, 1 = Minimal flow, 2a = Partial flow <50% of expected territory, 2b = Partial flow >50% of expected territory, 3 = Complete flow.

The relationship between HAS and angiography stratified by stroke severity (NIHSS) in IST-3 is shown in Figure 7-1. Note that there were more true positives and fewer true negatives as stroke severity increased (χ^2 = 84, 12 degrees of freedom, p<0.001). There was however little change in false negative or false positive rates with increasing stroke severity but all false positive cases had an NIHSS less than 15.

Figure 7-1 *Relationship between the presence of a HAS and the results of angiography in IST-3 stratified by stroke severity at onset*



Footnote: Stroke severity defined using NIHSS (National Institutes of Health Stroke Scale).
χ^2 = 84, df = 12, p<0.001

Table 7-3 compares the effect of scan, obstruction and patient characteristics on HAS sensitivity and specificity. When baseline non-contrast CT scans were dichotomised by slice thickness (greater than 3 mm, n=108 or equal to/lesser than 3 mm, n=162), HAS was more frequently detected on the thin-slice images but the difference was not significant (27% versus 21%, p=0.276). There was improved sensitivity but not specificity on the thin versus thick slices (62% versus 41%, p=0.031 and 98% versus 92%, p=0.089, respectively). There was no difference in the prevalence of HAS by location of arterial obstruction: proximal, n=91, sensitivity 55% versus distal, n=23, sensitivity 52% (p=0.814). More extensive angiographic obstruction, i.e. involving more than one named artery (n=48) versus obstruction of one named artery (n=66) did not influence with the sensitivity of HAS (58% versus 52%, p=0.475). Further analyses stratified by time from stroke onset and patient age did not meaningfully alter accuracy of the results.

Table 7-3 *Effect of CT, obstruction and patient characteristics on the sensitivity and specificity of HAS in IST-3*

		HAS Prevalence, n (%)	Sensitivity	Specificity
Non-Contrast CT Scan Characteristics				
CT Slice Thickness	≤ 3 mm	44/162 (27%)	62%	98%
	> 3 mm	23/108 (21%)	41%	92%
p-value for difference		0.276	0.031	0.089
Obstruction Characteristics				
Location of Obstruction	Proximal vessel	50/91 (55%)	55%	-
	Distal vessel	12/23 (52%)	52%	-
p-value for difference		0.814	0.814	-
Extent of Obstruction	1 segment	34/66 (52%)	52%	-
	2-3 segments	28/48 (58%)	58%	-
p-value for difference		0.475	0.475	-
Patient Characteristics				
Time from Stroke Onset	≤ 180 minutes	34/151 (23%)	49%	97%
	> 180 minutes	35/122 (29%)	61%	94%
p-value for difference		0.245	0.221	0.500
Age	≤ 80 years	30/136 (22%)	57%	96%
	> 80 years	39/137 (28%)	53%	96%
p-value for difference		0.225	0.709	0.977

Footnote: n=273. Proximal vessels: internal carotid artery, mainstem of the middle cerebral artery, vertebral and basilar arteries. Distal vessels: anterior cerebral artery, sylvian branches of the middle cerebral artery and posterior cerebral arteries. For *Obstruction Characteristics*, there were no false positive results as all included patients had genuine obstruction; hence specificity was not calculable.

7.2 Systematic Review and Meta-Analysis of HAS Sensitivity and Specificity

7.2.1 Methods

This systematic review and meta-analysis was undertaken in compliance with PRISMA (Preferred Reporting Items for Systematic review and Meta-Analysis) 2009.[1]

Search strategy

I concurrently searched the Embase and Medline databases using the search strategy outlined in Figure 7-2. Keywords pertaining to the topic areas of hyperattenuating arteries (in any intracranial location) and angiography (to include CT, MRI and catheter angiography) were combined within each topic area using the Boolean operator OR. Results from these topic area searches were then combined using the Boolean operator AND. As noted in Chapter 2, hyperattenuating arteries in stroke were first described in the early 1980s,[2] therefore all articles from 1980 to the date at time of search (September 2013) were included. Reference lists from the returned articles were hand searched and cross checked to identify any further relevant work.

Figure 7-2 *Search strategy for systematic review of HAS sensitivity and specificity*

1.	hyperdens*.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, an, sh, tn, dm, mf, dv, kw]
2.	hyper-dens*.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, an, sh, tn, dm, mf, dv, kw]
3.	hyperatten*.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, an, sh, tn, dm, mf, dv, kw]
4.	hyper-atten*.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, an, sh, tn, dm, mf, dv, kw]
5.	1 or 2 or 3 or 4
6.	arter*.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, an, sh, tn, dm, mf, dv, kw]
7.	vessel*.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, an, sh, tn, dm, mf, dv, kw]
8.	vascula*.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, an, sh, tn, dm, mf, dv, kw]
9.	6 or 7 or 8
10.	5 and 9
11.	hmcas.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, an, sh, tn, dm, mf, dv, kw]
12.	10 or 11
13.	angiogra*.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, an, sh, tn, dm, mf, dv, kw]
14.	arteriogra*.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, an, sh, tn, dm, mf, dv, kw]
15.	cta.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, an, sh, tn, dm, mf, dv, kw]
16.	mra.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, an, sh, tn, dm, mf, dv, kw]
17.	13 or 14 or 15 or 16
18.	12 and 17

Primary inclusion and exclusion criteria

I screened abstracts according to the following criteria to determine the final article list for subsequent data extraction. Only peer reviewed original articles published entirely in English were included. Articles had to contain data on ischaemic stroke patients assessed for hyperattenuating arteries who underwent invasive or non-invasive angiography (CT-, MR- or digital subtraction angiography, DSA).

Secondary exclusion criteria and data extraction

I performed quality assessment for each article that met primary inclusion criteria based on a modified version of the STARD (STAndards for Reporting Diagnostic accuracy) 2003 checklist.[3] This quality assessment checklist is shown in Table 7-4. For inclusion in subsequent meta-analysis, all essential criteria had to be met.

Articles were also excluded if imaging was not performed within 24 hours of stroke onset or if the number of patients undergoing angiography was fewer than 20. This time limit for imaging was chosen to allow inclusion of articles assessing HAS in the posterior fossa where longer delays before treatment are often deemed acceptable.

Table 7-4 *Quality assessment checklist used as secondary exclusion criteria for entry into meta-analysis. All essential criteria had to be met*

Essential	**Desirable**
Description of patient selection process	Prospective with sequential patients Randomised Inclusion/exclusion criteria provided
Image acquisition details provided	Scanner used (manufacturer and model, number of detector rows) Scan parameters (especially slice thickness) Time from stroke onset to imaging Time from non-contrast CT to angiography
Description of image analysis	Details of those analysing images Blinded to clinical details and treatment allocation (if any) Reproducibility data provided Hyperattenuating artery sign defined using previously described criteria

Two observers (me and a neuroradiology colleague, Dr Elena V Boyd) independently extracted data from included publications to allow calculation of true and false positive and negative rates as our primary outcome; data were extrapolated from tables and figures where necessary. We only meta-analysed papers where sensitivity or specificity (ideally both) could be calculated. We also recorded time from stroke onset to imaging and location and/or extent of angiographic obstruction as our secondary outcome. Extracted data were added to a preconfigured spreadsheet (Appendix 11, Chapter 11.4.4). Disagreements were resolved by consensus.

Meta-analysis

Sensitivity and specificity values were calculated for articles included in the final meta-analysis. Cumulative rates were also obtained using total patient numbers from those studies where all relevant data were available. Sensitivity and specificity were calculated as above (7.1.1).

Sensitivity and specificity results were meta-analysed with a random effects model in R2.8.1 (http://cran.r-project.org/), using the DiagMeta function, modelling within-study variation as a binomial proportion[4] (joint meta-analysis of sensitivity and specificity was not possible due to estimation problems).

7.2.2 Results

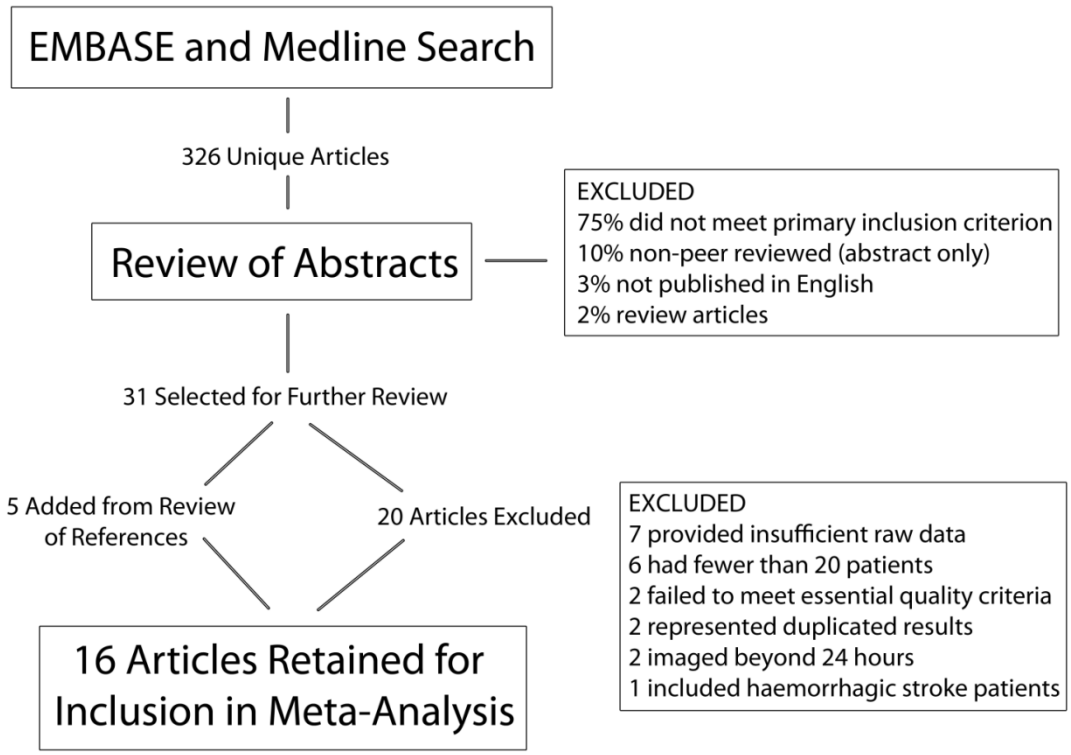
Search results

The flowchart in Figure 7-3 details the results of the systematic database search and the final number of articles retained for inclusion in the meta-analysis.

The database search identified 326 unique articles. An abstract review excluded the majority of these (approximately 75%) for not meeting the primary inclusion criterion: must contain original data pertaining to the hyperattenuating artery sign in ischaemic stroke patients who underwent angiography. Most of those excluded articles discussed CT hyperattenuation in locations other than intracranial arteries, e.g. solid abdominal organs. The next most common reason for exclusion was publication only in abstract (approximately 10%). Finally, some review articles and articles published in languages other than English were also excluded.

Abstract review identified 31 articles for more in-depth assessment using the secondary exclusion criteria. In addition, five further articles were identified from the reference lists of these 31 giving a total of 36 articles for full review.

Figure 7-3 *Flowchart showing results of systematic search and effect of exclusion criteria on final number of articles included in meta-analysis*



Following application of the secondary exclusion criteria, 16 original articles (total n=902) remained for meta-analysis.[5-20] Of the 20 excluded articles, seven provided insufficient raw data, six had fewer than 20 patients with angiography, two failed to meet all essential quality criteria (Table 7-4), two were duplicates, in two articles patients were imaged beyond 24 hours of stroke onset and one article included non-ischaemic strokes.

Quality assessment of articles retained for meta-analysis

The 16 articles identified in systematic review (n=902, not including IST-3) had a median of 52 patients (range 20-105). The majority of articles included in the meta-analysis (14/16, 88%) used data that were collected prospectively. None of the final 16 articles included randomised-controlled trial data. Only seven articles (44%) provided specific inclusion and/or exclusion criteria.

Most articles provided scan parameters including time from stroke onset to scan (15/16, 94%). Catheter angiography was the commonest technique (9/16, 56%). CTA and MRA were equally common (5/16, 31% and 4/16, 25%, respectively) and used almost exclusively since 2003. Most articles declared explicitly the experience level or professional position of those analysing images (14/16, 88%); in total there were 24 neuroradiologists and nine neurologists in the range 1-6 observers per article (median two). Image assessors were blinded to other data in 11/16 (69%) articles. In 12/16 articles a standardised definition for HAS was used. Reproducibility data for HAS were provided in only four articles (median kappa-statistic for the detection of HAS was 0.85, range 0.53-0.91) but none assessed reproducibility for the assessment of angiography.

Meta-analysis results

Meta-analysis of data from the 16 articles identified after systematic review is displayed in Figure 7-4 and Table 7-5. Both include IST-3 data presented above in 7.1.2.

Amongst a total of 1175 patients with angiography including IST-3, 769 had arterial obstruction and there were 405 HAS. The random effects summary estimate of sensitivity, based on 771 patients (384 true positive plus 387 false negative), was 52.4% (95% CI 41.2-63.4%). The random effects summary estimate of specificity, based on 493 patients, (468 true negative plus 25 false positive), was 94.9% (92.5-96.6%). Four studies with missing data were omitted from specificity analysis, Figure 7-4.

Figure 7-4 *Systematic review data for the 16 selected articles and IST-3*

Author	Year	CT Slice Thickness	Angiography		HAS	Abnormal Angiography	TP	TN	FP	FN	Calculated Results		Forest Plot (95% CI)	
			Type	n							Sensitivity	Specificity	Sensitivity	Specificity
Tomsick[5]	1990	10mm	Catheter	20	6	16	6	4	0	10	38%	100%		
Bastianello[6]	1991	8mm	Catheter	36	18	30	18	6	0	12	60%	100%		
Tomsick[7] *	1992	10mm	Catheter	38	15	29	14	9	1	15	48%	90%		
Wolpert[8]	1993	10mm	Catheter	60	16	60	16		0	44	27%			
von Kummer[9]	1994	8mm	Catheter	53	25	53	25			28	47%			
Flacke[10]	2000	8mm	Catheter/MRA	23	6	20	6	3	0	14	30%	100%		
Koga[11]	2003	10mm	Catheter	105	27	101	27	4	0	74	27%	100%		
Garg[12]	2004		CTA	65	12	16	10	47	2	6	63%	96%		
Agarwal[13]	2004	7mm	Catheter/CTA	39	15	29	14	9	1	15	48%	90%		
Barber[14] †	2004	5mm	MRA	100	25	47	18	140	11	31	37%	93%		
Kim[15] ‡	2005	1.25mm	CTA	51	45	45	45	6	0	0	100%	100%		
Schellinger[16]	2005	8mm	MRA	31	10	25	10	6	0	15	40%	100%		
Assouline[17]	2005		MRA	29	16	25	15	3	1	10	60%	75%		
Kim[18]	2008	5mm	CTA	90	46	78	46			32	59%			
Goldmakher[19]	2009	5mm	CTA	95	12	14	10	79	2	4	71%	98%		
IST-3[21]	2012	0.5-10mm	CTA/MRA	273	69	114	62	152	7	52	54%	96%		
Froehler[20]	2013	4.8mm	Catheter	67	42	67	42			25	63%			
TOTAL (95% Confidence Interval)				**1175**	**405**	**769**	**384**	**468**	**25**	**387**	**52.4%** (41.2-63.4%)	**94.9%** (92.5-96.6%)	0.2 0.6 1	0.2 0.6 1

Footnote: Data from individual studies included only if at least sensitivity or specificity could be calculated. Unless stated otherwise, CT slice thickness refers to the thickest slices used (i.e. in studies with more than one scanner). CTA = CT angiography. MRA = MR angiography. HAS = Hyperattenuating Artery Sign. TP = True positive. TN = True negative. FP = False positive. FN = False negative. CI = Confidence Interval.
* One patient had both a false positive HAS and a true occlusion without HAS (FN) in contralateral arteries. As such 39 results are reported from 38 angiograms.
† Data for proximal and distal middle cerebral artery were presented separately providing assessment of 200 arterial segments from 100 angiograms.
‡ Thin-slice CT data are presented. Thick-slice (5mm) data for the same angiography is also available.

Table 7-5 presents subgroup analyses assessing the relationships between the presence of HAS, the location and extent of arterial obstruction and time from stroke onset to scan. HAS was more common with angiographic obstruction in proximal arteries (189/402, 47%) rather than distal (92/247, 37%) and this difference was significant (p=0.015). The number of obstructed arterial segments showed a trend for increased rates of HAS (59% if two segments or more were obstructed compared with 49% if only one segment was obstructed) but this difference was non-significant (p=0.160). The time from stroke onset to scan was not associated with HAS incidence; 27% had HAS if scanned ≤180 minutes from stroke onset versus 25% if >180 minutes, p=0.682. Three studies with missing data were excluded from analyses of arterial obstruction characteristics and time from stroke onset.

Table 7-5 *Systematic review data assessing how characteristics of arterial obstruction (location and extent) and time from stroke onset affect the frequency of HAS*

Author	Year	Angiography n	Location of Arterial Obstruction		Number of Obstructed Arterial Segments		Time from Stroke Onset to Scan	
			Proximal	Distal	1	≥2	≤180 mins	≥180 mins
Assouline[17]	2005	39	8/16 (50)	6/7 (86)	11/17 (65)	4/8 (50)	-	-
Barber[14]	2004	100	7/25 (28)	11/24 (46)	-	-	-	-
Bastianello[6]	1991	36	12/17 (71)	6/13 (46)	-	-	-	-
Flacke[10]	2000	23	6/10 (60)	0/10 (0)	-	-	-	-
Froehler[20] *	2013	67	15/20 (75)	23/43 (53)	-	-	-	-
Garg[12]	2004	65	-	-	-	-	1/8 (13)	8/57 (14)
Kim[15] †	2005	51	11/38 (29)	7/31 (23)	-	-	-	-
Kim[18]	2008	78	36/56 (64)	10/22 (45)	-	-	-	-
Koga[11]	2003	105	21/63 (33)	6/38 (16)	-	-	-	-
Tomsick[5]	1990	20	4/9 (44)	2/7 (29)	3/10 (30)	3/6 (50)	-	-
Tomsick[7]	1992	38	7/14 (50)	5/12 (42)	8/22 (36)	6/7 (86)	-	-
von Kummer[9]	1994	53	-	-	-	-	20/43 (47)	5/10 (50)

Wolpert[8]	1993	60	12/43 (28)	4/17 (24)	-	-	-	-
IST-3[21]	*2012*	*273*	*50/91 (55)*	*12/23 (52)*	*34/66 (52)*	*28/48 (58)*	*34/151 (23)*	*35/122 (29)*
TOTAL			**189/402 (47)**	**92/247 (37)**	**56/115 (49)**	**41/69 (59)**	**55/202 (27)**	**48/189 (25)**
p-value for difference			**0.015**		**0.160**		**0.682**	

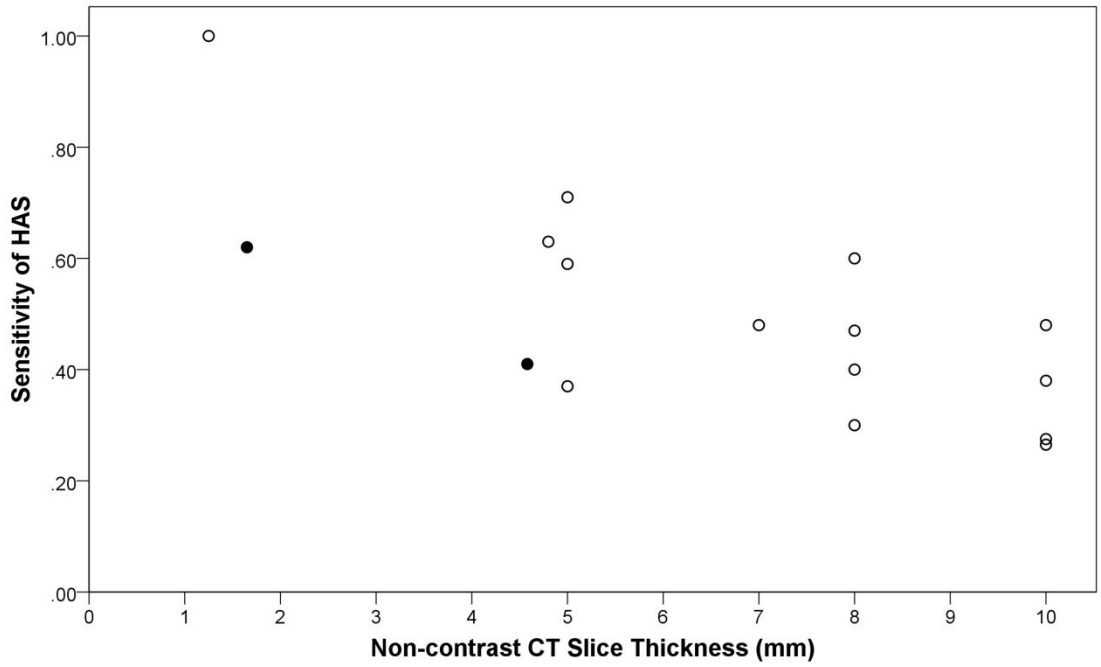
Footnote: Results represent number of HAS within each total (%). Data were only included when results were available for both sides of the equation (e.g. proximal and distal); three articles had incomplete data and are not included. Unless otherwise stated, proximal arterial locations include internal carotid artery, main-stem of the middle cerebral artery (MCA), vertebral and basilar arteries. Distal arterial locations include sylvian branches of the MCA, and anterior and posterior cerebral arteries.

* Proximal and distal arteries are defined as internal carotid and middle cerebral arteries, respectively.

† Thick-slice (5mm) CT data are presented here. Thin-slice data are also available.

Using combined data from the meta-analysis and IST-3, the slice thickness of the non-contrast CT used to identify HAS was significantly associated with the sensitivity of the sign (Figure 7-5, r = -0.73, p=0.001) but not with specificity of HAS. CT slice thickness was also inversely proportional to the year of article publication (r = -0.80, p=0.001, Figure 7-4).

Figure 7-5 *Relationship between the sensitivity of a HAS for arterial obstruction and non-contrast CT slice thickness*



Footnote: Closed dots represent IST-3 data (thin-slice ≤3mm, mean 1.65mm; thick-slice >3mm, mean 4.5mm). Open dots represent results from systematic review.

Two dots have been fractionally altered to reveal identical results (sensitivity = 0.27, slice thickness = 10mm). Correlation is r=-0.73, p=0.001.

7.3 Discussion of HAS Sensitivity and Specificity

In this, the first meta-analysis assessing the accuracy of HAS as a non-contrast CT marker of arterial obstruction in acute ischaemic stroke, I have confirmed that HAS is highly specific and moderately sensitive for angiographically demonstrated arterial obstruction with overall specificity 95% and sensitivity 52%. IST-3, as the largest individual study of HAS sensitivity and specificity, contributes 30% more data (273/902 patients, new total 1175) than was previously available. These results are widely applicable and provide substantial confidence that there is very high likelihood of arterial obstruction when HAS is present, particularly with more severe strokes (there were no false positives in IST-3 with NIHSS >15). However, absence of HAS does not predict normal arterial patency; in acute ischaemic stroke patients without HAS, approximately half will have arterial obstruction on angiography. It remains to be seen whether the presence (or absence) of acute arterial obstruction is important for intravenous thrombolysis treatment decisions, I will address this issue in Chapter 9.

Traditionally, HAS was thought to indicate complete arterial occlusion but data from IST-3 suggest that most arterial segments with HAS (90% in IST-3) are not completely occluded. However, with the CTA technique applied in IST-3, I was not able to determine flow direction. It is possible therefore that in some patients, contrast agent bypassed the arterial obstruction via retrograde collateral flow.[22] Nevertheless, these data still demonstrate that intravenous alteplase should be able to reach beyond the obstructed segment and facilitate lysis in the vast majority of cases; I explore relationships between the completeness of arterial obstruction on angiography and response to intravenous alteplase in Chapter 9.

My meta-analysis confirmed that HAS is more commonly identified with thinner CT slices. Thinner slices do not increase the specificity of HAS, perhaps as HAS is already highly specific for obstruction.[15;23;24] The mean diameter of intracranial arteries is less than 3mm. A slice thickness above this value, as used in the majority of IST-3 patients and most of the other studies reported herein, may impair HAS sensitivity (especially in smaller arteries) by volume averaging intra-luminal thrombus and surrounding CSF space. Volumetric thin-slice CT is becoming more

available; as suggested by the strong and highly significant inverse relationship between the year of publication and CT slice thickness in the systematic review. Allowing for the rising availability of volumetric CT, HAS sensitivity in current routine clinical practice may be higher than I report here.

Meta-analysis also confirmed that HAS is more likely to be identified within proximal rather than distal arteries probably reflecting the larger calibre of proximal arteries and therefore the greater volume of thrombus or embolus required to obstruct them.[18] Other factors I tested in meta-analysis (extent of obstruction and time from stroke onset) were not significantly related to the frequency of HAS.

Strengths and limitations

IST-3 was conducted in many centres so inevitably includes variability in scan parameters and protocols. However, IST-3 represents 'real world practice' and, combined with the systematic review, provides results that are widely applicable to centres assessing acute stroke with a range of CT scanners. Angiography in IST-3 was performed in about 10% of centres and may have been influenced by local practice, so has limitations. Nevertheless, IST-3 angiography remains the largest complete dataset of its kind, the only one performed in the standardised context of a randomised-controlled trial, and increases the available data by almost one-third. In terms of angiographies performed, I found only one larger dataset[25] but it only included patients with a HAS precluding assessment of sensitivity or specificity.

I used a qualitative measure to identify HAS in IST-3 which reflects routine practice. In Chapter 6 I explored whether measuring intra-arterial thrombus density quantitatively interacts with treatment response.

The sensitivity and specificity of HAS changes depending on how angiographic 'abnormality' is defined. I chose to include *any luminal narrowing* (TICI <3) as my measure of abnormality because this maximised the specificity of the sign without unduly undermining HAS sensitivity; a cut-off level at TICI <=2a would have maximised sensitivity with a minor reduction in specificity and could arguably have been chosen instead. Another consideration is that my method of dichotomising angiography results may have included some patients with chronic atheroma in the

'obstructed' group. This could erroneously raise the number of false negative HAS cases and thereby appear to reduce the sensitivity of HAS. However this is a general problem in acute stroke, no other studies that I identified in the literature had addressed this point, and I decided that the opposite approach (to only consider patients with occluded arteries as abnormal) would have been less accurate by having the same effect on HAS specificity but also by excluding patients with genuine non-dense thrombus/embolus from my analyses entirely.

By following PRISMA criteria and adopting a STARD checklist, I maintained a high quality systematic review and meta-analysis of HAS. My search identified a large number of papers, most not relevant but I assert that evaluating several hundred abstracts was preferable to missing relevant work. Excluding abstract-only and non-English publications may have reduced the completeness of this analysis and led to publication bias, but research published only in abstract provided insufficient raw data for the analyses.

The final 16 articles retained for meta-analysis were of moderate to high quality according to my criteria. In particular, most of the data were collected prospectively and the methods were described in sufficient detail to be replicated. More standard definitions for HAS and more consistent reporting of factors such as blinding of image assessment would improve future research.[26]

Conclusions

The high specificity of HAS provides substantial confidence for its use as a surrogate marker of angiographic obstruction and to confirm the diagnosis of acute ischaemic stroke. The moderate sensitivity means that absence of HAS cannot be used alone to indicate that angiography will be normal. Sensitivity of HAS is significantly improved with thin-slice volumetric CT.

7.4 References for Chapter 7

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Chapter 8 Observer Reliability for the Assessment of CTA in Stroke

In this chapter, I use imaging of IST-3 patients with CT angiography (CTA) at baseline to create multi-reader CTA observer reliability analyses.

This chapter addresses **Thesis Aim 4).**

This work is also published in a peer reviewed journal, the complete draft of which is included as Appendix 7, see Chapter 11.3.5:

> Mair G, von Kummer R, Adami A, White PM, Adams ME, Yan B, Demchuk AM, Farrall AJ, Sellar RJ, Ramaswamy R, Mollison D, Boyd EV, Rodrigues MA, Samji K, Baird AJ, Cohen G, Sakka E, Palmer J, Perry D, Lindley R, Sandercock PAG, Wardlaw JM, The IST-3 Collaborative Group. Observer reliability of CT angiography in the assessment of acute ischaemic stroke: data from the Third International Stroke Trial. *Neuroradiology*. 2015;57:1–9.

8.1 Methods for Observer Reliability of CTA

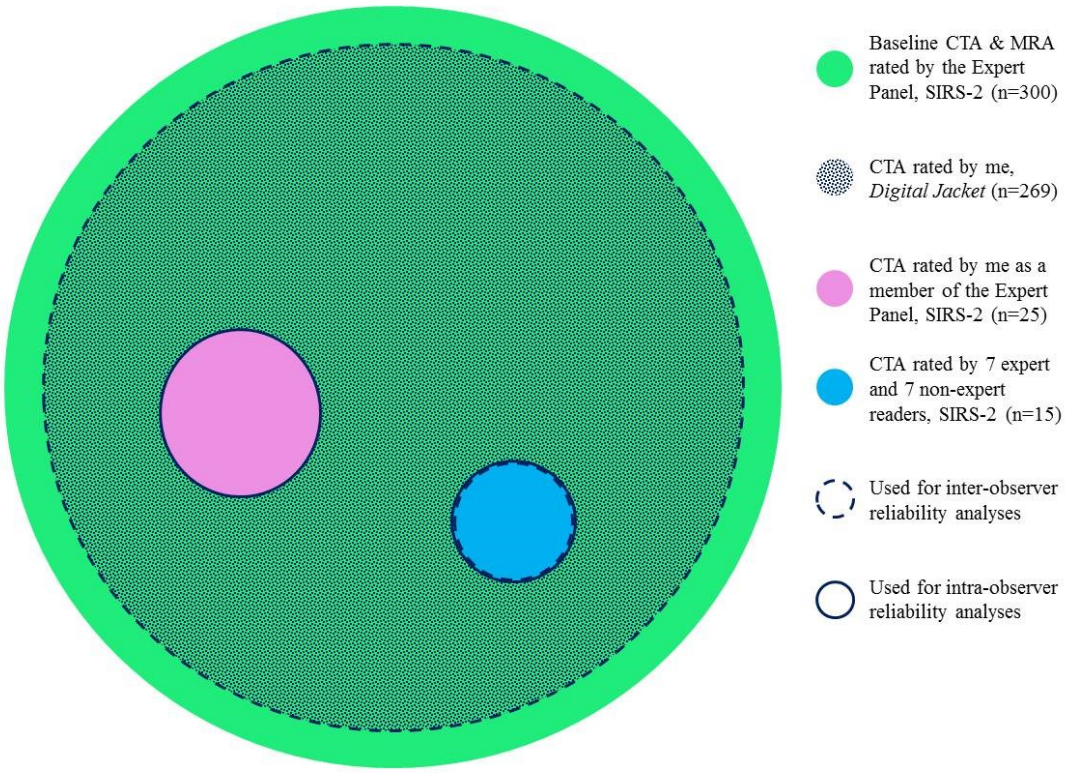
In order to most efficiently test both inter- (same scan, different reader) and intra-observer reliability (same scan, same reader) for the assessment of CTA performed acutely after ischaemic stroke, I performed two tasks:

1. I selected 15 representative CTA cases from IST-3 and recruited a number of expert and non-expert readers to each perform independent rating of all 15 scans online via SIRS-2 (see 3.4.2). As all of the recruited experts had previously served as members of the IST-3 angiography expert panel, a number of these scans were rated in an identical fashion by the same individual on two separate occasions.
2. I identified when IST-3 CTA scans had already been formally evaluated on more than one occasion and I matched and compared the assessment data from each episode. Essentially, this means I collated results from my own assessment of IST-3 baseline angiography (see Chapter 4.3) with that of the

IST-3 expert panel. On both occasions the same scan reading proforma was used, however, the expert panel performed reads online via SIRS-2 while I performed reads locally on the server using *Digital Jacket* (V5.0, DesAcc Inc, Terre Haute, IN, USA). As I also previously served as a member of the expert panel, a small number of scans were rated by me on two different occasions using two different viewing platforms. A secondary benefit of this comparison therefore was to evaluate whether choice of viewing platform significantly affects the results of image assessment.

Note that both task 1 and task 2 provided data for both inter- and intra-observer analyses. An overview of the different datasets and reader groups available for observer reliability analysis is provided in Figure 8-1.

Figure 8-1 *Different image reading subgroups from among all patients in IST-3 with angiography performed at baseline and how these subgroups were used for inter and intra-observer analyses.*



Footnote: SIRS-2 = Systematic Image Review System 2.

For all observer reliability analyses, users were presented only with imaging performed concurrently (non-contrast CT and CTA) i.e. readers were blinded to all other imaging and to any previous reports for that imaging, to clinical and to treatment allocation data. Note therefore that as CTA was always rated in the presence of concurrent non-contrast CT, observer reliability data for non-contrast CT were automatically compiled in parallel with CTA. The results of both are displayed below (Chapter 8.2).

8.1.1 Inter-observer reliability analyses

As discussed above, inter-observer reliability for the assessment of CTA, was tested in two separate analyses: the first used 15 representative CTA cases from IST-3 rated by 14 individuals of varying experience, specifically for the purpose of this observer reliability analysis (i.e. new data); the second compared ratings that had already been collected for the assessment of all baseline CTA in IST-3 from both the expert panel and from me.

Assessment of 15 cases rated by expert and non-expert readers

Selection of cases: I identified a subgroup of IST-3 patients (n=15) who had both non-contrast CT and CTA at baseline. These 15 cases were chosen to represent a range of angiographic findings (e.g. presence/absence of arterial obstruction in variable locations, clot burden) based on the consensus opinion of three senior neuroradiologists, Table 8-1. In three of these cases, CTA was deemed to be normal. In the remaining 12 cases, arterial obstruction of varying severity (TICI 1-2b) was identified in an internal carotid artery (n=4), in a middle cerebral artery (n=7), or in the basilar artery (n=1). Clot Burden scores ranged from 1-10.

Selection of readers: I identified 14 readers comprised of seven (of the original ten) expert IST-3 angiography panel members (each with greater than five years of experience in assessing CTA in acute stroke – the *expert group*) and also by seven non-expert readers (radiology trainees with less than two years of experience assessing CTA – the *non-expert group* – but this group included some with and some

without senior neuroradiology training). This approach enabled me, with approximately two years of experience in the interpretation of CTA, to include myself in the *non-expert group* (radiology trainee) which was most appropriate for the present analysis. This is however slightly counter to the fact that I had also previously served as a member of the IST-3 expert panel for angiography.

Scan rating: All 15 cases for this reliability analysis were independently rated by the 14 readers using SIRS-2 in an identical manner as described in Chapter 3.4.2 for the original IST-3 assessment of imaging. These scan ratings (of both non-contrast CT and CTA) were performed purely to assess reader reliability and were undertaken in addition to, and separate from, scan ratings performed during the main IST-3 trial and the IST-3 angiography subgroup analysis.

First inter-observer analysis: Three distinct inter-observer analyses were performed using the 15 cases:

1. The *expert group* inter-observer reliability analysis compared seven observers, i.e. maximum 315 pairs of readings for each imaging characteristic assessed (21 reader pairs x 15 cases).
2. The *non-expert group* (n=7) were assessed in a separate but identical analysis as for the *expert group* (maximum 315 pairs of readings).
3. To ascertain whether additional neuroradiology training might improve the inter-observer reliability of non-expert CTA readers, the results of neuroradiology specialist trainees (n=3) from within the *non-expert group* were separately examined as a subgroup, i.e. maximum 45 pairs (3 x 15) of readings for each imaging characteristic.

Comparison of IST-3 angiography cases rated by the expert panel and by me

Cases and readers: As discussed in Chapter 3.4.2, all baseline angiography routinely acquired for patients recruited into IST-3 was collected and centrally assessed by the IST-3 angiography expert panel of 10 readers using the SIRS-2 platform. As discussed in Chapter 4.3, baseline angiography in IST-3 was also separately and

independently assessed by me at a different time using *Digital Jacket* software. I did not have access to expert panel results during my own assessment of the imaging.

Scan rating: These two datasets were scored using the same imaging assessment proforma (Appendix 9, Chapter 11.4.1) including the scan rating characteristics previously discussed (Table 3-3).

Second inter-observer analysis: I matched and compared CTA ratings for all patients assessed by both the IST-3 expert panel using SIRS-2 and by me using *Digital Jacket* software. Where expert panel assessment was also performed by me, these results were included in the intra-observer reliability analysis, see Chapter 8.1.2.

When I assessed IST-3 angiography using *Digital Jacket* software, central trial office validation of angiographic imaging was incomplete. Following central validation, the initial classification of some scans (based on information provided by recruiting centres) was changed; i.e. the initial scan classification changed from pre-randomisation (baseline) to post-randomisation and vice versa in a few cases based on the time difference between non-contrast CT and angiography (had to be <30 minutes to be considered baseline). The initial classification of scans which I used to direct my own imaging assessment included 269 patients with CTA (compared with 271 in the final centrally validated classification, see Chapter 3.8). Of these 269, I also provided the expert panel assessment for 25 cases (included in the intra-observer analyses). There was therefore a maximum of 244 pairs of readings for each imaging characteristic assessed in these inter-observer analyses. Note that in each case a comparison is being made between my assessments of the imaging versus a variable member of the IST-3 expert panel's assessment of the same imaging.

Table 8-1 *Consensus opinion of three senior neuroradiologists for CTA results of the 15 cases selected for inter-observer analysis*

Scan	**CTA Abnormal** (yes/no)	**Location of CTA Abnormality**	**TICI Score** (0-3)	**IST-3 Angiography Score** (0-4)	**Clot Burden Score** (10-0)	**Collaterals** (good, moderate, poor)	**CTA-SI Deficit** (yes/no)
1	Yes	ICA	1	1	7	Poor	Yes
2	No	-	3	4	10	Good	No
3	Yes	M1	2a	2a	7	Moderate	Yes
4	Yes	M1	2b	2b	8	Good	Yes
5	Yes	M2	2a	2a	9	Moderate	Yes
6	Yes	ICA	2b	3	8	Good	Yes
7	Yes	ICA	1	1	1	Good	Yes
8	Yes	M1	2b	3	8	Good	No
9	No	-	3	4	10	Good	No
10	Yes	M1	2a	2a	7	Moderate	No
11	Yes	ICA	2b	2b	7	Good	Yes
12	Yes	Basilar	2b	3	9	Good	Yes
13	Yes	M2	1	1	8	Moderate	Yes
14	Yes	M1	2b	2b	8	Good	Yes
15	No	-	3	4	10	Good	Yes

Footnote: TICI = Thrombolysis in Cerebral Infarction. CTA-SI = CT Angiography Source Image. ICA = Internal Carotid Artery. M1 = Horizontal segment of middle cerebral artery. M2 = Sylvian branch of middle cerebral artery.

8.1.2 Intra-observer reliability analyses

Intra-observer reliability for the assessment of CTA was also tested in two separate analyses, as discussed above in Chapter 8.1.1: the first used the 15 representative CTA cases specifically selected from IST-3 for reliability analysis; the second used 25 cases, which were rated twice by me.

In all cases for intra-observer analysis, the user had no knowledge of their previous scan assessment or even, in most cases, that a previous assessment had taken place. All paired scan reads for intra-observer analyses were separated in time by at least four weeks, but in many cases, up to one year passed between scan reads.

Assessment of 15 cases rated by expert and non-expert readers

Each of the 15 IST-3 scans specifically selected for this observer reliability analysis had already been independently but otherwise identically (as per 3.4.2) rated by one member of the expert panel for the primary IST-3 angiography substudy assessment. When the angiography substudy assessment and the rating collected for reliability analysis were performed by the same individual, these were matched and compared; i.e. maximum 15 pairs of readings for each imaging characteristic assessed.

Comparison of 300 cases rated by IST-3 expert panel and by me

While serving as a member of the IST-3 expert panel for angiography, I assessed 25 baseline CTA cases using SIRS-2. At a different time and without knowledge of case specifics, I again assessed these cases as part of my own assessment of IST-3 angiographic imaging using the *Digital Jacket* software. Results from these two separate scan rating roles were matched and compared, i.e. maximum 25 pairs of readings for each imaging characteristic assessed.

8.1.3 Observer reliability of non-contrast CT

In addition to examining CTA scans, in all cases concurrent non-contrast CT scans were simultaneously provided to the readers and rated using the non-contrast CT components of the CT rating proforma (Appendix 9, see Chapter 11.4.1); the IST-3 method for assessing non-contrast CT is discussed more fully in Chapter 4.3.

In order to most efficiently use all collected data, the results of these non-contrast CT assessments were also examined in an identical manner as for CTA to provide observer reliability data for the assessment of non-contrast CT performed acutely after ischaemic stroke; this was particularly relevant for the assessment of HAS.

8.1.4 Statistical testing

All reliability analyses were performed using Krippendorff's alpha (K-alpha) with 1000 bootstrap samples for each. K-alpha results range from -1.0 to +1.0 where +1.0 equates to perfect agreement, 0.0 means no agreement and -1.0 implies perfect disagreement.[1] I have adopted the Landis and Koch approach for interpreting these results: K-alpha 0.00-0.20 = slight agreement, 0.21-0.40 = fair agreement, 0.41-0.60 = moderate agreement, 0.61-0.80 = substantial agreement and 0.81-1.00 = almost perfect agreement.[2]

Differences in K-alpha between expert and non-expert groups (including between neuroradiology specialist trainees and others), between imaging characteristics assessed on non-contrast CT and CTA or between assessments performed on different image viewing platforms were not tested for significance.

Statistical software

All analyses were performed using IBM SPSS Statistics software, version 20.0 (IBM Corporation, Armonk, NY, USA). SPSS does not provide native support for K-alpha; an appropriate macro was therefore applied, available at: http://afhayes.com/spss-sas-and-mplus-macros-and-code.html. This macro automatically provides 95% confidence interval data when used in bootstrapping mode.

8.2 Results for Observer Reliability

Inter-observer reliability analyses for CTA

Inter-observer reliability analyses for the assessment of CTA among expert and non-expert readers (n=7 for both groups) are displayed in Table 8-2. Expert readers had moderate to substantial agreement for all CTA measures (K-alpha in range 0.56-0.70) except identification of a perfusion deficit on CTA source images (CTA-SI, K-alpha = 0.32). Non-expert readers had only fair to moderate agreement (K-alpha in range 0.25-0.61) for all CTA variables. However among non-experts, neuroradiology specialist trainees' (n=3) agreement compared more favourably with the expert group (K-alpha in range 0.36-0.78).

Inter-observer reliability results comparing me as a single reader using Digital Jacket directly on the local server versus a variable member of the expert panel using SIRS via the web, were also generally comparable to the inter-observer results of the expert readers using SIRS alone (Table 8-2).

Inter-observer agreement among experts and non-experts with additional training was greatest for assessing whether CTA was 'normal' or 'abnormal' (i.e. any intracranial arterial obstruction or occlusion). For assessing the extent of angiographic abnormality, IST-3 scoring performed better than TICI in all groups although the 95% confidence intervals overlap so this difference is unlikely to be significant. Expert reader TICI scores for all 15 cases are presented in Table 8-3. Note that while there are some discrepancies, most of the disagreements do not differ by more than two scalar points.

The assessment of CTA collateral supply and the identification of a perfusion deficit on CTA-SI scored lowest for inter-observer reliability in all groups.

Table 8-2 *Observer reliability analyses for CT Angiography among expert and non-expert readers*

	Expert Readers		**Non Expert Readers**			
	n=7		n=7	Neuroradiology Trainees, n=3	Single Reader	
Total Stroke Cases	**15 Cases**				**269 Cases**	
Inter or Intra-Observer	**Inter** (315 pairs)	**Intra** (15 Pairs)	**Inter** (315 pairs)	**Inter** (45 pairs)	**Inter** (244 pairs)	**Intra** (25 Pairs)
CTA Abnormal (y/n)	0.70 (0.52-0.85)	0.54 (0.08-1.00)	0.49 (0.32-0.66)	0.78 (0.56-0.94)	0.59 (0.41-0.74)	0.82 (0.55-1.00)
TICI Score[3] (0-3)	0.60 (0.53-0.66)	0.60 (0.12-0.94)	0.39 (0.28-0.50)	0.63 (0.42-0.81)	0.62 (0.51-0.71)	0.75 (0.57-0.90)
IST-3 Angiography Score[4] (0-4)	0.66 (0.60-0.70)	0.63 (0.16-0.91)	0.43 (0.32-0.52)	0.72 (0.52-0.87)	0.62 (0.51-0.71)	0.78 (0.58-0.92)
Clot Burden Score[5] (10-0)	0.63 (0.56-0.70)	0.33 (-0.28-0.87)	0.61 (0.52-0.69)	0.76 (0.60-0.90)	0.76 (0.68-0.83)	0.86 (0.68-0.98)
Collaterals[6] (Good, moderate, poor)	0.56 (0.44-0.67)	0.72 (0.54-0.91)	0.30 (0.14-0.42)	0.36 (0.06-0.64)	0.43 (0.23-0.63)	0.56 (0.17-0.83)
CTA-SI Deficit (y/n)	0.32 (0.13-0.52)	0.57 (-0.29-1.00)	0.25 (0.07-0.45)	0.60 (0.35-0.80)	-	-

Footnote: Both inter and intra-observer results are provided. Results represent K-alpha (95% confidence interval). Maximum number of possible result pairs in each calculation is provided.

CTA = CT Angiography. TICI = Thrombolysis in Cerebral Infarction. CTA-SI = CT Angiography Source Images.

Table 8-3 *Comparison of angiography scores from 15 scans as assessed by 7 members of the IST-3 expert panel*

Scan	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7
1	2a	1	1	2b	0	2b	1
2	1	0	2b	1	0	0	0
3	3	3	3	3	3	3	3
4	2a	0	1	2a	0	0	0
5	2b	0	3	2b	0	0	1
6	2b	2b	1	2b	3	2b	3
7	1	1	1	2b	0	0	1
8	2b	3	3	3	3	3	3
9	3	3	3	3	3	3	3
10	2b	0	0	2b	0	0	0
11	1	3	3	2b	1	0	3
12	1	0	0	1	0	0	0
13	2b	1	2b	2b	0	0	2a
14	2a	3	3	3	3	3	3
15	2a	0	0	2a	0	1	2a

Footnote: K-alpha for this inter-observer analysis (315 pairs of readings) is 0.60 (95%CI = 0.53-0.66). Angiography scored using TICI = Thrombolysis in Cerebral Infarction: 0= No flow/patency, 1= minimal flow/patency, 2a= Partial flow/patency of <50% of expected territory, 2b= Partial flow/patency of >50% of expected territory, 3= Complete flow/patency.

Intra-observer reliability analyses for CTA

My own intra-observer agreement was generally greater (K-alpha in range 0.56-0.86) than that of the expert readers (K-alpha in range 0.26-0.72) however the wide 95% confidence intervals for intra-observer analysis among the expert readers indicate

that these results are underpowered (Table 8-2). Consider also that for the expert readers, there were relatively few scan rating pairs for any individual reader.

These results also indicate that reading the scans using different viewing platforms (SIRS-2 versus Digital Jacket) did not adversely affect my own intra-observer reliability compared with other expert panel members who used the same viewing platform (SIRS-2) on both occasions.

Reader reliability for CTA versus non-contrast CT

Table 8-4 presents inter and intra-observer reliability analyses for the expert readers' assessment of non-contrast CT. Most imaging characteristics were well represented by the 15 selected cases. These results show fair to moderate agreement for most imaging characteristics. Identification and classification of ischaemia (using either ASPECTS or the IST-3 Ischaemia score) showed the best agreement (K-alpha 0.56-0.66 for both inter- and intra-observer analyses).

Identification of a HAS showed only fair inter-observer agreement but almost perfect intra-observer agreement (K-alpha 0.37 and 0.83, respectively).

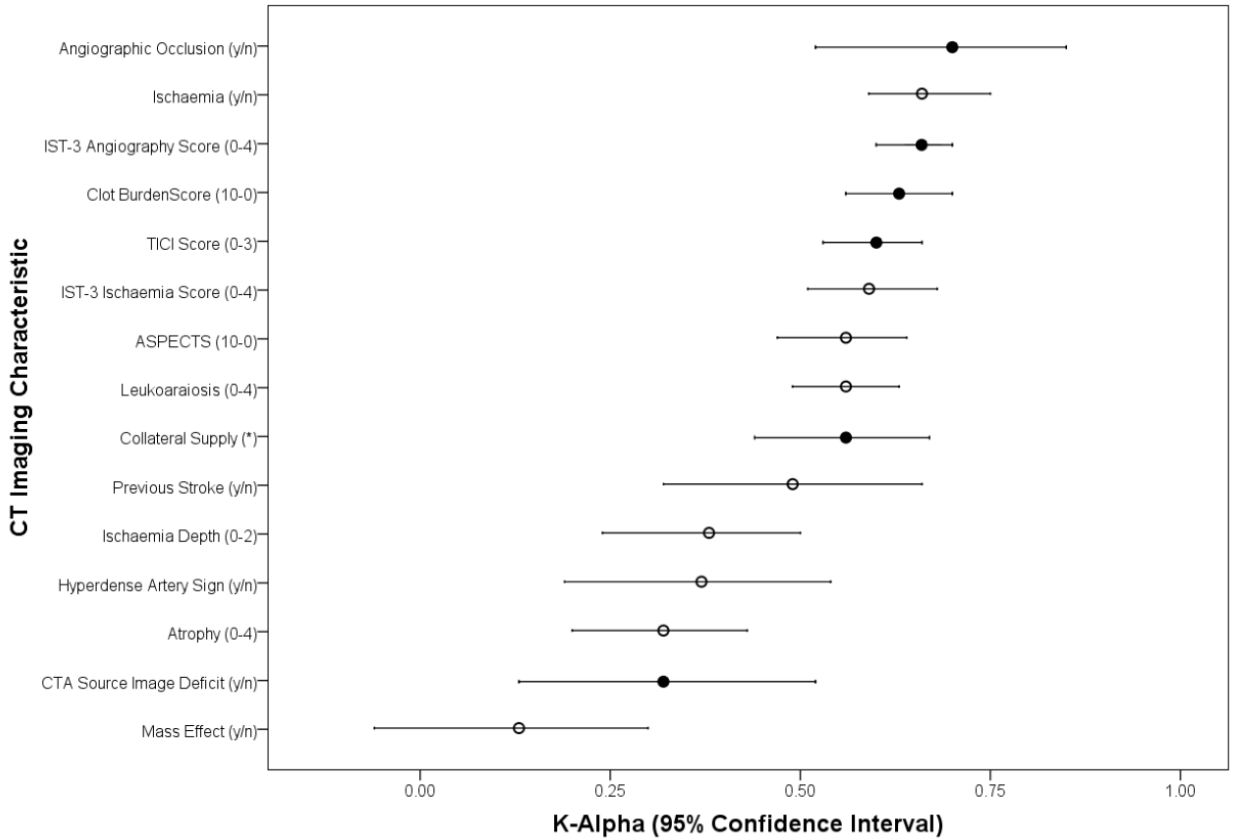
Table 8-4 *Results of expert readers' inter and intra-observer analyses for a range of imaging characteristics assessed on non-contrast CT*

Imaging Characteristic	**Consensus Opinion of Three Senior Neuroradiologists**	**Expert Reader Reliability**	
		Inter-observer	**Intra-observer**
Acute features on non-contrast CT			
Ischaemia	Yes = 75%	0.66 (0.59-0.75)	0.66 (0.39-0.93)
ASPECTS[7] (10-0)	7 (5-9)	0.56 (0.47-0.64)	0.64 (0.42-0.82)
IST-3 Ischaemia Score[8] (0-4)	2 (1-3)	0.59 (0.51-0.68)	0.61 (0.32-0.84)
Ischaemia Depth (0-2)	0 = 20% 1 = 80%	0.38 (0.24-0.50)	0.54 (0.02-0.90)
Mass Effect	Yes = 62%	0.13 (-0.06-0.30)	0.39 (-0.02-0.80)
HAS	Yes = 67%	0.37 (0.19-0.54)	0.83 (0.58-1.00)
Chronic features on non-contrast CT			
Atrophy[9] (0-4)	1 (1-2)	0.32 (0.20-0.43)	0.46 (0.17-0.70)
Leukoaraiosis[10] (0-4)	2 (1-3)	0.56 (0.49-0.63)	0.69 (0.34-0.92)
Previous Stroke	Yes = 47%	0.49 (0.32-0.66)	0.47 (0.17-0.78)

Footnote: Consensus results represent median (inter-quartile range) or percentage (total n=15). Reliability results represent K-alpha (95% confidence interval). Ischaemia defined as loss of grey-white matter differentiation or parenchymal hypoattenuation. ASPECTS = Alberta Stroke Program Early CT Score. Ischaemia Depth = degree of hypoattenuation (0 = none, 1 = grey matter appears indistinct from white matter, 2 = grey and/or white matter of lower attenuation than normal white matter). HAS = Hyperattenuating Artery Sign.

Figure 8-2 stratifies the expert readers' inter-observer reliability for all imaging characteristics of both non-contrast CT and CTA. Agreement was generally greater for the assessment of CTA features (K-alpha 0.32-0.70) than for non-contrast CT features (K-alpha 0.13-0.66) although the ranges were similar. In addition, four of the top five agreement scores were for imaging characteristics assessed on CTA.

Figure 8-2 *Expert reader inter-observer reliability results for the assessment of imaging characteristics of both non-contrast CT and CTA*



Footnote: K-Alpha of 1.0 = perfect agreement, 0.0 = no agreement, -1.0 = perfect disagreement. *Collateral supply is ranked as Good, Moderate or Poor. Closed circles represent imaging characteristics identified on CTA. Open circles represent imaging characteristics identified on non-contrast CT. Ischaemia defined as loss of grey-white matter differentiation or parenchymal hypoattenuation. TICI = Thrombolysis in Cerebral Infarction. ASPECTS = Alberta Stroke Program Early CT Score.

8.3 Discussion of Observer Reliability Results

In this analysis where 14 observers with differing levels of experience, assessed a purposive sample of 15 examinations, I show that CTA features have slightly higher levels of agreement than non-contrast CT features. Imaging characteristics that are likely to have the greatest clinical impact (e.g. the presence and severity of arterial occlusion) have the highest inter-observer agreement, both for experienced (K-alpha > 0.60) and inexperienced observers. There was less agreement over arterial collateral supply and use of CTA-SI to identify perfusion deficits, even among experienced observers (K-alpha 0.30-0.60). Despite being comparatively inexperienced, the participating radiology trainees that had undertaken additional neuroradiology training (neuroradiology fellows) performed as well as experts in the assessment of CTA. This implies that, with adequate training, CTA can be reliably assessed even by readers with less case-based experience.

Somewhat surprisingly, I found only fair inter-observer agreement for the identification of HAS (K-alpha 0.37) but near perfect intra-observer agreement (K-alpha 0.83; this was the second highest level of agreement for any of the features assessed in either inter- or intra-observer analyses). My interpretation of these results is that the identification of HAS is highly consistent for individuals but that there remains some discrepancy in the definition of what represents a true HAS between different observers.

I also show that my personal inter and intra-observer reliability for assessing CTA was consistent with equivalent results from the IST-3 expert panel as a whole. This provides confidence that my own results are meaningful and applicable to the work but also that my ability to interpret the imaging was not clearly affected by using a different image viewing platform. I suspect this latter observation reflects that reporting radiologists are routinely required to be adaptable to different viewing conditions and given a particular viewing platform will quickly adjust it to suit their own needs and preferences. This observation also provides some assurance that if radiological imaging assessment for multicentre research projects cannot be standardised, variability in image viewing techniques and conditions may be less important than previously supposed.

The IST3 angiography score is an adaptation of earlier scores (TICI, Mori).[3;11] It is designed to overcome the limitations of using a catheter angiography score for the assessment of CTA by primarily assessing residual arterial calibre at the point of stenosis and contrast penetration into the major distal vessels only and makes no attempt to assess distal tissue perfusion.[4] The present work represents the first external testing of observer reliability for the IST-3 angiography score and it compares favourably with TICI.

To the best of my knowledge, there are only a few previous studies of CTA reliability in stroke; all had fewer than seven observers and none tested all the CTA characteristics assessed in my analysis. Knauth et. al. reported an inter-reader kappa=0.78 for two readers identifying the correct location of occlusion on CTA in acute ischaemic stroke.[12] Suh et. al. compared TICI versus a modified TICI score and found both scales were moderately repeatable (intra-class coefficients, ICC, 0.67 and 0.73, respectively) across five readers.[13] IST-3 expert readers did not replicate inter- and intra- observer reliability demonstrated by Puetz and colleagues in their original report of the Clot Burden Score (six readers, ICC=0.87 and 0.96, respectively) despite similar reader numbers.[5] Similarly, in the original report defining their classification of collateral status, Miteff and colleagues demonstrated an inter-observer reliability of kappa=0.93 for two observers.[6] IST-3 expert readers did not replicate those findings but our results are more consistent with other methods of assessing leptomeningeal flow as demonstrated on systematic review (0.49-0.87).[14] Neither did IST-3 expert readers replicate the results from three recent articles, each with four readers that demonstrated improved detection of infarct using CTA-SI over non-contrast CT alone: Hopyan et. al. improved reader agreement from kappa 0.28-0.44 to 0.34-0.57;[15] Finlayson et. al. showed an increase in ICC from 0.83 to 0.88;[16] while van Seeters and colleagues improved their ICC range from 0.54-0.62 to 0.57-0.76.[17]

These studies report a mixture of kappa statistics and ICC and are not directly comparable with my K-alpha results; any comparisons should be treated with caution. Nevertheless, kappa, ICC and K-alpha work on the same numerical scale and are therefore broadly similar. Following discussion with a local medical

statistician, I opted to use K-alpha for several reasons. Cohen's Kappa is only suitable for assessing two observers rating nominal data, adjusted Kappa techniques are required to handle ordinal data or comparisons of more than two observers and even then Kappa may not be the most suitable test;[18;19] I had up to seven observers per analysis and a mixture of nominal and ordinal data. K-alpha has been shown to provide a more robust measure of observer variance than kappa or ICC and provides several advantages to the user; it allows comparisons between any number of observers, it can handle categorical and ordinal data, it is less prone to the influences of observer bias and result prevalence and it can still be computed in the presence of missing data.[1;20]

Strengths and limitations

Other strengths of my work include; a greater number of readers than in previous studies; calculation of both inter- and intra-observer reliability; use of a robust, standardised image analysis platform, previously shown to provide consistent multi-user reporting;[21-23] complete blinding of readers to all clinical information and to any other scan assessments; and use of representative cases from a multicentre trial which increases the generalisability and real world relevance of my results.

My work also has some limitations. Firstly, in contrast to previous work[23] I did not formally produce a single reference standard for the 'correct' interpretation of the 15 scans to compare with other readers. Use of a reference standard would have allowed me to assess reader accuracy in addition to reader reliability. The results in Table 8-1 represent the consensus opinion of three senior neuroradiologists but are nevertheless still open to interpretation error. By confirming high observer agreement among a group of seven experienced readers, including several senior neuroradiologists, I believe that my results are as informative as reader comparisons set against any reference standard created from the same data. I do however acknowledge the possibility that the expert readers were reliable in making false diagnoses but feel this is highly unlikely. Secondly, several of the characteristics I tested in intra-observer analyses are probably underpowered, evidenced by the wide confidence intervals. It is possible that if I had used more than 15 representative CTA cases, there might have been less variability in the results of these intra-observer

analyses. However, I decided upon 15 as this number allowed me to include a wide range of imaging characteristics without over-burdening the expert readers. Finally, the 15 cases were not deliberately chosen to be representative of the range of imaging features identifiable on non-contrast CT which may have affected the levels of agreement here. Nevertheless, most variables included a good range of values.

Conclusion

Experienced observers report CTA performed for the assessment of acute ischaemic stroke with substantial levels of agreement for most imaging characteristics. Non-expert readers perform well if given specialist training. The IST3 angiography score is reported as reliably as TICI and has some face validity and practical advantages for the assessment of CTA.

8.4 References for Chapter 8

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Chapter 9 CT and MR Angiography in IST-3

In this chapter, I use two datasets: first, data from my own assessment of IST-3 imaging of patients with non-contrast CT and concurrent CTA performed at baseline; second, existing data from the IST-3 expert panel's assessment of baseline CTA and MRA. These datasets are used to undertake three separate but related analyses examining the importance of angiographic imaging as a baseline investigation for patients presenting acutely with ischaemic stroke:

1. Diagnostic and prognostic benefits of adding CTA to routine stroke imaging and determination of the extra time needed to add CTA (9.1)
2. Relationships between CTA/MRA angiography results, response to thrombolysis and outcome in IST-3 (9.2)
3. Meta-analysis of randomised controlled trial data testing for interactions between angiography findings, treatment with intravenous thrombolysis and outcome after stroke (9.3)

This chapter addresses **Thesis Aims 5) and 6).**

The first part of this chapter was presented in two posters at the European Stroke Conference in London, 28-31 May 2013. The finalised posters are included as Appendices 13 and 14 (Chapter 11.5).

> Mair G, Wardlaw JM, Sandercock P, Lindley R, von Kummer R. Combining CT angiography with non-contrast CT to predict infarct on follow up CT in acute ischaemic stroke. Substudy analysis of imaging from the Third International Stroke Trial (IST-3). *Cerebrovascular Diseases* 2013;35(Suppl 3):237 (Abst.15)

> Mair G, Wardlaw JM, Sandercock P, Lindley R, von Kummer R, Farrall AJ. Association of non-contrast CT and CT angiography with baseline clinical deficit and functional outcome. Substudy analysis of imaging from the Third International Stroke Trial (IST-3). *Cerebrovascular Diseases* 2013;35(Suppl 3):405 (Abst.226)

The second and third parts of this chapter are published in a peer reviewed journal, the complete draft of which is included as Appendix 8, see Chapter 11.3.6:

> Mair G, von Kummer R, Adami A, White PM, Adams ME, Yan B, Demchuk AM, Farrall AJ, Sellar RJ, Sakka E, Palmer J, Perry D, Lindley RI, Sandercock PAG and Wardlaw JM and for the IST-3 Collaborative Group. Arterial Obstruction on Computed Tomographic or Magnetic Resonance Angiography and Response to Intravenous Thrombolytics in Ischemic Stroke. *Stroke* 2017;48:353-360

9.1 Baseline CTA for Improving Stroke Detection and Predicting Outcome, Time Needed for CTA

These analyses were designed to test whether the extra information provided from CTA performed acutely after ischaemic stroke can improve a readers' ability to identify relevant imaging features consistent with ischaemic stroke (i.e. whether the addition of CTA improves the rate of accurate diagnosis of ischaemic stroke using imaging alone) and also whether this extra information from CTA can be used to better predict prognosis (baseline stroke severity and six-month functional outcome) for a given patient compared with non-contrast CT imaging alone.

9.1.1 Methods

As discussed in Chapter 4.3, among IST-3 patients with concurrent non-contrast CT and CT angiography at baseline, I had assessed these scans (and the associated follow-up scans) for any acute imaging abnormality indicative of ischaemic stroke. Relevant abnormality on non-contrast CT included loss of normal grey-white matter differentiation, the presence of parenchymal hypoattenuation or a HAS. Relevant abnormality on CTA was arterial obstruction or occlusion.

Testing effect of CTA results on infarct detection and prognosis prediction

Non-contrast CT and CT angiography (CTA) data were used to determine the sensitivity and specificity of different combinations of abnormal imaging appearances at baseline for predicting the presence of infarct on 24-48 hour follow-up imaging.

Sensitivity and specificity were calculated for:

- Acute abnormality of non-contrast CT alone
- Acute abnormality of CTA alone
- Acute abnormality of either non-contrast CT or CTA
- Acute abnormality of both non-contrast CT and CTA

The sensitivities and specificities of these different combinations of imaging abnormality were compared graphically using likelihood ratios to determine whether the addition of CTA improved the ability of CT at baseline to predict infarct at 24-48 hour follow-up.

Non-contrast CT and CTA data were also used to determine whether adding CT angiography to standard non-contrast CT improved the ability of baseline imaging to predict clinical stroke severity at onset and functional outcome 6-months after stroke. NIHSS and OHS were compared between those with normal baseline imaging and the groups with abnormality of non-contrast CT alone and those with abnormality of either non-contrast CT or CTA. As before, relevant abnormality on non-contrast CT was the presence of acute ischaemia or HAS. Relevant abnormality on CTA was arterial obstruction or occlusion.

Additional time needed for CTA versus time needed for non-contrast CT alone

Among patients with non-contrast CT and CTA performed at baseline, I recorded the date and time of both scans from the DICOM headers. I did not record the scan times if non-contrast CT and CTA were clearly performed during separate trips to the scanner (e.g. two different studies separated by more than 30 mins). I noted if CT perfusion had also been concurrently performed.

I calculated the time difference in minutes between non-contrast CT and CTA.

Statistical testing

After deriving true and false positive and negative results (TP, FP, TN, FN, respectively) for the combinations of imaging abnormality described above, I calculated sensitivity and specificity as before and as follows:

- Sensitivity (%) was calculated as TP / (TP + FN) x 100
- Specificity (%) was calculated as TN / (TN + FP) x 100

Decision matrices were derived from sensitivity and specificity data to allow a comparison of the diagnostic capabilities of the different non-contrast CT and CTA imaging combinations. Chi-square statistics (χ^2) were used to determine if matrix differences were significant.[1]

I derived likelihood ratios from sensitivity and specificity data comparing non-contrast CT with and without the addition of CTA for predicting infarct on follow-up imaging by plotting the true versus false positive rates.[2] Thus a graphical comparison of the diagnostic capabilities of different imaging combinations was created.

Mann-Whitney U testing was used to compare median NIHSS (National Institutes of Health Stroke Scale) and OHS (Oxford Handicap Scale, see Chapter 3.3 for both) between groups with and without imaging abnormality for the different combinations of imaging modality assessed.

9.1.2 Results

Non-contrast CT and CTA were performed concurrently at baseline in 271 IST-3 patients. Nearly half of these patients (119, 44%) were male with a median age of 81 years (inter-quartile range, IQR 71-85). Non-contrast CT was performed at median 170 minutes from stroke onset (IQR 110-252). Follow up non-contrast CT was performed within 48 hours for 92%.

Baseline non-contrast CT was acutely abnormal (ischaemia or HAS) in 36% (95% confidence interval, 95%CI 30-42%). CTA was acutely abnormal (arterial obstruction or occlusion) in 42% (95%CI 36-48%). Either non-contrast CT or CTA were abnormal in 50% (95%CI 44-56%); both were abnormal in 28% (95%CI 23-33%).

Sensitivity and specificity of CT and/or CTA to predict infarct on follow-up imaging

Table 9-1 displays the sensitivities and specificities for different combinations of abnormal non-contrast CT and/or abnormal CTA at baseline to predict infarct on

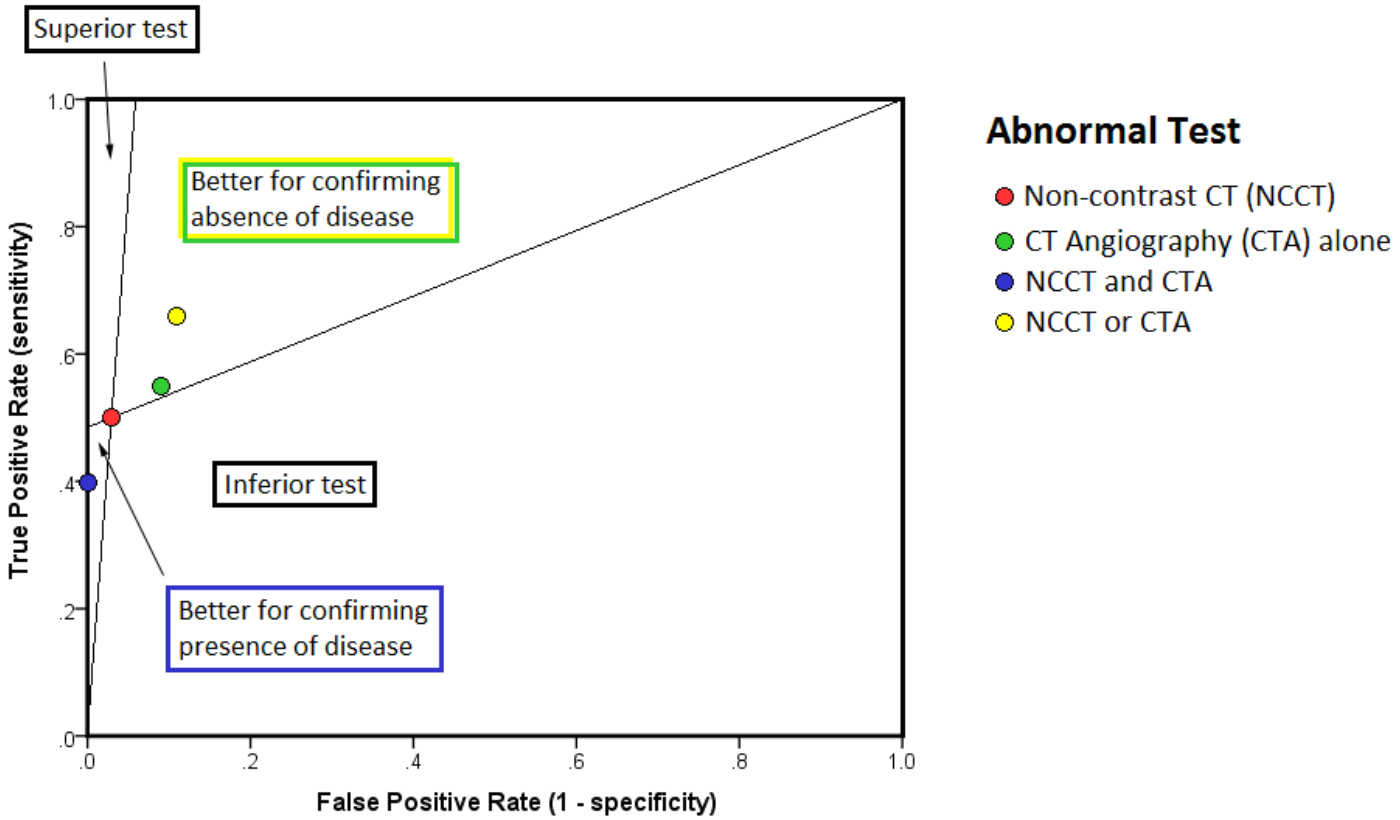
short-term follow-up imaging. Identifying abnormality on *either* non-contrast CT or CTA improved the sensitivity of imaging to predict infarct at follow-up ($\chi^2 = 27$, $p<0.001$) compared with non-contrast CT alone. Identifying abnormality on *both* non-contrast CT and CTA improved the specificity compared with non-contrast CT alone ($\chi^2 = 22$, $p<0.001$). Figure 9-1 displays these results graphically as likelihood ratios.

Table 9-1 *Sensitivity and specificity of various baseline imaging combinations for predicting infarct on follow-up imaging*

Imaging identified as abnormal	**Sensitivity**	**Specificity**
Non-contrast CT (NCCT) alone	50%	97%
CT angiography (CTA) alone	55%	91%
Either NCCT or CTA*	66%	89%
Both NCCT and CTA*	40%	100%

Footnote: * Significantly different from NCCT alone, $p<0.001$.

Figure 9-1 *Likelihood ratios comparing non-contrast CT and/or CTA at baseline for predicting infarct at follow-up*



Footnote: Sensitivity and specificity of an abnormal (ischaemia or HAS) baseline CT in predicting infarct on follow-up imaging is plotted in red. The slopes of the connecting lines represent likelihood ratios for this test. The addition of CT angiography to baseline CT in the acute setting improves either sensitivity or specificity but not both.

Prediction of stroke severity and outcome using non-contrast CT and/or CTA

Table 9-2 compares median NIHSS and OHS between patients with normal baseline imaging and those with abnormality on non-contrast CT alone versus those with abnormality on either non-contrast CT or CTA. Identifying imaging abnormality predicted more severe stroke and worse outcome for both groups but there was no significant difference between abnormal non-contrast CT alone and abnormal non-contrast CT +/- abnormal CTA for NIHSS or OHS results (p=1.000). Figure 9-2 shows the similarity of NIHSS distribution between the groups with abnormality on non-contrast CT alone versus those with abnormality on non-contrast CT or CTA. Figure 9-3 compares the distribution of OHS between these same imaging abnormality subgroups.

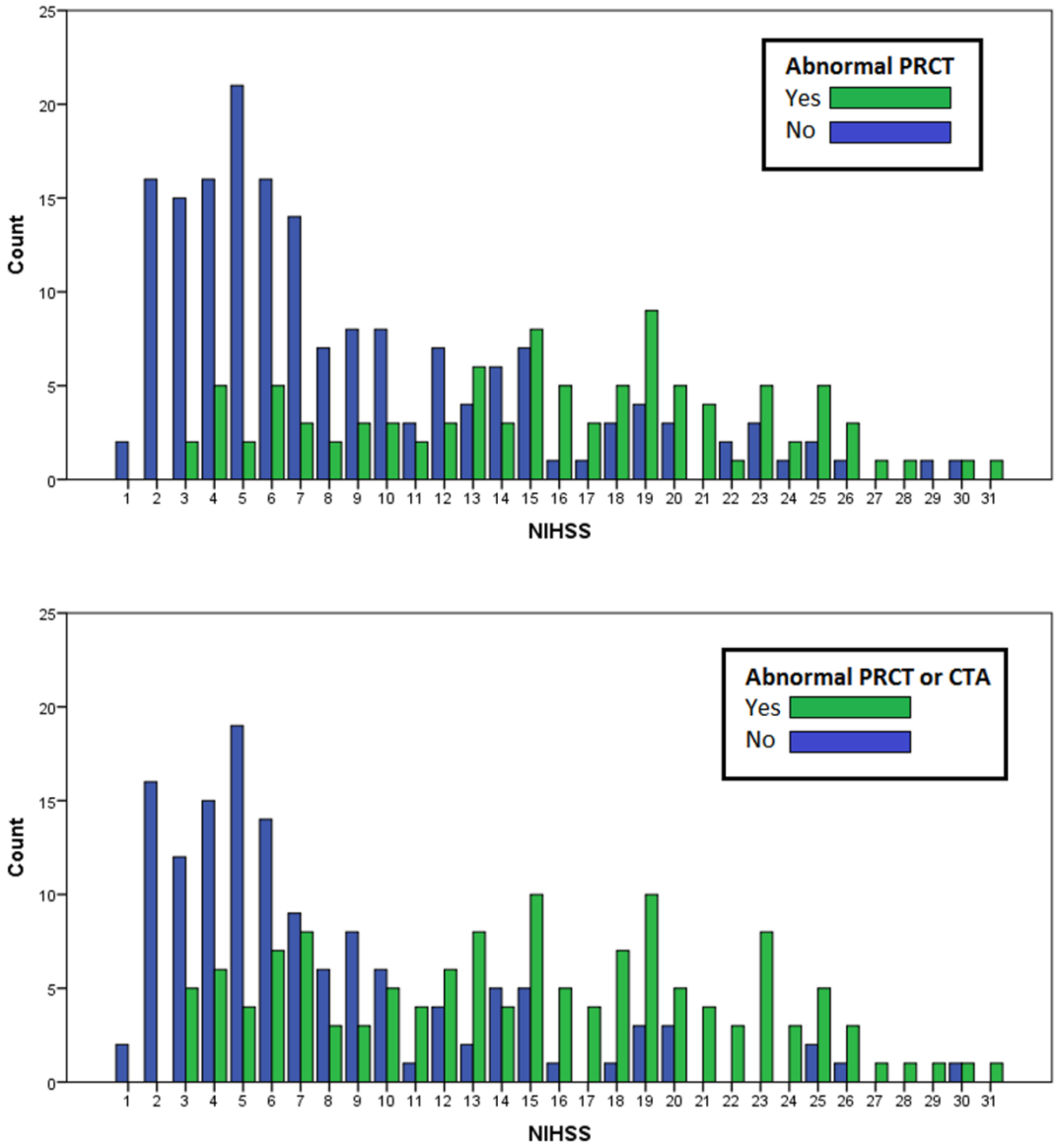
Table 9-2 *Stroke severity and outcome categorised by the findings on non-contrast CT and CTA*

Imaging test normal/abnormal	NIHSS	OHS
Normal non-contrast CT and CTA	6	2
Abnormal non-contrast CT alone	16*	5*
Abnormal non-contrast CT or CTA	15*	5*

Footnote: NIHSS = National Institutes of Health Stroke Scale (stroke severity). OHS = Oxford Handicap Scale (6-month functional outcome). Results are median NIHSS and OHS.

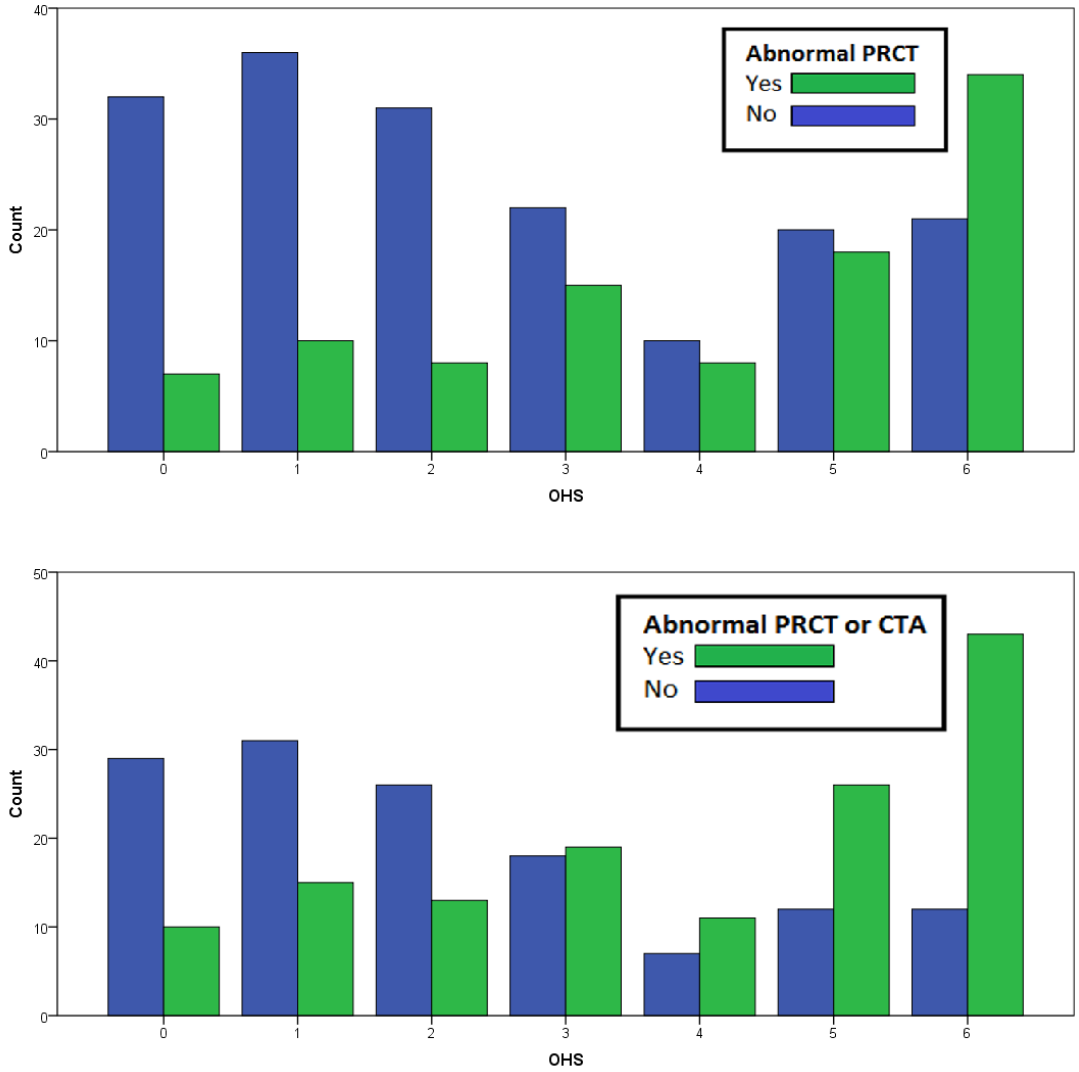
* Significantly different from those with no abnormality, $p<0.001$

Figure 9-2 *Comparison of baseline stroke severity for those with and without imaging abnormality*



Footnote: NIHSS = National Institutes of Health Stroke Scale. PRCT = Pre-randomisation CT, i.e. baseline non-contrast CT. CTA = CT angiography.

Figure 9-3 *Comparison of 6-month functional outcome for those with and without imaging abnormality*

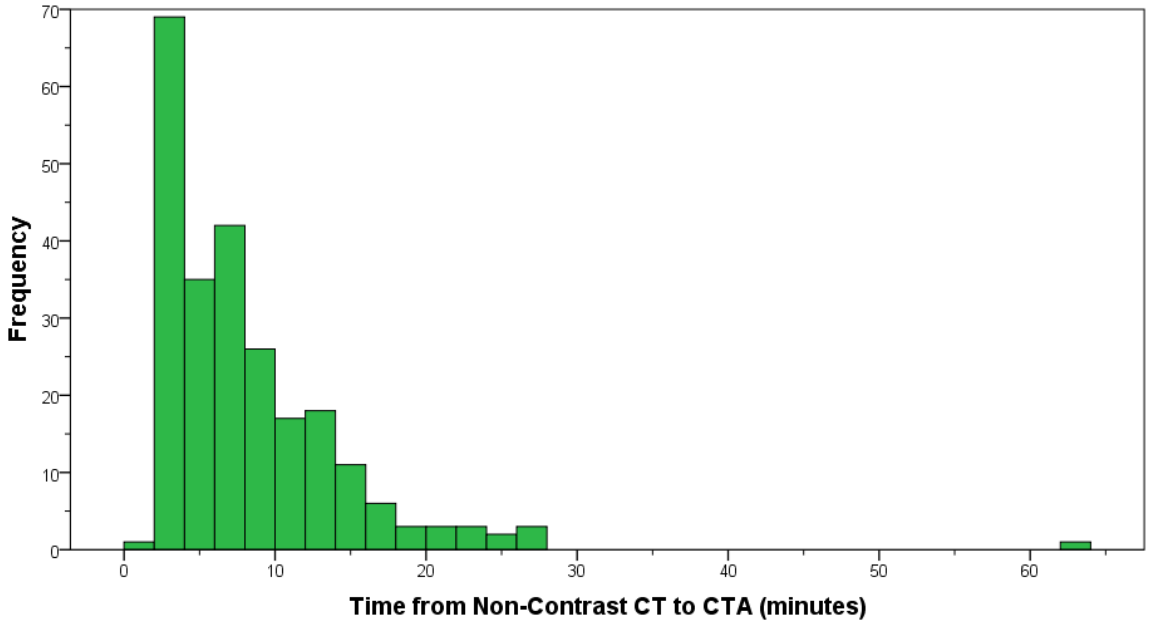


Footnote: OHS = Oxford Handicap Scale (0 = Normal, 1 = Minor symptoms, 2 = Minor handicap, 3 = Moderate handicap, 4 = Moderate to severe handicap, 5 = Severe handicap, 6 = Dead). PRCT = Pre-randomisation CT, i.e. baseline non-contrast CT. CTA = CT angiography.

Time needed for the addition of CTA to standard baseline stroke imaging

As an estimate of the delays incurred by performing CTA at baseline among patients presenting acutely with ischaemic stroke, I found that the median time between non-contrast CT and CTA in IST-3 was 6 minutes (IQR 3-10), n=240. The minimum delay incurred through the addition of CTA to standard imaging was 1 minute while the greatest delay was 63 minutes, see Figure 9-4. The results were slightly improved if patients with concurrent CT perfusion were excluded (n=52): median 5.5 minutes (IQR 3-9).

Figure 9-4 *Time delay between baseline non-contrast CT and CTA in IST-3, n=240*



9.2 Analysis of IST-3 Expert Panel Assessment of Angiography Data

These analyses were designed to assess the importance of baseline angiography findings in IST-3 in relation to baseline stroke severity and functional outcome at six months. I also tested angiogram characteristics individually to look for treatment interactions between normal versus abnormal angiography findings and response to intravenous alteplase.

9.2.1 Methods

In these analyses I used existing data from the IST-3 angiography expert panel's assessment of CT or MR angiography when either imaging technique was performed at baseline (n=300). The IST-3 expert panel assessment of imaging is discussed more fully in Chapter 3.4.2; Table 3-3 lists the angiography scoring options available to the IST-3 expert panel while the entire imaging assessment proformas for CT and MRI are presented as Appendices 9 and 10 (Chapter 11.4), respectively.

Angiography was categorised as 'normal' or 'abnormal' (any arterial occlusion or obstruction). Scan readers recorded the presence, location and severity of all arterial obstructions noting the largest affected artery first plus up to two additional segments. Locations were ICA (internal carotid artery), MCA (middle cerebral artery), ACA (anterior cerebral artery), PCA (posterior cerebral artery), VA (vertebral artery) or BA (basilar artery). I grouped affected arterial segments as proximal (ICA, main stem MCA, VA, BA) or distal (Sylvian branch of MCA, ACA, PCA) in an identical fashion to HAS coding (see Chapter 5.1).

The main focus of arterial obstruction was coded using a modified version of the Thrombolysis in Cerebral Infarction (TICI) and the IST-3 angiography scores.[3;4] Both are scalar and range from occlusion (0) through lesser grades of obstruction to normal (3 for TICI, 4 for IST-3).

Readers also coded the Clot Burden Score in patients with anterior circulation (i.e. ICA, MCA or ACA) obstruction (subtracts the sum of the number of arterial segments affected from 10, where a score of 0 means all segments are affected and a score of 10 means no segments are affected.[5]

The collateral vessel supply was recorded for patients with ICA or MCA obstruction; categorised as good where the entire MCA distal to obstruction reconstituted with contrast, moderate where there was some MCA branch reconstitution or poor if only distal superficial branches reconstituted.[6]

CTA source images (CTA-SI), collected on a single arterial pass (compared with 4-D angiography or true perfusion imaging collected over multiple passes) were used as a surrogate of arterial perfusion of brain tissue;[7] CTA-SI were assessed for deficits in contrast perfusion (any extent or location), by comparison with adjacent or equivalent contralateral brain.

For each of the angiography characteristics assessed in these analyses, I dichotomised the results into subgroups with more obstructed versus more patent angiography appearances (i.e. worse versus better appearances, respectively). I then used these dichotomies to test for differences in the alteplase treatment effect on outcome, i.e. to test for treatment interaction. Dichotomies included: angiography 'abnormal' versus 'normal'; obstructed arterial segment number 1 versus >1; obstruction location proximal versus distal and ICA versus MCA; IST-3 angiography score 0-2b versus 3-4, TICI 0-2a versus 2b-3; Clot Burden Score 0-7 versus 8-10; Collateral score *Good* versus *Moderate-Poor*; CTA-SI perfusion deficit versus no deficit.

Statistical testing

For univariate analyses testing for associations between angiography results, baseline stroke severity (NIHSS) and six month functional outcome (OHS), I used Mann-Whitney U statistics to compare angiography dichotomies and Spearman's rank coefficient to test for correlation with angiography scales. I used a multifactor analysis of variance (ANOVA) to look for interactions between combinations of angiographic findings and OHS.

To test for associations between angiography findings, alteplase versus control and outcome, I used multivariable ordinal regression to calculate common odds ratios (ORs) with OHS at 6 months as the dependent variable. First I tested the impact of different angiography characteristics on outcome individually, controlled for patient

age, NIHSS, time from stroke onset to scan, and alteplase treatment allocation (here, OR <1 indicates worse outcome with alteplase). I then compared alteplase treatment effect on outcome (here, OR <1 favours control) for the aforementioned dichotomies (more obstructed versus more patent) of each angiography characteristic individually, controlled for patient age, NIHSS, and time from stroke onset to scan and tested for treatment interactions by comparing ORs between dichotomies.

To stabilise ordinal regression estimates, I again used the same approach as the original IST-3 analysis of functional outcome, where the more severe grades of OHS (4-6) were grouped as one, leaving 5 groups for ordinal analysis (0, 1, 2, 3 and 4-6).[8] Similarly, I grouped the variable *time from stroke onset to baseline scan*, into 6 one-hour segments (0-60, 61-120, 121-180, 181-240, 241-300 and 301-360 minutes).

Comprehensive Meta-Analysis software (Biostat, Englewood, NJ, USA) was used to perform tests of interaction. All other analyses were performed using IBM SPSS Statistics software, version 20.0 (IBM Corporation, Armonk, NY, USA).

A p-value <0.05 was considered significant.

9.2.2 IST-3 angiography findings

In IST-3, angiography was abnormal (obstruction or occlusion) in 146/300 (48.7%) patients according to the expert panel (Table 9-3); the ICA (47/146, 32.2%) or the MCA mainstem (57/146, 39.0%) were most frequently affected. For those with arterial obstruction/occlusion, >1 arterial segment was affected in approximately half (77/146, 52.7%), 2 segments in 54 of 146 (37.0%), and 3 segments in 15 of 146 (10.3%) patients. From 288 patients with an IST-3 Angiography score, 104 (36.1%) scored 0 to 2b (occluded/moderate-severely obstructed), whereas 184 (63.9%) scored 3 to 4 (mildly obstructed or normally patent). See Table 9-3 for concurrent TICI scores. The median clot burden score was 8 (2 segments affected). Similar proportions had good (48/135, 35.6%), moderate (37/135, 27.4%), or poor (50/135, 37.0%) collateral scores. A perfusion deficit on CTA-SI was identified in 69 of 186 (37.1%) patients where assessable.

Table 9-3 *Angiography findings for 300 patients in IST-3 with CTA or MRA at baseline*

Angiography Type	CTA	271 (90.3)
	MRA	29 (9.7)
Angiography Abnormal (any obstruction or occlusion)	146 (48.7)	
Number of Abnormal Arterial Segments (n=146)	1	77 (52.7)
	2	54 (37.0)
	3	15 (10.3)
Largest Arterial Segment Affected (n=146)	ICA	47 (32.2)
	MCA Mainstem	57 (39.0)
	MCA Sylvian Branch	31 (21.2)
	Basilar	5 (3.4)
	Vertebral	3 (2.1)
	Other	3 (2.1)
	ACA	0
	PCA	0
Location of Angiographic Abnormality (142)	Distal Vessel Only	29 (20.4)
	Proximal Vessel Only	63 (44.4)
	Proximal & Distal Vessels	50 (35.2)
IST-3 Angiography Score (n=288)	4	155 (53.8)
	3	29 (10.1)
	2b	16 (5.6)
	2a	12 (4.2)
	1	32 (11.1)
	0	44 (15.3)
TICI Score (n=288)	3	163 (56.6)
	2b	32 (11.1)

	2a	17 (5.9)
	1	29 (10.1)
	0	47 (16.3)
Clot Burden Score (n=278)	10	154 (55.4)
	9	39 (14.0)
	8	29 (10.4)
	7	14 (5.0)
	6	10 (3.6)
	5	9 (3.2)
	4	8 (2.9)
	3	7 (2.5)
	2	5 (1.8)
	1	2 (0.8)
	0	1 (0.4)
Collateral Score (n=135)	Good	48 (35.6)
	Moderate	37 (27.4)
	Poor	50 (37.0)
CTA-SI Perfusion Deficit (n=186)	69 (37.1)	

Footnote: Numbers represent n (%). Variability in the total patient numbers reflects missing data in some categories; clot burden and collateral scores were calculated only with anterior circulation obstruction. Obstruction was obscured or too peripheral for calculation of an IST-3 angiography, TICI or clot burden score in 12 cases. Not all patients with normal IST-3 angiography scores had normal TICI scores and vice versa. CTA = CT Angiography, MRA = MR Angiography, ICA = Internal Carotid Artery, MCA = Middle Cerebral Artery, ACA = Anterior Cerebral Artery, PCA = Posterior Cerebral Artery, TICI = Thrombolysis in Cerebral Infarction, CTA-SI = CTA Source Images.

Angiography, stroke severity and outcome in IST-3

On univariate analysis, all categories of angiographic abnormality were associated with significantly worse baseline stroke severity and poorer 6-month outcome ($p<0.0001$ for most), see Table 9-4. A 3-way ANOVA found no interaction between any combination of the effects of TICI, clot burden, and collateral scores on OHS ($F=0.814$; $p=0.564$), see Table 9-5.

Table 9-4 *Univariate associations between angiography findings, stroke severity and outcome*

Angiography Measure	NIHSS	OHS
Angiography Abnormal (n=146) Angiography Normal (n=154)	14 (8-20)* 6 (4-10)	5 (2-6)* 2 (1-4)
Abnormal Arterial Segment Number (n=146)	0.34*	0.37*
Abnormal Proximal Artery (n=113) Abnormal Distal Artery Only (n=29)	16 (9-21)** 12 (7-15)	5 (2-6)*** 3 (1-5)
IST-3 Angiography Score (n=288)	-0.52*	-0.34*
TICI Score (n=288)	-0.51*	-0.36*
Clot Burden Score (n=278)	-0.56*	-0.39*
Collateral Score (n=135)	0.41*	0.36*
CTA-SI Deficit (n=69) CTA-SI No Deficit (n=117)	15 (9-20)* 7 (4-12)	5 (2-6)* 2 (1-5)

Footnote: Numbers represent median (interquartile range) or Spearman's correlation. * $p<0.0001$, ** $p=0.023$, *** $p=0.002$. NIHSS = National Institutes of Health Stroke Scale. OHS = Oxford Handicap Scale (six-months). TICI = Thrombolysis in Cerebral Infarction. CTA-SI = CTA Source Images. Proximal arteries include the internal carotid, middle cerebral mainstem, vertebral and basilar arteries. Distal arteries include the sylvian branch of middle cerebral, anterior cerebral and posterior cerebral arteries. Variability in the total patient numbers reflects missing data in some categories; Clot Burden Score and Collateral Score was not calculated in all cases. Not all patients with normal IST-3 angiography score had normal TICI score and vice versa.

Table 9-5 *Three way analysis of variance for interactions between combinations of angiographic findings and six-month functional outcome in IST-3*

Angiographic Finding Combination	Degrees of Freedom	F	p-value
Clot Burden Score * TICI	18	1.306	0.217
Clot Burden Score * Collateral Score	9	1.480	0.176
TICI * Collateral Score	6	1.416	0.223
Clot Burden Score * TICI * Collateral Score	6	0.814	0.564

Footnote: Due to collinearity between some of the angiographic measures, only one feature assessing extent of obstruction (Clot Burden Score rather than arterial segment number) and one feature assessing completeness of obstruction (TICI rather than IST-3 angiography score) were included in this ANOVA based on the strength of their associations with OHS on univariate analysis (Table 9-4). There was also strong collinearity between location and extent of obstruction with very few multi-segment obstructions sited distally rather than proximally; location of obstruction was therefore also excluded from this ANOVA. ANOVA = Analysis of Variance. OHS = Oxford Handicap Scale. TICI = Thrombolysis in Cerebral Infarction.

In multivariable ordinal regression analysis, controlled for age, NIHSS, time from stroke onset to scan and alteplase treatment allocation; having a greater number of obstructed arterial segments (OR 0.41; 95%CI 0.22–0.76; p=0.005), a worse clot burden score (OR 0.78; 95%CI 0.62–0.97; p=0.026), or poorer collaterals (OR 0.53; 95%CI 0.32–0.85; p=0.009) were all independent predictors of worse 6-month outcome, but not proximal versus distal (or ICA versus MCA) arterial obstruction, or the residual arterial calibre at the point of obstruction on IST-3 angiography or TICI scores (Table 9-6).

Table 9-6 *Multivariable ordinal regression analyses testing for independent associations between angiographic findings and six-month functional outcome*

	Odds Ratio	**95% Confidence Interval**	**p-value**
Angiography Abnormal vs Normal (n=300)	0.81	0.50-1.32	0.394
Increased Abnormal Arterial Segment Number (n=146)	0.41	0.22-0.76	0.005
Abnormal Proximal vs Distal Artery (n=142)	0.45	0.20-1.02	0.055
Abnormal ICA vs MCA (n=135)	0.85	0.40-1.81	0.679
Worse IST-3 Angiography Score (n=288)	0.90	0.78-1.04	0.145
Worse TICI Score (n=288)	0.85	0.71-1.01	0.062
Worse Clot Burden Score (n=278)	0.84	0.72-0.98	0.023
Poor Collateral Score (n=135)	0.53	0.32-0.85	0.009
CTA-SI deficit vs No Deficit (n=186)	0.97	0.50-1.88	0.923

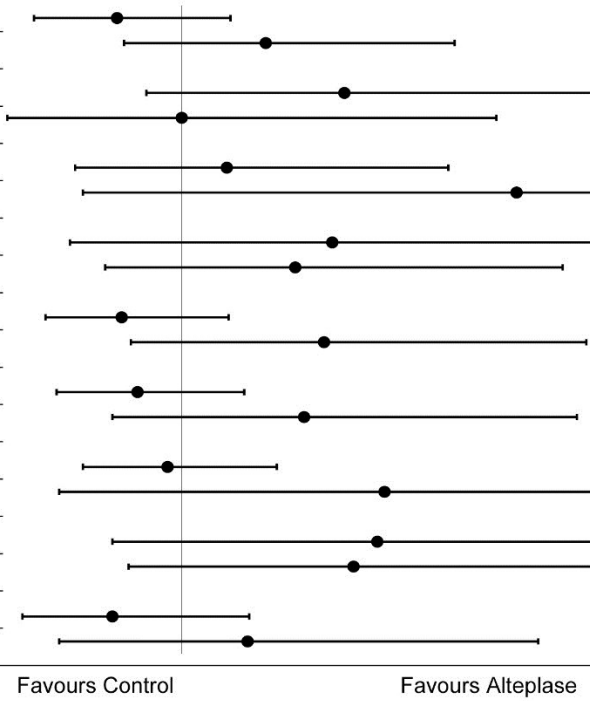
Footnote: An odds ratio <1 indicates a worse outcome. Six-month Oxford Handicap Scale (OHS) was the dependent variable for each separate model. In each case, results were controlled for the effects of patient age, time from stroke onset to scan, stroke severity (National Institutes of Health Stroke Scale) and treatment allocation. ICA = Internal Carotid Artery. MCA = Middle Cerebral Artery. CTA-SI = CTA Source Images. Variability in the total patient numbers reflects missing data in some categories; Clot Burden Score and Collateral Score was not calculated in all cases. Not all patients with normal IST-3 angiography score had normal TICI score and vice versa.

Angiography and effect of intravenous alteplase in IST-3

I found no significant interactions between any individual angiogram feature and the effect of alteplase on 6-month OHS (Figure 9-4). There was a nonsignificant trend toward better outcomes after alteplase versus control among patients with obstructed (OR 1.86; 95%CI 0.76–4.53; p=0.171) versus patent (OR 0.72; 95%CI 0.42–1.25; p=0.241) arteries on the IST-3 angiography score, p=0.075 for interaction (Figure 9-5 and Figure 9-6).

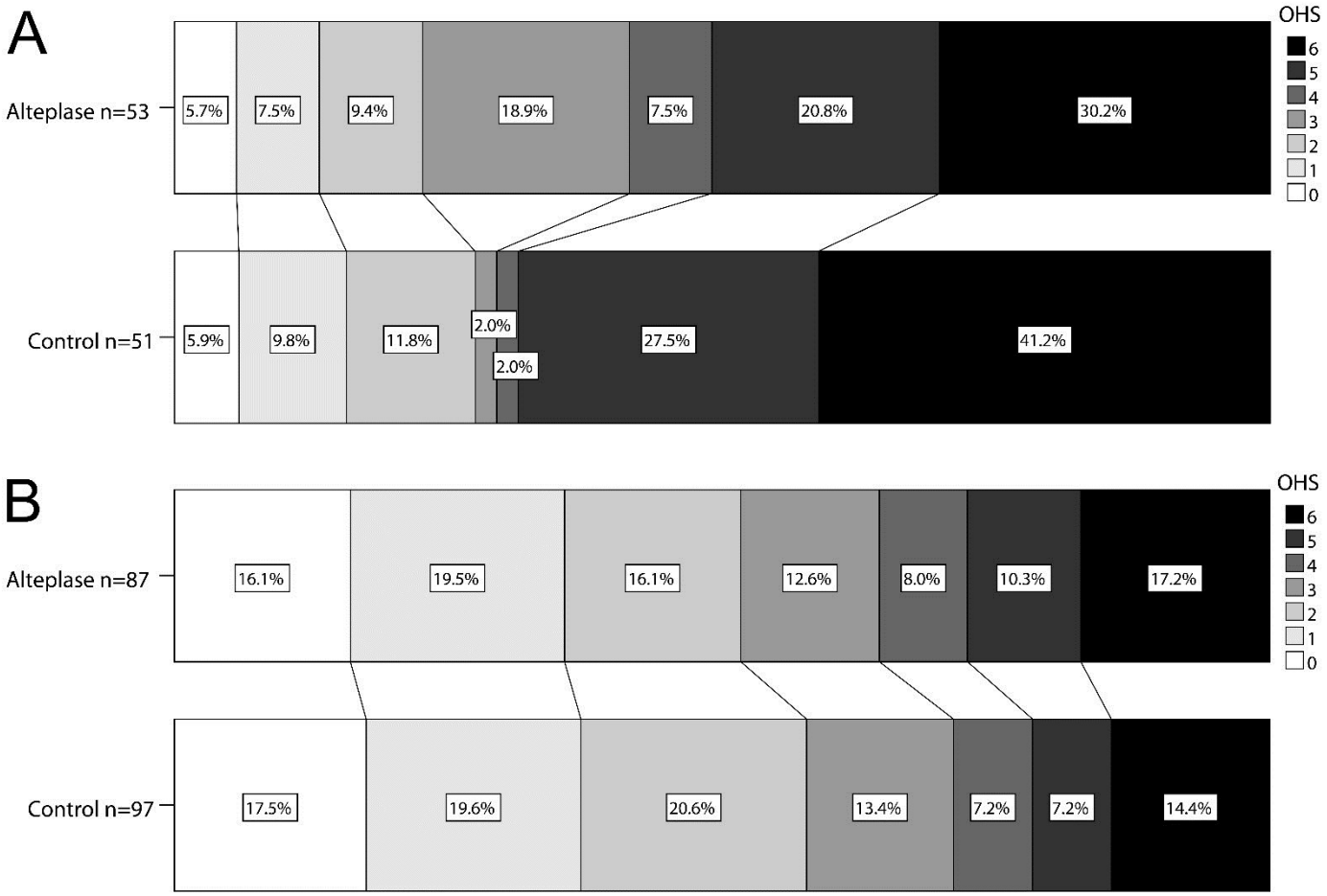
Figure 9-5 *Ordinal regression analyses comparing alteplase treatment effect on outcome (OHS as the dependent variable) in patients with more versus less normal results for the different angiography characteristics in IST-3*

Imaging Characteristic		Alteplase	Control	Odds Ratio (95% Confidence Interval)	Test for Interaction
Angiography (n=300)	Normal	75	79	0.70 (0.38-1.26)	p = 0.115
	Abnormal	72	74	1.47 (0.73-2.97)	
Abnormal Arterial Segments (n=146)	1	35	42	2.01 (0.83-4.88)	p = 0.367
	>1	37	32	1.00 (0.29-3.41)	
Location of Abnormal Segment (n=143)	Proximal	56	57	1.24 (0.53-2.91)	p = 0.304
	Distal	15	15	3.64 (0.56-23.41)	
Location of Abnormal Segment (n=135)	ICA	27	20	1.92 (0.51-7.21)	p = 0.860
	MCA	40	48	1.66 (0.65-4.21)	
IST-3 Angiography Score (n=288)	3-4	87	97	0.72 (0.42-1.25)	p = 0.075
	0-2b	53	51	1.86 (0.76-4.53)	
TICI Score (n=288)	2b-3	92	103	0.79 (0.46-1.34)	p = 0.156
	0-2a	48	45	1.72 (0.68-4.40)	
Clot Burden Score (n=278)	8-10	108	114	0.93 (0.56-1.54)	p = 0.283
	0-7	28	28	2.33 (0.47-11.52)	
Collateral Score (n=135)	Good	22	26	2.27 (0.68-7.59)	p = 0.914
	Poor-Mod	45	42	2.08 (0.75-5.80)	
CTA-SI (n=186)	No Deficit	54	63	0.68 (0.34-1.37)	p = 0.284
	Deficit	33	36	1.36 (0.47-3.90)	



Footnote: Ordinal regression analyses, of the effect of alteplase treatment on outcome (Oxford Handicap Scale [OHS] as the dependent variable) in patients with more versus less normal results by different angiography features. Results represent odds ratio of better (right of vertical line) or worse (left of line) 6-month outcome with alteplase. Adjusted for age, time from stroke onset to scan, and stroke severity (National Institutes of Health Stroke Scale). ICA = Internal Carotid Artery. MCA = Middle Cerebral Artery. TICI = Thrombolysis in Cerebral Infarction. CTA-SI = CTA Source Images.

Figure 9-6 *Bar charts comparing Oxford Handicap Scale (OHS) distribution for alteplase and control groups in those with (A) and without (B) arterial obstruction in IST-3.*



9.3 Meta-Analysis

This meta-analysis was designed to set the IST-3 angiography results from Chapter 9.2 in context and, where possible, to strengthen the dataset through the addition of comparable angiography data from other randomised-controlled trials which tested intravenous thrombolytics for ischaemic stroke. It was hoped that trends identified in IST-3 toward treatment interaction with angiography findings might be replicated using data from other trials and that the combined results might show statistically significant interactions which could ultimately be used to help guide treatment decisions in stroke.

9.3.1 Methods

In order to identify relevant trials for meta-analysis I decided against performing a systematic review. Rather, I used a recently updated Cochrane Collaboration systematic review to identify appropriate trials. This route was chosen for efficiency and since Prof Wardlaw, one of my MD project supervisors, was the primary author of the Cochrane review in question, its robustness and accuracy had been vouched for.

Identification and collection of relevant trial data

The Cochrane Collaboration systematic review on Thrombolysis for Acute Ischaemic Stroke,[9] was recently updated in 2014 and includes 27 randomised-controlled trials which tested thrombolytics for the acute treatment of ischaemic stroke. This systematic review was used to identify relevant randomised-controlled trials for my meta-analysis. I included trials if they had compared *intravenous* thrombolytics with control among patients with ischaemic stroke who had angiography performed at baseline. I extracted data directly from the original full-text trial publications. I sought results for any of the angiography features already assessed in IST-3 and collected both raw data and summary statistics (e.g. odds ratios with 95% confidence intervals). Chief investigators were contacted to obtain additional data where necessary.

Statistical testing

I calculated adjusted odds ratios (ORs) for the effect of intravenous thrombolytic treatment on outcome, by logistic regression, separately in patients with:

a) Patent versus obstructed arteries; and
b) ICA versus MCA obstruction location.

Specifically, I meta-analysed trial data using the random effects model to create summary ORs for the effect of intravenous thrombolytics (any formulation or dose) on outcome (OHS or mRS at 3-6 months) in:

a) Patients with occlusion/ severe arterial obstruction (IST-3 angiography score 0-2b or Thrombolysis In Myocardial Infarction – TIMI[10] 0-1 or 0-2, i.e. worse results) versus patent arteries (IST-3 angiography score 3-4 or TIMI 2-3, i.e. better results); and in a separate analysis
b) Patients with ICA versus MCA obstruction.

Finally, I tested for interactions between intravenous thrombolytics and arterial status on functional outcome. I tested for between-study heterogeneity using I^2 statistics. I followed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines for meta-analysis reporting.[11]

Statistical software

I used Comprehensive Meta-Analysis software (Biostat, Englewood, NJ, USA) to compute summary ORs and to test for interactions.

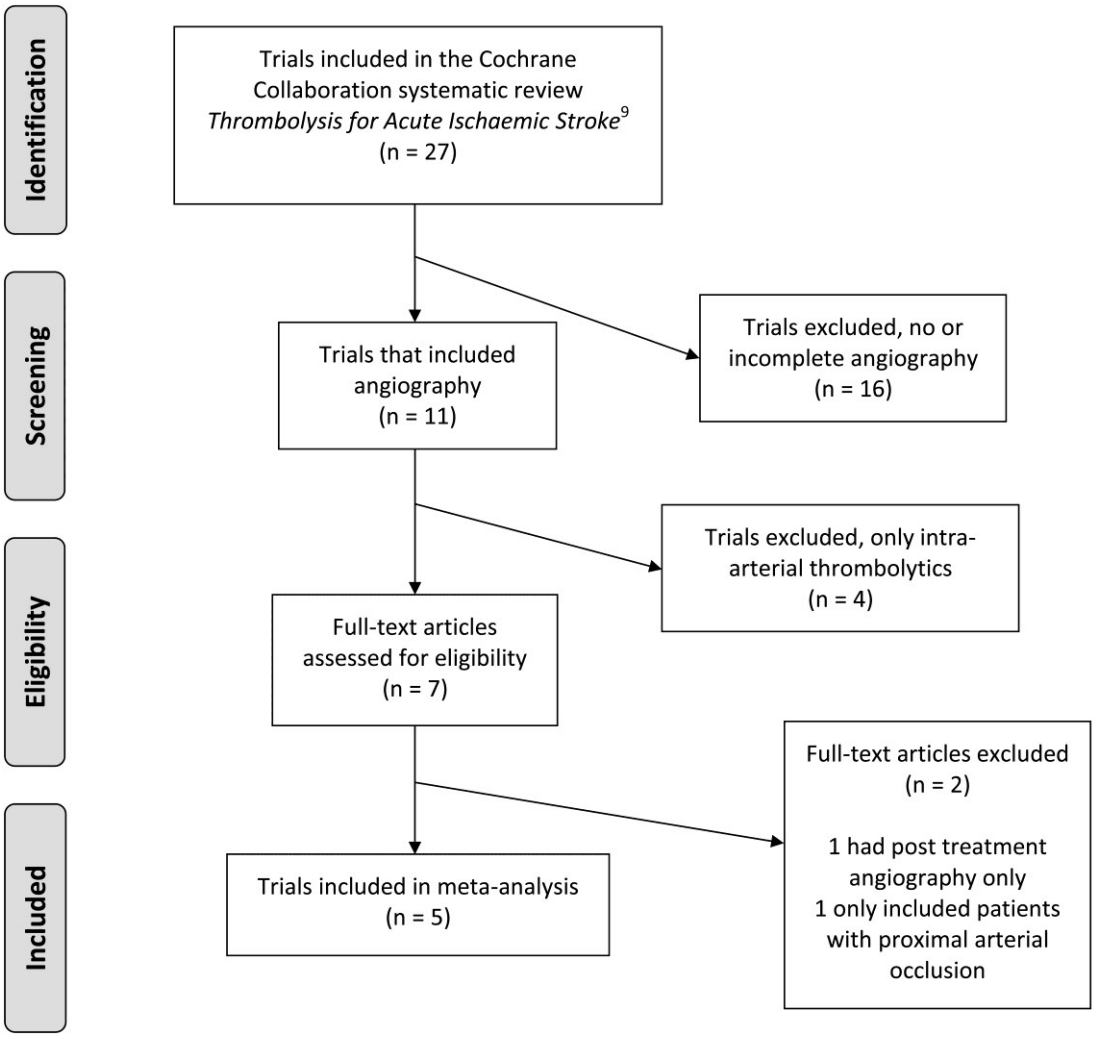
9.3.2 Results

From 27 trials included in the Cochrane Collaboration systematic review, baseline angiography data were available from four trials of intravenous thrombolytics in addition to IST-3, Figure 9-7. Sixteen trials were excluded for absence of angiography. Four trials were excluded as intra-arterial rather than intravenous thrombolytics were used. One trial was excluded as angiography was performed at

follow-up rather than baseline. One trial was excluded as it only recruited patients with proximal arterial obstruction.

The final five trials selected for meta-analysis (total n=591) included two trials of intravenous alteplase (288 patients from IST-3 and 87 from EPITHET)[12] and three trials of intravenous desmoteplase (216 patients from DIAS, DIAS-2 and DEDAS).[13-15]

Figure 9-7 *Flow chart of randomised –controlled trial selection for meta-analysis*



Testing interaction between completeness of arterial obstruction and response to intravenous thrombolytics

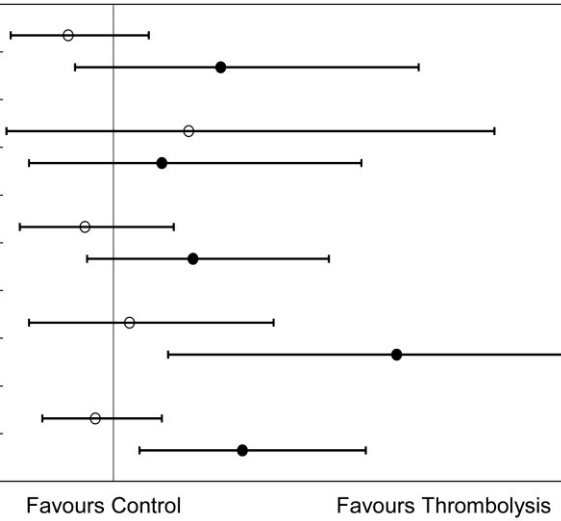
Patients with severe arterial obstruction or occlusion were significantly more likely to have better functional outcome with intravenous thrombolytics versus control (OR 2.07, 95%CI 1.18-3.64, p=0.011, I^2 0%) than were patients with patent arteries (intravenous thrombolytics versus control, OR 0.88, 95%CI 0.58-1.35, p=0.566, I^2 16%), p for interaction 0.017 (Figure 9-8).

Testing interaction between location of arterial obstruction and response to intravenous thrombolytics

For this analysis, data were only available from IST-3 and EPITHET (n = 135 + 54 = 189). I did not identify any difference in the response to intravenous thrombolytics between patients with ICA versus MCA obstruction (ICA obstruction, alteplase versus control, OR 0.65, 95% CI 0.06-6.89, p=0.720, I^2 74%; MCA obstruction, alteplase versus control OR 1.92, 95% CI 0.90-4.09, p=0.090, I^2 0%), p for interaction 0.391 (Figure 9-9).

Figure 9-8 *Meta-analysis of IST-3, EPITHET (alteplase) and the DIAS, DIAS-2 and DEDAS (desmoteplase) trials comparing the effect of intravenous thrombolytics on functional outcome between patients with arterial patency versus obstruction*

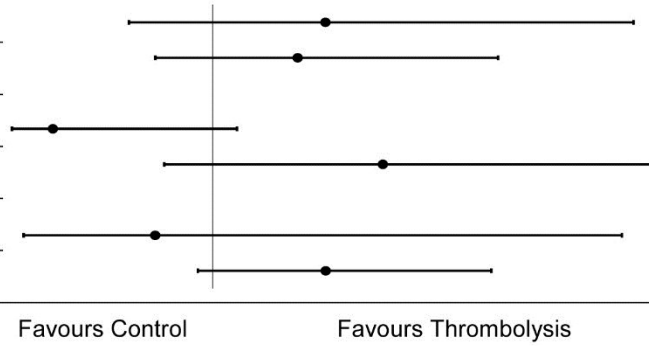
Trial	Angiography Grading		Thrombolysis	Control	Odds Ratio (95% Confidence Interval)	Test for Interaction
IST-3 (n=288)	IST-3 Score	3-4	87	97	0.72 (0.42-1.25)	p=0.075
		0-2b	53	51	1.86 (0.76-4.53)	
EPITHET (n=87)	TIMI	3	21	12	1.57 (0.40-6.12)	p=0.860
		0-2	25	29	1.35 (0.51-3.57)	
Alteplase Trials (n=375)	Normal		108	109	0.82 (0.46-1.45)	p=0.128
	Obstructed		78	80	1.61 (0.83-3.10)	
Desmoteplase Trials (n=216)	TIMI	2-3	143	73	1.11 (0.51-2.41)	p=0.050
		0-1			4.14 (1.40-12.2)	
Combined Result (n=591)	Normal		329	262	0.88 (0.58-1.35)	p=0.017
	Obstructed				2.07 (1.18-3.64)	



Total patient numbers in each trial are provided, n. Raw treatment data were unavailable for the Desmoteplase trials. Results represent odds ratio (95% confidence interval) of better (right of vertical line) or worse (left of vertical line) outcome after thrombolytics. Open circles represent patients with arterial patency (test for heterogeneity, $I^2 = 0\%$). Closed circles represent severe arterial obstruction / occlusion ($I^2 = 16\%$). TIMI = Thrombolysis in Myocardial Infarction.

Figure 9-9 *Meta-analysis of IST-3 and EPITHET comparing the effect of intravenous alteplase on outcome between patients with ICA versus MCA obstruction*

Trial	Location of Angiographic Obstruction	Alteplase	Control	Odds Ratio (95% Confidence Interval)	Test for Interaction
IST-3 (n=135)	ICA	27	20	1.92 (0.51-7.21)	p=0.860
	MCA	40	48	1.66 (0.65-4.21)	
EPITHET (n=54)	ICA	9	13	0.17 (0.02-1.17)	p=0.028
	MCA	16	16	2.54 (0.70-9.18)	
Combined Result (n=189)	ICA	36	33	0.65 (0.06-6.89)	p=0.391
	MCA	56	64	1.92 (0.90-4.09)	



Footnote: Total patient numbers in each trial are provided, n. Results represent odds ratio (95% confidence interval) of better (right of vertical line) or worse (left of vertical line) outcome after thrombolytics. ICA = Internal Carotid Artery (test for heterogeneity, $I^2 = 74$ %). MCA = Middle Cerebral Artery ($I^2 = 0$%).

9.4 Discussion on the use of Baseline Angiography in the Assessment of Ischaemic Stroke

In this chapter, I have examined three inter-related lines of research to explore relationships between the clinical presentation of stroke, findings on angiography performed at imaging baseline as soon as possible after symptom onset and the effect of intravenous thrombolytic therapy on functional outcome:

1. I looked at the diagnostic and prognostic benefits (and extra time needed) of performing CTA as part of the routine imaging work up of patients presenting acutely with stroke.
2. I used data from the expert panel assessment of imaging in one of the largest randomised-controlled trials of intravenous alteplase for ischaemic stroke (IST-3) to test for treatment interaction between intravenous alteplase and various angiographic imaging characteristics.
3. I compounded the results of the IST-3 analysis in 2, by setting them in the context of a meta-analysis including angiography data from other relevant randomised-controlled trials.

Diagnostic and prognostic benefits of performing CTA at ischaemic stroke baseline, additional time needed

I have demonstrated using IST-3 data that combining CTA with non-contrast CT for the baseline imaging assessment of patients with ischaemic stroke significantly increases the sensitivity (if either non-contrast CT or CTA are abnormal) or specificity (if both non-contrast CT and CTA are abnormal) for predicting the presence of infarction on follow-up imaging. While the inclusion of CTA therefore increases the number of patients with abnormal imaging, this extra information does not alter the prediction of stroke severity or functional outcome at six-months over non-contrast CT alone.

Patients recruited in IST-3 after CTA or MRA were randomised and treated, on average, ten minutes later after stroke onset than were those with only plain scans (see Table 3-4). By comparing the DICOM recorded time of non-contrast CT and

CTA in patients where these scans were performed in immediate succession, I confirmed that most of this delay (6 minutes) was attributable to acquiring the extra angiography. The steep time dependency of alteplase effects on outcome mean that even this small delay could reduce the potential benefit of treatment,[16] particularly on a population level if angiography is performed non-discriminately in all ischaemic stroke patients. Efforts should be directed at minimising delays attributable to obtaining angiography in stroke; some IST-3 centres were able to perform CTA only one minute after non-contrast CT.

Testing for interaction between the results of angiography and response to intravenous alteplase in IST-3

In my analysis of CTA and MRA data from IST-3, I demonstrated that having a greater number of obstructed arterial segments or poor collateral supply was associated with poor six-month functional outcome, independent of stroke severity and alteplase treatment; in these patients, alteplase appeared less likely to improve outcome. Those with a greater number of obstructed segments may, therefore, have the most to gain from endovascular therapy, which several RCTs have recently shown to be superior to intravenous alteplase alone among patients with angiography-confirmed proximal intracranial arterial obstruction.[17] Consistent with my results, post hoc analysis of one of these endovascular treatment trials demonstrated that patients with poor collateral supply had poorer outcomes than those with good collaterals.[18]

Despite trends toward improved outcome after alteplase versus control among patients with abnormality in one of several angiographic characteristics, I was not able to demonstrate a significant treatment interaction between alteplase and CT/MR angiography results in IST-3. That is, there was no significant difference in the treatment response between patients with abnormal angiography versus those with normal angiography, no matter how angiography was assessed. However, the wide confidence intervals indicate imprecise estimates of angiography findings on alteplase effects and that the sample is underpowered. It is for this reason that the subsequent meta-analysis was performed.

Other strengths and weaknesses of this IST-3 analysis are as follows. Angiographic imaging was not a requirement of IST-3 but some centres performed baseline CT or MR angiography for ischaemic stroke routinely; therefore, this angiography subgroup analysis was pre-planned to make most use of all available data and maximise knowledge gained from an RCT. The results of angiography were not used to determine trial eligibility, and at the time of IST-3 enrolment, there were virtually no data on how angiography results should be used in this context. Therefore, clinical uncertainty as to whether treatment with alteplase would be beneficial existed even for patients with large artery obstruction. Angiography was however performed in only 10% of IST-3 centres, reflecting local practice at the time, and so is limited by small sample size, the potential for selection bias and reduced generalizability of the findings to all current stroke centres. Nevertheless, IST-3 angiography represents 'real world' practice where approximately 50% had angiographic obstruction[19] and with 300 patients, is the largest angiography dataset from an RCT of intravenous alteplase in ischaemic stroke. IST-3 used robust, validated methods for scan management and scoring angiograms,[4] with blinding of readers and moderate to substantial inter-observer agreement.[20]

Meta-analysis of angiography results from randomised-controlled trials testing intravenous thrombolytics to identify treatment interaction

My meta-analysis of CTA and MRA data from five RCTs (including IST-3) of intravenous thrombolytics (alteplase and desmoteplase), and comprising 591 patients treated within six hours of stroke onset, indicates that patients with severe angiographic obstruction/ occlusion benefit significantly from intravenous thrombolytics ($p=0.011$) and this result was significantly different to the thrombolytic response of patients with patent arteries ($p=0.017$). Therefore, where endovascular therapy is not available,[21] intravenous thrombolytics remain an important treatment option. The significant interaction between angiography appearance and intravenous thrombolytics does not mean that patients with normal baseline angiography gain no benefit from intravenous thrombolytics;[22] the meta-analysis was neutral in this group, showing neither benefit or harm, but the sample is too small to exclude modest benefit or harm. It is important to remember that patients

with normal angiograms may have arterial obstruction(s) too small to see on CTA/MRA, and intravenous thrombolytics may be beneficial in these patients.

I could not confirm in the meta-analysis that patients with MCA versus ICA obstruction responded differently to thrombolytics, but the sample available for this comparison was very small. In general, I was unable to identify available comparable trial data for most of the other angiography characteristics assessed in IST-3. Similarly, it would have been interesting to test further the other non-significant trends toward improved outcome after alteplase hinted at by several of the other angiography features assessed in IST-3 as discussed above. It is possible that control arm data from the recently published endovascular treatment trials,[23-33] when pooled, could elucidate further the associations between baseline angiography and outcome after ischaemic stroke but only among patients treated with intravenous thrombolytics (since all patients in these trials received intravenous alteplase as the standard medical therapy). To better understand thrombolysis–angiography interactions, future RCTs of acute stroke therapy with CTA or MRA should examine location, extent, completeness of arterial obstruction, and adequacy of collaterals, to refine how these findings could help treatment decisions.

I used dichotomies of *better* versus *worse* angiography scoring to simplify scalar data in IST-3. I acknowledge that different dichotomies of the same scalar data could provide different results, but I felt that this approach nevertheless provided a meaningful and useable summary. For the meta-analysis, slight inconsistencies in angiogram scoring between trials mean that there is some overlap between categories (e.g. TIMI=2 is included in the *better* category for EPITHET but the *worse* category for the desmoteplase trials). Efforts to standardize angiogram rating in future trials should be encouraged because this will facilitate between-trial comparisons and meta-analysis.[34]

Conclusions

Among patients presenting acutely with stroke, identification of angiographic obstruction at baseline helps to correctly classify those with ischaemic aetiology and independently predicts poor functional outcome at six months.

Intravenous thrombolytic therapy is significantly more effective in improving functional outcome in patients with ischaemic stroke who have arterial obstruction than in those with apparently patent arteries on CTA or MRA. Intravenous thrombolytics, therefore, remain an important treatment option. The data are too sparse to determine, in this analysis, whether patients without apparent arterial obstruction benefit from intravenous thrombolytics, bearing in mind that these patients may have obstruction too small to detect on CTA or MRA.

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Chapter 10 Summary and Discussion

10.1 Basics of Ischaemic Stroke, Imaging & Treatment

In Chapter 1, I described that stroke is a major global health concern affecting both developed and under-developed nations. The majority of strokes are ischaemic and occur following a disruption of arterial blood supply to the brain, while a minority are secondary to brain haemorrhage. Emboli from the heart or thrombotic obstruction at the site of an atheromatous plaque in an artery supplying the brain are the commonest underlying causes of ischaemic stroke. Patients with hypertension, atrial fibrillation, severe carotid stenosis, hypercholesterolaemia, diabetes or a history of smoking are at most risk of stroke.

The clinical presentation of stroke can be highly variable but a neurological deficit with an abrupt onset is commonest. To be differentiated from a transient ischaemic attack (TIA), stroke symptoms must last more than 24 hours. Since the clinical presentation of ischaemic and haemorrhagic strokes can be identical but treatment options for the two stroke subtypes are very different, an early non-contrast CT scan of the brain is usually performed (MRI is sometimes infrequently used instead but CT has many practical advantages in this context) as an effective means of identifying acute haemorrhage (and other structural mimics, e.g. tumour). Treatment for ischaemic stroke can begin even in the absence of positive evidence for ischaemia (changes indicative of ischaemia in the brain parenchyma, hyperattenuation within an artery suggesting acute thrombus or embolus i.e. the hyperattenuation artery sign or HAS); in fact the lack of an acute abnormality on CT is common for patients presenting to hospital within a few hours of stroke symptom onset.

If an early non-contrast CT of the brain excludes haemorrhage or another structural mimic, then treatment to restore arterial blood flow is considered. Note that it has not been common practice to routinely image the major arteries supplying the brain before initiating treatment with an intravenous thrombolytic such as alteplase. However, with the advent of mechanical thrombectomy (physical removal of the clot

via a catheter) as an additive means of restoring arterial blood supply in some patients,[1] routine use of CT angiography (or MR angiography, CTA and MRA, respectively) for this purpose will increase. New guidance aimed at delivering effective thrombectomy services clearly advocates arterial imaging. Both the American Heart Association guidelines for thrombectomy and a very recent commissioning statement from NHS England state that the treatment should be offered in the presence of a proximal arterial occlusion.[2;3] Treatment for ischaemic stroke needs to be initiated as soon as possible to maximise benefit since there is an inverse relationship between time and the volume of potentially salvageable brain and therefore the likelihood of a given patient gaining benefit from treatment.

After ischaemic stroke, approximately one third of patients return to normal or at least retain their independence, one third are disabled in the long term and one third die. A worse long-term outcome is more likely if: stroke severity is worse at baseline (commonly measured using the National Institutes of Health Stroke Scale, NIHSS); if a large area of brain is affected (often directly correlated with stroke severity); or if patients suffer from symptomatic haemorrhagic transformation of infarct or significant brain swelling/ mass effect, or hydrocephalus in the short term (days to weeks). The modified Rankin Scale (mRS) provides an ordinal assessment of functional outcome after stroke and is commonly used in stroke trials.[4-6]

10.2 The Third International Stroke Trial

In recruiting 3035 patients, the Third International stroke trial (IST-3) was the largest single randomised-controlled trial which tested intravenous alteplase for ischaemic stroke.[7] The trial recruited patients from 156 stroke centres in 12 different countries and data collection, including standard imaging for the trial, was reflective of routine stroke care at the time.

The IST-3 dataset provided a unique and unparalleled opportunity to address questions relating to whether and how acute brain imaging appearances after stroke might affect functional outcome for patients given intravenous alteplase. As a randomised-controlled trial which compared patients given alteplase with controls

who did not receive alteplase, the IST-3 dataset provides a robust means of attempting to answer such questions with the minimum of bias.

10.3 Background for HAS and CTA/MRA Research in Stroke

In Chapter 2, I describe two literature reviews undertaken by me at the outset of this MD research project. The first looked at HAS and the second looked at CTA and MRA, both of which have been used to image arterial patency in ischaemic stroke. These reviews increased my understanding of HAS and CTA/MRA research and helped to direct subsequent analyses.

Systematic review and meta-analysis of HAS

In this review I summarised HAS prevalence in the published literature. I noted that HAS prevalence was affected by the location and size of the obstructed artery (most common in large, proximal vessels), was easier to identify on thin-slice CT and was naturally a transient phenomenon. I identified reasons for false positivity and techniques for improving accurate identification of the sign.

I also identified research articles where results for HAS sensitivity, specificity, positive and negative predictive value were reported. Again these data were heterogeneous but in general less than half of all patients were reported to have the sign but if present, HAS was highly likely to indicate a genuine ischaemic stroke. However these data were limited by the lack of angiographic imaging to confirm arterial obstruction in some cases.

Across several measures of short and long-term outcome after stroke, patients with HAS are likely to do poorly. Many of the studies identified in my search showed that associations between HAS and outcome occurred independently of other recognised prognostic factors such as age, sex and baseline stroke severity. However, these results were not always replicated and so it was not possible to be definitive in this regard.

While there was evidence that patients with HAS gain benefit from intravenous alteplase, the limited randomised-controlled trial data could not identify an interaction between alteplase treatment and HAS, i.e. patients with and without the sign did not respond differently. There was some evidence suggesting that HAS are predominantly composed of red blood cells (rather than white blood cells, platelets or fibrin). However given that clots with a high red blood cell composition are said to be more likely than other types of clot to respond to thrombolytics, this observation is in contrast with the limited evidence suggesting that patients with and without HAS respond similarly. I concluded that this might relate to the fact that many studies (especially older ones) have conflated the presence of HAS with large proximal arterial obstructions since HAS is more likely to be identified if it is large and proximal. Finally, it was not clear from the assembled evidence whether the presence of HAS meaningfully altered the risk of brain haemorrhage after intravenous alteplase.

Review of CT and MR angiography

In this review I identified that routine use of CTA for the acute assessment of ischaemic stroke has been increasing and is likely to continue to do so. This rise in CTA use will be compounded by the advent of endovascular thrombectomy as a major treatment option for ischaemic stroke.

I noted that while CTA (and MRA) provide an accurate ‘snap-shot’ representation of arterial patency, these techniques do not indicate speed or directionality of arterial flow. Unfortunately, many of the scales used for grading CTA and MRA in research are therefore not fully appropriate in this context as they were originally developed for assessing catheter angiography which is a dynamic test. Greater standardisation is required.

I identified very little data for two aspects worthy of consideration when using CTA or MRA for the acute assessment of ischaemic stroke. Firstly, the observer reliability for the assessment of angiographic imaging was poorly reported. Secondly, there was limited data on exactly how much extra time CTA adds to a conventional non-contrast CT imaging protocol.

While there was evidence that CTA and MRA provide additional information over standard brain imaging alone, it was less clear whether this extra information can be used usefully for effective diagnosis, whether the information might help front-line clinicians make treatment decisions more effectively and ultimately be used to help provide more accurate prognostic information to patients and their families.

Major observations from HAS and CTA/MRA reviews

The follow points were identified as being inadequately addressed by the literature at the time of my HAS and CTA/MRA reviews:

- The prevalence of HAS was likely to be higher than previously reported when thin-slice contiguous CT scans were routinely used for assessment
- Data addressing the sensitivity and specificity of HAS as a surrogate of arterial obstruction in ischaemic stroke were heterogeneous and derived using different gold standards
- The effect of HAS on stroke severity, response to intravenous alteplase and functional outcome after stroke was unclear
- It was not clear how accurately CTA and/or MRA can be scored in an acute setting if and when these techniques are implemented as routine practice for the assessment of ischaemic stroke
- How much extra time is needed to include CTA to a standard non-contrast CT protocol for imaging ischaemic stroke was under reported
- It was not clear whether the extra information provided by angiography improves the diagnosis of ischaemic stroke or ability to provide prognostic information during the early hours after stroke onset
- Clarity was needed whether specific features of angiography such as the presence or absence of obstruction, the location and extent of obstruction, or the adequacy of collateral supply alters the response to intravenous alteplase and therefore functional outcome after stroke.

10.4 Summary of Main Results Mapped to Thesis Aims

This summary includes an up to date context for my results where substantial new data were available (to December 2016) and where possible explores the potential impact of my work.

Thesis aims are provided as subheadings.

Quantify the prevalence and observer reliability of HAS on CT performed for the initial imaging assessment of patients with suspected ischaemic stroke

In my systematic review of prior HAS research (Chapter 2) I identified 54 relevant research articles dated between 1989 and 2012 and from over 20,000 non-contrast CT brain scans performed acutely for the assessment of ischaemic stroke, HAS was identified 21% of the time although the data were heterogeneous.

In IST-3, HAS was present in 24% of all non-contrast CT brain imaging performed at baseline (Chapter 5). As anticipated this result is slightly higher than the 21% from my systematic review since IST-3 data were more recently collected (recruitment ran from the year 2000 to 2011). However, a large proportion of IST-3 imaging was still not based on thin-slice CT acquisition, particularly among patients recruited earlier in the trial. Unfortunately, it was not possible to perform an analysis of only the thin-slice CT data from IST-3 since studies were never classified in this way. I have not identified any recently published results looking specifically at thin-slice CT in a large cohort or as an analysis of multiple datasets.

In Chapter 8, I used data from the IST-3 expert panel's assessment of imaging to derive observer reliability results for the identification of HAS. Somewhat surprisingly, inter-observer agreement was only *fair* (K-alpha = 0.37) while intra-observer agreement was near perfect (K-alpha = 0.83). I think these results tell us that even among experts, the definition of what constitutes a true HAS varies but that for an individual reader, the sign (however defined) is readily and consistently identified. Greater consistency among readers is therefore required.

On the basis of HAS research performed for this MD, I was invited to contribute to an international collaborative discussing stroke imaging research priorities with the aim of developing a 5 year research plan. The STIR (Stroke Imaging Research) group's *Acute Stroke Imaging Research Roadmap II* was published in 2013; in this context I conveyed the importance of using thin-slice CT for accurately detecting HAS.[8]

Determine how intravenous alteplase affects HAS and whether the presence or absence of HAS influences the response to intravenous alteplase and outcome and whether the characteristics of HAS such as attenuation, location and extent are important

In Chapter 5, I calculated that the presence of HAS on baseline non-contrast CT was strongly associated with increased stroke severity at baseline and worse 6-month outcome in IST-3. This association with outcome occurred independent of age, baseline stroke severity, time from stroke onset to scan, and treatment allocation (i.e. patients with HAS did worse in both the alteplase and control groups). I also found that outcome was even worse if HAS was more extensive (true on adjusted multivariable analyses) or if HAS was proximally located (true only on univariate analyses). In Chapter 6, I investigated the importance of assessing the baseline attenuation of arterial obstructions in ischaemic stroke. This included direct measures of attenuation (actual Hounsfield Units, HU or as a derived ratio compared with a coincidentally normal artery) and in a dichotomy of hyperattenuating versus iso-attenuating obstructions. Neither method of assessing the attenuation of arterial obstructions was independently predictive of functional outcome at 6 months.

Treatment with alteplase in IST-3 was associated with shrinkage and/or disappearance of HAS (and similarly, a non-significant greater reduction in attenuation of the obstruction in the alteplase versus control groups). This association between alteplase and HAS removal was independent of age, baseline stroke severity, and time between baseline and follow-up scans. In addition, I demonstrated that alteplase was associated with HAS removal both for those with large and proximal HAS and for those with small and distal HAS. Nevertheless, complete clearance of small and distal HAS was more common, as others have also now demonstrated.[9] Associations between alteplase and short term changes in HAS are

important as I also showed that functional outcome is improved when HAS shrinks or disappears. My analyses also offer an important counter argument to a widely held view that alteplase is ineffective against more extensive HAS; a previous report by Riedel et. al. stated that obstructions over 8 mm could not be removed by alteplase alone.[10]

I tested for a variety of HAS-alteplase treatment interactions using different subgroups; namely, presence versus absence of HAS, proximal versus distally sited HAS, more versus less extensive HAS and finally the measured attenuation of arterial obstructions in the treatment versus control groups. I did not demonstrate a differential response to alteplase in any of these comparisons, i.e. for each HAS characteristic, both subgroups responded similarly to treatment with alteplase. In other words, there was no treatment interaction between alteplase and HAS. This indicates that on the basis of one of the largest randomised-controlled datasets, HAS should not be used to select patients with ischaemic stroke for treatment with intravenous alteplase.

My work should therefore provide confidence that while complete removal of more extensive HAS is less likely, such patients can still benefit from treatment with alteplase.

Calculate the sensitivity and specificity of HAS for arterial obstruction

In a first-of-its-kind meta-analysis incorporating data from 17 research articles including IST-3 and incorporating data from 1175 patients, I calculated that HAS is 95% specific and 52% sensitive for true arterial obstruction (as demonstrated using angiography) during the acute phase of ischaemic stroke (Chapter 7). HAS was more commonly identified if proximally sited and there was a trend for greater identification of HAS if it was more versus less extensive. Sensitivity was improved if thin-slice CT was used.

My meta-analysis was highlighted by the *EvidenceUpdates* service provided by the BMJ Group and McMaster University (https://plus.mcmaster.ca/EvidenceUpdates) and scored as 6/7 for *Relevance to Practice* and 6/7 for *Is this News?* An extract from the site is included as Appendix 15 (Chapter 11.6).

Using IST-3 data alone, I showed that while use of thin-slice CT improved sensitivity and there was a non-significant trend for improved specificity of HAS, other features of the obstruction (its location or extent) or of the patient (time from stroke onset to scan or patient age) did not significantly alter sensitivity or specificity results.

A recent analysis of over 11,000 patients with baseline angiography from the SITS (Safe Implementation of Treatments in Stroke) International Stroke Thrombolysis Register (http://www.sitsinternational.org/), investigated whether thresholds of NIHSS could be used to predict arterial occlusion at baseline. They found that NIHSS = 11 optimally predicted arterial occlusion.[11] This aligns well with my assessment of IST-3 imaging where there were no false positive HAS and far fewer true negative HAS with NIHSS greater than 11-15 (Figure 7-1).

Most arterial segments with HAS in IST-3 (90%) were not completely occluded on angiography, rather they were obstructed; i.e. contrast (and thrombolytic agent) could bypass the obstruction to reach more distally occluded vessels and there was therefore an increased surface area of clot for thrombolytic action. This is counter to conventional thinking that the presence of HAS indicates occlusion and might help to explain the observation discussed above that alteplase does appear to work for these patients even when HAS is extensive. Some very recent analyses using data from the MR CLEAN (Multicenter Randomized CLinical trial of Endovascular treatment of Acute ischemic stroke in the Netherlands) thrombectomy trial[12] and from the Dutch Acute Stroke Study (DUST)[13] would seem to support my observations from IST-3. These secondary analyses of MR CLEAN and DUST tested whether correlative non-contrast CT and CTA can be used to assess the perviousness of obstructions (that is the extent to which they can be penetrated by fluid) by measuring the change in the attenuation of obstructions before and after the administration of intravenous contrast; obstructions which show an increase in attenuation on the latter scan, are presumed to be more permeable to contrast.[14;15] Their results showed that perviousness is strongly associated with higher recanalisation rate, smaller infarct volume at follow-up and better outcome. Data from MR CLEAN showed that these results were independent of treatment allocation; in other words, improved outcome

was demonstrated similarly in both the intravenous alteplase and thrombectomy groups.[14] Finally, DUST data showed that the effect of perviousness on outcome occurred independent of recanalisation status; i.e. also demonstrating that even a partial reduction in the extent of obstruction is beneficial.[15]

Quantify observer reliability for the assessment of CTA performed routinely as part of the initial CT imaging assessment of patients presenting with ischaemic stroke

In my analysis of observer reliability for the assessment of CTA (Chapter 8), I found moderate to substantial agreement between expert readers for all CTA measures except identification of parenchymal hypoperfusion on CTA source images. Agreement was less between non-expert readers but those with extra relevant training (i.e. neuroradiology trainees) were more comparable with the expert group. Reassuringly, imaging features that are likely to have the most clinical impact were reported with the most reliability (for example, the presence and extent of arterial obstruction).

My own personal inter- (me versus a variable member of the expert panel) and intra-observer reliability scores were consistent with those of the expert panel. In addition, my results seemed to be platform independent. This provides confidence that other work in this thesis derived from those results (Chapters 6, 7 and 9) is meaningful.

There remains very little published data assessing the reader reliability of non-invasive angiography performed for the assessment of stroke with which to compare my results. One recent analysis demonstrated that the inter-observer agreement for detection of occlusion increased when multiphase CTA (synonymous with time-resolved or 4-D CTA; imaging is performed intermittently during the entire phase of the contrast bolus as it flows in and out of brain, it is therefore more like catheter angiography) was compared with standard CTA.[16]

Determine if the addition of CTA to standard plain CT improves ischaemic stroke detection, and prognosis prediction, by CT when performed as part of the initial imaging assessment and determine how much extra time is needed to perform CTA in this context

In order to predict the presence of infarct on 24-48 hour follow-up imaging among IST-3 stroke patients (Chapter 9), I determined that combining results of non-

contrast CT and CTA at baseline provided the best means of improving the sensitivity (if either non-contrast CT or CTA were abnormal) and specificity (if both non-contrast CT and CTA were abnormal) over non-contrast CT alone. However, I did not find a corresponding improvement in the prediction of stroke severity or outcome for these patients.

In a recent very similar analysis testing whether CTA or CT perfusion improve the prediction of infarct presence and infarct volume on follow-up imaging, van Seeters et. al. found that the addition of baseline CTA to non-contrast CT was superior for predicting both the presence and the volume of infarct over non-contrast CT alone.[17] Their study also plans to model outcome prediction for non-contrast CT alone versus non-contrast CT plus CTA but the results are not yet published.[13]

In IST-3, performing CTA in addition to non-contrast CT took an extra six minutes which corresponds well with the extra ten minutes from stroke onset to randomisation identified among the angiography subgroup compared with the full trial. Although this may not seem a significant delay, *time is brain* and every minute counts.[18;19] It was clear from IST-3 data that some centres were able to minimise the delay attributable to CTA acquisition to around 1 minute. Such rapidity implies these centres are offering an efficient service that is fully protocol driven; one minute between scans does not allow for any deliberation over the need for CTA while a patient is still on the CT table. Notwithstanding, it is probably inappropriate to perform CTA on every patient suspected of having an ischaemic stroke. The ideal approach then must be to develop clear and robust (evidenced based) imaging protocols that allow the correct scanning to be performed in the correct patients without unnecessary delay.

Determine whether the presence or absence of arterial obstruction on angiography performed acutely after ischaemic stroke influences the response to intravenous alteplase and outcome and whether characteristics of obstruction such as location, completeness and extent are important

In my analyses of the IST-3 angiography expert panel data (Chapter 9), both the extent of arterial obstruction and a poor collateral supply at baseline were

independently associated with poor functional outcome at 6 months. This association with collateral supply has since been replicated elsewhere.[20;21]

There were trends in the IST-3 angiography data for different treatment responses across subgroups with and without a variety of angiographic abnormalities, but none of these differences were significant, i.e. there was no treatment interaction with angiography in IST-3. However, when I combined IST-3 data with data from four other randomised-controlled trials in a meta-analysis, I found that patients with severe angiographic obstruction or occlusion responded differently to intravenous thrombolytics compared to those with minimally obstructed or normal arteries. The former group with severe arterial obstruction showed benefit with improved outcome after treatment (versus control) while the latter group with normal arterial patency showed neither treatment benefit nor harm. Therefore while the difference in response between these two groups was significant, their responses to treatment are not necessarily conflicted; it may simply be that one group achieved a greater benefit than the other, i.e. there is no evidence from my analyses that patients with patent arteries do not benefit from alteplase (a common assumption). Indeed in my earlier analyses, patients without HAS demonstrated conclusive benefit from alteplase (Figure 5-4). However as discussed above, perhaps 50% of these patients would still have an arterial obstruction on angiography. But similarly, a 'normal' angiogram cannot exclude obstruction of arteries with a calibre below the spatial resolution of the scanner on which it was obtained (0.6 mm is a current standard minimum resolution for CT). I therefore have to assume there is an acute arterial obstruction at some level in every ischaemic stroke patient; hence there is always the potential for benefit from intravenous alteplase even in the presence of a 'normal' angiogram. Arguably if this is the case, such patients with 'small and distal' obstructions may actually have the most to gain from alteplase. It is also worth noting that the significant result from my HAS analyses in Figure 5-4 is powered by a much larger number of patients (2245 versus 154 with a normal angiogram).

I could not confirm in the meta-analysis that patients with proximal versus distal arterial obstruction responded differently to intravenous thrombolytics.

On the basis of these results, which as a meta-analysis of randomised-controlled trial data represent the best currently available evidence, I conclude that angiography is not necessary to select patients for intravenous thrombolytic therapy.

10.5 Strengths and Limitations of My Work

IST-3 used robust and validated methods for scoring imaging data which I replicated in my own analyses of IST-3 imaging. All image analysis was performed blind to other imaging (except if the other imaging was required; for example when measuring the attenuation of arterial obstructions on non-contrast CT using CTA as a guide to target attenuation measurements), to all other clinical information and to treatment allocation.

I adopted best practice statistical analysis techniques where possible, particularly by using multivariable ordinal regression analyses to assess for associations with six-month functional outcome and other ordinal scalar data, and to look for imaging interactions with alteplase. Similarly I used a relatively unknown but significantly more appropriate method (than Kappa) for assessing observer reliability when there are more than two observers and data types are mixed.

I have secured peer reviewed publication as first author for six discrete articles (published between July 2014 and December 2016) [22-27] and three scientific abstracts (two were presented at the *European Stroke Conference* in London, May 2013; one was presented at the *International Stroke Conference* in San Diego, Jan 2014) [28-30] which form the backbone of this thesis. Some of my results were also presented at the *American Society of Neuroradiology* meeting in San Diego, May 2013 and at the *British Society of Neuroradiology* meeting in Oxford, September 2013. In addition, I am a co-author on two further peer reviewed publications directly as a consequence of this work.[8;31] I have thereby demonstrated that my work is clinically relevant and scientifically robust.

Several of my analyses were probably underpowered, evidenced by wide confidence intervals. The full IST-3 trial was powered to detect outcome differences in the groups given alteplase versus control. Therefore any subgroup analyses using

reduced numbers of patients are likely to be underpowered to demonstrate differences in the alteplase treatment effect.

For some of my analyses (particularly, measuring the attenuation of arterial obstruction) thin-slice CT acquisition for all patients would have been ideal but it was available in only a minority.

A recent analysis concluded that manual measurements of attenuation in arterial obstruction in patients with ischaemic stroke probably oversimplify the true heterogeneity of attenuation within these obstructions by sampling in only a few discrete locations.[32] That analysis was based on a comparison with an automated method of assessing the attenuation of obstructions in their entirety. However the same authors separately concluded, in an analysis of observer variability for measuring the attenuation of obstructions that the greatest observer agreement was obtained by taking three manual region-of-interest measurements (the approach I adopted) rather than only one.[33]

A recent analysis by Seker et. al. concluded that the collateral score developed by Miteff et. al.[34] (as used in IST-3) correlated less well with the extent of infarct core and penumbral volume on perfusion imaging than other scores.[35] Nevertheless, inter-observer reliability for Miteff collateral scoring was moderate in IST-3 and I was able to demonstrate a robust association between Miteff collateral scoring and outcome for patients with arterial obstruction on angiography.

Other limitations of IST-3 have been previously discussed but include:[7] the open design of the trial (treating physicians and patients were aware of their allocation to treatment or control) could have led to bias; in order to achieve the best rate of enrolment, IST-3 did not unduly restrict scanning protocols and allowed centres to use MRI rather than CT for example (although this was uncommon), and to include advanced imaging with angiography or perfusion if that was routine local protocol. While this approach led to variability in the imaging dataset, this is offset by the fact that the imaging represented ‘real-world’ practice and any results are therefore more widely applicable.

10.6 Residual Unanswered Questions or Where More Work is Needed

In my analyses investigating treatment interaction between alteplase and imaging features of arterial obstruction, many of the subgroups were underpowered. IST-3 data have nevertheless allowed me to provide robust recommendations that imaging evidence of arterial obstruction should not be used to influence alteplase treatment decisions. Further randomised-controlled trial data would be required to more adequately power these analyses, but those data are currently not available. Future randomised-controlled trials of intravenous thrombolytics should also include validated scoring for imaging characteristics of arterial obstruction. I plan to update my angiography meta-analysis (and perhaps derive a similar meta-analysis for HAS) in future if this becomes possible. See Table 10-1 for the future research implications of my work.

Table 10-1 *Implications for future research*

Although I have been able to draw conclusions from some underpowered analyses, it is possible that with larger RCT datasets, the end results would be different. Future RCTs of ischaemic stroke should robustly assess brain and vascular imaging using validated assessment methods similar to those in IST-3 which will allow more powerful meta-analyses to be subsequently performed.
All of the imaging features I have investigated in this thesis may also be important for selecting patients most suitable for thrombectomy. Future thrombectomy trials should aim to standardise the collection and assessment of brain and vascular imaging. In particular, those undertaking thrombectomy trials should agree upon the most appropriate CTA/MRA grading system to avoid the same heterogeneity I encountered when meta-analysing CT and MR angiography among patients treated with intravenous thrombolytics (Chapter 9.3).
There is continued interest in the use of automated systems for image analysis. IST-3 data, including some of my own image assessments, are already being used to test the performance of such systems against the 'expert human rater' gold standard. Carefully collected and rigorously human-reader assessed datasets such as IST-3 are likely to continue to provide 'ground truth' for future developments in computational image analysis.

Footnote: RCT = randomised controlled trial.

There were insufficient numbers of patients in IST-3 with haemorrhagic transformation of infarct after alteplase to perform analyses exploring associations between HAS or angiographic obstruction and haemorrhagic transformation. Recent work has shown that the presence of HAS can independently predict haemorrhagic transformation[36] but not necessarily symptomatic haemorrhage;[37;38] and the latter is arguably more important.

IST-3 had only limited data with which to assess whether an arterial obstruction or HAS was more likely embolic or thrombotic, for example embolic obstructions are probably more common in the presence of atrial fibrillation. Recently in a large retrospective analysis of patients treated with alteplase, those with HAS due to large artery atherosclerosis were associated with lower rates of HAS disappearance compared with HAS secondary to cardio-embolic stroke.[39] There were insufficient data in IST-3 for a robust analysis of ischaemic stroke aetiology and any potential associations with brain imaging appearances. However in Chapter 6 I found no difference in the CT attenuation of arterial obstruction among patients with and without atrial fibrillation, one of the primary causes of cardio-embolic stroke. Another recent analysis has suggested that use of antiplatelets may increase the likelihood of an arterial obstruction being hyperattenuating;[40] this is something I could have tested using IST-3 data.

Certain assumptions have been made about the composition of arterial clots in ischaemic stroke based on minimal pathological data and evidence from ex-vivo phantom studies using blood products.[41-45] But a recent systematic review did conclude that HAS are more likely to be rich in red blood cells.[46] In this new stroke treatment era of thrombectomy, it should be possible to get better information on the true pathological composition of a large number of clots, I suspect this might become more common in the near future as neuro-interventionalists strive for a better understanding of whether the imaging characteristics of arterial obstruction should alter their decision to offer treatment. Whether it is feasible to consistently collect arterial clots after thrombectomy and then to provide an accurate histological analysis of those clots remains uncertain. The findings I describe here between HAS, composition of obstruction, response to treatment with intravenous alteplase and

outcome might also be applicable to patients being considered for mechanical thrombectomy, but this has so far been largely untested.[47-49] Unfortunately, due to the standard design of the major thrombectomy trials, it will not be possible to address similar issues for patients with angiographic obstruction using these trial data since patients without proximal obstruction were not enrolled in the major trials.

10.7 Conclusions

When identified on non-contrast CT performed soon after stroke onset, the presence of a hyperattenuated artery is a powerful surrogate indicator for arterial obstruction. However, when HAS is absent approximately 50% of patients may still have an unrecognised arterial obstruction.

Angiography does provide more confirmatory evidence of an ischaemic stroke diagnosis (by identifying the other 50% with an arterial obstruction but no HAS) but does not alter prognosis prediction. That is, it does not improve our ability to identify those patients most likely to do well or poorly following treatment; severely affected individuals are rarely those that require a CTA for confirmation of stroke.

My data provide confidence that CTA can be interpreted consistently by a range of individuals who differ in their experience level and that the reader reliability for CTA is similar to that for non-contrast CT. Delays inherent in the process of adding CTA to standard imaging can and should be minimised through the development of robust imaging protocols, these in turn should be derived from clear evidence that the addition of CTA is beneficial to any given patient.

I showed that arterial obstruction on baseline angiography (or its imaging surrogate on non-contrast CT – HAS – described in a much larger, more powerful dataset) is associated with more severe stroke at baseline and worse functional outcome at six-months independent of other known outcome predictors. I also showed that alteplase works to remove HAS regardless of its location or extent (but small distal HAS are more likely to be removed completely following treatment) and that HAS disappearance is associated with a better outcome. Unfortunately, IST-3 did not contain sufficient follow-up angiograms to check for the alteplase effect on

angiographic recanalisation, however it is plausible and likely that HAS clearance is a surrogate for recanalisation.

Despite these associations with stroke severity and outcome, I did not identify any interactions between imaging appearances of arterial obstruction at baseline (either HAS on non-contrast CT or obstruction on CT/MR angiography) and response to intravenous alteplase in IST-3. It was only after combining IST-3 results in meta-analysis that I identified a significant treatment interaction (with severity of arterial stenosis). While it is reassuring to discover that patients with severe arterial obstruction do benefit from intravenous thrombolytics, the lack of a significant outcome (i.e. neither benefit nor harm) in the subgroup with patent arteries means that even these results are non-contributory for alteplase treatment decision making. In other words, these data suggest that angiography results should not be used to decide whether patients do or do not receive intravenous alteplase. See Table 10-2 for a summary of my major findings and the clinical practice implications of my work.

Table 10-2 *Summary of main findings and their implications for clinical practice*

Summary of main findings	**Implications for clinical practice**
• HAS is a highly specific surrogate for arterial obstruction on non-contrast CT, present in around 25% of all patients presenting acutely with ischaemic stroke • The presence of HAS indicates that the patient is likely to be suffering a severe stroke and is likely to have poor functional outcome	If clinicians are mindful of these findings they can more effectively diagnose, correctly triage and provide evidenced-based prognosis to patients with ischaemic stroke
• The use of CTA for the acute assessment of ischaemic stroke is rising (and is likely to continue rising now that thrombectomy has been proven effective in the presence of a proximal arterial obstruction) • CTA can be added to a standard stroke imaging protocol with around 6 minutes of extra time in the scanner • The addition of CTA at baseline makes it more likely that ischaemic stroke is correctly diagnosed • Angiographic abnormalities are associated with severe strokes and poor functional outcomes • CTA can be assessed consistently by a range of users if they are adequately trained	Increased use of CTA will improve the recognition of ischaemic stroke My results provide confidence that CTA can be used efficiently and effectively
• The majority of arterial obstructions causing ischaemic stroke are not fully occluded, which might explain how intravenous alteplase is able to reduce and remove even large and proximally sited arterial obstructions • Shrinkage or removal of arterial obstructions improves functional outcome • Using the best currently available evidence (meta-analysis of RCT data), there is no proof that the presence versus absence of arterial obstruction (or specific characteristics of the obstruction) materially alters the beneficial effect of intravenous alteplase • Therefore, neither the presence/absence of arterial obstruction on CTA nor the presence/absence of a HAS should be used to select patients for treatment with intravenous alteplase	My results should provide stroke physicians with confidence to use intravenous alteplase even for patients with obstruction of large proximal arteries This might prove to be most relevant when thrombectomy is unavailable or contra-indicated

Footnote: RCT = randomised controlled trial.

While associations between imaging findings of arterial obstruction (however assessed), stroke severity, outcome and interaction with alteplase may seem contradictory, they can be explained by consideration of the statistical tests used in these analyses. Ordinal regression analyses of alteplase treatment effect on outcome look for step-wise shifts along the outcome scales compared between treatment and control groups. In this way, a patient who moves one point down the severity scale is considered improved no matter where on the scale this movement occurred; i.e. in these analyses a change from death to severe disability (mRS 6 to 5) is considered an improvement no different to a change from dependent to independent living (mRS 3 to 2). However, clinically and socio-economically we must acknowledge that these alternative 'improved' outcomes are not similar. Therefore, the correct interpretation of these results is that while alteplase offers benefit equally to different imaging subgroup patients (e.g. those with and without proximal arterial obstruction), those that have a severe stroke at outset remain likely to have a poor outcome, only this outcome is less poor following treatment.

Therefore my analyses provide evidence from a large randomised-controlled trial, counter to some popular opinion, that alteplase is effective across all of these different subgroups of ischaemic stroke patients and therefore should not be denied (or delayed) on the basis of imaging. Where a thrombectomy service is available, alteplase is the first step in this treatment pathway. Where thrombectomy is unavailable or contra-indicated, alteplase remains an effective means of improving the likelihood of a better outcome.

10.8 References for Chapter 10

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Chapter 11 Appendices

11.1 Appendix 1 - IST-3 Collaborative Group

For a complete list of all committees, please see the IST-3 primary publication in The Lancet (The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomized controlled trial. Lancet 2012;379:2352-63).

IST-3 was conceived by the co-chief investigators, Peter Sandercock (University of Edinburgh, Scotland), Richard I Lindley (Sydney Medical School – Westmead Hospital and The George Institute for Global Health, University of Sydney, Australia), and Joanna M Wardlaw (University of Edinburgh, Scotland).

11.1.1 CT and MRI reading panel

Joanna M Wardlaw, Andrew Farrall (University of Edinburgh, Scotland), Zoe Morris (University of Edinburgh, Scotland), Rüdiger von Kummer (Dresden University Stroke Centre, Germany), Lesley Cala (University of Western Australia, Crawley, Australia), Anders von Heijne (Dandyred Hospital, Stockholm, Sweden), Alessandro Adami (Sacro Cuore-Don Calabria Hospital, Verona, Italy), Andre Peeters (Cliniques Universitaires Saint-Luc, Bruxelles, Belgium), Gillian Potter (Salford Royal NHS Foundation Trust, England), Nick Bradey (Neuroradiology, James Cook University Hospital, South Tees Hospital NHS Trust, Middlesborough, UK).

11.1.2 Angiography reading panel

Joanna M Wardlaw, Rüdiger von Kummer, Andrew Farrall, Robin Sellar (University of Edinburgh, Scotland), Alessandro Adami, Philip White (Newcastle University, UK), Andrew Demchuk (University of Calgary, Canada), Matthew Adams (Great

Ormond Street Hospital, London, UK), Grant Mair (University of Edinburgh, Scotland), Bernard Yan (The Royal Melbourne Hospital, Parkville, Australia).

11.1.3 Trial steering committee

Independent chairmen: Colin Baigent (University of Oxford, UK); David Chadwick (University of Liverpool, UK). Independent member: Pippa Tyrrell (University of Manchester, UK); Gordon Lowe (University of Glasgow, UK). Co-principal investigators: PS; RIL. Chief investigator for Neuroradiology: JMW; Martin Dennis (University of Edinburgh, Scotland). Statistician: Geoff Cohen (University of Edinburgh, Scotland). Trial Co-ordinator: Karen Innes (University of Edinburgh, Scotland). Lay representative: Heather Goodare.

11.1.4 National coordinators and associate national coordinators

Australia: RIL, Graeme J Hankey (Royal Perth Hospital, Perth). **Austria:** Karl Matz (Landesklinikum Donauregion Tulln, Tulln), Michael Brainin. **Belgium:** AP. **Canada:** Gord Gubitz (Dalhousie University and Queen Elizabeth II Health Sciences Centre, Halifax), Stephen J Phillips (Dalhousie University and Queen Elizabeth II Health Sciences Centre, Halifax). **Italy:** Stefano Ricci (Department of Neurology ASL1, Ospedale, Citta' di Castello). **Mexico:** Antonio Arauz (Instituto Nacional de Neurologia, Mexico City). **Norway:** Eivind Berge (Oslo University Hospital, Oslo), Karsten Bruins Slot (Oslo University Hospital, Oslo). **Poland:** Anna Czlonkowska (Institute of Psychiatry and Neurology, Warsaw, and Medical University of Warsaw, Warsaw), Adam Kobayashi (Institute of Psychiatry and Neurology, Warsaw, Poland). **Portugal:** Manuel Correia (Hospital Geral de Santo Antonio, Porto). **Switzerland:** Phillippe Lyrer (University Hospital Basel, Basel), Stefan Engelter. **Sweden:** Veronica Murray (Karolinska Institutet, Stockholm), Andreas Terent, Bo Norrving, Per Wester: **UK:** Graham Venables (Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK).

11.1.5 IST-3 collaborative group and participating hospitals in each country

Figures in parentheses are the number of patients recruited in the country or by the centre.

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11.1.6 Centres in IST-3 that performed angiography

Australia

Austin Health - Repatriation Campus: Prof Helen Dewey.

Box Hill Hospital (Monash University): Prof Chris Bladin

Gosford Hospital: Dr Jonathan Sturm

John Hunter Hospital: Dr Chris Levi

Nambour General Hospital: Dr Rohan Grimley

Royal Brisbane and Women's Hospital: Dr Stephen Read

Royal Perth Hospital: Dr Graeme J. Hankey

Austria

Landesklinikum Donauregion Tulln: Dr Karl Matz

Belgium

Cliniques Universitaires St. Luc: Dr Andre Peeters

Canada

QEII Health Sciences Centre: Dr Gord Gubitz

Italy

Nuovo Ospedale Civile: Dr Federica Casoni

Ospedale Citta di Castello: Dr Silvia Cenciarelli

Ospedale di Branca (Ospedale di Gubbio): Dr Tatiana Mazzoli

Ospedale di Cattinara – Trieste: Dr Fabio Chiodo Grandi

Ospedale Maggiore: Dr Gaetano Procaccianti

Ospedale Valduce di Como: Dr Nicoletta Checcarelli

Universita degli Studi di Genova, Dipartimento di Neuroscienze Oftalmologia e Genetica: Prof Carlo Gandolfo

Norway

Aalesund Sjukehus: Dr Yngve Müller Seljeseth

Harstad Sykehus: Dr Odd Kildahl-Andersen

St Olavs Hospital, University Hospital of Trondheim: Dr Bent Indredavik

Ullevål University Hospital: Dr Eivind Berge

University Hospital Northern Norway: Dr Stein Harald Johnsen

Poland

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Institute of Psychiatry & Neurology, Medical University of Gdansk: Prof Walenty Michal Nyka, Dr Dariusz Gasecki

Military Medical Institute SPZZOZ w Sandomierzu: Prof A Stepien, Dr Piotr Sobolewski

Portugal

Centro Hospitalar de Trás-os-Montes e Alto Douro: Dr Mário Silva

Sweden

Danderyds Sjukhus: Dr Veronica Murray

Hassleholm Hospital: Dr Magnus Esbjornsson

University Hospital of Northern Sweden: Prof Per Wester

Uppsala University Hospital: Dr Erik Lundström

Switzerland

Universitätsspital Basel: Prof Philippe Lyrer

Universitätsspital Zürich: Prof Andreas Luft

United Kingdom

Addenbrookes Hospital: Dr Liz Warburton

City Hospital, Sandwell & West Birmingham Hospitals NHS Trust: Dr David Nicholl

Countess of Chester Hospital: Dr K Chatterjee

Guy's & St.Thomas' Hospital: Prof Anthony Rudd

Hammersmith Hospitals & Imperial College: Dr Pankaj Sharma

King's College Hospital: Professor Lalit Kalra

Leeds General Infirmary: Dr Ahamad Hassan

Norfolk and Norwich University Hospital NHS Trust: Dr Kneale Metcalf

Nottingham City Hospital: Dr Wayne Sunman

Queen Elizabeth the Queen Mother Hospital: Dr Gunaratnam Gunathilagan

Queen's Hospital, Barking, Havering & Redbridge Hospitals NHS Trust: Dr Khaled Darawil

Royal Hallamshire Hospital: Prof Graham Venables

Southend University Hospital: Dr Paul Guyler

St George's Healthcare NHS Trust: Dr Geoffrey Cloud

The National Hospital for Neurology & Neurosurgery: Prof Martin Brown

The Royal London Hospital, Barts and The London NHS Trust: Dr Patrick Gompertz

University Hospital Aintree: Dr Ramesh Durairaj

University Hospital of North Staffordshire: Prof Christine Roffe

University Hospitals Coventry & Warwickshire NHS Trust: Dr Anthony Kenton

Western General Hospital: Prof Peter Sandercock

William Harvey Hospital: Dr David Hargroves.

11.2 Appendix 2 - Funding for IST-3

The start-up phase of IST-3 was supported by a grant from the Stroke Association, UK (TSA 04/99). The expansion phase was funded by the Health Foundation UK (2268/1282). The scan reading development was funded by Chest, Heart Stroke Scotland (R100/7).

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Boehringer-Ingelheim GmbH donated drug and placebo for the 300 patients in the double-blind phase, but thereafter had no role whatsoever in the trial.

The UK Stroke Research Network (SRN study ID 2135) adopted the trial in 01/05/2006, supported the initiation of new UK sites, and in some centres, and, after that date, data collection was undertaken by staff funded by the network or working for associated NHS organisations.

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11.3 Articles Published in Peer Reviewed Journals

All of the following articles are derived directly from work contained within this thesis.

11.3.1 Appendix 3 - Article 1

Mair G and Wardlaw JM.

Imaging of acute stroke prior to treatment: Current practice and evolving techniques.

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REVIEW ARTICLE

Imaging of acute stroke prior to treatment: current practice and evolving techniques

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ABSTRACT

Standard imaging in acute stroke is undertaken with the aim of diagnosing the underlying cause and excluding stroke mimics. In the presence of ischaemic stroke, imaging is also needed to assess patient suitability for treatment with intravenous thrombolysis. Non-contrast CT is predominantly used, but MRI can also exclude any contraindications to thrombolysis treatment. Advanced stroke imaging such as CT and MR angiography and perfusion imaging are increasingly used in an acute setting. In this review, we discuss the evidence for the application of these advanced techniques in the imaging of acute stroke.

STROKE PREVALENCE AND AETIOLOGY

Stroke is defined conventionally as a focal neurological deficit that persists for more than 24 h owing to interruption of the blood supply to the brain. This definition is used to differentiate stroke from a transient ischaemic attack (TIA), which does not persist beyond 24 h but can be otherwise clinically identical. Stroke is a major health concern worldwide, but it is a particular problem in less developed countries where there are increased risk factors and less dedicated care. But even in developed nations, stroke remains a significant concern among their increasingly aged populations. Recent figures from the World Health Organization show that in the UK, cerebrovascular disease causes approximately 10.6% of all deaths and has a death rate of 45.6 per 100,000 of population. This places the UK 34th in world rankings (19th within Europe), which range from 25.0 to 249.4 per 100,000; the worldwide median death rate from stroke is 101.8 per 100,000 of population.[1]

Advances in care over the past 20 years have greatly improved the outcome for stroke patients with a reduction in deaths and more patients ultimately achieving independence. Amongst several possible changes, three factors are largely responsible for this improvement. First was the emergence of dedicated stroke units that offer both acute and rehabilitative care for the various and variable needs of stroke patients; a systematic review of randomized trial data has shown that specialized stroke unit care improves the long-term outcome for stroke patients by reducing both death and dependency.[2] Second was the introduction of intravenous thrombolysis, which represented the first effective treatment for acute ischaemic stroke (aspirin has a modest effect as described below).[3,4] Third is a better understanding among the general public at large that stroke represents a potentially treatable medical emergency. The FAST (Face, Arm, Speech, Time) campaign has been shown to improve public awareness of stroke.[5] Despite these advances, however, many stroke survivors remain disabled; in general, around one-third of stroke patients die, one-third remain disabled long term and one-third regain or retain their independence. Stroke-related disability has enormous implications and costs for both the patient and for society as a whole. It is estimated that stroke costs the UK economy around £9 billion per year. Up to 50% of this is billed directly to the National Health Services, 30% is for the cost of informal care, while loss of productivity is estimated to account for the remaining 20%.[6,7]

The majority of strokes are ischaemic (approximately 80%) and most of these relate to problems with arterial blood flow to the brain; ischaemic stroke secondary to venous insufficiency is much less common. Haemorrhagic stroke accounts for the remaining 20%. The aetiologies of ischaemic and haemorrhagic stroke are usually quite different, but ischaemic strokes are often complicated by secondary haemorrhagic transformation. Stroke aetiology also varies with patient age. In middle aged to elderly adults, large artery ischaemic stroke (affecting both the

11.3.2 Appendix 4 - Article 2

Mair G, von Kummer R, Morris Z, von Heijne A, Bradey N, Cala L, Peeters A, Farrall AJ, Adami A, Potter G, Cohen G, Sandercock PAG, Lindley RI and Wardlaw JM for the IST-3 Collaborative Group.

Effect of alteplase on the CT hyperdense artery sign and outcome after ischemic stroke.

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Effect of alteplase on the CT hyperdense artery sign and outcome after ischemic stroke

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ABSTRACT

Objective: To investigate whether the location and extent of the CT hyperdense artery sign (HAS) at presentation affects response to IV alteplase in the randomized controlled Third International Stroke Trial (IST-3).

Methods: All prerandomization and follow-up (24–48 hours) CT brain scans in IST-3 were assessed for HAS presence, location, and extent by masked raters. We assessed whether HAS grew, persisted, shrank, or disappeared at follow-up, the association with 6-month functional outcome, and effect of alteplase. IST-3 is registered (ISRCTN25765518).

Results: HAS presence (vs absence) independently predicted poor 6-month outcome (increased Oxford Handicap Scale [OHS]) on adjusted ordinal regression analysis (odds ratio [OR] 0.66, $p < 0.001$). Outcome was worse in patients with more (vs less) extensive HAS (OR 0.61, $p = 0.027$) but not in proximal (vs distal) HAS ($p = 0.420$). Increasing age was associated with more HAS growth at follow-up (OR 1.01, $p = 0.013$). Treatment with alteplase increased HAS shrinkage/disappearance at follow-up (OR 0.77, $p = 0.006$). There was no significant difference in HAS shrinkage with alteplase in proximal (vs distal) or more (vs less) extensive HAS ($p = 0.516$ and $p = 0.580$, respectively). There was no interaction between presence vs absence of HAS and benefit of alteplase on 6-month OHS ($p = 0.167$).

Conclusions: IV alteplase promotes measurable reduction in HAS regardless of HAS location or extent. Alteplase increased independence at 6 months in patients with and without HAS.

Classification of evidence: This study provides Class I evidence that for patients within 6 hours of ischemic stroke with a CT hyperdense artery sign, IV alteplase reduced intra-arterial hyperdense thrombus. ***Neurology*® 2016;86:1–8**

GLOSSARY

ACA = anterior cerebral artery; **CI** = confidence interval; **HAS** = hyperdense artery sign; **HU** = Hounsfield units; **ICA** = internal carotid artery; **IST-3** = Third International Stroke Trial; **MCA** = middle cerebral artery; **NIHSS** = NIH Stroke Scale; **OHS** = Oxford Handicap Scale; **OR** = odds ratio; **PCA** = posterior cerebral artery; **SIRS** = Systematic Image Review System.

Arterial hyperattenuation on noncontrast CT, the hyperdense artery sign (HAS), is a consistently recognized CT sign of acute ischemic stroke.[1] HAS is highly specific and moderately sensitive for intracranial arterial obstruction by thrombus.[2] HAS is associated with increased stroke severity at presentation and worse long-term outcomes.[3–5] There are, however, limited data on how the location, extent, or persistence of HAS relates to functional outcome following stroke, and importantly whether patients with (vs without) HAS benefit differently from IV thrombolysis with alteplase.[6–9]

Supplemental data at Neurology.org

From the Division of Neuroimaging Sciences (G.M., Z.M., A.J.F., G.C., J.M.W.) and the Division of Clinical Neurosciences (P.A.G.S.), University of Edinburgh, UK; the Department of Neuroradiology (R.v.K.), Dresden University Stroke Centre, Germany; Danderyd Hospital (A.v.H.), Stockholm, Sweden; Neuroradiology (N.B.), James Cook University Hospital, Middlesborough, UK; School of Pathology and Laboratory Medicine (L.C.), University of Western Australia, Perth; Cliniques Universitaires St Luc (A.P.), Neurologie, Belgium; Stroke Center (A.A.), Sacro Cuore-Don Calabria Hospital, Negrar, Italy; the Department of Neuroradiology (G.P.), Salford Royal NHS Foundation Trust, Manchester, UK; and the Westmead Hospital Clinical School and The George Institute for Global Health (R.I.L.), University of Sydney, Australia.

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Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article. The Article Processing Charge was paid by the MRC.

11.3.3 Appendix 5 - Article 3

Mair G, von Kummer R, Lindley RI, Sandercock PAG, Wardlaw JM.

Effect of X-ray attenuation of arterial obstructions on intravenous thrombolysis and outcome after ischemic stroke.

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RESEARCH ARTICLE

Effect of X-Ray Attenuation of Arterial Obstructions on Intravenous Thrombolysis and Outcome after Ischemic Stroke

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Data Availability Statement: All data used in these analyses can be made available on request to the IST-3 Data Access Committee. Unregulated access to study data is not possible. A general principle is that data users must not undertake research using personally-identifiable data unless there is consent in place to do so. In IST-3, information was received in confidence from study participants, and they were told that information and biological samples would be treated with absolute confidentiality and used only for medical research. The IST-3 Data Access Committee has the responsibility to ensure that data and

Abstract

Objective

To assess whether the x-ray attenuation of intra-arterial obstruction measured on non-contrast CT in ischemic stroke can predict response to thrombolysis and subsequent functional outcome.

Methods

The Third International Stroke Trial (IST-3) was a multicenter randomized-controlled trial of intravenous thrombolysis (rt-PA) given within six hours of ischemic stroke. Ethical approval and informed consent were obtained. In a subgroup of 109 IST-3 patients (38 men, median age 82 years), a single reader, masked to all clinical and other imaging data, manually measured x-ray attenuation (Hounsfield Units, HU) on non-contrast CT at the location of angiographically-proven intra-arterial obstructions, pre-randomization and at 24–48 hour follow-up. We calculated change in attenuation between scans. We assessed the impact of pre-randomization arterial obstruction attenuation on six-month functional outcome.

Results

Most arterial obstructions (64/109, 59%) were hyperattenuating (mean 51.0 HU). Compared with control, treatment with rt-PA was associated with a greater, but non-significant, reduction in obstruction attenuation at follow-up (-8.0 HU versus -1.4 HU in patients allocated control, p = 0.117). In multivariable ordinal regression analysis controlled for patient age, stroke severity, location and extent of obstruction, time from stroke onset to baseline scan and rt-PA treatment allocation, the attenuation of pre-randomization arterial obstruction was not independently associated with six-month outcome (odds ratio = 0.99, 95% confidence interval = 0.94–1.03, p = 0.516).

11.3.4 Appendix 6 - Article 4

Mair G, Boyd EV, Chappell FM, von Kummer R, Lindley RI, Sandercock P, Wardlaw JM, The IST-3 Collaborative Group.

Sensitivity and specificity of the hyperdense artery sign for arterial obstruction in acute ischemic stroke.

Stroke 2015;46:102-7.

Accepted for publication on the 30th October 2014.

Sensitivity and Specificity of the Hyperdense Artery Sign for Arterial Obstruction in Acute Ischemic Stroke

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Background and Purpose—In acute ischemic stroke, the hyperdense artery sign (HAS) on noncontrast computed tomography (CT) is thought to represent intraluminal thrombus and, therefore, is a surrogate of arterial obstruction. We sought to assess the accuracy of HAS as a marker of arterial obstruction by thrombus.

Methods—The Third International Stroke Trial (IST-3) was a randomized controlled trial testing the use of intravenous thrombolysis for acute ischemic stroke in patients who did not clearly meet the prevailing license criteria. Some participating IST-3 centers routinely performed CT or MR angiography at baseline. One reader assessed all relevant scans independently, blinded to all other data; we checked observer reliability. We combined IST-3 data with a systematic review and meta-analysis of all studies that assessed the accuracy of HAS using angiography (any modality).

Results—IST-3 had 273 patients with baseline CT or MR angiography and was the largest study of HAS accuracy. The meta-analysis (n=902+273=1175, including IST-3) found sensitivity and specificity of HAS for arterial obstruction on angiography to be 52% and 95%, respectively. HAS was more commonly identified in proximal than distal arteries (47% versus 37%; P=0.015), and its sensitivity increased with thinner CT slices (r=−0.73; P=0.001). Neither extent of obstruction nor time after stroke influenced HAS accuracy.

Conclusions—When present in acute ischemic stroke, HAS indicates a high likelihood of arterial obstruction, but its absence indicates only a 50/50 chance of normal arterial patency. Thin-slice CT improves sensitivity of HAS detection.

Clinical Trial Registration—URL: http://www.controlled-trials.com/ISRCTN25765518. Unique identifier: ISRCTN25765518. **(*Stroke*. 2015;46:102-107. DOI: 10.1161/STROKEAHA.114.007036.)**

Key Words: angiography ■ meta-analysis ■ stroke

Noncontrast computed tomography (CT) remains the primary imaging modality for hyperacute assessment of stroke in most centers.[1] Identifying features of acute ischemic stroke on CT, therefore, remains important for routine practice. Hyperattenuation of a cerebral artery on noncontrast CT in acute ischemic stroke is thought to represent acute thrombus or embolus; the presence of the Hyperdense Artery Sign (HAS), therefore, is a surrogate of arterial obstruction and may provide useful confirmation of the diagnosis of acute ischemic stroke. The sign has been defined as any artery that subjectively appears transiently denser than adjacent or equivalent contralateral vessels[2,3] although objective measures have also been applied.[4] When compared with angiography, previous studies have shown that the HAS is a specific (although false-positives are described)[5] but not sensitive indicator of arterial obstruction.[6,7] To our knowledge, no systematic review and meta-analysis of HAS sensitivity and specificity have been published.

The Third International Stroke Trial (IST-3) was a multicenter, randomized controlled trial, which tested intravenous thrombolysis (Alteplase) given within 6 hours of ischemic stroke.[8] Baseline (prerandomization) and follow-up (within 48 hours) brain imaging (predominantly noncontrast CT) was performed for all IST-3 patients (n=3035). In some centers, CT or MR angiography (CTA and MRA, respectively) were also routinely obtained prerandomization as part of their local stroke imaging protocol.[9]

In a prespecified analysis, we investigated the diagnostic accuracy of HAS for arterial obstruction detected with CTA or MRA and assessed if characteristics of the noncontrast CT scan (slice-thickness), the corresponding angiographic

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*IST-3 Principal Investigators who contributed imaging for these analyses are listed in Appendix I in the online-only Data Supplement.

†The complete IST-3 Collaborative Group is listed in Appendix II in the online-only Data Supplement.

The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.114.007036/-/DC1.

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Stroke **is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.114.007036**

11.3.5 Appendix 7 - Article 5

Mair G, von Kummer R, Adami A, White PM, Adams ME, Yan B, Demchuk AM, Farrall AJ, Sellar RJ, Ramaswamy R, Mollison D, Boyd EV, Rodrigues MA, Samji K, Baird AJ, Cohen G, Sakka E, Palmer J, Perry D, Lindley R, Sandercock PAG, Wardlaw JM, The IST-3 Collaborative Group.

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Neuroradiology
DOI 10.1007/s00234-014-1441-0

DIAGNOSTIC NEURORADIOLOGY

Observer reliability of CT angiography in the assessment of acute ischaemic stroke: data from the Third International Stroke Trial

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Received: 19 August 2014 / Accepted: 22 September 2014

Abstract
Introduction CT angiography (CTA) is often used for assessing patients with acute ischaemic stroke. Only limited observer reliability data exist. We tested inter- and intra-observer reliability for the assessment of CTA in acute ischaemic stroke.
Methods We selected 15 cases from the Third International Stroke Trial (IST-3, ISRCTN25765518) with various degrees of arterial obstruction in different intracranial locations on CTA. To assess inter-observer reliability, seven members of the IST-3 expert image reading panel (>5 years experience reading CTA) and seven radiology trainees (<2 years experience) rated all 15 scans independently and blind to clinical data for: presence (versus absence) of any intracranial arterial abnormality (stenosis or occlusion), severity of arterial abnormality using relevant scales (IST-3 angiography score, Thrombolysis in Cerebral Infarction (TICI) score, Clot Burden Score), collateral supply and visibility of a perfusion defect on CTA source images (CTA-SI). Intra-observer reliability was assessed using independently repeated expert panel scan ratings. We assessed observer agreement with Krippendorff's-alpha (K-alpha).

Electronic supplementary material The online version of this article (doi:10.1007/s00234-014-1441-0) contains supplementary material, which is available to authorized users.

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11.3.6 Appendix 8 - Article 6

Mair G, von Kummer R, Adami A, White PM, Adams ME, Yan B, Demchuk AM, Farrall AJ, Sellar RJ, Sakka E, Palmer J, Perry D, Lindley RI, Sandercock PAG and Wardlaw JM and for the IST-3 Collaborative Group.

Arterial Obstruction on Computed Tomographic or Magnetic Resonance Angiography and Response to Intravenous Thrombolytics in Ischemic Stroke.

Stroke 2017;48:353-360.

Accepted for publication on the 11th November 2016.

Original Contribution

OPEN

Arterial Obstruction on Computed Tomographic or Magnetic Resonance Angiography and Response to Intravenous Thrombolytics in Ischemic Stroke

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Background and Purpose—Computed tomographic angiography and magnetic resonance angiography are used increasingly to assess arterial patency in patients with ischemic stroke. We determined which baseline angiography features predict response to intravenous thrombolytics in ischemic stroke using randomized controlled trial data.

Methods—We analyzed angiograms from the IST-3 (Third International Stroke Trial), an international, multicenter, prospective, randomized controlled trial of intravenous alteplase. Readers, masked to clinical, treatment, and outcome data, assessed prerandomization computed tomographic angiography and magnetic resonance angiography for presence, extent, location, and completeness of obstruction and collaterals. We compared angiography findings to 6-month functional outcome (Oxford Handicap Scale) and tested for interactions with alteplase, using ordinal regression in adjusted analyses. We also meta-analyzed all available angiography data from other randomized controlled trials of intravenous thrombolytics.

Results—In IST-3, 300 patients had prerandomization angiography (computed tomographic angiography=271 and magnetic resonance angiography=29). On multivariable analysis, more extensive angiographic obstruction and poor collaterals independently predicted poor outcome ($P<0.01$). We identified no significant interaction between angiography findings and alteplase effect on Oxford Handicap Scale ($P\geq0.075$) in IST-3. In meta-analysis (5 trials of alteplase or desmoteplase, including IST-3, n=591), there was a significantly increased benefit of thrombolytics on outcome (odds ratio>1 indicates benefit) in patients with (odds ratio, 2.07; 95% confidence interval, 1.18–3.64; $P=0.011$) versus without (odds ratio, 0.88; 95% confidence interval, 0.58–1.35; $P=0.566$) arterial obstruction (P for interaction 0.017).

Conclusions—Intravenous thrombolytics provide benefit to stroke patients with computed tomographic angiography or magnetic resonance angiography evidence of arterial obstruction, but the sample was underpowered to demonstrate significant treatment benefit or harm among patients with apparently patent arteries.

Clinical Trial Registration—URL: http://www.isrctn.com. Unique identifier: ISRCTN25765518.

(*Stroke*. 2017;48:00-00. DOI: 10.1161/STROKEAHA.116.015164.)

Key Words: arteries ■ brain infarction ■ cerebral angiography ■ meta-analysis ■ stroke

Computed tomographic angiography (CTA) and magnetic resonance angiography (MRA) are increasingly used to assess arterial patency in patients with ischemic stroke.[1,2] Angiography may also improve selection of patients with ischemic stroke for treatment with intravenous thrombolytics. However, it is unclear if arterial obstruction, or collateral

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Guest Editor for this article was Louis Caplan, MD.

A list of all IST-3 Collaborative Group participants is given in the Appendix I in the online-only Data Supplement.

The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.116.015164/-/DC1.

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***Stroke* is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.116.015164**

11.4 Data Collection Forms

11.4.1 Appendix 9 - CT rating form for IST-3

The following form was developed by the IST-3 Chief Investigators, but primarily Joanna Wardlaw, and was used for assessing all CT brain imaging collected for patients included in the angiography and perfusion imaging substudies of IST-3.

The questions on this form were replicated exactly within the SIRS-2 imaging analysis platform.

IST-3 Perfusion-angiography study CT Reading form 20120225

CT image interpretation form

PATIENT ID:

DATE OF READING: **DATE OF SCAN:**

SCAN QUALITY: Good ☐ Moderate ☐ Poor ☐ **Comment:**

READER ID:

TYPE OF SCAN: (tick all that apply) **CT Plain:** ☐ **CTP:** ☐ **CTA:** ☐

TYPE OF PERFUSION AVAILABLE: **MTT:** ☐ **CBV:** ☐ **TMAX:** ☐

CBF: ☐ **TTP:** ☐ **Other:** ☐

Please tick Yes or No. Please do not leave blanks. Thank you.

1.	Are all the scan sequences completely normal?	**Y** ☐	**N** ☐	***If YES stop here***
2.	**Ischaemic Changes** Is there any sign of acute ischaemic change on any sequence? If in doubt as to whether acute or old, code as acute.	**Y** ☐	**N** ☐	***If No go to Q.7***
3.	Which side of the brain shows ischaemic change?	**R** ☐	**L** ☐	***Tick R and L if both***
4.	Classify signs of ischaemic change in the main lesions (if more than one recent lesion). (**see examples)**	**Y** ☐	**N** ☐	**N/A**
	a) Loss of grey/white matter cortex definition.	☐	☐	☐
	b) Loss of basal ganglia outline.	☐	☐	
	c) Hypodensity present (i.e. more than in a or b so that the lesion appears less dense than white matter).	☐	☐	

IST-3 Perfusion-angiography study CT Reading form 20120225

d) PWI lesion visible. (tick one box for each row that applies). The 20% refers to volume.

	⇑ or ⇓	N	<20%<CT	Same as CT	>20%>CT
CBFr	☐	☐	☐	☐	☐
CBVr	☐	☐	☐	☐	☐
MTTr	☐	☐	☐	☐	☐
TTPr	☐	☐	☐	☐	☐
ATFr	☐	☐	☐	☐	☐
FWHMr	☐	☐	☐	☐	☐
PTFr	☐	☐	☐	☐	☐
Cmaxr	☐	☐	☐	☐	☐
CBFq	☐	☐	☐	☐	☐
CBVq	☐	☐	☐	☐	☐
MTTq	☐	☐	☐	☐	☐
Tmaxq	☐	☐	☐	☐	☐
Raw data	☐	☐	☐	☐	☐
Other	☐	☐	☐	☐	☐

IST-3 Perfusion-angiography study CT Reading form 20120225

5. Classify site and size of ischaemic lesion on plain CT **(see examples)**

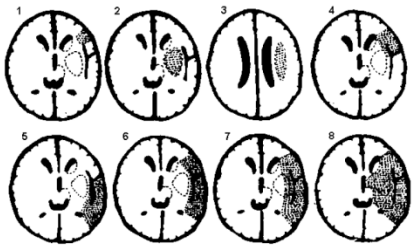
a) site (enter most appropriate code in box) 1° ☐ 2° ☐

M =MCA* = any lesion in the MCA territory
AS =Infarct of up to half of ACA territory
AL =Infarct of more than half of ACA territory
PS =Infarct of up to half of PCA territory
PL =Infarct of more than half of PCA territory
MAS=M+AS*
MAL=M+AL*
MPS=M+PS*
MPL=M+PL*
MAP=Infarct of whole MCA, ACA and PCA territories
L =Lacune*
B =Borderzone*
C =Cerebellum*
S =Brainstem*
CS =Cerebellum and brainstem

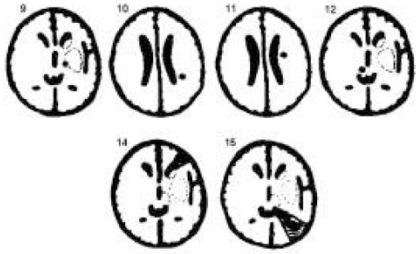
* code sub-territory sites in b

b) sub-territory sites 1° ☐ 2° ☐

MCA sub-territory codes
1=small cortical infarct
2=basal ganglia infarct (>2x2x2cm)
3= infarct of white matter lateral to the lateral ventricle (>2x2x2cm)
4=infarct of anterior half of peripheral MCA territory– a=not involving and b=involving part of basal ganglia
5=infarct of the posterior half of peripheral MCA territory – a= not involving and b=involving part of basal ganglia
6=infarct of the whole of peripheral MCA territory
7=6+infarct of lateral part of basal ganglia
8=infarct of whole of MCA territory



Lacunar/Borderzone sub-territory codes
9=lacune in internal capsule/lentiform
10=lacune in internal border zone
11=lacune in centrum semiovale
12=lacune in thalamus
13=lacune in brainstem, inc. pons (not shown)
14=anterior (mainly) border zone
15=posterior (mainly) border zone

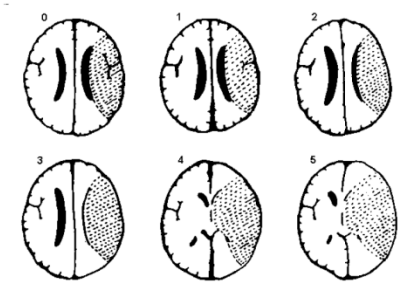


Cerebellum sub-territory codes
16=small cortical (not shown)
17=<1/2 hemisphere (medium) (not shown)
18=>1/2 hemisphere (not shown)

Brainstem sub-territory codes
11=small, i.e.<1/2 medulla (not shown)
12=extensive, i.e. pons + medulla (not shown)

c) degree of mass effect on plain CT 1° ☐ 2° ☐

Mass effect grading
0=no swelling
1=effacement of the sulci overlying the infarct
2=1+minor effacement of adjacent lateral ventricle
3=1+complete effacement of lateral ventricle
4=1+effacement of the lateral and third ventricle
5=4+shift of the midline away from the side of the ventricle
6=5+effacement of the basal cisterns



Note this form is based on an adaptation of the scan reading for developed by and copyright © J Wardlaw, University of Edinburgh, for IST-3 and related projects. This form should not be used in other projects without permission. 3

IST-3 Perfusion-angiography study CT Reading form 20120225

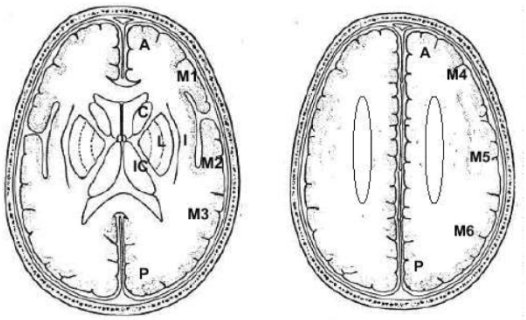
6. ASPECT Score lesion:

Please enter 'U' for unscoreable areas*, '0' for normal areas, '1' for ⇓flow areas, '2' for ⇑ flow areas

	Plain CT		CBFr	CBVr	MTTr	TTPr
	Swelling	Hypoattenuation				
N/A	☐	☐	☐	☐	☐	☐
Caudate (C)	☐	☐	☐	☐	☐	☐
Lentiform (L)	☐	☐	☐	☐	☐	☐
Insula (I)	☐	☐	☐	☐	☐	☐
Internal Capsule (IC)	☐	☐	☐	☐	☐	☐
MCA1 (M1)	☐	☐	☐	☐	☐	☐
MCA2 (M2)	☐	☐	☐	☐	☐	☐
MCA3 (M3)	☐	☐	☐	☐	☐	☐
MCA4 (M4)	☐	☐	☐	☐	☐	☐
MCA5 (M5)	☐	☐	☐	☐	☐	☐
MCA6 (M6)	☐	☐	☐	☐	☐	☐
A	☐	☐	☐	☐	☐	☐
P	☐	☐	☐	☐	☐	☐

***'unscoreable' = areas not included on CTP**

Diagrams and score taken from Lancet 2000;355:1670-1674



IST-3 Perfusion-angiography study CT Reading form 20120225

6 continued – additional PWI parameter scores

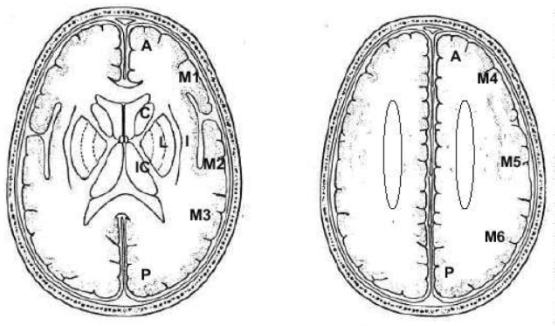
6. ASPECT Score lesion:

Please enter 'U' for unscoreable areas*, '0' for normal areas, '1' for ⇓flow areas, '2' for ⇑ flow areas

	ATFr	FWHMr	PTFr	Cmaxr	CBFq	CBVq	MTTq	Tmaxq	Raw data
N/A	☐	☐	☐	☐	☐	☐	☐	☐	☐
Caudate (C)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lentiform (L)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Insula (I)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Internal Capsule (IC)	☐	☐	☐	☐	☐	☐	☐	☐	☐
MCA1 (M1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
MCA2 (M2)	☐	☐	☐	☐	☐	☐	☐	☐	☐
MCA3 (M3)	☐	☐	☐	☐	☐	☐	☐	☐	☐
MCA4 (M4)	☐	☐	☐	☐	☐	☐	☐	☐	☐
MCA5 (M5)	☐	☐	☐	☐	☐	☐	☐	☐	☐
MCA6 (M6)	☐	☐	☐	☐	☐	☐	☐	☐	☐
A	☐	☐	☐	☐	☐	☐	☐	☐	☐
P	☐	☐	☐	☐	☐	☐	☐	☐	☐

***'unscoreable' = areas not included on CTP**

Diagrams and score taken from Lancet 2000;355:1670-1674



IST-3 Perfusion-angiography study CT Reading form 20120225

7. CT hyperattenuated/Abnormal Vessel Sign

a) Is there a hyperattenuated artery on plain CT — **Y** ☐ **N** ☐

b) Is there an abnormal/occluded artery on CTA? — **Y** ☐ **N** ☐

c) Name abnormal artery. If 'Y' to either a) or b), indicate which artery(ies). List most important (largest) abnormal artery first (1) and least important (smallest) last (3) if more than one.

1. ☐ **2.** ☐ **3.** ☐

1) ICA
2) MCA main stem
3) MCA Sylvian branch
4) PCA
5) ACA
6) BA
7) VA
8) 1+2+3
9) 1+2
10) 2+3
11) 6+7
12) other

8. If abnormal artery on CTA, indicate the degree of obstruction:

a) TICI score for abnormal artery: ☐
TIMI: NEJM 1985;312:932-6
TICI: Stroke 203;34:e109
Reviewed in Stroke 2005;36:2400-3

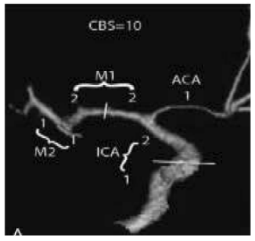
Grade	Criteria on arteriography
0	No flow/patency
1	Minimal flow/patency
2a	Partial flow/patency of <50% of expected territory
2b	Partial flow/patency of >50% of expected territory
3	Complete flow/patency

b) Score for abnormal artery ☐

Grade	Criteria on arteriography
0	No patency
1	Minimal patency – some contrast penetrates obstruction but no/minimal enters distal artery
2	Patency of <50% of the lumen and some filling of branches of the affected artery
3	Patency of >50% of the lumen and filling of most branches of the affected artery
4	Complete patency – normal artery

c) Clot Burden Score ☐

AJNR 2009;30:525-31
(Fig 1)

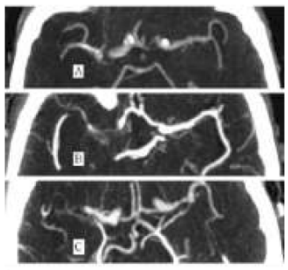


From a total score for normal arteries of 10, two points are subtracted for thrombus found on CTA in the supraclinoid ICA and each of the proximal and distal halves of the MCA trunk. One point is subtracted for thrombus found in the infraclinoid ICA and A1 segment and for each affected M2 branch.

In occluded ICA/MCA ONLY:

d) Score for Collateral Status ☐

Brain 2009;132:2231-2238
(Fig 2)



A 'Good' = the entire MCA distal to the occluded segment reconstituted with contrast.
B "Moderate" = if some of the MCA branches reconstituted within the Sylvian fissure.
C "Poor" = if only the distal superficial MCA branches reconstituted with contrast.

Figures in 8 c and d were extracted from the respective citations.

IST-3 Perfusion-angiography study CT Reading form 20120225

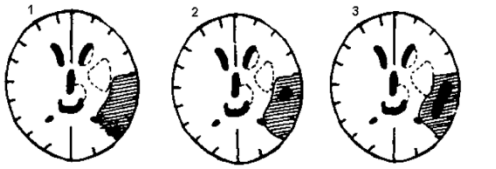
9. Haemorrhagic Changes *

	Y	N	
Is there any haemorrhage anywhere?	☐	☐	***If No go to Q.11***

10. Classify haemorrhage (if more than one haemorrhage, tick all present – indicate order of significance) :

	Y	N	**Order** (insert 1 (most important), 2, 3 (least important) to indicate your estimate of the order of clinical importance)	**Size of Haematoma** (tick box for max diam.): <3cm	3-5cm	5-8cm	>8cm
a) petechial haemorrhage (example 1 or 2 below)	☐	☐	☐	☐	☐	☐	☐
b) significant haemorrhagic transformation of infarct (i.e. underlying infarct still visible) (example 3 below)	☐	☐	☐	☐	☐	☐	☐
c) parenchymal haematoma (i.e. no infarct visible)	☐	☐	☐	☐	☐	☐	☐
d) parenchymal haematoma clearly remote from infarct	☐	☐	☐	☐	☐	☐	☐
e) subdural haematoma	☐	☐	☐	☐	☐	☐	☐
f) subarachnoid haemorrhage	☐	☐	☐				
g) extradural haemorrhage	☐	☐	☐	☐	☐	☐	☐

i) In your opinion, is the haemorrhage a major component of the infarct which is likely to have worsened mass effect or involved more brain in the damage present and so worsened symptoms, or if remote from the infarct, likely to have contributed significantly to the burden of brain damage? ☐ ☐



Haematoma with no or only slight mass effect

Haematoma with definite mass effect compressing

Note this form is based on an adaptation of the scan reading for developed by and copyright © J Wardlaw, University of Edinburgh, for IST-3 and related projects. This form should not be used in other projects without permission.

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IST-3 Perfusion-angiography study CT Reading form 20120225

11. Reduction in brain tissue volume

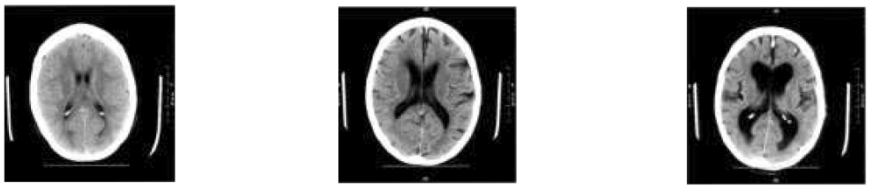
Is there any reduction in brain tissue volume? **Y** ☐ **N** ☐ *If No go to Q.13*

12. Classify atrophy (see examples and pick nearest likeness):

Central **None** ☐ **Mod** ☐ **Severe** ☐

CENTRAL reduction in brain tissue volume

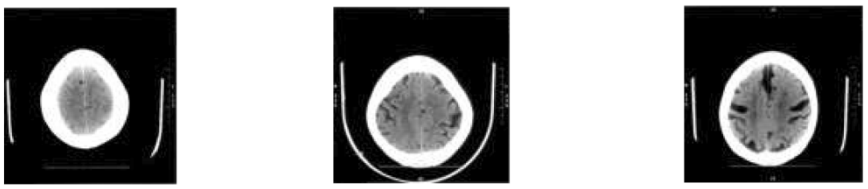
None **Modest** **Severe**



Cortical **None** ☐ **Mod** ☐ **Severe** ☐

CORTICAL reduction in brain tissue

None **Modest** **Severe**



Approach validated in Eur Radiol 2008;19:177-183

Note this form is based on an adaptation of the scan reading for developed by and copyright © J Wardlaw, University of Edinburgh, for IST-3 and related projects. This form should not be used in other projects without permission. 8

IST-3 Perfusion-angiography study CT Reading form 20120225

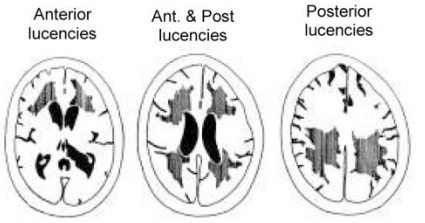
PERIVENTRICULAR LUCENCIES

13. Are there any periventricular lucencies? Y ☐ N ☐ ***If No go to Q.15***

14. Classify extent of white matter lucency

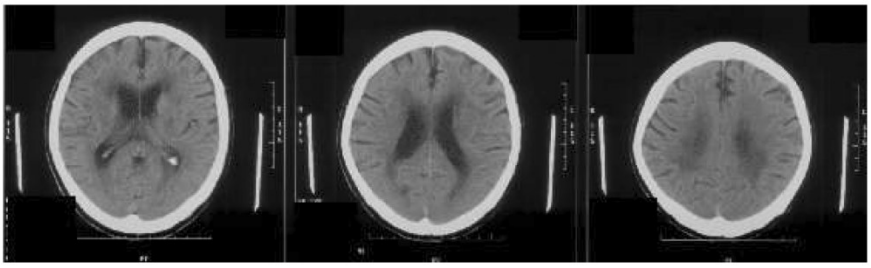
a. Anterior white matter **0,1,2** ☐
0= no lucency
1= lucency restricted to region adjoining ventricles
2= lucency covering entire region from lateral ventricle to cortex

b. Posterior white matter **0,1,2** ☐
0= no lucency
1= lucency restricted to region adjoining ventricles
2= lucency covering entire region from lateral ventricle to cortex

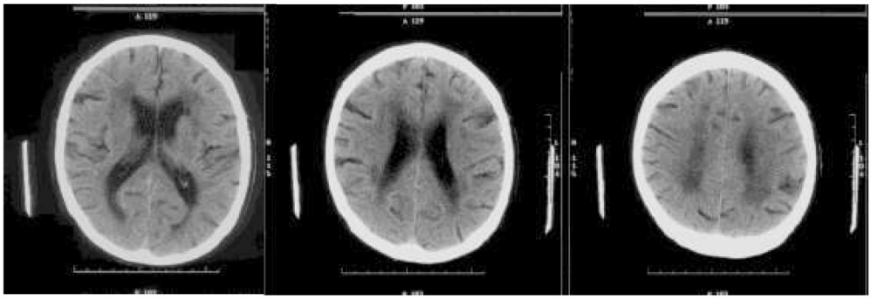


(diagram from van Swieten et al. JNNP 1990;53:1080-1083)

AWM = 1 PWM = 0



AWM = 2 PWM = 1



IST-3 Perfusion-angiography study CT Reading form 20120225

OLD VASCULAR LESIONS

		Y	N	
15.	Are there any old vascular lesions?	☐	☐	***If No go to Q.17***
16.	Classify old vascular lesion(s):	**Y**	**N**	
	a) old cortical infarct(s)	☐	☐	
	b) old striatocapsular infarct(s)	☐	☐	
	c) old borderzone infarct(s)	☐	☐	
	d) old lacunar infarct(s)	☐	☐	
	e) old brainstem/cerebellar infarct(s)	☐	☐	
	f) probable old haemorrhage	☐	☐	

NON-STROKE LESIONS

		Y	N	
17.	Is there a non-stroke lesion, which could have accounted for the patient's stroke syndrome?	☐	☐	***If No go to Q.19***
18.	Classify non-stroke lesion:	**Y**	**N**	
	a) cerebral tumour	☐	☐	
	b) encephalitis	☐	☐	
	c) cerebral abscess	☐	☐	
	d) other (e.g. contusion)	☐	☐	**Specify Other:** ☐

19. COMMENT:

11.4.2 Appendix 10 - MRI rating form for IST-3

The following form was developed by the IST-3 Chief Investigators, but primarily Joanna Wardlaw, and was used for assessing all MR brain imaging collected for patients included in the angiography and perfusion imaging substudies of IST-3.

The questions on this form were replicated exactly within the SIRS-2 imaging analysis platform.

IST-3 Perfusion-angiography study MR Reading form 20120630

MR image interpretation form

PATIENT ID:

DATE OF READING: **DATE OF SCAN:**

SCAN QUALITY: Good ☐ Moderate ☐ Poor ☐ **Comment:**

READER ID:

TYPE OF SCAN: (tick all the apply)
Diffusion: ☐ **Perfusion:** ☐ **MRA:** ☐
GRE/T2*: ☐ **T2/FLAIR:** ☐

TYPE OF PERFUSION AVAILABLE:
MTT: ☐ **CBV:** ☐ **TMAX:** ☐
CBF: ☐ **TTP:** ☐ **Other:** ☐

Please tick Yes or No. Please do not leave blanks. Thank you.

1.	Are all the scan sequences completely normal?	**Y** ☐	**N** ☐	***If YES stop here***
2.	**Ischaemic Changes** Is there any sign of acute ischaemic change on any sequence? If in doubt as to whether acute or old, code as acute.	**Y** ☐	**N** ☐	***If No go to Q.7***
3.	Which side of the brain shows ischaemic change?	**R** ☐	**L** ☐	***Tick R and L if both***
4.	Classify ischaemic change on DWI, T2/FLAIR.			
	a) Faint hyperintensity on DWI but no lesion visible on T2/FLAIR.	☐	☐	
	b) Bright hyperintensity on DWI but no/pale lesion visible on T2/FLAIR.	☐	☐	
	c) Lesion clearly visible on T2/FLAIR as well as on DWI.	☐	☐	
	d) Lesion visible on T2/FLAIR only.	☐	☐	

 1

IST-3 Perfusion-angiography study MR Reading form 20120630

e) PWI lesion visible. (tick one box for each row that applies). The 20% refers to volume.		⇑ or ⇓	N	<20%<DWI	Same as DWI	>20%>DWI
	CBFr	☐	☐	☐	☐	☐
	CBVr	☐	☐	☐	☐	☐
	MTTr	☐	☐	☐	☐	☐
	TTPr	☐	☐	☐	☐	☐
	ATFr	☐	☐	☐	☐	☐
	FWHMr	☐	☐	☐	☐	☐
	PTFr	☐	☐	☐	☐	☐
	Cmaxr	☐	☐	☐	☐	☐
	CBFq	☐	☐	☐	☐	☐
	CBVq	☐	☐	☐	☐	☐
	MTTq	☐	☐	☐	☐	☐
	Tmaxq	☐	☐	☐	☐	☐
	Raw data	☐	☐	☐	☐	☐
Other		☐	☐	☐	☐	☐

IST-3 Perfusion-angiography study MR Reading form 20120630

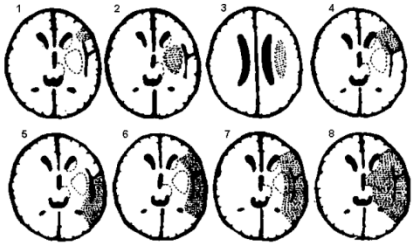
5. Classify site and size of ischaemic lesion on DWI **(see examples)**

a) site (enter most appropriate code in box) **1°** ☐ **2°** ☐

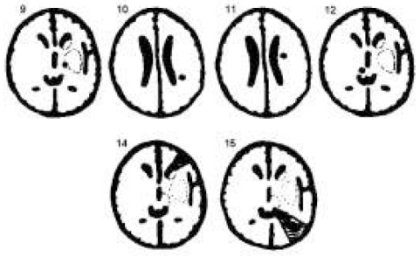
M =MCA* = any lesion in the MCA territory
AS =Infarct of up to half of ACA territory
AL =Infarct of more than half of ACA territory
PS =Infarct of up to half of PCA territory
PL =Infarct of more than half of PCA territory
MAS=M+AS*
MAL=M+AL*
MPS=M+PS*
MPL=M+PL*
MAP=Infarct of whole MCA, ACA and PCA territories
L =Lacune*
B =Borderzone*
C =Cerebellum*
S =Brainstem*
CS =Cerebellum and brainstem

* code sub-territory sites in b

b) sub-territory sites **1°** ☐ **2°** ☐



MCA sub-territory codes
1=small cortical infarct
2=basal ganglia infarct (>2x2x2cm) - striatocapsular
3=striatocapsular infarct lateral to the lateral ventricle (>2x2x2cm)
4=infarct of anterior half of peripheral MCA territory – a=not involving and b=involving part of basal ganglia
5=infarct of the posterior half of peripheral MCA territory – a= not involving and b=involving part of basal ganglia
6=infarct of the most or whole of peripheral MCA territory not including basal ganglia
7=6+infarct of lateral part of basal ganglia
8=infarct of whole of MCA territory

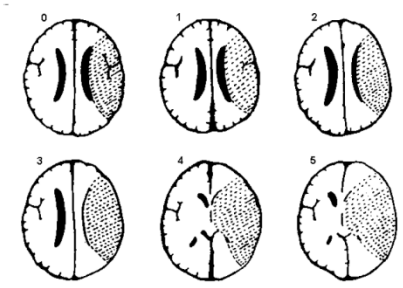


Lacunar/Borderzone sub-territory codes
9=lacune in internal capsule/lentiform
10=lacune in internal border zone
11=lacune in centrum semiovale
12=lacune in thalamus
13=lacune in brainstem, inc. pons (not shown)
14=anterior (mainly) border zone
15=posterior (mainly) border zone

Cerebellum sub-territory codes
16=small cortical (not shown)
17=<1/2 hemisphere (medium) (not shown)
18=>1/2 hemisphere (not shown)

Brainstem sub-territory codes
11=small, i.e.<1/2 medulla (not shown)
12=extensive, i.e. pons + medulla (not shown)

c) degree of mass effect on DWI/T2/FLAIR **1°** ☐ **2°** ☐



Mass effect grading
0=no swelling
1=effacement of the sulci overlying the infarct
2=1+minor effacement of adjacent lateral ventricle
3=1+complete effacement of lateral ventricle
4=1+effacement of the lateral and third ventricle
5=4+shift of the midline away from the side of the ventricle
6=5+effacement of the basal cisterns

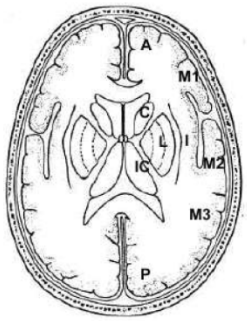
IST-3 Perfusion-angiography study MR Reading form 20120630

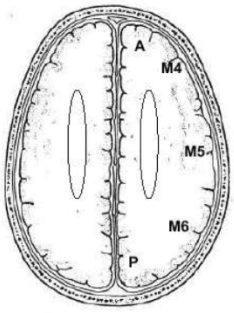
6. ASPECT Score lesion:

Please enter 'U' for unscoreable areas*, '0' for normal areas, '1' for ⇓flow areas, '2' for ⇑ flow areas

	DWI		CBFr	CBVr	MTTr	TTPr
	Signal	Swelling				
N/A	☐	☐	☐	☐	☐	☐
Caudate (C)	☐	☐	☐	☐	☐	☐
Lentiform (L)	☐	☐	☐	☐	☐	☐
Insula (I)	☐	☐	☐	☐	☐	☐
Internal Capsule (IC)	☐	☐	☐	☐	☐	☐
MCA1 (M1)	☐	☐	☐	☐	☐	☐
MCA2 (M2)	☐	☐	☐	☐	☐	☐
MCA3 (M3)	☐	☐	☐	☐	☐	☐
MCA4 (M4)	☐	☐	☐	☐	☐	☐
MCA5 (M5)	☐	☐	☐	☐	☐	☐
MCA6 (M6)	☐	☐	☐	☐	☐	☐
A	☐	☐	☐	☐	☐	☐
P	☐	☐	☐	☐	☐	☐

***'unscoreable' = areas not included on imaging acquisition**
Diagrams and score taken from Lancet 2000;355:1670-1674





IST-3 Perfusion-angiography study MR Reading form 20120630

6 continued – additional PWI parameter scores

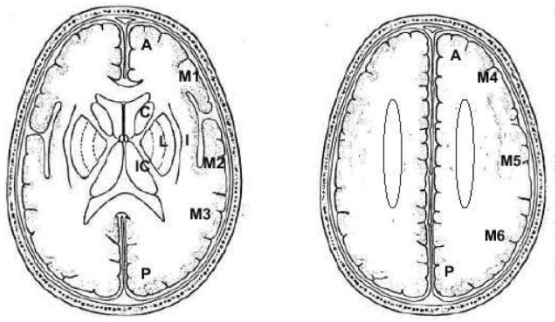
6. ASPECT Score lesion:

Please enter 'U' for unscoreable areas*, '0' for normal areas, '1' for ⇓flow areas, '2' for ⇑ flow areas

	ATFr	FWHMr	PTFr	Cmaxr	CBFq	CBVq	MTTq	Tmaxq	Raw data
N/A	☐	☐	☐	☐	☐	☐	☐	☐	☐
Caudate (C)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lentiform (L)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Insula (I)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Internal Capsule (IC)	☐	☐	☐	☐	☐	☐	☐	☐	☐
MCA1 (M1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
MCA2 (M2)	☐	☐	☐	☐	☐	☐	☐	☐	☐
MCA3 (M3)	☐	☐	☐	☐	☐	☐	☐	☐	☐
MCA4 (M4)	☐	☐	☐	☐	☐	☐	☐	☐	☐
MCA5 (M5)	☐	☐	☐	☐	☐	☐	☐	☐	☐
MCA6 (M6)	☐	☐	☐	☐	☐	☐	☐	☐	☐
A	☐	☐	☐	☐	☐	☐	☐	☐	☐
P	☐	☐	☐	☐	☐	☐	☐	☐	☐

***'unscoreable' = areas not included**

Diagrams and score taken from Lancet 2000;355:1670-1674



IST-3 Perfusion-angiography study MR Reading form 20120630

7. MR absent flow void/Abnormal Vessel Sign

a) Is there a hyperintense artery (ie acutely occluded) on FLAIR/T2/T2*?	**Y** ☐	**N** ☐
b) Is there an abnormal/occluded artery on MRA?	**Y** ☐	**N** ☐

c) Name abnormal artery. If 'Y' to either a) or b), indicate which artery(ies). List most important (largest) abnormal artery first (1) and least important (smallest) last (3) if more than one.

1. ☐ **2.** ☐ **3.** ☐

1) ICA 2) MCA main stem 3) MCA Sylvian branch 4) PCA 5) ACA 6) BA 7) VA 8) 1+2+3 9) 1+2 10) 2+3 11) 6+7 12) other

8. If abnormal artery on MRA, indicate the degree of obstruction:

a) TICI score for abnormal artery: ☐
TIMI: NEJM 1985;312:932-6
TICI:Stroke 203;34:e109
Reviewed in Stroke 2005;36:2400-3

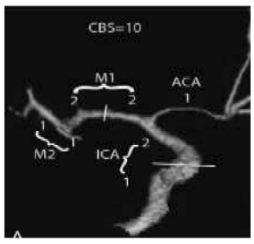
Grade	Criteria on arteriography
0	No flow/patency
1	Minimal flow/patency
2a	Partial flow/patency of <50% of expected territory
2b	Partial flow/patency of >50% of expected territory
3	Complete flow/patency

b) Score for abnormal artery ☐

Grade	Criteria on arteriography
0	No patency
1	Minimal patency – some contrast penetrates obstruction but no/minimal enters distal artery
2 a or b	Patency of <50% of the lumen at the point of obstruction and a) only partly filling (<½) or b) incomplete filling but ≥ ½ of the major branches of the affected artery;
3	Patency of >50% of the lumen and filling of most branches of the affected artery
4	Complete patency – normal artery

c) Clot Burden Score ☐

AJNR 2009;30:525-31
(Fig 1)

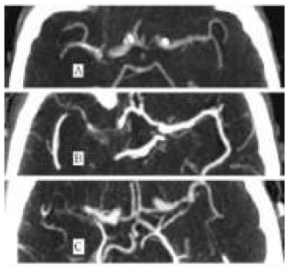


From a total score for normal arteries of 10, two points are subtracted for thrombus found on CTA in the supraclinoid ICA and each of the proximal and distal halves of the MCA trunk. One point is subtracted for thrombus found in the infraclinoid ICA and A1 segment and for each affected M2 branch.

In occluded ICA/MCA ONLY:

d) Score for Collateral Status ☐

Brain 2009;132:2231-2238
(Fig 2)



A 'Good' = the entire MCA distal to the occluded segment reconstituted with contrast.
B "Moderate" = if some of the MCA branches reconstituted within the Sylvian fissure.
C "Poor" = if only the distal superficial MCA branches reconstituted with contrast.

Figures in 8 c and d were extracted from the respective citations.

 6

IST-3 Perfusion-angiography study MR Reading form 20120630

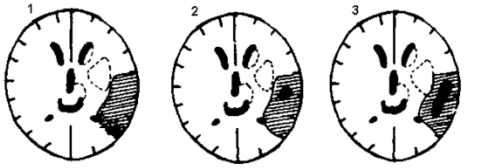
9. Haemorrhagic Changes On GRE/T2*

	Y	N	
Is there any haemorrhage anywhere?	☐	☐	***If No go to Q.11***

10. Classify haemorrhage (if more than one haemorrhage, tick all present – indicate order of significance) :

	Y	N	**Order** (insert 1 (most important), 2, 3 (least important) to indicate your estimate of the order of clinical importance)	**Size of Haematoma** (tick box for max diam.): <3cm	3-5cm	5-8cm	>8cm
a) petechial haemorrhage (example 1 or 2 below)	☐	☐	☐	☐	☐	☐	☐
b) significant haemorrhagic transformation of infarct (i.e. underlying infarct still visible) (example 3 below)	☐	☐	☐	☐	☐	☐	☐
c) parenchymal haematoma (i.e. no infarct visible)	☐	☐	☐	☐	☐	☐	☐
d) parenchymal haematoma clearly remote from infarct	☐	☐	☐	☐	☐	☐	☐
e) subdural haematoma	☐	☐	☐	☐	☐	☐	☐
f) subarachnoid haemorrhage	☐	☐	☐				
g) extradural haemorrhage	☐	☐	☐	☐	☐	☐	☐

i) In your opinion, is the haemorrhage a major component of the infarct which is likely to have worsened mass effect or involved more brain in the damage present and so worsened symptoms, or if remote from the infarct, likely to have contributed significantly to the burden of brain damage? ☐ ☐



Haematoma with no or only slight mass effect

Haematoma with definite mass effect compressing

j) Are there any microhaemorrhages? ☐ ☐

If yes, number of microhaemorrhages: ☐

 7

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11. Reduction in brain tissue volume on T2/FLAIR

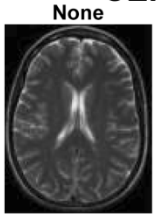
Is there any reduction in brain tissue volume? Y ☐ N ☐ *If No go to Q.13*

12. Classify atrophy (see examples and pick nearest likeness):

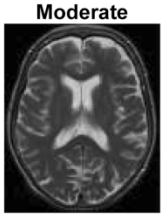
Central None ☐ Mod ☐ Severe ☐

CENTRAL reduction in brain tissue volume

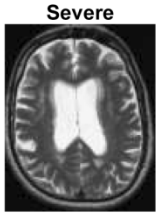
None



Moderate



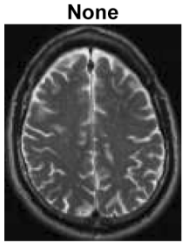
Severe



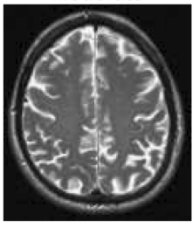
Cortical None ☐ Mod ☐ Severe ☐

CORTICAL reduction in brain tissue

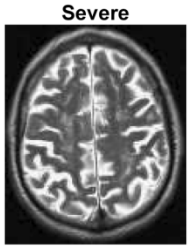
None



Moderate



Severe



Approach validated in Eur Radiol 2008;19:177-183

Note this form is based on an adaptation of the scan reading for developed by and copyright © J Wardlaw, University of Edinburgh, for IST-3 and related projects. This form should not be used in other projects without permission. 8

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13. Periventricular Hyperintensities

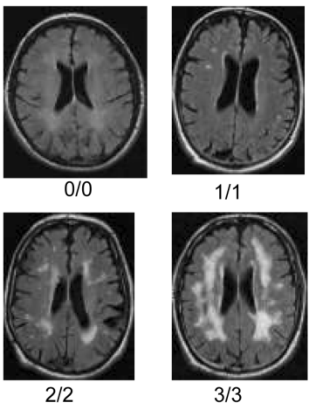
	Y	N
Are there any periventricular hyperintensities?	☐	☐

14. Classify extent of white matter hyperintensity

Fazekas et al (1987) MR signal abnormalities at 1.5T in Alzheimer's disease and normal aging. AJNR, 8:421-426.

a) Periventricular white matter **0,1,2,3** ☐

b) Deep white matter **0,1,2,3** ☐



0/0 1/1 2/2 3/3

PVH/DWMH ratings

15. Old Vascular Lesions

	Y	N
Are there any old vascular lesions?	☐	☐

16. Classify old vascular lesion(s):

	Y	N
a) old cortical infarct(s)	☐	☐
b) old striatocapsular infarct(s)	☐	☐
c) old borderzone infarct(s)	☐	☐
d) old lacunar infarct(s)	☐	☐
e) old brainstem/cerebellar infarct(s)	☐	☐
f) probable old haemorrhage	☐	☐

17. Is there a non-stroke lesion which could have accounted for the patient's stroke syndrome? Y ☐ N ☐

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18. Classify non-stroke lesion:

	Y	N	
a) cerebral tumour	☐	☐	
b) encephalitis	☐	☐	
c) cerebral abscess	☐	☐	
g) other (e.g.	☐	☐	**Specify Other:** ☐

19. COMMENT:

11.4.3 Appendix 11 - Spreadsheet for HAS and angiography assessment

This spreadsheet was developed by me and was derived from the pre-validated IST-3 CT rating form (see Appendix 9) but additionally includes sections for recording intra-arterial attenuation measurements.

Patient/ Scan Details	Patient ID	
	Date & time of scan	
	Date of reading	
	Scan quality	
	Normal or abnormal?	
Ischaemia/ Infarct	Acute ischaemia?	
	Side of affected brain	
	IST-3 ischaemia score	
	Mass effect	
	ASPECTS	
	Old infarcts?	
	IST-3 ischaemia score for old infarcts	
Hyperdense Artery Sign (HAS)	Present?	
	Location	
	Length	
Intra-arterial attenuation measures (1)	Basilar 1	
	Basilar 2	
	Basilar 3	
	Left MCA 1	
	Left MCA 2	
	Left MCA 3	

	Right MCA 1	
	Right MCA 2	
	Right MCA 3	
Angiography	CTA or MRA?	
	Abnormality on angio?	
	Matching HAS location?	
	Largest artery affected	
	Middle artery	
	Smallest artery	
	TICI score	
	IST-3 angiography score	
	Clot Burden Score	
	Collateral status	
Intra-arterial attenuation measures (2) – if CTA abnormal but no HAS	Measure 1	
	Measure 2	
	Measure 3	
Follow-up scans As above, plus:	Time interval	
	Clearance of HAS?	
	Haemorrhage?	
	Size of haemorrhage	
	Type of haemorrhage	
	Haemorrhage likely affected outcome?	

Footnote: ASPECTS = Alberta Stroke Program Early CT Score. MCA = middle cerebral artery. TICI = Thrombolysis in Cerebral Infarct. All variables were scored as per IST-3 image rating forms (see Appendices 9 & 10, Chapters 11.4.1 and 11.4.2).

11.4.4 Appendix 12 – Spreadsheet for HAS sensitivity and specificity meta-analysis

This spreadsheet was developed by me, Joanna Wardlaw and Elena Boyd based on the inclusion criteria and main areas of interest in the systematic review of HAS sensitivity and specificity.

Article	Author	
	Journal	
	Year	
	Reference	
Imaging	CT slice thickness	
	Angio type	
Totals	Angio total (n)	
	HAS total (n)	
	Abnormal angio (n)	
HAS Accuracy	True Positive	
	True Negative	
	False Positive	
	False Negative	
HAS Location (n/total occlusions)	Proximal	
	Distal	
HAS Extent (n/total occlusions)	1 segment	
	2 or more segments	
Time from onset (n HAS/total for given time frame)		
Notes		

NOTES FOR COMPLETION

CT SLICE THICKNESS - should relate to non-contrast scan on which hyperdense arteries were assessed

ANGIO TYPE - CTA, MRA or catheter

ANGIO TOTAL - all patients in article who had angiography

HYPERDENSE TOTAL - all patients in article who had a hyperdense artery (in any intracranial location - i.e. group MCA mainstem and sylvian branches into one total)

ABNORMAL ANGIO - i.e. 'occlusion', if abnormality is graded include all those with any luminal narrowing - e.g. group TICI 0,1,2a and 2b

TRUE POS - from the total angiography group, number who had hyperdense artery and angiographic abnormality

TRUE NEG - number who had neither hyperdense artery nor angiographic abnormality

FALSE POS - hyperdense artery with normal angiography

FALSE NEG - no hyperdense artery but angiographic abnormality

HYPERDENSE LOCATION - number of hyperdense arteries/number of occlusions in proximal (ICA, MCA mainstem, vertebral arteries, basilar) and distal (ACA, MCA sylvian branch, PCA) vessels

HYPERDENSE EXTENT - number of hyperdense arteries/number of occlusions where 1 or 2+ segments (named vessels as for location) are involved

TIME FROM ONSET - number of hyperdense arteries/number of occlusions within a given time frame. May need to provide 2 counts, i.e. for those more and those less than a given time point (e.g. <180min, >180mins).

11.5 Posters Presented at the European Stroke Meeting in London 28-31 May 2013

11.5.1 Appendix 13 – Poster 1

Combining CT angiography with non-contrast CT to predict infarct on follow-up imaging in acute ischaemic stroke

Substudy analysis of imaging from the Third International Stroke Trial (IST-3)

Grant Mair, Joanna M Wardlaw, Peter Sandercock, Richard Lindley, Ruediger von Kummer and Andrew J Farrell

The IST-3 Collaborative Group and The University of Edinburgh, UK

Background

- IST-3 is a multicentre, randomised controlled trial testing IV thrombolysis within 6 hours of ischaemic stroke[1]
- Pre-randomisation and follow-up brain imaging were performed for all patients and CT angiography (CTA) was additionally obtained in some centres
- This analysis aimed to identify if CTA better predicted infarct on follow-up imaging over non-contrast CT alone

Methods

- We included IST-3 patients who had pre-randomisation non-contrast CT (PRCT) and CTA and follow-up imaging (CT or MRI, 24-48 hours after stroke)
- A single observer (GM) analysed the images sequentially, blinded to subsequent imaging
- We recorded ischaemia (IST-3 score) and hyper-attenuated arteries (HAS) on PRCT, and arterial obstruction on CTA
- We calculated the sensitivity and specificity of abnormal PRCT +/- abnormal CTA for predicting infarction (IST-3 score) on follow-up imaging

Results

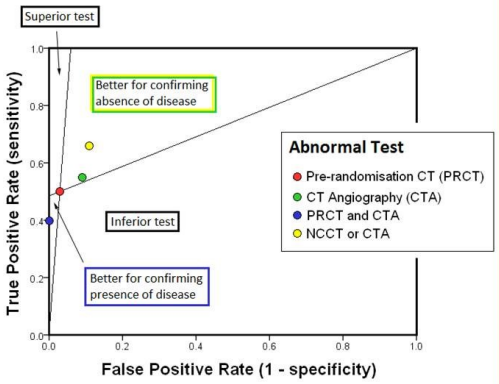
- We included 271 patients
  - 44% male, median age 81 years (IQR 71-85)
- PRCT was performed at median 170 minutes from stroke onset (IQR 110-252)
- Follow-up imaging was performed within 48 hours for 92%
- PRCT was abnormal (acute ischaemia or HAS) in 36% (95%CI 30-42%)
- CTA was abnormal in 42% (95%CI 36-48%)
- Either PRCT or CTA were abnormal in 50% (95%CI 44-56%)
- Both PRCT and CTA were abnormal in 28% (95%CI 23-33%)
- Sensitivity and specificity of these imaging combinations for predicting infarct are compared in Table 1 and Figure 1

Table 1. Sensitivity and specificity of various pre-randomisation imaging combinations for predicting infarct on follow-up imaging

Imaging identified as abnormal	Sensitivity	Specificity
Pre-randomisation CT (PRCT) alone	50%	97%
CT angiography (CTA) alone	55%	91%
Either PRCT or CTA*	66%	89%
Both PRCT and CTA*	40%	100%

* Significantly different from PRCT alone, p<0.001

Figure 1. The sensitivity and specificity of an abnormal pre-randomisation CT (PRCT) in predicting infarct on follow-up imaging is plotted in red. The slope of the connecting lines represent likelihood ratios for this test[2]. The addition of CT angiography to PRCT in the acute setting improves either sensitivity or specificity but not both



Conclusion

- Combining CTA with PRCT in acute stroke significantly increases sensitivity (if either PRCT or CTA are abnormal) or specificity (if both are abnormal) for predicting infarct on follow-up imaging

References:
1. The IST-3 collaborative group. The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. Lancet 2012; 379: 2352-2363
2. BJ Biggerstaff. Comparing diagnostic tests: a simple graphic using likelihood ratios. Statistics in Medicine 2000;19:649-663

IST-3 was generously supported by:

11.5.2 Appendix 14 – Poster 2

Association of non-contrast CT and CT angiography with baseline clinical deficit and functional outcome

Substudy analysis of imaging from the Third International Stroke Trial (IST-3)

Grant Mair, Joanna M Wardlaw, Peter Sandercock, Richard Lindley, Ruediger von Kummer and Andrew J Farrell

The IST-3 Collaborative Group and The University of Edinburgh, UK

Background

- IST-3 is a multicentre, randomised controlled trial testing IV thrombolysis within 6 hours of ischaemic stroke[1]
- Pre-randomisation and follow up brain imaging were performed for all patients and CT angiography (CTA) was additionally obtained in some centres
- This analysis aimed to identify if the additional CTA better predicted acute clinical deficit and late functional outcome over non-contrast CT alone

Methods

- We included IST-3 patients who had pre-randomisation non-contrast CT (PRCT) and CTA
- A single observer (GM) analysed the images sequentially, blinded to subsequent imaging and clinical findings
- We recorded ischaemia (IST-3 score) and hyper-attenuated arteries (HAS) on PRCT, and arterial obstruction on CTA
- We compared abnormal PRCT with abnormal PRCT +/- abnormal CTA for baseline stroke severity (National Institutes of Health Stroke Scale – NIHSS) and clinical outcome (Oxford Handicap Scale – OHS) at 6 months

Results

- We included 271 patients:
  - 44% male, median age 81 years (IQR 71-85)
- PRCT was performed at median 170 minutes from stroke onset (IQR 110-252)
- CTA was performed within 30 minutes of PRCT for 93%
- PRCT was abnormal in 36% (95%CI 30-42%)
- CTA was abnormal in 42% (95%CI 36-48%)
- Either PRCT or CTA were abnormal in 50% (95%CI 44-56%)
- The groups, abnormal PRCT and abnormal PRCT +/- CTA, were both associated with worse NIHSS and OHS compared to those with normal scans (Table 1)
- Abnormal PRCT alone provided very similar results to abnormal PRCT +/- CTA; there was no significant difference between these groups for NIHSS or OHS scores:
  - p=0.10 in both cases
  - Figures 1 and 2 show the similarity of NIHSS

Table 1. Median NIHSS and 6 month OHS categorised by the findings on non-contrast CT and CTA

Imaging test normal/abnormal	NIHSS	OHS
Both PRCT and CTA normal	6	2
Pre-randomisation CT (PRCT) abnormal	16*	5*
Either PRCT or CTA abnormal	15*	5*

* Significantly different from those with no abnormality, p<0.001

Figure 1. Comparing NIHSS scores in those with and without abnormal PRCT

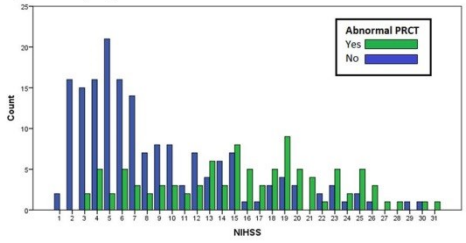
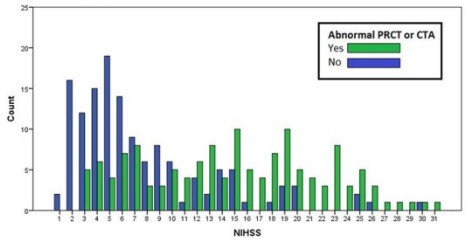


Figure 2. Comparing NIHSS scores in those with and without abnormal PRCT or CTA



Conclusion

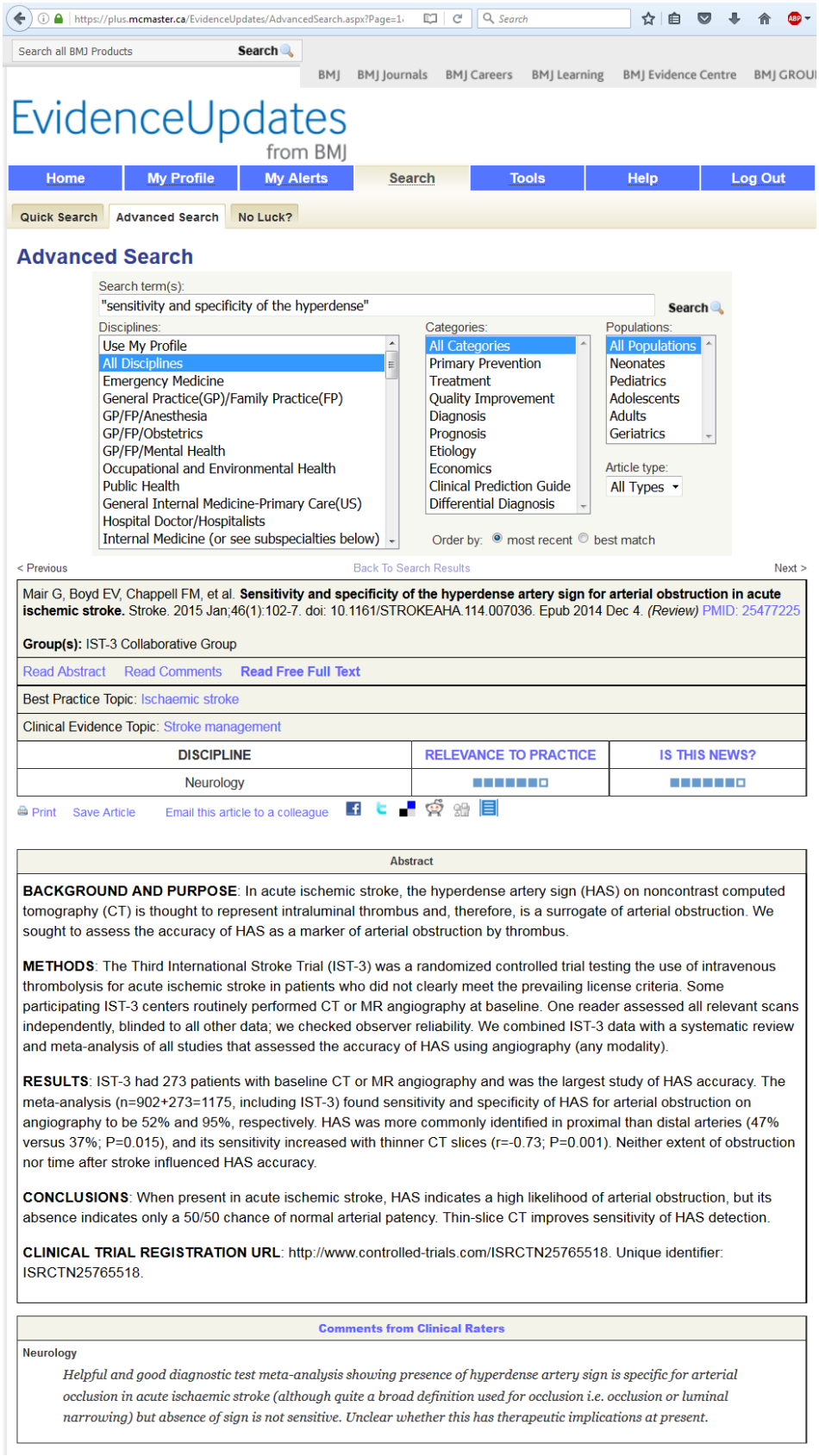
- Including CTA in the imaging assessment of acute stroke identifies more patients with abnormal scans but this information does not alter the prediction of stroke severity or functional outcome

Reference: 1. The IST-3 collaborative group. The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. Lancet 2012; 379: 2352-2363

IST-3 was generously supported by:

MRC Medical Research Council; NHS National Institute for Health Research; Regione Umbria; Dalhousie University; The Research Council of Norway; Hjärt Lungfonden; Australian Government National Health and Medical Research Council; Stroke association; The Health Foundation; Schweizerische Herzstiftung Fondation Suisse de Cardiologie Fondazione Svizzera di Cardiologia; afa Försäkring; Heart Foundation; Chest Heart & Stroke Scotland

11.6 Appendix 15 – Extract from *EvidenceUpdates*



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Mair G, Boyd EV, Chappell FM, et al. **Sensitivity and specificity of the hyperdense artery sign for arterial obstruction in acute ischemic stroke.** Stroke. 2015 Jan;46(1):102-7. doi: 10.1161/STROKEAHA.114.007036. Epub 2014 Dec 4. *(Review)* PMID: 25477225

Group(s): IST-3 Collaborative Group

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Abstract

BACKGROUND AND PURPOSE: In acute ischemic stroke, the hyperdense artery sign (HAS) on noncontrast computed tomography (CT) is thought to represent intraluminal thrombus and, therefore, is a surrogate of arterial obstruction. We sought to assess the accuracy of HAS as a marker of arterial obstruction by thrombus.

METHODS: The Third International Stroke Trial (IST-3) was a randomized controlled trial testing the use of intravenous thrombolysis for acute ischemic stroke in patients who did not clearly meet the prevailing license criteria. Some participating IST-3 centers routinely performed CT or MR angiography at baseline. One reader assessed all relevant scans independently, blinded to all other data; we checked observer reliability. We combined IST-3 data with a systematic review and meta-analysis of all studies that assessed the accuracy of HAS using angiography (any modality).

RESULTS: IST-3 had 273 patients with baseline CT or MR angiography and was the largest study of HAS accuracy. The meta-analysis (n=902+273=1175, including IST-3) found sensitivity and specificity of HAS for arterial obstruction on angiography to be 52% and 95%, respectively. HAS was more commonly identified in proximal than distal arteries (47% versus 37%; P=0.015), and its sensitivity increased with thinner CT slices (r=-0.73; P=0.001). Neither extent of obstruction nor time after stroke influenced HAS accuracy.

CONCLUSIONS: When present in acute ischemic stroke, HAS indicates a high likelihood of arterial obstruction, but its absence indicates only a 50/50 chance of normal arterial patency. Thin-slice CT improves sensitivity of HAS detection.

CLINICAL TRIAL REGISTRATION URL: http://www.controlled-trials.com/ISRCTN25765518. Unique identifier: ISRCTN25765518.

Comments from Clinical Raters

Neurology

Helpful and good diagnostic test meta-analysis showing presence of hyperdense artery sign is specific for arterial occlusion in acute ischaemic stroke (although quite a broad definition used for occlusion i.e. occlusion or luminal narrowing) but absence of sign is not sensitive. Unclear whether this has therapeutic implications at present.