OPTIMISING OUTCOMES OF INTRAVENOUS THROMBOLYSIS IN ACUTE ISCHAEMIC STROKE

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Abstract

Intravenous thrombolysis is an established treatment for selected patients with acute ischaemic stroke. Outcome after intravenous thrombolysis is dependent upon a number of modifiable and non-modifiable factors. This thesis explored three themes in addressing how outcome after intravenous thrombolysis can be optimised:

- The potential for tenecteplase to be a superior thrombolytic drug to alteplase

- Improved patient selection through CT perfusion studies identifying groups at risk of poor outcome or greater benefit

- The effect of patient heterogeneity on outcomes in trials of acute ischaemic stroke

Tenecteplase is emerging as an alternative thrombolytic to alteplase in acute ischaemic stroke. Tenecteplase was studied in a prospective imaging substudy of a large multi-centre randomised controlled trial of tenecteplase versus alteplase in patients conventionally eligible for intravenous thrombolysis. Tenecteplase was found, in patients treated with intravenous thrombolysis within 4.5 hours of ischaemic stroke onset, to have similar outcomes to alteplase in efficacy and safety in cases of large vessel occlusion; similar efficacy in those with mismatch of CT perfusion imaging; similar rates of early recanalisation; and similar degree of penumbral salvage. A meta-analysis of trials comparing tenecteplase and alteplase found superiority of tenecteplase in functional outcomes and similar safety outcomes.

Tenecteplase was also studied in retrospective analyses of a combined cohort from two previous randomised controlled trials comparing alteplase and tenecteplase. CT perfusion studies examined the effect of varying rates of progression in ischaemia after the onset of acute ischaemic stroke; and differences in blood brain barrier permeability and how this relates to haemorrhagic transformation. Ischaemic progression rate was found to be a predictor of follow up infarct volume and infarct growth and a non-significant

trend towards lower follow up infarct volumes in fast progressors treated with tenecteplase compared to alteplase was found. Measures of blood brain barrier permeability were found to be predictive of haemorrhagic transformation and tenecteplase was associated with higher blood brain barrier permeability in cases of haemorrhagic transformation than alteplase.

Thirdly, the effect of heterogeneity in participants in clinical trials of stroke and its effect on outcomes was studied. Follow up infarct volume was found to mediate a larger proportion of the relation between clinical outcomes and recanalisation than previously suggested. Heterogeneity in ischaemic progression rate of participants in trials of intravenous thrombolysis and mechanical thrombectomy was explored to highlight the effect of heterogeneity on trial outcome.

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Acknowledgement

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Author's Declaration

I declare that that this thesis 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.

Name: Ammad Mahmood

Signature:

Definitions/Abbreviations

ADC – apparent diffusion coefficient

AIF – arterial input function

AIS – acute ischaemic stroke

AF – atrial fibrillation

AMI – acute myocardial infarction

ASL – arterial spin labelling

ASPECTS - Alberta stroke programme early CT score

ATP – adenosine triphosphate

BBB – blood brain barrier

BI – Barthel index

CBF – cerebral blood flow

CBV – cerebral blood volume

CMRO2 - cerebral metabolic rate of oxygen

CPP – cerebral perfusion pressure

CT – computed tomography

CTA – computed tomography angiogram

CTP – computed tomography perfusion

DNA – deoxyribonucleic acid

DT – delay time

DSC - dynamic susceptibility contrast

DWI – diffusion weighted imaging

E – extraction fraction

EEG - electroencephalogram

EVT – endovascular thrombectomy

FIV – follow up infarct volume

FLAIR - fluid attenuated inversion recovery

GPVI – glycoprotein VI

GRE – gradient recalled echo

HERMES - Highly Effective Reperfusion Evaluated in Multiple Endovascular Stroke Trials

HIR – hypoperfusion intensity ratio

HT – haemorrhagic transformation

ICA – internal carotid artery

ICH – intracerebral haemorrhage

IG – infarct growth

IPR – ischaemic progression rate

IQR – inter quartile range

IVT - intravenous thrombolysis

K_{trans} – transfer constant

LACI – lacunar infarct

LVO – large vessel occlusion

mITT – modified intention to treat

MeVO – medium vessel occlusion

M1 – first branch of the middle cerebral artery

M2 – second branch of the middle cerebral artery

MRA – magnetic resonance angiography

MRI – magnetic resonance imaging

mRS – modified Rankin scale

MT – mechanical thrombectomy

MTT – mean transit time

MCA – middle cerebral artery

NBO – normobaric oxygen

NCCT – non-contrast computed tomography

NET - Neutrophil extracellular traps

NIHSS - National Institutes of Health Stroke Scale

OEF – oxygen extraction fraction

OR – odds ratio

PACI – partial anterior circulation infarct

PCI - percutaneous coronary intervention

PET - positron emission tomography

PFO – patent foramen ovale

PH – parenchymal haemorrhage

POCI – posterior circulation infarct

PROBE - prospective randomised open-label blinded endpoint

PS - permeability-surface area product

PWI – perfusion weighted imaging

RAPID – ‘RApid processing of PerfusIon and Diffusion’ software

RBC – red blood cell

RCT – randomised controlled trial

RFA – Rankin focused assessment

ROI – region of interest

rt-PA – recombinant tissue plasminogen activator

SAH – subarachnoid haemorrhage

sICH – symptomatic intracranial haemorrhage

SSS – Scandinavian stroke scale

SWI – susceptibility weighted imaging

TACI – total anterior circulation infarct

TAFI - thrombin-activated fibrinolysis inhibitors

TIA – transient ischaemic attack

TICI - Thrombolysis in Cerebral Infarction score

TIMI - Thrombolysis in Myocardial Infarction score

Tmax – time to maximum

TNK - tenecteplase

TOAST - Trial of ORG 10172 in Acute Stroke Treatment (TOAST)

VOF – venous output function

VPS – volume of penumbra salvaged

VWF – von Willebrand factor

1 Introduction

Stroke is a clinical syndrome of presumed vascular origin involving rapidly developing signs of focal or global cerebral dysfunction (1). Symptoms lasting less than 24 hours are labelled transient ischaemic attack (TIA). Stroke subtypes include:

- Ischaemic stroke – caused by focal cerebral, spinal or retinal cell death due to infarction following vascular occlusion or stenosis

- Haemorrhagic stroke – due to focal collection of blood in the brain parenchyma or ventricular system (intracerebral haemorrhage (ICH)) or bleeding into the arachnoid space that is atraumatic (subarachnoid haemorrhage (SAH)), though the clinical presentation of SAH differs to that of ischaemic stroke or intracerebral haemorrhage

1.1 Epidemiology

Stroke was the second leading cause of death and the third leading cause of death and disability globally in 2019, with an incidence of 12.2 million (11.0-13.6) and prevalence of 101 million (93.2-111). Globally ischaemic stroke, ICH and SAH accounted for 62.4%, 27.9% and 9.7% of strokes respectively, though rates of ICH were lower in higher income countries compared with others (15.8% vs 29.5%). There is considerable disparity in the global burden of stroke with mortality 3.6 (3.5-3.8) times higher and stroke related disability adjusted life years (DALY) 3.7 (3.5-3.9) times higher in low income countries compared to high income countries. The leading modifiable risk factors for stroke in 2019 were high systolic blood pressure, high body mass index, high fasting plasma glucose, ambient particulate matter pollution and smoking (2).

1.2 Classification of ischaemic stroke

Several systems classify stroke into subtypes using either suspected aetiology or clinical presentation.

1.2.1 Oxfordshire Community Stroke Project classification

The Oxfordshire Community Stroke Project (OCSP) (3) classified stroke presentations into 4 clinical subtypes – lacunar infarcts (LACI); total anterior circulation infarcts (TACI) with cortical and subcortical involvement; partial anterior circulation infarcts (PACI) with predominantly cortical infarcts; and posterior circulation infarcts (POCI) of the vertebrobasilar territories. The clinical presentations are summarised in table 1-1. This classification system proposed to distinguish types of stroke with differing natural histories – patients in the TACI group had high morbidity and mortality; PACI was associated with lesser mortality but a higher risk of early recurrent stroke; LACI patients were seen to have significant post stroke disability despite the commonly held view that lacunar strokes were mild; and POCI patients had the highest chance of good functional outcome but were at high risk of recurrent stroke in the first year. Imaging features were not considered in this classification.

1.2.2 TOAST classification

The Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification separated stroke subtypes into 5 categories depending on suspected aetiology – large artery atherosclerosis, cardioembolism, small artery occlusion, other determined cause or undetermined cause (4).

1.2.2.1 Large Artery Atherosclerosis

Large artery disease may cause ischaemia more commonly through embolism (artery-to-artery embolism) or less frequently by reduction of blood flow (5). It accounts for around 30% of all ischaemic stroke. Emboli from large artery disease are usually thrombus or platelet aggregates formed on atherosclerotic plaques. The most common site of symptomatic atherosclerosis is the bifurcation of the common carotid artery into the external and internal carotid arteries. Carotid or vertebral artery occlusion may occur silently without symptoms due to good collateral circulation from the Circle of Willis. While extracranial arterial disease is more common in Caucasian populations, intracranial atherosclerosis is more common in Asian and African American populations.

Lacunar infarct (LACI)	One of the major recognized lacunar syndromes: pure motor, pure sensory, sensorimotor, ataxic hemiparesis, and dysarthria-clumsy hand syndrome
Total anterior circulation infarct (TACI)	Ischemia in both the deep and superficial territories of the middle cerebral artery causing higher cerebral dysfunction (dysphasia, dyscalculia, visuospatial disorder; or homonymous visual field defect); and ipsilateral motor and/or sensory deficit of at least two areas of the face, arm, and leg
Partial anterior circulation infarct (PACI)	Clinical syndrome that includes only two of the three components of the TACI syndrome, with higher cerebral dysfunction alone, or with more restricted sensorimotor deficit than those classified as LACI
Posterior circulation infarct (POCI)	Clinical syndrome that includes ipsilateral cranial nerve palsy with contralateral motor and/or sensory deficit; bilateral motor and/or sensory deficit; disorder of conjugate eye movement; cerebellar dysfunction without ipsilateral long-tract deficit (i.e., ataxic hemiparesis); or isolated homonymous visual field defect

Table 1-1 Clinical presentations of stroke subtypes as per Oxfordshire Community Stroke Project (6)

1.2.2.2 Cardioembolic stroke

Cardioembolic disease accounts for 25-35% of ischaemic strokes (5). The most common cardioembolic source is non-valvular atrial fibrillation (AF), particularly with increasing age – the prevalence of AF in people aged >65 is 5-6%, rising to 12% in those aged >75. Presence of non-valvular AF increases the risk of ischaemic stroke 5-fold however this varies amongst individuals dependent on the presence of further risk factors such as age, female sex, hypertension, previous stroke or TIA, vascular disease and congestive heart failure – these can be assessed for using the CHADS-VASC score to estimate individual risk and guide treatment decisions (7). Other high risk cardioembolic sources include prosthetic heart valves, endocarditis, and intracardiac thrombus (due to recent anterior myocardial infarction or dilated cardiomyopathy).

Low risk cardioembolic sources include patent foramen ovale (PFO) (5). The exact mechanism by which PFO causes stroke is unclear and evidence comes from statistical associations – the prevalence of PFO in young ischaemic stroke patients is twice that of the general population (40% vs 20%). Stroke mechanism may be through paradoxical embolism involving venous or pulmonary embolism and a right-to-left shunt. Long term risk of recurrent stroke from PFO is 1.3% and reduced by half after endovascular occlusion of PFO (8).

1.2.2.3 Small vessel disease

Lacunar infarcts are small (<15mm diameter) subcortical infarcts resulting from occlusion of a single penetrating artery. They represent around 25% of ischaemic stroke (5). They are usually located in the basal ganglia, thalamus, internal capsule, corona radiata and brainstem. Lacunes form part of the spectrum of radiological lesions seen on MRI including white matter hyperintensities, perivascular spaces, cerebral microbleeds and superficial siderosis that constitute small vessel disease (9).

1.3 Pathophysiology of ischaemic stroke and the ischaemic penumbra

Ischaemic stroke results from a critical reduction of cerebral blood flow beyond a critical duration caused by one of the mechanisms highlighted above (10). This results in ischaemic infarction of brain tissue; the location, morphology and extent of this depending upon the vessel occluded, the mechanism of occlusion and the quality of collateral supply to the affected area of brain. Single vessel occlusions produce characteristic patterns of territorial infarction, whilst stenotic lesions producing low flow in distal areas of arterial supply and failure to wash out micro-emboli can result in 'border-zone infarction' (11). Haemorrhagic transformation can occur in early infarction when bleeding occurs from damaged blood vessels in the area of infarcted tissue, or due to reperfusion of severely ischaemic tissue – this can range from small areas of petechial haemorrhage to large areas of haemorrhage.

The fate of ischaemic brain tissue depends upon the severity and duration of reduced blood flow – tissue that is irreversibly damaged is termed 'ischaemic core' while the surrounding area at threat of infarction whilst remaining salvageable is termed 'ischaemic penumbra'. Cellular death in the ischaemic core occurs after a cascade of events detailed in 1.3.1. The conceptualisation, definitions and therapeutic targeting of the ischaemic penumbra is examined in 1.3.2.

1.3.1 The ischaemic cascade

The ischaemic cascade is a complex pathophysiological process involving multiple interconnected pathways activated by hypoxia and hypoglycaemia. Pathways involved include glutamate-mediated toxicity, calcium signalling, oxidative stress and inflammation involving peripheral immune cells migrating to the brain or the microglia and endothelium of the neurovascular unit (12). Some of these interlinked processes are summarised in figure 1-1.

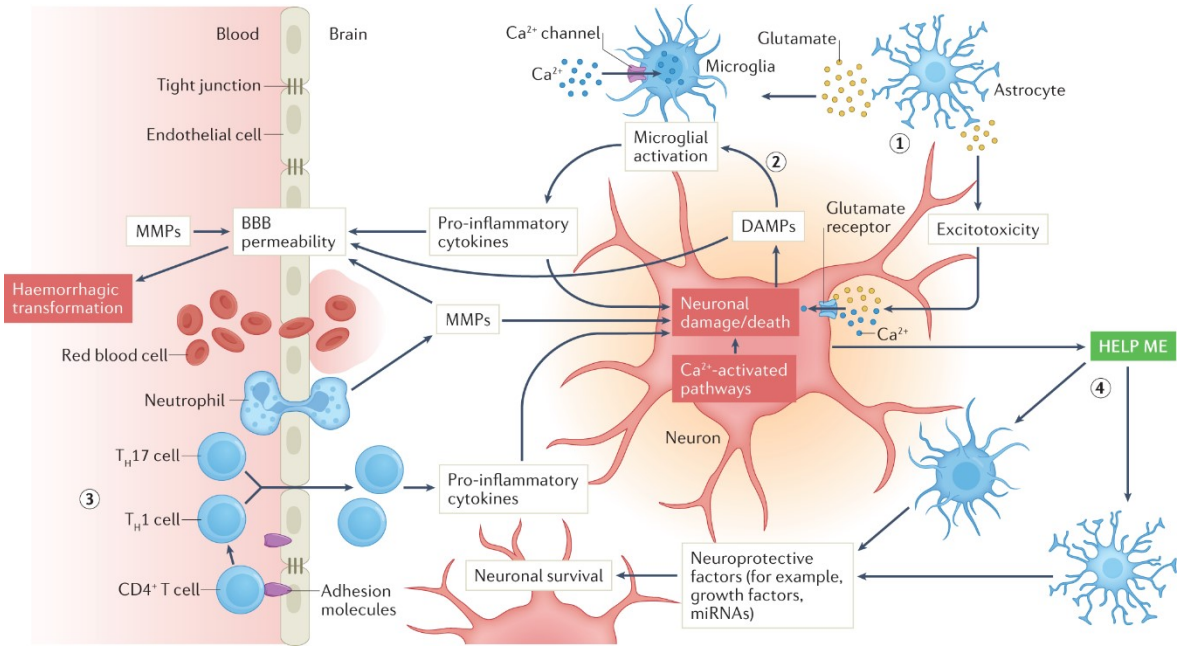


Figure 1-1 - Ischaemic cascade in acute ischaemic stroke

Reproduced from Fisher et al (12) with permission

(1) Hypoxia and hypoglycaemia lead to glutamate release from astrocytes

(2) Glutamate activates excitotoxic signalling in neurons leading to cell injury and death and activation of microglia

(3) Microglia release pro-inflammatory cytokines leading to neuronal damage, endothelial activation and blood brain barrier permeability allowing leucocyte migration into the ischaemic region

(4) Neurons release signalling to activate astrocytes and microglia

1.3.2 Emergence of the penumbra as a therapeutic target

Early descriptions of the penumbra in cerebral ischaemia by Astrup, Siesjo and Symon (13) were based on studies (14, 15) of cerebral blood flow in baboons and the relation of reduced blood flow to electroencephalogram (EEG), and to extracellular potassium concentration as a marker of pump failure and cellular damage. They demonstrated that somatosensory evoked potentials could be arrested if flow decreased below 15ml/100g/min in baboons, with other studies demonstrating a similar flow threshold in cats (16) and humans (17) causing electrical failure. Although it was initially presumed that electrical failure was due to pump failure causing efflux of potassium from the cell, Astrup and Symon's studies demonstrated that pump failure did not occur until a lower threshold of ~10ml/100g/min. They conceptualised that as blood flow decreased it first crossed a higher threshold causing electrical failure and then a lower threshold causing potassium efflux and failure of energy production in the cell. Tissue trapped in between these thresholds would be found in the areas surrounding the core of infarcted tissue following cerebral ischaemia and was termed the penumbra.

This depiction led to attempts to identify a therapeutic approach that could restore blood flow to the penumbra thereby restoring function to tissue which was not yet structurally damaged. Various approaches were investigated in animal models (18) including increasing blood pressure; reducing cerebrovascular resistance; reducing blood viscosity with fibrinolytics; inhibiting metabolic activity to limit oxygen use; or improving mitochondrial efficiency to optimise energy supply. None of these approaches was found to be beneficial, leading to the conclusion that the life span of the penumbra was short and it remained viable for a maximum of a few hours but typically less (19).

The cascade of events occurring as blood flow reduces was expanded beyond the two threshold model initially described (20). The first biochemical process to be disrupted is protein synthesis, followed first by an increase and shortly thereafter marked decrease in glucose utilisation. This coincides with accumulation of lactate and acidosis and as flow rates decrease further, reduction in adenosine triphosphate (ATP) and finally anoxic depolarisation causing efflux of potassium. Cell osmolality increases during this process causing

a shift of water from extracellular to intracellular compartments accounting for the changes seen on diffusion weighted MRI. Functional changes were seen to cascade in a similar manner with EEG suppression followed by disappearance of evoked potentials, reversible loss of function and finally irreversible damage. Flow thresholds at which these events occur have been studied across a number of species and models therefore exact estimates aren't possible, ranges of values are summarised in Figure 1-2.

Demonstration of the ischaemic penumbra in humans was undertaken by Baron et al using positron emission tomography (PET) imaging to measure the oxygen consumption of the brain. In 1981 (21) they described a case of 'misery perfusion syndrome' in a patient with an occluded internal carotid artery leading to recurrent TIAs treated by extracranial-intracranial bypass surgery. PET scanning pre-operatively showed decreased cerebral blood flow (CBF) and increased oxygen extraction fraction (OEF) which then normalised when bypass surgery was performed to reperfuse the affected brain. This demonstrated reversibility of metabolic changes taking place in an area of under perfused brain when CBF was restored. Further to this, the group undertook PET imaging within 5-18 hours of stroke onset in 30 consecutive patients with ischaemic stroke due to middle cerebral artery (MCA) occlusion with follow up PET and computed tomography (CT) imaging to determine the fate of the initial lesion (22). In the acute stage they identified areas of potential penumbra with markedly reduced CBF and increased OEF but essentially unchanged cerebral metabolic rate of oxygen (CMRO2) which was seen to be reduced in the predicted ischaemic core. At follow up these areas had reduced CMRO2 and were visible as infarcted tissue on follow up CT. They also noted considerable variability in the length of time that penumbra persisted, with some cases having penumbral tissue detected at 16 hours after onset whilst others had no penumbra within 5 hours. The authors highlighted the need to view the penumbra as a dynamic concept, changing in volume over time and offered the following as an updated definition of penumbra: "a severely ischaemic, functionally impaired tissue at risk of infarction, that will be saved if reperfused before it is irreversibly damaged, but that otherwise will be progressively recruited into the core until maximum infarct extension is reached". This marked a change in the understanding of the penumbra from a biochemical and functional concept to a definition based

around the penumbra as a therapeutic target (potentially definable by imaging) sparking further research into imaging of the penumbra and using imaging selected patients for clinical trials of acute stroke therapies.

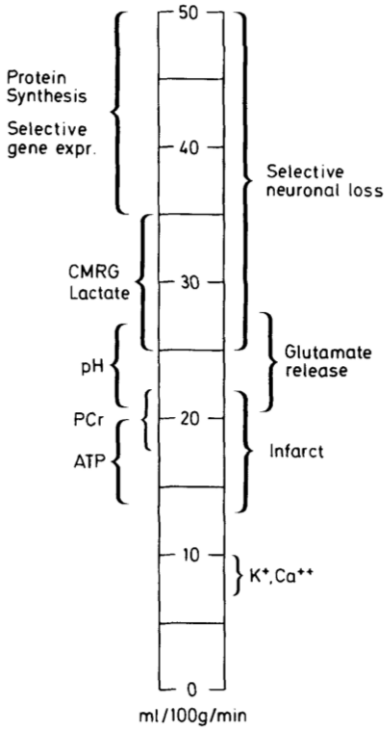


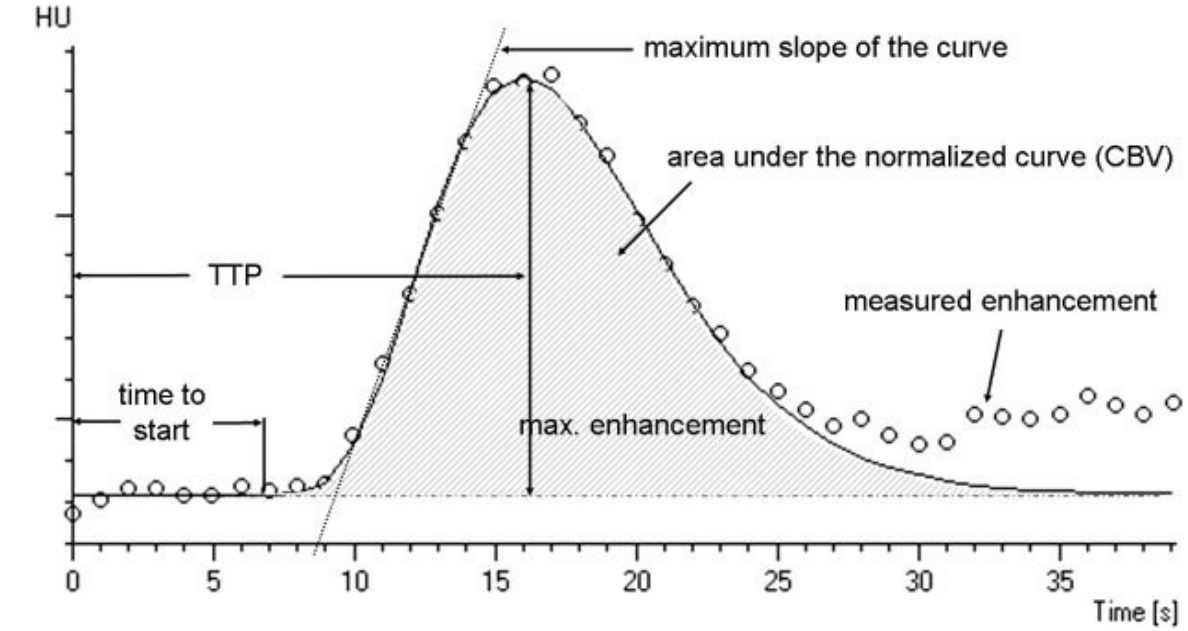
Figure 1-2 – Cerebral blood flow thresholds for functional and metabolic dysfunction from Hossman (20) with permission

1.4 Imaging the penumbra

Perfusion imaging techniques using CT and MRI rely on first pass bolus tracking, also known as dynamic susceptibility contrast (DSC) in MRI. This technique involves calculation of several parameters which are related by the central volume principle which states that 'CBF is the ratio of the blood volume within all blood vessels in a given volume of tissue (cerebral blood volume - CBV) to the mean transit time (MTT) of the agent from the arterial input to the venous drainage within the volume being evaluated (CBF=CBV:MTT)'(23). Values are obtained by imaging following injection of a contrast agent. In the case of CT changes in tissue density are observed on repeated scanning over approximately one minute. Changes in attenuation as contrast travels through the vascular bed are computed into time-density curves which are deconvolved with reference to the arterial input function (AIF). The AIF is the time density curve of a proximal artery and by correcting for this with deconvolution extracerebral factors such as extracranial vascular disease or slow infusion rates which may influence the calculated parameters are adjusted for. Eastwood et al (24) validated the deconvolution method in patients using CT perfusion (CTP) allowing for slower infusion rates. The resulting curves, before and after deconvolution, are shown in Figure 1-3 adapted from Tomandl et al (25) and Kim (26).

The parameters can be used to make deductions about the state of the cerebral tissue to identify areas of core and penumbra (26):

- CBV = area under the deconvolved curve = blood volume within the target area
    - Initially increases after vascular occlusion due to vasodilatation in an attempt to maintain CBF however quickly decreases as ischaemia progresses and infarction occurs
- CBF = curve height at maximum intensity = blood supply to brain tissue
    - Used to identify viable tissue



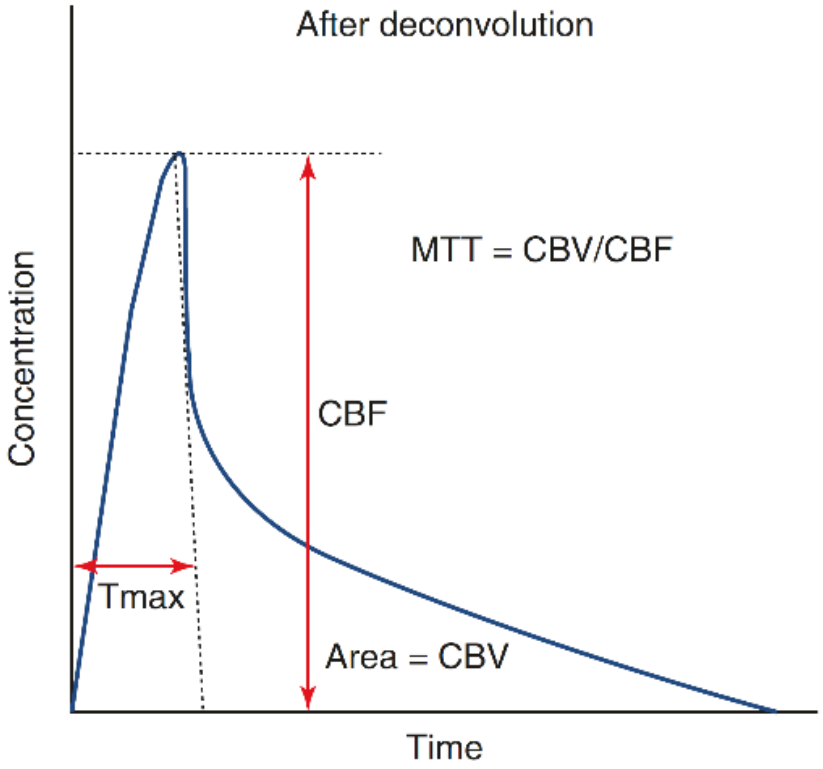


Figure 1-3 - Time density curves in CTP analysis before and after deconvolution adapted from Tomandl et al and Kim with permissions

- MTT = CBV/CBF = transit time of blood through the capillary bed
    - Most sensitive measure of perfusion deficit but overestimates ischaemia
- Tmax = time to maximum intensity in the deconvolved curve
    - Favoured as a measure of the penumbral tissue by several techniques
    - A further parameter which is Tmax corrected for the delay and dispersion of contrast is commonly referred to as ‘delay time’ (DT)

The progression of changes as perfusion pressures fall following vascular occlusion is as follows (27); the speed and extent of progression will depend upon the duration of ischaemia and magnitude of reduction in cerebral perfusion pressure (CPP). The stages of progression are demonstrated in Figure 1-4 from Gonzalez et al (27):

A - If the reduction in CPP is only mild, vasodilation will lead to increased CBV to maintain CBF, MTT will increase slightly and OEF and CMRO2 are maintained

B - As reduction in CPP progresses vasodilation (and increased CBV) is unable to maintain CBF which falls. MTT increases resulting in blood spending longer in gas permeable capillaries allowing increased OEF and maintenance of CMRO2 thus tissue is under-perfused but maintaining energy production

C - If OEF is maximised, CMRO2 begins to fall with lactate accumulation as a result of anaerobic metabolism. However, a small drop in CMRO2 does not cause clinically detectable electrical dysfunction nor lead to tissue damage. Up to this stage the tissue has progressed to benign oligaemia only

D - As CPP and CBF fall further CMRO2 continues to fall until a threshold of electrical failure is crossed and a neurological deficit can be expected. However, as the threshold for tissue viability is lower this tissue can persist as penumbra and be salvaged by reperfusion

E - Tissue infarction occurs with a further decrease in CBF. CBV increases to a peak but then fails, presumably as blood vessels collapse or very slow CBF leads to intravascular thrombosis

F - An alternative state to consider is if reperfusion occurs, either spontaneously or secondary to treatment. Due to persistence of compensatory vasodilation after perfusion is restored CBF and CBV are elevated to supra-normal levels, MTT can be increased or decreased.

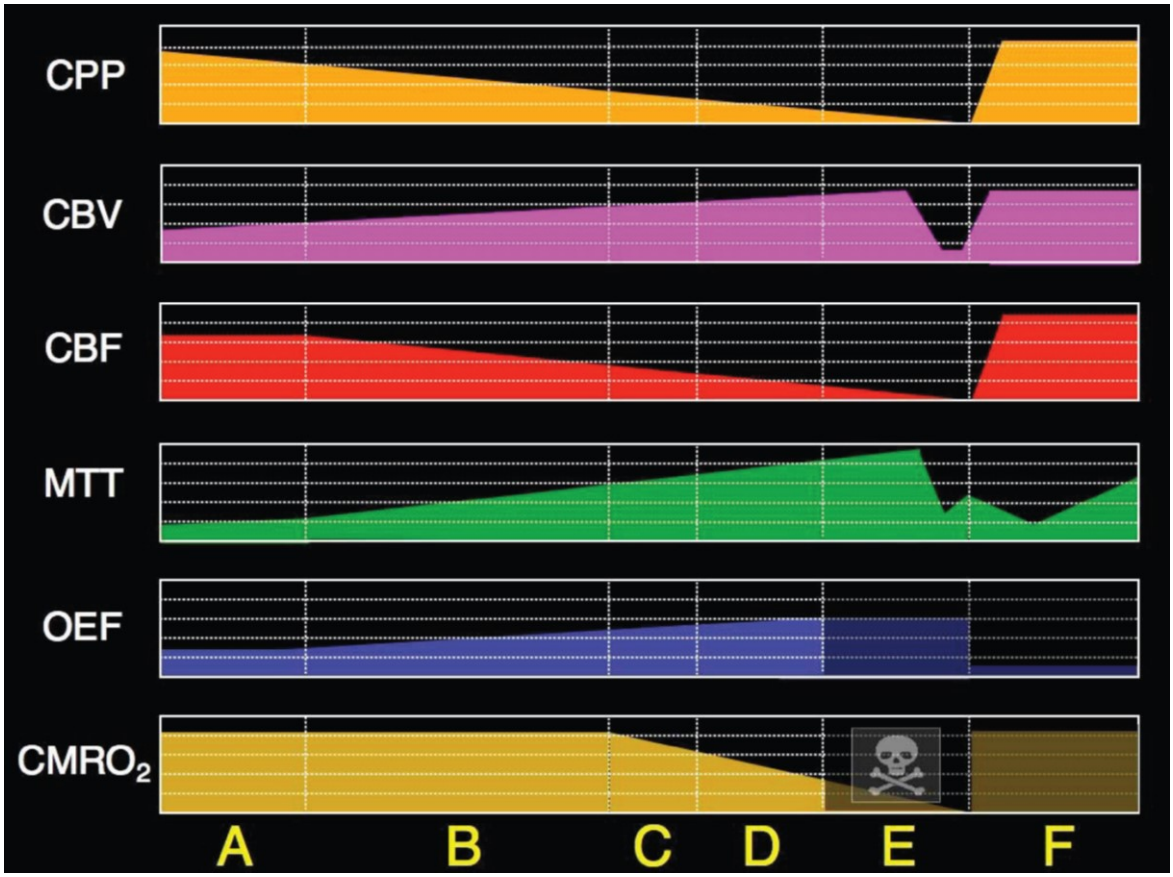


Figure 1-4 - Changes in metabolic parameters during ischaemia – from Gonzalez et al(27) with permission

An alternative to first pass bolus tracking in CT perfusion is the slow-infusion whole brain method which involves obtaining a non-enhanced and then enhanced scan after injection of contrast over 40 seconds. This technique only allowed CBV calculation and although it allowed whole brain coverage, it has been surpassed by techniques which now allow for greater coverage than previously when the area of scanning was more limited (23).

MRI perfusion weighted imaging (PWI) relies on the DSC technique and the change in T2* signal caused by the passage of gadolinium contrast through capillary beds. Repeated scanning is utilised and maps of CBF, CBV, MTT and Tmax constructed as with CTP (28). Signal change with contrast is greater in MRI than in CT but is not quantifiable as it relates to concentration of contrast in a non-linear fashion.

1.4.1 Perfusion imaging in acute ischaemic stroke patients

Wintermark and colleagues (2002) studied a series of 22 patients with acute ischaemic stroke performing CT perfusion at baseline and follow up MRI at 3 days along with clinical scores (29). They demonstrated that CT perfusion could be used to calculate maps of CBF, CBV and MTT used to predict penumbra and core. In the absence of recanalisation, the ischaemic lesion at baseline and the subsequent diffusion weighted imaging (DWI) lesion on MRI 3 days later were found to correlate well. When recanalisation did occur, either spontaneously or with thrombolysis, the DWI lesion was seen to be smaller than the initial perfusion lesion. This was interpreted to indicate that without recanalisation the predicted penumbra degraded over time until the entire lesion was infarcted, whereas when recanalisation occurred the penumbra that was still viable at the time of recanalisation was salvaged. They also found correlation of clinical scores such as the National Institutes of Health Stroke Scale (NIHSS) (30) with the size of the penumbra lesion found at baseline though correlation with follow up imaging was not found to be significant.

Creation of colour-coded maps of the parameters discussed above relies on thresholding to delineate normal from strata of under-perfused tissue. Ideally the chosen thresholds for predicting the ischaemic penumbra and core should correlate well with measures of tissue damage such as restricting lesions on MRI

DWI or final infarct volumes on CT at follow up. This is made challenging by the dynamic nature of the relationship between CBF and ischaemia, namely that ischaemia is a function of both severity and duration of reduced CBF (31). This reflects that perfusion imaging is a study of blood flow rather than tissue state and thus conclusions about the fate of regions of brain tissue based on perfusion imaging must be nuanced and examined in context. In the Wintermark study (29) the cut off chosen to identify predicted ischaemic core was regional CBV (rCBV) of <2.5ml per 100g; and for predicted ischaemic penumbra regional CBF (rCBF) of <34% relative to the corresponding region in the unaffected contralateral hemisphere. These cut offs were arbitrarily chosen based on several early papers in the clinical use of CTP (32-34).

Research has been carried out since to determine the optimum thresholds for CTP parameters however there has been considerable variability in the results. A review by Dani et al (35) in 2011 highlighted the differences in defining what thresholds represented as well as differences in thresholds themselves. In 69 studies involving CT (20 studies) or MRI (49 studies) perfusion scans within 24 hours of stroke onset, there was a wide variety of definitions for what constituted ‘non-viable’ (predicted core) and ‘at risk’ (predicted penumbra) tissue shown in table 1-2.

Normal tissue was most commonly defined as a mirrored region of interest (ROI) in the contralateral hemisphere though alternative areas of presumed normal tissue in the ipsilateral hemisphere were also used and it was also common for no definition to be offered.

These definitions were used in turn to inform the choice of optimum thresholds and expectedly this also showed considerable variation. Figure 1-5 from Dani et al (35) summarises CBF and MTT thresholds from studies in both CT and MRI. The thresholds examined are varied, with some studies studying grey and white matter separately, but most together as one entity. The ‘non-viable/at risk’ threshold represents the division between predicted core and penumbra and the ‘at risk/not at risk’ threshold represents the division between predicted penumbra and tissue predicted to fully recover regardless of reperfusion outcome.

	Predicted core	Predicted penumbra
MRI	Acute DWI lesion	Region of DWI lesion expansion
	Follow up DWI lesion	Region of T2/FLAIR lesion not on acute DWI/ADC
	Follow up T2, FLAIR or CT lesion	Final infarct on CT, T2 or FLAIR
	Tissue in both DWI and final infarct	Acute PWI lesion
	Region of ADC hypoattenuation	DWI/PWI mismatch
	Abnormality on DWI and ADC	DWI/PWI mismatch within final infarct
	80% reduction on ADC map	DWI/PWI mismatch outside final infarct
	CBV <2ml/100g	PWI lesion outside final infarct
		Acute DWI lesion that is not in final infarct
CT	Subacute or follow up DWI lesion	Region of hypoperfusion based on MTT
	Final infarct volume at follow up	Region of hypoperfusion based on CBF
	CBF ≤12ml/100g/min	Region of hypoperfusion based on MTT and CBV
	Reduced CBF and CBV within the final infarct	Region of tissue present within the final infarct
		Region of hypoperfusion based on CBF, CBV and TTP
		Region of hypoperfusion based on TTP and CBV
		Region of CTP hypoperfusion minus final infarct
		Region of CTP hypoperfusion minus acute DWI lesion
		Tissue within final infarct that had normal CBV and reduced CBF on acute CTP
		Normal tissue at follow up which had reduced CBF but normal CBV on acute CTP
		Proportion of tissue which is normal on 24-hour CTP but hypoperfused on acute CTP

Table 1-2 – Definitions of predicted core and penumbra in MRI and CT studies

ADC = apparent diffusion coefficient

T2 = T2 weighted MRI imaging

FLAIR = fluid attenuated inversion recovery

PWI = perfusion weighted imaging

Adapted from Dani et al (35) with permission

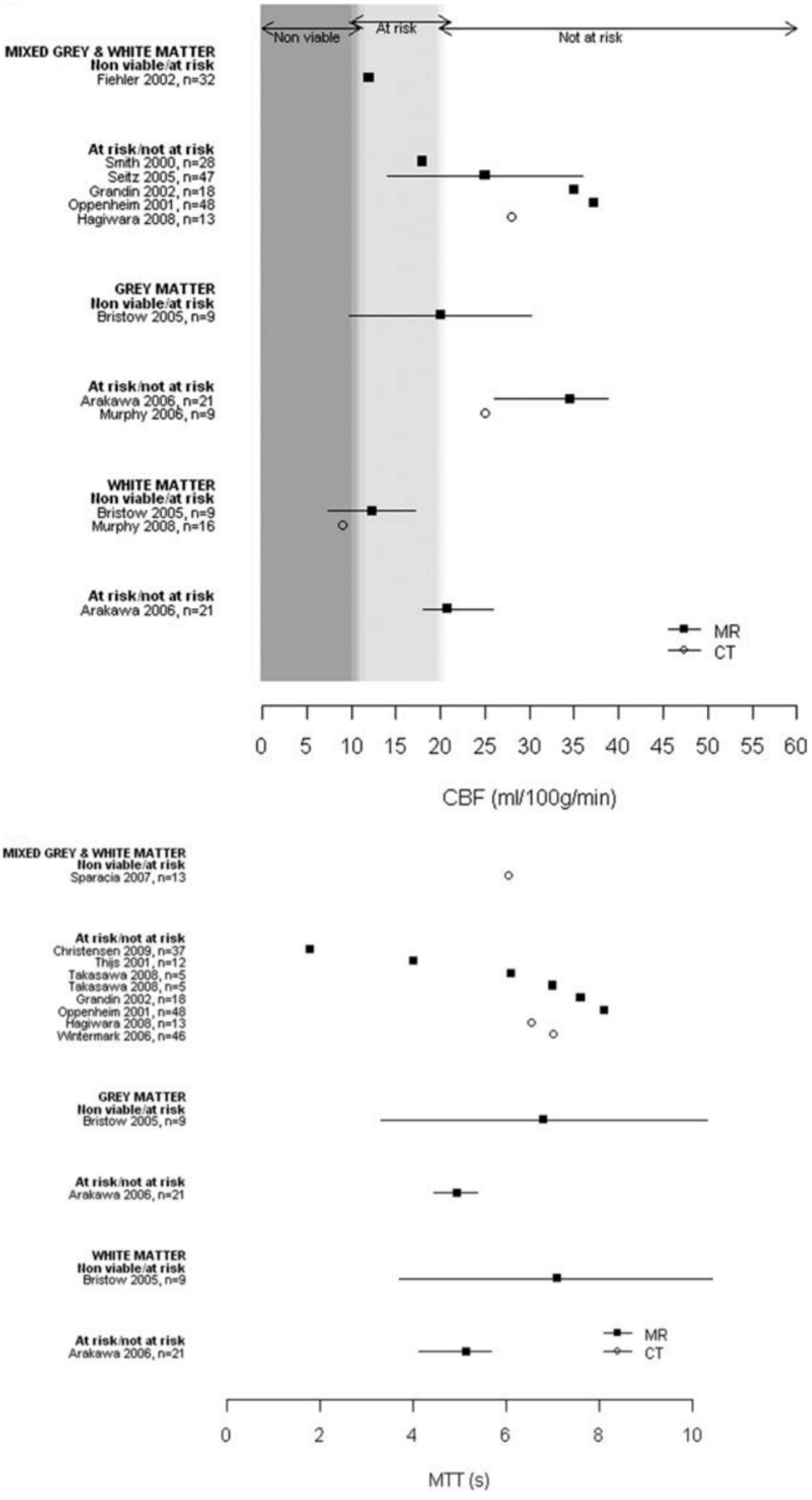


Figure 1-5 - CBF and MTT thresholds from studies in CT and MRI from Dani et al (35) with permission

1.4.2 Incorporation of perfusion imaging into clinical trials – defining a target population

Whilst acknowledging this variability, studies aiming to examine the relationship of perfusion-diffusion mismatch to treatment effect were undertaken. They hypothesised that patients with a favourable profile of small diffusion or predicted core lesion and large perfusion or predicted penumbra lesion would be most likely to benefit from reperfusion therapy such as intravenous thrombolysis (IVT). The Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution (DEFUSE) study (36) studied 74 patients treated with IVT 3-6 hours after symptom onset who underwent baseline and 3-6 hour follow up MRI scans. DWI and apparent diffusion coefficient (ADC) maps were used to identify predicted core lesions and predicted penumbra was defined as Tmax >2s on PWI. Patients were grouped into pre-determined profiles:

- Target mismatch – patients with a PWI lesion ≥10ml and ≥120% of the volume of the DWI lesion and not meeting malignant profile criteria
- No mismatch – PWI lesion <120% of the volume of the DWI lesion
- Small lesion – DWI and PWI lesions both <10ml
- Malignant profile – baseline DWI lesion of ≥100ml and/or PWI lesion with Tmax >8s of ≥100ml (this profile was identified during an interim analysis based on cases with poor outcome after perfusion and severe intracranial haemorrhage)

A favourable clinical response, defined as NIHSS of 0-1 or a reduction of ≥8 at 30 days, was associated with having a perfusion/diffusion mismatch and was more likely in the 'target mismatch' profile group. Conversely, in the absence of a mismatch there was no association with favourable clinical response; and in the malignant profile group there was an association with fatal intracranial haemorrhage.

The Effects of alteplase beyond 3 h after stroke in Echoplanar Imaging Thrombolytic Evaluation Trial (EPITHET) (37) studied a similar population with

101 patients with acute stroke randomised to Alteplase or placebo 3-6 hours after stroke onset undergoing MRI at baseline, 3-5 days and 90 days. MRI was not used to inform randomisation. Primary outcomes were infarct growth attenuation in mismatch patients measured by different methods. Alteplase was associated with a non-significant trend towards lower infarct growth. There was no significant difference between treatment groups in functional and neurological outcome. A third of patients in the Alteplase group fit the malignant profile criteria and 8% of Alteplase patients had a symptomatic intracerebral haemorrhage (sICH) compared to none in the placebo group. Alteplase was associated with reperfusion and patients who achieved reperfusion had significantly lower infarct growth and better neurological and functional outcomes overall.

Secondary analysis of the DEFUSE and EPITHET trials suggested that the use of Tmax >2s was overestimating the area of predicted penumbra and the optimal threshold was Tmax 4-6 seconds (38). This would have resulted in predicted penumbra including regions of benign oligaemia. By increasing the Tmax threshold the area would be limited to the tissue at risk of infarction only.

The DEFUSE 2 study (39) studied patients undergoing endovascular treatment for acute ischaemic stroke within 12 hours and used perfusion imaging to examine treatment effect in patients with and without ‘target mismatch’ criteria. Hypoperfused tissue predicted to be penumbra was now considered to have Tmax >6 seconds. Target mismatch criteria included:

- Ratio of ≥1.8 of predicted penumbra to predicted core with an absolute difference of >15ml
- Predicted ischaemic core volume <70ml
- Volume of Tmax>10 seconds (severely hypoperfused tissue) <100ml

Reperfusion was associated with a better clinical outcome assessed by NIHSS, better functional outcome assessed by day 90 modified Rankin scale (mRS) and attenuation of infarct growth at day 5 in the target mismatch group but not in the non-mismatch group.

A number of trials since have investigated the utility of perfusion imaging in patient selection for revascularisation therapies. The EXTEND study (40) used CT perfusion in acute ischaemic stroke presenting 4.5-9 hours after onset, or in patients wakening with symptoms within 9 hours of the midpoint of sleep, to identify patients with salvageable brain tissue and randomising to either alteplase or placebo. Thresholds used were Tmax >6 seconds and CBF<30% based on a study of acute CTP and MRI DWI (41). Despite the study stopping early due to loss of equipoise, a benefit of alteplase on functional outcome was detected. The ECASS-4 study (42) used MRI perfusion in the 4.5-9 hour population to compare alteplase to placebo but was stopped early due to slow recruitment with no difference found between groups in functional outcome. A meta-analysis (43) of EXTEND, ECASS-4 and EPITHET found benefit of alteplase over placebo on functional outcome in the 4.5-9 hour post symptom onset ischaemic stroke population with perfusion mismatch.

Trials of endovascular thrombectomy (EVT) have also used perfusion imaging to select target populations and demonstrated significant treatment benefit in those with salvageable brain tissue. The EXTEND-IA study (44) studied patients receiving alteplase within 4.5 hours of stroke onset who had a target mismatch profile and randomised to EVT + Alteplase vs Alteplase alone, and demonstrated significant benefit of EVT + Alteplase on early neurological improvement and long term functional outcome. Perfusion imaging has also shown benefit of EVT in selected patients in later time windows. The DEFUSE-3 study (45) selected patients with a target mismatch profile who presented 6-16 hours after symptom onset and demonstrated a benefit of endovascular therapy plus medical therapy over medical therapy alone on functional outcome and mortality.

Most studies have relied on the use of automated perfusion imaging processing software which is increasingly making its way into clinical practice. The most oft used in the clinical trials mentioned is ‘RApid processing of PerfusIon and Diffusion’ (RAPID) software (46) which analyses CT or MRI perfusion data to produce maps of predicted penumbra and core. RAPID uses thresholds optimised in the DEFUSE and EXTEND studies with predicted penumbra defined by Tmax >6 seconds and predicted core defined on CT by CBF<30% and on MRI by ADC<620. The software calculates a mismatch volume and ratio automatically to aid

decision making. MIStar software (47) uses slightly different parameters namely relative delay time of >3 seconds for predicted penumbra and relative CBF <40% with delay time >3 seconds for predicted core. Other software packages include Brainomix and Olea amongst others (48).

1.5 Intravenous thrombolysis for acute ischaemic stroke

In the first hours after onset, management of potentially disabling acute ischaemic stroke (AIS) focuses on early reperfusion of ischaemic brain. Reperfusion can limit the extent of brain tissue death by rescuing ischaemic tissue with residual perfusion that is sustained by collateral flow, which typically remains viable for a period of a few hours only. Intravenous thrombolysis (IVT) is an established treatment in eligible patients which aims to lyse the causal clot and recanalise the occluded vessel, thus re-establishing perfusion of the affected area of brain (49).

Alteplase is a recombinant tissue plasminogen activator (rt-PA) which has been the drug of choice for intravenous thrombolysis since the first positive trials of IVT in the 1990s (50). Other intravenous thrombolytic agents have been investigated to a limited extent. Streptokinase investigation was abandoned after it did not improve outcomes and was associated with higher bleeding risk in AIS within 6 hours of onset in 2 trials in the early 1990s; desmoteplase was investigated in later time windows (3-9 hours after onset) in imaging-selected patients but was not found to be effective in a series of small trials (51). Alteplase significantly improves the outcome from AIS but has several drawbacks which may make an alternative thrombolytic agent desirable. These include the method of drug administration which is open to error and delay in an emergency setting, and the propensity for thrombolysis-related intracerebral haemorrhage (52). Tenecteplase is a genetically engineered tissue plasminogen activator which has potential to be an alternative to alteplase (51).

1.5.1 Alteplase

Alteplase is a recombinant form of tissue plasminogen activator, a protease found in endothelial cells which catalyses the conversion of plasminogen to plasmin which in turn breaks down the fibrin components of a thrombus (51).

Alteplase has a very short circulating half-life and therefore is administered as a bolus (10% of dose) followed by infusion of the remaining drug over one hour. Plasma concentration declines rapidly after the initial bolus administration, and delays of greater than 5 minutes in commencing the infusion mean that plasma concentration increases only slowly and may never achieve target concentration (53). Delays between the bolus and infusion of alteplase are common in routine clinical use, an average of 9 minutes being documented in one single centre study (54), potentially compromising alteplase efficacy.

The landmark National Institute of Neurological Disorders and Stroke (NINDS) trial published in 1995 (50) demonstrated significantly greater improvement in disability-free recovery for AIS patients treated with alteplase within 3 hours after symptom onset compared to placebo. The absolute benefit for excellent functional outcome was around 12%. This led to the regulatory approval for the use of alteplase for AIS in addition to its uses in myocardial infarction and pulmonary embolism. Trials of alteplase in different time windows, more varied populations, and in imaging-selected patients have followed. An individual-level meta-analysis of 9 trials involving 6756 patients concluded that alteplase treatment within 4.5 hours, irrespective of age or stroke severity, significantly increased the likelihood of a good functional outcome in patients with AIS (55), with the caveat that benefit declines steeply with increasing delay from stroke onset to treatment.

1.5.2 Tenecteplase

Tenecteplase is a third generation thrombolytic produced with recombinant DNA technology as a modified form of alteplase with alterations in the protein structure at 3 sites (modified amino acid sites designated by the letters T, N and K leading to the drug's alternative name of TNK). These changes prolong the half-life of tenecteplase and allow greater binding affinity for fibrin than alteplase (51). Tenecteplase is administered as a single bolus making it an attractive alternative in the management of AIS to alteplase. Preparation of a single bolus is simpler and less time consuming in an emergency setting, particularly as AIS patients may require transfer to another hospital site for access to mechanical thrombectomy and avoid the bolus-infusion delay issue that compromises therapeutic plasma concentrations of alteplase. Avoidance of

ongoing infusions may reduce the need for medical supervision during transfer and improve door to needle time in hospitals that utilise MRI rather than CT-based acute stroke imaging protocols.

Animal studies in a rabbit model of embolic stroke found tenecteplase had a wider dose range and longer therapeutic window than alteplase, and was associated with better performance in a post-stroke behavioural rating scale (56).

Tenecteplase superseded alteplase in the management of acute myocardial infarction (AMI) after the ASSENT-2 trial demonstrated its non-inferiority (57). In trials of AMI, tenecteplase (0.5mg/kg dose) was found to improve recanalisation significantly prior to percutaneous coronary intervention (PCI); however this came at a cost of a higher rate of major adverse events including intracerebral haemorrhage (58). In subsequent trials at earlier timepoints, it was found that rates of intracerebral haemorrhage were highest in those aged >75, prompting a reduction in the dose of tenecteplase to 0.25mg/kg in this group (59). Subsequently no intracerebral haemorrhages occurred at this dose whilst efficacy in AMI treatment and mortality were comparable (60). Trials of tenecteplase 0.25 mg/kg in AMI prior to PCI are ongoing (61).

1.5.3 Tenecteplase vs Alteplase in AIS: Published studies

The first randomised, controlled trial of tenecteplase in AIS compared alteplase with tenecteplase at 3 different doses, 0.1, 0.25 and 0.4 mg/kg (62). The study was halted early due to funding and recruitment issues. The trial used an adaptive design with combined safety and early efficacy assessments guiding recruitment at each dose level, and recruitment to the 0.4mg/kg tenecteplase dose was discontinued after only 19 patients. The low numbers recruited meant no firm conclusions were possible.

Two further phase II trials comparing alteplase and tenecteplase followed. An Australian study compared standard dose alteplase with tenecteplase at either 0.1 or 0.25 mg/kg in patients with AIS presenting within 6 hours of symptom onset and specific imaging criteria (vessel occlusion on CT angiography and an ischaemic lesion ≥20% greater than core lesion on CT perfusion) (63). In 75

patients they found the 0.25 mg/kg was superior to lower dose tenecteplase and to alteplase in reperfusion of the ischaemic lesion, clinical improvement at 24 hours and excellent recovery at 90 days. The ATTEST study compared alteplase with tenecteplase at 0.25mg/kg in patients eligible for IVT presenting within 4.5 hours of symptom onset (64). In 104 patients no differences were found between treatments for proportion of penumbra salvaged or any other imaging or clinical outcome.

Individual patient data pooled analysis of these 3 trials found no significant differences between alteplase and tenecteplase at all doses investigated, but suggested that the 0.25 mg/kg dose warranted further investigation as it had the greatest odds of achieving early neurological improvement, excellent functional outcome and avoiding intracerebral haemorrhage compared with alteplase (65). Subgroup analyses suggested potentially superior efficacy among patients with large vessel occlusion (occlusion of the intracranial internal carotid artery or first part of the middle cerebral artery) (66, 67).

The NOR-TEST trial compared alteplase with tenecteplase 0.4mg/kg in patients eligible for IVT presenting within 4.5 hours of symptoms; or those presenting within 4.5 hours of awakening with stroke symptoms and displaying DWI-FLAIR mismatch on acute MRI imaging; or those eligible for IVT as a bridging therapy to mechanical thrombectomy. Although 1100 patients were recruited, there was a large proportion of stroke mimics (17%), and the majority of patients had mild stroke (median National Institutes of Health Stroke Scale (NIHSS) score of 4). No difference was found between groups in clinical or safety outcomes. In recognition of the confounding issues of mild stroke, the subsequent NOR-TEST-2A trial (68) was undertaken in patients with AIS of minimum severity (defined as a National Institutes of Health Stroke Scale [NIHSS] score >5). The trial was discontinued at interim safety review after a four-fold excess of symptomatic intracerebral haemorrhage was observed in the tenecteplase arm (16% tenecteplase versus 4% alteplase), although there were several imbalances in prognostic markers between groups - with tenecteplase arm participants being older (median age 73.2 vs 68.6), less likely to have a stroke mimic diagnosis (3% vs 11.5%), and lower levels of baseline function (40% mRS ≥1 vs 26.9%). Plans for

further investigation of the 0.4mg/kg dose have been abandoned in favour of the 0.25mg/kg dose.

The EXTEND IA-TNK trial enrolled patients eligible for mechanical thrombectomy within 4.5 hours of symptom onset and randomised to alteplase or tenecteplase 0.25mg/kg (69). In 202 patients, the primary outcome of reperfusion of ≥50% of the vascular territory or absence of the initial occluding thrombus occurred in significantly more patients in the tenecteplase (22%) than the alteplase (10%) group (adjusted odds ratio 2.6 (1.1-5.9) p=0.02). There were no significant differences in patients achieving independent recovery (defined as modified Rankin scale 0-2 at 90 days) or in early neurological improvement between groups. A follow up study, EXTEND IA TNK part 2 (70), compared tenecteplase 0.25 and 0.4 mg/kg in a similar study design. No significant differences were found in the radiological primary outcome or other clinical or safety outcomes, inferring that the 0.4mg/kg offered no advantage over the lower 0.25mg/kg dose in patients eligible for mechanical thrombectomy.

Superior reperfusion with tenecteplase 0.25mg/kg compared with alteplase was reported among patients treated in a mobile stroke unit setting in the TASTE-A trial (71) in Melbourne, Australia.

1.5.4 Tenecteplase – a non-inferior thrombolytic?

In a 2019 meta-analysis of the 5 published alteplase versus tenecteplase trials to that date, Burgos and Saver concluded that non-inferiority had been demonstrated (72). They calculated the proportions achieving disability-free survival after AIS of 57.9% with tenecteplase and 55.4% with alteplase with a risk difference of 4% (−1 - 8) on meta-analysis. A pre-specified non-inferiority margin of 6.5% as well as secondary margins of 5% and 1.3% were met. However, weaknesses of the meta-analysis included a heterogenous population in terms of stroke severity, a high number of stroke mimics (from the NOR-TEST data), differing doses of tenecteplase, and results largely being driven by trials selecting patients with large vessel occlusion. The primary non-inferiority margin selection was criticised for being drawn from a trial comparing two doses of alteplase (49).

Encouraged by the trial and meta-analysis results to date, some stroke centres have elected to implement routine use of tenecteplase. Zhong et al report routine use of tenecteplase compared with alteplase at 3 stroke centres in New Zealand as being feasible with similar clinical and safety outcomes (73). Warach et al report plans for a prospective study of use of tenecteplase for all IVT eligible patients presenting within 4.5 hours of symptom onset in a 9 hospital network in Texas (74). In France, the Tenecteplase Treatment in Ischemic Stroke (TETRIS) study group (75) also reported safety, efficacy and recanalisation rates using tenecteplase routinely to be in line with published results. The move to adopt tenecteplase as routine thrombolytic agent of choice has been spurred on by the COVID-19 pandemic which placed unprecedented pressure on emergency departments globally, motivating calls for use of tenecteplase over alteplase as an easier and more quickly administered alternative (76). However, this in turn led to shortages of tenecteplase for the management of acute myocardial infarction due to supply issues of the drug in some countries (77).

The Alteplase compared to Tenecteplase (ACT) trial, the first large trial comparing tenecteplase 0.25mg with alteplase, was presented in May 2022 (78). In 1600 patients randomised within 4.5h of AIS onset, tenecteplase exhibited an increase in excellent recovery of 2.1% compared to alteplase, meeting the pre-specified non-inferiority margin. The trial found no significant differences in any outcome measure. A subgroup analysis in patients with large vessel occlusion, a trend towards superiority of tenecteplase was seen.

The TWIST trial (79) compared tenecteplase 0.25mg/kg against non-IVT standard of care for patients with wake-up stroke presenting within 4.5 hours of wakening, enrolled on the basis of a non-contrast CT compatible with IVT. The trial stopped short of its planned sample size and was unable to establish non-inferiority or superiority of tenecteplase for any outcome measure.

A biocopy of tenecteplase was used in two trials based in China. The TRACE trial (80) studied tenecteplase at 3 doses (0.1, 0.25 and 0.32mg/kg) compared to standard dose alteplase in patients conventionally eligible for IVT, finding no major differences in safety outcomes or day 14 NIHSS. The TRACE-2 trial (81) enrolled 1430 participants and randomised to 0.25mg/kg tenecteplase or

0.9mg/kg alteplase for patients conventionally eligible for IVT within 4.5 hours but ineligible for mechanical thrombectomy (MT). Results showed non-inferiority of tenecteplase compared to alteplase in achieving mRS 0-1 at 90 days.

1.5.5 Current guidelines and ongoing clinical trials of tenecteplase in AIS

A number of clinical trials of tenecteplase in AIS are ongoing covering a variety of IVT indications and timeframes. Current guidelines for different clinical scenarios and future potential applications of tenecteplase currently being investigated in these scenarios are summarised below.

1.5.6 Disabling stroke <4.5 hours

European Stroke Organisation (ESO) guidelines for IVT published in 2021 maintained recommendation of alteplase over tenecteplase for patients routinely eligible for IVT, however recommended tenecteplase 0.25mg/kg over alteplase for patients receiving bridging IVT prior to mechanical thrombectomy (49). American Heart Association (AHA)/American Stroke Association guidelines published in 2019 similarly approved tenecteplase as a suitable alternative to alteplase in the pre-mechanical thrombectomy population (82). Guidelines from both ESO and AHA acknowledged the low quality of the evidence available at the time of writing and recommendations were weak. The addition of the AcT trial is likely to strengthen the recommendation of tenecteplase 0.25g/kg as an alternative to alteplase.

Trials comparing tenecteplase and alteplase in this patient population are ongoing. The ATTEST-2 trial (NCT02814409) is a randomised controlled trial comparing tenecteplase 0.25mg/kg and alteplase for patients routinely eligible for IVT presenting within 4.5 hours of symptom onset. The TASTE trial (ACTRN12613000243718) is comparing tenecteplase 0.25mg/kg and alteplase for patients with AIS presenting within 4.5hours of symptom onset who have favourable baseline imaging characteristics (confirmed CT perfusion mismatch with ischaemic core <70ml).

1.5.7 Disabling stroke with onset beyond 4.5 hours

Patients with AIS may be eligible for thrombolysis beyond the 4.5 hour window on the basis of brain imaging. CT angiogram (CTA) can identify patients with large vessel occlusion and CT perfusion can identify patients with small ischaemic core (irreversibly damaged, severely hypoperfused tissue) and larger volumes of penumbra (hypoperfused but potentially rescuable tissue), a pattern referred to as 'target mismatch'. The EXTEND trial, and a subsequent meta-analysis of the EXTEND, EPITHET and ECASS-4 trials, demonstrated the efficacy of alteplase in patients with target mismatch on CT perfusion imaging up to 9 hours after symptom onset in improving functional outcome after AIS (83, 84). Current European Stroke Organisation guidelines (49) support the use of alteplase in patients with target mismatch up to 9 hours after symptom onset in whom mechanical thrombectomy is not planned.

The TIMELESS trial (85) compared tenecteplase 0.25mg/kg and placebo for patients with AIS presenting between 4.5 and 24 hours of symptom onset in whom imaging confirms an anterior circulation stroke (ICA, M1 or M2 occlusion) with favourable perfusion imaging (mismatch with ischaemic core <70ml). The majority of patients underwent mechanical thrombectomy and results showed no significant difference between tenecteplase and placebo. The ETERNAL trial (NCT04454788) in Australia will also compare tenecteplase and alteplase in patients with LVO and target mismatch.

1.5.8 Wake-up stroke

Hyperacute MRI can identify patients with potentially recent onset of ischaemia based on the presence of a lesion on diffusion-weighted imaging (DWI) lesion that is not yet abnormal on a T2 fluid-attenuated inversion recovery (FLAIR) sequence (DWI-FLAIR-mismatch) (86). The WAKE-UP trial demonstrated the efficacy of alteplase in patients with wake-up strokes with DWI-FLAIR mismatch on acute MRI (87). The EXTEND trial also recruited patients with target mismatch on CT perfusion if they presented with symptoms on wakening up to 9 hours from the midpoint of sleep. Accordingly European guidelines support the use of alteplase in patients fulfilling these imaging criteria if thrombectomy is not planned (49).

1.5.9 Minor stroke and TIA

There is currently no standard definition of “minor stroke,” and total scores on clinical assessments scales such as the NIHSS do not reliably distinguish disabling from non-disabling deficits. Trials of alteplase generally required the presence of a disabling neurological deficit and excluded patients with non-disabling or rapidly improving deficits, but these terms were open to individual interpretation. European and American guidelines recommend treatment of minor stroke with disabling symptoms with alteplase (49, 82) as this group accounted for around 10% of patients in the alteplase trials (55). American guidelines suggested tenecteplase 0.4mg/kg as a potential alternative to alteplase in patients with minor neurological impairment and no major intracranial occlusion (82), based on the NOR-TEST findings, with a weak recommendation, but this is likely to be superseded by NOR-TEST-2A results. However, IVT is not recommended with minor symptoms which are non-disabling by either guideline, since such patients were excluded from the main alteplase trials. The PRISMS trial randomised patients with non-disabling symptoms and NIHSS 0-5 to aspirin or alteplase within 3 hours of symptom onset and found no benefit of alteplase, although the trial was terminated early with only around one third of the planned sample size (88). The ARAMIS trial compared dual antiplatelet therapy with aspirin and clopidogrel to alteplase for minor stroke within 4.5 hours and recruited 760 participants in China, finding non-inferiority of dual antiplatelet therapy compared to alteplase (89). Presently guidelines recommend dual antiplatelet therapy with aspirin and clopidogrel in this patient group (90, 91).

The TEMPO-1 study was a dose escalation study of tenecteplase in patients with minor stroke or TIA (NIHSS 0-5, non-disabling symptoms) and confirmed LVO (92). Tenecteplase was found to be safe at 0.1 and0.25 mg/kg doses. Complete recanalisation was strongly predictive of subsequent excellent functional outcome. The TEMPO-2 trial (NCT02398656) is comparing tenecteplase 0.25mg/kg against standard of care in patients presenting with minor stroke or TIA (NIHSS 0-5) who have a confirmed LVO, though trial recruitment has recently been stopped after interim analysis.

1.6 Adjunctive approaches to intravenous thrombolysis

Current ESO guidelines (49) do not advocate the use of any adjunctive therapy in addition to intravenous thrombolysis. Antiplatelets within the first 24 hours after IVT have been shown to increase the risk of symptomatic intracranial haemorrhage (93). Correction of physiological parameters such as blood pressure and blood glucose are recommended though only conclusively when they are elevated such that they are contraindications to IVT such as blood pressure of ≥185/110 mmHg or blood glucose of ≥22.2 mmol/l (due to risk of stroke mimic).

1.6.1 Adjunctive approaches under investigation

1.6.1.1 Physiological interventions

1.6.1.1.1 Blood pressure

Current guidelines advocate the lowering of blood pressure in AIS for patients undergoing IVT and mechanical thrombectomy to below 185/110 mmHg. However routine lowering of blood pressure in patients not treated with reperfusion therapies as well as intensive lowering of blood pressure in treated patients is not recommended (94). Evidence from clinical trials has not shown any benefit in functional outcome of lowering blood pressure beyond the above limits (95). The ENCHANTED trial showed a reduction in intracranial haemorrhage after reduction of blood pressure to 130-140mmgHg within one hour of alteplase treatment in AIS but this did not translate to a functional benefit (96).

1.6.1.1.2 Oxygen

Normobaric oxygen therapy has been studied in low doses to prevent desaturation in the hours to days after onset of AIS, and at high dose in the hours after stroke as a neuroprotectant. Previous large trials have predominantly used low dose oxygen beyond the hyperacute period and have shown no effect on early neurological recovery, mortality or functional outcomes (97). More recently the PROOF trial (98) trialled high dose oxygen therapy in patients eligible for MT but was halted early due to futility with no differences found in infarct growth or early neurological recovery. A single centre in China in a

similar population found smaller infarct volumes and improved functional outcome with high dose oxygen in combination with MT (99). A larger multi-centre trial is planned (100).

1.6.1.1.3 Remote ischaemic conditioning

Remote ischaemic conditioning involves inducing ischaemia in a remote limb using a blood pressure cuff for a few minutes which theoretically will promote humoral and neural responses in the body which may aid in stroke recovery. Trials thus far have not established an optimal protocol for treatment during the post stroke period though the treatment appears safe whilst not showing definitive long term functional benefit (101).

1.6.2 Neuroprotectants

Trials of neuroprotectants in AIS have failed to show any benefit to outcome after AIS over several decades despite promising results in animal models. However, there is renewed interest in the potential of neuroprotectant agents to be of benefit in the hyperacute period after AIS in the era of highly effective reperfusion therapies. Neuroprotectants in this context may allowing 'freezing' of the ischaemic penumbra, preventing further tissue infarction until reperfusion can be achieved (102).

1.6.2.1 Nerinetide

Nerinetide is a peptide that inhibits neuronal excitotoxicity and downstream neurotoxic signalling. The ESCAPE-NA1 trial (103) compared nerinetide with placebo in AIS patients treated with thrombectomy who had moderate-to-good collaterals on CTA and absence of extensive early infarction on CT. Although the primary outcome was not met, in the subgroup which did not receive thrombolysis the likelihood of favourable functional outcome was significantly higher in the nerinetide group. Plasma levels of nerinetide were lower in those receiving thrombolysis and subsequent studies found an interaction between nerinetide and alteplase. Animal model studies of a modified form of nerinetide avoided this interaction and maintained its neuroprotective effect (104). The ESCAPE-NEXT trial (105) studied a population eligible for EVT but not IVT, but found no difference in likelihood of functional independence after AIS between

nerinetide and placebo. The FRONTIER trial (105) studied nerinetide in the pre-hospital population, patients suspected of having stroke were randomised to nerinetide or placebo within 3 hours of symptom onset. Nerinetide was associated with greater odds of functional improvement than placebo in the entire population and in the AIS group only.

1.6.3 Intra-arterial thrombolysis

Successful recanalisation of large vessels in AIS results in disability freedom at 90 days in 27% of patients, despite 71% achieving successful reperfusion (106). Recovery may be hampered by incomplete reperfusion of the cerebral microcirculation termed the 'no-reflow' phenomenon which was seen in 1 in 4 cases of successful recanalisation in a pooled analysis of the EXTEND-IA and EXTEND-IA TNK part 1 and 2 trials (107). The CHOICE trial (108) randomised patients with successful recanalisation to receive either intra-arterial alteplase or placebo including patients who had received intravenous alteplase. The trial was halted early due to slow recruitment and lack of availability of the placebo due to the COVID-19 pandemic, however encouraging results were seen with intra-arterial alteplase associated with a greater likelihood of mRS 0-1 after 90 days compared with placebo (59.0% vs 40.4%, p=0.047). However, results are taken cautiously given the small sample size, wide confidence intervals and the protocol for intravenous alteplase which could be halted at the discretion of the investigators prior to full dosing (109). A meta-analysis of intra-arterial alteplase with MT found no change in recanalisation rate but improvement in functional outcomes though the CHOICE trial was the only randomised controlled trial included (110). A trial of intra-arterial tenecteplase administered prior to first pass of MT found higher rates of recanalisation compared to a retrospective control group (111); a larger randomised controlled trial is planned (112).

1.6.4 Adjunctive antiplatelet drugs

1.6.4.1 Glycoprotein VI blockade

Glenzocimab is a humanized antibody fragment targeting platelet glycoprotein VI (GPVI) to block platelet activation without inducing bleeding (113). The ACTIMIS study was a phase IIa study comparing glenzocimab to placebo in patients with AIS treated with IVT and MT (114). In 166 patients, glenzocimab

was associated with reduced symptomatic ICH rate and mortality compared to placebo. A larger phase II/III study is ongoing (115).

1.6.4.2 Glycoprotein IIb/IIIa inhibitors

Current guidelines do not support the use of glycoprotein IIb/IIIa inhibitors in routine care (82). Trials of tirofiban and eptifibatide have suggested it is safe in AIS patients (116, 117). A large trial of abciximab was stopped early due to excess symptomatic or fatal intracranial haemorrhages in the abciximab group (118). A subsequent Cochrane review concluded that glycoprotein IIb/IIIa inhibitors should not be used for AIS patients in routine practice (119). The ARAIS trial compared argatroban and alteplase versus alteplase alone in ischaemic stroke patients presenting within 4.5 hours, finding no additional benefit of argatroban over alteplase alone in terms of functional outcome at 90 days (120). Further trials of eptifibatide and argatroban are planned (121).

1.6.5 Targeted treatments based on clot composition

Thrombi causing AIS can be heterogenous in structure with a core that is variably comprised of red blood cell (RBC) rich areas or platelet rich areas; and an outer covering rich in platelets. Platelet rich areas can make thrombi resistant to thrombolytics due to presence of platelet derived fibrinolysis inhibitors, von Willebrand factor (vWF) multimers and neutrophil extracellular traps (122).

1.6.5.1 Fibrinolysis inhibitor antagonists

Animal studies of antagonists of thrombin-activated fibrinolysis inhibitors (TAFI) in transient occlusion models of AIS have found reduced ischaemic lesion growth when combined with reduced dose alteplase compared to full dose alteplase alone (123). A phase I trial in patients with AIS found it was well tolerated in 32 participants though one participant developed subarachnoid haemorrhage (124).

1.6.5.2 von Willebrand factor (vWF) multimers

vWF multimers are involved in platelet cross linking during thrombosis (122). A study of thrombi from AIS patients found around 20% of the composition of thrombi was vWF. The authors then tested a vWF-cleaving metalloprotease

(ADAMTS13) in a mouse model of MCA occlusion involving vWF-rich thrombi and found these clots were resistant to alteplase but dissolved by ADAMTS13 (125). N-acetylcysteine, a mucolytic drug in clinical use, also cleaves vWF multimers (126) and a trial of N-acetylcysteine in combination with thrombolysis is ongoing (NCT04920448).

1.6.5.3 Neutrophil extracellular traps (NETs) and extracellular DNA

NETs are fibrous networks of extracellular DNA which provide a scaffold for thrombus formation through binding of platelets and RBCs (122). Ex-vivo studies of thrombi recovered from patients undergoing MT for AIS have shown NETs were detected in all thrombi with a higher proportion in those of cardioembolic origin. Ex vivo lysis of thrombi using alteplase was more successful with the addition of DNase 1 (127). A trial using dornase alfa, a DNase 1 used in the treatment of cystic fibrosis, in combination with MT for patients with AIS is underway (NCT04785066).

1.7 Optimising outcomes of intravenous thrombolysis in acute ischaemic stroke

Outcome after IVT in AIS depends upon several modifiable and non-modifiable factors. Treatment with alteplase is associated with better 90-day functional outcome after AIS irrespective of age and stroke severity, however treatment delay is associated with poorer outcome (55). Strategies to optimise outcome after IVT may include addressing treatment pathways (128), improving patient selection (129), a more effective thrombolytic agent (130), or strategies to sustain the ischaemic penumbra for longer until reperfusion is achieved (102). This thesis will examine some of these strategies and propose future directions.

Three themes will be explored:

- The potential for tenecteplase to be a superior thrombolytic drug to alteplase

- Improved patient selection through CT perfusion studies identifying groups at risk of poor outcome

- The effect of patient heterogeneity on outcomes in trials of acute ischaemic stroke

Tenecteplase is emerging as an alternative thrombolytic to alteplase in acute ischaemic stroke with a particular interest in selected patient groups which in whom tenecteplase may be superior to alteplase. The evidence for use of tenecteplase in acute ischaemic stroke to date will be examined. Imaging subgroups based on CT perfusion analysis will be examined in a retrospective cohort of patients from two clinical trials comparing alteplase and tenecteplase in AIS. A prospective cohort in a large multi-centre randomised controlled clinical trial comparing tenecteplase and alteplase will examine treatment effects in imaging subgroups. Finally the effect of trial population heterogeneity on outcome in AIS trials will be examined.

2 Retrospective datasets from two trials comparing tenecteplase and alteplase

2.1 Introduction

Data from 2 previous trials comparing tenecteplase with alteplase were used in this thesis for secondary analyses. Both studies carried out baseline CT perfusion imaging in all patients though this informed patient inclusion only in one trial. The combined data is characterised in this chapter for reference in later chapters.

2.2 Methods

2.2.1 Study population

We used pooled individual data from two randomised controlled trials (RCTs) comparing alteplase versus tenecteplase in AIS. The ATTEST trial (6) was a single centre phase II RCT based in Scotland involving 104 participants with AIS randomised to receive IVT with either alteplase 0.9mg/kg or tenecteplase 0.25mg/kg. Participants had non-contrast CT, CT perfusion and CT angiogram performed at baseline and non-contrast CT and CT angiogram at 24 hours, treatment decisions were based on non-contrast CT only. A second phase II RCT based in 3 centres in Australia (7) involved 75 patients with AIS who had a favourable profile on CT perfusion defined as penumbral lesion ≥20% greater than the core lesion with a volume of ≥20ml. Patients meeting these criteria were randomised to IVT with alteplase 0.9mg/kg or tenecteplase at either 0.25mg/kg or 0.1mg/kg. Follow up imaging was with MRI rather than CT.

Two cohorts were identified for analyses (figure 2-1). A cohort of M1 occlusions only was used in chapters 3, 4 and 9. Cases without an ischaemic lesion on CTP were also excluded as were cases without follow up imaging. A separate cohort including any ischaemic lesion regardless of vascular occlusion site but excluding inadequate CTP scans was used for the blood-brain barrier permeability analysis in chapter 5. Inadequate CTP scans included those with <45 seconds of data as a minimum duration specified for blood brain barrier permeability analysis.

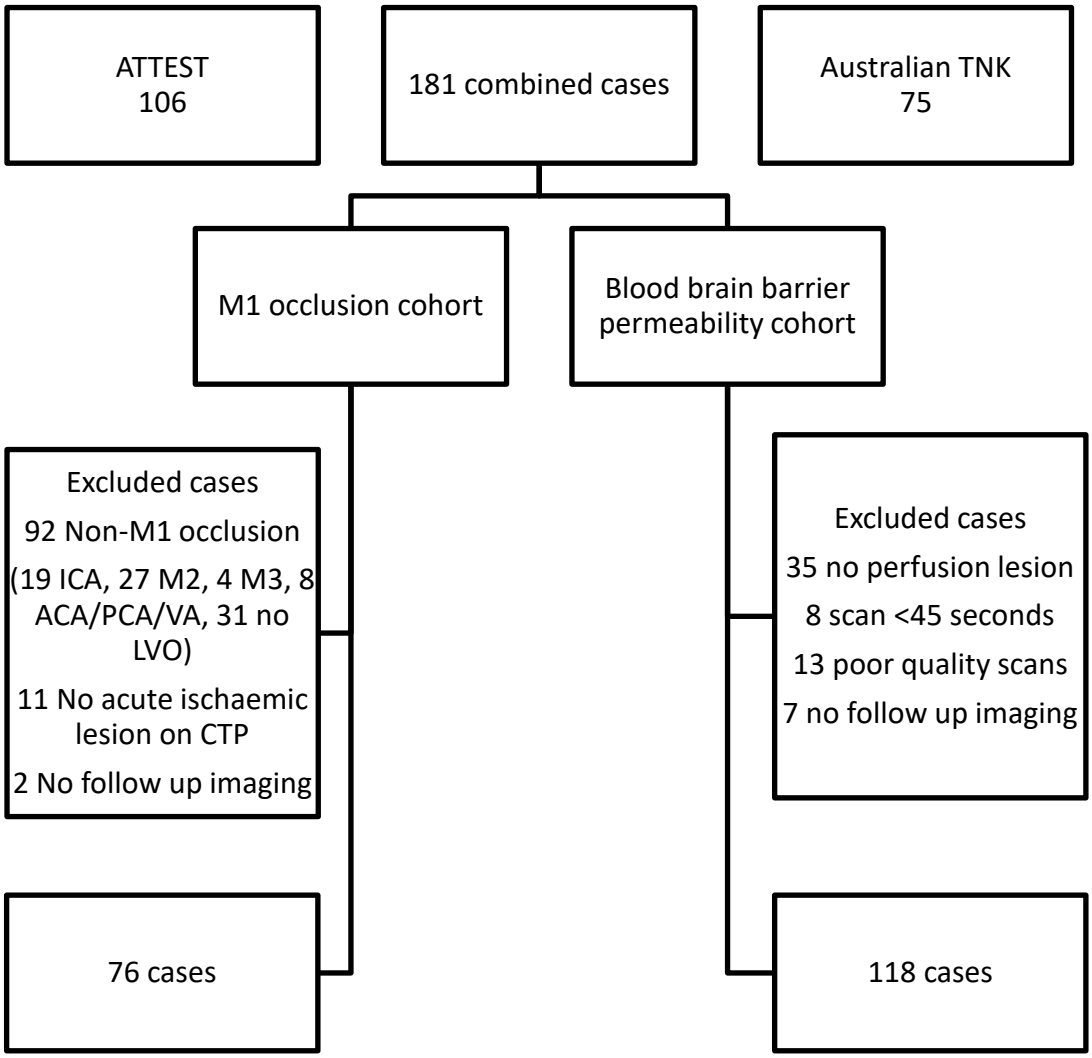


Figure 2-1 Flow chart of patient cohort selection

2.2.2 Imaging outcomes

Imaging outcomes were either extracted from the individualised data in each trial for follow up infarct volume (FIV) and recanalisation; or calculated post-hoc as detailed below. All imaging analyses were carried out using MiStar image processing software (Melbourne, Australia). In all cases imaging analysts were blinded to treatment allocation.

FIV was calculated in the ATTEST cohort using the average value of volumetric analysis performed twice each by two researchers.

Recanalisation was determined on 24-hour follow up imaging using CTA or MRA using TIMI scoring.

Ischaemic progression rate (IPR) was calculated in ml/hr by dividing the ischaemic core volume by the time from symptom onset to scan.

Infarct growth (IG) was calculated as the final infarct volume minus the baseline core volume, where this was negative it was considered as zero.

Volume of penumbra salvaged (VPS) was calculated as baseline penumbra minus the difference between FIV and core, where this value was negative it was considered as zero. A percentage was calculated for VPS, values below zero and above 100 were considered as zero and 100 respectively.

Data were tested for normal distribution using the Kolmogorov-Smirnov test and data that were non-normally distributed were transformed using log transformation and normality was confirmed using a Q-Q plot.

2.3 Results

2.3.1 Baseline characteristics

2.3.1.1 M1 occlusion cohort

In 76 cases of M1 occlusion, 28 were treated with alteplase, 32 and 16 with 0.25mg/kg and 0.1mg/kg tenecteplase respectively. Baseline characteristics are

described in table 2-1. Median NIHSS was 15 (13-18) indicating a moderately severe stroke population.

2.3.1.2 Blood brain barrier permeability cohort

A total of 118 cases were identified which fulfilled our criteria, 53 treated with alteplase and 65 with tenecteplase (49 0.25mg/kg, 16 0.1mg/kg). Baseline clinical and imaging characteristics are displayed in table 2-2.

Baseline characteristics	Mean±SD, Median (IQR), or proportion
Age	71±11
Baseline NIHSS	15 (13-18)
NIHSS change at 24h	-6.5 (-10 – 0)
IPR (ml/hr)	7.5 (4.5-18.2)

Table 2-1 M1 occlusion cohort baseline data

Baseline characteristics	Mean±SD, Median (IQR), or proportion
Age	71±12
Male sex	63/118 (53%)
Baseline NIHSS	14.5 (11-18)
Baseline systolic pressure	146±21mmHg
Baseline diastolic pressure	77±16 mmHg
History of hypertension	65/118 (55%)
History of antiplatelet use	62/118 (53%)

Table 2-2 - Blood brain barrier permeability cohort baseline data

3 Is outcome after recanalisation in ischaemic stroke mediated by follow up infarct volume?

3.1 Introduction

Early recanalisation aims to restore blood flow to the ischaemic brain prior to infarction thus limiting the volume of tissue progressing from ischaemic penumbra to infarct. However, whether the benefit of recanalisation is entirely explained by the reduction of volume of infarcted tissue is less clear. Benefit may be measured in terms of functional outcome or early neurological recovery. Infarct volume measurement is open to variability in practice with imaging modality, time since onset of infarction, degree of brain oedema, and inter-rater discordance all affecting measurements. Furthermore the eloquence of the affected brain regions may impact outcome over and above the volume of infarction alone. A study of data from the MR-CLEAN trial concluded that only 14% of the beneficial effect of MT on outcome was mediated by follow up infarct volume (FIV) (131). A larger study of the HERMES cohort similarly attributed 12% of the benefit of MT to be mediated by FIV (132). More recent analyses have found higher proportions of mediation, a further study of MR CLEAN data measuring benefit on early neurological recovery found mediation of 34% by FIV (133), and a study involving the German Stroke Registry found FIV mediated 56% of the benefit on outcome of recanalisation with MT (134).

Given the use of FIV as a marker of outcome in this thesis, we sought to explore the extent to which FIV mediates outcome in our study population. In this analysis we studied a cohort of AIS patients receiving IVT and assessed to what extent the effects of recanalisation were mediated by FIV.

3.2 Methods

We used data from the M1 occlusion cohort described in chapter 2. One case was excluded due to missing data on recanalisation.

Mediation analysis was performed on SPSS using the PROCESS macro (135, 136). Mediation analysis involves introducing a mediating variable, M, to a linear regression involving 2 variables, predictor variable X and outcome variable Y. X

may have an effect on Y either directly or through the mediating variable M. The relationships between X, Y and M are shown in figure 3-1 along with the relationships between the variables denoted a, b and c'.

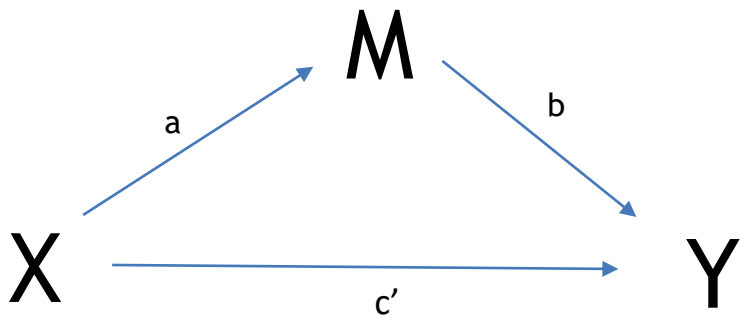


Figure 3-1 Mediation - variable relationships

Mediation is significant when zero falls outside of the lower and upper limit confidence intervals, calculated using bootstrapping, for the indirect effect of X on Y through M. A p value for mediation was calculated using Sobel's test (137).

In this analysis we chose recanalisation as the predictor variable X and FIV as the mediator M. Outcome variables (Y) included the modified Rankin scale (mRS) at 90 days as a measure of functional outcome, and National Institute of Health Stroke Scale (NIHSS) change at 24 hours as a measure of early neurological improvement. mRS was dichotomised into 0-2 as good outcome and 3-6 as poor outcome and assessed ordinally. NIHSS change was calculated as the difference between baseline and 24-hour NIHSS or dichotomised into those with or without 'major NIHSS change' defined as a decrease in NIHSS of ≥8 points or an improvement to 0-1. Co-variates included in the modelling were age, thrombolytic drug, ischaemic progression rate (IPR), and for the mRS models only, baseline NIHSS.

3.3 Results

75 cases of patients with M1 occlusions treated with thrombolysis were included. Baseline characteristics are described in table 3-1. Median NIHSS was 15 (13-18). 56 cases achieved recanalisation, defined as TIMI score 2 or 3 on repeat CTA at 24 hours. Median FIV was 32ml (16.1-75.7). 38 cases were mRS 0-2 at day 90.

Recanalisation was a significant predictor of all the outcome variables tested (likelihood of mRS 0-2, mRS, major NIHSS change, 24hr NIHSS change) when accounting for the stated covariates. Addition of FIV as a mediating variable resulted in recanalisation becoming non-significant for all variables except likelihood of mRS 0-2 for which it remained significant. After adjusting for age and drug allocation, recanalisation and FIV were independently predictive of the likelihood of mRS 0-2 (Recanalisation OR 14.9 (1.6-139.3) p=0.018, FIV OR 9.4 (9.1-9.7) per 10ml change, p=0.000).

FIV was a significant mediator of the relationship between recanalisation and all 4 outcomes, as shown in table 3-2. FIV accounts for 63%, 44%, 74% and 56% of the effect of recanalisation on likelihood of mRS 0-2, mRS, major NIHSS change, 24hr NIHSS change respectively. Results are shown in figure 3-2.

Baseline characteristics	Alteplase N=28	Tenecteplase N=47	p-value
Age (Years)	69±14	71±10	0.388
Baseline NIHSS	15.5	15	0.262
NIHSS change at 24h	-1	-9	0.007
Major NIHSS change	7 (25%)	28 (60%)	0.004
Recanalisation	19 (68%)	37 (79%)	0.295
IPR (ml/hr)	16.2±11.0	10.9±13.8	0.089
Baseline core volume (ml)	36.6±27.9	24.8±27.6	0.077
Baseline penumbra volume (ml)	63.3±29.8	47.5±28.4	0.025
Follow up infarct volume (ml)	98.0±107.4	53.7±70.0	0.034
Collateral status[1] (Tan score)	Good 10 (40%) Moderate 9 (36%) Poor 6 (24%)	Good 9 (22%) Moderate 22 (54%) Poor 10 (24%)	0.248
mRS 0-2 at day 90	10 (36%)	28 (60%)	0.046

Table 3-1 Baseline characteristics
Age, ischaemic progression rate (IPR) and imaging volumes are shown as mean±standard deviation
Baseline National Institute of Health Stroke Scale (NIHSS) and change in NIHSS shown as median and interquartile range
Major NIHSS change, recanalisation, collateral status and modified Rankin scale (mRS) shown as proportions
[1] Collateral status data only available for 66 cases

Outcome variable	Odds ratio of direct effect	p value	Mediating effect coefficient	p value	Mediator effect (%)
mRS 0-2	70.3 (2.8-1741.7)	0.009	7.22 (2.80 - 682.41)	0.03	62.9%
mRS (ordinal)	0.5 (0.2-1.2)	0.106	-0.56 (-1.04 - -0.16)	0.02	44.0%
Major NIHSS change	3.2 (0.0-23.4)	0.247	3.36 (0.98 - 11.41)	0.03	74.2%
NIHSS change	0.2 (0.0-3.0)	0.213	-2.38 (-4.01 - -0.82)	0.01	55.9%

Table 3-2 Prediction of outcome by recanalisation
The predictor variable is recanalisation and mediating variable is FIV in all cases

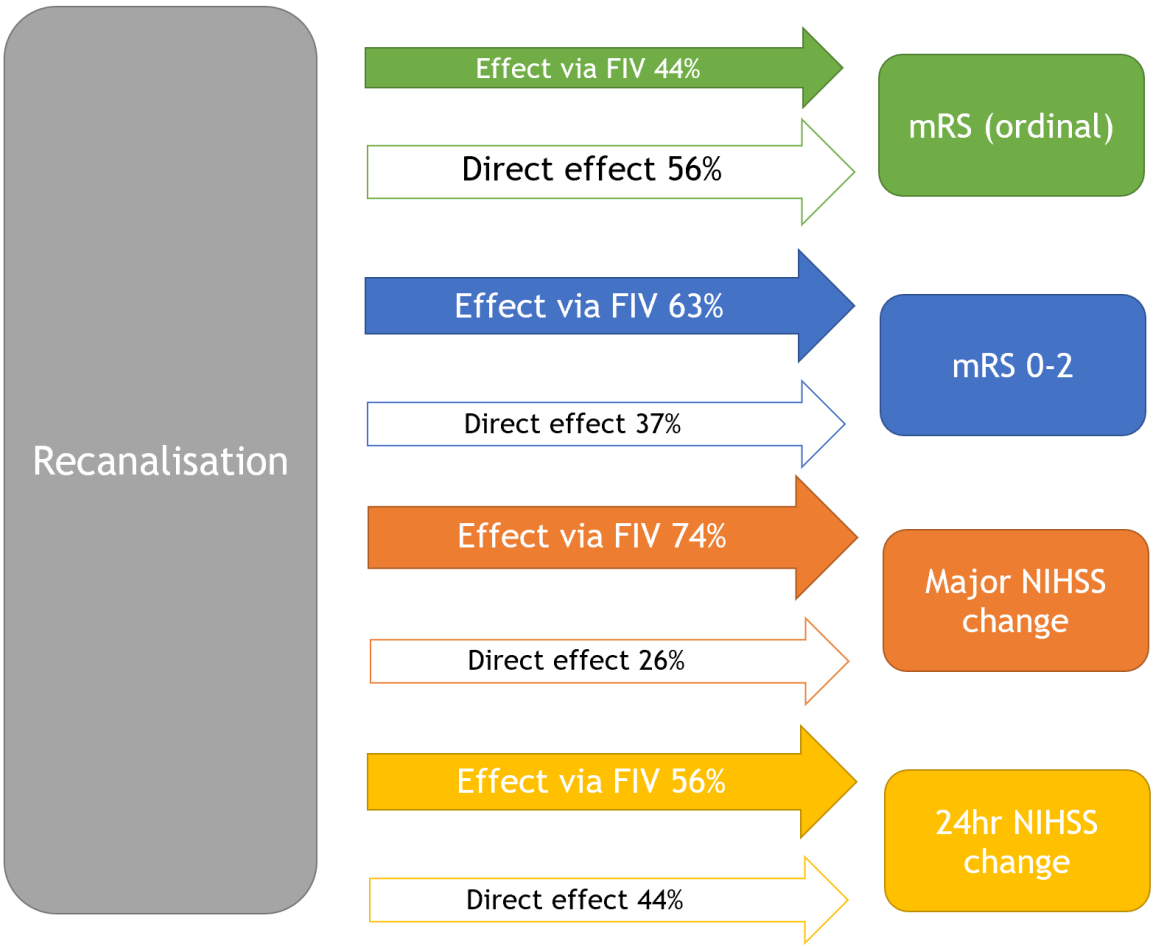


Figure 3-2 Effect of recanalisation on outcomes directly and via FIV

3.4 Discussion

FIV relates to clinical outcome in a non-linear manner, with moderate to large (>15ml) infarcts predicting poor clinical outcome with increasing FIV but small infarcts not being predictive (138). Successful recanalisation may result in improved clinical outcomes through the reduction in FIV (139). Previous research explored the relationship between endovascular treatment (regardless of recanalisation status) and outcome, and reported a mediation effect of FIV of only 12-14% (131, 132). Our results suggest that FIV mediates a high proportion – between 44 and 74% - of the effect of recanalisation on early neurological recovery and day 90 functional outcome after acute ischaemic stroke in the context of treatment with thrombolytic drugs. These results are in line with more recent analyses which have found higher proportions of mediation. A further study of MR CLEAN data measuring benefit on early neurological recovery, rather than functional outcome at 90 days, found mediation of 34% by FIV (133). A study involving the German Stroke Registry found FIV mediated 56% of the benefit on outcome of recanalisation with MT (134). A recent analysis of HERMES data found that differences of 2, 10 and 20ml in FIV at 48 hours correlated with 1%, 5% and 10% absolute increases in likelihood of achieving functional independence after MT (140).

There are several potential explanations for these differing results. Firstly, our study had a much smaller sample size than studies based on larger randomised trials of endovascular thrombectomy, though the magnitude of difference in mediation effect may not be explained by differences in sample size alone.

Secondly, as in the German Stroke Registry study, we used recanalisation from studies of IVT as our predictor variable, as opposed to previous studies which compared MT-treated groups with those receiving best medical therapy. Thus, we were comparing cases of successful versus non-successful recanalisation rather than comparing groups which differed based on allocated treatment group. Our findings are relevant to the biological question of interest, exploring the relationship of infarct volume to early and late recovery from stroke in relation to recanalisation. Previous investigations that used randomised allocation to thrombectomy or control groups may obscure the biological relationship since around 25% of patients randomised to thrombectomy do not

achieve satisfactory reperfusion, and a proportion of those in the control arm (around 90% of whom received thrombolytic drugs as standard of care) may have recanalised between the time of trial entry and follow-up imaging. The relationship of FIV and treatment effect are potentially relevant in considering whether FIV represents a useful biomarker of early outcome in a trial context.

Thirdly, the differing results may reflect the non-standardised manner of collecting FIV data. Routinely, infarct volume is assessed on 24-hour follow up imaging as was the case in our study. In cases with slowly progressive infarcts, the area of infarction seen on imaging at 24 hours may not represent a final infarct volume which would require to be assessed at a later timepoint, as demonstrated by infarct growth rates up to day 5 after onset in the DEFUSE-2 study (141). Infarct volumes measured by FLAIR imaging continue to evolve past day 5, owing to lesion oedema the volume typically peaks around day 3-8 and then subsides, approaching the final volume by day 30 (142). This reflects that any measure of follow up infarct volume is a single snapshot of a dynamic process. Volumetric assessment methods may also vary, as will the modality of imaging at follow up. Assessment of CT in the early time period is complicated by difficulty in clearly delineating areas of hypoattenuation, oedema and chronic background changes, leading to lower inter-rater reliability (143). Patterns of infarction have an effect on outcome – scattered infarction, grey matter involvement alone and sparing of corticospinal tracts are all associated with better clinical outcome regardless of total FIV (144). Confinement to grey matter alone was not significantly related to outcome when using only CT, presumably as grey-white matter differentiation is easier on MRI, indicating the importance of the follow up imaging modality.

In our mediation models, the direct effect of recanalisation on outcomes when FIV was included as a mediator was only found to be significant for likelihood of mRS 0-2 and not for other variables. Whilst significance of direct effect was previously thought to be required to show mediation, contemporary literature concludes that mediation can be demonstrated either when only the indirect effect is significant (full mediation) or when both direct and indirect effects are significant (partial or complementary mediation) (145).

3.5 Conclusion

FIV is a significant mediator of the relationships between recanalisation and functional outcome or early neurological recovery. The degree to which FIV mediates these relationships was significantly higher in our study than previous literature. FIV and recanalisation are independently predictive of likelihood of functional independence at 90 days in our study.

4 Treatment effect of Alteplase versus Tenecteplase in slow and fast progressors

4.1 Introduction

Early reperfusion after the onset of large vessel occlusion (LVO) improves functional outcome after acute ischaemic stroke (AIS) (146). Infarct growth following LVO progresses at different rates in different individuals (147) depending upon factors such as collateral circulation (148). In some the rate at which the volume of infarction enlarges is rapid, termed 'fast progressors', and in 'slow progressors' significant volumes of tissue remain viable for a longer period. Reperfusion remains beneficial up to 24 hours after symptom onset among patients with small core volumes and LVO (149, 150). A secondary analysis of the DEFUSE-2 study (141) found slower growth of DWI lesions to be associated with improved likelihood of good functional outcome following endovascular reperfusion.

Early time window (<6 hours) trials suggest tenecteplase may be non-inferior to alteplase in terms of functional outcome (72, 151). Where intravenous thrombolysis (IVT) is given prior to endovascular treatment, tenecteplase was associated with increased early recanalisation in the EXTEND IA TNK trial (69), however this was not in a LVO subgroup of the AcT trial (152), or in registry data from the TETRIS and ETIS registries (153, 154).

Modified randomisation in clinical trials commonly stratifies treatment allocation to ensure balance of key prognostic variables such as age or stroke severity among groups. Trials with small sample sizes, however, are vulnerable to imbalances in key physiological features that may not be immediately available, such as the proportion of patients with LVO, ischaemic core volume or different collateral status. Statistical analyses can adjust for unbalanced representation of prognostic variables, but failure to include relevant prognostic variables in analysis may result in treatment effects potentially being missed or misattributed to the trial intervention rather than between-groups physiological differences. We hypothesised that infarct growth rate, or ischaemic progression rate (IPR), was one such variable and examined whether its effect on outcome is modified by treatment allocation to alteplase or tenecteplase.

4.2 Methods

We included data for patients with M1 occlusion as described in chapter 2. The following parameters were collected from the datasets: drug allocation; time from symptom onset to scanning; recanalization at 24 hours; age; NIHSS at presentation; baseline blood glucose; diabetic status; baseline ischaemic core volume (defined as relative cerebral blood flow<30% and Tmax>6s); baseline penumbra volume and follow-up infarct volume (FIV). Ischaemic progression rate (IPR) was calculated in ml/hr by dividing the ischaemic core volume by the time from symptom onset to scan. Infarct growth (IG) was calculated as the final infarct volume minus the baseline core volume, where this was negative it was considered as zero. Volume of penumbra salvaged (VPS) was calculated as baseline penumbra minus the difference between FIV and core, where this value was negative it was considered as zero. A percentage was calculated for VPS, values below zero and above 100 were considered as zero and 100 respectively. Data were tested for normal distribution using the Kolmogorov-Smirnov test and data that were non-normally distributed were transformed using log transformation.

In comparison of baseline clinical and imaging characteristics, comparison of means was calculated using T-tests or Mann-Whitney tests for parametric and non-parametric variables respectively, and Pearson Chi-Square used to calculate differences in proportions.

Linear regression using the enter method was used to assess the relationships between variables. FIV, IG and VPS were chosen as the dependent variables because the two clinical trials were designed to detect a difference in imaging outcome and not functional benefit. Predictor variables included IPR, recanalization at 24h, thrombolytic agent allocation and baseline penumbra volume. Age, collateral score, baseline glucose and diabetes were also assessed as predictors of IPR.

In a subgroup analysis we compared slow and fast progressors only, defining slow and fast progressors as those with IPR <5ml/hr and >15ml/hr respectively, based upon similar definitions used in the literature (155). Linear regression was used to assess factors predictive of NIHSS change at 24 hours and logistic regression

was used to assess factors predictive of functional independence at 90 days (modified Rankin Scale ≤2).

4.3 Results

The ATTEST trial involved 104 participants with 52 each randomised to alteplase and tenecteplase. The Australian TNK study recruited 75 patients with 25 each randomised to alteplase or either of two doses of tenecteplase. After exclusion of cases without M1 occlusion and non-thrombolysed cases, 29 cases from ATTEST (13 alteplase, 16 tenecteplase) and 47 cases from the Australian TNK study (15 alteplase, 16 at each dose of tenecteplase) were included in the analysis.

In 76 cases of M1 occlusion, 28 were treated with alteplase, 32 and 16 with 0.25mg/kg and 0.1mg/kg tenecteplase respectively. Groups were well matched for age, median NIHSS and time from symptom onset to scan (table 4-1). There were significantly more patients with diabetes in the tenecteplase group than the alteplase group (27% vs 7% p=0.035). There was a higher mean ischaemic progression rate in the alteplase group (mean IPR 16.2ml/hr vs 10.8ml/hr for alteplase and tenecteplase respectively, p=0.04). Tenecteplase-treated patients exhibited a significantly greater improvement in NIHSS at 24 hours and a trend towards greater likelihood of functional independence at 90 days compared to alteplase-treated patients. Patients receiving alteplase had significantly larger baseline core and penumbra volumes and final infarct volumes.

Regression results are shown in table 4-2. FIV was significantly associated with IPR and 24h recanalisation. Drug allocation did not significantly modify the interaction of FIV, IPR and recanalization. This remained the case when a sensitivity analysis was undertaken that included only those patients who received tenecteplase 0.25mg/kg. Baseline penumbra volume, diabetes, baseline blood glucose and an interaction variable including both IPR and thrombolytic agent were not significantly predictive of FIV. A non-significant trend towards lower FIV in cases with faster IPR treated with tenecteplase compared with alteplase was seen (figure 4-1).

IG was significantly predicted by IPR and 24h recanalisation but not by drug allocation. Percentage VPS was predicted by recanalisation but not by IPR or drug allocation.

Collateral status was predictive of IPR when accounting for age, diabetes and baseline glucose.

In subgroup analysis (slow and fast progressors only), functional independence at 90 days was predicted by IPR ($p=0.030$). Drug allocation was found to be predictive of 24 hr NIHSS change ($p=0.003$).

	tPA 0.9mg/kg n=28	TNK either dose n=48	P-value		TNK 0.25mg/kg n=32	TNK 0.1mg/kg n=16
Age	69 ±14	72 ±10	0.34		71 ±10.7	73 ±7.2
Baseline NIHSS	15.5 (12 – 18)	15 (13 – 16)	0.87		15 (13 – 16)	15 (13 – 17)
Diabetes	2/28 (7%)	13/48 (27%)	0.035		7/32 (22%)	6/16 (38%)
Symptom onset to scan (mins)	154.4 ±58.0	153.9 ±50.9	0.97		156.4 ±50.8	148.9 ±52.5
IPR (ml/hr)	16.2 ±11.0	10.8 ±13.7	0.04		13.9 ±15.7	4.8 ±4.1
Recanalisation	19/28 (68%)	37/47 (79%)	0.295		25/32 (78%)	12/15 (80%)
24 h NIHSS improvement	1 (-2-8.5)	8.5 (3-11.75)	0.001		9 (1.5-12)	7.5 (3-10)
mRS 0-2 at 90 days	10/28 (36%)	28/48 (58%)	0.057		18/32 (56%)	10/16 (63%)
Collateral score	Good 9 (32%) Moderate 9 (32%) Poor 6 (21%) N/A 3 (11%)	Good 9 (19%) Moderate 22 (46%) Poor 10 (21%) N/A 7 (15%)			Good 7 (22%) Moderate 15 (47%) Poor 6 (19%) N/A 4 (13%)	Good 2 (13%) Moderate 7 (44%) Poor 4 (25%) N/A 3 (19%)
Mortality (deceased at day 90)	4/28 (14%)	5/48 (10%)	0.44		3/32 (9%)	2/16 (13%)
ICH	Nil 16/28 (57%) HI1 6/28 (21%) HI2 1/28 (4%) PH1 1/28 (4%) PH2 4/28 (14%)	Nil 30/48 (63%) HI1 9/48 (19%) HI2 7/48 (15%) PH1 1/48 (2%) PH2 1/48 (2%)			Nil 22/32 (69%) HI1 7/32 (22%) HI2 3/32 (9%) PH1 0/32 (0%) PH2 0/32 (0%)	Nil 8/16 (50%) HI1 2/16 (13%) HI2 4/16 (25%) PH1 1/16 (6%) PH2 1/16 (6%)
Baseline core volume (ml)	29 (13-55)	25 (6-26)	0.016		20 (13-44)	9 (4-17)
Baseline penumbra volume (ml)	69 (37-83)	39 (29-62)	0.034		44 (28-73)	37 (30-39)
Follow-up infarct volume (ml)	58 (24-141)	26 (13-65)	0.028		36 (16-65)	22 (8-57)
Infarct growth (ml)	64.2 ±85.2	31.2 ±51.0	0.039		36.8 ±59.5	19.2 ±22.0

Table 4-1 - Baseline results – tPA = alteplase, TNK = tenecteplase, ± = mean±SD, NIHSS + volumes = median (IQR)

Dependent	Independent	R^2 adj	Significance
Final infarct volume	Recanalisation IPR Drug	0.369	0.004 0.000 0.222
Final infarct volume	Recanalisation IPR Baseline penumbra volume	0.364	0.007 0.000 0.332
Final infarct volume	Recanalisation IPR Diabetes	0.356	0.003 0.000 0.802
Final infarct volume	Recanalisation IPR Baseline glucose	0.358	0.003 0.000 0.559
Final infarct volume	Recanalisation IPR	0.364	0.007 0.000 0.332
Infarct growth	Recanalisation IPR Drug	0.200	0.011 0.014 0.157
Percentage volume of penumbra salvaged	Recanalisation IPR Drug	0.374	0.003 0.923 0.333
Ischaemic progression rate	Age Collateral status Baseline glucose Diabetes	0.105	0.227 0.010 0.558 0.065
NIHSS change	Slow or fast progressor Drug	0.236	0.620 0.003
Functional independence (mRS 0-2)	IPR Drug	0.238	0.030 0.447

Table 4-2 - Regression results - R^2 adj = adjusted R squared

Functional independence was assessed using logistic regression. The remaining rows are results of linear regression using enter method

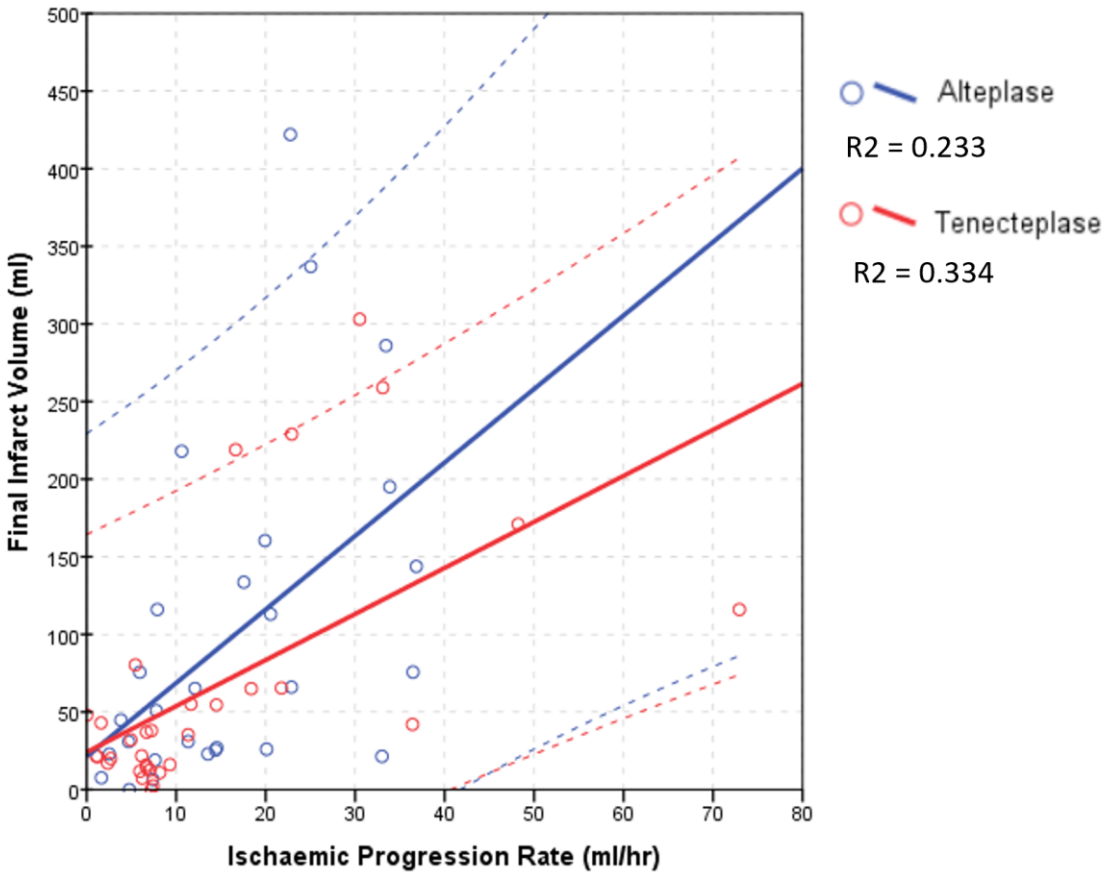


Figure 4-1 - Plot of IPR vs FIV in drug groups - R2 = R squared

4.4 Discussion

In this analysis of pooled individual patient data from two RCTs comparing alteplase with tenecteplase in AIS, we confirmed previously reported associations of tenecteplase with better recanalisation and clinical outcome in the subgroup of patients with large vessel occlusion and favourable perfusion imaging (69). FIV and IG was predicted by recanalisation in our analysis and a trend towards lower FIV in patients treated with tenecteplase adjusted for IPR was seen compared to alteplase-treated patients.

We found IPR to be an independent predictor of FIV and IG. This is consistent with previous observations such as the DEFUSE-2 study (141) in which patients with MRI-defined slower infarct growth rates had an increased likelihood of favourable clinical response at 30 days (improvement in National Institute of Health Stroke Scale (NIHSS) by 8 points or score of 0-1) and good functional outcome at 90 days (modified Rankin scale 0-2). In multivariate analysis, the initial growth rate was an independent predictor of poor functional outcome with a odds ratio of 1.51 (1.06-2.14) for each ml/hr increase in growth rate. Arising from this, the concept of “fast progressors” recognises a subgroup of patients with greater vulnerability to ischaemic damage. Many trials deploy selection criteria that will exclude a proportion of fast progressors, such as the presence of extensive CT hypoattenuation (low Alberta stroke programme early CT (ASPECTS) score), or large core volume on perfusion imaging. However, several of these are subjective, may not be reliably interpreted at early time points, and, since they do not explicitly include time, offer limited prediction of individual ischaemic lesion evolution. While imaging factors associated with fast progression may be incorporated into selection or adjustment variables, IPR potentially integrates these factors into a single quantifiable index of ischaemic vulnerability.

We believe our results demonstrate the potential value of including IPR as an adjustment variable in evaluation of reperfusion therapies. In patients with middle cerebral M1 occlusions and favourable perfusion profiles (small core and large penumbra volumes), tenecteplase was associated with higher rates of early recanalisation than alteplase. Characterisation of recanalization rates in a population that includes fast progressors will be informative both with respect to

efficacy and safety, since delayed reperfusion of large ischaemic core patients is associated with higher likelihood of symptomatic intracerebral haemorrhage.

IPR may be relevant in evaluating baseline populations entering clinical trials, particularly phase 2 trials with smaller sample sizes that include imaging. Exclusion of patients with large ischaemic core selects against fast progressors in study populations, meaning efficacy and risks specific to fast progressors may be under-reported. Whilst fast progressors may be excluded from late window trials, early window trials recruiting fast progressors will be informative about treatment benefit and risk in this population.

Effective neuroprotective agents which aim to freeze the ischaemic penumbra (156) may be most beneficial in fast progressors as a bridge to reperfusion therapy. Neuroprotectant therapies in ischaemic stroke have frequently shown promise in animal studies but none have yet shown efficacy in human trials (157). IPR may also be informative in this setting. Efficacy of neuroprotectants may be best demonstrated in fast progressors in whom degradation of the penumbra to core will occur quickly before reperfusion unless slowed by a neuroprotectant. Reperfusion in later time windows may continue to be beneficial in fast progressors treated with neuroprotectants. Participants in the ESCAPE NA-1 trial (103) of nerinetide in AIS included 62% and 65% with good collateral status in the placebo and intervention groups respectively. The majority of participants are therefore likely to be slow progressors in whom the neuroprotective effect may be less pronounced.

This study makes some assumptions about the nature of the ischaemic core. Firstly, we assume that the initial growth rate of the ischaemic core is uniform and linear starting from zero at the time of symptom onset, allowing a ml/hr calculation of the IPR to be made. Linear growth in the first 24-72 hours has been suggested in previous studies using serial MRI (141, 158). Secondly we assume that the areas of core perfusion reflect tissue destined to infarct. The exact correlation of early radiological ‘core’ lesions with final infarcts is not definite. DWI lesion reversal with early reperfusion has been noted in the DEFUSE-2 and EPITHET studies though this can be transient and not correlate with clinical change (159, 160). Similarly core identified by CT perfusion assessments of cerebral blood volume <1.5 mL/100 mL (161) or relative cerebral

blood flow <30% (162) were derived from studies predominantly undertaken in the pre-thrombectomy era when rapid and complete reperfusion was uncommon, and core by these definitions was found to be larger than final infarct volumes following early reperfusion, leading to the coining of the term ‘ghost core’. A recent review (163) proposed a revision of the term ischaemic core to “severely ischemic tissue with uncertain viability” (SIT-uv), citing a lack of a gold standard definition of ischaemic core; as yet poorly understood relationships between the length of reduced cerebral blood flow and progression of ischaemia; and differing response to ischaemia among brain cell types leading to incomplete infarctions. The authors also highlight the need to incorporate IPR in treatment decision making and highlight extraction of IPR from imaging data as an area requiring further research.

Limitations of our study include a small sample size, retrospective analysis of the data, and use of tenecteplase at two different doses. However, the regression results were not significantly altered by limiting the data to only tenecteplase 0.25mg/kg. The 0.1 mg/kg is lower than the doses used in most recent stroke trials of tenecteplase. A higher dose of 0.4mg/kg has been trialled but appears to offer no significant benefit over 0.25mg/kg in patients being treated with thrombolysis prior to endovascular treatment (70).

4.5 Conclusion

In two RCTs of alteplase and tenecteplase in AIS we found IPR to be an independent predictor of FIV and IG and found a non-significant trend towards lower FIV in fast progressors treated with tenecteplase over alteplase. IPR may be an informative variable to examine in trials of reperfusion and neuroprotective therapies. Tenecteplase through earlier recanalisation may be a superior treatment option for fast progressors. Further RCTs of tenecteplase are ongoing and present an opportunity to evaluate IPR as an adjustment variable.

5 Differences between alteplase and tenecteplase in prediction of haemorrhagic transformation by blood brain barrier permeability

5.1 Introduction

Haemorrhagic transformation (HT) following acute ischaemic stroke is associated with poorer outcome (164). Previous studies have found factors predictive of risk of haemorrhagic transformation to include size of the hypoattenuating lesion on non-contrast CT; prior anticoagulation; blood glucose; haemoglobin; degree of mass effect; presence of FLAIR lesion on acute MRI; and more severe decreases in apparent diffusion coefficient (ADC), cerebral blood flow (CBF) and cerebral blood volume (CBV) (165-167). In an animal model, disruption of the blood brain barrier (BBB) demonstrated by gadolinium enhancement was found to predict areas of subsequent thrombolysis induced haemorrhage (168).

Bivard et al used measures of BBB permeability derived from CTP scans to examine their diagnostic utility versus previously reported predictors of HT (169). These included extraction fraction (E) – the fraction of contrast agent removed during first pass; permeability-surface area product (PS) – the flow of molecules through capillary membranes; and the transfer constant (K_{trans}) – a combination of tissue blood flow and PS. Analysis of 1407 scans revealed a threshold of 30% of E within the acute perfusion lesion to be most reliable measure of HT prediction, and to be superior to conventional perfusion measures such as ischaemic core volume, very low CBV and severely delayed perfusion.

Tenecteplase has greater fibrin specificity (170) than alteplase and thus may be less likely to cause HT in AIS.

We examined whether HT incidence differed amongst patients with AIS treated with alteplase or tenecteplase whilst accounting for measures of BBB permeability.

5.2 Methods

The dataset is described in chapter 2. Scans were analysed using MIStar image processing software (Melbourne, Australia) to generate maps and calculate volumes of ischaemic core (relative cerebral blood flow (rCBF) <30% and <20%); ischaemic penumbra (delay time >3seconds and rCBF >30% or >20%); E above a threshold of 30% (E30); K_{trans} and PS above a threshold of 70ml/min/100g (K_{trans}70 and PS70 respectively). The proportion of the ischaemic lesion, expressed as a percentage, which exceeded the thresholds for each of the BBB permeability measures was calculated (E30%, K_{trans}70% and PS70%). Other data collected included age, sex, drug allocation, history of hypertension, baseline blood pressure, history of antiplatelet use, National Institute of Health Stroke Scale (NIHSS) at baseline and 24 hours; modified Rankin scale (mRS) at 90 days; onset to treatment time; and ICH at follow up defined as per ECASS-2 criteria (171).

Stepwise logistic regression was used to determine factors predictive of HT. Models were adjusted to include variables of clinical interest and relevance. A two block analysis was used with variables of interest in the first block and drug allocation in the second. Models were examined comparing any HT vs none; significant HT (HI2/PH1/PH2) vs non-significant HT (no ICH or HI1); any parenchymal haemorrhage (PH) vs no PH; and symptomatic ICH (sICH - ICH with worsening of NIHSS by ≥4) vs non-symptomatic ICH.

5.3 Results

A total of 118 cases were identified which fulfilled our criteria, 53 treated with alteplase and 65 with tenecteplase. Baseline clinical and imaging characteristics are displayed in table 5-1 and were similar between groups. Compared to the alteplase group the tenecteplase group trended towards a higher baseline NIHSS though the absolute difference of 1 point was of minimal clinical significance.

HT occurred in 22/53 (41%) of alteplase and 19/65 (29%) of tenecteplase cases. There were 5/53 PH2 haemorrhages and 4/53 symptomatic ICH (all PH2) in the alteplase group compared with 2/65 of each in the tenecteplase group. Comparing HT and non-HT cases, a higher E30% was seen with tenecteplase (mean difference +15.9%, p=0.004) but not alteplase (+9.4% p=0.110) as seen in

figure 5-1, suggesting tenecteplase was associated with HT only in cases with significantly higher BBB permeability, as opposed to alteplase being associated with HT across in cases of low and high E30%. K_{trans}70 and PS70 were found to be mostly identical, therefore only K_{trans}70 was interrogated in regression modelling.

Logistic regression results are shown in table 5-2. In prediction of HT versus no HT a number of imaging markers were closely correlated – core volume (CBF<30), E30% and K_{trans}70%. The variable with lowest p-value in logistic regression, E30% (p=0.076), was chosen over CBF<30 (p=0.654) and K_{trans}70% (p=0.356) for further modelling.

Baseline NIHSS and E30% were found to be independent predictors of occurrence of any HT when adusting for age, systolic blood pressure, history of antiplatelet use and onset to treatment time. Each unit increase of NIHSS corresponded to 13% increased risk of HT (p=0.012) and each 10% increase of E30% corresponded to 30% increased risk of HT (p=0.027). After adjustment for all the above variables there was a trend for reduced HT incidence with tenecteplase compared to alteplase (OR 0.44(0.19-1.02), p=0.056).

In prediction of significant HT (nil/HI1 versus HI2/PH1/PH2) systolic blood pressure at admission was a predictor in a model adjusting for age, antiplatelet use, onset to treatment time and baseline NIHSS; each 10mmHg increase of systolic BP corresponded to 30% increased risk of HT (p=0.032). E30% was not predictive of significant HT in this model. Drug allocation to tenecteplase was significantly associated with lower risk of significant HT (OR 0.33(0.12-0.92), p=0.033).

In prediction of any PH vs no PH, history of antiplatelet use was strongly associated with risk of PH (OR 8.15(1.46-45.44), p=0.017), systolic blood pressure at baseline was also predictive of risk of PH with each unit increase corresponding to a 4% increased risk of PH (p=0.036). Drug allocation to tenecteplase was significantly associated with lower risk of PH (OR 0.22(0.05-0.94), p=0.041). Due to the low numbers of symptomatic ICH in both groups no significant predictive relationships were found in logistic regression.

	Alteplase (n=53)	Tenecteplase (n=65)	P value	TNK0.25 (n=49)	TNK0.1 (n=16)
Age	71 ± 12	70 ± 11	0.30	70 ± 12	71 ± 8
Male Sex	27 (51%)	36 (55%)	0.59	26 (53%)	10 (62%)
History of hypertension	31 (59%)	34 (52%)	0.47	24 (49%)	10 (62%)
History of antiplatelet use	25 (47%)	37 (57%)	0.28	29 (59%)	8 (50%)
Systolic BP	147 ± 20	146 ± 23	0.31	147 ± 25	143 ± 14
Diastolic BP	75 ± 16	78 ± 16	0.59	77 ± 17	81 ± 13
NIHSS0	14 (10-18)	15 (12-18)	0.05	15 (12-18)	15 (13-17)
NIHSS change	-2 ± 7	-5 ± 7	0.68	-5 ± 7	-6 ± 5
Onset to treatment (mins)	183 ± 47	181 ± 42	0.13	179 ± 39	186 ± 53
Scan length	58 ± 9	56 ± 10	0.64	58 ± 10	51 ± 4
ICH	Nil 31 (59%) HI1 8 (15%) HI2 6 (11%) PH1 3 (6%) PH2 5 (9%)	Nil 46 (71%) HI1 10 (15%) HI2 5 (8%) PH1 2 (3%) PH2 2 (3%)	0.10	Nil 37 (76%) HI1 9 (18%) HI2 1 (2%) PH1 1 (2%) PH2 1 (2%)	Nil 9 (56%) HI1 1 (6%) HI2 4 (25%) PH1 1 (6%) PH2 1 (6%)
sICH	4/53 (8%)	2/65 (3%)	0.25	1/49 (2%)	1/16 (6%)
Core volume CBF<20	6 (1-18)	6 (2-13)	0.74	6 (2-15)	5 (0-12)
Penumbra volume CBF <20	51 (30-79)	49 (32-83)	0.57	47 (28-77)	61 (35-104)
Core volume CBF<30	16 (6-32)	15 (6-27)	0.48	15 (7-29)	14 (6-21)
Penumbra volume CBF <30	42 (27-65)	44 (24-71)	0.72	42 (20-67)	47 (27-96)
E30 volume	21 (8-35)	20 (10-36)	0.95	19 (7-32)	26 (13-49)
E30 percentage	39 (26-56)	45 (29-60)	0.90	47 (29-60)	42 (25-58)
Ktrans70 volume	14 (7-28)	15 (7-27)	0.47	15 (6-27)	17 (10-35)
Ktrans70 percentage	29 (18-45)	34 (25-47)	0.56	36 (27-48)	26 (23-40)

PS70 volume	14 (7-28)	15 (7-27)	0.49	15 (6-27)	17 (10-36)
PS70 percentage	29 (18-45)	34 (25-47)	0.54	36 (27-48)	28 (23-40)

Table 5-1 - Baseline clinical and imaging characteristics

n ± x = mean ± standard deviation

n(%) = number and proportion

n (x-y) = median (interquartile range)

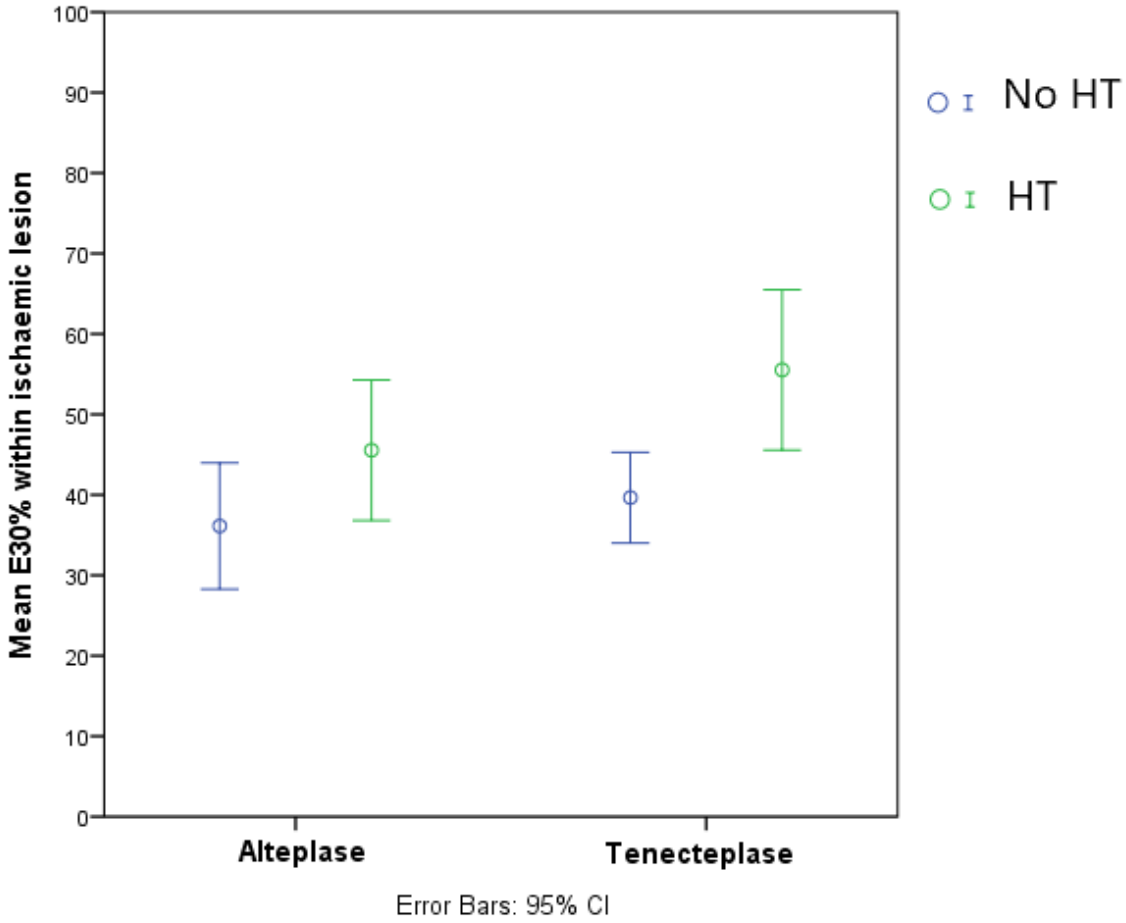


Figure 5-1 – Comparison of mean E30% in cases with and without HT

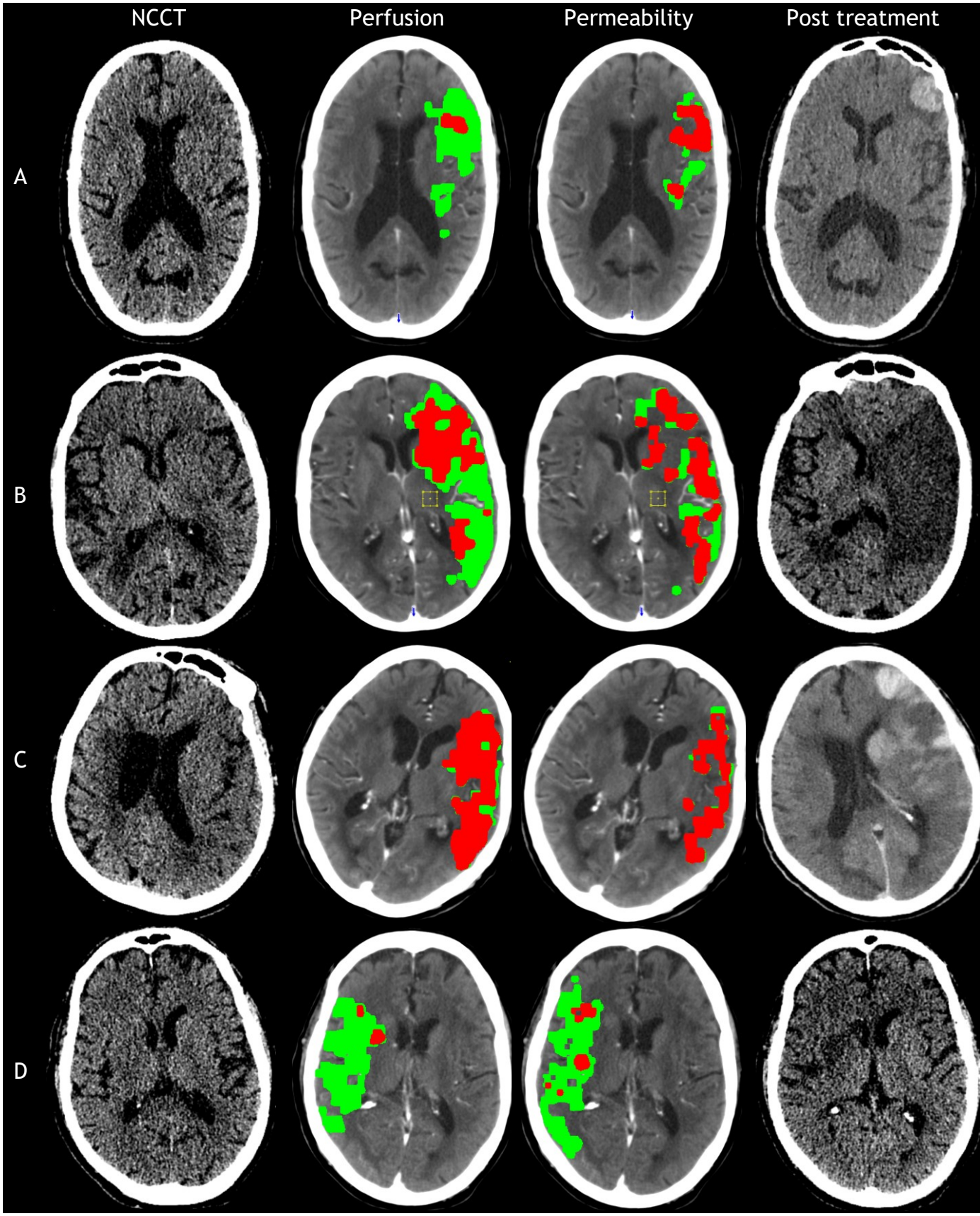


Figure 5-2 – Examples of HT prediction using BBB permeability measures

A – NIHSS 16, Alteplase at 150 mins. Core 3ml Penumbra 49ml, E30% 53%. PH1, mRS 2
B – NIHSS 25, Tenecteplase at 170 mins. Core 63ml Penumbra 70ml, E30% 73%. No HT, mRS 5
C – NIHSS 21, Tenecteplase at 110 mins. Core 83ml, Penumbra 14ml, E30% 84%. PH1, mRS 6
D – NIHSS 10, Alteplase at 267 mins. Core 5ml, Penumbra 63ml, E30% 12%. No HT, mRS 0

	Odds ratio	95% confidence interval	p-value
Any HT vs No HT			
Age	0.99	0.95 - 1.03	0.614
Systolic BP	1.02	1.00 - 1.04	0.087
History of antiplatelets	0.98	0.41 - 2.31	0.953
Baseline NIHSS	1.13	1.03 - 1.24	0.012
Onset to treatment time	1.00	0.99 - 1.01	0.609
E30%	1.03	1.00 - 1.05	0.027
Drug allocation to Tenecteplase	0.44	0.19 - 1.02	0.056
Significant HT (nil/HI1 vs HI2/PH1/PH2)			
Age	0.98	0.93 - 1.02	0.326
Systolic BP	1.03	1.00 - 1.05	0.032
History of antiplatelets	2.69	0.94 - 7.65	0.064
Baseline NIHSS	1.07	0.96 - 1.18	0.218
Onset to treatment time	1.00	0.99 - 1.01	0.945
E30%	1.01	0.99 - 1.04	0.406
Drug allocation to Tenecteplase	0.33	0.12 - 0.92	0.033
Any PH vs No PH			
Age	0.99	0.93 - 1.06	0.809
Systolic BP	1.04	1.00 - 1.08	0.036
History of antiplatelets	8.15	1.46 - 45.44	0.017
Baseline NIHSS	1.13	0.98 - 1.31	0.094
Onset to treatment time	1.00	0.99 - 1.02	0.986
E30%	1.01	0.98 - 1.04	0.494
Drug allocation to Tenecteplase	0.22	0.05 - 0.94	0.041

Table 5-2 - Logistic regression results

HT – haemorrhagic transformation

PH – parenchymal haemorrhage

5.4 Discussion

Our analysis highlights the potential utility of BBB permeability measures in prediction of HT and as an adjustment variable in determining the HT risk associated with treatments or interventions. We found reduced risk of significant HT or PH with tenecteplase compared to alteplase, with a trend towards reduced risk of any HT. Whilst we did not demonstrate the ability of BBB permeability measures to differentiate significant HT or PH from more minor types of HT, this may be demonstrable in a larger cohort.

Meta-analysis of the first 5 alteplase-tenecteplase trials found no difference in rates of symptomatic ICH after thrombolysis between treatments though low event rates compromise any statistical analysis (72). Registry data from the TETRIS cohort found lower rates of parenchymal haemorrhage in tenecteplase treated patients compared to alteplase (7.9% vs 12.3%) (75). Symptomatic ICH rates in the AcT trial were comparable between groups (tenecteplase 3.4%, alteplase 3.2%) (151). Our analysis suggested a lesser propensity for HT with tenecteplase than alteplase demonstrated by lower rates of HT in tenecteplase-treated groups, as well as higher likelihood of alteplase-related HT in cases at lower levels of BBB permeability whilst tenecteplase was associated with HT in cases with higher BBB permeability.

Thrombolysis treatment decisions in acute ischaemic stroke are multi-faceted with various clinical and radiological factors considered rapidly. Increasingly conventional time-limited treatment windows are being stretched and an individualised ‘tissue window’ determined using neuroimaging (172). Risk of HT must be considered prior to thrombolysis though quantification of this risk is complex with no single determining factor. Whilst evidence demonstrates that the risk of symptomatic ICH is outweighed by the benefit of IVT in trial populations, individual clinical decisions in IVT must take into consideration factors such as age, stroke severity and imaging features to judge individual risk. In our analysis, measures of BBB permeability were shown to be more reliable indicators of any HT than other factors such as core volume, NIHSS, onset to treatment time or systolic blood pressure. Whilst not predictive of significant HT or PH in our models, this could be explained by low event rates. Examination of BBB permeability measures in clinical trial settings may help to identify

populations in whom HT risk with thrombolysis is high. Examples shown in figure 5-2 highlight how BBB permeability imaging can be useful in predicting HT, and also highlights cases demonstrating possible lower propensity for HT with tenecteplase.

Limitations of our study include use of a small retrospective data set though individualised data was used. As with other studies of HT the number of events is low, particularly those of high clinical significance (PH2 or sICH), making predictive modelling difficult. Tenecteplase was used at two doses, though most patients received the higher dose.

5.5 Conclusion

Measures of BBB permeability are predictive of HT after thrombolysis and could be useful as an adjustment variable in clinical trials. Tenecteplase was associated with fewer cases of HT in our dataset. Tenecteplase-related HT occurred in cases with higher BBB permeability as compared to alteplase-related HT which occurred at lower and higher levels of BBB permeability.

6 ATTEST-2 imaging substudy

6.1 Introduction

The ATTEST-2 study was a UK-based, multi-centre, prospective randomised open-label blinded endpoint (PROBE) design clinical trial comparing alteplase versus tenecteplase in patients with acute ischaemic stroke who were eligible for intravenous thrombolysis as determined by usual standard of care. Whilst the main ATTEST-2 study was a pragmatic design with open inclusion criteria aiming to recruit a population reflective of clinical practice, other trials of tenecteplase such as the TASTE study (173) have used more specific inclusion criteria with imaging in particular used to provide an enriched population entering the trial. Previous trials have built evidence for the superiority of tenecteplase over alteplase in certain subgroups. The EXTEND IA TNK trial found greater early recanalisation with tenecteplase (69). The TASTE-A trial found greater early reperfusion with tenecteplase for patients treated in a mobile stroke unit (71). The TAAIS trial found superior reperfusion at 24 hours in patients with perfusion mismatch treated with alteplase (63). The ATTEST-2 imaging substudy therefore provided the opportunity to examine populations with particular imaging characteristics to aid in secondary analyses.

The ATTEST-2 imaging substudy aimed to assess imaging from participants of the ATTEST-2 trial to address 3 hypotheses:

- Tenecteplase will be associated with greater probability of early recanalisation compared to alteplase

- Tenecteplase will be associated with greater salvage of ischaemic tissue compared to alteplase

- The clinical effect (defined as day 90 mRS distribution) of tenecteplase compared to alteplase will be greater among patients with imaging features of large vessel occlusion in conjunction with a small ischaemic core

6.2 Methods

6.2.1 Study visits

The ATTEST-2 study took place across 5 visits – pre-randomisation, randomisation, 24 hour, day 5 and day 90. Study procedures are summarised in Table 6-1.

6.2.1.1 Visit 1 – Pre-randomisation – screening and consent

The ATTEST-2 trial recruited male and female patients aged ≥18 years who presented with symptoms of stroke evaluated according to each centre's standard of care and the clinical judgment of attending medical staff. Eligible patients were randomised (1:1) to receive either alteplase or tenecteplase. Inclusion and exclusion criteria are listed in the appendix.

Consent was taken separately for inclusion to the main ATTEST-2 study and inclusion in the imaging substudy. Consent to the main study included consent for analysis of routinely acquired imaging. Consenting procedures are listed in the appendix.

Baseline data collected included vital signs, medical history, concomitant medications, physical examination measured by the National Institute of Health Stroke Scale (NIHSS), blood glucose +/- INR if applicable, and pre-stroke functional status assessed by the modified Rankin scale (mRS).

6.2.1.2 Visit 2 – Randomisation and Treatment

Randomisation took place using an interactive voice response system and computer allocation by a minimisation algorithm based on age, stroke severity and onset-to-treatment time.

Treatment was initiated as soon as possible with either alteplase 0.9mg/kg, given as a bolus of 10% of the volume followed by the remaining 90% as an infusion over one hour; or tenecteplase 0.25mg/kg given as a single bolus. The participant and treating physicians were not blinded to the treatment allocation, however end point assessors were.

Study Procedure	Visit 1	Visit 2	Visit 2b	Visit 3	Visit 4	Visit 5
	Pre - Randomisation	**Randomisation**	**≤4hr**	**24hr (22-48 hrs)**	**Day 5 (or hospital discharge if earlier)**	**Day 90 (±7)**
Consent /Assent [#]	✓					
Review Inclusion Criteria	✓					
CT or MRI brain	*			*		
CTA ± CTP [1]	x		x(CTA)			
DSA or MRA	x		*			
Blood Pressure	*	*		*		
Medical History	*					
Concomitant medication	*			✓	✓	✓
Physical Examination – NIHSS	*			*	X	
Bloods	*					
Pregnancy Test	✓					
IVRS		✓				
Drug administration		✓				
mRS	*					✓
Adverse Events			✓	✓	✓	✓
Barthel Index						✓
EuroQOL						✓
Completion of eCRF	✓	✓	✓	✓	✓	✓

Table 6-1 ATTEST-2 schedule of visits

✓ study-specific procedure.

[#] Substudy consent to be taken simultaneously with main study consent, however if not possible, sub study consent can be taken after randomisation and before visit 4 as permission is being requested to use routinely acquired scans

*** clinically routine procedure (data captured for study)**

× procedure clinically routine in some patients

[1] Baseline CTA ± CTP can be acquired either prior to drug administration or within 15 minutes of bolus administration.

6.2.1.3 Visit 2b - <4 hours

CT angiogram (CTA) and/or CT perfusion (CTP) were performed. Results of these scans did not affect inclusion and exclusion criteria but were available to the treating clinician in deciding whether to proceed with IVT. Patients taken for thrombectomy had DSA performed at the time of MT.

6.2.1.4 Visit 3 – 24 hours

A non-contrast CT brain scan (NCCT) was undertaken at 24 hrs (22-36 hrs) to determine safety outcomes. NIHSS assessment was repeated, concomitant medications recorded and adverse events assessed for and reported.

6.2.1.5 Visit 4 – Day 5

At day 5 (+/-1) or on the day of hospital discharge if earlier the NIHSS was repeated, concomitant medications recorded and adverse events assessed for.

6.2.1.6 Visit 5 – Day 90

A blinded assessor carried out the day 90 (+/-7) visit using the Rankin Focused Assessment (RFA) tool to determine the mRS. Other measures of functional capacity and quality of life at this visit included Barthel Index and EuroQol respectively.

6.2.2 Acquisition and collation of scans

Participants in the ATTEST-2 trial required at minimum a NCCT at screening to confirm eligibility and a 24 hour NCCT to determine safety outcomes. The imaging substudy in addition aimed to undertake the following scans in participants:

- Baseline CTA of carotid and intracranial vessels to define arterial occlusion site and collateral status

- Baseline CT perfusion (CTP) to define areas of predicted ischaemic core and penumbra

- Early repeat CTA at 2-4 hours post-treatment to define early recanalisation rates

- Subacute follow-up MRI brain (22-48 hours after treatment) to measure follow-up infarct volume

At outset sites participating in the imaging substudy undertook the above scans in each participant in the substudy as was feasible according to local provision of scans and clinical suitability. However, following withdrawal of funding for the substudy shortly after recruitment commenced, only routinely acquired clinical scans were used for the substudy.

CTP required 45-60 seconds of acquisition in cine mode. Minimum z-axis coverage of 8cm was preferred.

CTA consisted of a complete aortic arch to vertex acquisition at baseline and intracranial-only CTA at 2-4h follow-up. Maximal intensity projection images derived from a CT perfusion study were an acceptable alternative.

MRI brain included as a minimum diffusion weighted imaging (DWI), T2-weighted-Fluid-Attenuated Inversion Recovery (T2 FLAIR), and a blood-sensitive sequence such T2*/gradient recalled echo (GRE), or susceptibility weighted imaging (SWI). Optional additional imaging at sites according to availability would include perfusion imaging using either gadolinium-enhanced dynamic susceptibility contrast perfusion imaging or arterial spin labelling (ASL), and intracranial MR angiography (MRA), either time-of-flight or contrast enhanced.

Scans acquired at each site were transferred to the central imaging office at the University of Edinburgh. Anonymised scans in DICOM format were uploaded to a web cloud or sent on CD.

6.2.3 Image processing and analysis

Analysis of scans took place either through the Systematic Imaging Review System 2 (SIRS-2) based at the University of Edinburgh or by researchers at the

Imaging Centre of Excellence, University of Glasgow based at the Queen Elizabeth University Hospital, Glasgow.

6.2.3.1 Presence and site of intra-cranial and extra-cranial arterial occlusion

CTA were reviewed to determine sites of intra-cranial occlusion. Non-contrast CT was also examined for hyperdense vessels.

6.2.3.2 ASPECTS score

Baseline non-contrast CT brain imaging were analysed using RAPID software to calculate the Alberta Stroke Programme Early CT Score (ASPECTS) which assesses the presence of early ischaemic changes such as loss of grey-white matter differentiation or focal hypoattenuation in 10 specified segments of brain. These are 6 areas of the territory of the middle cerebral artery (MCA) M1-M6, insula, lentiform, internal capsule and caudate, as shown in figure 6-1. For each region showing ischaemic changes one point is subtracted, such that a score of 10 represents normal appearances. Ideally analysis used reconstructions with 5mm slices, if this was not available the slice thickness closest to 5mm was chosen. An example of the RAPID ASPECTS output is shown in figure 6-2.

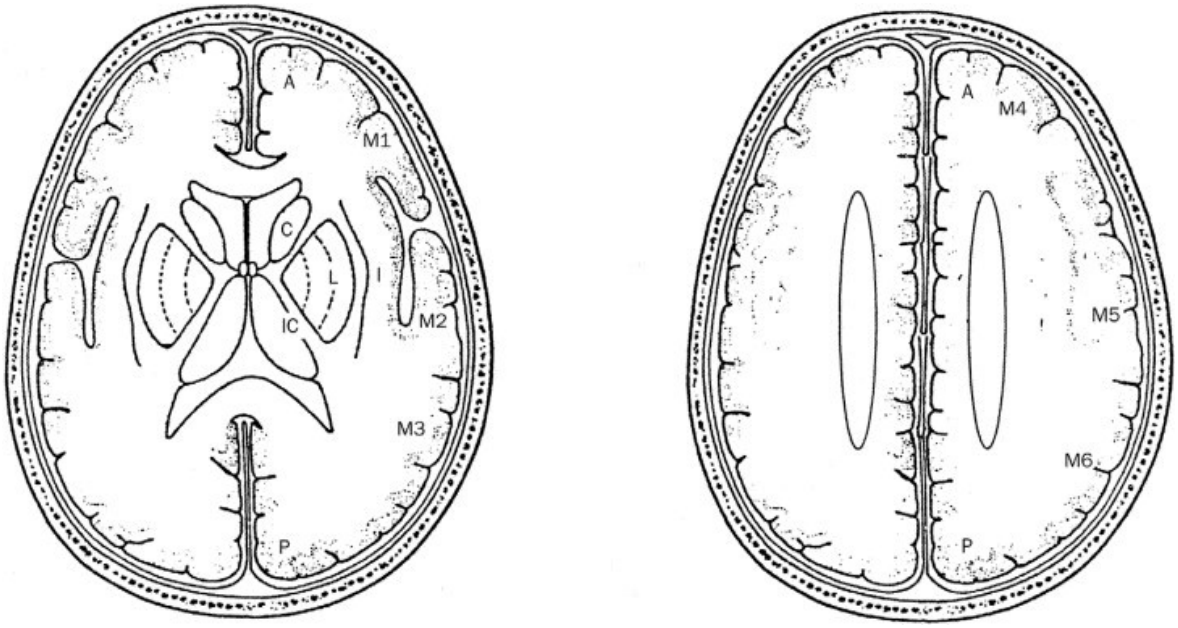


Figure 6-1 - Alberta Stroke Programme Early CT Score (ASPECTS) segments (174)

M1-M6 – segments within the territory of the middle cerebral artery, C – caudate, L – lentiform, IC – internal capsule, I - insula

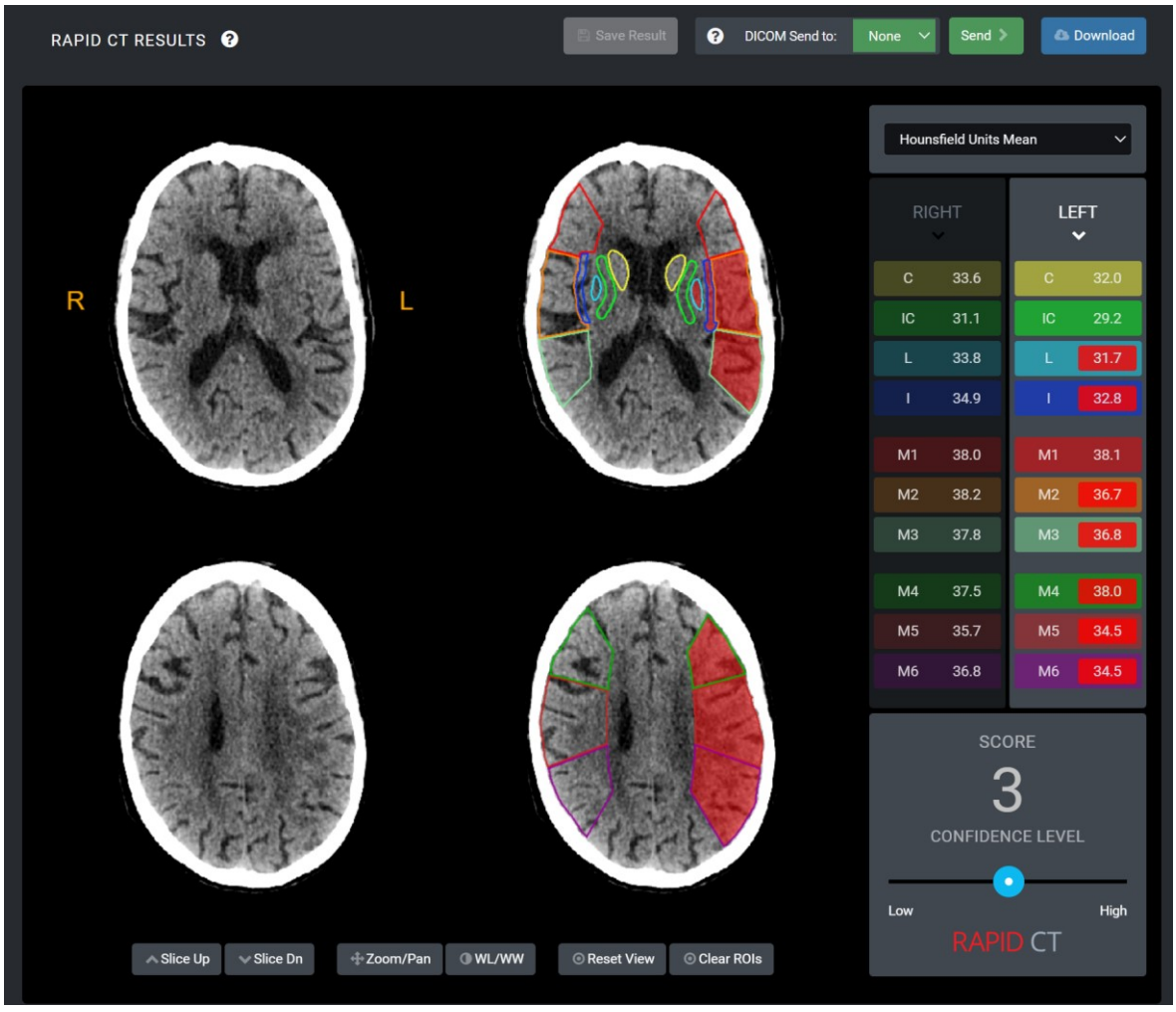


Figure 6-2 - Example output from RAPID CT ASPECTS

6.2.3.3 Thrombolysis in Cerebral Infarction (TICI) score

The TICI score (175), representing the degree of occlusion, was calculated as follows based on CT angiogram:

- Grade 0 – No perfusion – no anterograde flow beyond the point of occlusion
- Grade 1 – Penetration with minimal perfusion – contrast passes the area of occlusion but fails to opacify the entire bed distal to the occlusion
- Grade 2 – Partial perfusion – contrast passes the area of occlusion and opacifies the arterial bed distal to the occlusion
    - 2a – partial filling (<50%) of the vascular territory is visualised
    - 2b – partial filling (≥50%) of the vascular territory is visualised
- Grade 3 – Complete perfusion

6.2.3.4 Collateral score

Collateral score was determined by baseline CTA. Automated assessment using Brainomix software (Oxford, UK) (176) which calculated collateral scoring using the CTA-CS score (176) which grades collateral between 0-3:

- 0 - no collateral filling
- 1 - ≤50% but >0 of the occluded MCA territory
- 2 - >50% but <100% of the occluded MCA
- 3 - 100% collateral supply of the occluded MCA territory

An example of the automated output is shown in figure 6-3.

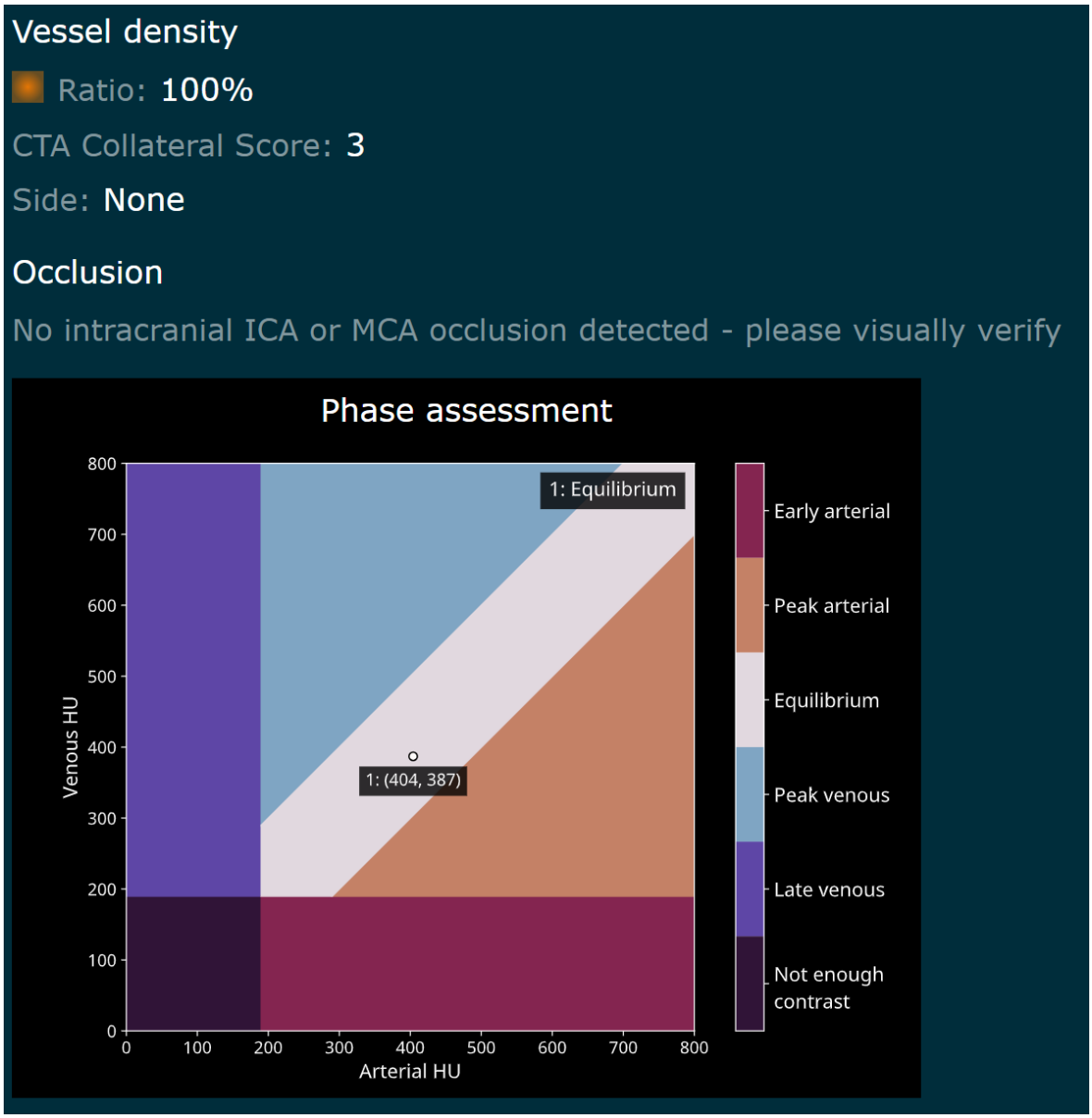


Figure 6-3 - Example of output from Brainomix software assessing the CTA collateral score

6.2.3.5 Perfusion Imaging

CT perfusion scans were analysed using RAPID software produced by iSchemaView (California, USA). Perfusion images were uploaded to the RAPID server at the Imaging Centre of Excellence, University of Glasgow which produced maps of cerebral blood flow (CBF), cerebral blood volume (CBV), mean transit time (MTT) and time to maximum (Tmax). Criteria from the DEFUSE-3 study (150) were applied to determine areas of suspected ischaemic core (CBF < 30%) and suspected ischaemic penumbra (Tmax >6 seconds) and the area and ratio of mismatch between core and penumbra – these were calculated automatically by the RAPID software. Hypoperfusion intensity ratio (HIR) was also calculated automatically and defined as volume of tissue with Tmax >10 seconds divided by volume of tissue with Tmax >6 seconds. Favourable collateral status is defined by a ratio <0.4 and poor collaterals as >0.4. Quality of imaging was examined through visual assessment of arterial input function (AIF) and venous output function (VOF) curves, motion estimate graphs and maps of CBV, CBF, MTT and Tmax. Incomplete AIF or VOF curves or severe motion which resulted in suspected artefactual findings were identified as poor quality imaging. Examples of the RAPID perfusion output are shown in figure 6-4.

If RAPID failed to read perfusion scanning data, we used MiStar software (Apollo Medical Imaging Technology, Melbourne, Australia) to process CTP scans. Parameters used for MiStar were CBF<30% for ischaemic core and delay time (DT) >3 seconds for ischaemic penumbra. Delay time is a validated alternative measurement of penumbral volume (177).

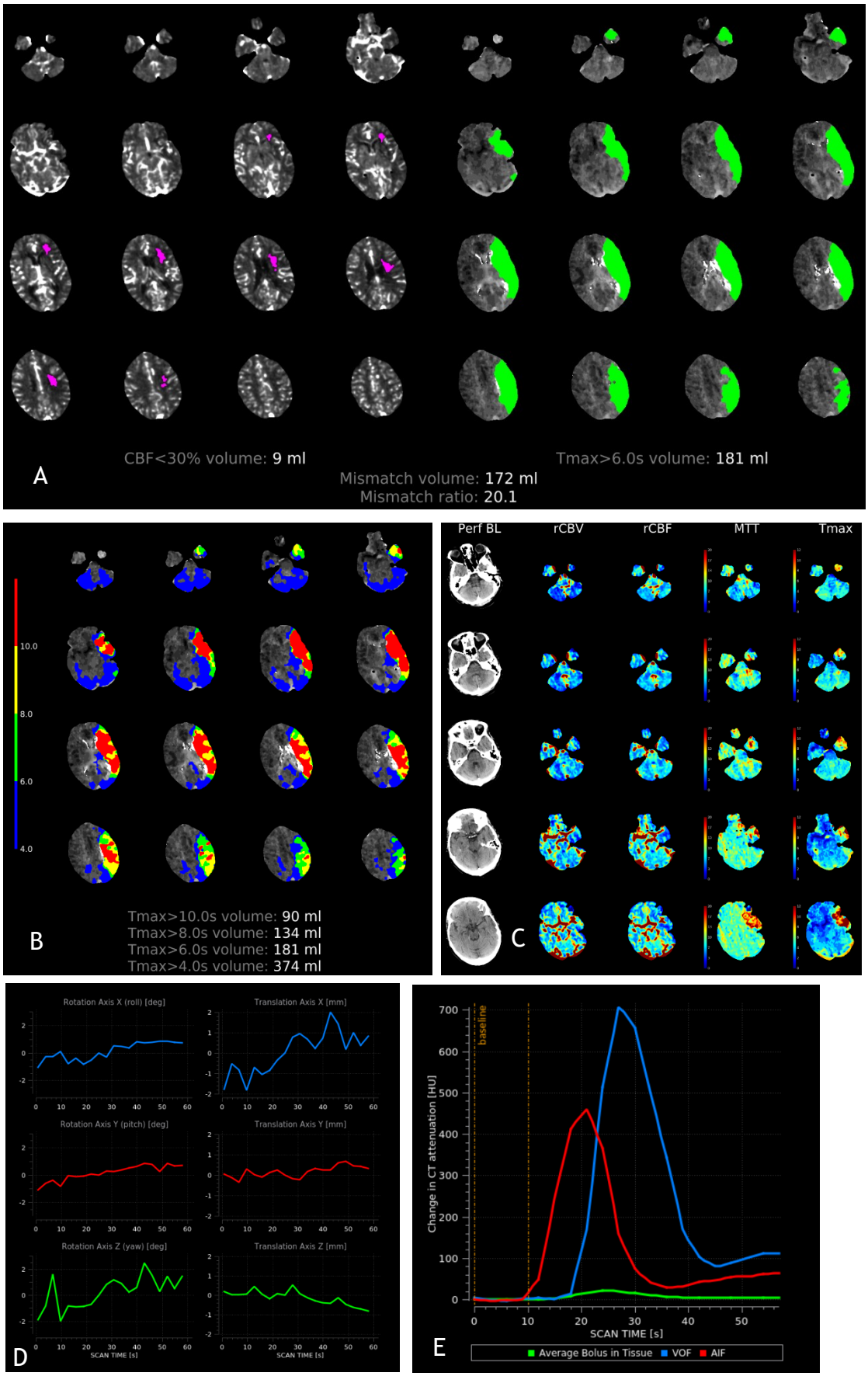


Figure 6-4 - RAPID CTP output example

A – RAPID Summary, B – Tmax threshold maps, C – Columned view of perfusion maps, D – motion estimates, E – AOF and VOF curves

6.2.3.6 Follow up MRI

Follow up infarct volumes were calculated using DWI imaging and Mango software (Research Imaging Institute, UTHSCSA). The area of hyperintensity was highlighted using thresholding and manual editing and a volume calculated. Calculations were made independently by two observers and a mean volume calculated. Time from baseline imaging to MRI was also calculated in hours. An example of the output from Mango software is shown in figure 6-5.

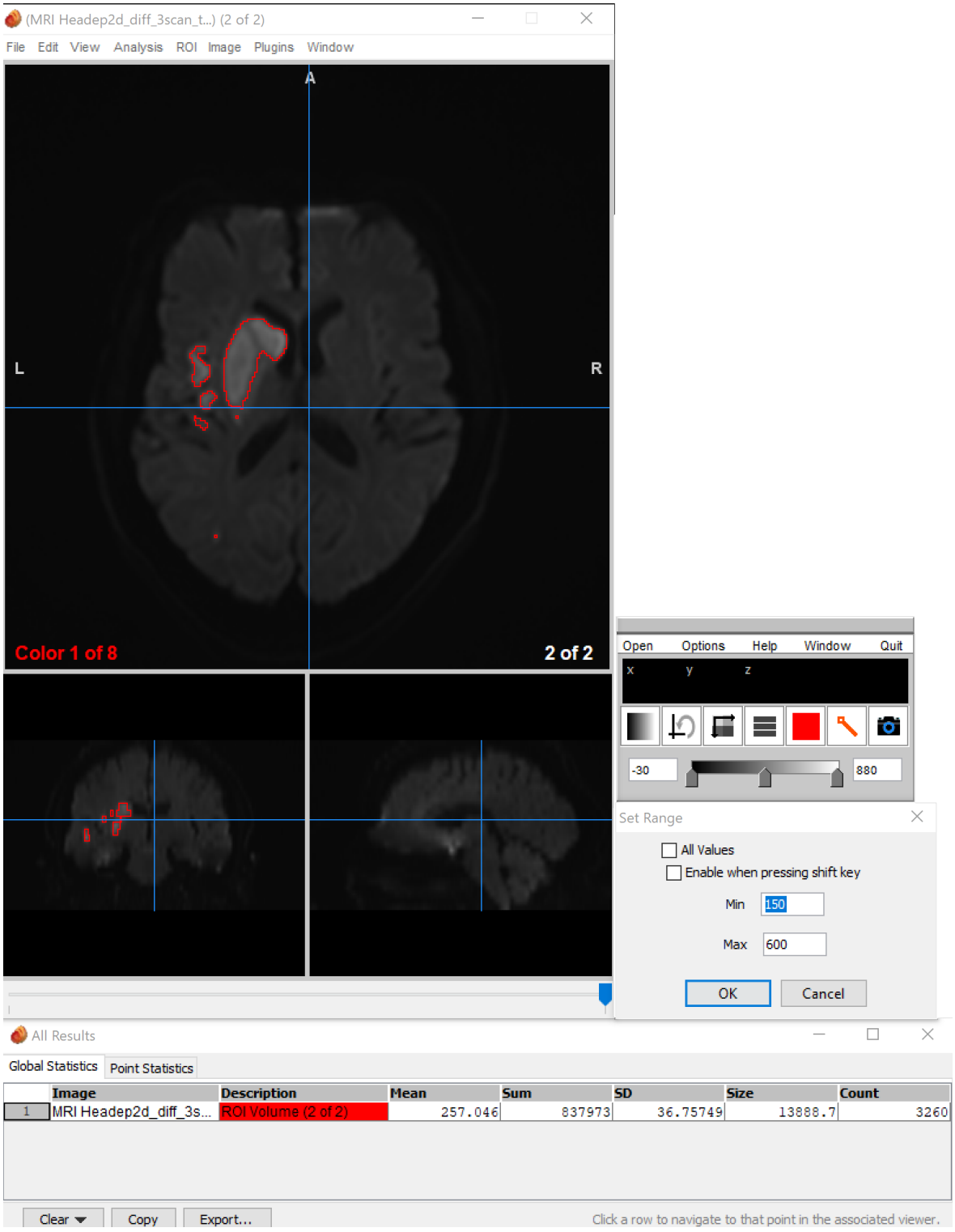


Figure 6-5 - Example of MRI DWI follow up infarct volume calculation using Mango software

6.2.3.7 Follow up CT

Follow up infarct volumes from non-contrast CT scans were calculated using MIStar software (Apollo Medical Imaging Technology, Melbourne, Australia) for cases with no follow up MRI. Scans with 5mm slice thickness derived using multiplanar reconstruction were loaded into the ROI tool. Window width was adjusted to 50 Hounsfield units and window level was adjusted to 40 Hounsfield units. Areas of hypoattenuation were manually traced on the ROI tool on each slice. No reference was made to the patient's perfusion imaging. Measurements from all slices on which hypoattenuation was visible were used to calculate volume. Time from baseline imaging to follow up CT was also calculated in hours. An example of the process is shown in figure 6-6.

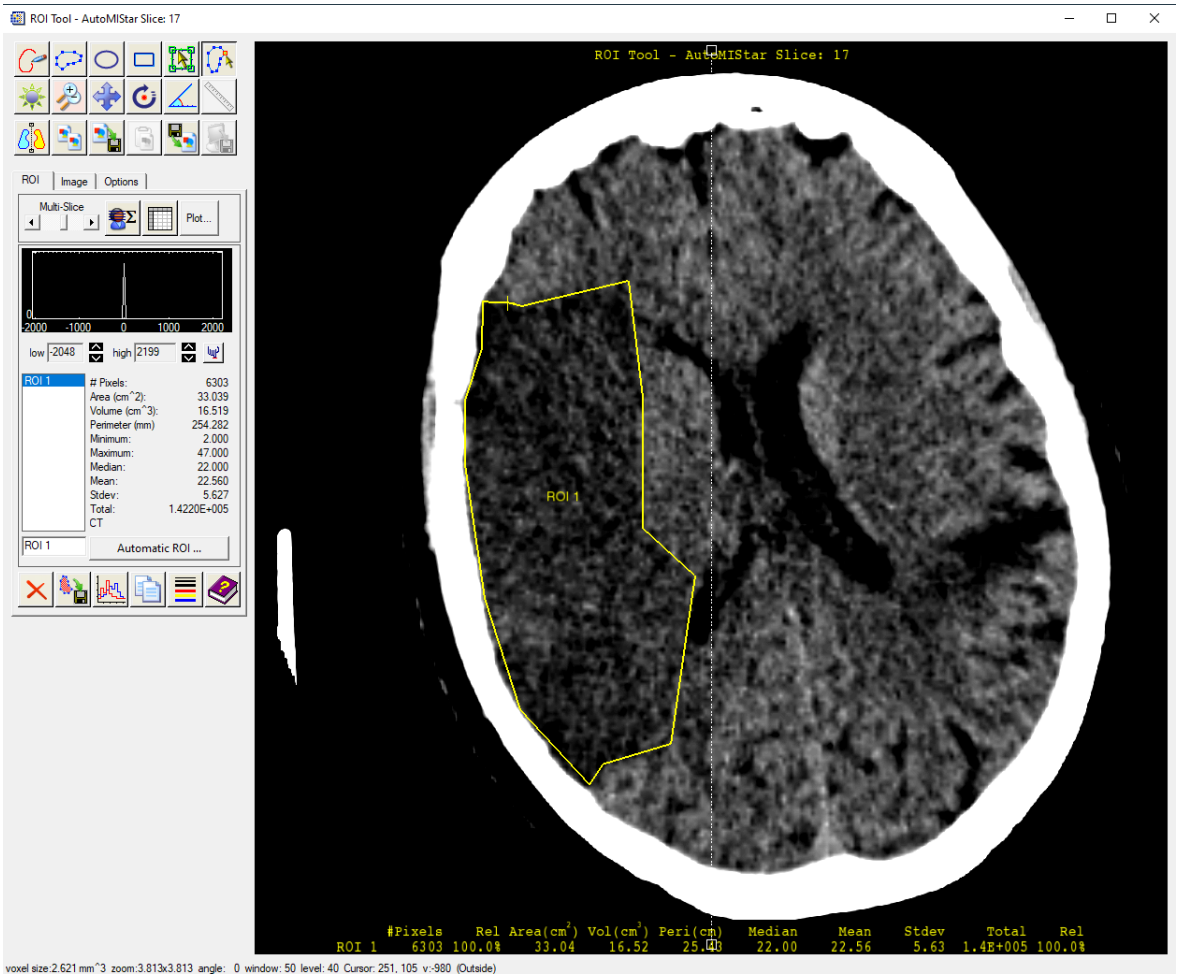


Figure 6-6 Example of infarct volume calculation on non contrast CT using MiStar software

6.2.3.8 Inter- and intra-rater reliability

Inter-rater reliability was determined for MRI readings of DWI lesions by two researchers and intra-rater reliability was determined for non-contrast CT measurement of follow up infarct volume. For intra-rater reliability, a sample of 20 scans were read twice by the same reader at two different time points. Weighted kappa and intraclass correlation coefficients were calculated using SPSS.

6.2.3.9 Data entry

For scan rating on SIRS-2, readers had access to all imaging acquired concurrently within a single study (e.g. NCCT and CTA at baseline) but no access to imaging from other time points or to clinical data. Scans were viewed in parallel with the rating questionnaire. Submitted responses were immediately stored on a secure server within the University of Edinburgh. Data submission occurred with each page transition of the questionnaire therefore incomplete responses were not lost.

At the Imaging Centre of Excellence, data were entered into a spreadsheet in CSV format and saved on secure servers of the University of Glasgow.

6.2.4 Outcome assessment

The primary outcome of the trial was the functional outcome at 90 days as measured by the modified Rankin scale. Secondary outcomes included full neurological recovery at 90 days, functional independence at 90 days, early neurological improvement, need for thrombectomy and quality of life scores. Safety outcomes included mortality, symptomatic intracranial haemorrhage rates and presence of any intracranial haemorrhage at follow up.

6.2.4.1 Functional outcomes

Functional outcomes were measured by the modified Rankin scale and Barthel Index. The modified Rankin scale (mRS) (178, 179) is an ordinal scale assessing functional capacity in patients recovering from acute stroke ranging from 0 (no symptoms) to 6:

Structured assessment can be made using the Rankin Focused Assessment (RFA) to improve interrater reliability (180). mRS assessments were made on screening to determine eligibility and again at day 90 (±7) for the primary outcome.

The Barthel Index (181) assesses ten activities of daily living such as feeding, bathing and grooming and was assessed at visit 5.

6.2.4.2 Neurological recovery

Neurological status at enrolment and subsequent improvement or deterioration was assessed using the National Institute for Health Stroke Scale (NIHSS) (182). A focused neurological examination eliciting signs of acute stroke can be quantified using the NIHSS which scores 11 aspects of neurological assessment, scores are shown in table 6-2. At randomisation, stroke severity was considered mild for NIHSS 1-8, moderate for NIHSS 9-16 and severe for NIHSS ≥17. At 24 hours improvement of the NIHSS by 8 points or to a score of 0-1 was considered major neurological improvement; while a deterioration of >3 points was considered an adverse event.

6.2.4.3 Intracerebral haemorrhage

Post thrombolysis intracerebral haemorrhage was classified using the European-Australasian Acute Stroke Study (ECASS II) classification (171). Symptomatic intracerebral haemorrhage (sICH) was defined as per the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST) definition (183) as PH2 haemorrhage on any scan up to 36 hours post treatment, associated with either death or a deterioration in NIHSS of >3 from baseline or from its lowest point in the first 24 hours.

6.2.5 Hypotheses testing

6.2.5.1 Large vessel occlusion

Cases with confirmed intracranial occlusion were classified as having large vessel occlusion (LVO) when involving the internal carotid artery (ICA) or proximal branches of the middle cerebral artery (MCA) – namely the M1 and proximal M2 branches. Occlusions of the distal M2 were classified as medium vessel occlusion

(MeVO). Definition of vessel occlusion was by CTA alone or by CTA and hyperdensity on NCCT. The efficacy of tenecteplase compared to alteplase in was assessed in these subgroups.

6.2.5.2 Perfusion mismatch

Cases with a mismatch profile were identified. Two definitions of mismatch profile were used as per the DEFUSE-3 and EXTEND trials.

DEFUSE-3 (150) definition of mismatch:

- ischaemic core (CBF<30% and Tmax>6 secs) <70ml
- penumbra (CBF>30% and Tmax>6 secs) : core ratio >1.8
- absolute penumbra volume ≥15ml

EXTEND (84) definition of mismatch:

- ischaemic core (CBF<30% and Tmax>6 secs) <70ml
- penumbra (CBF>30% and Tmax>6 secs) : core ratio >1.2
- absolute penumbra volume ≥10ml

Efficacy and safety outcomes were assessed among these subgroups, specifically assessing the efficacy of tenecteplase compared to alteplase in patients with mismatch profile.

6.2.5.3 Recanalisation

Recanalisation was assessed by identification of large vessel occlusion on baseline imaging and subsequent recanalisation on digital subtraction angiography prior to mechanical thrombectomy. The proportion of patients achieving early recanalisation with tenecteplase was compared to alteplase.

6.2.5.4 Penumbral salvage

Baseline CTP data was compared with the follow up infarct volume on MRI or CT. Cases with no perfusion lesion were excluded from this analysis. Infarct growth was calculated as the difference between FIV and baseline ischaemic core volume. The volume of penumbral salvage was calculated as baseline ischaemic penumbra volume minus the volume of infarct growth (184). The proportion of penumbra salvaged was calculated as the volume of penumbral salvage divided by baseline penumbra volume expressed as a percentage. The extent of penumbral salvage with tenecteplase was compared to that with alteplase using Mann-Whitney U test and linear regression. For regression, nonparametric data underwent log transformation before entry into the model.

6.2.6 Statistics

All statistics were performed on SPSS (136). Differences between groups were calculated using independent T-test for parametric data or Mann-Whitney U test for non-parametric data. Differences in proportions were calculated using crosstabulation and Pearson Chi square tests. Regression was performed using either logistic, ordinal, or stepwise linear regression as appropriate.

6.3 Results

The ATTEST-2 trial recruited 1,858 patients across 39 sites in the UK between January 2017 and May 2023. 1,777 were included in the modified intention to treat (mITT) analysis. 884 received tenecteplase and 893 received alteplase. Non-inferiority of tenecteplase compared to alteplase was seen in terms of functional outcome assessed by day 90 mRS distribution, and also the proportion of patients with excellent recovery (mRS 0-1) (185).

Of the mITT population, 306 patients underwent CTP imaging and were included in the imaging substudy, of whom 156 received tenecteplase and 150 received alteplase.

6.3.1 Baseline clinical data

Baseline clinical data are summarised in table 6-2. Clinical parameters were well matched between tenecteplase and alteplase groups. Mean age was 71 and 70 respectively, gender was balanced with 52% male participants in the tenecteplase arm and 51% in the alteplase arm. Median blood pressure was 152/80 and 147/80 in tenecteplase and alteplase groups respectively, in keeping with an acute stroke population. Median baseline NIHSS was 8 in both groups, reflecting a population of mild-moderate stroke severity. Median baseline mRS was 0 in both groups. Thrombectomy was undertaken in 22/155 tenecteplase cases and 25/148 alteplase cases, the 15.5% rate of thrombectomy being higher than the 12.4% in the main trial mITT population. At final diagnosis, 6/151 tenecteplase cases and 10/148 alteplase cases were stroke mimics.

6.3.2 Baseline imaging data

Baseline imaging data are summarised in table 6-3. There were no statistically significant differences in baseline imaging features. Median ASPECTS score was 7 (6-9) in the tenecteplase group and 8 (6-9) in the alteplase group. When defined by CTA alone, respectively in tenecteplase and alteplase cases, LVO was seen in 34/156 and 36/150, M2 occlusion in 27/156 and 20/150, other vascular occlusion in 2/156 and 6/150, no occlusion in 73/156 and 67/150 and no baseline CTA was available in 20/156 and 21/150 cases. When defined by CTA or by a hyperdense vessel sign on NCCT, respectively in tenecteplase and alteplase cases, LVO was seen in 37/156 and 40/150, M2 occlusion in 28/156 and 21/150, other vascular occlusion in 2/156 and 6/150 and no occlusion in 89/156 and 83/150. Collateral scoring for tenecteplase and alteplase respectively showed no collateral filling (grade 0) for 1/124 and 1/122 cases, 0-50% filling (grade 1) for 7/124 and 10/122 cases, 50-100% filling (grade 2) for 27/124 and 29/122 cases, and 100% filling (grade 3) for 89/124 and 82/122 cases.

Of the 306 patients who underwent CTP imaging, 49 scans were excluded from analysis due to poor quality CTP imaging and one due to unavailability of imaging, leaving 256 CTP scans included in the CTP analyses (129 tenecteplase, 127 alteplase). There were no significant differences in CTP parameters such as baseline core and penumbra volume, or proportions with mismatch or no

perfusion lesion. Median baseline ischaemic core volume was 0ml in both groups and median baseline penumbra volume was 20ml in both groups. Around half of patients met mismatch criteria in both groups, 50 % and 58% met DEFUSE-3 or EXTEND criteria respectively in the tenecteplase group, with 47% and 50% respectively in the alteplase group. No ischaemic lesion on CTP was found in 26% of the tenecteplase group and 30% of the alteplase group.

6.3.3 Efficacy outcomes

In the mITT population, functional outcome at 90 days assessed by mRS was not significantly different between the two treatments in ordinal regression when adjusted for age, baseline NIHSS, baseline core volume and baseline penumbra volume. All co-variates were independently predictive of day 90 mRS, in a combined model of all co-variates age, baseline NIHSS and baseline core volume was predictive of day 90 mRS but baseline penumbra volume was not. Results are shown in table 6-4. Functional independence at 90 days (mRS 0-2) occurred in 97/144 and 85/136 of tenecteplase and alteplase cases respectively (p=0.39). Excellent outcome at 90 days (mRS 0-1) occurred in 60/144 and 60/136 of tenecteplase and alteplase cases respectively (p=0.68). Major neurological improvement at 24 hours was seen in 75/155 and 70/147 participants for tenecteplase and alteplase respectively (p=0.89). Major neurological improvement at 5 days was seen in 85/140 and 79/121 participants for tenecteplase and alteplase respectively (p=0.45). Results are shown in figures 6-7 and 6-8.

In binary logistic regression for prediction of functional independence at 90 days age (p=0.006), baseline NIHSS (p=0.001) and baseline ischaemic core volume (p=0.017) were all predictive of mRS 0-2 when including treatment allocation and baseline ischaemic penumbra volume. Baseline ischaemic core volume was not however predictive of mRS 0-1 (p=0.25) though age (p=0.03) and baseline NIHSS (p=<0.001) remained predictive.

6.3.4 Safety outcomes

In the mITT population, sICH occurred in 4/155 and 2/150 tenecteplase and alteplase cases respectively (p=0.43). Mortality by 90 days occurred in 6/144 and 10/136 tenecteplase and alteplase cases respectively (p=0.25).

	Tenecteplase n=156	Alteplase n=150	p-value
Age	71+/-11.3	70+/-12.8	0.55
Male sex	81/156	80/150	0.81
Systolic BP	152 (135-166)	147 (135-164)	0.32
Diastolic BP	80 (71-91)	80 (71-89)	0.74
Baseline mRS	0 (0-0)	0 (0-1)	0.26
Baseline NIHSS	8 (5-14)	8 (5-15)	0.61
NIHSS severity	Mild (1-8) 56% Moderate (9-16) 27% Severe (≥17) 17%	Mild (1-8) 53% Moderate (9-16) 25% Severe (≥17) 21%	0.58
Thrombectomy	22/155	25/148	0.52
Stroke mimic	6/151	10/148	0.285

Table 6-2 Baseline clinical data

	Tenecteplase	Alteplase	p-value
Acute CTA performed	136/156	131/150	0.97
Vessel occlusion (by CTA only)	LVO 34/156 M2 27/156 Other 2/156 No occlusion 73/156 No CTA 20/156	LVO 36/150 M2 20/150 Other 6/150 Nil 67/150 No CTA 21/150	0.52
Vessel occlusion (CTA or hyderdensity on NCCT)	LVO 37/156 M2 28/156 Other 2/156 No occlusion 89/156	LVO 40/150 M2 21/150 Other 6/150 No occlusion 83/150	0.36

ASPECTS	7 (6-9)	8 (6-9)	0.41
Collateral score	0 – 1/124 1 – 7/124 2 – 27/124 3 – 89/124	0 -1/122 1 – 10/122 2 – 29/122 3 – 82/122	0.83
Ischaemic core (CBF<30)	0ml (0-13)	0ml (0-11)	0.79
Ischaemic penumbra (CBF>30, Tmax>6)	20ml (0-68)	20ml (0-76)	0.88
Mismatch by DEFUSE-3 criteria	64/129 (50%)	58/127 (47%)	0.53
Mismatch by EXTEND criteria	75/129 (58%)	64/127 (50%)	0.21
No perfusion lesion	33/129 (26%)	38/127 38 (30%)	0.44
Follow up MRI performed	35/156	34/150	0.96
Time from baseline imaging to follow up CT	27.8 hrs ± 12.4	27.4 hrs ± 12.5	0.80
Time from baseline imaging to follow up MRI	49.9 hrs ± 45.4	42.2 hrs ± 32.5	0.48
Follow up infarct volume (CT or MRI)	20.8ml ±45.5	27.6ml ±68.9	0.86
Follow up infarct volume (MRI only)	6.9ml ±13.5	12.0ml ±24.8	0.68
Volume of penumbra salvaged	11.9ml (0-39)	11.0ml (0-66.8)	0.54

Table 6-3 - Baseline imaging data

Dependent variable	Independent variable	p-value	Co-variates	p-value
Day 90 mRS	Treatment allocation	0.57	Age Baseline NIHSS	<0.001 <0.001
Day 90 mRS	Treatment allocation	0.63	Age Baseline core volume	<0.001 <0.001
Day 90 mRS	Treatment allocation	0.76	Age Baseline penumbra volume	<0.001 <0.001
Day 90 mRS	Treatment allocation	0.63	Age Baseline core volume Baseline penumbra volume	<0.001 <0.001 0.003
Day 90 mRS	Treatment allocation	0.45	Age Baseline NIHSS Baseline core volume Baseline penumbra volume	<0.001 0.002 <0.001 0.087

Table 6-4 - Ordinal regression results for the mITT population

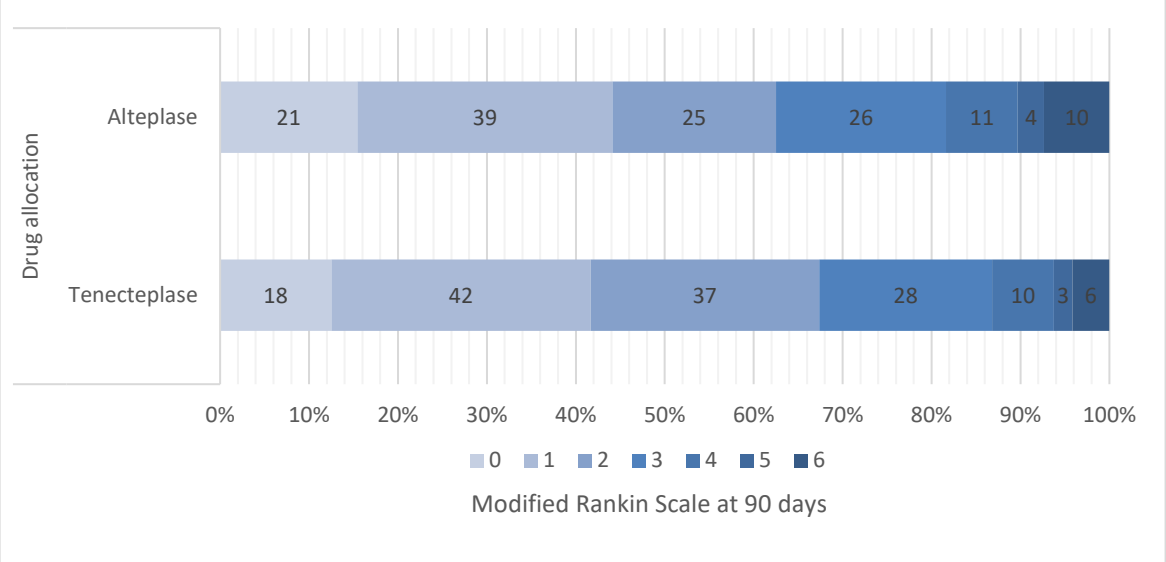


Figure 6-7 - Functional outcome in the mITT population

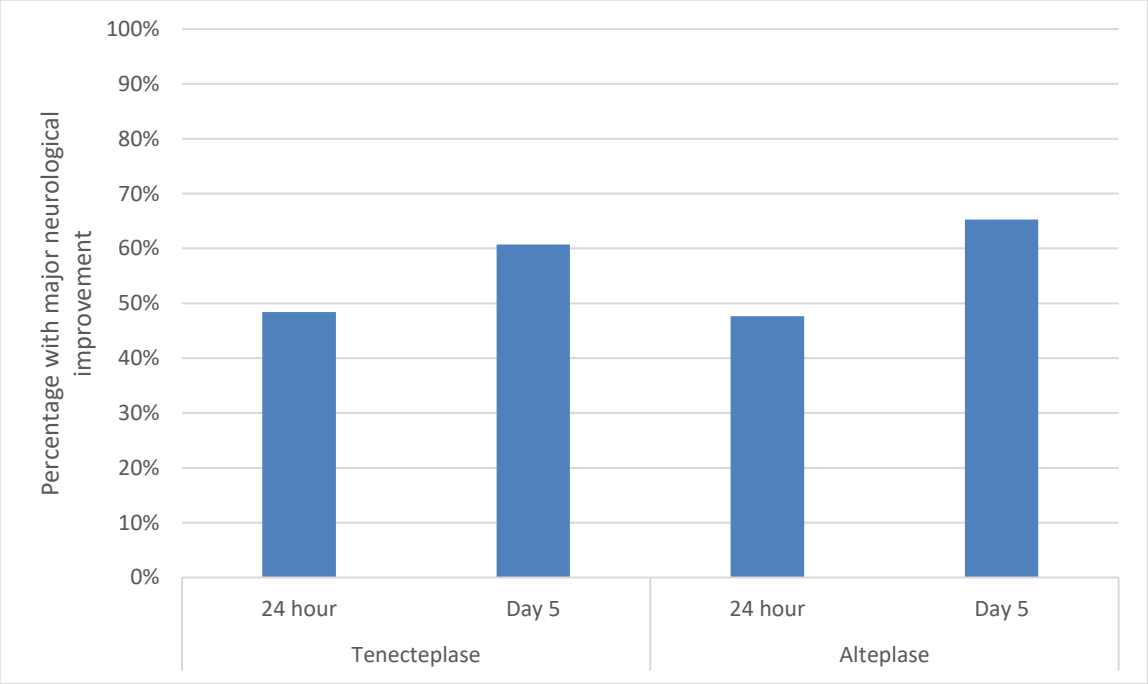


Figure 6-8 - Major neurological improvement in the mITT population

6.3.5 Large vessel occlusion (LVO) and medium vessel occlusion (MeVO)

In the mITT imaging substudy population using CTA alone there were 70 cases of LVO, 34 in the tenecteplase group and 36 in the alteplase group. In ordinal regression, day 90 mRS score was not predicted by treatment allocation when adjusted for age, baseline NIHSS, baseline core volume or baseline penumbra volume. Baseline NIHSS and baseline core volume were independently predictive of day 90 mRS when adjusting for age but baseline penumbra volume was not. In a combined model with all co-variates, baseline core volume was the only predictor of day 90 mRS. Results are shown in table 6-5. Functional independence at 90 days was seen in 13/31 tenecteplase cases and 15/33 (p=0.78). Excellent outcome, mRS 0-1, was seen in 9/31 tenecteplase cases and 8/33 (p=0.67). Major neurological improvement at 24 hours was seen in 15/33 and 18/34 tenecteplase and alteplase cases respectively (p=0.54). Major neurological improvement at day 5 was seen in 19/32 and 21/30 tenecteplase and alteplase cases respectively (p=0.38). Results are shown in figures 6-9 and 6-10.

When defining vessel occlusion by CTA or NCCT there were 77 cases of LVO, 37 in the tenecteplase group and 40 in the alteplase group. Efficacy outcomes were comparable with those for LVO defined by CTA only and are shown in figures 6-11 and 6-12.

A further analysis was carried out with combined LVO and MeVO data. In the mITT imaging substudy population using CTA alone there were 47 cases of MeVO, 27 in the tenecteplase group and 20 in the alteplase group. In total there were 117 cases of LVO or MeVO, 61 in the tenecteplase group and 56 in the alteplase group. In ordinal regression, day 90 mRS score was not predicted by treatment allocation when adjusting for age, baseline NIHSS, baseline core volume and baseline penumbra volume. Baseline NIHSS and baseline core volume were independently predictive of day 90 mRS when adjusting for age but baseline penumbra volume was not. In a combined model with all co-variates age and baseline core volume were predictive of day 90 mRS but baseline NIHSS and baseline penumbra volume were not. Results are shown in table 6-6. Functional independence at 90 days was seen in 32/55 tenecteplase cases and 24/49

(p=0.35). Excellent outcome, mRS 0-1, was seen in 20/55 tenecteplase cases and 15/49 (p=0.54). Major neurological improvement at 24 hours was seen in 29/60 and 31/54 tenecteplase and alteplase cases respectively (p=0.33). Major neurological improvement at day 5 was seen in 34/57 and 31/43 tenecteplase and alteplase cases respectively (p=0.20). Results are shown in figures 6-13 and 6-14.

Data on LVO and MeVO cases was also available for the mITT population of the main ATTEST-2 trial. Defined by CTA only, in the tenecteplase arm there were 124/882 LVO and 107/882 MeVO cases, and in the alteplase arm 129/888 LVO and 80/888 MeVO cases. A large number of cases had no CTA performed at baseline (320/882 for tenecteplase and 340/888 for alteplase). The proportion of LVO cases undergoing thrombectomy was 69/123 in the tenecteplase arm and 75/127 in the alteplase arm (p=0.64).

For LVO cases, functional independence (mRS 0-2) was achieved by 55/115 tenecteplase cases and 53/117 alteplase cases (p=0.70). Excellent outcome (mRS 0-1) was achieved by 30/115 tenecteplase cases and 33/117 alteplase cases (p=0.72). Major neurological improvement at 24 hours was seen in 48/120 tenecteplase cases and 59/123 alteplase cases (p=0.21). Major neurological improvement at day 5 was seen in 61/111 tenecteplase cases and 74/114 alteplase cases (p=0.13). Results are shown in figures 6-15 and 6-16.

For LVO and MeVO cases combined, functional independence (mRS 0-2) was achieved by 121/213 tenecteplase cases and 89/187 alteplase cases with a non-significant trend in favour of tenecteplase (p=0.07). Excellent outcome (mRS 0-1) was achieved by 75/213 tenecteplase cases and 55/187 alteplase cases which was not significantly different (p=0.22). Major neurological improvement at 24 hours was seen in 96/226 tenecteplase cases and 92/202 alteplase cases (p=0.52). Major neurological improvement at day 5 was seen in 112/207 tenecteplase cases and 111/182 alteplase cases (p=0.17). Results are shown in figures 6-17 and 6-18.

Dependent variable	Independent variable	p-value	Co-variates	p-value
Day 90 mRS	Treatment allocation	0.54	Age Baseline NIHSS	0.123 0.015
Day 90 mRS	Treatment allocation	0.58	Age Baseline core volume	0.087 <0.001
Day 90 mRS	Treatment allocation	0.997	Age Baseline penumbra volume	0.072 0.158
Day 90 mRS	Treatment allocation	0.52	Age Baseline core volume Baseline penumbra volume	0.072 <0.001 0.305
Day 90 mRS	Treatment allocation	0.47	Age Baseline NIHSS Baseline core volume Baseline penumbra volume	0.079 0.717 <0.001 0.427

Table 6-5 - Ordinal regression results for cases with LVO defined by CTA only

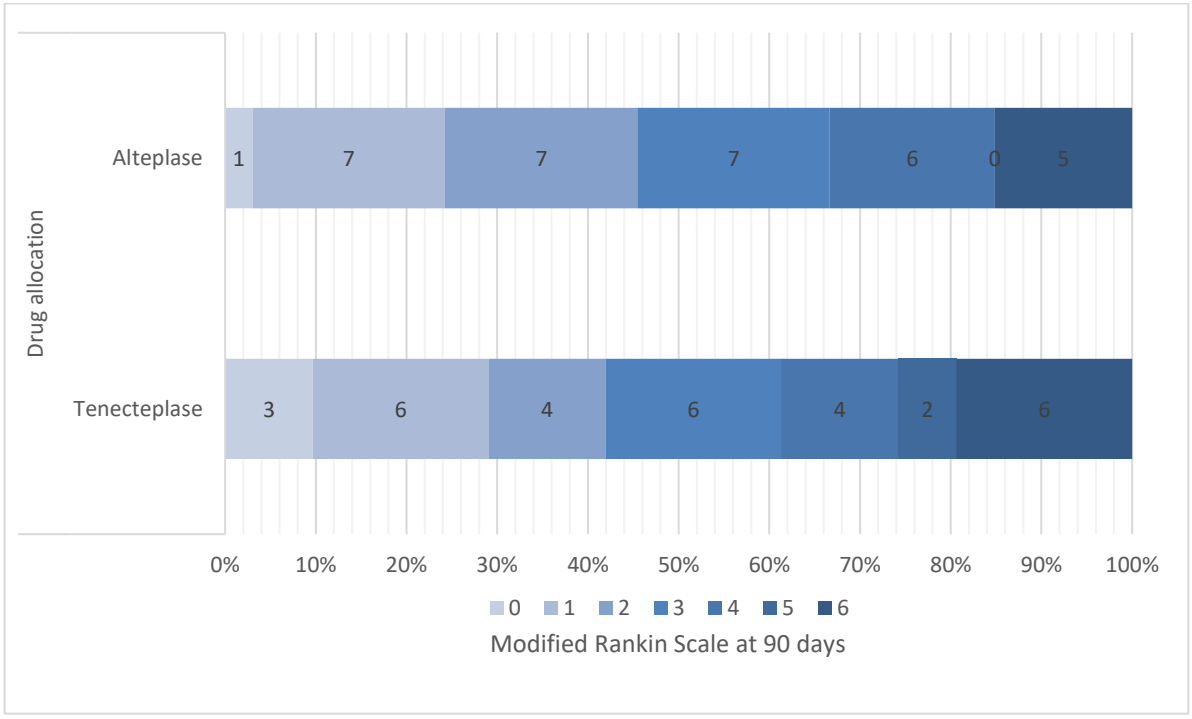


Figure 6-9 - Functional outcome for cases with LVO defined by CTA only

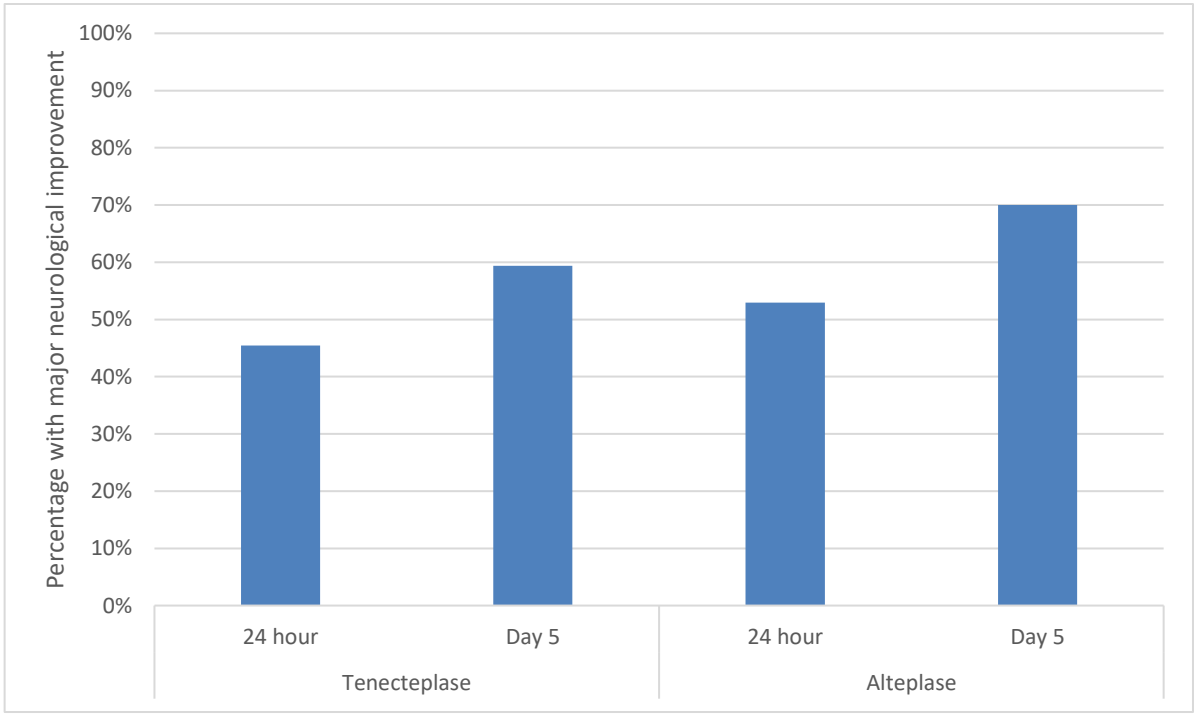


Figure 6-10 - Major neurological improvement for cases with LVO defined by CTA only

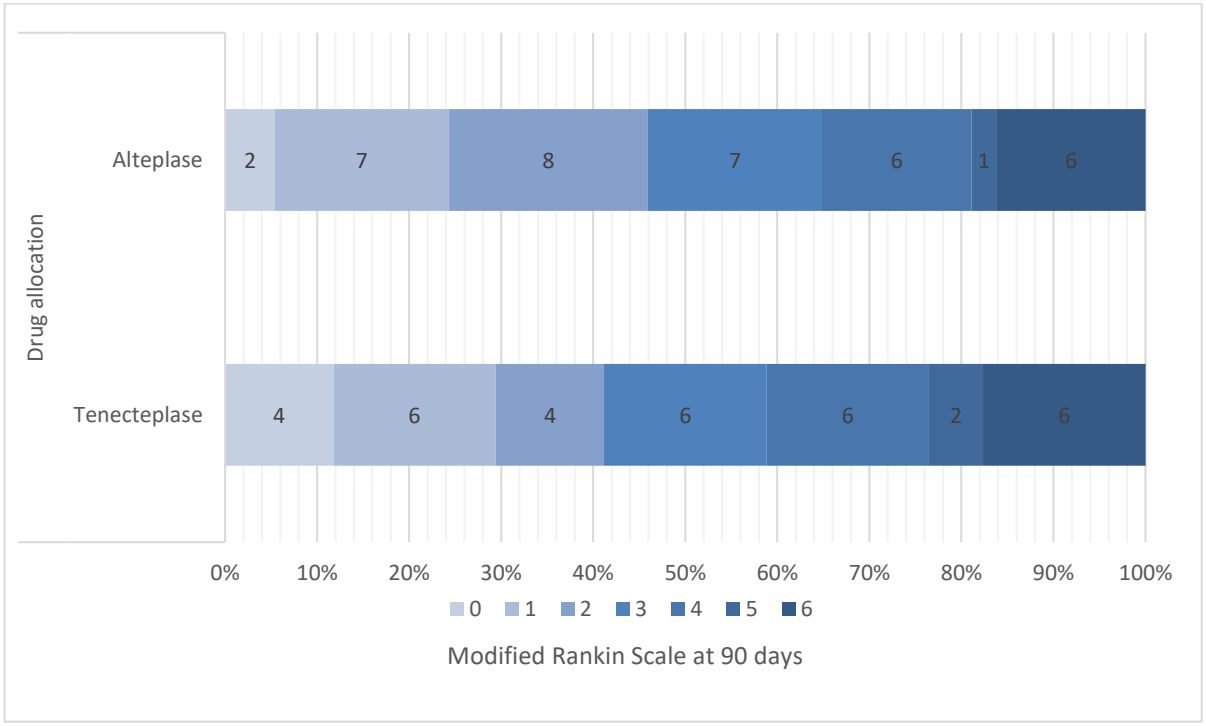


Figure 6-11 - Functional outcome for cases with LVO defined by CTA or NCCT

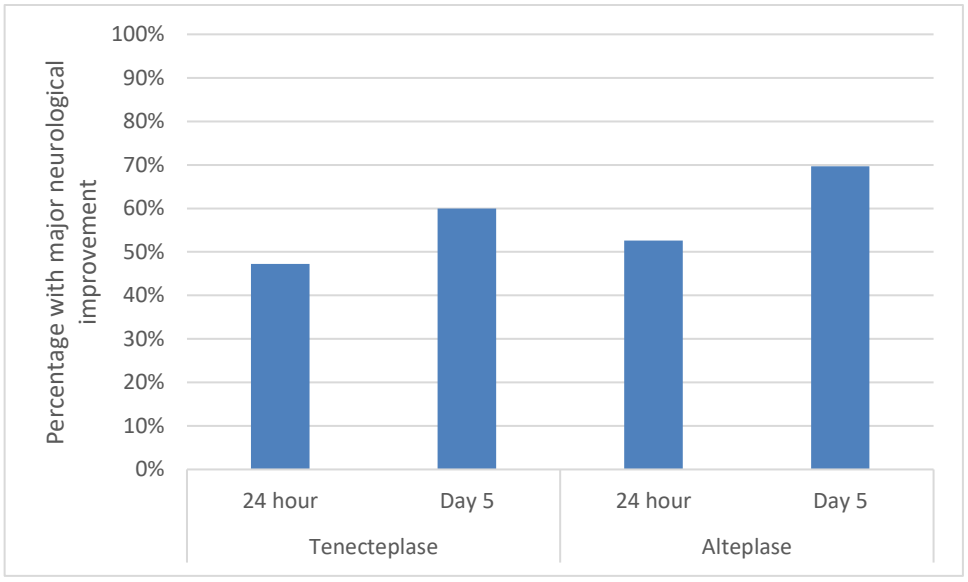


Figure 6-12 - Major neurological improvement for cases with LVO defined by CTA or NCCT

Dependent variable	Independent variable	p-value	Co-variates	p-value
Day 90 mRS	Treatment allocation	0.72	Age Baseline NIHSS	0.024 <0.001
Day 90 mRS	Treatment allocation	0.80	Age Baseline core volume	0.018 <0.001
Day 90 mRS	Treatment allocation	0.59	Age Baseline penumbra volume	0.033 0.06
Day 90 mRS	Treatment allocation	0.86	Age Baseline core volume Baseline penumbra volume	0.028 <0.001 0.165
Day 90 mRS	Treatment allocation	0.84	Age Baseline NIHSS Baseline core volume Baseline penumbra volume	0.027 0.152 0.003 0.383

Table 6-6 - Ordinal regression results for cases with LVO or MeVO defined by CTA

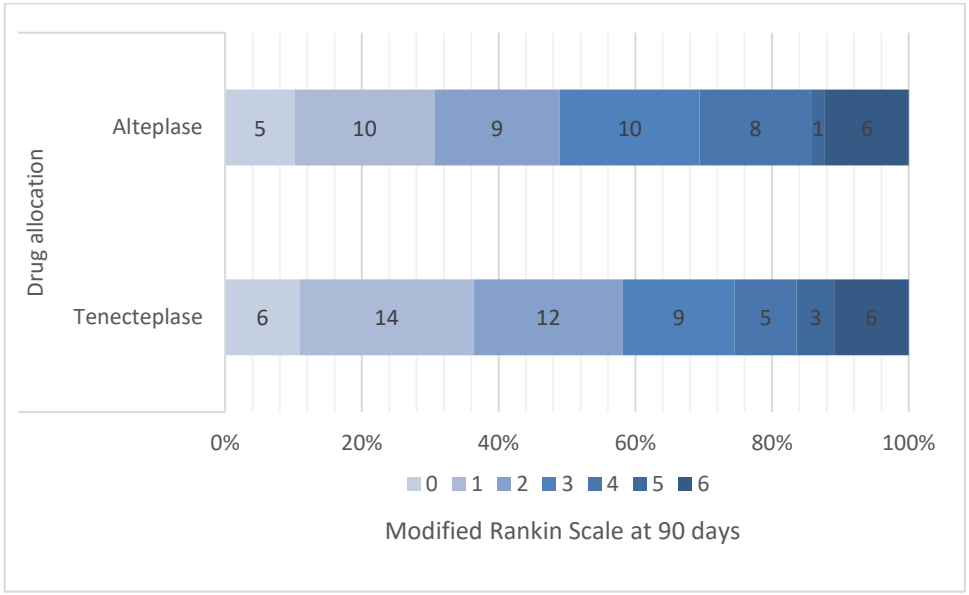


Figure 6-13 - Functional outcome for cases with LVO or MeVO defined by CTA

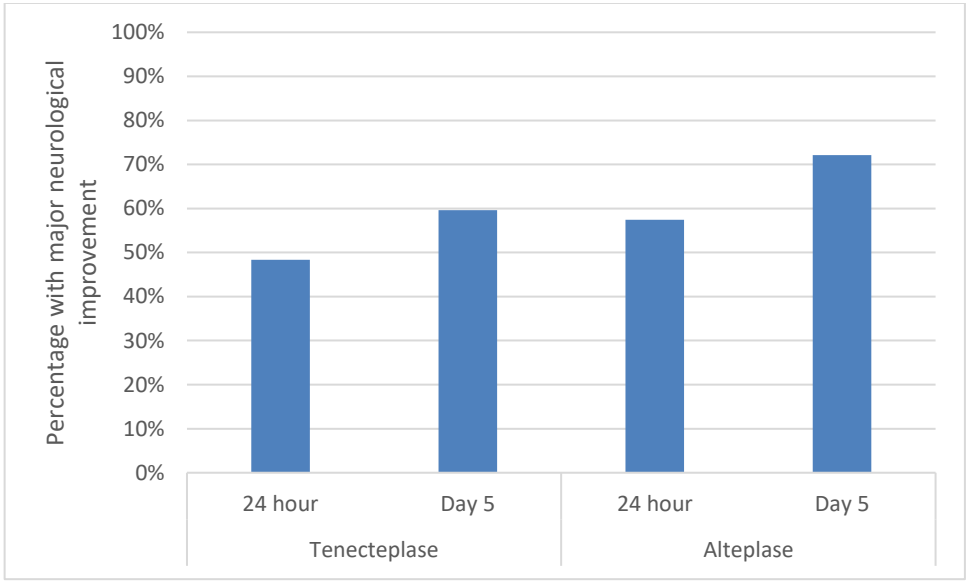


Figure 6-14 - Major neurological improvement for cases with LVO or MeVO defined by CTA

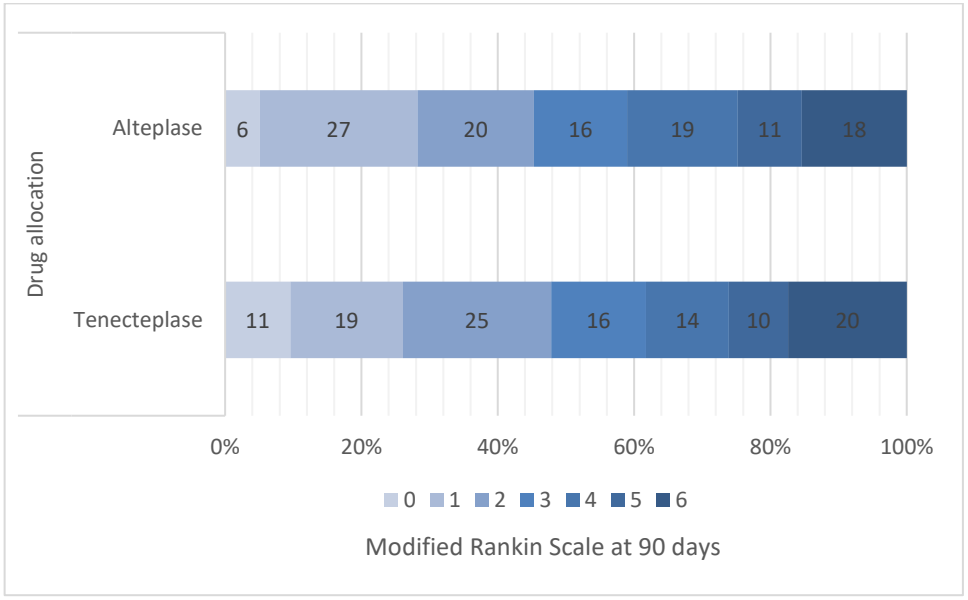


Figure 6-15 - Functional outcome in LVO cases in the ATTEST-2 trial

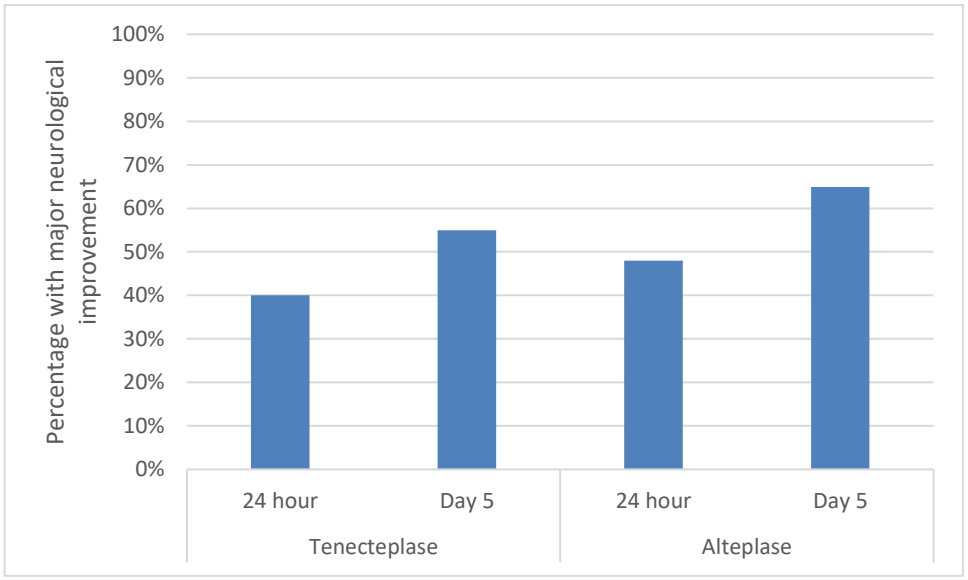


Figure 6-16 - Major neurological improvement in LVO cases in the ATTEST-2 trial

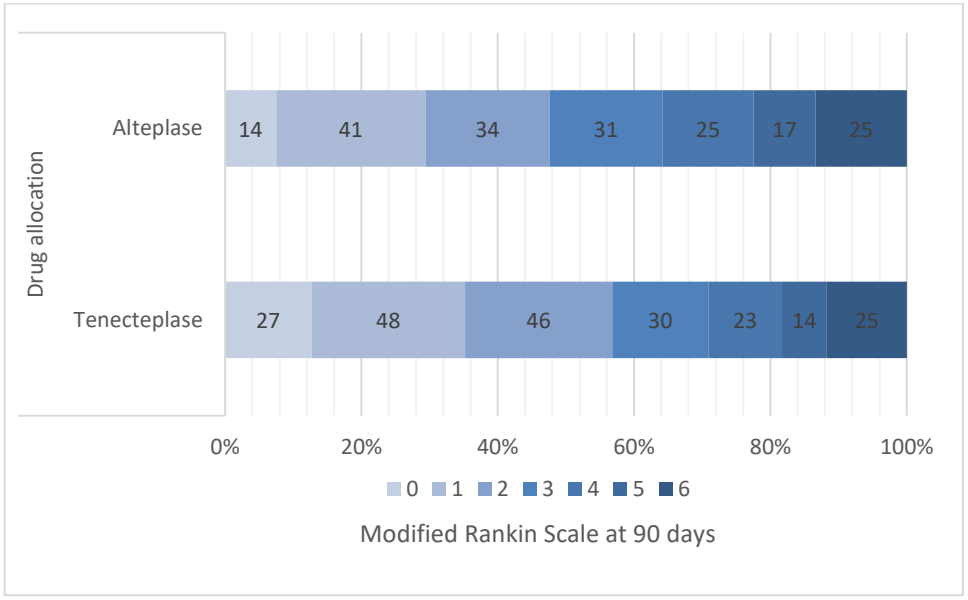


Figure 6-17 - Functional outcome in LVO and MeVO cases in the ATTEST-2 trial

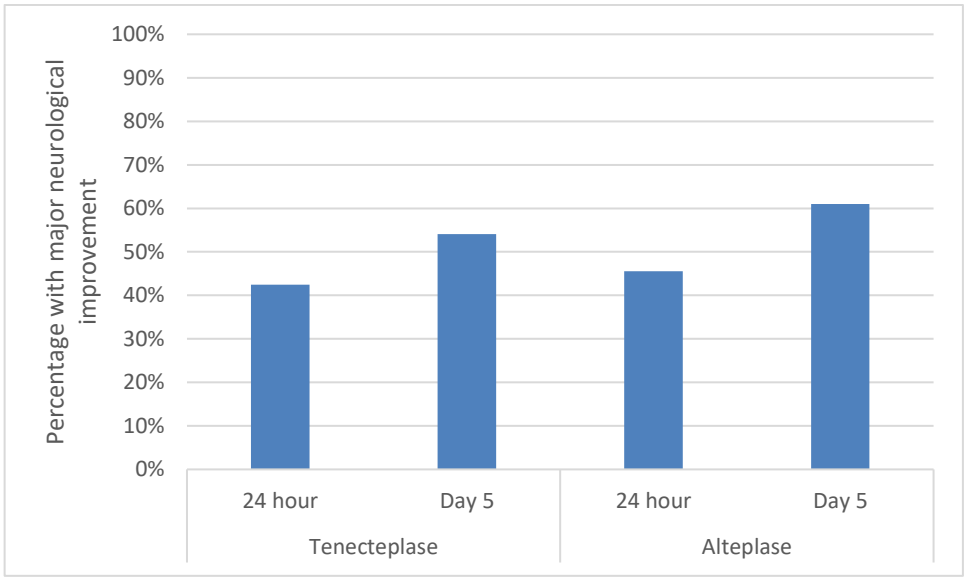


Figure 6-18 - Major neurological improvement in LVO and MeVO cases in the ATTEST-2 trial

6.3.6 Perfusion mismatch

6.3.6.1 DEFUSE-3 criteria

In the population who met DEFUSE-3 mismatch criteria age (71±11 in the tenecteplase group and 70±12 in the alteplase group) and baseline NIHSS (9.5 (5-15) for tenecteplase and 10 (7-18) for alteplase) were well matched between tenecteplase and alteplase groups (p=0.86 and p=0.31 respectively). Baseline ischaemic core volumes were similar, 7ml (0-19) in the tenecteplase group and 7ml (0-13) in the alteplase group (p=0.71). Baseline penumbra volumes were smaller in the tenecteplase group (45ml (27-109.5)) than the alteplase group (70.5ml (34.5-125.5)) but the difference was not statistically significant (p=0.19). The mismatch ratio was also smaller in the tenecteplase group (9.4 (4.3-infinite)) than the alteplase group (15.9 (5.7-infinite)) but again not statistically significant (p=0.40).

Functional outcome at 90 days assessed by mRS was not significantly different between the two treatments in ordinal regression when adjusting for age, baseline NIHSS, baseline core volume and baseline penumbra volume. All co-variates were predictive of day 90 mRS independently, in a combined model of all co-variates age and baseline penumbra volume were predictive of day 90 mRS but baseline NIHSS and baseline core volume were not. Results are shown in table 6-7. Functional independence at 90 days (mRS 0-2) occurred in 40/60 and 35/54 of tenecteplase and alteplase cases respectively (p=0.84). Excellent outcome at 90 days (mRS 0-1) occurred in 29/60 and 22/54 of tenecteplase and alteplase cases respectively (p=0.42). Major neurological improvement at 24 hours was seen in 30/64 and 34/57 participants for tenecteplase and alteplase respectively (p=0.16). Major neurological improvement at 5 days was seen in 31/57 and 34/47 participants for tenecteplase and alteplase respectively which was a trend in favour of alteplase but not statistically significant (p=0.06). Results are shown in figures 6-19 and 6-20.

There was 1 sICH in the tenecteplase group and none in the alteplase group (p=0.34). Mortality by day 90 occurred in 3 tenecteplase cases and 1 alteplase case (p=0.36).

In a binary logistic regression model to predict functional independence at 90 days, baseline ischaemic penumbra volume was the only predictive factor of mRS 0-2 (p=0.01) when including age, treatment allocation, baseline NIHSS and baseline ischaemic core volume.

6.3.6.2 EXTEND criteria

In the population who met EXTEND mismatch criteria age (70±11 for tenecteplase and 71±13 for alteplase) and baseline NIHSS (9 (5-15) for tenecteplase and 10 (7-18) for alteplase) were well matched between tenecteplase and alteplase groups (p=0.73 and p=0.29 respectively). Baseline ischaemic core volumes were similar, 7ml (0-22) in the tenecteplase group and 7ml (0-13) in the alteplase group (p=0.72). Baseline penumbra volumes were smaller in the tenecteplase group (41ml (23-102)) than the alteplase group (60.5ml (29-113)) but the difference was not statistically significant (p=0.15). The mismatch ratio was also smaller in the tenecteplase group (8.5 (3.7-infinite)) than the alteplase group (15.3 (5.1-infinite)) but again not statistically significant (p=0.44).

Functional outcome at 90 days assessed by mRS was not significantly different between the two treatments in ordinal regression when adjusting for age, baseline NIHSS, baseline core volume and baseline penumbra. All co-variates were independently predictive of day 90 mRS. Age, baseline core volume and baseline penumbra volume were predictive of day 90 mRS in a combined model. In a model containing all co-variates age was the only predictor of day 90 mRS. Results are shown in table 6-8. Functional independence at 90 days (mRS 0-2) occurred in 44/70 and 39/59 of tenecteplase and alteplase cases respectively (p=0.70). Excellent outcome at 90 days (mRS 0-1) occurred in 30/70 and 25/59 of tenecteplase and alteplase cases respectively (p=0.96). Major neurological improvement at 24 hours was seen in 35/75 and 37/63 participants for tenecteplase and alteplase respectively (p=0.16). Major neurological improvement at 5 days was seen in 38/67 and 38/53 participants for tenecteplase and alteplase respectively which was a trend in favour of alteplase but not statistically significantly (p=0.09). Results are shown in figures 6-21 and 6-22.

There was 1 sICH in the tenecteplase group and none in the alteplase group (p=0.35). Mortality by day 90 occurred in 4 tenecteplase cases and 1 alteplase case (p=0.24).

In a binary logistic regression model to predict functional independence at 90 days, baseline NIHSS was the only predictor of mRS 0-2 (p=0.04) when including age, treatment allocation, baseline ischaemic core volume and baseline penumbra volume. Baseline penumbra volume was predictive of functional independence (p=0.04) in a model with age, baseline core volume and treatment allocation.

6.3.6.3 Perfusion mismatch with LVO

There were 20 cases in each treatment group of perfusion mismatch by DEFUSE-3 criteria and LVO on CTA. Baseline NIHSS was 13 (5-21) for tenecteplase, and 22 (16-24) for alteplase which was a significant difference (p=0.049); baseline ischaemic core volume was 7ml (0-21.5) for tenecteplase, and 11.5ml (5.5-34) for alteplase (p=0.19); and baseline penumbra volume was 116ml (48-145) for tenecteplase and 138ml (76.5-185) for alteplase (p=0.17). Day 90 mRS did not differ between groups in ordinal regression when adjusting for age, baseline NIHSS, baseline core volume and baseline penumbra volume. Baseline core volume and baseline penumbra volume were predictive of day 90 mRS when adjusting for age but baseline NIHSS was not. In a combined model, baseline core volume was the only predictive factor of day 90 mRS. Results are shown in table 6-9. Functional independence (mRS 0-2) was seen in 10/19 cases in each treatment group (p=1.0) and excellent outcome (mRS 0-1) by 8/19 in the tenecteplase group and 6/19 in the alteplase group (p=0.50). Major neurological improvement was seen at 24 hours in 10/20 tenecteplase cases and 11/19 alteplase cases (p=0.62) and at day 5 in 12/20 and 12/16 alteplase cases (p=0.34). Results are shown in figures 6-23 and 6-24.

Using EXTEND criteria instead results were similar. There were 22 tenecteplase cases and 21 alteplase cases of perfusion mismatch and LVO on CTA. Baseline NIHSS was 13 (6-20) for tenecteplase, and 22 (16-24) for alteplase which was a significant difference (p=0.03); baseline ischaemic core volume was 8ml (0-27) for tenecteplase, and 11ml (4.5-33.5) for alteplase (p=0.38); and baseline

penumbra volume was 107.5ml (40-141.5) for tenecteplase and 136ml (73-177) for alteplase (p=0.15). Day 90 mRS did not differ between groups in ordinal regression when adjusting for age, baseline NIHSS, baseline core volume and baseline penumbra volume. Baseline NIHSS and baseline core volume were independently predictive of day 90 mRS but baseline penumbra was not when adjusting for age. In a combined model of all co-variates baseline core volume was the only predictor of day 90 mRS. Results are shown in table 6-10. Functional independence (mRS 0-2) was seen in 10/21 tenecteplase cases and 10/19 alteplase cases (p=0.75) and excellent outcome (mRS 0-1) by 8/21 in the tenecteplase group and 6/19 in the alteplase group (p=0.67). Major neurological improvement was seen at 24 hours in 11/22 tenecteplase cases and 12/20 alteplase cases (p=0.52) and at day 5 in 14/22 and 13/17 alteplase cases (p=0.39). Results are shown in figures 6-25 and 6-26.

Dependent variable	Independent variable	p-value	Co-variates	p-value
Day 90 mRS	Treatment allocation	0.50	Age Baseline NIHSS	0.01 <0.001
Day 90 mRS	Treatment allocation	0.88	Age Baseline core volume	0.009 0.001
Day 90 mRS	Treatment allocation	0.53	Age Baseline penumbra volume	0.012 <0.001
Day 90 mRS	Treatment allocation	0.64	Age Baseline core volume Baseline penumbra volume	0.009 0.046 0.004
Day 90 mRS	Treatment allocation	0.49	Age Baseline NIHSS Baseline core volume Baseline penumbra volume	0.011 0.125 0.193 0.048

Table 6-7 - Ordinal regression results for the DEFUSE-3 mismatch population

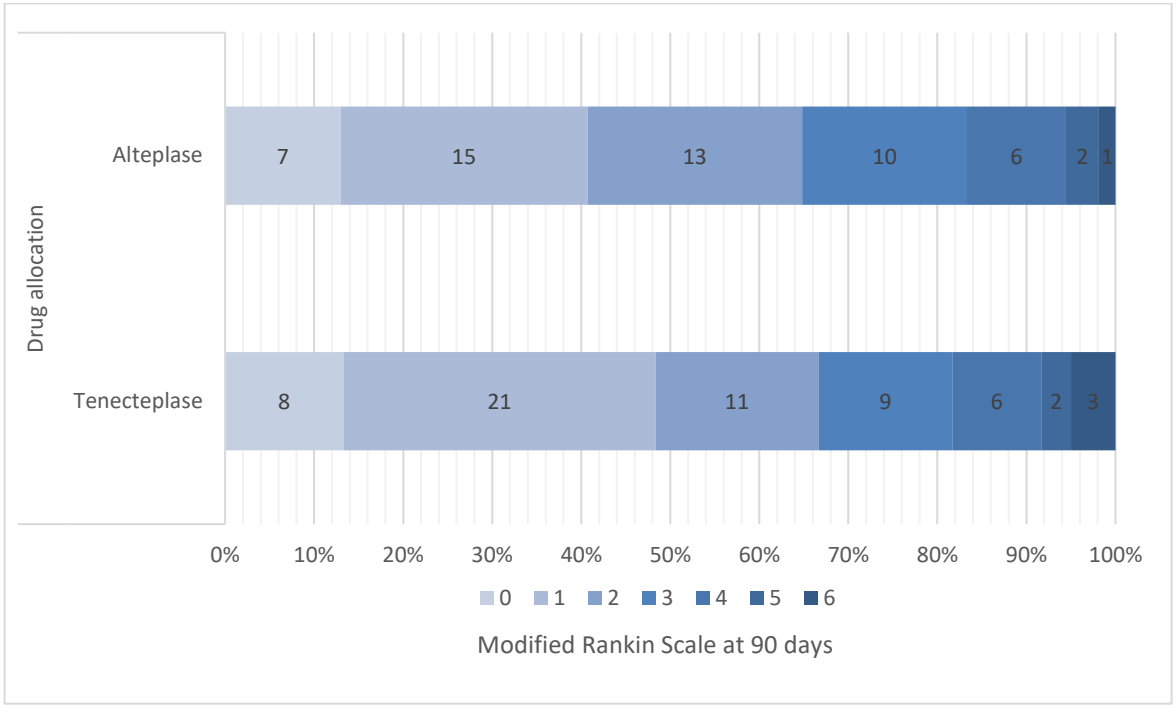


Figure 6-19 - Functional outcome in the DEFUSE-3 mismatch population

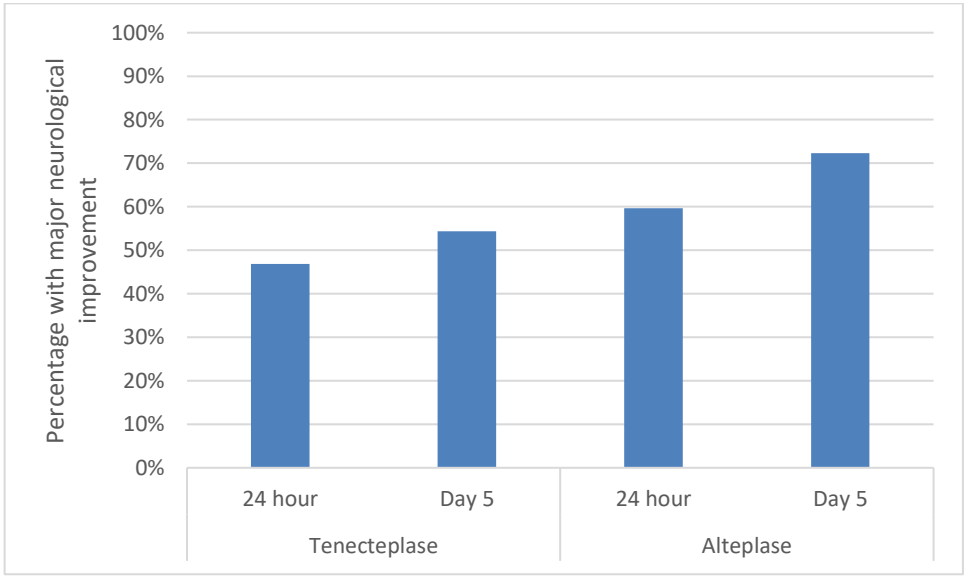


Figure 6-20 - Major neurological improvement in the DEFUSE-3 mismatch population

Dependent variable	Independent variable	p-value	Co-variates	p-value
Day 90 mRS	Treatment allocation	0.18	Age Baseline NIHSS	0.042 <0.001
Day 90 mRS	Treatment allocation	0.61	Age Baseline core volume	0.035 <0.001
Day 90 mRS	Treatment allocation	0.21	Age Baseline penumbra volume	0.035 <0.001
Day 90 mRS	Treatment allocation	0.29	Age Baseline core volume Baseline penumbra volume	0.031 0.012 0.007
Day 90 mRS	Treatment allocation	0.19	Age Baseline NIHSS Baseline core volume Baseline penumbra volume	0.035 0.087 0.123 0.100

Table 6-8 - Ordinal regression results for the EXTEND mismatch population

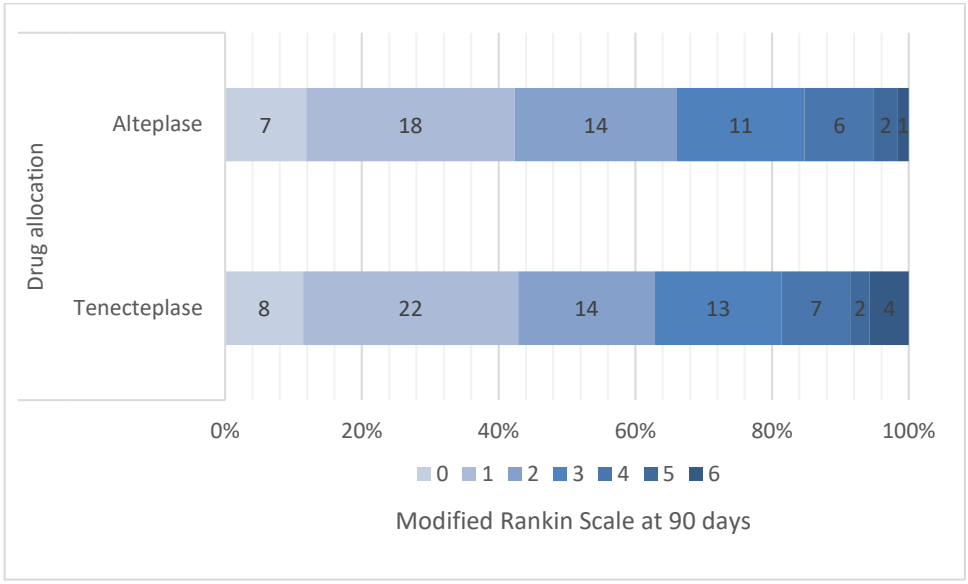


Figure 6-21 - Functional outcome in the EXTEND mismatch population

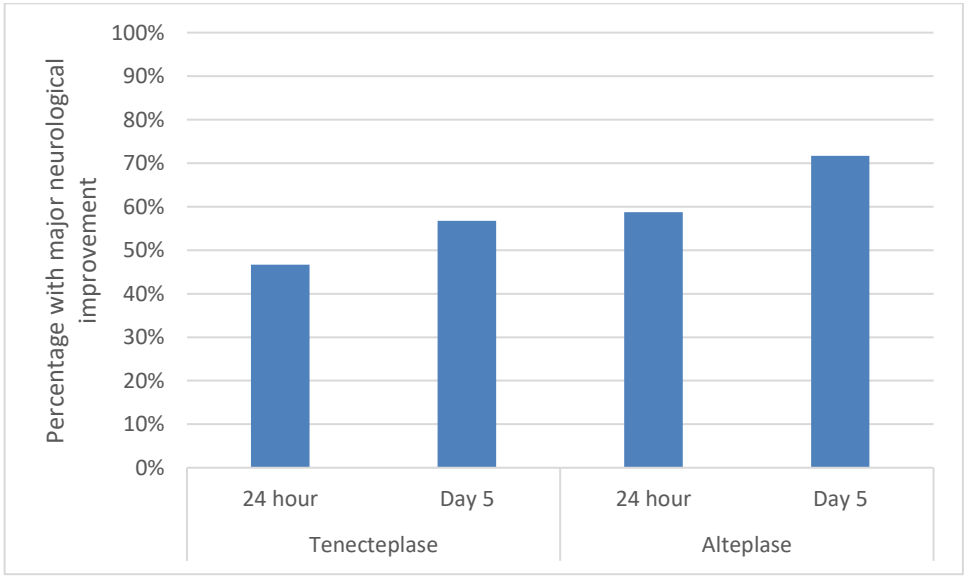


Figure 6-22 - Major neurological improvement in the EXTEND mismatch population

Dependent variable	Independent variable	p-value	Co-variates	p-value
Day 90 mRS	Treatment allocation	0.48	Age Baseline NIHSS	0.205 0.053
Day 90 mRS	Treatment allocation	0.89	Age Baseline core volume	0.044 0.012
Day 90 mRS	Treatment allocation	0.69	Age Baseline penumbra volume	0.109 0.042
Day 90 mRS	Treatment allocation	0.59	Age Baseline core volume Baseline penumbra volume	0.048 0.043 0.166
Day 90 mRS	Treatment allocation	0.60	Age Baseline NIHSS Baseline core volume Baseline penumbra volume	0.059 0.965 0.066 0.193

Table 6-9 - Ordinal regression results for the DEFUSE-3 mismatch LVO population

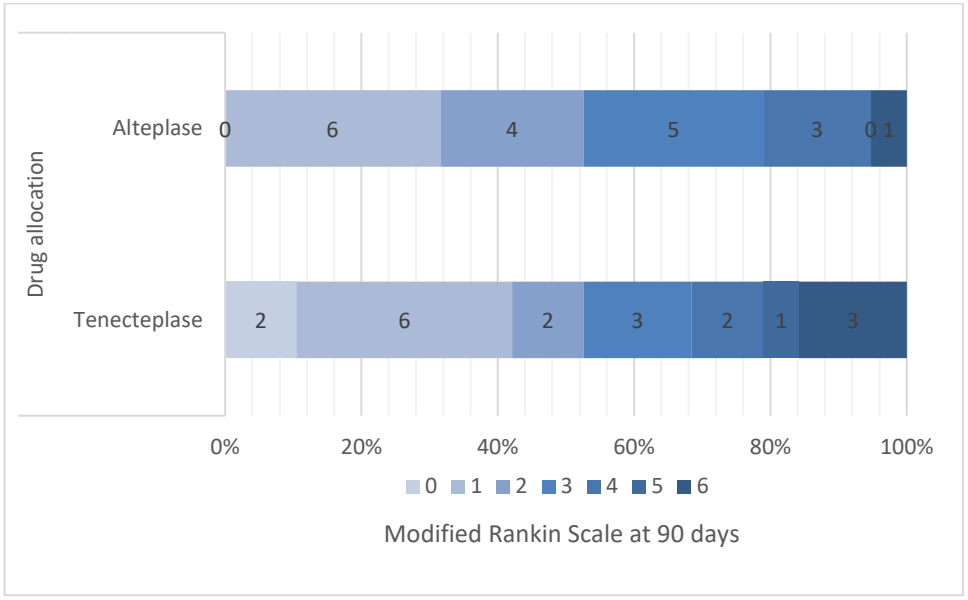


Figure 6-23 - Functional outcome in the DEFUSE-3 mismatch LVO population

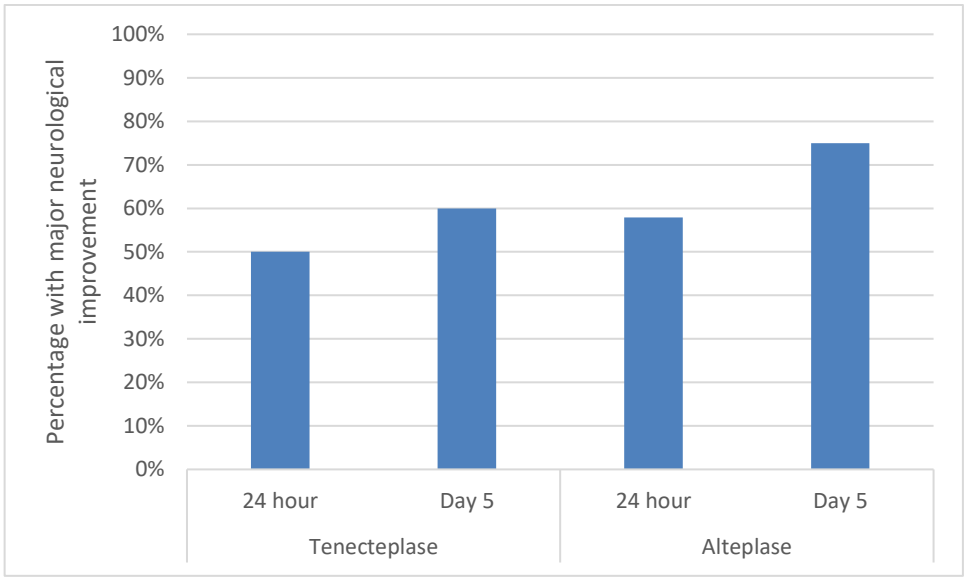


Figure 6-24 - Major neurological improvement in the DEFUSE-3 mismatch LVO population

Dependent variable	Independent variable	p-value	Co-variates	p-value
Day 90 mRS	Treatment allocation	0.27	Age Baseline NIHSS	0.180 0.039
Day 90 mRS	Treatment allocation	0.64	Age Baseline core volume	0.037 0.003
Day 90 mRS	Treatment allocation	0.50	Age Baseline penumbra volume	0.106 0.094
Day 90 mRS	Treatment allocation	0.44	Age Baseline core volume Baseline penumbra volume	0.039 0.008 0.325
Day 90 mRS	Treatment allocation	0.43	Age Baseline NIHSS Baseline core volume Baseline penumbra volume	0.050 0.891 0.017 0.377

Table 6-10 - Ordinal regression results for the EXTEND mismatch LVO population

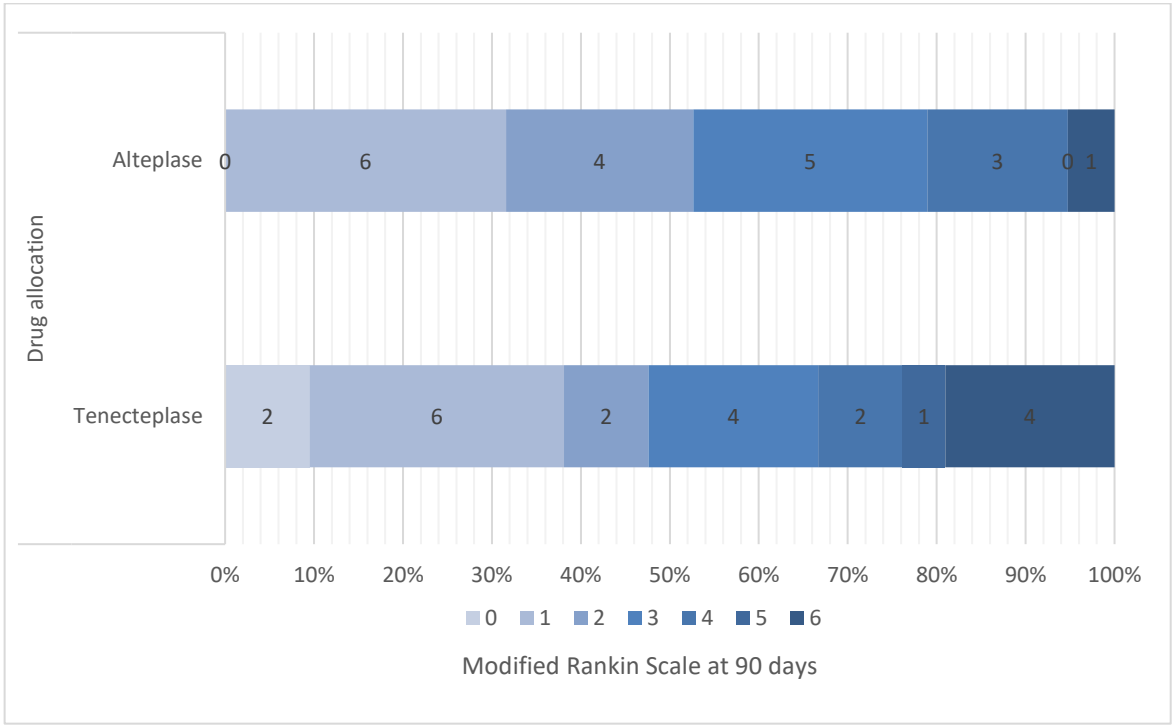


Figure 6-25 - Functional outcome in those with perfusion mismatch by EXTEND criteria and LVO on CTA

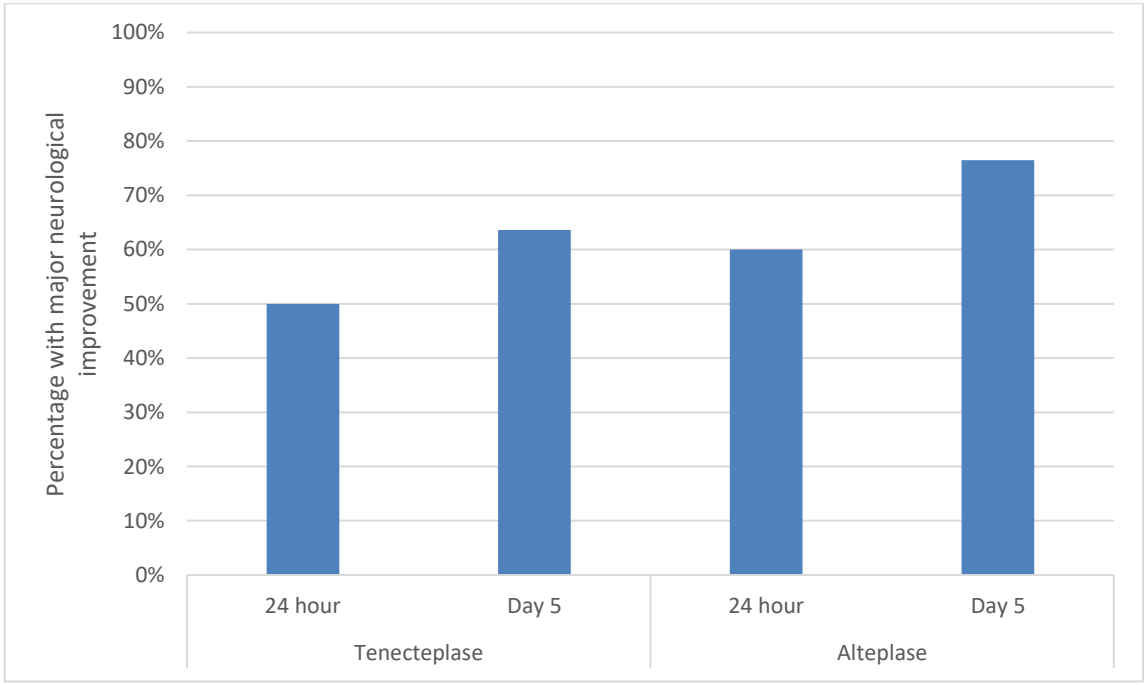


Figure 6-26 - Major neurological improvement in those with perfusion mismatch by EXTEND criteria and LVO on CTA

6.3.7 Recanalisation

In the imaging substudy mITT population, defining LVO by CTA alone, early recanalisation occurred in 1/13 tenecteplase cases and 1/17 alteplase cases (p=0.84). Defining LVO by CTA or NCCT, early recanalisation occurred in 1/13 tenecteplase cases and 1/18 alteplase cases (p=0.82).

In the main ATTEST-2 cohort, defining LVO by CTA alone, early recanalisation occurred in 6/70 tenecteplase cases and 2/82 alteplase cases, with a non-significant trend towards tenecteplase (p=0.09). Defining LVO by CTA or NCCT did not alter this result.

6.3.8 Penumbral salvage

Follow up infarct volumes (FIV) on CT and MRI were smaller than expected with a high proportion of imaging with no infarct seen at follow up (139/303 cases). This was reflected in mean FIV of 20.8ml ±45.5 for tenecteplase and 27.6ml ±68.9 for alteplase; but median FIV of 0.9ml (0-17.2) for tenecteplase and 1.2ml (0-20.5) for alteplase. For MRI only data, mean FIV was 6.9ml ±13.5 for tenecteplase and 12.0ml ±24.8 for alteplase with median FIV 1.0ml (0-7.7) for tenecteplase and 1.4ml (0-10.8) for alteplase. Follow up CT was carried out at a mean of 27.8 hours after baseline imaging for tenecteplase cases and 27.4 hours for alteplase cases. Follow up MRI was carried out at a mean of 49.9 hrs after baseline imaging for tenecteplase cases and 42.2 hrs for alteplase cases.

The median volume of penumbra salvaged (VPS) was 11.9ml (0-39) for tenecteplase and 11.0ml (0-66.8) for alteplase. There was no significant difference between groups of VPS (p=0.45), including when adjusted for baseline core volume (p=0.28). The median proportion of volume of penumbral salvaged (VPS%) was 97% (63-100) for tenecteplase and 100% (81-107) for alteplase (p=0.19). VPS in different populations is shown in figure 6-27 along with FIV, baseline core and penumbra volumes.

Negative values of VPS reflected infarct growth greater than baseline penumbra volume, indicating visibly abnormal tissue beyond the initial ischaemic lesion. In a sensitivity analysis, negative VPS values were interpreted as 0. Median VPS

values were unchanged and no significant difference was found between groups of VPS (p=0.09).

Box-plots for VPS in both analyses are shown in figures 6-28 and 6-29.

For regression analysis, VPS underwent log transformation to achieve normal distribution. In linear regression, treatment allocation was not associated with VPS (p=0.87) when adjusting for age, baseline core and baseline penumbra volumes. Baseline penumbra volume was predictive of VPS in this model (p=<0.001) with each 1ml increase in baseline penumbra volume equating to 0.8ml of VPS. In a model with age and treatment allocation but without baseline penumbra volume, baseline core volume was predictive of VPS (p=<0.001) with each 1ml increase in baseline core volume associated with 0.4ml of VPS.

In a sensitivity analysis, VPS was examined in those with LVO. In those with LVO, median baseline core volume was 9ml (0-40) for tenecteplase and 22ml (7-80) for alteplase; and baseline penumbra volume was 91ml (25.5-139.5) for tenecteplase and 118ml (69-154) for alteplase. Median VPS was 44.1ml (2-143.6) for tenecteplase and 112.5ml (11-157.6) for alteplase which was not significantly different (p=0.24). In linear regression, treatment allocation was not associated with VPS (p=0.59) when adjusting for age, baseline core and penumbra volumes.

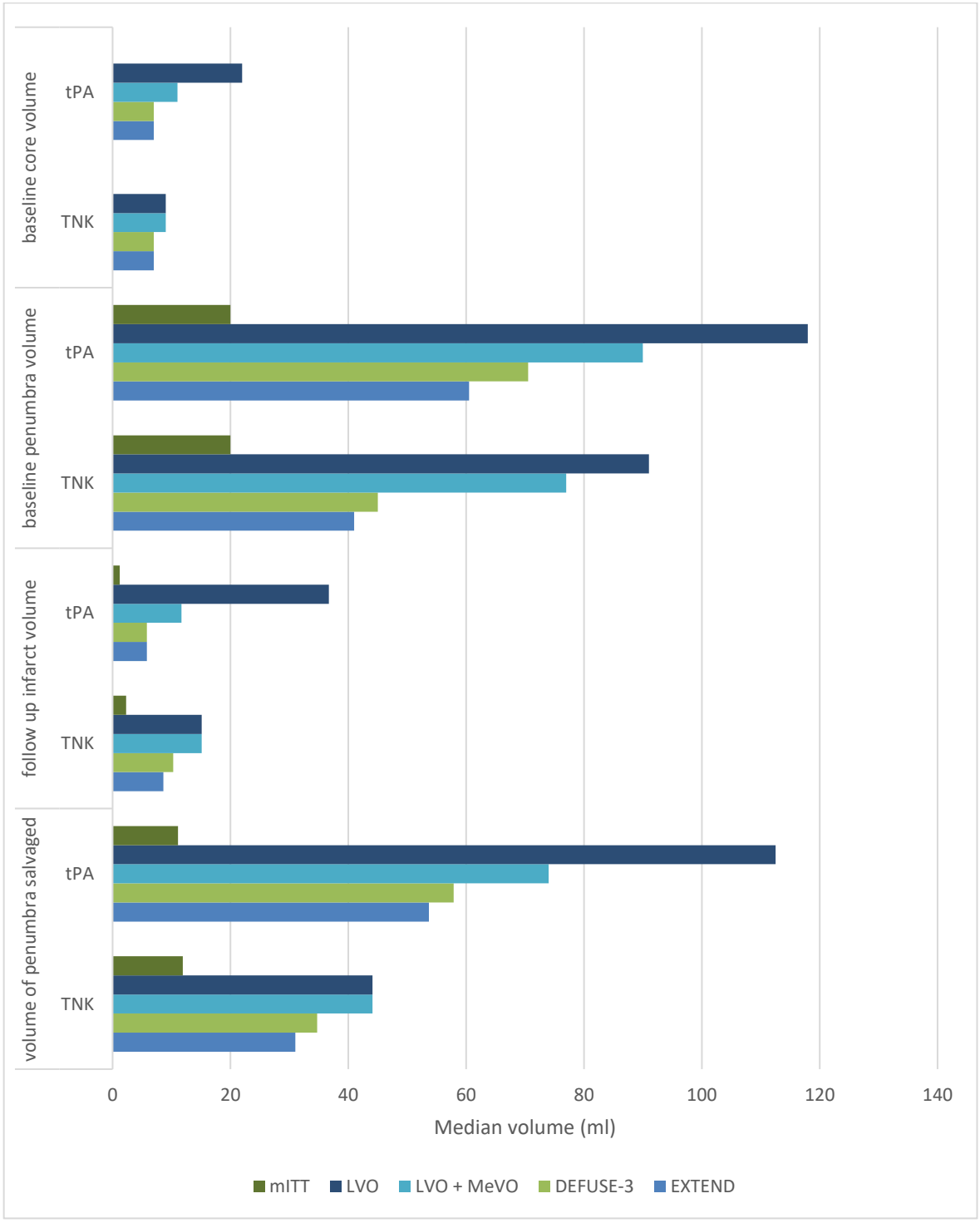


Figure 6-27 - Imaging volumes in different populations

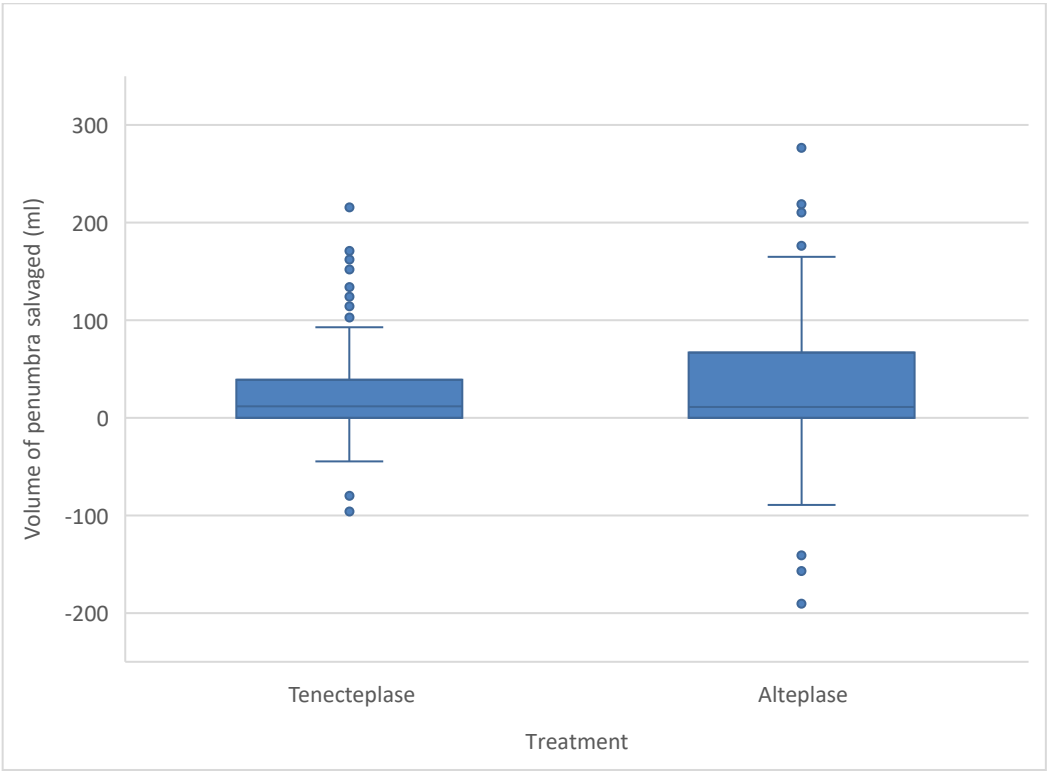


Figure 6-28 - Boxplots of VPS in each treatment group

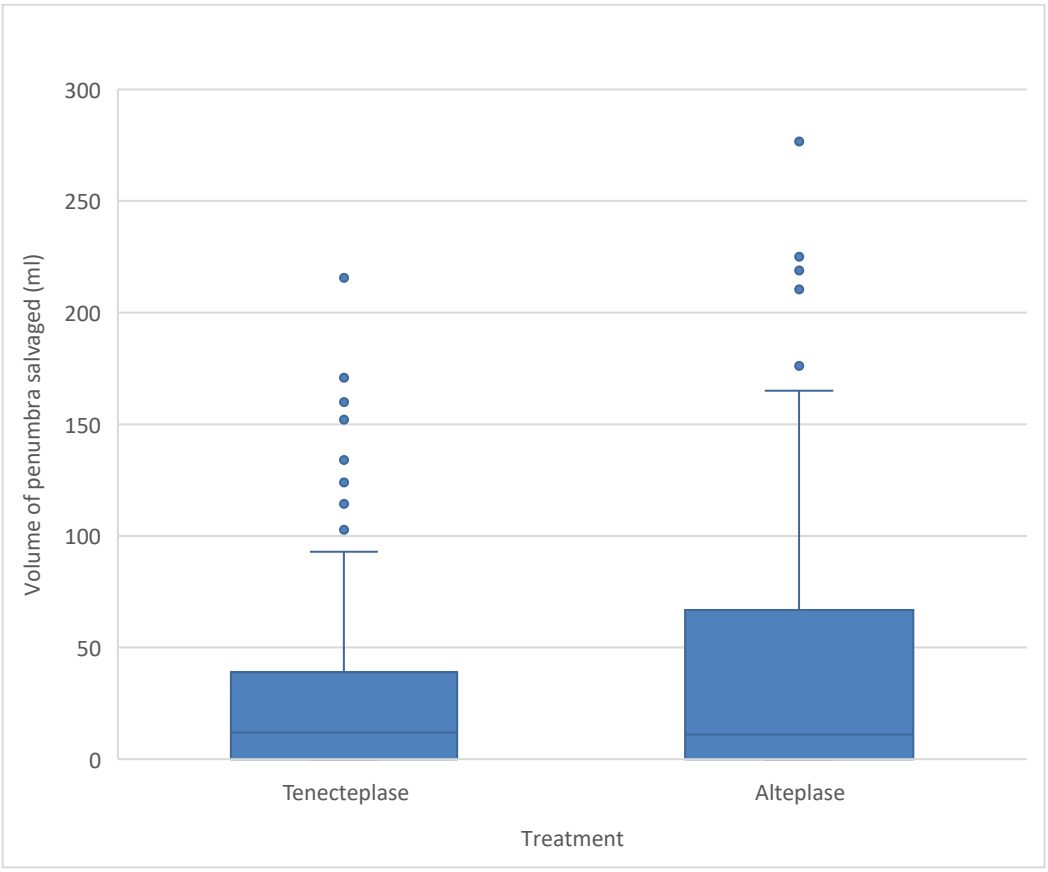


Figure 6-29 - Boxplots of VPS sensitivity analysis

6.3.9 Inter- and intra-rater reliability

Inter-rater reliability for MRI DWI volume calculation had a kappa coefficient of 0.94 (0.92-0.96) (p=<0.001) and intraclass correlation coefficient of 0.997 (0.996-0.998) (p=<0.001). Intra-rater reliability for non-contrast CT infarct volume calculation had a kappa coefficient of 0.81 (0.73-0.89) (p=<0.001) and intraclass correlation coefficient of 0.993 (0.983-0.997) (p=<0.001). These represent good to excellent reliability (186).

6.4 Discussion

In the ATTEST-2 imaging substudy we found no significant difference between tenecteplase and alteplase efficacy and safety outcomes in the overall population and a number of pre-specified subgroups. In the mITT population there was no difference in functional outcome, major neurological recovery, sICH rate or mortality. In logistic regression, functional independence (mRS 0-2) was predicted by age, baseline NIHSS, and baseline ischaemic core volume but not treatment allocation, age and NIHSS predicted mRS 0-1 but not baseline core volume.

Those with LVO and those with either LVO or MeVO showed no difference in functional outcome or major neurological recovery. Those with perfusion mismatch by DEFUSE-3 or EXTEND criteria had no significant difference in functional outcome, major neurological improvement, sICH rate or mortality. Analysis of data for those with perfusion mismatch in combination with LVO was complicated by higher baseline NIHSS in the alteplase cohort but no significant differences were seen in functional outcome or major neurological improvement. Baseline NIHSS was the only predictor of day 90 mRS in the EXTEND perfusion mismatch group but baseline ischaemic penumbra volume was predictive of day 90 mRS in the DEFUSE-3 perfusion mismatch group. Early recanalisation did not differ between treatments. There was no significant difference in terms of penumbral salvage, and in regression baseline core and penumbra volumes were predictive factors for volume of penumbral salvage.

There was a non-significant trend in favour alteplase for achieving major neurological improvement by day 5 in the mismatch populations, this may be

explained by the larger penumbra volumes seen in the alteplase groups though this was also non-significant. It did not translate into improved functional outcome at 90 days.

Assessment of early recanalisation involved small numbers due to the lack of follow up CTA imaging at 2-4 hours which had been planned at outset but was not possible after funding withdrawal. Relying on DSA at the time of MT only produced small numbers of cases. Examining the whole trial population, there were 6 and 2 cases respectively for tenecteplase and alteplase of early TICI 2b/3 recanalisation in cases of confirmed LVO which showed a non-significant trend in favour of tenecteplase. Larger numbers of cases may have shown significantly higher rates of early recanalisation with tenecteplase as seen in the EXTEND IA TNK trial (69).

Overall, we were unable to confirm our 3 hypotheses regarding the superiority of tenecteplase in terms of outcome in mismatch patients, early recanalisation and penumbral salvage. The superiority of tenecteplase in imaging outcomes such as early recanalisation and early reperfusion found in the EXTEND IA TNK, TAAIS and TASTE-A trials were not replicated in our study (63, 69, 71). Our findings correlate with registry data from TETRIS and ETIS who found comparable early recanalisation between tenecteplase and alteplase (153, 154). Our findings are also in keeping with the results of the main ATTEST-2 study showing non-inferiority of tenecteplase compared to alteplase, as well as subgroup analyses of the AcT trial (152).

Several weaknesses were present in the study. Firstly, the cessation of funding early on in the study meant that the original design of the study with NCCT, CTP, pre- and post-treatment CTA and 48 hour MRI in 400 patients was not possible. Thus the data collected thereafter were from routinely acquired scans only. Primarily this impacted the acquisition of post treatment CTA to assess for early recanalisation and 48 hour MRI to assess for infarct volume. Whilst assessment of infarct volume was possible with NCCT, this is less robust and generally was undertaken at an earlier 24 hour timepoint which may not reflect the final volume of infarction. This is likely reflected in the high proportion of follow up scans with no infarct despite the low rate of stroke mimics (5%). Furthermore, as MRI was mostly acquired from routine clinical care, usual

practice likely dictated that MRI was selectively used in cases where no infarct was seen on the initial CT meaning cases with MRI may have selected out those with minor stroke or suspicion of a stroke mimic specifically.

The proportion of CTP scans of poor quality necessitating withdrawal from the analysis was high with 1 in 6 scans not used in the CTP analyses. This reflects the challenge of undertaking CTP in acute stroke patients where motion artefact disrupting scan quality is common in clinical practice. It may also reflect the recent introduction of CTP to clinical practice in the UK as evidenced by the higher numbers of CTP scans obtained later in the trial, meaning levels of experience with acquiring CTP scans may have been low.

The profile of ischaemic strokes in the imaging substudy were characterised by high ASPECTS scores, good collateral status and small ischaemic core and larger ischaemic penumbra volume. Whilst this is not unexpected in a trial of IVT, it may have limited our ability to demonstrate differences between groups. As found in the earlier chapters, tenecteplase may be more effective in fast progressors and less likely to cause haemorrhagic transformation. These findings would be best addressed in a population containing fast progressors with poorer collateral status and larger ischaemic core. Whilst in clinical practice the efficacy of MT in this population is increasingly recognised following recently published trials (187), whether IVT with tenecteplase can be effective in this population is of interest particularly given the lack of availability of EVT globally.

6.5 Conclusion

Tenecteplase has similar efficacy and safety to alteplase in subgroups such as those with LVO and perfusion mismatch. Better early recanalisation and greater penumbral salvage was not confirmed in this study though the availability of relevant imaging likely contributed to this. With supporting evidence from amongst others the ATTEST-2 trial of non-inferiority of tenecteplase, guideline recommendation and regulatory approval of tenecteplase as an alternative to alteplase for acute ischaemic stroke is reasonable, including for such subgroups.

7 Tenecteplase compared to alteplase in the early window in acute ischaemic stroke: meta-analysis of randomised controlled trials

7.1 Introduction

Several trials have shown non-inferiority or superiority of tenecteplase 0.25mg/kg compared to alteplase 0.9mg/kg in acute ischaemic stroke (AIS) patients treated <4.5 hours from onset. We carried out a meta-analysis of published trial results.

7.2 Methods

Medline and EMBASE databases were searched using MeSH terms and Boolean operators for randomised controlled trials (RCT) comparing intravenous tenecteplase 0.25mg/kg to alteplase 0.9mg/kg for AIS treated <4.5hrs from symptom onset. Search criteria are shown in the appendix. Manual screening of titles and abstracts excluded publications not meeting the inclusion criteria such as secondary analyses, non-stroke trials, trials studying alternative doses or routes of tenecteplase or studying other AIS populations. Data were collected on functional outcome measured by modified Rankin Scale (mRS), rate of symptomatic intracranial haemorrhage (sICH - as per individual trial definition) and mortality. Random-effects binary outcome meta-analysis was carried out using StatsDirect software on these four outcomes (188). Risk of bias was assessed with the Cochrane risk of bias assessment tool (189).

7.3 Results

A database search yielded 1775 results, after exclusion of non-RCT and non-human trials, 51 studies were manually searched as detailed in figure 7-1. Eight published RCTs comparing intravenous tenecteplase 0.25mg/kg to alteplase 0.9mg/kg for AIS treated <4.5hrs from symptom onset were identified (62-64, 69, 71, 80, 81, 151), a further unpublished RCT for which data was available was included (ATTEST-2, NCT02814409).

Summary data for all trials is shown in table 7-1. A total of 5417 participants were recruited to the 9 studies. A biocopy of tenecteplase was used in 2 studies (80, 81). Most trials enrolled patients conventionally eligible for IVT and within 4.5 hours of AIS onset, 2 trials enrolled up to 3 hours from onset (62, 80) and one trial recruited participants with evidence of CTP mismatch within 6 hours (63), though only 3 patients in this study were treated beyond 4.5 hours. Three studies included tenecteplase at multiple doses in their design (0.1, 0.25 or 0.4mg/kg), data from participants receiving doses other than 0.25mg/kg were excluded from the analysis (62, 63, 80). Median age ranged from 64 to 76 and median baseline NIHSS from 7 to 17. Studies were generally of high quality with low risk of bias as per the Cochrane risk of bias assessment tool shown in table 7-3. Other than in one trial, all other trials were open label with no blinding of clinicians or patients but only of outcome assessors as is common in acute stroke trials. Blinding of mRS outcome assessors in the TNKS2B trial was unknown.

Outcome data used for meta-analysis is shown in table 7-2. In meta-analysis, tenecteplase had significantly greater likelihood of achieving excellent clinical outcome, mRS 0-1, compared to alteplase across all trials, odds ratio (OR) 1.15 (1.03-1.28), p=0.01. There was a strong trend towards a greater likelihood of functional independence, mRS 0-2, with tenecteplase compared to alteplase across all trials, OR 1.19 (1.00-1.41), p=0.05. Mortality, OR 0.96 (0.78-1.18) p=0.70, and rates of symptomatic ICH, OR 1.11 (0.78-1.58) p=0.57, did not differ between tenecteplase and alteplase. Forest plots are shown in figures 7-2, 7-3, 7-4 and 7-5.

A sensitivity analysis excluding 2 trials using a tenecteplase biocopy showed similar results. Likelihood of excellent outcome showed a strong trend towards tenecteplase, OR 1.14 (1.00-1.30) p=0.05, and likelihood of functional independence was significantly greater with tenecteplase, OR 1.29 (1.01-1.66) p=0.04. Forest plots are shown in figures 7-6 and 7-7.

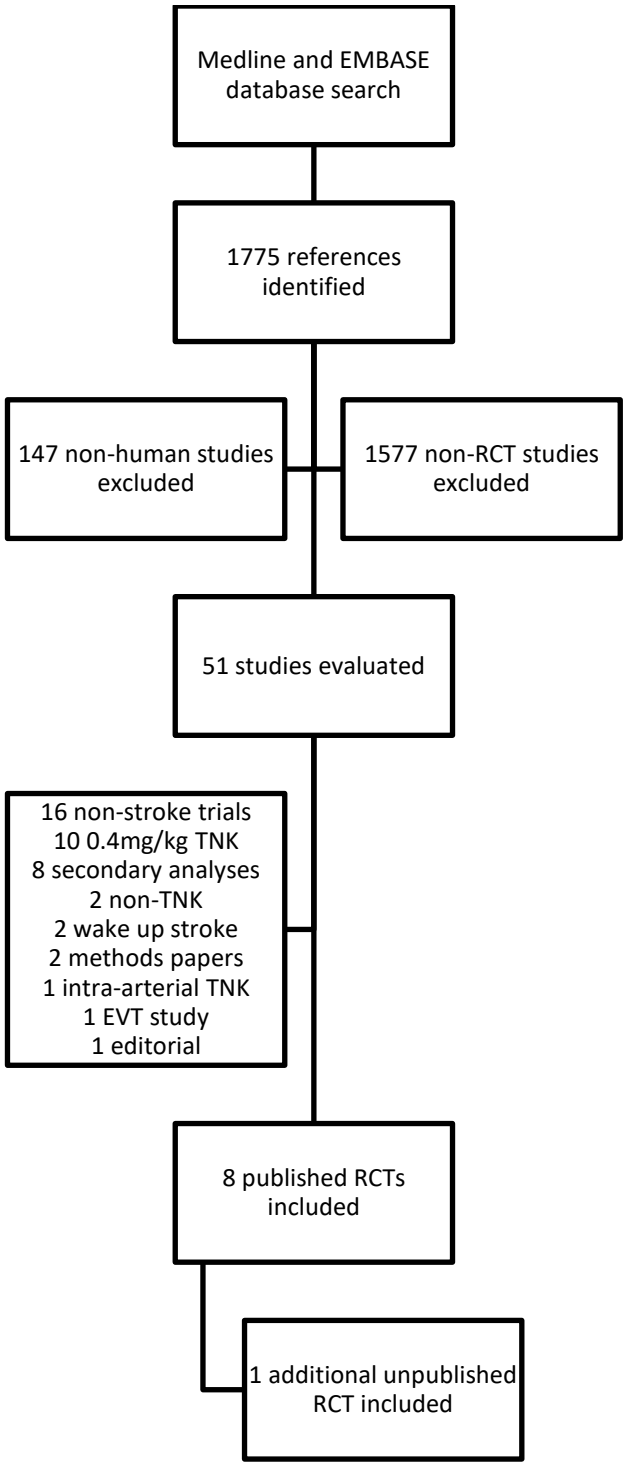


Figure 7-1 - Flow diagram of database search

Title	Author, Journal and Year	N	Trial design	Age (median+IQR or mean±SD)		Baseline NIHSS (median+IQR or mean±SD)		LVO (%)	
				TNK	tPA	TNK	tPA	TNK	tPA
AcT	Menon et al, Lancet 2022	1577	AIS <4.5hrs, conventionally eligible for IVT	74 (63-83)	73 (62-83)	9 (6-16)	10 (6-17)	24.5%	25.5%
ATTEST	Huang et al, Lancet Neurology 2015	104	AIS <4.5hrs, conventionally eligible for IVT	71±13	71 (12)	12 (9-18)	11 (8-16)	75%	61%
TAAIS	Parsons et al, NEJM 2012	50	CTP mismatch with LVO <6hrs after AIS onset	68±10	70±8	15±2	14±2	72%	76%
EXTEND IA TNK	Campbell et al, NEJM 2018	202	AIS <4.5hrs conventionally eligible for IVT and MT	70±15	72±14	17 (12-22)	17 (12-22)	100%	100%
TASTE-A	Bivard et al, Lancet Neurology 2022	104	AIS <4.5hrs, conventionally eligible for IVT	76 (60-84)	73 (61-80)	8 (5-14)	8 (5-17)	49%	39%
TNK S2B	Haley et al, Stroke 2010	62	AIS <3hrs, conventionally eligible for IVT	69±15	72±16	10 (6-15)	13 (5-17)	-	-
TRACE	Li et al, Stroke & Vascular Neurology, 2022	112	AIS <3hrs, conventionally eligible for IVT using TNK biocopy	64±13	67±13	8 (5-12)	8 (5-12)	-	-
TRACE 2	Wang et al, Lancet 2023	1430	AIS <4.5hrs, conventionally eligible for IVT using TNK biocopy, MT candidates excluded	67 (58-73)	65 (58-72)	7 (5-10)	7 (6-10)	4%	
ATTEST2	Unpublished	1776	AIS <4.5hrs, conventionally eligible for IVT	70±12	70±13	7 (5-13)	7 (5-12)	14%	14.5%

Table 7-1 - Summary of trial data

Only data for TNK 0.25mg/kg and tPA 0.9mg/kg has been included.

AIS – acute ischaemic stroke, CTP – CT perfusion, LVO – large vessel occlusion, IVT – intravenous thrombolysis, MT – mechanical thrombectomy, mRS – modified Rankin scale, sICH – symptomatic intracerebral haemorrhage, TNK – tenecteplase, tPA - alteplase

Title	mRS 0-1	mRS 0-2	sICH	Mortality
AcT	TNK 296/802 tPA 266/765	TNK 452/802 tPA 425/765	TNK 27/800 tPA 24/763	TNK 122/796 tPA 117/763
ATTEST	TNK 13/47 tPA 10/49	TNK 17/47 tPA 19/49	TNK 1/52 tPA 2/51	TNK 8/47 tPA 6/49
TAAIS	TNK 18/25 tPA 10/25	TNK 21/25 tPA 11/25	TNK 1/25 tPA 3/25	TNK 1/25 tPA 3/25
EXTEND IA TNK	TNK 52/101 tPA 43/101	TNK 65/101 tPA 52/101	TNK 1/101 tPA 1/101	TNK 10/101 tPA 18/101
TASTE-A	TNK 23/55 tPA 20/49	TNK 36/55 tPA 26/49	TNK 0/55 tPA 0/49	TNK 5/55 tPA 5/49
TNK S2B	TNK 15/31 tPA 13/31	TNK 18/31 tPA 13/31	TNK 2/31 tPA 1/31	TNK 7/31 tPA 8/31
TRACE	TNK 35/57 tPA 35/59	TNK 42/57 tPA 43/59	TNK 0/57 tPA 1/59	TNK 1/57 tPA 6/59
TRACE 2	TNK 439/705 tPA 405/696	TNK 516/705 tPA 502/696	TNK 15/711 tPA 13/706	TNK 46/711 tPA 35/706
ATTEST2	TNK 369/885 tPA 352/891	TNK 565/885 tPA 536/891	TNK 20/885 tPA 15/891	TNK 68/885 tPA 75/891

Table 7-2 Outcome data for meta-analysis

	Random sequence generation	Allocation concealment	Blinding of participant	Blinding of outcome assessment	Incomplete outcome	Selective reporting
AcT	Low	Low	High	Low	Low	Low
ATTEST	Low	Low	High	Low	Low	Low
TAAIS	Low	Low	High	Low	Low	Low
EXTEND IA TNK	Low	Low	High	Low	Low	Low
TASTE-A	Low	Low	High	Low	Low	Low
TNK S2B	Low	Low	Low	Unknown	Low	Low
TRACE	Low	Low	High	Low	Low	Low
TRACE 2	Low	Low	High	Low	Low	Low
ATTEST2	Low	Low	High	Low	Low	Low

Table 7-3 - Risk of bias assessment

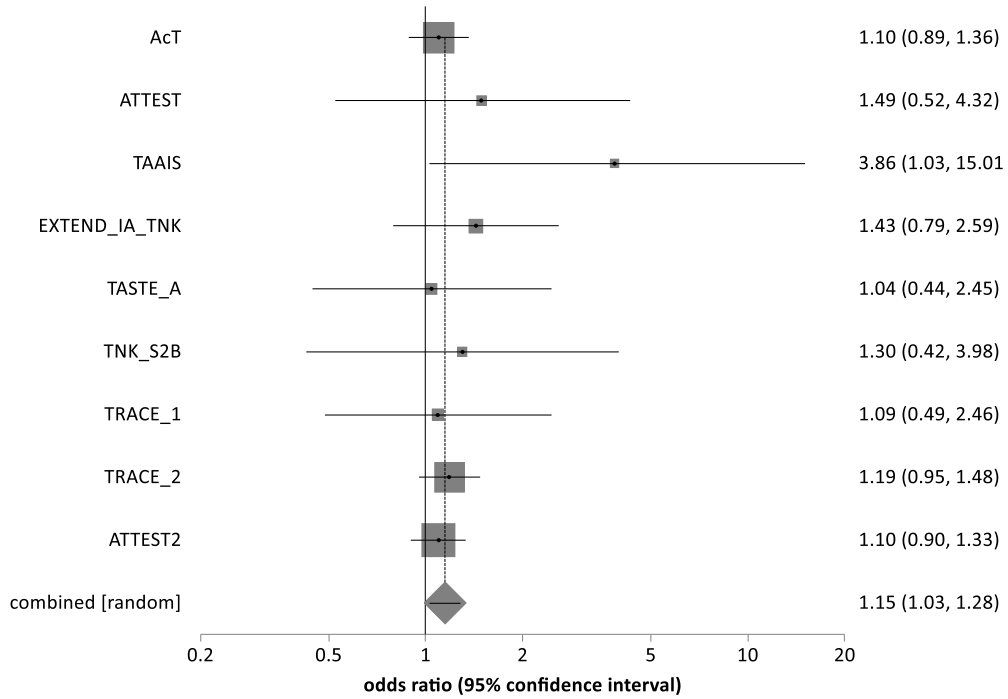


Figure 7-2 - Forest plot of mRS 0-1 across all trials $I^2 = 0\%$

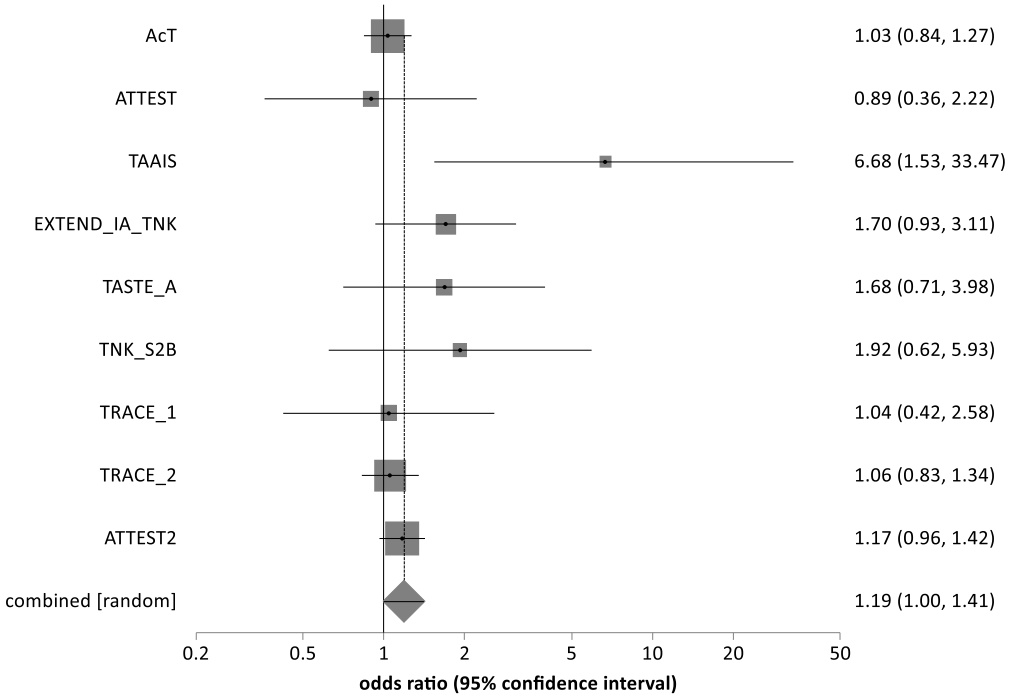


Figure 7-3 - Forest plot of mRS 0-2 across all trials $I^2 = 35.8\%$

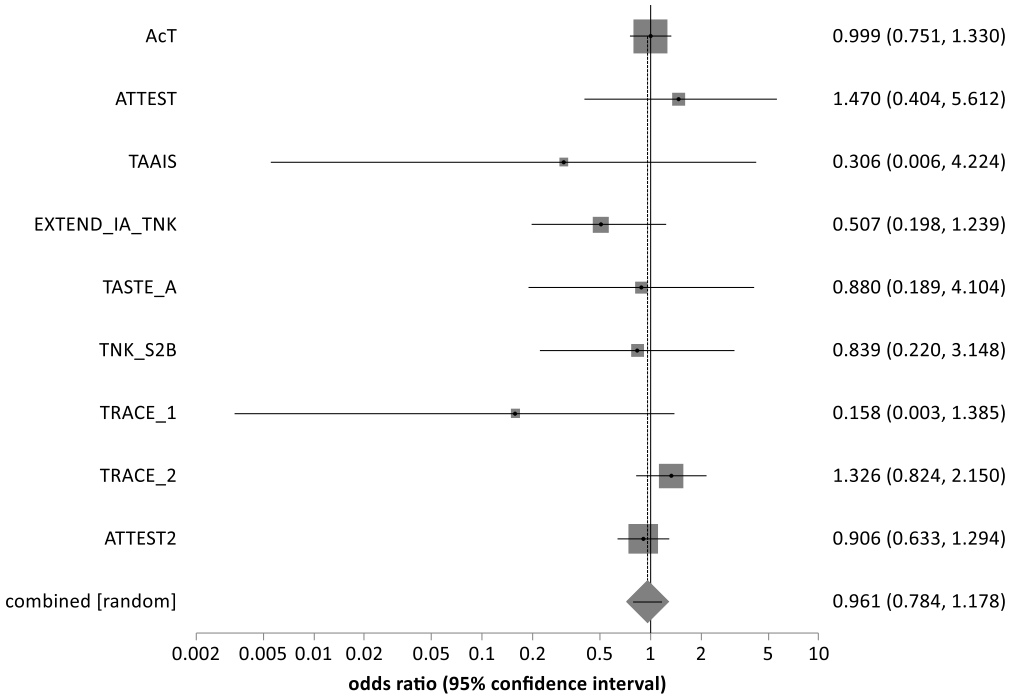


Figure 7-4 - Forest plot of mortality across all trials I^2 = 7.8%

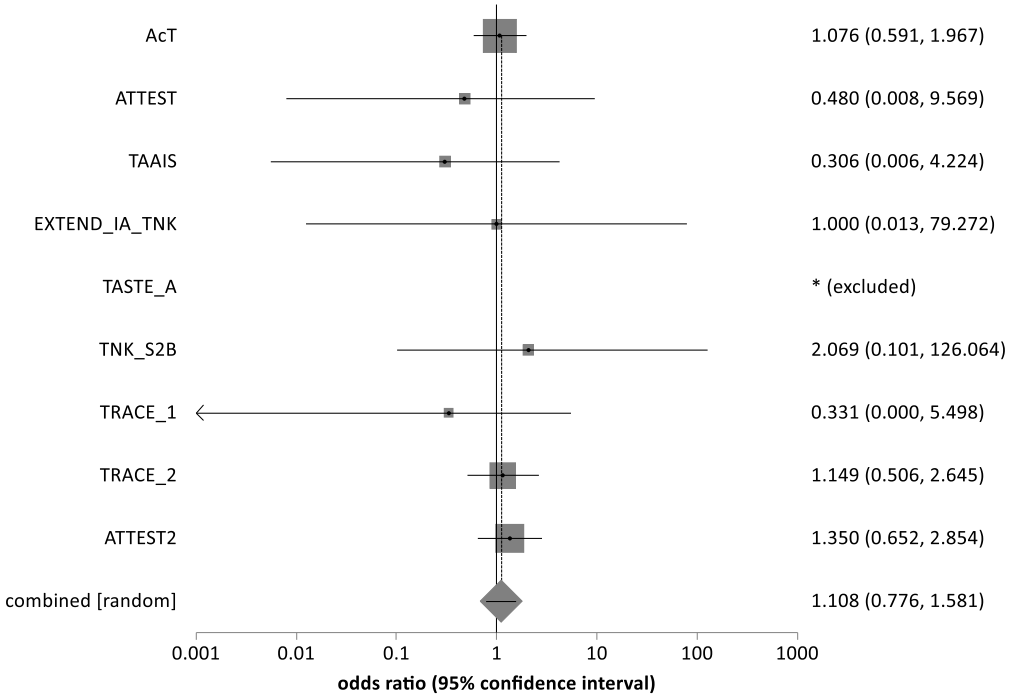


Figure 7-5 - Forest plot of symptomatic ICH rate across all trials I^2 = 0%

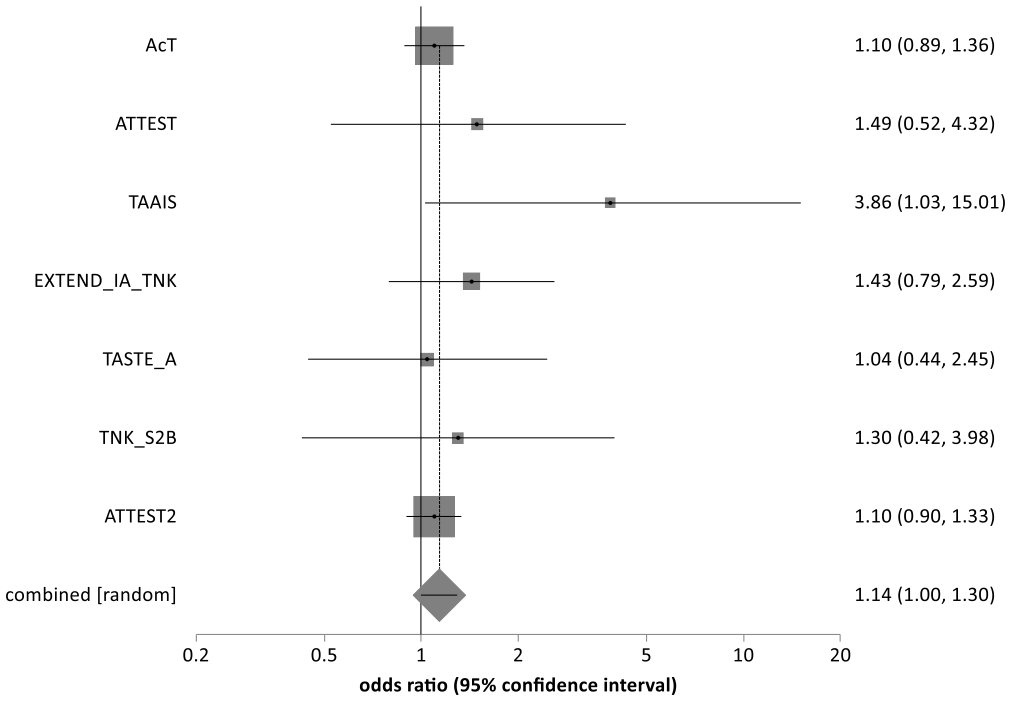


Figure 7-6 - Forest plot of mRS 0-1 in trials with no biocopy $I^2 = 0\%$

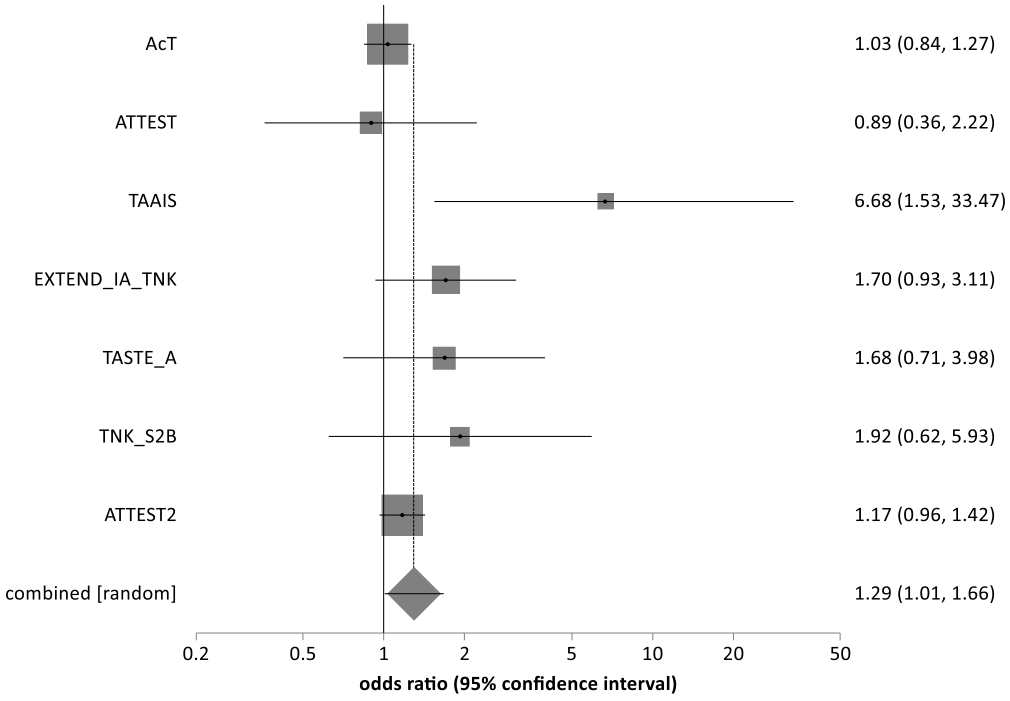


Figure 7-7 - Forest plot of mRS 0-2 in trials with no biocopy $I^2 = 49.6\%$

7.4 Discussion

The non-inferiority of tenecteplase compared to alteplase in AIS has been demonstrated individually in three separate trials (80, 81, 151) and is reflected in the latest European (190) and American (82) guidelines which endorse tenecteplase as a suitable alternative to alteplase for AIS. An expert consensus statement in the European guidelines advocates for preference of tenecteplase over alteplase due to the ease of administration. Registry data from the TETRIS registry and from New Zealand have found routine use of tenecteplase to be feasible, associated with faster processing times, potentially better safety and comparable efficacy to alteplase (73, 75).

Superiority of tenecteplase has been shown for imaging outcomes such as recanalisation in EXTEND IA TNK and early reperfusion in the TASTE-A trial. We carried out a meta-analysis to compare clinical outcomes between tenecteplase and alteplase in AIS. Superiority of tenecteplase over alteplase was found for achieving excellent clinical outcome across all trials and a strong trend towards tenecteplase for achieving functional independence. A similar result was seen when trials of tenecteplase biocopy were excluded with superiority for achieving functional independence and a strong trend for achieving excellent outcome both in favour of tenecteplase.

We included only trials using tenecteplase at the 0.25mg/kg dose as the 0.4mg/kg dose was found in the NOR-TEST2 trial to be inferior to standard dose alteplase in safety and functional outcome (68) and is not advocated in guidelines.

Tenecteplase has pharmacological properties that suggest potential advantages over alteplase. Since it is desirable to initiate thrombolysis as rapidly as possible, delaying thrombolytic administration until after CT angiography confirms LVO is not ideal, however, and challenging for optimal clinical workflow. There would be clear benefit from a single thrombolytic agent being recommended for all AIS patients.

Considerable enthusiasm exists for use of tenecteplase in AIS to replace alteplase as the thrombolytic agent for routine practice, driven primarily by the

practical advantages over alteplase in ease of administration by single bolus that are especially advantageous in the common scenario of inter-hospital transfer. If guideline recommendations move in favour of tenecteplase for thrombolysis in all AIS patients, regulatory approval for tenecteplase would be advantageous compared to widespread off-label use (191). Tenecteplase is currently packaged for the higher dose used in acute myocardial infarction management, including weight-graduated syringes and dose instructions, leading to the potential for dosing errors in the AIS population (192). In addition, a large amount of drug is inevitably wasted when stroke doses – maximum of half of a 50mg vial - are prepared. Manufacture of a stroke-specific dose with appropriate packaging, and secure drug supply sufficient for widespread AIS use, may depend on regulatory approval of tenecteplase for the AIS indication.

Further extension of tenecteplase indications in stroke may follow in future based on the multiple ongoing clinical trials in situations including extended time windows, minor stroke and TIA.

7.5 Conclusion

In this meta-analysis of AIS trials comparing 0.25mg/kg tenecteplase and 0.9mg/kg alteplase, we found tenecteplase was superior to alteplase for achieving excellent clinical outcome and found a strong trend towards superiority of tenecteplase for achieving functional independence. Further individual patient level meta-analysis would be of interest. Regulatory approval for tenecteplase may be forthcoming on the basis of recent trial evidence.

8 The effect of population ischaemic progression rates on sample size estimates in acute ischaemic stroke trials

8.1 Introduction

Greater clinical heterogeneity amongst acute ischaemic stroke (AIS) populations entered into clinical trials can lead to underestimation of sample size and reduce the likelihood of a positive outcome (193). Trials of neuroprotectants in acute ischaemic stroke have to date been unsuccessful in identifying an effective agent (157). Neuroprotectants may be able to reduce infarct size after ischaemic stroke by 'freezing' the penumbra as a bridge to recanalisation by intravenous thrombolysis (IVT) or mechanical thrombectomy (MT) (156). Recent neuroprotectant trials, such as the ESCAPE-NA1 trial (103), have selected patients with favourable imaging characteristics such as higher ASPECTS and collateral scores, selecting patients likely to have a slow ischaemic progression rate (IPR).

Reductions in infarct volume by potential neuroprotectants have been frequently demonstrated in animal models of stroke but this has not translated into human studies (194). Demonstrating a neuroprotectant can effectively reduce infarct size in a clinical trial may be more challenging in slow progressors already able to sustain the penumbra whilst awaiting recanalisation through good collateral supply. However, in fast progressors destined to have larger infarct volumes, an effective neuroprotectant which sustained the penumbra until recanalisation may produce larger differences between experimental and control groups in a trial setting. Recent analyses of the HERMES data found that differences of 2, 10 and 20ml in FIV at 48 hours correlated with 1%, 5% and 10% absolute increases in likelihood of achieving functional independence after MT (140).

We hypothesised that cohorts with larger proportions of fast progressors would lead to smaller sample size estimates powered to demonstrate relative reduction in follow up infarct volume (FIV). We used data from published trials of IVT and MT to construct hypothetical sample size calculations to investigate this.

8.2 Methods

We used data from the M1 occlusion cohort described in chapter 2.

We collected data on ischaemic progression rate (IPR), defined as the ischaemic core volume (cerebral blood flow <30%) divided by the time from symptom onset to CT perfusion scan; follow up infarct volume (FIV) from CT or MRI; presence of recanalisation; and functional independence defined as modified Rankin scale (mRS) 0-2 at 90 day follow up. Fast progressors were defined by two cut-offs IPR >15ml/hr or >25ml/hr as per definitions used in literature (155, 195). When considering published trial data which was not individualised, those without target mismatch on CT perfusion were taken to be fast progressors.

Data were also extracted from 5 trials of MT in AIS – MR CLEAN, SWIFT PRIME, EXTEND IA, DEFUSE-3 and DAWN (149, 150, 196-198). Where possible trial populations were divided into subgroups of treatment and control, and those with mismatch on CT perfusion (taken to be slow progressors) and those without mismatch (taken to be fast progressors). Median IPR and FIV were extracted or calculated for each subgroup.

Hypothetical sample size estimates were calculated for relative risk reduction (of 20%, 30% and 50%) in terms of follow up infarct volume for slow and fast progressors and for patients with and without recanalisation. Populations within the thrombolysis trials cohort that met eligibility criteria for the MT trials were identified and hypothetical sample size estimates calculated for relative risk reductions as above. Hypothetical populations consisting of varying proportions of slow or fast progressors, and recanalised or non-recanalised patients were constructed and used to estimate sample sizes. Sample size calculations were performed using StatsDirect (188).

8.3 Results

8.3.1 Pooled thrombolysis data

Baseline characteristics for slow and fast progressors are shown in table 9-1. Using the 15ml/hr cut off for fast progressors, age, time from symptom onset to CT, prevalence of diabetes, and baseline glucose did not differ significantly

between slow and fast progressors. Slow progressors had significantly smaller baseline core, baseline penumbra and follow up infarct volumes; higher rates of recanalisation; higher proportions of functionally independent participants at 90 days; and smaller NIHSS at baseline with greater recovery in NIHSS at 24 hours.

Slow progressors had a median IPR of 5.9ml/hr (IQR 2.6-8.0) versus 24.0ml/hr (20.1-34.5) in fast progressors. FIV was significantly different, 22.9ml (11.7-43.9) in slow progressors and 138.7ml (65.3-236.5) in fast progressors (p=0.000). In the absence of recanalisation, higher FIV was seen in each group, +28.3ml in slow progressors (p=0.023) and +85.2ml in fast progressors (p=0.075) (see figure 9-1).

The proportion of patients functionally independent at 90 days (mRS 0-2) was significantly different in slow (74%) and fast (31%) progressors who achieved recanalisation (p=0.004), but this was not the case in the absence of recanalisation (slow 10%, fast 11%, p=0.937) (see figure 9-2).

Similar baseline characteristics based on the 25ml/hr cut off for fast progressors are shown in table 9-2.

8.3.2 Published thrombectomy data

Data are shown in table 9-3 and figure 9-3. IPR varied considerably across the trial populations, ranging from 0.6ml/hr in slow progressors randomised to MT in the SWIFT PRIME trial to 25.4ml/hr in fast progressors randomised to control in the MR CLEAN trial.

	Slow Prog 0-15ml/hr n=54	Fast Prog >15ml/hr n=22	p-value
Age	70 ± 11	70 ± 11	0.421
Time from symptom onset to CT	158 ± 54	153 ± 49	0.880
IPR	5.9 (2.6-8.0)	24.0 (20.1-34.5)	0.000
Baseline core volume	13.3 (6.5-20.1)	59.5 (48.8-87.4)	0.000
Baseline penumbra volume	37.3 (28-61.7)	75.5 (49.8-91.5)	0.000
Recanalisation	43/53 (81%)	13/22 (59%)	0.046
Follow up infarct volume	22.9 (11.7-43.9)	138.7 (65.3-236.5)	0.000
Proportion mRS 0-2 at 90d	33/54 (61%)	5/22 (23%)	0.002
NIHSS at baseline	14.5 (12-16)	18 (15-21)	0.000
NIHSS change at 24 hours	-8 (-11 – -3)	-1 (-9 – 4)	0.014
Collateral grade	Good 7/44 (16%) Moderate 23/44 (52%) Poor 14/44 (32%)	Good 12/22 (55%) Moderate 8/22 (36%) Poor 2/22 (9%)	0.003
Baseline glucose	6.5 ± 1.5	7.1 ± 1.6	0.224
Diabetes	11/54 (20%)	4/22 (18%)	0.828

Table 8-1 - Baseline characteristics – 15ml/hr cut off

	Slow Prog 0-25ml/hr n=65	Fast Prog >25ml/hr n=11	p-value
Age	71 ± 11	72 ± 10	0.687
Time from symptom onset to CT	160 ± 53	139 ± 49	0.316
IPR	6.7 (3.5-12.2)	33.9 (33.0-36.8)	0.000
Baseline core volume	15 (7-25)	83 (59-96)	0.000
Baseline penumbra volume	39 (29-71)	78 (44-99)	0.024
Recanalisation	51/65 (79%)	5/11 (46%)	0.016
Follow up infarct volume	26 (13-65)	171 (76-286)	0.000
Proportion mRS 0-2 at 90d	36/65 (55%)	2/11 (18%)	0.022
NIHSS at baseline	15 (13-16)	20 (15-25)	0.008
NIHSS change at 24 hours	-7 (-10- -1)	0 (-11-2)	0.390
Collateral grade	Good 13/55 (24%) Moderate 27/55 (49%) Poor 15/55 (27%)	Good 6/11 (54%) Moderate 4/11 (36%) Poor 1/11 (9%)	0.100
Baseline glucose	6.7 ± 1.6	6.8 ± 1.3	0.783
Diabetes	14/65 (22%)	1/11 (9%)	0.337

Table 8-2 - Baseline characteristics - 25ml/hr cut off

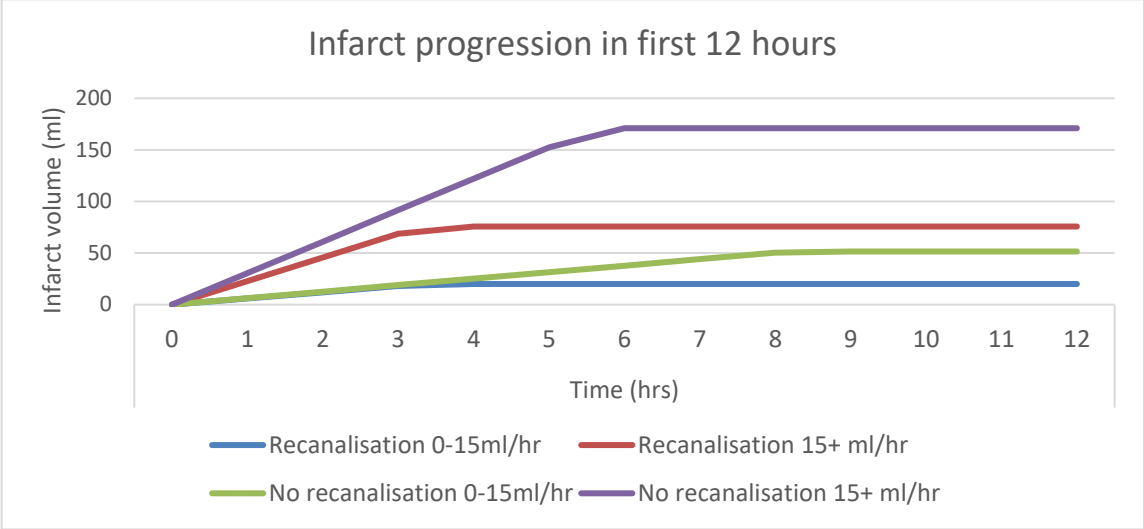


Figure 8-1 - Infarct progression in first 12 hours

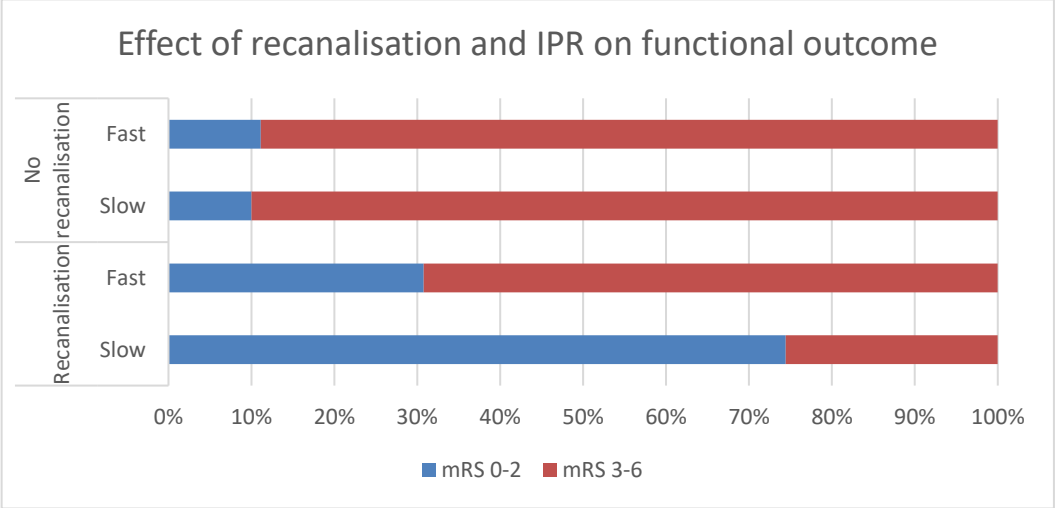


Figure 8-2 - Effect of recanalisation and IPR on functional outcome

Trial	Group	IPR (median) (ml/hr)	FIV (median) (ml)
MR CLEAN	Control with mismatch (slow)	8.4	79
	Control with no mismatch (fast)	25.4	79
	MT with mismatch (slow)	9.7	49
	MT with no mismatch (fast)	20.2	79
SWIFT PRIME	Control with mismatch (slow)	0.8	14.2
	Control with no mismatch (fast)	6.7	152.6
	MT with mismatch (slow)	0.6	14.5
	MT with no mismatch (fast)	6.9	83.2
DEFUSE-3	All	0.95	38
EXTEND IA	Control	13.5	-
	MT	9.2	-

Table 8-3 - IPR and FIV in subgroups of the MT trials

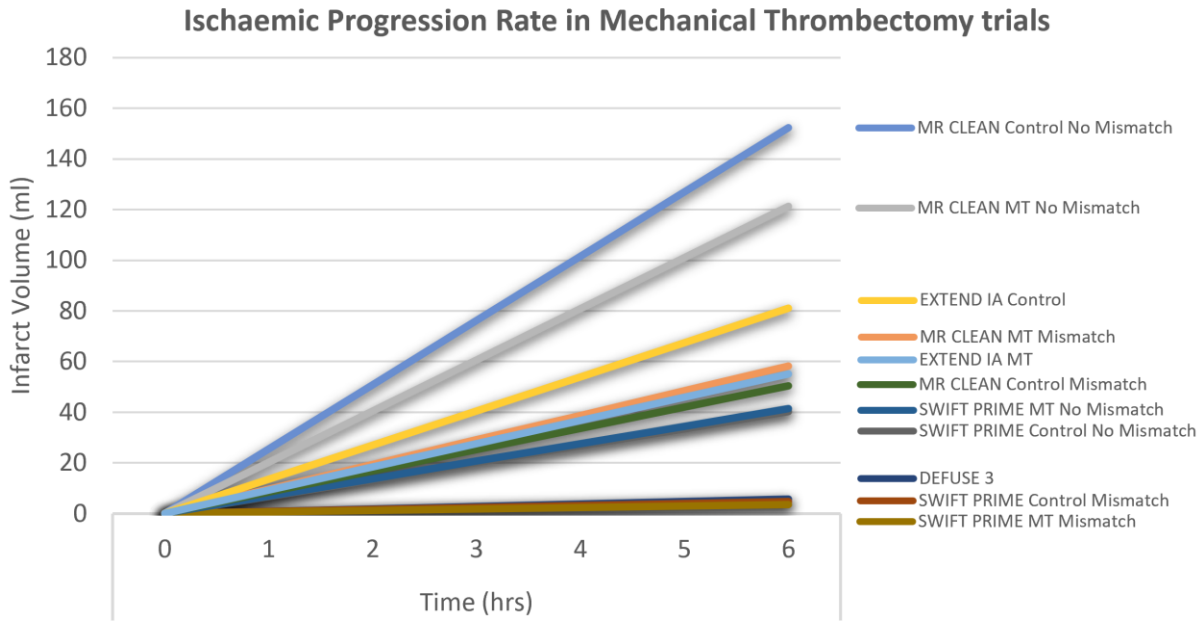


Figure 8-3 - IPR variation in subgroups of the MT trials

8.3.3 Sample size calculations

Sample size estimates for relative risk reduction in FIV were smaller for fast progressors compared to slow progressors, by around 4-fold using a cut off of 15ml/hr and 17-fold using a cut-off of 25ml/hr (table 9-4). Populations with larger proportions of fast progressors and of non-recanalised patients were associated with smaller sample size estimates (figure 9-4). Amongst populations eligible for MT trials, DAWN criteria had the smallest sample size estimate and MR-CLEAN criteria the largest (table 9-4).

Eligibility criteria for trial population	Median IPR (ml/hr)	Total sample size required for different effect sizes in terms of reduction in follow up infarct volume		
		RRR 20% of FIV	RRR 30% of FIV	RRR 50% of FIV
MR CLEAN eligible	7.5	7912	3518	1268
EXTEND IA eligible	7.3	4138	1840	664
DEFUSE-3 eligible	7.2	3756	1670	602
DAWN eligible	5.8	3174	1412	510
IPR 0-15ml/hr	5.9	2584	1150	416
IPR >15ml/hr	24.0	668	298	108
IPR 0-25ml/hr	6.7	7334	3262	1176
IPR >25ml/hr	33.9	426	192	70

Table 8-4 - Sample size estimates for reduction in FIV

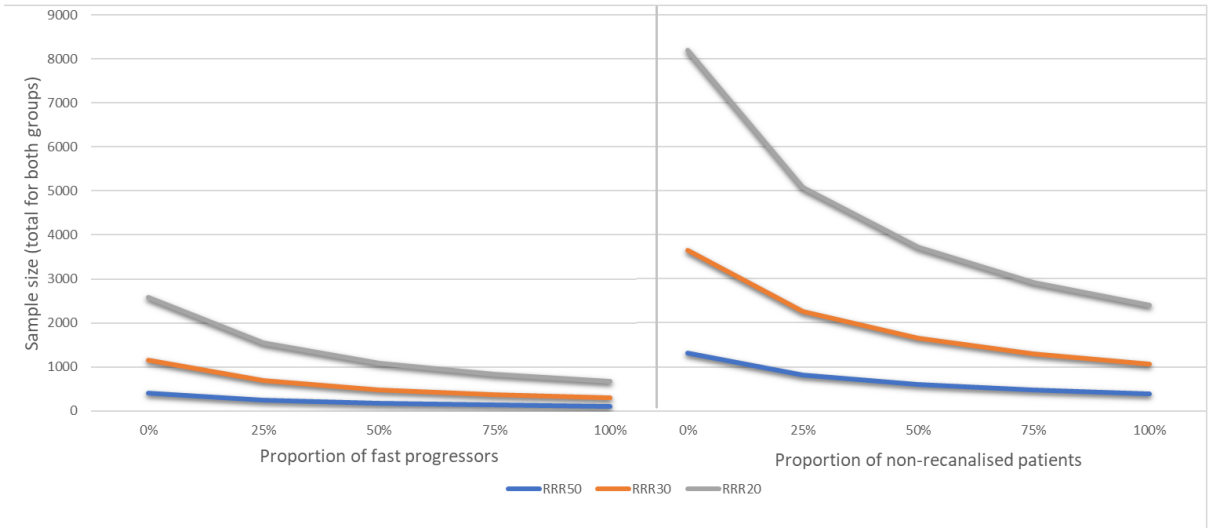


Figure 8-4 - Sample size variation by proportion of fast progressors and non-recanalised patients

8.4 Discussion

Ischaemic progression rate varied widely in the populations we examined entered into AIS trials of IVT and MT. Fast progressors were seen to have higher FIV and those with no recanalisation found to have worse outcome. In hypothetical sample size calculations, higher proportions of fast progressors and non-recanalised patients produced smaller sample size estimates powered to show a relative reduction in FIV.

Our findings may be of particular relevance to trials of neuroprotective agents in AIS. Inclusion of slow progressors capable of sustaining an ischaemic penumbra for many hours and destined to have smaller FIV in trials of neuroprotectants may affect the likelihood of demonstrating a positive effect of any experimental agent. In a randomised controlled trial, an effective neuroprotectant would best be demonstrated in a population of fast progressors at a very early time point before the infarct has progressed thus resulting in a greater difference between experimental and control groups. Consequently, smaller sample size estimates could be expected.

It was noteworthy that amongst MT-eligible cohorts, cohorts with a slower median IPR such as those eligible for DAWN were associated with smaller sample size estimates than cohorts with faster median IPR such as those eligible for MR-CLEAN, contradicting our other findings. This was felt to reflect selection of a more homogenous cohort through more exclusive selection criteria, with smaller standard deviation of the sample producing smaller sample size estimates.

Whilst our findings suggest selecting fast progressors may be beneficial in trial design, there are some drawbacks to this approach. There is no agreed definition of fast progressors and dichotomising all cases using a single cut off may be an oversimplification. Calculating IPR assumes a linear rate of infarct growth, for which there is some evidence from MRI studies (141, 158). IPR also relies on using a predicted ischaemic core volume, a concept which itself is controversial in terms of its definitions and clinical utility (163). FIV was used as an imaging marker of efficacy however the extent to which it explains outcome is debated as discussed in chapter 3. Selecting a homogenous population requires highly selective criteria which can lead to slow recruitment and requires a large

number of screened patients. Lastly, although we found smaller sample size estimates could be expected with inclusion of more non-recanalised patients, in practice a neuroprotectant aiming to sustain the ischaemic penumbra as a bridge to recanalisation may be ineffective in the absence of recanalisation.

8.5 Conclusion

Cohorts with higher proportions of fast progressors produce smaller sample size estimates powered to detect relative reductions in follow up infarct volume. Selecting more fast progressors to enter trials of potential neuroprotective agents may increase the likelihood of a positive outcome.

9 Conclusion

This thesis explored three themes in researching the optimisation of outcomes of intravenous thrombolysis in acute ischaemic stroke – tenecteplase and its potential superiority to the current standard thrombolytic for stroke treatment alteplase; use of CT perfusion to identify subgroups at risk of harm or with greater chance of benefit; and examination of the effect of patient heterogeneity in trial populations affecting outcomes.

Tenecteplase was found to have similar efficacy and safety outcomes to alteplase in imaging subgroups such as those with large vessel occlusion or perfusion mismatch, and similar rates of early recanalisation and penumbral salvage in an imaging substudy of a large clinical trial comparing tenecteplase and alteplase. In meta-analysis of early window trials of tenecteplase versus alteplase, tenecteplase was found to be superior to alteplase in some functional outcome measures with similar safety outcomes.

Using CT perfusion we identified two at-risk subgroups in acute ischaemic stroke, those with fast ischaemic progression rate at risk of larger infarct volumes and those with high blood brain barrier permeability at higher risk of haemorrhagic transformation. Tenecteplase showed a non-significant beneficial trend in fast progressors trending to smaller follow up infarct volumes, and was associated with lesser chance of haemorrhagic transformation compared to alteplase when accounting for blood brain barrier permeability. A mediation analysis also found that follow up infarct volume mediated a larger proportion of the benefit of recanalisation than previously suggested.

Patient heterogeneity in clinical trials of intravenous thrombolysis and mechanical thrombectomy in acute stroke was examined to assess its effect on outcomes. Higher proportions of fast progressors in acute stroke trials were found to lead to smaller sample sizes powered to detect differences in follow up infarct volume.

Several future directions are proposed. Tenecteplase is now established as non-inferior to alteplase in the early window after stroke, including in imaging subgroups, while a signal towards superiority in meta-analysis has been found.

An individual participant data meta-analysis of large trials of tenecteplase versus alteplase is planned and would be helpful in this regard. Regulatory and guideline approval for tenecteplase to be used as an alternative to alteplase is anticipated and its ease of use and potential superiority will be welcomed in the stroke community.

Future trials of tenecteplase may wish to explore specific patient subgroups such as those in the late window; patients at higher risk of poor outcome based on imaging parameters such as faster progression rate or high blood brain barrier permeability or those ineligible for mechanical thrombectomy either due to occlusion location or availability. Whilst mechanical thrombectomy is a highly effective treatment, issues with global availability and affordability will likely remain, accentuating the importance of optimising outcomes after intravenous thrombolysis which is more widely available.

The utility of CT perfusion in evaluating eligibility for mechanical thrombectomy has become contentious (199), particularly in the early time window (200), and increasingly in the late time window with the emergence of evidence of benefit in patients selected on the basis of collateral circulation on CTA in the late time window (201). However, CT perfusion provides several advantages in clinical practice such as improved detection of distal occlusions (202), superior prognostic information compared to NCCT for patients with large ischaemic core (203), greater interobserver agreement in assessing ischaemic core than NCCT (204) and aids in identifying potential stroke mimics. Experience in our centre has found a patient pathway incorporating CT perfusion alongside NCCT and CTA at baseline in all potential stroke patients being evaluated for reperfusion therapies aids in rapid diagnosis whilst reducing treatment delays involved in arranging CT perfusion as an additional scan.

An effective neuroprotectant agent remains an as yet unachieved goal in acute stroke. Trials of neuroprotectants in stroke may benefit from targeting at risk groups such as fast progressors in whom demonstration of benefit may be more evident than lower risk groups at lesser chance of poor outcomes.

Appendices

Chapter 6 – Inclusion and exclusion criteria for the ATTEST-2 trial

Inclusion criteria:

- Eligible for IV thrombolysis as per local standard of practice
- Male or non-pregnant female ≥18 years of age
- <4.5 hours after symptom onset
- Consent of patient or legal representative
- Independent prior to the stroke (estimated modified Rankin Scale 0-2)

Exclusion criteria:

- Contraindications to thrombolytic therapy:
    - Evidence of intracranial haemorrhage or significant non-stroke intracranial pathology likely to account for clinical presentation or represent a risk of intracerebral haemorrhage (eg CNS neoplasm) on pre-treatment CT
    - Stroke within the previous 14 days, thrombolytic therapy within the past 14 days, or hypodensity on pre-treatment CT consistent with recent cerebral ischaemia other than the presenting event
    - Systolic blood pressure >185 or diastolic BP >110 mm Hg, or aggressive management (intravenous pharmacotherapy) necessary to reduce BP to these limits
    - Clinical history suggestive of subarachnoid haemorrhage even if no blood is evident on CT

- High risk of haemorrhage, including major surgery, trauma or gastrointestinal or urinary tract haemorrhage within the previous 21 days; arterial puncture at a non-compressible site within the previous 7 days; prolonged cardiopulmonary resuscitation (> 2 minutes) within the previous 14 days; 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; known history of haemorrhagic stroke; known defect of clotting or platelet function (other than antiplatelet therapy)

- Hypo- (< 50 mg/dL or <2.8 mmol/L) or hyperglycaemia (>400 mg/dL or >22.2 mmol/L) sufficient to account for neurological symptoms

- Seizure at onset of symptoms unless brain imaging identifies positive evidence of significant brain ischaemia (eg early ischaemic change or hyperdense vessel on plain CT, CTA confirmed arterial occlusion)

- Pregnancy (for women of child-bearing potential a negative pregnancy test will be required prior to randomisation)

    - Women of non-childbearing potential are defined as those defined as women who are post-menopausal or permanently sterilised (e.g. hysterectomy, tubal occlusion, bilateral salpingectomy).

- Inadequate haemostasis:

    - Taking warfarin and INR >1.3

    - Taking a Direct Oral Anticoagulant (dabigatran, rivaroxaban, apixaban, edoxaban) unless known to be >12 hours since last dose and with normal coagulation assays

  - Low molecular weight heparin (at doses other than prophylaxis of venous thromboembolism) administered within the preceding 48 hours

  - Unfractionated heparin administered within the previous 48 hours and APTT is prolonged

- Any major medical condition likely to limit survival to day 90

- Unavailable for day 90 follow-up

Eligible participants were approached for consent prior to potential randomisation and treatment. Informed consent from the participant was sought using patient information sheets and a discussion with a member of the research team. Only when the participant lacked capacity was consent be sought from a legal representative. The hierarchy of sources of informed consent for incapacitated patients was different in Scotland to England, Wales and Northern Ireland as listed below:

- Scotland
    - Personal legal representative
        - 1A. Any guardian or welfare attorney who has power to consent to the adult's participation in research.
        - 1B. If there is no such person, the adult's nearest relative as defined in section 87(1) of the Adults with Incapacity (Scotland) Act 2000.
    - Professional legal representative.
- England, Wales and Northern Ireland
    - Personal legal representative
        - A person not connected with the conduct of the trial who is: (a) suitable to act as the legal representative by virtue of their relationship with the adult, and (b) available and willing to do so.
    - Professional legal representative.

Chapter 6 – Breakdown of National Institute of Health Stroke Scale (NIHSS) scoring

Component	Score
1a. Level of consciousness (LOC)	0 = Alert; keenly responsive 1 = Not alert; but arousable by minor stimulation to obey, answer, or respond 2 = Not alert; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped) 3 = Responds only with reflex motor or autonomic effects, or totally unresponsive, flaccid, and areflexic
1b. LOC questions – month and age	0 = Answers both questions correctly 1 = Answers one question correctly 2 = Answers neither question correctly
1c. LOC commands – eye closure and grip	0 = Performs both tasks correctly 1 = Performs one task correctly 2 = Performs neither task correctly
2. Best gaze	0 = Normal 1 = Partial gaze palsy; gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present 2 = Forced deviation, or total gaze paresis is not overcome by the oculocephalic manoeuvre
3. Visual	0 = No visual loss. 1 = Partial hemianopia. 2 = Complete hemianopia. 3 = Bilateral hemianopia (blind including cortical blindness)
4. Facial palsy	0 = Normal symmetrical movements. 1 = Minor paralysis (flattened nasolabial fold, asymmetry on smiling). 2 = Partial paralysis (total or near-total paralysis of lower face). 3 = Complete paralysis of one or both sides (absence of facial movement in the upper and lower face)
5. Motor arm 5a. Left 5b. Right	0 = No drift; limb holds 90 (or 45) degrees for full 10 seconds. 1 = Drift; limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support. 2 = Some effort against gravity; limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity. 3 = No effort against gravity; limb falls. 4 = No movement. UN = Amputation or joint fusion

6. Motor leg 6a. Left leg 6b. Right leg	0 = No drift; leg holds 30-degree position for full 5 seconds. 1 = Drift; leg falls by the end of the 5- second period but does not hit the bed. 2 = Some effort against gravity; leg falls to bed by 5 seconds but has some effort against gravity. 3 = No effort against gravity; leg falls to bed immediately. 4 = No movement. UN = Amputation or joint fusion
7. Limb ataxia	0 = Absent. 1 = Present in one limb. 2 = Present in two limbs. UN = Amputation or joint fusion
8. Sensory	0 = Normal; no sensory loss. 1 = Mild-to-moderate sensory loss; patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched. 2 = Severe or total sensory loss; patient is not aware of being touched in the face, arm, and leg
9. Best Language	0 = No aphasia; normal. 1 = Mild-to-moderate aphasia; some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response. 2 = Severe aphasia; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response. 3 = Mute, global aphasia; no usable speech or auditory comprehension
10. Dysarthria	0 = Normal. 1 = Mild-to-moderate dysarthria; patient slurs at least some words and, at worst, can be understood with some difficulty. 2 = Severe dysarthria; patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric. UN = Intubated or other physical barrier
11. Extinction	0 = No abnormality. 1 = Visual, tactile, auditory, spatial, or personal inattention, or extinction to bilateral simultaneous stimulation in one of the sensory modalities. 2 = Profound hemi-inattention or extinction to more than one modality; does not recognize own hand or orients to only one side of space

- haemorrhagic infarction type 1 (HI1)
    - petechial haemorrhages at the infarct margins
- haemorrhagic infarction type 2 (HI2)
    - petechial haemorrhages throughout the infarct
    - no mass-effect attributable to the haemorrhages
- parenchymal haematoma type 1 (PH1)
    - ≤30% of the infarcted area
    - minor mass effect attributable to the haematoma
- parenchymal haematoma type 2 (PH2)
    - >30% of infarct zone
    - substantial mass effect attributable to the haematoma

Chapter 6 – Modified Rankin Scale

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

Chapter 7 – Literature search criteria

Ovid MEDLINE(R) ALL <1946 to January 04, 2024>

Embase <1996 to 2023 Week 52>

1 exp stroke/ or exp brain infarction/ or exp brain stem infarctions/ or exp lateral medullary syndrome/ or cerebral infarction/ or exp infarction, anterior cerebral artery/ or exp infarction, middle cerebral artery/ or exp infarction, posterior cerebral artery/ or exp stroke, lacunar/ or exp Ischemic Attack, Transient/ or exp basal ganglia cerebrovascular disease/ 600406

2 (stroke or cerebrovascular accident or cerebral infarct$ or cerebral isch?emi$ or transient isch?emi$ attack or TIA or focal cerebral isch?emi$).tw. 869596

3 (isch?emi$ adj6 (stroke$ or cerebral vasc$ or cerebrovasc$ or cva or attack$)).tw.264506

4 ((brain or cerebr$ or cerebell$ or vertebrobasil$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$)).tw. 310456

5 tenecteplase.mp. [mp=ti, bt, ab, ot, nm, hw, fx, kf, ox, px, rx, ui, an, sy, ux, mx, tn, dm, mf, dv, dq] 4400

6 1 or 2 or 3 or 4 1133164

7 5 and 6 1775

8 limit 7 to humans 1628

9 limit 8 to RCTs 53

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