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Tenecteplase and alteplase in acute ischaemic stroke thrombolysis – clinical and imaging study

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Submitted in fulfilment of the requirements for the

Degree of Doctor of Philosophy

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Abstract

Introduction

Intravenous thrombolysis in acute ischaemic stroke with alteplase improves clinical outcomes, but it has limited efficacy and is associated with increased risk of intracranial haemorrhage. An improved tissue plasminogen activator, tenecteplase, was evidenced to be at least equally effective with lower risk of haemorrhage in acute myocardial infarction thrombolysis. To date, two completed phase II randomised controlled studies comparing tenecteplase and alteplase in acute ischaemic strokes showed variable results.

Methods

A literature review of thrombolytic agents used in myocardial infarction and acute ischaemic stroke was performed, followed by a retrospective investigation of the bolus-to- infusion delay of alteplase administration.

The main focus of this thesis is the report of our single centre phase II randomised controlled trial that compared tenecteplase (0.25mg/kg, maximum 25mg) and alteplase (0.9mg/kg, maximum 90mg, 10% as the initial bolus, following by one hour infusion with the rest of the dose) in acute ischaemic stroke thrombolysis using advanced imaging as biomarkers. Imaging comprised baseline computed tomography (CT), CT perfusion (CTP) and CT angiography (CTA), and CT+CTA at 24-48 hours. The primary end-point was penumbral salvage (CTP-defined penumbra volume minus follow-up CT infarct volume).

A sub-study of coagulation and fibrinolysis analysis of the two agents was performed by comparing a group of coagulation variables measured pre-treatment, 3-12 hours, and 24±3 hours post thrombolysis.

An individual patient data (IPD) meta-analysis was carried out using all three completed tenecteplase/alteplase comparison studies in stroke thrombolysis. We compared clinical outcomes including modified Rankin scale at 3 months, early neurological improvement at 24 hours, intracerebral haemorrhage rate and mortality at 3 months between all three tenecteplase doses (0.1mg/kg, 0.25 mg/kg, and 0.4mg/kg) examined and standard

alteplase. Imaging outcomes including penumbra salvage, recanalisation rates were also compared using the data from the two studies that had advance imaging carried out.

Results

Delay between the initial bolus and the subsequent infusion in administration of alteplase is common. This may reduce the likelihood of achieving a good functional outcome.

Among the 104 patients recruited in ATTEST trial, 71 contributed to the imaging primary outcome. No significant differences were observed for penumbral salvage [68 (SD 28) % tenecteplase vs 68 (SD 23) % alteplase], mean difference 1% (95% confidence interval -10%, 12%, p=0·81) or for any secondary end-point. The SICH incidence (1/52, 2% vs 2/51, 4%, by SITS-MOST definition, p=0·55; by ECASS-2 definition, 3/52, 6% tenecteplase vs 4/51, 8% alteplase, p=0.59) did not differed significantly. There was a trend towards lower ICH risk in the tenecteplase group (8/52 tenecteplase, 15% vs 14/51 alteplase, 29%, p=0·091).

Compared to baseline, alteplase caused significant hypofibrinogenaemia (p=0.002), prolonged Prothrombin Time (PT) (p=0.011), hypoplasminogenaemia (p=0.001) and lower Factor V (p=0.002) at 3-12 hours after administration with persistent hypofibrinogenaemia at 24h (p=0.011), while only minor hypoplasminogenaemia (P=0.029) was seen in the tenecteplase group. Tenecteplase consumed less plasminogen (p<0.001) and fibrinogen (p=0.002) compared with alteplase.

In a pooled analysis, tenecteplase 0.25mg/kg had the greatest odds to achieve early neurological improvement (OR [95%CI] 3.3 [1.5, 7.2], p=0.093), excellent functional outcome (mRS 0-1) at three months (OR [95%CI] 1.9 [0.8, 4.4], p= 0.28), with reduced odds of ICH (OR [95%CI] 0.6 [0.2, 1.8], P=0.43) compared with alteplase. Only 19 patients were treated with tenecteplase 0.4mg/kg, which showed increased odds of SICH compared with alteplase (OR [95% CI] 6.2 [0.7, 56.3]). In the two studies where advanced imaging was performed, the imaging outcomes did not differ in the IPD analysis.

Conclusion

Tenecteplase 0.25 mg/kg has the potential to be a better alternative to alteplase. It can be given as a single bolus, does not cause disruption to systemic coagulation, and is

possibly safer and more effective in clot lysis. Further phase III study to compare tenecteplase and alteplase in acute ischaemic stroke is warranted.

Acknowledgements

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Author’s declaration

I declare that, except where explicit reference is made to the contribution of others, this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Signature _____

Printed name _____

Publications and Presentations

Papers

- **Alteplase versus tenecteplase for thrombolysis after ischaemic stroke (ATTEST): a phase 2, randomised, open-label, blinded endpoint study.** Huang X.; Cheripelli BK, Lloyd SM, Kalladka D, Moreton FC, Siddiqui A, Ford I, Muir KW, Lancet Neurol. 2015 Apr;14 (4):368-376
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Additional platform presentation

- **Analysis of coagulation and fibrinolytic activity of tenecteplase and alteplase in acute ischaemic stroke** Charles Warlow Prize Presentation Australian Stroke Conference 2015

Definitions/abbreviations

3D	3 Dimensional
4D	4 Dimensional
α2-AP	α2-Antiplasmin
α2-M	α2-Macroglobulin
ACA	Anterior Cerebral Artery
AE	Adverse Event
AF	Atrial Fibrillation
AIF	Arterial Input Function
AIS	Acute Ischaemic Stroke
AMPA	Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxanole Propionate
ANOVA	Analysis of Variance
Apo	Apolipoprotein
APSAC	Anisoylated Plasminogen-streptokinase Activator Complex
APTT	Activated Partial Thromboplastin Time
ASD	Atrial Septal Defect
ASL	Arterial Spin Labelling (MRI)
ASPECTS	Alberta Stroke Program Early CT Score
ASSENT	Safety Assessment of Single-bolus Administration of TNK Tissue-plasminogen Activator in Acute Myocardial Infarction

ATLANTIS	Alteplase Thrombolysis for Acute Noninterventional Therapy in Ischemic Stroke
ATP	Adenosine Triphosphate
ATTEST	Alteplase – Tenecteplase Trial Evaluation for Stroke Thrombolysis
BBB	Blood Brain Barrier
BID	Bolus-infusion Delay
BP	Blood Pressure
CAPTORS	Collaborative Angiographic Patency Trial of Recombinant Staphylokinase
CBF	Cerebral Blood Flow
CBV	Cerebral Blood Volume
CI	Confidence Interval
CLOTBUST	Combined Lysis of Thrombus in Brain ischemia Using Transcranial Ultrasound and Systemic tPA
CMRglu	Cerebral Metabolic Rate of Glucose
CMRO2	Cerebral Metabolic Rate of Oxygen
CNS	Central Nervous System
CPP	Cerebral Perfusion Pressure
CSF	Cerebro-Spinal Fluid
CT	Computed Tomography
CTA	CT Angiography
CTP	CT Perfusion
CVR	Cerebrovascular Resistance

DEFUSE Evaluation	Diffusion and Perfusion Imaging Evaluation for Understanding Stroke
DICOM	Digital Imaging and Communications in Medicine
DWI	Diffusion Weighted Imaging (MRI)
DSA	Digital Subtraction Angiography
DSPA	Desmodus Salivary Plasminogen Activator
DT	Delay Time
DUS	Doppler Ultrasound
ECASS	European Cooperative Acute Stroke Study
ECG	Electrocardiogram
EGF	Epidermal Growth Factor
eGFR	estimated Glomerular Filtration Rate
ELISA	Enzyme-Linked ImmunoSorbant Assay
ENCHANTED	Enhanced Control of Hypertension and Thrombolysis Stroke Study
EPITHET	Echoplanar Imaging Thrombolytic Evaluation Trial
EXTEND	Extending the Time for Thrombolysis in Emergency Neurological Deficits
F1+2	Prothrombin Fragment 1+2
FAST-MAG	Field Administration of Stroke Therapy – Magnesium
FDP	Firbin(ogen) Degradation Productions
FLAIR	Fluid-Attenuated Inversion Recovery imaging (MRI)
FOV	Field of View
GABA	Gamma-aminobutyric Acid

GI	Gastrointestinal
GRE	Gradient Echo (MRI)
GUSTO	Global Utilisation of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries
HI	Haemorrhagic Infarction
HMW	High-molecular-weight
HU	Hounsfield Unit
IA	Intra-arterial
ICA	Internal Carotid Artery
ICH	Intracerebral Haemorrhage
INR	International Normalised Ratio
IPD	Individual Patient Data
IQR	Interquartile Range
IST3	International Stroke Trial 3
IV	Intravenous
IVRS	Interactive Voice Response System
K1	Kringle 1
K2	Kringle 2
LMW	Low-molecular-weight
MASIS	Multicentre Acute Stroke Imaging Study
MCA	Middle Cerebral Artery
MDCT	Multi-Detector CT

MEDDRA Medical Dictionary for Regulatory Activities

MHRA Medicine and Healthcare products Regulatory Agency

MI Myocardial Infarction

MIP Maximum Intensity Projection

MRA MRI Angiography

MR CLEAN Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands

MRI Magnetic Resonance Imaging

MR RESCUE Mechanical Retrieval and Recanalization of Stroke Clots Using Embolectomy

mRS modified Rankin Scale

MR WITNESS A Study of Intravenous Thrombolysis with Alteplase in MRI-Selected Patients

MTT Mean Transit Time

NCCT Non-Contrast Computed Tomography

NIHSS National Institute of Health Stroke Scale

NINDS National Institute of Neurological Disorders and Stroke

NMDA N-methy1-D-asapartate

NNT Number Needed to Treat

NOR-TEST Study of Tenecteplase versus Alteplase for Thrombolysis in Acute Ischaemic Stroke

NVU Neurovascular Unit

OEF Oxygen Extraction Fraction

OR	Odds Ratio
OTT	Onset-to-treatment Time
PA	Plasminogen Activator
PAI-1	Plasminogen Activator Inhibitor-1
PCA	Posterior Cerebral Artery
PET	Positron Emission Tomography
PFO	Patent Foramen Ovale
PH	Parenchymal Haemorrhage
POSH	Post Stroke Hyperglycaemia
PRACTISE	Penumbra and Recanalisation Acute Computed Tomography in Ischaemic Stroke Evaluation
PROBE	Prospective Randomised Open Blinded End-point
PT	Prothrombin Time
PWI	Perfusion Weighted Imaging (MRI)
RAPID	Rapid Processing of PerfusIon and Diffusion
ROI	Region of Interest
rtPA	recombinant tissue Plasminogen Activator
SAE	Serious Adverse Events
SAH	Subarachnoid Haemorrhage
scu-PA	Single-chain Urokinase Plasminogen Activator
SD	Standard Deviation
SICH	Symptomatic Intracerebral Haemorrhage

SITS-MOST	Safe Implementation of Thrombolysis in Stroke Monitoring Study
SK	Streptokinase
SWI	Susceptibility Weighted Imaging (MRI)
SVD	Singular Value Decomposition
TAFI	Thrombin Activatable Fibrinolysis Inhibitor
TASTE	Tenecteplase versus Alteplase for Stroke Thrombolysis Evaluation trial
TAT	Thrombin-antithrombin Complex
TCD	Transcranial Doppler
TEMPO	TNK–Tissue-Type Plasminogen Activator Evaluation for Minor Ischemic Stroke with Proven Occlusion
TIA	Transient Ischaemic Attack
TICI	Thrombolysis in Cerebral Infarction
TIMI	Thrombolysis in Myocardial Infarction
Tmax	Time to Maximum
TNFα	Tumour Necrosis Factor Alpha
TNK	Tenecteplase
TOAST	Trial of ORG 10172 in Acute Stroke Treatment
TOE	Trasoesophageal Echocardiogram
TOF	Time of Flight Imaging (MRA)
TP	Time Point
tPA	Tissue Plasminogen Activator
UK	Urikinase

VOF	Venous Output Function
WAKE-UP	Efficacy and Safety of MRI-based Thrombolysis in Wake-up Stroke

Chapter 1 Introduction

Traditionally, Stroke was defined as a syndrome characterised by rapidly developing neurological deficits attributed to an acute focal injury to the central nervous system secondary to a vascular cause that lasts more than 24 hours[1], including ischaemic stroke, Intracerebral Haemorrhage (ICH) and Subarachnoid Haemorrhage (SAH). However, advances in imaging confirmed that permanent brain tissue damage can occur even when symptoms resolve fully in less than 24 hours. Driven by the development in hyperacute stroke management, an updated definition of ischaemic stroke has recently been published by the American Stroke Association, in which cerebral, spinal and retinal infarction based on imaging evidence were included, along with recognised clinical and pathology evidence[2].

1.1. Stroke epidemiology

Stroke is the second leading cause of death worldwide in the adult population, and the fourth leading cause of disease burden (as measured in disability – adjusted life years)[3]. Ischaemic stroke comprises more than 80% of all strokes in Western countries[4]. Over the past four decades, age adjusted stroke incidence rates decreased by 42% in high-income countries, whereas it more than doubled in low to middle-income countries according to a recent systematic review[5]. However, this comparison was made with a relatively small proportion of all studies included from limited geographic regions and countries at different times. As an age dependent disease, it has been predicted that the number of strokes will increase in parallel with the increasingly aged population[6].

Risk factors for stroke are divided into two categories. Old age, male gender, family history of stroke, and low birth weight are non-modifiable risk factors. Although it is well known that ICH is more frequent in China[7], and that SAH is more common in Finland and Sweden[8], no particular ethnic pattern is observed in stroke mortality globally[9]. The traditional modifiable risk factors, such as diabetes, hypertension, smoking and atrial fibrillation account for 60-80% of population attributable risk for ischaemic strokes[10]. More recently identified risk factors including waist-to-hip ratio, ratio of apolipoprotein

(Apo) B to A1, diet, physical activity, psychosocial stress and depression, along with the traditional factors, account for more than 90% of all strokes[11].

1.2. Aetiology of ischaemic stroke

The aetiology of ischaemic stroke can be largely classified into atherosclerosis, cardioembolic and lacunar syndrome resulting from small vessel occlusion[12]. In published large stroke registries, 25-39% of ischaemic strokes never had their definite causes identified[13]. This group of strokes is known as cryptogenic stroke. The proportion of this type of stroke is nevertheless decreasing due to the wide use of improved diagnostic tools, (e.g. Magnetic Resonance Angiography [MRA], Computed Tomography Angiography [CTA], Transoesophageal Echocardiogram [TOE]), and prolonged cardiac monitoring) and raised awareness of early investigation. Extracranial carotid and vertebral artery dissection account for only 2% of ischemic strokes overall, but in the less than 45 years old age group, dissection is responsible for approximately 20% of ischaemic strokes[14]. Other rare causes of ischaemic stroke such as vasculitis, and hypercoagulopathy account for less than 5% of strokes[12]. Positive anti-phospholipid antibodies, which can be present in more than 40% of ischaemic stroke patients may not have any clinical significance, and have not been shown to increase the risk of stroke reoccurrence[15].

Atherosclerosis is the most common pathology causing the occlusion of intra and extracranial arteries. The development of atherosclerosis is a chronic process which is caused, or at least accelerated partly by hypertension, smoking, diabetes and hyperlipidaemia[16]. The commonest sites affected by atherosclerosis that are relevant to stroke are the carotid bifurcation and the vertebral artery origins[17].

The risk of major thrombotic and thromboembolic complications of atherosclerosis is related to the stability of the plaque rather than the severity of stenosis[18]. Patients with irregular or ulcerated plaques on carotid angiography have higher risk of ischaemic stroke irrespective of the degree of stenosis of the vessel lumen[19,20]. The key feature of rupture-prone unstable plaques are a thinned fibrous cap overlying a large necrotic core in the setting of active inflammatory infiltration[21]. Breakdown in the fibrous cap or plaque erosion, allows the intensely pro-coagulant lesion centre to contact the flowing blood, resulting in acute thrombosis[22].

Atherosclerosis can lead to ischaemic stroke either via complete occlusion of intracranial carotid artery (ICA) with a thrombus resulting from the sluggish blood flow in the progressive narrowing of ICA (atherothrombosis), or "artery to artery" embolism – the thrombus embolises to block a more distal artery (atherothromboembolism)[23].

Emboli from cardiac sources are mainly from the left heart. The Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification[12] grouped cardiac sources of embolism into high and medium risk. High risk groups included Atrial Fibrillation (AF), sick sinus syndrome and coronary artery disease related conditions, while Patent Foramen Ovale (PFO) was classified as in the medium risk group. PFO is found in approximately 50% of cryptogenic strokes, but one third of them may be incidental[24]. Furthermore, the risk of stroke recurrence may not be significantly higher for patients with PFO compared to those without PFO[25]. Besides, no study to date has demonstrated that percutaneous PFO closure is superior to medical management[26,27].

1.3. Cerebral Blood Flow (CBF) and pathophysiology of Acute Ischaemic Stroke (AIS)

1.3.1. Physiology of CBF

The human brain has a high energy demand, and uses glucose as the main substrate of energy supply. Unable to store energy, a constant supply of oxygenated blood is required to meet the high metabolic demand. A healthy adult's CBF is about 50-55 mL/100g of brain/min, 800 mL/min for the whole brain. The values of CBF differ among different parts of the brain. Grey matter (75 mL/100g/min) has a higher rate of CBF than white matter (45 mL/100g/min). A well-developed intracranial collateral circulation and physiological response (cerebral autoregulation) constitutes the protective mechanism against cerebral ischaemia.

1.3.2. Autoregulation

In a physiological condition, CBF is determined by the Cerebral Perfusion Pressure (CPP) and by the Cerebrovascular Resistance (CVR) imposed by blood viscosity and the size of the intracranial vessels. When resting CPP is constant, CBF is closely matched to the metabolic demands of the brain tissue. Therefore, CBF, Cerebral Blood Volume (CBV), Cerebral Metabolic Rate of Oxygen (CMRO2) and Cerebral Metabolic Rate of Glucose

(CMRglu) are all coupled[28]. Under normal conditions, the brain has the ability to maintain CBF at a relatively constant level despite the change of CPP, which is termed "autoregulation"[29].

When AIS occurs, the occluded vessel causes a fall in CPP which can initially be compensated by autoregulation by dilatation of the collateral and distal vessels, increased CBV, and reduced CVR. When the mean arterial pressure falls below 40-50 mmHg (Figure 1-1.), compensatory mechanisms become exhausted, CBF falls along with Blood Pressure (BP), and cerebral ischaemia occurs[30]. At this stage, oxygen supply to the brain is still adequate to maintain usual metabolic activities by increasing the oxygen extraction from blood, a stage termed "misery perfusion"[31]. Once CBF falls below 20 mL/100g brain/min, Oxygen Extraction Fraction (OEF) becomes maximal, and CMRO2 begins to fall[32].

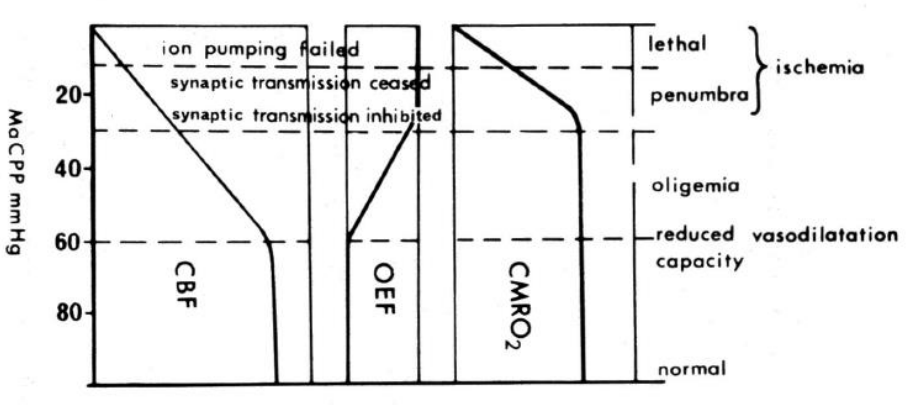


Figure 1-1. Flow metabolism and perfusion pressure diagram under the physiological and pathological condition. Reproduced from "Pathophysiology of brain ischaemia as it related to the therapy of acute ischaemic stroke" Clinical Neuropharmacology 1990[33]

The autoregulatory curve shifts to the right in chronic hypertension[34]. This population is therefore potentially more vulnerable to cerebral hypoperfusion if blood pressure is reduced aggressively. Similarly, an ischaemic brain with impaired autoregulation may be particularly vulnerable to hypoperfusion. If BP is aggressively controlled, it is potentially subject to further ischaemia.

Many studies have examined the steady-state and dynamic components of cerebral autoregulation in acute ischaemic stroke settings using various imaging modalities (Transcranial Doppler [TCD], Single-Photon Emission Computer Tomography [SPECT] or

Positron Emission Tomography [PET]). None has shown clear evidence of global impaired autoregulation in acute ischaemic brains, except perhaps in infarcted tissue[35,36].

1.3.3. Mechanisms of neuronal injury and neuronal death in ischemic brains

At the molecular level, the development of ischaemic neuronal injury is complex and heterogeneous. At the early stage of ischaemia, in the worst affected region with energy depletion, the process "excitotoxicity" is triggered to cause acute cell death (necrosis), and to initiate molecular events that lead to apoptosis, a delayed type of neuronal death which mainly occurs in penumbral tissue[37]. Glutamate that acts as a gateway for this complex cascade accumulates in the extracelluar space in energy depleted neurones, leading to the opening of the calcium channel and an influx of calcium, which is aggravated by the activation of N-methy1-D-asapartate (NMDA) and Alpha-amino-3-hydroxy-5-methyl-4-isoxanolepropionic Acid (AMPA) receptors. Influx of sodium ions results in the passive increase of water intracellularly, which results in the subsequent cerebral oedema, a major factor which contributes to early death in AIS.

The overload of neuronal calcium is related to a series of post-ischaemic inflammatory responses by releasing cytokines, such as Tumour Necrosis Factor Alpha (TNFα) and interleukin-1β; the expression of adhesion molecules on to endothelium; the migration of leucocytes, macrophages and monocytes into the ischaemic brain further contribute to ischaemia[37].

Repeat repolarisation and depolarisation is observed in the penumbral region, which is associated with infarct growth[38]. This phenomenon is called "peri-infarct depolarisations"[39].

Apoptosis, programed cell death, is linked with caspase activation[37], that cleaves protein, and modifies homeostasis and proteins which are directly responsible for cell death.

1.3.4. The Neurovascular unit (NVU)

The NVU has become an increasingly recognised concept in AIS pathophysiology. Comprising of neurones, interneurones, astrocytes, basal lamina covered with smooth muscular cells and pericytes, endothelial cells and extracellular matrix, it is considered the functional structure that interconnects neurones and the capillary vasculature, and that

plays an important role in the regulation of CBF[40,41]. Ischaemic injury to the brain can damage many components of the NVU[42] (Figure 1-2.)

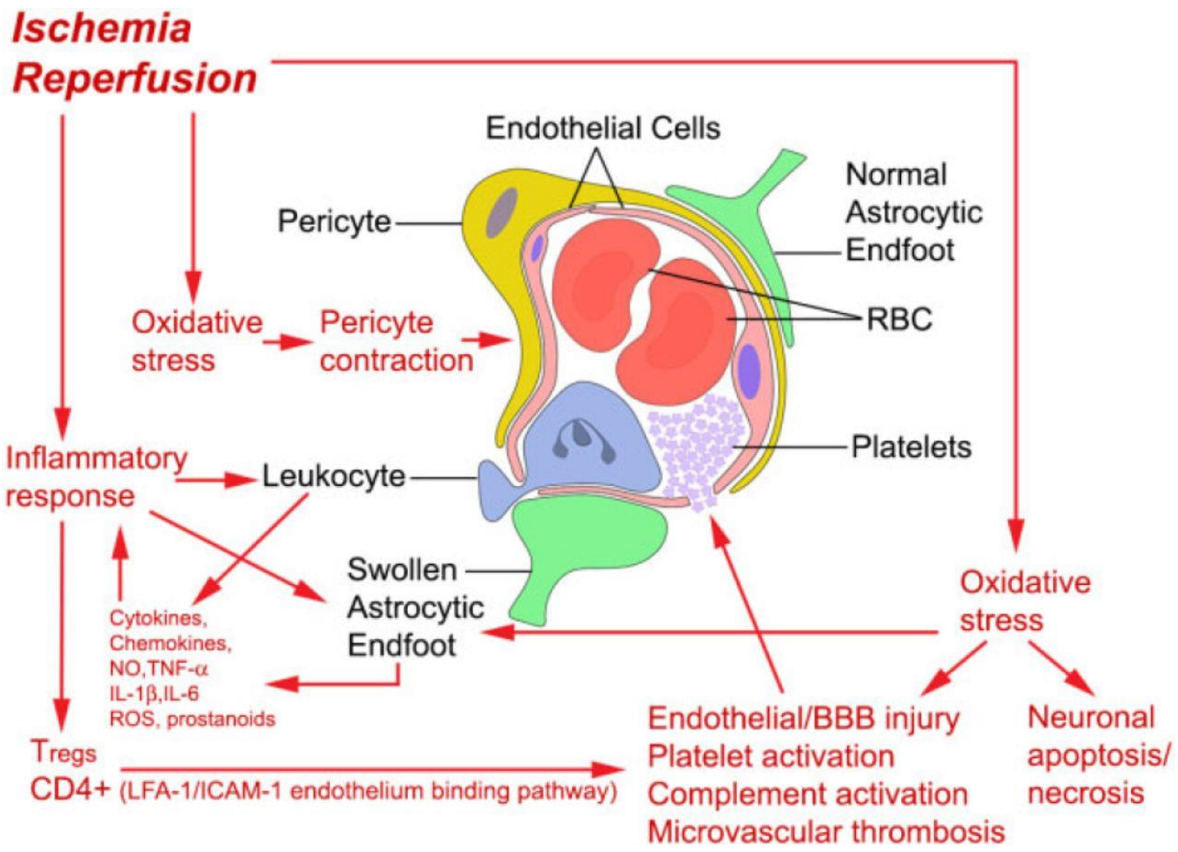


Figure 1-2. Schematic model of neurovascular mechanism of postischemic reperfusion injury. Reproduction from "Revisiting cerebral postischaemic reperfusion injury: new insights in understanding reperfusion failure, haemorrhage, and oedema" International Journal of Stroke 2015[42]

An animal study[43] showed that the reperfusion of an ischaemic brain induced an excessive amount of autophagy accumulation within endothelial cells in the stroke rat model, which may result in cell death by degrading the normal cell component[44]. Pericytes are contractile cells that have functions similar to smooth muscle cells that can regulate CBF by constricting or dilating the capillaries[45]. Ischaemia leads to constriction and death of pericytes, which may irreversibly constrict capillaries and damage the Blood Brain Barrier (BBB)[45]. The role of astrocytes in acute ischaemia and stroke recovery differs. In the acute post ischaemia phase, astrocytes promote neuronal death by transporting and releasing inflammatory factors[46], whereas in stroke recovery, they are cytoprotective by increasing cellular proliferation and facilitating the neurovascular remodelling[47]. Continuing efforts have been made to developed strategies targeting non-

neuronal components of the NVU in AIS, but, in clinical studies, neuroprotection has not shown any benefit.

1.3.5. Ischaemic Penumbra

In the presence of an arterial occlusion, the downstream CPP falls below the capacity of cerebral autoregulation, and CBF continues to fall passively along with CPP as a result. Critical CBF thresholds were established in nonhuman primates and human studies[48-50] to describe discrete stages of tissue ischaemia. These findings formed the basis for the concept "ischaemic penumbra". Astrup and colleagues[51] described "penumbra" as severely ischaemic, functionally impaired brain tissue that is at risk of infarction. Its fate depends on whether CBF is restored timeously, either to be salvaged or to progress to infarction. The original concept[52] that the ischaemic area comprises of a central homogenous core tissue surrounded by a potentially salvageable penumbra provides the theoretical foundation for thrombolysis. This notion has been refined recently to the extent that what was believed to be the homogenous core probably contains pockets of "mini-cores" and "mini-penumbras" in the early minutes of ischaemia which evolve dynamically and heterogeneously depending on local differences in microvessel perfusion[53].

Ischaemic brain tissue is arbitrarily divided into three stages using physiological and metabolic parameters: an ischaemic core, penumbral tissue and an area of benign oligaemia (the area that is mildly hypoperfused , but not at risk of infarction)[54,55]. Except for CBF and electrophysiological parameters, characteristics of penumbra tissue can be described at the molecular level, and include decreased protein synthesis that can recover; preserved Adenosine Triphosphate (ATP) concentration; synthesis of heat-shock proteins; and possibly a successful unfolded protein response[53]. Clinically, the combination of core and penumbral volume correlates better with neurological deficits than do each of them individually[56,57]; the salvage of penumbra is highly correlated with neurological improvement[56,58].

The penumbra is dynamic; its fate is influenced by many factors:

Time: Timely reperfusion is the crucial principle in AIS management. Without reperfusion, penumbra tissue will ultimately become irreversibly damaged, although exceptions exist in that some penumbra appears to be preserved for up to 24 hours or more[54,59]. In most

cases, the entire region of the penumbra is recruited into core within 6 hours. The natural history of AIS suggests that in each minute after a stroke, without treatment, 1.9 million neurones die[60]. The importance of time in AIS management is evidenced by the failure of early thrombolysis studies in which patients were treated late. Thrombolysis in stroke only became a success when the time issue was addressed in the National Institute of Neurological Disorders and Stroke (NINDS) study. The meta-analyses to date and the evolution of intra-arterial studies repeatedly proved time is the key.

Collateral circulation: With intracranial or extracranial occlusion, collateral supply is the only way to perfuse the ischaemic tissue, which will be discussed in detail below.

Temperature: Hyperthermia is associated with worsening brain injury in the ischaemic brain[61]. In an animal model of Middle Cerebral Artery (MCA) occlusion, a rise of body temperature of three degrees increased infarct volume threefold histologically[62]. An experiment with hypothermia therapy[63] as a neuroprotective strategy is underway in a phase III study.

Hyperglycaemia: It is established by many studies that hyperglycaemia is associated with poor functional outcomes and infarct growth in AIS[64-66]. The risk of death and ICH is higher even with mildly raised serum glucose levels post stroke[67]. However, aggressive blood glucose control in AIS has not demonstrated any improvement in functional outcomes. On the contrary, it increases the risk of hypoglycaemia[68]. In a Magnetic Resonance Imaging (MRI) spectroscopy study[65], acute hyperglycaemia was associated with greater lactate production irrespective of baseline diabetes status, which in turn, was independently associated with reduced salvage of penumbral tissue.

Age: Age is one of the unmodifiable risks for stroke. Recent literature[69,70] confirmed that elderly patients benefit at least as much as younger patients from IV rtPA. However, older patients do suffer from more severe strokes, and have poorer outcomes[71]. A recent study[72] using whole brain CT perfusion to examine penumbra in acute ischaemic stroke suggested age is negatively correlated with penumbra volume despite similar haemodynamics and better recruitment of collaterals. Animal[73] and human[74] studies indicated the alternation of autoregulation with aging. There may be an intrinsic tissue process with aging that influences penumbra.

1.3.6. Brain oedema and reperfusion injury

Both animal[75] and human[76] studies have now firmly established that reperfusion of the ischaemic but potentially salvageable region timeously, causes marked infarct volume reduction. Delayed reperfusion into the already infarcted area is considered to be harmful and potentially fatal by inducing haemorrhagic transformation, and worsening cerebral oedema, a concept named "reperfusion injury"[77].

The mechanism involved in reperfusion injury is a complex process with multiple parallel and crosslinked pathways involved. During early ischaemic injury, cytotoxic oedema develops as the consequence of cellular dysfunction. The influx of calcium after the depolarisation of cell membrane results in an accumulation of intracellular calcium and lipolysis. Once delayed reperfusion occurs, the lipolytic break down products react with oxygen, producing large amounts of reactive oxygen species which are involved in mitochondrial mediated cell death[42]. In addition, protein synthesis is suppressed in vulnerable neurons. Post ischaemic reperfusion worsens the disruption of BBB permeability, possibly by reintroducing blood flow, increased production of free radicals, and matrix metalloproteinases mediated attack on the basal lamina in cerebral capillaries[78], which results in vasogenic oedema. In addition, neurotoxicity, neuroinflammation and leucocyte recruitment also play a role in causing haemorrhagic transformation[79] (Figure 1-3.).

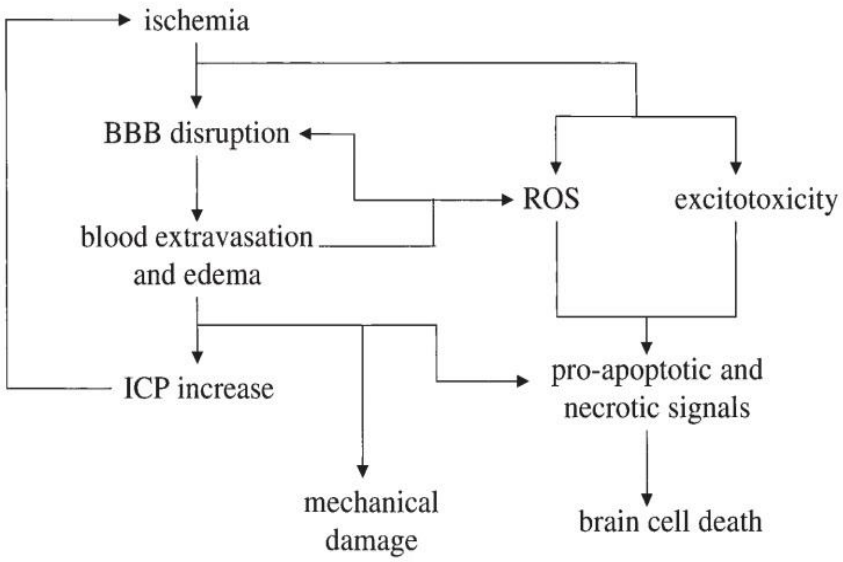


Figure 1-3. Simplified schematic representing the multiple cascades of tissue injury that are initiated after hemorrhage (Reproduction from "Triggers and

mediators of haemorrhagic transformation in cerebral ischemia" Molecular Neurobiology 2003[79])

1.3.7. Collateral circulation

Leptomeningeal vessels provide alternative routes for CBF in acute arterial occlusions[80]. Good collateral status is associated with better clinical outcomes after acute ischaemic strokes, higher rates of recanalisation, better reperfusion and less infarct growth[81-83]. Patients with proximal MCA occlusion who had adequate collateral flow did as well as those with no visible occlusion, while patients with occlusion and absent collaterals had a 10-fold increased risk of severe worsening measured with the National Institute of Health Stroke Scale (NIHSS) score, or death[82]. Poor pial collateral status is an independent predictor for post thrombolysis haemorrhage in intra-arterial therapy[84]. The effectiveness of collateral flow varies significantly in acute ischaemia. The mechanism of vasculogenesis remains unclear. Animal studies have suggested genetic and environmental factors may play a role[85], as does vascular endothelial growth factor[86]. In humans, it was noted that elevated pre-treatment systolic blood pressure and a history of hypertension are associated with poor collateral status[87]; the use of Statins enhances collateral supply, possibly by increasing nitric oxide synthesis and endothelial progenitor cell growth[88]. Chronic progressive atherosclerotic disease affecting cerebral perfusion allows collateral recruitment over time[89,90]. Hyperglycaemia may also reduce collateral flow[80]. Collateral flow is dynamic. Following arterial occlusion, there seems to be a time-dependant recruitment of collateral flow, especially within the first hour of post-ictal, although the ability and speed varies considerably[82].

1.3.8. Imaging penumbra

Penumbra is defined with neurophysiological and metabolic characters as discussed above. As the only non-invasive imaging modality that provides quantitative measurements of physiological and metabolic parameters including CBF, OEF, regional cerebral metabolic rate of oxygen and glucose, PET has been used initially to map the human penumbra and subsequently has become the gold-standard for verification of MRI-based Perfusion Weighted Imaging (PWI) and CT perfusion (CTP). The absolute flow values for thresholds established in humans vary among different research groups[91],

partly because the determination of values requires arterial blood sampling, and partly because of the variability in measurements of tracer concentration. The time of the measurement post-ictus is another essential factor contributing to the variability[54]. Flumazenil, a central benzodiazepine receptor ligand that binds to the Gamma-aminobutyric Acid (GABA) receptor is later used as a PET marker of neuronal integrity[54] . It can identify ischaemic changes without invasive arterial sampling, and is not affected by time after itcus or variability in blood flow. The method can map the early ischaemic change more accurately if combined with the use of $H_2{}^{15}O$ to determine flow. In a study[92] which imaged 10 patients within 2-12 hours of stroke symptoms onset, flumazenil binding thresholds of 3.4 identified ischaemic core, and CBF <14.1mL/100g/min defined potentially salvageable tissue – the penumbra. The complexity and restrictions on the availability of PET nevertheless prevents its use in acute stroke management clinically. It remains a research tool.

1.4. Advanced imaging in acute ischaemic stroke

Current guidelines[93] advise that decision making for Intravenous (IV) thrombolysis should be based on clinical diagnosis of acute ischaemic stroke and non-contrast Computed Tomography (NCCT) excluding ICH[93], but diagnosis of stroke cannot always be achieved directly from clinical information. Advance multimodal imaging has been used in many centres to improve the sensitivity and specificity of stroke diagnoses and provide essential information to aid decision making and predict outcomes. Both multimodal MRI and CT are used as clinical and research tools; each has its advantages and limitations. In particular, there are unsolved issues in PWI-MRI and CT perfusion, and further validation is required[94]. The combination of NCCT/ Diffusion Weighted Imaging (DWI), angiogram and perfusion imaging addresses the crucial questions in IV thrombolysis decision-making: the presence of haemorrhage, the occlusion site, and the extent of irreversible and reversible tissue injury.

1.4.1. Magnetic resonance imaging

1.4.1.1. Diffusion Weighted Imaging

DWI measures the random Brownian motion of water molecules within a voxel of tissue. As a result of arterial occlusion, cytotoxic oedema develops due to the increase of

intracellular water causing a restriction of water molecule diffusion, which is evident as a reduced Apparent Diffusion Coefficient (ADC) on the ADC map, and a hyperintensity on DWI[95,96]. DWI is very sensitive in detecting early ischaemia (probably greater than 95% within the first hour). It is positive as early as two minutes after ischaemia in experimental animals and 40 minutes in humans[97], and can readily discriminate acute ischaemia from chronic ischaemia. The DWI signal gradually decreases over 7-10 days post stroke, therefore needs to be performed quickly in order to identify acute stroke. DWI can also be positive in pathologies such as focal seizures, encephalitis and migraines, but only an acute infarction causes a bright signal on DWI and a dark area on the corresponding ADC map.

The positive DWI lesion in AIS within the first few hours of symptoms onset is considered to represent ischaemic brain tissue that is irreversibly damaged (the ischaemic core). It is recognised that some DWI lesions may be reversible[98], especially if CBF is restored timeously, but this is rare, the extent of tissue reversal is small, and rarely alters the DWI/PWI mismatch ratio[99,100].

1.4.1.2. Perfusion weighted imaging and Arterial Spin Labelling (ASL)

Hypoperfused brain tissue can be imaged using PWI, which is obtained by bolus tracking the passage of IV gadolinium contrast. The DWI/PWI mismatch was hypothesised to represent core/penumbra[101] mismatch. PWI identifies penumbra with high sensitivity, but tends to overestimate penumbra by including benign oligaemiac tissue[102,103]. The parameters proposed to identify an optimal threshold accurately defining PWI lesion remain variable, and most of them were not prospectively evaluated[104]. Currently, Time to Maximum (Tmax)>5-6 seconds defines “tissue at risk” most accurately[105-107] .

PWI is the most validated penumbra imaging method against PET. Many clinical studies use MRI to evaluate penumbra; however, about 10 – 15% of patients suspected of acute stroke cannot undergo MRI because of contraindications[108]. The duration of scanning is long, and may not be tolerated by those with severe symptoms; where scans are possible, the quality of imaging is often suboptimal.

ASL imaging evaluates brain perfusion by using radiofrequency pulses labelling the protons in arterial blood as an endogenous contrast, and therefore does not require IV

contrast. It has the potential to replace PWI, but has the disadvantages[109] of low signal to noise ratio, limited spatial resolution, and is a time consuming sequence to quantify tissue blood volume. Higher magnetic field MRI significantly improves the quality of ASL. As a relativly new technique, it lacks standardisation of acquisition, post-acquisition optimisation and automation. Compared with PWI, it overestimates hypoperfused tissue[110,111] by underestimating CBF when there is a delay in contrast arrival. One study[110] suggested a threshold of CBF<40% for defining penumbra correlates well with Tmax>6s in PWI imaging.

1.4.1.3. T2 Fluid-attenuated Inversion Recovery Imaging (FLAIR)

T2 FLAIR reveals an infarcted area as a hyperintensity area by suppressing the Cerebro-Spinal Fluid (CSF) signal, and is more sensitive to detecting small infarcts adjacent to CSF spaces compared to conventional T2 sequencing. In contrast to DWI, the bright signal on FlAIR in AIS appears 3-6 hours post stroke[112], hence these signals are used as "a tissue clock" when the stroke onset time is unclear. The hyperintense vessel sign on FLAIR is an indicator of insufficient collateral circulation[113], has a high specificity (86%) for predicting proximal vessel occlusion, and is associated with a larger ischaemic lesion and clinically more severe strokes[113,114].

1.4.1.4. Magnetic resonance angiography (MRA)

MRA can be acquired as Time of Flight (TOF) imaging or post gadolinium contrast imaging including intracranial and extracranial vessels. Compared with CTA, 3 Dimensional (3D) TOF imaging has the advantage of being non-invasive, but the acquisition time is much more prolonged, resulting often therefore in suboptimal quality. It is flow dependent and signal loss due to turbulent flow (stenosis) or slow flow may overestimate degree of stenosis. Gadolinium contrast MRA can be acquired rapidly, but has poor spatial resolution compared to TOF. As with CTA, MRA is a reliable alternative for the provision of vessel assessment to Digital Subtraction Angiography (DSA).

1.4.1.5. Gradient Echo (GRE) and Susceptibility Weighted Imaging (SWI)

Gradient Echo and SWI sequences are both blood sensitive sequences that are used to detect haemorrhage. GRE is sensitive to blood break down products, such as deoxygenated haemoglobin, and intracellular methemoglobin, haemosiderin within

perivascular macrophages, due to their paramagnetic effect that results in signal loss[115]. GRE can therefore detect micro haemorrhages that are not visible on NCCT, and is possibly comparable to NCCT in detecting acute ICH[116].

SWI was designed with the aim of enhancing contrast in MRI imaging. It provides identification of tissue which has a different susceptibility to its surrounding structure, including blood break down products and minerals[117]. It has higher sensitivity in detecting blood than GRE and NCCT[118]. Systematic reviews[119-121] did not find the presence of micro haemorrhage to significantly increase the risk of post rtPA haemorrhage, although in the case of people with severe micro haemorrhages, who may have a significant higher risk of ICH, the evidence is lacking.

The susceptibility sign on GRE and SWI[122] indicating the presence of a proximal arterial clot has a high sensitivity (87%) and specificity (100%), and is the equivalent of a hyperdense vessel on NCCT.

1.4.2. Multimodal CT imaging in acute ischaemic stroke

1.4.2.1. Non Contrast CT

In many parts of the world, NCCT is the only imaging undertaken in AIS patients prior to administering IV recombinant tissue Plasminogen Activator (rtPA). It is sensitive in excluding haemorrhage, some tumours and established infarcts. It measures X-Ray beam attenuation proportional to tissue density quantified in Hounsfield Units (HU), which are a linear density scale with water valued zero[123].

A normal CT does not exclude AIS. Hyperdense vessels on the lesional side and early ischaemic changes on NCCT assist diagnosis of AIS. The hyperdense vessel sign is highly specific for AIS (90-100%), but it only presents in approximately 30% of patients[124]. The sensitivity of detecting hyperdense vessels increases with thin slice CT[125]. Early ischaemic changes on CT are present in 60-80% of patients within three hours from symptoms onset in MCA strokes in thrombolysis studies[126,127], but in practice, the inter-observer agreements were low for detection of hypodensity of more than 1/3 MCA territory. Using the Alberta Stroke Program Early CT score (ASPECT score)[128], a systematic approach to NCCT interpretation that deducts a point for abnormality in each of ten defined regions at two levels of a scan, the intraclass correlation coefficient improved to 0.69-0.86[129,130]

from 0.14-0.49, but to achieve this, training is required. In contrast, CTP and CTA have consistently higher inter-rater agreement in ASPECT score readings[130]. DWI sequences can detect acute ischaemic change with high sensitivity within less than 40 minutes post-ictus[97], with the advantages of differentiating acute and chronic lesions without contrast, and the inter-rater agreements for experienced radiologist (K=0.84) and novel readers were similar (K=0.76)[131].

1.4.2.2. CT perfusion

CTP is the most convenient imaging modality currently available to image tissue status. It can typically be acquired in 60-90 seconds. The large majority of CT scanners currently in service are still the generation limiting CTP to 2-4 cm coverage of brain; but the new generation of 16cm detector ("320-slice" or similar) CT scanners can provide whole brain coverage[132].

Compared with MRI, multimodal CT is much more widely available, less time consuming, and better tolerated with fewer contraindications. The use of iodine contrast may be limited in patients with impaired renal function. The occurrence of contrast induced nephropathy is generally low, being approximately 2-7 % in the stroke population who underwent acute evaluation with CTA regardless of whether baseline renal function was known prior to contrast scan[133]. If contrast scan is necessary, the risk of iodine contrast is smaller than that of gadolinium in patients with borderline renal function (estimated Glomerular Filtration Rate [eGFR] 45-60)[134].

There has been concern that the use of iodine contrast may impair the effectiveness of thrombolysis, or that the interaction between the contrast and alteplase may increase the risk of ICH. Neither has been proven to date.[135,136] Ischaemic core and penumbra are included in the same map in CTP. Mismatch can be visualised directly by readers.

CTP provides quantifiable information due to the linear relationship between the contrast concentration and CT attenuation , which is not achievable with PWI that relies on the non-linear T2 effect induced in adjacent tissues by high concentrations of intravenous gadolinium.[134]

The disadvantages of CTP over MRI-PWI include limited coverage for most current scanners, radiation exposure and more complex post-processing. Acute stroke MRI

sequences can also detect microbleeds with GRE sequence or SWI, which is not achievable with CTP.

CTP post-processing

CTP imaging raw data requires post-processing to generate interpretable parametric maps. A few algorithms have been developed for this purpose, but resulting quantitive maps differ significantly[137]. New evidence suggested that a delay-corrected Singular Value Decomposition (SVD) method probably provides the most accurate quantification of core and penumbra[138].

An Arterial Input Function (AIF) is required for the calculation of CBF. The choice of AIF and laterality was shown to have no significant impact on the calculation of the CBF and CBV values[139]. In practice, contralesional Anterior Cerebral Artery (ACA) is the common chosen AIF in order to minimise partial volume effects that can arise from use of arteries in the plane of section.

The accuracy of CBF calculation is based on the assumption that there is no delay or dispersion. In reality, significant AIF delay exists in clinical situations such as AF, severe carotid stenosis, poor left ventricular function, or proximal intracranial occlusion with poor collateral circulation. The presence of these result in underestimation of CBF and overestimation of Mean Transit Time (MTT)[140], and hence overestimate the volume of penumbra. The overestimation can be minimised by using a delay-insensitive algorithm[137], which also appears to be less sensitive to the laterality of AIF selection compared with the use of standard processing software[141].

Parameters used to define tissue status

Multiple parameters derived from various post-processing algorithms have been proposed to define the ischaemic core and penumbra post-processing. Using CTP to assess tissue status quantitatively is much less studied than using MRI, and many of the available studies are not of good quality, with limited numbers of patients[104].

Recent evidence has emerged that CBF may be the more accurate measurement of non-viable tissue[142]. The physiology of haemodynamic changes occurring in acute stroke supports the view that CBF may be a more desirable parameter for estimating the ischaemic core across different times post-ictus and recanalisation status[134]. Relative

values performed better than absolute values due to the variability in normal values on the contralesional side[134]. The actual CBF value to define ischaemic core varies depending on the post-processing algorithms[138,143]. The other widely used parameter to define irreversible tissue is CBV[144]. CBV has the advantage over CBF of being relatively delay-invariant[145], and is not influenced by AIF selection[146]. However, the normal CBV value varies in each individual, the difference between the CBV values defining irreversible tissue and the normal white matter is small. An absolute CBV value to define core may not represent the true ischaemic core.

It has been demonstrated that Delay Time (DT) may be a more accurate parameter to define critical hypoperfused tissue[147]. DT, which is similar to Tmax –- the current preferred parameter to define penumbra in PWI-MRI,[148] is derived from a vascular transport model correcting for delay and dispersion, and may identify tissue at risk more accurately than Tmax[147]. The previously preferred variable MTT, although relatively reliable as its value does not differ significantly between normal white and grey matter, and is less likely to be affected by extracranial factors, still overestimates penumbra by including "benign oligaemia", especially under the circumstances of poor cardiac function or severe extracranial carotid stenosis[145]. DT is nevertheless not a commonly used parameter in most post-processing software. To increase the reliability of defining ischaemic core and penumbra, standardised post-processing software such as "Rapid Processing of PerfusIon and Diffusion (RAPID)" is the necessary next step to develop the utility of CT perfusion in respect of clinical and large scale research.

1.4.2.3. CT angiography

The current most widely used protocol for CTA allows visualisation of vessels from the aortic arch to the vertex. Alongside the advantages of availability, low cost and fewer contraindications, a modern Multi-detector CT (MDCT) can perform CTA in less than a minute, and is therefore better tolerated than MRA, and is a preferred choice by patients.[149] CTA provides true anatomic, non-flow dependent data in assessing the severity of stenosis, residual lumen diameters, and calcification. This is not achievable with flow-dependent imaging, such as MRA and Doppler Ultrasound (DUS).

DSA is considered the gold-standard imaging modality for vasculature assessment. Its invasive nature and availability have ensured that it is mainly reserved for patients who

undergo interventional procedures. CTA is an excellent non-invasive alternative that can provide similar information.

Endarterectomy in patients who have symptomatic significant ipsilateral extracranial carotid stenosis is a standard practice to reduce the risk for recurrent stroke[93]. DUS is most commonly used in practice to identify candidates likely to benefit from this procedure. There is no sufficient direct evidence to suggest whether CTA is superior to DUS in the evaluation of carotid stenosis, but a recent study comparing DUS, CTA, and MRA against DSA suggested that CTA is the most accurate modality for evaluating carotid stenosis and is significantly more accurate than DUS (97% vs. 76%)[150]. A systematic review suggested that with MDCT, the sensitivity and specificity of CTA to diagnose significant carotid stenosis is close to 100%[151]. The inter-observer agreement is much higher in CTA interpretation, while DUS is more operator dependant, and the result is less reproducible[152]. CTA is also superior to DUS in detecting plaque ulceration and vessel wall abnormalities[153].

1.4.2.4. The use of multimodal CT in clinical settings

Stratification for ischaemic stroke and stroke mimics

In many centres, CT perfusion is used in routine work-up for hyperacute stroke patients. It has a higher sensitivity in detecting stroke than NCCT, (77.6% vs. 69.2%; P< 0.01), with an improved specificity (75.6-92.7% vs.65%; P<0.01) and a much better rate of inter-observer agreement[154]. Multimodal CT (NCCT, CTP and CTA) improved the sensitivity for stroke determination by 18.2% over NCCT alone[155]. Using NCCT and clinical information only to make therapeutic decision, 1-16% of stroke mimics received IV thrombolysis depending on the experience of centres[156]. The SICH risk of treating stroke mimics may be low (approximately 1%)[156], but improving the diagnostic accuracy and treating fewer stroke mimics is nevertheless still desirable.

A small percentage of clinically diagnosed stroke patients have normal multimodal CT, mainly accounted for by lacunar or infratentorial strokes[157]. Lacunar infarcts of less than 1.5cm in diameter are not reliably detectable by CTP, probably due to poor signal to noise ratio, with accompanying low spatial resolution[158]. Although limited coverage of CTP is thought to be responsible for reducing the sensitivity of CTP in detecting stroke, after

excluding infratentorial and lacunar stroke, the sensitivity of CTP in detecting stroke is 94%[157].

CTA is highly sensitive and specific in detecting thrombi especially those within proximal large vessels. Compared with good standard DSA, CTA has a sensitivity of 98.4%, and a specificity of 98.1% for the detection of large vessel occlusion[159]. It has a higher positive predictive value than that of MRA for detecting both stenosis and occlusion[160].

CTA and MRA are comparable in the diagnosis of cervical arterial dissection[161]. A wide range of sensitivities have been identified in studies (60-100%). Arterial luminal irregularities are more commonly identified by CTA, whilst intramural haematoma is better detected by MRI when a cervical axial fat-suppressed sequence is included[162].

Predicting clinical outcomes

Both hyperdense vessel signs and early ischaemic changes on NCCT also have prognostic value. Clot lengths measuring more than 8mm[163] are unlikely to be recanalised by IV rtPA, and are therefore associated with poor outcomes, and has been used as one of the selection criteria for intra-arterial therapy[164]. Hypodensity on a plain CT correlates with ischaemic core on CTP[165], PET[166] and MRI DWI lesion[167]. This is an independent predictor of poor outcomes[168], and a risk factor for Symptomatic ICH (SICH) if it is more than 1/3 of MCA territory[169,170].

Baseline infarct core volumes have been shown to be strongly correlated to the clinical outcome by multiple studies using various imaging modalities[134]. A core volume of > 70mls, measured by DWI on admission is a strong predictor for a poor outcome regardless of the application of reperfusion therapy[171]. Core size is also the best predictor for clinical outcomes at three months[172].

The baseline core volume, along with the Onset-to-treatment Time (OTT), systolic high blood pressure, admission glucose level, and the initial NIHSS score are independent predictors for risk of haemorrhage following IV rtPA[173]. In the absence of hypodensity on the plain CT, regions with very low CBF[174] or very low CBV [175,176] were found to be at high risk of haemorrhage post reperfusion therapy.

The location of thrombus is independently associated with the clinical outcome and recanalisation status following IV rtPA. Proximal occlusion (distal ICA, M1, M2 or basilar

artery) has been demonstrated to be a significant independent predictor for poor outcome[177]. In addition, the combination of the clinical assessment (NIHSS score) and the CTA score is highly predictive. 77.6% of patients with NIHSS>10 and proximal occlusion had a poor outcome, compared with only 21.5% of patients with NIHSS<10 and distal or no occlusion on CTA ($p<0.0001$) regardless of treatment. Clinical meaningful recanalisation is significantly less likely in patients with proximal occlusions (ICA, $p=0.005$; proximal M1, $p=0.021$)[178]. Proximal occlusion generates a larger CBV defect; more of the penumbra can be salvaged if the occlusion is located distally[179]. In basilar strokes[180] treated with IV thrombolysis, the frequency of recanalisation was also location-dependent, with top-of-basilar only clot significantly correlating with recanalisation ($p=0.004$). Distal small branch or perforating artery occlusions can be difficult to visualise on CTA. In this group of patients, a good clinical outcome is expected after IV rtPA treatment, and the associated risk of haemorrhage is much lower[181].

Except for the location of the thrombus, the extent of the clot is another significant contributor to the outcome of revascularisation therapy. Several clot burden scores have been developed providing a combined assessment of clot location and extent, which are independently associated with final infarct volume, clinical outcomes, and the risk of haemorrhage.[177,182,183] Measuring clot length/burden nevertheless remains problematic using either CTA or NCCT. The outline of thrombus on CTA is highly dependent on the degree of collateral flow. The current standard CTA is a single phase study. The lack of contrast opacification in distal arteries may represent delayed filling rather than intraluminal thrombus[182]. Although the hyperdense vessel sign is highly specific, the prevalence is less than 30% of the stroke population[124], and its value in measuring clot burden is very limited. Furthermore, measurement is only possible when there is a segment of proximal hyperdense artery, rather than a dot sign in the M2 region.

Current decision making for IV rtPA is still based exclusively on non- contrast CT[184]. Whether multimodal CT can be advantageous remains to be answered by a well-designed study that examines this hypothesis. Some experts argue that the combination of non-contrast CT and CT angiography can provide enough information about tissue fate and outcomes[81,185]. A newly published retrospective study[186] emphasised that CT perfusion adds a unique evaluation on the determination of penumbra, which cannot be obtained from either non-contrast CT, CT angiography or clinical assessment. Others worry that

multimodal CT assessment in a hyperacute stroke setting will defer the thrombolysis treatment. Our centre's experience[187] was that multimodal CT takes approximately 15 minutes to acquire. Whether the post-processing, interpretation and subsequent decision making will delay IV rtPA significantly, and whether the benefit of extra knowledge gained from multimodal CT will outweigh the delay is being examined in the ongoing Penumbra and Recanalisation Acute Computed Tomography in Ischaemic Stroke Evaluation (PRACTISE) trial.

Imaging based selection for reperfusion therapy candidates

Despite much exciting research exploring new technologies or drug treatments in acute stroke, the only approved management currently remains intravenous rtPA within 4.5 hour from the onset of symptoms[188]. Because of the narrow treatment time window, only 3% of European stroke patients received thrombolysis in 2005[189]. Although the benefit of extending IV rtPA up to 6 hours is unclear[70], it is known that core/penumbra mismatch can persist for up to 24 hours from symptoms onset[190].

Advanced imaging with MRI or CT using probabilistic perfusion thresholds allows assessment of the extent of viable (penumbral) and non-viable (core) tissue, and it has been hypothesised that this may allow individualised treatment decisions, including delayed intervention in individuals with favourable brain imaging features[191]. The pioneer studies testing this hypothesis were the Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evaluation (DEFUSE)[192] and Echoplanar Imaging Thrombolytic Evaluation Trial (EPITHET)[193] studies, which demonstrated that in patients presenting within 3-6 hours from symptoms onset who had a "DWI/PWI mismatch" (i.e. where penumbra volume exceeds core volume by an arbitrarily defined ratio), early reperfusion is significantly associated with favourable clinical response.

A small number of completed studies [184,192-198] have used penumbral mismatch to select patients, predominantly in extended time windows between 3-9 hours. Early meta-analyses[199,200] found that baseline favourable imaging features were better associated than unfavourable imaging features with increased recanalisation, reperfusion and better outcomes. However, functional outcomes did not differ from control (non-thrombolysed) groups, and treatment was associated with increased mortality and ICH risk. More recently, the Mechanical Retrieval and Recanalization of Stroke Clots Using Embolectomy

(MR RESCUE) [196] study found no interaction between imaging features and treatment effect from delayed Intra-arterial (IA) thrombectomy: outcomes were good in those with good scans, and poor in those with poor scans, regardless of intervention. In contrast, MRI profile and response to endovascular reperfusion in the non-randomised DEFUSE 2 study[195] found that MRI "target mismatch" was associated with better outcomes following IA treatment. The populations in these two studies had very different characteristics (e.g. median core volume in MR RESCUE was 60mL compared with 15mL in DEFUSE 2), indicating different selection biases. Imaging parameters to define core and penumbra remain highly heterogeneous in published literature[104] and better validated and platform-independent software tools[201] are not yet in widespread use, although current MRI-based trials will test one of these. The Extending the Time for Thrombolysis in Emergency Neurological Deficits Study **(**EXTEND) (NCT00887328)[202] uses the RAPID [201]software package in a 4.5-8h window.

The optimal mismatch ratio also remains uncertain. In one of the first studies testing the imaging selection hypothesis,the DEFUSE study[192], mismatch was arbitrarily defined as a PWI lesion volume ≥120% accompanied with a DWI lesion volume with a minimum of 10mL of mismatch. A subsequent sub-analysis[203] showed that favourable clinical outcome rates associated with early reperfusion are related to the degree of mismatch. However, the greater the mismatch ratio applied in patient selection, the fewer patients will be eligible for treatment. A ratio of 1.8 (ie perfusion lesion >180% of DWI lesion) was considered a better ratio to be applied in future studies as only 15% of patients would be excluded, while the ratio for good clinical outcomes would still be doubled compared with the mismatch ratio of 1.2. Other ongoing studies however, use different mismatch ratios. The European Cooperative Acute Stroke Study (ECASS)-4 (Eudra-CT: 2012-003609080) uses a mismatch ratio of 1.6 in its patient selection, while the Tenecteplase versus Alteplase for Stroke Thrombolysis Evaluation trial (TASTE)[204] still uses the 1.2 mismatch ratio. Further research and validation are required to reach a consensus on the most suitable mismatch ratio.

In addition, the absolute volume of penumbra and core was suggested to be a better criterion for the selection of candidates. Patients with "malignant profile"[192] (baseline DWI lesion ≥100mL, and/or PWI lesion≥100mL with Tmax delay≥8 seconds) were

associated with SICH or poor outcomes following reperfusion therapy, despite the fact that a large mismatch ratio was present in this population.

A post-hoc analysis of DEFUSE 2 data[205] suggested that the assessment of occlusion sites and ischaemic cores size are sufficient in providing information for patient selection. In patients who had ICA or M1 occlusion and DWI lesion <50mL, reperfusion was associated with good outcomes (Odds Ratio [OR] [95% Confidence Interval [CI]] 8.5 [2.3-31.3]). In recent completed endovascular studies[197,198,206-208], the imaging selection criteria varied considerably. It is worth noting that although not included in the inclusion criteria, many patients in some of these studies had undergone perfusion imaging prior to the treatment, indicating a possible selection bias. This has also highlighted the urgent need for further work to define a unified advantageous imaging profile definition to be utilised in future studies, and more importantly clinical practice.

In summary, using advance imaging to select patients for acute reperfusion therapy is probably the better way forward, especially in respect of endovascular therapy. Nevertheless, uncertainties exist in many aspects, including: the use of MRI or CT; the tissue status definition and mismatch ratio; whether the additional time involved is worthwhile; the post-processing and analysis of imaging; and what imaging is required for selection.

Imaging selection for wake-up stroke

Approximately one quarter of strokes are recognised on waking up. They are excluded from the current thrombolysis treatment protocols due to the unknown onset time. But "wake-up" stroke patients and conventional thrombolysis candidates are similar in clinical presentation and in imaging characteristics[209,210]. Evidence suggests that a large proportion of these strokes seem to have occurred shortly before waking up[211]. A systemic review[212] of all available wake-up stroke reperfusion studies revealed that only a small number of patients are treated, and that the selection criteria and imaging modality used varied considerably. No evidence, to date, supports treating patients in this category.

Advance imaging has been utilised as a tissue clock to identify those who presented with wake-up stroke, but who are within the potential treatment time window, and are eligible

for thrombolysis. Two main imaging criteria are used for this purpose. One is the traditional core/penumbra mismatch, which is used in ECASS 4[213] and EXTEND[214]. Both studies are investigating IV rtPA in patients presenting 3-9 hours from symptoms onset. Wake-up strokes are also included, the onset time being defined as the midpoint of last known well and symptoms recognition. A large multicentre observational study[215] found that DWI/FLAIR mismatch can identify stroke within 4.5 hour onset with 62% (95% CI 57-67) sensitivity, 78% (72-84) specificity, and 83% (79-88) positive predictive value. This concept has subsequently been used in the Efficacy and Safety of MRI-based Thrombolysis in Wake-up Stroke study (WAKE-UP)[216] and A Study of Intravenous Thrombolysis With Alteplase in MRI-Selected Patients (MR WITNESS)[217], two studies which examine IV rtPA in wake-up strokes only. In both studies, patients have to receive treatment within 4.5 hours from symptoms recognition.

Biomarkers in revasculisation studies

Advanced imaging can quantify the consequences of thrombolysis by assessing reperfusion and recanalisation. It has been used as a biomarker in reperfusion studies as these are closely correlated to clinical outcomes[192,193]. In DEFUSE[192] and EPITHET[193], early reperfusion and infarct growth were used as endpoints in assessing efficacy. Early reperfusion and reduced infarct growth were correlated with good clinical outcomes. Phase II studies have used this strategy to reduce sample size[218]. It is clear now that reperfusion is the better independent predictor for clinical outcomes[219,220], while recanalisation does not necessarily lead to reperfusion[221]; reperfusion can occur without recanalisation[220], and predicts penumbra salvage, less infarct growth, smaller final infarct volume and good clinical outcomes.

Traditionally, studies have used PWI-MRI post-revascularisation therapy to quantify reperfusion[192,193]. CT perfusion studies have shown that neurological recovery in the early stage of stroke is predominantly driven by penumbral salvage[58], hence the reperfusion of the ischaemic area. The proportion of penumbra salvaged representing reperfusion can be used as a biomarker for clinical outcomes.

Recanalisation remains an important clinical outcome biomarker[222], and is a commonly used imaging outcome measurement in acute stroke revasculisation studies. Several scoring systems have been developed for the assessment of vessel patency based on

different imaging modalities and have been used in various revascularisation therapy trials[223]. However, to date there is no consensus on what grading system is the best to describe the degree of recanalisation[224]. The commonly used and most validated scales[225], such as the Thrombolysis in Cerebral Infarction (TICI) scale and the Thrombolysis in Myocardial Ischaemia (TIMI) scale were developed for conventional angiography. Both allow description for distal vessel reperfusion as well as vessel patency[226]. Without dynamic information available, these scales are difficult to apply in non-invasive studies using CTA to evaluate vessel status. Radiologists still use a simple grading system (complete, partial, and no recanalisation). Given that in most studies using imaging endpoints, recanalisation is usually dichotomised in data analysis[227], the need for a complicated grading scale is questionable.

Visualisation of collateral flow

Various grading systems to describe the extent of collateral flow exist, but few have been validated. Studies using MRI to quantify collaterals have more diversity in sequences used, definitions and grading of collateral flow than do studies using CTA. None of them has been replicated. In comparison to MRA, leptomeningeal flow is better visualised by CTA using DSA as reference[228], probably because it shows the anatomy of leptomeningeal vessels.

The use of CTA, however, is inadequate because CTA only provides a snap shot in time, and does not give any dynamic information. It is difficult to differentiate antegrade flow resulting from an incomplete occlusion from retrograde flow acquired from collateral circulation with standard single phase CTA. CTA may also underestimate collaterals by not showing delayed contrast arrival. CT perfusion can complement CTA by providing dynamic "4 Dimensional" (4D) angiography[81]. Assessing collaterals with the combination of CTP and CTA is more accurate and reliable[229].

The combination of NCCT, CTP and CTA – multimodal CT – provides a complete assessment for acute ischaemic stroke patients, allowing the evaluation of the stroke severity, and prediction of the outcome of reperfusion therapy. It does take extra time, and there are many unresolved issue, in particular in CT perfusion, which prevent it becoming adopted widely as a valid clinical tool . In addition to ensuring continuing development in imaging technology, we still require a well-designed study to

demonstrate that decision-making based on multimodal CT is advantageous compared with the current CT only approach.

1.5. Conclusion

AIS causes significant disability. Research in its complicated pathophysiology has made important progress over the years. However, this has not translated into clinical treatment, except in respect of IV and IA reperfusion.

The technology to assess and manage AIS continues to evolve. Advanced imaging comprises an important part of AIS evaluation, but with the expense of extra time. In clinical research, it is used increasingly to select potential responders to treatment. The concept nonetheless has not been validated in a clinical trial. Acute stroke MRI remains the most sensitive in detecting early infarct, and gives additional information of microbleeds, but multimodal CT has improved in coverage, and in defining tissue status. It remains the most time-efficient, well-tolerated with fewer contraindications. Its use in clinical and research setting has increased.

Advanced imaging is used increasingly to select patients in research studies, although it has not itself been validated in a clinical trial.

Chapter 2 Bioproperties of thrombolytic agents and their use in acute ischaemic stroke

Acute ischaemic stroke results from intracranial artery occlusion. Timely recanalisation by IV thrombolysis or endovascular treatment restores the CBF, limits the damage to brain tissue, and improves functional outcomes. In this chapter, I review the mechanism of thrombolysis, thrombolytic agents, their pharmacological properties and clinical use.

2.1. Mechanism of thrombolysis

In the event of endothelial injury, collagen in the subendothelial matrix becomes exposed to flowing blood, and triggers the accumulation and activation of platelets. Concomitantly, tissue factor initiates blood coagulation and generates thrombin. Thrombin plays a crucial role in clot formation. It not only converts fibrinogen to fibrin, which forms the scaffolding for the thrombus, but also activates platelets. In addition, platelet activation is necessary for thrombus formation under arterial flow conditions and accompanies thrombin-mediated fibrin formation [230].

Recent research has suggested that two independent pathways of platelet activation exist [231,232]. In one pathway, platelet activation is initiated by the collagen exposed from the injured vessel wall. Platelets bind to collagen with collagen specific receptors – glycoprotein VI and glycoprotein Ib-V-IX. This adhesion is facilitated by the Von Willebrand factor (a marker of endothelial damage). In the other pathway, endothelial injury is not necessary. Tissue factor from the exposed vessel wall or present in flowing blood generates thrombin, which in turn activates platelets[233]. By cleaving to the surface of the platelet, thrombin initiates a positive feedback mechanism, which activates more platelets and leads to further fibrin network formation. The process of thrombus

formation is influenced by many other factors, such as shear stress, flow, turbulence and the number of platelets in circulation.

The endogenous thrombolytic system comprises plasminogen, Plasminogen Activators (PAs) and their inhibitors. Figure 2-1 shows a simplified pathway of thrombolysis. When a thrombus is formed in the physical environment, thrombin, a key player in clot formation, is directly involved in the conversion of plasminogen to plasmin by inducing localised generation of PAs from endothelial cells, thereby activating the endogenous fibrinolysis system. Plasmin formation is essential to the lysis of thrombi. It occurs in two places; in the plasma or on reactive surfaces such as thrombi or cells. The fibrin network provides the scaffold for plasminogen activation.

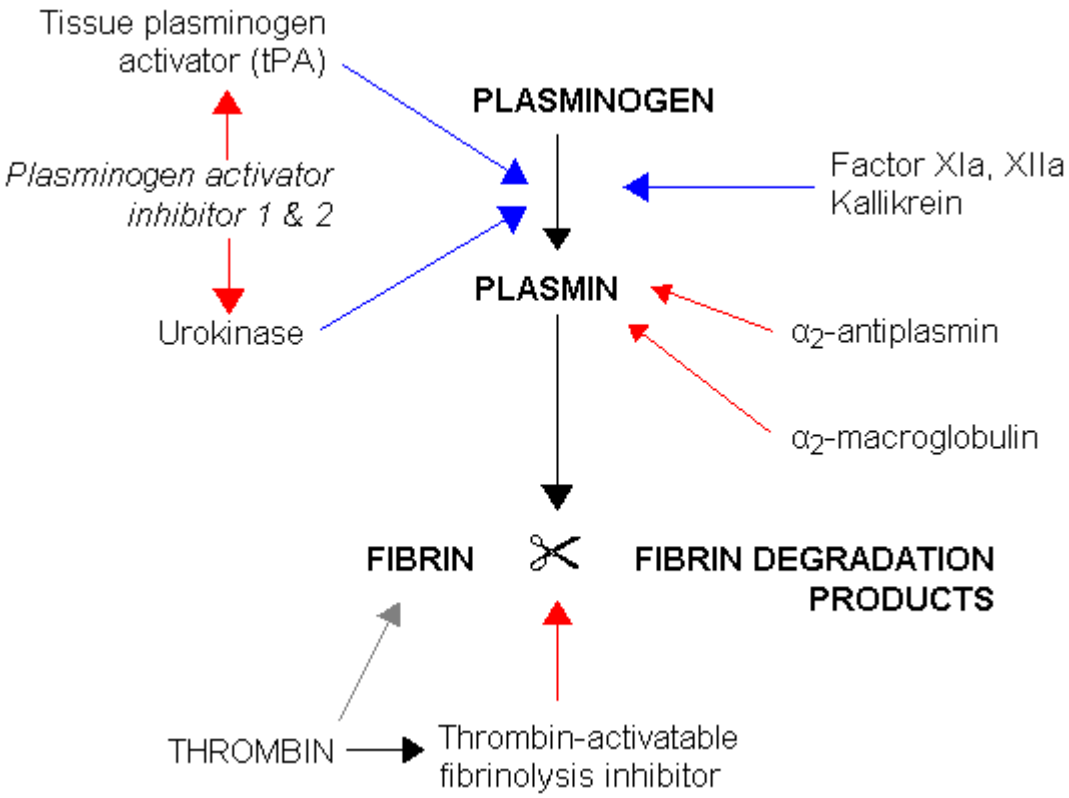


Figure 2-1. A simplified illustration demonstrates fibrinolysis from Wikipedia with blue arrows denoting stimulation, and red arrows inhibition.

The activation of plasminogen follows two different pathways. One is as an activator-catalysed conversion of Glu-plasminogen into Glu-plasmin followed by an autocatalytic conversion of Glu-plasmin into Lys-plasmin. The other is as a plasmin-catalysed conversion of Glu-plasminogen into Lys-Plasminogen followed by an activator-catalysed

conversion of Lys-plasminogen into Lys-plasmin[234]. Fibrin accelerates its own re-solution by serving as a cofactor for the activation of plasminogen[235]. Partial degradation of the fibrin network also provides a positive feedback mechanism to promote further local fibrinolysis by increasing the rate of conversion of activator-catalysed Glu-plasminogen to Glu-plasmin regardless of the activator type[236].

In the circulation, α2-Antiplasmin (α2-AP) is the main inhibitor of fibrinolysis by inhibiting plasmin directly, whereas Thrombin Activatable Fibrinolysis Inhibitor (TAFI) prevents further plasmin activation from forming a plasmin-fibrin complex[237]. Excess plasmin is inactivated by α2-Macroglobulin (α2-M). The most important inhibitor of tPA and Urokinase (UK) is Plasminogen Activator Inhibitor-1 (PAI-1), which is mainly synthesised by platelets, vascular endothelial cells and hepatocytes[238]. Its plasma concentration is low, but platelets are a reservoir of PAI-1, account for 93% circulating PAI-1[239], and release large amount of PAI-1 during aggregation. Several animal studies have suggested that PAI-1 is the major determinant of the resistance of platelet-rich thrombi lysis by tPA[240,241]. Agents with a feature of PAI-1 resistance therefore may have advantages in the lysis of platelet-rich clots.

Thrombolytic agents given at pharmacological dose significantly affect haemostasis by inducing hypofibrinogenaemia and increasing the amount of Fibrin(ogen) Degradation Products (FDPs) in circulation, inhibiting fibrin polymerization[242], reducing factors V and VIII, and prolonging the Activated Partial Thromboplastin Time (APTT) and the thrombin time[243]. As a result, the newly formed fibrin is dissolved rapidly by the active fibrinolytic system, impairing the formation of new haemostatic plugs[244], which may introduce the complication of bleeding. This disruption of haemostasis is most prominent in the first generation of PAs, such as Streptokinase (SK) and urokinase (UK).

2.2. Thrombolytic agents, and their biochemical properties (Table 2-1)

Plasminogen activators include both endogenous plasminogen activators, which are involved in physiological fibrinolysis, and exogenous plasminogen activators. Tissue type plasminogen activators and UK are considered to be endogenous PAs, whereas streptokinase (SK), Anisoylated Plasminogen-streptokinase Activator Complex (APSAC, Anistreplase), staphylokinase, desmoteplase and other variant tPAs are exogenous PAs.

So far, SK, UK, tPA, desmoteplase and tenecteplase have been examined in ischaemic stroke.

Table 2-1. Biochemical properties of common plasminogen activators

	Streptokinase	Urokinase	rtPA (Alteplase)	Tenecteplase	Desmoteplase
Direct Plasminogen activator	No	Yes	Yes	Yes	No
Plasma half-life	8-25 mins	7-18 mins	4-9 mins	15-19 mins	2.8 hours
Administration	Infusion	Infusion	Bolus followed by infusion	Bolus	Bolus
ß-amyloid stimulation	No	No	Yes	?Yes	No
Fibrin specificity	_	_	+	++	+++
Antigenicity	+++	_	_	_	+
Inhibition by PAI-1	+	+++	+++	+	+
Pro-coagulant effect	Yes	Yes[245]	Yes	No	?
Neurotoxicity		Yes[246]	Yes	?Yes	No

2.2.1. Streptokinase

Before the development of rtPA, streptokinase was the most widely used thrombolytic agent. It is a 47kDa single-chain polypeptide derived from group C β-Haemolytic streptococci[247]. When SK makes contact with human plasminogen, a 1:1 complex is formed which contains an active site allowing the conversion of circulating plasminogen to plasmin[248]. The complex itself is converted to a SK-plasmin complex via intramolecular activation[249].The SK-plasmin complex is however 10^5 times less reactive towards α2-antiplasmin than plasmin[250]. The uninhibited complex and free plasmin in circulation consume fibrinogen, fibrin and inactivate prothrombin, factors V and VIII, resulting in fibrinogen depletion (a systemic lytic state)[251].

The human body contains antistreptococcal antibodies, which probably form following previous infection with β-haemalytic streptococci. A sufficient amount of SK must be

infused to neutralise the antibodies before a thrombolytic activation is obtained[252]. The usual dose required to neutralise antibodies is at least $3.5x10^5$ U SK. The anamnestic response is generally maximal 4-7 days following the initiation of the SK infusion[230].

Streptokinase has been shown to reduce mortality in acute Myocardial Infarction (MI)[253] by recanalising the coronary artery[254]. Its use in acute ischaemic stroke proved to be unsuccessful due to the high ICH rates and mortality[255].

2.2.2. Urokinase

Urokinase is a trypsin-like serine protease composed of two polypeptide chains connected by a single disulfide bridge[256]. There are two molecular forms, High-molecular-Weight (HMW) UK (54kDa) and Low-molecular Weight (LMW) UK (31kDa). Both forms demonstrate fibrinolytic activity. During thrombolysis, there is a continuous conversion of HMW-UK into LMW-UK[257,258]. The plasma half-life of both forms is 9-12 min[230]. Urokinase is secreted as a Single-chain Urokinase Plasminogen Activator (scu-PA) inactive proenzyme form by endothelial cells, renal cells, and certain malignant cells. It converts to fully active two-chain UK following limited digestion with plasmin[259]. Scu-PA has been demonstrated to possess more fibrin specificity than two-chain UK, as significant clot lysis can be obtained without the systemic activation of the fibrinolytic system with scu-Uk, while extensive fibrinolytic activation does occur with two-chain UK in order to achieve significant clot lysis[260]. Scu-UK is inactive in human plasma due to a competitive inhibitor. The inhibition is abolished however in the presence of fibrin, resulting in the formation of fibrin-associated plasmin. This demonstrates a different mechanism of fibrin-specificity from that of tPA, which seems to be due to the fact that fibrin neutralises the competitive inhibitor rather than the fibrin enhanced activation of tPA[247].

The clinical evidence in relation to scu-UK showed that after a short lag phase, substantial amounts of the substance are converted into UK, which results in significant fibrinogen break down[261,262]. As for UK, conclusions from limited evidence are that it achieves similar efficacy and safety to tPA in treating acute MI patients, and that reocclusion during the first 24 hours may be less frequent[263].

2.2.3. Recombinant Tissue Plasminogen Activator

2.2.3.1. Alteplase

rtPa is a 70kDa single-chain glycosylated serine protease[264,265] (Figure 2-2). It consists of four domains, a finger (F-) domain, an Epidermal Growth Factor (EGF) domain, two kringle regions (K1&K2) and a serine protease domain[266]. The finger domain residues 4-50 and the K2 domain are responsible for fibrin affinity. The COOH-terminal serine protease domain contains the active site for plasminogen cleavage[230]. With limited plasmin action, the single-chain form can be converted to a double-chain form linked by disulfide bonds[267,268]. Both forms are enzymatically active and have relatively fibrin-selective properties[268,269]. It has been suggested that physiological fibrinolysis induced by native one-chain t-PA nevertheless occurs mainly via the two-chain derivative[269]. tPA is cleared by the liver[270].

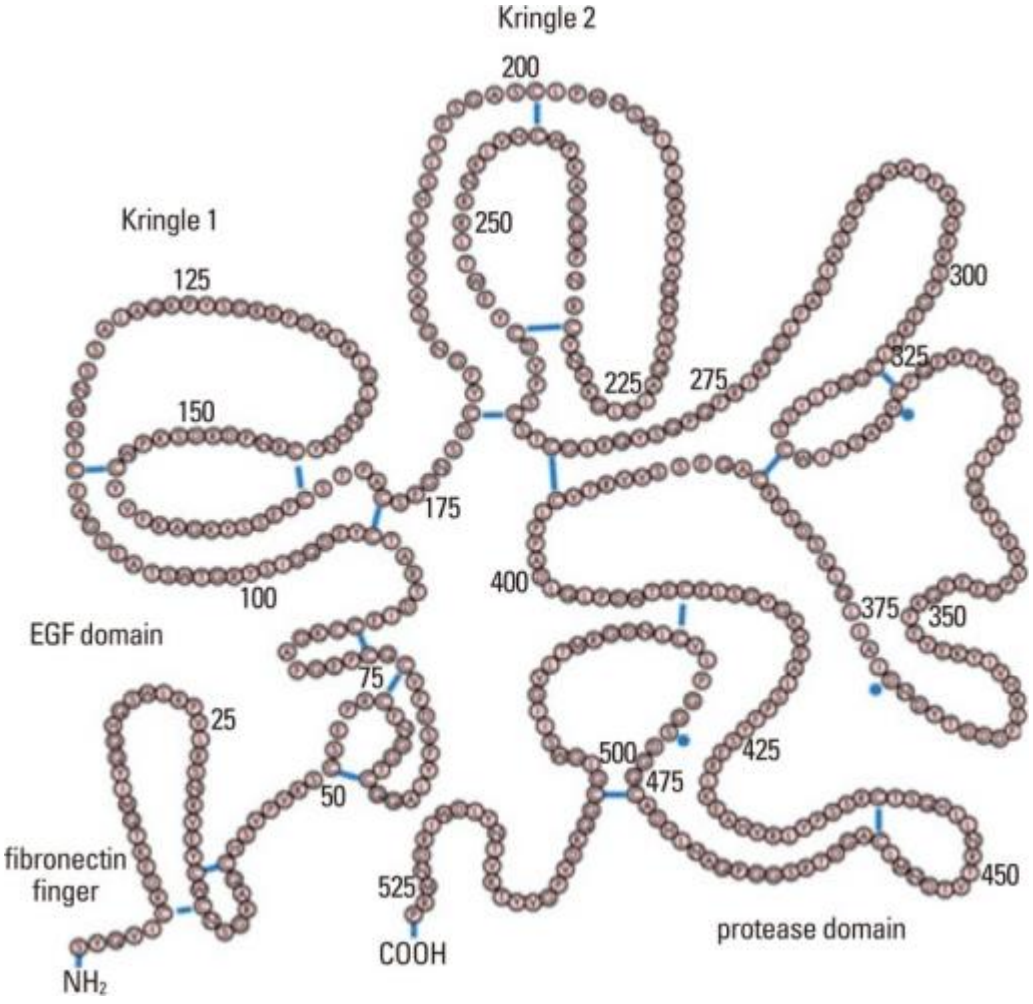


Figure 2-2. Biochemical structure of alteplase (reproduction from "Review of stroke thrombolytics" Journal of Stroke 2013[271])

Alteplase has a certain degree of fibrin affinity, a well-established character that results in less disruption to the haemostasis system, and is hence a safer option than the first generation thrombolytics[272]. It is a poor activator in plasma in the absence of fibrin. By

interacting with exposed lysine residues in partially degraded fibrin with lysine binding sites located in both tPA and plasminogen, its ability to activate plasminogen significantly increases[273].

Prolonged infusion of alteplase is required to maintain plasma concentration due to its short plasma half-life of 4-9 minutes[270,274,275]. As a result acute MI investigators have taken steps to identify the optimal infusion regime to lyse clot[275]. The paradoxical procoagulant effect[276] of alteplase is undesirable in clinical practice. It is also susceptible to PAI-1 inhibitation[277] by forming a stable complex with PAI-1.

It has also been suggested that alteplase is linked to the development of cerebral oedema secondary to its property of increasing BBB permeability, and is associated with neurotoxicity leading to neuronal death[278]. These observations flowed from animal studies, and have not been replicated in human studies.

Extensive clinical evidence supported the replacement of streptokinase with alteplase as the main thrombolytic agent in acute MI patients. The Global Utilisation of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) study[279] compared four IV thrombolysis strategies in MI and showed that accelerated alteplase plus IV heparin reduced mortality at 30 days with a net clinical benefit in comparison to other groups. The haemorrhagic stroke rate in this group was nevertheless higher than the two streptokinase groups (2 more ICH in every 1000 patients treated).

The initial ground-breaking NINDS study[280], the results of which were confirmed by subsequent studies[70], demonstrated that timely thrombolysis with a lower dose of alteplase in AIS improves functional outcomes. Alteplase is now the only approved treatment for AIS.

2.2.3.2. Tenecteplase

Tenecteplase is a third-generation thrombolytic agent that was specifically bioengineered to improve on a number of features compared with the native molecule. It is a 65kDa single chain glycoprotein with modification in three regions of alteplase: substitution of threonine to asparagine at amino acid 103 (T103N); replacement of asparagine at position 117 with glutamine (N117Q); and a tetra-alanine substitution at amino acids 296 to 299 (KHRR296- 299AAAA)[281] (Figure 2-3). This resultant agent has a 15-fold higher

fibrin specificity, an 80-fold reduced binding affinity to PAI-1 and a 4-fold prolonged plasma half-life (18 vs 4 minutes) in comparison to alteplase[282]. Fifteen minutes following injection, 50% of tenecteplase and 1% of alteplase respectively still remains in circulation[283]. Tenecteplase can be administrated as a single bolus and can potentially be more effective in platelet rich thrombi due to its high resistance to PAI-1. Theoretically, it should induce minimised systemic plasminogen activation while targeting the thrombi, and is therefore safer compared to other thrombolytic agents due to its high fibrin specificity. Animal experiments[283] showed that tenecteplase induces 50% lysis in one-third of the time required by alteplase when both drugs were given at the same dose (0.18mg/kg). It is 8- and 13-fold more potent in rabbits than alteplase towards whole blood clots and platelet-enriched clots respectively[283]. Compared with alteplase, time to reperfusion is significantly less with tenecteplase, and the duration of recanalisation is greater[284]. It also achieves more complete lysis of blood clots than alteplase with fewer overall bleeding complications. Evidence suggests that tenecteplase lacks the procoagulant effect, which may cause early reocclusion[285] seen with other thrombolytic agents. In an acute MI study, there was no increase in Thrombin-antithrombin Complex (TAT) after administration of tenecteplase, in contrast to a four-fold increase following streptokinase and a doubling after tPA[286]. In addition, a rabbit model of carotid stenosis showed that tenecteplase does not generate collagen-induced aggregation of platelets in vitro as alteplase does[284].

Tenecteplase has been studied extensively in acute MI. The major studies are: TIMI 10A[287], and the Safety Assessment of Single-bolus Administration of tenecteplase Tissue-Plasminogen Activator in Acute Myocardial Infarction (ASSENT) 1 study[288], both phase I dose ranging trials; TIMI 10B[289], a phase II non-blinded comparison of fixed doses of intravenous bolus tenecteplase and front-loaded infusion of alteplase; and ASSENT II[290], a phase III comparison of body weight adjusted tenecteplase bolus and alteplase infusion (Table 2). Evidence from these studies suggested that there is no significant difference between the two agents in terms of the rate of achieving TIMI 3 flow. The ICH rates are similar in both agents, but the rate of overall bleeding complication was much lower in the tenecteplase group[290].

Two phase II studies have compared whether tenecteplase has advantages over alteplase in AIS thrombolysis, but the study population varied between studies. Currently, a few

phase III studies are investigating the use of tenecteplase in the treatment of acute ischaemic strokes.

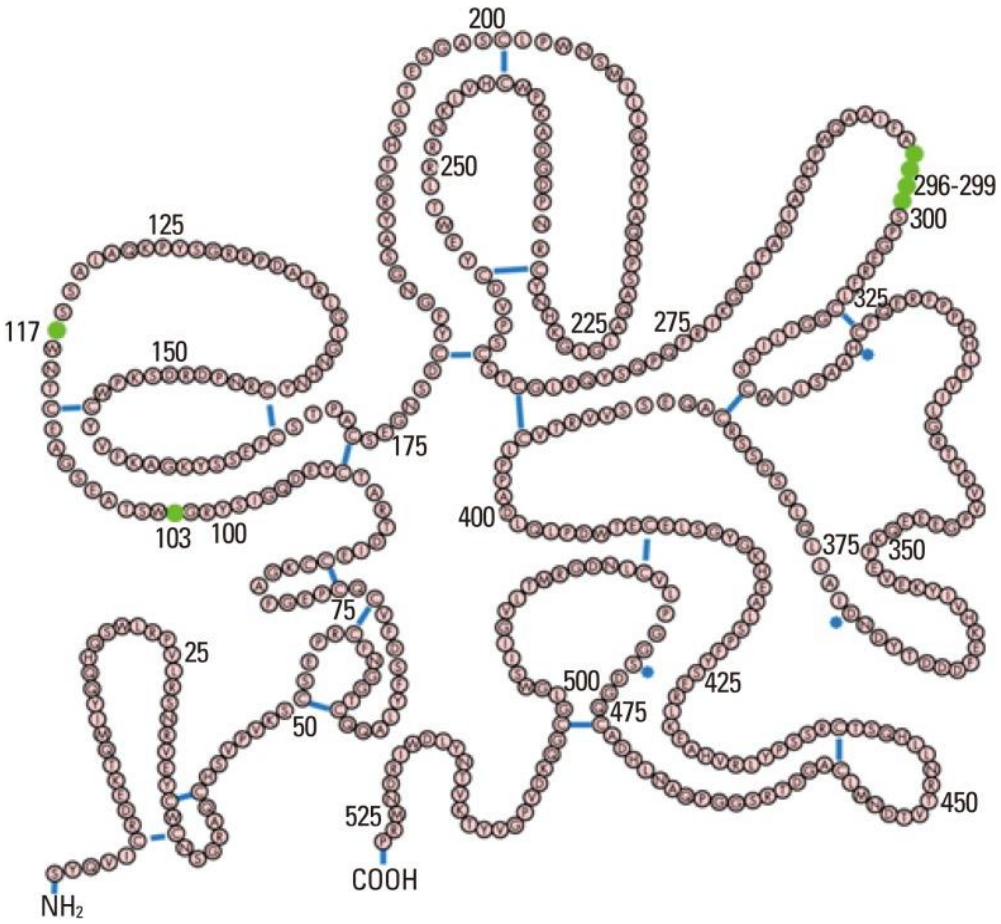


Figure 2-3. Biochemical structure of Tenecteplase (Reproduction from "Review of stroke thrombolytics" Journal of Stroke 2013[291])

2.2.4. Desmoteplase

Saliva of the vampire bat (*desmodus rotundus*) contains a family of four plasminogen activators called Desmodus Salivary Plasminogen Activators (DSPAs). Of these, DSPAα1 (Desmoteplase) has the most homologous structure to human tPA except that it only has one kringle structure and does not have the plasmin cleavage site which is necessary for conversion into a two-chain structure[292]. Desmoteplase is highly fibrin specific. It is almost inactive in the absence of fibrin, but with the presence of fibrin the activity of desmoteplase is 105,000 times higher than where fibrin is absent, whereas tPA's activity is only 550 times higher[293]. Because fibrinogen, a co-factor of plasminogen activation by tPA, does not have an effect on desmoteplase, the factor of fibrin selectivity expressed as the quotient of activity in the presence of fibrin versus activity in the presence of fibrinogen is 12,900 for DSPA but only 72 for rtPA[294]. In comparison to rtPA, desmoteplase

is neither stimulated by the native nor aggregated amyloid β-peptide analogues[295], which is thought to be a factor that contributes to rtPA induced intracranial haemorrhages[293]. Desmoteplase also lacks the neurotoxic effects of rtPA[296] and has a significantly longer plasma half-life of 2.8 hours[297]. The thrombolytic efficacy is not affected by the presence of platelets or platelet-rich clots[297].

Like streptokinase, desmoteplase could potentially stimulate antibody formation due to its non-human origin. A study with healthy volunteers suggested that unlike streptokinase, the induction of antibodies is infrequent, dose-dependent, and only observed with a higher frequency after repetitive administrations.[298]

Although the animal studies showed promising results compared to tPA, the use of desmoteplase in acute MI has been limited. A phase II open label dose finding study has taken place, but, any conclusions regarding safety and efficacy were limited by the small patient number (26 patients)[293]. Investigation in AIS thrombolysis in an extended window of 3-9 hours[299,300] has been very comprehensive, although the results were neutral[301].

2.2.5. Other tPAs

Another agent, staphylokinase – a protein produced by a certain strain of staphycoccus aureus, is completely fibrin selective. Like streptokinase, it forms a 1:1 stoichiometric complex with plasminogen. Two clinical studies, the Collaborative Angiographic Patency Trial of Recombinant Staphylokinase (CAPTORS) study [302]and CAPTORS II[303] showed promising results, however, due to safety concerns, the dose (0.05mg/kg) that can achieve similar efficacy to tPA was not evaluated.

Other third generation tPAs, such as reteplase and lanoteplase have been studied in acute MI patients. Reteplase has been shown to have an efficacy equal to alteplase, but there are concerns that it is associated with a greater incidence of reocclusion in comparison to alteplase[292] and these have limited its clinical use. Lanoteplase was not approved for clinical use due to the evidence that there was an increased rate of haemorrhagic stroke in a lanoteplase treated group.[304] Neither of these two agents has been studied in ischaemic stroke patients.

The main thrombolysis studies in acute MI using the above agents are summarised in Table 2-2. Many of them are well-conducted studies with large sample sizes, using both

angiography and clinical endpoints. The accompanying pharmacokinetic study and coagulation assays provided robust evidence for dose selection and safety evaluation.

Table 2-2. Summary of major acute MI thrombolysis studies

	Primary outcome	***Recanlisation rate (%)***	***Bleeding /reocclusion rate (%)***	***All-cause mortality (%)***	***Percentage of Fibrinogen fall (%)***
TIMI I[305] rtPA vs SK N=290	TIMI 2/3 flow at 90 mins	62% vs 31% (p<0.001)	Comparable	4.9% vs 8.2%	33% vs 58%
European Co-operative Study[306] rtPA vs SK N=129	Vessel patency at 75-90 mins	70% vs 55%	Comparable	4.7% vs 4.6%	39±65% vs 92±89%
GUSTO[307,308] rtPA vs SK N=41021	All-cause mortality at day 30	81% vs 60%	Comparable	6.3% vs ≥7.0% (p=0.001)	
TIMI 10B[289] rtPA vs TNK N=837	TIMI grade 3 flow at 90 mins	62.8% vs 62.7%	Comparable.	No significant difference	40% vs 5-10%
ASSENT II[290] rtPA vs TNK n=16949	All-cause mortality at 30 days		ICH risk comparable; Overall bleeding: 28.95% vs 26.43%, p<0.001	6.2 vs 6.2%	

rtPA, recombinant tissue Plasminogen Activator; SK, Streptokinase; TNK: Tenecteplase; TIMI, Thrombolysis in Myocardial Infarction; ICH: Intracerebral haemorrhage.

2.3. Revascularisation therapy in acute ischaemic stroke

2.3.1. Intravenous thrombolysis

Thrombolysis in ischaemic stroke differs from that in MI because of the difficulty in symptom recognition with associated delay in presentation, larger clot burden, and heterogeneity in thrombus composition. The risk of ICH is higher in ischaemic brain tissue. The three streptokinase studies[255] terminated early because of higher mortality and ICH rates rate in the SK group, highlighted important lessons: minimising time to treatment is essential[309]; individualised dose adjustment by weight is safer; and the concomitant use of anti-platelet agents increases risk of ICH[255].

2.3.1.1. Alteplase

The NINDS study[280] in which alteplase was compared with placebo demonstrated that thrombolysis in acute ischaemic stroke can improve functional outcomes if it is given

within three hours from symptom onset. Other large studies (ECASS[310], ECASS II[311], Alteplase Thrombolysis for Acute Non-interventional Therapy in Ischemic Stroke [ATLANTIS] A[312] & B[313]) nevertheless did not show that the treatment effect can be extended up to six hours from symptom onset, despite using similar methods and dose regimes (Table 2-3). The meta-analysis[314] using the combined data from the above trials confirmed that time is the critical factor in revascularisation treatment. Treatment benefit diminishes as time elapses. The subsequent ECASS III study[315] demonstrated that the treatment window can be extended up to 4.5 hours from symptoms onset. However the Number Needed To Treat (NNT) increases from 4.5 when treatment is within the the first 90 minutes of symptoms onset to 14 if the OTT is between 180-270 minutes of symptom onset[76]. One can deduce that NNT increases by 1 with each 20 minutes delay in treatment. In addition, evidence[316] suggests thrombus becomes more resistant to tPA with the increase of time. The benefit of treating patients who present beyond 4.5 hours but less than 6 hours remains unclear. The most updated meta-analysis[70] including all the large IV thrombolysis studies to date demonstrated the time dependent efficacy of rtPA administration within 4.5 hours, but no strong evidence to support treatment beyond this time window. Patients aged over 80 or who have minor neurological deficits on presentation benefit from treatment with no increasing risk of ICH.

The haemorrhagic complication rate related to IV alteplase was found to be from 2.7-15.7% in IV thrombolysis trials[317], which resulted from different definitions of symptomatic haemorrhage applied in different studies. It is understood now that haemorrhage on post thrombolysis CT is not always the culprit for neurological deterioration, which could be due to reasons such as extension of the infarction or cerebral oedema secondary to ischaemic infarction. Small haemorrhagic transformation within the infarction does not affect the clinical outcome. When stricter symptomatic ICH definition is applied (the ECASS III definition or the Safe Implementation of Thrombolysis in Stroke Monitoring Study [SIT-MOST] definition), symptomatic ICH prevalence was approximately 2% overall[315,318]. The most recent meta-analysis[70] suggests that the relative risk of ICH may not be time dependent as was previously thought, nor is it related to age, but the absolute risk of alteplase related haemorrhage was higher among patients who had more severe strokes.

IV rtPA's efficacy varies depending on the occlusion site[319] and recanalization occurs more frequently in distal branches[320]. Early recanalisation (defined as recanalisation occurring within two hours from the start of treatment) can be achieved in 42.9% of patients with M2 occlusion, in 30.7% of patients with M1 occlusion, in 11.4% of patients with basilar occlusion, and in 12.9% of patients in terminus ICA or tandem ICA/M1 occlusion[319,321]. Reocclusion is relatively common within the first two hours[322] with alteplase.

The narrow 4.5 hour treatment window is one factor that has led to gross underuse of IV thrombolysis despite intensive efforts undertaken to raise public awareness and improve acute stroke facilities. Only 10.3% of acute ischaemic stroke patients received IV thrombolysis in 2011-2012 in the UK[323].

In 2014, Medicine and Healthcare Products regulatory Agency (MHRA) launched an investigation in response to the concern of the safety of alteplase in acute ischaemic stroke thrombolysis. However, it is evident from the individual study data and especially the most recent meta-analysis[324], which was performed with individual patient data, that the benefit of thrombolysis outweighs the risk of symptomatic ICH in patients presenting within 4.5 hours from symptoms onset.

The main focus of research currently is to seek an alternative pharmacological agent or mechanical methods to reperfuse the brain more effectively in the current treatment time window, and investigate the potential of advanced imaging technology to identify suitable candidates to be treated in an extended time window.

Table 2-3. Main randomised controlled studies comparing rtPA with placebo (summarised from individual study data)

Study (year)	Number		rtPA Dose	Time window (hours)	Method	Main results*	
	rtPA	Placebo				Functional outcomes (%)	SICH (%)
NINDS[280] 1995	312	312	0.9mg/kg (Max 90mg)	0-3	Double blind	mRS 0-1 at 90 d: 43% vs 27%	13% vs 1%
ECASS[310] 1995	313	307	1.1mg/kg (Max 100mg)	0-6	Double blind	mRS 0-2 at 90 d: 29% vs 36%	6% vs 2%
ECASS II[311] 1998	409	391	0.9mg/kg (Max 90mg)	0-6	Double blind	mRS 0-1 at 90 d: 40% vs 37%	8.8% vs 3.4%
ATLANTIS A[312] 2000	71	71	0.9mg/kg (Max 90mg)	0-6	Double blind	mRS Median at 90 d: 5 vs 2	11% vs 0
ATLANTIS B[313] 1999	272	275	0.9mg/kg	3-5	Double blind	mRS 0-1 at 90 d: 41% vs 42%	6.7% vs 1.3%
ECASS III[315] 2008	418	403	0.9mg/kg (Max 90mg)	3-4.5	Double blind	mRS 0-1 at 90 d: 52% vs 45%	2.4% vs 0.2%
EPITHET[193] 2008	52	49	0.9mg/kg (Max 90mg)	3-6	Double blind	mRS 0-1 at 90 d: 36% vs 21%	7.7% vs 0
IST3[69] 2012	1515	1520	0.9mg/kg (Max 90mg)	0-6	Open label	OHS 0-1 at 180 d: 22% vs 24%	7% vs 1%

* SICH rates quoted use the individual study's own definition; IST3 International Stroke Trial 3; mRS Modified Rankin Scale.

2.3.1.2. Tenecteplase

Tenecteplase is an attractive alternative because of the theoretical advantages and the presence of extensive evidence from MI studies. All published and ongoing tenecteplase studies in AIS are shown in Table 2-4. After dose finding studies[325,326], a randomised study[327] comparing doses of 0.1, 0.25, and 0.4 mg/kg to standard alteplase within three hours discontinued recruitment to the 0.4 mg/kg dose due to a higher SICH incidence and less frequent major neurological improvements at 24 hours. Another randomised study[194] demonstrated that tenecteplase (0.1 mg/kg and 0.25 mg/kg) was superior to alteplase in achieving reperfusion, recanalisation and mean NIHSS score improvement at 24 hours in a selected group of patients with favourable perfusion lesion patterns and intracranial occlusions within six hours of onset. Since advanced imaging selection criteria were used in this study, the result is restricted to a highly selected population. To investigate tenecteplase's efficacy in the general acute ischaemic stroke population, further study is required.

Table 2-4. Summary of all completed and ongoing tenecteplase studies in AIS

Study (year) n	Design	Number		Time window (hour)	Inclusion	Efficacy Outcome (%)	SICH (%)
		TNK	Alteplase				
Haley et al[325] (2005) 88	Dose escalation	0.1mg/kg 25; 0.2mg/kg 25; 0.4mg/kg 25; 0.5mg/kg 13	Historical control in NINDS	0-3	Conventional patient selection	0-1: 36% vs 32% vs 32% vs 46% vs 13%	0 vs 0 vs 0 vs 15% vs 36%
Parsons et al[326] (2009) 20	Controlled	0.1mg/kg 15	35	3-6	Target mismatch and intracranial occlusion	0-1: 60% vs 35%	0 vs 2.9%
Molina et al[328] (2008) 122	Controlled	0.4mg/kg 42	80	0-3	MCA occlusion	0-2: 66% vs 52%	2.1% vs 3.7%
Haley et all[327] (2010) 112	Double blinded, Phase IIB/3	0.1mg.kg 31; 0.25mg/kg 31; 0.4mg/kg 19;	31	0-3	Conventional patient selection	0-1: 45% vs 48% vs 32% vs 39%	0 vs 6.5% vs 15.8% vs 3.2%
Parsons et al[194] (2012) 75	PROBE, phase IIIB	0.1mg/kg 25; 0.25mg/kg 25;	5	0-6	Target mismatch and intracranial occlusion	0-1: 36% vs 72% vs 40%	4% vs 4% vs 12%
TEMPO-1[329] (2015) 50	Single arm cohort	0.1mg/kg 25; 0.25mg/kg 25		0-12	NIHSS<5, and proximal occlusion	0-1: 76% vs 56;	4% vs 0%
NOR-TEST[330] (ongoing) 954	PROBE Phase III	0.4mg/kg		0-4.5; 0-4.5 from wake-up; 0-6 for embolectomy	Conventional patient selection, wake-up/embolectomy: target mismatch/proximal occlusion		
TASTE[204] (ongoing) 1024	PROBE Phase III	0.25mg/kg		0-4.5	Target mismatch and intracranial occlusion		
TEMPO-2[331] 1274	PROBE Phase III	0.25mg/kg	antiplatelets	0-12	NIHSS<6, ASPECT>5, and proximal occlusion		

The appropriate dosage of tenecteplase that should be used in stroke thrombolysis remains unclear. An initial dose escalation study[325] discarded doses of 0.5mg/kg and higher because of the high incidence of ICH. The subsequent phase II study[327] comparing 0.1, 0.25, 0.4mg/kg against standard alteplase (0.9 mg/kg) discarded a 0.4 mg/kg dose for failing to show superiority based on an adaptive sequential design with combined early neurological improvement and ICH rates. The ICH rate nevertheless was not significantly higher than for other dose groups. The study terminated before it could distinguish the efficacy between the 0.1 and 0.25 mg/kg doses. Parsons et al[194] however did demonstrate the superiority of a 0.25 mg/kg dose to 0.1mg/kg in delivering reperfusion and clinical benefits.

Two phase III studies are currently recruiting: the Tenecteplase versus Alteplase for Stroke Thrombolysis Evaluation (TASTE) trial[204] (ACTRN12613000243718) uses imaging based patient selection examining 0.25mg/kg TNK, while the study of tenecteplase versus alteplase for thrombolysis in acute ischaemic stroke (NOR-TEST)[330] (NCT01949948) compares 0.4mg/kg TNK with standard alteplase.

2.3.1.3. Desmoteplase

Desmoteplase studies pioneeered the use of imaging-based criteria in assisting patient selection with the application of the mismatch concept in a much later time window of 3-9 hours after stroke. Two initial dose-escalation studies in a 3-9h window population selected using MRI mismatch criteria[332,333] suggested a dose-response effect. However, a subsequent trial testing two doses against a placebo failed to confirm efficacy. An excess of less severe strokes, low rates of vessel occlusion at baseline, mixed imaging modalities for patient selection, and small sample sizes may explain the discordant findings[334]. Revised to select patients on the basis of arterial occlusion rather than MRI mismatch, desmoteplase did not appear to be superior to placebo in achieving good functional outcomes at three months in patients presenting between 3-9 hours from symptoms onset in DIAS 3[301].

2.3.2. Endovascular reperfusion therapy

Endovascular reperfusion therapy with devices or IA thrombolysis achieves much higher recanalisation rates in comparison with IV rtPA[335]. In 2012, the first three randomised trials[196,336,337] comparing its efficacy against IV therapy disappointingly showed no benefit over IV therapy. A pooled analysis[338] of earlier endovascular trials suggested that almost half of recanalisations were "futile" – ie not associated with favourable clinical outcome. A time to treatment stratum of 3-6 hours was associated with futile recanalisation. Compared with IV therapy, delays are common in endovascular therapy, such that the benefit of the high recanalisation rates may be cancelled[336]. Other factors that had a possible impact on the failed studies included the use of first and second generation devices, which have much lower recanalisation rates compared with the recent stent retriever. It has been suggested that if IV therapy is started within two hours from symptom onset, and IA therapy can be initiated within 90 minutes after the start of IV therapy, endovascular treatment may be beneficial[337]. Following the Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands (MR CLEAN)[206], the first successful RCT to compare modern endovascular treatment with standard care, similar studies around the world overwhelmingly showed the superiority of intra-arterial treatment. (Table 2-5). The common feature of these studies was that the median onset to femoral puncture times were all less than 4.5 hours, notwithstanding that some studies permitted inclusion of patients up to 12 hours from symptom onset. The patient selection methods varied among these studies. Some used vascular imaging and collaterals, some used additional tissue imaging. In MR CLEAN, target mismatch was not one of the selection criteria, but two-thirds of patients had perfusion imaging.

It is evident that endovascular treatment is a highly effective therapy which is only suitable for a particular group of AIS patients, but the treatment time window that ensures benefit, and the patient selection criteria remain unclear. To apply intra-arterial therapy in clinical practice, in addition to the requirement of significant service reorganisation, it is crucial that we have solutions to these issues. Even so, it is likely to be only available in highly specialised stroke centres and in a highly selected populations. The search for a better pharmacological agent will remain relevant for a majority of ischaemic stroke patients.

Table 2-5. Randomised controlled studies for endovascular therapy to date

Study N (IAT vs CTL)	Selection criteria	Treatment arms	Device	Onset to Puncture Time (mins)	Results	
					mRS 0-2 at 90 day	SICH
IMS 3[337] 656 (434 vs 222)	NIHSS≥10, or intracranial occlusion with NIHSS≥8 0-3 hours	IV rtPA+ intervention vs IV rtPA alone	1st & 2nd generation		43% vs 40%	6.2% vs 5.9%
MR Rescue[196] 127 (70 vs 57)	Target mismatch 0-8 hours	Intervention vs standard care	1st & 2nd generation		41% vs 49%	9% vs 6%
Synthesis[336] 362 (181 vs 181)	Eligible for IV rtPA 0-4.5 hours	Intervention vs Iv rtPA	1st & 2nd generation	225	42% vs 46%	6% vs 6%
MR CLEAN[206] 500 (233 vs 267)	Proximal occlusion 0-6 hours	Intervention + standard care vs standard care alone	Majority used stent retriever (86.1%)	260	33% vs 19%	7.7% vs 6.4%
ESCAPE[207] 315 (165 vs 150)	ASPECT 6-10, Proximal occlusion 0-12 hours	Intervention + standard care vs standard care alone	Stent retriever	220	53% vs 29%	3.6% vs 2.7%
SWIFT PRIME[197] 196 (98 vs 98)	Proximal occlusion, and target mismatch 0-6 hours	Intervention + IV rtPA vs IV rtPA	solitaire	224	60% vs 36%	0 vs 3%
EXTEND-IA[198] 70 (35 vs 35)	Proximal occlusion, and target mismatch 0-6hours	Intervention + IV rtPA vs IV rtPA	solitaire	210	71% vs 40%	0 vs 6%
REVASCAT[208] 206 (103 vs 103)	Proximal occlusion, ASPECT>7 on CT, or ASPECT>6 on DWI 0-8 hours	Intervention + standard care vs standard care alone	solitaire	269	44% vs 28%	1.9% vs 1.9%

2.3.3. Other alternatives to standard IV thrombolysis, or adjunctive therapies

2.3.3.1. Modified alteplase regimes

While alteplase 0.9mg/kg is the RCT-supported dose, some Asian countries advocate a reduced dose of 0.6mg/kg, asserting ethnic differences and possibly lower haemorrhagic risk[339], as well as greater affordability. Low dose alteplase (0.6mg/kg) is the only approved stroke thrombolysis treatment in Japan, but is supported only by small, non-randomised series[340,341] and observational data[342,343], and disagreement exists even within local medical communities[344,345]. The Enhanced Control of Hypertension and Thrombolysis Stroke study (ENCHANTED) trial is the first large RCT to address rtPA dose (as well as addressing BP lowering), which showed that the low dose alteplase is not inferior to the standard dose, but has significant lower risk of SICH[346].The majority of the study recruitment was drawn from an Asian population, such that while the result will support the use of low dose alteplase in Asian country, it is unlikely to change the practice in the West.

2.3.3.2. Liposomal tPA

Drug delivery direct to the thrombus has the potential advantages of enhancing clot lysis and reducing adverse events by allowing smaller doses[347]. Liposome[348]-encapsulated PA has a longer half-life due to the avoidance of premature systemic release and inactivation by circulating inhibitors[349], and in animal models liposomal PAs digested significantly more thrombi in a shorter time than their native forms[350,351]. Adjunctive transcranial doppler ultrasound, by disrupting liposomes, may offer targeted local drug release at the site of arterial occlusions.

2.3.3.3. Sonothrombolysis

Diagnostic frequency 2MHz TCD ultrasonography has microstructural effects on fibrin strands and agitates rtPA and blood at the thrombus surface, thereby enhancing thrombolysis by IV rtPA[352]. Adjunctive TCD was associated with higher recanalisation rates and a greater likelihood of functional independence in five clinical trials involving 233 patients[353,354]. The recently completed phase III RCT Combined Lysis of Thrombus With

Ultrasound and Systemic Tissue Plasminogen Activator for Emergent Revascularization (CLOTBUST-ER) in Acute Ischemic Stroke study tested an ultrasound-delivery headset that is largely operator-independent as an adjunct to IV rtPA (NCT01098981), but was terminated on grounds of futility at a planned iterim analysis. In a small RCT, one dose of 1.4 ml of microspheres, in addition to rt-PA, and insonation showed increased recanalisation compared to rt-PA alone, but 2.8mL of microspeheres was associated with higher ICH incidence[355].

2.4. Neuroprotective agents

Aiming to "freeze the penumbra" or prevent reperfusion injury, neuroprotectants include a variety of antioxidants, anti-inflammatory drugs and other therapies. Despite success in animal models, none of the early neuroprotectives has demonstrated efficacy in human trials. Many reasons have been put forward for the failure to translate the success in preclinical experiments to humans[356]. Among those, the time window of drug administration is an essential factor. In an analysis of six large neuroprotectants studies, 92% of patients were enrolled beyond three hours[357], and animal studies indicated that most neuroprotectives are ineffective when administrated more than four hours after occlusion[356]. The Field Administration of Stroke Therapy – Magnesium **(**FAST-MAG) study[358] addressed this issue by giving treatment in a pre-hospital setting within two hours from symptoms onset. It nonetheless did not show any benefits over placebo. High dose IV albumin[359] was also shown not to be beneficial. Therapeutic hypothermia[63] is currently under investigation.

2.5. Other evidence based management for acute ischaemic stroke

2.5.1. Stroke unit care

Early admission to a specialist stroke unit reduces mortality, shortens the hospital stay and improves independence[360]. The recent updated systematic review indicated that a dedicated stroke ward is significantly better in all outcomes in comparison to a mobile stroke team or a mixed rehabilitation ward[361]. There is a suggestion[362] that a comprehensive stroke unit (combining acute and rehabilitation units) may reduce the length of stay, mortality, and dependency compared to other forms of stroke unit. To

date, no randomised controlled trial has been conducted to demonstrate this. Patients with ICH benefit at least as much as ischaemic stroke patients[363].

2.5.2. Antiplatelets

Early administration of aspirin in acute ischaemic stroke (≤48 hours) has been shown to significantly reduce the risk of recurrent strokes, improving survival and functional outcomes, with a small increased of risk of ICH (0.2%) compared with control[364,365]. Early anti-coagulation, on the other hand, compared with aspirin, is associated with a significant increase in mortality and a risk of haemorrhage, with no net benefit on long term functional outcome[366]. A recently completed Chinese study[367] suggested that short-term use of dual anti-platelet agents (aspirin and clopidogrel) prevents early recurrent stroke in patients who had a minor stroke or a Transient Ischaemic Attack (TIA). An ongoing similar study[368] elsewhere is examining the same question.

2.6. Conclusions

Among the many thrombolytic agents, tenecteplase and desmoteplase demonstrate the best pharmacological profile and theoretical advantages, but the current approved therapy is still IV alteplase within 4.5 hours from symptoms onset in AIS. Studies investigating tenecteplase are ongoing. Desmoteplase did not prove to be beneficial compared with placebo in AIS patients with evidence of vessel occlusion between 3-9 hours from symptoms onset.

Intra-arterial therapy is superior to IV therapy alone in treating severe stroke due to larger artery occlusion in the anterior circulation, but its application will be limited to a highly selected group of patients in specialised centres, and the selection criteria remain unclear from currently available studies. IV thrombolysis will remain a significant part of recanalisation therapy for majority of patients. Seeking a better alternative thrombolytic agent is worthwhile.

Chapter 3 Materials and methods

3.1. Introduction

This chapter provides a detailed description of the general methods used in the studies presented in this thesis. Methodology, protocol and endpoints relevant to individual studies are described in each study presented.

Patient assessment, recruitment, follow-up, and clinical data collection was carried out by the stroke research team in the Southern General Hospital (now the Queen Elizabeth University Hospital), Glasgow, Scotland. Imaging data were collected with the assistance of radiographers. Imaging post-processing analysis was performed by the author of this thesis. Part of the analysis was repeated by a second clinical research fellow whose main project was based on CT based imaging analysis. Inter-observer agreement was calculated in these cases.

3.2. The ATTEST study

The Alteplase – Tenecteplase Trial Evaluation for Stroke Thrombolysis (ATTEST) pilot phase is a Prospective Randomised Open label Blinded End-Point (PROBE) study that compares markers of biological activity to inform the design of a larger definitive trial comparing the efficacy and safety of alteplase and tenecteplase as thrombolytic agents in eligible patients with acute ischaemic stroke.

The study was performed between January 2012 and September 2013. All patients were recruited from the Acute Stroke Unit, Southern General Hospital, Glasgow, Scotland. The study was funded by the Stroke Association.

The primary and secondary outcomes are shown in Table 3-1.

Patients were eligible for recruitment into the study if they had a clinically diagnosed supratentorial acute ischaemic stroke with a measurable deficit on the NIHSS[369], were within 4.5 hours of symptoms onset, were aged ≥18 years, were living independently pre-stroke, and were considered eligible for IV thrombolysis according to clinical guidelines. Table 3-2 shows the full inclusion and exclusion criteria. Anticoagulant and antiplatelet drug treatments (including aspirin, clopidogrel, heparin and warfarin) should be avoided for at least 24 hours after thrombolytic drug treatment, and usually until after follow-up brain imaging. In lactating patients breast milk must be discarded and not used for feeding within the first 24 hours after thrombolytic therapy.

Table 3-1. Primary and secondary outcomes for the ATTEST study

Primary Endpoint • Percent penumbral salvage at 24-48 hours (initial CTP-defined penumbra volume versus 24-48 hour CT infarct volume)
Secondary endpoints • Proportion of patients exhibiting recanalisation (measured by CTA) 24-48 hours post treatment • Early Clinical improvement (NIHSS score reduced by 8 points, or 0 or 1) 24 hours post treatment • Proportion of patients with symptomatic ICH (SICH) on 24-48h CT: • SITS-MOST definition - PH2/PHr2 + NIHSS deterioration by 3-4 points at 24h • Any ICH • Distribution of functional outcome scores (mRS) at Day 30 and Day 90 • Proportion of patients with favourable clinical outcome (mRS 0-1) at Day 30 • and Day 90 • Average “home time” (number of nights spent in non-institutional private residence) by Day 90 • Mortality at Day 90

Table 3-2. Inclusion and exclusion criteria for the ATTEST study

Inclusion Criteria	
1	Clinical diagnosis of supratentorial acute ischaemic stroke with score of ≥1 on the NIH Stroke Scale
2	Male or non-pregnant female ≥18 years of age
3	Within 4.5 hours of onset as defined by time since last known well
4	CTP and CTA examinations acquired prior or immediate after the initiation of treatment
5	mRS ≤2 pre-admission
Exclusion Criteria	
1	Contraindications to thrombolytic drug treatment for stroke • Evidence of intracranial haemorrhage or significant non-stroke intracranial pathology (including CNS neoplasm, aneurysm or Arteriovenous malformation) on pre-treatment CT • Established hypodensity on pre-treatment brain CT of more than one third of the MCA territory or ASPECT score <4 (sulcal effacement or loss of grey white differentiation in cortical territories alone are not counted towards ASPECT score) • Hypodensity consistent with recent cerebral ischaemia other than the presenting event • Very severe stroke (eg NIHSS>25) • Systolic blood pressure> 185 or diastolic BP> 110 mm Hg, or aggressive management (intravenous pharmacotherapy) necessary to reduce BP to these limits • If on warfarin, INR >1.4 • Current prescription of non-warfarin oral anticoagulant drugs; • Significant abnormality of coagulation parameters pre-treatment (prolonged INR or APTT, or platelet count <100,000/mm3) • Administration of heparin within the previous 48 hours and a thromboplastin time exceeding the upper limit of normal for laboratory, or use of therapeutic dose low molecular weight heparin within 48h • Clinical history suggestive of subarachnoid haemorrhage even if no blood is evident on CT • Risk of bleeding (Major surgery within previous 1 month; intracranial or spinal surgery; recent trauma to the head or cranium; prolonged cardiopulmonary resuscitation (> 2minutes) within the past 2 weeks; acute pericarditis and/or subacute bacterial endocarditis; acute pancreatitis; severe hepatic dysfunction, including hepatic failure, cirrhosis, portal hypertension (oesophageal varices) and active hepatitis; active peptic ulceration; any known history of haemorrhagic stroke or stroke of unknown origin; arterial aneurysm and known arteriovenous malformation) • Dependent (mRS 3-5) pre-stroke • Blood glucose <2 mmol/l or >18 mmol/l • Seizure at onset of symptoms unless brain imaging identifies positive; evidence of significant brain ischaemia (eg CTA confirmed arterial occlusion, early ischaemic change on plain CT, hypoperfusion on CTP) • Pregnancy

2	Known history of impaired renal function with an eGFR <30 mL/min pre-treatment precluding contrast CT
3	Known allergy to radiological contrast
4	History of allergies to active substances in either trial medication, or to excipients including Gentamicin
5	Severe concurrent medical condition that would prevent participation in study procedures (e.g. cardiac failure with severe pulmonary oedema) or with life expectancy ≤ 3 months.
CNS Central Nervous System; INR International Normalised Ratio	

Patients presenting with infratentorial ischaemic strokes were not included due to the limited coverage of CT perfusion. Our exclusion criteria reflected the current clinical interpretation of thrombolysis contraindication in stroke[70,370], and the availability of extended imaging studies at the time of randomisation. We therefore allowed the inclusion of those aged over 80 years, with minor neurological deficits, a previous history of stroke and concomitant diabetes, or seizure at the onset of stroke if advanced imaging confirmed the presence of an ischaemic lesion. Patients with other standard contraindications for alteplase[311,315] were excluded, as were those with contraindications for radiological iodinated contrast, including eGFR < 30 mL/min or with a previously identified allergy to contrast. The protocol was approved by the ethical committee responsible for trials involving adults with incapacity (Scotland A REC, Reference: 11/SS/0039); this study's clinical trial registration number is NCT01472926.

All patients who were referred for possible thrombolysis to the acute stroke unit were screened by a member of the clinical research team. Once the decision in favour of thrombolysis was made by a clinician, patients or their representatives (if patients did not have the capacity to consent) were approached for study consent. Witnessed consent and consent by an independent physician was permitted if the patient could not write due to physical impairment or in the event of the patient lacking capacity and no representative being available. There was no restriction of time during the day for patient recruitment. During the study period, all patients suitable were approached for study inclusion.

Patients were able to withdraw from the study at any time for any reason with an option of continuing clinical follow-up offered for those who were unwilling to undergo further imaging. Patients could also be withdrawn at the discretion of the clinical research staff if

they were found to have an alternative diagnosis to that required by the study protocol. All data collected prior to withdrawal were retained.

All patients who arrived in the acute stroke unit received a full clinical assessment including the symptom onset time, past medical history, family history, and medication. The severity of the stroke was evaluated with the NIHSS. The pre-morbidity functional status was recorded using a modified Rankin Scale. Baseline blood pressure, pulse, temperature, capillary glucose and weight were measured. There was no study specific laboratory test. As determined by routine clinical procedures, all patients had a baseline electrocardiogram (ECG), urea and electrolytes, eGFR, blood glucose, full blood count, and coagulation. The study specific procedures included the assessments of the NIHSS scale at 24 hours, 72 hours and day-7 (or at discharge if earlier); modified Rankin Scale assessments were carried out at days 30 and 90. Capillary glucose was monitored every four hours in the first 48 hours from admission; vital signs were checked as per stroke unit protocol. The full study schedule is shown in Table 3-3. mRS assessment was performed at 30 days and 90 days via telephone interview. All clinical assessments were performed by trained observers. Whilst observers performing clinical assessments were not informed of treatment allocation at the time of follow-up, as a single-centre trial, blinding to treatment allocation for clinical end-points could not be guaranteed.

Concomitant medications were recorded, as were all adverse events occurring from the point of study entry until 90 days post stroke, the defined study completion time.

Treatment decisions were made by a clinician based on clinical judgement and the NCCT excluding ICH. Following written informed consent, a central computerised system which used a minimisation algorithm to balance allocation by age group (≤80, >80) and baseline NIHSS score (1-9, 10-15, ≥16) randomly assigned patients in a 1:1 ratio to standard dose alteplase (0·9mg per kilogram to a maximum 90mg, with 10% of dose as initial bolus, followed by 90% over one hour infusion) or tenecteplase (0·25mg per kilogram, to a maximum 25mg as a single bolus). Clinicians responsible for routine clinical care were aware of treatment assignment since additional infusion over 1 hour is required for alteplase administration. Patients were not informed of treatment allocation. The majority lacked capacity at the time of treatment, and would only have been aware of treatment allocation if they had prior knowledge of the different modes of drug administration (Figure 3-1)

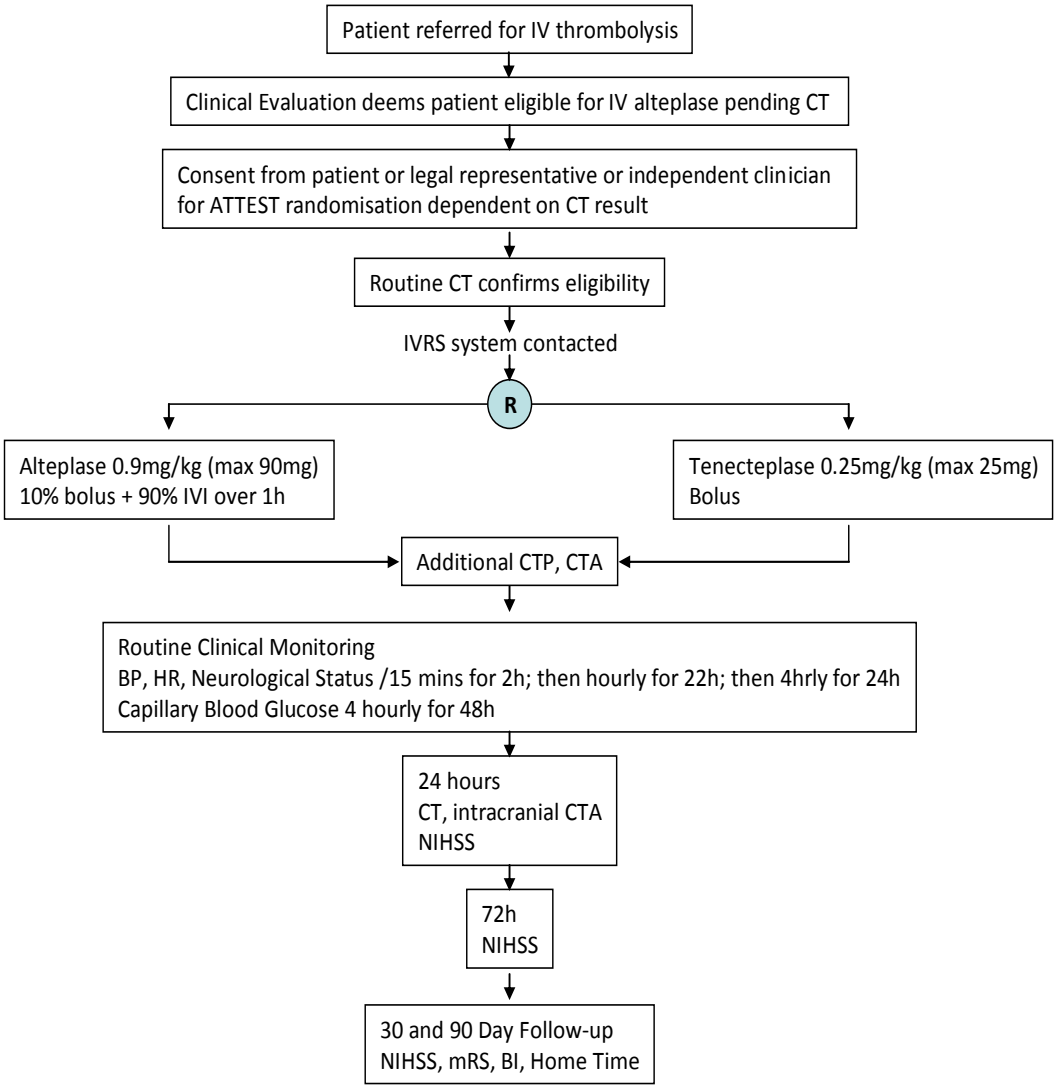


Figure 3-1. ATTEST study flow chart

Table 3-3. Scheduled assessments for the ATTEST study

Study Procedure	Pre- Randomisation	Randomisation	0-2h	2-24h	24-48h	72 h	Day 7 (±2)	Day 30 (±5)	Day 90 (±7)
Obtain Informed Consent or assent from next of kin	√								
Review Inclusion/Exclusion Criteria	√								
CT brain	*				×				
CT perfusion	X								
CT Angiography	X				√				
Vital Signs (temperature, blood pressure, pulse, respiration rate)	*				√	√	√		
Post-thrombolysis observations (BP, pulse)			*[1]	*[2]					
Capillary Blood Glucose	*		*[3]	*[3]					
Physical Examination-NIHSS	*				×	√	√		
Weight	*								
Bloods - Haematology and Biochemistry	*								
IVRS		√							
Pregnancy Test (female patients of child bearing potential)	√								
Trial Drug		√							

Administration									
mRS	√							√	√
Adverse Events Evaluation					√	√	√	√	√
Home time evaluation									√

√study-specific procedure; *clinically routine procedure (data captured for study); ×procedure clinically routine in some patients

[1] BP and pulse monitored every 15 minutes for 2h after starting IV thrombolysis

[2] BP and pulse monitored hourly from 2-24h after starting IV thrombolysis

[3] Capillary thrombolysis for 48 hours blood glucose monitored 4 hourly after starting IV

3.3. Definition of outcomes

We defined the volumes of tissue fulfilling characteristics of the ischaemic penumbra (tissue that is hypoperfused but potentially salvageable) and core (hypoperfused tissue that will inevitably infarct) based on established probabilistic thresholds[147] for CT perfusion. The primary outcome measure was the percentage of penumbral salvage at 24-48 hours post treatment[56,58] using the baseline CTP-defined penumbra plus core volume minus final infarct volume on follow-up NCCT.

Recanalisation was defined as TIMI grade 2-3[225] on follow-up CTA[371]; early clinical improvement was defined by a reduction of the NIHSS score of eight points or more, or an NIHSS score of zero or one at 24-48 hours post treatment; patients who achieved mRS 0-1 at 30 and 90 days[76,315] were deemed to have had an excellent outcome. Safety outcomes were the proportion of patients with Symptomatic Intracerebral Haemorrhage (SICH) at 24-48 hours post treatment defined by: i) the SITS-MOST[318] criteria (Parenchymal haemorrhage [PH] type 2 or remote PH2 on 24-48h NCCT, plus neurological deterioration of ≥4 points NIHSS score); and ii) the ECASS II definition[311] (any ICH on follow-up NCCT with clinical deterioration); and iii) any ICH.

3.4. Study data recording and transfer

All patients were identified by a study number in the form of 01-xxx, with 01 as the site number, and the subsequent three digits as the patient number.

All clinical data were recorded on a paper based study workbook which was stored at our research office and subsequently transferred onto a web-based electronic data system. The electronic data system was designed and maintained by the Robertson centre for biostatistics, University of Glasgow, who oversaw the data management and was responsible for statistical analysis.

3.5. Study imaging

Baseline imaging comprised NCCT, and CT perfusion and CTA. Since CTP and CTA were study imaging only, treatment decisions were not made on the basis of this information. They can be acquired either before or immediately after the study drug administration.

Follow-up imaging included NCCT and an intracranial CTA at 24-48 hours post thrombolysis. Additional imaging could be carried out at the clinician's discretion.

All scans were carried out on a Philip 64 slice multidetector scanner. Whole brain NCCT was acquired first, (5mm slice thickness FOV 218 x 218mm, 120kv, 171mA or 0.9mm slice thickness, Field of View [FOV] 250x250mm, 120kV, 404mA) followed by CTP with 40mm slab coverage from the basal ganglia (8x5mm slices, FOV 25cm, 80kVp, 476mAs, 2 second cycle time, 30 cycles) using a 50mL contrast bolus administered at 5mLs/second (350 Xenetix) via a large-gauge cannula. The subsequent CTA covering from the aortic arch to the top of the lateral ventricles (0.67mm slice thickness, 120kV, 475mA) was acquired during the first arterial pass of contrast (Xenetix 350, 60mLs, followed by 30mLs of saline bolus, both given at 5mL/sec).

3.6. Imaging processing and analysis

CT workstations were only used to undertake processing of CTP and CTA for clinical reporting and, if required, to inform clinical management.

Study imaging records were transferred from clinical scanners or radiology archives after removal of individual identifiers from the Digital Imaging and Communications in Medicine (DICOM) file (patient name, date of birth, hospital number or similar unique identifier) and were identified with the study number. All imaging analysis was undertaken on separate secure computer workstations using a commercially available software package MIStar (Apollo Medical Imaging Technology, Melbourne, Australia).

All imaging was analysed by two clinical research fellows (XH and BC) separately after the study was completed in order to minimise the likelihood of linking scans with treatment allocation. Inter-observer agreement was assessed for all parameters analysed. Volumetric analysis was carried out by each research fellow twice. The average of the four readings was taken as the final reading for analysis. Disagreements in categorical readings were resolved by the consensus of experienced neurologists or neuroradiologists who were also blinded to treatment allocation. The adjudication results of ICH on follow-up NCCT according to the ECASS II radiological definition[311] were used for the final safety outcome analysis.

Baseline NCCT was assessed for early ischaemic changes using the ASPECT score[128] with 5mm thickness slices imaging, and the presence or absence of hyperdense vessels with 0.5mm thickness slices imaging.

The MIStar software package provides four parameters when processing CTP: cerebral blood flow, cerebral blood volume, mean transit time, and delay time. Arterial input function and Venous Output Function (VOF) were manually selected from a Region of Interest (ROI) in the contralesional anterior cerebral artery and superior sagittal sinus respectively when possible. Motion correction was applied automatically using package provided options. A noise elimination technique "closing clustering" of <5mm^2 was used to minimise the small artifactural pixels probably induced by noise[142]. After AIF and VOF selection, a Hounsfield Unit filter was applied in order to mask CSF (15) and bone (700), leaving only brain parenchyma as far as possible. Voxels with CBV values >90cm^3/100g were then masked, as arterial voxels mimic tissue areas of high perfusion and their exclusion improves the accuracy of CTP relative to PET[372] (Figure 3-2 to 3-4).

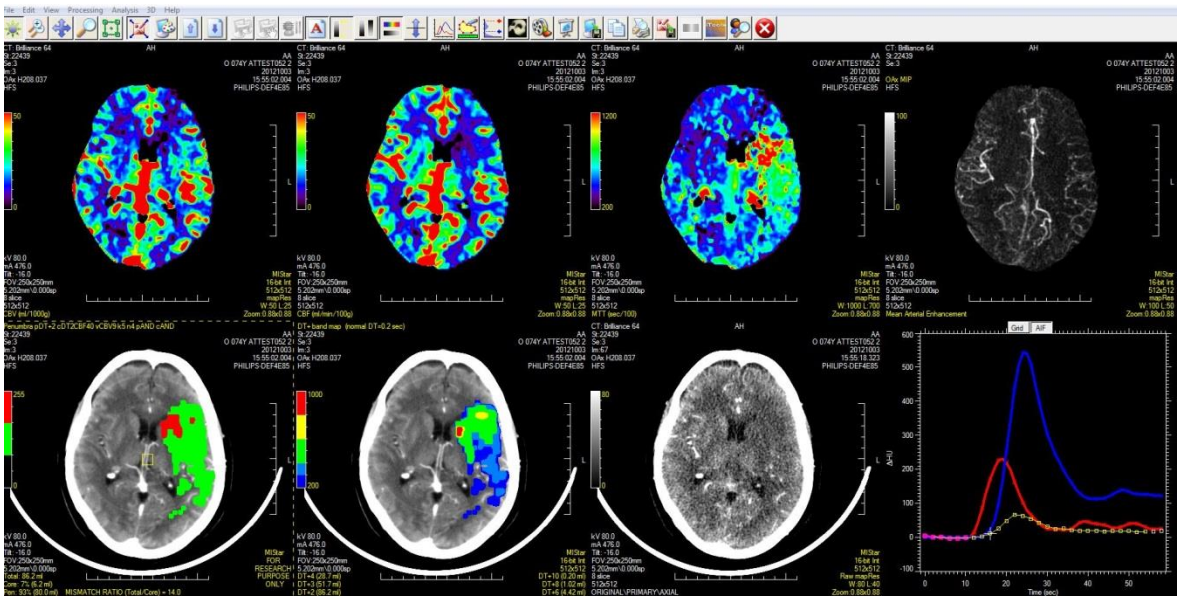


Figure 3-2. CTP maps generated with MIStar software (Top four panels: CBV, CBF, MTT, Angiography; Lower four panels: penumbral map, DT, contrast CT, and time-attenuation curve for arterial input function [red], and venous output function [blue]).

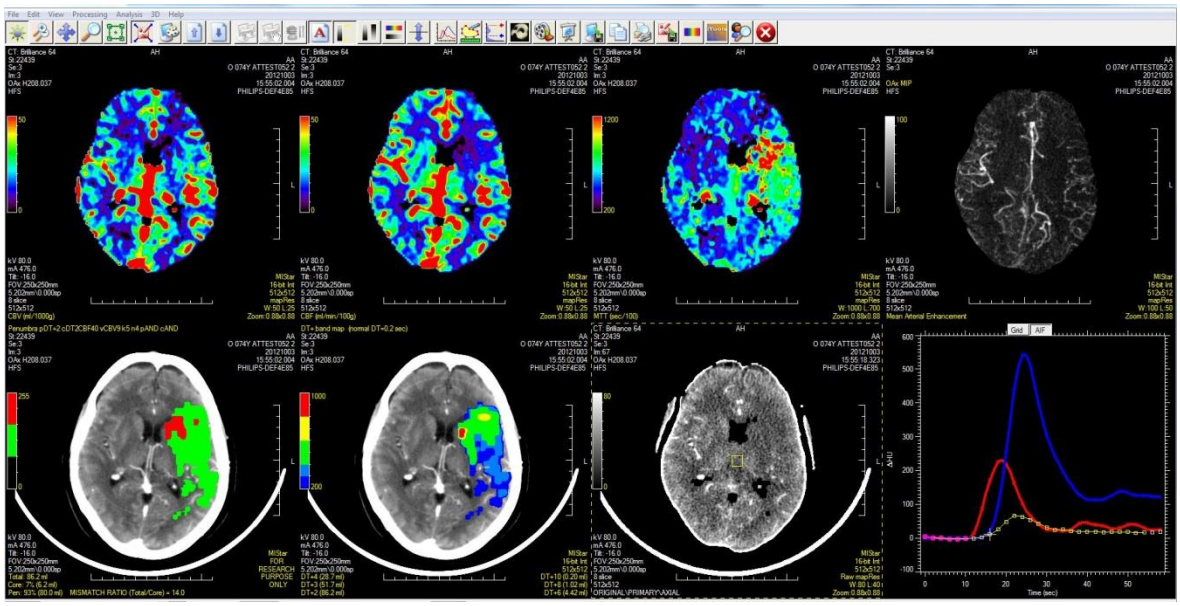


Figure 3-3. After applying thresholds masking CSF and bone

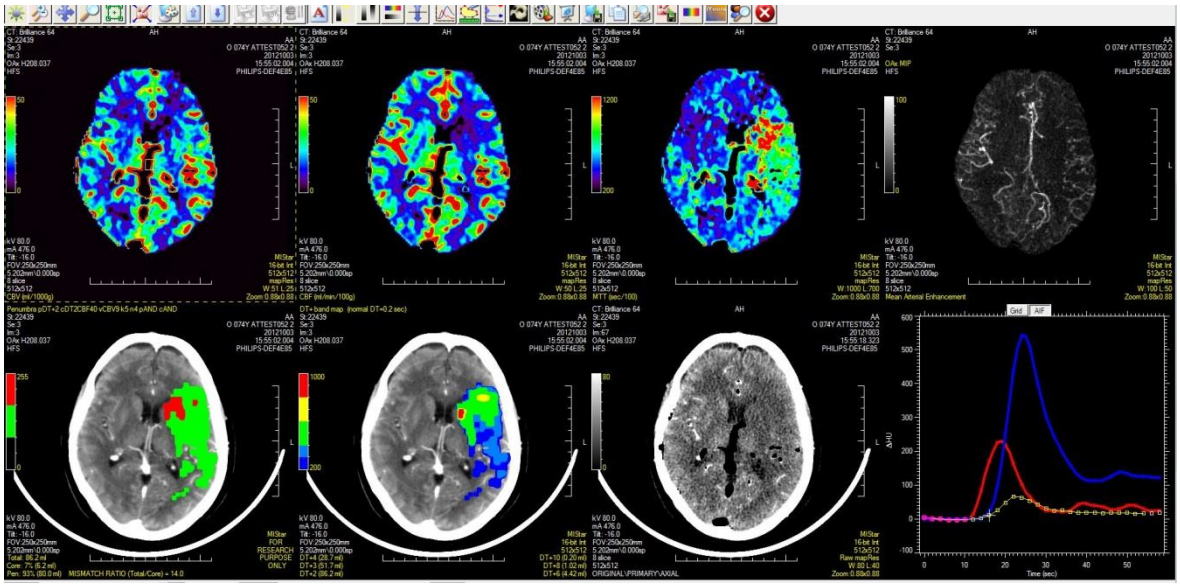


Figure 3-4. After applying thresholds masking voxels with CBV value >90cm^3/100g)

A modified SVD deconvolution was used to obtain the tissue enhancement curve and AIF with compensation for the effects of arterial delay and dispersion[373]. A series of delay time values, DTi ranging from 0 to Tmax were applied and for each delay time value a modelled arterial transport function was convolved with the measured global AIF to produce the AIFi which was used for SVD deconvolution of the tissue curve to produce an impulse residue function IRFi with its maximum appearing at Tmax (i). DT was determined as the minimal DTi value which produces Tmax (i) =0. CBF and CBV were calculated from

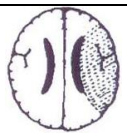
the peak height and area under the curve of the tissue enhancement curves respectively with MTT =CBV/CBF.

Core volume was defined as tissue with reduced CBF (relative CBF < 40% that of the contralesional hemisphere) and prolonged DT (relative DT < 2 sec)[147]; penumbra volume was defined as tissue with prolonged DT only (relative DT > 2 sec). Total CTP lesion volume was calculated as the combination of core volume and penumbra volume (Figure 3-5).

The presence and location of arterial occlusions were determined using the baseline CTA. An occlusion visualised using CTA source images and 3D Maximum Intensity Projection (MIP) reconstructions was defined as the absence of contrast filling in a vessel separating the proximal and distal portions of the vessel[81]. The degree of carotid stenosis was collected as part of the clinical data from clinical radiological reports.

The follow up NCCT was evaluated for the presence or absence of ICH, the degree of brain swelling (Table 3-4) and the ASPECT score for the quantification of infarction.

Table 3-4. The classification of the degree of brain swelling post thrombolysis (Adapted and modified from IST-3 imaging analysis protocol[374])

Brain swelling	
No Swelling (grade 0)	
Effacement of adjacent lateral ventricle (grade 1)	
Effacement of the lateral and the third ventricle (grade 2)	
Midline shift (grade 3)	

Because of the limited coverage of CTP, the co-registered infarct volume was used to calculate penumbral salvage. Both follow up NCCT and CTP scans were loaded side by side into a fusion tool function using MIStar. A rigid body 3D transformation was used to register the follow up structural imaging to the perfusion scan. Structural CT sequences were reformatted manually and visually verified to match the orientation of the original CTP (Figure 3-6). Reformatted structural CT slices resulting from co-registration were used to measure the final infarct volume with manually drawn ROIs using visual inspection (Figure 3-7 to 3-9). The combination of the co-registered infarct volume and the infarct volume beyond the co-registered slices produces the total infarct volume.

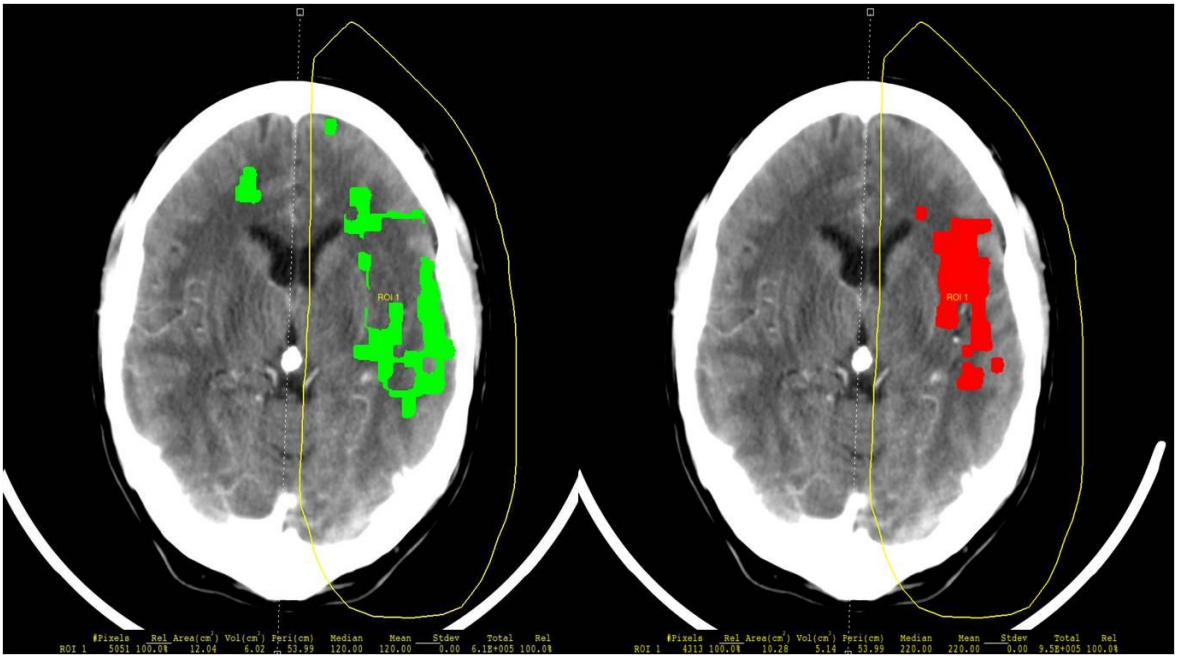


Figure 3-5. An example of Ischaemic core and penumbra measurement (The left panel showed penumbra region [green area], the right panel showed ischaemic core [red area], the yellow circle was region of interest).

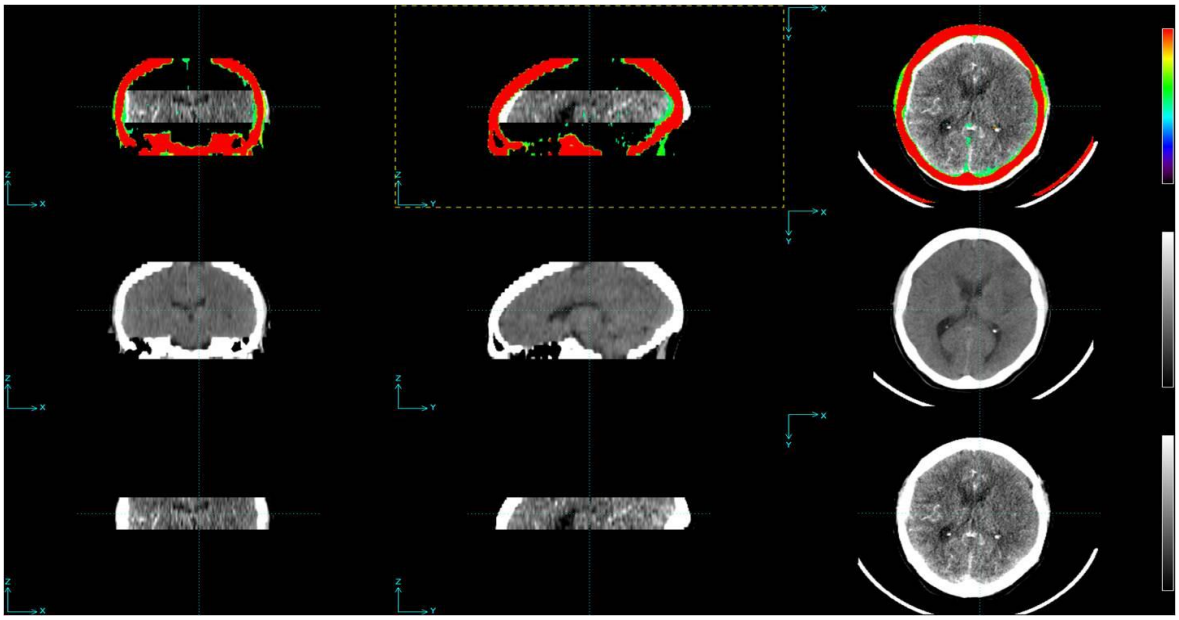


Figure 3-6. Co-registration (The top three panels: CT perfusion with superimposed [red] 24 hour CT following co-registration; middle three panels: 24 hour CT at the same anatomical level; lower three panels: CT perfusion at the same anatomical level)

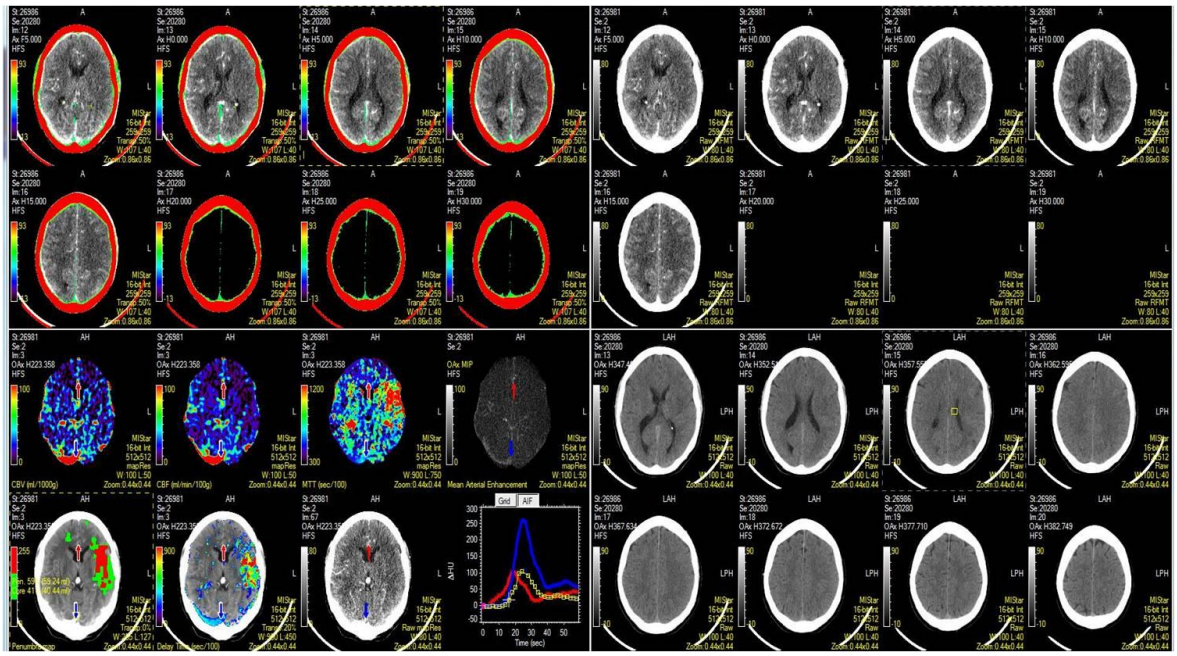


Figure 3-7. Reformatted 24 hours CT (with the red colour overlay – top left eight panels) presented in a same window as the original 24 hours CT (lower right eight panels) and baseline CTP (lower left eight panles). (CT perfusion angiography – top right eight panels).

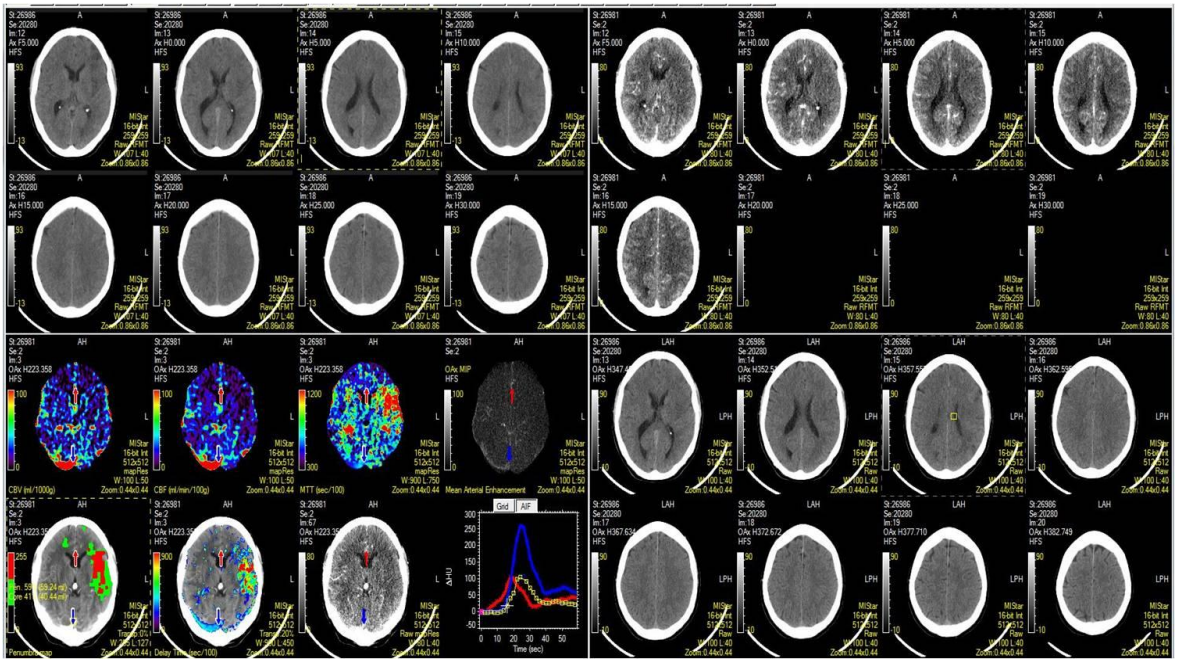


Figure 3-8. Reformatted 24 hours CT (with the red colour overlay turned off – top left eight panels) presented in a same window as the original 24 hours CT (lower right eight panels) and baseline CTP (lower left eight panels). (CT perfusion angiography – top right eight panels).

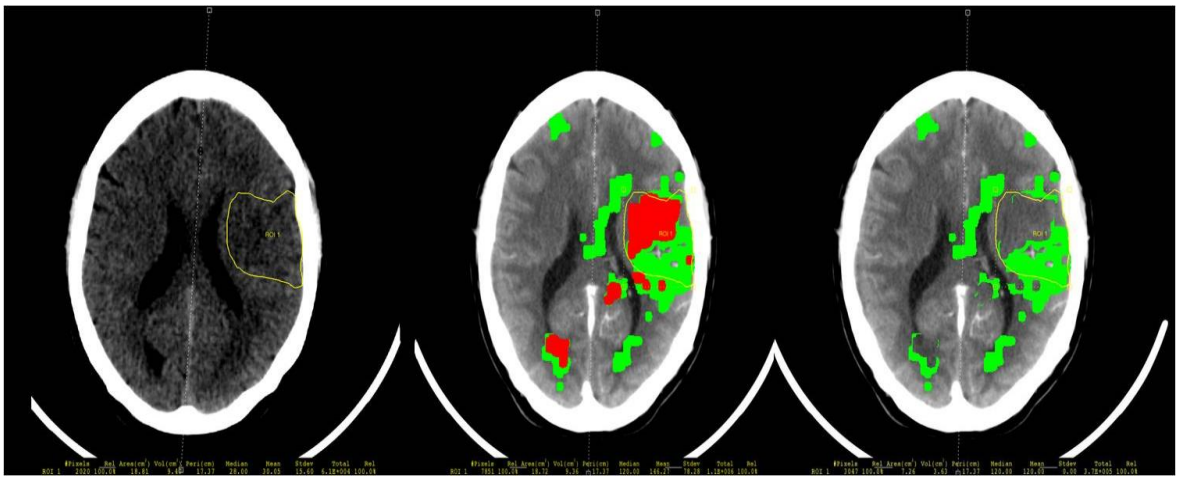


Figure 3-9. The measurement of co-registered final infarct volume (left panel: manually drawed ROI [region within the yellow line] of final infarct volume; middle panel: the ROI transposed onto the corresponding penumbra map [core-red, penumbra-green]; right panel: core area was excluded by applying threshold, left with penumbra only. The penumbra volume infarcted is the green area within the ROI).

To minimise the opportunity of including false positive pixels, ROIs drawn for final infarct volume measurement were transposed onto the corresponding CTP slice (Figure 3-9). Manual adjustment was applied when necessary in order to match to the same anatomical region. The penumbra volume that was infarcted was calculated from the ROI on the corresponding CTP slice. The following equations were used to determine relevant values:

- Infarct Growth = Co-registered infarct volume - Core volume
- Penumbra volume salvaged = penumbra volume – penumbra volume that infarcted;
- Percentage of penumbra salvaged = (penumbra salvage/penumbra volume) x100

Recanalisation was determined on follow up CTA using a TIMI scale[371], a grading system adapted from cardiologists to describe recanalisation and reperfusion in revasculisation studies. We also used the TICI scale[375], but found its application to be difficult due to the lack of dynamic information on the CTAs.

3.7. Statistics

The statistical analysis presented in this thesis was performed mainly with IBM SPSS statistics (SPSS Chicago, Illinois, USA v.19) and SAS (SAS Software limited, United Kingdom v.9.3); StatsDirect 2.8 was used in inter-observer agreement calculation and meta-analysis.

3.8. Assessment and Scales

3.8.1. NIH Stroke Scale

The National Institute of Health Stroke Scale (Appendix 1) is a 42 point scale developed to provide quantitative objective measurements of neurological deficit caused by stroke. A score of zero indicates no neurological deficit, whilst a score of 42 indicates death. The NIHSS is probably the most frequently used scale in stroke studies and in clinical practice due to its simplicity and reasonable reliability across different professional groups. However, certain items assessed using the NIHSS such as facial palsy, gaze and ataxia were shown to demonstrate poor inter-rater reliability. Non-dominant hemisphere infarcts can be underscored due to its emphasis on language and the abililty to follow commands[376]. The severity of posterior circulation strokes is poorly represented using the NIHSS. The result of various factors is that the overall scale does not always reflect the severity of a stroke. Patients with isolated dysphasia or hemianopia are underestimated on the scale despite their significant functional disability. If only part of the limb is affected, motor deficits can be underestimated using the NIHSS. The baseline NIHSS score has been shown to be a strong predictor for clinical outcomes[377]. In reperfusion studies, NIHSS ≤ 1, or improvement in NIHSS ≥ 8 points at 24 hours post treatment are commonly considered as good clinical outcomes. This may however still represent a significant functional disability if the baseline NIHSS score is high.

3.8.2. Modified Rankin Scale

The modified Rankin scale is an ordinal hierarchical grading from zero (no symptoms) to six (death) for measuring the degree of disability or dependence in daily activity (Table 3-5). It is the most used functional outcome measurement in stroke studies. However, the descriptions given for the categories of the mRS are too broad and leave ample spaces for subjective interpretation, which results in relatively lower inter-rater agreement

compared with other scales[378]. Inter-observer reliability improved moderately with the more recently developed structured mRS interview and video training system[378,379]. The more recent Rankin Focused Assessment provided a much clear criteria for scale assignment and requires much less time in assessment[380], which may make it more popular in the future studies.

Table 3-5. Modified Rankin Scale

Scale	Description
0	No symptoms at all.
1	No significant disability despite symptoms; able to carry out all usual duties and activities.
2	Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance.
3	Moderate disability; requiring some help for more complex tasks (eg finances), but able to walk without assistance. Able to manage alone at home for at least 1 week.
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance. Able to be left alone for at least a few hours during the day.
5	Severe disability; bedridden, incontinent and requiring constant nursing care and attention.
6	Dead.

3.8.3. ASPECT Score[128]

ASPECT score is a 10 points scoring system developed to quantify early ischaemic changes on presenting NCCT for MCA stroke. It segments the MCA vascular territory, and deducts one point if hypodensity or swelling is presented (Figure 3-10). The use of ASPECT improved the inter-rater agreement in the assessment of early ischaemic changes on NCCT with training, and is a commonly used score in AIS trial patient selection.

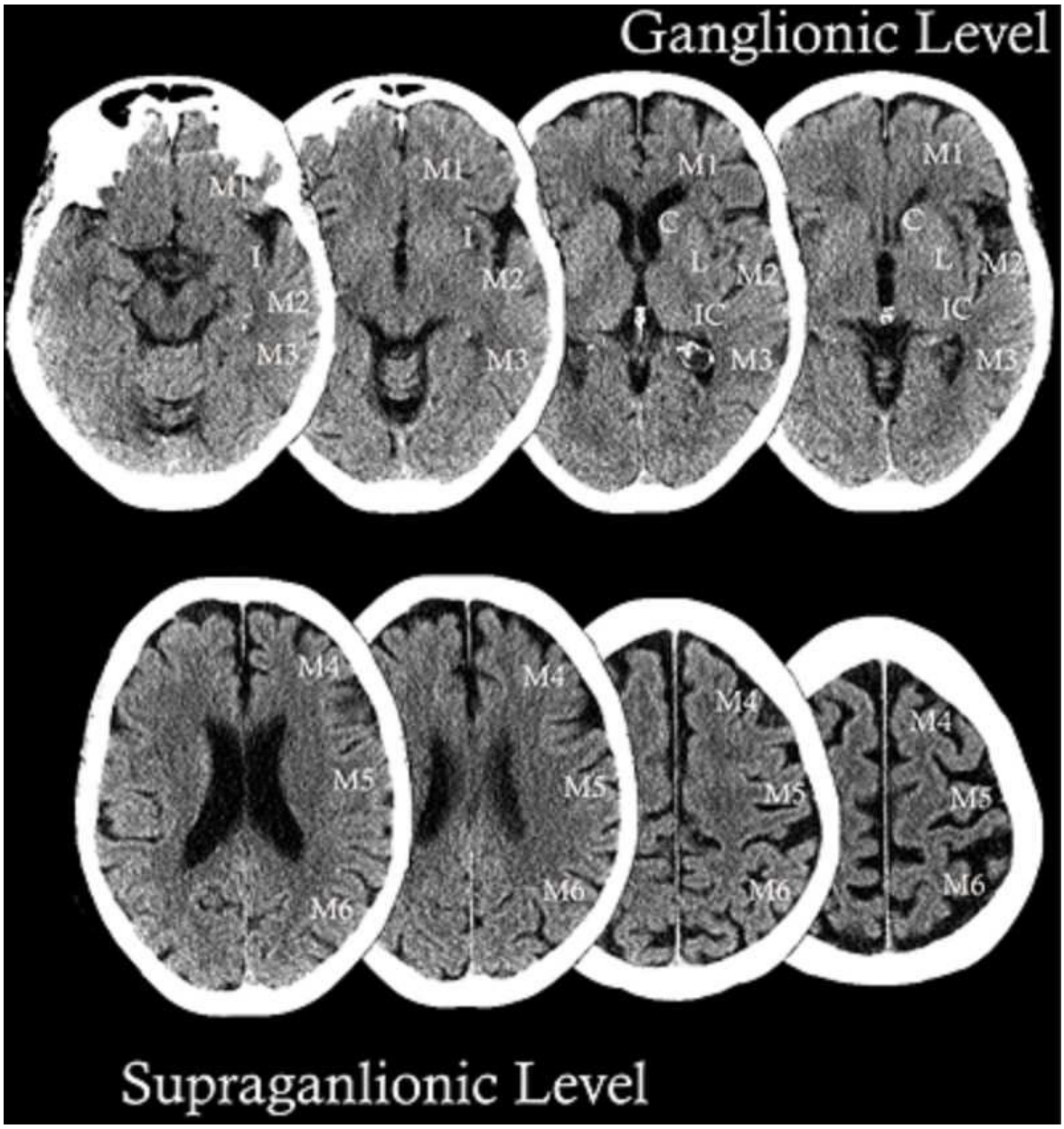


Figure 3-10. Axial NCCT images showing the MCA territory regions as defined by ASPECTS. C – Caudate, I – Insularribbon, IC – Internal Capsule, L – Lentiform nucleus, M1 – Anterior MCAcortex, M2 – MCA cortex lateral to the insular ribbon, M3 – Posterior MCA cortex, M4, M5, M6 are the anterior, lateral and posterior MCA territories immediately superior to M1, M2 and M3, rostral to basal ganglia. Subcortical structures are allotted 3 points (C, L, and IC). MCA cortex is allotted 7 points (insular cortex, M1, M2, M3, M4, M5and M6).

3.8.4. Scales to describe vessel patency (TIMI, TICI) (Table 3-6)

The TIMI scale was initially used to grade the severity of vessel occlusion, ranging from zero (no flow) to three (normal flow), but was then subsequently used widely in cardiology revasculisation studies to describe vessel patency. Stroke physicians have

recognised that the TIMI scale does not give a description for distal branch perfusion. The TICI scale[223] was subsequently developed to account for that, and has become popular in cerebral endovascular intervention studies. However, the TICI score was designed for conventional angiography, and dynamic information is crucial in order to use it accurately. Since most CTAs currently only provide a single-phased image, the TICI scale is unlikely to describe recanalisation more accurately than the TIMI scale.

Table 3-6. TIMI and TICI scale

Grade	TIMI	TICI	
0	No reperfusion	No perfusion beyond the occlusion	
1	Penetration without perfusion	Penetration but no perfusion, contrast penetration exists past the initial obstruction but with minimal filling of the normal territory	
2	Partial reperfusion	a	Partial perfusion with incomplete distal branch filling of <50% of the expected territory
		b	Partial perfusion with incomplete distal branch filling of ≥50-99% of the expected territory
		c	Near complete perfusion without clearly visible thrombus but with delay in contrast run-off
3	Complete reperfusion	Full perfusion with normal filling of all distal branches of the expected territory in a normal haemodynamic fashion	

3.8.5. ECASS Classification of haemorrhagic transformation

The haemorrhagic transformation following ischaemic strokes with or without reperfusion therapy is classified radiologically into four types[381]: Haemorrhagic Infarction (HI) 1 - Small petechiae along the margins of the infarct; HI2 - Confluent petechiae within the infarcted area but no space occupying effect; PH1 - Blood clots in ≤30% of the infarcted area with space occupying effect; PH2 - Blood clots in >30% of the infarcted area with space occupying effect (Figure 3-10).

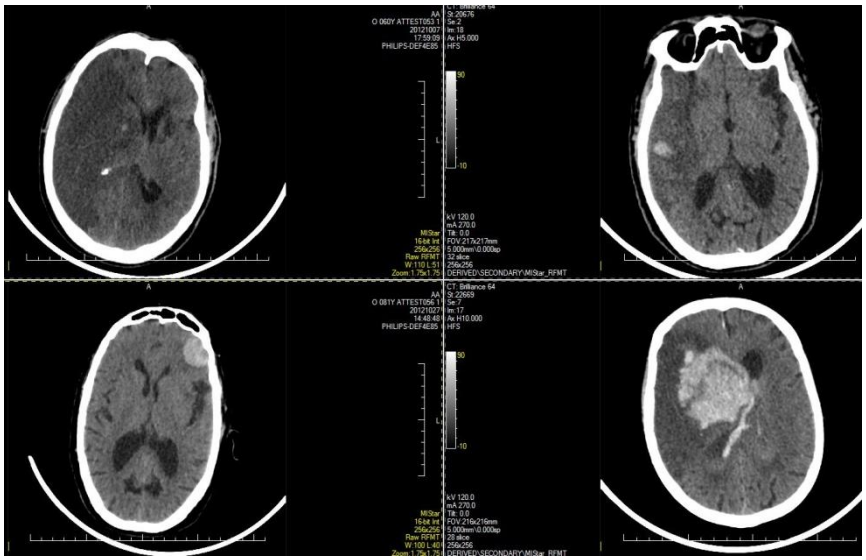


Figure 3-11. Examples of ECASS 2 radiological classification of ICH from the ATTEST study imaging (from above left clockwise: HI1, HI2, PH1, PH2)

3.8.6. The SITS-MOST definition of symptomatic intracerebral haemorrhage

As discussed in Chapter 2, a number of definitions of symptomatic ICH have been used in stroke thrombolysis studies as safety outcomes. Among them, the SITS-MOST definition[318] ensures the neurological deterioration is from the result of an ICH rather than from the extensive infarction or associated swelling. It uses the combination of the deterioration of the NIHSS scale of more than four from the baseline or the lowest subsequently recorded score within a time frame of less than 36 hours from treatment and the ECASS radiological criteria of PH2 to define symptomatic ICH. It is now widely used in stroke reperfusion studies.

Chapter 4 Does the delay between bolus and infusion in thrombolysis affect outcome?

4.1. Introduction

Timely recanalization is the goal in the management of acute ischaemic strokes. The odds of achieving functional independence diminishes the later the treatment is initiated[70]. The current approved therapy, the administration of IV rtPA within 4.5 hours from symptoms onset improves functional outcomes[382], but its efficacy is limited to a small proportion of patients.

rtPA has a very short half-life (4-9 minutes)[274] requiring an hour-long continuous infusion following a front-loaded bolus to maintain therapeutic plasma concentration. Large randomised trials such as the NINDS study assumed no significant delays in the commencement of maintenance infusion dosing.

Recanalization is achieved in only 50% or less of patients who receive IV rtPA[319]. Factors that contribute to this include patient factors such as the origin and the extent of the clot, the adequacy of collateral flow, and possibly blood glucose, but drug factors including pharmacokinetics may also be relevant. The emphasis of door-to-needle, or onset-to-treatment times focuses on the delays in the start of treatment. However, delays between bolus and maintenance infusion, and interruptions of maintenance infusion, are common. We questioned whether delays in the completion of treatment affected functional outcomes.

4.2. Methods

We performed a retrospective study in a single tertiary neurology referral centre. Patients included were those who received IV rtPA treatment in 2010, and patients who received thrombolysis between 2008 and 2011 from two local imaging study databases; the Multicentre Acute Stroke Imaging Study (MASIS) and the Post Stroke Hyperglycaemia (POSH) study, two studies that performed CT or MRI angiography at baseline and 24-72 hours. We collected information for stroke onset time, bolus time, infusion start and finish time, any interruption during the infusion, along with clinical information, such as baseline and 24 hours NIHSS scores, baseline occlusion, recanalisation status and day 90 mRS. We compared clinical outcomes and recanalization rates in patients who had bolus-infusion delay (BID) and those who had not. We defined BID as a delay between bolus and infusion start of more than nine minutes (based on one plasma half-life of rtPA). An early neurological improvement in 24 hours was defined as a reduction of NIHSS score of ≥ 8 points, or an NIHSS score = 0 or 1 at 24 hours; a favourable functional outcome was defined as mRS 0 – 1 at day 90.

Imaging assessment comprised pre-treatment CTs, with CTA covering from the aortic arch to the top of the lateral ventricles, with follow-up CT at 24 hours, and intracranial CTA at 24-72 hours. The detailed procedure of acquiring imaging is described in Chapter 3 above.

CTA occlusion and recanalization were determined by a single reader (FM). The vessel patency on follow-up CTA was defined as none, partial, or complete recanalization. SICH was defined as any intracerebral haemorrhage on follow-up non-contrast CT with clinical deterioration using the ECASS II definition[311].

Baseline characteristics in the two groups were compared using the independent t test, the Mann-Whitney *U* test for continuous variables, Chi-square, and Fisher's test for categorical variables. Binary logistic regression was used to investigate whether there was an association between the BID, clinical outcomes and recanalization rates. mRS distribution ("ordinal shift") analysis was carried out with ordinal logistical regression.

All statistical analysis was carried out with SPSS 19 and SAS 9.3.

4.3. Results:

118 patients with documented bolus times and infusion start times were included in this study, of which 63 patients had CTA assessments: 49 of these had occlusion on pre-treatment CTA (Figure 4-1). The baseline demographic information is shown in Table 4-1.

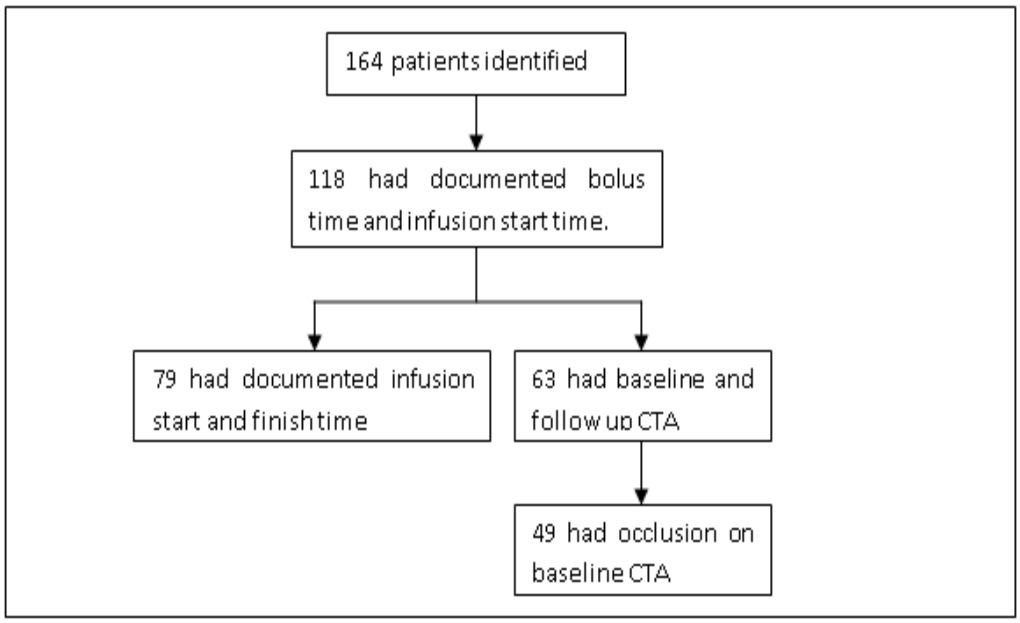


Figure 4-1. Study CONSORT chart

Table 4-1. Baseline demographics for all patients in the study

	Group with BID	Group without BID	p Value
	N=90	N=28	
Age year Mean (SD∗)	70 (17)	68.5 (17)	0.47
Sex male no. (%)	50 (55.6)	21 (75)	0.07
Baseline NIHSS Median (IQR®)	14 (8-19)	13 (7-18)	0.74
Basline mRS Median (IQR)	0 (0-1)	0 (0-1)	0.74
OTT (minutes) Mean (SD)	170 (70)	173 (77)	0.92
Co-morbidities			
Hypertension no. (%)	59 (66%)	15 (54%)	0.25
Diabetes no. (%)	13 (14%)	6 (21%)	0.38
Previous MI no. (%)	10 (11%)	2 (7%)	0.54
Atrial Fibrillation no. (%)	20 (22%)	7 (25%)	0.76
Previous stroke or T.I.A., no. (%)	19 (21%)	5(18%)	0.71
Hyperglycaemia (Random glucose >7.0mmol/l) no. (%)	24 (27%)	9 (32%)	0.57
Hyperlipidaemia no. (%)	20 (23%)	4 (14%)	0.43
Peripheral vascular disease n. (%)	3 (3%)	1 (3%)	1
∗SD Standard Deviation; ®IQR Interquartile Range			

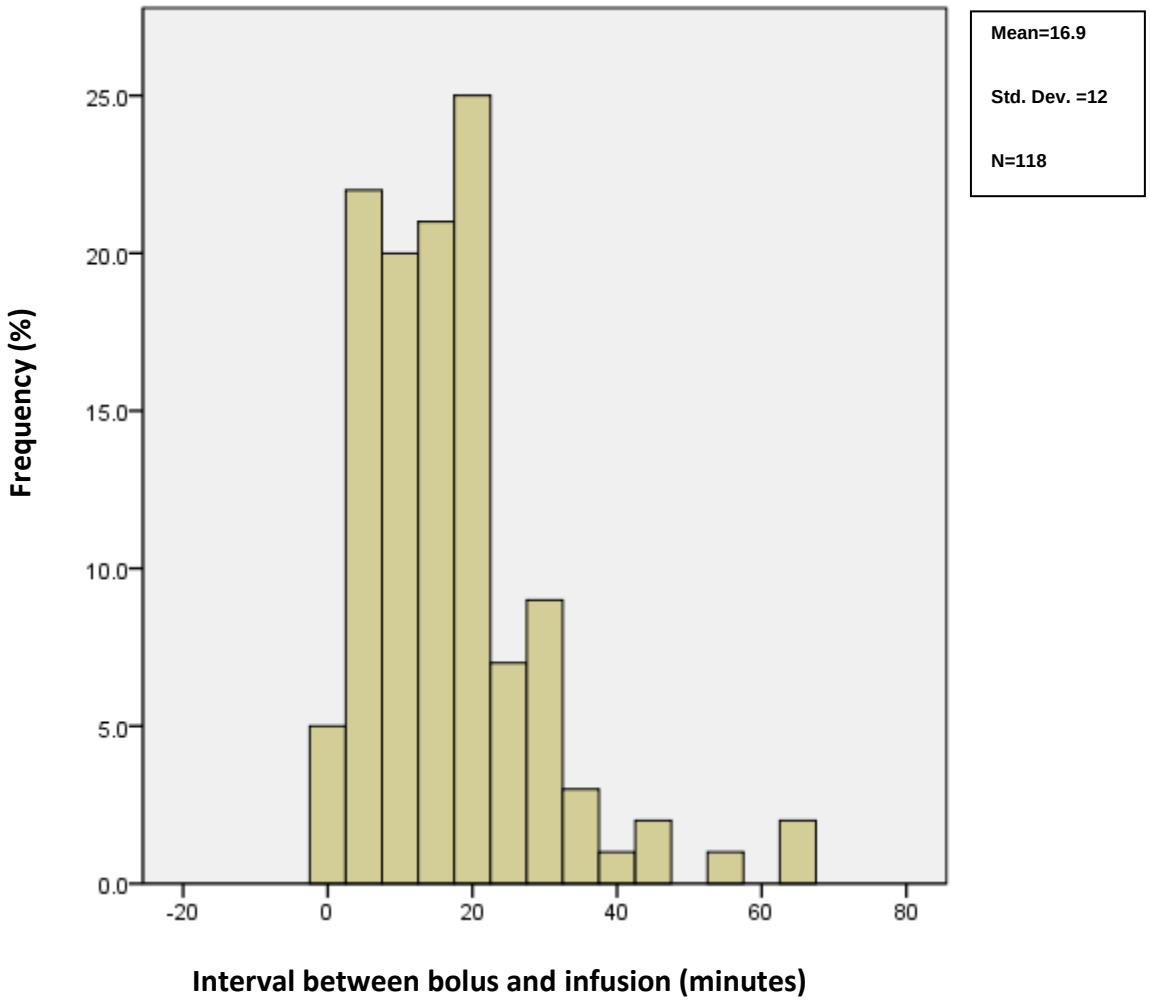


Figure 4-2. Distribution of all 118 patients with/without bolus infusion delay

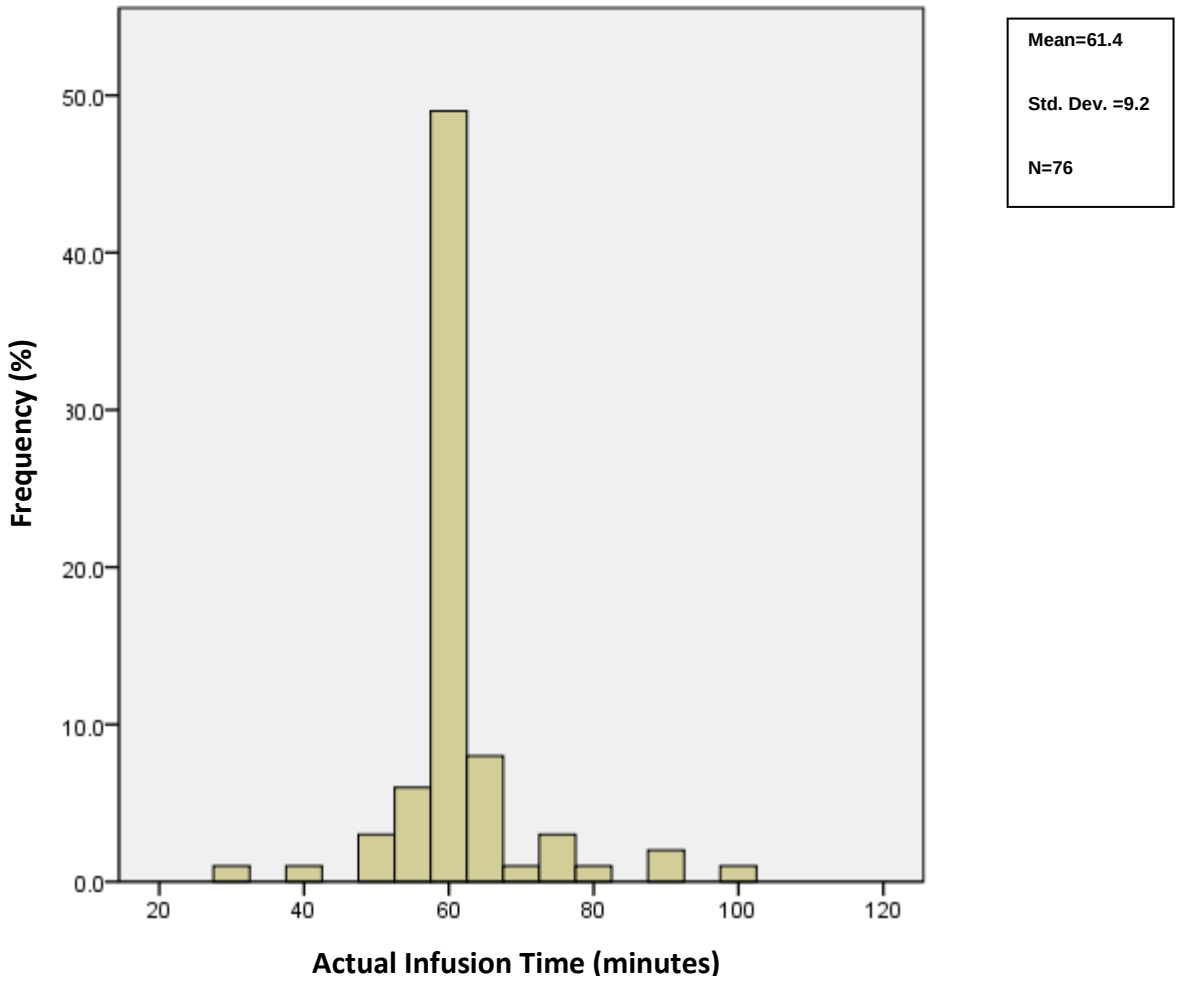


Figure 4-3. Distribution of infusion time of 76 patients who completed infusion and had documented infusion start and finish time.

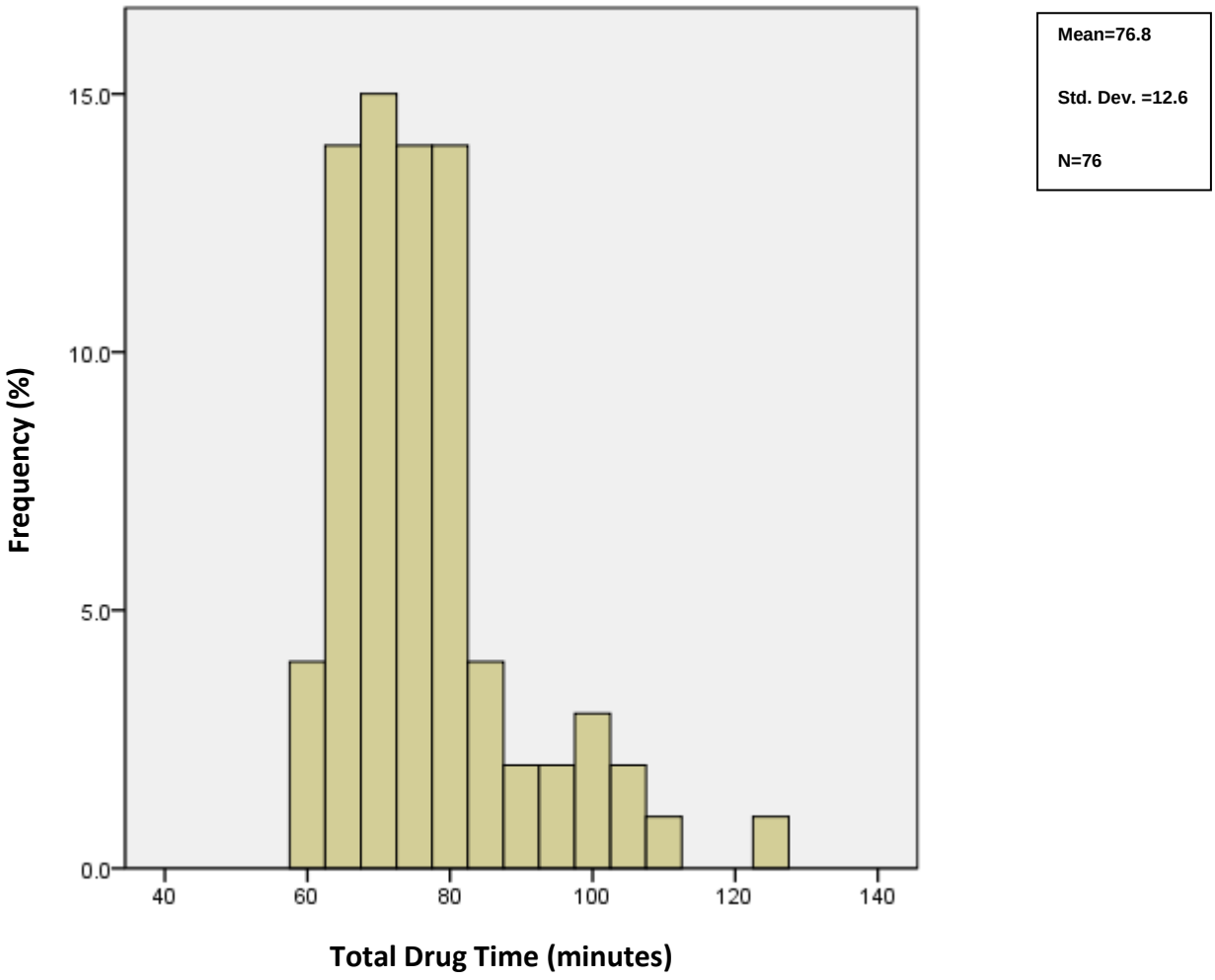


Figure 4-4. Distribution of total treatment time from initial bolus to the completion of infusion in 76 patients who completed infusion and had documented infusion start and finish time.

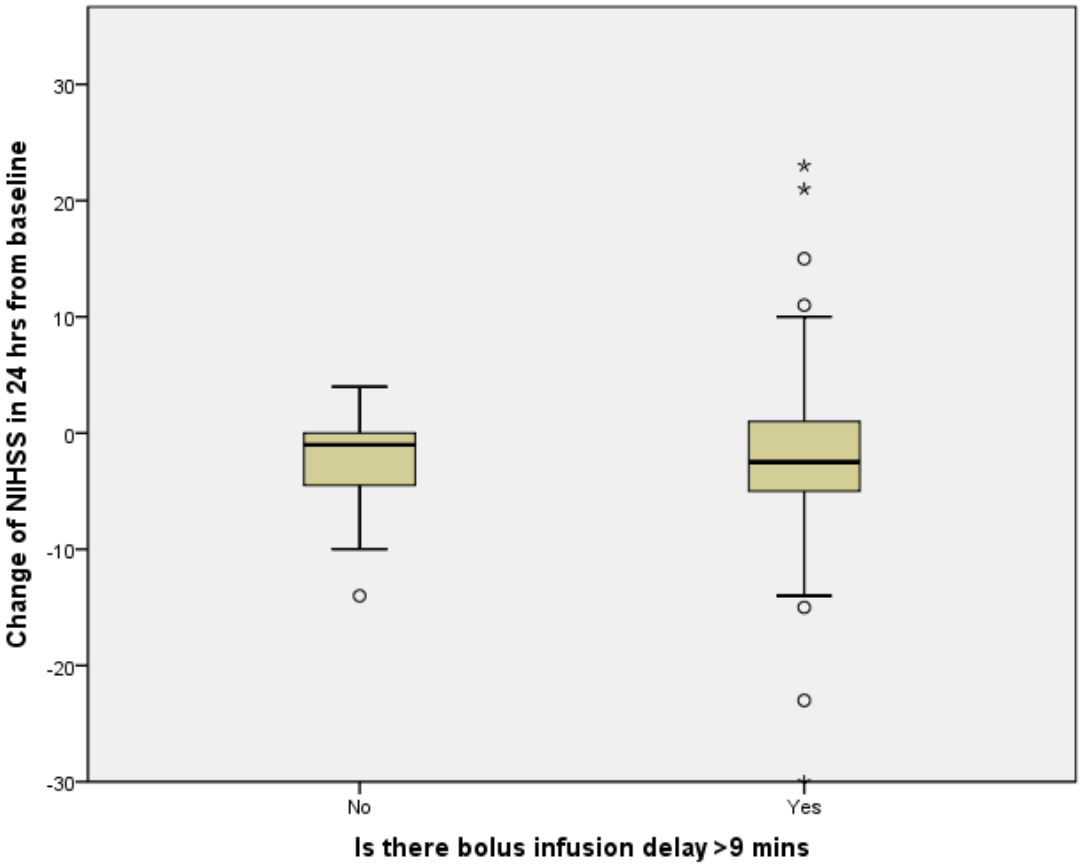


Figure 4-5. Change of NIHSS in 24 hours in the group with BID and the group without BID.

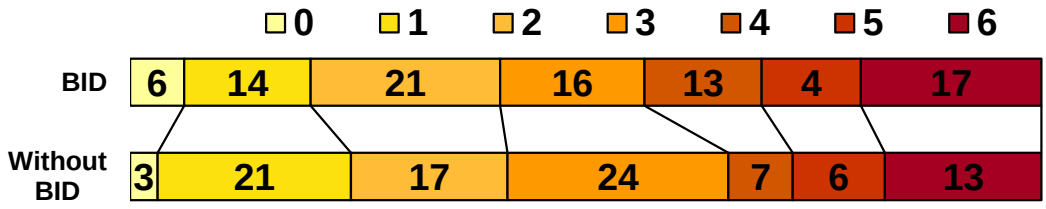


Figure 4-6. mRS at day 90 between the two groups.

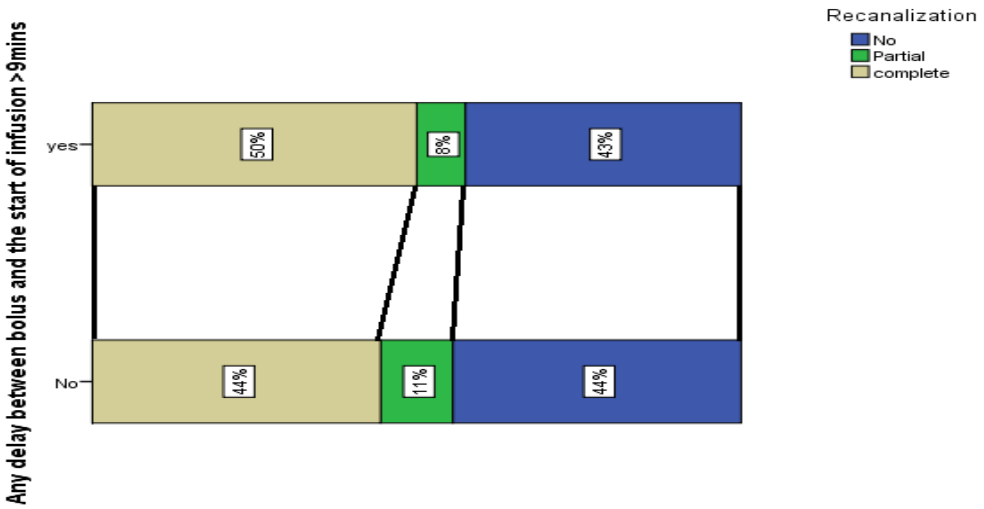


Figure 4-7. The comparison of Recanalization rates between the group with BID and the group without BID.

There were only four patients who had no documented bolus infusion delay. The range of delays between bolus and infusion was 2 to 65 minutes (median [IQR] 15 [10- 20]) (Figure 4-2). Using our definition of BID, 89 (75%) patients had a delay. In 79 patients who had documented infusion start and finish times, 58 (73%) completed the infusion within 60 minutes (Range 30 – 100 minutes, median [IQR] 60 [60-60]) (Figure 4-3); 3 (4%) infusions were discontinued prematurely due to clinical deterioration. One patient's infusion finished in 30 minutes, and another's completed in 40 mintues. No reason was documented in the clinical notes for the shortened infusion. There were 8 (10%) patients whose infusion duration ≥ 65 minutes, of which one was due to the interruption of scan, one due to clinical deterioration, and another one due to delayed blood results. For the remaining five patients, no reason was documented for the extended infusion time. Total treatment times ranged between 60 to 125 minutes (median [IQR] 75 [69-80]) (Figure 4-4). The differences in outcome measures including early neurological improvement, mRS at day 90 and recanalization rates between the two groups are shown in Figures 4-5 to 4-7. The 90 day mRS distributions show higher mortality and reduced proportions of patients with favourable outcomes in the group with BID. However the mRS shift analysis adjusted for the baseline NIHSS score and age showed no significant statistical shift of day 90 mRS between the two groups (P=0.92).

In a binary logistic regression model adjusted for known predictors of outcome (age, initial NIHSS score, OTT and baseline glucose), no significant association between early

neurological improvement (P=0.73), day 90 favourable clinical outcomes (P=0.9) and BID was found. In those 49 patients who had vessel occlusion on the initial CTA, no significant association was found between the recanalization rate and BID (P=0.92). The same analysis was performed between the group who had BID of more than 15 minutes, and the group with BID<15minutes. No significant association was demonstrated between the early neurological improvement (p=0.3), favourable clinical outcomes at day 90 (p=0.34), recanalisation (p=0.83) and BID of more than 15 minutes.

15 (13%) out of the total 118 patients had intracerebral haemorrhages on the 24 hour CT scan. Three (10%) were from the group without BID, none had SICH; 12 (13%) were from the BID group, of whom three (3%) who were PH type 2 also fulfilled the SICH criteria, but no significant difference in SICH rates was demonstrated between the two groups (P=1.00).

4.4. Discussion:

This retrospective study of tPA administration showed that, in practice, the extended administration of tPA is common, mainly due to delays between the initial bolus and subsequent infusion commencement. In some cases, the delays were quite prolonged.

Experiments in rabbits showed that the rate of elimination of tPA in vivo is rapid (16.1ml/min/kg), especially within the first hour (plasma half-life in rabbits is three minutes). 15 minutes after IV bolus administration, only 1% of the tPA still remained in circulation[283]. In humans, an early pharmacokinetic study of a large single bolus dose (50mg over 2 minutes) compared with a front-loading dose regime (10mg as bolus, followed by 50mg infusion in 1 hour, and 30mg in 1.5 hour—60mg given within the first 60 minutes) in acute MI thrombolysis showed that 10 minutes after the bolus, less than 50% remained in the system[383,384] . It is worth noting that the purpose of this study was to compare a high single bolus regime with the conventional infusion regime. In this study, the initial recanalization rates at 60 minutes determined by angiography were similar in the two groups, with a higher rate of recurrent ischaemic events and reocclusions in the former group. It is thought that the initial high plasma concentration was probably responsible for the higher recanalization rate; whereas the principal function of the maintenance infusions was probably the prevention of reooclusion[385].

The pharmacokinetic studies of IV alteplase carried out in healthy populations and MI patients demonstrated that the front-loading regime with an initial bolus followed by an infusion led to much quicker attainment of steady state concentration (4 minutes)[386], rather than about 20 minutes for infusion only dosing[387]. A study[388] which simulated the effect of rtPA bolus-infusion delays using a mathematic model showed a significant drop of plasma concentration to 25% with a bolus infusion delay of 15 minutes (the approximate mean delay time in our sample). As expected, it also affected the speed of achieving therapeutic plasma concentration (which took about 36 minutes in the model, as opposed to 3 minutes in the situation with no delay).

No pharmacokinetic study has been performed in which tPA has been studied in relation to acute ischaemic stroke. The front-loading regime we currently use was presumably adapted from cardiology studies. However, fundamental differences between acute MIs and ischaemic strokes exist. Coronary arteries are of smaller diameter than the major intracranial vessels (the average diameter of the left main stem of the coronary artery is 0.004-0.005cm[389]; the average diameter of the middle cerebral artery ranges from 0.3-0.5cm[390]) and the pathological process of MI is one of in-situ plaque rupture and thrombosis; most strokes arise from the embolism of clots formed more proximally, and of varying composition, and in many instances the clot volume is much larger. The total dosage of rt-PA used in the treatment of acute ischaemic strokes is smaller compared with that used in acute MIs, with 10% of dose as initial bolus and 90% of dose infused within one hour, defined by ascending dose studies in stroke that escalated dose until ICH was encountered. The initial plasma concentration is lower in comparison with the standard accelerated infusion regime used in acute MIs[275]. If there is a delay between bolus and the subsequent infusion, the speed of recanalisation and subsequent attempts to prevent reocclusion can both be affected. In our sample, we did not demonstrate any difference in clinical, imaging or safety outcomes between the two groups.

The strength of the present study is that both clinical outcomes and imaging outcomes were assessed. We confirmed significant BID in our routine clinical practice. A clinical focus on shortening onset-to-treatment time emphasises the initiation of treatment but perhaps at the expense of timely commencement of the maintenance infusion. Systemic delays include relative ease of bolus injection in the CT scanning environment compared

to set-up of an infusion pump; the need to transfer patients from CT to the stroke unit environment; and competing nursing priorities once in the stroke unit.

This study was retrospective and reflects a single centre experience. The sample size was small, although the stroke characteristics were well-balanced and similar to most general acute stroke patients; only around half of the subjects had a CTA assessment, and even fewer contributed useful data on the basis of having an initial occlusion identified. In the statistic model we used, established factors which correlated with outcomes, such as OTT[391], the initial NIHSS score[391], and high serum glucose [392] (defined as glucose > 7.0 mmol/l) did not show any significant association with outcomes, except for the initial NHISS, which was significantly correlated with day 90 favourable clinical outcomes ($P<0.001$). However, another similar study[393] with a larger sample size (n=229) failed to show any significant difference in functional outcomes in patients who were treated with BID and those who did not have BID. The complexity of pathophysiology in ischaemic strokes ensures that the determination of successful recanalisation is multifactorial. To demonstrate a significant difference in clinical or imaging outcomes influenced by bolus infusion delay alone may require a very large sample size.

Given that a zero delay between bolus and infusion is difficult to achieve, newer thrombolytic agents with a longer plasma half-life which enable single bolus injection may be a solution in clinical practice.

4.5. Conclusion:

Extended IV tPA treatment is common in clinical practice, mainly due to delays between initial bolus and following infusion. This may affect clinical and imaging outcomes, but this has not been demonstrated in the present sample. Further study in this area is warranted.

Chapter 5 The ATTEST study, demographics and main outcomes

5.1. Introduction

As discussed in Chapter 2, alteplase significantly improves the likelihood of disability-free recovery, but it has limited fibrinolytic efficacy, achieving arterial recanalisation in fewer than 50% of patients[319]. Among those who recanalise, only about half do so within two hours of drug administration[394]. Clinically, the most recent meta-analysis[70] included all major thrombolysis studies using alteplase showed that only 33.8% (1145/3391) treated within six hours achieved independence (mRS 0-1) at three months; in practice, if treated within three hours, 38.9% (2386/6136) of patients recovered completely at three months in the SIT-MOST database[318]. Tenecteplase is purposely modified to improve efficacy[283] through higher affinity binding to fibrin, greater resistance to inactivation by PAI-1, lack of procoagulant effects, and longer free plasma half-life.

In animal models, tenecteplase took significantly less time to reperfuse compared with alteplase, with greater recanalisation rates and reduction of thrombus burden, and significantly less blood loss from existing wounds[284]. In acute MI, tenecteplase demonstrated a similar therapeutic efficacy and lower overall bleeding risk than alteplase[290], the ICH rates were not different in the two groups probably due to the small number of occurrence of ICH in MI. There was a suggestion that tenecteplase treated patients might have a greater likelihood of early recanalisation than was the case with the alteplase group at 60 minutes, although this was not statistically significant[395].

Investigation of new thrombolytic agents in acute stroke has, until recently, concentrated on time periods beyond the 4.5 hour time window from symptom onset established for alteplase in order to treat more patients. However, given that more than 60% alteplase treated patients according to the current guideline[93] still remained disabled, seeking a

better, alternative, thrombolysis agent is an essential element of the search to improve outcomes of stroke patients. Dose-ranging studies of tenecteplase in acute stroke have been reported[325,326]. Parsons and colleagues[194] reported superiority of tenecteplase over alteplase in terms of both imaging-defined reperfusion and clinical outcomes in a selected group of patients with large artery occlusions and favourable brain perfusion patterns defined on CT. However, it is unclear how the effect sizes observed in a highly selected population will translate into more general populations. We undertook a phase II randomised, controlled clinical trial comparing alteplase with tenecteplase in a thrombolysis-eligible acute ischaemic stroke population, using imaging criteria as an exploratory outcome measure rather than as a selection criteria, in order to define possible effect sizes in a general stroke population eligible for intravenous thrombolysis.

5.2. Methods

The detailed methodology of the ATTEST study was described in Chapter 3. The trial was conducted at a regional neurosciences centre providing a comprehensive stroke service to a population of approximately 450,000, with some additional secondary transfers for thrombolysis assessment from seven regional stroke services. Total admissions are approximately 900 per annum, of whom approximately 600 are confirmed to have had ischaemic strokes. Thrombolysis numbers average 100 per annum. Clinical research staff recruited to the trial and additionally supervised multimodal CT acquisitions, including out of hours.

All eligible patients were screened and approached for consent to the study once the treating physician deemed they were candidates for thrombolysis. All patients were weighed when they arrived in the unit. The patient or the relatives were given the study information sheet, and the study was discussed by the research team with the patient/relative. Immediately following the NCCT confirming eligibility for thrombolysis, consent was taken. All efforts were made to avoid delaying treatment by recruiting patients into the study. In the rare situation (on two occations during the whole study) when thepatient had no capacity, and there was no relative available, an independent clinician's consent was sought. The whole process from screening to consent typically took approximately 15 minutes.

5.2.1. Inter-rater agreements

The methods of imaging analysis to derive the primary and secondary imaging outcomes were detailed in Chapter 3. All imaging parameters were analysed by two research fellows (XH and BC). The inter-rater agreements were assessed initially in a test set of scans and then evaluated in the full set of the ATTEST study scans.

5.2.1.1. Agreement in test scans

The test scan set (n=18) was randomly taken from our imaging archive including the ATTEST study and other studies which have used advanced CT imaging at our site.

The inter-rater agreements for continuous variables were assessed with an intra-class correlation coefficient, and categorical variables were examined with weighted kappa using StatsDirect.

For the ischaemic core, the intra-class correlation coefficient was 0.87 (95% limits of agreement -9.95-23mL) (Figure 5-1).

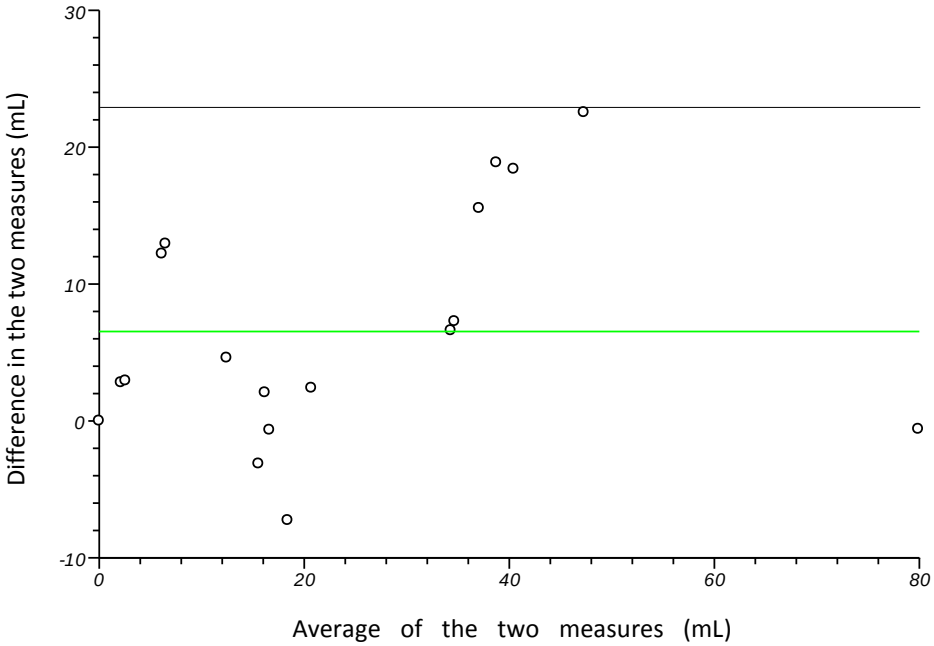


Figure 5-1. Bland-Altman agreement plot for inter-rater agreement in baseline core volume (test scan set) (mean[green]±1.96SD[black]).

For penumbral volume, the intra-class correlation coefficient was 0.92 with 95% limits of agreement of -20.8-11.3mL (Figure 5-2).

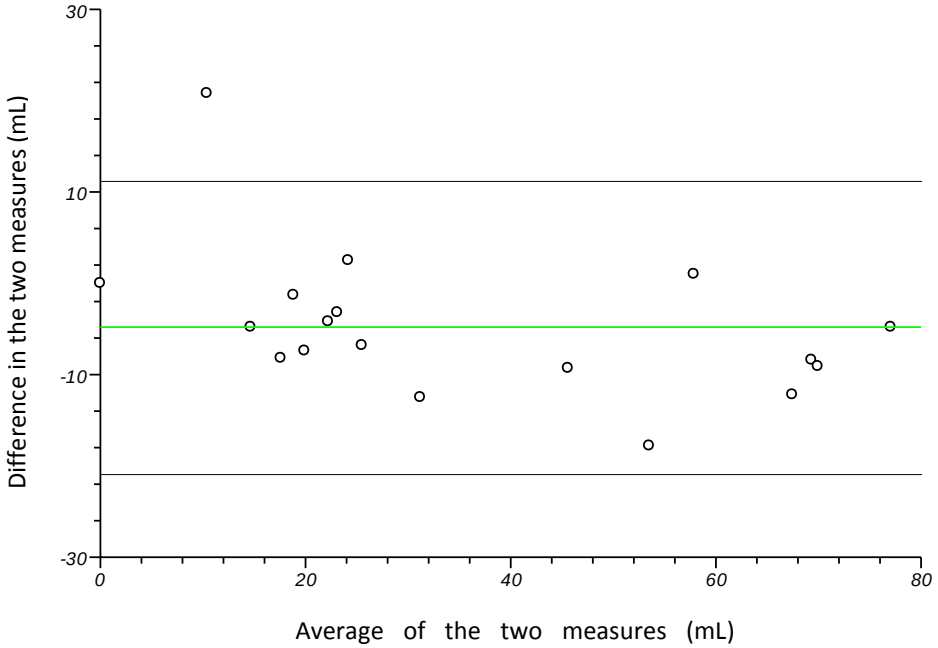


Figure 5-2. Bland-Altman agreement plot for inter-rater agreement in baseline penumbra volume (test scan set) (mean[green]±1.96SD[black]).

The correlation coefficient for co-registered infarct volume was 0.8 (95% limits of agreement -31-73.4mL) (Figure 5-3).

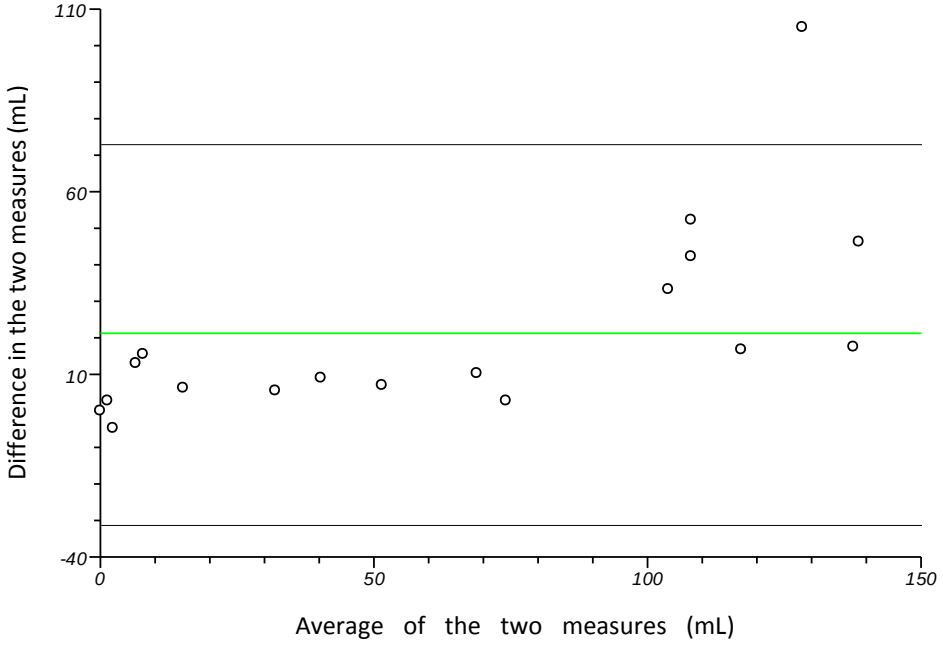


Figure 5-3. Bland-Altman agreement plot for inter-rater agreement in co-registered infarct volume at 24-48 hours (test scan set) (mean[green]±1.96SD[black]).

The correlation coefficient for total infarct volume was 0.94 (95% limits of agreement -22.2- 70.2mL) (Figure 5-4).

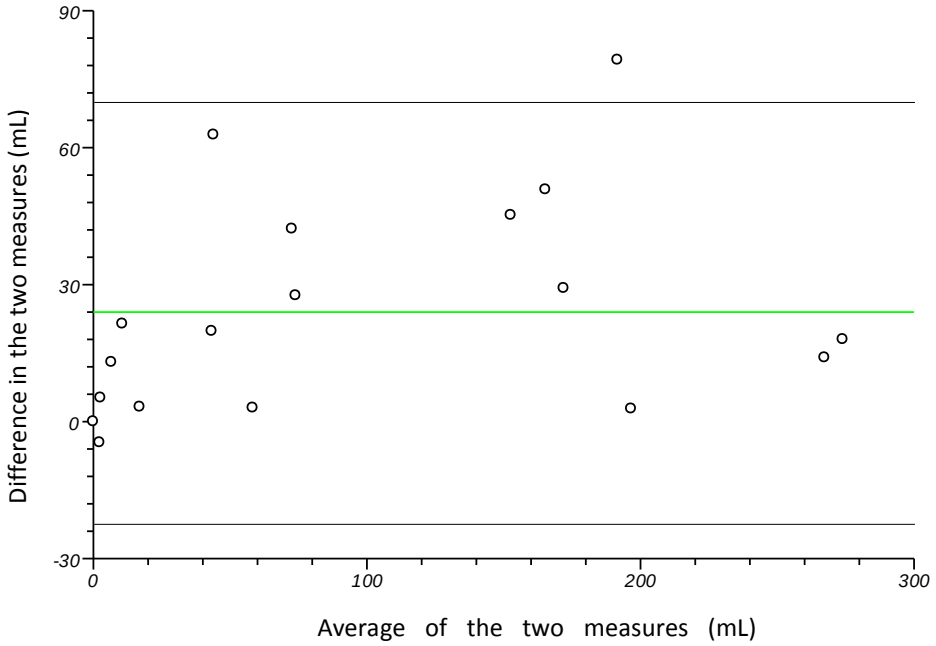


Figure 5-4. Bland-Altman agreement plot for inter-rater agreement in total infarct volume at 24-48 hours (test scan set) (mean[green]±1.96SD[black]).

Overall, one of the raters consistently measured the volume as being larger than did the other rater.

The weighted kappa for arterial patency (n=20) was 0.46 (95% CI 0.2-0.7).

5.2.1.2. Inter-rater agreement in the ATTEST study scans

For baseline irreversible tissue volume, the correlation coefficient was 0.96 (95% limits of agreement -16.2-19.9mL) (Figure 5-5).

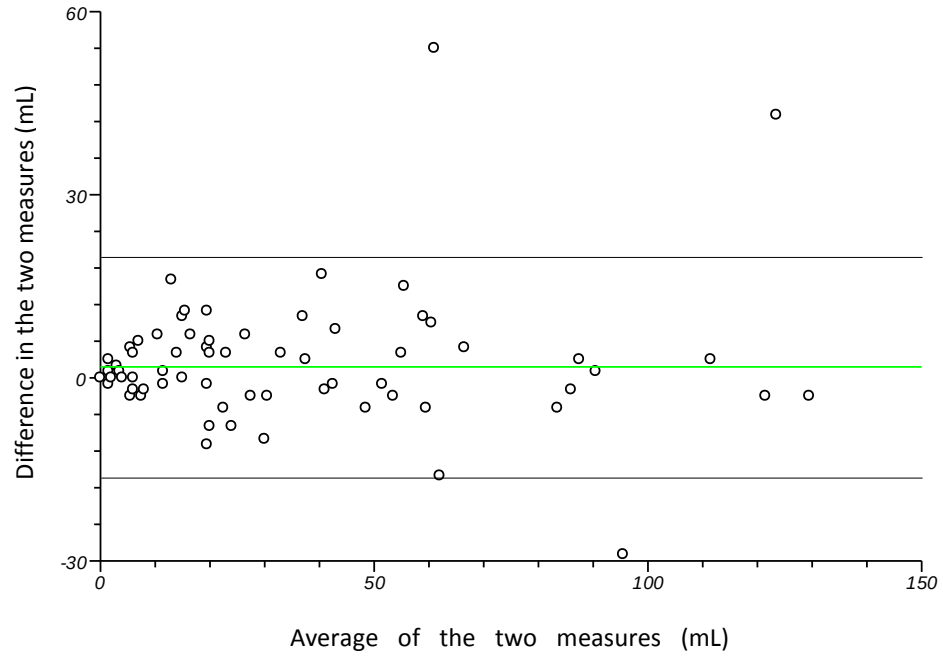


Figure 5-5. Bland-Altman agreement plot for inter-rater agreement in baseline core volume (mean[green]±1.96SD[black]).

For penumbra volumes, the correlation coefficient was 0.91 with 95% limits of agreement of -30.3-30.1mL (Figure 5-6).

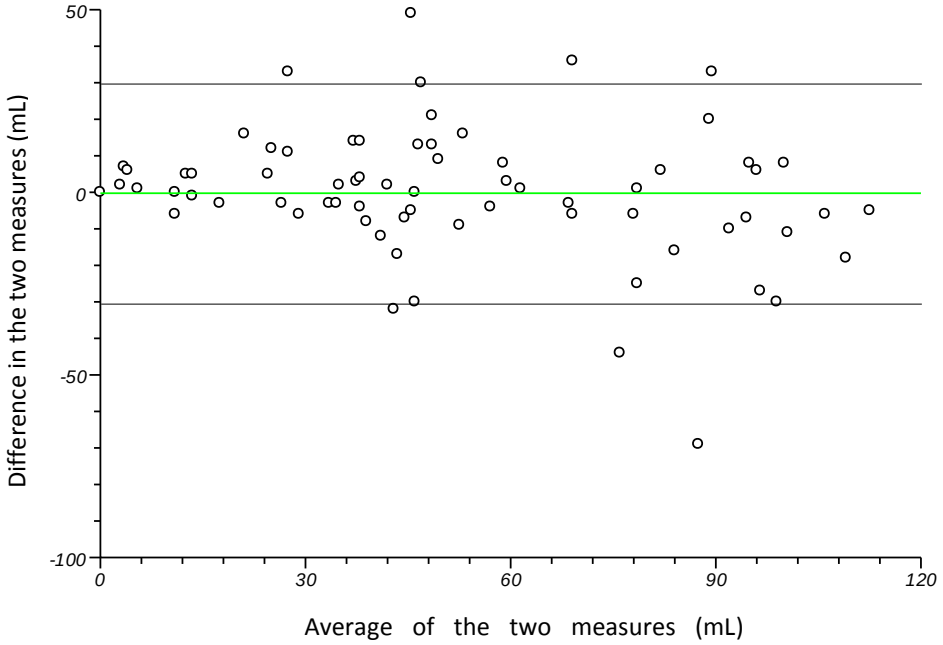


Figure 5-6. Bland-Altman agreement plot for inter-rater agreement in baseline penumbra volume(mean[green]±1.96SD[black]).

The correlation coefficient for the co-registered infarct volume was 0.91 (95% limits of agreement -57.3-26.4mL) (Figure 5-7).

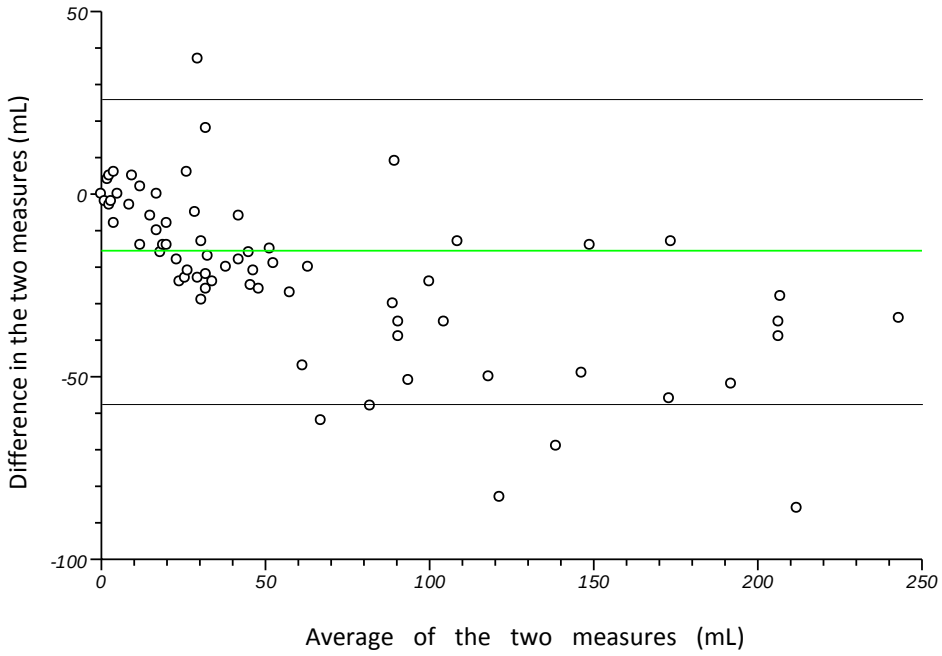


Figure 5-7. Bland-Altman agreement plot for inter-rater agreement in co-registered infarct volume at 24-48 hours (mean[green]±1.96SD[black]).

For total infarct volumes, the correlation coefficient was 0.91 with 95% limits of agreement of -92.9-47.3mL (Figure 5-8).

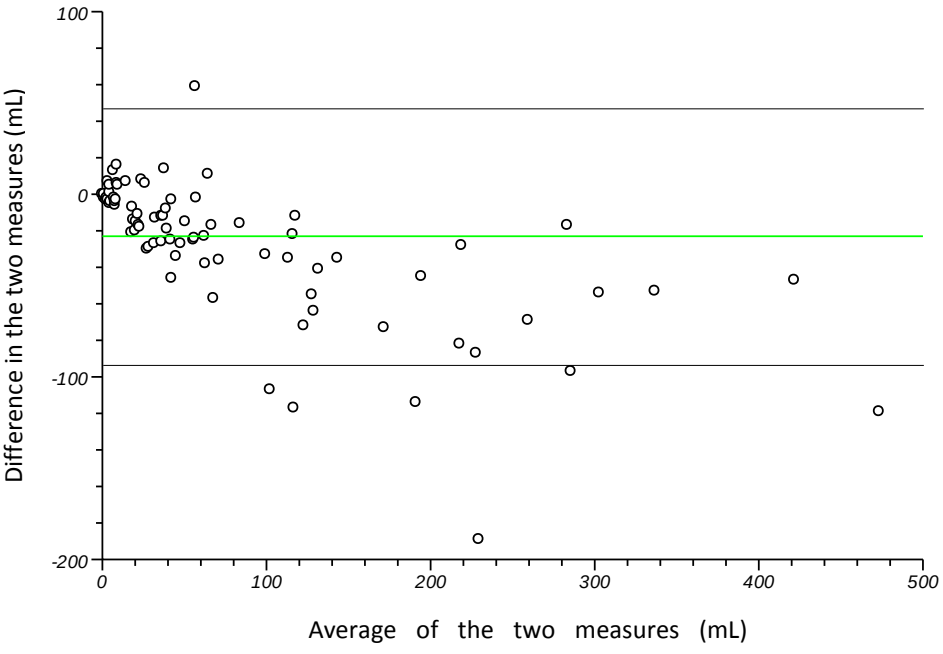


Figure 5-8. Bland-Altman agreement plot for inter-rater agreement in total infarct volume at 24-48 hours (mean[green]±1.96SD[black]).

Again, one of the raters consistently produced higher measures than did the other rater.

The weighted kappa for baseline occlusion sites was 0.63 (95%CI 0.49-0.77); for arterial potency at 24-48 hours, kappa=0.68 (95%CI 0.48-0.89) using the TIMI scale, and 0.7 (95% 0.5-0.89) using the TICI scale.

Other imaging parameters assessed included brain swelling at 24-48 hours (k=0.5, 95% CI 0.36-0.65), the radiological classification of ICH according to ECASS 2[311] (k=0.75, 95% CI 0.6-0.91), hyperdense vessel sign on baseline NCCT (k=0.61, 95% CI 0.42-0.8), the ASPECT score on baseline NCCT (k=0.41, 95% CI 0.29-0.53) and on follow-up CT (k=0.69, 95% CI 0.57-0.81).

Given the low agreement in categorical variables, the discrepancies in baseline occlusion and recanalisation were resolved by referencing the reports of radiologists who were not aware of the treatment allocation. The ICH classification was adjudicated by experienced external readers from the University of Calgary. Any remaining categorical parameters disagreements were settled by readings from Professor. Muir.

5.2.2. Statistical analysis

The trial was primarily intended to inform the design of a larger definitive study by yielding information on potential recruitment rates, incidence of relevant imaging abnormalities, and the distribution of outcome events; however, a sample size based on imaging parameters was determined, assuming that tenecteplase would exhibit a 15% absolute superior recanalisation rate compared to alteplase[326,327] with quicker recanalisation. We estimated a potential 25% reduction in mean infarct volume (38 mL vs 49 mL, standard deviation 20mL)[396], equating to 52 subjects per group for 80% power at a 5% level of significance. The findings were expected to assist in deciding whether a definitive trial based on imaging selection criteria or clinical features alone would be preferable.

The primary outcome and other continuous variables were analysed with a linear regression model adjusted for the stratification variables of age and baseline NIHSS scale. Binary variables were tested using logistic regression models, adjusted for the same stratification variables. P values for differences between treatment groups have been extracted from these models. In the subgroup analyses, when the numbers were small, univariate regression was used. The results presented below use per protocol analysis population comprising all patients who were randomised and had a final diagnosis of

stroke, as pre-specified in the statistical plan. This was selected as appropriate for a phase II exploratory study reliant on a primary imaging end-point. Normally distributed variables are described as mean and standard deviation (SD), ordinal variables as median and interquartile range (IQR) and categorical variables as number and percentage per category.

The primary outcomes and secondary outcomes listed in the study protocol were analysed by the study statistician from the Robertson centre for Biostatistics in the University of Glasgow, the rest of the analysis was performed by the author of this thesis.

5.3. Results

Between January 2012 and September 2013, 355 patients were screened (Figure 5-9), of whom 157 were eligible for IV thrombolysis and 104 patients were enrolled, 52 being assigned to each treatment group. The study recruitment completed earlier than scheduled (Figure 5-10). Reasons for exclusion are detailed in Figure 5-9. Eight patients ultimately had a diagnosis of non-stroke conditions and were excluded from the protocol-defined analysis. Groups were well-balanced for clinical baseline characteristics and co-morbidities (Table 5-1), had moderate stroke severity (median NIHSS score 11 to 12), and similar onset-to-treatment time at just over three hours. Participants randomised to tenecteplase had a larger median core volume (20 mL [IQR 2-55] vs 15 mL [IQR 3-40]), and a higher proportion presented with large artery occlusion (internal carotid artery or proximal middle cerebral artery occlusion in 26/35 [75%] tenecteplase-treated vs 23/38 [61%] alteplase treated) on baseline CTA, although these differences were not statistically significant.

One patient assigned to alteplase did not receive the study drug after randomisation, following a clinician review of the baseline CT querying minor ICH; all other participants received the full dose of the study treatment. Excluding those with no baseline perfusion lesion or vessel occlusion, 71 had technically satisfactory imaging for the primary outcome analysis, and 67 were suitable for recanalisation assessment. Patients who did not contribute to the primary imaging analysis (n=33) were younger (mean age 66 years, SD 13) and had less severe strokes (median NIHSS score 9, IQR 6-12) than those with analysable imaging.

All patients with a confirmed stroke were followed-up for three months after entry into the study.

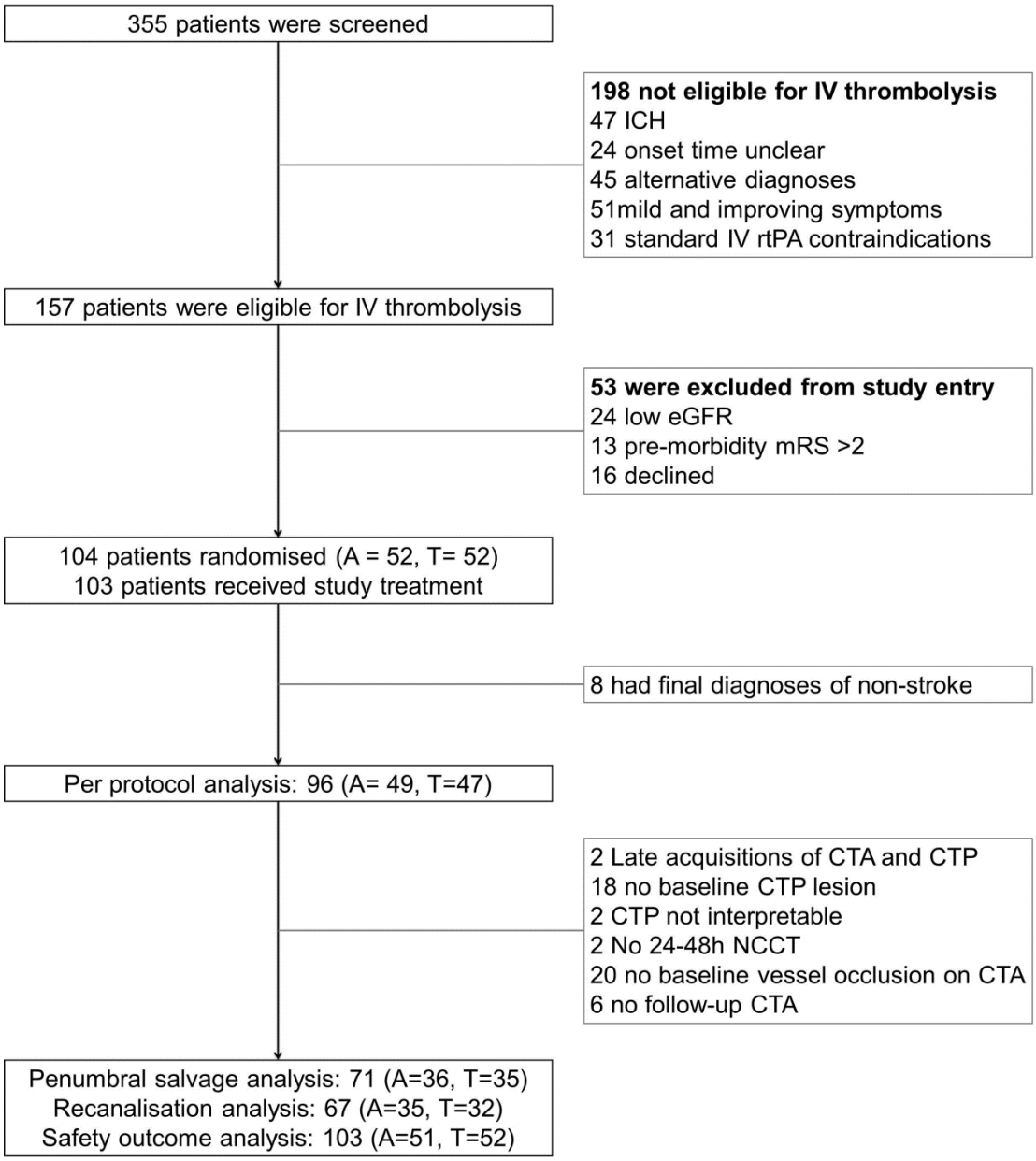


Figure 5-9. Study CONSORT chart

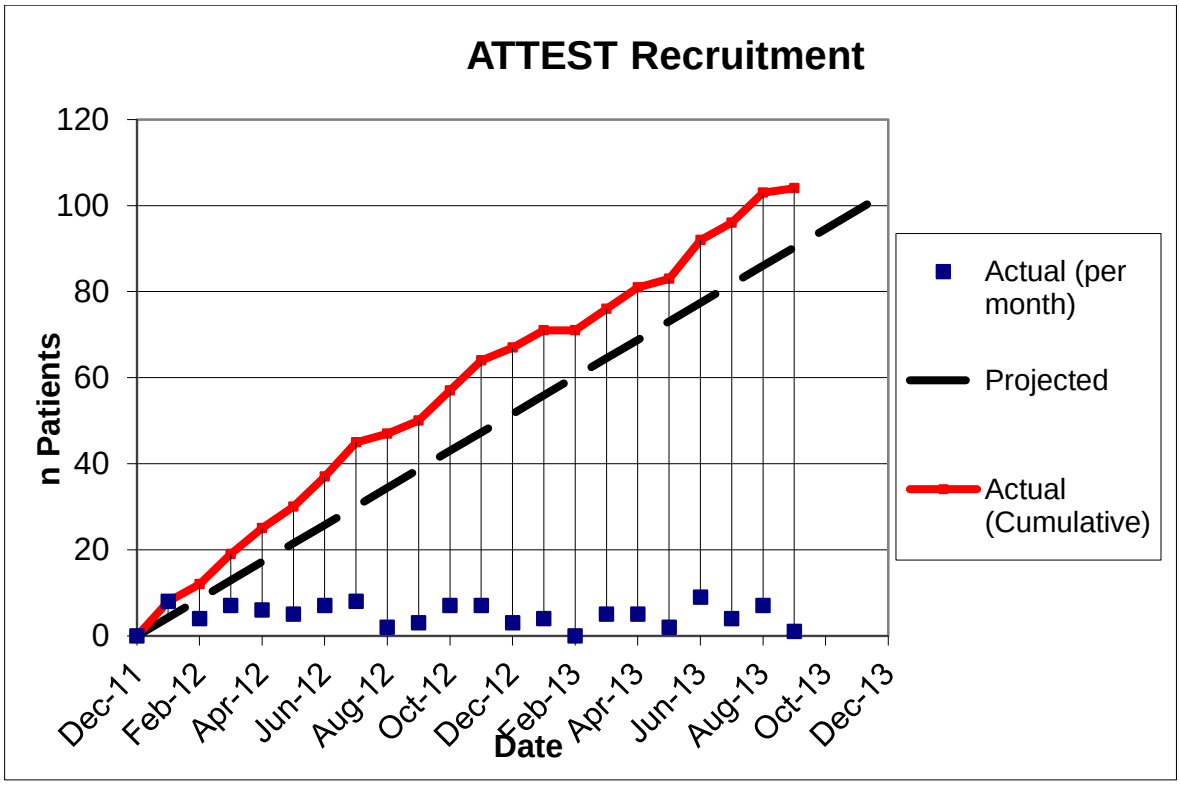


Figure 5-10. Graph of study recruitment progress

Table 5-1. Demography, risk factors and stroke characteristics in the per protocol population

		Tenecteplase (n=47)	Alteplase (n=49)
Clinical			
Age years mean(SD*)		71 (13)	71 (12)
Male (n, %)		30, 64%	31, 63%
Dominant hemisphere stroke (n, %)		24, 51%	26, 53%
Baseline NIHSS (Median, IQR)[min-max]		12 (9-18)[2-26]	11 (8-16)[3-27]
Onset-to-treatment Time min	mean(SD)	184 (44)	192 (45)
	Median (IQR)	180 (156, 215)	200 (160, 220)
Time between Initial and Follow-up Imaging (hours) mean (SD)		28.5 (7.1)	27.3 (7.5)
Door to Needle Time min mean(SD)		42(17)	38 (19)
Previous stroke/TIA (n, %)		12, 26%	11, 22%
Hypertension (n, %)		20, 43%	28, 57%
Diabetes (n, %)		7, 15%	7, 14%
Blood glucose mmol/L mean (SD)		7 (1)	7 (2)
Atrial Fibrillation (n, %)		19, 40%	15,31%
Hyperlipidaemia (n, %)		4, 9%	7, 14%
Smoker (n,%)		13, 28%	10,20%
Stroke clinical syndrome			
TACS (n, %)		27, 57%	28, 57%
PACS (n, %)		16, 34%	16, 33%
LACS (n, %)		2, 4%	3, 6%
POCS (n, %)		2, 4%	2, 4%
ASPECT score mean (SD)		7 (2)	7 (2)
Imaging			
Penumbra Volume ml	Median (IQR)	40 (4-62)	37 (9-69)
	Mean (SD)	53 (31)	49 (30)
Core Volume ml	Median (IQR)	20 (2-55)	15 (3-40)
	Mean (SD)	32 (36)	24 (29)
Occlusion (n,%)		35/47, 74%	38/49, 78%
Tandem/ICA		10/35,29%	8/38, 21%
M1§		16/35, 46%	15/38, 40%
M2§		6/35, 17%	11/38, 29%
M3§		1/35, 3%	3/38, 8%
ACA/PCA		2/35, 6%	1/38, 3%

*SD, standard deviation; NIHSS: National Institute of Health Stroke Scale; IQR: Interquartile Range; TIA: Transient Ischaemic Attack; TACS; Total Anterior Circulation Syndrome; PACS: Partial Anterior Circulation Syndrome; LACS: Lacunar Syndrome; POCS: Posterior Circulation Syndrome; ASPECT score: Alberta Stroke Program Early CT score; ICA: Internal Carotid Artery; § Middle Cerebral Artery M1, M2, M3 segment; ACA: Anterior Cerebral Artery; PCA: Posterior Cerebral Artery.

5.3.1. Efficacy

There were no significant differences in the primary endpoints of the percentage of penumbra salvaged (mean difference 1%, 95%CI -10% to 12%, p=0.81), or of secondary endpoints, either for imaging or for clinical outcomes (Table 5-2, Figure 5-11 to 5-12).

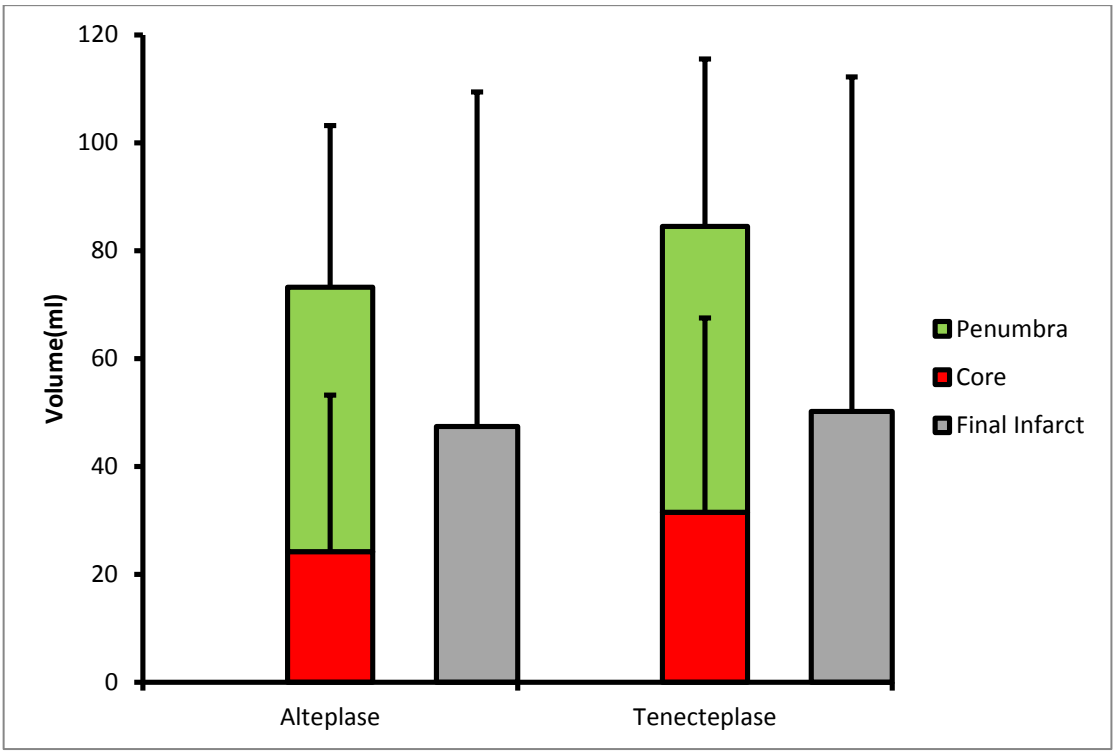


Figure 5-11. Baseline CT perfusion lesion segmented into core and penumbra and co-registered final infarct volumes at 24-48 hours. Error bars show standard deviation.

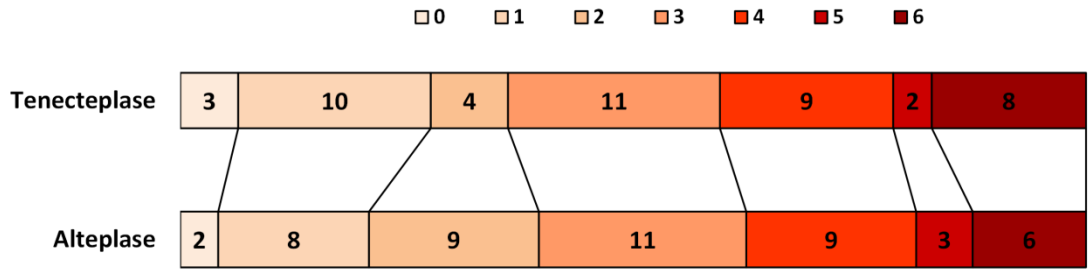


Figure 5-12. Distribution of Modified Rankin scale at 90 days

Adding core volumes and occlusion sites to the regression models as a post-hoc analysis, did not affect either the primary outcomes (mean difference 2.5%, 95%CI -8%-13%, p=0.65) or recanalisation rates (OR 0.7, 95%CI 0.2-2.2, p=0.65) (Figure 5-13).

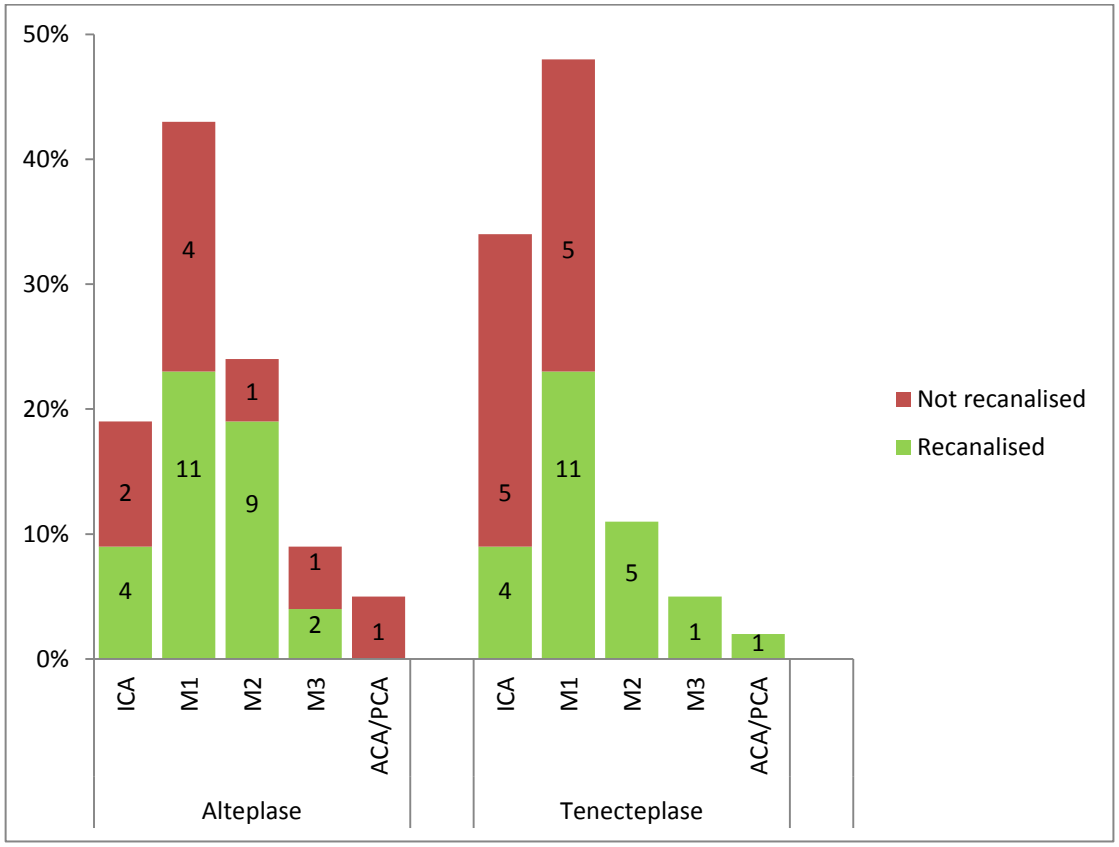


Figure 5-13. Recanalisation rates according to treatment groups and occlusion sites (percentage within each treatment group shown).

The volume of infarct growth (mean difference -6mL, 95% CI -21-9, p= 0.43), and the volume of penumbra salvage (mean difference 5mL, 95% CI -7-17, p=0.43) at 24-48 hours did not differ between the two groups. When core volumes and occlusion sites were added as adjusted variables, the results were still similar (mean difference -7mL, 95% CI -21-7, p=0.33; and mean difference 1mL, 95% CI -10-11, p=0.88 respectively).

5.3.2. Safety

The safety population included 52 patients treated with tenecteplase and 51 treated with alteplase. ICH of any kind was seen in eight patients in the tenecteplase group and 14 in the alteplase group (OR 0.4, 95%CI 0.2-1.2, p=0.091); only one incidence of PH[311] occurred in the tenecteplase group, compared to five in the alteplase group. The symptomatic ICH incidence, using either the SITS-MOST definition or the ECASS II definition, did not differ (Table 5-2).

Table 5-2. Study outcomes in the protocol-defined population.

	Tenecteplase (n=47)	Alteplase (n=49)	p value¢	Mean difference (95%CI)	Odds ratio (95%CI)
Primary outcome					
Percent penumbral salvage at 24-48 h mean (SD)*	68% (28)	68% (23)	0.81	1.3% (-9.6, 12.1)	-
Secondary imaging outcomes					
Co-registered final infarct volume at 24-48 h mL mean (SD)#	50 (62)	47 (62)	1.00	0.1 (-20, 20)	-
Total infarct volume at 24-48 h mL mean (SD) ‖	75 (101)	66 (91)	0.75	5 (-26, 35)	-
Volume of infarct growth at 24-48 h mL mean (SD)	18 (39)	22 (42)	0.43	-6 (-21, 9)	-
Volume of penumbra salvaged at 24-48 h mL mean (SD)	36 (27)	32 (22)	0.43	5 (-7, 17)	-
Recanalisation at 24-48 h (n, %)§	21/32, 66%¤	26/35, 74%¤	0.38	-	0.6 (0.2-1.8)
Secondary clinical outcomes					
Early neurological improvement at 24 h (n, %) ¶	19/47, 40%	12/49, 24%	0·10	-	2.1 (0.9-5.2)
Improvement in NIHSS between baseline and 24 h mean (SD)	3 (6)	2 (6)	0.74	-0.4 (-3.1-2.2)	-
mRS 0-1 at 30 days (n, %)	7/47, 15%	7/49, 14%	0.89	-	1.1 (0.3-3.5)
mRS 0-1 at 90 days (n, %)	13/47, 28%	10/49,	0.28	-	1.8 (0.6-5.5)

		20%			
Home by 90 days (n, %)	30/47, 64%	36/49, 73%	0.36	-	0.6 (0.2-1.8)
Days at home by 90 days mean (SD)	45 (39)	50 (36)	0.64	-3.1 (-15.8-9.7)	-
Mortality at 90 days (n, %)	8/47, 17%	6/49, 12%	0.51	-	1.3 (0.4-3.7)
Safety outcomes (A=51, T=52)					
Any ICH (n, %)	8/52, 15%	14/51, 27%	0.09	-	0.4 (0.2-1.2)
Any parenchymal haemorrhage (n, %)	1/52, 2%	5/51, 10%	0.12		
Parenchymal haemorrhage type 2 (n, %)	0/52, 0%	3/51, 6%	0.94		
Symptomatic ICH (ECASS II[311] definition) (n, %)	3/52, 6%	4/51, 8%	0.59	-	0.6 (0.1-3.2)
Symptomatic ICH (SITS-MOST[318] definition) (n, %)	1/52, 2%	2/51, 4%	0.50	-	0.4 (0.04-5.1)

*SD, standard deviation; NIHSS: National Institute of Health Stroke Scale; IQR: Interquartile Range; ICH: Intracerebral haemorrhage; mRS: Modified Rankin Scale;

¢ p-values were calculated from linear of logistic regression models that adjust for stratification variables and are a test for difference between groups.

Co-registered infarct volume was defined as infarct volume measured on 24-48 h CT slices co-registered to baseline CT perfusion;

‖Total infarct volume was defined as total infarct volume measured on follow-up CT at 24-48 h;

§ Recanalisation was defined as TIMI[371] 2-3;

¤ The percentages for recanalisation were derived from the number of subjects with an occlusion.

¶ Early neurological improvement at 24 h was defined as NIHSS reduction ≥ 8 points or 24 h NIHSS 0-1.

There were 32 Serious Adverse Events (SAEs) in 22 (42%) patients given tenecteplase and 16 SAEs in 16 (31%) patients given alteplase, including ICH events fulfilling the criteria for seriousness. Within seven days of randomisation, six SAEs were documented in the tenecteplase group and four in the alteplase group (excluding SICHs, shown in detail in Table 5-3). Full details of all SAEs are shown in Tables 5-3 to 5-4 splitting into prior to day seven and post day seven of treatment, a brief summary of non-serious AEs is given Table 5-5. Probable treatment-related SAEs other than ICH were anaphylactoid reactions (one case in the tenecteplase group), and epistaxis resulting in hypotension (one case in the tenecteplase group). Three other cases of bleeding in the tenecteplase group (one each of post-menopausal bleeding [53 days after treatment], rectal bleeding [70 days after treatment] and upper gastrointestinal bleeding [60 days after treatment]) all occurred beyond the first month after study drug administration, and were therefore deemed to be unrelated.

Table 5-3. Summary of serious adverse events occurring on or prior to Day 7 (subjects with at least one event)

System Organ Class Preferred Term	Tenecteplase (N=52)	Alteplase (N=51)	All (N=103)
Total number of events on or prior to Day 7	8	9	17
No. subjects with at least one adverse event on or prior to Day 7	8 (15%)	9 (18%)	17 (17%)
General disorders and administration site conditions	1 (2%)	1 (2%)	2 (2%)
Chest pain	1 (2%)	0 (0%)	1 (1%)
General physical health deterioration	0 (0%)	1 (2%)	1 (1%)
Infections and infestations	1 (2%)	0 (0%)	1 (1%)
Pulmonary sepsis	1 (2%)	0 (0%)	1 (1%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0 (0%)	1 (2%)	1 (1%)
Malignant glioma	0 (0%)	1 (2%)	1 (1%)
Nervous system disorders	4 (8%)	5 (10%)	9 (9%)
Cerebral haemorrhage	0 (0%)	5 (10%)	5 (5%)
Embolic stroke	1 (2%)	0 (0%)	1 (1%)
Haemorrhage intracranial	1 (2%)	0 (0%)	1 (1%)
Ischaemic stroke	1 (2%)	0 (0%)	1 (1%)
Subarachnoid haemorrhage	1 (2%)	0 (0%)	1 (1%)
Respiratory, thoracic and mediastinal disorders	2 (4%)	2 (4%)	4 (4%)
Epistaxis	1 (2%)	0 (0%)	1 (1%)
Pneumonia aspiration	1 (2%)	2 (4%)	3 (3%)
Skin and subcutaneous tissue disorders	1 (2%)	0 (0%)	1 (1%)
Angioedema	1 (2%)	0 (0%)	1(1%)

Table 5-4. Summary of serious adverse events occuring after Day 7 (subjects with at least one event)

System Organ Class Preferred Term	Tenecteplase (N=52)	Alteplase (N=51)	All (N=103)
Total number of events after Day 7	24	7	31
No. subjects with at least one adverse event after Day 7	18 (35%)	7 (14%)	25 (24%)
Cardiac disorders	3 (6%)	1 (2%)	4 (4%)
Atrial fibrillation	3 (6%)	1 (2%)	4 (4%)
Gastrointestinal disorders	3 (6%)	0 (0%)	3 (3%)
Abdominal pain	1 (2%)	0 (0%)	1 (1%)
Constipation	1 (2%)	0 (0%)	1 (1%)
Diarrhoea	1 (2%)	0 (0%)	1 (1%)
Rectal haemorrhage	1 (2%)	0 (0%)	1 (1%)
Upper gastrointestinal haemorrhage	1 (2%)	0 (0%)	1 (1%)
General disorders and administration site conditions	2 (4%)	0 (0%)	2 (2%)
Chest pain	2 (4%)	0 (0%)	2 (2%)
Non-cardiac chest pain	1 (2%)	0 (0%)	1 (1%)
Infections and infestations	2 (4%)	1 (2%)	3 (3%)
Bronchopneumonia	1 (2%)	0 (0%)	1 (1%)
Gastroenteritis viral	0 (0%)	1 (2%)	1 (1%)
Urosepsis	1 (2%)	0 (0%)	1 (1%)
Injury, poisoning and procedural complications	1 (2%)	1 (2%)	2 (2%)
Fall	1 (2%)	1 (2%)	2 (2%)
Investigations	1 (2%)	0 (0%)	1 (1%)
Biopsy uterus	1 (2%)	0 (0%)	1 (1%)
Hysteroscopy	1 (2%)	0 (0%)	1 (1%)
Metabolism and nutrition disorders	1 (2%)	0 (0%)	1 (1%)

System Organ Class Preferred Term	Tenecteplase (N=52)	Alteplase (N=51)	All (N=103)
Dehydration	1 (2%)	0 (0%)	1 (1%)
Musculoskeletal and connective tissue disorders	0 (0%)	1 (2%)	1 (1%)
Mobility decreased	0 (0%)	1 (2%)	1 (1%)
Nervous system disorders	4 (8%)	2 (4%)	6 (6%)
Cerebrovascular accident	2 (4%)	1 (2%)	3 (3%)
Ischaemic stroke	2 (4%)	1 (2%)	3 (3%)
Psychiatric disorders	0 (0%)	1 (2%)	1 (1%)
Depression	0 (0%)	1 (2%)	1 (1%)
Intentional self-injury	0 (0%)	1 (2%)	1 (1%)
Renal and urinary disorders	3 (6%)	0 (0%)	3 (3%)
Renal failure acute	1 (2%)	0 (0%)	1 (1%)
Renal impairment	2 (4%)	0 (0%)	2 (2%)
Reproductive system and breast disorders	1 (2%)	0 (0%)	1 (1%)
Postmenopausal haemorrhage	1 (2%)	0 (0%)	1 (1%)
Respiratory, thoracic and mediastinal disorders	3 (6%)	0 (0%)	3 (3%)
Epistaxis	1 (2%)	0 (0%)	1 (1%)
Pneumonia aspiration	1 (2%)	0 (0%)	1 (1%)
Pulmonary embolism	1 (2%)	0 (0%)	1 (1%)
Surgical and medical procedures	3 (6%)	1 (2%)	4 (4%)
Endarterectomy	1 (2%)	1 (2%)	2 (2%)
Gastrointestinal tube insertion	1 (2%)	0 (0%)	1 (1%)
Hospitalisation	1 (2%)	0 (0%)	1 (1%)
Joint resurfacing surgery	1 (2%)	0 (0%)	1 (1%)
Vascular disorders	2 (4%)	0 (0%)	2 (2%)
Circulatory collapse	1 (2%)	0 (0%)	1 (1%)
Orthostatic hypotension	1 (2%)	0 (0%)	1 (1%)

Table 5-5. Total Adverse Events (AE) (including serious)

	Tenecteplase	Alteplase
All AEs to day 90	110	76
Probably or definitely related to study drug	3	7
Subjects with at least one AE *n (%)*	42 (81%)	36 (71%)
MEDDRA classification	Incidence (n AEs)	
Blood	1	0
Cardiac	6	6
Congenital (ASD)	1	0
Eye	0	1
GI	8	6
General Disorders	3	5
Hepatobiliary	0	1
Infections	13	7
Injury	5	5
Investigations	3	0
Metabolic/ Nutritional	3	0
Musculoskeletal	3	5
Neoplasms	0	1
CNS	17	17
Psychiatric	4	2
Renal & Urinary	5	1
Respiratory	11	9
Skin	1	1
Procedures	4	2
Vascular	7	2

MEDDRA Medical Dictionary for Regulatory Activities; ASD Atrial septal Defect; GI Gastrointestinal.

5.3.3. Correlations between baseline variables and outcomes

The baseline NIHSS score was significantly associated with the baseline penumbra volume (p=0.002, 95% CI [0.6-2.8]. Itwas found to be significantly inversely associated with excellent functional outcomes at 90 days (OR [95%CI] 0.7 [0.6-0.9], p<0.001) in this sample, but there was no association with early neurological improvement (p=0.59). The baseline NIHSS score was correlated with the proportion of penumbra salvaged (p<0.001, 95%CI [-2.7-- -0.9]) (Figure 5-14), and infarct growth (p<0.001, 95%CI [1.5-4.1]) (Figure 5-15), but not with the volume of penumbra salvaged (p=0.4). No significant association was found between the baseline NIHSS score and recanalisation (p=0.7). More severe strokes were associated with a higher risk of ICH (OR [95%CI] 1.1 [1-1.2], p=0.014), but not with SICH (SITS-MOST definition).

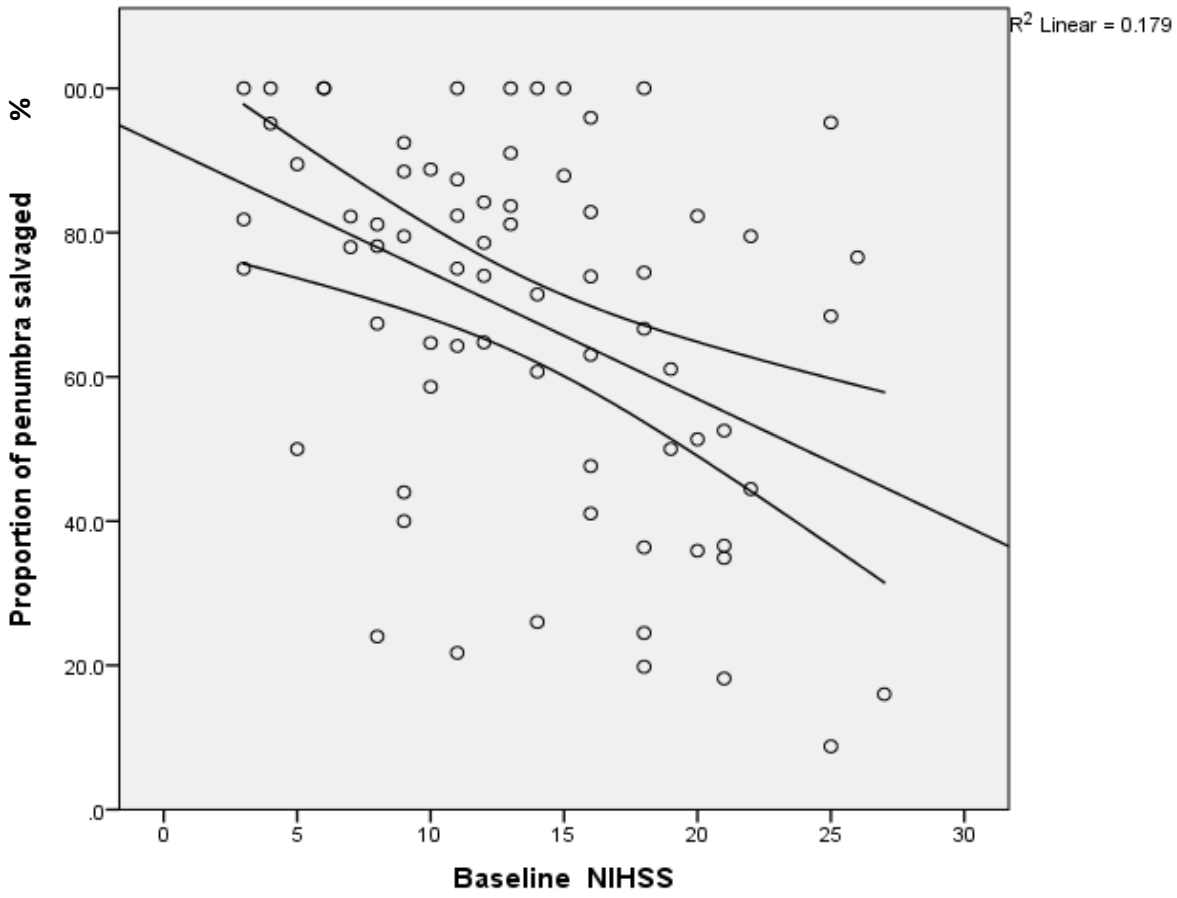


Figure 5-14. The association between the baseline NIHSS score and the proportion of penumbra salvaged

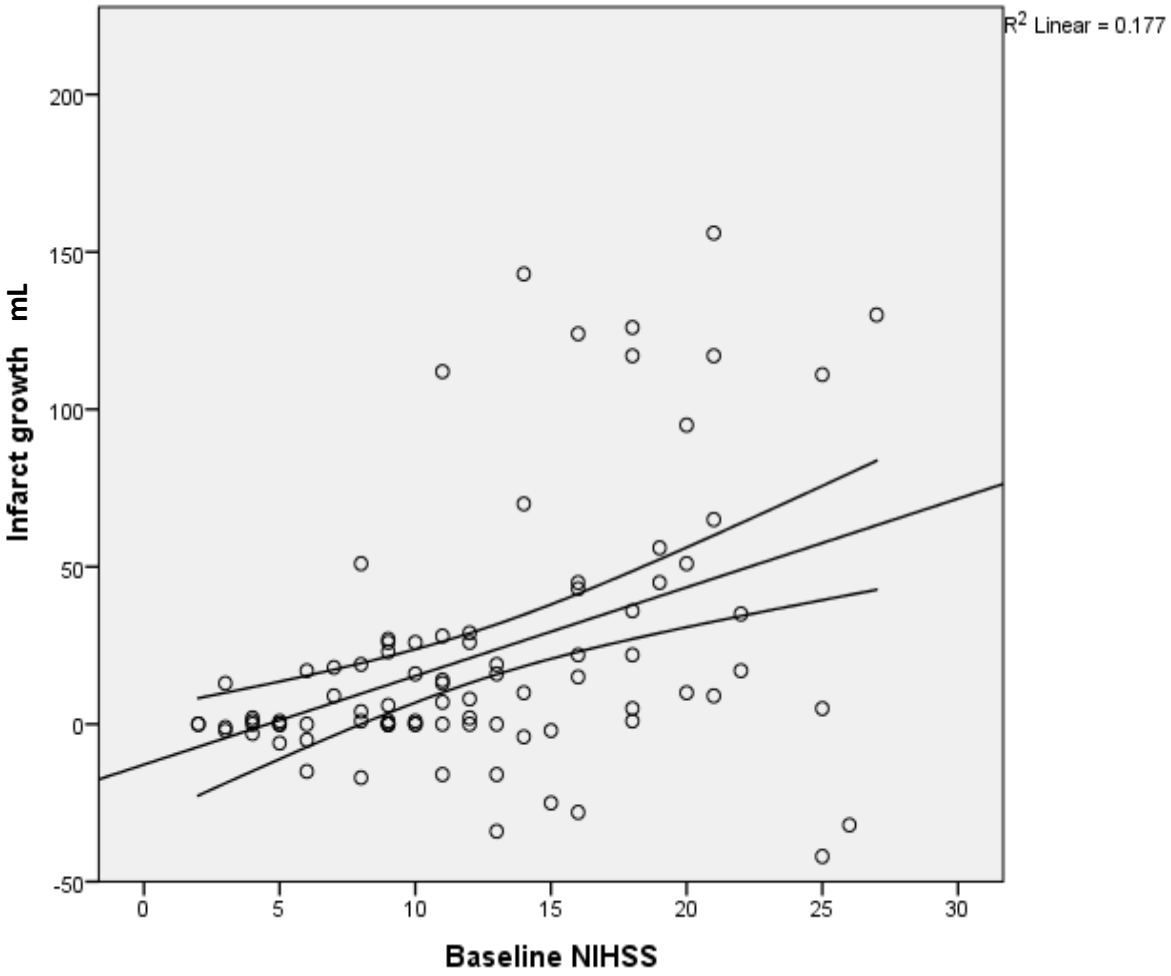


Figure 5-15. The association between the baseline NIHSS score and Infarct growth

There was no association shown between age or OTT and any of the main outcomes.

Baseline glucose levels on admission were significantly associated with the volume of the penumbra salvaged (p=0.006, 95%CI [-6.6- -1.2]) (Figure 5-16), but not with the percentage of penumbral salvage (p=0.45) or infarct growth (p=0.62). The baseline glucose level has no correlation with recanalisation (p=0.24), haemorrhage (p=0.25), functional outcomes at 90 days (p=0.3) or early neurological improvement (p=0.89).

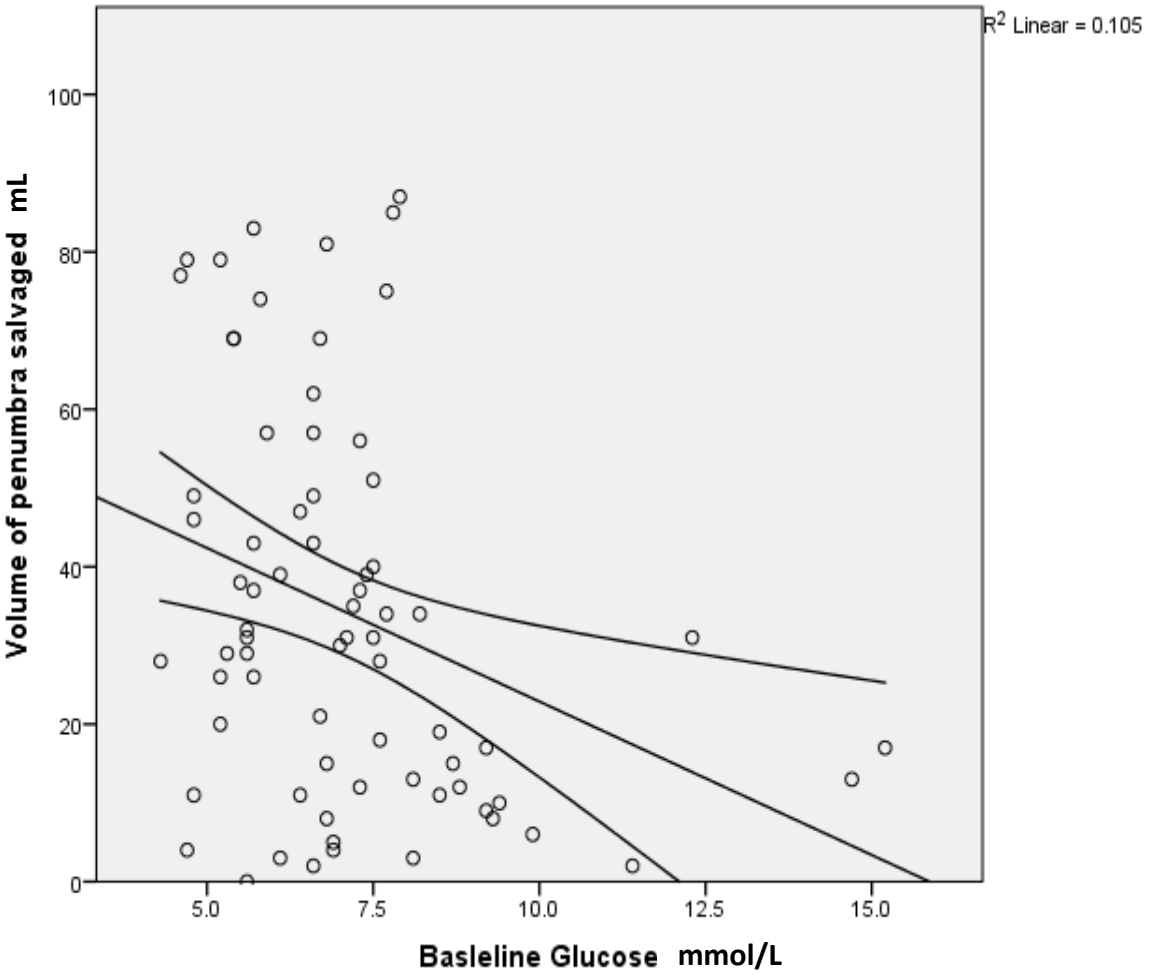


Figure 5-16. The association between the baseline glucose and volume of penumbra salvaged

Baseline systolic BP was shown to be associated with the change of the NIHSS score at 24 hours (p=0.047, 95%CI [0-0.1]) (Figure 5-17), and there was a near-significant association with early neurological improvement (OR 95%CI 0.98 [0.96-1], p=0.073). It had no correlation with excellent functional outcomes at day 90 (p=0.26), penumbral salvage (p=0.4), infarct growth (p=0.47) or recanalisation (p=0.41). High baseline BP was associated with higher ICH risk (OR [95%CI] 1.03 [1-1.1], p=0.043), but not with SICH (SITS-MOST) (p=0.14).

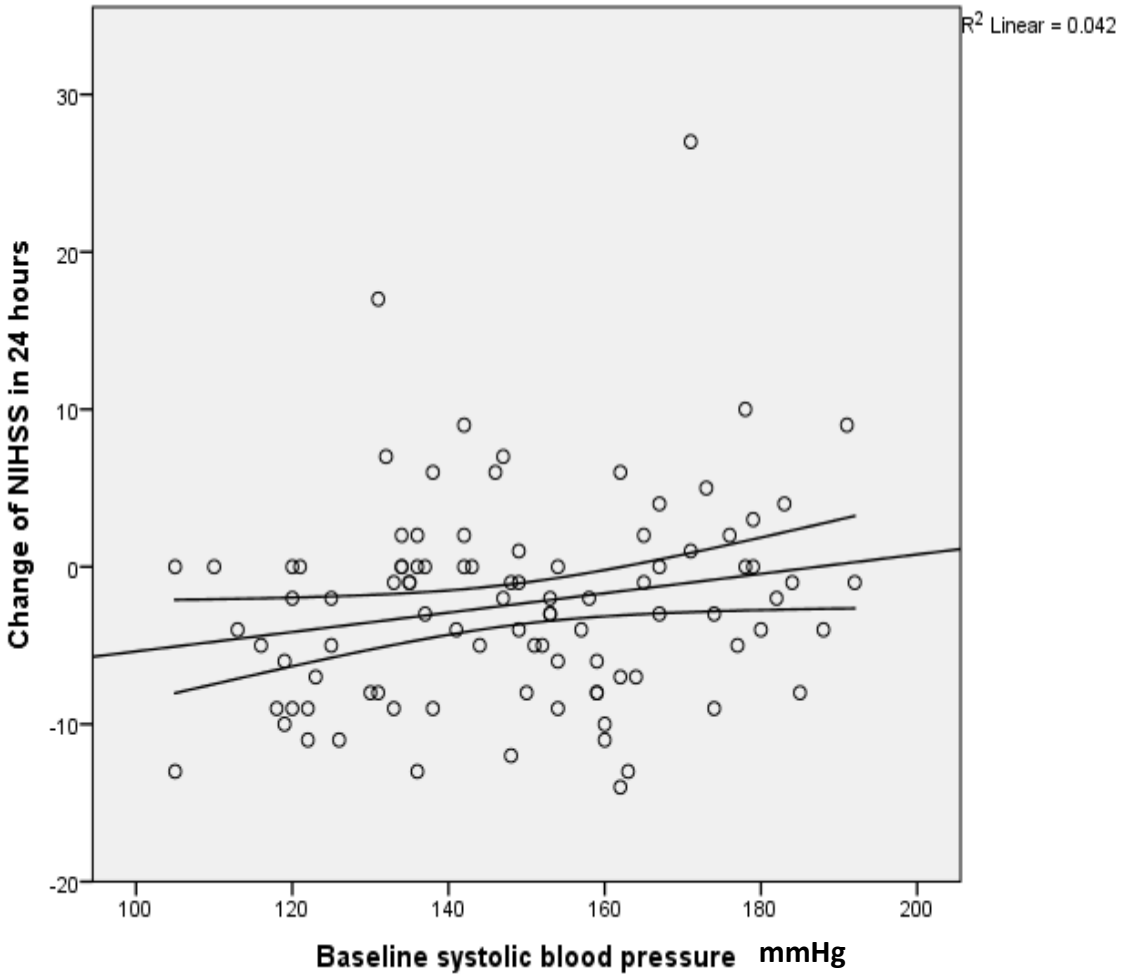


Figure 5-17. The association between baseline systolic blood pressure and change of the NIHSS score in the first 24 hours post thrombolysis26 out of 96 (27.1%) patients had confirmed cardioembolic stroke, 18 (18.8%) had confirmed artherosclerotic stroke. The proportion of patients who had hypersense vessel sign on baseline NCCT did not differ between the two groups [17(65.4%) vs 11(61.1%), p=0.77]. Compared with artherosclerotic stroke, with thein regression model adjusted for age, baseline NIHSS and OTT, cardioembolic stroke was associated with significantly more early neurological improvement at 24 hours (p=0.031, 95%CI[1.2-21.7]), but not mRS 0-1 at 90 days (p=0.77), nor any ICH (p=0.58). None of these 44 patients had SICH according to the SITS-MOST criteria. No difference was found in the percentage of penumbra salvaged (p=0.73), the volume of penumbra salvaged (p=0.36), infarct growth (p=0.95), or recanalisation (p=0.94) at 24 hours.

5.3.4. Sub-group analysis

5.3.4.1. Oxford stroke classification

Of the 96 patients included, 55 (57%) presented with total anterior circulation syndromes, 32 (33%) with partial anterior circulation syndromes, 5 (5%) with lacunar syndromes, and 4 (4%) with posterior circulation syndromes. This sample provided minimal information for IV thrombolysis in lacunar stroke.

5.3.4.2. Age

71 (74%) out of 96 patients were aged less than 80, and 25 (26%) were aged 80 or over. Between the tenecteplase- and alteplase-treated groups, the imaging outcomes including the percentage of penumbra salvaged, the volume of penumbra salvaged, infarct growth and recanalisation rates at 24 hours did not differ. No differences were found in early neurological improvement, in excellent functional outcomes at 90 days, or in any ICH or SICH between tenecteplase- and alteplase-treated patients (Table 5-6).

5.3.4.3. Stroke severity

14 (15%) out of 96 patients had a baseline NIHSS score of 1-5, 48 (50%) of the subjects' NIHSS scores were between 6-14, 29 (30%) between 15-24, and 5 (5%) were 25 and above. The main outcomes following IV thrombolysis in these groups are shown in Table 5-7. In the subgroup of moderate severe stroke (NIHSS score 6-14), significantly more patients who received tenecteplase achieved early neurological improvement at 24 hours ($p=0.048$). All other clinical and imaging outcomes were similar. In the severe stroke group (NIHSS score 15-24), no difference was demonstrated in any outcomes. In the groups of patients with mild stroke (NIHSS score 1-5) and very severe stroke (NIHSS score ≥25), the numbers were too small to provide any useful comparison.

Table 5-6. Comparison of the main imaging and clinical outcomes between tenecteplase and alteplase treated patients in subgroups of patients<80 or ≥80 years of age

Age TNK vs Alteplase	**Percentage of penumbra salvaged** % mean difference (95%CI)	**Volume of penumbra salvaged** mL mean difference (95% CI)	**Infarct growth** mL mean difference (95%CI)	**Recanalisation** n (%), OR (95%CI)	**Early neurological improvement** n (%), OR (95%CI)	**mRS 0-1 at 90 days** n, %, OR (95%CI)	**Any ICH** n (%), OR (95%CI)	**SICH (SITS-MOST)** n (%), OR (95%CI)
<80 34 vs 37	5% (-4, 17), p=0.22	10 (-5,25), p=0.2	-12 (-30, 5),p=0.17	13 (38%) vs 19 (51%), 0.7 (0.2, 2.4), p=0.56	14 (41%) vs 9 (25%), 2 (0.7, 5.6), p=0.2	9 (27%) vs 8 (22%), 1.8 (0.5, 6.7), p=0.39	5 (15%) vs 9 (24%), 0.4 (0.1, 1.5), p=0.4	1 (3%) vs 1 (3%), 0.7 (0.03, 14.1), p=0.66
≥80* 13 vs 12	-12% (-45-20), p=0.44	-7 (-29-14), p=0.48	20 (-72-58), p=0.83	8 (62%) vs 7 (58%)	5 (38%) vs 3 (25%), 1.9 (0.3-10.4), p=0.47	4 (31%) vs 2 (17%), 2.2 (0.3-15.2), p=0.42	2 (15%) vs 5 (42%), 0.2 (0.03-1.5), p=0.12	0 vs 1 (8%)
* Univariate regression model used due to small sample size.								

Table 5-7. Comparison of main outcomes between tenecteplase and alteplase treated patients in stroke severity subgroup

Stroke severity (NIHSS) TNK vs Alteplase	**Percentage of penumbra salvaged** % mean difference (95%CI)	**Volume of penumbra salvaged** mL mean difference (95% CI)	**Infarct growth** mL mean difference (95%CI)	**Recanalisation,** n (%), OR (95%CI)	**Early neurological improvement** n (%), OR (95%CI)	**mRS 0-1 at 90 days** n, %, OR (95%CI)	**Any ICH** n (%), OR (95%CI)	**SICH (SITS-MOST)** n (%), OR (95%CI)
1-5∗ 7 vs 7	-3% (-39-45), p=0.85	0.3 (-32-31), p=0.98	-0.7 (-4-6),p=0.67	1(14%) vs 2 (29%)	4 (57%) vs 3 (43%), 4.6 (0.8-87.7), p=0.35	6 (86%) vs 5 (71%), 11.8 (0.8-744), p=0.24	1 (14%) vs 1 (14%)	0 vs 0
6-14 27 vs 21	7% (-9-21), p=0.4	7 (-13-26),p=0.49	-7 (-26-10),p=0.39	10 (48%) vs 27(52%), 0.2 (0.02-1.6), p=0.13	9 (43%) vs 5 (19%), 11.8 (1-20.5), p=0.048	5 (24%) vs 5 (19%), 1.4 (0.3-6,3), p=0.63	1 (5%) vs 6 (22%), 0.1 (0.01-1.2), p=0.07	0 vs 1(4%), p=1
15-24∗ 13 vs 16	3% (-29-15), p=0.51	3 (-23-21), p=0.92	-5 (-33-56), p=0.59	9 (56%) vs 8 (62%), 0.5 (0.03-9.5), p=0.66	4 (25%) vs 3 (23%), 1.1 (0.2-6.2), p=0.9	2 (13%) vs 0, p=1	5 (31%) vs 5 (38%), 0.6 (0.1-3), p=0.57	1 (6%) vs 1 (8%), 0.7 (0.04-13), p=0.83
≥25∗ 3 vs 2	11 (-116-138), p=0.8	-5 (-69-59), p=0.83	-25 (-292-243), p=0.8	1 (33%) vs 2 (100%)	2 (67%) vs 1 (50%)	0 vs 0	0 vs 2 (100%)	0 vs 0
∗ Univariate regression model used due to small sample size.								

5.3.4.4. Onset-to-treatment time

No patients were treated less than 90 minutes from the onset of symptoms. 44 (46%) were treated within 180 minutes, and 52 (54%) were treated between 181-270 minutes. In the group treated within 180 minutes, all clinical and imaging outcomes were similar between the tenecteplase and the alteplase groups. In the subgroup of patients who were treated between 181- 270 minutes, those who had tenecteplase had a significantly higher rate of early neurological improvement (p=0.014), and a near-significant trend of lower proportion of ICH rate (p=0.053) (Table 5-8).

Table 5-8. Comparison of main outcomes between tenecteplase and alteplase treated patients in OTT subgroup

OTT TNK vs Alteplase	**Percentage of penumbra salvaged** % mean difference (95%CI)	**Volume of penumbra salvaged** mL mean difference (95% CI)	**Infarct growth** mL mean difference (95%CI)	**Recanalisation,** n (%), OR (95%CI)	**Early neurological improvement** n (%), OR (95%CI)	**mRS 0-1 at 90 days** n, %, OR (95%CI)	**Any ICH** n (%), OR (95%CI)	**SICH (SITS-MOST)** n (%), OR (95%CI)
<180 24 vs 20	12 (-25-19), p=0.78	-2 (-25-20), p=0.8	12 (-24-33), p=0.77	11 (46%) vs 10 (50%), 0.7 (0.08-5.3), p=0.69	8 (33%) vs 8 (40%), 0.7 (0.2-2.6), p=0.55	6 (25%) vs 6 (30%), 2.8 (0.5-16.6), p=0.27	6 (25%) vs 6 (30%), 0.4 (0.07-2), p=0.25	1 (4%) vs 0, p=1
181-270 23 vs 29	8 (-11-17), p=0.67	10 (-7-24), p=0.26	-16 (-29-14), p=0.35	10 (43%) vs 16 (55%), 0.4 (0.09-1.6), p=0.19	11 (48%) vs 4 (14%), 5.7 (1.4-22.7), p=0.014	7 (30%) vs 4 (14%), 2 (0.4-10.9), p=0.41	1 (4%) vs 8 (28%), 0.1 (0.01-1), p=0.053	0 vs 2 (7%), p=1

5.3.5. Comparative study using different definitions for imaging analysis

5.3.5.1. Volumetric analysis for CT perfusion using two definitions

As previously discussed, the definition of tissue status in perfusion imaging varies depending on the analysis methods and processing algorithms used[104,137]. When the ATTEST study was planned, the most recognised thresholds to define irreversible and potential reversible tissue were from Wintermark's group[144]. When the study started in 2011, further evidence emerged that delay-corrected SVD with DT>2 seconds most accurately defined the penumbra, and a double core threshold with DT>2 seconds and CBF< 40% provided the most accurate definition of the infarct core among all the available post-processing algorithm and viability thresholds[138]. Since we used both definitions in our analysis, we compared the results generated.

Intra-observer agreement for volume measurements using the two definitions

During the volumetric analysis, although there appeared to be a greater variability in the volumes measured using Wintermark's (MW) definition compared with those using Bivard's (AB) definition, the intra-observer agreements between the two measurements for each definition were both excellent with AB's definition faring slightly better.

Ischaemic core:

The intra-class correlation coefficient for MW definition was 0.96 (95% limits of agreement -24-20mL) (Figure 5-18).

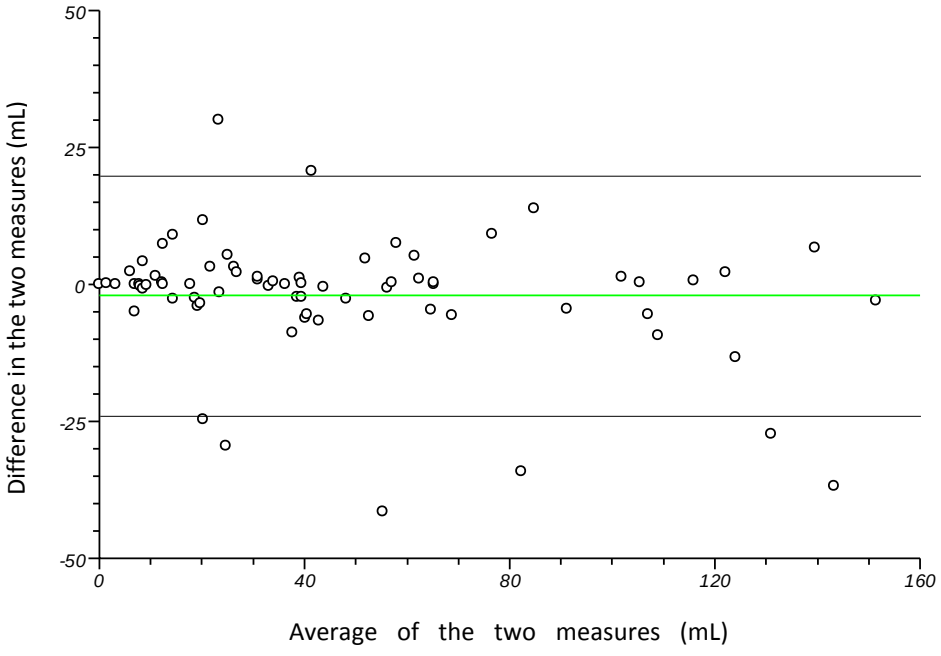


Figure 5-18. Intra-observer Bland-Altman agreement plot for ischaemic core volume using Wintermark's definition (mean[green]±1.96SD[black]).

The intra-class correlation coefficient for AB definition was 0.99 (95% limits of agreement -9-11mL) (Figure 5-19).

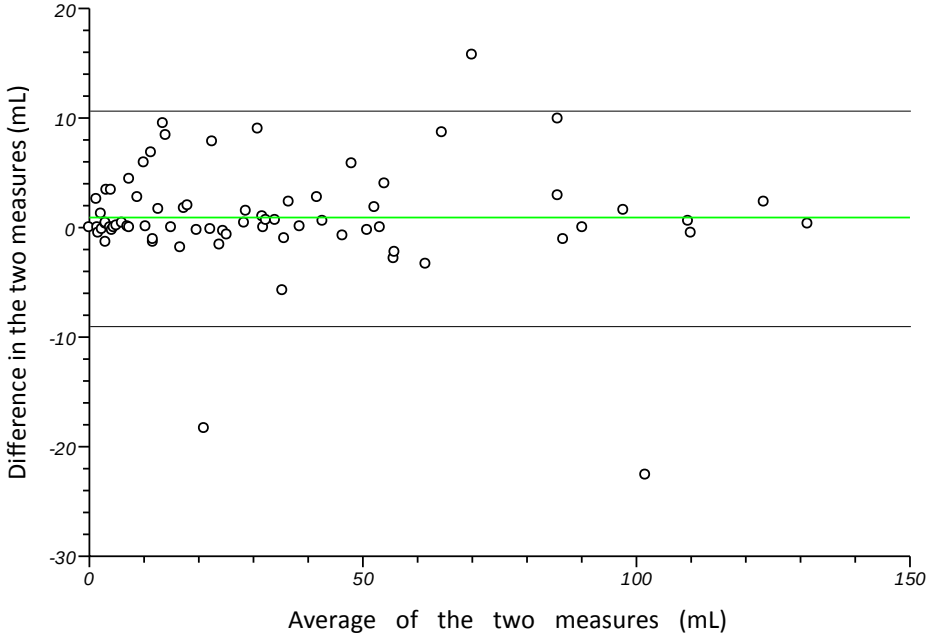


Figure 5-19. Intra-observer Bland-Altman agreement plot for ischaemic core volume using Bivard's definition (mean[green]±1.96SD[black]).

Penumbra:

The intra-class correlation coefficient for MW definition was 0.91 (95% limits of agreement -23-21mL) (Figure 5-20).

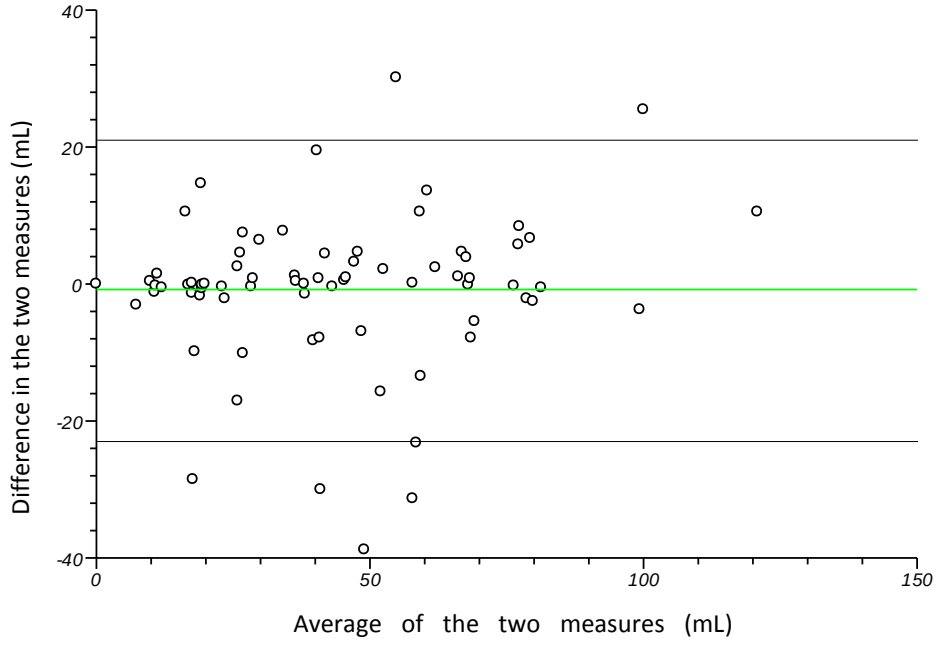


Figure 5-20. Intra-observer Bland-Altman agreement plot for penumbra volume using Wintermark's definition (mean[green]±1.96SD[black]).

The intra-class correlation coefficient for AB definition was 0.98 (95% limits of agreement -13-13mL) (Figure 5-21).

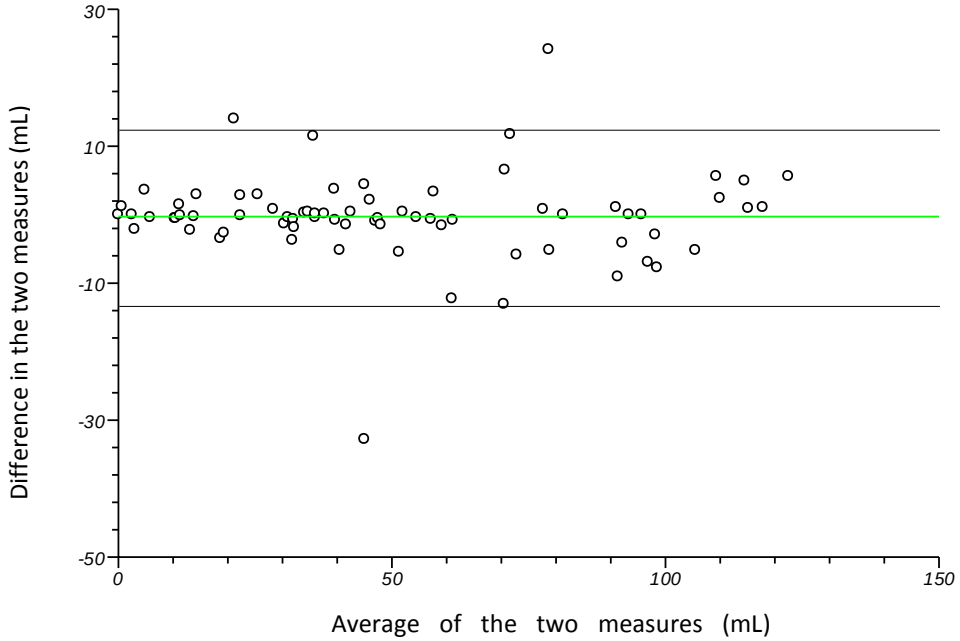


Figure 5-21. Intra-observer Bland-Altman agreement plot for penumbra volume using Bivard's definition (mean[green]±1.96SD[black]).

The comparison of CT perfusion lesion volumes measured using the two definitions was shown in Table 5-9. The core sizes measured with the two definitions (mean 26mL vs 33mL, $p<0.001$) were significantly different, the penumbra volumes (mean 38mL vs 33mL, $p=0.058$) were not significantly different. The absolute volumes of penumbra salvaged (mean 34mL vs 29mL, $p=0.043$) were significantly different, but the proportion of penumbra salvaged was not different (mean 68% vs 70%, $p=0.2$). However, the mismatch ratio calculated using the two definitions (mean 4.2 vs 2.5, $p<0.001$) were significantly different.

Table 5-9. The comparison of the perfusion lesion volume measured with two widely used definitions

		AB definition[147]	MW definition[144]	P Value*
Ischaemic core mL	Mean (SD)	26 (32)	33 (37)	P<0.001
	Median (IQR)	14 (0-41)	25 (0-47)	P<0.001
Penumbra mL	Mean (SD)	38 (34)	33 (30)	P=0.058
	Median (IQR)	37 (0-62)	31 (0-59)	P=0.091
Volume of penumbra salvaged mL	Mean (SD)	34 (25)	29 (19)	P=0.043
	Median (IQR)	31 (12-49)	17 (0-36)	P=0.094
Percentage of penumbra salvaged %	Mean (SD)	68 (25)	70 (25)	P=0.2
	Median (IQR)	75 (50-88)	78 (51-85)	P=0.32
Mismatch Ratio	Mean (SD)	4.2 (3.7)	2.5 (0.9)	P<0.001
	Median (IQR)	2.9 (1.9-5)	2.3 (1.8-2.9)	P<0.001
* p Value was calculated using paired T tests for mean and related-samples Wilcoxon Singed rank tests.				

5.3.5.2. Recanalisation status using TIMI[371] and TICI[375]

Both the TIMI and the TICI scales are popular in acute ischaemic stroke recanalisation studies for describing the reperfusion status. As discussed previously, both scales were designed for endovascular study, where reperfusion can be observed directly during the procedure in a 4D manner. CTA, on the other hand, can only provide a snapshot of the contrast passing through a vessel, in which case delayed perfusion can be missed. Neither the TIMI nor the TICI scales is ideal to describe vessel patency or reperfusion on CTA. TICI, which categorises reperfusion in a more detailed fashion, did not show any benefit over TIMI on CTA reading during our analysis.

In 5.2.1, I have discussed the inter-observer agreement for TIMI and TICI between the two readers for the ATTEST study, which were similar (0.68 vs 0.7). The percentage of patients that recanalised according to the TIMI definition (47%) was similar to that using TICI (53%) (p=0.55) using a Chi-Square test.

5.4. Discussion

Despite baseline imbalances such that the tenecteplase group included a higher proportion of patients with large artery occlusion (and therefore a lower probability of favourable response to treatment[319]) and a larger ischaemic core (and therefore a higher risk of haemorrhagic complications[173]), neither radiological nor clinical outcomes were significantly different with IV tenecteplase 0·25 mg/kg compared to alteplase 0·9 mg/kg, the current standard of care. In addition, there was a trend towards fewer intracerebral haemorrhages of all kinds in the tenecteplase group, and, notably, fewer parenchymal haematomas, a complication most strongly associated with treatment-related neurological deterioration[397]. In two subgroups (NIHSS score 6-14 and OTT 181-270), tenecteplase-treated patients had significantly higher rates of early neurological improvement. This nevertheless was not a pre-specified analysis, numbers were small, and needs to be interpreted with caution. Because of the modest sample size, some of the known predictors for stroke outcomes, such as OTT and age, were not shown to be associated with any outcome in this sample. Even if efficacy does not differ, the greater ease of administration alone may offer a significant advantage for tenecteplase over alteplase, for which delays in respect of the latter between the initial bolus and the initiation of maintenance infusion are common and may compromise effectiveness; the

possibility of improved safety with respect to fewer intracerebral haemorrhages is promising, meriting further investigation of tenecteplase for acute ischaemic stroke.

The study population in the ATTEST study is similar to that of previous alteplase trials, with respect to age, comorbidities and stroke severity.[70] Except for the treatment time window (4.5 hours compared to 3 hours), our inclusion criteria were similar to those of the phase IIB tenecteplase study conducted by Haley and colleagues[327]. In contrast to the ECASS trials, we applied no upper age limit or minimal baseline NIHSS score. Patients with history of previous stroke and concomitant diabetes (four patients), or seizure at onset of stroke (although none were recruited in this study) were not excluded from our study based on current clinical practice and the availability of advanced imaging to support a clinical diagnosis of stroke[70,370].

We selected a dose of tenecteplase of 0·25 mg/kg based on available data[194,325-327] although numbers of randomised subjects in previous studies were small. One previous study[327] terminated investigation of a higher dose (0·4 mg/kg) due to possible increased SICH incidence (3 in 19 patients). The same study suggested more frequent good outcomes with 0·25 mg/kg compared to 0·1 mg/kg, and Parsons and colleagues[194] also found superior efficacy of 0·25 mg/kg compared to 0·1 mg/kg, with no additional haemorrhagic risk.

We used advanced CT imaging for outcome analysis to offer insights into biological efficacy with a modest sample size, but did not use imaging for patient selection. This differs from the approach of Parsons and colleagues[194], who demonstrated the superiority of tenecteplase over alteplase for both reperfusion and clinical outcomes in a small randomised controlled trial that compared two different tenecteplase doses to standard alteplase. Their study used multimodal CT imaging to identify what is widely considered to be the optimal "responder" population in acute stroke, with favourable core/penumbra "mismatch" ratios, small ischaemic cores, and intracranial vessel occlusions, a strategy that has been expected to reduce sample size in phase II trials[218], but which is likely to have limited general application. In contrast, our patients had larger core (median [IQR] 14 mL [0-41] compared to 10 mL [5-17] and smaller penumbra volumes (median [IQR] 53 mL [0-110] compared to 79 mL [56-100], with only 64% (67/104) with baseline vessel occlusion. In our study, 14% of patients had either acute ICA occlusion or a thrombus involving the terminal ICA extending to the proximal MCA

segment, while these patients were excluded from the Parsons study. These factors will certainly contribute to the difference in outcomes between these two studies. However, it is notable that both studies identified a trend towards lower ICH risk with tenecteplase.

The application of advanced imaging in acute stroke trials has strengths and limitations. Limitations of this study included the use of non-contrast CT to quantify final infarct, restricted brain coverage for CT perfusion imaging, and reliance on probabilistic thresholds to define core and penumbra that have only limited validation[104,398]. The analysis in our study using the two widely accepted thresholds demonstrated that the results generated using different definitions can be significantly different. Although it would not have affected our primary outcome (the percentage of penumbra salvaged). The core/penumbra mismatch ratio was significantly different. Perfusion mismatch has been used widely as an essential criterion for trial entry in trials examining thrombolysis in extended time windows[213,299,300], or in identifying potential responders[194-196,204] within the current treatment time window. The optimal mismatch ratio remains controversial. The additional variability in the mismatch ratios generated with different thresholds adds the uncertainty of the quantitive analysis of perfusion imaging.

Various deconvolution algorithms have been used in different post-processing commercial software packages[145], an approach which may require different tissue status thresholds to be validated. We used the same software from which AB's thresholds were derived and additionally had derived similar thresholds using a different imaging dataset independently[399]. The algorithm used in MIStar is a modified SVD with compensation for the effects of arterial delay and dispersion[373], whereas the MW thresholds were derived with software based on the central volume principle[144]. It is possible that MIStar is not optimised to process perfusion imaging using the MW definition. Further literature[138] has suggested that AB's threshold using delay-corrected SVD is the most accurate one among all those thresholds used in currently available post-processing algorithms, which again emphasises the importance of standardising the post-processing of perfusion imaging, and caution is required in utilising perfusion imaging in clinical settings.

MRI has a high sensitivity in defining the extent of infarction[400], and greater lesion conspicuity at early time points than CT, but this advantage is at the expense of lower follow-up rates, and the introduction of additional bias in patient selection. Large detector CT systems which are able to acquire entire brain volumes for perfusion studies

remain largely confined to research environments. While we did not utilise advanced imaging for patient selection, studies that do so may introduce delay in treatment initiation to allow for acquisition, processing and interpretation of additional scans, and thus may compromise treatment efficacy. However, when used as a biomarker for relevant outcomes (reperfusion, recanalisation, and tissue salvage) additional imaging offers valuable insights in phase II evaluation. In our study, imaging identified baseline imbalance in important markers of potential treatment response and haemorrhagic risk between groups that was not evident using clinical criteria alone. This in itself is a powerful argument in favour of acquiring additional imaging at this stage of research. With respect to definitive trial design, additional imaging analyses may lead to significant loss of evaluable subjects from a trial. Even with a single centre study familiar with the techniques, 30% of per protocol recruited patients did not contribute to imaging analyses, the great majority of these being due to the absence of an initial perfusion lesion or vessel occlusion. In any multicentre trial, the proportion of non-evaluable subjects is likely to be increased further. While baseline imaging characteristics offered additional insights into stroke severity beyond clinical criteria, the potential detriment of delaying treatment initiation in order to review imaging findings as a selection criterion pre-randomisation needs to be considered.

5.5. Conclusions

The non-inferiority in efficacy and improved safety compared to standard thrombolytic treatment, despite imaging features indicative of greater baseline severity, support the further investigation of IV tenecteplase in acute ischaemic stroke.

Chapter 6 Secondary imaging analyses of the ATTEST study data

6.1. Introduction

In Chapter 5, the main results of the ATTEST study were presented, along with the sub-group analyses of age, stroke severity and OTT. In this chapter, I will discuss further findings from the imaging analysis.

It was noted in the main analysis that some of the imaging characteristics identified imbalances that were not reflected by clinical scales. Furthermore, well recognised imaging parameters that influence outcomes such as collateral flow and the length of thrombus were not taken into account. In this chapter, these two variables are included in further analysis of the data, and the association between them is explored. In addition baseline known confounders and clinical and imaging outcomes are investigated.

Brain oedema in acute ischaemic stroke is a poor prognostic predictor. There have been suggestions that alteplase exacerbates cerebral oedema post thrombolysis[278] by augmenting blood brain barrier permeability. To date only sparse data examining post thrombolysis brain swelling are available. We assessed the presence and the severity of cerebral oedema post thrombolysis in the ATTEST study patients, and compared the occurrence of brain swelling in patients who received the two different thrombolytic agents.

The Australian tenecteplase study[194] which used imaging selection is the the only study among the three completed RCTs that compared tenecteplase and alteplase in AIS to show the superiority of tenecteplase. In this chapter, a sub-group of the ATTEST study patients who fulfilled the inclusion criteria of Parsons et al[194] was examined to determine whether tenecteplase is superior to alteplase in this population.

6.2. Methods

6.2.1. Additional imaging variables

6.2.1.1. Measurement of clot length

Clot length was derived from non-contrast CT. We used a method that was modified from Riedel and colleagues[125] to measure the clot length.

All baseline non-contrast CTs and corresponding CTAs were processed offline on a university computer as described in Chapter 3, using MIStar. NCCT was reformatted with a slice thickness of 2.5mm. Hyperdense vessels were identified by two research fellows (XH and BC). Disagreements were resolved by an experienced neurologist (KM). The occlusion was confirmed with a concurrent CTA. All hyperdense vessels with confirmed occlusions including intracranial ICA, M1 and M2 sections were included as long as the length was measurable.

To measure the clot length, a region of interest was drawn around the hyperdense vessel area. MIP was applied (with a slice thickness between 2.5-10mm depending on the extent of clot) to include the whole length of the clot. Hounsfield unit thresholds were subsequently applied to segment the clot, starting with 55-80, followed by including all imaging pixels in the ROI with a threshold of 45-80 (Figure 6-1 to 6-2). Clot length was then measured using the segmented pixels. To reduce measurement error, two measurements were taken on two separate occasions by XH. Intra-observer agreement was assessed, and the average of the two measurements was used in the final analysis.

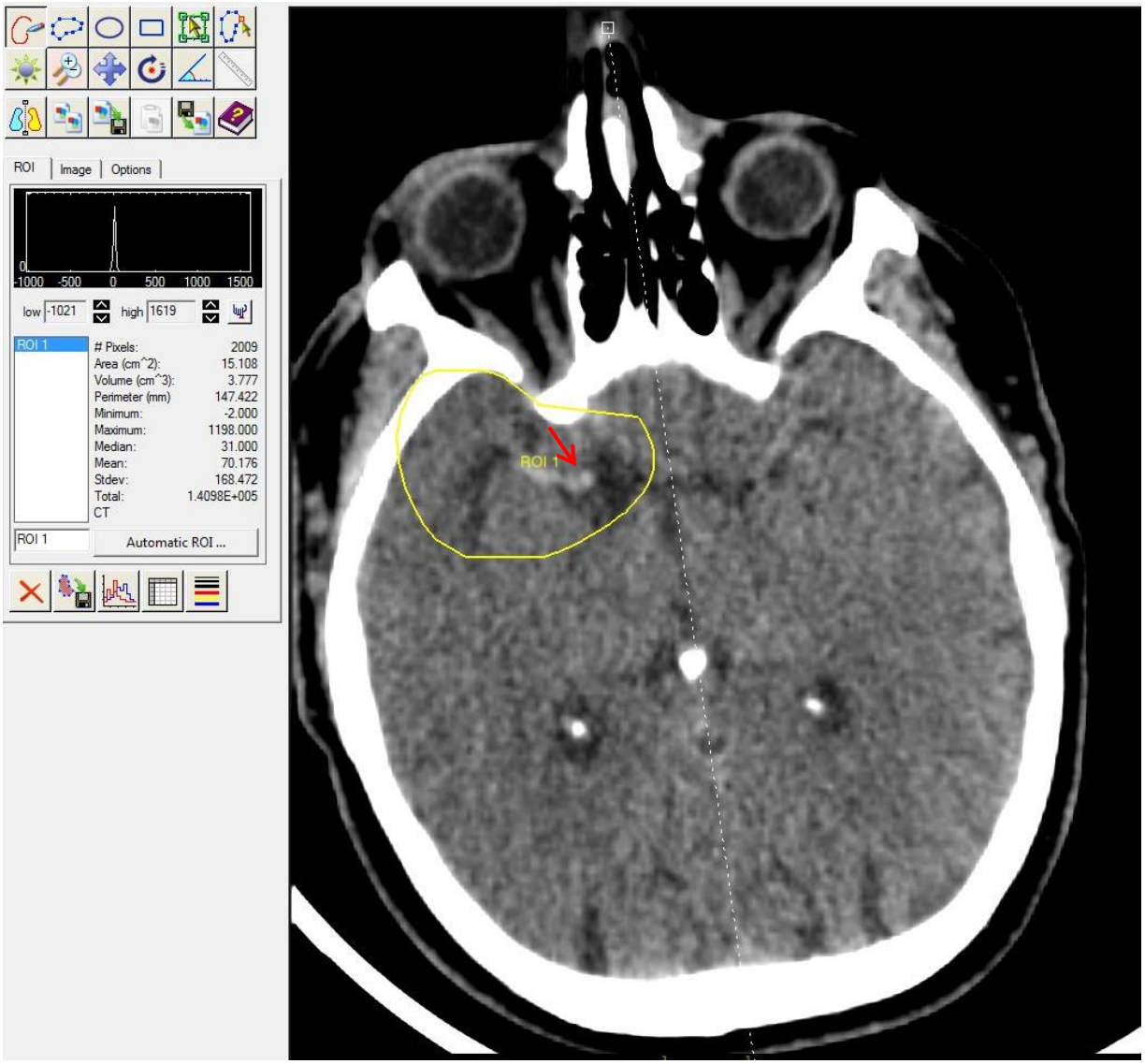


Figure 6-1. ROI (yellow circle) with hyperdense vessel on the lesional side

(Arrow: hyperdense vessel)

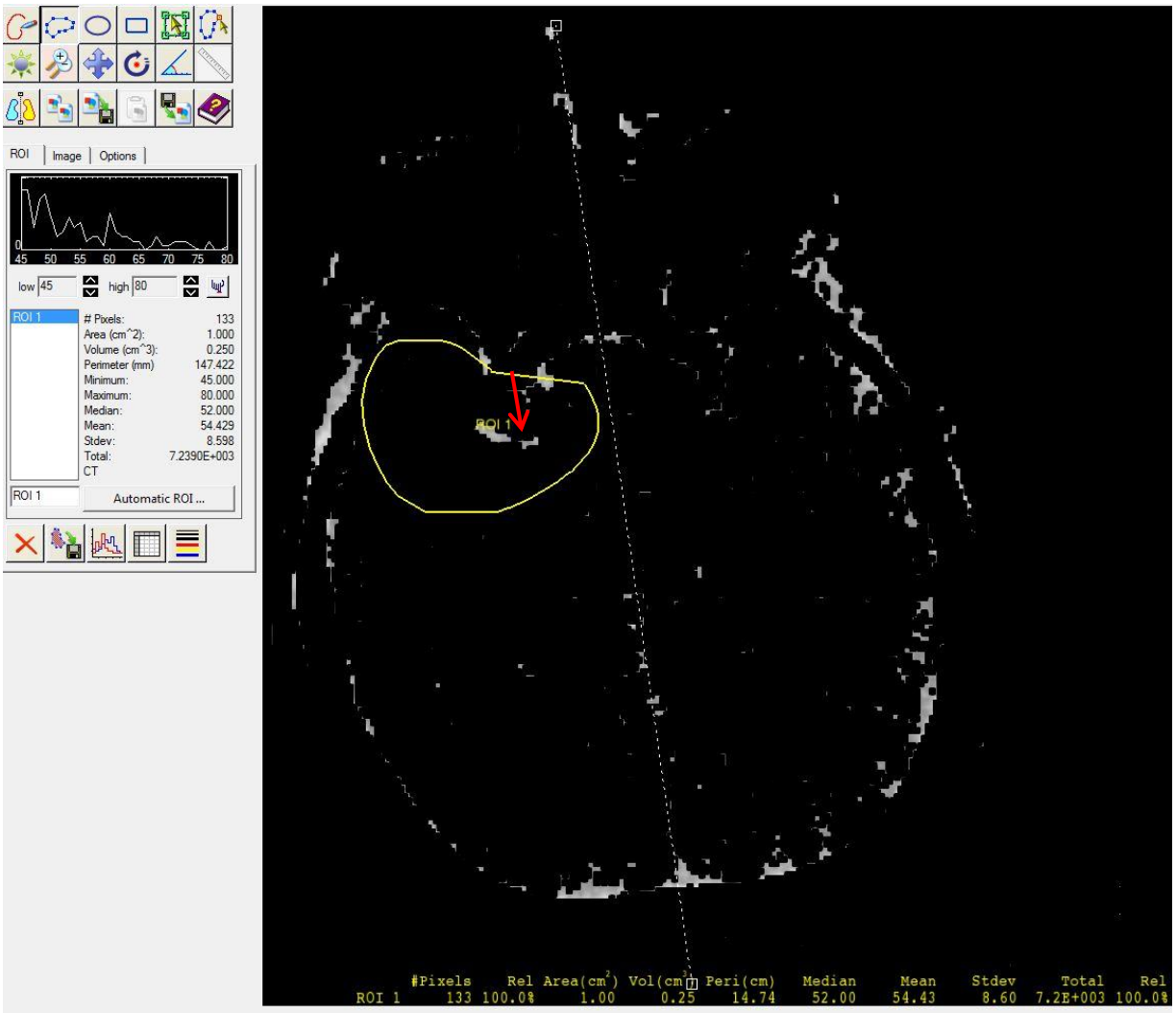


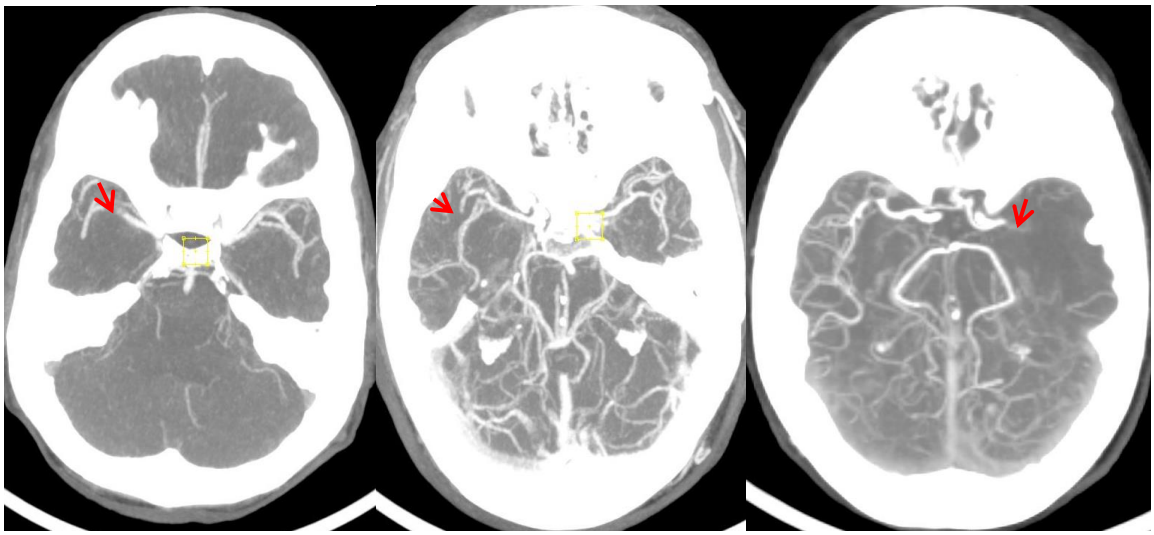
Figure 6-2. Same ROI after applying HU threshold (45-80) (Arrow: hyperdense vessel)

6.2.1.2. Assessment of collateral status

Collateral flow was assessed using a modified method derived from the one used by Miteff and colleagues[81]. Baseline CTP and CTA were required for the assessment. First of all, CTA imaging was reviewed to determine the presence of terminal ICA, M1 or M2 occlusions using a MIP with a slice thickness of between 10-20mm. Once the occlusion was confirmed, we used CT perfusion to assess the direction of the downstream flow beyond the occlusion site. CT perfusion was processed with the method described in Chapter 3 producing CBF, CBV, DT, and MTT maps. A 4D angiography MIP image of the 4cm CTP slab was viewed in cine mode in order to evaluate the flow direction. Those with antegrade flow were considered as partial occlusions and were excluded from the analysis. Only those with retrograde flow were deemed as being supplied by leptomeningeal collateral flow and proceeded to collateral status evaluation. The

intracranial baseline CTA imaging was subsequently displayed as axial MIP imaging with 40mm slab thickness. Depending on the downstream flow, the collaterals were catogorised into three grades: good, moderate and poor. The definitions of the three categories are as follows[81]:

Good: showed the entire M1 or M2 distal to the occluded segment reconstituting with contrast. CTA MIP reconstructions clearly demonstrated the MCA branches, with abrupt termination of the reconstituted vessels at the distal end of the occlusion within the M1 or M2 segments (Figure 6-3). M3 occlusions were not included as all of them had good collaterals. The original Miteff method did not include M2, but we noticed frequent poor regional collaterals in the ischaemic territory.



Good Moderate Poor

Figure 6-3. Examples of collateral supply with three grade respectively (Arrowed area: occlusion site). Left panel: contrast can be visualised distal to the occlusion; middle panel: some contrast can be seen distal to the occlusion partially in M2 branch ; right panel: No contrast seen distal to the occlusion site and the downstream perfusion area.

Moderate: the distal M1/M2 reconstituted only partially to ischaemic territory, but not distal to occlusion (Figure 6-3).

Poor: only the distal superficial MCA branches reconstituted (Figure 6-3).

6.2.1.3. The severity of brain swelling

The methods of assessing cerebral oedema and the scales used were detailed in Chapter 3.

6.2.2. Subgroup analyses using all patients who fulfilled Parsons and colleagues'[194] imaging selection criteria

We applied the inclusion criteria of Parsons' group in all of the ATTEST study patients: baseline NIHSS scores >4; the presence of intracranial occlusion in ACA, MCA or Posterior Cerebral Artery (PCA) (ICA or basilar artery occlusions are excluded); infarct core <1/3 of MCA or ½ of ACA/PCA territory; Penumbral volume>20mL with a mismatch ratio >1.2.

In our centre, CTP has a limited coverage of 4cm, and a single CTP study was undertaken in order to minimise radiation exposure, whereas Parsons and colleagues used two contiguous CTP acquisitions to cover most of the brain. To account for the difference, the minimal core and penumbral volume for study entry were reduced proportionately. The proportion was derived as follow:

The proportion = the mean co-registered final infarct volume (non-recanalised patients)/the mean total infarct volume = 0.715

The CTP lesion covered by a 4cm Z axis is therefore approximately 70% of the whole brain coverage.

The corrected core and penumbral volume criteria are: infarct core <70mL in MCA territory (1/3 of MCA territory is approximately 100mL), <50mL in ACA/PCA territory; penumbra volume>14mL.

Patients who had good or moderate collateral gradings were considered to have good collateral status, and those had poor collateral grading were deemed to have poor collateral status.

Other definitions of imaging and clinical outcomes are as described in Chapter 3, as are the calculations of the percentage of penumbra salvaged, and infarct growth.

6.2.3. Statistical methods

The continuous variables are compared using the independent t test, the Mann-Whitney *U* test, and categorical variables with Chi – square and Fisher's test. We used a

generalised linear model, and linear and logistic regressions to assess the association between imaging and clinical variables, and the comparison of imaging and clinical outcomes was adjusted for variables including age, OTT and the baseline NIHSS score. All statistical analyses were carried out with SPSS 21.

6.3. Results

6.3.1. Clot length

63(66%) (T=35, A=28) out of 96 patients had hyperdense vessel signs on the baseline CT brain, of which 47/96 (49%) (T=26, A=21) were measurable. No significant difference was found in the mean clot length between the two groups (13±11mm vs 14±11mm, p=0.63). 25 patients had a clot length of more than 8mm (T=14 [54%], A=11 [52%], p=1).

The intra-class correlation coefficient for the two clot length measurements performed by XH was 94.8%, with 95% limits of agreement of -6.1 - 7.7 (Figure 6-4)

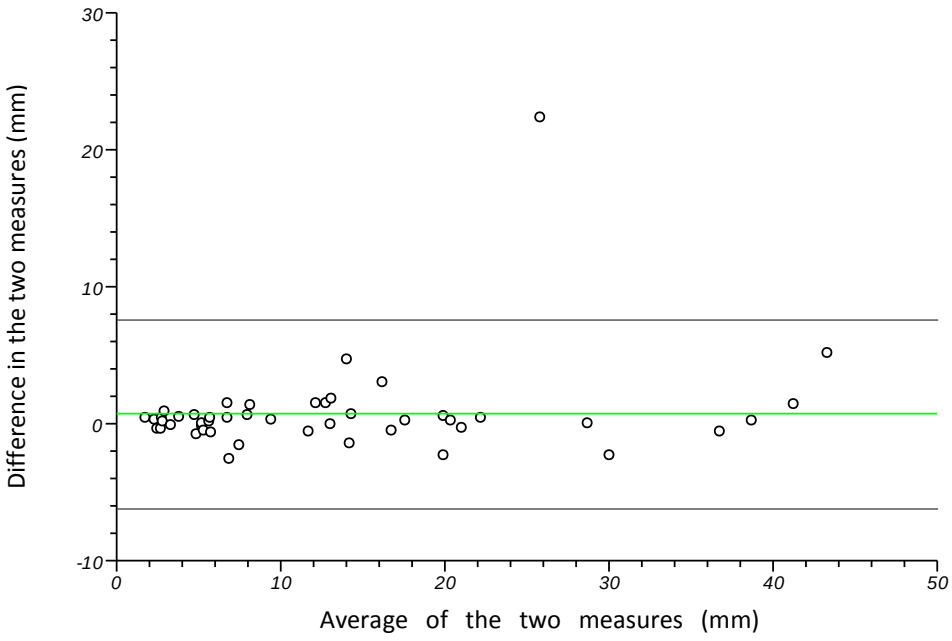


Figure 6-4. Bland-Altman agreement plot between two measurements for clot length (mean[green]±1.96SD[black]).

The mean clot lengths that resulted in recanalisation were similar between the tenecteplase and alteplase groups using a univariate Analysis of Variance (ANOVA) (12.8±11.1mm vs 12±13.1mm, p=0.87) (Figure 6-5). In this sample, the length of thrombus did not correlate with the baseline stroke severity measured by the NIHSS score (p=0.74). 22 out of the 46 patients (46.8%) had confirmed cardioembolic stroke, and the

clot length was reversely associated with cardioembolic stroke (p=0.005, 95%CI [-14.6 – -2.7]). When adjusted with age, OTT and baseline NIHSS, the significance persisted (p=0.016, 95%CI [-13.6 – -1.5]). Patients with longer clots were more likely to have symptomatic ICH (ECASS 2 definition[311]) (p=0.033, 95%CI [1-23.2]). When adjusted for age, OTT and baseline NIHSS, the significance persisted (p=0.034). No association existed between clot length, the change of the NIHSS score at 24 hours (p=0.13), and early neurological improvement (p=0.46), mRS 0-1 (p=0.66) or mRS 0-2 (p=0.58) at 90 days; but there was an independent association between increased mortality at 90 days and those who had longer thrombi on presenting CT scan (p=0.01, OR 1.2, 95%CI [1.1-2]).

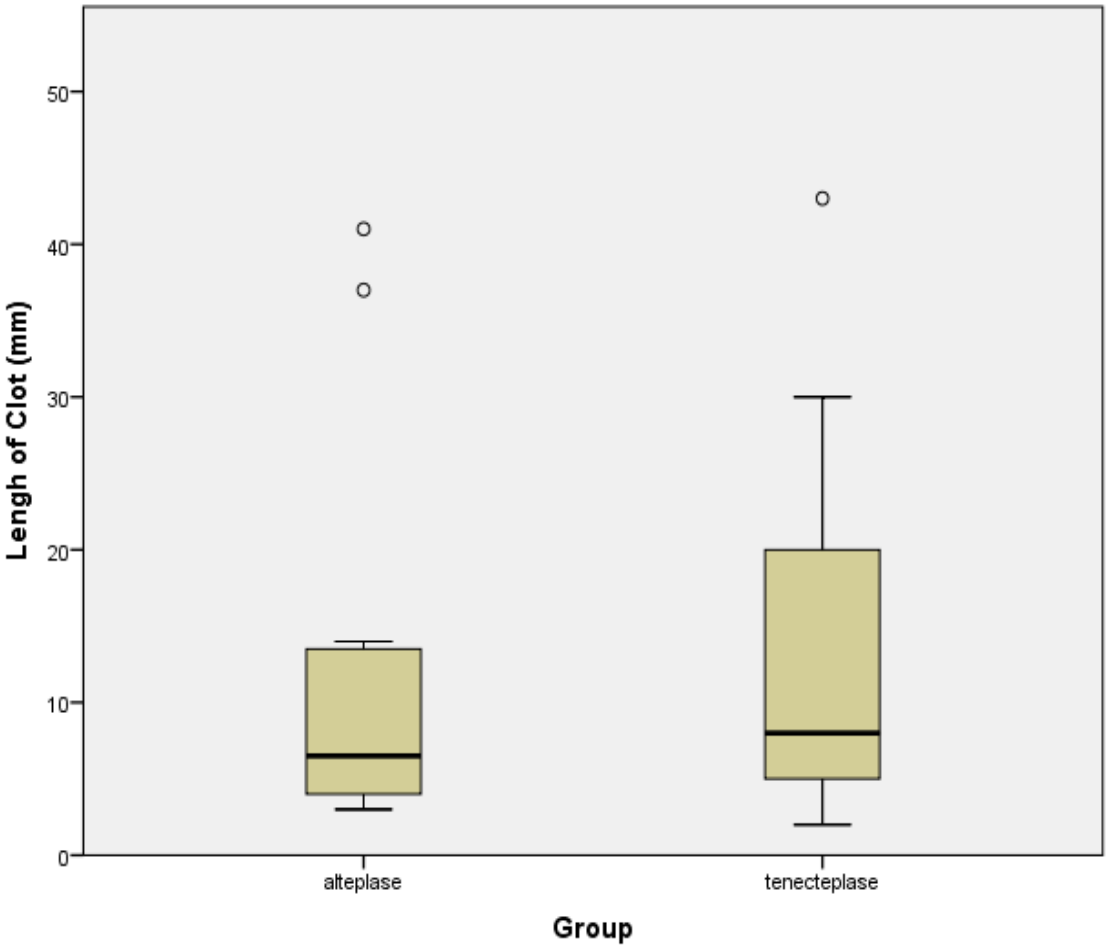


Figure 6-5. The mean length of clot that recanalised by tenecteplase and alteplase

No association was found between clot lengths and baseline CTP ischaemic core volumes (p=0.25), or the proportion (p=0.54) or the volume (p=0.19) of penumbra salvaged at 24 hours, but patients with longer clots had a trend towards larger co-registered (p=0.07, B-

coefficients 1.7, 95%CI [-0.1-3.5]) and total infarct volumes (p=0.055, B-coefficients 2.8, 95%CI [-0.1-5.5]). In the subsequent multiple linear regression model, the significances did not persist when adjusted with age, OTT and baseline NIHSS scores.

No association was found between clot length and the likelihood of recanalisation (p=0.89) (Figure 6-6). A trend towards a reduced likelihood of recanalisation was evident nevertheless in those who had a thrombus measured longer than 8mm (p=0.071, OR 0.3, 95%CI [1.3-437.4]). In the multiple regression model, the near-significant trend continued (p=0.054, OR 0.2, 95%CI [1.1-735.3]).

6.3.2. Collateral flow

49 (T=22, A=27) patients had retrograde flow on the baseline CT perfusion, and qualified for collateral assessment. The proportion of patients who were considered to have good collaterals did not differ between the two treatment groups (T=15, A=20, p=0.76).

In the univariate regression model, good collaterals were associated with less severe strokes (p=0.021, OR 0.9, 95%CI [0.5-0.95]), smaller ischaemic core volumes (p=0.001, OR 0.96, 95%CI [0.9-0.95]), and better ASPECT scores (p=0.002, OR 2.2, 95% CI [2-36.6]). We did not find any association between collateral status and other potential predictors including penumbra volumes (p=0.82), age (p=0.98), OTT (p=0.69), cardio-embolic stroke (p=0.11), history of hypertension (p=0.59), diabetes (p=0.12), hyperlipidaemia (p=0.32), the baseline systolic BP (p=0.58), glucose (p=0.92), ipsilateral carotid stenosis (p=0.63) or previous use of statin (p=0.72).

There was a near-significant association between collaterals and mRS 0-2 at 90 days (p=0.068, OR 7.3, 95% CI [0.7-13.7]), but not with mRS 0-1 (p=1), early neurological improvement at 24 hours (p=0.3), or mortality (p=0.17). Good collaterals were associated with a reduced risk of SICH (ECASS 2 definition) (p=0.041, OR 0.1, 95% CI [0.001-0.8]) or any ICH (p=0.005, OR 0.1, 95%CI [0.001-0.3]). Significant correlations were found between good collaterals, larger proportions of penumbra salvaged (p=0.024, B-coefficients 18.4, 95% CI [2.9-34.9]) (Figure 6-6), smaller co-registered infarct volumes (p=0.001, B-coefficients -72.3, 95% CI [-112.2 – - 32.8]) and total infarct volumes (p=0.001, B-coefficients -115.8, 95% CI [-184- -50.2]), but not with recanalisation (p=0.47).

When adjusted for age, OTT and the baseline NIHSS score, collateral flow was independently associated with a risk of SICH (ECASS 2 definition), any ICH, co-registered and total infarct volumes (Table 6-1).

Table 6-1. Good collateral status as a prognostic factor in multivariate regressions adjusted for age, OTT and baseline NIHSS scores for clinical and imaging outcomes

	B-coefficients or OR (95% CI)	P Value
SICH (ECASS 2 definition)	0.1 (0.02-0.9)	0.044
Any ICH	0.1 (0.03-0.6)	0.012
mRS 0-2 at 90 days	1.7 (0.1-22)	0.67
Early neurological improvement at 24 hours	2 (0.4-9.5)	0.38
Mortality at 90 days	0.3 (0.07-1.8)	0.2
Proportion of penumbra salvaged	12.381 (-4.4-29.2)	0.15
Co-registered infarct volume	-48.883 (-87.6- -10.2)	0.015
Total infarct volume	-80.559 (-147.5- -13.6)	0.02

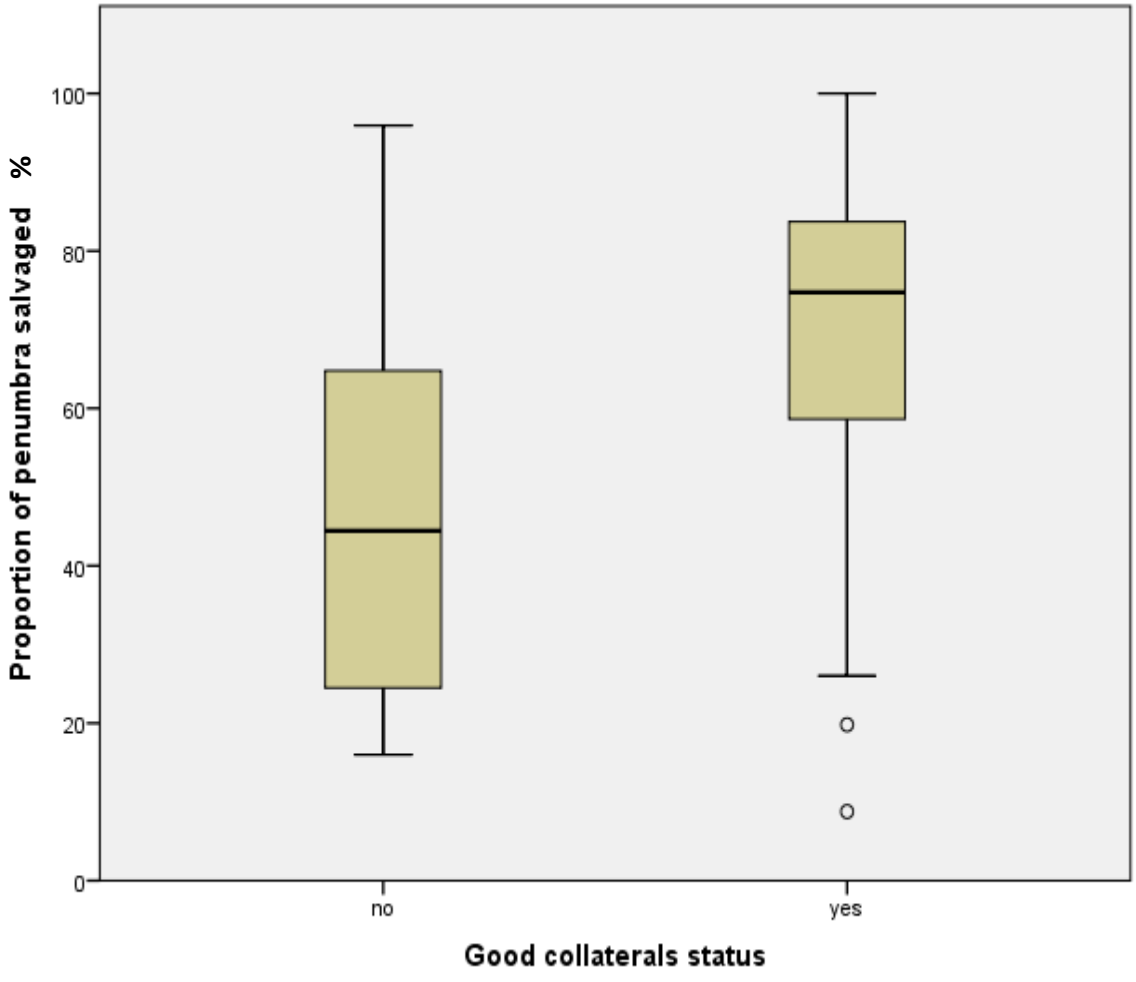


Figure 6-6. The proportion of penumbra salvaged according to collateral circulation

6.3.3. Cerebral oedema and clinical outcomes

35 out of 96 patients (36.5%) (Table 6-2) had brain swelling on a 24-hour CT post thrombolysis. No difference was found in the distribution of brain swelling severity between the two groups (p=0.97). In the univariate logistic regression model, many clinical and imaging variables were associated with postthrombolysis brain swelling (Table 6-4). In the subsequent multiple regression model which included all variables that had a significance level <0.3, all associations became insignificant.

Table 6-2. Brain swelling in tenecteplase and alteplase treated patients

Severity of brain swelling n, %	Tenecteplase N=47	Alteplase N=49
No swelling	28 (60%)	30 (61%)
Effacement of adjacent lateral ventricle	11 (23%)	10 (20%)
Effacement of the lateral and the third ventricle	4 (9%)	4 (8%)
Midline shift	4 (9%)	3 (6%)

Table 6-3. Univariate logistic regression to predict post thrombolysis brain swelling

Variables	OR (95%CI)	P value
Age	1 (0.97-1)	0.92
Baseline NIHSS	1.2 (1.1-1.3)	<0.001
OTT	1 (0.99-1)	0.8
Baseline glucose	0.9 (0.7-1.1)	0.3
Baseline systolic BP	1 (0.99-1)	0.18
AF	1.7 (0.7-4)	0.23
Clot length >8mm	2.9 (0.9-10)	0.085
Baseline ASPECT	0.6 (0.5-0.8)	<0.001
Core volume	1.05 (1-1.1)	<0.001
Large vessel occlusion (ICA/M1)	4.6 (1.5-13.6)	0.006
Collateral status	0.2 (0.05-0.6)	0.007
Hyperdense vessel sign	7 (2.2-22.3)	0.001

In univariate regressions, brain oedema was associated with all clinical and imaging outcomes, except for recanalisation (p=0.43) and mRS 0-1 at 90 days (p=0.056) (Table 6-4). When adjusted for age, OTT and the baseline NIHSS score, brain oedema was still associated with a high risk of haemorrhage (Figure 6-7), and poor clinical and imaging outcomes as was expected (Table 6-5). The distribution of mRS at 90 days according to the severity of cerebral oedema is shown in Figure 6-8.

Table 6-4. Univariate regression for cerebral oedema as a poor prognostic predictor for clinical and imaging outcomes

	B-coefficients/OR (95% CI)	P Value
SICH (ECASS 2 definition)	4.3 (1.9-9.9)	0.001
Any ICH	4 (2.2-7.6)	<0.001
Early neurological improvement at 24 hours	0.4 (0.2-0.9)	0.017
mRS 0-1 at day 90	0.5 (0.2-1)	0.056
mRS 0-2 at day 90	0.3 (0.2-0.7)	0.004
Mortality at day 90	3.6 (1.9-6.8)	<0.001
Recanalisation	0.8 (0.5-1.4)	0.43
Proportion of penumbra salvaged	-17.867 (-22.5- -13.2)	<0.001
Co-registered infarct volume	54.561 (46.1-63)	<0.001
Total infarct volume	80.662 (66-95.3)	<0.001

Table 6-5. Multivariate regression for cerebral oedema as a poor prognostic predictor for clinical and imaging outcomes

	B-coefficients/OR (95% CI)	P Value
SICH (ECASS 2 definition)	11.1 (2.3-54)	0.003
Any ICH	5.1 (2.2-11.6)	<0.001
Early neurological improvement at 24 hours	0.4 (0.2-0.8)	0.009
mRS 0-1 at day 90	1.3 (0.5-3.4)	0.6
mRS 0-2 at day 90	0.6 (0.3-1.3)	0.19
Mortality at day 90	3.8 (1.6-9.1)	0.002
Recanalisation	0.7 (0.3-1.3)	0.26
Proportion of penumbra salvaged	-16.665 (-22- -11.3)	<0.001
Co-registered infarct volume	44.698 (35.1-54.2)	<0.001
Total infarct volume	66.752(50.3-83.2)	<0.001

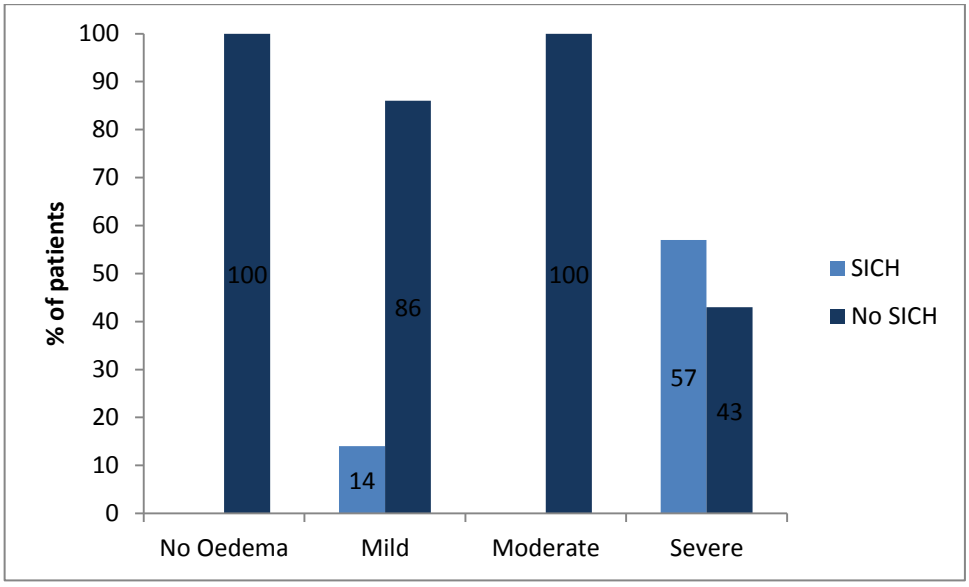


Figure 6-7. The distribution of SICH using ECASS 2 definition across the different severity of brain swelling

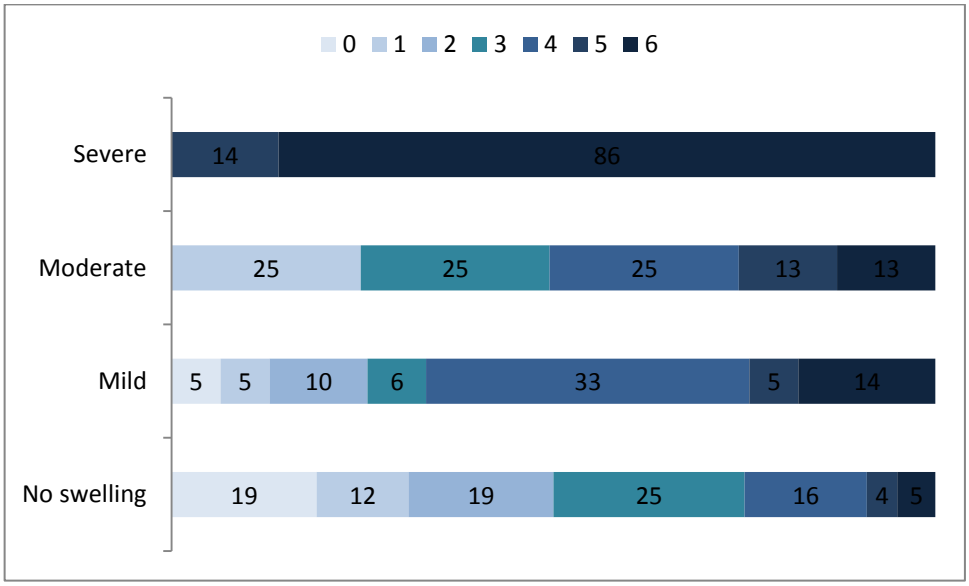


Figure 6-8. The distribution of mRS at 90 days according to the severity of brain swelling

6.3.4. Subgroup analysis of patients fulfilled imaging selection used in the Australian TNK study

From 104 patients, 38 (36.5%) fulfilled the imaging selection criteria described above; 21 (55.3%) received alteplase and 17 (44.7%) received tenecteplase. The baseline clinical and imaging characteristics were well balanced in the two groups (Table 6-6), but patients who received tenecteplase had a significantly higher percentage of the presence of hyperdense vessel signs compared with those who received alteplase (p=0.024).

6.3.4.1. Imaging outcomes (Table 6-7)

The mean percentage of penumbra salvaged at 24 hours post-thrombolysis (75±26% vs 70±23%, p=0·62, mean difference [95%CI] 5% [-20-12]) did not differ between tenecteplase treated patients and those who received alteplase. Similarly, the mean volume of the penumbra salvaged was similar (p=0.1, mean difference [95%CI] 14mL [-29, 2]) (Figure 6-9). The volumes of infarct growth (p=0.74), co-registered infarct volumes (p=0.83) or total infarct volumes (p=0.92) between the two treatment groups were not different. The recanalisation rates were 76% in both groups respectively (p=0.48, OR [95%CI] 0·5 [0·08-3.3]).

6.3.4.2. Clinical outcomes (Table 6-7)

47% and 29% respectively of patients achieved early neurological improvement at 24 hours (p=0·27, OR [95%CI] 2.2 [0.5-9.3]). The proportion of patients who achieved functional independence (mRS 0-1) and mortality rates at 90 days was similar, and the mRS ordinal shift test was not significantly different (Figure 6-10) in the two groups.

6.3.4.3. Safety outcomes (Table 6-7)

ICH rates of any kind were significantly lower in patients who received tenecteplase (12% vs 29%, p=0.048, OR [95%CI] 0.07 [0-1]). The rates of SICH using either the SITS-MOST definition[318] (p=0·46, OR [95%] 0·3 [0·02-6]) or the ECASS 2 criteria[311] (p=0·46, OR [95%] 0·3 [0·02-6]) were nevertheless similar, probably due to the very small number of events.

Table 6-6. Baseline clinical and imaging characteristics of the subgroup of patients who fulfilled imaging selection criteria

		Tenecteplase (n=17)	Alteplase (n=21)	P Value
Clinical				
Age years mean(SD*)		77 (9)	72 (13)	0.15
Male (n, %)		9, 53%	9, 43%	0.75
Dominant hemisphere stroke (n, %)		6, 35%	13, 62%	0.096
Baseline NIHSS score (Median, IQR)[min-max]		12 (9-15)[5-21]	12 (10-16)[4-27]	0.79
Onset-to-treatment Time min	mean(SD)	187 (50)	195 (50)	0.64
	Median (IQR)	197 (145, 218)	197 (153, 235)	0.69
Previous stroke/TIA (n, %)		5, 29%	4, 19%	0.7
Hypertension (n, %)		9, 53%	12, 57%	1
Diabetes (n, %)		4, 24%	4, 19%	1
Blood glucose mmol/L mean (SD)		7 (2)	7 (3)	0.36
Atrial Fibrillation (n, %)		10, 59%	9, 43%	0.52
Hyperlipidaemia (n, %)		12, 71%	10, 48%	0.2
Smoker (n,%)		1, 6%	3, 14%	0.61
Stroke clinical syndrome				
TACS (n, %)		12, 71%	14, 67%	1
PACS (n, %)		5, 29%	7, 33%	
LACS (n, %)		0, 0%	0, 0%	
POCS (n, %)		0, 0%	0, 0%	
Imaging				
ASPECT score mean (SD)		7 (2)	7 (2)	0.68
Hperdense sign (n, %)		17, 100%	15, 71%	0.024
Penumbra Volume mL	Median (IQR)	53 (38-94)	45 (35-66)	0.28
	Mean (SD)	61 (29)	50 (21)	0.16
Core Volume mL	Median (IQR)	20 (9-40)	19 (12-38)	0.82
	Mean (SD)	26 (20)	25 (19)	0.87
Mismatch Ratio mean (SD)		5.6 (5)	4.5 (3.4)	0.47
Occlusion				
M1§		12, 71%	11, 52%	0.51
M2§		5, 29%	9, 43%	
M3§		0, 0%	1, 5%	
ACA/PCA		0, 0%	0, 0%	

*SD, standard deviation; NIHSS: National Institute of Health Stroke Scale; IQR: Interquartile Range; TIA: Transient Ischaemic Attack; TACS; Total Anterior Circulation Syndrome; PACS: Partial Anterior Circulation Syndrome; LACS: Lacunar Syndrome; POCS: Posterior Circulation Syndrome; ASPECT score: Alberta Stroke Program Early CT score; ICA: Internal Carotid Artery; § Middle Cerebral Artery M1, M2, M3 segment; ACA: Anterior Cerebral Artery; PCA: Posterior Cerebral Artery.

Table 6-7. Study outcomes in this sub-group of patients who fulfilled imaging selection criteria

	TNK (n=17)	Alteplase (n=21)	p value¢	Mean difference (95%CI)	Odds Ratio (95%CI)
Primary outcome					
Percent penumbral salvage at 24-48 h mean (SD)*	75% (26)	70% (23)	0.62	5% (-20-12)	-
Secondary imaging outcomes					
Co-registered final infarct volume at 24-48 h ml mean (SD)#	44 (42)	49 (53)	0.83	-5 (-27, 37)	-
Total infarct volume at 24-48 h ml mean (SD)‖	65 (71)	66 (78)	0.92	-1 (-48, 51)	-
Volume of infarct growth at 24-48 h ml mean (SD)	18 (36)	24 (42)	0.74	-6 (-20, 32)	-
Volume of penumbra salvaged at 24-48 h ml mean (SD)	48 (29)	34 (18)	0.1	14 (-29, 2)	-
Recanalisation at 24-48 h (n, %)§	13, 76%¤	16, 76%¤	0.48	-	0.5 (0.08-3.3)
Secondary clinical outcomes					
Early neurological improvement at 24 h (n, %) ¶	8, 47%	6, 29%	0.27	-	2.2 (0.5-9.3)
Improvement in NIHSS score between baseline and 24 h mean (SD)	4 (6)	2 (8)	0.43	2 (-3-6)	-
mRS 0-1 at 30 days (n, %)	2, 12%	2, 10%	0.84	-	1.3 (0.1-10.8)
mRS 0-1 at 90 days (n, %)	4, 24%	4, 19%	0.84	-	1.2 (0.2-8.5)
mRS ordinal shift			0.73		1.2 (-1-1.4)
Mortality at 90 days (n, %)	3, 18%	2, 10%	0.46	-	3.1 (0.2-64.4)
Safety outcomes (A=51, T=52)					
Any ICH (n, %)	2, 12%	6, 29%	0.048	-	0.07 (0-1)
Any parenchymal haemorrhage (n, %)	0, 0%	2, 10%	1		
Parenchymal haemorrhage type 2 (n, %)	0, 0%	1, 5%	1		
Symptomatic ICH (ECASS II[311] definition) (n, %)	1, 6%	2, 10%	0.46	-	0.3 (0.02-6)
Symptomatic ICH (SITS-MOST[318] definition) (n, %)	1, 6%	2, 10%	0.46	-	0.3 (0.02-6)

*SD, standard deviation; NIHSS: National Institute of Health Stroke Scale; IQR: Interquartile Range; ICH: Intracerebral haemorrhage; mRS: Modified Rankin Scale;

¢ p-values were calculated from linear of logistic regression models that adjust for stratification variables and are a test for difference between groups.

Co-registered infarct volume was defined as infarct volume measured on 24-48 h CT slices co-registered to baseline CT perfusion;

‖ Total infarct volume was defined as total infarct volume measured on follow-up CT at 24-48 h;

§ Recanalisation was defined as TIMI[371] 2-3;

¤ The percentages for recanalisation were derived from the number of subjects with an occlusion.

¶ Early neurological improvement at 24 h was defined as NIHSS reduction ≥ 8 points or 24 h NIHSS 0-1.

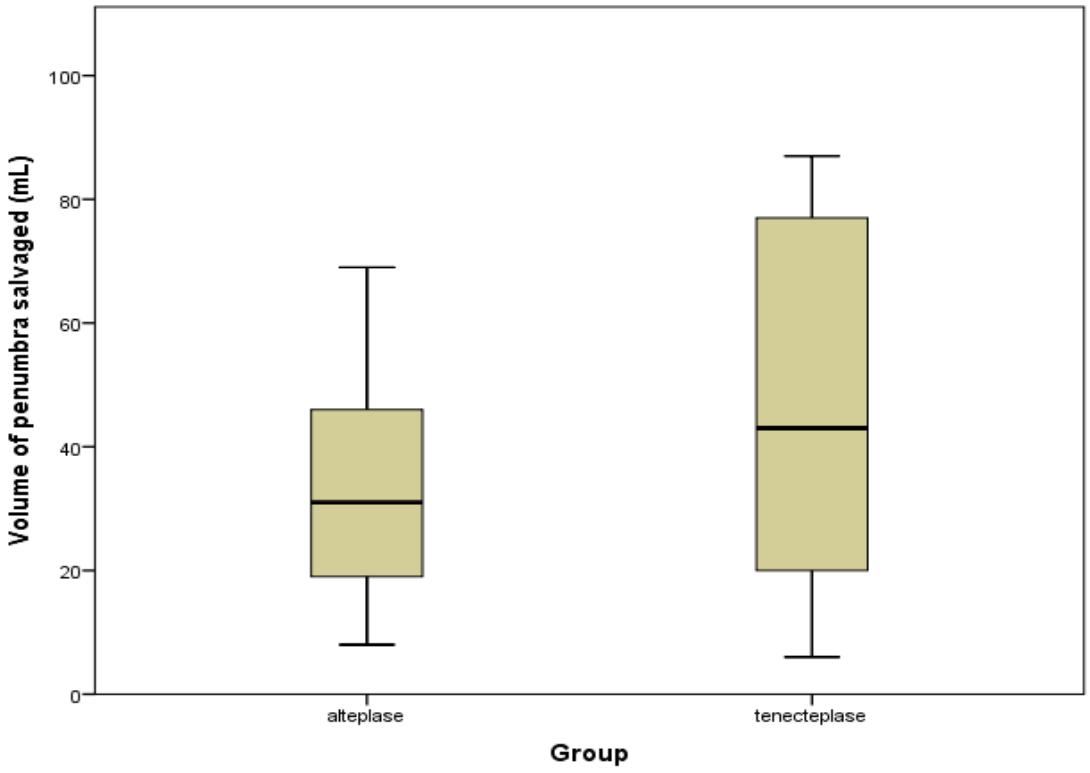


Figure 6-9. The mean volume of penumbra salvaged at 24 hours post thrombolysis in the two groups

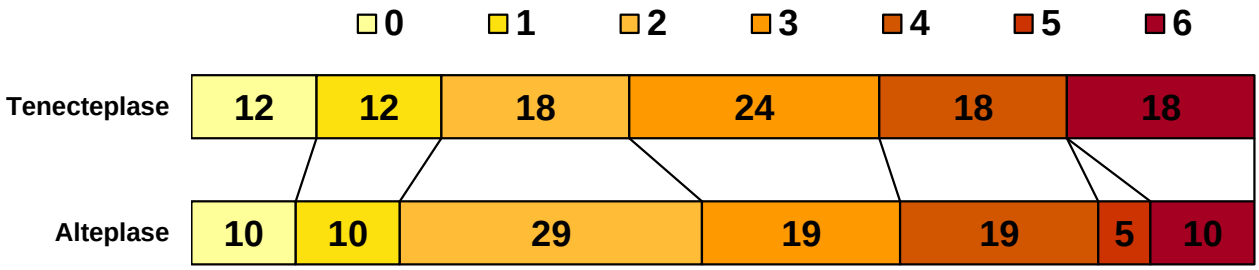


Figure 6-10. mRS in tenecteplase and alteplase treated patients at 90 days (expressed as percentage)

6.4. Discussion

In further imaging analysis, we have assessed the effects of length of thrombi and collateral flow.

No difference was found in the mean clot length that was recanalised in the tenecteplase and alteplase groups.

In this sample, no association between the size of the thrombus and the likelihood of recanalisation was found, which is probably due to the small sample size. However, we did find that those with a thrombus longer than 8mm had a trend towards reduced odds of recanalisation, which is consistent with the finding from Riedel's group[163].

The size of thrombus was associated with an increased risk of symptomatic ICH, a trend towards higher mortality and larger infarct volumes.

Clot length has been considered to be one of the factors when determining the triage of patients for IV or IA recanalisation therapy. However, the methods of measuring thrombi using either the single phase CTA or plain CT have their limitations. The measurements on thin slice CT derived by Riedel and colleagues have not been reproduced by other groups. Using the modified method, we found that the method is easy to apply, but that only about 50% of patients had a measurable hyperdense vessel.

Many methods have been proposed to assess collateral status in acute ischaemic strokes[229], but without consistency. We used the methods developed by Miteff and colleagues[81], as this takes into account true retrograde (ie leptomeningeal collateral) flow and is easy to use. We have however extended the assessment to M2 occlusions because poor collaterals are often present in this population.

Previous literature has described several factors that may influence the recruitment of collaterals including a history of hypertension, the presence of carotid stenosis, hyperglacaemia and the previous use of statin. However, we did not demonstrate the existence of any association between collateral status and those factors, which may be again due to the small sample size.

Collateral status is an important predictor for outcomes, and has been used as one of the selection criteria for endovascular treatment by some investigators[207]. In our sample, we have demonstrated that similar associations arise with imaging outcomes and ICH risk.

36.5% of the ATTEST study patients developed cerebral oedema post thrombolytic therapy, and approximately 10% had detrimental oedema that was potentially life threatening. This overall rate is higher than previously reported[401] in the thrombolysed population and those who did not receive treatment[402] although methods for grading brain oedema are not standardised and this findings is reported inconsistently. Unfortunately, we did not have an untreated control group with which to make a comparison. The proportion of severe brain swelling found was similarly to previous findings[403].

Post-thrombolysis brain swelling was associated with a higher risk of ICH and mortality, a poor neurological outcome at 24 hours, less penumbra salvage, and larger infarct volumes. We did not find any correlation between post-thrombolysis brain swelling and recanalisation. As far as we are aware, this is the first study to have explored the relationships between post-thrombolysis oedema and imaging outcomes. A larger sample is warranted for further investigation.

Late reperfusion may hypothetically augment BBB permeability, and worsen cerebral oedema[404,405]. However, our study was limited since recanalisation was assessed at 24-48 hours, and the study did not therefore provide information regarding early recanalisation and the development of brain oedema after treatment.

Some literature has suggested that alteplase neurotoxicity might induce cerebral oedema by increasing neurovascular unit permeability[278]. No similar finding had been reported for tenecteplase. However, given that the structure of tenectplase is rather similar to alteplase, except for the three points of modification, we presume that tenecteplase has a similar effect on the blood brain barrier caused by an interaction with the low-density lipoprotein-receptor-related protein. In our sample, we did not find any difference in the proportion of brain oedema in tenecteplase or alteplase treated patients.

In the comparison of the subgroup of the ATTEST study patients who fulfilled the imaging selection criteria used by Parsons and colleagues, with the exception of significantly less

ICH in tenecteplase treated patients, no difference was found in other efficacy or safety outcomes.

Compared with the patients in the Parsons study, ours had a larger median core volume (19-20mL vs 8-13mL), and a smaller penumbra (45-53mL vs 76-80mL) indicating a selection bias. The baseline NIHSS scores (12 vs 14) were similar, but OTTs (187-195 vs 162-186 minutes) were longer in our patients. The larger cores and smaller penumbras may contribute to the neutral results in our sample, but other factors such as clot length or collateral flow may also play a role.

To date, AIS reperfusion studies which have used imaging selection have generated heterogeneous results (Tabel 6-8). The studies[194,195] which have yielded positive results all had a small core (11-18mL) and a large penumbra. On the other hand, MR RESCUE[196], the only study to date examining the concept of penumbra selection that has failed to demonstrate its benefit had a much larger median core of 60mL, although the median penumbra volume was 177mL. The main reasons for the neutral result are believed to be the selection bias of large core volumes, together with the device (1st generation, therefore low efficacy) used, and delayed reperfusion. Our subgroup analysis supported the view that selection bias can occur with current tissue selection criteria. Together with other unresolved issues in perfusion imaging, the study demonstrated that advanced imaging selection requires further refinement of criteria, and ideally validation in a well-designed RCT.

In addition, in order to ensure that intra-arterial intervention is beneficial, careful patient selection may be necessary. The recent success of the series of endovascular studies is a significant progress in AIS management, but the variable selection criteria used in these studies makes establishing criteria for clinical application difficult. If the current tissue criteria are used, treatment benefits may be smaller than current evidence suggests due to the potential selection bias.

Table 6-8. Studies using imaging criteria for patient selection

Study	Therapy	Imaging modality	Inclusion Criteria					Core (Median, mL) (treatment vs control)	Penumbra (Median, mL) (treatment vs control	Favourable clinical outcomes at 90 days* (treatment vs control)
			Time window (hour)	Tissue (mL)		Mismatch Ratio	Occlusion Sites			
				Core	Penumbra					
DIAS[332]	IV vs Placebo	MRI	3-9	<1/3 of MCA territory	>2cm in diameter involving gray matter	>1.2		17.8 vs 20.4		39% vs 22%
DEDAS[333]	IV vs Placebo	MRI	3-9		>2cm in diameter involving gray matter	>1.2	ICA occlusion excluded	22.2 vs 35.1		45% vs 25%
DIAS2[184]	IV vs Placebo	MRI&CT	3-9	<100		>1.2		11.3 vs 7.9 vs 12.3	66.2 vs 51.9 vs 48.8	49% vs 54% vs 57%
TNK[194]	TNK vs alteplase	CT	<8	<100	>20	>1.2	MCA/ACA/PCA	11 vs 8 vs 13	79 vs 80 vs76	72% vs 44%
EXTEND-IA[198]	IA+IV vs IV	MRI&CT	<6	<70	>10	>1.2	ICA/M1/M2	12 vs 18	106 vs 115	71% vs 40%
SWIFT PRIME[197]	IA+IV vs IV	MRI&CT	<6	age18-79: MRI<50, CT<40 Age80-85: MRI<20, CT<15	>15	>1.8	ICA/M1			60% vs 35%
ESCAPE[207]	IA+Standard care/standard sare	CT	<12	>1/3 of MCA territory			ICA /M1/>2 M2 occlusions			53% vs 29%

* Trail's own definition.

The analyses carried out in this chapter have limitations. They were secondary analyses based on a single centre small phase II study. The sample size was small. The results need further verification in a large study.

6.5. Conclusion

In secondary analyses, we found patients with a thrombus of more than 8mm in length have a trend towards a reduced likelihood of recanalisation. In patients who had achieved recanalisation, the size of clot did not differ with tenecteplase or alteplase treatment.

Good collateral circulation was associated with smaller infarct volumes, and with the possibility of more penumbra salvage.

Post-thrombolysis cerebral oedema is associated with an increased risk of haemorrhage, less penumbra salvage, a larger infarct volume and possiblly worse clinical outcomes. Further focused analyses using a larger sample size is warranted to establish this.

In a sub-group of patients who had favourable imaging profiles, no difference was found in efficacy between the tenecteplase and alteplase groups. Tenecteplase treated patients have a lower risk of ICH post-treatment. Advance imaging based patient selection is appealing in conducting studies. However, the method is not verified, requiring further examination in RCT. Tissue status criteria require further refinement to avoid selection bias.

Chapter 7 Analysis of coagulation and fibrinolytic activity of tenecteplase and alteplase in acute ischaemic stroke

7.1. Introduction

IV thrombolysis with alteplase in acute ischaemic strokes improves clinical outcomes, but is associated with a 2.7% risk of fatal ICH (relative risk compared to placebo 6.8%, OR [95%CI]7.14 [3.98-12.79])[70]. The efficacy of alteplase varies depending on the occlusion site[319], and approximately 15% of those recanalised reoccluded within the first two hours of receiving treatment[322].

The potentially beneficial pharmacological properties of tenecteplase have prompted investigations of tenecteplase in acute stroke thrombolysis. In chapter 5, we demonstrated a trend towards fewer ICH complications among tenecteplase-treated patients. This finding is consistent with that of Parsons and colleagues[194]. Coagulation and fibrinolysis assays have been performed in several studies comparing thrombolytic agents in MI [306,395], but there are fewer data from acute strokes. Those studies[406,407] that have examined the fibrinolytic system after IV thrombolysis in strokes have done so to investigate mechanisms and predictions of ICH, and have suggested that early fibrinogen depletion is relevant, and that significant hypofibrinogaemia (a decrease of 2g/L or 50% from baseline) is about one-fifth among patients receiving intravenous alteplase[406].

tPA activates plasminogen to plasmin, which breaks fibrin down to fibrin-degradation products. Non-fibrin-selective agents, such as streptokinase or UK significantly affect haemostasis by breaking down circulating fibrinogen as well as the fibrin in thrombi[242].

rtPA has some ability to selectively bond to fibrin on the surface of a clot, and to activate mainly fibrin-bound plasminogen, which results in relatively controlled fibrinolysis, and hence gives rise to fewer instances of bleeding complication and in more targeted lysis. This property of selectively bonding to clot fibrin is termed "fibrin specificity or affinity", and is more prominent in tenecteplase than in alteplase (15-fold)[283]. The expectation that tenecteplase should decrease haemorrhagic incidence without comprising efficacy has been evidenced by MI thrombolysis studies[290].

The incidence of spontaneous ICH after thrombolysis in acute MI nevertheless is extremely low, and mechanisms are likely to differ from those after acute ischaemic stroke. Studies in MI therefore offer limited insight for stroke thrombolysis. Direct comparisons using ischaemic stroke patients may be informative.

As a sub-study of the ATTEST trial, we compared the effects of the two agents on coagulation and the fibrinolytic system, and explored associations with ICH and recanalisation.

7.2. Methods

The study protocol amendment for this sub-study was approved by the ethical committee in December 2012, with additional funding awarded from the NHS endowments fund. This sub-study was initiated part-way through the main trial. All trial participants were approached about the sub-study after it commenced.

Venous blood samples were collected on citrate (final concentration 0.109 M, Greiner Bio-One, Austria) at baseline (pre-thrombolysis) (Time Point [TP] 1), 3-12 hours (TP2) and 24±3 hours (TP3) after the initiation of thrombolytic drug treatment from each patient. Plasma was obtained by centrifugation immediately after sampling and then stored at -80 °C until analysis. The following assays were performed at the Haematology laboratory in Glasgow Royal infirmary; Prothrombin Time (PT), APTT, Fibrin(ogen) Degradation Products (FDP), plasminogen, D-Dimer, Factor V, PAI-1 activity and Prothrombin Fragment 1+2 (F1+2) (Table 7-1). The reference ranges for PT, APTT, fibrinogen and Factor V were derived locally, while the others were determined by the kit manufacturer.

Table 7-1. Detailed laboratory methods for coagulation assays

Analyte	Assay method & analyser	Assay Kit	Reference Limits	Assay CV
Prothrombin Time	Clot-based [ACL TOP700 CTS]	HemosIL® ReCombiPlasTin2G *	9-13s	1.8-2.6%
APTT	Clot-based [ACL TOP700 CTS]	HemosIL® SynthASil *	27-38s	2.3-2.5%
Fibrinogen [Clauss][408]	Clot-based [ACL TOP700 CTS]	HemosIL® Fibrinogen-C XL *	2.0-4.1 g/L	7.2-8.3%
Factor V	Clot-based [ACL TOP700 CTS]	HemosIL® Factor V deficient plasma *	66-167 iu/dL	6.8%
Fibrin D-dimer	Latex immunoassay [ACL TOP700 CTS]	HemosIL® D- Dimer HS *	< 243 ng/mL	5-7%
Fibrin(ogen) degredation products (FDP)	Latex immunoassay [ACL TOP700 CTS]	HemosIL® FDP *	< 2.01 ug/mL	3.4%
Plasminogen activity	Chromogenic assay [ACL TOP700 CTS]	HemosIL® Plasminogen *	73-140 u/dL	5%
PAI-1 activity	ELISA **	ZYMUSEST PAI-1 Activity, HYPHEN BioMed	< 5ng/mL	6.6-11.2%
Prothrombin Fragment 1+2	ELISA **	Enzygnost® 1+2 [monoclonal], SIEMENS	62-229 pmol/L	2.3-12.5%

7.2.1. Statistical analysis

The baseline value was expressed as the mean ± SD, the changes within groups at TP2 and TP3 were expressed as the mean ± SD percentage change from the baseline. Time and group interactions were sought using a repeated measure general linear model.

A related samples Wilcoxon signed rank test was used to examine the within-groups differences (TP2 versus TP1, TP3 versus TP1), and any significant global differences explored further for between-group effects using an independent Mann-Whitney test.

A univariate binary logistic regression model was used to explore the potential association between coagulation variables, ICH, and recanalisation.

7.3. Results

There were 104 participants in the main ATTEST trial, 30 of whom took part in this sub-study (alteplase = 14, tenecteteplase = 16) (Figure 7-2). The median sampling times did not differ significantly between the alteplase and tenecteplase groups for either TP2 (5.3 vs 4.4 hours, P=0.27) or TP3 (23.8 vs 23.9 hours, P=0.62), nor did the proportion of samples acquired in the morning[409] (5am-10am) (7% vs 31%, P=0.3). Key baseline characteristics were similar between patients who received alteplase and those treated with tenecteplase (Table 7-2). The baseline mean values (SD) of all coagulation variables and the proportion of their changes at TP2 and TP3 are summarised in Table 7-3.

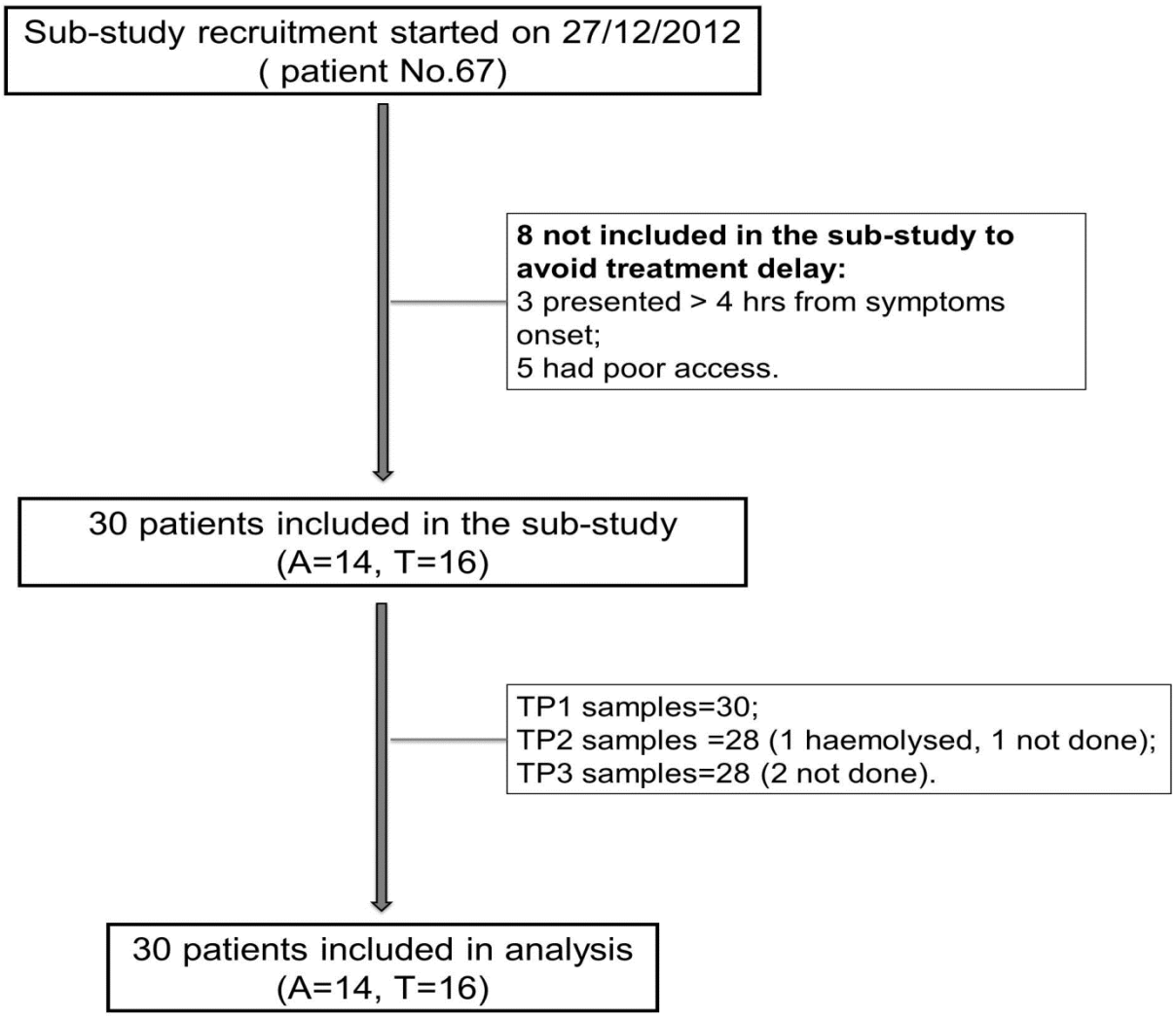


Figure 7-1. Coagulation and fibrinolysis essay sub-study CONSORT chart.

Table 7-2. Key demographic and stroke characteristics of the 30 patients

	Alteplase **N=14**	Tenecteplase **N=16**	P value
Age year (mean±SD)	70±12	69±15	0.95
Male **(n, %)**	10 (71%)	10 (63%)	0.71
OTT mins **(mean±SD)**	187±52	181±47	0.75
Baseline NIHSS score **(median, IQR)**	10 (6-15)	11 (8-17)	0.58
Cardioembolic stroke **(n, %)**	8 (57%)	8 (50%)	0.7
Baseline vessel occlusion **(n, %)**	9 (64%)	8 (50%)	0.34
Large vessel occlusion (ICA, M1) (n, %)	6 (43%)	6 (38%)	0.7
Sampling time for TP2 hours **(median, IQR)**	5.3(4.8-10.1)	4.4(3.9-11.8)	0.27
Sampling time for TP3 hours **(median, IQR)**	23.8(23.1-24.6)	23.9(23.5-24.6)	0.62
Diurnal Sampling for TP2 **(n, %)**	1 (7%)	5 (31%)	0.3

ICA Internal Carotid Artery; M1 Meddle Cerebral Artery M1 Segment; TP Time point; Frequencies were compared using Chi-squared test and Fisher's test; Mean or median values were compared using independent T test and Mann-Whitney U test respectively.

Table 7-3. Percentage change of coagulation and fibrinolytic variables in alteplase and tenecteplase group within the first 24 hours post thrombolysis

	Baseline¢			% change TP 2 versus TP1			% change TP3 versus TP1		
	Alteplase	**TNK**	**#P value**	**Alteplase** *ϕP value*	**TNK** *ϕP value*	**#P Value**	**Alteplase** *ϕP value*	**TNK** *ϕP value*	**#P value**
PT ***9-13 secs**	12±1	12±2	0.47	+13±16% *0.011*	+1±11% *0.67*	0.037	+11±14% *0.005*	+1±10% *0.86*	0.031
APTT ***27-38 secs**	30±3	29±3	0.26	+5±14% *0.31*	+1±11% *0.72*	0.16	-2±13% *0.73*	-3±9% *0.41*	1
Fibrinogen ***2.0-4.1 g/L**	3.1±0.6	3.3±0.7	0.53	-29±26% *0.002*	- 3 ± 17% *0.64*	0.002	-18±30% *0.021*	-8±21% *0.26*	0.011
FDPs ***< 2.01ug/mL**	3.8±3.8	8.3±19.5	0.39	+1048±810% *0.001*	+908±1204% *0.003*	0.39	+212±175% *0.002*	+146±144% *0.009*	0.29
Plasminogen ***73-140 U/dL**	84±16	79±11	0.32	-39±14% *0.001*	-8±15% *0.029*	<0.001	-24±13% *0.001*	-2±16% *0.62*	0.001
PAI-1 activity ***<5 ng/mL**	1.2±1.3	0.9±0.5	0.49	+600±1191% *0.093*	+104±314% *0.87*	0.24	+58±142% *0.62*	+11±54% *1*	0.73
D Dimer ***<243 ng/mL**	570±631	934±2419	0.59	+716±815% *0.003*	+1140±1385% *0.001*	0.59	+107±117% *0.064*	+148±119% *0.008*	0.40
Factor V ***66-167 IU/dL**	88±21	89±18	0.93	-18±18% *0.002*	↑4±16% *0.65*	0.002	+1±29% *0.45*	+6±20% *0.48*	0.48
F1+2 ***62-229 pmol/L**	308±173	413±518	0.48	+64±87% *0.087*	+66±89% *0.074*	0.83	+28±142% *0.68*	-10±39% *0.58*	0.91

Baseline values were expressed as mean±SD, the changes at TP2 and TP3 were expressed as mean ±SD % changes from baseline; *laboratory reference value; ¢Baseline values were expressed as mean±SD; #P value between the groups; ϕ*P value* within the group; TP Time point; PT Prothrombin time; APTT Activated Partial Thromboplastin Time; FDP Fibrin(ogen) degradation products; PAI-1 Plasminogen Activator Inhibitor-1; F1+2 Prothrombin Fragment 1&2.

7.3.1. Changes in coagulation and fibrinolysis within treatment groups (Figure 7-2)

Alteplase treatment was associated with prolongation of PT, reduced fibrinogen, plasminogen levels, elevated FDP and D-Dimer at 3-12 hours post-thrombolysis and at 24±3 hours. Factor V level also dropped significantly at TP2, but did return to the baseline at TP3. The rise of FDP at TP2 was significantly greater than that of D-Dimer (p=0.039).

In the tenecteplase group, only elevations of FDP and D-Dimer at TP2, TP3, and transiently reduced plasminogen at TP2 were seen. The proportion of the FDP rise was significantly smaller compared to that of D-Dimer (p=0.005).

7.3.2. Between-Groups comparison of coagulation and fibrinolytic variables (Figure 7-2)

Alteplase was associated with a significantly greater change from the baseline of PT (P=0.037), fibrinogen (P=0.002), plasminogen (P<0.001) and Factor V (P=0.002) at TP2 compared to tenecteplase, and differences remained significant at 24 hours for PT (P=0.031), fibrinogen (P=0.011) and plasminogen (P=0.001).

7.3.3. Association between ICH, depletion of fibrinogen, Factor V and the production of FDP, D-Dimer

Six out of the 30 patients had a haemorrhage post-thrombolysis, four of which were classified as HI1[311], one as HI2, and one had a small SAH. None was considered symptomatic using either the ECASS 2[311] or the SITS-MOST criteria[318].

Fibrinogen levels dropped below 1g/L at TP2 in two patients, both of whom received alteplase (2/30, 14%). This low level persisted at TP3 in one, whose follow-up CT revealed HI2; The fibrinogen rise to 1.4g/L at TP3 in the other patient who had postthombolysis SAH. A binary logistic regression found no association between ICH and the change of fibrinogen between TP2 and TP1 (p=0.37), or of D-Dimer (P=0.89). But a near-significant association was shown between the magnitude of Factor V drop and ICH (P=0.077).

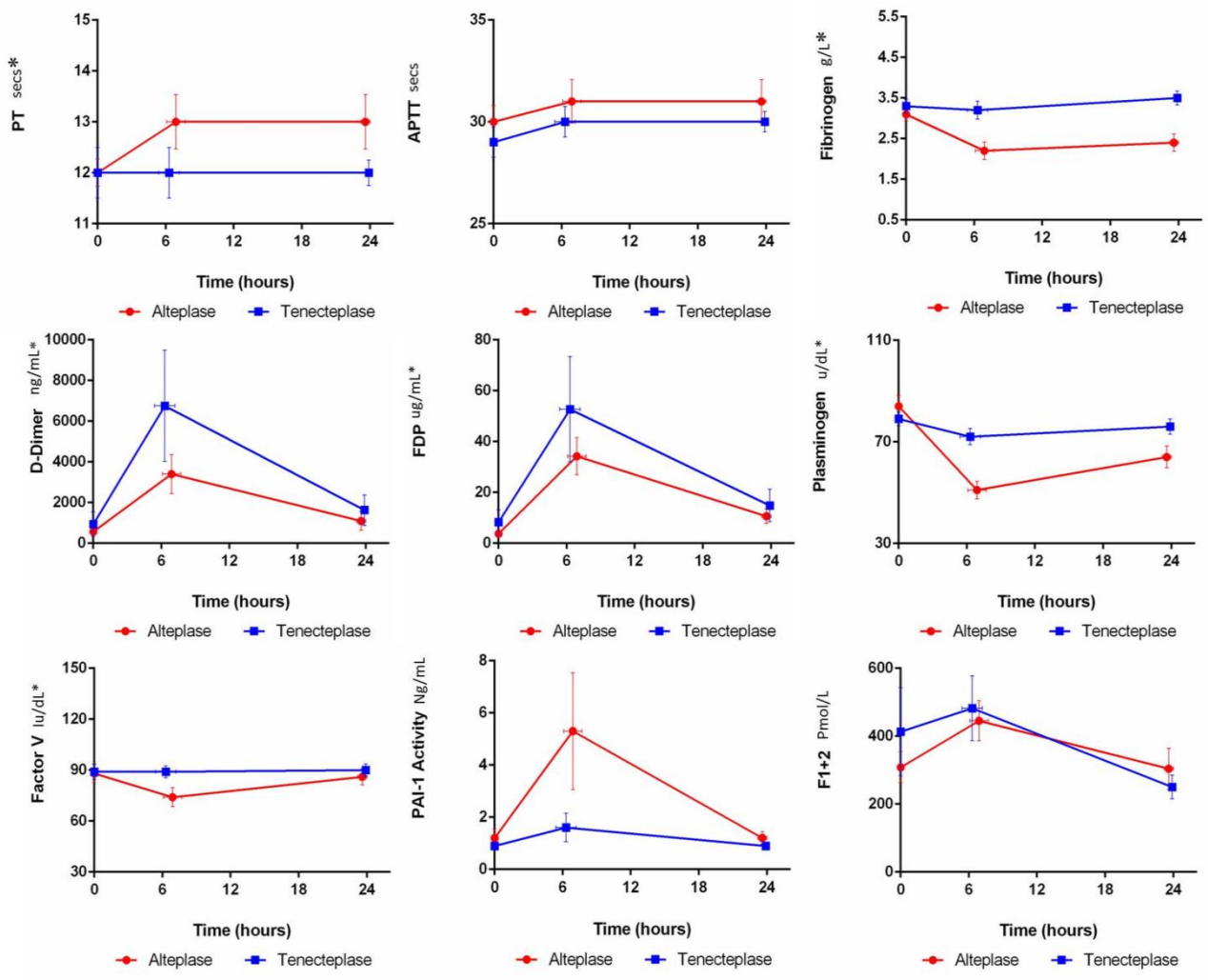


Figure 7-2. The changes of coagulation and fibrinolytic variables in alteplase and tenecteplase treated stroke patients from baseline to 24 hours post thrombolysis. *Statistical significant difference within or between groups.

A rise of FDP of 10-fold or more at TP2 was significantly correlated with the incidence of ICH (P=0.046, OR [95% CI] 10.7, [1.05-109.8]); the very wide confidence interval probably reflected the very small sample size.

7.3.4. Recanalisation and the change of FDP, fibrinogen, D-Dimer and PAI-1 activity

Within this subset of 30 patients, 17 had a baseline occlusion, of whom 10 recanalised at 24-48 hours. No association was found between recanalisation status and D-Dimer, FDP or PAI-1 activity at TP2 in this sample. The fibrinogen level of the recanalised group was non-significantly lower than that of non-recanalised patients (2.2±0.9 vs 2.9±1, P=0.144) at TP2. Within the 10 patients who recanalised, 7 received alteplase and 3 had tenecteplase; the fibrinogen levels were 2.1±0.9 and 2.7±0.6 respectively (P=0.25).

7.4. Discussion

Tenecteplase is reported to have 15-fold higher fibrin specificity, and 80-fold lower binding affinity to PAI-1 compared to alteplase[282]. High fibrin affinity is a desirable property of a thrombolytic agent as this ought to translate into greater potency for thrombus lysis, while preserving the integrity of systemic coagulation[410]. By bonding mainly to clot surface fibrin, the fibrin-specific agent ensures that the activation of plasminogen is limited to the clot surface. The systemic plasminogen pool is preserved, serving a continuous supply to the thrombus surface, leading to a more effective lysis. Animal models[284] and MI thrombolysis studies[289,290] have found fewer bleeding complications with tenecteplase while retaining thrombolytic efficacy. Trials in acute ischaemic strokes suggest that tenecteplase may also be associated with a lower ICH risk with similar or superior recanalization compared to alteplase[194 411]. In this small sample of stroke patients who were treated with standard alteplase or 0.25mg/kg tenecteplase, alteplase caused significant fibrinogen depletion and consumption of plasminogen, systemic activation of coagulation evidenced by degradation of Factor V[412], and activation of PAI-1. In contrast, tenecteplase caused minimal disruption to systemic coagulation with no hypofibrinogenaemia, while breaking fibrin down at least equally effectivly as suggested by the significant rise in FDP and D-Dimer at TP2 and TP3.

Early degradation of fibrinogen is associated with the occurrence of ICH. Matosevic et al[406] reported that within six hours post-thrombolysis, a decrease of ≥ 2g/L in fibrinogen

levels was an independent predictor for bleeding of all kinds. Significant hypofibrinogaemia (a decrease of 2g/L or 50% from baseline) occurs in about one-fifth of those receiving IV alteplase, whereas a tenecteplase dose escalation study[325] showed no severe hypofibrinogenaemia (fibrinogen <1g/dL) in any dose (0.1-0.5mg/kg) tested. Similarly, in our sample, tenecteplase treatment did not cause hypofibrinogenaemia using either of these criteria. We could not replicate an association between hypofibrinogenaemia and ICH, probably due to the small sample, but we found that a significant rise in the FDP level at TP 2 (>10 fold) was associated with the incidence of ICH.

In our sample, the average drop of Factor V was 18% at TP2 for alteplase treated patients, but was not severe enough to cause APTT prolongation. The decline of Factor V is a marker of systemic fibrinogenolysis and may have a role in the development of bleeding complications[412], as demonstrated by the near-significant association between the drop of Factor V and the occurrence of ICH in our sample. In acute MI[413], intravenous alteplase caused a much greater drop (average 40%) in circulating Factor V 90 minutes after the initiation of treatment, but the co-administration of heparin confounds interpretation.

Significant disruption of systemic coagulation by alteplase was further evidenced by the greater proportion of the rise in FDP than the rise in D-Dimer at TP2. D-Dimer is a specific degradation product of cross-linked fibrin, while FDP also includes the degradation products of fibrinogen. A greater ratio of FDP to D-Dimer rise suggests degradation of fibrinogen (as seen with alteplase), whereas a lower ratio suggests that the majority of the FDP were degraded fibrin with minimal fibrinogen breakdown (as seen with tenecteplase).

PAI-1 is the main inhibitor of alteplase, and increased PAI-1 activity post-thrombolysis after MI was thought to be one of the main reasons for the limited potency of alteplase[414]. We observed a similar, although non-significant, pattern in the alteplase group, and a smaller rise in the tenecteplase group, consistent with greater resistance to PAI-1 inhibition. Increased PAI-1 activity was also found to be associated with persistent occlusion in acute MI[415], but we could not confirm this observation, possibly because of the very small sample size, although other factors such as onset-to-treatment time, clot burden and collateral flow may have greater relevance than PAI-1 alone on recanalization in ischaemic strokes.

F1+2 is produced during the process of prothrombin activation by the cleavage of factor Xa and the generation of thrombin. The increase of F1+2 during thrombolysis reflects thrombin activation. An MI thrombolysis study suggested that the level of F1+2 increase can be reduced by concomitant heparin infusion, which implies that the thrombin activity during thrombolysis is at least partly due to prothrombin activation[416]. The level of F1+2 reflects the procoagulant effect and can serve as a biomarker for monitoring the process of reocclusion. In our sample, at TP2, the percent of F1+2 increase was very similar between alteplase and tenecteplase. At 24 hours, it returned close to the baseline level in the tenecteplase group, but was still elevated in the alteplase group. None of these changes was statistically significant. Thrombin activation can also be evaluated by measuring the thrombin-antithrombin complex and fibrinopeptide A[417], but we did not include these variables in our study. No data in a human thrombolysis study comparing the procoagulant effect of alteplase and tenecteplase exists to date. It is certainly worthwhile investigating whether tenecteplase is less likely to cause reocclusion than alteplase in an ischaemic stroke setting, given that concomitant heparin or large dose aspirin can be potentially harmful[418].

The significant increase in D-Dimer and FDP post-thrombolysis is well described[419,420]. One early study reported that arterial recanalisation was associated with a greater rise of D-Dimer post-thrombolysis than with those who had persistent occlusion:[421] We could not replicate similar results in our small sample.

Previous investigators[413,422] observed that in MI and pulmonary embolism thrombolysis, there was more extensive fibrinogenolysis activity in those who had successful recanalisation than the non-recanalised group. It was further suggested that some degree of firbinogenolysis may be necessary for recanalisation, as it produces adequate amounts of FDP in circulation, which inhibits further fibrin production, and therefore prevents the ongoing reformation of clots during the thrombolysis process. Therefore when fibrin-affinity increases beyond a certain limit, the lysis ability cannot rise further[410]. Whether the fibrinogenolysis requirement in recanalisation plays a role in limiting the lysis ability of fibrin-specific agents is unknown. However, even if the efficacy does not increase, the simplicity of administration and the better safety profile of agents with high fibrin-affinity still warrant further investigation in acute ischaemic strokes.

Limitations of our study include the small sample size, a variable sampling time at TP2 and TP3, and a low incidence of serious ICH (none having parenchymal haemorrhage, or clinical deterioration because of the haemorrhage). Since only 17 patients contributed to the analysis of recanalization, these findings should also be interpreted with caution. We were unable to adjust for known confounding factors such as onset-to-treatment time, baseline NIHSS score and age because of the small sample size. Since a repeat CTA was undertaken only at approximately 24 hours, we also lack information on the early evolution of vessel status, and clot lysis is known to be a dynamic process. Nonetheless, we found significant changes in coagulation and fibrinolysis after IV alteplase was administered, and significantly less disruption with tenecteplase, consistent with potentially a better safety profile for tenecteplase with the retention of fibrinolytic efficacy.

7.5. Conclusions

In acute ischaemic stroke, tenecteplase caused significantly less disruption to the coagulation and fibrinolytic systems compared to alteplase. This finding was consistent with the trend towards a reduced incidence of ICH observed in the ATTEST trial. The early significant rise of FDP was associated with the occurrence of ICH, and it may have a role in predicting ICH.

Chapter 8 Tenecteplase versus Alteplase in acute ischaemic stroke: individual patient data meta-analysis of randomized controlled trials

8.1. Introduction

Intravenous thrombolysis for acute ischaemic strokes with rtPA (alteplase) significantly improves functional outcomes if given with 4.5 hours from symptom onset[315]. Alteplase is recognised as having a number of limitations as discussed in previous chapters. In clinical use, IV alteplase achieves recanalisation in fewer than 50% of patients[319], with especially limited efficacy for larger volume occlusions in major cerebral arteries. While much effort has been expended on clinical trials to extend the treatment time window or the clinical indications for alteplase[423-425], better pharmacological revascularisation strategies may offer significant benefits for the large proportion of patients who present in the current treatment time window. Tenecteplase[283] demonstrated improved clot lysis, faster recanalisation, and a lower bleeding risk compared with alteplase in a rabbit carotid thrombosis model[284]. In acute myocardial infarctions, tenecteplase[290] showed superior safety compared to alteplase with no difference in efficacy.

A small number of preliminary studies have compared tenecteplase and alteplase in ischaemic strokes. We undertook a meta-analysis using individual patient data from randomised studies to examine the current available evidence for the efficacy and safety of tenecteplase compared with alteplase.

8.2. Methods

8.2.1. Search strategy

A comprehensive internet search was conducted using the MEDLINE, Embase, Cochrane databases and clinicaltrials.gov using the term "tenecteplase" (search year 1996-2014). The search was then refined by combining these with the terms "stroke", or "cerebrovascular accident". The latest search was performed in December 2014. By reading titles and abstracts, we selected the completed randomised studies. We also searched the reference lists of these articles for additional articles. Non-randomised studies were not included in analysis.

8.2.2. Data extraction

Study principal investigators were contacted to request individual patient data from the studies selected. A standardised form was developed for data extraction. Authors were contacted for any additional information and clarification. The primary outcome was defined as an excellent functional outcome at three months; secondary outcomes included a good functional outcome and the mRS shift test at three months, and early neurological improvement at 24 hours as efficacy outcomes, as well as safety outcomes of ICH, SICH and mortality at three months.

We defined an excellent functional outcome at three months as an mRS 0-1; a good functional outcome as an mRS 0-2; early neurological improvement at 24 hours as an improvement on the NIHSS of 8 or more points, or where the NIHSS score was 0 or 1 at 24 hours post-treatment. ICH was defined as being any intracerebral haemorrhage on follow-up CT post-thrombolysis. We defined SICH as being the presence of a PH2 according to the ECASS 2 radiological classification for ICH[311] accompanied by a clinical deterioration of four points or more in the NIHSS score if available, or used the investigator-reported SICH if original CT data were not available for review.

8.2.3. Statistical analyses

All analyses were performed on an intention-to-treat basis using all randomised patients in the selected studies. Since some studies examined more than one dose tier of tenecteplase[194,327], we compared the effect size in each dose group against the entire alteplase control group. We first performed traditional meta-analysis using the DerSimonian-Laird test, and used the Breslow-Day test to evaluate the heterogeneity

between studies with I^2 for inconsistency. Random effects models were undertaken to account for study heterogeneity. Outcomes were expressed as OR and their 95% CI.

An individual patient data analysis was then performed using a random effects logistic regression model, and an ordinal regression model adjusting for study, age, OTT, and the baseline NIHSS score. Group level meta-analysis was performed with StatDirect; individual patient data meta-analysis was conducted with SAS 9.3.

8.3. Results

8.3.1. Studies and patients

Seven studies investigating the use of tenecteplase in stroke treatment were identified. Five of these were excluded, one being a single arm dose escalation study[325], three[326,328,426] being non- randomised comparisons, and one[427] has not started recruitment. Three completed randomised studies[194,327,411] were included in the analysis, with a total of 291 patients.

8.3.2. Comparative analysis of the studies

8.3.2.1. Eligibility criteria

The design features and key characteristics of the study populations are described in Table 8-1. Only one trial was double blind; the other two used a PROBE design. Imaging methods dictated trial populations, with the ATTEST study including only supratentorial strokes due to the limited coverage of CTP, and Parsons and colleagues used imaging based selection to identify a responder population.

None of the three studies applied an upper age limit. Haley et al did not have a minimal NIHSS score limit, but did require significant neurological deficits such as language or motor deficit if the score was 1 at baseline. No maximum NIHSS score was exclusionary in this study. Similarly, the ATTEST study allowed the inclusion of patients with an NIHSS score between 1 and 25. Parsons et al required a minimal NIHSS score of 4 or above for study entry. The ATTEST study and Parsons et al also required a pre-stroke estimated functional independence (mRS 0-2) for study entry.

While studies varied in the application of guideline or licence contraindications, all three shared common exclusion criteria, including ICH on baseline NCCT, contraindications for

thrombolysis such as recent surgery, trauma, bleeding disorders, haemostasis problems and a high baseline blood pressure.

8.3.2.2. Study treatment

All studies used the standard dose of alteplase (0.9mg/kg to a maximum of 90mg, 10% of dose as the initial bolus, followed by a one-hour infusion with 90% of dose) as the comparator. Haley et al examined three different dose tiers of tenecteplase (0.1mg/kg, 0.25mg/kg, and 0.4mg/kg) using an adaptive sequential design scoring each dose according to the occurrence of early neurological improvement at 24 hours and SICH. The 0.4mg/kg dose was eliminated early in the study with only 19 patients in this group because it met the pre-specified criterion for elimination of unpromising performance (a score of six fewer units than the dose group with the leading score). When the study terminated prematurely due to slow recruitment, there were 31 patients respectively in the 0.1mg/kg, 0.25mg/kg and alteplase groups. Two tier doses of tenecteplase were tested in Parsons et al (0.1mg/kg and 0.25mg/kg), while the ATTEST study examined only 0.25mg/kg.

8.3.2.3. Outcome measures

The primary outcome differed in each study. Haley et al terminated prematurely while the primary end-point remained the occurrence of early neurological improvement and SICH: the original plan to compare three month functional outcomes for the selected tenectplase dose did not proceed. The co-primary endpoint of the proportion of reperfused perfusion lesion measured by perfusion weighted MRI and the extent of neurological improvement at 24 hours in the NIHSS score was used by Parsons et al. The ATTEST study compared penumbral salvage at 24-48 hours using the baseline CT perfusion, defined as the perfusion lesion minus the final infarct volume on the follow-up CT.

Clinical outcome measures (NIHSS scores at baseline and 24 hours, three month mRS, mortality) were common to all three studies. All studies recorded the ICH incidence on follow-up CT at 24-54 hours post treatment, but different definitions of SICH were applied. Recanalisation status, penumbral salvage and infarct volumes at 24 hours were additionally available for two studies.

8.3.2.4. Heterogeneity in baseline characteristics

Consistent with different selection criteria, baseline demographics varied significantly across the three studies (Table 8-1), notably for OTT ($p<0.001$) and the baseline NIHSS scores ($p<0.001$). There were nine subjects with a baseline mRS of 3-4 in Haley et al. No differences were found in age and the main co-morbidities, except for the proportion of patients with hypertension or previous stroke/TIA. In the two studies with baseline perfusion imaging, ischaemic core volumes did not differ, but the median penumbra volume was larger in Parsons et al (79ml, IQR 56-100 vs 53ml, IQR 0-110; $p=0.008$).

Table 8-1. Studies included in the meta-analysis and comparison of key baseline characteristics.

	Haley et al[327] (2010)	Parsons et al[194] (2012)	ATTEST[411] (2015)	Combined	
				TNK	**Alteplase**
Study characteristics					
Treatment arms Active vs control	TNK# 0.1mg/kg vs 0.25mg/kg vs 0.4mg/kg vs alteplase 0.9mg/kg	TNK 0.1mg/kg vs 0.25mg/kg vs alteplase 0.9mg/kg	TNK 0.25mg/kg vs alteplase 0.9mg/kg		
Double blind	Yes	Blinded endpoints	Blinded endpoints		
Number	31 vs 31vs 19 vs 31	25 vs25 vs 25	52 vs 52	183	108
Key inclusion criteria	NIHSS>0; If NIHSS=1, requires significant deficit; Symptoms onset <3 hours	First stroke (not brain stem stroke); NIHSS≥4; Symptoms onset <6 hours; mRS 0-2; Core volume< 1/3 of MCA or 1/2 ACA/PCA territory; Perfusion volume > 120% core , and ≥20mls; Occlusion of MAC/ACA/PCA.	Supertentorial stroke, NIHSS 1-25; Symptoms onset <4.5 hours; mRS 0-2.		
Key difference in exclusion criteria	Stroke in previous 3 months; Seizures at the onset of stroke.	Stroke in previous 3 months; eGFR<15mls/min; Contraindication for MRI.	Recent stroke on NCCT; Hypodensity of >1/3 of the MCA territory; ASPECT score ≤4; Glucose >18mmol/L; eGFR<30mls/mins; Allergy to Iodinated contrast.		
Definition of SICH	Any clinically important neurological worsening attributable to new hemorrhage by independent neurology adjudication	SITS-MOST[318]¤	SITS-MOST; ECASS 2[311]§.		
Demographics and Clinical Characteristics					
Age Year (mean±SD) P=0.69*	70±17	71±10	70±12	69±14	71±13
Male n (%)	58 (52)	38 (51)	65 (63)	100 (55)	61 (56)

P=0.18*					
Baseline NIHSS (Median, IQR) P<0.001*	10(5-16)	15(12-16)	11(8-18)	12 (7-16)	12 (8-17)
OTT mins (mean±SD) P<0.001*	141±28	176±46	176±46	167±43	170±50
Current Smoker n (%) P=0.07*	16 (14)	15 (20)	27 (26)	38(21)	20 (19)
Hypertension n (%) p<0.001*	89 (80)	47 (63)	50 (48)	121 (66)	65 (60)
Atrial Fibrillation n (%) P=0.22*	27 (24)	28 (37)	33 (32)	56 (31)	32 (30)
Hyperlipidaemia n (%) p=0.89*	56 (50)	37 (49)	53 (51)	96 (52)	50 (46)
Previous Stroke/TIA n (%) p<0.001*	2 (2)	0	25 (24)	16 (9)	11 (10)
Diabetes n (%) p=0.7*	21 (19)	15 (20)	16 (15)	40 (22)	12 (11)
Blood Glucose mmol/L (mean±SD) P=0.91*		6.9±1.7	7±1.9	6.9±1.7	6.9±2
Imaging Characteristics (TNK=104, alteplase=79)					
Ischaemic core volume ml (median, IQR) P=0.5*		15(12-16)	15(12-16)	10 (3-23)	13 (4-28)
Penumbra volume ml (median, IQR) P=0.008*		79(56-100)	53(0-110)	73 (43-106)	59 (23-101)
Vessel occlusion n (%) P<0.001*		72 (96)	73 (70)	83 (80)	61 (77)
Large vessel occlusion n (%) P<0.001*		58 (77)	49 (47)	66 (63)	41 (52)
***P values quoted were from ANOVA analysis (continuous variables), and Chi Square test (categorical variables) between studies; OTT=Onset-to-treatment time; NIHSS=National Institutes of Health Stroke Scale; TNK=tenecteplase; TIA=Transient Ischaemic Attack; NCCT=Non-Contrast Computed Tomography; eGFR=Estimated Glomerular filtration rate; ASPECT score=Alberta Stroke Programme Early CT Score; ¤ PH type 2 or PH remote type 2 on 24-48 hours Non-contrast CT post treatment, plus neurological deterioration defined by increase of NIHSS score by 4 or more points; § any ICH on follow-up NCCT with clinical deterioration.**					

8.3.3. Meta-analysis results

Of 291 patients randomised, 108 were allocated to 0.25mg/kg TNK, 56 to 0.1mg/kg TNK, 19 to 0.4mg/kg TNK and 108 to standard dose alteplase.

8.3.3.1. Group level data

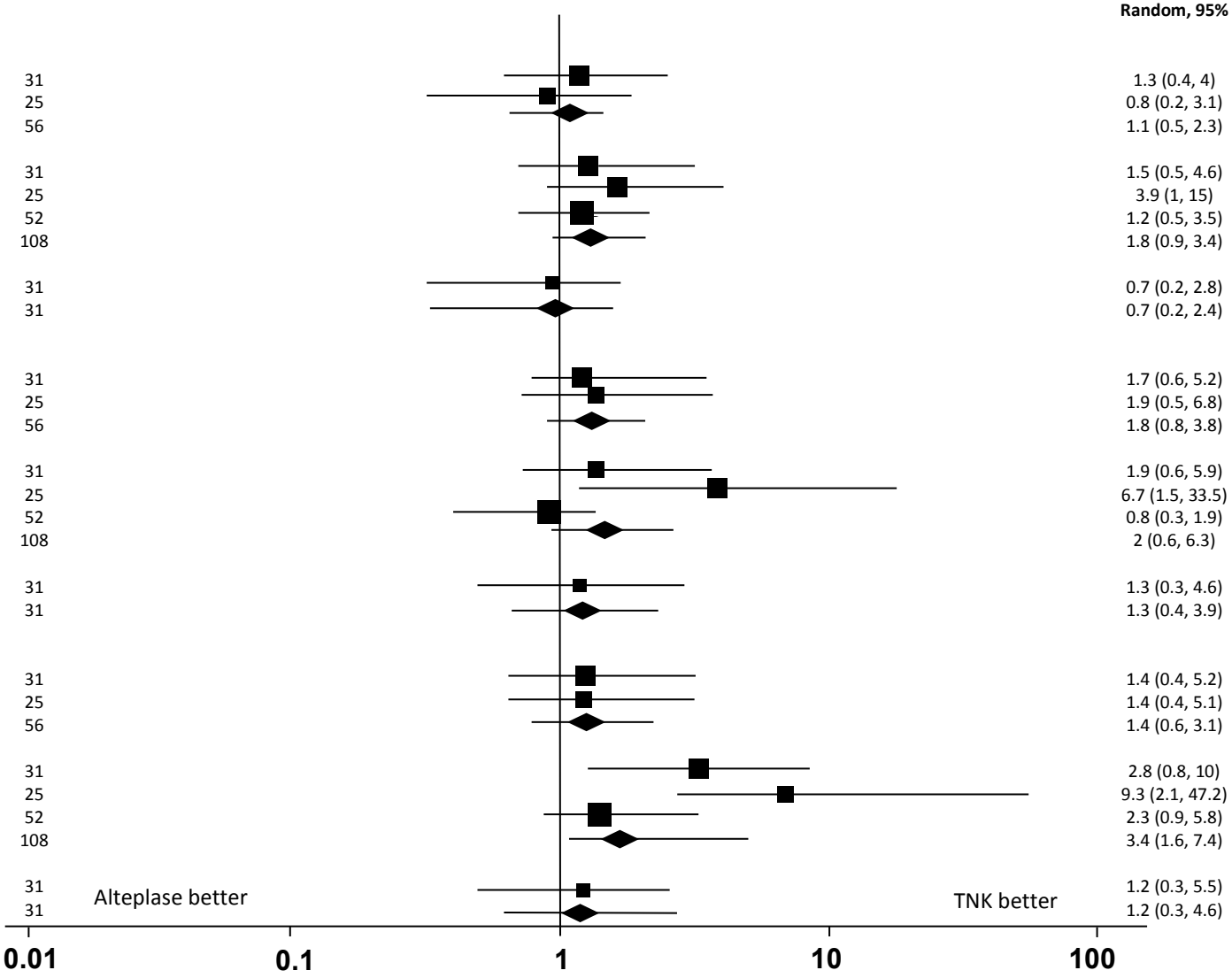
The detailed results for the comparison between alteplase treated patients and those who received one of the three dose tiers of tenecteplase respectively are summarised in Figure 8-1.

The 0.25mg/kg TNK group showed a significantly greater odds ratio to achieve early neurological improvement at 24 hours with an odds ratio for the combined data of 3.4 (95% CI, 1.6-7.4, P=0.002). The excellent (OR=1.8, 95% CI, 0.9-3.4, P=0.08) or good (OR=2, 95% CI, 0.6-6.3, P=0.26) functional outcome at three months, the risk of ICH (OR=0.6, 95% CI, 0.3-1.3, P=0.2), SICH (OR=0.6, 95% CI, 0.2-2.1, P=0.39), and the mortality rate at three months (OR=0.9, 95% CI, 0.4-2, P=0.89) were not significantly different between patients treated with 0.25mg/kg TNK and alteplase. No significant heterogeneity was detected in these three studies in all outcomes with I^2 for inconsistency ranging from 0-36.1%, except for good functional outcomes (p=0.02, I^2=73.8%, 95%CI 0-90.1%).

No significant differences were found in any outcome between 0.1mg/kg TNK and alteplase treated patients.

Only 19 patients received TNK 0.4mg/kg and outcomes did not differ from alteplase

Study	Tenecteplase Event	Total	Alteplase Event	Total	Odds Ratio M-H, Random, 95%
Excellent Functional Outcome (mRS 0-1) at 3 months					
0.1mg/kg					
Haley 2010	14	31	12	31	1.3 (0.4, 4)
Parsons 2012	9	25	10	25	0.8 (0.2, 3.1)
Subtotal (95%CI)		56		56	1.1 (0.5, 2.3)
0.25mg/kg					
Haley 2010	15	31	12	31	1.5 (0.5, 4.6)
Parsons 2012	18	25	10	25	3.9 (1, 15)
ATTEST 2015	13	52	11	52	1.2 (0.5, 3.5)
Subtotal (95%CI)		108		108	1.8 (0.9, 3.4)
0.4mg/kg					
Haley 2010	6	19	12	31	0.7 (0.2, 2.8)
Subtotal (95%CI)		19		31	0.7 (0.2, 2.4)
Good Functional Outcome (mRS 0-2) at 3 months					
0.1mg/kg					
Haley 2010	17	31	13	31	1.7 (0.6, 5.2)
Parsons 2012	15	25	11	25	1.9 (0.5, 6.8)
Subtotal (95%CI)		56		56	1.8 (0.8, 3.8)
0.25mg/kg					
Haley 2010	18	31	13	31	1.9 (0.6, 5.9)
Parsons 2012	21	25	11	25	6.7 (1.5, 33.5)
ATTEST 2015	17	52	20	52	0.8 (0.3, 1.9)
Subtotal (95%CI)		108		108	2 (0.6, 6.3)
0.4mg/kg					
Haley 2010	9	19	13	31	1.3 (0.3, 4.6)
Subtotal (95%CI)		19		31	1.3 (0.4, 3.9)
Early Neurological Improvement					
0.1mg/kg					
Haley 2010	9	31	7	31	1.4 (0.4, 5.2)
Parsons 2012	11	25	9	25	1.4 (0.4, 5.1)
Subtotal (95%CI)		56		56	1.4 (0.6, 3.1)
0.25mg/kg					
Haley 2010	14	31	7	31	2.8 (0.8, 10)
Parsons 2012	21	25	9	25	9.3 (2.1, 47.2)
ATTEST 2015	21	52	12	52	2.3 (0.9, 5.8)
Subtotal (95%CI)		108		108	3.4 (1.6, 7.4)
0.4mg/kg					
Haley 2010	5	19	7	31	1.2 (0.3, 5.5)
Subtotal (95%CI)		19		31	1.2 (0.3, 4.6)



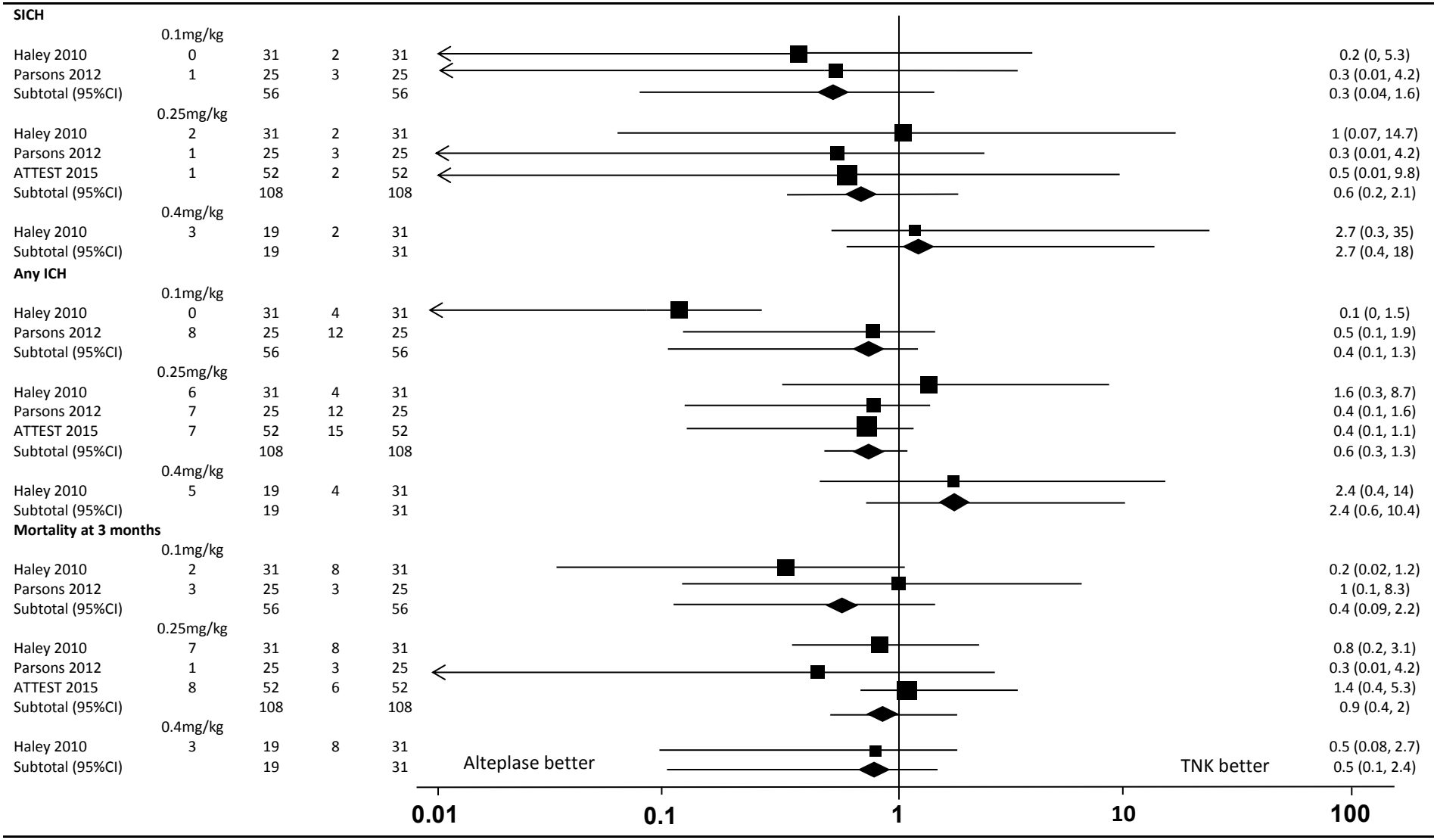


Figure 8-1. Effect of Tenecteplase (all doses) compared with alteplase in all efficacy and safety outcomes in a traditional meta-analysis.

8.3.4. Individual patient data analysis (Figure 8-2)

8.3.4.1. Comparison of tenecteplase and alteplase

We performed pooled analysis using random effects logistic regression and ordinal regression models. Individual studies were treated as an independent variable for adjustment, as were other key prognostic variables including age, OTT and baseline NIHSS scores. Imaging variables were not included, since these were only available in two studies.

TNK 0.25mg/kg treated patients had greater odds of early neurological improvement (OR [95%CI] 3.3 [1.5, 7.2], p=0.093). Other outcomes, including mRS shift analysis (Figure 8-3), did not differ significantly from alteplase. Other tenecteplase doses did not differ significantly from alteplase for any end-point.

	Tenecteplase (event/total)	**Alteplase** (event/total)	Odds Ratio (95%CI)
Excellent Functional Outcome at 3 months	0.1 mg/kg (23/56)	33/108	0.7 (0.3, 1.9)
	0.25mg/kg (46/108)	33/108	1.9 (0.8, 4.4)
	0.4 mg/kg (6/19)	33/108	0.4 (0.09. 2.2)
Good Functional Outcome at 3 months	0.1 mg/kg (32/56)	44/108	1.5 (0.5, 4.7)
	0.25mg/kg (56/108)	44/108	1.9 (0.5, 7.2)
	0.4 mg/kg (9/19)	44/108	1.2 (0.3, 5.3)
mRS at 3 months (Ordinal shift)	0.1 mg/kg (56)	108	1.2 (0.6, 2.4)
	0.25mg/kg (108)	108	1.4 (0.5, 4.3)
	0.4 mg/kg (19)	108	0.9 (0.3, 2.6)
Early Neurological Improvement	0.1 mg/kg (20/56)	28/108	1.5 (0.6, 3.4)
	0.25mg/kg (56/108)	28/108	3.3 (1.5, 7.2)
	0.4 mg/kg (5/19)	28/108	1.3 (0.3, 5)
SICH	0.1 mg/kg (1/56)	7/108	0.2 (0.02, 2)
	0.25mg/kg (4/108)	7/108	0.7 (0.2, 2.5)
	0.4 mg/kg (3/19)	7/108	6.2 (0.7, 56.3)
Any ICH	0.1 mg/kg (8/56)	31/108	0.2 (0.03, 2.1)
	0.25mg/kg (20/108)	31/108	0.6 (0.2, 1.8)
	0.4 mg/kg (5/19)	31/108	4.6 (0.9, 24.3)
Mortality at 3 months	0.1 mg/kg (5/56)	17/108	0.5 (0.1, 1.8)
	0.25mg/kg (16/108)	17/108	1.1 (0.5, 2.5)
	0.4 mg/kg (3/19)	17/108	1.1 (0.2, 5.8)

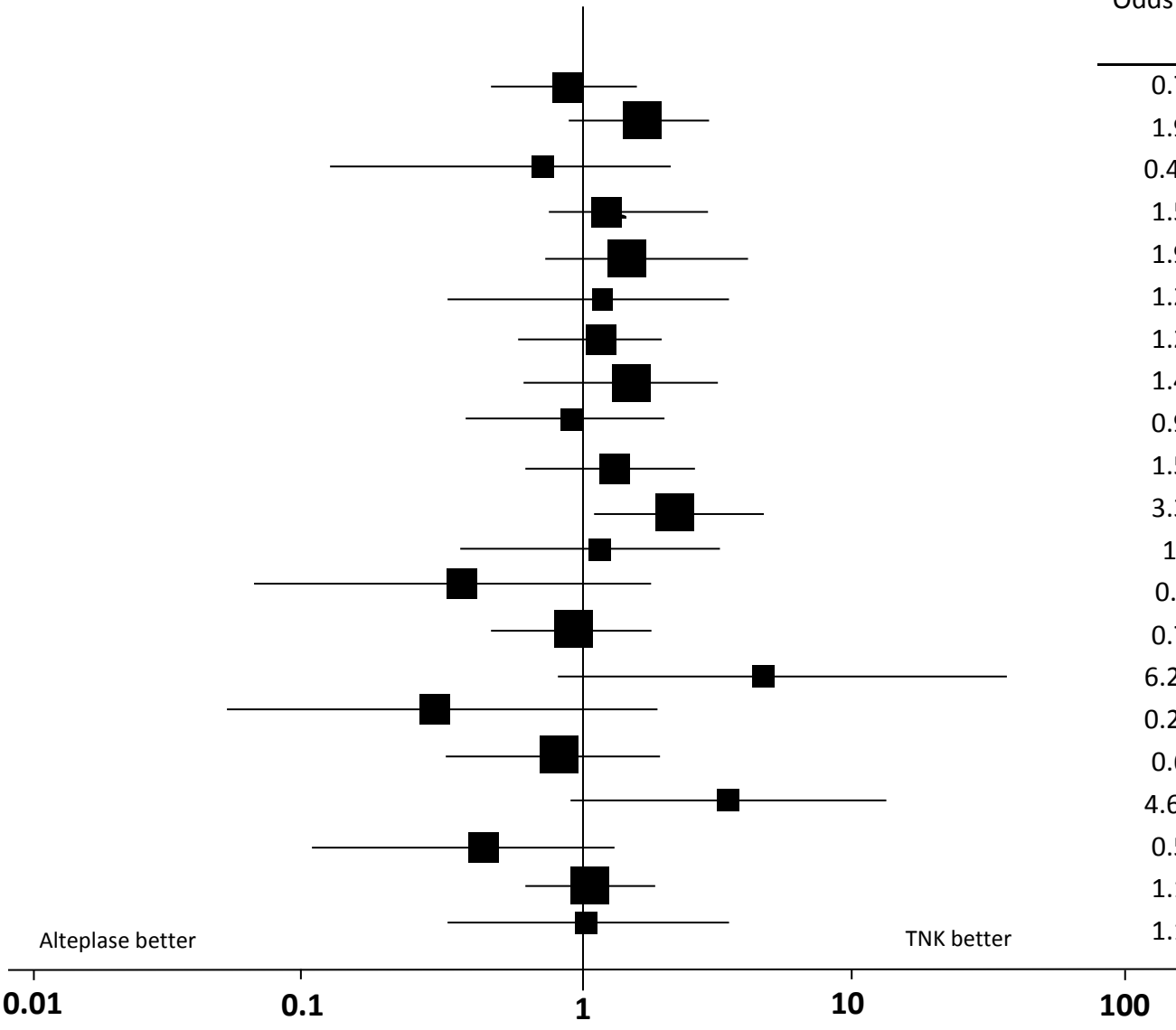


Figure 8-2. Pooled analysis comparing efficacy and safety outcomes between tenecteplase and alteplase treated patients using random effect logistic and ordinal regression models adjusted for studies, baseline NIHSS score, Onset-to-treatment Time and age.

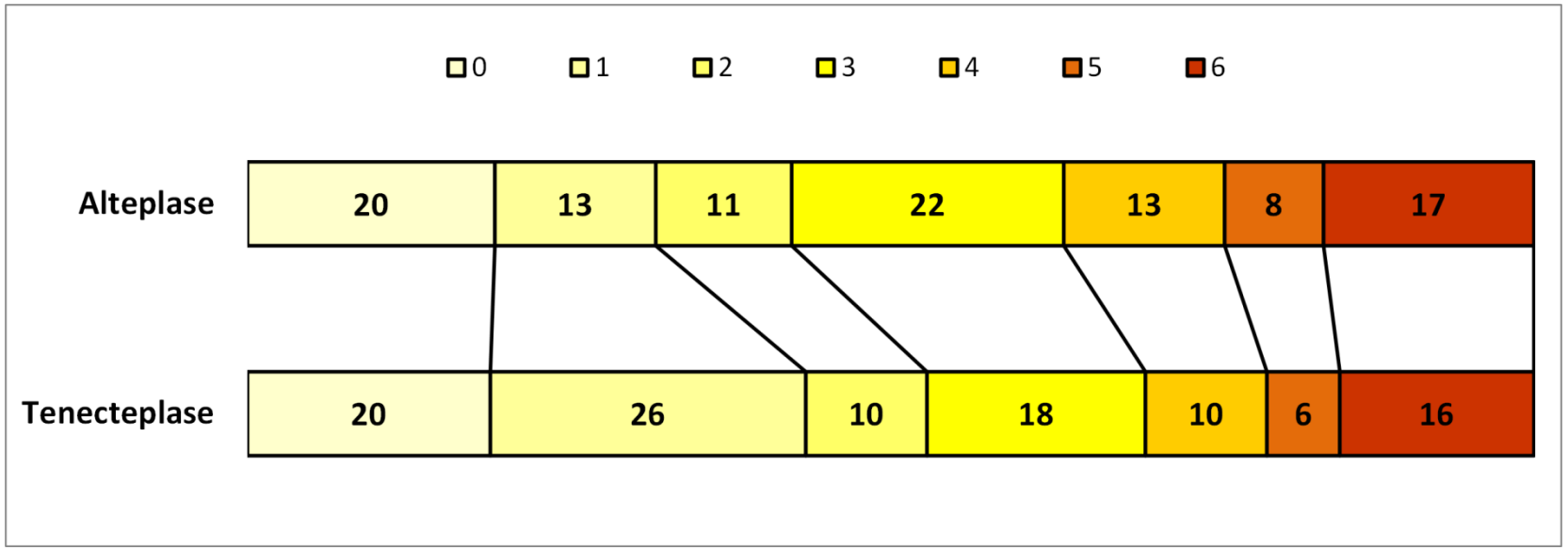


Figure 8-3. Distribution of Modified Rankin scale at 90 days for patients treated with tenectepkase 0.25 mg/kg and alteplase.

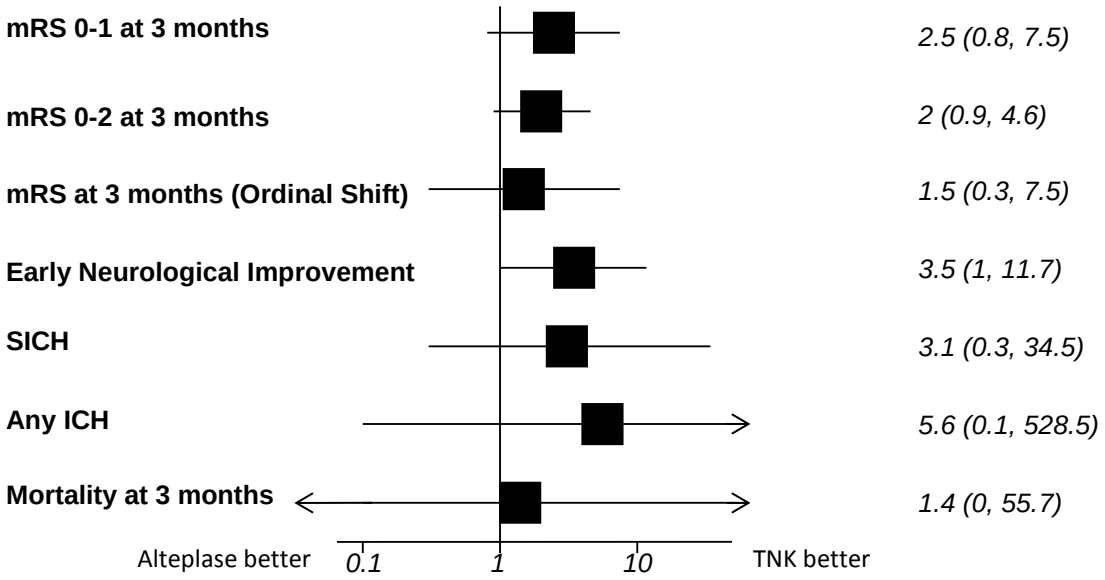


Figure 8-4. Pooled analysis comparing efficacy and safety outcomes between tenecteplase 0.25mg/kg and 0.1mg/kg treated patients using random effect logistic and ordinal regression models adjusted for studies, baseline NIHSS score, Onset-to-treatment Time and age.

8.3.4.2. Comparison of TNK dose 0.25mg/kg and 0.1mg/kg (Figure 8-4)

We compared the efficacy and safety of TNK 0.1mg/kg and 0.25mg/kg using the same outcomes. We did not include 0.4mg/kg TNK in the comparison due to the small sample size. No significant differences were observed between these doses, although point estimates of efficacy suggest that the 0.25mg/kg dose was associated with a greater probability of improvement at 24 hours (OR [95%CI] 3.5 [1, 11.7], P=0.29), and excellent functional outcomes (mRS 0-1) at three months (OR [95%CI] 2.5 [0.8, 7.5], P=0.36).

8.3.5. Individual patient data analysis using Haley et al and the ATTEST study only (Figure 8-5)

There is no significant difference in all outcomes between patients treated with TNK 0.25mg/kg and alteplase, except that TNK 0.25mg/kg has a greater likelihood of achieving early neurological improvement compared with alteplase (OR 2.4, 95%CI 1.4-4.7, p=0.24).

The 0.1mg/kg dose was not analysed, since it was only studied in Haley et al.

	Tenecteplase (event/total)	Alteplase (event/total)	Odds Ratio (95%CI)
Excellent Functional Outcome at 3 months	28/83	23/83	1.3 (0.6, 2.7)
Good Functional Outcome at 3 months	35/83	33/83	1.0 (0.5, 2)
mRS at 3 months (Ordinal shift)	83	83	0.9 (0.5, 1.6)
Early Neurological Improvement	35/83	19/83	2.4 (1.2, 4.7)
SICH	3/83	4/83	0.9 (0.2, 4.5)
Any ICH	13/83	19/83	0.8 (0.1, 8.2)
Mortality at 3 months	15/83	14/83	1.4 (0.5, 3.4)

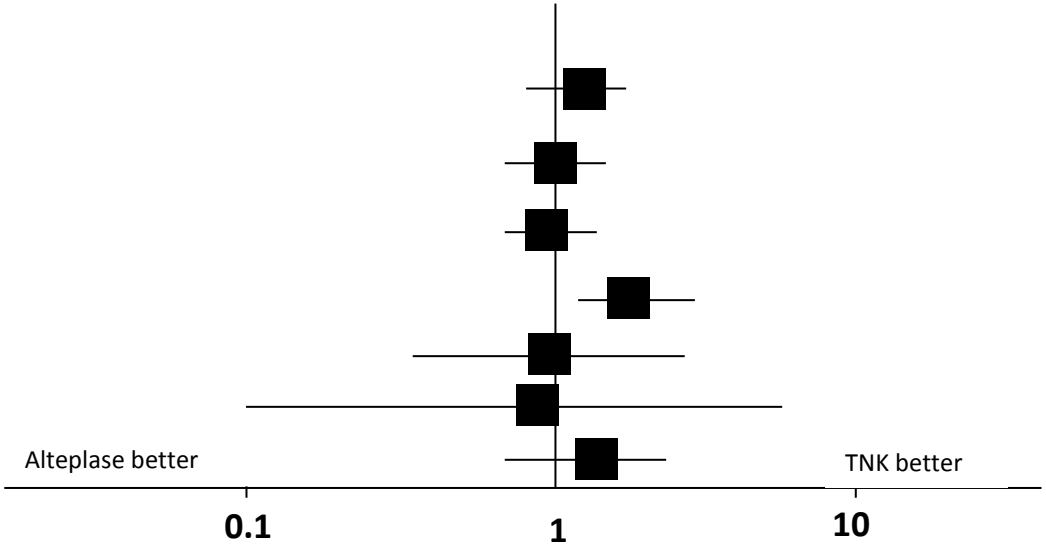


Figure 8-5. Comparison of TNK 0.25mg/kg and alteplase using Haley et al and ATTEST data only

8.3.6. Discussion

Both conventional meta-analysis using group level data and individual patient data analysis showed a greater likelihood of early major neurological improvement with tenecteplase 0.25mg/kg, and point estimates suggested a potentially greater likelihood of functional independence at 90 days. Point estimates for clinical outcomes were, in contrast, neutral for both 0.1mg/kg and 0.4mg/kg doses. Experience with tenecteplase in acute stroke remains limited, however, with fewer than 300 patients contributing to the randomised controlled trial data. Comparisons of different dose tiers are therefore very limited, particularly for the 0.4mg/kg TNK dose group.

While our analyses support the possible existence of a dose-dependent ICH risk, the small numbers of outcome events mean that no analysis achieves statistical significance. The evaluation of the 0.4mg/kg dose was very limited, with only 19 patients receiving this dose prior to termination of this dose level. While the estimated higher ICH risk of 0.4mg/kg TNK compared with alteplase is non-significant, the unfavourable point estimate likely contributed to the elimination of this dose in the relevant trial due to the combination of efficacy and safety factors. Further trial data with this dose will be important in order to test this association more rigorously.

Historically, dose escalation studies in stroke thrombolysis included only small numbers in each tier[325,332,428,429]. The ascending dose design produced a bias towards early dose abandonment on the basis of incident ICH since investigators expect greater risk as the trial progressed. Early rtPA trials[280,310] explored only a narrow range of alteplase doses, and abandoned dose escalation on the basis of a small number of ICH events. Efforts to select the optimal tenecteplase dose were methodologically rigorous, but premature trial discontinuation limits the reliability of dose selection,[327] and likely contributes to the variability of doses being investigated in current and upcoming tenecteplase studies. Availability of individual patient data allowed us to apply common definitions for outcomes (with the exception of SICH) and also to adjust for heterogeneous onset-to-

treatment time and baseline stroke severity. The selection of patients with an imaging-defined target profile (favourable penumbra: core ratio, small core, and intracranial vessel occlusion) in Parsons et al, may have resulted in overestimation of the overall ORs for efficacy outcomes, although the heterogeneity analysis was not significant across studies. When adjusted for baseline prognostic variables including individual study in the pooled analysis of all three studies, or only Haley et al and the ATTEST study, the results were similar.

Advanced imaging characterisation of acute stroke may identify heterogeneity that is not captured adequately by clinical variables, and further exploration of imaging-characterised populations in the ATTEST study and Parsons studies may be informative.

Currently three[204,330,430]ongoing studies are investigating the use of teneteplase in acute ischaemic stroke, using either 0.25mg/kg or 0.4mg/kg TNK dose. The ongoing study of tenecteplase versus alteplase for thrombolysis in acute ischaemic stroke[330] (NOR-TEST) (NCT01949948) (clinicaltrials.gov) chose 0.4mg/kg and will thus provide more data for this dose when completed. Other ongoing studies have chosen 0.25mg/kg. The TNK–Tissue-Type Plasminogen Activator Evaluation for Minor Ischemic Stroke With Proven Occlusion (TEMPO-1)[329] trial suggested that both 0.1mg/kg and 0.25mg/kg TNK were safe in treating minor strokes with intracranial occlusion within 12 hours of symptom onset. TEMPO-2[430] will randomise patients fulfilling the same criteria to 0.25mg/kg TNK or placebo. Similarly, the Tenecteplase versus Alteplase for Stroke Thrombolysis Evaluation (TASTE)[204] trial is comparing 0.25mg/kg TNK and alteplase in an imaging-selected group, whereas the ATTEST-2 study will examine the same dose in all thrombolysis eligible patients on the basis of non-contrast CT alone. The data of over 2000 patients will be available when these three studies are complete. Dose selection is influenced by the trial populations, since in minor stroke (eg TEMPO-2) safety may be a more compelling basis for dose selection than for major stroke where efficacy considerations may dominate.

8.3.7. Conclusions

Tenecteplase 0.25mg/kg was associated with greater odds of early major neurological improvement and a trend for better functional outcome at three months. Further investigation of tenecteplase in acute ischaemic stroke patients is warranted.

Chapter 9 Tenecteplase versus alteplase in acute ischaemic stroke: imaging outcomes – an individual patient data meta-analysis

9.1. Introduction

Advance imaging in acute ischaemic stroke plays an important role in providing biomarkers and in assisting patient selection in many recent studies. In the ATTEST study[411], penumbra salvage was used as the primary outcome to reduce the sample size. In the Australian tenecteplase study[194], advance imaging was utilised for both patient selection and in defining one of the co-primary endpoints. The clinical outcomes in tenecteplase- and alteplase-treated ischaemic stroke were analysed in the pooled analysis reported in Chapter 8. To investigate whether these two thrombolytics yield any difference in imaging outcomes, we performed a pooled analysis of the imaging outcomes from the two studies using advanced imaging to determine outcome measures.

9.2. Methods

9.2.1. Data acquisition

Study data of the Australian tenecteplase study was acquired as described in Chapter 8. We used the investigator's own data for the imaging variables including baseline CTP lesion volumes, penumbra salvaged at 24 hours, final infarct volumes at 24 hours, and baseline occlusion and recanalisation status at 24 hours. To acquire additional imaging variables that were absent from their original data, the team was contacted for the transfer of the raw study imaging, which was obtained via a secure online file sharing system www.box.com. All imaging had previously been anonymised by the investigator and studies were only indentified by study numbers.

9.2.2. Imaging acquisition

The imaging procedure for the ATTEST study was discussed in detail in Chapter 3.

The Australian tenecteplase study[194] was a multicentre study. Its baseline imaging included NCCT, CTP and CTA. Imaging was performed with multidetector scanners (16- or 64-slice) before randomisation. NCCT was followed by CT perfusion imaging, comprising two 60-second series, each performed after an intravenous bolus of 40mL of iodinated contrast agent at a concentration of 370 mg per millilitre, followed by a 40mL saline flush at a rate of 6mL per second. Each perfusion series covered an axial section of 24 to 40mm, acquired as adjacent slices of 5 to 8mm. Subsequently, CTA was performed from the carotid bifurcation to the top of the lateral ventricles.

MRI was performed with the use of 1.5 tesla scanners. Standardized sequences were obtained 24 hours after treatment and included an axial gradient-echo T2-weighted series, a DWI, a PWI, an echoplanar spin-echo sequence, a TOF MRA, and a FLAIR sequence. At 90 days, a FLAIR sequence was performed for final infarct volume.

9.2.3. Imaging analysis

The procedure for volumatic analysis and recanalisation for the ATTEST study was described in Chapter 3.

The software MIStar (Apollo Medical Imaging Technology, Melbourne, Australia)[373] was used for all imaging analysis in both studies. A slightly different tissue status definition was used in the Australian study: ischaemic core was defined as the area with a CBF <40% of the contralesional side[194], whereas the ATTEST study applied additionally a relative DT>2s. The penumbra tissue imaged by both CTP or PWI was defined as the area with MTT>145% on the contralesional side[194], but the ATTEST study used the relative DT>2s definition.

Additional imaging variables including clot length and collateral flow were assessed by XH. The methods are as detailed in Chapter 6. The clot length was measured with a method modified from Riedel et al[125]; and collateral status was assessed using a method modified from Miteff et al[81]. Analyses were performed twice at separate occasions to minimise the risk of error. An average of the continuous variable was taken as the final reading, whereas a third reading was carried out on

another occasion for the discrepancy in the categorical reading. The result demonstrated on the two agreed readings was used as the final reading.

9.2.4. Outcome measures

We defined the primary outcome as the percentage of penumbra salvaged at 24 hours. Secondary outcomes were infarct growth, final infarct volumes, and the rate of recanalisation at 24 hours. We also assessed the length of clot that was recanalised in the two groups. Recanalisation was defined as TIMI2-3[225,371] on a 24 hour CTA. Good collateral status was defined as collateral flow of good or moderate[431]. The final infarct volume was defined as the total infarct volume measured on NCCT or DWI at 24 hours. The calculations of the percentage of penumbra salvage and infarct growth are as described in Chapter 3.

9.2.5. Statistical analysis

The analysis was performed on the basis of intention-to-treat. All randomised patients were included in the analysis. Traditional meta-analysis was performed first using the Dersimonian-Laird test. The heterogeneity of the studies was evaluated with Cochran Q. Inconsistency and bias could not be assessed as there were too few strata.

The individual patient data pooled analysis was subsequently carried out with a random effect generalised linear model for continuous variables, and with a random effect binary model for categorical variables. The random effect model was used to account for the heterogeneity of the two studies. The significance level was set at 0.05. Many confounding factors exist that could potentially influence the results. The essential imaging ones include the ischaemic core, the baseline occlusion site, collateral status, and clot length, in addition to the clinical variables such as age, OTT and the baseline NIHSS score. In this analysis, the study itself is also a potential significant confounder due to the obvious difference in the study populations. Considering the small sample size, a univariate regression model was used to identify the potential imaging confounders. The variable was considered to be a confounder if $p<0.4$ in the univariate regression model. The final confounders included in the random effect model were age, OTT, the baseline NIHSS score, and the study. Additionally, the ischaemic core, and collaterals were adjusted for in analyses of the percentage of penumbral salvage, infarct growth, and final infarct volumes; baseline occlusion site in recanalisation; ischaemic core and baseline occlusion site in clot length. Outcomes were expressed as the effect size for continuous variables, the odds ratio for categorical variables, and their 95% confidence intervals.

Group level meta-analysis was performed with StatsDirect 2.8. Individual patient data meta-analysis was performed with SAS 9.3.

9.3. Results

9.3.1. Comparative analysis of the studies

The extent of the differences in patient selection and baseline characteristics were discussed in detail in the last chapter, especially the significant difference in the baseline penumbra volumes in the two studies.

No significant difference in clot length and collateral status at baseline was found between the two studies (Table 9-1).

Table 9-1. The difference in clot length and collateral status in the two studies according to study groups

	Clot Length n (%)∗ mean (SD) mm			Good Collaterals n (%)∗, ***n (%)***		
	Parsons et al	ATTEST	p value	Parsons et al	ATTEST	p value
TNK 0.1mg/kg	9 (36%) 10 (8)			10 (40%) ***7 (28%)***		
TNK 0.25mg/kg	10 (40%) 13 (13)	26 (48%) 13 (11)	0.95	12 (48%) ***10 (40%)***	37 (69%) ***29 (54%)***	0.53
Alteplase	7 (28%) 8 (8)	21 (39%) 14 (11)	0.1	8 (32%) ***8 (32%)***	36 (67%) ***27 (50%)***	0.18
∗ The number (percentage) of imaging that can be evaluated for clot length or collateral status; ***n (%)*** the number (percentage) of patients with good collaterals; p value for continuous variables was derived with independent t test, and Chi-square test for categorical variables.						

9.3.2. Group level data

Since the TNK 0.1 mg/kg dose was only examined in one study, it was not included in the group level meta-analysis. The results for TNK 0.25 mg/kg versus alteplase were shown in Table 9-2 and Figure 9-1 to 9-4. The primary outcome did not differ in the two groups. There was a significant difference in the final infarct volume at 24 hours between the two treatment groups, with a standardised mean difference of -0.4 (95%CI -0.8- -0.1, p=0.02). The heterogeneity test for final infarct volumes was not significant (p=0.25). No difference was found in other outcomes.

Table 9-2. Group level analysis for TNK 0.25 mg/kg vs alteplase

	n		Standardised Mean difference	95%CI
	TNK	Alteplase		
Percent of penumbra salvaged (%)				
Parsons et al	25/25	24/25	1	0.4, 1.6
ATTEST	36/54	36/54	0.2	-0.3, 0.7
Total	61/79	60/79	0.6	-0.2, 1.3
p=0.15 Cochran Q p=0.042				
Final Infarct Volume (mL)				
Parsons et al	25/25	24/25	-0.6	-1, -0.1
ATTEST	51/54	46/54	-0.3	-0.7, -0.1
Total	76/79	70/79	-0.4	-0.8, -0.1
p=0.022 Cochran Q: p=0.25				
Infarct Growth (mL)				
Parsons et al	25/25	24/25	-0.7	-1.3, -0.1
ATTEST	51/54	46/54	-0.1	-0.5, 0.3
Total	76/79	70/79	-0.4	-1, 0.2
p=0.17 Cochran Q: p=0.12				
Recanalisation				
Parsons et al	24/25	15/25	11.2	1.2, 525.2
ATTEST	22/54	25/54	0.8	0.2, 2.6
Total	46/79	40/79	2.5	0.2, 34.3
p=0.49 Cochran Q: p=0.03				

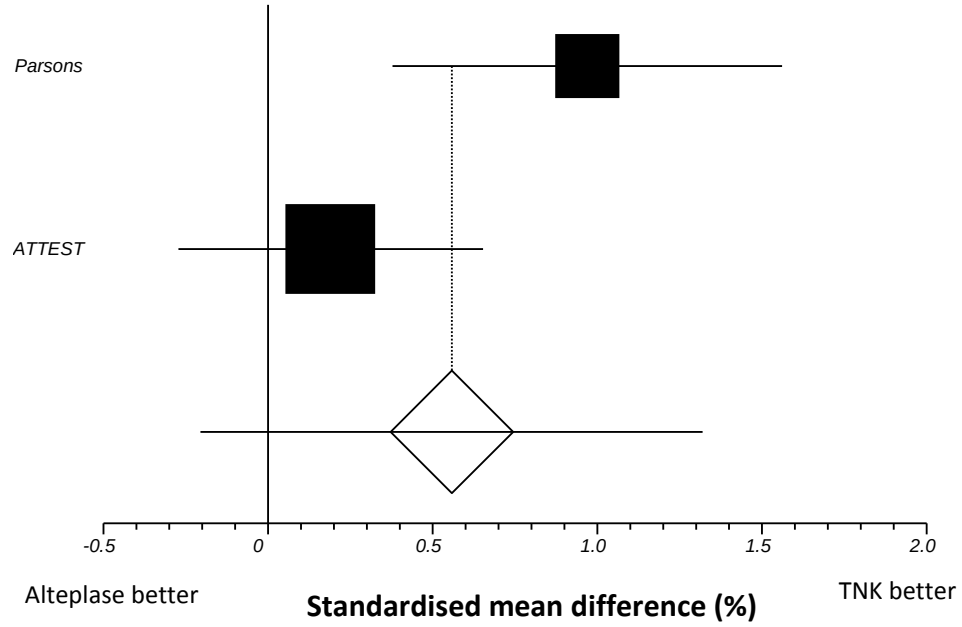


Figure 9-1. Forest plot comparing the percentage penumbra salvaged (random effect) between TNK 0.25 mg/kg and alteplase treated patients

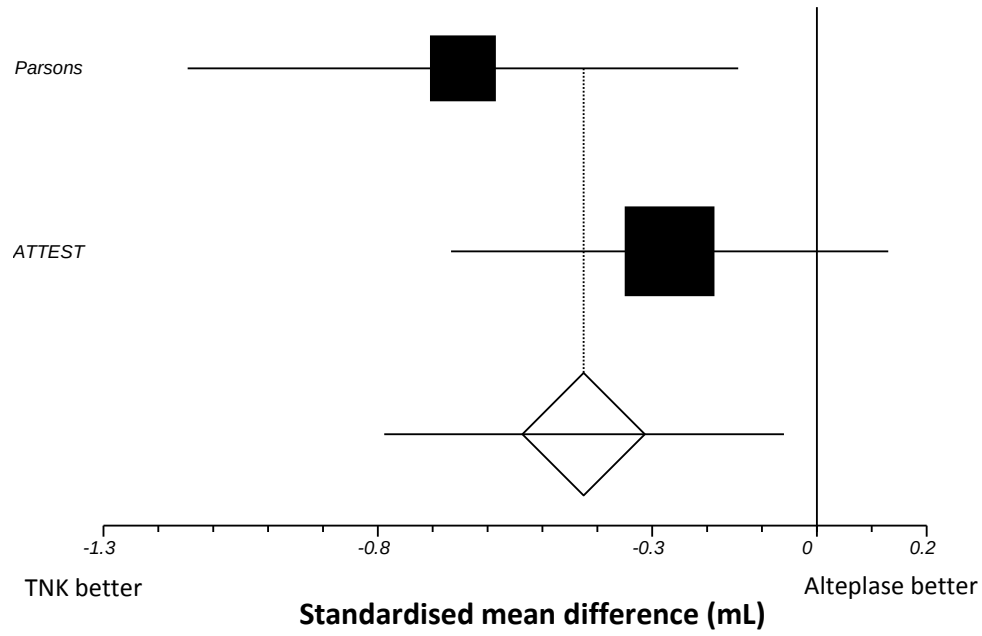


Figure 9-2. Forest plot for final infarct volume at 24 hours (random effect) between TNK 0.25 mg/kg and alteplase treated patients

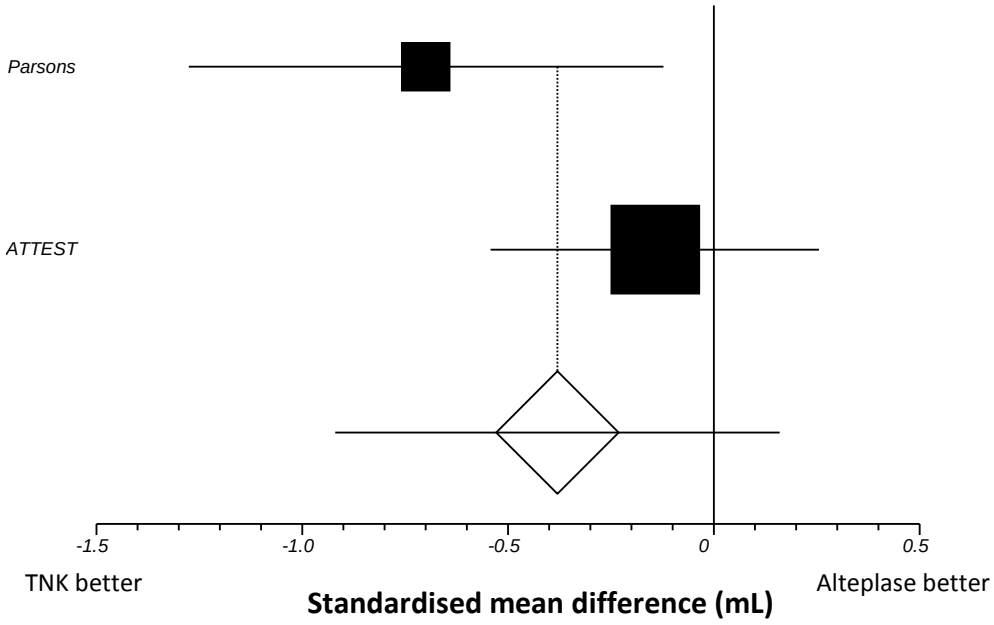


Figure 9-3. Forest plot for infarct growth at 24 hours (random effect) between TNK 0.25 mg/kg and alteplase treated patients

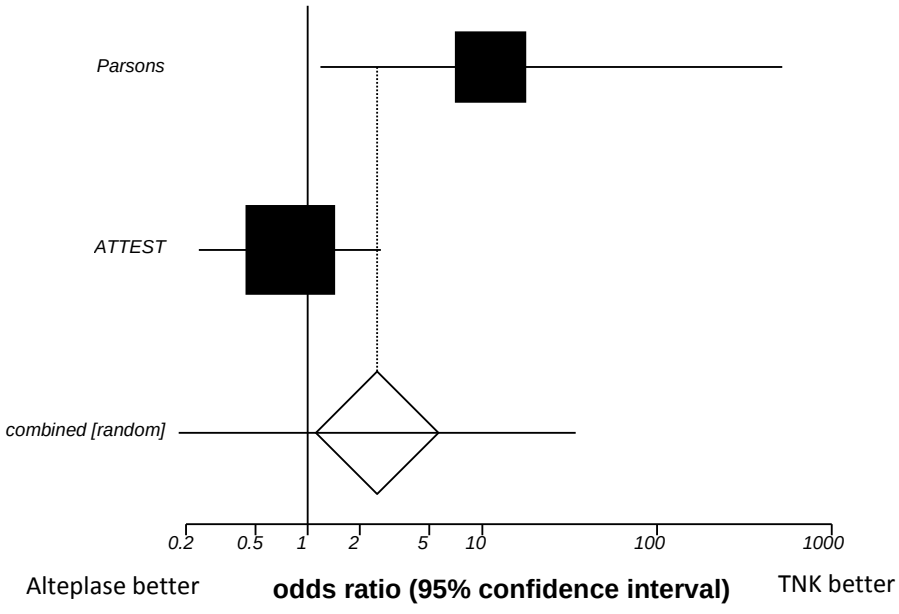


Figure 9-4. Forest plot for recanalisation at 24 hours (random effect) between TNK 0.25 mg/kg and alteplase treated patients

9.3.3. Individual patient data analysis (Figure 9-5)

Patients who had TNK 0.25 mg/kg had a mean 19% (95%CI -11.8, 49.8, p=0.43) more penumbra salvaged at 24 hours compared with alteplase treated patients, but without statistical significance. No difference was found in other outcomes in these two groups of patients.

Between TNK 0.1 mg/kg- and alteplase-treated patients, all outcomes were similar between the two groups.

Between patients who received TNK 0.25 mg/kg and those who had TNK 0.1mg/kg, no difference was found in any outcomes.

9.3.4. Mean difference in clot length in recanalised patients

Within the total 179 patients, 104 achieved recanalisation at 24 hours, of whom 48 had a visible clot on NCCT that could be measured (TNK 0.1 mg/kg 5, TNK 0.25 mg/kg 26, alteplase 17). We assessed the mean difference in the length of clot that was recanalised at 24 hours between the TNK 0.25 mg/kg and alteplase groups using the random effect generalised linear model. A non-significant mean difference of 4.2 mm (95%CI -2.8, 11.2, p=0.25) was demonstrated.

Clot length and recanalisation status was available for 65 out of the 179 patients. In a univariate binary logistic regression, clot length more than 8mm was inversely correlated with recanalisation (p=0.013) (Figure 9-6). When adjusted with confounders age, the baseline NIHSS score, OTT, the study, treatment, baseline core and occlusion sites, the statistical significance remained (p=0.018).

9.4. Discussion

In this meta-analysis of imaging outcomes from two thrombolysis studies comparing tenecteplase and alteplase in the treatment of acute ischaemic stroke, we found a significant reduction in final infarct volumes at 24 hours using group level data. However, in the pooled analysis, when adjusted for confounders including study, age, the baseline NIHSS score, OTT, ischaemic core volume and collaterals, significance could no longer be demonstrated. Using group level data or IPD analysis, the primary outcome of percentage of penumbra salvaged at 24 hours did not differ; neither did other outcomes including infarct growth and recanalisation.

Discussion of the heterogeneity among the three RCTs of tenecteplase was presented in Chapter 8. Although differences were found in the baseline NIHSS score, and in OTT across the three studies, the heterogeneity test did not show any significant differences. Between the ATTEST study and the

Australian study, additional differences in penumbra size and baseline vessel occlusion sites were evident. When analysing imaging outcomes, significant heterogeneity was demonstrated by the Cochran Q test on two outcomes (percentage of penumbra salvaged and recanalisation). When we performed the pooled analysis, the difference in the two studies was taken into account by adjusting the cofounding variable study, but the results still need to be interpreted carefully due to the differences in the two studies.

In addition, we explored other imaging characteristics including clot length and collateral status. No significant difference was found between the two studies. However, the overall rate of hyperdense vessel sign was lower in the Australian study (35% vs 45%). The presence of the hyperdense vessel sign is associated with poor clinical outcomes[432]. The lower prevalence of hyperdense vessels in the Australian study, the significantly larger penumbra volumes and the absence of carotid occlusion may account for the better outcomes in that study compared to the ATTEST study.

Tenecteplase is more fibrin selective than alteplase, which should translate into better lysis ability. We did not find any difference in the mean length of clot that was lysed between tenecteplase 0.25 mg/kg and alteplase in a small group of patients who achieved recanalisation and had a measurable clot length on NCCT. This result flows from a secondary analysis in a small heterogenic group of patients. A large sample is required to examine this further.

In the secondary analysis of the ATTEST study, we did not find any association between clot length and the recanalisation rate. In this larger sample, we demonstrated that those with a clot longer than 8mm on NCCT have significantly less likelihood of recanalisation by IV thrombolysis. This finding is consistent with the results from Riedel's group[163].

The limitations of this study include the small sample size with a total of 179 patients. Furthermore, since the analysis was performed for imaging outcomes, there is sizable missing data due to the group of patients who had no perfusion lesion (19%) or no occlusion on the baseline CTA (24%) which has had an impact on the results of the intention-to-treat analysis. This group of patients was mainly from the ATTEST study. The small sample also limited our ability to adjust for essential cofounding imaging variables.

The other important limitation is, as discussed in detail above, the heterogeneity of the two studies included. Unfortunately, the design of the few ongoing phase III studies comparing tenecteplase and alteplase in the treatment of acute ischaemic stroke remain heterogenetic. NOR-TEST and TASTE have different patient selection methods and use different tenecteplase doses, partly as the result of

the uncertainty arising from the previous dose selection studies[194,325,327]. This also reflects the evolution in acute stroke research, and the wish to extend the current treatment window to patients with wake-up stroke or to those who present late. Future study design should keep this in mind, and collaborate in advance for pre-planned meta-analysis.

9.5. Conclusions

In the pooled analysis, there was no difference in the percentage of penumbra salvaged or in other imaging outcomes between tenecteplase- and alteplase-treated patients in the two phase II studies which applied advance imaging. This analysis was limited by the small sample size, missing data and significant heterogeneity in the two studies. When designing future phase III studies, this should be taken into account or meta-analysis should be pre-planned to achieve better quality evidence.

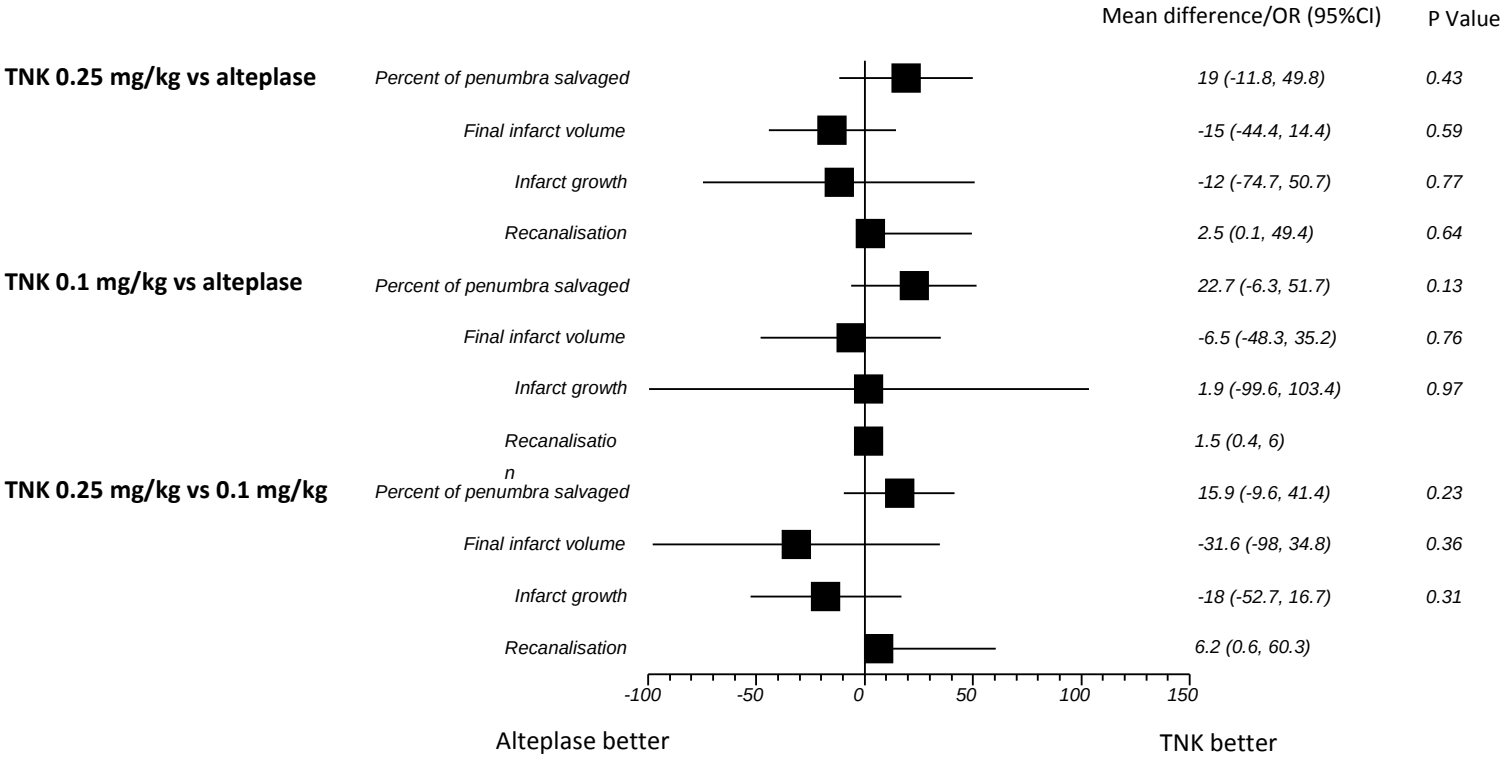


Figure 9-5. IPD results for all outcomes (mean difference [95%CI] for continuous variables, and OR [95%CI] for categorical variables)

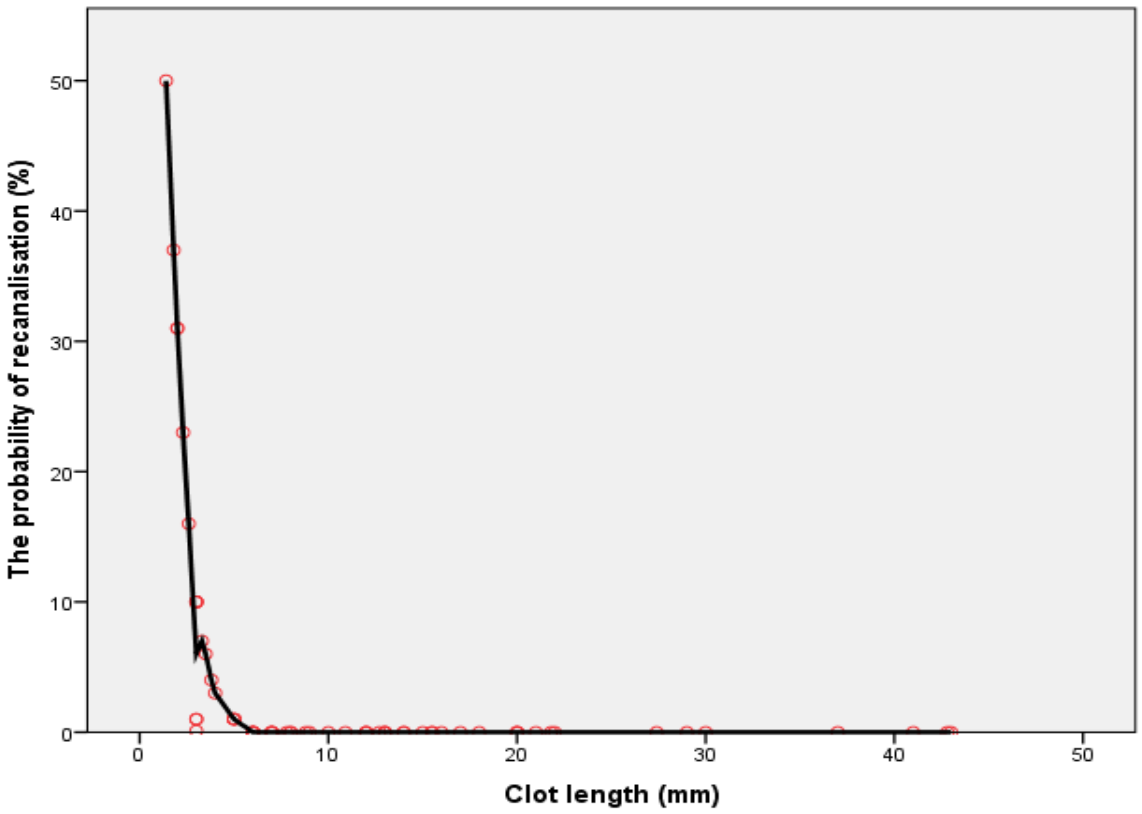


Figure 9-6. Sigmoid plot of the probability of recanalisation depending on clot length

Chapter 10 Conclusions

Twenty years on since the milestone NINDS study[280] was published, the recent success of intra-arterial studies[197,198,206-208] has been another significant step in the development of reperfusion therapy in hyperacute stroke. However, endovascular intervention only benefits a small group of patients with the right imaging profile. Meanwhile, the remaining majority of acute ischaemic stroke patients still only have the option of intravenous alteplase, which is advantageous in approximately one-third of the population[433] if given within three hours. The treatment is associated with 2-3% risk of symptomatic ICH[315,318]. Looking for alternative thrombolytic agent with better efficacy and safety profiles is essential in improving the outcomes in those who are not suitable for, or who do not have access to IA therapy.

Alteplase has a short plasma half-life of 4-9 minutes[270,274,275]. An hour-long infusion is required following the initial bolus to maintain the plasma level in acute ischaemic stroke thrombolysis. In practice, delay between the bolus and the subsequent infusion is common, which may affect its efficacy.

Tenecteplase[283], an improved tPA with better fibrin specificity, slower plasma clearance, resistance to PAI-1, and without a procoagulant effect, has been examined in a small number of phase II studies in the treatment of acute ischaemic strokes. In a single centre phase II randomised controlled study (ATTEST) that compared tenecteplase 0.25mg/kg and standard alteplase in acute ischaemic stroke thrombolysis using imaging biomarkers as endpoints, we demonstrated that tenecteplase 0.25 mg/kg has a trend toward a lower risk of ICH compared to alteplase, with no difference in the percentage of penumbra salvaged at 24 hours post thrombolysis or in clinical outcomes at 90 days.

In a subset of patients from the ATTEST study, tenecteplase 0.25 mg/kg was shown to give rise to minimal disruption to the coagulation and fibrinolytic systems, whereas alteplase caused significant hypofibrinogenaemia. This is consistent with the finding from the main study of a possibly reduced risk of ICH with tenecteplase 0.25mg/kg.

Using advanced imaging to identify candidates who are likely to respond to reperfusion therapy has become increasingly popular among investigators. However, studies using this strategy have produced mixed results[184,194]. Much uncertainty around methodology for analysis persists, especially in perfusion imaging, and this has hampered the further application of this method. Criteria for optimal imaging characteristics differ in different centres. In further analysis of the imaging data from the ATTEST study patients, we found that using different tissue definitions for penumbra and core significantly altered the mismatch ratio. This highlighted one of the potential problems that multicentre imaging selection studies face where there may be no consensus as to tissue definition, and where post processing software may not be in place.

The uncertainty in imaging selection study was further evidenced by analysing our subgroup of patients who fulfilled the selection criteria applied in Parsons et al.[194]; no difference was demonstrated in patients who received tenecteplase or alteplase.

To date, including the ATTEST study, three phase II randomised controlled studies which have compared tenecteplase and alteplase have been completed. The methodologies of the three studies are different, which resulted in significant differences in baseline patient characteristics including the baseline NIHSS score and OTT. In the two studies which acquired advance imaging, further differences in imaging characteristics were demonstrated. However, the individual patient data meta-analysis suggested that tenecteplase gave rise to potentially better efficacy, especially with the dose 0.25mg/kg, with a similar SICH risk. In the pooled analysis of the imaging data from the ATTEST study and Parsons et al[194], no differences in the percentage of penumbra salvaged or recanalisation rates at 24 hours were found.

Based on the above information, a phase III study to compare tenecteplase and alteplase in the treatment of acute ischaemic strokes is warranted. The design of the future phase III study should include all patients who are suitable for thrombolysis based on current guidelines, in order for the generalisation of the study results. Since it will be a multicentre study, a NCCT based study is more practical and will avoid some potential problems in using advance imaging for recruitment as discussed. Similarly, the primary outcome should be clinical outcomes, such as mRS at 90 days. However, bias is still possible as patients presenting with severe stroke are likely to undergo endovascular intervention in some centres rather than receiving intravenous thrombolysis. Efforts should be made to select participating centres carefully, in order to minimise this bias.

Appendices

A.1. National Institute of Health Stroke Scale

Instructions	Scale definition
1a. Level of consciousness	0=Alert 1=Not alert, but arousable with minimal stimulation 2=Not alert, requires repeated stimulation to attend 3=Coma
1b. LOC questions	0=Answers both questions correctly 1=Answers one question correctly 2=Answers neither question correctly
1c. LOC commands	0=Performs both tasks correctly 1=Performs one task correctly 2=Performs neither task correctly
2. Best gaze	0=Normal 1=Partial gaze palsy 2=Forced deviation
3. Visualfield	0=No visual loss 1=Partial hemianopia 2=Complete hemianopia 3=Bilateral hemianopia
4. Facial Palsy	0=Normal symmetrical movement 1=Minor paralysis (asymmetry on smiling) 2=Partial paralysis (total or near total of lower face) 3=Complete paralysis of one or both sides
5. Motor arm 5a. Left arm 5b. Right arm	0=Normal 1=Drift 2=Some effort against gravity 3=No effort against gravity 4=No movement UN=Untestable
6. Motor leg 6a. Left leg 6b. Right leg	0=Normal 1=Drift 2=Some effort against gravity 3=No effort against gravity 4=No movement UN=Untestable
7. Limb ataxia	0=Normal 1=Present in one limb 2=Present in two limbs UN=Untestable
8. Sensory	0=Normal

	1=Mild to moderate decrease in sensation 2=Severe to total sensory loss
9. Best language	0=No aphasia 1=Mild to moderate aphasia 2=Severe aphasia 3=Mute, global aphasia
10. Dysarthria	0=Normal articulation 1=Mild to moderate slurring of words 2=Near unintelligible or unable to speak UN=Intubated or other physical barrier
11. Extinction and inattention	0=Normal 1=Inattention or extinction to bilateral simultaneous stimulation in one of the sensory modality 2=Severe hemi-inattention to more than one modality

A.2. Consent forms

Alteplase-Tenecteplase Trial Evaluation for Stroke Thrombolysis (ATTEST) – Pilot Phase

CONSENT FORM

Please initial box

I confirm that I have read and understand the information sheet dated 2011 (version 1) for the above study and have had the opportunity to ask questions	☐
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
I understand that sections of any of my medical notes may be looked at by local researchers or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.	☐
I understand that anonymous data from the study, including CT scans, may be shared with other researchers. I give permission for data to be used in this way.	☐
I give my permission for the study doctor to contact my GP to inform him/her of my participation in this study	☐
I agree to take part in the above study.	☐

_____	_____	_____
Name of Patient (Print name)	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher (Print name)	Date	Signature

Alteplase-Tenecteplase Trial Evaluation for Stroke Thrombolysis (ATTEST) – Pilot Phase

FORM OF ASSENT for RELATIVES

Chapter 11 Please initial box

I confirm that I have read and understand the information sheet dated 2011 (version 1) for the above study and have had the opportunity to ask questions	☐
I understand that my relative's participation is voluntary and that I am free to withdraw them at any time, without giving any reason, without their medical care or legal rights being affected.	☐
I understand that sections of any of my relative's medical notes may be looked at by local researchers or from regulatory authorities where it is relevant to their taking part in research. I give permission for these individuals to have access to their records.	☐
I understand that anonymous data from the study, including CT scans, may be shared with the other researchers. I give permission for my relative's data to be used in this way.	☐
I give my permission for the study doctor to contact my relative's GP to inform him/her of their participation in this study	☐
I agree that my relative will take part in the above study.	☐

I am the nearest relative/ welfare guardian of the patient named below and I can confirm that there is neither a nearer relative or welfare guardian to the same said patient.

_____	_____	
Name of Patient (Print name)	Relationship to Patient	
_____	_____	_____
Name of Relative (Print name)	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher (Print name)	Date	Signature

Alteplase-Tenecteplase Trial Evaluation for Stroke Thrombolysis (ATTEST) – Pilot Phase

FORM OF CONSENT to Continuing Participation

Please initial box

I confirm that I have read and understand the information sheet dated 2011 (version 1) for the above study and have had the opportunity to ask questions	☐
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
I understand that sections of any of my medical notes may be looked at by local researchers or from regulatory authorities where it is relevant to this research study. I give permission for these individuals to have access to my records.	☐
I understand that data from the study, including scans, may be shared with the international MR Stroke Collaborative Group researchers. I give permission for my data to be used in this way.	☐
I give my permission for data already collected for this study to be used for all study purposes.	☐
I agree to my continued involvement in the above study.	☐

_____	_____	_____
Name of Patient (Print name)	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher (Print name)	Date	Signature

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