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Use Of Thrombolytic Therapy Beyond Current Recommendations For Acute Ischaemic Stroke

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M.B.B.S**

**This thesis is submitted in fulfilment of the requirements for the
degree of Doctor of Philosophy (PhD)
Cardiovascular and Medical Sciences
Faculty of Medicine
University of Glasgow
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Acknowledgement

In everyone's life, at some time, our inner fire goes out. It is then burst into flame by an encounter with another human being. We should all be thankful for those people who rekindle the inner spirit.

-- Albert Schweitzer

I wish to express my sincere gratitude to all those who influenced me shape my attitude and motivate to pursue career in Stroke Medicine. Had it not been for them, perhaps, I might not have succeeded in presenting this work. At the outset, I think of my parents, for they gave me values, encouragement and love, and thank them for their unrelenting struggle to give me an education. I dedicate this work to them. Similarly, I am grateful to Prof. Kennedy Lees who accepted me as a student to supervise this doctoral work. He has been a great teacher who over the last years has always encouraged and supported me. Also, I thank VISTA and SITS-ISTR research groups who contributed data and offered their suggestions/criticisms on my work. I am also mindful of the fact that had it not been for the training and support that I received during the initial days of my career from Professor Satish Khadilkar, Dr Shirish Hastak, Prof NV Dravid and Prof Jean Marie Annoni, I wouldn't have succeeded in accomplishing this task. I express my sincere gratitude to them. I also wish to thank Prof Thomas Kent and Dr Pitchhiah Mandava who discussed about their research on matching procedures while my stay in Houston. Special thanks to Matthew for his continued support at various stages of the PhD, Myzoon for her co-operation as a VISTA co-ordinator and Mrs Pamela McKenzie for assisting in several administrative matters. I also thank Jesse Dawson, Katharine Preedy, Dr Chris Weir, Dr Lilian Murray, Abhinav Sinha, Neeti Mishra, Niall McDougall, Irene Conway, Terry Quinn, Kate Gray, Tiago Moreira, Peter Higgins, Antonio Carota, Karim Hajjar and Rachael Fulton for their help and co-operation. I also wish to thank the University of Glasgow and the European Stroke Organisation because they funded my living and studies in Glasgow and sponsored the academic visits to the Europe or the United States.

Thanks also to all those whom I met over the last years as many of them have "rekindled my inner spirit".

Declaration

I declare that I am the sole author of this thesis entitled “*Use Of Thrombolytic Therapy Beyond Current Recommendations For Acute Ischaemic Stroke*”. This work has never previously been submitted for a higher degree.

This work involves tertiary analyses of anonymised data from VISTA and SITS-ISTR registry and therefore is not subject to the Research Medical Ethics Approval.

I conducted all research at the Division of Cardiovascular and Medical Sciences, University of Glasgow or in the department of our collaborators at the Karolinska University (SITS-ISTR), Stockholm and University of Texas Health Sciences Centre, Houston and always under the supervision of Prof. Kennedy R. Lees.

Dr Nishant Kumar Mishra

Date:

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Publications and Conferences

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- 12) NK **Mishra**, J Dawson, KR Lees. Prediction of Outcome Following Intracerebral Haemorrhage: A Systematic Review (Poster in European Stroke Conference, Stockholm, 2009)

Abstract

In Chapter 1, I introduce ischaemic stroke, thrombolytic therapy, thrombolysis trials and then discuss the rationale for exclusion criteria in stroke thrombolysis guidelines.

In Chapter 2, I describe methods for examining outcomes in patients that are currently recommended for exclusions from receiving alteplase for acute ischaemic stroke.

In Chapter 3, I examine Virtual International Stroke Trials Archive (VISTA) data to test whether current European recommendation suggesting exclusion of elderly patients (older than 80 years) from thrombolysis for acute ischaemic stroke is justified. Employing non-randomised controlled comparison of outcomes, I show better outcomes amongst all patients ($P < 0.0001$; OR, 1.39; 95% CI, 1.26 to 1.54), young patients ($P < 0.0001$; OR, 1.42; 95% CI, 1.26 to 1.59) and the elderly patients ($P = 0.002$; OR, 1.34; 95% CI, 1.05 to 1.70). Odds Ratios are consistent across all age deciles > 30 years. Outcomes assessed by National Institutes of Health Scale (NIHSS) score and dichotomised modified Rankin Scale score are consistently similar.

In Chapter 4, I compare thrombolysed patients in Safe Implementation of Thrombolysis in Stroke International Stroke Thrombolysis Register (SITS-ISTR) with VISTA non-thrombolysed patients ("comparators" or "controls") and test exactly similar question as in Chapter 3. Distribution of scores on modified Rankin scale are better amongst all thrombolysis patients than controls (odds ratio 1.6, 95% confidence interval 1.5 to 1.7; Cochran-Mantel-Haenszel $P < 0.001$). Association occurs independently amongst patients aged ≤ 80 (OR 1.6, 95% CI 1.5 to 1.7; $P < 0.001$; $n = 25,789$) and in those aged > 80 (OR 1.4, 95% CI 1.3 to 1.6; $P < 0.001$; $n = 3439$). Odds ratios are consistent across all 10 year age ranges above 30, and benefit is significant from age 41 to 90; dichotomised outcomes (score on modified Rankin scale 0-1 v 2-6; 0-2 v 3-6; and 6 (death) versus rest) are consistent with the results of ordinal analysis. These findings are consistent with results from VISTA reported in Chapter 3.

Age alone should not be a criterion for excluding patients from receiving thrombolytic therapy.

In Chapter 5, I employ VISTA data to examine whether patients having diabetes and previous stroke have improved outcomes from use of alteplase in acute ischaemic stroke. Employing a non-randomised controlled comparison, I show that the functional outcomes are better for thrombolysed patients versus nonthrombolysed comparators amongst non-diabetic ($P < 0.0001$; OR 1.4 [95% CI 1.3-1.6]) and diabetic ($P = 0.1$; OR 1.3 [95% CI 1.05-1.6]) patients. Similarly, outcomes are better for thrombolysed versus nonthrombolysed patients who have not had a prior stroke ($P < 0.0001$; OR 1.4 [95% CI 1.2-1.6]) and those who have ($P = 0.02$; OR 1.3 [95% CI 1.04-1.6]). There is no interaction of diabetes and prior stroke with treatment ($P = 0.8$). Neurological outcomes (NIHSS) are consistent with functional outcomes (mRS).

In Chapter 6, I undertake a non-randomised controlled comparison of SITS-ISTR data with VISTA controls and examine whether patients having diabetes and previous stroke have improved outcomes from use of alteplase in acute ischaemic stroke. I show that adjusted mRS outcomes are better for thrombolysed versus non-thrombolysed comparators amongst patients with diabetes mellitus (OR 1.45[95% CI 1.30-1.62], $N=5354$), previous stroke (OR 1.55[95% CI 1.40-1.72], $N=4986$), or concomitant diabetes mellitus and previous stroke (OR 1.23 [95% CI 0.996-1.52], $P=0.05$, $N=1136$), all CMH $p < 0.0001$. These are comparable to outcomes between thrombolysed and non-thrombolysed comparators amongst patients suffering neither diabetes mellitus nor previous stroke: OR=1.53(95%CI 1.42-1.63), $p < 0.0001$, $N=19339$. There are no interaction between diabetes mellitus and previous stroke with alteplase treatment (t-PA*DM*PS, $p=0.5$). Present data supports results obtained from the analyses of VISTA data in chapter 5. There is no statistical evidence to recommend exclusion of patients with diabetes and previous stroke from receiving alteplase.

In Chapter 7, I examine VISTA data to test whether exclusion of patients having a mild or severe stroke at baseline would be justified. Stratifying

baseline stroke severity for quintiles of NIHSS scores, I observe that there are significant associations of use of alteplase with improved outcomes for baseline NIHSS levels from 5 to 24 ($p < 0.05$). This association loses significance for baseline NIHSS categories 1 to 4 ($P = 0.8$; OR, 1.1; 95% CI, 0.3-4.4; $N = 8/161$) or ≥ 25 ($P = 0.08$; OR, 1.1; 95% CI, 0.7-1.9; $N = 64/179$) when sample sizes are small and confidence interval wide. These findings fail to provide robust evidence to support the use of alteplase in the mild or severe stroke patients, though potential for benefit appears likely.

In Chapter 8, I present a meta-analysis of trials that investigated mismatch criteria for patients' selection to examine whether present evidence supports delayed thrombolysis amongst patients selected according to mismatch criteria. I collate outcome data for patients who were enrolled after 3 hours of stroke onset in thrombolysis trials and had mismatch on pre-treatment imaging. I compare favourable outcome, reperfusion and/or recanalisation, mortality, and symptomatic intracerebral haemorrhage between the thrombolysed and non-thrombolysed groups of patients and the probability of a favourable outcome among patients with successful reperfusion and clinical findings for 3 to 6 versus 6 to 9 hours from post stroke onset. I identify articles describing the DIAS, DIAS II, DEDAS, DEFUSE, and EPITHET trials, giving a total of 502 mismatch patients thrombolysed beyond 3 hours. The combined adjusted odds ratios (a-ORs) for favourable outcomes are greater for patients who had successful reperfusion (a-OR=5.2; 95% CI, 3 to 9; $I^2=0\%$). Favourable clinical outcomes are not significantly improved by thrombolysis (a-OR=1.3; 95% CI, 0.8 to 2.0; $I^2=20.9\%$). Odds for reperfusion/recanalisation are increased amongst patients who received thrombolytic therapy (a-OR=3.0; 95% CI, 1.6 to 5.8; $I^2=25.7\%$). The combined data show a significant increase in mortality after thrombolysis (a-OR=2.4; 95% CI, 1.2 to 4.9; $I^2=0\%$), but this is not confirmed when I exclude data from desmoteplase doses that are abandoned in clinical development (a-OR=1.6; 95% CI, 0.7 to 3.7; $I^2=0\%$). Symptomatic intracerebral haemorrhage is significantly increased after thrombolysis (a-OR=6.5; 95% CI, 1.2 to 35.4; $I^2=0\%$) but not significant after exclusion of abandoned doses of desmoteplase (a-OR=5.4; 95% CI, 0.9 to 31.8; $I^2=0\%$). Delayed thrombolysis amongst patients selected according to mismatch imaging is associated with increased reperfusion/recanalisation.

Recanalisation/reperfusion is associated with improved outcomes. However, delayed thrombolysis in mismatch patients was not confirmed to improve clinical outcome, although a useful clinical benefit remains possible. Thrombolysis carries a significant risk of symptomatic intracerebral haemorrhage and possibly increased mortality. Criteria to diagnose mismatch are still evolving. Validation of the mismatch selection paradigm is required with a phase III trial. Pending these results, delayed treatment, even according to mismatch selection, cannot be recommended as part of routine care.

In Chapter 9, I summarise the findings of my research, discuss its impact on the research community, and discuss weaknesses inherent in registry data and limitation of statistical methods. Then, I elaborate the future directions I may take to further research on the theme of this thesis.

Chapter 1

Introduction

1 Introduction

1.1 Background

Stroke is defined as a *'rapidly developing clinical signs of focal (or global) disturbance of cerebral function with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than vascular origin'*.¹ It is classified into ischaemic stroke and haemorrhagic stroke. Ischaemic stroke occurs after reduction in cerebral blood flow leading to loss of neuronal functions.² Symptoms correspond to the vascular territory involved. If hypoperfusion persists, the involved tissue gradually undergoes infarction. Haemorrhagic stroke occurs due to rupture of a blood vessel in brain parenchyma.³

Stroke is the third leading cause of death.⁴ Survivors lead a life that is characterised by physical dependence and suffering due to several neurological symptoms (like cognitive decline, dementia, depression or seizures).⁵⁻⁷ Every year approximately 15 million people suffer from stroke. Of these, about 5 million die and another 5 million are left with residual disability.⁸ Costs involved in providing curative, preventive and rehabilitative measures to these patients are significant.^{9,10} The United States of America spends about 17 billion dollars a year in caring for these patients.^{11,12} For example, in 2000, Medicare for Stroke hospitalisations cost \$7.04 billion, the average being \$15,818 per person.¹¹ Europe is subject to suffer similar major costs.^{13,14} Developing (emerging) economies are no less affected. Here, stroke incidence is 7-10 times greater than the developed countries.¹⁵ Urgent measures to control the stroke epidemic are needed.^{16-18,19-22}

A patient who presents with an acute onset of ischaemic stroke is offered a therapy called thrombolytic therapy. At present, alteplase is the only thrombolytic agent that is licensed for use in ischaemic stroke.²³ As per guidelines, this drug should be given only to a selected group of patients.^{23,24} Table 1.1 shows that there are differences in the wordings of contraindications for alteplase use across different jurisdictions.²⁵

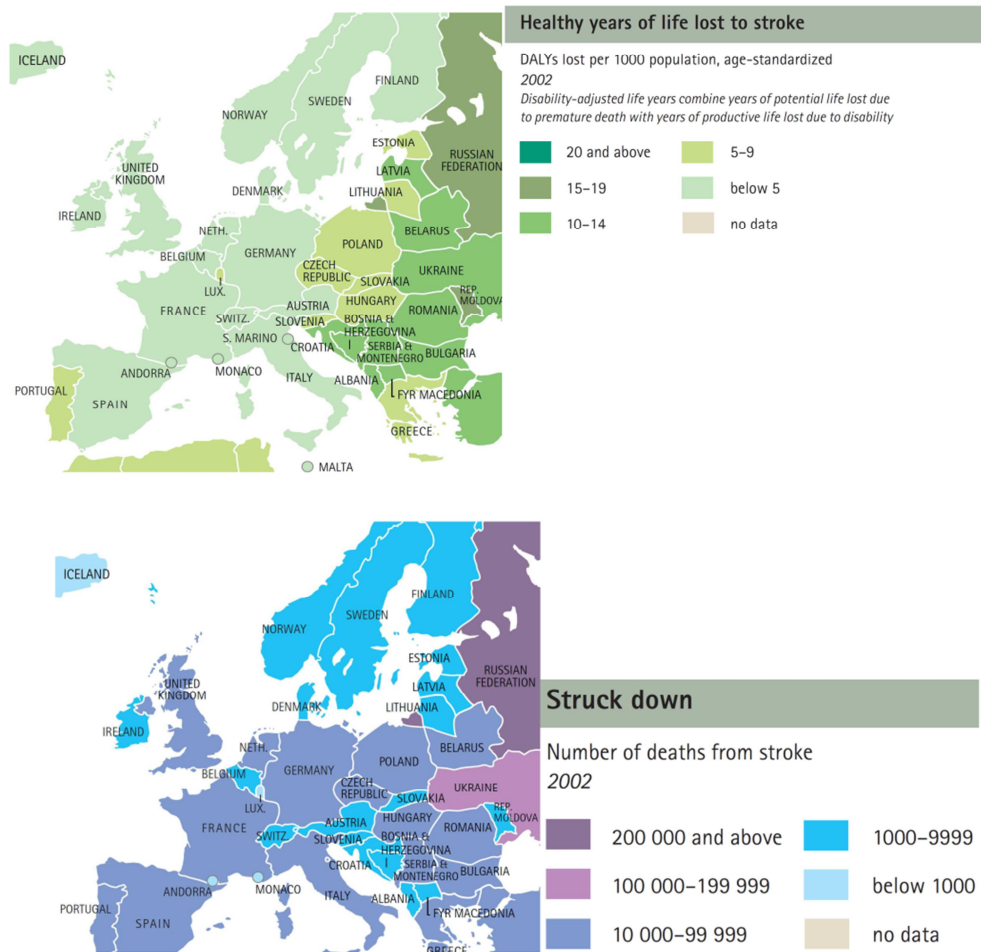


Figure 1-1 Loss of Disability Adjusted Life Years (above) and mortality rate (below) attributed to stroke within European Union (above)

Source: www.who.int (Permissions obtained from World Health Organization Press WHP (Permissions Management and Reprint Rights) 20 Avenue Appia, Office 4152 CH-1211 Genève 27, Switzerland; ID number 82115)^{13,14}

Table 1-1 Contraindications to the use of alteplase in acute ischaemic stroke in European Union, United States and Canada

<i>List of contraindications</i>	European License for t-PA use in Stroke²³	United States Guidelines²⁶	Canadian Guidelines²⁷
<i>If Brain imaging shows intracranial haemorrhage</i>	Contraindicated	Contraindicated	Contraindicated
<i>Age group <18 years or >80 years of age</i>	Contraindicated		
<i>If onset of symptoms occurred >4.5 hours¹</i>	Contraindicated	Contraindicated	Contraindicated
<i>Severe stroke (e.g. NIHSS >25)</i>	Contraindicated		
<i>If patient has a minor deficit or symptoms rapidly improving</i>	Contraindicated	Contraindicated	Contraindicated
<i>If heparin was given ≤48 hours ago and patient has an elevated APTT</i>	Contraindicated	Contraindicated	Contraindicated

¹ Use of rtPA in the time period of 3 to 4.5 hours after stroke has received a Class I Recommendation, Level of Evidence B by the American Heart Association. In these patients, exclusion criteria include (a) those that are employed for treatment in 0-3 hours and (b) those incorporated within ECASS III trial: age > 80 years, patients receiving anticoagulant with an INR of >1.7 baseline NIHSS >25 or presence of diabetes mellitus and previous stroke²⁸. Del Zoppo GJ, Saver JL, Jauch EC, Adams HP, Jr. Expansion of the time window for treatment of acute ischemic stroke with intravenous tissue plasminogen activator: a science advisory from the American Heart Association/American Stroke Association. *Stroke* 2009;40:2945-8.

List of contraindications	European License for t-PA use in Stroke²³	United States Guidelines²⁶	Canadian Guidelines²⁷
<i>If platelet count $\leq 100\ 000/mm^3$</i>	Contraindicated	Contraindicated	Contraindicated
<i>If patient is receiving oral anticoagulants</i>	Contraindicated	and if INR >1.7, then contraindicated	and if INR >1.7, then contraindicated
<i>If a severe stroke is demonstrated by brain imaging</i>	Contraindicated	Contraindicated in case of >1/3 cerebral hemisphere	Contraindicated in case of >1/3 MCA territory
<i>If seizure occurred at stroke onset</i>	Contraindicated	Contraindicated	Contraindicated
<i>If patient has a manifest or recent severe or dangerous bleeding</i>	Contraindicated	If it occurred in prior 21 days, then alteplase is contraindicated	If it occurred in prior 21 days, then alteplase is contraindicated
<i>If there is a history of intracranial haemorrhage</i>	Contraindicated	Contraindicated	Contraindicated
<i>If there is a suspected subarachnoid haemorrhage</i>	Contraindicated	Contraindicated	Contraindicated
<i>Prior stroke within the last 3 months</i>	Contraindicated	Contraindicated	Contraindicated
<i>Bacterial endocarditis</i>	Contraindicated		
<i>Myocardial infarction in the past 3 months</i>		Contraindicated	Contraindicated

List of contraindications	European License for t-PA use in Stroke²³	United States Guidelines²⁶	Canadian Guidelines²⁷
<i>Pericarditis</i>	Contraindicated		If post myocardial, then t-PA is contraindicated
<i>If patient has a history of recent puncture of a non-compressible blood vessel</i>	Contraindicated	If in past 7 days, then t-PA is contraindicated	If in past 7 days, then t-PA is contraindicated.
<i>If patient underwent a major surgery</i>	If in past 3 months, t-PA is contraindicated	If in past 14 days, then t-PA is contraindicated	If in past 14 days, t-PA is contraindicated.
<i>If patient's systolic blood pressure >185 or diastolic >110 mm Hg or if there is a need of aggressive management to reduce blood pressure to these limits</i>	t-PA is contraindicated	t-PA is contraindicated	t-PA is contraindicated
<i>Prior stroke and concomitant diabetes</i>	Contraindicated		
<i>Intracranial neoplasm, arteriovenous malformation, or aneurysm</i>	Any history of CNS damage, then t-PA is contraindicated	t-PA is contraindicated	t-PA is contraindicated
<i>If patient suffered a significant trauma in past 3 months</i>	contraindicated	If head trauma, t-PA is contraindicated	If head trauma, t-PA is contraindicated

<i>List of contraindications</i>	European License for t-PA use in Stroke²³	United States Guidelines²⁶	Canadian Guidelines²⁷
<i>Acute pancreatitis</i>	Contraindicated		
<i>Blood glucose levels (mmol/L)</i>	Contraindicated if levels are <2.7 or >22.2	Contraindicated if levels are <2.7	Contraindicated if levels are <3 or >22
<i>Ulcerative gastrointestinal disease during the last 3 months, oesophageal varices, arterial-aneurysm, arterial/venous malformations</i>	Contraindicated		
<i>History of Obstetrical delivery</i>	Contraindicated		
<i>Neoplasm with increased bleeding risk</i>	Contraindicated		
<i>Severe liver disease, including hepatic failure, cirrhosis, portal hypertension (oesophageal varices) and active hepatitis</i>	Contraindicated		
<i>Recent (<10 days) traumatic external heart massage</i>	Contraindicated		
<i>Other illness that could limit effectiveness or increase risk of</i>			Contraindicated

<i>List of contraindications</i>	European License for t-PA use in Stroke²³	United States Guidelines²⁶	Canadian Guidelines²⁷
<i>bleeding in the judgment of the physician</i>			

Adapted from Jacques De Keyser, Zuzana Gdovinová, Maarten Uyttenboogaart, Patrick C. Vroomen, Gert Jan Luijckx Intravenous Alteplase for Stroke: Beyond the Guidelines and in Particular Clinical Situations, Stroke 2007 38(9):2612-2618 (Permissions obtained from Wolters Kluwer Health, License Number 2783781278803)²⁵

1.2 Pathophysiology of Ischaemic Stroke

Ischaemic stroke occurs following occlusion of vessel(s) that supply blood to brain tissue. Clots destined to occlude the cerebral blood vessel originate *either* in circulation and then reach the brain vessels, e.g., from an artery or heart in case of artery-to-artery embolism or cardio-embolism respectively, *or* develop locally (like an atheroma) and reduce the distal cerebral blood flow (e.g., following arterial stenosis due to the occlusion of arteries or the arterioles).³ Occlusion of the brain vessels results in an abrupt reduction of cerebral perfusion which then results a mismatch of the brain's requirement of oxygen and the amount that is actually available.²⁹

Normal rate of blood flow in grey matter is 0.8 ml/g/minute. Brain functions are preserved as long as blood flows at a rate above 0.25 ml/g/minute. When it falls below 0.15 ml/g/minute, neurons enter a phase of irreversible morphological change leading to their death.^{29,30} As a result, at stroke onset, there is often an ischaemic core (that is irreversibly damaged) which is surrounded by an area of hypo-perfused brain parenchyma ("penumbra"; that is not yet infarcted). The penumbra is an ischaemic tissue that is functionally impaired and is likely to undergo an infarction if it is not salvaged before a certain duration by using reperfusion or/and other strategies. If it is not salvaged, penumbra undergoes progressive recruitment into an infarct core. In a time-dependent manner, it expands and approximates the maximal volume that was originally at risk.^{29,30} Penumbra may derive some blood supply from surrounding collateral circulation, such as the Circle of Willis, ophthalmic artery or the leptomenigeal arteries. However, because these patients differ in the amount of possible collateral circulation, a similar degree/type of occlusion of brain vessels leads to a variable amount of ischaemic insult. Penumbra is present in most patients until 3 hours of stroke onset, and then, the proportion of patients with penumbra diminishes to about 40% by 23 hours.³¹

Cerebral ischaemia also results in a time-dependent cascade of events at molecular level leading to cell death.³²

1.3 Preclinical studies on thrombolytic therapy for acute ischaemic stroke

Meyer et al showed successful thrombolysis in embolic stroke models of cats and monkeys.³³ They used intravenous or intra-arterial bovine or human plasmin for thrombolysis.³³ Here, the thrombolysis was not associated with excess haemorrhagic infarctions.³³

Del Zoppo studied neurological outcomes (defined by a neurological function measured quantitatively by employing a neurologic scale, computerised tomography based estimation of cerebral infarction volume and carotid angiography) after the intracarotid administration of urokinase in baboons that suffered stroke in the right corpus striatum.³⁴ In these models, stroke was induced by compression of the right middle cerebral artery (just before the branching of the lenticulostriate arteries) lasting 3 hours. Also, six animals were employed as concurrent untreated controls.³⁴ The controls underwent occlusion of middle cerebral artery but did not receive the urokinase infusion.³⁴ The study demonstrated improved neurologic outcomes and reduction in infarction volume amongst those baboons that received urokinase compared to those that did not.³⁴ The study was significant because it showed that “thrombolytic therapy given *within 3 hours* of experimental thrombotic occlusion may salvage neurologic function and reduce cerebral infarction volume without CT scan detectable intracranial bleeding.”³⁴

Zivin et al studied neurological outcomes in embolic stroke models of rabbits that were first injected with numerous small clots in their carotids and then given tissue plasminogen activator.³⁵ Use of tissue plasminogen activator was associated with significant improvement in neurological outcomes.³⁵ Further, it was also shown that the concentrations used in in-vitro lysis of clots were similar to those that were anticipated in vivo situations.³⁵ No haemorrhages related to the use of the drug were seen.³⁵ In summary, the study showed that the tissue plasminogen activator could be used early after the stroke onset.³⁵

Experiments indicated that the use of thrombolytic therapy was associated with excess haemorrhages if thrombolysis was given in delayed time after the stroke onset; and the rates were lower if it was given within 3.5 hours.^{36,37} But, Lyden et al showed that there were no differences in the rates of cerebral haemorrhage if rabbits were thrombolysed with alteplase at 10 minutes, 8 hours or 24 hours.³⁸ Therefore, they concluded that “tPA treatment successfully causes thrombolysis of cerebral emboli without causing an increase in the incidence of cerebral haemorrhage in rabbits”.³⁸ Later, Lyden et al also showed that the streptokinase (and not the t-PA) was associated with significantly higher rates (and also size) of ICH.³⁹ Sundt et al showed that ischaemia could be tolerated for 3 hours, and maybe longer and vascular occlusion did not immediately lead to death of all neurons fed by the supplying artery.⁴⁰ Similarly, Harvey et al showed that in case of monkeys, it takes about 50 minutes for ischaemia to last so that it leads to the infarction of the entirely affected parenchyma.⁴¹

These preclinical experiments indicated that the use of thrombolytic therapy was an option to treat ischaemic stroke patients, and that the treatment should be offered as soon as possible after the onset of cerebral ischaemia. Therefore, when Brott et al⁴² undertook to pursue a dose finding study for t-PA in humans, they decided to treat patients as soon as possible.

1.4 Thrombolytic therapy in acute ischaemic stroke

From the previous discussion, it is apparent that recruitment of ischaemic penumbra into the core of cerebral ischaemia progresses in a time-dependent manner.^{29,32,40,41} If this progression is to be halted, a logical treatment would be to achieve rapid reperfusion of ischaemic tissue before it gets fully infarcted. By achieving this, one expects to see better outcomes in these patients.

1.5 Thrombolytic agents

Thrombolytic agents include recombinant tissue plasminogen activator (rt-PA), desmoteplase, urokinase, anisoylated plasminogen streptokinase activator

complex, staphylokinase, streptokinase, recombinant pro-Urokinase and tenecteplase.⁴³

Amongst these, only rt-PA, also referred to as alteplase, activase or actilyse, is licensed for use in acute ischaemic stroke.²³

Some physicians also use desmoteplase, urokinase or alteplase in an extended time window based on their local experience or in the settings of clinical trial.⁴⁴⁻

⁴⁷ Streptokinase was investigated in the past and the trials had to be prematurely terminated because the drug was associated with excess complications.⁴⁸⁻⁵⁰ To date, alteplase is the only thrombolytic agent that has a proven efficacy in patients suffering acute ischaemic stroke; desmoteplase has potential, and is being investigated in delayed time windows.

1.5.1 Tissue Plasminogen Activator (t-PA)

Tissue Plasminogen Activator (t-PA) is an endogenous fibrin specific serine protease that releases plasmin from plasminogen by lysing arginine-valine bond.⁵¹ In addition to a thrombolytic effect, t-PA also has various pleiotropic effects: excitotoxicity,^{52,53} proteolysis of extracellular matrix (contributes in neuronal migration, e.g. by neurite and axonal extension),⁵⁴⁻⁵⁷ long term potentiation (enhanced memory formation),⁵⁸⁻⁶¹ vasoactive effect (vasodilation at lower concentrations of t-PA and vice versa),^{62, 63} and enhanced expression of metalloproteinases (like MMP-9).⁶⁴⁻⁷¹

Before stroke physicians began to use it, alteplase was already in use for the treatment of acute myocardial infarction (AMI). A clot can occlude the coronary arteries and lead to AMI. Thrombolytic agents are used in these patients to recanalise the occluded blood vessels.. Patients that suffer AMI are treated with either a *3-hour infusion regimen*⁷²⁻⁷⁵ or an *accelerated regimen*.⁷⁶⁻⁷⁸ In the former regimen, a dose of 100mg (6-10% of dose administered bolus, followed by 50-54% as infusion over one hour and then 20% over each succeeding hour) is given to the patients (Dose is 1.25 mg/kg if the patients weight is less than 65 kg).⁷²⁻⁷⁵ In case of the latter regimen, dose is 100mg for those weighing more than 67 kg (15mg intra venous bolus, then 50mg in half an hour infusion and then 35mg over the next hour).⁷⁶⁻⁷⁸ For those who weigh less than 67 kg, the dose

used is 15mg bolus, then 0.75 mg/kg in half an hour infusion and finally 0.5 mg/kg during next one hour.^{77,78} In case of AMI, aim of the thrombolytic therapy is to achieve recanalisation of the coronaries and salvage the ischaemic cardiac parenchyma. This leads to improved outcomes.²³

Hoping for a similar phenomenon to occur in ischaemic stroke patients and guided by findings from animal models (see section 1.5) NINDS investigators initiated dose finding studies for t-PA in ischaemic stroke patients (NINDS pilot study part 1 and part 2).⁷⁹ In part 1 of the study, 74 patients were investigated within 90 minutes of symptoms onset after the administration of alteplase in doses ranging from 0.35 mg/kg (maximum dose of 25 mg) to 1.08 mg/kg . Here, none of the 58 patients treated with a dose $\leq 0.85 \text{ mg/kg}$ developed cerebral haematoma.⁴² In part 2 of study, patients were treated between 90 minutes and 180 minutes of symptoms onset with alteplase at a dose of 0.6 mg/kg (maximum dose: 60 mg, N=8), 0.85 mg/kg (maximum dose: 90 mg, N=6) or 0.95 mg/kg (maximum dose: 90 mg, N=6). Here, the risk of cerebral haematoma was 17% for patients receiving a dose $\geq 0.85 \text{ mg/kg}$.⁸⁰

1.5.2 Urokinase (u-PA)

Urokinase is a trypsin-like enzyme that is produced in the kidney and excreted in human urine. It acts as a plasminogen activator (Molecular Weight 54000).⁸¹⁻⁸⁴ Several investigators have examined outcomes from its use in ischaemic stroke patients either in a non-randomised design or by comparing outcomes with the use of other thrombolytic agents,^{44,47,85-92} or in a randomised controlled trials design.⁹³⁻⁹⁸

In a double blind study design, Abe et al (1981) randomised patients that suffered from presumed thrombotic strokes within 2 weeks. Those that suffered presumed embolic stroke or severe stroke were excluded. Patients received urokinase at the dose 60000 units/day for 7 days (n=57) or received a placebo (n=56). Outcomes were measured at 4 weeks after the start of treatment and included measurement of global improvement rating and safety assessment. Patients underwent a CT scan at baseline and a repeat CT examination if they suffered neurological worsening. Clinical improvement occurred in 70.4%

thrombolysed patients compared to 56% non-thrombolysed patients. Haemorrhages did not occur in both arms.⁹⁹

Atarashi et al (1985) examined outcomes in patients that had a presumed diagnosis of cerebral arterial thrombosis of 5 days.⁹⁴ Patients received urokinase (n=191, two groups comprising high dose urokinase 240 000 u/day IV for 7 days and low dose urokinase 60 000 u/day for 7 days) or placebo (n=94) in a double blind design.⁹⁴ Where possible, the patients also underwent an angiography.⁹⁴ Those that suffered a presumed embolic stroke or a severe neurological deficit were excluded from enrolment. Outcome measures included clinical improvement described as per a final global improvement rating (done at 4 weeks after the treatment was begun) and safety (defined by absence of any side effects).⁹⁴ Thrombolysis was offered in a 120 hours' time window. Rates of clinical improvement were similar in both arms: 45% in the thrombolysis group and 43.6% in placebo group. Rates of haemorrhage were 1% and 1.1% the two arms respectively.⁹⁴

Australasian Urokinase Stroke Study (AUST) was a multicentre randomised controlled trial that investigated outcomes from the use of intra-arterial urokinase in patients suffering posterior circulation stroke.⁹⁵ Patients were either given urokinase (in increments of 10^5 IU to a maximum dose of 10^6 IU) within 24 hours of symptoms onset or entered into the control arm. 20 patients were screened, 16 randomised, 8 received urokinase. Primary outcome at 6 months (defined by combined morbidity (Barthel Index and Rankin Score)) occurred in 7/8 of the patients that received only anticoagulation and 4/8 of the patients that also received urokinase (OR 0.14, 95%CI 0.02-1.43).⁹⁵ Odds for disability free survival were 1.33 (95% CI 0.07-26.62). Outcome data are shown in figure 1.2.⁹⁵

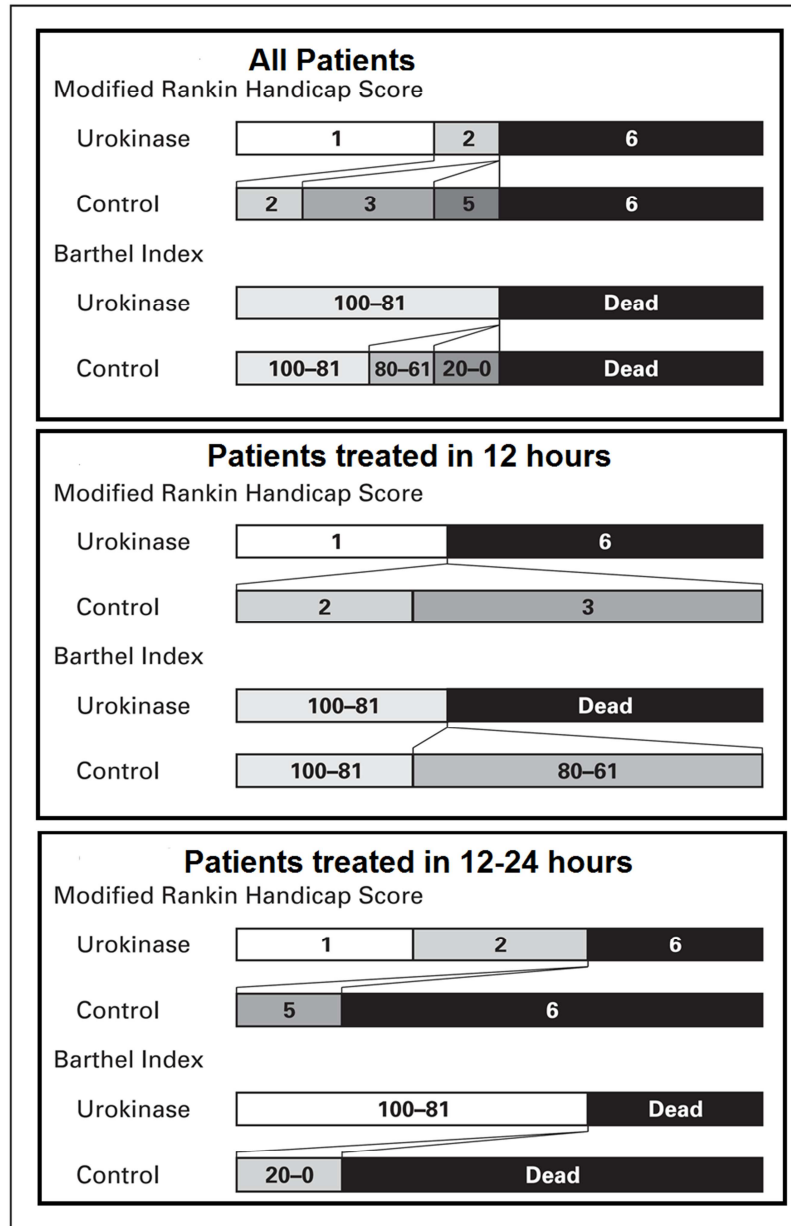


Figure 1-2 Figure showing outcomes in patients enrolled in AUST trial that randomised patients with urokinase and anticoagulants (heparin and warfarin) or only the anticoagulants

(Source: *Modified from* Macleod MR, Davis SM, Mitchell PJ, Gerraty RP, Fitt G, Hankey GJ, Stewart-Wynne EG, Rosen D, McNeil JJ, Bladin CF, Chambers BR, Herkes GK, Young D, Donnan GA. Results of a multicentre, randomised controlled trial of intra-arterial urokinase in the treatment of acute posterior circulation ischaemic stroke. *Cerebrovasc Dis.* 2005; 20(1):12-7; Permissions for use obtained from S. Karger AG, Basel)⁹⁵

Middle Cerebral Artery Embolism Local Fibrinolytic Intervention Trial (MELT) selected 114 patients (intra-arterial urokinase N=57 or placebo N=57) with symptoms of ischaemic stroke for last 6 hours and a demonstrable occlusion of middle cerebral artery (M1/M2) on CT angiography.⁹⁷ The study had to be stopped when tissue plasminogen activator received approval for use in ischaemic stroke in Japan. Non-significantly higher rates of favourable outcomes (m-RS 0-2) were observed on day 90 in the group that received thrombolytic therapy compared to the placebo arm (49.1% vs. 38.6%, p=0.3). Secondary outcome defined by m-RS 0-1 on day 90 was achieved by 42.1% and 22.8% of the patients in the respective treatment arms (p=0.045). Neurological improvement (NIHSS 0-1 at day 90) occurred more frequently after the use of urokinase (p=0.02). The study reported mortality rates of 5.3% and 3.5% in thrombolysis and control arm (p=1.000). Rates of intracerebral haemorrhage at 24 hours of treatment occurred in 9% and 2% of the patients (p=0.2).⁹⁷

1.5.3 Prolyse

Prolyse (nasaruplase), a glycosylated 411-amino acid single chain molecule (r-proUK), is a precursor of urokinase. In the presence of fibrin associated plasmin, it gets activated into double chain urokinase (UK) on the surface of a thrombus. Heparin enhances the thrombolytic effect of urokinase, either by the release of tPA from the endothelium or by neutralisation of thrombin. Urokinase is an endogenous plasminogen activator ($t_{1/2}$ = 9 to 12 minutes).¹⁰⁰⁻¹⁰³

Prolyse was investigated in two intra-arterial thrombolysis trials called Prolyse in acute cerebral thromboembolism (PROACT) I and II in a 6 hour time window.^{104,105} The drug was administered intra-arterially in the proximal 1/3rd (or close to the proximal tip) of the clot in the middle cerebral artery (MCA 1 or MCA 2). Prolyse was used at a dose of 6 mg in PROACT I and 9 mg in PROACT II; low dose iv heparin was given to all patients for four hours at the start of angiography in both PROACT I and PROACT II. PROACT I, a phase II randomised study, showed recanalisation efficacy (2P=0.02) and the safety (SICH rates, 2P=0.6) from the use of intra-arterial thrombolysis in a 6 hour time window.¹⁰⁴ Later, PROACT II study showed significantly better functional outcomes in thrombolysed patients compared to the controls (90 day m-RS 0-2 was achieved by 40% of the patients

in treatment arm compared to 25% in the control arm, $p=0.04$; $N=180$, patients randomised in proportions of 2:1 between treatment and control arms). Rates of recanalisation were 66% vs. 18%, $p<0.05$ and SICH within 24 hours 10% vs. 2%, $p=0.06$ between the two groups respectively. Despite showing an improvement in functional outcomes, the therapy failed to obtain approval from regulatory authorities.¹⁰⁵

1.5.4 Desmoteplase

Desmoteplase is a novel plasminogen activator that is derived from saliva of *Desmodus rotundus*. It differs from alteplase in that it lacks the 2nd kringle site in its molecular structure; does not need to be cleaved by plasmin and is active in its single chain form. It has reduced neurotoxicity and limited passage through the blood-brain-barrier. DSMA has a theoretical advantage over rt-PA as it is almost non-functional if fibrin is absent.¹⁰⁶⁻¹⁰⁸ Investigators have been examining its efficacy and safety for use in acute ischaemic stroke patients in a delayed time window.¹⁰⁸

Desmoteplase in Acute Ischaemic Stroke (DIAS) was a placebo controlled double blind dose finding phase II study which enrolled patients (baseline stroke severity on NIHSS 4-20) for thrombolysis in 3 to 9 hours' time window (Total patients: 104 patients). After treating 47 patients with a fixed dose regimen (25 mg, 37.5mg or 50 mg) of desmoteplase or placebo (part 1), dosing pattern were modified because there occurred excessive symptomatic intracerebral haemorrhages (26.7%). Investigators switched over to part II of the study in which a lower weight adjusted dose of 62.5 microgram/kg, 90 microgram/kg and 125 microgram/kg were used. In the part II of the study, rates of symptomatic intracerebral haemorrhages were 2.2%; no SICH occurred in the placebo arm. Reperfusion rates were 71.4% in patients treated with 125microgram/kg compared to 19.2% in the placebo arm. Favourable 90% clinical outcome occurred in 60% of patients treated with 125 microgram/kg of desmoteplase, 13.3% patients with a dose of 62.5microgram/kg of desmoteplase and 22.2% of placebo treated patients. The study showed a strong correlation of early reperfusion with improved clinical outcomes ($P=0.0028$).¹⁰⁹

Dose Escalation of Desmoteplase for Acute Ischaemic Stroke (DEDAS) study was another placebo-controlled double blind dose escalation phase II randomised study. It employed weight adjusted doses similar to DIAS and treated patients in a 3 to 9 hour time window. In this study, 8 patients received placebo and 29 received desmoteplase (90 microgram/kg; n=14). No symptomatic intracerebral haemorrhage occurred. Rate of reperfusion was 37.5% in the placebo arm, 18.2% in the desmoteplase arm that used a dose of 90microgram/kg and 53.3% in desmoteplase arm that used a dose of 125 microgram/kg arm. 25% of the placebo arm patients, 28.6% of 90 microgram/kg arm patients and 60% of 125 microgram/kg desmoteplase had good clinical outcomes on day 90.¹¹⁰ Desmoteplase at doses of 90 and 125 microgram per kg was shown to be associated with a strong dose-response relationship in a phase II design.^{109,110}

Between 2005 and 2007, Hacke et al randomised 193 patients in a dose ranging double blind placebo controlled trial in which patients that suffered acute stroke were selected based on presence of tissue at risk visible on either CT or MRI.¹¹¹ 123 patients entered the desmoteplase arm (n=57 for dose 90 microgram/kg and n= 66 microgram/kg) and 63 received placebo.¹¹¹ Median NIHSS at baseline was 6(IQR 6-14) and only 30% patients had a visible occlusion at baseline brain imaging.¹¹¹ In the three groups of patients that received desmoteplase at dose of 90microgram/kg, 120 microgram/kg or placebo, the respective clinical response rates on day 90 (defined by composite NIHSS improvement of 8 or more points, or NIHSS of 0 or 1, m-RS of 0-2 points, Barthel Index 75-100) were 47%,36% and 46%; change in lesion volume between baseline and day 30 were 14%,10.8% and -10%; rates of symptomatic intracerebral haemorrhage were 3.5%,4.5% and 0%; and mortality rates were 5%,21% and 6%. The study failed to show benefit.¹¹¹

Dose finding studies allow investigators to select a dose for further investigation in randomised controlled settings.

1.5.5 Tenecteplase

Tenecteplase is a mutant form of plasminogen activator which is characterised by delayed clearance, longer half-life, greater fibrin selectivity, greater resistance to plasminogen activator inhibitor and greater ability to lyse a thrombus compared to alteplase. This drug appears to be safe for use in acute

ischaemic stroke.¹¹²⁻¹¹⁴ Chapman et al undertook to compare thrombolysis from use of wild t-PA and tenecteplase.¹¹⁴ They used a model of New Zealand White rabbits who suffered ischaemic stroke due to injection of radio labelled blood clots.¹¹⁴ t-PA was administered to 57 animals, tenecteplase to 70 animals (43 received a dose of 0.6mg/kg, the rest received a dose of 1.5 mg/kg) and 37 animals were used as controls. The study found recanalisation rates that were similar between the animals that received tenecteplase or alteplase.¹¹⁴ The study concluded: “while tPA increases haemorrhage rate, the haemorrhage associated with TNK treatment is not statistically different compared with controls or the tPA group.”¹¹⁴

In a pilot dose escalation study, Haley et al showed that tenecteplase at a dose of 0.1-0.4 mg/kg could safely be used in the acute ischaemic stroke patients: there occurred no symptomatic intracranial haemorrhages in these dose ranges.¹¹⁵ A phase IIb/III trial of tenecteplase had to be prematurely terminated.¹¹⁶ The study involved an “adaptive, sequential dose selection procedure that used major neurological improvement at 24 hours balanced by risk (Symptomatic Intra-cerebral Haemorrhage)” and allowed investigators to exclude the dose of 0.4mg/kg as inferior, but because the trial did not finish, an optimal dose between the other doses (0.1 and 0.25mg/kg) could not be identified.¹¹⁶ Alteplase-Tenecteplase Trial Evaluation for Stroke Thrombolysis- (ATTEST) trial is a pilot phase study that would compare outcomes between patients who would receive tenecteplase (dose: 0.25mg/kg; maximal dose 25mg) or alteplase (dose: 0.9 mg/kg administered as 10% bolus and then 90% of the dose as IV infusion over 1 hour; maximum dose 90 mg) in a prospective, randomised, blinded outcome evaluation clinical trial design.¹¹⁷

1.6 Clinical trials to evaluate therapy for acute ischaemic stroke

The invention of CT technology led to better stroke trials: physicians could select those patients for thrombolytic therapy who did not have intracerebral haemorrhage on CT brain.

In 1992, del Zoppo and colleagues, examined outcomes in ischaemic stroke patients who received alteplase in an 8 hours' time window for cerebral artery occlusion. The study reported 40% rate of recanalisation, 9.6% symptomatic intracerebral haemorrhages and 12.5% mortality (N=139).¹¹⁸ Later, Brott and Haley showed that dose < 0.95 mg/kg was safe for use in these patients.^{42,80}

Mori et al showed that use of thrombolytic therapy was associated with excess recanalisation rates.¹¹⁹ Finally a "bridging trial", in a randomised double blind controlled design (n=27, 20 treated in 90 minutes and 7 in 91-180 minutes; dose used 0.85 mg/kg) indicated the efficacy of alteplase in improving outcomes at 24 hours. The study highlighted the need of a larger RCT.¹²⁰

1.7 Randomised controlled trials for alteplase use in acute ischaemic stroke

1.7.1 European Australasian Collaborative Acute Stroke Study (ECASS) I

In ECASS I trial, patients were treated with a dose of 1.1 mg/kg in a 0-6 hours' time window. The target Population (TP) excluded protocol violators (17.4% of patients). This study failed to show a significant difference in primary endpoints (Barthel Index and m-RS on day 90) in Intention-to-Treat (ITT) analysis, but showed significant improvement in outcomes in per-protocol analysis (P=0.035), analyses of secondary end points (a combination of Barthel index and Rankin scale) (P<0.001, in both ITT and TP analyses), and also analyses of neurological outcomes (P=0.03, TP analyses only). The rate of recovery was significant until 7

days for all patients and until a month for the target population. Hospital stays were shortened in the alteplase arm and the mortality and ICH rates were similar in both arms.¹²¹ Investigators concluded: “intravenous thrombolysis in acute ischemic stroke is effective in improving some functional measures and neurologic outcome in a defined subgroup of stroke patients with moderate to severe neurologic deficit and without extended infarct signs on the initial CT scan.”¹²¹

1.7.2 National Institutes of Neurological Diseases (NINDS) study

The NINDS study (291 patients in part 1, 333 in part 2) was the first randomised controlled trial that showed safety and efficacy from use of alteplase in acute ischaemic stroke patients (dose:0.9 mg/kg, maximal dose of 90, time window: within 3 hours of symptoms onset)

Part I of the study defined early treatment response as improvement in NIHSS scores by ≥ 4 or a complete recovery at 24 hours.²⁴ Whereas analysis of early treatment response failed to show a statistically significant improvement, a subsequent post-hoc analysis noted improvement on median NIHSS scores at 24 hours (8 vs. 12, $p < 0.02$). Global outcome scores improved in patients that received active treatment in part 2 (OR 1.795% CI: 1.2-2.6). The study reported an 11-13% absolute increase in excellent outcomes and a non-significant reduction in mortality (17% vs. 21%, $p = 0.30$). The rates of symptomatic haemorrhage were significantly greater in the treatment arm compared to placebo (6.4% vs. 0.6%, $p < 0.001$).¹²² It was the first study to demonstrate efficacy and safety of alteplase in a 3 hour window that led to its FDA approval for use in acute ischaemic stroke.¹²³

1.7.3 European Australasian Collaborative Acute Stroke Study (ECASS) II

ECASS II examined outcomes in ischaemic stroke patients and used a dose of 0.9 mg/kg within 6 hours of symptoms onset. Though it failed to confirm a statistically significant benefit ($p = 0.2$, $n = 800$) when data were examined for patients that achieved a Rankin score of 0-1, in a post-hoc analysis better outcomes were observed when data were examined for patients that achieved

functional outcomes defined by m-RS = 0-2 (54.3% vs. 46%, $p=0.02$). Rates of symptomatic haemorrhages were 8.8% in thrombolysed patients and 3.4% in the non-thrombolysed group.¹²⁴

1.7.4 Streptokinase Trials

Three streptokinase trials, the Multicentre Acute Stroke Trial in Europe (MAST-E, 1996), the Multicentre Acute Stroke Trial in Italy (MAST-I, 1995) and the Australian Streptokinase Study (ASK, 1996) were all terminated prematurely because of complications.⁴⁸⁻⁵⁰

The MAST-E study showed greater mortality ($p=0.06$) and a trend towards reduced disability (and also a shorter stay in a rehabilitation centre/nursing home) if the patient received streptokinase.⁴⁹

The MAST-I study failed to show benefit in any of its treatment compared to placebo arms. Symptomatic haemorrhages were more common in the streptokinase arm.⁴⁸

The ASK study was characterised by higher mortality, worse clinical outcome and increased ICH rates. Analyses of patients who received streptokinase therapy within 3 hours of symptoms onset ($N=70$) with patients who were treated beyond 3 hours ($N=270$) showed that the former group had better outcomes.^{50,125}

1.7.5 Alteplase Thrombolysis for Acute Noninterventional Therapy In Ischaemic Stroke Study (ATLANTIS)

The ATLANTIS study started recruiting patients in August 1991 and had originally aimed to enroll patients within 6 hours of the onset of symptoms'. In December 1993, the time window was modified to 0 to 5 hours of symptom onset. After the publication of NINDS trial, the time window was narrowed down to treatment within 3 hours. Owing to these changes, the ATLANTIS trial was reported in two parts: part A (patients enrolled until December 1993, $N=142$) and part B.¹²⁶⁻¹²⁸

85% of patients in part A of the ATLANTIS trial received alteplase beyond three hours of symptoms' onset.¹²⁸ There was a significant increase in proportion of patients that achieved 4 points improvement in NIHSS scale at 24 hours (40% vs.

21% patients, $P=0.02$). There was a significant increase in proportion of SICH by tenth day of stroke (11% vs. 0%, $P<0.01$) and mortality by three months (23% vs. 7%, $P<0.01$).^{127,128} The outcomes in 61 patients treated within 3 hours of symptom onset were similar to the NINDS trial showing a 35% absolute increase in favorable outcomes.¹²⁶

The part B of the study found no significant difference in the excellent neurologic outcome (defined by NIHSS of 0 or 1 at day 90), between the treatment and control arm (34% vs. 32%, $P=0.65$, $N=613$).¹²⁷

1.7.6 European Australasian Collaborative Acute Stroke Study (ECASS) III

ECASS III was the second positive t-PA trial that showed efficacy of t-PA in the 3-4.5 hour time window (52.4% vs. 45.2%; OR 1.34, 95% CI 1.02-1.76, $P=0.04$, $N=821$).¹²⁹ The trial was mandated by EMEA in order for the drug to receive the license within the European Union.²³ Though there occurred excess ICH (27% vs. 17.6%, $p=0.001$) in the alteplase arm, the proportion of patients that suffered symptomatic ICH was smaller (2.4% vs. 0.2%, $P=0.008$). Mortality rates were similar between the alteplase and the placebo group (7.7% vs. 8.4%, $P=0.68$).¹²⁹

1.8 Findings from pooled data analyses and meta-analyses of t-PA trials

1.8.1 First pooled analyses of t-PA stroke trials

Pooled analyses of data from ECASS, NINDS, ATLANTIS ($N=2775$) showed an inverse relationship between the time since symptom onset and improved outcomes: OR=2.8 (95% CI 1.8–4.5) for 0–90 minutes, 1.6 (1.1–2.2) for 91–180 minutes, 1.4 (1.1–1.9) for 181–270 minutes, and 1.2 (0.9–1.5) for 271–360 minutes ($P=0.005$). The hazard ratios for time windows 0-90, 91-180, 181-270 were 1.0, and for time window 271-360, 1.45 (95% Confidence Interval 1.02–2.07). The risk of cerebral haemorrhage had a significant association with the use of t-PA (5.9% vs. 1.1%, $p<0.0001$). Whereas the time since symptom onset

was not associated with occurrence of cerebral haematoma, use of alteplase ($p < 0.0001$) and age ($p < 0.0001$) were.¹³⁰

1.8.2 Second pooled analyses of alteplase trials

A recent pooled analysis of t-PA trials (2010) by Lees et al reconfirms the efficacy of t-PA in 4.5 hours of symptoms onset.¹³¹

1.8.3 Cochrane review meta-analyses

The Cochrane group reports a meta-analysis of clinical trial data by examining the safety and efficacy of thrombolytic therapy in acute ischaemic stroke patients. These meta-analyses are just not limited to alteplase trials (that constitute 55% of its data), but also include trials that investigated streptokinase, urokinase, desmoteplase and pro-urokinase (N= 26 trials, 7152 patients). About 0.5% of these patients are older than 80 years of age. Odds for death or dependency (m-RS 3-6) in patients thrombolysed in 3-6 hours after stroke onset are 0.8, 95% CI 0.73-0.90. In a 6 hour window, t-pa therapy is associated with significant odds of 3.5 for symptomatic haemorrhage; for mortality, these are OR 1.31, $P < 0.05$. In a 3 hour time window, there occurred a significant reduction in the rates of death or dependency with odds of 0.7; for death, OR=1.13, 95%CI 0.86-1.48. Odds for all-cause mortality within 10 days were 1.8 for all patients (95%CI 1.4-2.2, N=4423); for those who got t-PA, OR=1.2, 95% CI 0.9-1.7, N=2500.⁹²

1.9 Observational data on use of alteplase

After the NINDS trials were reported, many physicians were still not convinced. They argued that the study was conducted in specialised stroke centres and therefore data were not generalisable to other settings.¹³²⁻¹³⁴ Hence, there was felt a need to examine outcomes in community hospitals. I report a summary of various observational studies that examined t-PA for acute ischaemic stroke.

1.9.1 Houston community experience

Chiu et al reported outcomes in 30 patients treated with alteplase between December 1995 and December 1996 (dose of 0.9mg/kg): rate of symptomatic intracerebral haemorrhage: 7%; rate of fatal ICH: 3%; 37% patients recovered to full independence (Barthel Index 95-100) and 30% with m-RS 0-1. The rate of mortality on day 90 was 20%.¹³⁵

1.9.2 Cologne community experience

Grond et al described their experience from Cologne (n=100): door to needle time: 48 minutes; stroke onset to arrival time: 78 minutes; day 90 Barthel Index of 95-100 achieved by 53% patients; 40% achieved a m-RS of 0-1, NIHSS score of 0-1: 42%; symptomatic ICH rate of 5%; and mortality rate of 12%.¹³⁶

1.9.3 Oregon community experience

33 patients received alteplase. The result indicated that t-PA-use was “feasible and efficacious”: mortality rates=18.2%; 36.4% patients achieved m-RS of 0-1 at 3 months.¹³⁷

1.9.4 Cleveland community experience

By employing a chart review of 3948 patients enrolled in 29 different hospitals in Cleveland, Ohio, between 1997-1998, Katzen et al aimed to estimate rates at which t-PA was used in the community.¹³⁸ This study highlighted that many patients were not getting t-PA because the time window of t- 3 hours was too short: only 17% of the patients could be hospitalised within the 3 hour time limit and only 1.8% finally received alteplase. NIHSS was recorded in 405 of patients, median 12. This study highlighted that t-PA was associated with excess poor outcomes: symptomatic haemorrhages in 15.7% of the patients (a rate that was 2.5 times that of the NINDS study). A large proportion of patients (50%) had deviated from the t-PA guidelines. The deviations included use of antithrombotic agents within 24 hours of alteplase use (in 37.1% of patients; heparin in 16 patients, aspirin in 7 patients, their combination in 2 patients and ticlopidine in 1 patient), thrombolysis beyond 3 hours (even those treated within 3 hours 5 minutes were considered eligible, N=4 in these extra 5 minutes; 12.9% treated

between 3 hours 10 minutes and 6 hours 13 minutes) and raised blood pressure. Excess deaths occurred if they received alteplase compared to those who did not receive alteplase (15.7% vs. 5.1%, $p < 0.01$). These findings differ from experiences of other groups: STARS (discussed in subsequent section) reported rates of symptomatic haemorrhage = 3%, which were 5 times lower than what Cleveland experienced. Excess adverse events in Cleveland were attributed to practices that deviated from t-PA guidelines. The Cleveland study differed from others: these were retrospective chart reviews; patients were identified based on the ICD system; data were collected on a 6 monthly basis; nurses would look for cases after these had already occurred (case ascertainment bias); a population that was different in characteristics compared to other centres that reported earlier (19.4% valvular heart disease in Cleveland cohort compared to only 8.3% in NINDS) and importantly deviations from guidelines.¹³⁸

A subsequent paper from the Cleveland group described patients that were admitted to their Clinical Health System between 2000 and 2001. This time investigators practiced performance monitoring, frequently reviewed data, provided round-the-clock access to the stroke team by introducing a stroke pager and raising the education level regarding stroke management. Now, 18.8% of the patients reached hospitals within 3 hours; the rate of symptomatic ICH was 6.4%; and protocol violations occurred in 19% of the patients.¹³⁹

These two studies from Cleveland highlighted that there was a need to educate participants in “chain of delivery” and strictly follow the t-PA guidelines.^{138,139}

1.9.5 Standard Treatment with Alteplase to Reverse Stroke (STARS)

STARS was an American study that aimed to examine outcomes in ischaemic stroke patients that received alteplase between February 1997 and December 1998 (N: 389 patients, centre participation: 24 academic and 33 community centres). Median onset to treatment time was 2 hours 44 minutes; and, baseline NIHSS score 13. On day 30, mortality rates were 13%; Rankin score 0-1 35%, Rankin score 0-2 43%; rate of symptomatic haemorrhage 3.3% (N=13) and mortality attributed to symptomatic haemorrhage N= 7. STARS showed that t-PA could be safely administered in university and community settings.^{122,129,140}

1.9.6 Vancouver Experience

Chapman et al report outcomes in 46 patients who were given t-PA in a 3 hour time window and based on NINDS protocol. Here, SICH rates were 2.2 % (at 36 hours); outcomes at 13 months were mortality 22%, 43% reaching a m-RS of 0-1 and 48% a Barthel Index of 95-100. ¹⁴¹

1.9.7 Calgary Experience

Over half of the patients in Calgary data suffered ischaemic stroke (1168/2165; duration: 1996 to 1999). Delay in patients' arrival accounted for 73% of the patients' exclusion. Only 84 of the 314 patients received alteplase despite presenting within t-PA time window; overall, only 4.7% patients received alteplase. Outcomes of thrombolysed patients on day 90 were comparable to randomised data: 54% of reached m-RS of 0-2 and 7.1% patients developed Symptomatic ICH. Reasons why some patients were excluded despite presenting within 3 hours include: presence of mild stroke (13.1%) or rapidly improving stroke (18.2%) at baseline; delay in referral (8.9%); and, patient suffered other illnesses that would have adversely affected outcomes (8.3%). It should be noted that, about 32% of those patients who suffered a mild or rapidly improving stroke were still dependent upon discharge or had died while they were still in the hospital. The Calgary experience highlights that mild or rapidly improving stroke patients do not always have a spontaneous resolution of symptoms or better outcomes. ¹⁴² In chapter 7, I examine this question.

1.9.8 Berlin Experience

Koennecke reported data collected over two years during which 75 patients (9.4%) received alteplase. 2.7% suffered cerebral haemorrhages. 40% achieved m-RS of 0-1, 32% a m-RS of 2-3 and 13% a m-RS of 4-5; 15% of the patients died. The investigators highlighted that performance improved with time, and gradually more and more patients could be enrolled). ¹⁴³

1.9.9 Houston Experience

Grotta et al reported improvement in neurological outcomes of 269 patients that received alteplase (baseline NIHSS 14.4+/- 6.1 to 7+/-7 at discharge) between January 1996 -June 2000. SICH rates were 4.5 %.¹⁴⁴

1.9.10 Canadian Activase for Stroke Effectiveness Study

The Canadian group examined 1099 patients who received t-PA during February 1999 to June 2001. Stroke severity was NIHSS=15. 46% patients achieved m-RS of 0-2 on day 90 and the rate of ICH was 4.6%. Baseline Stroke severity, baseline ASPECT scores, age, atrial fibrillation and baseline blood sugar levels predicted outcomes in this population. Raised blood pressure and hyperglycaemia at baseline were predictors of ICH occurrence. This study suggested that IV rt-PA could safely be used in Canada.¹⁴⁵

1.9.11 The Safe Implementation of Thrombolysis in Stroke studies

Following the NINDS trial, alteplase was approved in the USA (in 1996) and Canada (in 1999) for use in acute ischaemic stroke.¹²³ But, European drug authorities were hesitant to permit its use in the European Union.²³ Alteplase was finally approved for use in ischaemic stroke patients in 2002 subject to the fulfilment of two conditions by the study sponsor (i.e. Boehringer Ingelheim): (a) to conduct a pan-European observational study with strict monitoring of outcomes in patients receiving t-PA and (b) to conduct a randomised controlled trial evaluating outcomes beyond 3 hours of symptoms onset.²³ In order to fulfil the first requirement, the Safe Implementation of Thrombolysis in Stroke Monitoring Study (SITS-MOST) a study was conducted, and for the second requirement, the ECASS III.^{129,146}

The primary aim of the Safe Implementation of Thrombolysis in Stroke Monitoring Study (SITS-MOST) was to assess the safety and efficacy of alteplase in patients treated within the European Union, Iceland and Norway in a 3 hour time window. The study enrolled 6483 patients between 25 December, 2002 and 30 April, 2006 from 285 centres spread across 14 member states. The study employed some monitoring procedures for data collection. For example, national

co-ordinators and professional monitors in collaboration with the study sponsor, Boehringer Ingelheim, source verified sample source data.¹⁴⁶ The study reported that “a minimum of 10% of patients recruited in SITS-MOST were monitored and in UK source data verification was done by independent clinical staff under the direction of the UK national coordinators who also checked for the completeness of registrations at all sites.”¹⁴⁶ About half of the centres that participated had little prior experience with thrombolysis. The baseline characteristics were similar between the SITS-MOST patients and the randomised trials. At 24 hours, the SICH rate was 1.7% per SITS-MOST definition. On day 7, 7.3% patients suffered SICH per Cochran definition (8.6% in randomised trials). Mortality by 90 days was 11.3% compared to 17.3% as observed in the randomised trials. Thus, the SITS-MOST study showed that the alteplase could be safely administered within three hours of symptom onset.¹⁴⁶

The Safe Implementation of Treatments in Stroke (SITS) group compared the outcomes in patients treated in the 3-4.5 hour time window (n=664) with patients treated in the <3 hours' time window (n=11 865) (enrolled between December 25, 2002 and November 15, 2007) employing data that were recorded in the prospective International Stroke Thrombolysis Registry (ISTR).¹⁴⁷ Outcomes did not differ significantly between the two groups: the rates of symptomatic intracerebral haemorrhage within 24 h (haemorrhage type 2 associated with National Institutes of Health Stroke Scale [NIHSS] ≥ 4 points deterioration) were 2.2% versus 1.6%, $p=0.24$; mortality at 3 months: 12.7% versus 12.2%, $p=0.72$ and adjusted $p=0.053$; and independence (modified Rankin Score 0-2) at 3 months 58.0% versus 56.3%, $p=0.42$ and adjusted $p=0.18$.¹⁴⁷ The findings offered some data to show that alteplase could safely be given in a 3-4.5 hour time window after ischaemic stroke.¹⁴⁷ Later the ECASS III trial showed that use of alteplase was indeed associated with improved outcomes when patients were treated in a 3-4.5 hour time window.¹²⁹

1.10 Brain Imaging

1.10.1 Introduction

The invention of Computed Tomography (CT) technology was a major technological advance in the field of clinical medicine. It led to the award of a Nobel prize to Dr Hounsfield.¹⁴⁸ With a CT scan, a physician could now exclude an intracranial haemorrhage and the stroke mimics. It is a widely available imaging modality that is used by stroke physicians to image stroke patients.

Brain MRI is considered better because it provides the physicians with some additional information. For example, s/he can see the hyper acute ischaemic changes on the Diffusion Weighted Imaging (DWI) sequences, haemorrhages on Gradient Echo (GRE) sequences and predict the timing of stroke onset based on Fluid Attenuated Inversion Recovery (FLAIR) sequences (DWI-FLAIR mismatch).^{149,150}

Perfusion brain imaging gives information about the haemo-dynamic changes that occur after cerebral ischaemia (e.g. cerebral blood flow, volume and other perfusion parameters). CT Angiography (CTA) or MR Angiography (MRA) give the visual details of brain vessels (e.g. the occlusions). Physicians aim to achieve recanalisation/reperfusion, and therefore on being able to see a vessel occlusion on CTA or MRA, physicians can implement an appropriate recanalisation strategy for treatment of these patients.¹⁵¹ For example, patients having occlusion of “Carotid-T” occlusion of middle cerebral arteries are known to respond poorly to iv t-PA and these patients may be considered as candidates for intra-arterial or mechanical thrombolysis by some physicians.¹⁵²

1.10.2 Ischaemic Penumbra

That an ischaemic penumbra develops after a cerebral vessel occlusion was known to stroke neuroscientists long before t-PA therapy was introduced as a

treatment of acute ischaemic stroke.^{29,30,153,154} When a cerebral artery occludes the core of ischaemic lesion suffers irreversible injury and surrounding tissue is functionally silent because of hypo-perfusion. If the hypo-perfusion persists, the central core enlarges and gradually the whole of hypo perfused brain parenchyma undergoes infarction. However, if the perfusion is restored early enough, a large portion of surrounding hypo-perfused tissue can be prevented from undergoing infarction.²⁹

The rate at which penumbra recruits into the infarct core differs between individuals, for it is a dynamic process, and depends upon vasomotor response to ischaemia and collateral circulation that are specific to an individual and differ between individuals.²⁹ From the statistical analyses of randomised t-PA trials data, we know that the odds for improved outcomes are significantly >1 only until 4.5 hours after onset of the symptoms.¹³¹ The patients that were treated in the 4.5-6 hour time window had odds of (for better outcomes) 1.22 but here the confidence intervals were wide, i.e., 0.92 to 1.61.¹³¹ Current interest is, therefore, in the identification of those patients who may still have salvageable parenchyma despite delayed presentation. In the confidence interval for OR in the time interval 4.5-6 hours suggests that while some patients might benefit from t-PA, others would suffer excess harm.¹³¹ There is a potential to select patients despite delay in their arrival based on presence of penumbra.⁴⁶

1.10.3 *Multimodal Stroke Imaging*

Multimodal stroke imaging aims to provide information about the extent of salvageable brain tissue, site of vessel occlusion, state of the collaterals. It also helps exclude cerebral haemorrhage or a stroke mimic.¹⁵⁵⁻¹⁶²

Multimodal MRI protocol takes < ½ hour and includes sequences like T2-Weighted, Fluid Attenuated Inversion Recovery (FLAIR), T2*- and diffusion weighted imaging (DWI) along with the perfusion weighted imaging (PWI) and MR angiography.

Standard T1-, T2-weighted, and FLAIR sequences are sensitive to vasogenic oedema. After the stroke onset, these take hours to develop. T2-weighted images or FLAIR images provide information about tissue characteristics that help the physician to exclude stroke mimics. Diffusion weighted imaging is sensitive to regions of ischaemia and develops within minutes of symptoms onset. Hence, it allows detection of ischaemic areas soon after the stroke onset. This is because DWI reflects signal changes that occur due to relative restriction of intracellular protons resulting from the failure of ATP dependent Na^+/K^+ pumps. These pumps pump the intracellular water out of the cells. While ADC values return to normal within days of ischaemia, hyper intensity on DWI may persist for weeks.^{163,164}

Immediate visualisation of an ischaemic stroke lesion is an advantage of DWI imaging over CT imaging. However, a study based on analyses of DEFUSE trial data indicated that the DWI lesions might be reversible (referred to as “Reversible Acute Diffusion Lesion Already Reperfused (RADAR)”)¹⁶⁵ Recently, however, an analysis of DEFUSE and EPITHET data (n=119) indicated that a clinically relevant diffusion lesion reversal (DLR) is uncommon.^{166,167} The findings from the former study describing RADAR were attributed to errors in co-registration of images and/or infarct atrophy.^{166,167}

DWI is a useful tool and shows up hyper-acute ischaemic lesions as hyper intense signals on MR scans. Clinically, there are scenarios when a patient with a prior stroke presents with a recurrent stroke with symptoms suggesting a new infarct in the same territory. It is also likely that these patients may not have a new stroke but seizure activity from the scar of a previous lesion or symptoms due to deranged metabolic parameters or underlying infections. In order to decide whether new symptoms are due to a previous event or from a fresh infarct, the physician may want to use DWI sequence of MRI that can help confirm a fresh ischaemic lesion by the presence of bright hyper-intense signals. Also, DWI shows up smaller lesions that are very subtle on CT scans (e.g. lesions in posterior fossa).

Gradient Recalled Echo (GRE) sequences and other T2*W images are equal in sensitivity compared to CT images when detecting an acute ICH. T2*W images

also pick up signals from deposits of hemosiderin, thus allowing identification of patients that suffer vasculopathies and are prone to spontaneous bleeding.¹⁶⁸

Further, there appears to be a potential for FLAIR images in wake-up stroke: FLAIR negative DWI positive MRI may indicate a time window of less than 3 (or 4.5 hours) from symptoms onset (specificity 0.93, positive predictive value: 0.94; but, sensitivity 0.48 and negative predictive value 0.43).^{149,169}

Also, Fluid-attenuated inversion recovery (FLAIR) is the most sensitive MRI pulse sequence for detecting subarachnoid haemorrhage and shows the lesion as high signal intensity.^{170,171} MRI can also identify a cerebral venous sinus thrombosis. Acute thrombus contain deoxyhemoglobin which is visible on T2*W imaging as linear or dot shaped low signal areas of magnetic susceptibility in a blood vessel lumen.¹⁷²⁻¹⁷⁴

In addition, 3-D Time-of Flight (TOF) MRA imaging informs the state of vascular flow: when a saturation pulse is applied repeatedly, stationary protons in an excited plane get saturated while the flowing blood protons do not. Vascular contrasts are preferred because they have a relative independence from the flow dynamics and substantially reduce artefacts.¹⁷⁵ MRA also allows visualisation of vascular occlusion or a stenosis.

MR perfusion imaging refers to various techniques that allow non-invasive measurement of tissue perfusion and give haemodynamic data like tissue blood volume, blood flow and mean transit time. This technique involves serial measurement of signal changes that occur in surrounding tissue when a contrast bolus passes through its capillaries.^{29,176} Data so obtained are then transformed into relative tissue-concentration time course data. The haemodynamic parameters are measured based on a model that expresses the manner in which tracer passes through (or gets distributed within) the tissue. Variables that influence these parameters are the method of infusion (bolus vs. constant) and the pharmacokinetics of the contrast agent used for imaging (diffusibility of the agent, volume of distribution and half-life to reach equilibrium). The exogenous tracer method works on an assumption that the tracer stays restricted within the intravascular compartment (i.e. no diffusion into the extracellular space). The

endogenous tracer method works on an assumption that the tracer diffuses freely between the intravascular and extravascular compartment.^{177,178}

In the case of ischaemic stroke patients, the exogenous tracer method based Dynamic state MR perfusion technique is used. When a bolus of paramagnetic tracer passes through a capillary network, there occur rapid alterations of the local magnetic field of surrounding brain tissue. These signal changes are measured by ultrafast imaging techniques like Echo Planar Imaging MRI.^{178,179} Signal-time course data so obtained are then transformed into relative tissue-concentration time course data which then lead to the calculation of haemodynamic parameters like Mean Transit Time (MTT, average time taken by the contrast to pass through capillaries), cerebral blood volume (CBV; blood volume per unit of brain), relative cerebral blood flow (CBF; blood flow per unit brain mass per minute), time-to-peak (TTP; time to peak of the contrast agent in the vessel) and T-max (Time to peak of residue function). Because of the rapid passage of the bolus through the capillaries, a good temporal resolution can be achieved.^{177,178} Because CBF, CBV and MTT depend on variables like bolus injection (dose, rate of injection, paramagnetic property of contrast) or patient characteristics (total body vascular volume and cardiac output) that have intra- and inter-individual variability, semi-quantitative (relative) values obtained by using an internal reference point (like normal grey or white matter) are preferred.^{177,178} The Cerebral Blood Volume is calculated by measuring the area under curve of the tracer concentration time graph.^{144,145} Calculation of cerebral blood flow requires extensive processing of imaging data and includes steps like deconvolution of arterial input function.^{144,145} Cerebral Blood Flow then equals cerebral blood volume divided by mean transit time. The arterial input function varies between the voxels. Because most methods assume them to be a constant, there are chances of committing an error (e.g. when the middle cerebral artery is diseased on one side).^{177,180}

The concept of Diffusion Perfusion mismatch was recently tested in a few studies but failed to show success in achieving the primary end points.^{111,181} Investigators attributed the failure to trial design, e.g., in DIAS II mild strokes were recorded. Whereas these studies defined mismatch as a ratio of 1.2 between the perfusion volume and diffusion weighted lesion, analyses of DEFUSE

trials data later suggested that an optimal ratio should have been 2.6.¹⁸² There is a potential to select patients in a 3-6 hour time window based on MR diffusion perfusion mismatch criteria: in DEFUSE there was a significant association between a successful recanalisation and reduction in infarct growth in patients with mismatch (OR 5.4, P=0.04).¹⁸³

Contraindications to the use of MR based imaging include patients having pacemakers, metallic implants, electronic devices or obesity. Some patients may also need sedation for agitation or claustrophobia.

Further, thanks to the efforts of regulatory authorities that led to better clinical practices (e.g. screening patients and not using gadolinium when GFR is below 30ml/minute per 1.73 metre square) and likely new treatment(s), the incidence of nephrogenic systemic fibrosis have substantially reduced (≈ 0).¹⁸⁴⁻¹⁸⁶

Multimodal CT imaging includes non-contrast CT, a perfusion CT and a CT angiography. These take about ten minutes.

Non contrast CT is used to exclude haemorrhages and other stroke mimics. It is widely available and can be done rapidly. Non contrast CT scan can identify some early ischaemic changes like hyper dense vessels, insular ribbon sign, loss of clarity of the lentiform nucleus or loss of grey white matter differentiation; but these are subtle in the first 3 hours of stroke onset (sensitivity is only 25% compared to DWI).¹⁸⁷ Non-contrast CT provides structural information and no physiologic information; and hypo-attenuation is highly specific to infarction.

CT angiography provides detailed information on vessel characteristics like the site of occlusion and may confirm recanalisation after the drug use. In addition, it can be used to assess flow in collateral blood vessels.¹⁸⁸

In the **CT perfusion studies**, it is the entry and washout of iodinated contrast agent that is studied; and the signal density of contrast, while in its transit through the capillaries, is analysed for image interpretation. CT contrast agent and CT tissue density have a linear relationship and therefore it is simpler to calculate the quantitative brain perfusion data. Conventional CT scanners are

unable to image the whole brain as they scan a 2-4 cm section of brain parenchyma in each contrast bolus administered; but better newer CT scanners allow greater brain coverage.¹⁸⁸

Dynamic perfusion CT involves measurement of brain haemo-dynamics by employing first pass tracer methodology.¹⁸⁹ The method involves a continuous cine scanning lasting about 45 seconds (scan rate: 1 image per second). The patient is injected with \approx 50 cc of 350 mg/dL iodinated contrast material intravenously.¹⁹⁰ Because low kilovolts (about 90 kilovolt peaks) or milli amperes (about 150mA) protocols are used, patients are not exposed to excessive radiation.¹⁹⁰ Whereas an unenhanced CT brain results in radiation exposure of 2.5 mSv, perfusion CT leads to an exposure of 1.6-2.0 mSv.¹⁴⁹ Unless the contrasts are used in patients suffering renal failure or diabetes mellitus, the use of contrast is considered safe.¹⁹¹

In 2009, it was discovered that more than 200 patients had received a radiation overdose in Cedars Sinai Medical Centre, Los Angeles California over a period of 1.5 years.^{186 192} Subsequent investigation by the United States Federal Drug Agency led to the identification of a total of 385 patients from six hospitals that had been exposed to radiation overdose.^{192 193} The investigations failed to identify violations of the laws or regulations; and noticed that when the scanners were used according to the specifications given by the manufacturer, overexposure to radiation did not occur.¹⁹³ This incidence had occurred because of an error in the computer tomography console, and the overexposure was discovered when a patient reported suffering hair loss after receiving a CT scan.^{186 192} The levels of radiation were 8 times higher than the permissible limits.^{186 192} 40% of the patients suffered alopecia because of these exposures.¹⁹² To prevent recurrence of similar incidents, CT quality assurance programs are needed.¹⁸⁶ US-FDA had recommended that “(a) imaging facilities assess whether patients who underwent CT perfusion scans received excess radiation; (b) imaging facilities review their radiation dosing protocols for all CT perfusion studies to ensure that the correct dosing is planned for each study; (c) imaging facilities implement quality control procedures to ensure that dosing protocols are followed every time and the planned amount of radiation is administered; (d) radiologic technologists check the CT scanner display panel before

performing a study to make sure the amount of radiation to be delivered is at the appropriate level for the individual patient;(e)if more than one study is performed on a patient during one imaging session, practitioners should adjust the dose of radiation so it is appropriate for each study.”¹⁹² Wintermark and Lev, in an editorial, highlight that “while unnecessary radiation exposure should be avoided, a medically needed CT scan obtained with appropriate acquisition parameter has benefits that outweigh the radiation risks.”¹⁸⁶

Helical CT scanners, operating in cine mode are employed to obtain perfusion CT data. Multi-slice CT scanners are better because these allow for greater tissue coverage in each of the acquisitions.¹⁸⁹ Automatic injectors are used; rate of contrast injection: 3-4 cc/second; Catheter size employed ≥ 22 gauge.^{189,194,195}

Post-processing software used to process CT perfusion data (time taken: about 5 minutes) employ methods like *rate of upslope estimation* of cerebral blood flow (when the infusion rates are above about 6 cc per second) or *deconvolution analysis* (when the infusion rates are 4-5 cc per second); the latter provides quantitatively accurate rates.¹⁹⁶

CBF, CBV, MTT are read on the computer console allowing a physician appreciate visually the image at the same time as quantitatively analyse the region of interest. MTT are prolonged in areas where brain perfusion is reduced; and are sensitive to haemodynamic changes in the brain. Under perfusion they can be examined by comparing the CBV, CBF and MTT between the brain regions that are abnormal to their mirror images in the control area. Areas with prolonged MTT and increased CBV are considered to be the “tissue at risk”. Areas with reduced CBV with prolonged MTT correspond to an infarct core.¹⁹⁷

Table 1-2 Acquisition and Post processing Parameters recommended for CT and MR perfusion

Acquisition Parameter	Suggested by Roadmap for CT	Suggested by Roadmap for MR
Type of sequence	Not applicable	Single-shot gradient echo EPI
Duration	2-phase acquisition	90-120 s
Temporal resolution	1000 ms	$\leq 1500-2000$ ms
Other technical details	Peak kilo voltage 80kvp, current 100mAs	Echo time 1.5T (35-45 ms); 3T (25-30 ms), flip angle 1.5T (60-90°); 3T (60°)
Magnet strength	N/A	1.5 or 3.0T
Anatomic Coverage	20 mm (minimum) coverage per bolus	≥ 12 slices
Slice thickness	5-10 mm	5 mm
Field of view	about 24cm	about 24cm
Avoidance of lenses	above orbits	Not Applicable
Basal ganglia coverage	Yes	Not Applicable
Slice orientation stated	Yes	Yes
Contrast type	N/A	Gadolinium use stated
Contrast volume	35-50 mL	Not Applicable
Contrast concentration	350-370 mg/ml	Not Applicable

Injection delay	N/A	10s
Injection rate	4-6 mL/s	4-6 mL/s
Saline chaser described	Yes	Yes
Power injector used	Yes	Yes
Cannula gauge	18-20G	18-20G
Side of injection	Right	Right
Antecubital vein used	Yes	Yes
Post processing parameter	CT perfusion	MR perfusion
Use of deconvolution	Yes	Yes
Arterial input function selection lateralisation	Yes	Yes
Arterial input function selection artery	Yes	Yes
Venous output function site	Yes	N/A

[Source: Dani KA, Thomas RG, Chappell FM, Shuler K, Muir KW, Wardlaw JM. Systematic Review of Perfusion Imaging With Computed Tomography and Magnetic Resonance in Acute Ischaemic Stroke: Heterogeneity of Acquisition and Post processing Parameters: A Translational Medicine Research Collaboration Multicentre Acute Stroke Imaging Study. *Stroke*, 2011, e-pub doi:10.1161/STROKEAHA.111.629923. Permissions obtained from Wolters Kluwer Health, License Number 2783781063122.]¹⁹⁸

Multimodal brain imaging has potential for use in selecting patients for thrombolytic therapies. At the moment, however, there is a need for standardising the image processing methods.^{198,199}

1.11 Adverse events after use of thrombolytic therapy

Thrombolytic therapy is associated with adverse events like intracerebral haemorrhage, systemic bleeding or allergic reactions (like orolingual oedema, laryngeal oedema, anaphylaxis, or rash).

Intracerebral Haemorrhage is the most feared complication of thrombolytic therapy. Its rate of occurrence is examined as an outcome measure (for safety) in clinical trials of thrombolytic therapy. Symptomatic intracerebral haemorrhage (SICH) has various definitions. The **NINDS definition of SICH** is any haemorrhage that is associated with any neurological deterioration.¹²² The **ECASS II definition of SICH** is any haemorrhage with neurological deterioration, as indicated by an NIHSS score of 4 or more than the value at baseline or the lowest value within 7 days, or any haemorrhage leading to death.¹²⁴ In the **ECASS III** protocol, symptomatic intracranial haemorrhage was defined as “any apparently extravascular blood in the brain or within the cranium that was associated with clinical deterioration, as defined by an increase of 4 points or more in the score on the NIHSS, or that led to death and that was identified as the predominant cause of the neurologic deterioration”.¹²⁹ Hence, this definition is a modification of ECASS definitions in which it is further specified that the haemorrhage has to be identified as the predominant cause of the neurologic deterioration.¹²⁹ As per **SITS-MOST criteria**, the symptomatic intracerebral haemorrhage (SICH) is defined as a local or remote parenchymal haemorrhage type 2 on the 22 to 36 hour post imaging scan, combined with a neurological deterioration of 4 or more points compared with baseline NIHSS or the lowest NIHSS value between baseline and 24 hours.¹⁴⁶ Haemorrhagic infarction type 1 refers to small petechiae along the margins of the infarct. Haemorrhagic infarction type 2 refers to confluent petechiae within the infarcted area, but

without space-occupying effect. Parenchymal haematoma types 1 are the haematoma that occupy $\leq 30\%$ of the infarcted area with some slight space occupying effect. Parenchymal haematoma type 2 refers to dense haematoma occupying larger than 30% of the infarcted territory and occupies substantial space, or as any haemorrhagic lesion outside the infarcted area.

Orolingual oedema or the oedema of throat and mouth may occur from the use of intravenous t-PA.²⁰⁰⁻²⁰² Hill et al report 9 cases of orolingual angioedema in a series of consecutively enrolled 176 patients. The severity was reported as “mild, transient and contra lateral to the ischaemic hemisphere”.²⁰⁰ The authors reported a significantly increased risk of orolingual oedema with the use of angiotensin converting enzyme inhibitors (Relative Risk 13.6, 95% CI 3.0-62.7).²⁰⁰

1.12 Exclusion of patients from receiving alteplase

Investigators that design randomised controlled trials want to pick up the positive “signals” of treatment effect and not let the “noise” (effect of a heterogeneous study population that trials enrol) dilute it. In order to achieve this, the trialists a priori decide to exclude certain subgroups of the patient population that according to them would add to the “noise”. Hence, when t-PA trials were designed, certain subgroups of patients were excluded from getting t-PA. Investigators assumed that these patients were more likely to suffer poor outcomes (e.g. baseline severity >25 on NIHSS scale) or would get better any way (e.g. mild strokes). As a result, when the trials turned positive, those patients that were recommended for exclusion were not given approval in the drug license. This is because of lack of randomised data and not because the trial had shown poor outcomes in those patients.(See Table 1-3)

Table 1-3 Table showing various exclusion criteria that were incorporated in the t-PA trials

Exclusion criteria	NINDS ¹²²	ATLANTIS-A* ¹²⁶⁻¹²⁸	ECASS III ¹²⁹	EPITHET ¹⁸¹
Intracranial haemorrhage	Yes	Yes	Yes	Yes
Unknown time since symptoms onset	Yes	Yes	Yes	Yes
Rapidly improving symptoms or only minor before start of infusion	Yes	Yes; minor stroke < 4 points on NIHSS	Yes	Minor stroke defined as m-RS <5; rapidly improving stroke
Seizure at stroke onset	Yes	Yes, if known active seizure disorder; or first seizure within the 6 hours immediately before administration of study drug	Yes	Yes
Stroke or serious head trauma within previous 3 months	Yes	Head trauma in last 90 days; stroke in past 6 weeks	Yes	Yes
Combination of diabetes and	No	No	Yes	No

previous stroke				
Platelet < 100000/ml	Yes		Yes	Yes
Systolic BP>185 and Diastolic BP>110.	Yes, if aggressive blood pressure treatment was needed.	Yes; or if requiring aggressive treatment to bring down the BP to normal.	Yes, and if aggressive treatment was needed.	Yes, if aggressive treatment was needed.
Blood glucose level	Yes, if <50 and > 400 mg/dl	Yes, if <50 or >400 mg/dl	Yes if <40 or > 400 mg/dl	Yes if <40 or > 400 mg/dl
Symptoms suggestive of SAH even if CT is normal	Yes	Yes	Yes	Yes
Anticoagulant treatment	Yes, if received heparin in previous 48 hours and had an elevated PTT;PT>15 seconds		Yes	Yes, if received heparin in previous 48 hours and had an elevated PTT;PT>15 seconds
Major surgery or severe trauma in previous 3 months	Surgery within 14 days.	Surgery in last 30 days; or a biopsy of a parenchymal organ; or	Yes	Surgery in last 14 days

		trauma with internal injuries or ulcerative wounds;		
Gastrointestinal or Urinary Tract Haemorrhage	Yes, if within previous 21 days			Yes, if within last 21 days.
Arterial puncture on non-compressible site	Yes, if within previous 7 days			Yes, within previous 7 days
Other medical disorders associated with increased risk of bleeding		Hereditary or acquired haemorrhagic diathesis (raised APTT,PT;Coagulation factor deficiency; oral anticoagulant therapy with raised PT)	Yes	
Others		Coma, severe obtundation, fixed eye deviation or complete hemiplegia; previous intracranial haemorrhage,	Age <18 or > 80 years	If patient presents before 3 hours or after 6 hours; age below 18; non contrast CT scan showing

		neoplasm, arteriovenous malformation or aneurysm; presumed septic embolus, pericarditis, presence of vascular thrombus or aneurysm related to AMI; other serious advanced terminal illness; any other condition that investigator felt would cause significant harm to patients if the drug was given; if patient was participating in any other trial; CT Brain showing high density lesions suggesting		haemorrhage and major early ischaemic changes; inability to undergo MRI; history of ICH; if confounding neurological illnesses present like dementia or if patient suffered a life threatening illness.
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		haemorrhage, significant mass effect showing midline shift or SAH; pregnancy, lactation or parturition within last month.		
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*ATLANTIS B had similar exclusion criteria. In addition it considered the presence of parenchymal hypo density, loss of grey/white matter distinction, and/or effacement of cerebral sulci in 33% of the middle cerebral artery territory as exclusion criteria; these were based on data from ECASS trial.¹⁶¹

It is more than 15 years since the first positive t-PA trial, NINDS trial (two parts, published together), was reported.²⁴ So far only the time criteria of drug licensing has been examined in a randomised trial design in which the findings suggested that the time window could be extended until 4.5 hours of symptoms onset.¹²⁹ Many of the original exclusions continue to persist in routine clinical practice and prevent a significant proportion of patients from getting thrombolysis therapy.

Large proportions were excluded from the NINDS trial. 17367 patients were originally screened. Then, investigators had excluded 8708 (51.6%) patients because of delayed presentation, 10% (N=1749) due to rapidly improving symptoms, 7.8% (N=1306) due to intracranial haemorrhage, 6.6% patients (N=1106) due to minor symptoms, 6.1% (N=1021) because of age <18 or >80 years, 2.9% (N=490) due to serious illnesses, 2.3% (N=391) due to seizure at stroke onset, 2.2% (N=373) because they were not stroke, 1.3% (N=219) because of a recent stroke, 1.2% (N=210) due to prior use of oral anticoagulant and 1% (N=169) due to previous subarachnoid haemorrhage. Only 3.5 % of the screened patients were finally enrolled to the NINDS trial.^{122,123}

David Tong and colleagues reported Simplified Management of Acute Stroke Using Revised Treatment criteria (SMART) for thrombolytic therapy. In the absence of these criteria, a large proportion of patients would be excluded from getting t-PA (as shown in figure 1-2). According to SMART recommendations all ischaemic stroke patients are considered for use of thrombolytic therapy despite presence of common exclusions (see figure 1-2).^{203,204}

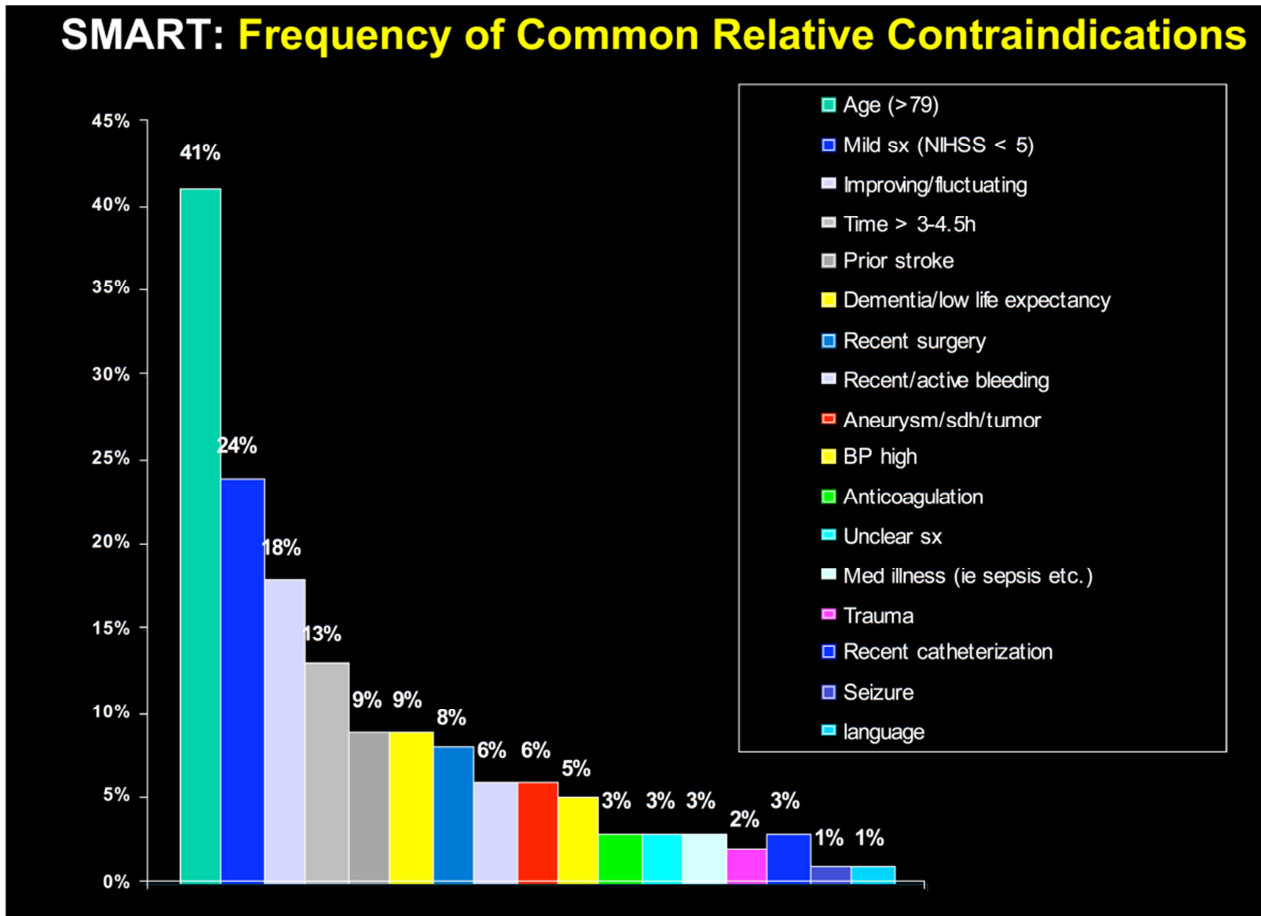


Figure 1-3 Figure shows frequency of common relative contraindications to the use of alteplase in acute ischaemic stroke.

Source: http://www.westernstroke.org/files/4_ExtendingTimeWindow_Tong.pdf (permissions obtained from Dr D Tong)^{203,204}

Because the various non-evidence-based (i.e. randomised data are lacking) exclusions are suggested by drug licensing agencies, many physicians strictly follow them. As a result, large proportions of patients who have ischaemic stroke and present within the therapeutic time window fail to receive thrombolytic therapy. In this thesis, I revisit the rationale behind these exclusions and then examine outcomes in those who are recommended for exclusion from getting t-PA. For this, I asked various t-PA trialists about the deliberations that took place during the period that preceded these trials (at the design phase), and got some information from NINDS investigator, Prof James Grotta. I mention them here.

t-PA was selected for use in the NINDS trial at a dose of 0.9mg/kg based on two underpowered studies.^{42,80} These studies had suggested that there were increased risks with the use of higher doses. Regarding exclusion criteria, there were no numerical cut-offs prescribed for baseline severity in the NINDS trial. Regarding a query about exclusions based on blood sugar levels, I was informed that these were based on consensus in the group: it was decided to exclude patients whose blood sugar levels were low enough to cause stroke-like symptoms or high enough to lead to neurological problems. Regarding appropriate cut-offs for platelet count or prothrombin time at baseline, a consensus was built based on the practices by cardiologists for myocardial infarctions. The purpose of exclusions was that the investigators wished to reduce risks, i.e., chance of bleeding, in most cases. Exceptions to this were the situations like seizures, rapid improvement of symptoms and high or low glucose level which were included to eliminate the stroke mimics. Rapidly improving or minor symptoms were excluded because investigators wished to exclude the TIAs. The source of many of the exclusions in the NINDS trial was from the exclusions prescribed for patients having myocardial infarctions e.g., patients having prior stroke or serious head trauma within preceding 3 months and major surgery in previous 14 days, gastrointestinal haemorrhage or urinary tract haemorrhage within the previous 21 days, arterial puncture at a non-compressible site within prior 7 days or platelet counts below 100,000 per cubic millimetre. The upper limit of systolic blood pressure was set at 185 mm of Hg

and diastolic blood pressure at 110 mm of Hg based on prior experience of Dr T Brott. Patients having symptoms which suggested that they suffered a subarachnoid haemorrhage were also recommended for exclusions. This was because at that time the CT imaging did not have great resolution and the investigators were afraid that they would miss a subtle SAH. Patients with seizures at symptoms onset were recommended for exclusions because if the patients had some of their symptoms because of Todd's paralysis, its resolution might be due to natural history and not related to improvement of stroke. There was no vascular imaging at that time that would have allowed visualisation of occluded vessels patients suffering ischaemic stroke and also have seizures at stroke onset. It was not, therefore, possible to include patients with seizure at stroke onset in the trial. Patients who were taking anticoagulants or who had received heparin within 48 hours preceding the onset of stroke and had an elevated partial-thromboplastin time were also recommended for exclusion. At that time, investigators did not know if re-occlusion was going to be a big problem. As is known, it is a problem in coronary recanalisation. If it occurred, then the investigators were going to be more inclined to allow the use of anticoagulants since that was being done after t-PA use for patients with myocardial infarction. It was an assignment meant for Dr J Grotta to examine evidence that re-occlusion occurred after stroke. He was unable to find any reports documenting re-occlusion. He acknowledges that there were no good angiographic studies of acute stroke to rely on. In addition, at that time there were no reports of improvement followed by deterioration which would have suggested recanalisation followed by re-occlusion. Therefore, the investigators didn't think that the evidence for reocclusion was worth the risk of causing bleeding by allowing the use of anticoagulants, so they were very strict about the levels of PT and PTT that they would allow. Investigators also prospectively built into the trial the objective of looking for deterioration following improvement (DFI) as a surrogate for reocclusion. The reason they wrote the 48 hour heparin use was that because of the half-life of heparin, they were very sure it all would be gone within 48 hours if the PTT was normal. Regarding exclusion criteria of prothrombin times, cut-off at 15 seconds was a guess and the reasoning was based on Myocardial Infarction (MI) data. Investigators had agreed to exclude patients from receiving alteplase if aggressive treatment was required to

reduce their blood pressure to the specified limits. This was based on the viewpoint of Dr Brott. Further by aggressive they meant IV infusions, in particular, nitroprusside. At that point, nicardipine was not in use, but within a short time Dr Grotta's department at Houston had begun using it and clarified with the steering committee that its use was allowed. (Source: personal communication with Prof James Grotta, UT Houston, USA)

Exclusion criteria in other trials were similar to NINDS trial (See table 1-3), and probably had a similar logic. ATLANTIS B considered little additional exclusion like the presence of parenchymal hypodensity, loss of grey/white matter distinction, and/or effacement of cerebral sulci in 33% of the middle cerebral artery territory based on data from the ECASS trial.¹²⁷ Exclusions resulted in serious reduction of sample size in each of these studies.

The EMEA'S document "summary information on a referral opinion following an arbitration pursuant to article 29 of directive 2001/83/EC for actilyse" prepared by European Agency for the Evaluation of Medicinal Products (EMA), London informs about the status of alteplase use within the European Union.²³ The document states that within EU, alteplase is indicated for acute ischaemic stroke patients presenting within 3 hours of symptoms. The drug should be used at a dose of 0.9 mg/kg (maximum dose 90 mg/kg, 10% given as bolus) and cannot be given concomitantly with the aspirin or heparin.²³

Interestingly, the exclusions differ between the jurisdictions reflecting regulatory acceptance of either NINDS criteria (US and Canada) or ECASS criteria (EMA). US guidelines include different restrictions for <3h and 3-4.5h use. (See table 1-1)²⁵

1.13 Utilisation of thrombolytic therapy in acute ischaemic stroke

In cardiology, a large volume of data is available to show that thrombolytic therapy reduces mortality: Gruppo Italiano per lo Studio della Streptochinasi

nell'Infarto miocardico (GISSI-1; N=11806)²⁰⁵, Intravenous Streptokinase in Acute Myocardial Infarction (ISAM; N=1741)²⁰⁶, APSAC Intervention Mortality Study (AIMS; N=1004)²⁰⁷, Second International Study of Infarct Survival (ISIS-2; N=17187)^{208,209}, Anglo-Scandinavian Study of Early Thrombolysis (ASSETS; N=13318)²¹⁰, Urochinas per via Sistemica nell'Infarto Miocardico (USIM; N=2531)²¹¹, Third International Study of Infarct Survival) Collaborative Group (ISIS-3, N=41299)²¹², Estudio Multicentrico Estreptoquinasa Republicas de America del Sur (EMERAS;N=4534)²¹³ and Late Assessment of Thrombolytic Efficacy (LATE; N=5711)²¹⁴. These trials showed strong and significant treatment effect and were published around a similar time period. The findings caught wide media attention and created enthusiasm and the thrombolytic agents were rapidly accepted by the cardiologists for the treatment of myocardial infarction. Large volume of these data on their own showed efficacy of thrombolytic therapy in AMI; and, when examined in meta-analysis reconfirmed the benefit (Mortality rate 9.6% vs. 11.5%, $p < 0.0001$, N=58600).²¹⁵

In the case of ischaemic stroke, the story has been different. Though, efficacy of alteplase in acute ischaemic stroke had been endorsed by several medical bodies (like American Academy of Neurology, American Heart Association or European Stroke Organisation), the claims for better outcomes were based on fewer data from only three randomised trials (the NINDS trial (two parts, N=291 and 333, data published together, in 1995) and ECASS III study (N=821, published in 2008))^{122,129}. Amongst the NINDS, ECASS, ECASS II and ATLANTIS, only NINDS trial was positive and reported efficacy of t-PA in acute ischaemic stroke in a 3 hours' time window.¹²² But, the NINDS trial was underpowered to detect any influence on mortality (t-pa vs. placebo 17% vs. 21%, $p=0.3$).¹²² The Streptokinase trials showed excess complications and therefore had to be abandoned.⁴⁸⁻⁵⁰ So far, only 7152 patients have been thrombolysed in 26 thrombolysis trials; and, only 3670 patients received t-PA.^{92,131} Fewer patients have been enrolled in thrombolysis trials compared to cardiology trials for acute myocardial infarction.²¹⁵ Despite FDA approval and upgrading of the level of evidence by American Heart Association (AHA, from Class IIb to Class I intervention i.e., from "optional" to "definitely recommended"), the drug was not widely accepted by physicians.^{122,123 216,217} Many physicians remained unconvinced by the data arguing that the data were few and were based on a

highly selected population; and, the treatment could only be given effectively by the experts.^{132-134,218}

Then there was a range of controversies about trial and AHA recommendations. In an article published in BMJ, in March 2002, Jeanne Lenzer, a medical investigative journalist, questioned the process leading to AHA recommendations for t-PA use in acute ischaemic stroke as it was based on just one single randomised trial.¹³⁴ In that BMJ paper, the following issues were raised¹³⁴: It was argued that the NINDS trial could have been positive merely by chance.^{218,219,134} Questions were raised around imbalances in baseline severity data of those patients that were treated in the 91-180 minutes time window of the NINDS trial.¹³⁴ So far, NINDS was the only positive study that had shown benefit while various others had failed to show benefit; and, had instead demonstrated excess mortality from use of thrombolytic agent.^{134,218-222} Based on chart reviews of consecutive patients examined in emergency departments (between 1990 and 1992), it was found that 19% of the patients were stroke mimics. Hence there was a logical worry that a physician might offer a t-PA therapy, which is associated with excess risk of haemorrhagic complications, to a patient who may not have a stroke.²²³ Further, the public health impact from the use of t-PA was predicted to be minimal.^{134,224} Emergency physicians favoured a more restrictive licensing than the one endorsed by the American Heart Association.^{132,221} Standard Treatment with Alteplase to Reverse Study (STARS) had indicated effectiveness of t-PA. But, there is a question as to whether a study can be considered to be an “effectiveness” study if the participating centres had already had experience of working as the sites for the ATLANTIS trial.¹³⁴ The ATLANTIS trial had received sponsorship of Genentech, a pharmaceutical company that manufactures alteplase.¹³⁴ Further, it was worrisome to see the reports from Cleveland study that had shown poor outcomes in patients treated with t-PA.¹³⁴ The American Heart Association had claimed that t-PA saved lives, but the data did not show reduction in mortality.¹³⁴ Lenzer highlighted that AHA had received massive funding from Genentech implying a strong conflict of interest.¹³⁴ In summary, there were some controversies about the manner in which the t-PA guidelines were framed or NINDS trial findings were promoted.¹³⁴

The European Medicines Agency did not license t-PA in European Union for several years, and when it did, it required the sponsors to show efficacy and safety by conducting what were later to be known as the SITS-MOST observational study and the ECASS III trial.²³ It was only after several observational studies (see section 1.9) and importantly the SITS-MOST and a positive phase III ECASS III trial (see section 1.7), (confirming effectiveness of alteplase until 4.5 hours of symptoms onset) that the scepticism around the use of alteplase got considerably reduced. But the body of randomised clinical data continues to remain small.^{92,131}

It is expected that a larger body of trials data would influence clinical practice.²²⁵ In the case of cardiology, Ketley and Woods undertook to examine the temporal profile of the manner in which the thrombolytic therapy for acute myocardial infarction was adopted in the Trent Regional Health Authority Area after the thrombolysis trials got published.²²⁵ They based their analyses on the supply of thrombolytic drugs from all sources (district pharmacy services or trial organisations) for the period of 1987-1992.²²⁵ The results of the study are shown in figure 1-3. The study showed that the supply was negligible for the first two years after the GISSI study but gradually increased after ISIS-2 and reached an approximate plateau at around 1991-1992.²²⁵

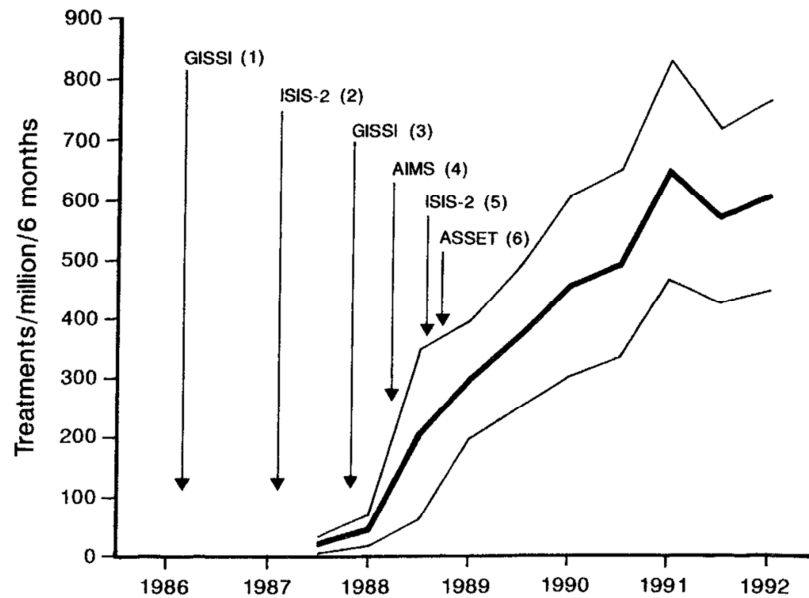


Figure 1-4 Figure shows the trend in the thrombolytic use over time (1987-1992) for patients suffering acute myocardial infarction in the Trent Regional Health Authority area of Central England. The central thick line shows the mean values, the other two lines show the standard deviation.

[Source: Ketley D, Woods KL. Impact of clinical trials on clinical practice: example of thrombolysis for acute myocardial infarction. *Lancet* 1993; 342:891-4. Permissions obtained from Elsevier Limited, The Boulevard, Langford Lane Kidlington, Oxford, OX5 1GB, UK; License number 2783780816358]²²⁵

Ketley and Wood's study shows that there was a lag phase after which thrombolytic therapy began to pick up its pace of usage in the community and then reached a plateau between 1991-1992.²²⁵

But, it has not always happened that the trial findings influence clinical practice (for example, despite a showing that diethylstilboestrol did not benefit, pregnant women continued to use it during the 1950s).^{226,227}

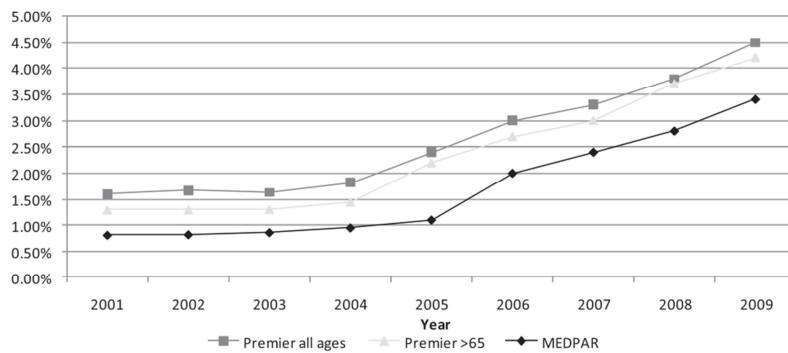


Figure 1-5 t-PA usage in the United States of America

(Source: Adeoye O, Hornung R, Khatri P, Kleindorfer D. Recombinant tissue-type plasminogen activator use for ischaemic stroke in the United States: a doubling of treatment rates over the course of 5 years. *Stroke* 2011; 42:1952-5. Permissions obtained from Wolters Kluwer Health 2783780982628)²²⁸

In case of thrombolysis trials for stroke, the manner in which their data influenced stroke practices in the past (prior to ECASS III) may only be speculated upon. Unlike cardiology, there were fewer data of stroke patients having better outcomes after receiving thrombolytic therapy. There was an original scepticism about the efficacy of t-PA. These factors may have contributed to initial poor usage of thrombolytic therapy. Another reason would have been that the stroke care is resource intensive and requires the setting up of a stroke unit, imaging facilities and access to an experienced clinical staff. These things take a while to get organised in hospitals. Adeoye et al report that, in USA, there has been a significant increase in the t-PA usage between the years 2005 and 2009 (1.1% to 3.4%, $p < 0.001$ for trend). The rates were stagnant between 2001 and 2005 (figure 1-4).²²⁸ The following reasons were cited for the increased rates of usage after 2005: financial incentive given to the hospital, Joint Commission certification of primary stroke centres, initiatives to standardise acute stroke care, and the aggressive Get With The Guidelines (GWTG) campaign.²²⁸

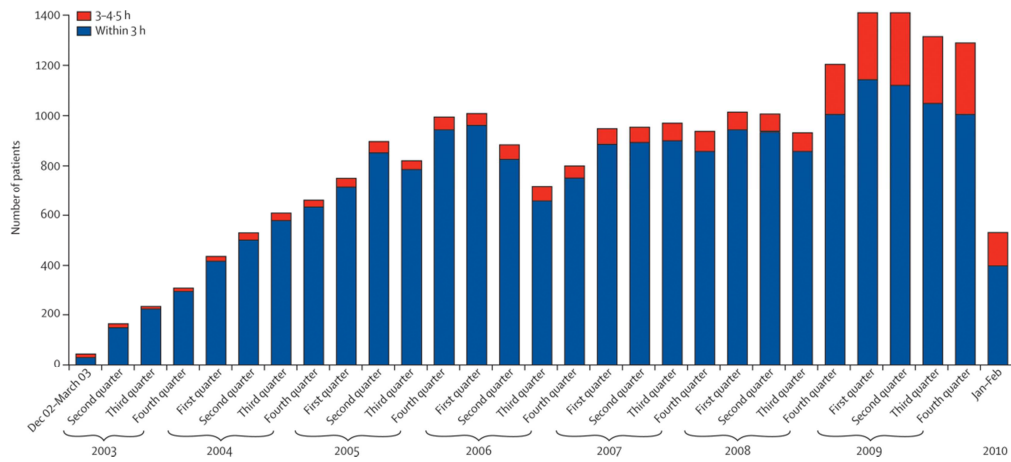


Figure 1-6 Figure shows number of patients recorded in SITS registry in each quarter

Source: Ahmed N, Wahlgren N, Grond M, et al. Implementation and outcome of thrombolysis with alteplase 3-4.5 h after an acute stroke: an updated analysis from SITSISTR. *Lancet Neurol* 2010; 9:866-74; Permissions obtained from Elsevier Limited The Boulevard, Langford Lane, Kidlington, Oxford, and OX5 1GB, UK, License number: 2783781166304.²²⁹

After the NINDS trial, it was only the ECASS III trial that was positive and has shown better outcomes amongst stroke patients (in 3-4.5 hours' time window).¹²⁹ So, did it influence the clinical stroke practice? SITS-ISTR registry recorded an "immediate and lasting" effect of ECASS III publication with no change in the presentation-to-hospital to initiation-of-treatment time.²²⁹ Similarly, a Swedish Stroke Registry has reported that after the publication of ECASS III trial, rates of t-PA utilisation increased from 0.5% to 2.1% (between 2008-2010 in patients treated within 3-4.5 hours' time window).²³⁰ The rates in 0-3 hours had increased from 0.9% in 2003 to 6.6% in late 2008 and then levelled at a thrombolysis rate of about 6%.²³⁰ There was no difference between the median time of arrival-to-hospital and the start-of-treatment ($p=0.06$).²³⁰ Here, the 0-3 hour time window thrombolysis program had developed over the years, but, the 3-4.5 hours t-PA use could be initiated soon after the ECASS III results went public. This was only possible because these centres had already developed the necessary infrastructure and the experienced physicians. Increased usage of t-PA after ECASS III trials suggests that the ECASS III trial had an impact on the clinical practice.²³⁰

Further, the Swedish noted a plateau effect for the use of alteplase. It was attributed to the fact that these hospitals had reached a saturation level for the rate of thrombolysis they could achieve within their jurisdiction.²³⁰ It may be speculated that the t-PA use in 3-4.5 hour time window might also level-off in a similar fashion when the hospitals would reach saturation levels for the use of t-PA. So, for the next rise in t-PA usage to commence, one would need additional high quality clinical evidence for the treatment of some more additional patients that at present do not get treated with t-PA. This is possible because, some of the Swedish centres that practice off label use of t-PA report a higher rates of thrombolysis ($\approx 10-15\%$).²³⁰

So it can be thought that in order for change in current clinical practice to occur and to treat more patients, we should have more randomised controlled clinical data. Only randomised controlled trials can provide the best quality of clinical evidence and assuage the fears of poor outcomes in stroke patients. Data on subgroups that are currently excluded would allow physicians to better select patients. But, conducting such a trial would be a difficult task because

physicians do not have uniform opinion regarding the effectiveness of t-PA therapy in the several patients' subgroups (e.g. physicians differ in their opinion regarding treatment of mild ischaemic strokes or the elderly patients that suffer an ischaemic stroke) or for treatment approach, (like, intravenous vs. intra-arterial); and, therefore there is a lack of equipoise. Alteplase is already an approved treatment for acute ischaemic stroke in most countries. Some centres employ practices in which they are extremely good (e.g. intra-arterial thrombolysis). Hence, participation in randomised controlled trials may be difficult for these physicians.²³¹ Also, the enrolment of patients in t-PA trials is a slow process that takes a while to reach the target sample size. About a decade ago, IST-3 trialists set out with a target of 6000 patients to be randomised within 6 hours of symptoms onset.²³² So far, they have only been able to enrol about 3000 patients.^{233,234} Recruitment would also be challenging because patients meant for exclusion constitute only a small fraction of those that get treated (e.g. in SITS-ISTR, 2.6% patients have concomitant diabetes and previous stroke, n/N=602/23062).

1.14 Why aren't many eligible patients treated with t-PA in the routine clinical practice?

In routine clinical practice, there are other variables that influence patients' selection: delay in arrival at a stroke treatment facility within the time window, lack of awareness (including amongst some physicians²³⁵)¹⁸, delay in admission to a stroke treatment facility and lack of local stroke care facility/expertise.²³⁶ Many physicians fear excessively about the bleeding complications and therefore they do not thrombolysed those patients that belong to any of the exclusion mentioned in the list of the t-PA exclusion criteria.^{236,237} Some expert centres practise off-label use of t-PA based on a physician's clinical judgement; but fear of litigations or causing harm (due to valid reasons that had originally influenced the drug authorities to considering patients for exclusion) prevent many others from using t-PA in scenarios that do not find clear mention in treatment protocol.^{203,204}

In those countries where the costs of health care are borne by the patients and relatives, t-PA may not be used, because it is an expensive therapy.²³⁸ The loss of disability adjusted life years incurred by underuse of thrombolytic therapy carries a large economic and societal cost. Hence, national governments need to initiate programs to treat acute stroke patients.^{18,20}

There is wide variation in the usage of t-PA for ischaemic stroke between centres. For example, an analysis of Healthcare Benchmarking Systems International (HBSI) EXPLORE database showed that 35% of the community hospitals (profit and non-profit hospitals throughout USA) did not use t-PA at all; and the variables that significantly influenced the use of t-PA in these hospitals were race, age and disease severity (N=137 centres, 23058 patients).²³⁹

There is an intra and inter country variability in the use of t-PA as well (figure 1-6, for UK contribution to SITS-Centres).^{240,241} The variation between centres could reflect the limited evidence that is available for the use of t-PA in various subgroups. Physicians are also concerned about the patients' safety and therefore might not treat those patients for whom, based on their clinical judgement, the potential for harm outweighs the possible benefit.



Figure 1-7 Figure shows UK SITS centres with size of circle denoting the number of patients recruited within the SITS registry from each of these centres

Source: Lees KR, Ford GA, Muir KW, et al. Thrombolytic therapy for acute stroke in the United Kingdom: experience from the safe implementation of thrombolysis in stroke (SITS) register. *QJM* 2008;101:863-9; Permissions obtained from Oxford University Press, License Number: 2783781419775²⁴⁰

Thrombolysis is a standard practice for the treatment of patients suffering myocardial infarction, but the acceptance rate is lower amongst physicians caring for acute stroke: only about 5% of the patients receive thrombolytic therapy.²²⁸ Alteplase is associated with absolute benefit of 10-15%, but because it is infrequently used, it hasn't had the kind of public health impact it is expected to have. There is a need to enhance community awareness about the need for emergent stroke care and encourage participation of community hospitals in providing stroke care.

Racial differences are known to influence stroke outcomes, and inter-ethnic differences in risk factor profiles are well described.²⁴²⁻²⁴⁴ The Japanese drug agency approved a lower dose of 0.6mg/kg in lieu of 0.9 mg/kg for the treatment of acute ischaemic stroke.²⁴⁵⁻²⁴⁹²⁵¹ The Chinese examined outcomes in patients treated with a lower dose of 0.6mg/kg (n=116) and standard 0.9mg/kg (n=125) and observed that there occurred excess symptomatic haemorrhages in the standard group (rates were, per NINDS, 10.4% vs. 5.2%; per ECASS definition, 8.0% vs. 2.6%; SITS-MOST definition, 5.6% versus 1.7%, respectively) and mortality at 90 days (12.8% versus 6.9%) in the patients that received the standard 0.9mg/kg dose.²⁵⁰ These differences may be attributed to the ethnic variability in the rate of fibrinolysis.²⁵¹⁻²⁵³ It is likely that certain ethnic groups respond differently to the use of t-PA; and, therefore these physicians might be cautious when extrapolating thrombolysis data on their population.

1.15 Present Thesis

When the NINDS trial proved the efficacy of alteplase, the US FDA promptly approved its use within its jurisdiction.¹²³ However, EMEA was not immediately convinced and argued whether findings based on a non-European trial that studied only over a thousand patients could be extrapolated onto the European population.²³ Proponents of alteplase argued that though European trials (ECASS I, II) had not shown favourable outcomes similar to the NINDS trial, failure could be attributed to differences in trial design.²³ An ad hoc expert group had also failed to show a definitive and a big treatment effect from the use of alteplase in elderly stroke patients, patients having diabetes or patients having a severe

stroke.²³ The expert group suggested that a randomised controlled trial in this subgroup of the patient population would be required to confirm the efficacy of use of the alteplase.²³ The Committee for Proprietary Medicinal Product (CPMP) felt the need to undertake a randomised dose comparator study for time window 0-3 hours, but conducting such a study was not considered feasible owing to ethical concerns.²³ Several centres had already incorporated alteplase in their local clinical practice.²³ Hence, it was proposed to study efficacy of alteplase in a 3-4 hour time window in a randomised controlled trial design and by undertaking a safety study of observational data for patients treated within the 3 hour time window.²³ While the ECASS III trial showed efficacy and safety of alteplase in acute ischaemic stroke patients when administered between 3 and 4.5 hours after the symptoms onset, the SITS-MOST study confirmed that i.v. alteplase is safe and effective in routine clinical use when used within 3 h of stroke onset.^{129,146}

When formulating CPMP approval, the EMEA recommended few exclusion criteria that were incorporated in ECASS III study.²³ After outcomes were shown to be better for the patients treated in 3-4.5 hour time window in the ECASS III trials, uncertainties continue to persist for those that were excluded from trials.²³ Now that alteplase is proven to be effective in ischaemic stroke, it is desirable to investigate if other exclusions are justified and if treatment could be extended to other patients that are included in current EMEA list of exclusions.²³

In the present thesis I have tackled following questions:

1. Should elderly patients receive alteplase for their acute ischaemic stroke? [Chapter 3 and Chapter 4]
2. Should patients having a concomitant diabetes and history of previous stroke receive alteplase therapy? [Chapter 5 and Chapter 6]
3. Should patients having mild or severe stroke receive alteplase for acute ischaemic stroke?[Chapter 7]
4. Should thrombolytic therapy be administered to stroke patients based on mismatch criteria and beyond 3 hours of symptoms onset? [Chapter 8]

Chapter 2

Methodology

2 Methodology

Here I describe the methodology that I have used with its merits and limitations.

2.1 Introduction

Clinical Investigation refers to the examination of clinical data for the association of intervention(s) with the outcomes in a study population. Expert opinions based on anecdotal evidence are likely to result in flawed conclusions.²⁵⁴ Clinical trials are the best means of investigating treatment interventions.²⁵⁴

I selected data from the Safe Implementation of Thrombolysis in Stroke-International Stroke Thrombolysis Register (SITS-ISTR) and Virtual International Stroke Trials Archive (VISTA) for this thesis for reasons of time and practicality and undertook to examine outcomes in patients' subgroups that were recommended for exclusion by the European drug authorities.^{147,255} I hoped that if my findings were consistent between the two datasets, I would have given a useful piece of evidence to support (or reject) thrombolytic therapy for those patients that are currently recommended for exclusions. These findings could be an important source of information for physicians until further confirmatory randomised data are available.

For SITS- and VISTA- analyses, blinding and randomisation techniques could not be employed. These data were already collected per the protocol of SITS group or VISTA trialists. I undertook a controlled comparison using controls from VISTA dataset. VISTA controls were compared with patients that received alteplase in VISTA trials or in SITS-ISTR registry.^{147,255}

2.2 Data Source

VISTA is a collaborative, not-for-profit, register of stroke trials.²⁵⁵ The treatments studied in these trials range from putative neuro-protectants through anticoagulants and thrombolytic agents to simple rehabilitation measures. All trials in VISTA hold necessary review board and regulatory approvals, and patients consented to participation; only anonymised data are held by VISTA and the trial source is not disclosed as per VISTA guidelines. All stroke patients were treated as per institutional practice and stroke guidelines acceptable at the point of trial conduct. Monitoring for protocol compliance was undertaken on behalf of sponsors for these trials. This implies that where thrombolysis was administered, this was in accordance with marketing authorisation for the relevant country, i.e. that treatment commenced within 3 hours of stroke onset; however, the onset to treatment delay is not recorded for thrombolysis in these trials. Further, note that the reason for withholding thrombolysis in each patient was not recorded in VISTA, but will include absence of marketing approval in the region at that time, clinical uncertainty over the use of thrombolysis for stroke generally, absence of treatment facilities for thrombolysis in the hospital at that time, and contraindications to thrombolysis for the individual patient. These data were derived mainly from Northern American (60%), European (16%) and Australasian (13%) centres.²⁵⁵

For my research, VISTA data were obtained from neuroprotection trials that were conducted between 1998 and 2007. These trials hold necessary review board and regulatory approvals. The patients had consented to participation. VISTA holds only anonymised data. These data were rigorously collected by the trialists. Some of the patients in the studies received an investigational neuroprotective agent. One or more of these neuroprotective agents could have interacted with thrombolysis; however each contributing trial has already tested for and excluded significant interaction. VISTA data handling procedures preclude further testing for effects of the original neuroprotection agent or identification of source trials. Hence, I cannot identify the trials that contributed to VISTA dataset, nor did I ever have access to these: I was given access to an anonymised dataset of 9665 patients from VISTA.

SITS-ISTR is an ongoing internet-based, academic-driven, interactive thrombolysis register registry (www.sitsinternational.org), held at Karolinska

University Hospital, Stockholm. The methodology of the SITS-ISTR including the procedure for data collection and management, patient identification and verification of source data has been described in their early publications. It is a prospective, open, multinational, observational monitoring registry for clinical centres using thrombolysis and other interventions for the treatment of acute ischaemic stroke. The registry is open to all countries, and collects data on patients who receive thrombolytic therapy for acute ischaemic stroke.^{147,256}

I was given SITS-ISTR data after their extraction from the SITS-ISTR and on my arrival at the Department of Neurology, Karolinska University Hospital. For the present thesis, data were extracted for a period December 2002 to November 2009. These data were then combined with a control group derived from untreated stroke patients (untreated for t-PA) within VISTA neuroprotection trials conducted between 1998 to 2007 and held within the Virtual International Stroke Trials Archive, VISTA (www.vista.gla.ac.uk). Referred to as “SITS-VISTA”, this combined dataset is employed for analyses in chapter 4 and 6.

I show the details of data extraction in the figure 2.1. to indicate completeness of data that were available for present analyses. Further, information on missing data for various co-variates can be inferred from the baseline characteristics that I report in each chapter.

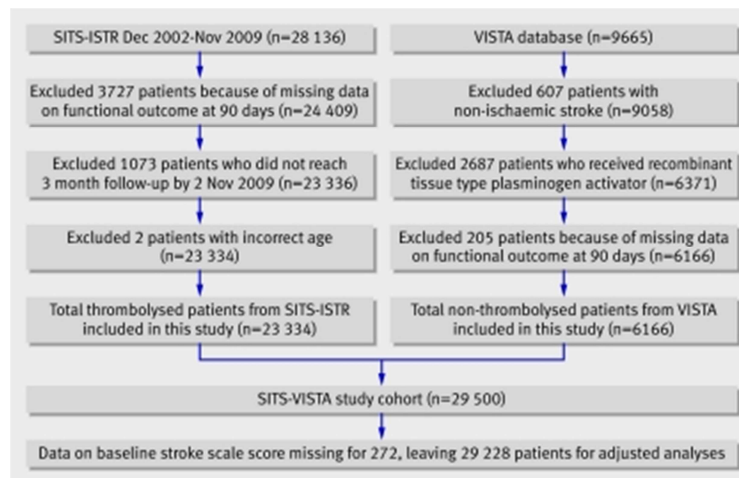
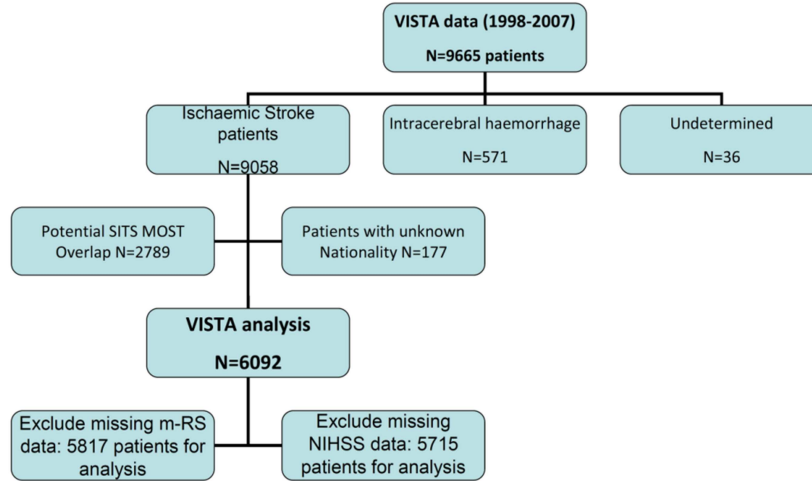


Figure 2-1 Flow chart showing the details of data extraction for VISTA (above) and SITS-ISTR (below) which are employed for analyses in the present thesis ²

² I published these figures in two papers: Mishra NK, Diener HC, Lyden PD, Bluhmki E, Lees KR; VISTA Collaborators. Influence of age on outcome from thrombolysis in acute stroke: a controlled comparison in patients from the Virtual International Stroke Trials Archive (VISTA). *Stroke*. 2010 Dec;41(12):2840-8. and Mishra NK, Ahmed N, Andersen G, Egido JA, Lindsberg PJ, Ringleb PA, Wahlgren NG, Lees KR; VISTA collaborators; SITS collaborators. Thrombolysis in very elderly people: controlled comparison of SITS International Stroke Thrombolysis Registry and Virtual International Stroke Trials Archive. *BMJ*. 2010 Nov 23;341:c6046. doi: 10.1136/bmj.c6046 respectively; Permissions were obtained from Wolters Kluwer Health (License Number- 2790451375765) and BMJ Publishing Group Limited (License Number- 2783790122184) respectively.

When reporting findings from the analyses, I describe the baseline characteristics of the data and also examine them for completeness of covariates (Figure 2.1).

Data on 9665 patients were collated from VISTA. Of these, 5342 (59%) were enrolled from non-European sites. To avoid dual publication with SITS-MOST, I excluded 2789 patients (28%) enrolled from European sites between 2002 and 2006, and 177 patients for whom the data lacked information on nationality.¹⁴⁶

2.3 Ethics Approval

Because clinical studies ought to be based on current ethical standards, and because there is a range of procedures that ought to be followed in order to obtain consent, it was necessary to clarify these requirements from VISTA and similarly later from SITS-ISTR. Ethical considerations are an important component of clinical investigations. In VISTA, participating patients had already consented to their participation in VISTA trials, and therefore it was deemed unnecessary to seek additional consents from the patients for the projects that had been proposed for the present thesis; it would also have been impractical since most by now would have died, even if they could somehow be identified.

In order to obtain an unbiased and blinded feedback from other experts, a study proposal was circulated between the VISTA steering committee members and similarly to the SITS-ISTR steering committee members. SITS Steering Committee members comprise members from all across Europe and the VISTA from all over the world. (See appendix)

2.4 Outcome Measures

Outcomes measured in randomised trials should be valid, reliable, robust and responsive. Common outcome measures that were employed in stroke research include the modified Rankin Scale, National Institute of Health Stroke Scales, Barthel Index and Scandinavian Stroke Scale.²⁵⁷

The Modified Rankin Scale (m-RS) was originally not meant for use in trials, but now, it is a commonly used measure of outcome assessment employed in Stroke Trials.²⁵⁷ It is an ordinal scale that scores patients for their functional status on a scale of 0 to 6 in which zero refers to the absence of any disability while a score of six means that the patient is dead. Rankin scores are associated with substantial inter-observer variability that is most apparent for the Rankin scores 1 to 4.²⁵⁸ Whereas one may find it easier to score a patient 0, 1, 5 or 6, it is often difficult to distinguish a 2 from 3 or 3 from 4 on this scale. An examination of a hundred paired assessment suggested that kappa statistics for inter-observer variability were lower than those for intra-observer variability (0.57 vs. 0.72).²⁵⁸ There was no significant difference observed between a structured interview method and a standard m-RS method.²⁵⁸ But, two previous studies have also shown that weighted kappa statistics for agreement between raters of m-RS scores were greater when a structured interview was used instead of the conventional method (k 0.91 vs 0.71 and 0.93 vs 0.78)^{259,260} In order to reduce bias arising from inter-observer variability, measures like structured interviews or video training are employed.

Scores on a modified Rankin scale²⁶¹

- 0=no symptoms from stroke
- 1=no severe disability, despite symptoms
- 2=slight disability in which patients are unable to do all previous activities but able to look after themselves without help
- 3=moderate disability that requires some help, but patients can walk by themselves
- 4=moderately severe disability in which patients are unable to walk without assistance and need help for bodily needs
- 5=bedbound patients who are incontinent or require personal attention
- 6=death

The National Institute of Health Stroke Scale (NIHSS) is a scale used to measure the neurological outcomes in stroke trials.²⁶² Its use is associated with increased power.²⁶³ Baseline stroke severity measured on NIHSS is an excellent predictor of outcomes.²⁶⁴ Age and baseline stroke severity account for maximal variance in stroke outcomes; which is why these are employed as covariates for adjusted analyses when comparing outcomes from use of t-PA in present analyses.²⁶⁵ A

patient having a left sided brain lesion scores higher on NIHSS compared to a similar right sided brain lesion.²⁶⁶ By combining the NIHSS scale with neuropsychological tests like line bisection tests and visual perception tasks, one can augment its sensitivity for right brain lesions as well.²⁶⁷

Item	Name	Response
1A	Level of consciousness	0=Alert 2=Not alert, obtunded 3=Unresponsive
1B	Questions	0=Answers both correctly 1=Answers one correctly 2=Answers neither correctly
1C	Commands	0=Performs both tasks correctly 1=Performs one task correctly 2=Performs neither task
2	Gaze	0=Normal 1=Partial gaze palsy 2=Total gaze palsy
3	Visual fields	0=No visual loss 1=Partial hemianopsia 2=Complete hemianopsia 3=Bilateral hemianopsia
4	Facial palsy	0=Normal 1=Minor paralysis 2=Partial paralysis 3=Complete paralysis
5	Motor arm	0=No drift
	a. Left	1=Drift before 10 seconds
	b. Right	2=Falls before 10 seconds 3=No effort against gravity 4=No movement
6	Motor leg	0=No drift
	a. Left	1=Drift before 5 seconds
	b. Right	2=Falls before 5 seconds 3=No effort against gravity 4=No movement
7	Ataxia	0=Absent 1=One limb 2=Two limbs
8	Sensory	0=Normal 1=Mild loss 2=Severe loss
9	Language	0=Normal 1=Mild aphasia 2=Severe aphasia 3=Mute or global aphasia
10	Dysarthria	0=Normal 1=Mild 2=Severe
11	Extinction/inattention	0=Normal 1=Mild 2=Severe

Table 2-1 Modified National Institutes of Health Stroke Scale

Source: Lyden P, Brott T, Tilley B, Welch KM, Mascha EJ, Levine S, Haley EC, Grotta J, Marler J. Improved reliability of the NIH Stroke Scale using video training. NINDS TPA Stroke Study Group. Stroke. 1994 Nov;25(11):2220-6. Permissions obtained from Wolter Kluwer Health, License Number: 2813100962854

For analyses presented here, from VISTA, I had access to two measures of outcome: modified Rankin scale and NIH stroke scale both measured on day 90 of the stroke occurrence. From SITS-ISTR, I had access to the baseline demographics and only m-RS score on day 90 of stroke as a measure of outcomes.

2.5 Errors and Bias

Before beginning to collect data, it is desirable to consider the measures that can reduce errors or bias in a study.

Errors are of two types: *random* errors and *systematic* errors. Random sampling error refers to chance variation that is observed between one study population and another. Bias refers to the deviation from truth of inferred results. *It* is a form of *systematic error* that is ubiquitous, results in erroneous estimates of association, and cannot be completely removed. Methods to prevent it should be thought out early during the design and execution phase of the study. When attempting to reduce bias, an investigator may introduce another bias compromising generalisability of the results. A good understanding of bias permits better interpretation of the results in a more meaningful way. Bias occurs because of various reasons. For example, selection bias (sampling bias) occurs when a sample does not represent the population under study. For instance, predicting the rates of cardiac disease in athletes would introduce selection bias because athletes are less likely to suffer cardiac illness. Berkson's bias refers to a bias that is introduced from the hospital stay. Measurement bias occurs when information gets distorted due to the unique characteristics of methodology by which the data were collected. These include instrument bias or insensitive measure bias. The Hawthorne effect refers to situations when attention is paid to the subjects under study that alters their behaviour and thus the outcomes. Intervention Bias results from abnormal or distorted interventions and leads to bias like contamination bias where the control group mistakenly receives treatment, co-intervention bias when some subjects receive treatment and an additional intervention, timing bias(es) when treatment is

provided for a longer duration, compliance bias when there are differences in the compliances of the patients enrolled in the study arms, withdrawal bias because some subjects withdrew from the study, proficiency bias that occurred when the interventions are not equally applied to the various subjects owing to differences in the proficiencies of the investigator and/or the differences in the resources or procedures employed at different centres.

Hypothetically, by increasing the size of data, it is possible to reduce random sampling error and bring it close to zero. On the other hand, *bias* has no relationship to population size of study. In order to reduce bias, one can take the following measures: incorporate a placebo arm; undertake a blinded study; consider a cross over study design; apply good randomisation techniques; train the investigators about various stages of the study (e.g. ECASS II study, in which the investigators were trained in reading CT scans). Despite measures to reduce bias, some bias may persist in a study; and, an exact degree of bias is never precisely known. An investigator may want to consider a trade-off between bias and variance, and logically, accept the measurement with the lowest mean square of standard error.²⁶⁸

For my thesis, I undertook analysis of data that were already lodged in VISTA and SITS-ISTR. I could only undertake a controlled comparison employing robust statistical techniques. It was not possible to employ principles of blinding or randomisation procedures in these datasets.

2.6 Data Analyses

The Global statistics method, responder analysis method and shift analysis method are often used to analyse data in stroke trials.^{261,269-274} In addition, simple analyses of dichotomised outcomes measures are also often used. In the global statistics method, one examines outcomes employing a combination of multiple outcomes scales.^{261,270} Responder analysis permits one to consider the prior disability when examining the outcomes.²⁶¹ Shift analysis has the merit in that it allows the investigator to capture signal indicating shift towards improved

(or poor) outcomes.²⁶¹ In the case of stroke, this is a meaningful method of analysing data because the therapy does not always cure the patients, but shifts them in one or the other direction of the outcome spectrum.^{261,271} Analysis of artificially dichotomised outcomes scales is a simple method but is associated with loss of information, confusing interpretation of results, an artificial creation of patients' groups that are considered good or poor and loss of study power.^{261,272,273} For example, Jeffrey Saver discusses that *“When the 7-level mRS is dichotomised as 0 to 2 vs. 3 to 6, a traditional breakpoint in binary trial analysis, the resulting analysis examines only one important transition in health state, from vocationally impaired, but able to live independently, to requiring assistance in daily living. However, this analysis places absolutely no value on other health state transitions that are pertinent to patients. For example, going from vocationally impaired (mRS=2) to no symptoms at all (mRS=0) is not counted as a clinically meaningful improvement, nor is going from dead (mRS=6) to moderately disabled and able to walk on one’s own (mRS=3). Binary outcome analyses prioritise only a single health state transition as clinically worthwhile, whereas patients naturally place great value on several health state transitions.”*²⁶¹ Nevertheless, dichotomisation has its advantages: analyses of ordinal data by dichotomisation of outcomes are simple and proportions so obtained can be easily converted to clinically intuitive values such as “number needed to treat”. Hence, the results so obtained are easy to interpret.²⁶¹ However, unlike dichotomisation where one examines a few arbitrarily selected health states, shift analyses examine all health states.^{261,269} For the present thesis, I decided to undertake “shift analyses” by employing ordinal analyses using proportional odds logistic regression analyses and a non-parametric Cochran-Mantel-Haenszel (CMH) test. In addition to this, I also analysed outcomes by dichotomising them.

CMH is a non-parametric approach that avoids invoking an assumption of proportional odds in which the odds ratios are common across all cut points of an ordinal outcome scale. Koch and Edwards described the relationship between the van Elteren test²⁷⁵ and the extended Mantel-Haenszel²⁷⁶ procedure.²⁷⁷ In SAS, the CMH test is done by employing proc freq procedure (see appendix). It is a powerful procedure available to SAS users. “cmh” in the code tells SAS software to run the extended Mantel Haenszel test (referred to as CMH in SAS software).

Modified ridits (used to calculate within-stratum standardised midrank scores) are employed to undertake transformations that are necessary to run the right calculations.

Mantel Haenszel methods can be employed in situations of $I \times J$ tables, where $I > 2$ and/or $J > 2$, that is, when in a case control study, the exposure levels and/or the outcome levels are more than 2.

In the present analyses, there were two possible exposures with respect to use of the use of alteplase: thrombolysis or no-thrombolysis (controls). Outcomes had 6 levels in case of m-RS outcomes and 9 levels in case of NIHSS categorised outcomes. The CMH test tested for an association of “use of t-PA” (C) and “outcomes” (D) by controlling by stratification for covariates (A and B, see an example code in the appendix) that were introduced in the model.²⁷⁵⁻²⁷⁷

The CMH test gives 3 different sets of correlation statistics: *Non-zero correlation*, *Row Means Scores Differ* and *general association*. If the p-value for *Non-zero correlation* is significant, results are interpreted as linear correlation between two ordinal variables for at least one stratum. If the p value for *Row Means Scores Differ (RMSD)* is significant, the interpretation is, for an ordinal column variable the mean CMH scores differ across the columns for at least one stratum. If the p-value for *general association* is significant, there is an association between the two variables for at least one stratum. The RMSD statistic when significant suggests that an effect across the m-RS spectrum is significant. RMSD is used for interpretation when the data are arranged such that only the columns have an order (in case of analyses presented in this thesis, the Rankin Scores or the NIHSS scores, that have an order).

In order to undertake adjusted analyses, covariates were introduced into the CMH test. As mentioned earlier, adjusted analysis involving the CMH test occurs by means of stratification of outcome data of thrombolysed and non thrombolysed patients for various covariates. Therefore, this is limited by the sample size and can preclude simultaneous adjustment for several variables. Hence, I prospectively planned to adjust for age and baseline NIHSS and to consider other variables only in exploratory analyses. The choice of baseline

factors for adjustment was based on two influences.²⁷⁸ First, age and baseline severity are the two most powerful prognostic factors for stroke and are usually included in outcome distribution analyses. Baseline NIHSS accounts for about 80% of the variance in outcome data of stroke patients.^{265,279,280} Second, age and NIHSS data were available for our entire sample, whereas some of the other factors of potential interest were incomplete. However, I also undertook a sensitivity analysis by considering the combined effect of the variables that differed significantly at baseline.

Because the CMH test does not measure the extent of association, I also undertook a proportional odds logistic regression analysis.

Ordinal Regression Analysis (Proportional Odds Logistic Regression Analysis) is employed when the distribution of scores is not normal and the majority of respondents scores are at extremes of the scale.^{281,282} SAS software allows an analysis that fits a proportional odds model based on ranked scores and computes common odds at each cut-off level of ordinal scores. The proportional odds assumption is tested based on a score test which if significant, indicates that the proportionality assumption does not hold. The score test for proportionality assumption tends to be extremely sensitive for larger data.^{281,282}

Analyses involved shift in outcomes (Rankin scores and NIHSS scores in case of VISTA only analysis and Rankin scores in case of SITS-VISTA (NIHSS data were unavailable in SITS-ISTR)) when patients received alteplase compared to those who did not receive alteplase. Choice of NIHSS as an additional measure of outcomes is supported by the European Medicines Evaluation Agency (EMA) Points to Consider for reporting trials.^{283,284} As per this document, the EMA allows the use of the full range of the Rankin scores and then further suggests that this can be supported by a secondary analysis of another outcome measure such as NIHSS.²⁸⁴ I analysed supporting endpoint, neurological outcomes measured by NIHSS scores, by grouping the NIHSS scores into categories: 0 (no measurable deficit), 1-4, 5-8, 9-12, 13-16, 17-20, 21-24, ≥ 25 (most severe neurological deficit) or dead. I then compared the distribution of patients across the NIHSS categories in a manner similar to one done for the mRS. Here again, to test for a significant association of outcome distribution with intervention/drug

exposure, I employed the Cochran-Mantel-Haenszel [CMH] test and calculated the odds ratio based on proportional odds logistic regression analyses. The ordinal outcomes can be compared, adjusting for covariates.

Chapter 8 of this thesis examines data by means of meta-analyses. For detailed methodology refer to chapter 8.

Chapter 3

Influence of age on outcome from thrombolysis in acute stroke

3 Influence of age on outcome from thrombolysis in acute stroke

3.1 Introduction

Thrombolysis for acute ischaemic stroke has proven benefits but randomised trial data in patients >80 years are limited.^{92,122} To date, the European Medicines Evaluation Agency (EMA) has not approved thrombolysis with alteplase amongst the very elderly.²³ Patients older than 80 years represent about 30% of acute stroke incidence.²⁸⁵⁻²⁸⁷ Many experienced centres treat the elderly but others observe the terms of product approval.^{240 237,288-292}

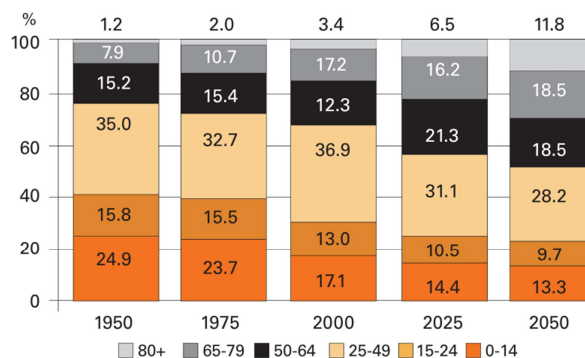


Figure 3-1 Figure showing rapid increase in the proportions of elderly in European Union

The coloured boxes represent age group with grey showing people older than 80 years, black showing age group 65 to 79 years and different shades of orange showing age group 0-14, 15-24 and 25 to 49. [Source: *Features and Challenges of Population Ageing: The European Perspective* by Asghar Zaidi; Permissions obtained by e mail from Dr Asghar ZAIDI, Director Research European Centre for Social Welfare Policy and Research (Affiliated to the United Nations) Berggasse 17, A-1090 Vienna, Austria]²⁹³

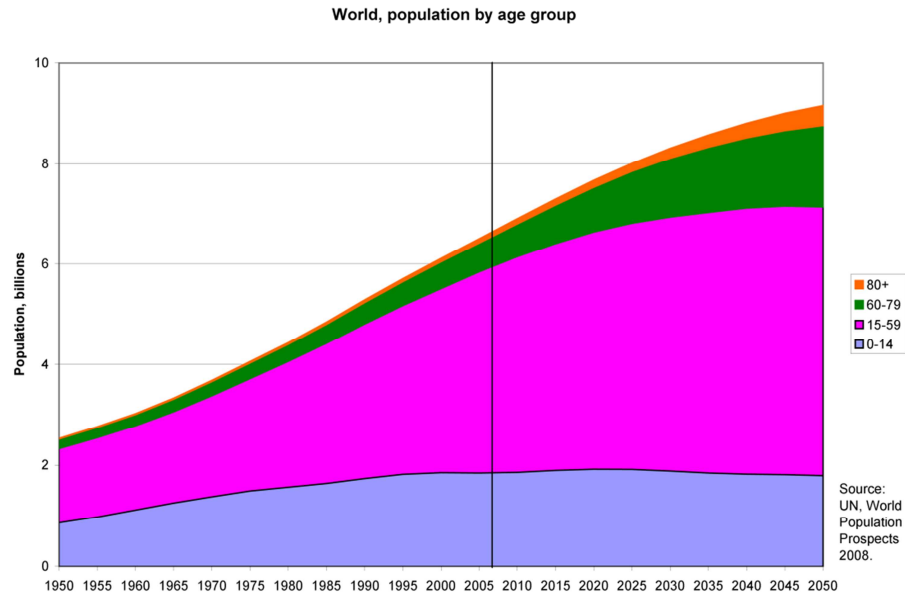


Figure 3-2 Projections for world population for age group 0-14, 15-59, 60-79 and 80 and above.

(Source: Implications of Population Ageing for Economic Growth by David E. Bloom, David Canning, and Günther Fink; *Permissions obtained from Oxford University Press for the publication “David E. Bloom, David Canning, Günther Fink, Oxford Review of Economic Policy content title Implications of population ageing for economic growth”, License Number 2784230886123*).²⁹⁴

The NINDS trial initially restricted enrolment to patients aged up to 80 years. The age criterion was lifted after enrolling 188 patients in part A of the trial but only 42 very elderly patients were enrolled.¹²² All ECASS trials applied an upper

³ I published Chapter 3 in *Stroke* (Mishra NK, Diener HC, Lyden PD, Bluhmki E, Lees KR; VISTA Collaborators. Influence of age on outcome from thrombolysis in acute stroke: a controlled comparison in patients from the Virtual International Stroke Trials Archive (VISTA). *Stroke*. 2010 Dec;41(12):2840-8.) Permissions were obtained from Wolters Kluwer Health (License Number: 2790451375765)

age limit of 80 years, and recent studies with desmoteplase also excluded the elderly.^{109-111,121,124,129}

The main reasons advanced for withholding treatment from the elderly patients in clinical practice are fears that advancing age is associated with poorer prognosis with greater risk for haemorrhage and in-hospital mortality.^{23,295,296} Conversely, a meta-analysis of pooled thrombolysis data concluded that the risks of symptomatic intra-cerebral haemorrhage ICH did not increase amongst the elderly despite less favourable outcomes.²⁹⁷ Less favourable outcomes are expected to occur in the elderly, mostly due to comorbidity.^{237,298}

The proportion of elderly is rising in our society.^{285-287,293,294} In the UK alone, the population aged >80 years has doubled since 1982.²⁹⁹ Effective treatments should not be withheld from the elderly in the absence of compelling data suggesting unacceptable risk or proven lack of benefit. It was therefore hypothesised that clinical practice over the last decade would have been sufficiently diverse to allow analysis of existing rigorously collected clinical data to construct a comparison of thrombolysis against matched controls, with the possibility of adjusting for any imbalance in severity. I anticipated that the use in the elderly would be sufficiently frequent to assess the influence of age on any association of stroke outcome with thrombolysis treatment.

3.2 Methods

3.2.1 Data Source and Patients

Details of data source are provided in Chapter 2. Briefly, I collated data on demographics, clinical data and measures of functional outcome from neuroprotection trials conducted in the period 1998 to 2007, held within the Virtual International Stroke Trials Archive, VISTA [www.vista.gla.ac.uk] ²⁵⁵

3.2.2 Statistical Analyses

I undertook a non-randomised, adjusted comparison of outcomes between patients who received rt-PA and patients who did not receive rt-PA (henceforth referred to as treated and control group respectively) amongst patients who met the age criterion for the European alteplase marketing authorisation. I repeated the comparison amongst patients aged ≥ 81 years. I then examined the association of thrombolysis treatment with outcome within each age decile to illustrate the strength of evidence across the full age range. For each contrast, I compared the overall distribution of all seven categories of day 90 mRS scores of the two groups, i.e. from 0 (asymptomatic) through 5 (bedbound and completely dependent), to 6 (dead). For analysis of the supporting endpoint, NIHSS, I grouped adjacent scores into categories: 0 (no measurable deficit), 1-4, 5-8, 9-12, 13-16, 17-20, 21-24, ≥ 25 (most severe neurological deficit) or dead. The distribution of patients across these categories was then compared between the groups as for mRS. To test for a significant association of outcome distribution with thrombolysis exposure I employed Cochran-Mantel-Haenszel [CMH] statistic, adjusting for both age and baseline NIHSS. Because CMH does not express the extent of the association, I also applied logistic regression analysis, also adjusted for age and baseline NIHSS, to estimate the odds ratio under the assumption of proportional odds and its associated 95% confidence interval. In addition to undertaking an age and baseline adjusted analyses, I also undertook a sensitivity analysis by considering the combined effect of the variables that differed significantly at baseline (as shown in table 3.1).

For comparison with prior randomised trial and registry reports, I also present dichotomised analyses of mRS, based on favourable outcome (mRS 0-1), independence (mRS 0-2) and survival; these analyses were expressed as odds ratios adjusted for age and bNIHSS, as for the primary and secondary endpoints. Proportional Odds ratios in these analyses express the common odds of an improved distribution of outcome in association with alteplase treatment. Reliable information on symptomatic haemorrhage was not available since post treatment imaging was not routinely applied in neuroprotection trials to patients who had not been treated with alteplase.

3.3 Results

3.3.1 Patient Sample

Complete data were available for analysis of mRS in 5817 patients and on NIHSS in 5715. (See figure 2.1 for information on completeness of VISTA data) Baseline characteristics are shown in table 3.1.

Of the 5817 patients with mRS outcome data, 1585 (27.2%) received thrombolysis. Baseline severity was higher by one NIHSS point amongst the younger patients who received thrombolysis therapy compared to our control group; amongst patients aged ≥ 80 , severity was equal between treated and control groups. The delay between stroke onset and initiation of alteplase was not recorded, but the delay to research enrolment and initiation of investigational product was shorter in the thrombolysis group than control patients irrespective of age (3.7 versus 5.1 hours, $p=0.0001$). Independently, baseline NIHSS accounted for 28% and age for 9.7% of the variation in 90 day outcome by mRS (both $p<0.0001$) and were included in all models, together explaining 33.5% of the variation.

Table 3-1 Baseline demographics of the VISTA data employed for present study

	Thrombolysis		Control		<i>P</i>
Age, mean years (range)					
All	71 (21–98)	N=1585	72 (21–101)	N=4232	<0.05
Young age ≤80	67 (21–80)	N=1284	69 (21–80)	N=3339	<0.05
Elderly age >80	84 (81–98)	N=301	84 (75–101)	N=893	0.58
Sex, male					
All	880/1585	55.52%	2226/4232	52.6%	0.05
Young age ≤80	750/1284	58.4%	1874/3339	56.1%	0.16
Elderly age >80	130/301	34.9%	352/893	39.4%	0.25
Baseline NIHSS					
All	14 (2–32)	N=1585	13 (2–37)	N=4232	<0.05
Young age ≤80	13 (2–30)	N=1284	12 (2–32)	N=3339	<0.05
Elderly age >80	15 (4–32)	N=301	15 (2–37)	N=893	0.70
Prior antiplatelets					
All	429/1078	39.8%	446/1306	34.2%	<0.05
Young age ≤80	323/881	(36.7%)	335/1049	31.9%	0.03
Elderly age >80	106/197	53.8%	111/257	43.2	0.02
Prior anticoagulation					
All	67/1078	6.2%	198/1306	15.2%	<0.05
Young age ≤80	49/881	5.6%	151/1049	14.4%	<0.05
Elderly age >80	18/197	9.1%	47/257	18.3%	0.01
Previous stroke					
All	319/1555	20.5%	1579/4076	38.7%	<0.05
Young age ≤80	248/1255	19.8%	1178/3201	36.8%	<0.05
Elderly age >80	71/300	23.7%	401/875	45.8%	<0.05
Congestive heart failure					
All	151/1262	12%	164/1409	11.6%	0.79
Young age ≤80	100/1019	9.8%	106/1136	9.3%	0.70
Elderly age >80	51/243	21%	58/273	21.3%	0.94
Diabetes mellitus					
All	342/1548	22.1%	992/3991	24.9%	0.03
Young age ≤80	293/1250	23.4%	816/3136	26%	0.08
Elderly age >80	49/298	16.4%	176/855	20.6%	0.12
Hypertension					
All	1030/1548	66.5%	2827/3991	70.8%	<0.05
Young age ≤80	813/1250	65.04	2163/3136	69%	0.01
Elderly age >80	217/298	72.8%	664/855	77.7%	0.09
Atrial fibrillation					
All	398/1548	25.7%	1274/3991	31.3%	<0.05
Young age ≤80	268/1250	21.4%	807/3136	25.7%	<0.05
Elderly age >80	130/298	43.6%	440/855	51.5%	0.02
Myocardial infarction					
All	278/1548	18%	691/3991	17.3%	0.57
Young age ≤80	227/1250	18.2%	529/3136	16.9%	0.31
Elderly age >80	51/298	17.1%	162/855	19%	0.48

3.3.2 Overall Outcome

Across the whole sample, the distribution of mRS scores was better amongst thrombolysed patients, CMH $p < 0.0001$; OR: 1.39 (95% CI: 1.26-1.54).

3.3.3 Outcomes amongst patients aged ≤ 80 years

Amongst the 4623 patients with 90 day mRS data, treatment with thrombolysis was associated with a significantly more favourable distribution of mRS scores: CMH $p < 0.0001$, adjusted odds ratio (a-OR) 1.4 (95%CI: 1.3-1.6). The unadjusted odds ratio was 1.2 (95% CI: 1.1-1.4). Dichotomised comparisons were also significant for independence (mRS 0-2 versus 3-6), OR=1.54 (95% CI 1.33-1.79, $p < 0.0001$); for favourable outcome (mRS 0-1 versus 2-6), OR= 1.31 (95%CI 1.12-1.53, $p = 0.0008$); and for survival OR=1.44 (95% CI 1.18-1.76, $p = 0.0004$).

The functional outcomes were supported by the secondary endpoint. The spectrum of NIHSS scores at 90 days was significantly better amongst the thrombolysed patients than controls: CMH $p < 0.0001$, a-OR = 1.6 (95% CI: 1.4-1.8), $n = 4537$. The unadjusted comparison yielded OR=1.3 (95%CI: 1.2-1.5).

The sensitivity analysis, in which I adjusted for age, baseline NIHSS, previous stroke, hypertension and atrial fibrillation, as these differed at baseline (see table 3-1) also yielded OR 1.4 (95% CI 1.2-1.6), $N = 4387$, CMH $p < 0.0001$.

3.3.4 Outcomes amongst patients aged ≥ 81 years

Amongst the 1194 very elderly patients with 90 day mRS data, treatment with thrombolysis was associated with a significantly more favourable distribution of mRS scores : CMH $p = 0.002$, a-OR= 1.34 (95%CI: 1.05-1.70). The unadjusted odds ratio was 1.26 (95% CI: 1.00-1.59), CMH $p < 0.05$. The dichotomised comparison was significant for independence (mRS 0-2): OR=1.52 (95% CI 1.06-2.17, $p = 0.022$). For favourable outcome (mRS 0-1), the OR was 1.46 (95%CI: 0.97-2.20, $p = 0.07$); and for survival the OR was 1.20 (95%CI: 0.90-1.65, $p = 0.20$).

The functional outcomes were supported by the secondary endpoint. The spectrum of NIHSS scores at 90 days was significantly better amongst the thrombolysed patients than controls: CMH $p=0.0004$, a-OR= 1.4 (95%CI: 1.1-1.8), $n=1178$. The unadjusted comparison yielded a similar estimate: OR=1.4 (95%CI: 1.1-1.7).

The sensitivity analysis, in which I adjusted for age, baseline NIHSS, hypertension, previous stroke and atrial fibrillation, also yielded OR= 1.2 (0.96-1.57), $N=1152$, CMH $p=0.02$.

A figure showing proportions of patients within different Rankin categories of SITS-MOST and the pooled RCT data are pasted below (source: from SITS-MOST study ¹¹⁷). Proportion of patients in randomised data shown here are similar to the raw data of VISTA patients as shown figure 3-5.

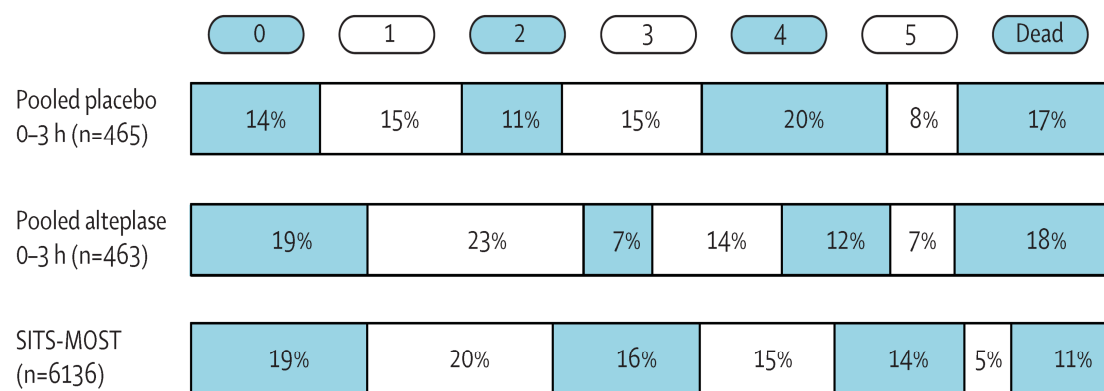


Figure 3-3 Figure shows proportions for patients belonging to various Rankin categories in the SITS-MOST data and also the pooled randomised data

Source: Wahlgren N, Ahmed N, Dávalos A, Ford GA, Grond M, Hacke W, Hennerici MG, Kaste M, Kuelkens S, Larrue V, Lees KR, Roine RO, Soenne L, Toni D, Vanhooren G; SITS-MOST investigators. Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST): an observational study. *Lancet*. 2007 Jan 27; 369(9558):275-82; *Permissions obtained* from Elsevier Limited The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, UK; License Number- 2783791420125¹⁴⁶

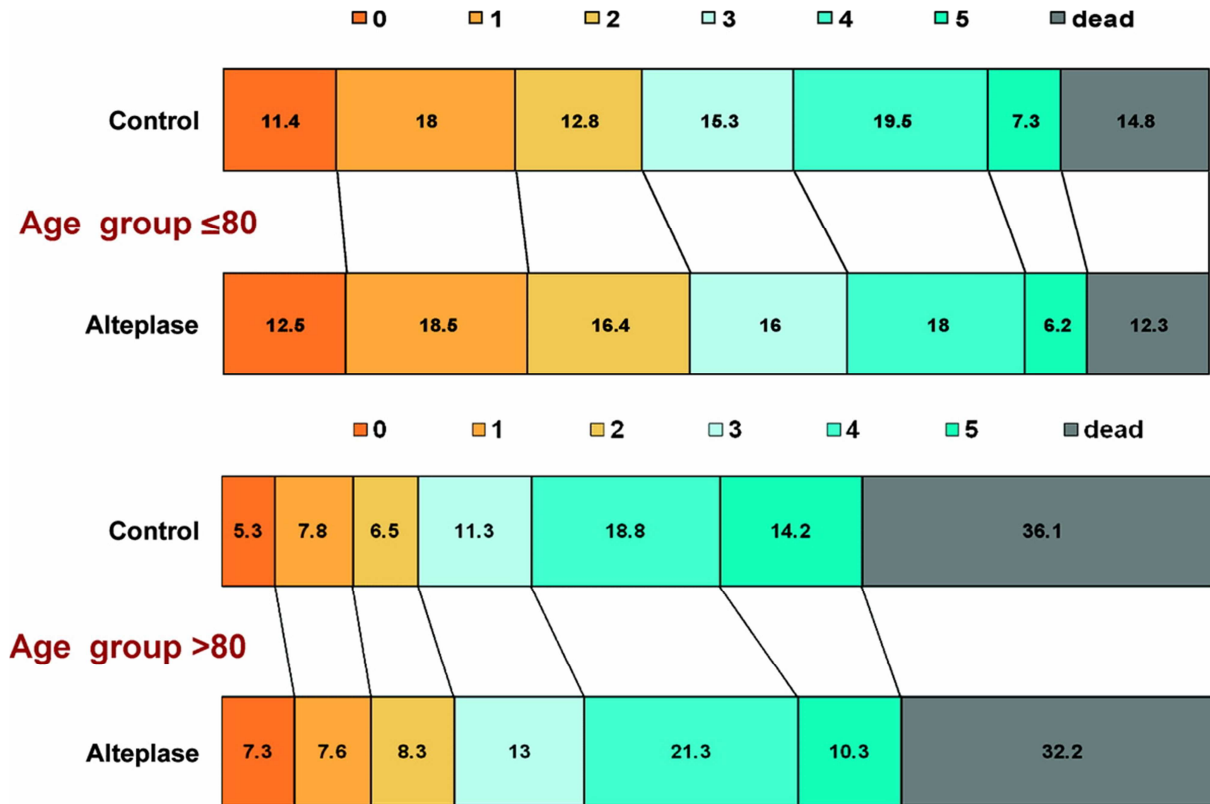


Figure 3-4 Functional outcomes after use of thrombolytic therapy in patients suffering ischaemic stroke

Diagram showing association of functional outcomes with use of rtPA in the younger patients (age ≤80 years) and elderly patients (age >80 years) having acute ischaemic stroke. Each box of the horizontal bar corresponds to the mRS category specified by the colour code. Upper horizontal bar belongs to control group of young and elderly patients and lower to the rtPA-treated patients in each age group. Numbers in each box denote the percent of total patients belonging to a specific treatment category (tPA or control) and representing the mRS score corresponding to the box.

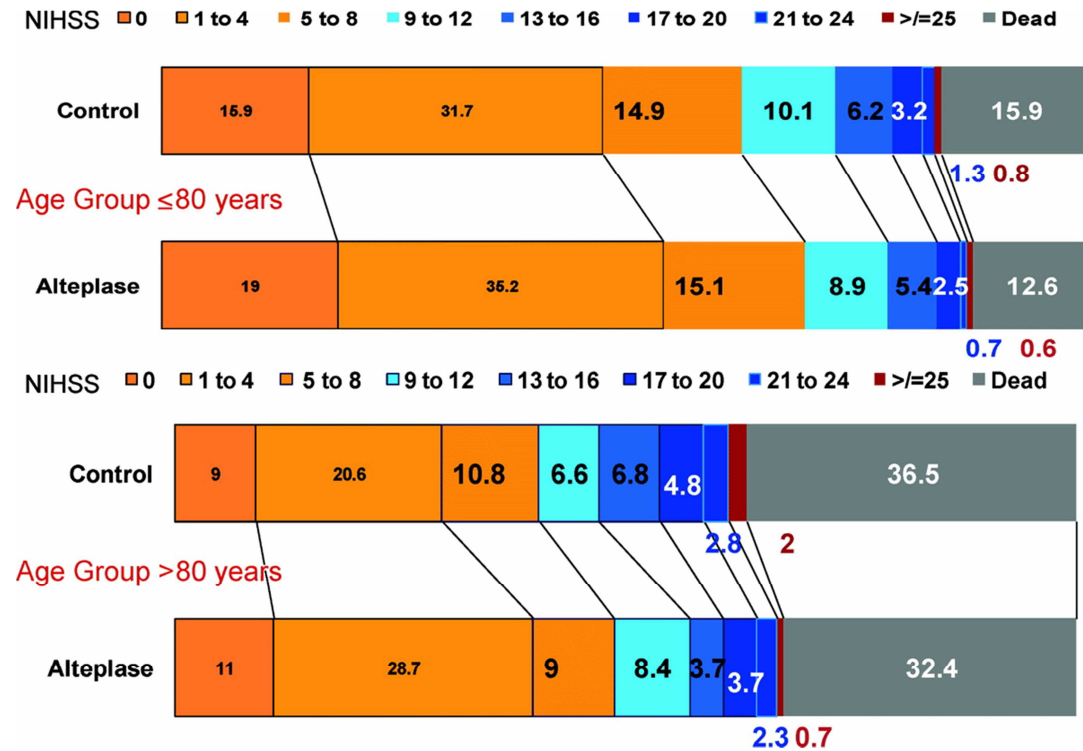


Figure 3-5 Neurological outcomes after use of alteplase in patients suffering acute ischaemic stroke

Diagram showing association of neurological outcomes with use of tPA in the younger patients (age ≤80 years) and elderly patients (age >80 years) having acute ischaemic stroke. Each box of the horizontal box corresponds to the mRS category specified by the colour code. Upper horizontal bar belongs to control group of young and elderly patients and lower to the tPA-treated patients in each age group. Numbers in each box denote the percent of total patients belonging to a specific treatment category (tPA or control) and representing the mRS score corresponding to the box.

3.3.5 Association of thrombolysis with outcome by age decile

Both functional outcome and neurological outcome were significantly better amongst thrombolysed patients than controls within each decile of age from 51 years to 90 years; and except amongst the small sample of 21-30 year old patients, point estimates for the adjusted odds ratios were consistent across all age groups (Fig 3-7 and 3-8)

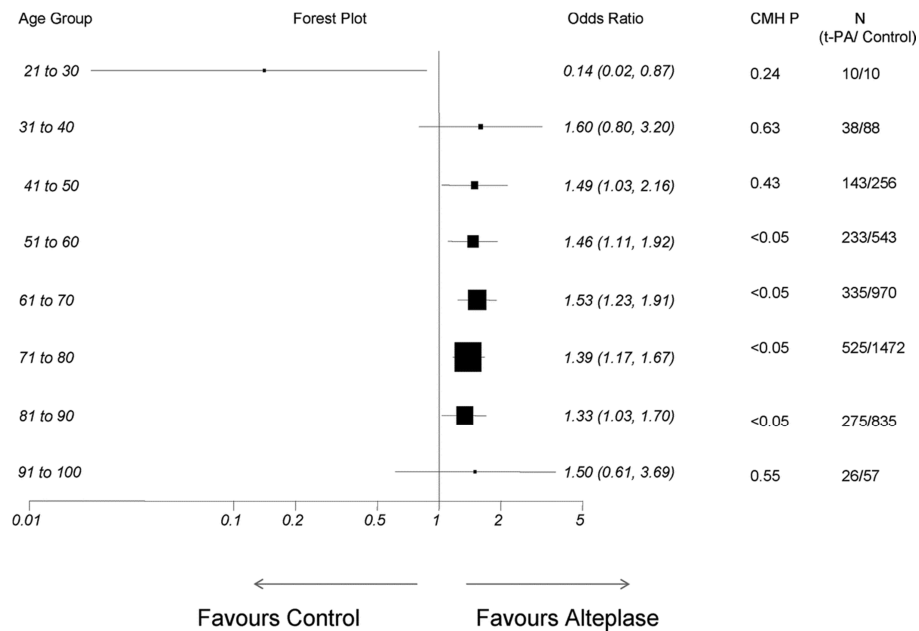


Figure 3-6 Functional outcomes after thrombolytic therapy

The Forest plot shows a significant association of use of alteplase with improved functional outcomes in age group from 41 to 50 until 81 to 90. Point estimates are indicative of improved outcomes even for age group 31 to 40 and 91 to 100. The odds ratio (OR) are shown for age groups stratified by age ranges of 10. CMH P refers to the test statistic for Cochran-Mantel-Haenszel Test and N refers to total sample size.

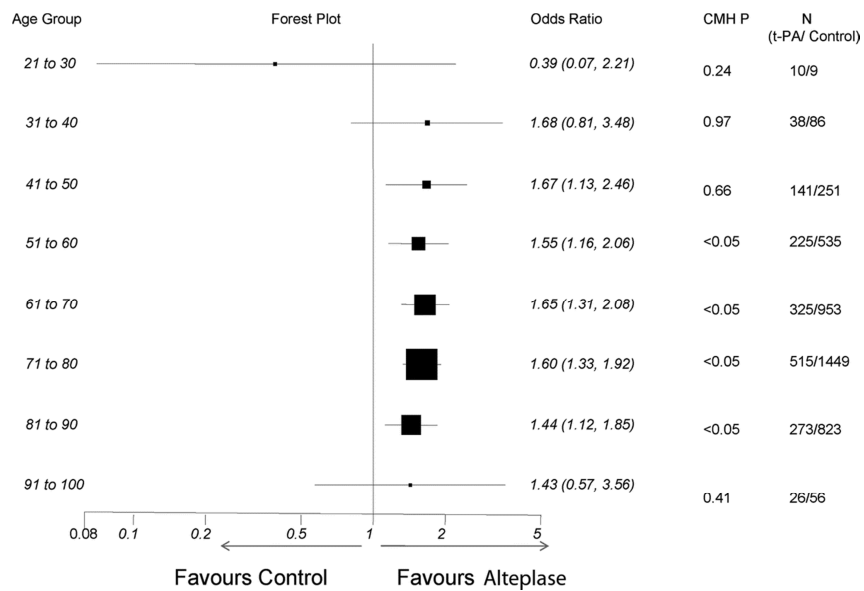


Figure 3-7 Neurological outcomes after thrombolytic therapy

The figure shows an association of neurological outcomes with improved outcomes in patients who received thrombolytic therapy. Patients are shown stratified by age groups and corresponding forest plot, OR, CI, CMH P, and sample size are marked.

3.4 Discussion

Analyses reported in this chapter demonstrate that the use of thrombolysis for acute stroke is associated with better functional and neurological outcomes, and probably lower mortality, in all adult patients who are treated irrespective of their age. It supports the limited randomised trial data and places data on safety from stroke registries in context.

These data draw validity from four origins. First, the source clinical trials of investigational medicinal products were undertaken under strict controls on reporting of concomitant treatments and outcomes, and on-site data verification procedures were in place for each trial. Second, attitudes to treatment of the very elderly vary amongst clinicians, some European clinicians strictly following the EMEA marketing authorisation, others in Europe and North America treating without regard to age.^{240,300} Third, the estimates of control outcomes correspond closely to those from the published RCTs of thrombolysis,^{130,131} the estimates of

outcomes amongst the treated group aged ≤ 80 years are similar to those of the RCTs* ,¹⁴⁶ and the estimate of treatment odds ratios in the patients aged ≤ 80 years closely corresponds to treatment effects demonstrated in the RCTs.¹²⁹⁻¹³¹ It should be acknowledged that dose finding studies have not been undertaken in the very elderly, however. Fourth, I chose as the primary endpoint the modified Rankin scale, which is the most prevalent outcome measure in recent stroke trials²⁵⁷ and I followed an approach to analysis that is described in the EMEA Points to Consider for interpretation of clinical trials in acute stroke.²⁸⁴ There, comparison of the distribution of the full range of the Rankin is proposed as acceptable, with supporting evidence from a secondary endpoint such as NIHSS: both are positive and give similar estimates of benefit in our comparison. Further, although the less powerful dichotomised analyses are not all significant amongst the very elderly, they each give point estimates for magnitude of association that correspond to the estimates derived in the young and from the full mRS or NIHSS distributions.

* I obtained the SITS-MOST data from the figure 3.3. I undertook an *unadjusted* comparison of the Rankin scores in the non-elderly patients SITS-MOST data with the VISTA data (age below 80 years) by employing proportional odds logistic regression analysis. The point estimates suggested a shift towards better outcomes when the pooled data of the thrombolysed patients recruited in the randomised controlled trials (RCT) were compared with the VISTA non-thrombolysed patients (OR 1.1, 95%CI 0.9-1.3). When the VISTA controls were compared with the RCT placebo group, OR=0.9, 95% CI 0.8-1.1. When SITS-MOST patients were compared with the VISTA thrombolysed patients, OR=1.3, 95% CI 1.2-1.5. It should be noted that these are unadjusted analyses: influence of age and baseline severity on outcomes in these comparisons could not be incorporated in the proportional odds logistic regression analysis model as I donot have access to the SITS-MOST raw data.

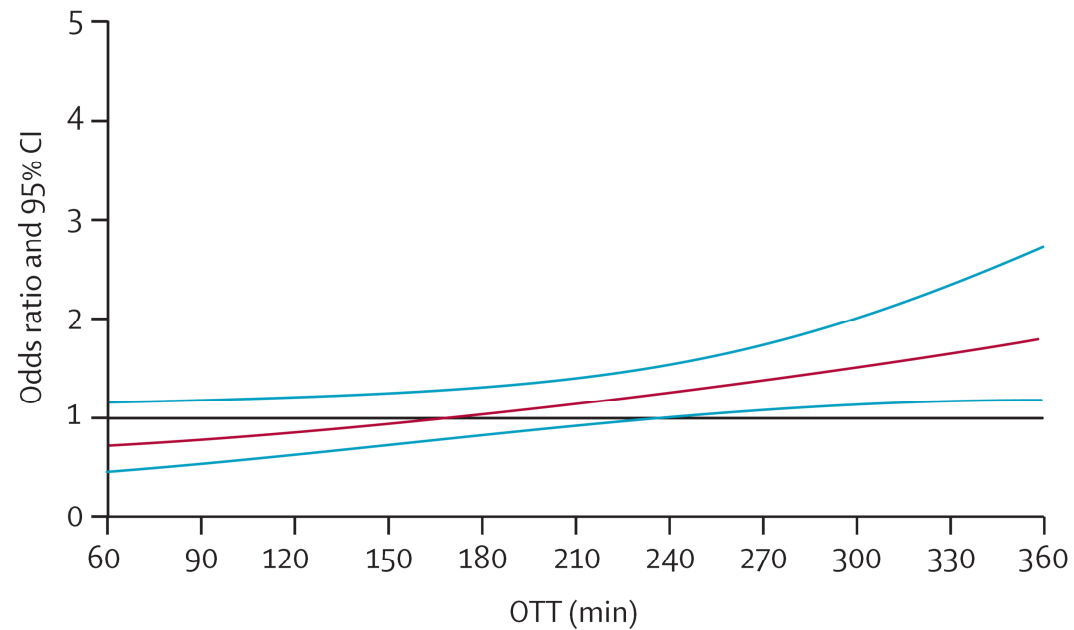


Figure 3-8 Figure shows the relationship of onset to treatment time with adjusted odds for mortality in a pooled analysis of patients from randomisation controlled trials that investigated t-PA (N=3670)

(Source: Lees KR, Bluhmki E, von Kummer R, Brott TG, Toni D, Grotta JC, Albers GW, Kaste M, Marler JR, Hamilton SA, Tilley BC, Davis SM, Donnan GA, Hacke W; ECASS, ATLANTIS, NINDS and EPITHET rt-PA Study Group, Allen K, Mau J, Meier D, del Zoppo G, De Silva DA, Butcher KS, Parsons MW, Barber PA, Levi C, Bladin C, Byrnes G. Time to treatment with intravenous alteplase and outcome in stroke: an updated pooled analysis of ECASS, ATLANTIS, NINDS, and EPITHET trials. *Lancet*. 2010 May 15; 375(9727):1695-703. Permissions obtained from Elsevier Limited, The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, UK; License Number: 2783800078653)¹³¹

Present data indicate a reduction in mortality of non-elderly (12.3% vs. 14.8%) and elderly (32.2% vs. 36.1%) patients (figure 3-4) and confirm improved survival (1.4 (95%CI 1.2-1.8) amongst non-elderly. Pooled analyses of all trials have seen the trends for favourable outcomes in t-PA arm (see figure 3-8) through modelling.¹³¹ Also, these findings are consistent with the meta-analysis data: while odds for death or dependency were OR 0.71 and 95% CI 0.52 to 0.96, the odds for death were 1.1 with a confidence interval that was wide, 95%CI 0.9 to 1.5.⁹² In the present analysis for the elderly, the odds for survival are 1.2 and confidence intervals wide, 95%CI 0.9-1.7 (N=301 patients in t-PA group). These data indicate benefit by reduction in mortality including amongst the elderly. Mortality was shown to have reduced owing to larger sample size, and thereby giving greater power to the subgroup age ≤ 80 years (N=1284).

Elderly patients usually do poorly after a stroke because of pre-existing illnesses and on-going poly-pharmacy. For example, elderly are more likely to suffer from cardiac diseases like atrial fibrillation, cardiac failure, coronary heart disease and hypertension. Prevalence of cardiac disorders increases with age: in the Framingham Heart Study, incidence for atrial fibrillation was 0.2/1000 for age group 30-39 while 39/1000 for age group 80-89 years.³⁰¹ With increasing age there is also a steady rise in the proportion of patients who suffer stroke because of arrhythmia (in age group 80 to 89 years, the rates are 36.2%).³⁰¹ Patients suffering cardio embolic stroke are more likely to have larger infarcts or suffer multiple infarctions involving different territories. Further, the cardio-embolic strokes are associated with greater risk of haemorrhagic transformation: it occurs in about a fifth of cardio-embolic stroke patients.³⁰² Further, some data indicate that patients suffering cardio-embolic strokes may fail to recanalise despite the use of alteplase.^{303,304} This could be due to a unique clot composition in these patients that renders itself unresponsive to alteplase and because cardio-embolism occurs more frequently amongst the elderly, these patients could be the ones more at risk of not responding to the use of alteplase. But, the data presented in this chapter support the contrary: the elderly responded to the use of alteplase. Outcomes improved not only on Rankin scores but also on the NIHSS scores in the age group >80 years.

It is known that compared to younger population, elderly patients have larger deposits of cerebral amyloid material.³⁰⁵ These deposits could have contributed to increased risk of post-thrombolysis intracerebral haemorrhage. Further, these patients have poorer cognitive reserve and may be having poorer abilities to employ neuroplasticity and regain functions.³⁰⁶ Further, their abilities to recruit collaterals after an ischaemic insult might be poorer than the younger patients. These biological substrates are more likely to result in poorer outcomes in the elderly age group. In the present analysis, I failed to show that t-PA use in elderly interacts differently compared to the non-elderly population. Instead, I show that despite excess poorer outcomes in the elderly, these patients responded to the t-PA use and showed improved outcomes. Further, there is no evidence from other disease areas, like myocardial infarction, to support this.²³⁷

There are also limitations to my study that must be considered. The data are based on a non-randomised comparison, and there is a high potential for selection bias for thrombolytic treatment. Although many of the usual descriptors of baseline prognosis are reasonably matched between the two groups, thrombolysis and controls, and although I have adjusted our analyses for the most important of these - age and baseline severity,^{265,307} which together account for 33.5% of the variation in outcome I could not adjust for every factor. I planned to adjust for all variables that differed at baseline but did not include presence of use of antithrombotic agents (prior use of antiplatelet and anticoagulant agents) in the sensitivity analyses. This is because on trying to adjust for antithrombotic agents, along with other variables that differed at baseline, the sample size for the analysis reduced dramatically both for elderly population (from 1193 to 454) and non-elderly (4624 to 1930) population. This occurred because observations got deleted (739 and 2694 respectively) by the program due to missing values for the response or explanatory variables. Further, atrial fibrillation, use of warfarin and prior diabetes were all less prevalent amongst the treated group. However, the magnitude of these differences was small, the absolute differences were equal for young versus very elderly, and the sensitivity analyses with adjustment for these additional factors also yielded significantly positive findings. This implies that although the estimate of the association of treatment with outcome may be imprecise, the estimates of trends in this measure across the age range are robust. Some of the

patients in this study received an investigational neuroprotective agent and it must be considered that one or more of these could interact with thrombolysis; however, each contributing trial has already tested for, and excluded, a significant interaction. VISTA data handling procedures preclude further testing for effects of the original investigational agent or identification of source trials.

The delay between stroke onset and treatment initiation in the thrombolysis group was not known, but the time of baseline NIHSS assessment is earlier in the thrombolysed than non-thrombolysed patients. Presentation delay is associated with outcome, but this is mediated through earlier presentation of more severe stroke, a factor that favoured our control group in the young only. Last is the possibility of systematic bias in other aspects of care and thus outcome, between centres that used thrombolysis routinely versus those that did not, or that restricted use in the elderly. It is difficult to counter criticism on this point, except to indicate that the contributing trials sought to minimise such effects through site selection, training of investigators and monitoring of care and of outcomes; and to point again to the correspondence of outcomes in each of the treatment groups with those from RCT and registry data.

Trials and registries of thrombolysis generally report three outcomes: functional attainment, mortality and symptomatic or serious intracranial haemorrhage.^{122,146} VISTA lacked data on the last of these, since patients who are not treated with thrombolysis generally do not undergo follow-up cerebral imaging for routine detection of haemorrhagic transformation. Fortunately, information on this aspect can be inferred from other sources: the rate of serious or symptomatic bleeding is very low amongst patients who do not receive thrombolysis -approximately 1%¹³¹ and registry data such as SITS inform us on the rate amongst treated patients and have found no significant increase in the very elderly compared to the young.^{292,308}

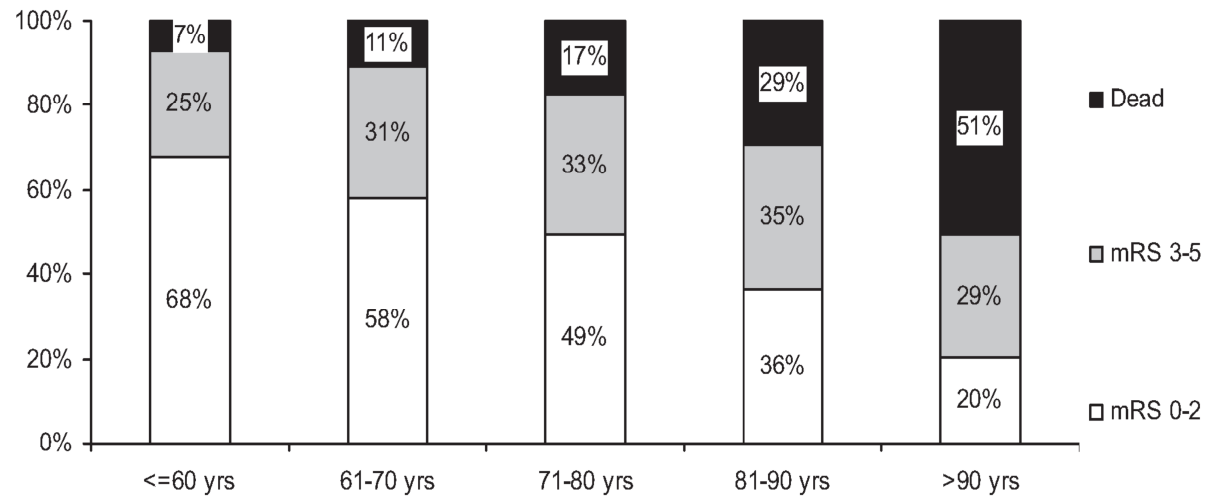


Figure 3-9 Figure shows the functional outcomes in patients for various age bands in SITS registry

Source: Ford GA, Ahmed N, Azevedo E, Grond M, Larrue V, Lindsberg PJ, Toni D, Wahlgren N. Intravenous alteplase for stroke in those older than 80 years old. Stroke. 2010 Nov;41(11):2568-74. Permissions obtained from Wolters Kluwer Health, License Number 2783791484358³⁰⁸

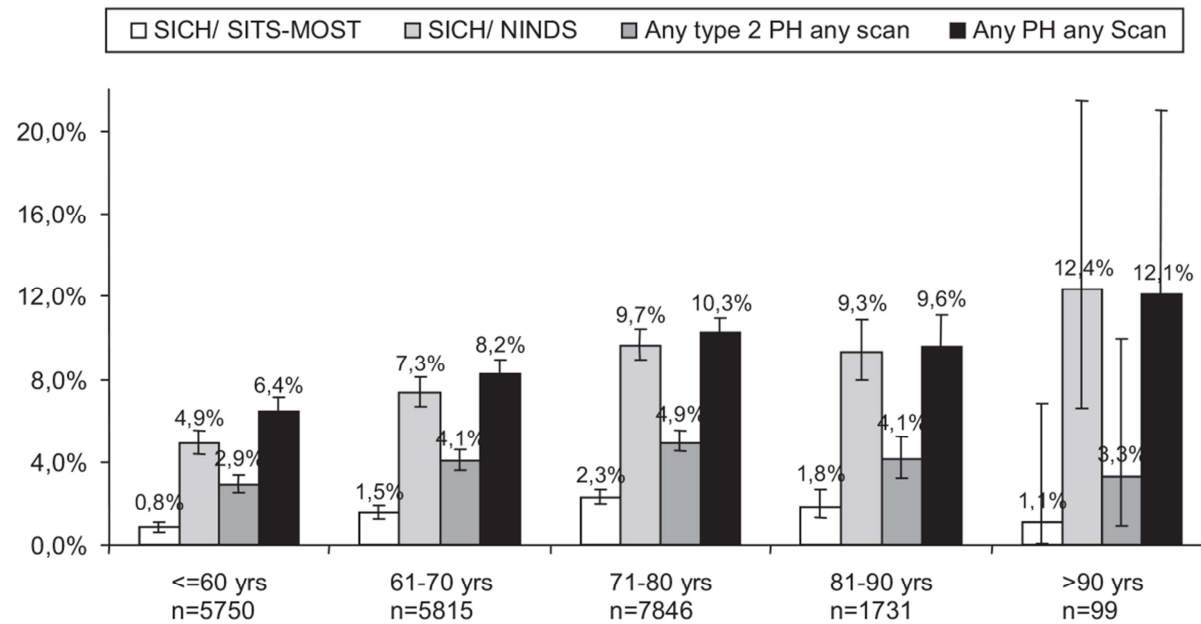


Figure 3-10 Figure shows rates of symptomatic intracerebral haemorrhages per SITS-MOST definition, NINDS definition and parenchymal haemorrhage (including type II) in the SITS registry.

Source: Ford GA, Ahmed N, Azevedo E, Grond M, Larrue V, Lindsberg PJ, Toni D, Wahlgren N. Intravenous alteplase for stroke in those older than 80 years old. Stroke. 2010 Nov;41(11):2568-74. Permissions Obtained from Wolters Kluwer Health, License Number 2783791484358³⁰⁸

A more important response on the issue of serious bleeding comes from our use of the full mRS distribution as our outcome measure. Bleeding is relevant only if it affects eventual functional outcome. Dichotomisation of mRS outcomes into 0-1 versus 2-6 or 0-2 versus 3-6 could conceal harmful results of serious bleeding reflected by higher proportions of severely disabled patients within the unfavourable outcome group (for example more mRS 5 amongst patients with mRS 3-5). The data and analysis approach exclude this possibility: even if haemorrhage were more common in the very elderly than young, which has been discounted,^{308,309} this does not translate into poorer functional outcomes after adjustment for age and stroke severity.

Data on stroke outcomes associated with thrombolysis use in the elderly come from 3 other sources. A meta-analysis of cohort studies by Ringleb and colleagues in 2007 found that the elderly experienced similar rates of symptomatic haemorrhage to the young (6.1% versus 5.1%) but higher mortality (32% versus 14%), with fewer attaining favourable outcome by 90 days (mRS 0-1: 26% versus 41%).³⁰⁹ However, within one of the largest studies in this analysis, the baseline severity of stroke was much higher in elderly than young patients (NIHSS 16 versus 13.9 respectively).²⁹² Outcomes of very elderly patients described by the Safe Implementation of Thrombolysis in Stroke (SITS) registry reinforce these findings: stroke severity was higher in the 643 elderly versus 6749 younger patients, NIHSS 15 v 13. Symptomatic ICH was no more common in the very elderly (2.0 [95% CI 1.1-3.5] versus 1.5 [1.2- 1.8] per cent), but 90-day mortality was higher (31 [27-36] versus 15 [14-16] per cent); and independence (mRS 0-2) was achieved less frequently (30 [26-34] versus 52 [51-53] per cent).³¹⁰ Interpretation of these uncontrolled registry findings is compromised by the known influence of age and stroke severity on outcome in the absence of thrombolysis treatment. Only 164 patients aged ≥ 80 years were included amongst the large randomised trials combined.³¹¹ The elderly group was again more severely affected at baseline than the younger patients, but there was also a severity imbalance amongst the elderly that favoured the controls. A pooled analysis of these data in the elderly (0-4.5 hours subgroup: N=137/2199) estimated odds ratios for independence (mRS 0-2 at day 90) and mortality under alteplase versus placebo of 1.09 and 1.28 respectively based on unadjusted data.

However, after adjustment for the demonstrable imbalance in baseline NIHSS the odds ratios respectively were 1.77 and 0.96.³¹¹ The sample size was small and none of these outcomes reached statistical significance. Thus, the present findings are entirely consistent with the randomised trial data, not only in terms of the estimated extent of benefit from treatment but also with regard to the influence of baseline severity on the interpretation of outcomes.

Treatment allocation in this study was not randomised, and a randomised controlled trial would more conclusively inform the influence of rt-PA on outcomes amongst elderly. Two trials currently aim to examine this topic.^{232,234,312} There is an Italian trial that has so far enrolled around 10% of the planned 600 patients, over a 2 year period. The International Stroke Trial-3 [IST-3] aims to examine outcomes amongst all patients who receive thrombolytic therapy and has prescribed no upper age limit.^{232,234} The trial has so far enrolled 3035 patients from the originally planned 6000.²³³ 1756 of these patients are very elderly and being treated within the time window of interest.^{233,313,314} Though the present analysis is not a randomised controlled trial, it is the only source of evidence to support the registry and RCT data that currently available.^{290,292,297}

In summary, outcome amongst patients treated with thrombolysis as standard of care within clinical research trials is more favourable than amongst patients who are not offered thrombolysis, and this apparent advantage to patients who are treated extends to patients aged 81 years and older. From this analysis, I not only fail to find evidence to support the present restriction of the European marketing authorisation for alteplase use in the elderly; I find positive evidence that alteplase is beneficial amongst patients aged 81-90 years and that this is likely to extend even to patients aged 91-100 years. These data support and extend the extensive uncontrolled data on outcomes from registries, and the limited randomised controlled trial data. Age is not a relevant factor when considering whether to use alteplase for acute stroke.

Chapter 4

Outcomes from thrombolytic therapy amongst elderly patients recruited in SITS

Registry

4 Outcomes from thrombolytic therapy amongst elderly patients recruited in SITS Registry: validation of VISTA findings on SITS-VISTA data

4.1 Introduction

In chapter 3, I described findings from analysis of VISTA dataset and showed that outcomes improve amongst elderly patients who receive alteplase for acute ischaemic stroke. The results were based on a robust statistical analysis. Findings were consistent on two different measures of outcomes, namely, the NIHSS scale and the modified Rankin Scale. Point estimates for the approved population were similar to the ones observed in randomised trials, thus allowing us to anchor our results on the elderly onto the randomised data. Because it was not a randomised study, which I acknowledge as a weakness, I also looked at the prospects of the two on-going randomised trials, the IST III^{232,233,313} and TEPSI³¹², but from the rate at which these studies are recruiting patients, it appears that clinicians will have to wait for a while before the randomised data are available. IST-3 originally targeted to recruit 6000 patients.²³⁴ Per September 2011 issue of IST-3 times, the total recruitment figures were 3035. Of these, 1756 were elderly.²³³

In order to confirm the findings from VISTA only analyses in previous chapter, I planned to validate results in another dataset. For this, I decided to examine outcomes in elderly patients whose data were held within the SITS-ISTR at Karolinska University Hospital. Below, I report methods and results from the data derived from SITS-ISTR registry.

4.2 Method

4.2.1 Data Source and patients

I describe the SITS-ISTR data in chapter 2. Briefly, I collated the data of stroke patients who received thrombolytic therapy through the SITS-ISTR between December 2002 and November 2009. The control group comprised untreated stroke patients from neuroprotection trials conducted between 1998 to 2007 held within the VISTA. From both of these sources, I collated the demographics, clinical data and information of functional outcome as measured with the modified Rankin scale (mRS) score after 90 days.

4.2.2 Patient Sample

Details of data extraction are shown in figure 2.1. The sample for this analysis comprised 29500. Data on baseline NIHSS for 272 of these patients were missing, reducing the sample for the baseline severity adjusted analysis

4.2.3 Statistical analysis

I compared 90 days outcome between patients who received intravenous thrombolysis and non-thrombolysed controls for the whole cohort. I repeated the comparison amongst patients aged ≤ 80 and >80 years. I then examined the association of thrombolysis treatment with outcome within each age decile to illustrate the strength of evidence across the full age range.

For each contrast, I compared the overall distribution of all seven categories of day 90 mRS scores of the two groups, i.e. from 0 (asymptomatic) through 5 (bedbound and completely dependent), to 6 (dead).

I published this chapter in BMJ (Mishra NK, Ahmed N, Andersen G, Egido JA, Lindsberg PJ, Ringleb PA, Wahlgren NG, Lees KR; VISTA collaborators; SITS collaborators. Thrombolysis in very elderly people: controlled comparison of SITS International Stroke Thrombolysis Registry and Virtual International Stroke Trials Archive. BMJ. 2010 Nov 23;341:c6046. doi: 10.1136/bmj.c6046.). Permissions were obtained from BMJ Publishing Group Limited 2783790122184

To test for a significant association of outcome distribution with thrombolysis exposure I used the Cochran-Mantel-Haenszel [CMH] statistic, adjusting for both age and baseline NIHSS as continuous variables. However, I also undertook a sensitivity analysis by considering the combined effect of the variables that differed significantly at baseline.

Reliable information on symptomatic intracerebral haemorrhage (sICH) was not available from VISTA controls since post treatment imaging was not routinely applied in neuroprotection trials to patients who had not been treated with alteplase. However, the rates of sICH for the definitions used within SITS (local or remote parenchymal haemorrhage type 2 on the imaging scan at 22-36 h after treatment, combined with a neurological deterioration of 4 or more points on the NIHSS from baseline, or from the lowest NIHSS score between baseline and 24 h, or leading to death) and the NINDS-study (any intracranial haemorrhage in the post-thrombolysis imaging scans if it was not seen on a previous imaging scan and any decline in neurological status) for younger and older patients of the SITS-ISTR registry was compared.

4.3 Results

4.3.1 Baseline Characteristics of the Patients

Baseline characteristics are shown in table 4.1. Baseline stroke severity was similar between thrombolysed versus non thrombolysed patients amongst both the >80 elderly ($p=0.6$) and ≤ 80 years age groups ($p=0.3$). Independently, baseline NIHSS accounted for 25.5% and age for 7.4% of the variation in 90 day outcome by mRS (both $p<0.001$) and were included in all models, together explaining 29.6% of the variation.

It is to be noted that the number of patients in control arm differs between the SITS-VISTA analyses reported in this chapter and those shown in previous chapter. This is because, in the previous chapter, I had excluded 2789 patients

that were likely to have been in SITS-MOST. I did not employ a similar exclusion criterion for the analyses presented in this chapter.

Table 4-1 Baseline characteristics of patients enrolled in SITS-VISTA data

	Thrombolysis	Control	P value
Mean (SD) and median (range) age (years)			
All	67.1 (12.4), 69 (10-98); n=23 334	70.1 (12.2), 72 (21-101); n=6166	<0.001
≤80	65.3 (11.63), 68 (10-80); n= 21 099	66.5 (10.7), 69 (21-80); n=4929	<0.001
>80	84.4 (3.25), 84 (81-98); n= 2235	84.84 (3.36), 84 (81-101); n=1237	<0.001
No (%) of men			
All	13 594/23 334 (58.3)	3271/6166 (53.0)	<0.001
≤80	12 744/21 099 (60.4)	2783/4929 (56.5)	<0.001
>80	850/2235 (38.0)	488/1237 (39.5)	0.41
Median (range) baseline score on National Institutes of Health stroke scale			
All	12 (0-42), n=23 062	12 (2-37), n=6166	0.14
≤80	12 (0-42), n=20 860	11 (2-32), n=4929	0.32
>80	14 (0-39), n=2202	14 (2-37), n=1237	0.61
No (%) who had previously taken antithrombotic agents			
All	8776/22 792 (38.5)	1267/2968 (42.7)	<0.001
≤80	7537/20 623 (36.5)	977/2414 (40.5)	<0.001
>80	1239/2169 (57.1%)	290/554 (52.3)	0.04
No (%) with known diabetes mellitus			
All	3962/22 968 (17.2)	1449/5896 (24.6)	<0.001
≤80	3570/20 784 (17.2)	1203/4704 (25.6)	<0.001
>80	392/2184 (17.9)	246/1192 (20.6)	0.06
No (%) with previous stroke			
All	3005/23 013 (13.1)	2014/5993 (33.6)	<0.001
≤80	2629/20 840 (12.6)	1521/4776 (31.8)	<0.001
>80	376/2173 (17.3)	493/1217 (40.5)	<0.001
No (%) with congestive heart failure			
All	1932/22 840 (8.5)	277/3167 (8.7)	0.59
≤80	1581/20 697 (7.6)	185/2579 (7.2)	0.39
>80	351/2143 (16.4)	92/588 (15.6)	0.67
No (%) with hypertension			
All	14331/22 875 (62.6)	4170/5896 (70.7)	<0.001
≤80	12687/20 683 (61.3)	3273/4704 (69.6)	<0.001
>80	1644/2192 (75)	897/1192 (75.3)	0.87
No (%) with atrial fibrillation			
All	5835/22 753 (25.6)	1712/5896 (29.0)	<0.001
≤80	4837/20 613 (23.5)	1147/4704 (24.4)	0.19
>80	998/2140 (46.6)	565/1192 (47.4)	0.67

*Of patients with baseline National Institutes of Health stroke scale score in SITS datasheet, two (0.32%) in age group 31-40, five (0.30%) in age group 41-50, nine (0.25%) in age group 51-60, six patients (0.1%) in age group 61-70, eight (0.09%) in age group 71-80, and two (0.1%) in age group 81-90 were coded as having baseline score of 0 and treated with alteplase. They were assumed to have neurological deficit considered potentially disabling but not measured by restricted rules of the scale (such as distal limb weakness). These few cases will have no material impact on findings.

4.3.2 Overall outcome

Across the whole sample, the distribution of mRS scores at 3 months was better amongst thrombolysed patients, $p < 0.0001$; OR: 1.59 (CI: 1.51-1.67). (Figure 4-2)

4.3.3 Outcomes amongst patients aged ≤ 80 years

Treatment with thrombolysis was associated with a significantly more favourable distribution of mRS scores at 3 months: CMH $p < 0.0001$, adjusted odds ratio (a-OR) 1.6 (95%CI: 1.5-1.7); N= 25789. (Figure 4-3) The unadjusted odds ratio was 1.5 (95% CI: 1.4-1.6) [N=26028]. Dichotomised outcomes were also significantly favourable for thrombolysed patients compared to control (mRS 0-2 versus 3-6), OR=1.87(95% CI 1.74-2.01); for excellent outcome (mRS 0-1 versus 2-6), OR= 1.55 (95%CI 1.44-1.67); and for mortality OR= 0.87 (95% CI 0.79-0.95).

The sensitivity analysis, in which in addition to age and baseline stroke severity, I also adjusted for sex, history of either diabetes or previous stroke, use of prior antithrombotic agents, and hypertension, for they differed at baseline, yielded CMH $p < 0.0001$ and proportional odds of 1.6 (95% CI 1.4-1.7), N= 22148 in favour of thrombolysis.

4.3.4 Outcomes amongst patients aged ≥ 81 years

Amongst the 3439 elderly patients above 80 years old with 90 day mRS and baseline stroke severity data, treatment with thrombolysis was associated with a significantly more favourable distribution of mRS scores at 3 months compared to control CMH $p < 0.0001$, a-OR= 1.43 (95%CI: 1.26-1.63), [N=3439] (Figure 4-4). The unadjusted odds ratio was 1.4 (95% CI: 1.23-1.58), CMH $p < 0.0001$ [N=3472]. The dichotomised outcome analysis was significantly in favour for thrombolysed patients compared to control, for favourable outcome (mRS 0-2): OR= 2.06 (95% CI 1.72-2.48). For excellent outcome (mRS 0-1), the OR was 1.87 (95%CI: 1.53-2.29); and for mortality the OR was 0.89 (95%CI: 0.76-1.04).

The sensitivity analysis, in which, in addition to baseline NIHSS and age, I also adjusted for sex, history of either diabetes or previous stroke, use of prior antithrombotic and hypertension, for they differed at baseline, yielded CMH

$p=0.003$ and proportional odds OR: 1.5, 95% CI: (1.3-1.8), $N=2593$ favourable for thrombolysis.

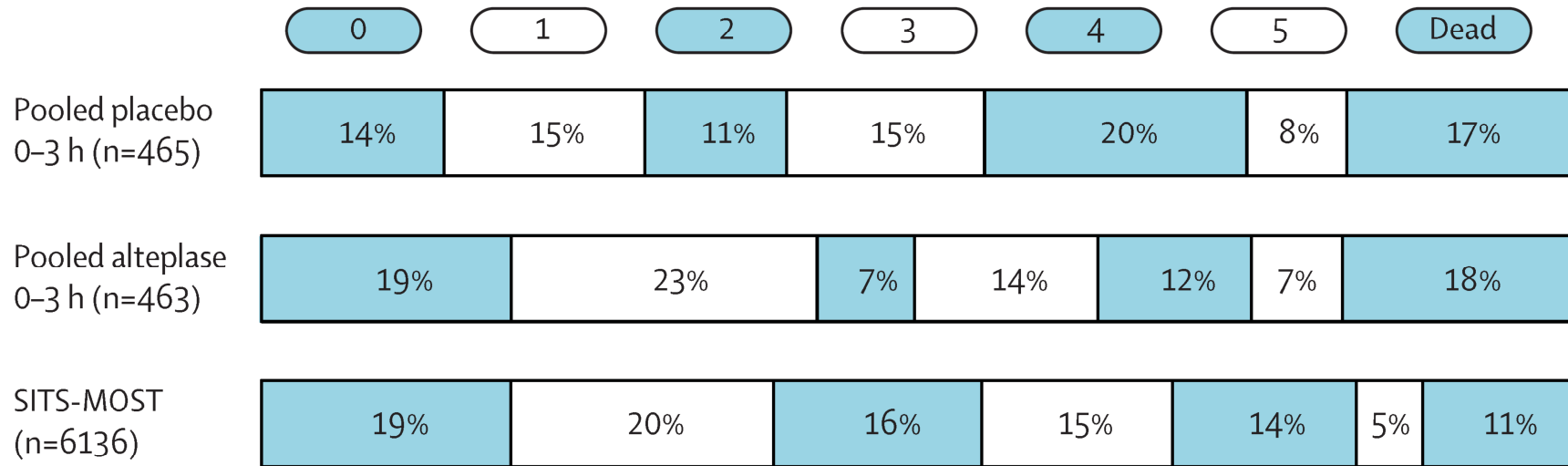


Figure 4-1Figure shows proportions for patients belonging to various Rankin categories in the SITS-MOST data and also the pooled randomised data

(Source: Wahlgren N, Ahmed N, Dávalos A, Ford GA, Grond M, Hacke W, Hennerici MG, Kaste M, Kuelkens S, Larrue V, Lees KR, Roine RO, Soenne L, Toni D, Vanhooren G; SITS-MOST investigators. Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST): an observational study. *Lancet*. 2007 Jan 27;369(9558):275-82; *Permissions obtained from Elsevier Limited The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, UK; License Number- 2783791420125*)¹⁴⁶



Figure 4-2 Outcomes after thrombolytic therapy in the entire population

Scores on modified Rankin scale (from 0=no symptoms from stroke to 6=death) at three months between patients who underwent thrombolysis with alteplase and controls, indicating shift towards improved outcomes with thrombolysis. Numbers within coloured cells are percentages. Total Patients in alteplase arm 23 3334 and control arm 6166.

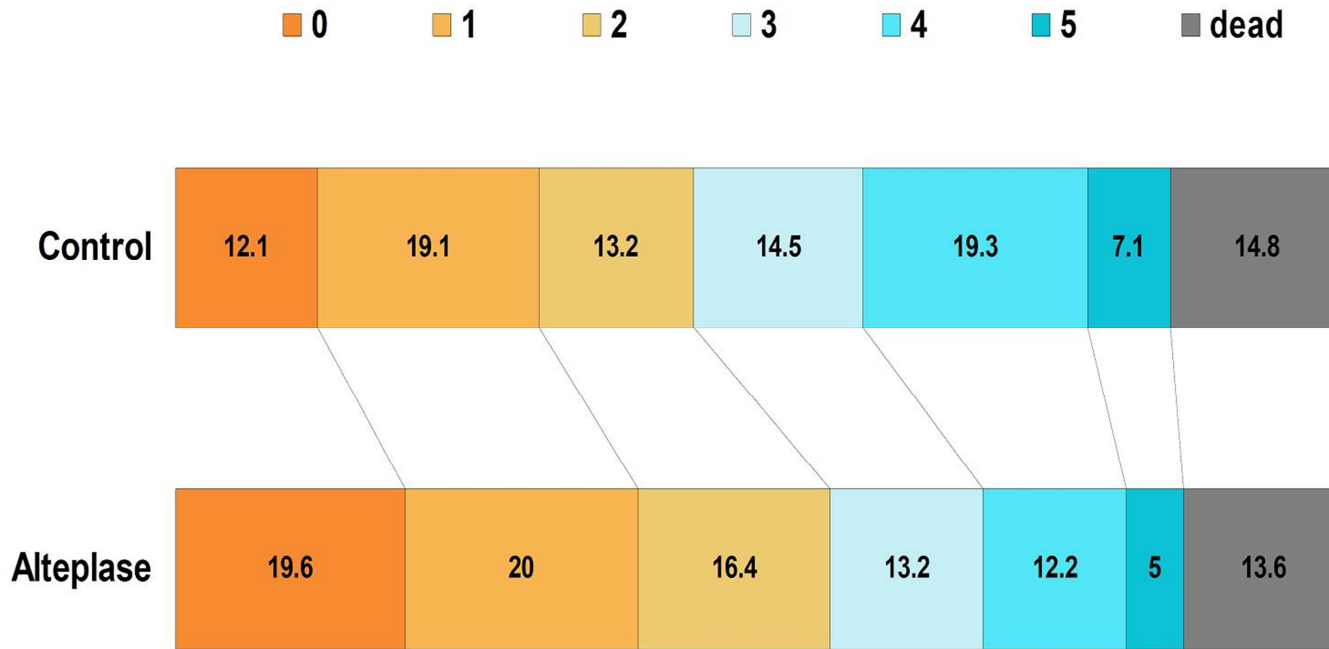


Figure 4-3 Outcomes after thrombolysis amongst the patients \leq 80 years

Scores on modified Rankin scale (from 0=no symptoms from stroke to 6=death) at three months between patients who underwent thrombolysis with alteplase and controls, indicating shift towards improved outcomes with thrombolysis. Numbers within coloured cells are percentage. Total patients in alteplase arm 21 099 and control arm 4929.

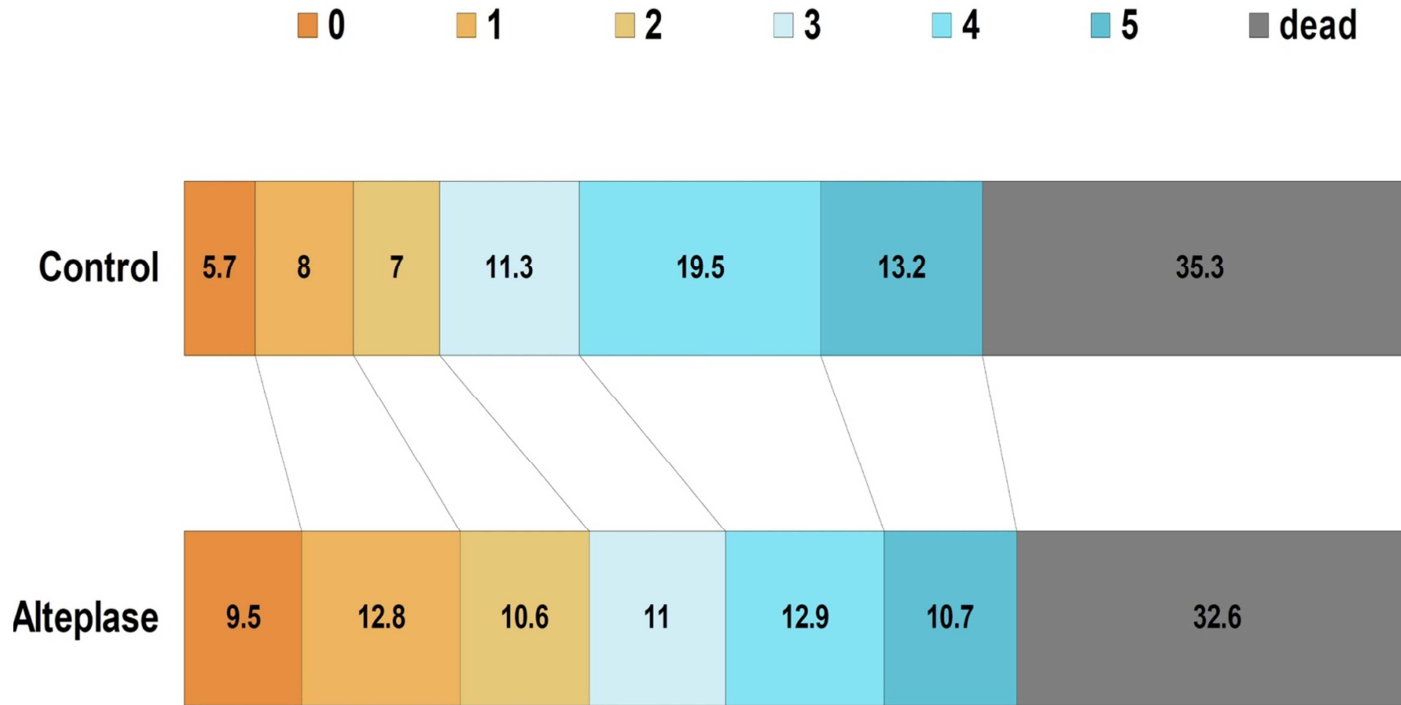


Figure 4-4 Outcomes after thrombolytic therapy amongst patients older than 80 years

Scores on modified Rankin scale (from 0=no symptoms from stroke to 6=death) at three months between patients who underwent thrombolysis with alteplase and controls, indicating shift towards improved outcomes with thrombolysis. Numbers within coloured cells are percentages. Total patients in alteplase arm 2235 and control 1237.

mRS distributions at 90 days were significantly better amongst thrombolysis patients than controls within each decile of age from 40 years to 90 years and, except amongst the small samples of patients younger than 30 years and older than 90 years, point estimates for the adjusted odds ratios were consistent across all age groups. (Figure 4-5)

Functional outcome measured by mRS 0-1, mRS 0-2 and survivor analysis showed similar results in favour of thrombolysis. (Figure 4-6, 4-7 and 4-8)

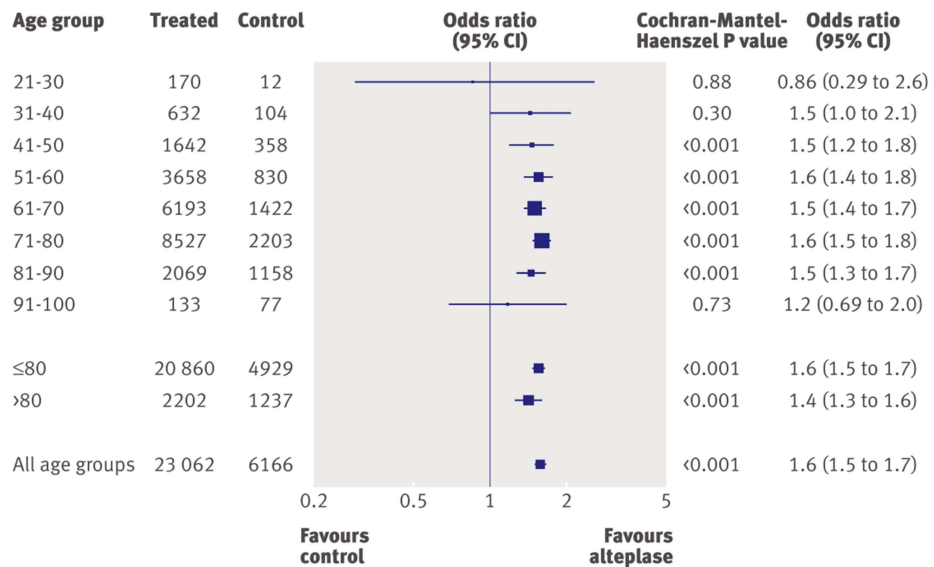


Figure 4-5 Shift towards better outcomes on mRS at 90 days adjusted for age and baseline severity

Number of patients shown for age groups do not add up to 29,228 because numbers of patients ages <21 (n=38) and >100 (n=2) were too low to allow any comparison. All patients aged <21 were from SITS and underwent thrombolysis; 15 patients reached a 90 day modified Rankin score of 0, 10 patients attained a score of 1, eight patients reached a score of 2, one patient achieved a score of 3, and two a score of 4; two died. Two patients aged 101 did not undergo thrombolysis in VISTA neuroprotection trials; they achieved modified Rankin score of 0 and 4 at 90 days

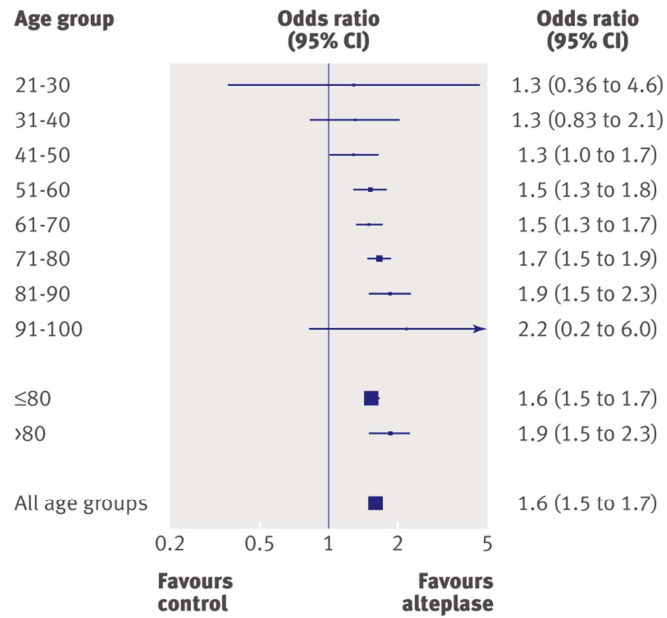


Figure 4-6 Age and baseline severity adjusted odds for the mRS score 0-1 at 90 days amongst patients treated with alteplase

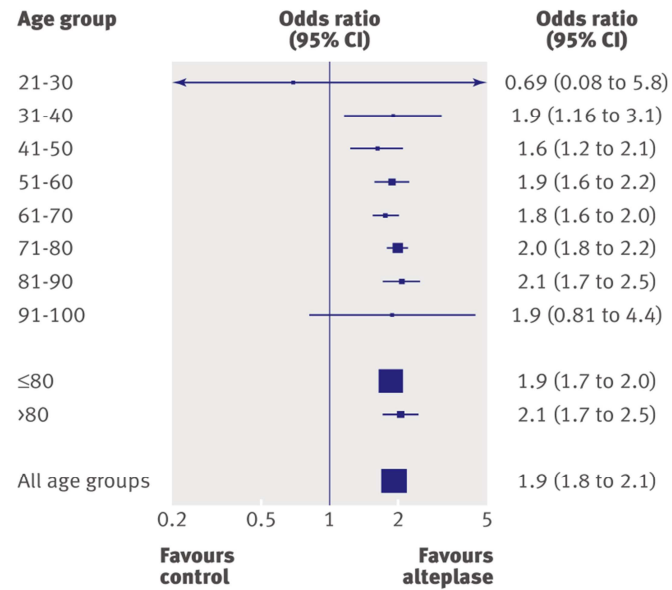


Figure 4-7 Age and baseline severity adjusted odds for the mRS score 0-2 at 90 days amongst patients treated with alteplase

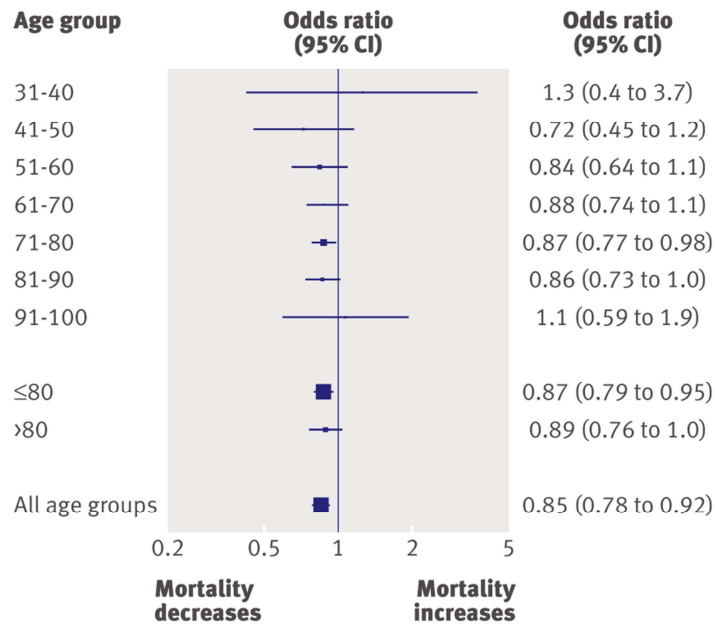


Figure 4-8 Age and baseline severity adjusted odds for mortality amongst patients treated with alteplase

4.3.5 Post Thrombolysis Intracerebral Haemorrhage

The symptomatic intracerebral haemorrhage (SICH) rate per SITS definition (4 or more point increase in NIHSS from baseline or death within 24 hours and PH2 or PHr2 haemorrhage at 22-36h imaging scans) was 2.5% (54/2163) among >80 years compared to 1.9% (398/ 20759) among ≤80 years, and thus insignificantly higher (OR 1.31, 95%CI 0.96-1.75, $p= 0.07$). The corresponding rates for SICH per NINDS definition (any increase in NIHSS from baseline and any parenchymal intracerebral haemorrhage) were significantly higher, 11.0% (229/ 2087) vs. 8.3% (1670/ 20220), (OR1.37, 95%CI 1.18-1.58, $p<0.0001$).

4.3.6 Onset to Treatment Time

The stroke onset to treatment time (OTT) of the administration of thrombolytic therapy to patients in SITS-ISTR was calculated. The median OTT was similar, 145 minutes ($p= 0.25$), between younger (≤ 80 years) and older (>80 years). Data on OTT for thrombolytic therapy were not collected in VISTA.

4.4 Discussion

Comparing patients from SITS-ISTR who were treated with alteplase at an average of 145 minutes after stroke onset against controls from VISTA who received no alteplase a more favourable outcome across the entire range of modified Rankin Scores with alteplase is observed (Cochran-Mantel-Haenszel $p < 0.0001$; Odds Ratio: 1.59 CI: 1.51 to 1.67). The nature and extent of this effect of alteplase is comparable to results from pooled analysis of randomised controlled trials, confirming the validity of the controlled but nonrandomised analysis.²⁸ I could therefore examine outcomes separately among nonelderly patients (aged ≤ 80 years) and elderly patients (age group > 80 years). In each subgroup a more favourable functional outcome was noted: Odds Ratios 1.6 (95%CI: 1.5 to 1.7), $N=25789$ and 1.4 (95%CI: 1.26 to 1.63), $[N=3439]$ respectively.

Extending the analysis to smaller subgroups of age, I found independently significant benefits from alteplase in each 10-year age range from 40 to 90 years. I found no interaction of age with alteplase efficacy and across the full age range from under 20 years to over 100 years. Only at under 30 years did the trend not favour outcomes after alteplase use.

In summary, I show that association between thrombolysis treatment and outcome is maintained in all patients, even in the elderly regardless of generally poorer outcomes in these age groups.

The analysis of SITS-VISTA data is based on almost 30,000 patients and confirms that improved outcomes occur after acute ischaemic stroke among patients who are offered thrombolytic therapy. The extent of the apparent benefit matches that from published randomised trials. These observations extend to the elderly age groups and it is only in a small group of patients in age group 91 to 100 (137 patients in alteplase group and 77 in the non thrombolysed group) that it fails to achieve a statistical significance where the point estimates are consistent but confidence intervals are wide. The point estimates for improved outcomes in age group 91 to 100 are also consistent with the published data.^{131,315120 247}

I undertook the primary analyses employing “shift analysis”, an analytical approach accepted by the European Drug Licensing agency.²⁸⁴ The Cochran-Mantel-Haenszel test is a non-parametric approach that avoids invoking an assumption of a common odds ratio (i.e. proportionality) across all cut points on the ordinal outcome scale. It provides a conservative estimate of statistical significance. Because it does not express the extent of the association, I also applied an ordinal logistic regression analysis to estimate a common odds ratio across modified Rankin scale categories. Again, I found significantly better outcomes, though the proportionality assumption was not satisfied. Whereas a non-significant test for proportionality would imply that common odds may be assumed, the converse does not necessarily apply.²⁸² The proportionality assumption test may be over-sensitive when applied to large sample sizes.³¹⁶ Further, it is a global test that cannot differentiate the heterogeneity resulting from alteplase or other covariates³¹⁶ The sample sizes were large and so I may still be justified in using the odds estimated from ordinal logistic regression. Even so, for final confirmation we tested using a less powerful dichotomised approach.^{272,273} With all three methods I reach similar conclusions.

There were improved outcomes among thrombolysis patients belonging to age groups encompassing 31 to 90 years. Analysis did not show improved outcomes for patients aged below 30 and above 90 years, but the small number of patients in these groups greatly reduced statistical power for these analyses and the trends mostly followed the same pattern as for intermediate ages.

I chose age and baseline NIHSS for adjusted analysis mainly because of their established roles of influence on stroke outcomes.²⁶⁵ However, I also undertook sensitivity analysis adjusting for differences in age, sex, history of either diabetes or previous stroke, use of prior antithrombotic, baseline National Institutes of Health Stroke Scale score and hypertension between the thrombolysed and non-thrombolysed groups.²⁷⁸ The adjusted analyses for these variables confirmed significant findings for improved outcomes from thrombolysis regardless of age.

The baseline demographic characteristics for the complete dataset give an advantage to the thrombolysis group. This influence does not extend to patients

aged >80 years, however. As a result, though the estimates of overall effect of alteplase may be biased, the relative differences between subgroups should remain reliable. I did not match patients by co-morbidity score or baseline functional status, which may be considered a limitation. However, premorbid functional status is difficult to establish reliably in stroke and the strong influence of baseline severity on outcome, for which I did adjust, is known and is discussed in chapter 7. A corresponding analysis examining the influence of baseline severity on outcomes in SITS and VISTA data is underway.³¹⁷

The conclusions derive merit from having been based on a huge patient population who were treated in routine clinical practice [N = 28136] and compared against controls from rigorously conducted neuroprotection trials: any bias in quality of care should favour the control group. The limitation of SITS-ISTR data has been discussed extensively in previous publications.^{146,147,229,318} In short, SITS-ISTR is a registry and therefore, it is impossible to guarantee completeness of inclusions and to exclude selection bias.^{146,147,229} The SITS-MOST publication reports: *“Sample source data verification was done by professional monitors working with national coordinators in collaboration with the study sponsor (Boehringer Ingelheim). A minimum of 10% of patients recruited in SITS-MOST was monitored. In the UK, source data verification was done by independent clinical staff under the direction of the UK national coordinators. This monitoring also checked for completeness of registrations at all sites.”*¹⁴⁶ The SITS-MOST investigators also report that: *“Incomplete data entries could be saved for later updating, but data were not deemed to be complete or included in report generation unless confirmed by the investigators by electronic signature. To be included in the SITS-MOST cohort, all baseline data entry had first to be confirmed by the investigator. SITS-MOST report generation was updated every 24 h via an automatic statistical package, which displayed main outcome details, demographic and baseline statistics, logistic information, and a recruitment report with indication of complete and delayed data. Registered centres could review statistical details for their own centres, and compare with country statistics and with the total SITS-MOST dataset.”*¹⁴⁶ Finally, the SITS-MOST publication reports that *“in the UK, source data verification was done by independent clinical staff under the direction of K R Lees and G A Ford”*.¹⁴⁶

In SITS-MOST, individual investigators' results are not published, limiting the incentive for selection; in contrast, the sharing of total enrolment numbers may act as an incentive to be inclusive.¹¹⁵ Almost identical main outcomes in SITS-MOST¹⁴⁶ and RCTs¹³¹ after adjustment for baseline differences suggests that the influence from such potential bias is limited. Subsequent studies based on SITS-ISTR data also show the similar outcome for the overall study population as compared to the SITS-MOST.^{129,229} None of the neuroprotective agents used for the patients in the VISTA control group has an influence on outcome, and over half of the VISTA cohort received only placebo.

Because VISTA lacks data on repeat brain imaging among non-thrombolysed patients, I had no data on SICH in the control group. Therefore, I compared the SICH rates between over 80 years and \leq 80 years old patients only with SITS data. There was no difference in SICH rates between over 80 years and \leq 80 years as per SITS-MOST definition but slightly higher as per NINDS definition. In a complementary per-protocol analysis of SITS-ISTR data (i.e. patients' selection based on SITS-MOST criteria¹¹⁵ except for its age criterion), no significant difference in SICH rate was noted amongst very elderly patients compared to the younger cohort [per SITS 1.8% VS. 1.7%, $P=0.70$, adjusted odds ratio (aOR)=0.90 (95%CI 0.73 to 1.09)³⁰⁸ The SITS patients in the SITS-VISTA dataset are unselected and therefore SICH rates are slightly higher in the current study. Regardless, I now show that even if there were any more haemorrhages amongst elderly patients who receive thrombolysis, based on a conservative definition, there appears to be no adverse influence on the distribution of outcomes. In fact, one observes a beneficial effect on mortality of these patients. Others have concluded that factors such as comorbidity rather than alteplase use are responsible for the observed increase in late case fatality among the elderly.³¹⁹⁻

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The present analyses reach the same conclusions as analyses employing VISTA data, as shown in previous chapter, or the limited pooled randomised trial data in the elderly (supplemental data).³¹¹ Elderly patients treated with thrombolysis in trials reported from VISTA in the previous chapter (N= 5817) had significantly better adjusted outcomes than nonthrombolysed comparators ($p=0.002$; Odds Ratio=1.34, 95%CI 1.05 to 1.70).³¹¹ Elderly patients treated in the pooled

randomised trials showed a trend towards better adjusted outcomes (modified Rankin score 0 to 2 versus 3 to 6) than non thrombolysed comparators (Odds Ratio 1.77; 95% CI 0.73-4.25, N=137). Previous studies have shown findings consistent with results in present chapter, but on very small data sets.^{290,292,297,315,320,322,323} Despite these points, treatment allocation in this study was not randomised. More extensive randomised controlled trial data could more conclusively answer this question. Two trials currently aim to examine this topic.^{232,312} There is an Italian trial that has so far enrolled around 10% of the planned 600 patients, over a 2 year period.³¹² A trial from UK, International Stroke Trial-3 [IST-3], aims to examine outcomes amongst all patients who receive thrombolytic therapy and has no upper age limit prescribed in its inclusion criteria.^{232,234,313} Over more than a decade, the trial has enrolled around 3035 patients from the originally planned 6000, 1756 patients are very elderly and being treated within the time window of interest.²³³

In this analysis, patients who were treated with intravenous alteplase had better outcomes than their untreated comparators, and this effect was not dependent on their age. In particular, patients aged over 80 years derived similar benefit from treatment as younger patients. The weight of evidence to date indicates a potential for benefit in the elderly and there is no *a priori* reason to suspect a diminished effect compared to the non-elderly. Furthermore there are reassuring safety data on the risk of intracerebral haemorrhage. It may be concluded that clinical treatment guidelines should be revised to remove the age restriction in use of intravenous alteplase for acute ischaemic stroke. Age alone should not be a barrier to treatment.

Chapter 5

**Presence of Diabetes and Previous Stroke in
Acute Ischaemic Stroke Patients: Is it a valid
exclusion criterion?**

5 Presence of Diabetes and Previous Stroke in Acute Ischaemic Stroke Patients: Is it a valid exclusion criterion?

5.1 Introduction

The European Medicines Agency (EMA) recommends that the ischaemic stroke patients suffering from hypo- or hyperglycaemia (blood glucose levels at baseline <50 and > 400 mg/dl respectively) should be excluded from receiving the thrombolytic therapy.²³ EMA also recommends that if a patient suffering from the onset of new ischaemic stroke has diabetes and a prior stroke, he should not be thrombolysed with the t-PA.²³ These recommendations often put a stroke physician into a difficult situation. This is because the hyperglycaemia occurs in about 70% of the ischaemic stroke patients and only 20% of these patients report suffering from diabetes mellitus.^{324,325} Hyperglycaemia at baseline may be associated with the diabetes (uncontrolled or unrecognised diabetes or poor glucose tolerance) or it may be a response to an acute stress (stress hyperglycaemia).³²⁵ It should be noted that, though overlapping, true diabetes and post-stroke hyperglycaemia are distinct entities.³²⁶ E Melamed describes transitory reactive hyperglycaemia in about a third of ischaemic stroke patients who had no history of diabetes mellitus and also in those who had history of diabetes prior to stroke.³²⁷ The following reasons have been attributed to the hyperglycaemic reaction to the acute ischaemic stroke: “a non-specific reaction to acute stress and tissue injury with the associated autonomic, hormonal and metabolic alterations; uncovering of underlying latent diabetes by the acute stroke; increased secretion of growth hormone due to stroke-induced hypothalamic dysfunction; and irritation of the glucose regulatory centres in the hypothalamus and brain stem by blood-laden cerebrospinal fluid or local ischaemia”.³²⁷

The thrombolytic therapy is the only proven therapy known to enhance outcomes in ischaemic stroke patients.^{122,129} Its mechanism involves a recanalisation and a rapid reperfusion of the critically hypoperfused brain tissue, which in turn restores the function of ischaemic brain parenchyma.^{122,328} Reperfusion induced improvement in outcomes depend on local factors in the ischaemic brain tissue such as the extent of neuronal damage, cerebral perfusion pressure (systemic blood pressure), oxidative stress, intracellular acidosis and mitochondrial dysfunction.^{328,329} Animal studies indicate that, following a cerebral ischaemia, hyperglycaemia enhances the super-oxide anion production, the local acidosis and the production of the advanced glycation end products that are damaging to the blood vessels.^{330,331,328,332-335} Superoxide production may predispose the brain to greater risk of blood brain disruption and thereby intracerebral haemorrhage.³³⁶⁻³³⁸ A systematic review of the animal studies that examined association of the infarct volume with the hyperglycaemia in the ischaemic stroke animal models (streptozotocin induced hyperglycaemia models, N=303 animals and dextrose induced hyperglycaemia models, N=356 animals), hyperglycaemia was associated with a 94% larger infarct size.³²⁵ Infarct volume in streptozotocin model was greater than the infarct volume in dextrose model (140% vs. 48% increase).³²⁵ However, in seven studies, there were non-significant reduction of infarct size if insulin were used.³²⁵ The authors concluded: “Although hyperglycaemia exacerbates infarct volume in MCAO models, studies are heterogeneous, and do not address the common clinical problem of post stroke hyperglycaemia because they have used either the streptozotocin model of type I diabetes or extremely high glucose loads. Insulin had a nonsignificant and significantly heterogeneous effect. Further studies with relevant models may inform clinical trial design.”³²⁵

Because post-stroke hyperglycaemia results in poor outcomes, trialists investigated the outcomes after treatment with the insulin in the patients having post-stroke hyperglycaemia. A meta-analysis of randomised controlled trials (16 studies, 2459 patients) showed that the use of insulin in these patients does not affect the mortality (OR 1.1, 95% CI 0.9 to 1.5, p=0.29, n= 1236) or the favourable functional outcome (OR 1.03, 95% CI 0.7 to 1.5, p=0.88, n=1217), and instead, may put the patient to the risk of hypoglycaemia (range: 8% to 80%).³³⁹

Clinical studies have largely shown adverse influence of hyperglycaemia on the outcomes of non-lacunar ischaemic stroke patients.^{328,329,340} Poorer response to thrombolysis in these patients is considered to be a result of increased plasminogen activator inhibitor-1 activity, resistance to antithrombotic agents, excessive up regulation of metalloproteinase and higher prevalence of atherosclerosis.^{328,340-342} Stroke recurrence is another major problem encountered in clinical practice, and prior stroke in patient is suspected to reduce the benefits attainable from thrombolytic therapy.³⁴³ Because these patients are already on antithrombotic therapy, several clinicians mostly worry about haemorrhagic complications when administering the thrombolytic therapy. An analysis of patients who were treated during 0-6 hours of stroke onset (N=2184) enrolled in 5 thrombolysis trials^{122,124,127,128} has shown that a previous stroke and diabetes mellitus may predict poor outcome (p for t-PA-prior stroke interaction <0.05; p for diabetes <0.0001, p for prior stroke= 0.06).³⁴³

Whereas the clinical trials have shown improved outcomes amongst patients who receive alteplase for acute ischaemic stroke, t-PA was granted marketing authorisation in Europe in 2002 and patients with prior stroke and concomitant diabetes mellitus were excluded from the approval.²³ The basis for this restriction is described in the review documents: “The therapeutic benefit is reduced in patients that had a prior stroke or in whom an uncontrolled diabetes is known, thus the benefit/risk ratio is considered less favourable, but still positive in these patients.”²³ These were based on unpublished results of data analysed by regulatory authorities. Hence, ECASS III trialists excluded patients having diabetes mellitus or a history of stroke when designing ECASS III study.²³ As per the recent press release of Boehringer Ingelheim dated 4th November,2011, “Actilyse® has been approved through a mutual recognition

I published Chapter 5 in Diabetes Care (Mishra NK, Davis SM, Kaste M, Lees KR; VISTA Collaboration. Comparison of outcomes following thrombolytic therapy among patients with prior stroke and diabetes in the Virtual International Stroke Trials Archive (VISTA). *Diabetes Care*. 2010 Dec;33(12):2531-7. Epub 2010 Sep 15.) As per *Diabetes Care* “The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.” Further see <http://creativecommons.org/licenses/by-nc-nd/3.0/>

procedure for 15 European countries* for thrombolytic treatment of acute ischaemic stroke up to 4.5 hours of onset of stroke symptoms and after prior exclusion of intracranial haemorrhage.”³⁴⁴ Now, the European drug labelling continues to retain those exclusion criteria that have not been formally tested.²³ For example, the use of rt-PA in acute stroke is not recommended in Europe amongst patients who suffer from concomitant presence of diabetes mellitus and previous stroke.²³ Regardless, physicians still treat these patients in routine clinical practice.³⁴⁵ In the current scenario, an ideal approach would be to conduct a randomised controlled trial and examine the influence of thrombolytic therapy on outcomes of these patients. But, running a clinical trial for this subgroup would not be feasible. Patient recruitment in stroke trials takes a long time. The IST-3 trialists started with an enrolment target of 6000 patients and have so far been able to enrol only 3035 patients.²³⁴ Further, these patients constitute a small proportion of all thrombolysed patients. Within SITS-ISTR, of all the patients that were recruited between December 2002 and November 2009 (N=23062), only 602 patients had suffered from both diabetes and previous stroke. To the best of my knowledge, there is not any plan of any trial group to conduct such a trial. Therefore, I planned for an alternative approach. Similar to the method adopted in chapter 3, I decided to examine outcomes of patients who received rt-PA against those who did not, from the data of the neuroprotection trials conducted between 1998-2007; and obtained these data from Virtual International Stroke Trials Archive [VISTA]. Employing an analytical approach recommended by EMEA, I compared outcomes amongst patients having diabetes, previous strokes and a combination thereof.²⁸⁴

5.2 Methods

5.2.1 Data Source and patients

I collated the demographics, clinical data and measures of functional outcome from neuroprotection trials conducted in the period 1998 to 2007, held within the Virtual International Stroke Trials Archive, VISTA. (See Chapter 2)

5.2.2 Statistical analysis

I compared outcome between patients who received thrombolysis and non-thrombolysed controls amongst patients who had diabetes, prior stroke or their combination. For each contrast, I compared the overall distribution of all seven categories of day 90 mRS scores of the two groups, i.e. from 0 (asymptomatic) through 5 (bedbound and completely dependent), to 6 (dead), and also NIHSS categories. For analysis of the supporting endpoint, NIHSS, I grouped adjacent scores into categories: 0 (no measurable deficit), 1-4, 5-8, 9-12, 13-16, 17-20, 21-24, ≥ 25 (most severe neurological deficit) or dead. To test for a significant association of outcome distribution with thrombolysis exposure I used the Cochran-Mantel-Haenszel [CMH] statistic, adjusting for both age and baseline NIHSS as continuous variables. Because CMH test does not express the extent of the association, I also applied logistic regression analysis, also adjusted for age and baseline NIHSS, to estimate the odds ratio under the assumption of proportional odds and its associated 95% confidence interval. I also undertook a sensitivity analysis by considering the combined effect of the variables that differed significantly at baseline, but if this resulted in excessive diminution of study sample, I did not quote the findings. The objective was mainly to undertake ordinal distribution or “shift” analysis. I also present dichotomised analyses of mRS, based on excellent outcome (mRS 0-1), favourable outcome (mRS 0-2), and survival; these analyses were expressed as odds ratios adjusted for age and bNIHSS, as for the primary and secondary endpoints. In order to remain consistent with the current age criteria of the EMEA, I examined if the interaction of age with t-PA had influence over outcomes; and further, estimated CMH p and proportional odds by ordinal logistic regression analysis for patients belonging to age group ≤ 80 .

Odds ratios in the ordinal analysis express the common odds of an improved distribution of outcome in association with alteplase treatment. Reliable information on symptomatic haemorrhage was not available since post treatment imaging was not routinely applied in neuroprotection trials to patients who had not been treated with alteplase.

5.3 Results

5.3.1 Patient sample

Complete data were available for analysis of mRS in 5817 patients and on NIHSS in 5711 (see figure 5.1). Baseline characteristics are shown in table 5.1.

5.3.2 Analysis of outcomes

I have shown the findings on the analyses of outcomes in patients having diabetes or no diabetes, outcomes in patients having previous stroke or no previous stroke and the outcomes in patients having concomitant diabetes and previous stroke or their absence in figures 5-2 to 5-6.

In a proportional logistic regression analysis adjusting for age and baseline NIHSS, we did not find any interaction of diabetes [$p=0.49$], prior stroke [$p=0.72$] and diabetes and prior stroke [$p=0.8$] with use of rt-PA. For each of the analyses above, I also considered the subgroup of patients aged ≤ 80 years; and also sought evidence of any interaction with age. I failed to find evidence of a differential effect or interaction with age.

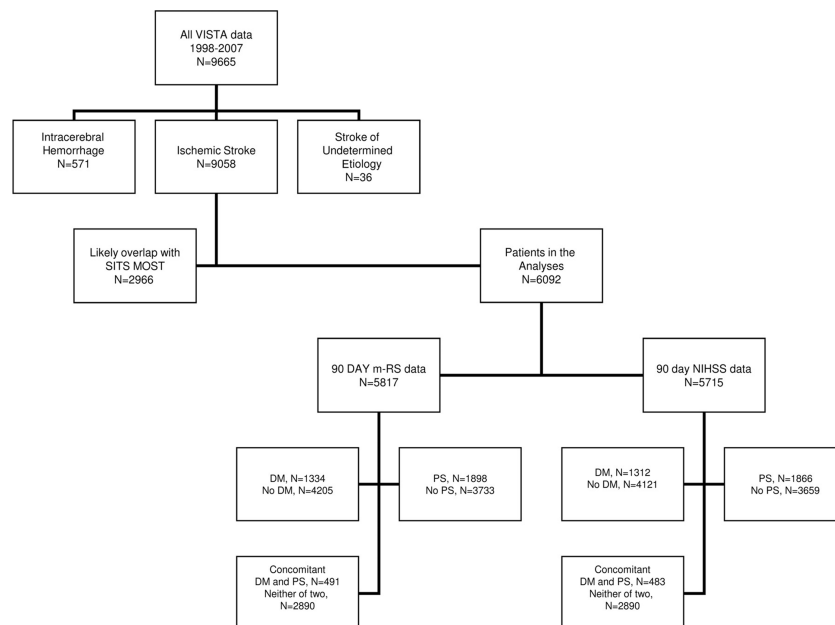


Figure 5-1 Flow chart showing the description of the VISTA data employed for present analyses

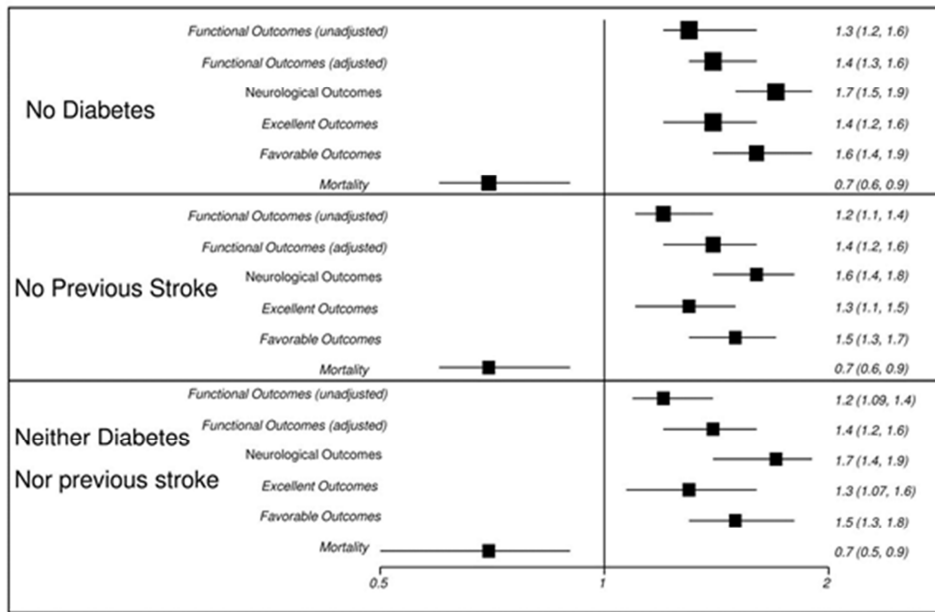


Figure 5-2 Outcomes in approved population (i.e. patients having absence of diabetes or previous stroke or both)

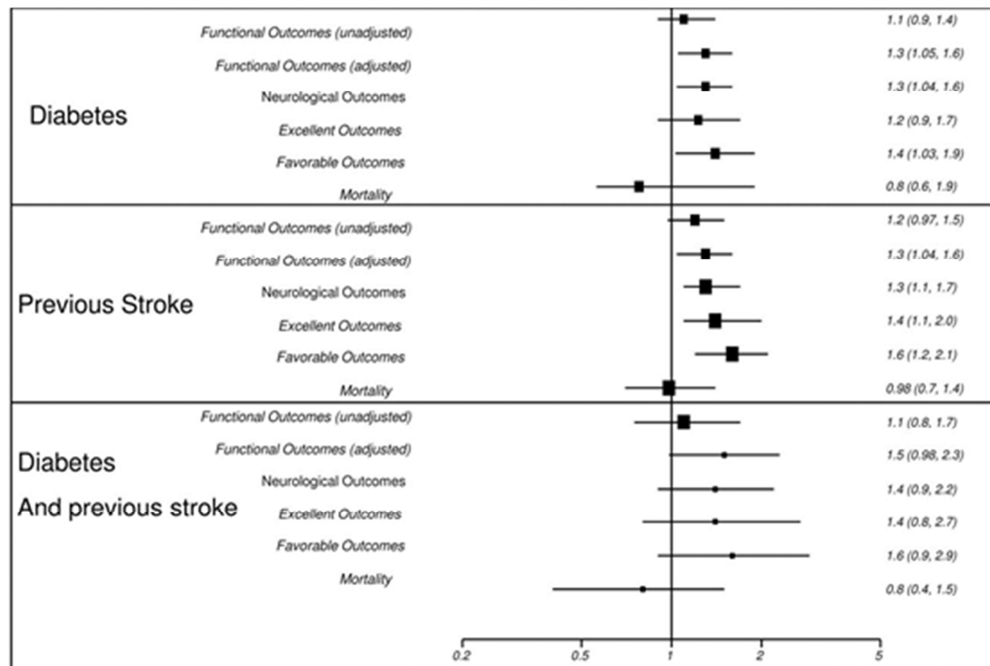


Figure 5-3 Outcomes in patients population having diabetes prior stroke or both

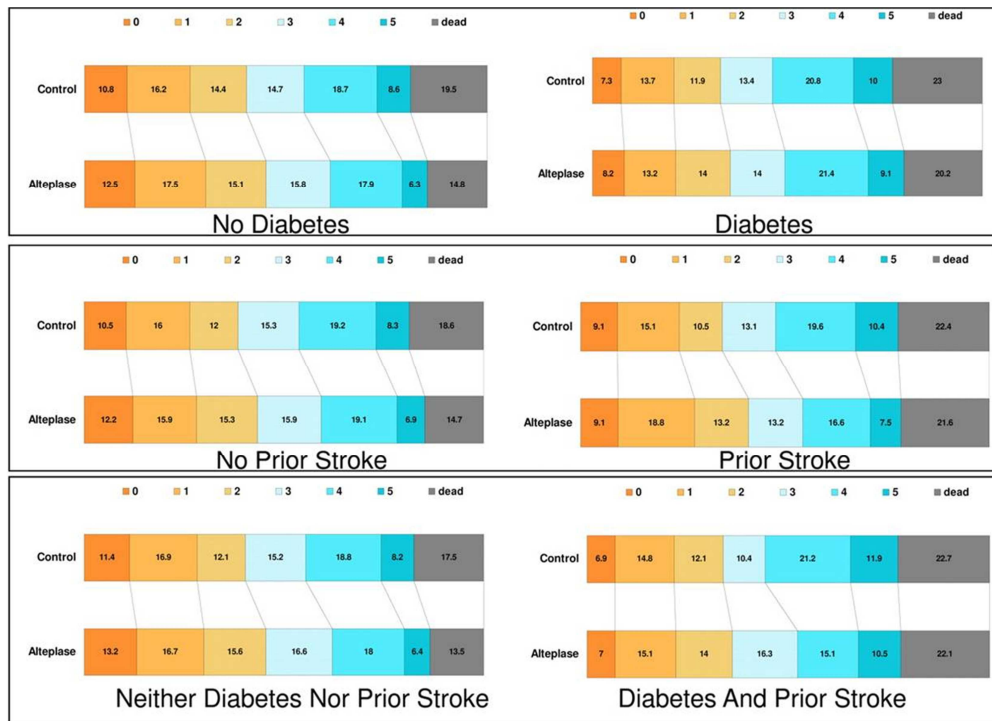


Figure 5-4 Figure shows outcomes in patients receiving alteplase or no alteplase in subgroups of patients belonging to subgroups diabetes or no diabetes, previous stroke or no previous stroke and presence of diabetes and previous stroke together and absence thereof

Table 5-1 Baseline characteristics of patients enrolled in the VISTA trials

	Group of diabetic patients		Group of prior stroke patients		Group of concomitant diabetes and prior stroke*	
	Alteplase	No alteplase	Alteplase	No alteplase	Alteplase	No alteplase
Age**						
Mean	69.5 (10.9), n = 342	70.3 (10.8), n = 992	70.6 (12.6), n = 319	73.2 (10.9), n = 1,579	71.2 (10), n = 86	71.8 (9.8), n = 405
Median	71 (33–98)	72 (30–96)	73 (21–98)	75 (30–101)	73 (46–98)	73 (37–90)
	P = 0.2		P = 0.0002		P = 0.6	
Sex (male)	198/342 (57.9%)	532/992 (53.6%)	180/319 (56.4%)	822/1,579 (52.1%)	54/86 (62.8%)	212/405 (52.3%)
	P = 0.17		P = 0.2		P = 0.07	
Baseline NIHSS	13 (5–30)	13 (2–29)	13 (3–30)	12 (2–37)	13.5 (5–30)	12 (2–29)
	P = 0.01		P = 0.02		P = 0.03	
Atrial fibrillation	82/342 (24%)	256/992 (34.5%)	82/312 (26.3%)	501/1,494 (33.5%)	18/86 (20.9%)	105/405 (25.9%)
	P < 0.05		P = 0.009		P = 0.3	
Hypertension	278/342 (81.3%)	795/992 (80.1%)	240/312 (76.9%)	1,162/1,494 (77.8%)	76/86 (88.4%)	332/405 (82%)
	P = 0.6		P = 0.7		P = 0.2	
Prior use of antiplatelets	120/241 (49.8%)	145/348 (41.7%)	119/186 (64%)	168/349 (48.1%)	37/51 (72.5%)	62/105 (59.0%)
	P = 0.053		P = 0.001		P = 0.09	
Prior anticoagulants	17/241 (7.1%)	44/348 (12.6%)	18/186 (9.7%)	77/349 (22.1%)	5/51 (9.8%)	20/105 (19.0%)
	P = 0.03		P < 0.05		P = 0.1	
Use of antithrombotics	129/241 (53.5%)	173/348 (49.7%)	128/186 (68.8%)	225/349 (64.5%)	38/51 (74.5%)	72/105 (68.6%)
	P = 0.04		P = 0.3		P = 0.4	
Myocardial infarction	94/342 (27.5%)	202/992 (20.4%)	79/312 (25.3%)	299/1,494 (20%)	26/86 (30.2%)	87/405 (21.5%)
	P = 0.009		P = 0.047		P = 0.1	
Congestive cardiac failure	46/272 (16.9%)	50/374 (13.4%)	40/197 (20.3%)	45/359 (12.5%)	11/53 (20.7%)	13/110 (11.8%)
	P = 0.2		P = 0.02		P = 0.2	

P value refers to the comparison between the treated and nontreated group corresponding to each variable. *The group comprising diabetes and prior stroke was compared specifically against those patients who had neither of the two factors. **Age is described with mean and SD (above) and median (range) (below).

5.4 Discussion

The present analysis reconfirms the occurrence of improved outcomes amongst patients who do not have diabetes and prior stroke, with significant odds of 1.4. These odds were comparable to the odds obtained from large thrombolysis trials,¹³¹ suggesting outcomes following thrombolysis are similar in these patients to those seen in the trials. The present analysis shows a significant association of improved outcomes with the use of thrombolytic therapy in patients having diabetes or prior stroke. However, I failed to confirm statistical significance for a small group of patients that concomitantly had previous stroke and diabetes [OR 1.5, 95% CI: 0.98-2.3]. I attribute this to a type 2 error in this smaller subgroup of sample size, N=491. The findings from shift analyses were also supported by similar results from the analyses of dichotomised outcomes. It should be noted that the European license restrictions for the use of t-PA in ischaemic stroke includes the subgroup of patients that have concomitant presence of diabetes and previous stroke (and not “only diabetes” or “only prior stroke”).²³ The analysis failed to show significant odds ratios at $p=0.05$ for this particular subgroup of patients.

These findings can be generalised not only because of the comparable odds to those obtained from the large trials data¹³¹ but also because these results were replicated on another analysis involving the comparison of thrombolysed patients [in SITS dataset] with the non-thrombolysed controls [from VISTA] (reported in chapter 6). In addition, I was informed of supportive findings that were available from the analyses of a collaborator, Prof Markku Kaste at Helsinki Thrombolysis Register. In their data, from 1200 consecutively treated patients, complete information was available for 1104 patients, 51% patients were treated despite licence contraindications. The analysis of 26 patients aged ≤ 80 years who had both DM and previous stroke suggested poorer favourable outcomes (mRS 0-2) in univariate analysis when compared to those treated according to the European licence of alteplase; but, not in multivariate analysis. These patients had more symptomatic intracerebral haemorrhages than those treated on-label in univariate analysis but 95% CI was not significant and in a multivariate analysis there was no trend for symptomatic haemorrhage. I could

not however examine the symptomatic brain haemorrhages in the VISTA patients, as these data were derived from neuroprotection trials, which had not routinely obtained imaging information of the untreated patients.

I based the conclusions on adjusted analyses, adjusting for age and baseline NIHSS, because prognostic variables differed at baseline; and, age and baseline severity have an established influence on stroke outcomes.¹³¹ However, I could not adjust for all variables, as data were incomplete. Despite robust analyses and adjustment for important variables, it needs to be acknowledged that a randomised study would have the best design. But, there are no such studies conducted yet, or are on-going. The present study is the only analysis that is based on the largest dataset on thrombolysis patients available up to the present date.(except the data that are presented in the following chapter). It employs an analytic approach recommended by EMEA, and replicates findings of functional outcomes on neurological outcomes.

It is known that patients with pre-morbid mRS >1 were excluded from the VISTA trials, but the pre-stroke functional status of individual patients was not recorded in VISTA. Hence, it is likely that any effect of pre-stroke disability in the patients having prior stroke(s) would only lead to an underestimation treatment effect in the analyses.

I acknowledge that there could be differences on outcomes owing to centre specific effects on treatment of patients. This would however occur if the patients were part of routine clinical care, and not enrolled in a rigorously controlled clinical trial. Because the patients in VISTA were enrolled in neuroprotection clinical trials during 1998-2007, I assume that they all received best standard of care at each participating centre. 60% of VISTA patients were from American centres where the thrombolytic therapy was approved soon after the NINDS trial results were published in 1995.¹²² During the next years, even other centres [including European (16% patients)] practised thrombolytic therapy based on clinicians' choice.¹⁴⁶ In 2002, the alteplase was approved in Europe as well.²³ This would imply that all those stroke patients who were eligible for thrombolysis in the physician's opinion received therapy. However, I could not adjust for centres, as centre-specific information was missing in the dataset.

Country	Hyperglycaemia is treated if blood sugar levels above (in mmol/l)
Austria	8
Belgium	11
Denmark	12 ^a
Finland	8
Germany	11
Iceland	9
Italy	11
Norway	10
Portugal	11
Sweden	10
Switzerland	12
UK	12
Croatia	14 ^a
Cyprus	7.4 ^a
Czech Republic	7.4
Estonia	12
Hungary	8
Israel	—
Latvia	8
Poland	14
Slovenia	10 ^a
Turkey	11

Table 5-2 Acute stroke treatment with respect to hyperglycaemia at base line in European Federation of Neurological Society member states

("a" refers to national estimates. The other numbers are from the stroke panel member centre.)

(Source: Thomassen L, Brainin M, Demarin V, Grond M, Toni D, Venables GS; EFNS Task Force on Acute Neurological Stroke Care. Acute stroke treatment in Europe: a questionnaire-based survey on behalf of the EFNS Task Force on acute neurological stroke care. *Eur J Neurol*. 2003 May;10(3):199-204. Permissions obtained from John Wiley and Sons, License number: 2811330661886)

It should however be noted that the European centres/countries differ in their practices with regard to the treatment of hyperglycaemia at the stroke onset (table 5-2).³⁴⁶ For example, some centres employ insulin infusion to control blood sugars when the patient presents with stroke with hyperglycaemia. Physicians may differ in their choice of drugs for the secondary prevention of diabetes during the follow-up period. These variables are likely to influence the outcomes on day 90. It can be guessed that the participating centres in the neuroprotection trials would have been inconsistent with regard to the recommendations for the control of hyperglycaemia at stroke onset and the subsequent secondary prevention treatments. Treatments are likely to have been different between the individual studies. In conclusion, the differences arising from the varying practices across the participating centres in the neuroprotection trials would have resulted in bias in my present study. Further because the data lack in the information on specific centre specific clinical practices with regard to control of hyperglycaemia, I could not control for the resulting bias.

In the present analysis, the 95% confidence interval for improved outcomes were 0.98 to 2.3 and marginally missed the level of significance at $p=0.05$. One must consider that analyses like these are based on probabilistic models where level of significance is arbitrarily chosen (and conventionally set at 5%), and decision to thrombolysis is left to the clinicians who determine their own limits regarding taking chances whilst offering the therapy. The group of patients having concomitant diabetes and previous strokes missed statistical significance, but had a favourable point estimate of 1.5. Had I considered a higher level of significance, the results would have been significant for outcomes amongst all subgroups of patients.

In summary, the analysis shows improved outcomes amongst patients with diabetes, prior stroke and those with diabetes and/or prior stroke; though marginally failed to show a statistically significant improvement in the patients who had both diabetes and prior stroke together. A potential benefit might exist for the combined group too, and withholding a proven therapy amongst these patients would not be justified. Treatment may be offered to a carefully

selected patient, and trialists should now be encouraged to consider examining these patients by conducting a randomised controlled trial. Finally, it can be recommended that withholding treatment from these patients would be unjustified.

Chapter 6

Presence of Diabetes and Previous Stroke in Acute Ischaemic Stroke Patients

A validation study to confirm findings from VISTA data

6 Presence of Diabetes and Previous Stroke in Acute Ischaemic Stroke Patients: A validation study to confirm findings from VISTA data

6.1 Introduction

Intravenous alteplase administration is a proven treatment for acute ischaemic stroke.^{122,129} In Europe, it was granted marketing authorisation in 2002 but patients with prior stroke and concomitant diabetes mellitus were excluded from approval.²³ The basis for this restriction is described in the review documents: “The therapeutic benefit is reduced in patients that had a prior stroke or in whom an uncontrolled diabetes is known, thus the benefit/risk ratio is considered less favourable, but still positive in these patients.”²³ Because of this restriction, the EMEA-mandated third European Cooperative Acute Stroke Study, ECASS-III, was required to exclude patients with prior stroke and concomitant diabetes from its protocol. In routine clinical practice many such patients are treated but others will be excluded due to confusion over the evidence.¹²⁹ In chapter 5, I reported the findings from data held within VISTA and suggested that the exclusion of patients based only on the basis of the presence of diabetes and previous stroke would be unjustified.

In the present chapter, I describe the findings from another controlled comparison, in which I examine thrombolysed patients from SITS-ISTR compared against control patients within VISTA. Here, I examine the treatment effect of intravenous alteplase in patients with diabetes, prior stroke and their combination, in order to clarify the validity of restriction on the latter group. For EMEA’s restriction to be valid, one ought to find that the benefit/risk would be diminished independently in each sub-group, or at least diminished within the combined group due to an interaction of diabetes with prior stroke.

6.2 Methods

6.2.1 Data source and patients

I collated the data of stroke patients who received thrombolytic therapy through the SITS-ISTR between December 2002 and November 2009, and the controls were derived from the non-thrombolysed control stroke patients from the neuroprotection trials of the duration 1998 to 2007 held within the Virtual International Stroke Trials Archive, VISTA (www.vistacollaboration.org)

6.2.2 Patient Sample

Patient sample employed for present analyses are shown in figure 2.1.

6.2.3 Statistical analysis

I compared the outcomes between patients who received thrombolysis and non-thrombolysed controls amongst patients who had diabetes, prior stroke or both together. For each contrast, I compared the overall distribution of all seven categories of day 90 mRS scores of the two groups, i.e. from 0 (asymptomatic) through 5 (bedbound and completely dependent), to 6 (dead). To test for a significant association of outcome distribution with thrombolysis exposure I used the Cochran-Mantel-Haenszel [CMH] statistic, adjusting for both age and baseline NIHSS. Then I also employed proportional odds logistic regression analyses in order to calculate age and baseline stroke severity adjusted odds for better outcomes in each subgroup of patients' population. Then, I undertook a sensitivity analysis by considering the combined effect of the variables that differed significantly at baseline (at $p < 0.05$). Adjusted analysis was expected to result in reduction of number of patients, owing to missing data for certain

I published Chapter 6 in Neurology (N.K.Mishra, N.Ahmed A.Davalos H.K.Iversen L.Soinne N.Wahlgren K.R.Lees Post thrombolysis outcomes amongst patients with prior stroke and diabetes: controlled comparison of SITS international registry (SITS-ISTR) versus Virtual International Stroke Trials Archive (VISTA) Neurology, 2011;77:1866-1872.) Permissions were obtained from Wolters Kluwer Health, License Number 2790960994499.

variables. Hence, when reporting results, I also quote the corresponding sample size.

In addition to calculating the proportional odds ratios (that express the common odds of an improved distribution of outcome in association with alteplase treatment), I also undertook an interaction test between the alteplase and presence of diabetes, previous stroke or both. Reliable information on symptomatic haemorrhage was not available since post treatment imaging was not routinely applied in neuroprotection trials to patients who had not been treated with alteplase.

6.3 Results

6.3.1 Baseline demographics

The data comprised 29500 patients for this analysis. Data on baseline NIHSS for another 272 patients were missing and had to be excluded leaving 29228 patients whilst undertaking the baseline severity adjusted analysis (Figure 2.1).²⁷¹ The baseline characteristics of the patients included for this analysis are shown in Table 6.1.

6.3.2 Analysis

I undertook comparison of outcomes for patients who suffered from diabetes, previous stroke or both concomitantly. The findings are shown in figures.

I found no interaction of variables prior stroke (t-PA * PS, $p=0.9$), diabetes (t-PA*DM, $p=0.19$). I also found no interaction of use of alteplase with presence of diabetes and previous stroke (t*PA*DM*PS, $p=0.5$).

Table 6-1 Baseline characteristics of the patients.

		Thrombolysis	Control	P-values
Age	All	67.1 (12.5),N=23062	70.1(12.2), N=6166	<0.0001
	DM	69.9(9.5), N=3905	70.5(10.4),N=1449	0.06
	No DM	66.5(12.9),N=18799	70.1(12.6),N=4447	<0.0001
	PS	70.1(10.5),N=2972)	72.9(10.8), N=2014	<0.0001
	No PS	66.6(12.7),N=19782	69(12.5),N=3979	<0.0001
	DM+PS	71.0(9.4), N=602	71.6(9.7), N=534	0.31
	Neither DM nor PS	65.9(13.1), N=16275	68.7(13), N=3064	<0.0001
Gender (Male)	All	13437/23062(58.3%)	3271/6166(53.1%)	<0.0001
	DM	2355/3905(60.3%)	781/1449(53.9%)	<0.0001
	No DM	10873/18799(57.8%)	2351/4447(52.9%)	<0.0001
	PS	1748/2972(58.8%)	1059/2014(52.6%)	<0.0001
	No PS	11521/19782(58.2%)	2121/3979(53.3%)	<0.0001
	DM+PS	363/602(60.3%)	285/534(53.4%)	0.02

	Neither DM nor PS	9410/16275(57.8%)	1625/3064(53.0%)	0.001
Baseline NIHSS*	All	12[0-42],N=23062	12[2-37],N=6166	0.14
	DM	13[0-40],N=3905	11[2-29], N=1449	0.04
	No DM	12[0-42], N=18799	12[2-37],N=4447	0.002
	PS	12[0-42],N=2972	11[2-37],N=2014	0.75
	No PS	12[0-40],N=19782	12[2-31],N=3979	0.02
	DM+PS	12[0-40],N=602	11[2-29],N=534	0.08
	Neither DM nor PS	12[0-40],N=16275	12[2-31], N=3064	0.002
Hypertension	All	14163/23062(61.4%)	4170/5896(70.7%)	0.001
	DM	3235/3878(83.4%)	1170/1449(80.8%)	0.02
	No DM	10736/18509(58%)	3000/4447(67.5%)	0.001
	PS	2247/2936(76.5%)	1491/1917(77.8%)	0.31
	No PS	11711/19414(60.3%)	2679/3979(67.3%)	0.001
	DM+PS	538/600(89.7%)	441/534(82.6%)	0.0005
	Neither DM nor PS	8918/16018(55.7%)	1950/3064(63.6%)	0.001
Atrial Fibrillation	All	5753/22493(25.6%)	1712/5896(29%)	0.001

	DM	1130/3814(29.6%)	365/1449(25.2%)	0.001
	No DM	4533/18397(24.6%)	1347/4447(25.2%)	0.001
	PS	799/2893(27.6%)	624/1917(32.6%)	0.0002
	No PS	4853/19353(25.1%)	1088/3979(27.3%)	0.003
	DM+PS	179/591(30.3%)	141/534(26.4%)	0.15
	Neither DM nor PS	3850/15948(24.1%)	864/3064(28.2%)	0.001
Congestive Heart Failure	All	1914/22583(8.5%)	277/3167(8.8%)	0.61
	DM	538/3802(14.2%)	79/800(9.9%)	0.001
	No DM	1349/18503(7.3%)	198/2367(8.4%)	0.06
	PS	312/2898(10.8%)	70/711(9.8%)	0.47
	No PS	1567/19444(8.1%)	207/2456(8.4%)	0.53
	DM+PS	92/584(15.8%)	19/222(8.6%)	0.008
	Neither DM nor PS	1108/16044(6.9%)	147/1878(7.8%)	0.14
Prior use of antithrombotic agents	All	8672/22543(38.5%)	1267/2968(42.7%)	0.001
	DM	1920/3795(50.6%)	367/751(48.9%)	0.39
	No DM	6649/18430(36.1%)	900/2217(40.6%)	0.001

	PS	2140/2863(74.7%)	442/687(64.3%)	0.001
	No PS	6397/19413(33%)	825/2281(36.2%)	0.002
	DM+PS	442/574((77%)	141/213(66.2%)	0.002
	Neither DM nor PS	4888/15998(30.6%)	599/1743(34.4%)	0.001
Systolic Blood Pressure	All	151.3(21.1),N=22 736	156.6(26.9),N= 6156	0.001
	DM	154.6(20.7), N=3848	159.7(27.5),N=1446	<0.0001
	No DM	150.5(21.1),N=18540	155.9(26.8),N=4440	<0.0001
	PS	152.1(20.4), N=2925	160.3(27.1),N=2013	<0.0001
	No PS	151.1(21.2), N=19510	155.1(26.7), N=3970	<0.0001
	DM+PS	154.0(20.5),N=589	163.2(27.8),N=534	<0.0001
	Neither DM nor PS	150.3(21.2), N=16052	154.3(26.6),N=3058	<0.0001
Diastolic Blood Pressure	All	82.6(13.5),N=22710	84.0(16.1),N=6156	<0.0001
	DM	81.9(13.4), N=3841	83.4(15.7),N=1446	0.0007
	No DM	82.7(13.4),N=18521	84.3(16.3),N=4440	<0.0001
	PS	82.4(13.4),N=2921	84.9(15.7),N=2014	<0.0001
	No PS	82.6(13.5),N=19489	83.6(16.3),N=3969	<0.0001

DM+PS	81.7(13.6),N=587	84.2(15.8),N=534	0.005
Neither DM nor PS	82.7(13.5),N=16036	83.8(16.5),N=3057	<0.0001

Numbers do not add to a total of 29228 due to missing data for some of the variables. () records standard deviation and [] the range, * p statistics were calculated by employing Wilcoxon non- parametric test.

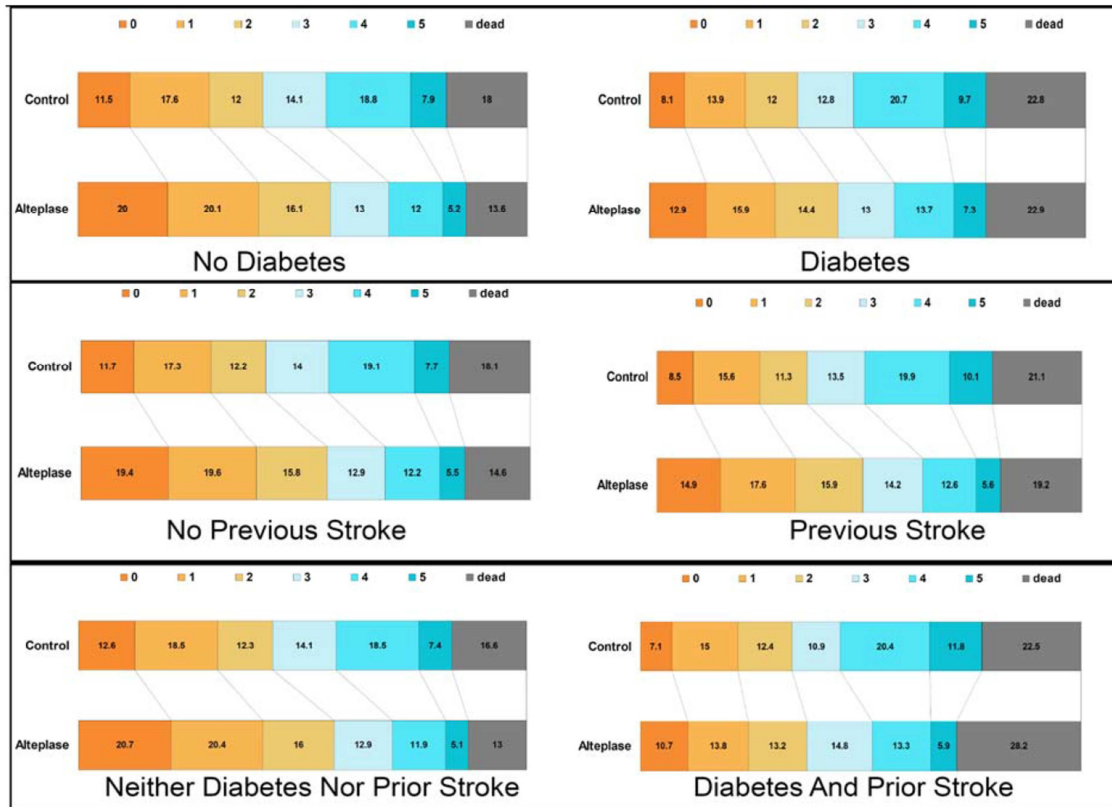


Figure 6-1 Bar Diagrams showing the distribution of patients in each risk factor category. The numbers in the box denote percentage. Colour codes refer to modified Rankin Scale.

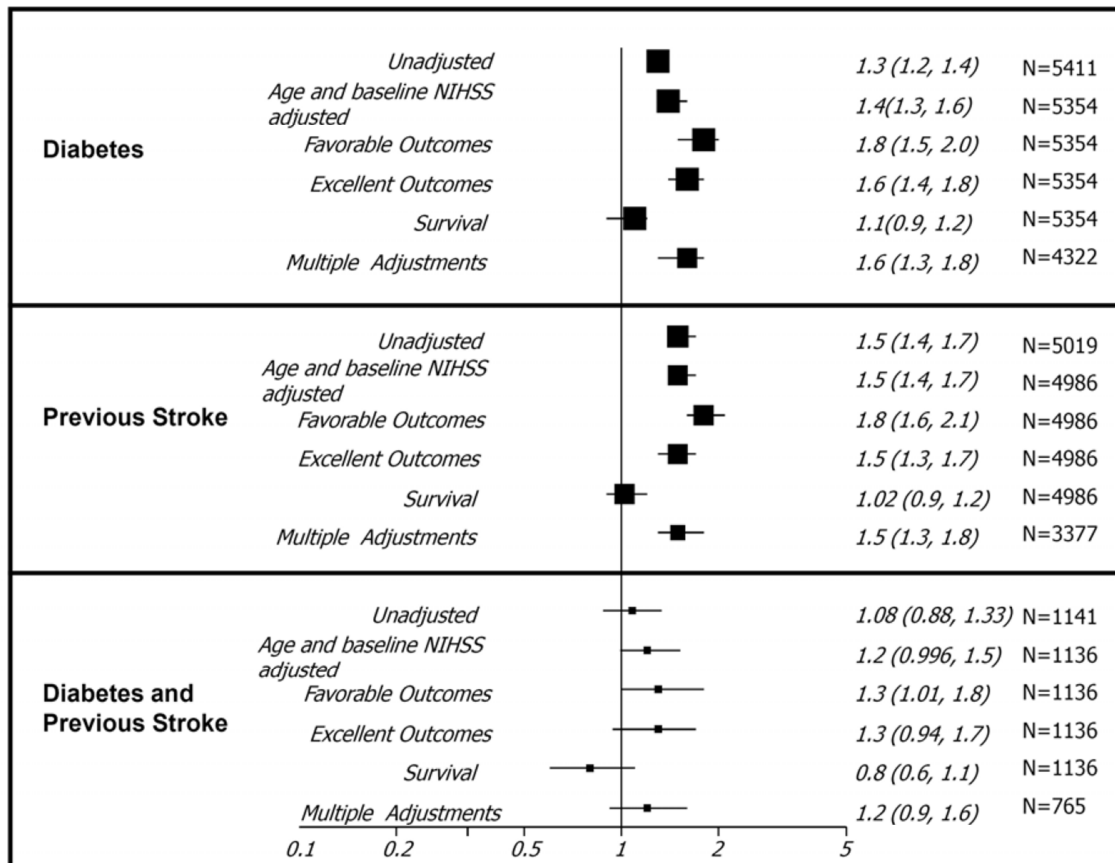


Figure 6-2 Forest Plot showing outcomes for various analyses in affected patients' population.

Unadjusted, and age and baseline adjusted analyses refer to ordinal regression analysis. Odds Ratio refers to common odds for improved outcomes at each Rankin category. Favourable outcomes refer to Rankin 0-2 whereas the Excellent outcomes refer to the Rankin score 0-1. All adjusted analyses refer to an adjusted analysis in which adjustments were made for all variables that differed at baseline.

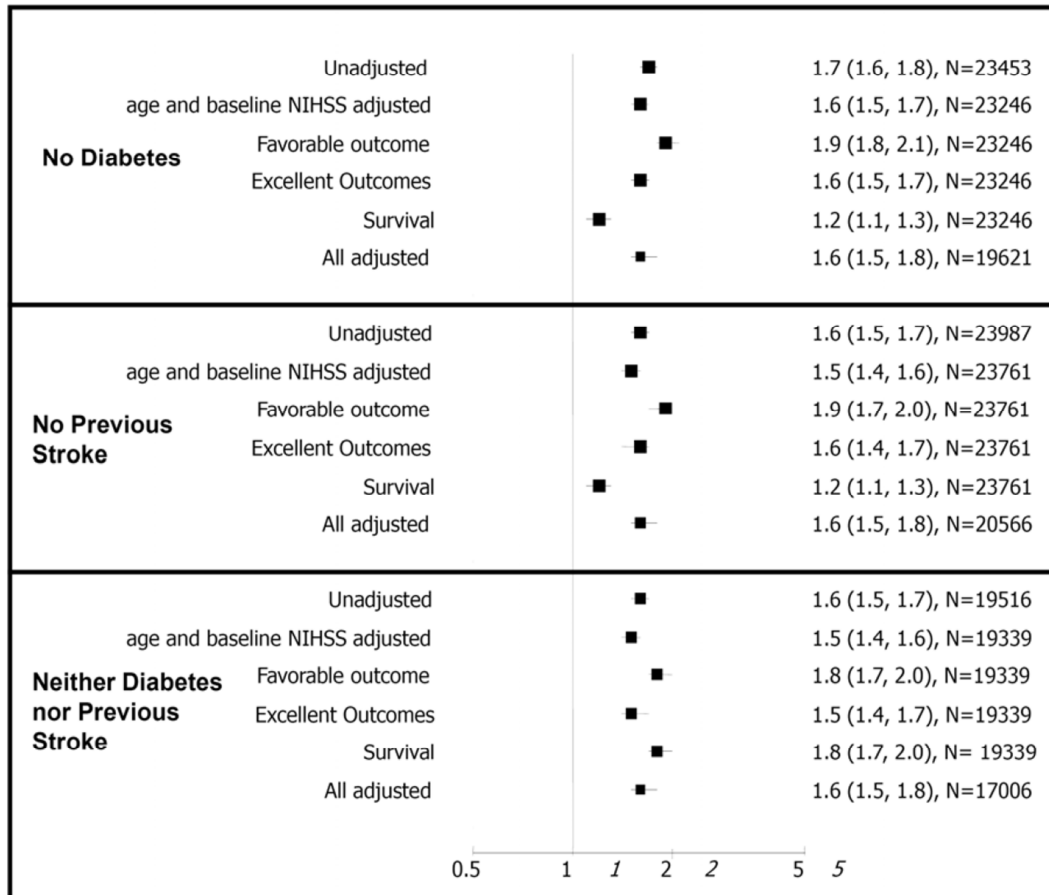


Figure 6-3 Forest Plot showing outcomes for various analyses in unaffected patients' population.

Unadjusted and age and baseline adjusted analyses refer to ordinal regression analysis. Odds refer to common odds for improved outcomes at each Rankin category. Favourable outcomes refers to Rankin 0-2 whereas the Excellent outcomes refers to the Rankin score 0-1. All adjusted analyses refers to an adjusted analyses in which adjustments were made for all variables that differed at baseline.

6.4 Discussion

The patients who suffer from the concomitant presence of diabetes and previous stroke are currently recommended for exclusion from receiving thrombolysis for acute ischaemic stroke. Employing a robust analytical methodology [shift analysis] and a large number of patients [N=29500], I have again shown improved outcomes across patients having diabetes, previous strokes or both who received thrombolytic therapy compared to those who were excluded from receiving this therapy.

I have first shown improved outcomes amongst patients who did not have diabetes and prior stroke, each having adjusted odds of 1.7 and 1.6 respectively, each of which is comparable to the odds for a similar time window obtained from the analysis of pooled data from all of the large trials conducted to date.¹³¹ This concordance of odds anchors my findings from patients having diabetes and previous stroke to established randomised control trial results.²⁹ In the populations of patients with diabetes, prior stroke or either condition, this better outcome amongst the group who received thrombolysis with intravenous alteplase versus untreated comparators is maintained. I confirm the finding using both ordinal and dichotomised analysis approaches, and whether or not the analyses are adjusted for baseline variables known to associate with prognosis. I find no interaction between diabetes and prior stroke with the effect of alteplase to justify further subgroup analysis. However, in the small group of patients having both diabetes and prior stroke, I no longer confirm a better distribution of outcome with alteplase [OR 1.2, 95% CI: 0.996-1.5] though dichotomisation reveals favourable outcomes [m RS 0-2], OR=1.3(95% CI 1.008-1.8)]. This discordance may arise from the smaller sample size, N=1141, which yields less precise point estimates and may be associated with a type II error. The point estimates remain consistent with those from the more reliable main analyses. This could also be explained by the fact that I did not account for the baseline disability amongst patients who had a previous stroke, and one does not expect the patients' outcomes would shift and get better than their pre-stroke disability.

In the present chapter, I have analysed a large dataset, N=29500. The findings are reassuring because these patients have a clinical profile similar to the one often seen in clinical practice. More patients in the diabetic group had hypertension, history of previous stroke, atrial fibrillation, and heart failure and used antithrombotic agents. Here the patients who received treatment had suffered a severe stroke at baseline compared to the untreated patients [NIHSS: 13 vs. 11]. Similarly, more patients having previous stroke(s) had hypertension, diabetes, atrial fibrillation, and heart failure, and use of antithrombotic agents before stroke.

In addition, this analysis supports the findings obtained from VISTA-only analysis [N=5817] reported in chapter 5, where employing a similar approach I compared the patients in VISTA who received thrombolytic therapy against those who did not. That analysis reported similar odds: patients having diabetes, OR= 1.3 [95%CI: 1.05-1.6]; patients having previous stroke, OR= OR: 1.3 [95%CI: 1.04-1.6], and both together, OR= p-OR: 1.5 [95%CI: 0.98-2.3].

A limitation of this study is that the data are non-randomised. However, I adjusted the analysis for age and baseline NIHSS scores.^{278,279,347,348} (and then also for other variables that differed at baseline) and employed robust statistical tools [Cochran-Mantel-Haenszel Test, proportional odds logistic regression; and analysis by dichotomisation of Rankin scores]. I believe that I have attained a reliable statistical result. One may anchor the findings on the fact that the odds in the group of patients who did not have DM or PS were comparable with the published randomised trial data [In NINDS, OR 1.6 (95%CI 1.2-2.1)]²⁶⁹, while acknowledging the caveat that there are no analyses of RCT data that specifically examined outcomes after excluding those patients that had diabetes or prior stroke. Next, although prior stroke patients who were offered thrombolysis may have had higher premorbid mRS than patients who had no prior stroke, it is known that patients with premorbid mRS >1 were excluded from the VISTA trials from which comparators for analysis are derived. This potential bias would be expected to lead to underestimation of any benefit from alteplase in that subgroup of patients.

Hyperglycaemia after stroke occurs in about 60% of patients and only 20% of these patients report a history of diabetes mellitus.³²⁴ As discussed previously in

the Chapter 5, hyperglycaemia and diabetes are overlapping but two different conditions. Hyperglycaemia at baseline may be associated with diabetes (uncontrolled or unrecognised diabetes or poor glucose tolerance) or it can be a response to the acute stress (stress hyperglycaemia). The EMEA recommends that patients suffering from blood glucose levels at baseline <50 and > 400 mg/dl should be excluded from receiving therapy. The purpose of analysis that I present in this chapter was to examine outcomes amongst patients who already had diabetes and prior stroke as these are distinct exclusion criteria specifically mentioned in the EMEA document. I realise that denominators in my analyses (“non-diabetic patients”) might be having hyperglycaemia (due to unmasking of diabetes, poor glucose tolerance, unknown case of diabetes or stress hyperglycaemia) at baseline. What proportions of non-diabetics had raised blood sugar levels in this dataset is unknown. Examination of outcomes for different levels of hyperglycaemia (with or without presence of diabetes/previous stroke) would require another analysis. In summary, the analysis shows improved outcomes amongst patients with diabetes, or prior stroke. The magnitude of benefit is comparable to that in other patient groups. This finding contrasts with EMEA’s justification for restricting use of intravenous alteplase. The analysis did not confirm a significant benefit in the small subgroup of patients who had concomitant diabetes and prior stroke but here the confidence intervals were wide, and there was no interaction between these two risk factors with the treatment effect of alteplase. Hence, I find no justification to exclude patients from receiving alteplase for acute ischaemic stroke if they suffer from a previous stroke and also have diabetes mellitus.

Chapter 7

**Should patients having mild or severe stroke receive
alteplase?**

7 Should patients having mild or severe stroke receive alteplase?

7.1 Introduction

Intravenous thrombolysis with alteplase is a proven therapy for acute ischaemic stroke patients presenting within 4.5 hours of symptom onset.^{122,129,131} However, some patients are denied therapy for fear of poor outcomes. European guidelines recommend that patients with baseline stroke severity NIHSS ≥ 25 and minor/rapidly improving strokes should not be given alteplase.²³ But, there is a lack of consensus on what defines a “mild stroke”.³⁴⁹ Heinrich Mattle and colleagues³⁴⁹ undertook a Medline search to look for papers published between 1950 and 2009 by using a search term “ Minor Stroke”. They extracted 670 papers and found that most of these had not defined a minor stroke.³⁴⁹ However, some of the definitions have been used to define mild stroke are:

1. Definition A: baseline NIHSS score of 0 or 1 in each NIHSS component except the items 1a, 1b and 1c that deal with the level of consciousness.^{349 350}
2. Definition B: lacunar strokes at baseline.^{349 350}
3. Definition C: presence of motor symptoms (including dysarthria or ataxia +/-sensory symptoms).^{349 350}
4. Definition D: NIHSS Score ≤ 9), excluding all patients with extinction or neglect, aphasia or having level-of-consciousness.^{349 350}
5. Definition E: maximum NIHSS score of 9.^{349 350}
6. Definition F: NIHSS ≤ 3 .³⁴⁹
7. Definition G: NIHSS < 5 .

Employing definition A to E NINDS investigators examined outcomes in 624 patients that were enrolled in the NINDS trial.³⁵⁰ They used a global test statistic

based on a logistic model to examine patients who had favourable outcome on day 90. In addition, they also examined the rates of symptomatic intracerebral haemorrhages. Data comprised N=28 patients as per definition A, N=81 per definition B, N=439 per definition C, N=173 per definition D and N=177 per definition E. Based on an Intention-to-treat analysis, investigators showed that the odds for better outcomes ≈ 2 for all the definitions ($p < 0.05$) (lower limit of confidence interval between 1.4 to 1.5; upper limit between 2.7 and 2.9). Symptomatic Intracerebral Haemorrhage occurred in 0-4% of patients per definition.³⁵⁰

Employing the definitions A to F, Fischer et al³⁴⁹ reported analysis of Bernese Stroke Registry data (N=760) and examined short term outcomes (patient goes home) and a favourable medium term outcomes (m-RS 0-2 on day 90) for patients. In addition, they also analysed data for various subgroups of patients defined by their sex, age, laterality of lesions (left-right) or circulation (anterior-posterior) and temporal profile of admissions (early versus late). This study reported that median NIHSS were lowest for the definitions A and F. Definition A and definition F were associated with most favourable short and medium term outcomes. Per definition C, more numbers of patients with anterior circulation infarction were discharged home compared to posterior circulation stroke ($p = 0.02$). Rankin scores on day 90 were less favourable for elderly patients per definition E ($p < 0.05$). Definitions A, D and F were not associated with differences on outcomes across the subgroups. Authors recommended for the need of a consensus definition of a mild stroke.³⁴⁹

Several stroke trials explicitly exclude patients with NIHSS of 5 or less (definition G).^{46,247,351,352}

Patients having rapidly improving stroke symptoms are also recommended for exclusion because these patients are assumed to have improved outcomes.¹²² But

I published Chapter 7 in Stroke (Mishra NK, Lyden P, Grotta JC, Lees KR; VISTA Collaborators. Thrombolysis is associated with consistent functional improvement across baseline stroke severity: a comparison of outcomes in patients from the Virtual International Stroke Trials Archive (VISTA). Stroke. 2010 Nov;41(11):2612-7.). Permissions were obtained from Wolters Kluwer Health, License Number: 2783790313323.

data indicate that this may not always be the case.^{142,353-356} Nedeltchev et al³⁵⁷ show that about 75% of patients that present with mild or rapidly improving stroke symptoms achieve a Rankin score of ≤ 1 by day 90. Those that have persistent proximal vessel occlusion and a baseline NIHSS of ≥ 10 suffer 7 times the excess risk of unfavourable outcomes.³⁵⁷ Persistent occlusion of blood vessels was shown to be associated with poor outcomes in patients.^{356,358-360} Additional data are needed so that one can reliably select phenotypes of those patients that would either undergo spontaneous recovery or need thrombolysis.

t-PA guidelines were framed under an assumption that many patients who show rapid improvement/ have minor strokes would not display residual deficit, but treatment with thrombolytic therapy would expose them to risk of complications such as cerebral haemorrhage.²³ Similarly, those patients who present with baseline NIHSS ≥ 25 are also supposed to have poorer outcomes because of excess symptomatic haemorrhages.²³ Baseline stroke severity (b-NIHSS) is known to affect outcomes amongst thrombolysed patients, and was therefore incorporated for patients' selection in ECASS III trial.²³ Though the regulatory authorities have recommended withholding thrombolytic therapy amongst patients with minor/rapidly improving strokes and for those with severe stroke at baseline, poorer response to therapy in these subgroups has never been demonstrated in randomised controlled trials.²³ Indeed, post hoc analyses of the NINDS and ECASS-III trials suggest equal efficacy across severity ranges, though power to examine subgroups is inevitably lower than that chosen for the primary analyses and patients at extremes of severity were under-represented.³⁶¹⁻³⁶³ The logistical challenges involved in generating randomised trial evidence for these limited subgroups militate against any prospect for producing a definitive answer in the foreseeable future. Therefore one must turn to alternative sources of evidence.

7.2 Methods

7.2.1 Data Source and patients

As discussed in Chapter 2, I collated the demographics, clinical data and measures of functional outcome from neuroprotection trials conducted in the period 1998 to 2007, held within the Virtual International Stroke Trials Archive, VISTA (www.vista.gla.ac.uk).

7.2.2 Statistical analysis

I compared outcomes between patients who received thrombolysis and patients who did not receive thrombolysis (controls) amongst categories of baseline NIHSS scores (below 4, 5-8, 9-12, 13-16, 17-20, 21-24 and ≥ 25). For each contrast, I compared the overall distribution of all seven categories of day 90 mRS scores of the two groups, i.e. from 0 (asymptomatic) through 5 (bedbound and completely dependent), to 6 (dead). For analysis of the supporting endpoint, NIHSS, I grouped the adjacent scores into categories: 0 (no measurable deficit), 1-4, 5-8, 9-12, 13-16, 17-20, 21-24, ≥ 25 (most severe neurological deficit) or dead. The distribution of patients across these categories was then compared between the groups as for mRS.

To test for a significant association of outcome distribution with thrombolysis exposure I used the Cochran-Mantel-Haenszel [CMH] statistic, adjusting for both age and baseline NIHSS as continuous variables. Further, I applied logistic regression analysis, also adjusted for age and baseline (b-) NIHSS, to estimate the odds ratio under the assumption of proportional odds and its associated 95% confidence interval. Reliable information on symptomatic haemorrhage was not available since post treatment imaging was not routinely applied in neuroprotection trials to patients who had not been treated with alteplase.

7.3 Results

7.3.1 Patient sample

Complete data were available for analysis of mRS in 5817 patients and on NIHSS in 5715 (see chapter 2 for details of VISTA data). Of the 5817 patients with mRS outcome data, 1585 (27.2%) received thrombolysis.

7.3.2 Does baseline stroke severity influence stroke outcomes?

In an ordinal logistic regression analysis, I found that baseline severity ($p < 0.0001$), use of rt-PA and age were significant predictors of outcomes. Then, in an age adjusted ordinal logistic regression analysis, I found that baseline stroke severity ($p < 0.0001$) and the interaction between severity and use of alteplase ($p = 0.04$) were associated with outcome from stroke, but I did not see an independent effect of alteplase ($p = 0.65$).

Supported by this interaction test, I classified the baseline stroke severity into seven baseline NIHSS score categories: 1-4, 5-8, 9-12, 13-16, 17-20, 21-24, and ≥ 25 , and undertook tests of association for thrombolysis with outcomes in each of these categories.

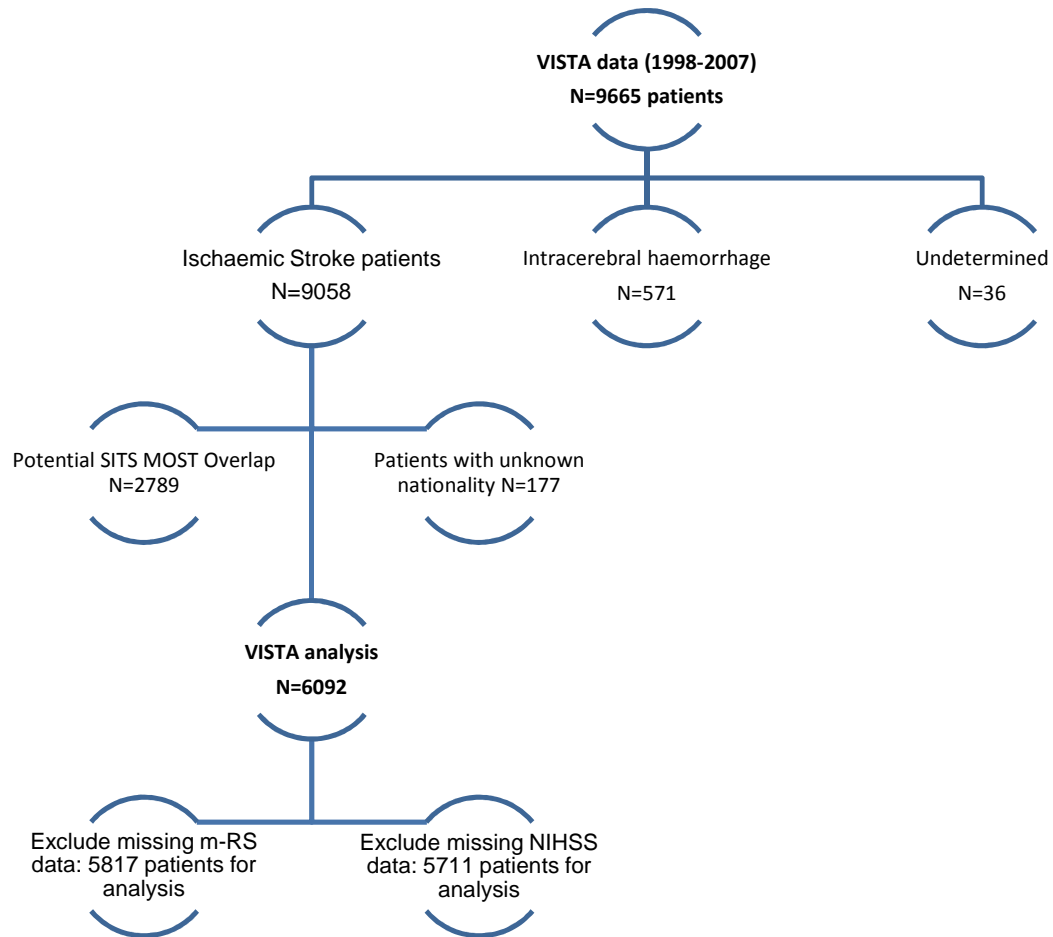


Figure 7-1 Figure showing details of selection of patients from VISTA for this analysis.

	Thrombolysis		Nonthrombolysed Controls		<i>P</i>
Age, median (range)	71 (21–98)	N=1585	72 (21–101)	N=4232	<0.05
Male	880/1585	55.52%	2226/4232	52.6%	0.05
Baseline NIHSS, median (range)	14 (2–32)	N=1585	13 (2–37)	N=4232	<0.05
Previous antiplatelet use	429/1078	39.8%	446/1306	34.2%	<0.05
Previous anticoagulation use	67/1078	6.2%	198/1306	15.2%	<0.05
Previous stroke	319/1555	20.5%	1579/4076	38.7%	<0.05
Congestive heart failure	151/1262	12%	164/1409	11.6%	0.79
Diabetes mellitus	342/1548	22.1%	992/3991	24.9%	0.03
Hypertension	1030/1548	66.5%	2827/3991	70.8%	<0.05
Atrial fibrillation	398/1548	25.7%	1274/3991	31.3%	<0.05
Myocardial infarction	278/1548	18%	691/3991	17.3%	0.57

NIHSS, National Institutes of Health Stroke Scale.

Table 7-1 Baseline characteristics of the patients studied in VISTA.

7.3.3 Are there improved outcomes across all baseline stroke severity categories?

7.3.3.1 Primary analysis: functional outcome by modified Rankin Scale.

I report the findings in figure 7-2 below.

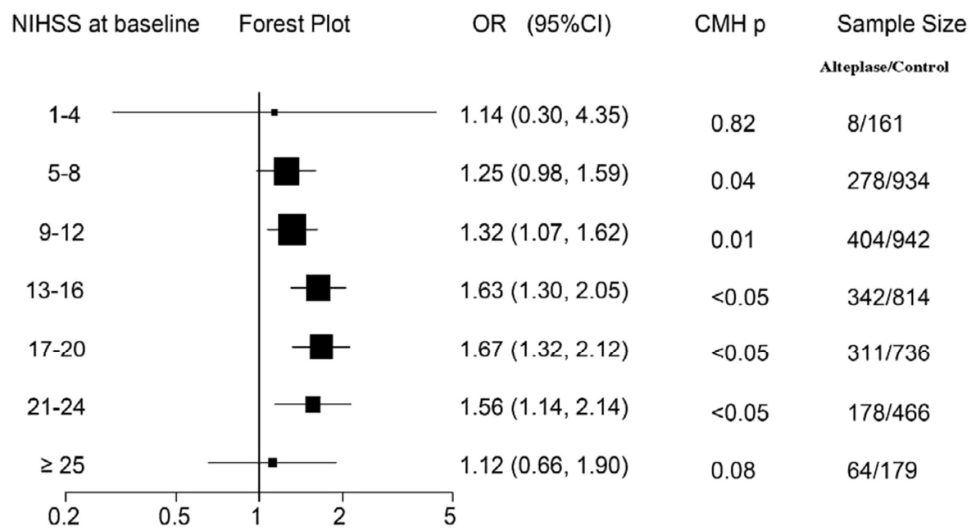


Figure 7-2 Forest plot showing age and baseline stroke severity adjusted outcomes across categories of baseline severity level

7.3.4 Secondary analysis: neurological outcomes by NIHSS.

Findings are shown in figure 7.3.

7.3.5 Sensitivity analysis

I also performed unadjusted analysis, and analysis in which I adjusted for additional factors. Results are shown in figure 7.3

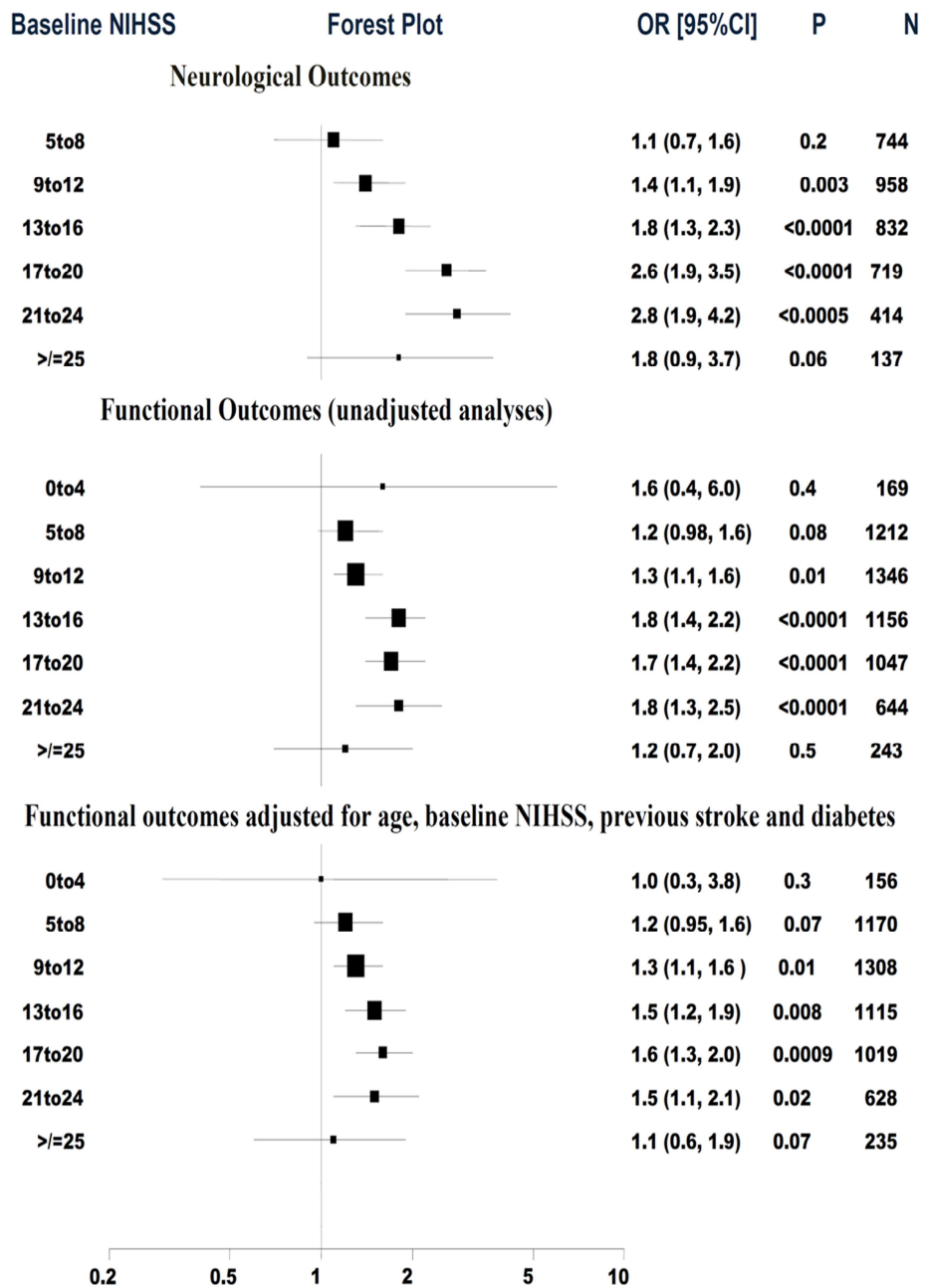


Figure 7-3 Findings from secondary and sensitivity analyses

I could not adjust for onset to treatment [OTT] time because time to initiation of thrombolytic therapy was not recorded within the source neuroprotection trials.

Fifty-nine per cent of records lacked coding for the variable “antithrombotic” (i.e. antiplatelet and anticoagulants) (N=3432), 4.8% for atrial fibrillation (N=278) and 3.2% for patients with prior strokes (N=186). Because the analyses were to be conducted within each stratum of baseline stroke severity, these differences were more pronounced limiting the analyses of data controlled for all variables that differed at baseline: age, baseline NIHSS, prior use of antithrombotic drugs, previous stroke and atrial fibrillation).

7.4 Discussion

Patients with mild and severe strokes are under-represented in randomised trials and post marketing analyses. The EMEA marketing authorisation for alteplase in acute ischaemic stroke lists minor neurological deficit or symptoms rapidly improving before start of infusion, and severe stroke as assessed clinically (e.g. NIHSS>25) and/or by appropriate imaging techniques, as contraindications.²³ Such patients do present to hospital services, however, and this places the physicians in a dilemma whether or not to offer treatment. Some experienced physicians treat such patients. For example, 12% of patients in the SITS-ISTR thrombolysis registry had a baseline NIHSS score in the range 0 to 4 and 4% had severe stroke with NIHSS \geq 25. Many more patients were probably not included for thrombolysis treatment: a Canadian series found that 31% of patients were considered too mild or improving too rapidly for treatment.¹⁴² About 20-46% of patients that present in emergency departments do not receive thrombolytic therapy because of presence of mild or rapidly improving stroke.^{142,364-367} This cannot be justified on the basis of observed outcome. In dataset of 93517 patients that suffered stroke, 29200 patients had mild or rapidly improving stroke. Of these, 28 % of the patients failed to go home on discharge. A similar proportion was unable to move around without assistance.³⁶⁸ Randomised trials to establish the existence or extent of benefit at extremes of baseline severity

may be difficult to conduct and delayed in execution. Other sources of evidence must be examined, and high quality registry data are the obvious choice.

In the present non-randomised comparison of data held in VISTA, outcomes after thrombolysis were significantly better than in untreated comparators across baseline NIHSS scores 5 to 24. This significant association was lost only at extremes of b-NIHSS (i.e. 1-4 and ≥ 25). Although the point estimates for both adjusted and unadjusted odds ratios remain favourable in the extreme groups, they are lower than those observed at other levels of stroke severity and confidence intervals include the possibility of significant harm as well as benefit.

In these extreme groups the small sample size seriously undermines the power of the statistical tests and with wide confidence intervals the true point estimate is not reliably indicated. There is a second statistical issue to consider, relating to the outcome measure that I used. By examining the full distribution of the modified Rankin scale I have used a test that is less dependent on case mix than dichotomisation: I was able to use the same test for patients with mild stroke as severe stroke and still detected benefit.

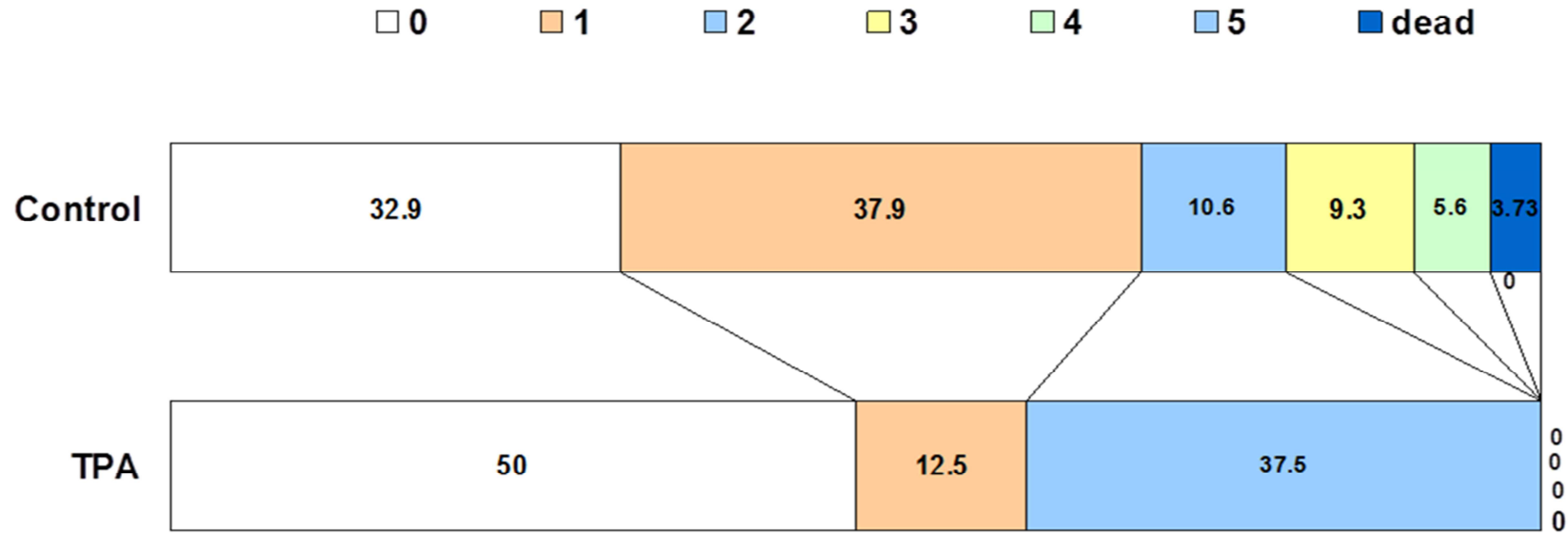


Figure 7-4 Distribution of outcomes in patients with baseline severity NIHSS score of 0-4

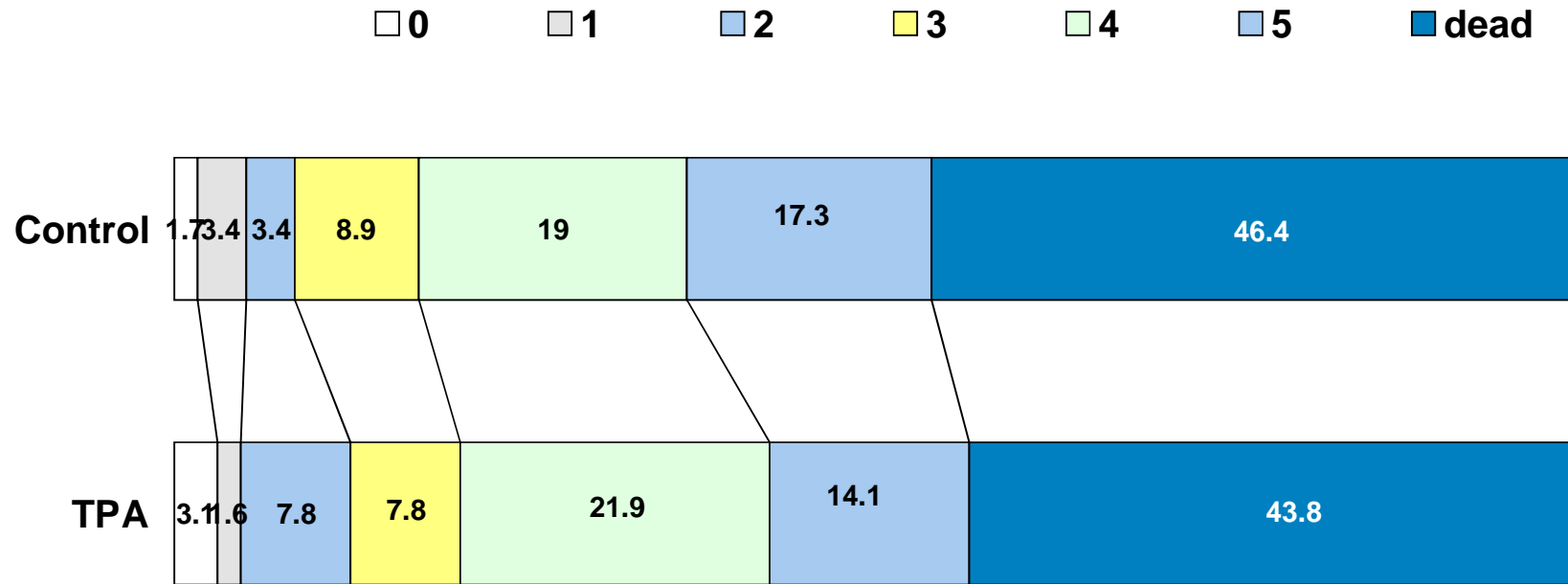


Figure 7-5 Outcomes in patients having baseline severity NIHSS ≥ 25

Even so, at the extremes of baseline severity, outcomes are generally so good or so poor that only a few mRS categories are well represented in the control groups. Both the Cochran-Mantel-Haenszel test and the proportional odds estimations will be compromised if some categories are not contributing to the analysis: effectively, the test of treatment effect will be diluted by the non-contributing groups. For CMH this means that it becomes harder to reach statistical significance but for the proportional odds tests, the basic assumption has been breached: the effect is not proportional. There is no easy solution to this problem: if case mix is altered to deliver a significant result then patients with mild or severe stroke must be excluded - the solution used by the trials. Conversely if the outcome measure is varied according to the sample case mix (the sliding dichotomy approach discussed by Murray et al)²⁸³ then interpretation is rendered difficult: is an odds ratio for achieving mRS 0 versus 1-6 equivalent to an odds ratio for achieving mRS 0-5 versus 6, i.e. is survival free from symptoms equivalent to survival at any cost?

Here, I have chosen to present one analytic approach for all severities of stroke but I also illustrate the range of outcomes at extremes of severity. From these, although the summary statistics show only a non-significant but favourable trend, I can draw further conclusions. Amongst patients with severe stroke, there appears to be a trend towards benefit across almost all boundaries of mRS (Figure 7-5). Amongst patients with mild stroke, all boundaries except 0-1 versus 2-6 show benefit but four of the mRS categories are entirely unrepresented (Figure 7-4). Findings derived from the VISTA data show no reason to withhold treatment from either group of patients but are not in themselves sufficient evidence to justify treatment.

My findings draw validity from the fact that the source clinical trials rigorously reported outcomes, and had strict on-site data verification procedures. However, the non-random allocation to treatment versus control groups is a significant weakness of our design. One cannot determine the degree and cause of exclusion of patients from our database; one can only consider factors known to associate with prognosis. Further, information on variables like do-not-

resuscitate order for severe strokes or complete list of concomitant treatments were not captured in the VISTA datasets. These could have introduced bias as well.

I have adjusted statistically for factors that have a large influence on outcome. One can also 'anchor' the findings by comparison of treatment associations for patients with moderate stroke severity in our study against known treatment effects in comparable patients from randomised trials. For example, I found an OR for favourable outcome of 1.3 - 1.6 for patients with b-NIHSS 9-12 and 13-16; the comparable estimate from treatment within 3 hours of stroke onset in RCT would be 1.64 and for 3-4.5 hours would be 1.34.¹³¹ The estimates are comparable and perhaps conservative.

The decay of benefit across later onset to treatment times raises a second issue. I do not have information on the onset to treatment delay for alteplase in the current analysis. Since the patients were permitted only one investigational drug in the participating VISTA trials, alteplase being used as standard of care, and since these trials were closely monitored by their sponsors, one can assume that patients were largely treated within 3 hours of stroke onset. I also assume that the onset-to-treatment time is comparable to those from the CASES and SITS-MOST registries (155 (130-175) minutes and 140 (115-165) minutes [n=6483] respectively).^{146,369} Unfortunately, the latency between stroke onset and recording of initial severity differed between the treatment group (3.7 hours) and controls (5.1 hours) in the VISTA data. Severity is associated with onset to hospital arrival time: patients with more severe stroke present earlier.³⁷⁰ I adjusted the analyses for stroke severity but it is conceivable that residual bias persists. Such a bias would cause underestimation of true initial severity amongst the controls and through the baseline adjustment would have led to overestimate treatment effect. It will influence all patients across the severity range but may be less evident at extremes of severity: the NIHSS criterion will be responsible for discouraging use of alteplase, and so the proportion of patients who are treated with alteplase will fall at extremes of NIHSS.

I lacked data on symptomatic haemorrhages, since patients who are not treated with thrombolysis generally do not undergo follow-up cerebral imaging for

routine detection of haemorrhagic transformation. However, the outcome measure that I used takes into account effects of haemorrhage or other adverse events on function.

I adjusted for age and baseline severity as these are the established most important variables known to influence outcomes.²⁶⁵ I could not adjust for all - age, baseline NIHSS, prior use of antithrombotic drugs, previous stroke and atrial fibrillation together - as one of the contributing trial programs did not record pre-treatment medications. However I was able to undertake an adjusted analysis for the variables that were found significant in ECASS III, namely diabetes and prior stroke, and the estimates remained consistent.

Some of the patients in this study received an investigational medicinal product. Each contributing trial has already tested for, and excluded, a significant interaction of that product with alteplase, both in vitro and in vivo.

Individual patient data analyses from pooled randomised data¹³¹ could be the most informative method to assess the influence of baseline severity on outcome response to alteplase. Unfortunately, the pooled data have a median baseline NIHSS of 11 and interquartile range of 7 to 16, that would weaken analyses to guide use of t-PA in the patients at the extremes of baseline NIHSS categories. Enrolment to extreme subgroups is generally limited.⁴¹ Pooja Khatri and colleagues have applied for funding to seek support a placebo-controlled trial of alteplase in patients suffering mild stroke. The trial would be called Potential of rtPA for Ischaemic Strokes with Mild Symptoms (PRISMS) (International Stroke Conference, Los Angeles, 2011).

Analyses of VISTA data imply that patients at extremes of NIHSS scores recorded at baseline may still benefit from treatment but the supporting evidence remains weak.

Chapter 8

Mismatch based delayed thrombolysis: a meta-analysis

8 Mismatch based delayed thrombolysis: a meta-analysis

8.1 Introduction

Thrombolysis is the principal therapy for acute stroke patients in the early hours after symptom onset but has a short treatment window.^{122,129} In a meta-analysis of data derived from 2775 patients (pooled from the ATLANTIS, ECASS, and NINDS trials), there was a gradually diminishing benefit toward 6 hours from stroke onset [(odds ratio [OR]=2.8; 95% CI, 1.8 to 4.5) for 0 to 90 minutes, 1.6 (95% CI, 1.1 to 2.2) for 91 to 180 minutes, 1.4 (95% CI, 1.1 to 1.9) for 181 to 270 minutes, and 1.2 (95% CI, 0.9 to 1.5) for 271 to 360 minutes].¹³⁰ Recently, the ECASS III trial (N=821; treatment vs. placebo 1:1; median time for administration of alteplase=3 hours, 59 minutes) confirmed clinical benefit within 4.5 hours of stroke onset. (OR=1.34; 95% CI, 1.02 to 1.76; $P=0.04$).¹²⁹ However, the wider 95% CI at 6 hours (0.9 to 1.5 for 271 to 360 minutes in the meta-analysis¹³⁰) have suggested that there may still be patients able to benefit from thrombolysis even beyond 4.5 hours. Conversely, others may be at increased risk from late treatment. The use of imaging approaches to select patients who have remaining salvageable tissue for delayed treatment has been proposed, most notably approaches that include magnetic resonance imaging (MRI) perfusion/diffusion “mismatch.”^{371,372}

Several trials have tested thrombolysis in patients selected after MRI. Even some centres have incorporated mismatch imaging and delayed thrombolysis into their routine clinical practice.^{45,373} Safety and efficacy data were reported for those patients that received thrombolysis beyond 3 hours of symptoms onset in a MR based selection paradigm (n=180) and also CT or MR based patients thrombolysis within 3 hours of their symptoms onset (714 and 316 respectively). These were pooled data from four German and one Spanish centres (N=1210). These data

showed that the rates of symptomatic haemorrhages or mortality were similar between the three groups ($p=0.2$ and 0.7 respectively).⁴⁵ Symptomatic haemorrhages were significantly reduced in case of patients selected by MRI. ($p=0.5$).⁴⁵

I undertook a meta-analysis of data in the public domain to examine whether extension of the treatment window among patients selected according to the presence of mismatch can be recommended for routine clinical practice.

8.2 Methods

8.2.1 Selection of Trials

I planned to include only relevant papers that described findings of studies which either undertook prospective enrolment of consecutive stroke patients with mismatch profile for delayed thrombolysis [beyond 3 hours of stroke onset] or had studied mismatch-based, delayed thrombolysis in a randomised controlled design. I excluded case reports, case series and studies restricted to specific anatomical brain locations.³⁷⁴ I defined (1) *mismatch profile* as a perfusion volume at least 1.2 times that of the infarction core utilising the imaging methodology available with the concerned trial centre, (2) *Symptomatic Intracerebral Haemorrhage (SICH)* as radiologically confirmed cerebral haemorrhage in association with clinical worsening following thrombolytic therapy [within 36 hours in case of t-PA and 72 hours in case of desmoteplase] (3) *reperfusion and/or recanalisation* according to the respective studies' definitions (4) *favourable clinical outcome* as an NIHSS improvement of 8 points from the baseline or attainment of NIHSS of 0 or 1 and / or m-RS of 0 or 1 and (5) *Mortality* as death (m-RS 6) in the 90 days following thrombolytic therapy.

I considered the rt-PA and desmoteplase together as both are thrombolytic agents.^{70,106-108,375,376} These differ in some features: desmoteplase lacks the 2nd

I published Chapter 8 in Stroke (Mishra NK, Albers GW, Davis SM, Donnan GA, Furlan AJ, Hacke W, Lees KR. Mismatch-based delayed thrombolysis: a meta-analysis. Stroke. 2010 Jan;41(1):e25-33.) Permissions were obtained from Wolters Kluwer Health, License Number: 2783790733330

kringle site in its molecular structure; does not need to be cleaved by plasmin; is active in its single chain form; has reduced neurotoxicity; limited passage through the blood-brain-barrier.^{70,106-108,375,376} Desmoteplase has a theoretical advantage over rt-PA as it is almost non-functional if fibrin is absent.^{70,106-108,375,376} Alteplase is already a proven therapy for treating stroke patients in the early hours of its onset [NINDS, ECASS III]^{122, 129} and doses that carry acceptable safety and efficacy have been identified¹⁰⁹⁻¹¹¹. Both desmoteplase and alteplase remain investigational for delayed thrombolysis. However, I undertook a sensitivity analyses for any differential effect between desmoteplase versus alteplase.

Until the DIAS II study the identification of ischaemic penumbra was based on magnetic resonance perfusion-diffusion weighted imaging (MR PI-DWI) mismatch.¹¹¹ For the first time, the DIAS II investigators were permitted to select patients based on the visual appreciation of mismatch on perfusion CT images as an alternative to MR perfusion studies depending upon the local expertise of the imaging centre. I included data from either method as reported in the DIAS II publication.¹¹¹

I included all trials that defined the mismatch profile as the perfusion volume being 1.2 times of the infarction core. I placed no restriction on the manner in which perfusion was measured in these trials. For example, in DIAS 2, the mismatch population was identified, based on either CT perfusion or MR perfusion according to centre preference. The determination of mismatch in DEFUSE and EPITHET was based on post-processed PWI data that included correction for arterial input and thresholding. In contrast, in the desmoteplase studies mismatch was determined “real time” without post-processing by the investigator using the “eyeball” technique.

8.2.2 Endpoints

Endpoints of interest for our meta-analysis were comparisons between thrombolysed and non-thrombolysed patients in (1) favourable outcome (2) reperfusion and/or recanalisation (3) mortality and (4) SICH. I also examined

the rates of favourable versus unfavourable clinical outcome amongst successfully reperfused patients.

8.2.3 Search

I first searched the “Web of Knowledge” for ten *broad terms* [“*Clinical Trial**”, “*Prospective Study*”, “*stroke trial**”, “*thrombolytic agent*”, “*desmoteplase*”, “*tissue plasminogen activator*”, “*recanalisation in stroke*”, “ *reperfusion therapy in stroke*”, “*penumbra in stroke*”, and “*mismatch hypotheses*”]. Then, I refined the search by combining these with terms that underline the mismatch hypotheses and thrombolysis. My last search was undertaken on 19th December, 2008. From review of the title and abstract I selected for further examination all relevant papers describing the original findings of studies that used the mismatch hypotheses and selected patients for thrombolysis despite delay beyond 3 hours of stroke onset. I checked whether any later paper or abstract offered supplemental data. Once selected, each paper was read completely and the relevant data extracted. I also searched the bibliography of each of these papers for further articles.

8.2.4 Statistical Analysis

For this meta-analysis, I retrieved “estimate(s) of effect” from the abstract(s). When relevant data were missing, I searched the full text and any supplementary articles.³⁷⁷ Primarily, I wished to analyse data derived from the patients with mismatch profile on an intention-to-treat (ITT) basis but where the ITT data were unavailable we accepted ‘per protocol’ data and described the underlying limitations. The comparisons were mainly planned between patients offered any dose of any thrombolytic agent against the corresponding placebo treated patients.³⁷⁷

I performed sub-group analysis amongst patients who were treated with thrombolytics at doses approved or still under clinical investigation, i.e. 90µg/kg desmoteplase or 0.9mg/kg of rt-PA. Comparisons (summary estimates) are expressed as odds ratio [OR] and their 95% confidence interval [CI]. Whereas I applied both fixed [inverse-variance weighting method] and random methods to

calculate the summary estimate [adjusted OR (a-OR)],³⁷⁷ I report only the findings of fixed method here. But, I have indicated the instances where the results have diverged. I assessed the heterogeneity using the tests statistics for the heterogeneity and I^2 for inconsistency supported by the examination of L'Abbé's plots.

The analysis included data derived from those patients who were selected (or could have been selected) based on mismatch profile. In order to assess if favourable outcomes (clinical outcomes at day 90) were more common amongst the patients who had successful reperfusion, I retrieved data on 242 patients for whom the reperfusion findings were available [the DIAS I trial¹⁰⁹ (N=97), the DEDAS trial¹¹⁰ (N=34), the EPITHET trial¹⁸¹ (N=77; "Good Neurological Outcome" with reperfusion : 30 and without reperfusion : 47 (for mismatch patients only) and the DEFUSE trial¹⁸³ (N=34; mismatch with early reperfusion: 18 patients, mismatch without early reperfusion: 16 patients)]. Corresponding information was not reported in the DIAS II trial.¹¹¹ Similarly, in order to answer if favourable clinical outcome occurred more frequently in the thrombolysed group of patients, information on 410 patients was available [DIAS I trial (N= 102), DIAS II trial (186), DEDAS trial (N=37) and the EPITHET trial (N=85; mismatch patients with/without "Good Neurological Outcome" in the thrombolysis group: 42; and placebo group: 43)] in which the patients were thrombolysed with any thrombolytic agent at any dosage. Next, to answer if reperfusion or recanalisation occurred more frequently amongst those who were thrombolysed, I retrieved data on 211 patients who received thrombolytic therapy at any dose [DIAS I: 97 patients; DEDAS (ITT): 37 patients, [Target Population, (TP) = 23 patients]; EPITHET: 77 patients]. To assess mortality between thrombolysed and non-thrombolysed patients, I extracted data on 410 patients [DIAS I=102, DIAS II=186, DEDAS= 37, EPITHET = 85 (mismatch only)]. To assess SICH between thrombolysed and non-thrombolysed patients, I extracted data on 405 patients [DIAS I=102, DIAS II=186, DEDAS= 37, EPITHET =80 (mismatch group only)]. Owing to mathematical difficulties involved in calculating OR when the numerator is zero, I combined the DEDAS data with DIAS I data for mortality analysis.

I undertook sensitivity (subgroup) analyses in which I compared the data after excluding the data from doses of desmoteplase that have been abandoned for

further evaluation. I also analysed differences in clinical outcome between the patients who were thrombolysed within 3-6 hours of stroke onset versus beyond 6 hours. Finally, I compared and contrasted the attributes of the studies and assessed their quality based on the manner in which patients were enrolled and the resulting baseline characteristics.

8.3 Results

8.3.1 Literature search

The literature search (Table 1) led to 13 citations on the DEFUSE trial (6 articles),^{183,378-385} two on the DEDAS trial (1 article),¹¹⁰ 6 on the DIAS trial,¹⁰⁹ 9 on the EPITHET trial (three articles)^{181,386-388} and 2 on DIAS II(1 article).^{111,389} The information on 502 patients was obtained from the five main articles^{109-111,181,183} describing the relevant trials [(DIAS (104 patients), DIAS II(186 patients), DEDAS (37 patients), DEFUSE (74 patients) and EPITHET (101 patients)] and the data corresponding to the patients with mismatch profile were retrieved for subsequent analysis.

8.3.2 Comparative analysis of the “Mismatch” Trials:

I compared the attributes that differed between the trials to highlight the underlying heterogeneity in the manner the selected trials were conducted DIAS II enrolled the least severely affected stroke patients (median NIHSS 9) and EPITHET the most severe (median NIHSS 14 in the treatment arm and 10 in the placebo arm). Median baseline NIHSS scores were 11.5 and 12 respectively in the DEFUSE and DIAS I trials. I also compared the time since stroke onset until thrombolysis [OTT] and we assessed qualitatively the proportion of patients treated in each trial after 4.5 hours. Detailed analysis of OTT could not be undertaken without raw data.

Table 8-1 Characteristics of the trials included for the meta-analysis

Attributes	DIAS I¹⁰⁹	DIAS II¹¹¹	DEFUSE¹⁸³	DEDAS¹¹⁰	EPITHE¹⁸¹
Drug	Desmoteplase	Desmoteplase	t-PA	Desmoteplase	t-PA
Doses	Fixed doses: 25mg, 37.5 mg, 50 mg and later weight adjusted doses of 62.5µg/kg, 90 µg/kg and 125 µg/kg.	90 µg/kg and 125µg/kg; permissible maximal dose: equivalent to 100 kg.	0.9mg/kg; 10% dose bolus, rest over one hour; no upper limit to dose.	90µg/kg and 125 µg/kg; no upper limit to the maximal dose.	0.9mg/kg;10% dose bolus, rest in 1 hour; permissible upper limit 90mg.
Age (years)	18 to 85	18 to 85	18 +	18 to 85	18+
NIHSS	8 TO 20	4 TO 24	≥5	4 TO 20	≥5
Eligibility criteria					
Method used to evaluate mismatch	Mean Transit Time (MTT)	CT&MRI; based on visual impression	selection on visual impression of the	MTT; based on visual impression	Tmax

investigator.

Primary Endpoints of the study	Reperfusion [†] in 4 to 8 hours post treatment and clinical outcome at day 90.	8 points improvement or score of 0-1 on the NIHSS scale; a score of 0 to 2 on mRS and BI score of 75-100.	8 points improvement or score of 0-1 on the NIHSS scale; a score of 0 to 2 on mRS and BI score of 75-100.	Reperfusion [†] in 4 to 8 hours post treatment and clinical outcome at day 90.	Infarct growth attenuation in mismatch patients between alteplase and placebo analysed.
SICH definitions	Any ICH associated with a worsening of 4 points or more on the NIHSS and confirmed by CT within 72 hours of treatment.	ICH confirmed by “appropriate imaging tool and clinical worsening of 4 or more points on NIHSS at 72 hours	Any degree of brain haemorrhage identified along with worsening on NIHSS greater than or equal to 2 with in 36 hours of t-PA [major SICH if on NIHSS and major SICH if the deterioration on NIHSS is 4 or more]	Any ICH associated with a worsening of 4 points or more on the NIHSS and confirmed by CT within 72 hours of treatment	(As per SITS-MOST criterion) Clinical deterioration of 4 or more on NIHSS scale within 36 hours of thrombolysis, parenchymal haemorrhage of grade 2 on CT scans.

Legend: [†]Reperfusion defined as either $\geq 30\%$ reduction of MTT volume of abnormality or 2 points improvement on the adapted Thrombolysis In Myocardial Infarction (TIMI) grading scheme using MRA.

Table 8-2: Baseline characteristics of Onset-to-Treatment time in the Mismatch Trials

Clinical Trial	OTT data
DIAS II	<ul style="list-style-type: none"> • 43 patients thrombolysed in 3-6 hours against 26 in placebo arm. • 80 patients thrombolysed in 6-9 hours against 37 in placebo arm.
DEDAS	<ul style="list-style-type: none"> • OTT (median) for treatment group (n=29): 7 h 29 min [Range: 3 h 42 min – 9 h 28 min] • OTT (median) for placebo group (n=8): 7 h 23 min [Range: 3 h 40 sec – 8 h 36 min].
DIAS I	<ul style="list-style-type: none"> • OTT (median) for treatment group (n=75) : 5 h 24 min • OTT (median) for placebo group (n=27) : 5 h 25 min • OTT (median) for total population : 5 h 25 min.
EPITHET	<ul style="list-style-type: none"> • OTT (mean) for the treatment group: 4 h 57 min [standard deviation (SD) = 42 min] • OTT (mean) for the placebo group: 4 hours 54 minutes [SD=50 min] • OTT (mean) mismatch profile group: 4 h 53 min. SD= 45 minute • OTT (mean) mismatch profile placebo group : 4h 51 min, SD = 51 min.

8.3.3 Statistical Analyses

8.3.3.1 Did reperfusion or recanalisation occur more frequently in the patients who were thrombolysed?

The data from 211 patients showed greater individual odds for reperfusion and/or recanalisation amongst the patients who received thrombolytic therapy [statistically significant in the DIAS I trial (OR = 4.1, 95% CI = 1.3-15.2) the

EPITHET trial (OR = 3.7, 95% CI = 1.3 - 10.8) but non-significant in DEDAS trial [(OR=0.9, 95% CI =0.1-6.9)]. Here, the combined data gave a greater adjusted odds for reperfusion / recanalisation for the patients who had thrombolytic therapy (at any dosage) [a-OR: 3.0; 95% CI = 1.6-5.8; $P < 0.05$, P for heterogeneity =0.26 and I^2 : 25.7%]

I repeated the analysis after excluding desmoteplase doses that have been abandoned for clinical development: the sub-analysis restricted to desmoteplase 90 $\mu\text{g}/\text{kg}$ or rt-PA gave a-OR=2.28; 95%CI =0.7-7.3, $P=0.165$ [*Random Method*] and a-OR=2.65, 95%CI = 1.3-5.5, $P=0.007$ [*Fixed Method*], P for clinical heterogeneity =0.13 and I^2 : 50.5%]. I examined the underlying heterogeneity by L'Abbé plot.

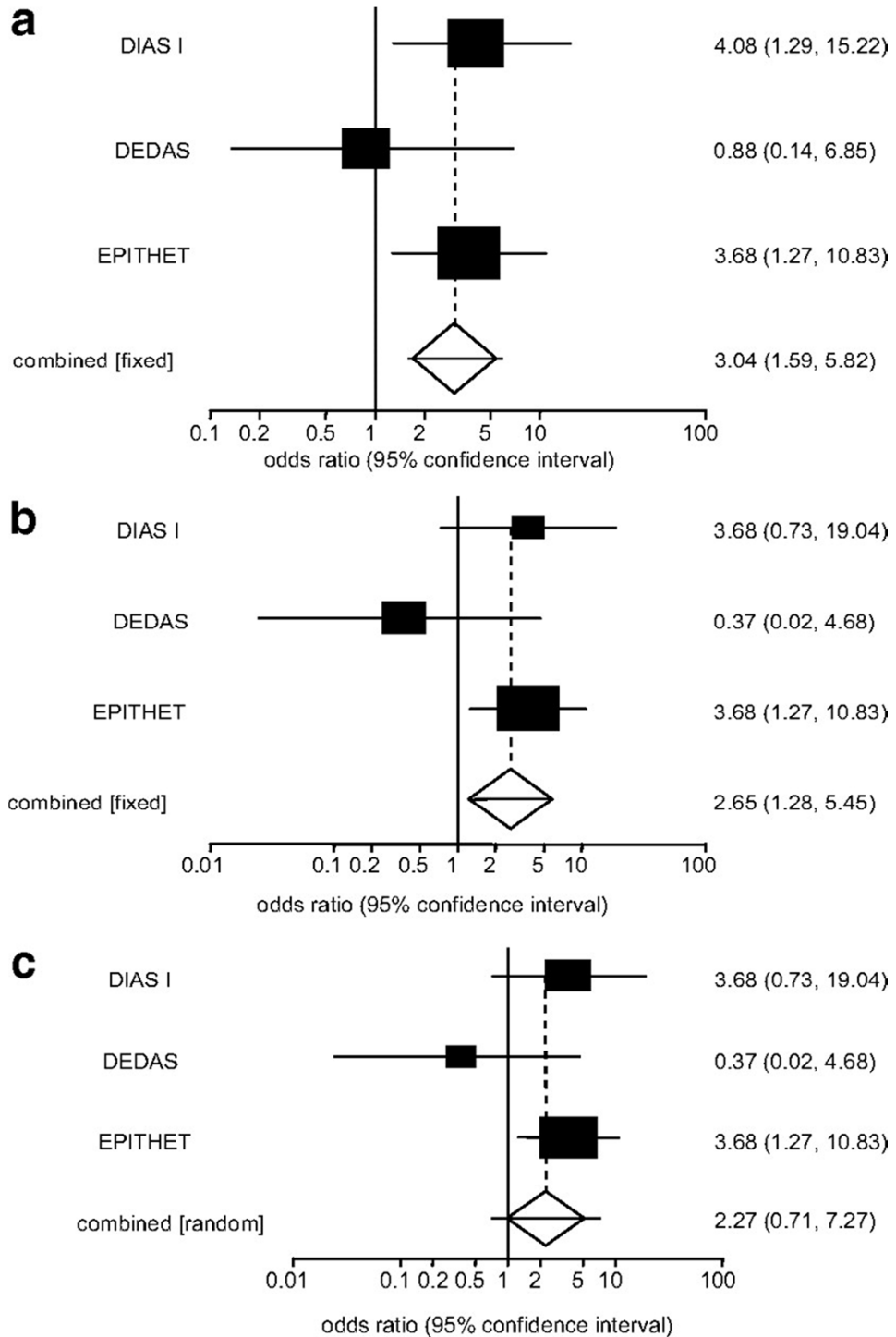


Figure 8-1 Did reperfusion or recanalisation occur more frequently in patients who were thrombolysed?

Findings are shown from the fixed-method analysis of combined data (a) after exclusion of abandoned doses by fixed (b) and random-method (c) analyses.

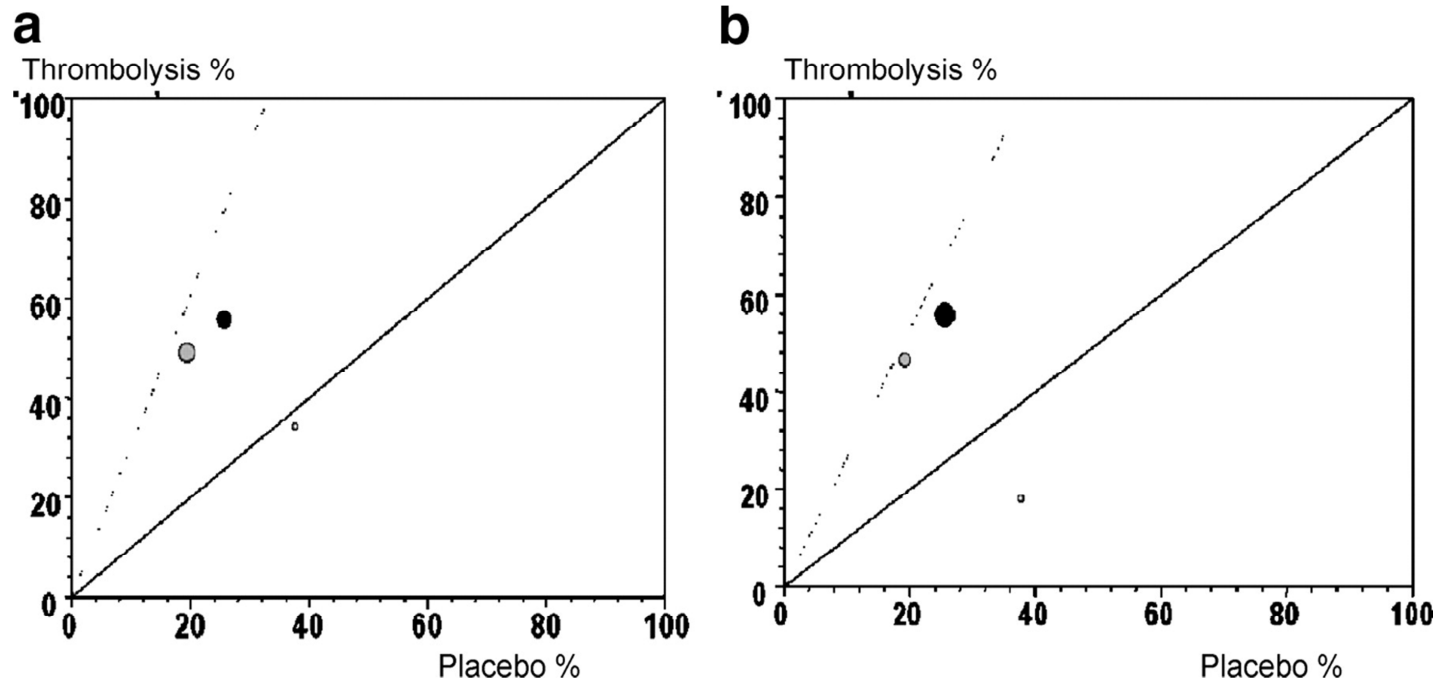


Figure 8-2 Did reperfusion or recanalisation occur more frequently in patients who were thrombolysed?

L'Abbé plot shows (a) the complete data set and (b) the abandoned doses excluded for heterogeneity. The circle size denotes the sample size; DIAS, grey circles; DEDAS open circles; and EPITHET, black circles.

8.3.3.2 Are favourable outcomes more common in the patients who had reperfusion?

The individual odds for favourable clinical outcome in the four studies reporting this endpoint were greater in the patients who had reperfusion as compared to those who did not reperfuse (DIAS I : OR = 3.4, 95% CI = 1.3 - 8.8; DEDAS : OR = 9.6, 95% CI = 1.5 - 64.6; EPITHET : OR=7.2, 95% CI :2.3 to 23.2; DEFUSE : OR = 5.4, 95% CI = 0.94 - 38.1). For all trials combined, the adjusted odds were greater for the patients who had successful reperfusion as compared to those who did not [a-OR = 5.2, 95% CI = 3 to 9.1], P for clinical heterogeneity = 0.60, I^2 :0%]

In a sensitivity analyses in which the DEFUSE trial data were excluded [as the DEFUSE was (unlike others) a non-randomised prospectively conducted study], the a-OR remained greater amongst patients with successful reperfusion (a-OR = 5.2, 95% CI = 2.8 to 9.5, P =0.00; heterogeneity statistics p: 0.4 and I^2 :0%)

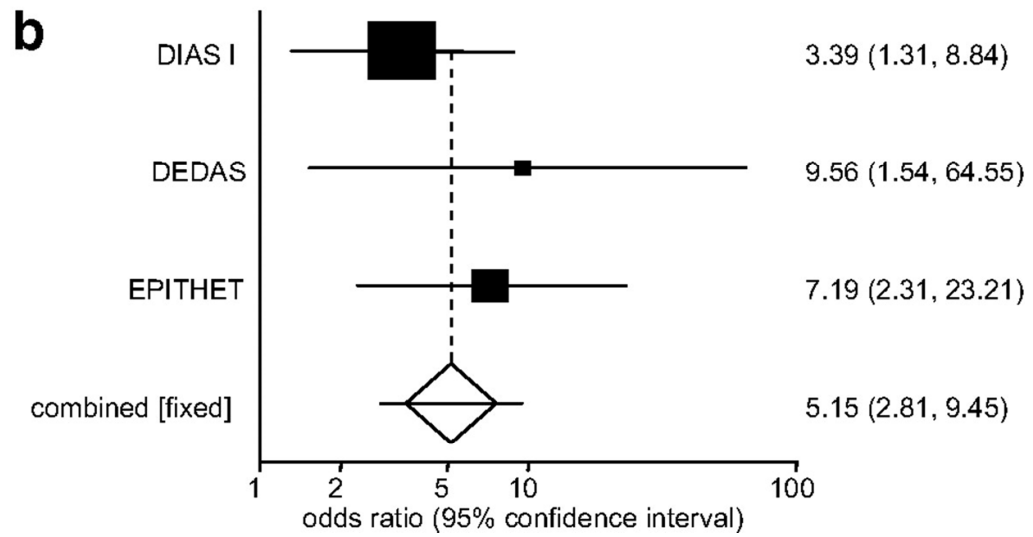
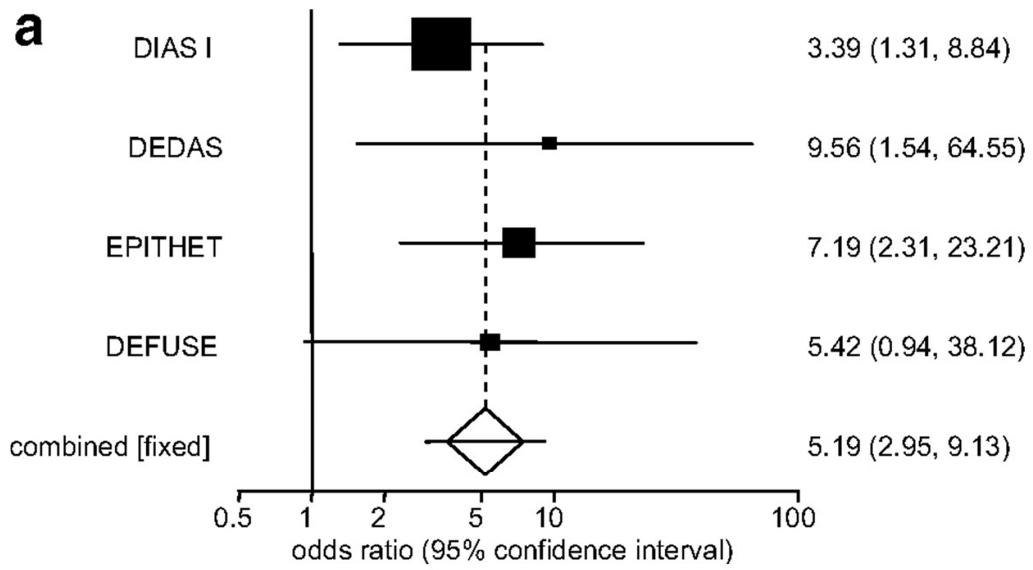


Figure 8-3 Are favourable outcomes more common in patients who underwent reperfusion?

Findings are shown from the fixed-method analysis of combined data (a) and after excluding DEFUSE data (b).

8.3.3.3 Did favourable clinical outcome occur more frequently in the thrombolysed group of patients?

With the exception of DIAS II, all trials had reported non-significantly improved odds of favourable clinical outcome in the thrombolysis group of patients. (DIAS I: OR = 2.2, 95% CI = 0.7 - 7.44; DEDAS ⁽¹⁹⁾: OR = 2.4, 95% CI = 0.4 - 28.0; EPITHET: OR = 1.7, 95% CI= 0.7-4.4; DIAS II: OR=0.8, 95% CI =0.4-1.6). The combined data analysis failed to show significant benefit (a-OR = 1.28 95% CI = 0.84-1.97), P for clinical heterogeneity = 0.28 and $I^2=20.9\%$. On excluding the DIAS II data, a-OR: 1.96, 95%CI: 1.06-3.63 and for clinical heterogeneity the $I^2:0\%$ and $P=0.89$.

I repeated the analysis after excluding desmoteplase doses that have been abandoned for clinical development: with 90ug/kg desmoteplase and rt-PA 0.9 mg/kg data alone, I found a-OR = 1.4; 95% CI = 0.9-2.3, $P=0.16$; for clinical heterogeneity $p=0.56$ and, $I^2:0\%$. On excluding the DIAS II data, OR=1.88, 95% CI: 0.95-3.72 and heterogeneity statistics: $I^2= 0\%$; and $p=0.69$. L'Abbé's plots were examined for the underlying heterogeneity in these analyses.

Under sensitivity analysis, no differential effect of desmoteplase versus alteplase was found, with the ratio of OR= 0.7 [95%CI: 0.24 to 1.920; $P: 0.46$].

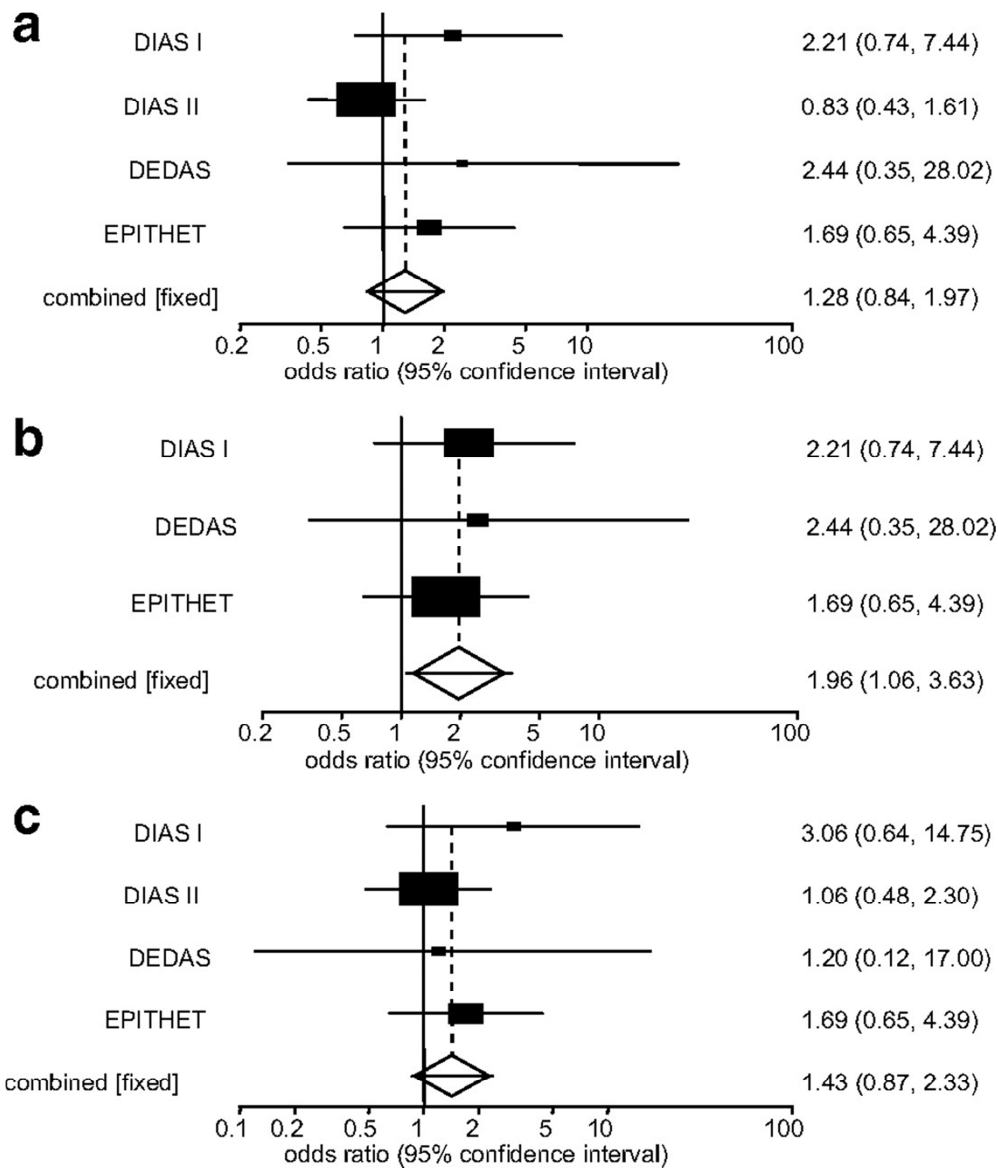


Figure 8-4 Did a favourable clinical outcome occur more frequently in the thrombolysed group of patients?

Findings are shown from the fixed method analysis of combined data (a), after exclusion of DIAS II data (b), and after exclusion of abandoned doses (c).

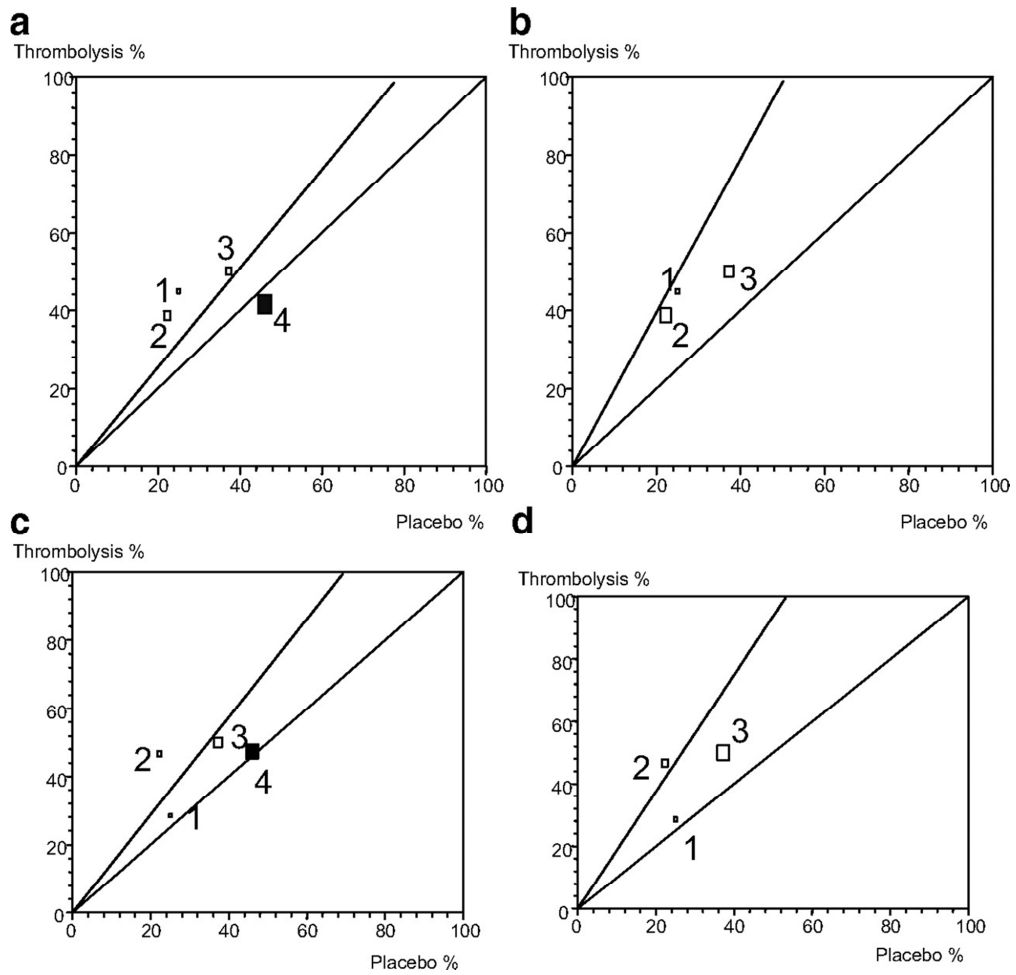


Figure 8-5 Did a favourable clinical outcome occur more frequently in the thrombolysed group of patients?

L'Abbé plot examining heterogeneity in the analysis (a) for complete data, (b) for DIAS II data excluded, (c) for complete data but abandoned doses excluded, and (d) for DIAS II data and abandoned-dose data excluded. The size of the square denotes the sample size. 1 indicates DEDAS; 2, DIAS I; 3, EPITHET; and 4, DIAS II (black rectangle).

8.3.3.4 Was there a greater probability of mortality in thrombolysed patients compared to those not thrombolysed?

Here, the individual odds for mortality were non-significant in the thrombolysis group. [DIAS II:OR= 2.4, 95% CI =0.7-10.1) ; DIAS I: OR= 3.6, 95%CI = 0.5-161.3;EPITHET trials (OR: 2.7, 95% CI: 0.8-10.9) and the DEDAS trial (OR: 0.5, 95% CI=0.0-34.9)]. The combined-data analysis found a significant increase in mortality in the thrombolysis group of patients compared to the placebo group [a-OR =2.4, 95% CI =1.2-4.9; P=0.02 and P for heterogeneity =0.67 and I²:0%].

Repeating the analysis after excluding data from abandoned desmoteplase doses, i.e. restricting the analysis to patients treated with 90 µg/kg of desmoteplase or 0.9 mg/kg rt-PA, then a-OR= 1.6 [95%CI: 0.7 - 3.7], P = 0.28 ; P for heterogeneity = 0.56 and I²:0%.

Under sensitivity analysis, no differential effect of desmoteplase versus alteplase was found, with the ratio of OR=. 0.8 [95%CI: 0.2 to 3.5; P:0.8]

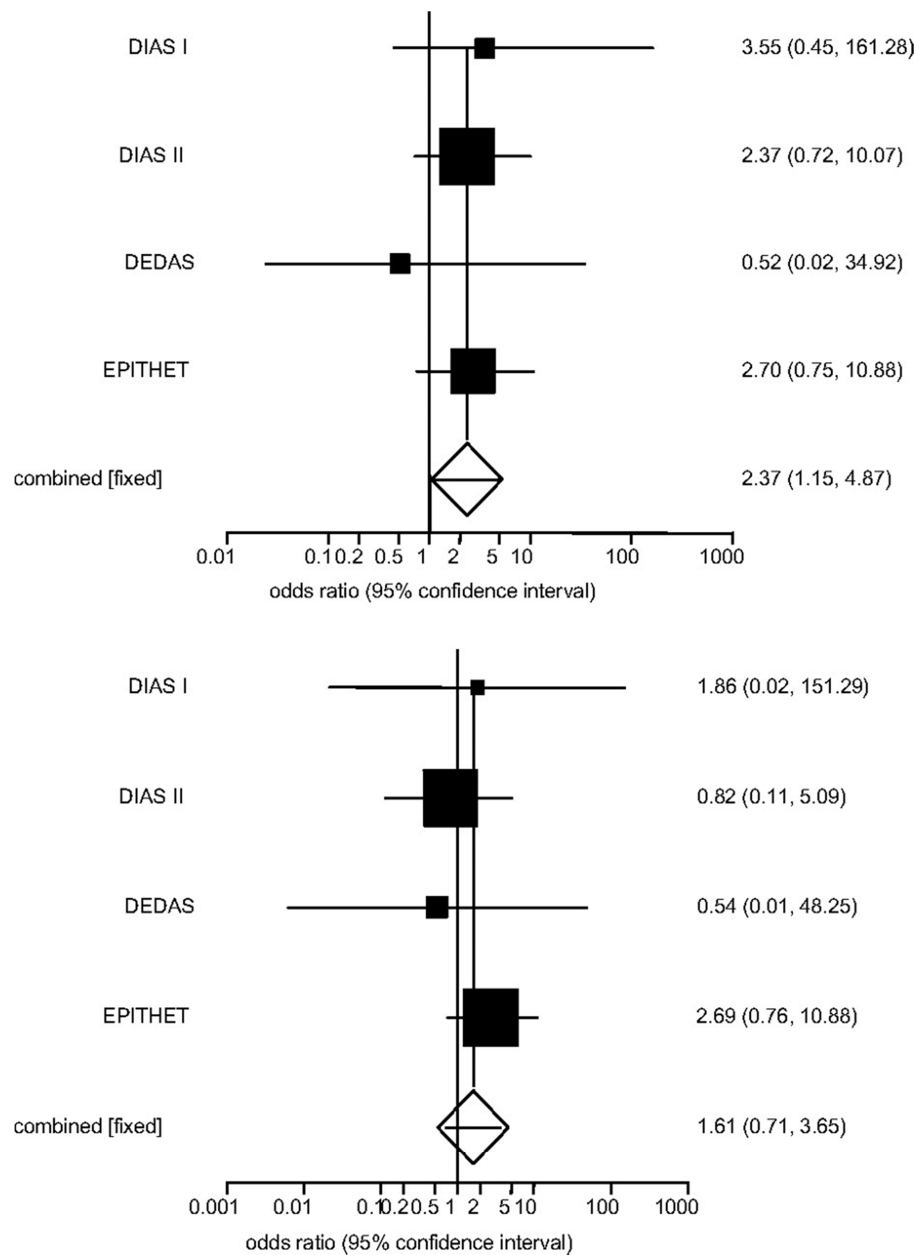


Figure 8-6 Was there a greater probability of mortality in thrombolysed patients compared with those not thrombolysed?

Findings are shown from the fixed-method analysis of combined data (a) and after exclusion of the abandoned-dose data (b).

8.3.3.5 Was there a greater probability of SICH in thrombolysed patients compared to those not thrombolysed?

The individual odds for SICH were non-significant for the combined data (DIAS I: OR=7.9, 95%CI =0.7-infinity; DIAS II: OR=5.9, 95%CI: 0.5-infinity; EPITHET: OR=152.6, 95%CI: 15.9-infinity) but the combined odds for SICH were significantly greater for the group that underwent thrombolytic therapy (a-OR: 24.7, 95%CI : 5.2-118.2. Heterogeneity statistics: I^2 :35.4%, and $P=0.2$). On combining data from DEDAS with DIAS I, the findings remained non-significant for the individual odds (DIAS I + DEDAS: OR=7.1, 95%CI=0.7-infinity) but were significant for the combined analysis [a-OR: 6.5, 95%CI: 1.2-35.4 and for clinical heterogeneity $p=1.0$ and I^2 :0%]

Repeating the analysis by excluding the data of abandoned doses, the findings were non-significant both for individual odds (DIAS I+DEDAS OR=3.7, 95%CI: 0.03-infinity; DIAS II: OR=5.7, 95%CI: 0.2-infinity; EPITHET: OR=6.5, 95%CI: 0.4-infinity) and by combining it with the DIAS I data (a-OR 5.4, 95%CI 0.9-31.8), P for heterogeneity = 0.97 and I^2 :0% but attained a marginal significance of the adjusted odds derived by excluding DEDAS trial (a-OR: 6, 95%CI 1.00-35.8; heterogeneity statistics: $p:1$ and I^2 : 0%).

There occurred no SICH in placebo arms and therefore a sensitivity analysis to assess any differential effect of desmoteplase versus alteplase could not be undertaken.

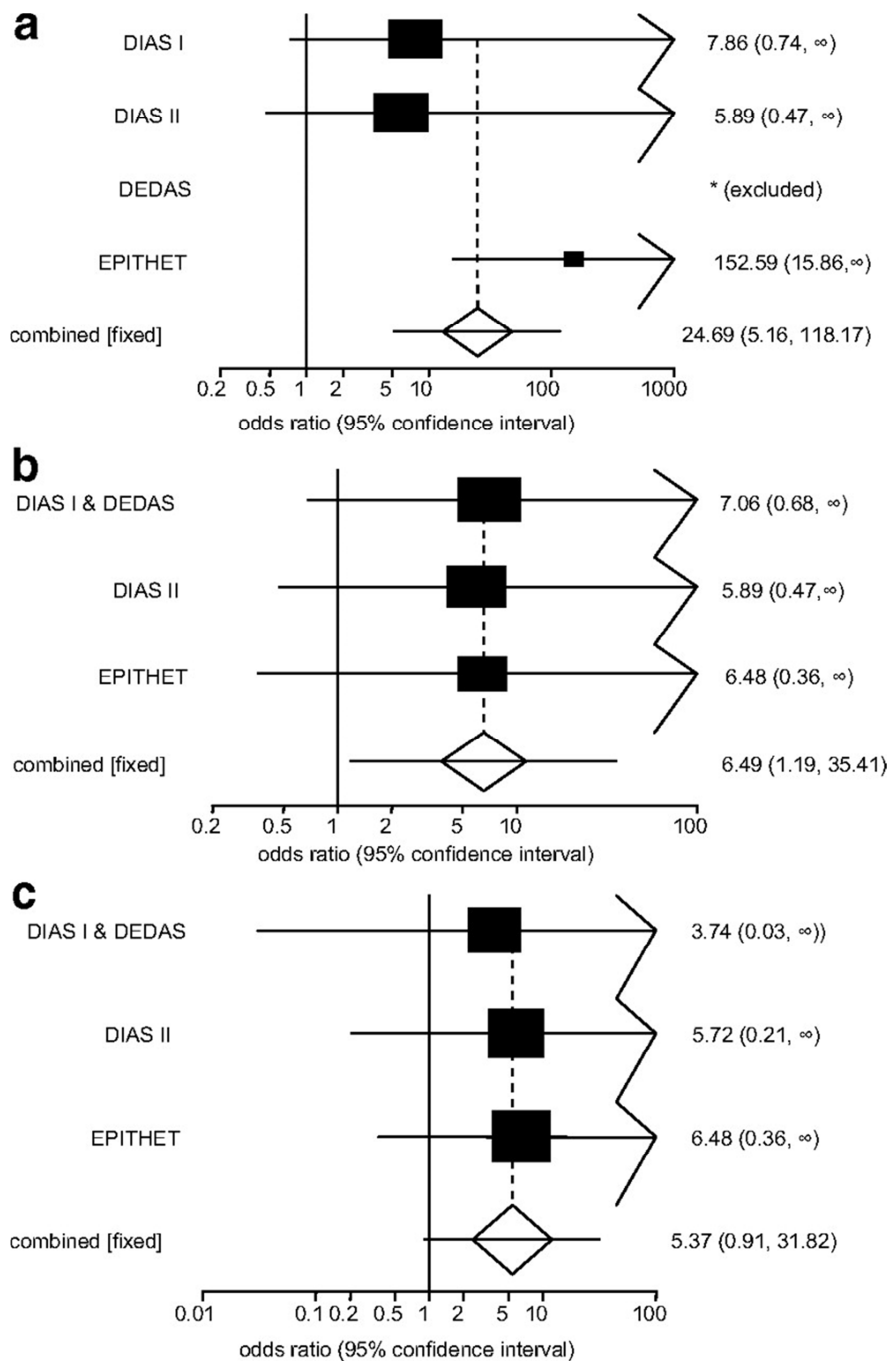


Figure 8-7 Was there a greater probability of SICH in thrombolysed patients compared with those not thrombolysed?

Findings are shown from the fixed-method analysis for all studies combined but with DEDAS data excluded (a), DEDAS combined with DIAS I data (b), and after exclusion of the abandoned-dose data (c).

8.3.3.6 Were there better clinical findings (outcomes or reperfusion) if treatment commenced within 3 to 6 hours versus 6 to 9 hours?

Limited data were available to examine OTT and neither the DIAS I ⁽²⁰⁾ nor DIAS II individually suggested significantly greater odds (DIAS I: OR= 1.07, 95%CI = 0.4-2.9, P= 0.9; DIAS II: OR= 0.8, 95%CI: 0.4- 1.8, P= 0.7). When combined, the a-OR = 0.9, 95%CI: 0.5- 1.7, p=0.8)

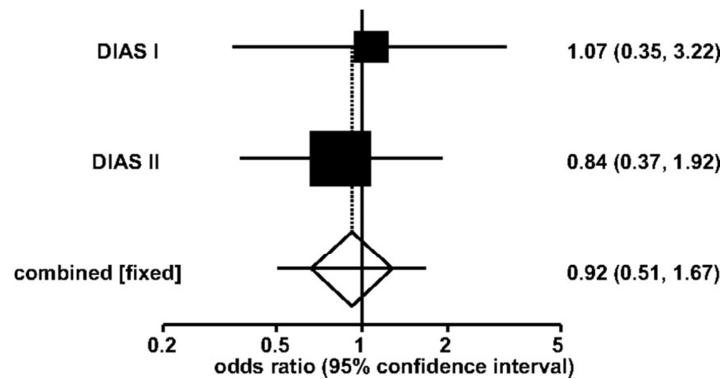


Figure 8-8: Were there better clinical findings (outcomes or reperfusion) when treatment was commenced within 3 to 6 hours vs. 6 to 9 hours?

8.3.4 Analysis of Mortality

In DIAS I trial, one placebo and two desmoteplase deaths occurred due to cardiac causes. In the DIAS II trial, only one of three deaths in the 90 µg/kg group and three of 14 deaths in 125 µg/kg were considered related to the trial medication. In the DEDAS trial, the sole death in the 90 µg/kg groups was due to aspiration pneumonia while that in the 125 µg/kg groups was due to evolving neurological deterioration of a left MCA infarct, leading to pneumonia.

8.4 Discussion

I undertook a meta-analysis of all previous studies that evaluated the principle of physiological selection for delayed thrombolysis, based on the presence of potentially viable tissue in the ischaemic penumbra.^{29,390,391} These trials utilised the mismatch hypothesis using either MRI (perfusion/diffusion mismatch) or CTP (perfusion/cerebral blood volume mismatch) as a signature of the putative penumbra.^{109-111,181,183} Apart from the recent DIAS-II trial¹¹¹, these trials had supported the physiological basis of the mismatch concept. The disappointing findings of the DIAS II trial have been attributed to limitations of the study and to chance.^{111,389} To test for consistency, I undertook a meta-analysis of the studies that studied the mismatch hypothesis to select and thrombolysed patients despite delays beyond 3 hours. Five trials (DIAS I, DIAS II, EPITHET, DEFUSE and DEDAS)^{109-111,181,183} were available for inclusion.

It was a priori decided that those studies would be excluded which did not undertake prospective enrolment of consecutive stroke patients with mismatch profile (for treatment in delayed time window, i.e., beyond 3 hours of stroke onset) or did not study mismatch-based delayed thrombolysis in a randomised controlled design. Hence, I did not include the study by Schellinger et al⁴⁵ as it was based on *pooled* data (and not a pre-planned prospective trial) from 5 different centres and lacked in a control arm that would have prevented calculation of odds ratio in the absence of a denominator. DEFUSE trial was included in this meta-analysis because it was a prospectively conducted clinical trial. Further, it reported reperfusion data that could be considered for the analysis.

The results from this metaanalysis indicate that reperfusion and/or recanalisation is more common with thrombolysis when all doses are considered together but the significance is lost with the exclusion of data on abandoned doses of desmoteplase that reduced the power of this analysis through effects on sample size.

Furthermore, favourable clinical outcome is more common amongst patients with successful reperfusion of the ischaemic parenchyma, despite delays beyond three hours from stroke onset. This conclusion is not influenced by inclusion of the non-randomised DEFUSE trial data. The DIAS II trial did not report reperfusion findings.

However, I did not find evidence from the current analysis that favourable clinical outcome was significantly improved in the group that underwent thrombolysis. Neither did I find a significant benefit if I excluded doses of desmoteplase that have been abandoned for clinical development. The confidence interval around the estimate of effect remains wide and would be consistent with a doubling of odds for favourable outcome, though in this respect DIAS-II suggests that the likely upper limit may be 1.5. Even so, odds of 1.5 remain greater than those achieved in unselected patients treated with rt-PA in the ECASS-III trial and have been regarded as sufficient to influence national and European stroke treatment guidelines (SIGN or ESO)³⁹².

Late treatment even among selected patients may carry some risk. I found a marginally significant increase in the odds of death among all treated patients, with a point estimate of 2.4. When I restricted the analysis to rt-PA 0.9 mg/kg and to the dose of desmoteplase that remains under development (90 µg/kg), the odds ratio for mortality fell to 1.6 and the risk was not significant. Higher doses of desmoteplase were clearly linked to excessive SICH and were abandoned for this reason. The current analysis does not take into account the attributed cause of death. Many deaths in DIAS-II and in EPITHET were considered unrelated to treatment. The attribution may be important for understanding of the mechanism of effect but caution is required when drawing conclusions from subjective assessments such as these. Treatment failure can contribute to late death, just as unrecognised excitotoxic damage may represent a potential mechanism. Regardless, if mortality is increased, this may be mediated via haemorrhagic transformation.

Despite lack of significance in the individual odds for the SICH in the patients given thrombolytic therapy, the adjusted OR indicates a statistically significant increase in SICH after delayed thrombolysis. Similarly, an increased risk of sICH

has long been recognised for time-based tPA in the established clinical windows, but this is offset by the improved clinical outcomes in the treated patients. On excluding doses of desmoteplase that have been abandoned for clinical development, the adjusted odds for SICH again lost significance.

Caution is required in interpreting these post-hoc sub-group analyses. Although the inclusion of data from all doses may give a falsely pessimistic view of the risk/benefit profile after mismatch-based thrombolysis, post-hoc exclusion of doses that have been abandoned in clinical development is a data-driven decision and raises statistical concerns of bias that can only be assuaged by further prospective trials.

I find no evidence that relatively earlier (3-6h) versus later (6-9h) treatment influence my findings. This is of particular relevance, since ECASS-III has recently shown unselected patients benefit from alteplase given within 4.5 h of stroke onset, and a small proportion of patients in the mismatch trials would now be considered eligible for such treatment: I cannot exclude the possibility that some of the potential benefit amongst mismatch patients may be time-dependent but it appears unlikely that this is sufficient to explain all effects.

Now that ECASS-III results are known, a further meta-analysis using individual patient data from the trials studied here should be undertaken to assess clinical and radiological outcomes for the patients who were thrombolysed beyond 4.5 hours of the stroke onset. Similarly, an additional analysis comparing outcomes in patients with mismatch versus without mismatch is desirable but was beyond the scope of our meta-analysis.

The current meta-analysis included data from five different trials of which DEFUSE could be considered only in the analysis of favourable clinical outcome amongst patients with reperfusion vs. no-reperfusion. DIAS II did not report the reperfusion findings and had to be excluded where these data were needed. The L'Abbe plot^{393,394} suggests that DIAS II contributes to the heterogeneity in the combined analysis of favourable outcomes in all thrombolysed patients, and the DEDAS trial to the analysis of reperfusion and recanalisation in patients thrombolysed with the abandoned doses excluded. Both these sources of

heterogeneity appeared to affect the results by virtue of the effects of sample size on the power of a study.

We know that the number needed to treat to achieve enhanced favourable outcome with alteplase may be as few as seven within 3h but that this has risen by 3-4.5 h to approximately fourteen. When treatment with alteplase is started between 4.5 hours and 6 hours OTT the number needed to treat rises to 25. Hence, the challenge is to establish whether any patients benefit or donot based on multimodal imaging approaches and delayed time wondow. The use of either MR imaging to identify perfusion/diffusion mismatch or a CT-based alternative is attractive. It is clear from current data that delayed thrombolysis amongst patients selected according to mismatch imaging is associated with increased reperfusion and/or recanalisation and that recanalisation and/or reperfusion is associated with improved outcomes. At present, whilst the data remain consistent with improved functional outcome from delayed thrombolysis amongst mismatch patients, statistically significant benefit on functional outcomes has not been confirmed. Although the pooled results suggest that mortality may be higher, the retention of excessive doses of desmoteplase in the analysis is likely to lead to overestimation of any risk.

It should be noted that existing methods for defining mismatch may be optimised in future resulting in greater power of the mismatch-based thrombolysis studies. For example, in this analysis, I considered 1.2 as the cut-off for defining a mismatch profile. However, a post-hoc analysis of the DEFUSE study has recently shown that highest sensitivity and specificity occur at a mismatch ratio of 2.6; suggesting that the previous studies were probably underpowered and lacked a sufficiently rigorous definition for mismatch ratio.¹⁸² Further, the 2-second threshold for T max is likely also suboptimal, as a post-hoc analysis of DEFUSE data have shown a significantly better correlation between infarct growth and penumbra salvage volume for PWI lesions defined by Tmax > 6 seconds.³⁸³ The EPITHET investigators have reported similar findings.³⁸⁶ It is now clear that both trials included significant volumes of benign oligemia in their mismatch assessments. Recently, automated online analysis of MR mismatch has been described that facilitates the rapid selection for delayed treatment. In summary,

the continued refinement in the definitions of different perfusion parameters would result in better choice of the best measure of perfusion [T max, TTP, MTT, CBV or CBF] and correction for arterial input functions.

Thus, the definitions used in the published trials to date have been generous, including many patients who had limited penumbral tissue and limited prospect of clinical improvement in response to thrombolysis. The recently formed Stroke Imaging Repository (STIR) collaboration has initiated a detailed examination of this topic.³⁹⁵ The diversity of mismatch definitions and large number of investigators involved in these studies weaken conclusions about the potential value of mismatch in future clinical management of patients with stroke.^{198,199} However, these weaknesses do not extend to our conclusions about the status of existing evidence for use of thrombolysis amongst mismatch patients: patients were selected according to the best intentions of the investigators under protocols which were state of the art when written, though they have already been overtaken. Prospective phase III trials are required to test whether thrombolysis is associated with a favourable risk/benefit ratio when used under modified circumstances. In Australia, the Extending the Time for Thrombolysis in Emergency Neurological Deficits (EXTEND) trial,³⁹⁶ which will use a phase III design and randomisation of patients 4.5-9 hours alteplase or placebo, using automated mismatch selection, will test this hypothesis.

These data show that delayed thrombolysis amongst patients selected according to mismatch imaging is associated with increased reperfusion/recanalisation and that recanalisation/reperfusion is associated with improved outcomes. But, not all patients that receive alteplase improve in their outcomes. From an analysis of combined DEFUSE and EPITHET dataset, treatment effect of t-PA was shown to be greater amongst those patients that had vessel occlusion at baseline.^{46,397} Further, in a meta-analysis, Rha and Saver have also shown strong association of recanalisation with improved outcomes and reduced mortality in a meta-analysis.¹⁵¹ It appears logical that the target of thrombolytic therapy should be to achieve recanalisation. Hence trialists now aim to select patients with “Vessel occlusion or high-grade stenosis in proximal cerebral arteries” for treatment until 9 hours of symptoms onset.⁴⁶

Meantime, while the concept of selection of patients based on their individual pathophysiology rather than a rigid time window remains attractive, delayed treatment according to mismatch selection cannot be recommended as part of routine care until or unless further trials show benefit.

Chapter 9

Conclusions and future directions

9 Conclusions and future directions

Thrombolytic therapy is the therapy of proven efficacy for the treatment of acute ischaemic stroke.¹³¹ However, it has not yet made a significant public health impact; very few patients receive this therapy.²²⁸ Many physicians fail to administer t-PA because they adhere strictly to the stroke guidelines and fear that any deviation would increase the risk of haemorrhage. It is known that most of the exclusion criteria are based not on negative evidence (showing harm from t-PA use), but on absence of data from the original trials. Many of the exclusions were based on expert opinion which was originally framed to assist recruitment of a homogeneous patient population for the trial. Soon after the NINDS trial was published, showing better outcomes in a 3 hour time window, the drug was approved in the USA.²⁴ The European Medicines Agency was reluctant to approve its use (because ECASS I, ATLANTIS and ECASS II failed to confirm the NINDS findings), but later did, subject to the fulfilment of conditions that the SITS-MOST study and ECASS III trials would be conducted by the sponsors.²³ While SITS-MOST showed that the outcomes from the t-PA use in 3-hours window were similar to the pooled RCT data (and can also be used by less experienced centres), the ECASS-III trial showed that t-PA use improved outcomes even in a 3-4.5 hour time window.^{129,146,147} After it was shown that t-PA is efficacious until a 4.5 hours' time window, there was a felt need to answer other exclusions that were listed in the EMEA document.²³

The purpose of this thesis was to examine those exclusion criteria, and therefore, I decided to examine the following questions: (a) Whether the benefit from t-PA use extends to those older than 80 years? (b) Whether those suffering from concomitant diabetes and previous stroke have improved outcomes after the use of alteplase? (c) Whether the patients having a mild stroke or a severe stroke should be treated with alteplase? In addition, I also planned to examine whether use of thrombolytic therapy beyond 3 hours and based on mismatch criteria could be recommended for routine clinical practice?

So in Chapter 3, I examine Virtual International Stroke Trials Archive (VISTA) data to test whether current European recommendation suggesting exclusion of

elderly patients (older than 80 years) from thrombolysis for acute ischaemic stroke is justified. Employing non-randomised controlled comparison of outcomes, I show better outcomes amongst all patients ($P < 0.0001$; OR, 1.39; 95% CI, 1.26 to 1.54), young patients ($P < 0.0001$; OR, 1.42; 95% CI, 1.26 to 1.59) and the elderly patients ($P = 0.002$; OR, 1.34; 95% CI, 1.05 to 1.70). Odds Ratios are consistent across all age deciles > 30 years. Outcomes assessed by National Institutes of Health Scale (NIHSS) score and dichotomised modified Rankin Scale score are consistently similar.

In Chapter 4, I compare thrombolysed patients in Safe Implementation of Thrombolysis in Stroke International Stroke Thrombolysis Register (SITS-ISTR) with VISTA non-thrombolysed patients ("comparators" or "controls") and test exactly similar question as in Chapter 3. Distribution of scores on modified Rankin scale are better amongst all thrombolysis patients than controls (odds ratio 1.6, 95% confidence interval 1.5 to 1.7; Cochran-Mantel-Haenszel $P < 0.001$). Association occurs independently amongst patients aged ≤ 80 (OR 1.6, 95% CI 1.5 to 1.7; $P < 0.001$; $n = 25,789$) and in those aged > 80 (OR 1.4, 95% CI 1.3 to 1.6; $P < 0.001$; $n = 3439$). Odds ratios are consistent across all 10 year age ranges above 30, and benefit is significant from age 41 to 90; dichotomised outcomes (score on modified Rankin scale 0-1 v 2-6; 0-2 v 3-6; and 6 (death) versus rest) are consistent with the results of ordinal analysis. These findings are consistent with results from VISTA reported in Chapter 3. Age alone should not be a criterion for excluding patients from receiving thrombolytic therapy.

In Chapter 5, I employ VISTA data to examine whether patients having diabetes and previous stroke have improved outcomes from use of alteplase in acute ischaemic stroke. Employing a non-randomised controlled comparison, I show that the functional outcomes are better for thrombolysed patients versus nonthrombolysed comparators amongst non-diabetic ($P < 0.0001$; OR 1.4 [95% CI 1.3-1.6]) and diabetic ($P = 0.1$; OR 1.3 [95% CI 1.05-1.6]) patients. Similarly, outcomes are better for thrombolysed versus nonthrombolysed patients who have not had a prior stroke ($P < 0.0001$; OR 1.4 [95% CI 1.2-1.6]) and those who have ($P = 0.02$; OR 1.3 [95% CI 1.04-1.6]). There is no interaction of diabetes and prior stroke with treatment ($P = 0.8$). Neurological outcomes (NIHSS) are consistent with functional outcomes (mRS).

In Chapter 6, I undertake a non-randomised controlled comparison of SITS-ISTR data with VISTA controls and examine whether patients having diabetes and previous stroke have improved outcomes from use of alteplase in acute ischaemic stroke. I show that adjusted mRS outcomes are better for thrombolysed versus non-thrombolysed comparators amongst patients with diabetes mellitus (OR 1.45[95% CI1.30-1.62], N=5354), previous stroke (OR 1.55[95% CI1.40-1.72], N=4986), or concomitant diabetes mellitus and previous stroke (OR 1.23 [95% CI 0.996-1.52], P=0.05, N=1136), all CMH $p < 0.0001$. These are comparable to outcomes between thrombolysed and non-thrombolysed comparators amongst patients suffering neither diabetes mellitus nor previous stroke: OR=1.53(95%CI 1.42-1.63), $p < 0.0001$, N=19339. There are no interaction between diabetes mellitus and previous stroke with alteplase treatment (t-PA*DM*PS, $p = 0.5$). Present data supports results obtained from the analyses of VISTA data in chapter 5. There is no statistical evidence to recommend exclusion of patients with diabetes and previous stroke from receiving alteplase.

In Chapter 7, I examine VISTA data to test whether exclusion of patients having a mild or severe stroke at baseline would be justified. Stratifying baseline stroke severity for quintiles of NIHSS scores, I observe that there are significant associations of use of alteplase with improved outcomes for baseline NIHSS levels from 5 to 24 ($p < 0.05$). This association lose significance for baseline NIHSS categories 1 to 4 ($P = 0.8$; OR, 1.1; 95% CI, 0.3-4.4; N = 8/161) or ≥ 25 ($P = 0.08$; OR, 1.1; 95% CI, 0.7-1.9; N = 64/179) when sample sizes are small and confidence interval wide. These findings fail to provide robust evidence to support the use of alteplase in the mild or severe stroke patients, though potential for benefit appears likely.

In Chapter 8, I present a meta-analysis of trials that investigated mismatch criteria for patients' selection to examine whether present evidence supports delayed thrombolysis amongst patients selected according to mismatch criteria. I collate outcome data for patients who were enrolled after 3 hours of stroke onset in thrombolysis trials and had mismatch on pre-treatment imaging. I compare favourable outcome, reperfusion and/or recanalisation, mortality, and symptomatic intracerebral haemorrhage between the thrombolysed and non-

thrombolysed groups of patients and the probability of a favourable outcome among patients with successful reperfusion and clinical findings for 3 to 6 versus 6 to 9 hours from post stroke onset. I identify articles describing the DIAS, DIAS II, DEDAS, DEFUSE, and EPITHET trials, giving a total of 502 mismatch patients thrombolysed beyond 3 hours. The combined adjusted Odds Ratios (a-ORs) for favourable outcomes are greater for patients who had successful reperfusion (a-OR=5.2; 95% CI, 3 to 9; $I^2=0\%$). Favourable clinical outcomes are not significantly improved by thrombolysis (a-OR=1.3; 95% CI, 0.8 to 2.0; $I^2=20.9\%$). Odds for reperfusion/recanalisation are increased amongst patients who received thrombolytic therapy (a-OR=3.0; 95% CI, 1.6 to 5.8; $I^2=25.7\%$). The combined data show a significant increase in mortality after thrombolysis (a-OR=2.4; 95% CI, 1.2 to 4.9; $I^2=0\%$), but this is not confirmed when I exclude data from desmoteplase doses that are abandoned in clinical development (a-OR=1.6; 95% CI, 0.7 to 3.7; $I^2=0\%$). Symptomatic intracerebral haemorrhage is significantly increased after thrombolysis (a-OR=6.5; 95% CI, 1.2 to 35.4; $I^2=0\%$) but not significant after exclusion of abandoned doses of desmoteplase (a-OR=5.4; 95% CI, 0.9 to 31.8; $I^2=0\%$). Delayed thrombolysis amongst patients selected according to mismatch imaging is associated with increased reperfusion/recanalisation. Recanalisation/reperfusion is associated with improved outcomes. However, delayed thrombolysis in mismatch patients was not confirmed to improve clinical outcome, although a useful clinical benefit remains possible. Thrombolysis carries a significant risk of symptomatic intracerebral haemorrhage and possibly increased mortality. Criteria to diagnose mismatch are still evolving. Validation of the mismatch selection paradigm is required with a phase III trial. Pending these results, delayed treatment, even according to mismatch selection, cannot be recommended as part of routine care.

In summary, by employing rigorous analysis techniques, I provide some data that would serve as a useful source of evidence to the practicing physicians.³⁹⁸

However, there are challenges that are associated with the use of historical controls (like VISTA non-thrombolysed patients as used in chapters 3-7). In some cases, it is possible to adjust for potential sources of bias or to interpret findings through internal controls. For example, in assessing effects of thrombolysis on

age, the results in the elderly could be compared to those in younger patients for whom RCT data are available to support the effect size estimate. Bias is less easy to control in the analyses for which comparable RCT data are unavailable. Owing to differences in population characteristics, one may observe excessive heterogeneity between patients' subgroups. If the contributing VISTA controls data used entry criteria for severity that did not fully encompass those of the thrombolysed group, then a systematic bias in outcomes would be likely. Further, geographical or temporal variation in management can generate bias as well. Although the historical outcomes in VISTA were rigorously collected, the SITS-ISTR data (Chapters 4 and 6) are open to bias through knowledge of treatment. Further, because SITS-ISTR is a registry, one cannot guarantee its completeness.

Bias may manifest as overly-estimated point estimate: bigger treatment effect (point estimates > 1.6 for improved outcomes)²⁶⁹ for patients with moderate b-NIHSS may indicate an imbalance in case mix (possibly leading to large heterogeneity between each subgroup) rather than a strong treatment effect from the use of t-PA. I undertook analyses adjusting for age and baseline severity, and then for those covariates that differed at baseline (if data were completely available), but, numerous other potential confounders remain unmeasured or unknown. For example, the time from stroke onset to use of t-PA could not be considered in these analyses. The onset to treatment time was known for SITS-ISTR but an equivalent value was not available in VISTA, where onset to randomisation for an investigational product was instead recorded (and was different between the t-PA and control groups). Further, these analyses did not control for the centre effect which may have influence on patients' outcomes.³⁹⁹ Baseline NIHSS accounts for about 80% of variance in outcomes of acute ischaemic strokes, and together with age should normally control for the variability in the adjusted analysis.^{265,280} The most compelling bias arises from the fact that the control patients were not given thrombolysis through clinician choice, implying that they may have been 'unsuitable' in an immeasurable or unrecorded way. The magnitude of these biases is unknown in these present analyses. Methods employed to adjust for differences at baseline do not confirm bias-removal.⁴⁰⁰ Despite availability of good prognostic data, residual

confounding may still persist.⁴⁰⁰ Even alternative methods like the propensity scores method do not guarantee removal of bias.

I based my adjusted analyses on CMH test and proportional odds logistic regression analyses. This approach to data analyses were applied by Lees et al in the SAINT trial²⁷¹ and have been tested and shown to give reliable estimates on other datasets.²⁶⁹ These also add to the power of the study.⁴⁰¹ The CMH test is limited by sample size, and if data on covariates are missing, it may not be able to stratify beyond a limit. For example, in chapter 7, fifty-nine per cent of records lacked coding for the variable “antithrombotic” (i.e. antiplatelet and anticoagulants) (N=3432). If the analyses were to be done within each stratum of the baseline stroke severity level, there would occur further diminution of the available data per stratum. Hence, an adjusted analysis for several covariates would be difficult within the stratum. In chapter 3, prior use of antiplatelet and anticoagulants was not used for adjusted analyses along with other co-variables that had differed at baseline, because, by including antithrombotic in the analyses, the effective size of dataset reduced from 4620 to 1930 for the non-elderly and from 1152 to 454 for the elderly. In chapter 4, adjusted analyses were possible for all variables that differed at baseline: the effective sample size reduced from 26028 patients to 22148 patients for the non-elderly age group and from 3472 to 2593 patients for the elderly age group. Adjustment by regression analysis relies on assumptions that there is a linear relationship between the covariates measured at baseline and outcomes at a later time point; and these can be expressed by regression equation. However, the assumption that the relationship is linear between the baseline stroke severity and outcomes may not always be true.^{280,402}

Despite these limitations, and pending randomised data, evidence presented in this thesis may be found useful by stroke physicians to guide therapy in their patients. International Stroke Trial (IST-3) has been enrolling patients in 0-6 hour time window for about a decade and will report subgroup analyses on some of the patients that are currently excluded from European drug label (like the elderly). Subgroup analysis of an open label trial spanning a period of >10 years will require careful scrutiny. These will be selected patients who may have been treated by centres that may have had doubts about their capability to deliver

alteplase safely or about treatment efficacy. Owing to dismal prospects of conducting large randomised trials that specifically test a hypothesis of outcomes in the specific subgroups of patients for which data are currently lacking (e.g. concomitant diabetes and previous stroke), data like those that are presented in this thesis may have relevance to the routine clinical practice. Clinicians should individualise the stroke therapy based on their clinical judgement.

In the future, I intend to expand this work by examining outcomes in remaining exclusion subgroups. In order to do this, I have initiated collaborations with colleagues at various centres. Working together, I will undertake systematic reviews and meta-analysis of data. Also, where sufficient data are available, I will compare outcomes between thrombolysed and non-thrombolysed groups for improvement in patients' outcomes. Whereas in this thesis, I employed adjusted analyses technique, adjusting for age and baseline stroke severity, for any further work, I may also consider matching procedures like propensity scores, the Mahalanobis method or Kent's method when dealing with non-random data like these.²⁸⁰

Appendix

Appendix

SAS 9.2 Codes for statistical analyses

A PROC FREQ function was employed to run CMH test. In SAS 9.2 software, analyses employing a code “proc freq; tables A*B*C*D / cmh; run;” was undertaken. Here, the analysis examines association of C with D when adjusted by stratification for covariates A and B.

A PROC LOGISTIC function was used to fit the proportional odds model.

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See: approval letter-

<http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm080822.pdf>

; Summary basis of approval -

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Clinical review -

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