

From the DEPARTMENT OF CLINICAL NEUROSCIENCE
Karolinska Institutet, Stockholm, Sweden

**INTRACEREBRAL HEMORRHAGE IN
PATIENTS TREATED WITH
INTRAVENOUS THROMBOLYSIS FOR
ACUTE ISCHEMIC STROKE**

Michael V. Mazya



**Karolinska
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*To my wonderful family - Amelie, Maximilian, Miranda - with love and gratitude,
and to my parents Tatyana and Vladimir - a dedication to your dedication*

*"Though a little one, the master-word looms large in meaning. It is the 'Open Sesame' to every portal, the great equalizer in the world, the true philosopher's stone which transmutes all the base metals of humanity into gold. The stupid man among you it will make bright, the bright man brilliant, and the brilliant student steady. With the magic word in your heart, all things are possible, and without it all study is vanity and vexation. The miracles of life are with it; the blind see by touch, the deaf hear with eyes, the dumb speak with fingers. To the youth it brings hope, to the middle-aged confidence, to the aged repose. True balm of hurt minds, in its presence the heart of the sorrowful is lightened and consoled. It is directly responsible for all advances in medicine during the past twenty-five centuries. And the master-word is **Work**, a little one, as I have said, but fraught with momentous sequences if you can but write it on the tablets of your hearts, and bind it upon your foreheads."*

Dr William Osler, British Medical Journal, 1903

ABSTRACT

Background. Nearly 30000 people suffer a stroke in Sweden every year. Stroke is the third most common cause of death after heart disease and cancer carrying a 17% mortality rate at three months. It is the most common cause of neurological disability in adults. Intravenous thrombolysis with alteplase is the only approved pharmacological therapy for acute ischemic stroke, improving neurological and functional outcome in one third of all treated patients. Meanwhile, thrombolytic treatment can in itself cause intracerebral hemorrhage. The aim of this thesis was to study risk factors associated with this complication, in a large cohort of ischemic stroke patients treated with intravenous alteplase.

Methods. All studies were based on patient data contained within the Safe Implementation of Treatments of Stroke - International Stroke Thrombolysis Register (SITS-ISTR). The main outcomes of interest were symptomatic intracerebral hemorrhage (SICH) by SITS-MOST, ECASS II and NINDS definitions, functional status at 3 months (modified Rankin Scale), and death at 7 days and 3 months.

Study 1. We aimed to develop a clinical scoring algorithm predicting the risk of SICH, using data from 31627 patients. Baseline and demographic factors associated with SICH were entered into a logistic regression model. Adjusted odds ratios (OR) were converted into points, summated to produce a risk score. We identified 9 predictors of SICH: stroke severity, plasma glucose, blood pressure, age, body weight, stroke onset to treatment time, aspirin or combined aspirin and clopidogrel, and history of hypertension. The overall rate of SICH was 1,8%. The score ranged from 0 to 12 points, showing a >70-fold increase in the rate of SICH for patients with a score ≥ 10 points (14,3%) compared to 0 points (0,2%), with an acceptable predictive performance, AUC-ROC = 0,70. We concluded that the SITS SICH Score is able to predict large thrombolysis-related SICH associated with severe clinical deterioration.

Study 2. The SEDAN score is another prediction algorithm for SICH. We assessed its predictive performance for two definitions of SICH. Odds ratios for SICH per one-point increase of the score were obtained using logistic regression. The predictive capability for SICH per ECASS II was moderate at AUC-ROC = 0,66. With rising scores, there was a moderate increase in risk for SICH ECASS II (OR 1,7 per point, $p < 0,001$), SICH rates between 1,6% for 0 points and 16,9% for ≥ 5 points. Prediction of SICH per SITS-MOST was weaker, AUC-ROC = 0,60, rates between 0,8% for 0 points and 5,4% for ≥ 5 points. We concluded that the predictive performance of the SEDAN was moderate for SICH per ECASS II and low for SICH per SITS-MOST.

Study 3. The European license for alteplase contraindicates its use in stroke patients treated with warfarin. Conversely, American guidelines accept it in patients with an international normalized ratio (INR) $\leq 1,7$. We studied the influence of warfarin on SICH, arterial recanalization, functional outcome and mortality in 768 patients with baseline warfarin treatment and $\text{INR} \leq 1,7$. They were older, had more comorbidities, and more severe strokes compared to patients without warfarin. There were no differences in SICH rates, mortality or functional outcome between warfarin and non-warfarin patients after adjustment for differences in age, stroke severity and co-morbidities. Arterial recanalization defined as the disappearance of a baseline hyperdense cerebral artery sign at 22-36 hour imaging was increased in warfarin patients at 63% vs 55%, $p = 0,022$.

Study 4. Hemorrhage following stroke thrombolysis can occur in brain parenchyma remote from acutely ischemic tissue (PHr), as well as in local relation to the infarct (PH). We investigated the risk factors, mortality and functional outcome in patients with the poorly understood complication of PHr, as well as PH, and concomitant occurrence of both. We compared baseline data in 970 patients (2,2%) with PHr, 2325 patients (5,3%) with local PH, and 39761 patients (91,4%) without PH or PHr. Independent risk factors were obtained by multivariate logistic regression. Increasing age and blood pressure were the only strong risk factors for PHr. High stroke severity, atrial fibrillation, CT hyperdense cerebral artery sign, *ie* factors indicating large artery occlusion, were associated with local PH. Functional independence at 3 months was more common in PHr than PH (34% vs 24%, $p < 0,001$), 3 month mortality was lower (34% vs 39%, $p < 0,001$). PH and PHr were equally often symptomatic. The better outcome in PHr is explained by PHr occurring in patients with milder strokes. We concluded that the differences in risk factors likely indicate an influence of underlying small vessel disease in PHr, and large vessel occlusion in PH.

LIST OF PUBLICATIONS

- I. Mazya M, Egido JA, Ford GA, Lees KR, Mikulik R, Toni D, Wahlgren N, Ahmed N.

Predicting the Risk of Symptomatic Intracerebral Hemorrhage in Ischemic Stroke Treated With Intravenous Alteplase: Safe Implementation of Treatments in Stroke (SITS) Symptomatic Intracerebral Hemorrhage Risk Score.

Stroke. 2012;43:1524-31.

Oral presentation at the European Stroke Conference in Lisbon, Portugal in May 2012. Received the Outstanding Young Research in Stroke Award at the same conference.

- II. Mazya MV, Bovi P, Castillo J, Jatuzis D, Kobayashi A, Wahlgren N, Ahmed N.

External validation of the SEDAN score for prediction of intracerebral hemorrhage in stroke thrombolysis.

Stroke. 2013;44:1595-600.

Poster presentation at the European Stroke Conference 2013 in London, UK.

- III. Mazya MV, Lees KR, Markus R, Roine RO, Seet RC, Wahlgren N, Ahmed N;
Safe Implementation of Thrombolysis in Stroke Investigators.

Safety of intravenous thrombolysis for ischemic stroke in patients treated with warfarin.

Annals of Neurology. 2013;74:266–274.

Oral and e-poster presentation at the European Stroke Conference 2013 in London, UK.

- IV. Mazya MV, Ahmed N, Ford GA, Hobohm C, Mikulik R, Paiva Nunes A, Wahlgren N.

Remote intracerebral hemorrhage – a poorly understood complication in stroke thrombolysis. Results from the SITS International Stroke Thrombolysis Register (SITS-ISTR).

Stroke. 2014;45:1657-1663.

Oral presentation at the European Stroke Conference 2014 in Nice, France.

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LIST OF ABBREVIATIONS

ACA	Anterior cerebral artery
Acom	Anterior communicating artery
ADL	Activities of daily living
AHA/ASA	American Heart Association / American Stroke Association
ATP	Adenosine tri-phosphate
BA	Basilar artery
CASES	Canadian Alteplase for Stroke Effectiveness Study
CBF	Cerebral blood flow
CHD	Congestive heart disease
DALY	Disability adjusted life year
ECA	External carotid artery
ECASS	European Cooperative Acute Stroke Study
ESO	European Stroke Organisation
GTWG	USA Get With The Guidelines – Stroke Registry
HASTA	Hyper Acute STroke Alarm
ICA	Internal carotid artery
ICH	Intracerebral hemorrhage
IST	International Stroke Trial
IV tPA	Intravenous recombinant tissue plasminogen activator
MCA	Middle cerebral artery
mRS	Modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
NNT	Number needed to treat
NNH	Number needed to harm
OR and aOR	Odds ratio and adjusted odds ratio
PCA	Posterior Cerebral Artery
PH	Parenchymal hemorrhage
PHr	Remote parenchymal hemorrhage
Pcom	Posterior communicating artery
RCT	Randomised controlled trial
SEK	Swedish Krona
SITS	Safe Implementation of Treatments in Stroke
SITS-MOST	Safe Implementation of Thrombolysis in Stroke – Monitoring Study
SITS-ISTR	Safe Implementation of Treatments in Stroke – International Stroke Thrombolysis Register
VA	Vertebral artery
VISTA	Virtual International Stroke Trials Archive
WHO	World Health Organisation

1 INTRODUCTION

1.1 STROKE – A BACKGROUND

The word “stroke” is derived from the Greek *apoplexia*, denoting the state of being “struck down and incapacitated”. The definition of stroke has been in evolution since the time of Hippocrates. During the last 40 years, the most widely used definition has been “rapidly developed clinical signs of focal (or global, if observed in patients with subarachnoid hemorrhage) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than of vascular origin”. This wording originated as an inclusion criterion in epidemiological studies on cerebrovascular disease coordinated by the World Health Organisation in the 1970s and has been in prominent use ever since.¹⁻³ The 24 hour cut-off has been useful for epidemiological purposes because it can be applied consistently in different places and times. For patients assessed within 24 hours, various other terms were proposed, such as “brain attack” and “acute stroke syndrome”.^{4,5} However, the relevance of the time-mark has declined with increasing understanding of the nature, timing and imaging of stroke. Strong calls have been made for an updated definition of stroke, based on what actually matters for patients and physicians – the interrelation of mechanisms, brain tissue, symptoms and clinical signs.⁶

Ischemia (Latin *ischaemia*, from Greek *iskhaimos*, meaning “stopping blood”) is a pathophysiological mechanism characterised by a deficient supply of blood to a body part that is due to obstruction of the inflow of arterial blood. Infarction is the term used for cell death caused by ischemia. Cerebral infarction is the mechanism behind 85% of all strokes in high-income countries, and 70% of strokes in low to middle income countries.⁷ The causes of focal cerebral ischemia are plentiful and include arterial thrombosis, thromboembolism and dissection, cardio-embolism, hemodynamic insufficiency, vasculitis, haematological diseases and many others.⁸⁻¹⁰ The remaining 15-30% of strokes are caused by intracerebral (within brain tissue) and subarachnoid hemorrhage (on the brain surface or within the ventricular system). To complicate matters, cerebral hemorrhage may follow an infarction, either spontaneously or after antithrombotic or thrombolytic therapy used to treat the initial ischemia. This may at times aggravate the clinical condition and lead to a worsened prognosis. Such hemorrhagic complications of ischemic stroke are the main topic of the present thesis.

Stroke continues to be a leading cause of neurological disability in adults worldwide.¹¹⁻¹⁴ In Sweden, around 20% of stroke survivors previously independent in their activities of daily living (ADL), require help with their daily needs three months after a stroke.¹⁵ Around 40% of stroke victims have some degree of long-term hemiparesis, 25% have a chronic walking impairment, and equally many have language difficulties.¹⁶⁻¹⁸ With almost 30000 people suffering

a stroke per year in Sweden and a 17% overall mortality at three months, it is the third largest cause of death after heart disease and cancer, while being fourth in the USA and Great Britain.¹⁹⁻²³ Moreover, it is the disease responsible for the highest consumption of hospital bed capacity in the country, with nearly a million hospital days per year.¹⁵ In spite of these numbers, stroke research has been underfunded in Europe and the USA compared to research on cancer and coronary heart disease.^{24,25} Numbers from the UK show that stroke receives only 4% of all funds directed toward research on cancer, vascular disease and dementia, with 74% going to cancer alone.²⁶ It is important for research funding to better reflect the burden of each disease to society, particularly in light of the projected increase in the worldwide impact of stroke, with a rising proportion of older people in society.²⁷⁻²⁹

1.1.1 Epidemiology

In 2010, an estimated 16,9 million cases of stroke took place worldwide (69% in low-income and middle-income countries), more than a third of which (5,9 million stroke deaths, 71% in low-income and middle-income countries) resulted in death. Given that there were 56,2 million deaths worldwide in 2010, stroke accounted for over 10% of all deaths. The global prevalence, or number of people who had survived a stroke, was 33,0 million (52% in low-income and middle-income countries).^{30,31} Estimates for the 2010 incidence of ischemic stroke across a selection of countries is given in Table 1, with a comparison of estimated numbers for the year 1990.

Country	Incidence 2010	Mortality 2010	Incidence 1990	Mortality 1990
Sweden	123	24	152	38
Finland	174	24	216	50
UK	85	24	108	46
USA	143	19	174	31
Russia	371	138	332	155
China	241	47	226	56
India	143	39	128	37
South Africa	164	38	156	57

Table 1. Ischemic stroke, age-standardised incidence and mortality per 100 000 person-years by country.³²

High income countries have seen a continuous decline in stroke incidence and mortality rates since the 1960s.^{7,30,33} Specifically, in wealthy nations, incidence has decreased by on average 42% during the last four decades. However, contrary to the main trend, the incidence of in particular ischemic stroke in patients aged 20 to 64 years has seen an increase between the years 1990 and 2010.³²

In contrast to the wealthier nations, stroke incidence in low to middle income countries has more than doubled during the same period.^{7,34} Whereas in the 1970s high income countries had a stroke incidence approximately triple that of low to middle income countries, the latter group now has a stroke incidence that has surpassed that of the most wealthy nations, as illustrated in Figure 1.^{7,30,35} Fortunately, presumably due to improved stroke care, mortality within one month after stroke (early case fatality) has decreased in both high and low income countries since the 1970s from 36% to 20%.^{7,36}

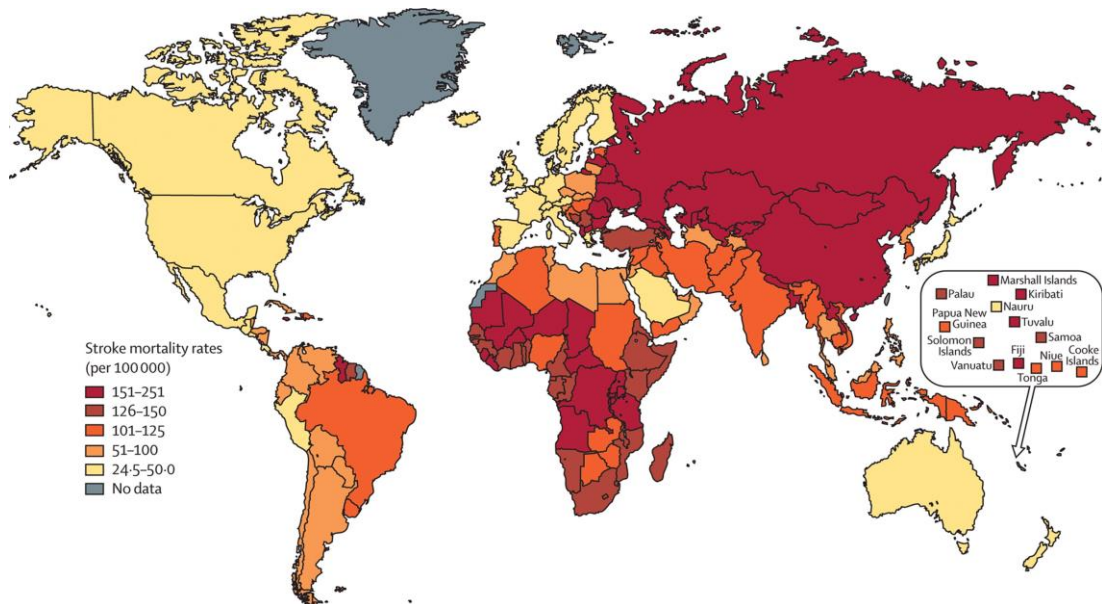


Figure 1. Age-adjusted and sex-adjusted stroke mortality rates 2009. From Johnston et al, 2009.³⁵ Permission for use obtained from Elsevier.

Assessing the burden of stroke to society by measuring mortality rates leaves out the great impact of neurological disability in stroke survivors. Disability adjusted life years (DALYs) is a concept widely used in epidemiological studies to reflect the burden of disease in a population level. DALYs are calculated as the sum of life years lost (YLL) due to premature mortality and years lived with disability (YLD), thus one DALY can be said to represent one year of healthy life lost.^{30,37} Stroke accounted for 4%, or around 100 million of the world's total number of DALYs lost in 2010. Similar to findings regarding mortality rates, there was a 10-fold difference in DALY loss between the most affected and the least affected countries, with the heaviest burden falling on the Eastern European and Northern Asian former member states of the Soviet Union.³⁵

Measures of disease burden can be made even more specific for individual countries. In 2012, the Swedish national stroke registry Riks-Stroke reported that the proportion of patients dependent of others for activities of daily living (ADL) at three months after stroke was 18,9%, the lowest since the opening of the registry in 1994. This reflects an absolute reduction of 4% during the last decade, despite an unchanged patient age and mean stroke severity.¹⁵ A possible explanation for decrease in the burden of functional disability could be improved hospital stroke care and rehabilitation in Sweden during this period.

With an increasing proportion of the population surviving to old age, the number of strokes occurring every year in wealthy nations is projected to rise faster than would be expected from pure population growth.^{27,28} Projections for the European region suggest that the proportion of the population aged 65+, in which most stroke events occur, will increase from 20% in 2000 to 35% in 2050, as the median population age will rise from 37,7 years in 2000 to 47,7 years in 2050. Assuming stable stroke incidence rates, the absolute number of annual strokes in the EU is projected to rise from 1,1 million per year in 2000 to over 1,5 million per year in 2025.^{38,39} A similar demographic redistribution of the “age pyramid” expected to occur in the USA is presented in Figure 2. Consequently, the number of strokes is estimated to expand by 2,25 times from the current 800 000 to over 1,3 million per year (Figure 3).²⁸

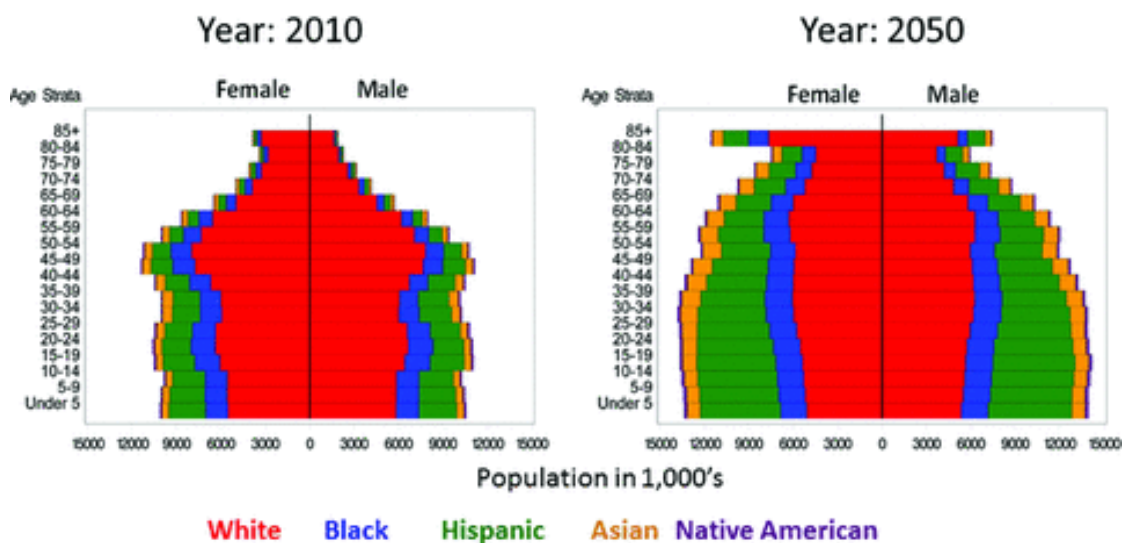


Figure 2. Projected redistribution of age categories in the USA from 2010 to 2050, shown by race/ethnicity and age. From Howard et al, 2012.²⁸ Permission for use obtained from John Wiley and Sons.

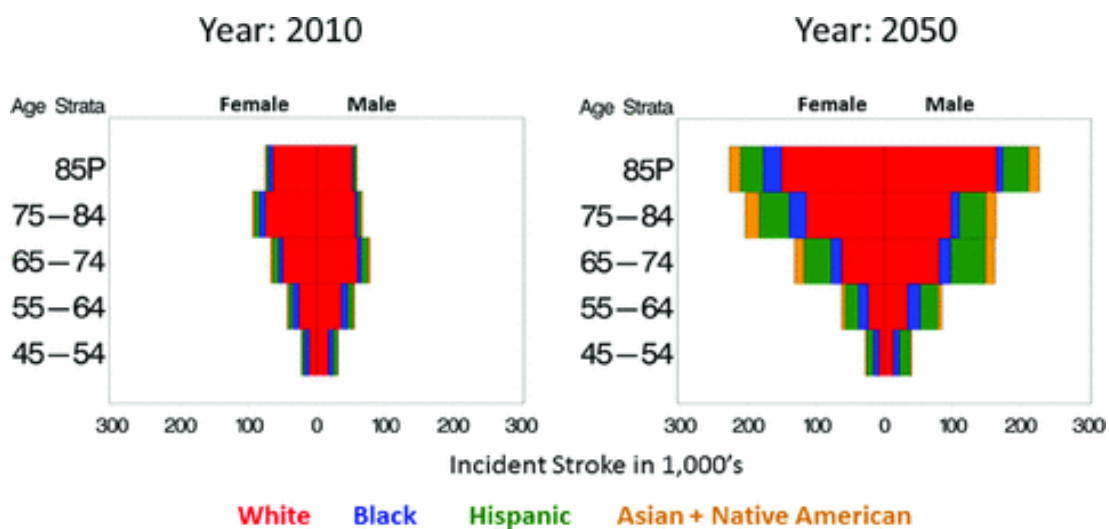


Figure 3. Projected increase in incident stroke numbers in the USA from 2010 to 2050, shown by race/ethnicity and age (for ages 45 and over). From Howard et al, 2012.²⁸ Permission for use obtained from John Wiley and Sons.

The projected trends for the coming decades highlight the importance of improving primary and secondary prevention to reduce stroke incidence, as well as develop acute treatment and rehabilitation methods in order to lower the burden of post-stroke disability.

In Sweden, the total cost of stroke occurring in 2009 has been estimated at 18,3 billion SEK, a mean cost of over 600 000 SEK per patient. These numbers are comprised of both direct costs (66% of total), i.e. inpatient and ambulatory care, rehabilitation, medication and living arrangements due to disability, and indirect costs, i.e. loss of income (34% of total). The mean cost per patient has decreased by 11% since 1997, largely comprised of a decline in costs for social services and living arrangements for patients with disability, in keeping with the lower proportion of patients with post-stroke ADL dependence reported by Riks-Stroke.^{15,40-42} The total annual cost per patient in Sweden can be compared to 340 000 SEK reported in the USA and 530 000 SEK the UK, however estimated with somewhat different methodology.^{21,43} In line with the projected increase in the incidence of stroke, the direct costs in the USA are projected to rise by over 150% until 2030. Since the increasing elderly (retired) population will account for the majority of the new strokes, indirect costs due to loss of income is expected to show a lower, but still substantial increase of 68% in the same period.²⁹

1.1.2 Cerebral vascular anatomy

The cerebral arteries are derived from the internal carotid and vertebral. These form at the base of the brain an anastomosis known as the circle of Willis. It is comprised in front of the anterior cerebral arteries (ACA), branches of the internal carotid (ICA), which are connected together by the anterior communicating (ACom); behind by the two posterior cerebral arteries (PCA), branches of the basilar (BA), which are connected on either side with the internal carotid by the posterior communicating (PCom) (Figure 4).⁴⁴ The internal diameter of the proximal cerebral arteries varies between 2 and 3 mm, while the communicating arteries measure closer to 1 mm.⁴⁵ The internal carotid arteries supply 80% of the total cerebral blood flow, while the basilar artery contributes the remaining 20%.⁴⁶ After entering the cranium, large arteries branch into progressively smaller arteries and arterioles that run in a vast network along the surface of the brain (pial arteries).⁴⁷

Pial vessels are surrounded by cerebrospinal fluid (CSF) in the so-called Virchow-Robin space, which is a continuation of the subarachnoid space. The penetrating arteries become parenchymal arterioles as they penetrate into brain tissue and become surrounded by astrocytic end-feet.⁴⁸ Pial vessel architecture forms an effective collateral network such that occlusion of one vessel does not necessarily decrease cerebral blood flow. However, penetrating and parenchymal arterioles are long and largely unbranched; thus, occlusion of an individual arteriole results in significant reductions in flow and infarction of the surrounding local tissue.⁴⁹

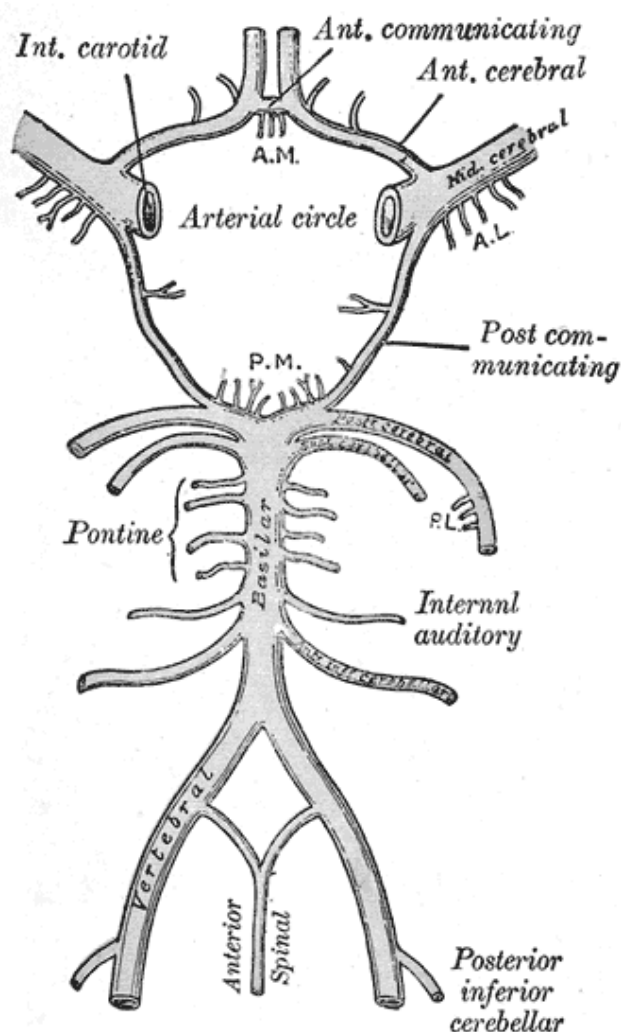


Figure 4. Diagram of the arterial circulation at the base of the brain. A.L. Antero-lateral. A.M. Antero-medial. P.L. Postero-lateral. P.M. Posteromedial ganglionic branches. *Anatomy of the Human Body*, H. Gray, 1918.⁴⁴ Image in the public domain.

The blood supply to the brain is unique because its major arteries form an equalising distributor, the circle of Willis, which can redistribute blood flow in the event of a sudden occlusion of a parent vessel (Figure 4). This anastomotic loop provides low-resistance connections, allowing reversal of blood flow to provide primary collateral support to the anterior and posterior circulations. The anatomy of the circle of Willis varies between patients: around 50% of individuals have a normal or complete configuration of the circle of Willis. The presence of any abnormalities, particularly absent or hypoplastic ACom or PCom arteries, can seriously compromise ability to compensate for sudden occlusions.⁵⁰

The network of pial or leptomeningeal arteriolar anastomoses (of Heubner) comprises secondary collaterals responsible for redistribution of flow when there is constriction or occlusion of an artery distal to the circle of Willis.⁵¹ In these vessels, blood can flow in both directions as a function of the hemodynamic and metabolic needs of the two territories that they connect. Thus the MCA is effectively joined with both the ACA and the PCA also at the cortical level.⁵²

A third collateral network connects the extracranial and intracranial circulations (Figure 5). Important collateral circuits include flow from the external carotid artery (ECA) through the ophthalmic and superficial temporal arteries to the

intracranial vessels normally supplied by the ICA. In the posterior circulation, many anastomoses exist between the vertebral arteries and cervical muscular branch arteries. The anterior and posterior spinal arteries also communicate with the intracranial posterior circulation arteries supplying the medulla and pons.^{52,53}



Figure 5. (A) Extracranial arterial collateral circulation. Shown are anastomoses from the facial (1), maxillary (2), and middle meningeal (3) arteries to the ophthalmic artery, and dural arteriolar anastomoses from the middle meningeal artery (4) and occipital artery through the mastoid foramen (5) and parietal foramen (6). Intracranial arterial collateral circulation in frontal (B) and lateral (C) views. Shown are the posterior communicating artery (1); leptomeningeal anastomoses between anterior and middle cerebral arteries (2) and between posterior and middle cerebral arteries (3); the tectal plexus between posterior cerebral and superior cerebellar arteries (4); anastomoses of distal cerebellar arteries (5); and the anterior communicating artery (6). From Shuaib et al, 2011.⁵² Permission for use obtained from Elsevier.

Having entered into brain tissue, parenchymal arterioles subsequently form the cerebral capillary network. It has been estimated that nearly every neuron in human brain has its own capillary.⁵⁴ Brain capillary structure is unique compared to other organs. It is distinguished by the extensive presence of tight junctions between adjacent endothelial cells (the walls of the capillaries), forming the blood-brain barrier (BBB). The BBB tightly regulates the active transport of ions, glucose and amino acids across the capillary wall. Moreover, it limits the entry of plasma components, red blood cells, and leukocytes into the brain.⁵⁵ If they cross the BBB due to an ischemic injury, intracerebral hemorrhage, trauma, neurodegenerative process, inflammation, or vascular disorder, this typically generates neurotoxic products that can compromise synaptic and neuronal functions.⁵⁶ Endothelial cells are covered by basal lamina which is continuous with astrocytic foot processes and pericytes, which ensheath the capillaries (Figure 6).⁴⁸ Astrocytes intimately influence capillary and arteriolar function, regulating cerebral blood flow, contributing to ion and water homeostasis, and interfacing directly with neurons.⁵⁷ Pericytes regulate capillary permeability and diameter, contribute to toxic metabolite clearance and influence cerebral angiogenesis.⁵⁸ In and around the capillary, there is complex cross-talk between all entities and cell types, which together form the “neurovascular unit”.

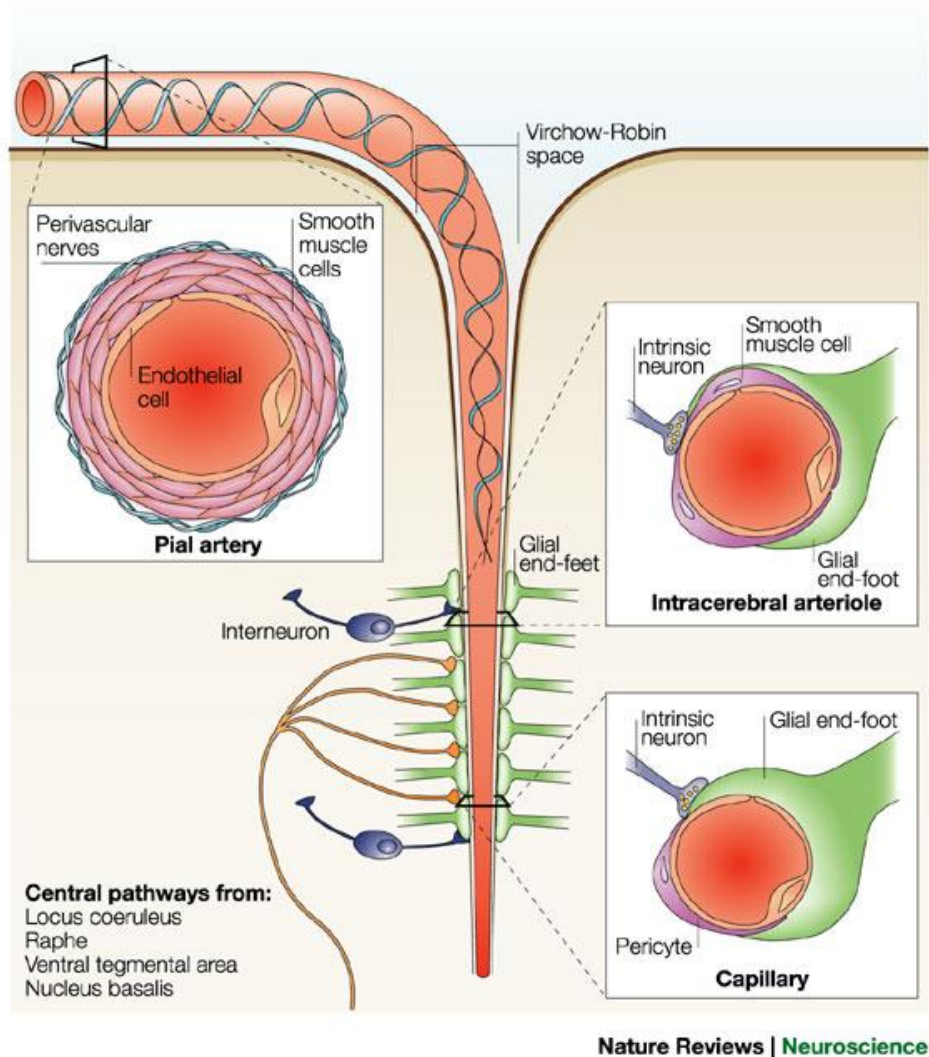


Figure 6. The pial penetrating and parenchymal arterioles, ending in capillaries, part of the neurovascular unit comprised of endothelium, astrocytic end-foot processes, pericytes and neurons. From Iadecola et al, 2004.⁴⁸ Permission for use obtained from Nature Publishing Group.

1.1.3 Pathophysiology of ischemic stroke

The energy demands of nervous tissue are very high, and therefore sufficient blood supply to the brain must be maintained. An adult brain contains approximately 80-120 billion neurons while comprising only around 2% of the body mass.^{59,60} It consumes at rest an impressive 20% of the body's total oxygen consumption, supplied by 15% of the cardiac blood output. The average cerebral blood flow (CBF) in the brain as a whole is 50-60 ml/100 g tissue/min, between 20 ml/100g/min in white matter and 80 ml/100g/min in cerebral cortex.⁶¹ Oxygen is used in the brain for the oxidative metabolism of glucose, which almost exclusively acts as the substrate for energy metabolism in the brain. Overall, only 15-20% of the total energy consumption is needed for processes not related to communication between neurons, such as protein and membrane synthesis and turnover.⁶² Of the remaining 80-85% the largest part, 87%, is used

for signalling, while 13% is expended in maintaining membrane resting potential.⁶³ From this follows that a reduction in local cerebral blood flow following the occlusion of a supplying artery will lead first to disturbed, then abolished signalling and only later to irreversible cellular damage. This assumption was experimentally confirmed in a number of animal studies in the 1970s.^{64,65} Monkeys gradually develop a neurological deficit progressing from mild weakness at a level of CBF of 22 ml/100 g/min to complete paralysis at 8 ml/100 g/min. When the ischemia (even if profound) is rapidly reversed, neurological function is regained. Whereas neuronal function is impaired immediately following a sufficient drop in blood flow, the development of irreversible tissue damage is time dependent.⁶⁵ Thus, the functional activity and destiny of neurons during a reduction of blood flow is tightly coupled with the degree and duration of ischemia (Figure 7).⁶⁶

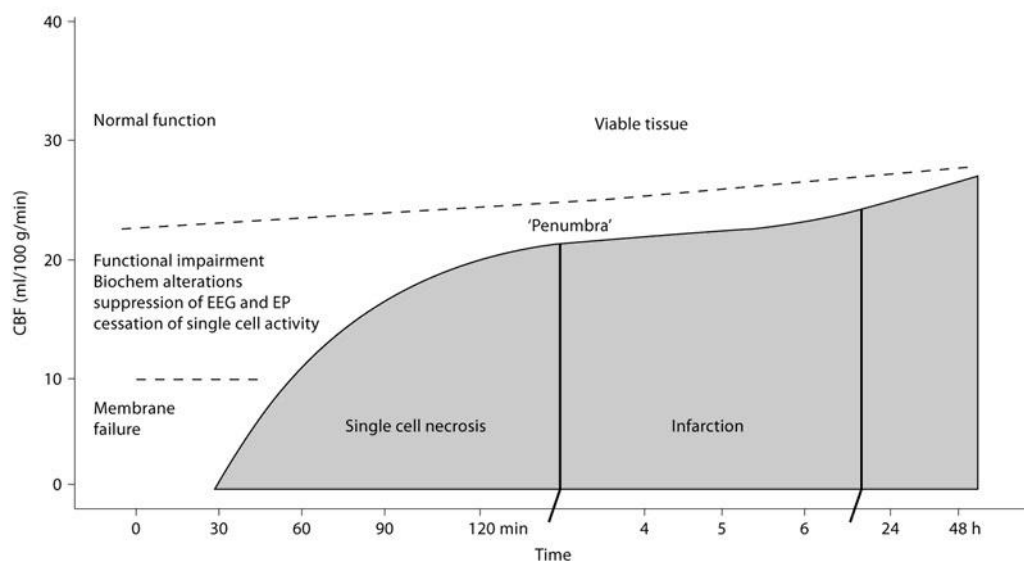


Figure 7. Diagram of CBF thresholds required for the preservation of function and morphology of brain tissue. Neuronal activity is blocked when flow decreases below a certain threshold (dashed line) and returns when flow is raised again above this threshold. The fate of the cells depends on the duration for which CBF is impaired below a certain level. The solid line separates structurally damaged from functionally impaired but intact tissue, the “penumbra”. The dashed line distinguishes viable from functionally impaired tissue. Modified from Heiss, 2011.⁶⁶ Permission for use obtained from Karger Publishers.

Cerebral blood flow less than around 10 ml/100 g/min even for a very limited amount of time is insufficient for neuronal viability.⁵¹ Neurons located furthest away from the nearest capillary suffer irreversible damage already at less severe levels of ischemia.⁶⁷ Within 2 minutes of anoxia, the neuronal stores of ATP are depleted, leading to failure of the Na^+/K^+ ATPase, the ubiquitous ion pump responsible for keeping a low intracellular concentration of sodium and high level of potassium, thus generating resting membrane potential. As energy is depleted, membrane potential is lost and neurons and glia depolarize.⁶⁸ Due to the depolarization, glutamate (the main excitatory neurotransmitter) is released

prompting further depolarization of yet more cells. Glutamate also activates specific receptors which dramatically increase intracellular Na^+ and Cl^- levels. Water follows the ions, leading to cellular oedema. Moreover, failure to clear the released glutamate leads to influx of Ca^{2+} , which acting as an intracellular messenger initiates a series of events that furthers the development of tissue damage, such as activation of proteolytic enzymes that degrade cytoskeletal as well as extracellular matrix proteins. Activation of phospholipase A2 and cyclooxygenase generates free-radical species, producing lipid peroxidation and membrane damage.⁶⁹ This process, leading up to cell lysis and early tissue necrosis, is named “excitotoxicity” due to the central role played by glutamatergic overactivation.

Cerebral microvessels (<0,1 mm in diameter) react just as quickly to ischemia as neurons, with coordinated responses varying with the degree of reduction in blood flow.⁷⁰ Structural alterations of the microvasculature start as early as in 30 – 90 min after experimental focal brain ischemia. Among the first things to happen is the expression on endothelial walls of molecules promoting adhesion of leukocytes.⁷¹ These interact with the ischemic endothelium increasing permeability of the BBB, whereupon tissue factor located in astrocyte end-feet comes into contact with plasma hemostatic factors, generating fibrin deposition in the lumen.⁷² The microvasculature becomes obstructed by leukocytes, fibrin and activated platelets, the endothelium itself undergoes a degree of ischemic swelling, while also being compressed by swollen metabolically compromised neurons and astrocytes.⁷³ This leads to the focal “no-reflow” phenomenon, *i.e.* impaired or outright failed tissue reperfusion even upon recanalization of the occluded supplying artery.⁷⁴

Within 1-2 hours of ischemia, as microglia and astrocytes come into contact with plasma proteins due to early BBB dysfunction, they begin to secrete proteinases which cleave components of the microvascular basal lamina, among them matrix metalloproteinases (MMPs).⁷⁵ These take part in remodelling of the extracellular matrix in a normal metabolic state, however in the setting of ischemia, MMPs play an important role in further loss of microvascular integrity.⁷⁶

Following the initial dysfunction of endothelial BBB and early degradation of the basal lamina, the normal barriers against the incursion of leukocytes into brain tissue are lifted. Within hours, inflammation ensues, fuelled by pro-inflammatory cytokines such as TNF-alpha and IL-1 beta released from every cell type in the neurovascular unit.⁷⁷ This is necessary for the removal of necrotic tissue and subsequent repair.⁷⁸ However in the initial phase, neutrophil leukocytes and microglia produce a chemical environment which further injures the ischemic tissue, including cells which may not be irreversibly damaged by hypoxia itself.⁷⁷

Far from all cells in an ischemic area are hypoxic enough to undergo necrosis within the first few hours. Protein synthesis reduction is the earliest metabolic response to ischemia. This occurs already after CBF reductions of around 50% and is not caused by failure of energy metabolism as ATP depletion is not observed until CBF decreases to 20%.⁷⁹ Failure to produce protein components

necessary for cellular upkeep, together with an increasingly toxic extracellular environment (increased glutamate, free oxygen radicals etc.) leads to a pathway of delayed, programmed cell death or apoptosis.⁸⁰ This process is detectable at 8-12 hours after onset of ischemia, peaks at 24-72 hours and is most prominent in neurons, however present also in glia and endothelial cells.^{81,82}

1.1.4 The ischemic penumbra

“In general there is now good evidence that in an ischaemic process in the primate brain, failure of function of neurones extends much more widely than ultimate infarction. This principle contains a profound therapeutic implication. [...] it provides a powerful clinical stimulus to the development of techniques whereby the 'grey zone' of failure of function surrounding the structureless zone of complete infarction may be adequately reperfused and function restored as a result.” Symon et al, *Journal of Clinical Pathology*, 1977.⁶⁴

The “grey zone” of Symon and colleagues was rechristened in 1981 (incidentally the birth year of the present author) as the “penumbra” by Astrup, Siesjö and Symon, in analogy to the partly illuminated area around the compact shadow of the moon in full solar eclipse (Figure 8).⁸³

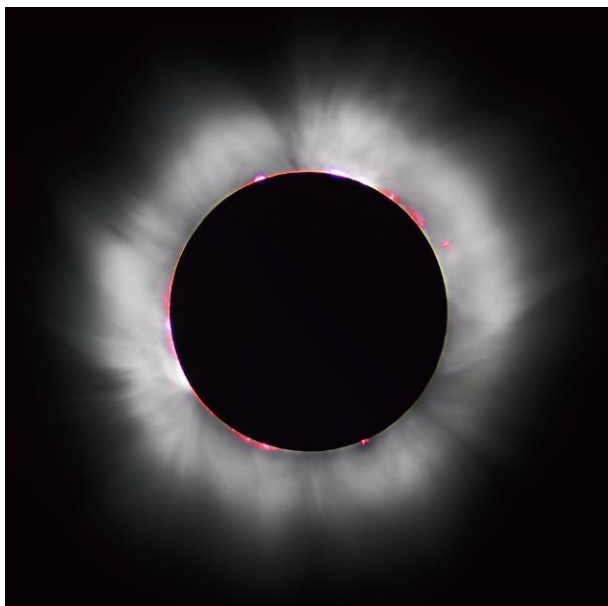


Figure 8. Full solar eclipse with the moon shadow across the sun surrounded by the partly illuminated penumbra. Photo: Luc Viatour, Creative Commons license.

The concept of the ischemic penumbra is closely tied to the concept of the “infarct core”, which is defined as irreversibly damaged tissue which cannot be rescued by reperfusion following recanalization of the occluded artery.⁸⁴ The penumbra is tissue surrounding the core, existing between the CBF threshold of infarction (<10 ml/100g/min) and the threshold of functional impairment (<20 - 25 ml/100g/min).^{83,85} Importantly, as the first hours of ischemia pass, penumbral tissue will gradually accumulate irreversible cellular damage enough to progress to infarction, unless reperfusion occurs (Figure 9).⁸⁵ The speed at which this occurs varies remarkably between individuals, some showing no salvageable penumbral tissue even after 1-2 hours from symptom onset, and others with significant surviving penumbra at 18-24 hours, which could regain function after

reperfusion.⁸⁶ Clinical recovery in a patient with acute ischemic stroke is determined by the fate of the tissue at risk, which in turn depends on a vast number of factors.⁸⁷ These include the anatomy and function of collateral circulation, systemic metabolic and physiological parameters, anatomy of the occluded vessel and genetics.⁸⁸

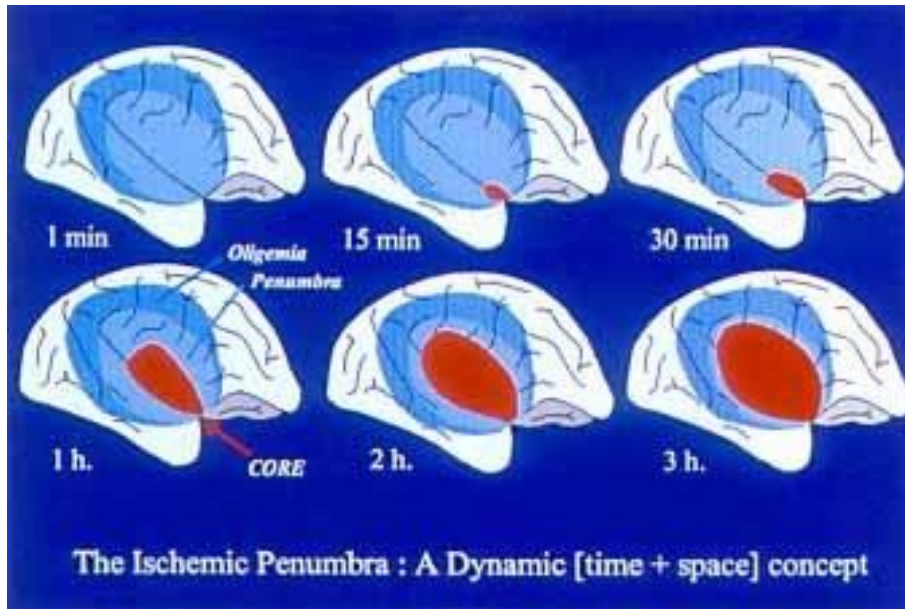


Figure 9. Idealised diagram of the brain showing the time course of infarct growth at the expense of the penumbra, from the situation immediately following MCA occlusion (top left), to 3 h later (bottom right). From Baron, 1999.⁸⁵ Permission for use obtained from Karger Publishers.

In 2006, Saver published an estimate on how much brain tissue is lost per unit of time in typical large vessel, supratentorial ischemic stroke. Guided by a systematic literature review, he established an average infarct volume of 54 ml (varying between 19 and 100 ml), an average duration of stroke evolution of 10 hours and an average number of neurons in the human forebrain of 22 billion. He was then able to calculate that a typical stroke causes a loss of 120 million neurons, 830 billion synapses, and 714 km of myelinated fibres per hour.⁸⁹

Thus, the penumbra concept has become the basis of stroke clinical pathophysiology. Under the adage “time is brain”, the penumbra is now firmly established as the main target for therapeutic attempts of reversing neurological deficit in patients with acute ischemic stroke.

1.1.5 Hemorrhagic infarct transformation

As follows from the above discussion, tissue reperfusion is a *sine qua non* for penumbral survival. However, given sufficient ischemic damage to the endothelium, basal lamina and other elements of the BBB, as regional blood flow is restored, blood extravasation into infarcted tissue can occur. This phenomenon is generally known as hemorrhagic transformation (HT) of the infarct.⁹⁰ Most commonly, HT can be seen as petechia in grey matter (due to its

more abundant vascularization) and on the border between necrotic and living tissue.⁹¹ However, large, confluent hematomas may also occur within an infarct. In 1951, C. Miller Fisher and Raymond D. Adams explained the occurrence of hemorrhagic infarction as the result of arterial recanalization and reperfusion of infarcted brain tissue. Their hypothesis was supported by the presence of “pale” infarctions downstream of still occluded and “red” infarctions downstream of recanalized large arteries. Moreover, they diagnosed an embolic infarct etiology in 63 of 66 deceased cases with post-mortem findings of HT, with only the remaining 3 infarcts suspected to be caused by local thrombosis of the ICA or MCA.⁹² Thus, breakup of an embolus occluding the proximal MCA would lead to HT in the reperfused, but already infarcted basal ganglia supplied by the lenticulostriate perforating arteries, but embolic fragments further out in the MCA territory would keep those infarcted areas in a non-reperfused state, leaving them “pale”. In a comment to the Fisher and Adams hypothesis, F. Hiller proposed that the above mechanism is not the only possible one; even in persisting occlusion, HT could be caused by blood supplied through patent anastomotic vessels.⁹² Both hypotheses, mutually non-exclusive, have subsequently been well supported in literature.^{93,94}

A first mention of microvascular contribution to hemorrhagic complications is found in a 1958 paper by J. S. Meyer, who described microvascular breakdown in areas of infarcted primate brain tissue which developed HT. In particular, perivascular and pericapillary hemorrhages were consistently found in animals which had been treated with anticoagulants (heparin and dicumarol), as well as with pharmacologically induced acute hypertension.⁹⁵ Subsequent research by del Zoppo, Hamann and Okada showed that HT occurs specifically in regions where ischemia causes a breakdown in the basal lamina.⁹⁶

In 1953, Globus and Epstein showed that the degree of confluence of perivascular petechia within an infarct depends on the number and proximity of affected vessels. In infarctions following the clipping of the MCA in experimental animals, perivascular blood extravasation could be seen extending along the course of the lenticulostriate arteries. These vessels displayed necrotic changes and frank defects in the vessel wall, “through which a column of blood appeared to pass without interruption into the surrounding tissue”.⁹⁷

From this, let us briefly examine the dynamics of events once hemorrhage has commenced. In a seminal paper from 1971, Fisher described a large number of ruptured arteries and arterioles from 0,06 to 0,2 mm in diameter, situated on the border of hemorrhage and normal tissue. He proposed that the smaller vessels ruptured as a result of mechanical disruption caused by blood escaping from the primary site of hemorrhage, likely being one of the two largest vessels (Figure 10). As several authors before him in preceding decades, Fisher described “hemostatic globes” consisting of fibrin and platelets, abutting the ruptured arteries and arterioles (Figure 11).⁹⁸

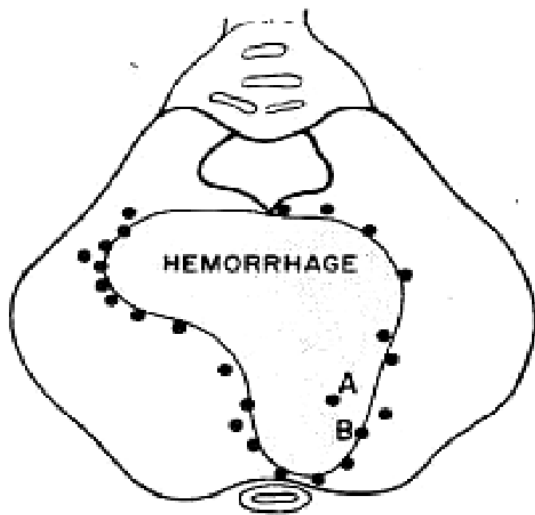


Figure 10. Pontine hemorrhage. The black dots denote 24 definite sites of arterial rupture. A and B are arteries 0,15 and 0,2 mm in diameter, with hemostatic globes measuring 5 mm in diameter each (as shown in Figure 11), one of them a likely site of initial hemorrhage. From Fisher, 1971.⁹⁸ Permission for use obtained from Wolters Kluwer Health.

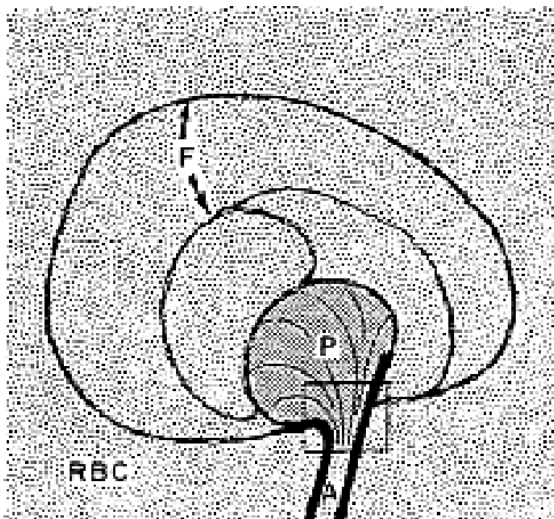


Figure 11. Diagram of the fibrin platelet hemostatic globe. The globe measures 5 mm in diameter. A: ruptured artery. RBC: mass of red blood cells within and around the fibrin globe. F: fibrin strands. P: platelet mass. From Fisher, 1971.⁹⁸ Permission for use obtained from Wolters Kluwer Health.

To summarize the findings outlined above, in reperfusion-related HT, the extent and location of blood extravasation can be seen as the product of interaction between the following major factors:

- Number and dimensions of ischemically damaged microvessels
- Degree of ischemic damage sustained by the vessel walls
- Extent of basal lamina disruption
- Pre-morbid condition of the now ischemic vessels
- Diameter of vessel which ruptures first
- Extent of the rupture site
- Hemostasis (platelets, coagulation cascade, mechanical tamponade)
- Blood pressure within the ruptured vessel(s)

Although perceived as a “common phenomenon” in clinical practice, the exact frequency of HT is difficult to express as a percentage which would be generally valid and meaningful for all settings. In literature, it varies between 0% and 85%, depending on the definition of HT, extent, etiology and vascular territory of the stroke, other characteristics of the studied population, as well as the timing and type of examination modality, be it autopsy, CT or MRI.⁹⁹

1.2 THROMBOLYTIC THERAPY WITH IV TPA

1.2.1 Pharmacological basis

Recombinant tissue-type plasminogen activator (tPA) is a virtually identical analogue of endogenous plasminogen activator produced by endothelial cells. It is a fibrin specific serine protease which binds to fibrin threads in a thrombus and converts the enmeshed plasminogen into plasmin, which in turn effectuates local fibrinolysis. In comparison to the older thrombolytic agents streptokinase and urokinase, tPA has a higher fibrin specificity and has only a limited effect on circulating coagulation factors.¹⁰⁰ The plasma half-life of unbound tPA is 4-6 minutes. Circulating tPA is rapidly inactivated by plasminogen activator inhibitor type 1 (PAI-1) produced by endothelial cells and platelets, and subsequently cleared by the liver. However, fibrin-bound tPA is less susceptible to inactivation and remains pharmacologically active at the thrombus site for several hours after its clearance from circulation.¹⁰¹

Pharmacologically induced recanalization should be viewed as a gradual process, since binding and activity of tPA depend on the area exposed to blood flow. As treatment starts, the thrombus softens and partially dissolves, allowing some degree of flow restoration. The restored bloodstream delivers more tPA to bind with fibrinogen inside the clot. This process maintains continual clot lysis and enhances blood flow until the clot breaks up under the pressure of arterial blood pulsations.

Without specific treatment, spontaneous arterial recanalization within 24 hours occurs in as few as 24% of patients, according to a meta-analysis from 2007. This rate nearly doubles to 43% in patients treated with IV thrombolysis.¹⁰² In the SITS-ISTR material, recanalization at 22-36 hours has been reported at 49%, with the higher rate possibly explained by somewhat later assessment.¹⁰³

From the point of view of penumbral salvage, knowledge of rates of early recanalization following IV tPA treatment is certainly of even greater importance. Vessel patency evaluation within the first 1-2 hours after treatment is usually only feasible by ultrasound technology, at least in consecutive patients reflecting routine care. Figure 12 shows recanalization rates at two hours specified by occlusion site, in the largest series of IV tPA treated stroke patients (n=335) examined to this end with transcranial and cervical Doppler ultrasound before and after treatment.^{104,105}

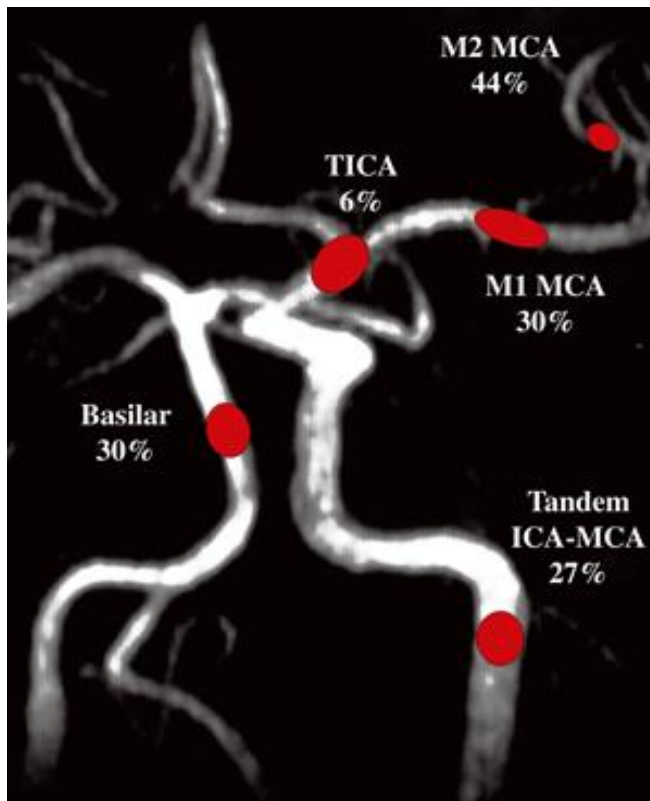


Figure 12. MR angiography of the human cerebral vasculature. Percentages indicate the proportion of patients with complete recanalization of an arterial occlusion at the respective site within 2 hours from the onset of IV rt-PA treatment, as measured by Doppler ultrasound. From Alexandrov, 2010.¹⁰⁵ Permission obtained from John Wiley and Sons.

It has historically been suggested that recanalization itself may have a driving influence on hemorrhagic infarct transformation. However, the 2007 meta-analysis by Rha and Saver showed overall rates of any HT of 13,7% for recanalized versus 12,5% for non-recanalized patients, the difference being non-significant.¹⁰² This finding suggested that the extent and severity of the ischemic injury to the BBB is an equal or greater determinant of hemorrhagic transformation, than whether reperfusion occurs under high pressure through a recanalized vessel or under lower, retrograde pressure through collaterals. This assumption has been supported by the latest SITS-ISTR findings of nearly equal risk of all types of HT in patients treated within 3 hours of stroke symptom onset, compared to those treated in the intervals 3-4,5 hours, and 4,5-6 hours.¹⁰⁶

Nevertheless, in addition to its thrombolytic effect, tPA has potential deleterious effects. In treatment non-responders, where early reperfusion is not achieved and a persistent ischemic state ensues, tPA has been suggested to potentially exacerbate ischemic damage by various mechanisms. The chief among these is likely to be activation of matrix metalloproteinases (MMPs), especially in ischemic vascular endothelium.¹⁰⁷ Matrix metalloproteinases are a family of zinc-binding proteolytic enzymes that normally remodel the extracellular matrix (ECM). MMP-2 and MMP-9 specifically attack type IV collagen, laminin, and fibronectin, which are the major components of the basal lamina around cerebral blood vessels.¹⁰⁸ Increased pre-thrombolysis plasma levels of MMP-9 have been shown to increase the risk of hemorrhagic infarct transformation and development of brain edema in stroke patients.¹⁰⁹ Well inside the CNS, after crossing a disrupted BBB, tPA further increases BBB permeability. This is mediated in part by cleaving and thus activating platelet-derived growth factor CC (PDGF-CC). This subsequently acts on the PDGF receptor alpha located on

astrocyte endfeet¹¹⁰. Blocking the PDGFR- α with the tyrosine kinase inhibitor Imatinib has been shown in mice to reduce cerebrovascular permeability and hemorrhagic infarct transformation following MCA occlusion.¹¹¹ This mechanism is currently targeted in the clinical trial iStroke coordinated by the Stroke Research Unit of the Karolinska University Hospital.

1.2.2 Clinical evidence of effect

In 1996, the US FDA approved treatment with IV tPA for acute ischemic stroke, for use in patients aged 18-80 years, within 3 hours of symptom onset. This was largely based on the results of the pivotal 2-part NINDS trial, enrolling a total of 624 patients. Active treatment was associated with an increase in the odds of complete or nearly complete neurological recovery at 3 months, with 40% versus 28% reaching excellent outcome (OR, 1,9; 95% CI, 1,2–2,9).¹¹² Four subsequent trials, the European Cooperative Acute Stroke Study (ECASS I and ECASS II)^{113,114} and the Alteplase Thrombolysis for Acute Noninterventional Therapy in Ischemic Stroke (ATLANTIS A and B)^{115,116}, enrolled subsets of patients in the ≤ 3 -hour time period and found largely similar effects in this time window, to those seen in the NINDS trial. These data subsets were subsequently pooled with data from the NINDS trial to show an overall benefit of IV tPA, with improved odds of favourable outcome at 3 months: OR 2,8 (95% CI 1,8-4,5) for treatment within 90 minutes and 1,6 (1,1-2,2) for 91-180 minutes.¹¹⁷

In the European Union, the European Medicines Evaluation Agency granted a licence for the use of IV tPA in acute stroke in 2002. This was done on two conditions; (1) establishment of a prospective registry of patient treatment experience for the purpose of conducting an observational safety study, the Safe Implementation of Thrombolysis in Stroke - Monitoring Study (SITS-MOST), to assess the safety of alteplase in routine clinical practice within 3 h of symptom onset, and (2) initiation of a new randomised trial, the ECASS III, with a therapeutic window extended beyond 3 hours.

Between 2002 and 2006, the SITS-MOST study enrolled 6483 patients from 285 centres (50% with little previous experience in stroke thrombolysis) in 14 countries. Published in the Lancet in 2007, the study showed that stroke thrombolysis with IV tPA in routine hospital care has a safety profile at least as good as that seen in RCTs and is an effective treatment when used within 3 h of stroke onset.¹¹⁸ The main results of the study are shown in Figure 13.

Thus, the evidence base for IV tPA use within 3 hours of stroke onset was firmly established. Both the American Stroke Association and the European Stroke Organisation have treatment within the early time window their strongest recommendation class (I), judging the evidence as being of the highest level, or grade A (based on multiple RCTs or meta-analyses).^{19,119}

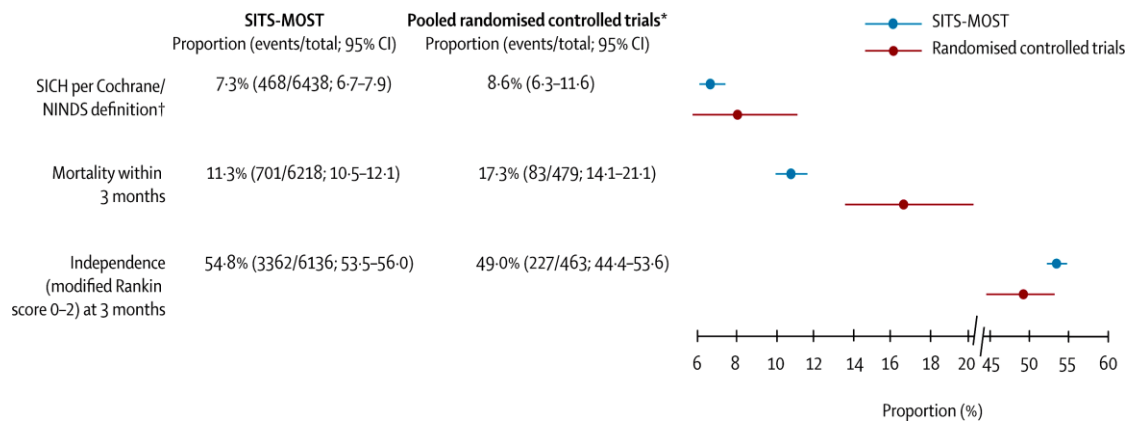


Figure 13. Main results of the SITS-MOST study. From Wahlgren et al, Lancet 2007.¹¹⁸ Permission for use obtained from Elsevier.

One year after SITS-MOST was published, in 2008, two concomitant publications in the New England Journal of Medicine and the Lancet reported results on IV tPA treatment in the time window between 3 and 4,5 hours from symptom onset. These were the randomized, placebo-controlled ECASS III trial (n=821) and the first observational SITS-ISTR publication (n=664).^{120,121}

The ECASS III trial was positive for its primary outcome of mRS 0-1 at 90 days, with 52,4% reaching this in the active arm versus 45,2% in the placebo arm; OR 1,34; 95% CI 1,02 – 1,76; P = 0,04. The SITS-ISTR publication in turn showed an insignificant increase in symptomatic intracerebral hemorrhage in the later time window versus the earlier (2,2% vs 1,6% for the SITS-MOST definition; 8,0% vs 7,3% for the NINDS definition) and the same for mortality at 3 months (12,7% vs 12,2%). There was no significant difference in rates of functional independence (mRS 0-2) or excellent outcome (mRS 0-1) at 3 months. However, following adjustment for baseline differences between the two populations, the p values for the odds ratios for became borderline significant, with adjusted OR for SICH per SITS-MOST at 1,32, p=0,052 and adjusted OR for mortality at 1,15, p=0,053.

Following the results of the ECASS III trial and the SITS-ISTR publication, the US guidelines were updated to recommend treatment also within the later time window of 3-4,5 hours, however with a slightly lower valuation of the level of evidence (category B, based on one RCT or several non-randomized studies).¹²² Meanwhile, the European Stroke Organization awarded the recommendation “class I, evidence level A” to the entire treatment window from 0 to 4,5 hours.¹²³

In 2012, the International Stroke Trial 3 (IST-3) was published, the hitherto largest RCT of stroke thrombolysis, enrolling 3035 patients in 12 countries. The trial sought to determine whether patients outside the established age (18-80 years) and time window range would benefit from treatment. Thus, 53% of all enrolled were over the age of 80 and 33% were treated between 4,5 and 6 hours from stroke onset. The trial was negative on the primary outcome measure dichotomising the Oxford Handicap Scale (OHS, nearly identical with the mRS)

at 0-2 versus 3-6 at 6 months. However, a secondary analysis showed an overall treatment benefit, if this was instead measured by shift in the outcome to any more beneficial state, OR 1,27, $p < 0,001$.¹²⁴

Simultaneously with the publication of the IST-3 results in 2012, Wardlaw et al concomitantly reported the largest meta-analysis of IV thrombolysis to-date, pooling 12 trials enrolling 7012 patients. In patients treated within 3 hours of symptom onset the benefit is highest, with 40,7% being alive and independent in activities of daily living at 3-6 months, versus 31,7% among controls.¹²⁵ Unfortunately, the meta-analysis pooled treatment between 3 and 6 hours into one tranche, without further subdividing at the 4,5 mark. This likely led to the finding that treatment at 3-6 hours is not significantly better than placebo or non-treatment. Meanwhile, more informative data on treatment between 3 and 4,5 hours can be found in the earlier pooled RCT data analysis by Lees et al from 2010, showing rates of favourable outcome (mRS 0-1) of 44,6% for IV tPA versus 38,2% for placebo, $p = 0,014$.¹²⁶

The number needed to treat (NNT), is a commonly used measure of the effectiveness of a medical intervention. It represents the number of patients that need to be treated for one to benefit compared with a control in a clinical trial. For IV tPA treatment within 0-3 and 3-4,5 hours, the NNT values have been calculated based on the NINDS and ECASS III trials respectively (Table 2).^{127,128}

Trial and definition (3 month outcomes)	NNT to benefit
NINDS (0-3h) – to achieve mRS 0-1	9
NINDS (0-3h) – to improve by at least 1 point on the mRS	3
ECASS III (3-4,5h) – to achieve mRS 0-1	13
ECASS III (3-4,5h) – to improve by at least 1 point on the mRS	7

Table 2. Number needed to treat (NNT) with IV tPA to benefit from treatment compared to controls in the early versus late treatment time windows (NINDS and ECASS III trials).

Only two days before this thesis was handed in to the printer's office, a most important publication for its topic appeared online in the Lancet. This was an individual patient data meta-analysis from all phase 3 randomised trials of alteplase for acute ischemic stroke hitherto performed, including 3391 patients receiving active treatment and 3365 patients receiving control. The authors confirmed the efficacy of IV tPA, as active treatment unequivocally resulted in more patients with an excellent neurological outcome at 3-6 months. This analysis is likely to put to rest previously lingering concerns on the level of evidence for effect of treatment in patients above the age of 80 years, in the later treatment time window of 3-4,5 hours and in patients with the least and most

severe strokes (Figure 14). The specific findings in these patient subgroups will be reviewed in more detail under the respective subsections of Chapter 1.4.¹²⁹

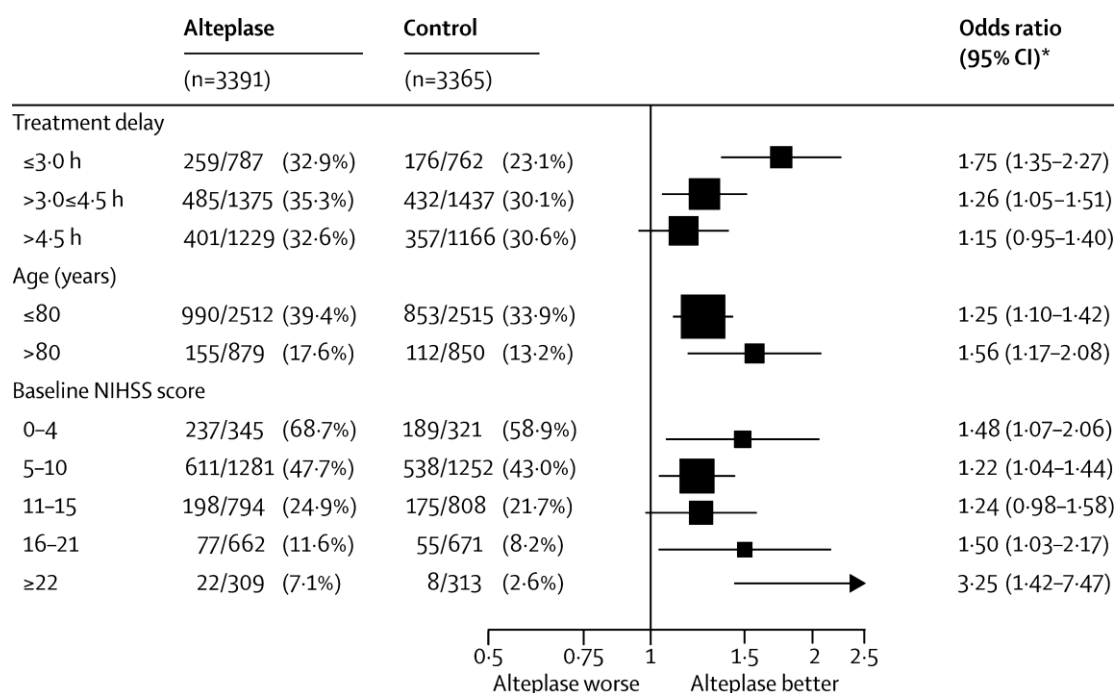


Figure 14. Effect of IV alteplase on excellent stroke outcome (mRS 0-1) by treatment delay, age, and stroke severity. From Emberson et al 2014. Reproduced under the Creative Commons BY license.

1.2.3 Contraindications

Current contraindications for treatment of acute ischemic stroke with IV tPA have been adopted by regulatory authorities and in part by professional organizations from inclusion and exclusion criteria used in thrombolysis RCTs. The aim of these criteria was to recruit trial patients with traits conferring the clearest potential for treatment benefit combined with optimal safety. The majority of these criteria were based on expert opinion, with very limited empirical support, such as from trials of thrombolysis in myocardial infarction. The more restrictive indications and contraindications of the European Cooperative Acute Stroke Studies I and II^{113,114} were taken up by the European product license, whereas the American license is more liberal due to the protocol of the National Institute of Neurological Disorders and Stroke (NINDS) trial (Table 3).¹¹²

Characteristic	Contraindication
Age & Medical history	
Aged > 80 years	EU-L
Aged > 80 years (if OTT 3-4,5 h)	US-G
Aged under 18 years	EU-L, US-G
Previous stroke within the last 3 months	EU-L, US-L, US-G
Previous stroke and diabetes mellitus	EU-L
Previous stroke and diabetes mellitus (if OTT 3-4,5 h)	US-G
Seizure at the onset of stroke	EU-L, US-L
Stroke severity and CT infarct size	
NIHSS >25	EU-L
NIHSS >25 (if OTT 3-4,5 h)	US-G
"Severe stroke as assessed by appropriate imaging"	EU-L
CT showing infarction of >1/3 of MCA territory	US-G
"Minor deficit or rapidly improving symptoms"	EU-L
Hemostasis	
Use of oral anticoagulation	EU-L, (US-L?)
Use of oral anticoagulation (if OTT 3-4,5 h)	US-G
Oral anticoagulation and INR ≤1,7	EU-L, (US-L?)
Oral anticoagulation and INR > 1,7	EU-L, US-L, US-G
International Normalized Ratio > 1,7	US-L
Platelet count <100 10 ⁹ /L	EU-L, US-L
Heparin within last 48 hours with elevated APTT	EU-L, US-L, US-G
Glucose & Blood pressure	
Glucose level > 22,2 mmol/L	EU-L
Glucose level < 2,8 mmol/L	EU-L, US-G
Systolic blood pressure ≥185 mm Hg	EU-L, EU-G, US-G
Diastolic blood pressure ≥110 mm Hg	EU-L, EU-G, US-G
IV blood pressure medication necessary	EU-L

Table 3. Contraindications to treatment with IV tPA for acute ischemic stroke. EU-G: European Stroke Organization Guidelines 2008-09.^{119,123} EU-L: EU License.¹³⁰ US-G: American Heart Association/American Stroke Association Guidelines 2013.¹⁹ US-L: USA FDA License.¹³¹ OTT: Onset to treatment time.

Depending on how individual physicians and hospital policies relate to these contraindications, the rate of stroke patients treated with IV thrombolysis can vary widely between centres, from as few as 5% to over 25%, as evidenced by numbers from Sweden and the Netherlands.^{15,132} A number of papers have reported treatment despite a wide range of contraindications, the largest single-centre series coming from Helsinki, Finland. The authors reported 985 thrombolysed patients with anterior circulation stroke, of whom 51% had one or more EU license contraindications. The off-label patients did not have more frequent hemorrhagic complications compared to the on-label population.¹³³

These findings were later confirmed by an even larger multicentre SITS-EAST observational registry study in 2013 by Karlinski et al, reporting 5594 patients from Central and Eastern Europe.¹³⁴ The state of the science on individual contraindications will be further described in detail in chapter 1.4.

1.3 CEREBRAL HEMORRHAGE IN IV THROMBOLYSIS

However one defines it, following administration of IV alteplase to stroke patients, there is an increase in the risk of cerebral hemorrhage. This has been a consistent finding in all major trials. Results from the recent large individual patient data meta-analysis in 2014 in the Lancet show that fatal large intracerebral hemorrhage (defined as parenchymal hemorrhage type 2, see below in 1.3.1, and death within 7 days) occurs in 2,7% of treated patients, compared with 0,4% of controls. This translates into an increase in total mortality within 7 days at 8,3% in treated and 6,1% in controls.¹²⁹

1.3.1 Radiological classification

The SITS-ISTR employs the classification of post-thrombolytic intracerebral hemorrhage introduced by Pessin et al in 1991 and later used by the ECASS trialists.^{113,135,136} Hemorrhagic infarction type 1 (HI1) is defined as small petechiae along the margins of the infarct; HI2, as confluent petechiae within the infarcted area without space-occupying effect; local, or intra-ischemic parenchymal hemorrhage type 1 (PH1), as blood clots in $\leq 30\%$ of the infarcted area with some slight space-occupying effect; and PH2, as blood clots in $>30\%$ of the infarcted area with a substantial space-occupying effect. Further analysis of ECASS II data emphasized the need to add extraischemic remote hematomas to the list of possible hemorrhages. These could be solitary or multifocal, with or without mass effect, visible on CT in brain regions without visible ischemic damage, *i e* remote from an infarct or in brains without any visible infarct lesions on CT.¹³⁷ Thus, two remote parenchymal hemorrhage (PHr) definitions were introduced to the SITS-ISTR for the SITS-MOST study.¹¹⁸ PHr type 1 (PHr1) were defined as small or medium sized blood clots located remote from the actual infarct, with mild space occupying effect; PHr2 were defined as large confluent dense blood clots in an area remote from the actual infarct, with substantial space occupying effect.

1.3.2 Symptomatic intracerebral hemorrhage - definitions

The first mention of the term “symptomatic intracerebral hemorrhage”, however without any definition, stems from the National Institutes of Health open-label study of rt-PA, reported by Brott, Haley and colleagues in 1992.^{138,139} In a further analysis of this study in 1994, Levy et al clarified that “contemporaneous neurological worsening” had to be present, but without further specifying the degree or timing in the deterioration.¹⁴⁰ The NINDS trialists subsequently defined SICH as any decline in neurological status (later interpreted as NIHSS ≥ 1) from baseline, and ICH on CT scans at 24 hours, 7-10 days and whenever else it was performed on clinical suspicion of hemorrhage (Table 4).¹¹² The NINDS trial was not only pivotal in establishing stroke thrombolysis as an effective therapy and a vital research field, but also started a still unresolved controversy on how to best define SICH, the most important safety outcome measure in trials of cerebral arterial recanalization therapy.

A discussion of the various definitions of SICH should take into account the two components of this notion: *i e* what does “symptomatic” entail and what

radiological findings should be classified as “intracerebral hemorrhage”. If any neurological worsening is used, such as in the NINDS, IST-3 and Cochrane definitions, there is a risk of falsely labelling patients with minor fluctuations in status. These are common in the first few days after stroke, may have a circadian explanation or be due to the small, but still non-negligible inter- and intra-rater variability of the NIHSS.¹⁴¹ Conversely, the ≥ 4 point NIHSS increase used by the PROACT II, ECASS III and SITS-MOST definitions, while more stringent and clinically significant, may label hemorrhages causing a 3 point deterioration is asymptomatic.

Regarding what should be viewed as an “ICH” for the purposes of defining SICH, the debate has stood between “any blood”, including petechial HI (see 1.3.1), and “parenchymal hemorrhage only”, excluding petechia. In the ECASS I and II studies, HI had no significant impact on the outcome at 3 months and was actually more common among control patients than among those treated with rt-PA.^{142,143} In both materials, only parenchymal hemorrhages were shown to be predictors of poor outcome defined as mRS 5-6. Moreover, Molina et al have shown that thrombolysis-related HI may be a marker of successful early arterial recanalization (within 6 hours) and that neurological improvement is actually more common in patients with HI than in those without.¹⁴⁴ However, these data were contradicted to an extent by an observational study of the Canadian CASES registry, where extensive, confluent HI (named HI-2) was a risk factor for poor 3 month outcome after adjustment for confounding variables, such as stroke severity, age and extent of ischemic changes on baseline CT.¹⁴⁵

For purposes of comparability of results, many researchers presently report SICH rates in their materials using a variety of definitions. This is the case for most publications using SITS-ISTR data (usually reporting three definitions), as well as the ECASS 3 study, which gave SICH rates according to as many as four different definitions.¹²⁰

Definition	Description
NINDS ¹¹²	To detect intracranial hemorrhage, CT scans were required at 24 hours and 7 to 10 days after the onset of stroke and when any clinical finding suggested hemorrhage. A hemorrhage was considered symptomatic if it was not seen on a previous CT scan and there had subsequently been either a suspicion of hemorrhage or any decline in neurologic status (NIHSS ≥ 1).
Cochrane ¹⁴⁶	Either symptomatic (temporally associated with a deterioration in the patient’s neurological state), or fatal (leading directly to death), and occurring within the first seven to 10 days.

ECASS II ¹¹³	The presence of blood at any site in the brain on the CT scan, documentation by the investigator of clinical deterioration or adverse events indicating clinical worsening or causing ≥ 4 point increase in the NIHSS, up to 7 days or leading to death. In case of doubt regarding whether edema or hemorrhage was the leading pathology, an association of the hemorrhage with the deterioration was assumed.
ECASS III ¹²⁰	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.
SITS-MOST ¹¹⁸	Local or remote parenchymal hemorrhage type 2 ($>30\%$ of the infarct area, with substantial mass effect) on the 22-36 h post-treatment imaging scan, combined with a neurological deterioration of 4 points or more on the NIHSS from baseline, or from the lowest NIHSS value, between baseline and 24 h, or leading to death.
PROACT II ¹⁴⁷	Hemorrhagic transformation causing ≥ 4 point increase in the NIHSS or ≥ 1 point increase in the level of consciousness within 24 hours, however the scale used for the latter was not specified in the original publication. Presumably, the Glasgow Coma Scale was used. Trial evaluated intraarterial, not intravenous tPA.
DEFUSE ¹⁴⁸	“Minor” SICH if these are associated with a worsening of 2 or 3 points on the NIHSS within 36 hours of intravenous thrombolysis and “major SICH” if associated with a worsening of 4 or more points within 36 hours of intravenous thrombolysis.
IST-3 ¹²⁴	Significant neurological deterioration accompanied by clear evidence of significant intracranial hemorrhage on the post-randomisation scan (or autopsy if not rescanned and death occurs after 7 days). Significant hemorrhage was present on any post-randomisation scan if the expert reader both noted the presence of significant hemorrhagic transformation of the infarct or parenchymal hematoma and indicated that hemorrhage was a major component of the lesion (or was remote from the lesion and likely to have contributed significantly to the burden of brain damage). This event included clinical events described as a recurrent stroke within 7 days, in which the recurrent stroke was confirmed to be caused by an intracranial hemorrhage.

Table 4. Various definitions of symptomatic intracerebral hemorrhage in studies of intravenous and intraarterial thrombolysis.

An analysis Strbian et al in 2011 of the association of various SICH definitions with poor outcome (mRS 3-6) and death showed that the SITS-MOST definition was the strongest predictor of both outcomes at 3 months, compared to the NINDS and ECASS II classifications, showing an adjusted RR for death of 4,8 (95% CI 2,8-8,2).¹⁴⁹ Subsequently, in 2012, researchers in Heidelberg compared the association with mortality between the NINDS, ECASS II, ECASS III and SITS-MOST definitions, as well as the interrater agreement between stroke neurologists for these classifications.¹⁵⁰ The conservative SITS-MOST definition, only counting large PH Type 2 as SICH had the strongest association with mortality, but was more difficult to use, reflected by the relatively low kappa value. The trade-off between specificity and practical simplicity is reflected by findings for the ECASS II definition: while easy to use (any ICH, worse by ≥ 4 NIHSS points), reflected by a high interrater agreement, it had the weakest association with fatal outcome (Table 5).

Definition	Kappa	OR for death	Rate of SICH
NINDS	0,57	5,7	7,7%
ECASS II	0,85	4,7	5,4%
SITS-MOST	0,65	14,4	3,5%
ECASS III	0,62	12,3	3,2%

Table 5. Odds ratios for mortality within 3 months for different definitions of SICH as compared with patients without hemorrhage. Kappa values denote interrater agreement. N=314 at the University Clinic of Heidelberg, Germany. From Gumbinger et al, 2012.¹⁵⁰ Permission from Wolters Kluwer Health.

An important aspect of the various SICH definitions is the strength of their relation to IV tPA treatment. If one considers the proportion of all SICHs which are not induced by active treatment but by placebo, it is 30% for NINDS, 24% for ECASS II, 11% for SITS-MOST and 9% for ECASS III. Thus, the SITS-MOST and ECASS III definitions, in the vast majority (9 out of 10 cases) denote a treatment related SICH. Conversely, the 1 in 3 NINDS and 1 in 4 ECASS II SICHs would have occurred even without IV thrombolysis (Table 6).¹²⁰

Definition	IV tPA N=418	Placebo N=403	OR (95% CI)	P
Any ICH	113 (27%)	71 (17,6%)	1,7 (1,2-2,4)	0,001
ECASS III	10(2,4%)	1 (0,2%)	9,9 (1,3-77,3)	0,008
ECASS II	22 (5,3%)	9 (2,2%)	2,4 (1,1-5,4)	0,02
SITS-MOST	8 (1,9%)	1 (0,2%)	7,8 (1,0-63,0)	0,02
NINDS	33 (7,9%)	14 (3,5%)	2,4 (1,3-4,5)	0,006

Table 6. Rates of ICH and SICH per various definitions in the ECASS III study. Modified from Hacke et al, NEJM 2008.¹²⁰ Copyright Massachusetts Medical Society.

1.4 FACTORS INFLUENCING SAFETY AND OUTCOMES OF STROKE THROMBOLYSIS

Below follows a presentation of common clinical, radiological and laboratory parameters relevant in the setting of thrombolytic therapy for acute ischemic stroke. They are also available in the SITS International Stroke Thrombolysis Register, which provided the data for the studies in the present thesis. There has been a great body of scientific evidence published based on SITS registry data, with peer-reviewed articles currently numbering around 40. Therefore the following review places special emphasis on the way various patient characteristics are registered in the SITS-ISTR, and on previously published SITS findings.

1.4.1 Age

Age is one of the best studied parameters influencing safety and functional outcomes in stroke thrombolysis. In the multivariate analysis of data from the SITS-MOST study, it was expectedly found to be independently associated with mortality and inversely associated with functional independence at 3 months.¹⁵¹ The same relation was also observed with SICH per SITS-MOST and per NINDS. Later, Ford et al showed that rates of parenchymal hemorrhage and SICH do not differ between age groups 71-80 years and 81-90 years (Figure 15).¹⁵² In 2010, Mishra et al used combined SITS-ISTR and VISTA data to show that stroke patients across all age deciles from 31 to 90 years significantly benefit from treatment with IV tPA, with a trend for benefit also beyond 90 years (Figure 16).¹⁵³ For some observers, these analyses based on observational data, were not sufficient to change the practice of withholding IV tPA treatment from patients over the age of 80 years, mandated by the USA and EU regulatory authorities.¹⁵⁴ Two years later, the Third International Stroke Trial (IST-3), a randomised controlled trial enrolling 1617 patients above the age of 80, confirmed that benefit of IV tPA indeed does not diminish in the elderly.¹²⁴

On the lower end of the age spectrum, in 2012, Toni et al analysed young stroke patients (aged 18-50) in the SITS-ISTR. Of main interest were the findings that the frequency of both SICH and mortality at 3 months was only around 1/3 of that in older patients (51-80 years), at 0,6% versus 1,9% for SICH per SITS-MOST, and 4,9% versus 14,4% for mortality. Moreover, the rate of functional independence was 72,1% in the younger versus 54,5% in the older group.¹⁵⁵

The individual patient data meta-analysis by Emberson and colleagues in the Lancet 2014 gave what is likely to be the most definitive estimate of treatment effect in the age group above 80 years. As shown in Figure 14, with 17,6% of thrombolysed elderly stroke patients reaching excellent outcome (mRS 0-1) versus 13,2% in the control arm, the OR for benefit was highly significant at 1,56 (95% CI 1,17-2,08).¹²⁹ Importantly, there was no evidence that age modified the proportional benefits or hazards of alteplase. Neither was there evidence that age shortened the period during which a benefit was seen (Figure 17), in line with recently published results from the VISTA database.¹⁵⁶

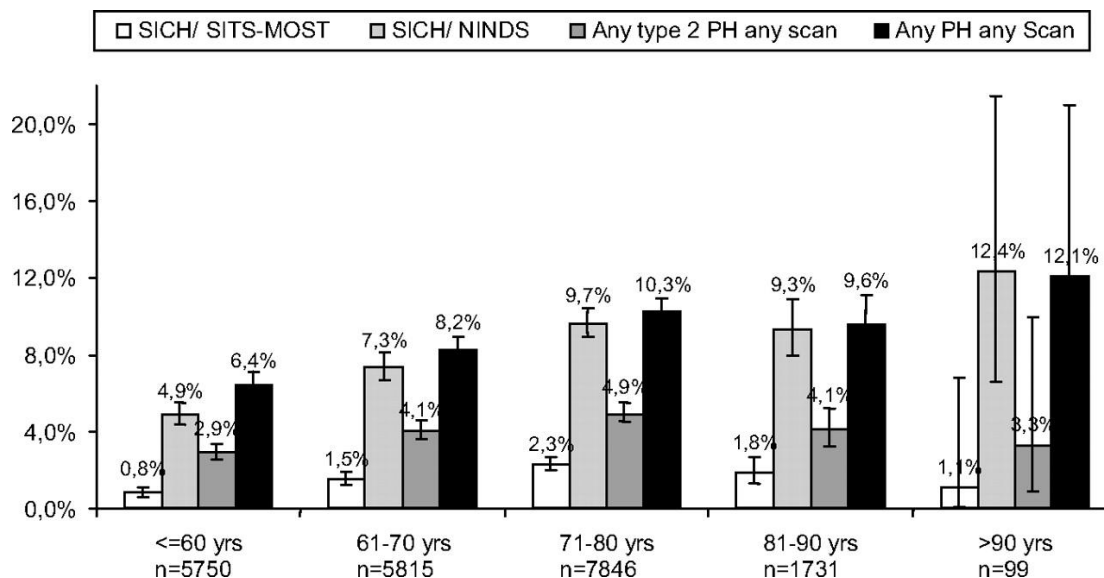


Figure 15. SICH and PH on any post-thrombolysis imaging for 10-year age strata. Hemorrhage rates between 71- to 80- and 81- to 90-year-old groups had no significant differences for any PH ($P=0.96$), SICH per SITS-MOST ($P=0.84$), and SICH per NINDS ($P=0.31$). Reproduced from Ford et al, Stroke 2010.¹⁵² Permission obtained from Wolters Kluwer Health.

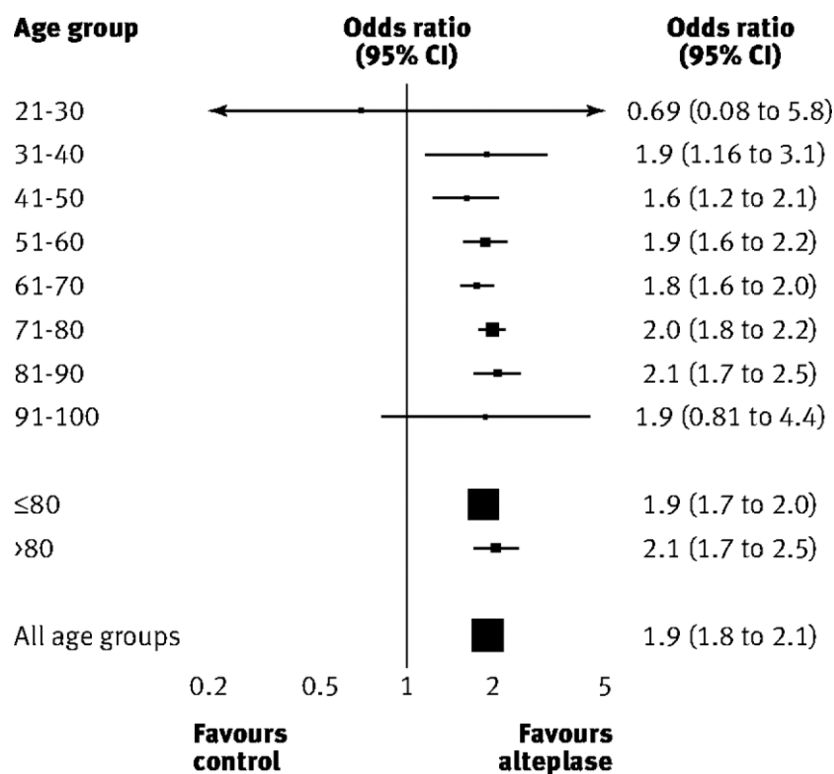


Figure 16. Odds ratios for score 0-2 on modified Rankin scale at three months adjusted for age and baseline National Institutes of Health stroke severity scale in patients who received thrombolytic therapy. Reproduced from Mishra et al, BMJ 2010, under the Creative Commons license.¹⁵³

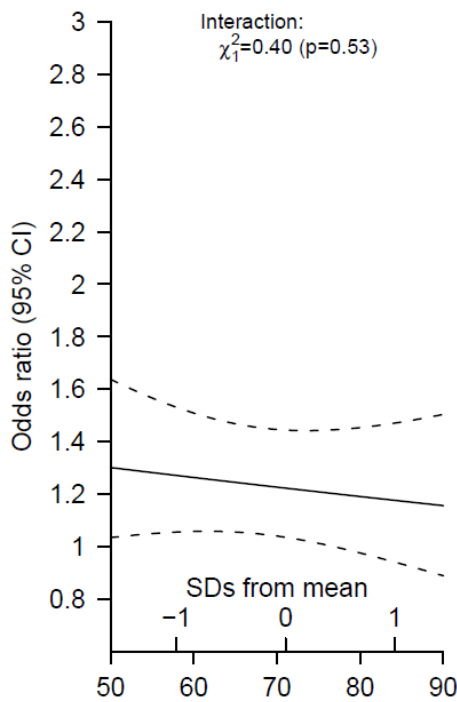


Figure 17. No significant interaction between alteplase treatment and age regarding the magnitude of the odds for excellent outcome (mRS 0-1). From Emberson et al 2014.¹²⁹ Reproduced under the Creative Commons BY license.

1.4.2 Sex

Longitudinal studies based on stroke registries suggested that women are more likely than men to have a stroke because of their higher life expectancy (by around 10 years), associated with the exponential rise in stroke incidence with age.¹⁵⁷ In addition, the natural course of stroke is worse in women having a higher probability to be functionally dependent and institutionalized.¹⁵⁸ In light of this, it is intriguing that a 2005 pooled analysis of RCTs on IV tPA reported no difference in outcomes between men and women among those treated.¹⁵⁹ The authors suggested that thrombolysis could reverse the sex differences usually observed in the spontaneous evolution of an ischemic stroke. The same conclusion has been drawn from analyses of data in the Canadian Alteplase for Stroke Effectiveness Study (CASES) register.¹⁶⁰ A recent study of SITS-ISTR data by Lorenzano et al (n=45079) lent further strength to this notion. After multivariate adjustment for confounding variables, the authors did not find any differences between sexes regarding excellent outcome (mRS 0-1) or functional independence (mRS 0-2) at 3-month follow-up (OR 1,03; 95% CI 0,97–1,09; P=0,39). However, male sex was independently, however weakly, associated with mortality (OR 1,19; 95% CI 1,10–1,29; P<0,001) and SICH according to all definitions.¹⁶¹ Similar results had previously been shown in the multivariable analysis of SITS-MOST data from 2008 by Wahlgren et al.¹⁵¹ An earlier meta-analysis (excluding SITS-MOST) also found no association of sex with favourable functional outcome and a slightly increased risk of SICH in men. However, this analysis differed from SITS-ISTR results on mortality, as no association between sex and death at 3 months was observed.¹⁶²

1.4.3 Stroke severity – the NIH Stroke Scale

The SITS-ISTR registers measurements of stroke severity employing the National Institutes of Health Stroke Scale.¹⁶³ Registration is mandatory at four time points: at baseline (immediately prior to administration of IV tPA) and at 2 hours, 24 hours, and 7 days after initiation of tPA infusion. The NIHSS is a 15-item impairment scale rated from 0 to 42 points, which provides a quantitative measure of key components of a standard neurological examination. The scale assesses level of consciousness, eye movements, visual fields, facial muscle function, extremity strength, sensory function, coordination (ataxia), language (aphasia), speech (dysarthria), and hemi-inattention (neglect).¹⁶⁴ An additional item that measures distal motor function in the upper extremities has been used in a few drug trials, but is now commonly omitted in research and in clinical practice. In expert users, the NIHSS has an excellent intra-observer (same observer and patient, comparing measurements obtained at a three month interval) and inter-observer (same patient, different observers) reliability, with weighted kappa values of 0,95 (values above 0,8 considered excellent).^{165,166} Following the introduction of a NIHSS video training and certification program on VHS in 1988, later on DVD and on the web, excellent reliability was confirmed in a 2009 study of 8214 raters from different venues (33% of all responses came from registered nurses, 23% from emergency department MD/other emergency department/other physicians, and 44% from neurologists), including 49% without previous NIHSS certification.¹⁶⁷

An important feature of the NIHSS scale is its correlation with cerebral infarct volume. This has been reported in several studies, using both CT and MRI, suggesting a high degree of validity.^{168,169} As a marker of the extent of ischemic tissue, the NIHSS has also consistently been shown to correlated strongly and independently with thrombolysis-related SICH. Following a large number of publications with consistent results, it was shown in a 2012 meta-analysis of 55 studies and 65 264 patients to correlate strongly with the risk of SICH following IV tPA, with low heterogeneity between studies.¹⁷⁰ Together with age, the NIHSS has consistently been shown to be the most important clinical determinant of outcome in stroke and is included in every published multivariate stroke prognostic model to-date.¹⁷¹ Importantly, IV tPA has been shown to be effective across a wide range of baseline stroke severity grades measured by the NIHSS (Figure 18).¹⁷²

Regarding the effect of alteplase on beneficial functional outcome, the recent individual patient data meta-analysis by Emberson et al in the Lancet 2014 definitively showed that IV thrombolysis is effective across all levels of stroke severity (Figure 14). This had previously been shown in a number of publications, among them by Mishra et al in Stroke, 2010, however some uncertainties existed among patients with the lowest and highest stroke severity levels (Figure 18).¹⁷² In fact, there was a trend for ($p=0,06$) for an interaction between treatment and stroke severity in the positive direction, *i e* of proportionally higher treatment effects in the highest, and somewhat also in the lowest stroke severity strata (Figure 19).

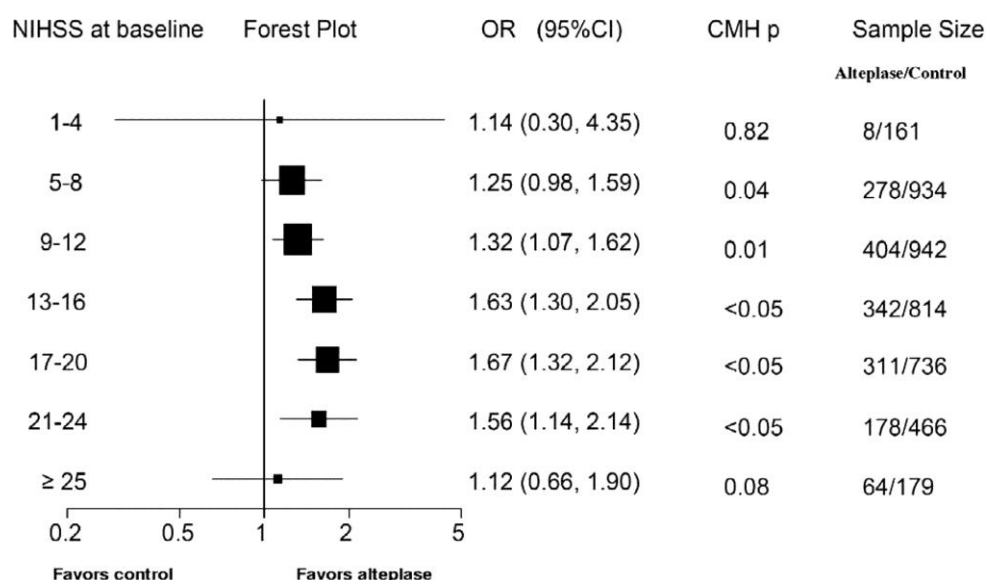


Figure 18. Age and baseline severity-adjusted analyses showing functional outcomes corresponding to various baseline NIHSS categories. Odds ratios are derived from proportional odds logistic regression analyses and refer to proportional (common) odds for shift toward better modified Rankin scale categories for patients who receive alteplase. From Mishra et al, 2010, with permission from Wolters Kluwer Health.

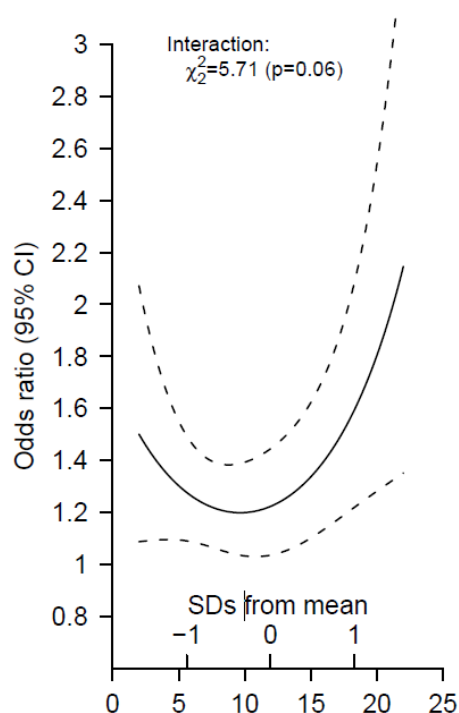


Figure 19. Trend, however non-significant ($p<0,06$) for a modification of the odds for excellent outcome (mRS 0-1) by the level of stroke severity. From Emberson et al 2014.¹²⁹ Reproduced under the Creative Commons BY license.

1.4.4 Body weight

Intravenous alteplase has a weight-based dose of 0,9 mg/kg with a maximum dose limit of 90 mg according to the European licensing criteria for its use in ischemic stroke. Thus, patient body weight is necessary for calculating the correct total drug dose. In the SITS-ISTR, two options exist for reporting body

weight: (1) estimated weight, including weight indicated by the patient or the family or estimated by the attending hospital staff and (2) measured weight. In the database, of all patients with a reported body weight, 15% of readings were measured, and the remaining 85% were estimated. This appears to be a common issue in stroke thrombolysis research, as for example the ECASS II study also reported that only a minority of patients had an actual measured weight.¹¹³ According to literature, patients' own estimations are fairly accurate with reported errors of approximately 4%, compared to nurses (8%) and physicians (11%).¹⁷³ In one study of prospectively included 178 consecutive stroke inpatients in Australia, 85% of patients were able to give their own estimation of their body weight.¹⁷⁴

The maximum dose limit of 90 mg for alteplase leads to a lower per kilogram dose in patients weighing above 100 kg. It has been unclear whether this would lead to less effective recanalization and poorer outcome in these patients. In 2011, Diedler et al analysed SITS-ISTR data on 1190 patients weighing >100 kg. They showed that after multivariate adjustment, there was no significant difference in major neurological improvement or functional independence between weight categories of >100 kg and ≤100 kg.¹⁷⁵ In this material, heavy patients were 8 years younger and had less severe strokes (by 2 points on the NIHSS) than those weighing ≤100 kg. Interestingly, despite these facts and the lower per kg alteplase dose, the incidence of SICH per SITS MOST was significantly higher in patients weighing >100 kg – a finding which persisted after adjustment for baseline imbalances. This is in keeping with results from the multivariate analysis of SITS-MOST data which identified body weight as an independent predictor of SICH.¹⁵¹

1.4.5 Dose of IV tPA

The dose of IV tPA is entered into the database as the total amount in milligrams received by the patient. The database automatically calculated the per kg dose using the reported patient body weight. All participating centres, including the ones in Asian countries, followed the standard dosage of 0,9 mg/kg iv tPA. By request from a reviewer for Stroke during the submission process for Study I, the mean tPA dose in the database was calculated to be 0,88 mg/kg (SD = 0,095). A lower per kg dose of alteplase has been associated with higher mortality at 3 months in the SITS-MOST study.¹⁵¹ The study by Diedler et al mentioned in section 1.4.4 also found that patients weighing >100 kg had the same crude 3 month mortality rate of 15% as lighter patients, which is unexpected, keeping in mind the large difference in age and stroke severity (see above).¹⁷⁵ The adjusted odds ratio for death in this study was 1,4 (95% CI 1,1-1,7) in the >100 kg group versus ≤100 kg. This cannot be explained entirely by the increased risk for SICH, as the absolute difference in SICH rates was small (however significant), at 2,3% versus 1,7%. Thus, the reasons for the increased adjusted odds for death in heavier patients are as yet unclear.

1.4.6 Blood pressure

Systolic and diastolic blood pressure (SBP and DBP) measurements in mm Hg, performed per clinical routine in participating centres, is reported at baseline (immediately prior to IV tPA bolus), at 2 hours, and 24 hours.

Elevated blood pressure occurs frequently during acute ischemic stroke. In a very large observational study of stroke patients in the USA, SBP was ≥ 140 mm Hg in 77% and ≥ 185 mm Hg in 15% of patients on arrival at the emergency department.¹⁷⁶ In the large International Stroke Trial (n = 17 398), a U-shaped relationship was shown between first hospital admission SBP, early death and late death or dependency: early death increased 18% for every 10 mm Hg below 150 mm Hg ($P < 0,001$) and 4% for every 10 mm Hg above 150 mm Hg ($P = 0,016$).¹⁷⁷ In spite of these findings, lowering of blood pressure has not been found to be associated with less death or disability after acute stroke in several randomized trials.¹⁷⁸⁻¹⁸¹

Hypotension is rare on presentation in acute ischemic stroke, with only around 4% presenting with an SBP < 120 mm Hg.¹⁷⁷ In the SITS-ISTR, 0,6% of patients are registered as having an SBP < 100 mm Hg at baseline.¹⁸² Hypotension has been associated with poor outcomes in multiple studies.^{177,183,184} Possible reasons for low BP include hypovolemia, sepsis, impaired cardiac output secondary to cardiac failure, arrhythmias or cardiac ischemia, and aortic dissection.¹⁸⁵ Due the heterogeneous etiology and the rarity of hypotension in acute ischemic stroke, there is no available trial data for its treatment.

The risk of SICH associated with uncontrolled severe hypertension is not known, since such patients were excluded from all stroke thrombolysis trials and clinical guidelines recommend that they be excluded from IV t-PA treatment in routine clinical practice. The European Summary of Product Characteristics contraindicates stroke thrombolysis in patients with SBP > 185 mm Hg and/or diastolic BP > 110 mm Hg. Current European Stroke Organization and American Stroke Association guidelines recommend treatment intervention if SBP exceeds 180 mm Hg or DBP exceeds 105 mm Hg during and early after IV tPA.^{19,119} In the presently used SITS-ISTR material, 2% of patients are entered as having a baseline SBP > 185 and 1% with DBP > 110 at baseline. Smaller observational case series have rendered conflicting results regarding the risk of SICH and poor outcomes in patients who are treated with IV tPA despite blood pressure protocol violations (i.e. thrombolysis in spite of BP $> 185/110$).^{133,186,187} Importantly, an analysis of VISTA data showed significant improvement of functional outcome in thrombolysed stroke patients with baseline BP $> 185/110$ versus controls with the same BP levels (OR 1,3, $p = 0,009$ if given IV tPA despite SBP > 185 and OR 1,7, $p = 0,008$ if given IV tPA despite DBP > 110).¹⁸⁸

In a detailed evaluation of blood pressure in patients registered in the SITS-ISTR, Ahmed et al found a similar U-shaped association with mortality and independence as the IST trialists (Figure 20).¹⁸² Systolic BP in the interval 141 to 150 mm Hg was associated with the most favourable outcomes. In contrast, a linear relationship between SBP and SICH was described. This is in line with

findings from the SITS-MOST study, where SBP was found to be an independent predictor of SICH, as well as other previous trials of thrombolysis for ischemic stroke and myocardial infarction.^{135,151,189-191}

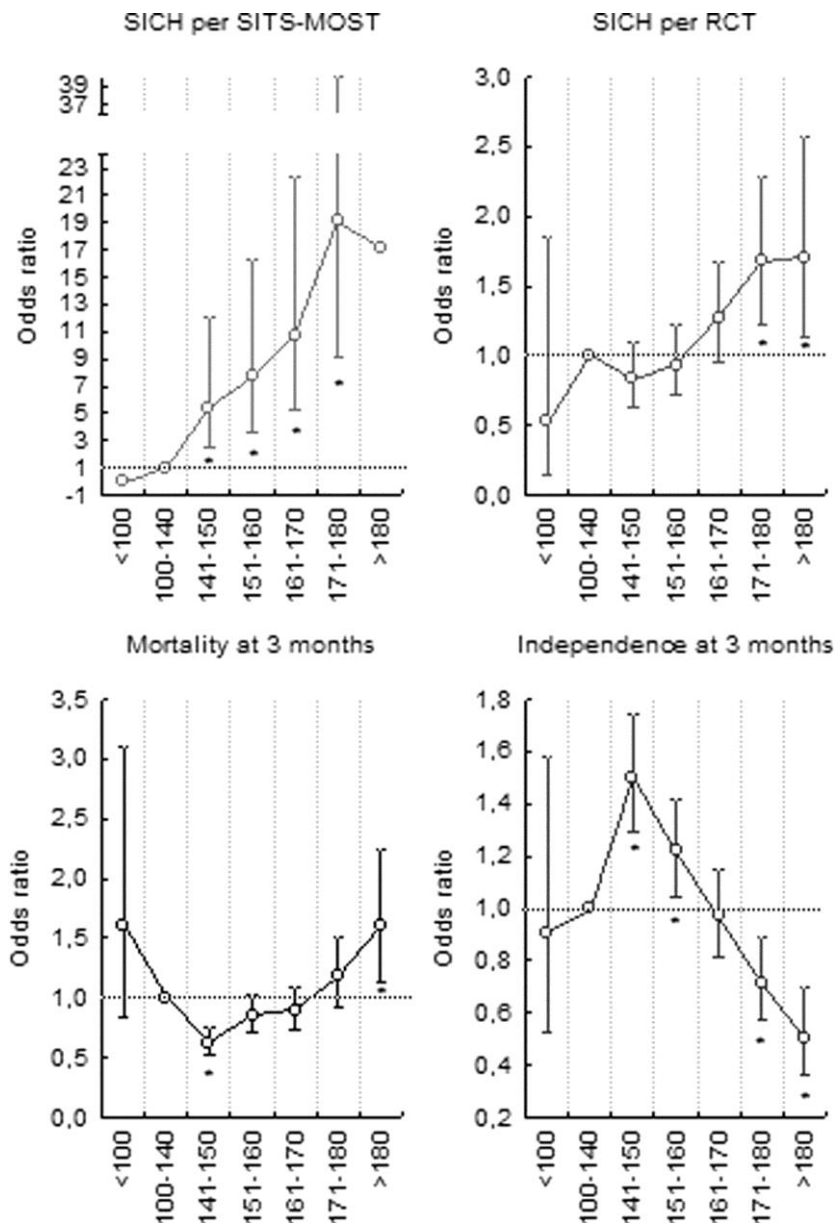


Figure 20. Adjusted OR (midpoints) and 95% CIs (vertical error bars) derived from multivariable analysis for main outcomes categorized by average post-thrombolysis systolic BP (SBP) within 24 hours. From Ahmed et al, Stroke 2009.¹⁸² Permission obtained from Wolters Kluwer Health.

1.4.7 Hypertension

This variable denotes whether the patient has a history of the diagnosis of hypertension, irrespective of whether it is under treatment or not.

Hypertension is the most important independent contributor to the burden of stroke worldwide. Of ten major stroke risk factors studied in the worldwide INTERSTROKE study reported in 2010 (n=3000), a self-reported history of

hypertension accounted for a third of the population-attributable risk (PAR) for ischemic, and nearly half of the PAR for hemorrhagic stroke, with odds ratios of 2,4 (99% CI 2,0-2,8) and 3,8 (99% CI 3,0-4,8) respectively.¹⁹²

The frequency of hypertension in patients enrolled in the SITS-ISTR has held consistently at 59-61% throughout the years.^{118,193} This level is very near the 57% reported in the pooled analysis of the NINDS, ATLANTIS, ECASS II, ECASS III, and EPITHET trials.¹²⁶

A previous diagnosis of hypertension in stroke patients treated with IV tPA has been found in a meta-analysis of 11 studies to confer an OR of 1,5 (95% CI 1,2-1,9) for SICH.¹⁷⁰ It should however be noted that only one study in the meta-analysis, albeit the one with the highest number of recruited patients (the SITS-MOST multivariable analysis)¹⁵¹ showed statistically significant association of hypertension with SICH in univariate analysis. This study also showed a history of hypertension to be a relatively weak, but statistically significant independent predictor of lower likelihood of functional independence at 3 months.

1.4.8 Antihypertensive therapy

Antihypertensive therapy is registered in the SITS-ISTR as two variables with different timepoints: (1) at baseline and (2) within 7 days from IV tPA treatment. Both variables can be registered as only oral, only IV or both oral and IV therapy.

In 2011, the authors of the Angiotensin-receptor blocker candesartan for treatment of acute stroke trial (SCAST) performed a meta-analysis of RCTs evaluating treatment of hypertension in the acute post-stroke phase in non-thrombolysed patients. There was no signal of effect on functional outcome or mortality.¹⁷⁹ Furthermore, the COSSACS trial, evaluating continuation versus suspension of chronic antihypertensive treatment in acute stroke patients, did not show any difference in clinical outcomes in the two study arms.¹⁹⁴

The 2009 SITS-ISTR blood pressure and hypertension paper by Ahmed et al, reported that providing antihypertensive therapy after intravenous thrombolysis in patients with a history of hypertension or high BP without a history of hypertension did not affect outcomes adversely. In contrast, stopping antihypertensive therapy in patients with a history of hypertension was associated with increased mortality, a high symptomatic hemorrhage rate, and a low rate of functional independence. This finding was confirmed after adjusting for other prognostic factors.¹⁸²

The same year, an analysis was published by Martin-Schild et al of 50 ischemic stroke patients who received IV antihypertensive treatment just prior to initiation of IV tPA, in order to lower severely elevated BP to levels below those mandated by guidelines. Those treated were compared to 128 patients who did not require BP lowering (mean BP in treated group 195/101, in controls 160/87). The authors found no significant differences between the groups on any outcome, including SICH, in-hospital mortality or good neurological

outcome. However, this could have been a low power issue, as rates of any grade of hemorrhagic infarct transformation, SICH, neurological deterioration and death were all higher in the treated group.¹⁹⁵ Similarly, the NINDS trialists did not detect any adverse effects of pre-randomization acute antihypertensive treatment (n= 56/624, 9%) on 3 month outcomes.¹⁹⁶ Here, it is relevant to note that baseline antihypertensive treatment prior to initiation of IV tPA has been shown to increase the door-to-needle time by around 10 minutes.¹⁹⁷ On the other hand, the same NINDS trial analysis found that post-randomization antihypertensive therapy for thrombolysis-treated patients was associated with less favourable outcomes, in comparison with those who were hypertensive and were not treated with antihypertensives. Similarly, Lindsberg et al (n=75) also found that using antihypertensive therapy after thrombolysis reduced the likelihood of favourable outcome.¹⁹⁸

Thus, there is strong evidence that high BP both at baseline and after IV tPA raises the risk of SICH. However, in the absence of sufficiently powered randomised trials, it remains an open question whether correction elevated BP mitigates the risk of this complication following stroke thrombolysis.

1.4.9 Onset-to-treatment time

The onset-to-treatment time (OTT) denotes the interval passing between the onset of stroke symptoms as reported by patients or family, and initiation of IV tPA infusion in the hospital. The benefit of alteplase is strongly time dependent, in keeping with the theory of recruitment of viable but non-functional ischemic penumbra into the irreversibly damaged infarct core. Although the number needed to treat (NNT) for one patient to achieve excellent recovery at 3 months (mRS 0–1) is small when treatment is initiated early, within 1,5 hours of symptom onset (NNT = 5), this drops in the 1,5 to 3 hour time frame (NNT = 9), and further on if the treatment is delayed until 3 to 4,5 hours from symptom onset (NNT = 15).^{117,126} The largest RCT to-date, the IST-3 (n=3035), confirmed previous findings showing that benefit with treatment was greatest within 3 hours, but the analyses did not have sufficient power to define the shape of the relation between benefit and time beyond 3 hours.¹²⁴ The subsequent individual patient data meta-analysis by Emberson et al in the Lancet 2014 confirmed definitively that the effect of tPA is time dependent and that statistical certainty of an effect persists up to 5,1 hours, which is the time point at which the lower 95% CI for the treatment effect crosses 1,0 (Figure 21).¹²⁹

In a 2012 meta-analysis of risk factors for SICH following stroke treatment with IV tPA by Whiteley et al, the association of dichotomous OTT (early, 0-3 hours versus late, 3-4,5 hours) with SICH was both nominally weak, and did not reach statistical significance (OR 1,08; 95% CI 0,97-1,20; p=0,16).¹⁷⁰ This is consistent with the findings in the pooled RCT analysis by Lees et al in 2010. Here, large parenchymal haemorrhages (type 2, >30% of infarct size) also showed a slight rising gradient with increasing OTT, but despite relatively large numbers of patients (n=3531), the analysis was underpowered to show statistical significance.^{126,199} One potential confounder in both analyses is that patients with

more severe strokes tend to be treated earlier.^{121,193} This could potentially shift patients with higher risk of SICH due to large ischemic lesions (see 3.3.1.3) to the earlier treatment time window. With increasing numbers of patients treated between 3 and 4,5 hours after onset, the updated SITS-ISTR late time study by Ahmed et al in 2010 (n=23 942) confirmed that the limited hemorrhage risk increase previously suspected in the late time window is indeed real (adjusted OR 1,44 per the SITS-MOST definition and adjusted OR 1,27 per the ECASS II definition, both p=0,02).¹⁹³

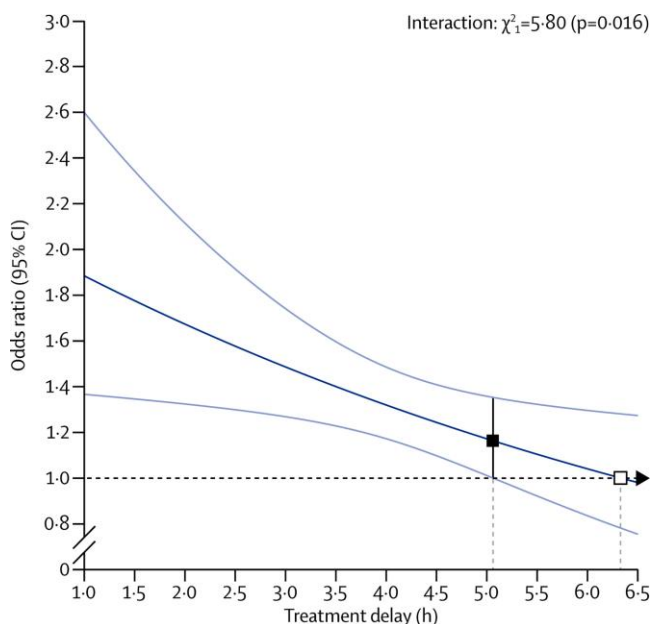


Figure 21. Effect of timing of alteplase treatment on good stroke outcome (mRS 0–1), adjusted for age and NIHSS. Solid line: the best linear fit between the odds ratio for mRS 0-1 in patients given alteplase vs controls. White box: point where the estimated treatment effect crosses 1. Black box: point where the lower 95% CI for the treatment effect crosses 1,0. From Emberson et al 2014.¹²⁹ Reproduced under the Creative Commons BY license.

In 2013 in Berlin, a Stroke Emergency Mobile Unit staffed by a stroke neurologist and equipped with a CT scanner and capability to administer IV tPA in the field was tested for its ability to shorten onset-to-treatment time (Figure 22).²⁰⁰ This succeeded, with 48% of studied stroke patients receiving treatment within 90 minutes from stroke onset, compared to 14% in the first SITS-ISTR study.¹²¹ However, this was a pilot feasibility study not powered for detection of effect on neurological outcomes. Therefore, it remains yet to be seen if prehospital thrombolysis, by reducing OTT, can improve patient outcomes and lower the risk of SICH following stroke thrombolysis.

1.4.10 Onset-to-door time

The time of stroke symptom onset was recorded as reported by patients or family members. “Door time” is the time point of patient arrival at the emergency department. The interval between these time points is calculated automatically by the database.

Stroke care begins in the pre-hospital phase. Current evidence of treatment benefit of IV tPA being limited (on a group level) to 4,5 hours from symptom onset means that delay at any phase up to infusion start is detrimental to the patient.¹²⁶ Onset-to-door time has been reported in large materials at 51 minutes

in the NINDS trial (n=624; 40 hospitals)²⁰¹, 68 minutes in the SITS-MOST study (n=6483; 285 hospitals)¹¹⁸ and 89 minutes in the Helsinki Stroke Thrombolysis Registry (n=1860; 1 hospital)²⁰².



Figure 22. Toward a faster delivery of thrombolytic therapy in stroke. Drs Tiago Moreira, Michael Mazya and Niaz Ahmed (left to right) on-board the Berlin Stroke Emergency Mobile Unit (STEMO). CT scanner in the background. Photo taken during the European Stroke Conference 2013 in London, UK.

The interval from symptom onset to first call for help is the main part of prehospital delay.²⁰³ Reasons for this include lack of awareness of stroke symptoms and recognition of their severity, but also denial of the disease and the hope that symptoms would resolve. In many cases contact is initially made by a family member.²⁰³ One approach to reducing pre-hospital delay has been educating the population to recognize stroke symptoms, and changing people's attitudes to acute stroke. Several studies have demonstrated beneficial effects on stroke recognition, delays and frequency of thrombolytic treatment using educational programmes directed at the public, paramedics and health professionals, using a pre-post design.^{204,205} However, there is evidence of a wearing off of this effect after the discontinuation of such programs.²⁰⁶ European and American guidelines suggest that public education should be maintained to sustain stroke awareness in the population.^{19,119} For further components and issues in prehospital acute stroke care, see Table 7.

Upon the initial contact with emergency medical services (EMS), making a possible stroke diagnosis is facilitated by standardized question algorithms and

test batteries such as the Face-Arm-Speech-Test.²⁰⁷ If the diagnosis is established, there is strong evidence that an ambulance unit should be dispatched to the patient with highest priority (same as trauma or suspected myocardial infarction) to shorten time to potential treatment. In 2012, Berglund et al published an RCT on whether elevating stroke to a level one emergency priority would improve patient access to acute care and whether this change would affect management of other life-threatening diseases, titled the Hyper Acute STroke Alarm (HASTA) Study. The intervention group (priority 1, immediate dispatch) reached hospital 26 minutes earlier than the control group (priority 2, within 30 minutes, more delay if another priority 1 call takes precedence) after the emergency call. IV tPA was given to 24% of the patients in the intervention group compared with 10% of the controls. Importantly, the higher priority level caused no negative effects on other critically ill patients needing priority 1 prehospital care.²⁰⁸

Links in the chain of recovery	Critical issues, possible solutions
Timely recognition of symptoms by patient or eyewitness and call for help.	<p>Patients fail to recognize symptoms as stroke - public education.</p> <p>Patients are alone and unable to call for help - alarm systems in high-risk individuals.</p>
Call center recognition of possible stroke symptoms and rapid ambulance dispatch.	<p>Failure to suspect stroke - protocols for identifying key words suggestive of stroke.</p> <p>Ambulances are dispatched at low priority - initiate code stroke with high priority.</p>
Initial evaluation and suspicion of stroke by ambulance personnel.	Failure to suspect stroke -training and protocols for rapid clinical evaluation of patients.
Transportation of patient to a hospital with acute stroke facilities and a rapid tPA protocol.	<p>Patient taken to a hospital without t-PA protocols, facilities, or personnel Consultation of receiving hospital ensures acute service availability.</p> <p>Patient taken to an unprepared emergency room - prenotification allows for ED to liberate resources and prepare for the patient's arrival.</p>

Table 7. Prehospital components of acute stroke care. From Meretoja and Kaste, Ann NY Acad Sci 2012.²⁰⁹ Permission obtained from John Wiley and Sons.

1.4.11 Door-to-imaging time

The door-to-imaging time is calculated as the difference between the time of first, diagnostic CT or MRI scan and the time of arrival at hospital. This time interval has mainly been reported as a component of the door-to-needle time in studies reporting successful single-centre protocols aiming to shorten total delays to treatment. Moving the patient from the ambulance stretcher immediately to the CT table, bypassing the emergency room bed, effectively cuts door-to-imaging time.^{202,210} This method was first employed in Sweden at the Norrland University Hospital in Umeå, contributing to bring door-to-needle time down to 27 minutes in 2011, the lowest in the country at the time.²¹¹ On a nation-wide level, data on over 125 000 patients from the USA Get With The Guidelines–Stroke program shows continuous improvement in this parameter, with rates of door-to-imaging time ≤ 25 minutes (per AHA/ASA guidelines) increasing from 33% in 2003 to 45% in 2009.²¹²

1.4.12 Door-to-needle time

Door-to-needle (DNT) time has become the prevalent term for the time interval between hospital arrival and initiation of IV tPA infusion. Whereas stroke physicians are rarely in a position to influence pre-hospital delays, the possibilities to study and optimise in-hospital processes are usually greater. Shortening the DNT is contingent on changing in-hospital infrastructure and logistic practices, as well as improving the flow of information from the pre-hospital to the in-hospital stage.²¹³ A large number of practices have been shown to shorten the DNT, such as:^{209,214}

- Telephone pre-notification of stroke physician on call by the ambulance staff
- Large-bore venous cannula inserted on-route in the ambulance into the antedecubital vein, if advanced imaging requiring contrast medium is used per local routine
- Electronic patient records read by stroke physician while ambulance is on-route and if possible, history taken from family members / witnesses by telephone prior to patient arrival in hospital
- CT scan requested based on pre-notification, prior to patient arrival
- Immediate availability of CT scanner and stroke team (stroke physician and nurse, radiologist, radiological and laboratory staff), waiting to receive the patient upon direct transfer onto the CT scanner from the ambulance
- Point-of-care coagulation parameter (International Normalised Ratio) and blood glucose testing
- Alteplase and infusion pump stored and prepared at the CT scanner, ready to initiate infusion immediately upon decision to treat

Using a simple stop-watch is an effective, low-cost way of successfully measuring component time intervals of the DNT (Dr Mia von Euler, personal communication, 2010). There are ongoing projects, such as the CLOQS trial, aiming to study the use of stop-watches formally in a multi-centre setting, with

the aim of increasing the proportion of stroke patients treated in accordance to best practice guidelines.²¹⁵

An ironic paradox has been observed by several authors, that the earlier a patient is admitted within the treatment time window, the longer the treating physician takes to initiate thrombolysis.^{193,216} This inverse correlation is mainly attributed to a psychological effect, with the physician feeling that there is no rush to treat, as there is ample time before the time window runs out. Being aware of this fact, implementing organizational improvements, as well as rigid documentation and reviewing of DTN times has been shown to eliminate this effect.²¹⁷

1.4.13 Hyperlipidemia

The presence of known hyperlipidemia is registered in the SITS-ISTR if the patient has a prior diagnosis of hyperlipidemia or hypercholesterolemia, regardless of subtype or treatment status.

Hyperlipidemia was reported in 35% of patients enrolled in the SITS-MOST study (n=6483).¹¹⁸ This data is consistent with a pooled analysis of 4012 patients from 11 large single-centre stroke thrombolysis registries, which reported a frequency of 38%.²¹⁸ Several papers reporting results of multivariate analyses have shown that hyperlipidemia does not have an independent influence on SICH, mortality and functional outcome in stroke thrombolysis.^{151,218,219}

1.4.14 Serum cholesterol

The SITS-ISTR allows registration of serum total cholesterol in milligrams. In 2012, the Lipid Profile in Thrombolysis Study Group (LPTSG) published a study of 1847 consecutive patients with detailed blood lipid profiles. They found that neither total cholesterol, LDL, HDL, nor triglycerides were independently associated with SICH per any definition.²²⁰ This is consistent with results from a the Japanese multicentre SAMURAI stroke thrombolysis registry (n=489)²²¹, but conflicts with a smaller single-centre case series showing an independent association of triglyceride levels (aOR 2,2 per mmol/L increase) with SICH per the NINDS definition, which however did not impact mortality or functional outcome.²²² These results are also at odds with the findings by the LPTSG study, which demonstrated that lower HDL and triglyceride levels were independently associated with mortality.

1.4.15 Statin

The SITS-ISTR allows registration of baseline statin use, however without specification as to which agent is used and at what dose. In 2008, data from the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) study evaluating atorvastatin 80 mg/day for secondary stroke prevention, showed an absolute risk reduction for recurrent stroke of 2% over 5 years and for any serious vascular event (including myocardial infarction) of 3,5% over 5 years.²²³ Together with similar findings from the Heart Protection Study (n >20000) published in 2004²²⁴, these results form the basis for current American,

European and Swedish recommendations on secondary stroke prevention, suggesting treatment for most patients with acute ischemic stroke.^{119,225,226}

The SPARCL study reported an increased risk of intracerebral hemorrhage in the active treatment arm (2,3% ICH with atorvastatin versus 1,4% with placebo). This was mainly noted in the subgroup of patients treated with high dose statin following an index primary intracerebral hemorrhage.²²⁷ A subsequent extensive meta-analysis of statin studies enrolling nearly a quarter of a million patients, published in late 2011, found no evidence of an association of statins with ICH. In particular, among 11 studies exclusively enrolling patients with cerebrovascular disease, SPARCL was the only one showing statistically significant increase in hemorrhage risk, while being third in size.²²⁸

A few months prior to the publication of the large meta-analysis discussed above, with a considerable debate on statins and ICH still ongoing, a small meta-analysis of three case series comprising 163 patients showed an increased risk of SICH following stroke thrombolysis in patients treated with statins (OR 2,0; 95% CI 1,0 – 3,8; p=0,04). This meta-analysis did not include patient level data, and thus multivariable risk factor adjustment was not performed.²²⁹ However, shortly thereafter, a much larger meta-analysis of 11 studies including 6438 patients found no association of baseline statin use with beneficial or detrimental effects in acute stroke thrombolysis, including SICH, functional outcome and mortality.²¹⁹ A year later, in 2013, the Get With The Guidelines – Stroke registry published confirmatory results from 22 216 stroke thrombolysis patients, reporting no independent association of lipid lowering medication on the risk of SICH.²³⁰

1.4.16 Diabetes mellitus

The presence of known diabetes mellitus is registered in the SITS-ISTR if the patient has a prior diagnosis of diabetes type 1 or 2, regardless of treatment status.

The frequency of diabetes in the SITS-ISTR has been reported previously at 17% (n=45079).¹⁶¹ This proportion lies between that reported from the Helsinki Stroke Thrombolysis Registry (14%, n=1398)²³¹ and in stroke thrombolysis patients in the US GWTG registry (25%, n=27928)²³⁰. For comparison, the frequency of diabetes mellitus among unselected ischemic stroke patients in Sweden is approximately 20%.^{15,232}

The large meta-analysis of risk factors for intracerebral hemorrhage in stroke thrombolysis by Whiteley et al included 12 studies reporting data on the influence of diabetes. The authors reported an unadjusted OR of 1,5 (95% CI 1,2-2,0) for SICH.¹⁷⁰ However, fewer studies have reported adjusted analyses demonstrating a history of diabetes to be an independent risk factor for SICH, the largest being the Multicentre rt-PA acute stroke survey (n=1205).²³³ Both the SITS-MOST multivariate analysis¹⁵¹, and the SITS-ISTR hyperglycemia and diabetes study from 2010 (n=16049)²³⁴ reported increased univariate odds ratios

for SICH in diabetic patients. However neither study showed the association to persist after adjustment for confounding factors. This was further confirmed in a large meta-analysis by Desilles et al in 2013, when the analysis of five studies reporting multivariate results was conducted both with and without the Ahmed glucose study.²³⁵ It would appear that acutely elevated blood glucose, rather than the presence of a previous diagnosis of diabetes, is the more important risk factor for thrombolysis-related SICH (see below in 1.4.17). Meanwhile, the Ahmed et al 2010 paper found that a history of diabetes was found to be an independent risk factor for mortality and lower likelihood of functional independence at three months, with an aOR for death of 1,3 and aOR for mRS 0-2 of 0,8 ($p<0,001$ and $p=0,005$ respectively).²³⁴ A finding of particular interest from this paper was that elevated blood glucose was a stronger driver of risk of SICH and mortality in patients without a known diagnosis of diabetes, compared to those with such a history. The reasons for this are as yet unclear.

1.4.17 Blood glucose

Levels of whole blood, serum or plasma glucose are entered into the SITS-ISTR as measured at baseline, i.e. on admission to hospital or in the pre-hospital phase (by point of care measurement in the ambulance). The unit of measure in the database is mg/dL. To convert this to the international standard using molar concentration, or mmol/L, the mg/dL value is divided by 18, the molecular weight of glucose being 180 g/mol.

Up to 60% of acute ischemic stroke (AIS) patients will experience some level of hyperglycemia, depending on its definition.²³⁶ Robust hyperglycemia after stroke is usually a short-lived phenomenon in patients without a history of diabetes, and often normalizes during the first 10 hours. However, around one in four of the non-diabetic patients enters a second, later hyperglycemic phase 2-3 days post-stroke.²³⁷ The negative effects of hyperglycaemia in ischaemic stroke animal models are due to a pro-oxidative, pro-inflammatory, and pro-coagulant state induced by the elevated blood glucose, along with increased BBB permeability and disruption by elevated matrix metalloproteinase activity.^{238,239} Hyperglycemia in the acute stroke phase has been shown in a large number of clinical studies to be an independent predictor of poor functional outcome and death.^{235,236} The large analysis of SITS-ISTR data by Ahmed et al in 2010 used both a continuous (receiver operating characteristic (ROC) curve analysis) and a stratified method of examining glucose levels. By both analyses, a detrimental effect of increasing baseline blood glucose values on long-term outcome was seen already above the 7 mmol/L level.²³⁴ This may in part also be mediated by detrimental effects of hyperglycemia on arterial recanalization in IV stroke thrombolysis.^{240,241} One potential mechanism behind this may be that hyperinsulinemia decreases fibrinolytic activity by increasing the production of plasminogen activator inhibitor 1 (PAI-1).²⁴²

Baseline hyperglycemia has been shown to be an important risk factor for SICH. Ahmed et al reported that the largest risk increase for SICH among SITS-ISTR patients occurred at glucose levels above 10 mmol/L, showing an adjusted OR

for SICH per SITS-MOST as high as 2,9 (95% 1,7-4,8, $p < 0,001$).²³⁴ Both meta-analyses by Whiteley 2012 (univariate OR)¹⁷⁰ and Desilles 2013 (multivariate OR)²³⁵ showed an increase in OR for SICH of 1,1 per mmol/L increase of baseline blood glucose. The elevated hemorrhage risk is reflected by the fact that six out of seven prognostic scores for SICH following stroke thrombolysis have included blood glucose as a parameter.^{171,243}

1.4.18 Smoking

Tobacco smoking is reported in the SITS-ISTR as two variables: currently smoking, or currently non-smoking but with a history of previous smoking.

In the SITS-MOST multivariate analysis study, current smoking was related to lower incidence of SICH per the NINDS definition, a result that had previously been observed in the National Institute of Neurological Diseases and Stroke (NINDS) study.¹⁵¹ A meta-analysis of unadjusted data from 13 studies showed current smoking to confer an OR of 0,7 for SICH by any definition.¹⁷⁰ Of further interest, a study by Kufner et al of 148 consecutive stroke thrombolysis patients showed current smoking to be an independent predictor of arterial recanalization and reperfusion, indicating that thrombolytic therapy acts more effectively in smokers.²⁴⁴ However, no study, including that by Kufner et al, has yet reported a statistically significant effect of smoking on long-term mortality or functional independence following adjustment age and other confounding factors. This is particularly important as smoking stroke thrombolysis patients are considerably younger than non-smoking.

The ICH-protective and recanalization-promoting effect of smoking in stroke patients, dubbed “the smoker’s paradox”, is likely a reflection of a phenomenon first observed in large trials of thrombolysis for myocardial infarction. There, current smoking was associated with lowered mortality²⁴⁵, explained by improved myocardial perfusion on imaging following thrombolysis in smokers, observed even after adjustment for age and other comorbidities.²⁴⁶ Although speculative, Ovbiagele and Saver proposed in 2006 the following explanation for this phenomenon in stroke thrombolysis: “The pathogenesis of vascular occlusions may be more thrombogenic than atherogenic in smokers compared to nonsmokers. In the latter occlusion may be more frequently due to rupture or ulceration of atheromatous plaque with formation of platelet-rich clot, on which thrombolysis has little effect. This may reflect a greater susceptibility of cerebral thrombi to fibrinolysis in smokers.”²⁴⁷ A potential explanation for the protective effect against ICH in stroke thrombolysis could be that smoking, by increasing exposure to reactive oxygen species, “preconditions” the endothelium, reducing its susceptibility to ischemia and reperfusion damage.²⁴⁸

1.4.19 Atrial fibrillation

Atrial fibrillation (AF) is the most common cardiac arrhythmia, affecting 1% of the general population, with a lifetime risk for development of 1 in 4 when ≥ 40 years of age.^{249,250} AF is an independent risk factor for stroke, increasing its incidence nearly 5-fold.²⁵¹ The prevalence of AF in unselected stroke patients

increases sharply with age, from 3% in patients aged <50 years to 39% in those aged >90 years (Table 8).²⁵²

Age	< 50 years	50-59 y	60-69 y	70-79 y	80-89 y	>90 y	All
% with AF	3,2%	5,5%	10,7%	20,7%	31,8%	39,3%	19,7%

Table 8. Frequency of atrial fibrillation among a total of 502 036 stroke patients in the US GWTG Stroke Registry.²⁵²

AF is present in >60% of all cardioembolic strokes and in 40% of all strokes in patients with two or more concurrent possible etiologies.²⁵³ The severity of cardioembolic stroke is higher than in other stroke etiologies, median NIHSS score of 8 (n=1286) versus 5 (n=1047) for large vessel disease and 3 (n=1028) for small vessel disease in a German multi-centre stroke registry.²⁵³ This is confirmed by stroke trial data collated VISTA database. This material is comprised of patients with a substantially higher overall NIHSS score, but still a clear separation between median NIHSS score 14 for AF-related stroke (n=1631) versus 12 for non AF-related stroke (n=5460, p<0,001).²⁵⁴

Age and stroke severity are well-established as having the strongest influence on crude rates of functional outcomes and as shown above, AF co-varies in a major way with both factors. This underlines the importance of adjusting for these, and other important confounders, which was not done in several previous studies.²⁵⁵⁻²⁵⁷ Employing more robust statistical methodology on large samples, neither the VISTA AF study by Frank et al²⁵⁴ nor the SITS-MOST multivariate analysis¹⁵¹ have found an independent effect of AF on functional outcome or mortality.

The influence of atrial fibrillation on post-thrombolytic cerebral hemorrhage is controversial. In review articles of risk factor for this complication, Lansberg et al from 2007²⁵⁸ mention the Multicenter rt-PA Acute Stroke Survey²³³ and the MAST-E study²⁵⁹ where AF was associated with SICH in univariate, but not multivariate analysis; Derex and Nighoghossian from 2008²⁶⁰ make no mention of AF at all; Weiser et al from 2013²⁶¹, list AF as a hemorrhagic risk factor, but cite only the iScore in support of this statement. However, the iScore is a predictive instrument for various outcomes in stroke thrombolysis, comprised of independent risk factors for *mortality* but not for SICH^{262,263}. The review by Jickling et al from 2013²⁴³ also erroneously cites the iScore in support of AF as a SICH risk factor, however they also cite the Whiteley et al 2012 univariate meta-analysis, which found an unadjusted OR of 1,9 (95% CI 1,5-2,3; p<0,001) for SICH.¹⁷⁰ We shall examine these results below. Lastly, a recent review by Álvarez-Sabín in 2013 refers to AF as a “strong risk factor” for SICH and parenchymal hemorrhage.²⁶⁴ To substantiate this, the paper cites analyses of data from the NINDS¹⁸⁹ and the ECASS²⁶⁵ trials, where AF was in fact not found to be an independent risk factor in multivariate analysis. Furthermore, the authors cite an observational study by Paciaroni et al in 2008, which recruited 65 thrombolysed stroke patients and analysed data after pooling these with 1060 non-thrombolysed patients. This paper reported AF as a risk factor for

parenchymal hemorrhage following multivariate adjustment.²⁶⁶ However, the low number of IV tPA patients, as well as the pooling procedure (no sub-group analysis was reported) makes conclusions on any interaction between AF, IV tPA and stroke impossible. Lastly, Álvarez-Sabín et al also cite the Whiteley meta-analysis.

A closer look at the 12 included studies in the AF sub-analysis in Whiteley et al, reveals that 8 of them report multivariate adjustment for confounders. Three of these found AF to be an independent risk factor for some type of cerebral hemorrhage following stroke thrombolysis. The largest, being the SITS-MOST multivariate analysis from 2008, reports an adjusted OR of 1,4 (95% CI 1,1-1,7) for SICH by NINDS. However, AF was not found to be a risk factor for the stricter SICH by SITS-MOST definition.¹⁵¹ The second study, by Tanne et al from 2002, used a multivariate model incorporating age, sex, hypertension, diabetes mellitus, atrial fibrillation, cigarette smoking, other cardiac disease, use of aspirin, other antiplatelets, baseline NIHSS score, and mean blood pressure. This resulted in an adjusted OR of 1,7 (95% CI 1,6-4,0) for any ICH, but no relation with symptomatic ICH. Moreover, when the authors added laboratory and radiological parameters (i.e serum glucose, platelet count, and early CT changes >33% and >33% of MCA), the independent association of AF with ICH disappeared.²³³ The last study is an analysis of EPITHET trial data, comprising 49 treated with IV tPA and 48 with placebo.²⁶⁷ All analyses were performed after pooling the two groups. In an unusual finding, the authors report that 79% of all patients with parenchymal hemorrhage (n=11 tPA and n=4 placebo) had atrial fibrillation. A multivariate analysis showed AF having an adjusted OR as high as 7,6 (95% CI 1,8 to 33,1) for PH. However, the same analysis did not show an independent association with hemorrhage for the omni-present risk factors NIHSS, blood glucose or age, which suggests that the patient material was neither representative of common clinical practice, nor large enough for multivariate logistic regression analysis to be meaningful.

1.4.20 Congestive heart failure

The presence of congestive heart failure (CHF) at baseline is registered in the SITS-ISTR based on self-reported history or existing patient medical records. It is not subject to severity classification per the NYHA system, neither is it possible to specify the left ventricular ejection fraction, the end-diastolic left ventricular diameter or other measures indicative of systolic or diastolic dysfunction.

Heart failure and atrial fibrillation frequently co-exist in the same population. Of those with a diagnosis of AF, about 40% will also develop a diagnosis of heart failure, the reverse being true as well.²⁶⁸ Estimates suggest that baseline annual stroke risk increases with severity of NYHA functional class: 0,5 % for mild, 1,5 % for moderate and up to 4% for severe heart failure.²⁶⁹ Moreover, in patients with AF, concomitant heart failure increases the risk of cerebral cardio-embolism, as reported by the designers of the widely used AF stroke prediction instruments CHADS₂ and CHA₂DS₂-VASC.^{270,271}

The SITS-MOST multivariate analysis¹⁵¹, as well as a smaller (n=130) study conducted in Florence, Italy by Palumbo et al²⁷², have shown that CHF is an independent predictor of 3 month mortality in stroke thrombolysis patients. Neither study showed an influence of heart failure in general on disability, however the more detailed Palumbo study found an independent association of CHF with NYHA class III to IV with 3 month disability. This is to be expected, as these severity grades confer a degree of disability in and of themselves. Class III is defined as “Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea”, while class IV is defined as “Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.”

In the univariate meta-analysis of SICH risk factors by Whiteley et al from 2012, CHF is reported to be associated with SICH, OR 2,0 (95% CI 1,3-2,9; p<0,001).¹⁷⁰ However, this number is derived from reports of unadjusted analyses, with a high degree of covariance with other hemorrhagic risk factors. No study has hitherto demonstrated CHF to be an independent predictor of SICH.

1.4.21 Previous stroke

A history of previous stroke, if present, is entered into the SITS-ISTR as one of two categories: (1) having occurred within the last three months, or (2) having occurred further back in time. The exact time point, initial severity or other data on the previous stroke are not registered.

Previous stroke within 3 months is a contraindication to acute stroke treatment with IV tPA according to the US FDA²⁷³⁻²⁷⁵ and EU EMEA¹³⁰ tPA licenses and the AHA/ASA guidelines.¹⁹ This follows from the exclusion criteria in the NINDS¹¹² and ECASS¹¹⁴ trials. Professor James Grotta, a leading NINDS trial investigator, recounted the deliberations in the design phase of the trial in a personal communication to Dr Nishant Mishra, as published in the latter's doctoral thesis²⁷⁶: “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...” However, the exact origin of the 3 month cut-off mark is unclear, as RCTs of myocardial infarction thrombolysis (using streptokinase, urokinase, tPA, and tenecteplase) used various exclusion criteria: “any previous stroke” in the ISAM²⁷⁷, AIMS²⁷⁸, GUSTO²⁷⁹, EMERAS²⁸⁰, LATE²⁸¹, ISIS-2²⁸² and all tenecteplase trials²⁸³, “within 6 months” in the ASSET²⁸⁴ and GISSI-2²⁸⁵ trials, and “within 2 months” in the GISSI²⁸⁶ and USIM²⁸⁷ trials.

During the course of patient enrolment into the NINDS trial between 1991 and 1994, 1,3% of all screened patients (219/17367) were excluded from randomization due to recent prior stroke.²⁷³ From this can be concluded that this particular contraindication has no major impact on patient enrolment. Subsequent stroke thrombolysis RCTs have used different cut-off time points:

ATLANTIS¹¹⁶ excluded patients with prior stroke within 6 weeks, IST-3 employed a much shorter 14 day period²⁸⁸ and the ECASS II did not exclude patients with prior stroke at all¹¹³. Neither these, nor the myocardial infarction trials cited above have published explanations for their choice of time points for this exclusion criterion, other than a presumed, unquantified, increase in hemorrhage risk.

In 2007, an interesting study was published in the Journal of Neurology, Neurosurgery and Psychiatry, querying a group of international stroke thrombolysis experts (n=30) on their perceptions of the clinical contraindications to thrombolytic treatment. In particular, the paper reports consensus at 1,5 months for the cut-off point for previous stroke, which was perceived not to compromise the effectiveness and safety of treatment.²⁸⁹

Previous stroke in general has been mentioned by the regulatory authorities as potentially conferring a reduction in the therapeutic benefit of stroke thrombolysis. However, the benefit / risk ratio was still considered as positive in these patients.^{130,273} Subsequently, Mishra et al demonstrated equal benefit of IV tPA in stroke patients with and without previous stroke in the VISTA and SITS-ISTR registries, with adjusted OR for any benefit (at least one step improvement in mRS) of IV tPA of 1,5.^{290,291} Moreover, the larger of these two studies (Mishra et al, Neurology 2011, n=29500) demonstrated that the European (EMA) contraindication for stroke thrombolysis in patients with concomitant history of previous stroke and diabetes¹³⁰ is unfounded.

The SITS-MOST multivariate analysis authors reported previous stroke (without the recent/non-recent subdivision) to be an independent risk factor for ICH per SITS-MOST, with an aOR of 1,7 (95% CI 1,0-2,9). This is in line with findings from a large American observational registry of thrombolysis in myocardial infarction, where an analysis of 31732 patients yielded a similar adjusted OR for intracerebral hemorrhage of 1,5 (95% CI 1,1-2,0, p=0,001). However, the unadjusted meta-analysis by Whiteley et al, including 8 studies reporting data on previous stroke and risk of ICH in stroke thrombolysis, did not find a risk increase, with an OR for ICH of 0,8 (95% CI 0,4-1,9, p=0,67), although with a high degree of heterogeneity between studies (I²=68%).¹⁷⁰

1.4.22 Pre-existing disability (baseline mRS)

The SITS-ISTR has an option for registering the baseline functional status prior to the onset of the currently treated stroke, employing the modified Rankin scale. The mRS grading is done according to the judgement of the treating physician, and any certification for use of the scale cannot be ascertained. Stroke thrombolysis despite pre-existing disability has not been the subject of much study. The ECASS trial expressly excluded patients with “pre-existing disabling neurological disease”.¹¹⁴ More specifically, in IST-3, pre-existing dependence in activities of daily living was listed as an exclusion criterion.²⁸⁸ The other stroke IV tPA trials do not clearly state exclusion of patients with prior disability,

however it seems likely that such patients generally would not be considered for trial enrolment even at the screening phase.

1.4.23 Aspirin

Ongoing medication with oral aspirin at baseline is a binary variable in the SITS-ISTR, without any option to register the current daily dose.

Following stroke or TIA, secondary prevention with oral aspirin (acetylsalicylic acid, ASA) reduces the risk of a new stroke by 15% regardless of dose, in the range of 50-1500 mg per day.²⁹² Due to the fact that mild gastrointestinal side effects (but not major bleeding events) of aspirin are dose-dependent and influence patient adherence to therapy,²⁹³ the lower dose interval is employed in many countries including Sweden. The NNT in order to prevent early death or non-fatal stroke (within 2-4 weeks) is 100-150 as shown in 1997 in the large IST and CAST trials,^{294,295} while the NNT to prevent death or dependency at 6 months may be as low as 70, as reported in the IST trial.

For IV tPA patients it is important to note that aspirin should be started 24 hours after alteplase, to avoid an excess SICH risk present when aspirin is instituted right after the end of the tPA infusion. This was demonstrated by the ARTIS RCT in 2012 (n=642), with SICH rates of 4,3% in the ultra-early aspirin arm versus 1,6% in the aspirin at 24 hours arm (p=0,04).²⁹⁶

In 2010, Diedler et al published an analysis of 11 865 patients in the SITS-ISTR, of which 3782 patients received one or two antiplatelet drugs at baseline, the majority (n=3016, 25% of total) were taking aspirin.²⁹⁷ This analysis did not show aspirin monotherapy to be an independent predictor of SICH by any definition, nor was it independently associated with mortality or functional outcome. Notably, patients taking aspirin were five years older and had a lot more comorbidities than the aspirin naïve: with 50% more hypertension and diabetes, around twice the frequency of hyperlipidemia and atrial fibrillation and three times the frequency of previous stroke and congestive heart failure. The authors concluded that aspirin in and of itself did not influence the safety and efficacy of stroke thrombolysis. This was corroborated by the IST-3 trial, where aspirin did not influence outcomes.¹²⁴ An analysis of VISTA data by Frank et al in 2013 also showed a significant benefit of IV tPA in patients treated with a single antiplatelet agent, the majority on aspirin.¹⁸⁸ The baseline imbalances between patients with and without baseline aspirin likely explain most of the increase in the unadjusted OR for ICH (2,1; 95% CI 1,5-3,0) reported in the Whiteley meta-analysis from 2012, which furthermore did not include the very large Diedler 2010, IST-3 or the VISTA materials.¹⁷⁰

1.4.24 Dipyridamole

Ongoing medication with oral dipyridamole at baseline is a binary variable in the SITS-ISTR, without any option to register the current daily dose, and whether the formula was for immediate or extended release.

Two large and several smaller RCTs of dipyridamole + aspirin (DP+ASA) versus aspirin alone have been published. The 1996 ESPS-2 and the 2006 ESPRIT trials (n=3299 and n=2739 respectively) showed that the combination was around 20% more effective at preventing recurrent stroke.^{298,299} Later Cochrane meta-analyses have confirmed this finding, importantly demonstrating no increase in intracerebral hemorrhage or myocardial infarction in the DP+ASA group.³⁰⁰ However, the combination is not superior to aspirin alone in preventing vascular death and total mortality.^{300,301}

Baseline treatment with dipyridamole + aspirin prior to IV tPA administration has not been shown to differ from aspirin alone regarding the risk of SICH.²⁹⁷

1.4.25 Clopidogrel

Ongoing medication with oral clopidogrel at baseline is a binary variable in the SITS-ISTR, without any option to register the current daily dose.

No secondary stroke prevention studies have compared clopidogrel with placebo. The CAPRIE trial³⁰² of clopidogrel versus aspirin and the PROFESS trial³⁰³ comparing it with DP+ASA have not clearly established any superiority. However, clopidogrel appears to be better tolerated than the aspirin + dipyridamole, with fewer adverse events leading to drug discontinuation (11% versus 16% in the PROFESS study).³⁰³

Secondary stroke prevention using a combination of clopidogrel + aspirin (CLP+ASA) has been compared with clopidogrel alone in the MATCH trial, with no proven difference in benefit and an excess of life-threatening hemorrhage in the combination group.³⁰⁴ Neither was the combination found to be more effective than aspirin alone in the CHARISMA trial, this study confirming the increased bleeding risk for CLP+ASA.³⁰⁵ Current EU and US guidelines put clopidogrel and combined DP+ASA on par with each other, both being regarded as first-line options for secondary stroke prevention.^{119,225}

Clopidogrel alone has not been shown in the SITS-ISTR material to independently increase the risk for SICH in stroke thrombolysis.¹⁵¹ However, Diedler et al showed an important finding in patients with combined CLP+ASA at baseline. With triple to quadruple rates of SICH per various definitions compared to antiplatelet-naïve patients, CLP+ASA was the only antiplatelet regiment to be associated with SICH after multivariate adjustment for confounders.²⁹⁷ This finding is in line with results from the SAINT I and II trials, which also showed this double antiplatelet therapy to be an independent risk factor for SICH.³⁰⁶

1.4.26 Other antiplatelets

Baseline treatment with antiplatelet agents other than aspirin, clopidogrel and dipyridamole is possible to register in the SITS-ISTR, however without specification of which particular agent was used. For this reason, patients may have been on ticagrelor, prasugrel, triflusal, cilostazol, or any other.

On a group level, in the SITS-ISTR, patients taking an “other” antiplatelet drug, have been shown to be about as common as those taking clopidogrel, CLP+ASA or DP+ASA, comprising around 1-2% of the database population. “Other” antiplatelets were not shown to independently predict SICH, functional outcome or mortality in the Diedler et al paper.²⁹⁷

1.4.27 Oral anticoagulants (warfarin)

Until 2008, the SITS-ISTR had three options for reporting baseline warfarin treatment: Yes, No and Unknown. In 2008, a fourth option was implemented, “warfarin with baseline INR $\leq 1,7$ ”. In 2011, the warfarin options became “INR $\leq 1,7$ ”, “INR $> 1,7$ ”, and “INR unspecified (n/a)”.

Warfarin for stroke prevention has been shown to confer an overall relative risk reduction of $>60\%$, with an annual NNT=37 in primary prevention and annual NNT=12 in secondary prevention.³⁰⁷ However, warfarin increases the annual risk for intracerebral hemorrhage by around 0,2% to 0,3-0,5%.³⁰⁸ In the recent trials of novel oral anticoagulants (dabigatran, rivaroxaban, apixaban and edoxaban) versus warfarin, the warfarin trial arms saw a frequency of intracranial hemorrhage of 0,5-0,7%.³⁰⁹⁻³¹²

The US FDA Alteplase license has the following wording in its list of contraindications: “Current use of oral anticoagulants (e.g., warfarin sodium) or an International Normalized Ratio (INR) > 1.7 or a prothrombin time (PT) > 15 seconds”.²⁷⁴ Literally, the first “or” in this sentence should mean that stroke IV thrombolysis is contraindicated in patients currently using warfarin, regardless of INR. The same document further states: “In patients without recent use of oral anticoagulants or heparin, Activase treatment can be initiated prior to the availability of coagulation study results. However, infusion should be discontinued if either a pre-treatment International Normalized Ratio (INR) $> 1,7$ or a prothrombin time (PT) > 15 seconds or an elevated activated partial thromboplastin time (aPTT) is identified”. The issue is clarified by the current AHA/ASA guidelines, which accept IV tPA treatment within 3 hours (but not in the 3-4,5 hour time window) for patients on warfarin, with an international normalized ratio (INR) $\leq 1,7$.¹⁹ The European Stroke Organization provides no guidelines on this matter.¹¹⁹ Meanwhile, the European license for Alteplase precludes its use in patients treated with oral anticoagulants altogether.¹³⁰

In 2012, Xian et al published results from the Get With The Guidelines-Stroke Registry, showing no increase in SICH among 1802 warfarin treated patients with INR $\leq 1,7$, after adjustment for confounding factors.³¹³ Prior to this, several single-centre and two multi-centre observational studies had been published, with numbers of warfarin treated patients at INR $\leq 1,7$ ranging between 9 and 125, showing widely divergent SICH rates (0-36%). Two meta-analyses of studies totalling 284 and 240 warfarin treated patients reported increased SICH risk, however without adjustment for confounders.^{314,315} An analysis of controlled VISTA data suggested that functional outcomes were improved by IV tPA

among 157 patients taking oral anticoagulation with $\text{INR} \leq 1,7$: unadjusted OR for improved mRS at 3 months was 2,2 (95% CI 1,1-4,3), $p=0,02$.¹⁸⁸

At INR values over 1,7, even less is known about stroke thrombolysis in warfarin treated patients. The STARS trialists reported ten patients with $\text{INR} > 1,7$, of whom none developed ICH.²¹⁶ Brunner et al showed no SICH in eight such patients in a single-centre material.³¹⁶ Among 33 patients with $\text{INR} > 1,7$ reported by Xian et al, there was one occurrence of SICH.³¹³ The VISTA analysis, based on 14 treated $\text{INR} > 1,7$ patients (no occurrence of SICH) and 134 non-thrombolysed controls offered a reassuring, however non-significant odds ratio for benefit of tPA regarding functional outcome: 1,5 (95% CI 0,6-4,2), $p=0,78$.¹⁸⁸

The reader is kindly referred to Study III and Chapters 3 to 5 of the present thesis for our own results on stroke thrombolysis in warfarin treated patients.

1.4.28 CT early infarct signs

Computed tomography (CT) of the head was the main baseline imaging requirement in the SITS-ISTR. Other imaging studies, including CT angiography, MRI and MR angiography, and transcranial Doppler ultrasound, were optional. Admission CT scans were evaluated for the presence of early signs of infarction and the HMCAS. Follow-up CT scans were performed within 22-36 hours of IV tPA initiation (or earlier if clinically indicated) and evaluated for the presence of intracerebral haemorrhage, infarct signs, infarct swelling and HMCAS. Other imaging modalities on follow-up were also optional, as well as repeated imaging after the mandatory 22-36 hour scan.

Non contrast CT scan can identify early cerebral infarct changes, such as focal hypoattenuation, insular ribbon sign, loss of clarity of the lentiform nucleus and loss of precise delineation of the grey-white interface of the basal ganglia, cortical sulcal effacement etc.³¹⁷ Early ischemic CT findings are subtle in the first 3 hours of stroke onset and have a sensitivity of only 25% compared to DWI MRI.³¹⁸ However, there is considerable variability in the interpretation of early CT findings, depending among other factors on the experience and medical specialty of the reader.³¹⁹

The presence of any early infarct signs on CT has been shown to be associated with long-term functional outcome and mortality.^{151,317} The influence of any early infarct signs, if their extent is not specified, on thrombolysis-related SICH, is controversial.^{320,321} Several studies beginning in the 1990s and up until 2002 reported such signs to be independent predictors of increased SICH risk. However, the scans during this early period likely picked up only the most severe manifest infarcts, which may explain why in later years, only singular studies report any infarct signs as a risk factor for SICH.¹⁷⁰ Notably, the latest (and largest) stroke thrombolysis RCT, the IST-3, did not find the presence of such imaging findings to be associated with reduced benefit of IV tPA in adjusted analysis.¹²⁴ The SITS-MOST multivariate analysis showed the presence

of any early ischemic signs to be independently associated with SICH per NINDS (aOR 1,5 (95% CI 1,2-1,9), but not with SICH per SITS-MOST.¹⁵¹

If quantified binarily by assessing whether early ischemic changes comprise more or less than one third of the MCA territory (for anterior circulation stroke), the picture becomes clearer. Such major early changes have been shown in several studies to be associated with SICH and large parenchymal hematomas after stroke thrombolysis.²⁶⁰

Both the US and EU labels for Alteplase issue warnings concerning stroke thrombolysis in patients with “major early infarct signs” specified as “substantial edema, mass effect, or midline shift” (US) and stating that “patients with extensive infarctions are at greater risk of poor outcome including severe haemorrhage and death” (EU) Neither however contraindicates treatment in such patients, both recommend that the risks should be weighed against potential benefits.^{130,274}

1.4.29 CT hyperdense cerebral artery sign

The term “hyperdense cerebral artery sign” (HCAS) in the SITS-ISTR implies, but is not limited to the hyperdense middle cerebral artery sign, corresponding to an occlusion of the M1 segment of the MCA.

Dense artery signs are manifestations of cerebral artery thrombosis on non-contrast CT and have been observed in all large intracranial arteries, *ie* the ICA, ACA, MCA, PCA and BA.³²²⁻³²⁶ The most commonly reported variant is the hyperdense middle cerebral artery sign (HMCAS). It is defined as density above 45 Hounsfield units of the proximal MCA, higher than that of any other visible artery or vein. CT criteria for interpretation include spontaneous visibility of the horizontal part of the MCA, attenuation of the MCA higher than that of surrounding tissue, disappearance of higher attenuation with bone window settings, and unilaterality.³²⁷ The sensitivity of the sign for MCA occlusion is 27-54%, but specificity approaches 100%.³²⁸⁻³³¹ The HMCAS is strongly associated with large MCA infarctions (>1/3 of the territory).³²⁶ According to findings from the SITS-ISTR by Kharitonova et al, the frequency of baseline HCAS is around 20%. Patients with hyperdense artery sign have a median NIHSS of 17 (versus 11 in the absence of the sign), almost double the mortality (23% vs 13%) and around half the frequency of functional independence at 3 months (31% vs 56%).³³² The same paper found that the sign is independently associated with SICH per NINDS (but not per SITS-MOST), as well as with mortality and lower chances of independence. Conversely, the disappearance of the HCAS on follow-up CT at 22-36 hours may be used as a surrogate marker for arterial recanalization, as shown in another SITS-ISTR paper by Kharitonova et al from 2009, being associated with early neurological improvement, good functional outcome and reduced mortality.³³³ Subsequent research has shown that with thin slice CT image reconstruction, it is possible to very closely match HMCAS and CTA measurements of thrombus size.³³⁴ The extent of the thrombus (*ie* the

HMCAS) has also been shown to be strongly associated with the odds of arterial recanalization following treatment with IV tPA (Figure 23).³³⁵

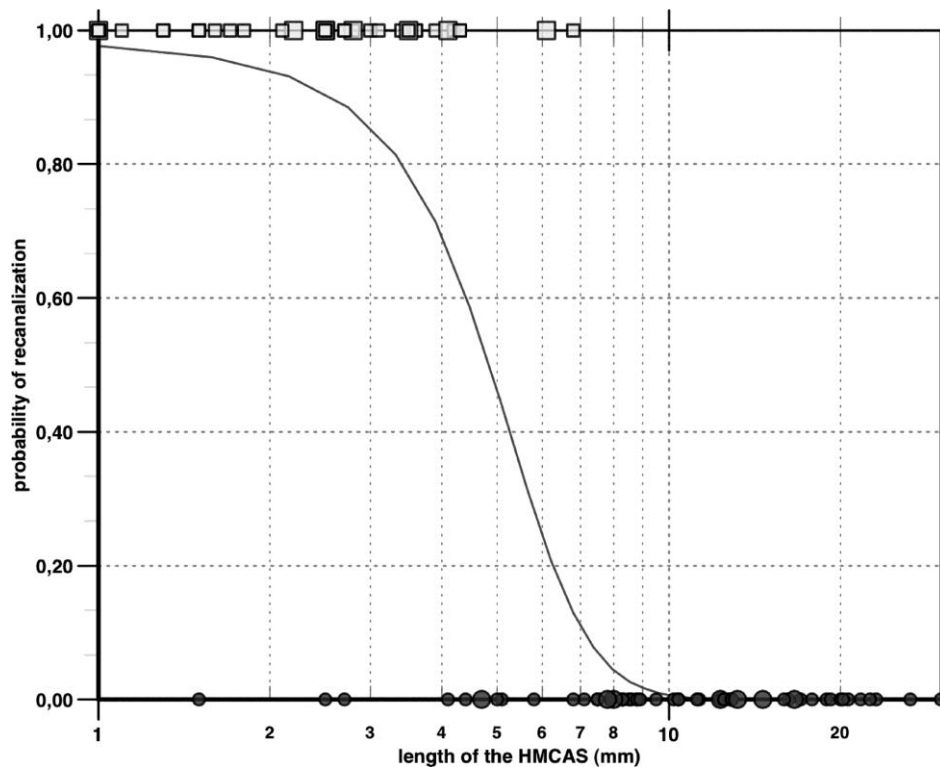


Figure 23. Logistic regression curve representing an estimate of the probability for successful recanalization of occluded vessels by intravenous thrombolysis (IVT) depending on thrombus length. From Riedel et al, 2011.³³⁵ With permission from Wolters Kluwer Health.

1.4.30 CTA / MRA occlusion

CTA and MRA occlusion can be registered in the SITS-ISTR at baseline and at 22-36 hour follow-up. Which vessel and segment is occluded is not specified. The diagnostic precision of CTA for arterial stenosis and occlusion has been shown to be a near perfect (99-100%) match that of digital subtraction angiography (DSA).^{336,337} CTA has been shown to be more diagnostically accurate than non-contrast time-of-flight (TOF) MRA, particularly for second-order intracranial arteries.³³⁷

Arterial recanalization visualised by repeated CTA or MRA at 22-36 hours has been shown to be an important prognostic marker of good functional outcome and mortality after stroke. This is seen in particular in patients with early clinical improvement, but also in patients in whom early neurological improvement does not occur (Figure 24).¹⁰³

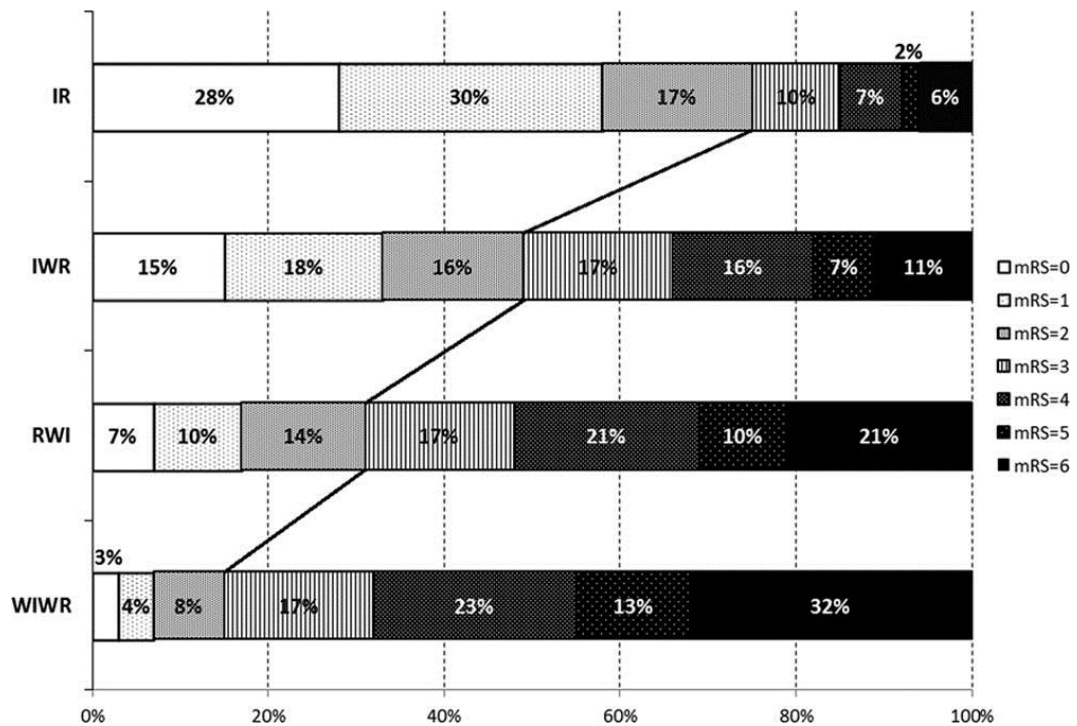


Figure 24. Three-month functional outcome (modified Rankin scale [mRS]) of patients with and without neurological improvement by 20% from baseline at 2 hours post-treatment (n=5324). IR indicates improvement with recanalization, improved at 2 hours, recanalized at 22–36 hours; IWR, improvement without recanalization, improved at 2 hours, occlusion at 22–36 hours; RWI, recanalization without improvement, not improved at 2 hours, recanalized at 22–36 hours; and WIWR, without improvement and without recanalization, not improved at 2 hours, occlusion at 22–36 hours. From Kharitonova et al, 2013.¹⁰³ With permission from Wolters Kluwer Health.

1.4.31 Advanced Magnetic Resonance techniques

Details of advanced MR imaging such as parameters of diffusion and perfusion sequences, measurements of cerebral blood volume, and microhemorrhages or superficial siderosis are as yet not possible to register in the SITS-ISTR. However, they are of interest for the topic of this thesis and will thus be discussed in brief.

Three imaging parameters have been associated with post-thrombolysis brain hemorrhage: diffusion lesion volume,³³⁸ very low cerebral blood volume (VLCBV),^{339,340} and volume of severely delayed blood flow indicated by time to maximum (Tmax)>8 and even >14seconds.^{339,341} VLCBV and volume of tissue with Tmax >8 seconds both reflect poor collateral blood flow with intense ischemia. Diffusion-weighted MRI lesions reflect areas of severe ischemic damage with disturbed energy metabolism.³⁴² A large study from 2008 of 536 thrombolysed patients showed a significant increase of SICH risk with increasing pre-treatment DWI lesion size, from about 2% in patients with very small lesions to about 12-16% in patients with large DWI lesions involving more than one-

third of the MCA territory (>100ml).³³⁸ The results were all the more robust as the authors reported them for several SICH definitions.

In 2013, Campbell and colleagues compared the predictive capability of the above three parameters for the development of parenchymal hematoma. VLCBV was defined, as in previous studies, as CBV below the 2,5th percentile of the CBV of the contralesional hemisphere, in a tissue volume >2 ml. The authors found that PH was very strongly associated with the restoration of blood flow into an area where VLCBV was detected, occurring in 44% of successfully recanalized patients with this finding. VLCBV had a sensitivity for PH of 96% and a specificity of 66%, with an AUC-ROC of 0,81 (see chapter 3.4). Importantly, VLCBV had the best performance in logistic regression modelling than other examined MRI parameters.³³⁹ However, subsequent work by Mishra et al showed that the optimal VLCBV threshold predicting PH is <30% of the contralateral CBV in a tissue volume >1,7 ml.³⁴³ It is encouraging that the sufficient tissue volumes have been found being nearly the same in these studies, however, the difference in optimally performing CBV threshold level is noteworthy and will doubtless be subject to further validation studies.

Cerebral microbleeds (CMBs) are small perivascular hemorrhages, appearing as well demarcated, rounded lesions with low signal intensity on MRI sequences sensitive to magnetic susceptibility. They are a marker of microangiopathy (*i.e.* hypertensive or cerebral amyloid angiopathy (CAA)).³⁴⁴ It has been proposed that CMBs could help to identify patients at high risk of ICH following IV thrombolysis and endovascular stroke therapy.³⁴⁵ Several studies have been performed, giving conflicting results regarding the possible risk of ICH after thrombolysis in individuals with CMBs. In 2013 a meta-analysis by Charidimou and colleagues included five studies and 790 patients. Among patients with CMBs, 10/135 (7,4%) had a symptomatic ICH after thrombolysis, compared to 29/655 (4.4%) patients without CMBs. The pooled relative risk of ICH was 1,90 (95% CI 0,92 to 3,93; p=0,082), thus the increased frequency of SICH in the CMB group was not statistically significant.³⁴⁶ A subsequent study from Switzerland published in 2014, reporting findings from 392 patients, showing that neither the existence of CMB, their burden, predominant location nor their presumed pathogenesis influenced the risk for symptomatic or asymptomatic ICH. However, a higher CMB burden marginally increased the risk for ICH outside the infarct.³⁴⁷

1.5 PREDICTION OF OUTCOMES

"And he will manage the cure best who has foreseen what is to happen from the present state of matters." – Hippocrates, The Book of Prognostics

Outcome prediction or prognosis was established as a principal concept in medicine already at the time of Hippocrates. It can be defined as the probability or risk of an individual developing a particular state of health (outcome), based on his or her clinical characteristics.³⁴⁸

1.5.1 From risk factors to risk scores

A single risk factor or variable rarely gives an adequate estimate of prognosis. Prognostic studies need a multivariable approach to determine predictors of the studied outcomes and to provide tools for the estimation of outcome probabilities given different sets of risk factors. These tools are called prognostic models, prediction models, prediction rules, or risk scores. Nonetheless, stroke treatment literature still has a strong focus on a single rather than multiple predictors, as evidenced by meta-analyses¹⁷⁰ and systematic reviews of risk factors for SICH and other clinical outcomes.^{258,260,349}

Component predictors in a risk score should be well defined, standardised, and reproducible to make the model generalizable and applicable in practice. Factors requiring subjective interpretation, for example imaging test results, can be problematic in this context, because there is a risk of studying the predictive ability of the observer rather than that of the predictors.

Guidelines have been put forward delineating three major steps in multivariable prognostic research. These are (1) developing the prognostic model, (2) validating its performance in new patients, and (3) studying its clinical impact.³⁴⁸ It is important for a prognostic model to be validated outside of its original development patient cohort. Otherwise, clinicians may not trust that the probabilities provided by the model will be applicable to his or her patient population. Like any scientific hypothesis, the generalizability of a prognostic system is established by being tested and being found accurate in different settings. The more patient groups in which the score is tested and found accurate, the more it is likely to work in an untried setting.

A number of studies have been conducted evaluating whether clinical decision support systems improve clinical practice. A 2005 meta-analysis by Kawamoto et al, examining 75 such studies, found that implementing such tools improved practice in 68% of the conducted trials. System features predicting independently associated with success were (1) automatic provision of decision support as part of clinician, (2) provision of recommendations rather than just assessments, (3) provision of decision support at the time and location of decision making, and (4) computer based decision support. If all four features were implemented, improved results were seen in 94% of the studied trials.³⁵⁰

There is no widely agreed approach to building a multivariable prognostic model from a set of candidate predictors. However, in an authoritative article series in the British Medical Journal of 2009, Moons, Royston, Altman et al give recommendation on the design, validation and use of these predictive instruments.^{348,351-353}

1.5.2 Prediction from a clinician's point of view

In recent years, there has been a proliferation of risk scores for acute stroke, all of which are designed to predict clinical outcome in ischemic stroke patients, in particular those who receive IV thrombolysis.³⁵⁴⁻³⁵⁷ Several more scores have been designed to estimate the risk of SICH after thrombolysis.³⁵⁸⁻³⁶⁰

A crucial question applies to all outcome prediction instruments designed for stroke thrombolysis. Should any score predicting very poor outcome despite thrombolysis, or a high risk of hemorrhage with thrombolysis, be deemed sufficient to withhold IV tPA administration?

In an editorial in the Archives of Neurology, Louis Caplan offered strongly worded critique toward the use of risk score systems. The main tenets were: (1) scores are primitive and cater to non-specialists, whereas it is entirely undesirable for stroke patients to be treated by such, (2) “complex conditions such as stroke cannot be made simple and cannot be homogenized without harming many patients who differ from the average statistic” and (3) there is a risk that scores may be used by healthcare administrators to impose limitations (regulatory or financial) on clinical management of patients with score levels prognosticating a high likelihood of poor outcome.³⁶¹

Nevertheless, scores offer valuable prognostic information and deserve attention. They may be useful for risk adjustment when outcome-based metrics are collected to assess hospital performance. Moreover, among clinicians still weary of using IV thrombolytic therapy in stroke due to an insufficiently informed perception of hemorrhagic risk, very low risk scores may encourage the use of this proven therapy. Furthermore, scores may also guide eligibility criteria for future trials.¹⁷¹

A practical aspect of risk score usage was alluded to in a recent letter to the editor of Stroke by the Taiwanese research team of Sung et al. Here, the authors respond to critique of SICH risk scores, reflecting on national reimbursement practices of off-label medication use:

“Because there are well-established guidelines for thrombolytic therapy, these SICH risk scores might seem redundant at first glance. Nevertheless, the clinical usefulness of risk prediction models largely depends on the context. In Taiwan, the current licensing criteria of intravenous tissue-type plasminogen activator exclude many patients with stroke from thrombolysis, including age >80 years, severe stroke, mild or rapidly improving stroke, or diabetes mellitus with prior stroke. Off-label drug use is expensive and could be burdensome for some

patients. In addition, adverse outcomes, such as death or SICH, could potentially lead to legal liability, especially for patients treated on an off-label basis. Applying risk score models to provide patients and families concrete information on the risk of post-thrombolysis SICH may help their decision-making. Particularly, appropriately informed patients whose predicted risk of SICH is low may be more willing to accept the therapy.”³⁶²

2 AIMS

The general aim of this thesis was to study risk factors for symptomatic intracerebral hemorrhage (SICH) following treatment with IV thrombolysis in patients with acute ischemic stroke. Knowledge of clinical parameters associated with this complication would potentially allow its prediction and prevention by use of a multivariate prediction model. Moreover, in government regulations and national treatment guidelines, certain patient characteristics are viewed as strong enough risk factors for ICH to preclude IV thrombolytic treatment. These contraindications originate from expert opinion in the process of clinical trial design and have been insufficiently validated. Further, risk factors associated with different types of thrombolysis-related ICH may guide generation of hypotheses on causative mechanisms behind this complication.

Based on the above, the specific objectives of the present thesis were:

1. To report risk factors for SICH following stroke thrombolysis and use them to develop a clinical scoring system for the risk of SICH in individual patients.
2. To perform an external validation study of a concurrently developed prediction system for SICH named the SEDAN Score.
3. To investigate the safety and outcome of IV thrombolysis in stroke patients treated with warfarin, with and without a history of atrial fibrillation.
4. To investigate whether warfarin treatment influences the rate of arterial recanalization in patients with treated with IV thrombolysis for acute ischemic stroke.
5. To report for the first time risk factors for remote (unrelated to current ischemic lesion) parenchymal hemorrhage and compare them with risk factors for local (related to current ischemic lesion) hemorrhage secondary to stroke thrombolysis.
6. To report short- and long-term outcomes in patients suffering from remote and local parenchymal hemorrhage following stroke thrombolysis.

3 MATERIALS AND METHODS

3.1 THE SITS INTERNATIONAL STROKE THROMBOLYSIS REGISTER

This thesis is based on patient data contained within the Safe Implementation of Treatments of Stroke - International Stroke Thrombolysis Register (SITS-ISTR, <http://sitsinternational.org/>). The design of all studies is observational. The SITS-ISTR is an Internet-based, non-profit, open, academic-driven international database of stroke thrombolytic treatment. The complete registry is owned by the SITS International Collaborative Group, represented by the SITS Scientific Committee (see the Acknowledgements). The Karolinska University Hospital in Stockholm, Sweden is the legal administrator of the registry. The first and pivotal major project based on the SITS-ISTR platform was the Safe Implementation of Thrombolysis in Stroke -Monitoring Study (SITS-MOST).¹¹⁸ In 2002, the EU license for use of tPA in for acute ischemic stroke was granted on the condition that an observational safety study was initiated. Between 2002 and 2006, 6483 patients were recruited from 285 centres in 12 EU countries plus Norway and Iceland. These data are embedded among the >100000 patients from over 40 countries registered in the SITS-ISTR as of spring 2014.

The limitations of SITS-ISTR data has been discussed extensively in previous publications.^{118,121,151,193} The main issue is that SITS-ISTR is a registry and thus, it is impossible to guarantee the completeness and veracity of inclusions and to exclude selection bias. To address this issue, consecutive reporting is a basic requirement for participation in the SITS-ISTR. This is agreed upon in the electronic contract between SITS and the centers. Patients are not selected or excluded based on treatment protocol, whether international, national or local. This means that the registry contains some patients treated beyond the established 4,5 hour time window, at stroke severity levels above NIHSS 25, with very high baseline blood pressure and blood glucose levels, high INR levels due to oral anticoagulation and other parameters generally regarded as contraindications to treatment.

Data is owned by the generating centres. Each participating hospital owns and has the right to publish its own data. The National Coordinator for the registry in each country has the right to publish country-specific data, and local centres can publish centre-specific data. Ownership is shared among the national coordinating group for national publications and the international coordinating group for international publications.

The National Coordinator (NC) is appointed by SITS as to be the responsible person for the SITS-ISTR in the specific country. The NC is generally a senior stroke researcher and physician, having an excellent overview of the stroke care infrastructure in the country. The role of the NC is to coordinate and support participating centers in the country. In addition to the ability to approve SITS centers in the country, the NC has access to complete data on all national centers. Moreover, National Coordinators are responsible for ensuring that data

is registered in accordance with ethical committee and data safety authority regulations in the individual countries.

A Local Coordinator (LC), is responsible for each SITS centre. The LC must be a stroke physician authorised by the medical head of the department responsible for the hospital's stroke unit.

All data is entered electronically and encrypted in order to maintain patient integrity in the registry. If preferred, there is also the possibility to download paper versions of the case report forms. Depending on the data safety regulations in individual countries, users are able to register patients in three different approaches:

1. Patients are identified completely by entering the full unique identity of patient (date of birth, surname, and name).
2. Patients are identified by entering date of birth and initials.
3. Patients are identified anonymously. In this case, a unique code in the SITS registry is correlated to the same unique code as on the paper ID-form at the participating hospital.

The IT infrastructure of the SITS-ISTR, including data storage and web interface is developed, maintained and upgraded by ZiteLab ApS, Copenhagen, Denmark. Full downloads of the database are provided to researchers several times per year, with anonymized case codes denoting individual patients according to the structure [Nation and Centre code][Date of initial data entry][Number from 01 and up, if several patients were entered by the centre on the same date]. For example SEKSJ 20030116 01 would denote the first patient entered on January 16, 2003 at the Karolinska University Hospital in Sweden. Date of registration is not required to be identical with the date of treatment or any date of clinical follow-up.

3.2 STUDY SUBJECTS

3.2.1 Study I

All patients recorded in the SITS-ISTR between 25 December 2002 and 1 March 2010 were included in this study. Patients were included if they presented with stroke symptoms and were treated with intravenous alteplase within and outwith license criteria. In total, 31627 patients with ischemic stroke treated with IV tPA were recorded in SITS-ISTR at 669 centers from 34 countries, of whom 93,3 % (29508 of 31627) were from Europe. Data was complete for all variables included in the final score model and the main outcome in 27804 patients (87,9 %), in the entire database. Follow-up imaging results at 22-36 hours were available in 96,4 % of cases. The score was developed in a population of 15814 patients with odd database entry numbers – a random pick, avoiding potential chronological and geographical confounding factors. Internal validation was performed on the remaining 15813 patients with even database entry numbers. In both populations, only patients with complete data for all score variables and outcomes were included in the analysis.

3.2.2 Study II

All patients recorded in the SITS-ISTR between December 25, 2002 and December 12, 2011 were included in this study, n=45074, with 95% contributed by European centres. Patients were included if they presented with stroke symptoms and were treated with IV tPA within or outwith license criteria. Data for all SEDAN score variables and outcomes were complete in 36027 (80%) patients for the ECASS II and 36127 (80%) patients for the SITS-MOST SICH definitions.

3.2.3 Study III

In this study, the same patient cohort and data file was employed as in Studies 2 and 4. Therefore, the recruiting period was the same and the number of patients was also 45074. Warfarin with $INR \leq 1,7$ was recorded in 768 (1,7%) patients. An additional 318 (0,7%) patients with warfarin had unspecified (n/a) INR values. IV tPA was also administered to 24 patients with $INR > 1,7$. Warfarin status was missing or unknown in 313 patients (0,7%). Data for symptomatic intracerebral hemorrhage was available in 98% of patients. Three month follow-up data on mortality and functional outcome were complete in 81% and 80% of patients, respectively.

3.2.4 Study IV

In this study, the same patient cohort and data file was employed as in Studies 2 and 3. Therefore, the recruiting period was the same and the number of patients was also 45074. Baseline and follow-up radiological data at 22-36 hours from start of IV tPA was available in 43494 (96,5%) patients, who were therefore eligible for this study. Pure remote (extra-ischemic) parenchymal hemorrhage was recorded in 970 patients (2,2%), while pure local (intra-ischemic) parenchymal hemorrhage occurred in 2325 patients (5,3%). In addition, 438

patients (1,0%) developed concomitant local and remote hemorrhages. Data on early (7 days post treatment) mortality was complete in 98% of patients, while 3 month outcomes had the same proportion of missing data as in study 3.

3.3 STUDY DESIGN

The design of all studies is observational. Figure 25 gives an overview of data gathering time points in the SITS-ISTR. The diagnosis of acute ischemic stroke was established on admission. Data were gathered at baseline (immediately prior to initiation of IV tPA infusion, set at 0 hours), at 2 hours, 24 hours and 7 days after start of IV tPA treatment. Head imaging (CT and/or MRI) at baseline and at 22-36 hours after treatment was required. Reporting other imaging was possible, such as in patients with neurological deterioration where emergent radiology was deemed necessary. In some patients, CT or MRI angiography data were also recorded. Functional outcome and mortality were assessed 3 months post-treatment (on day 90).

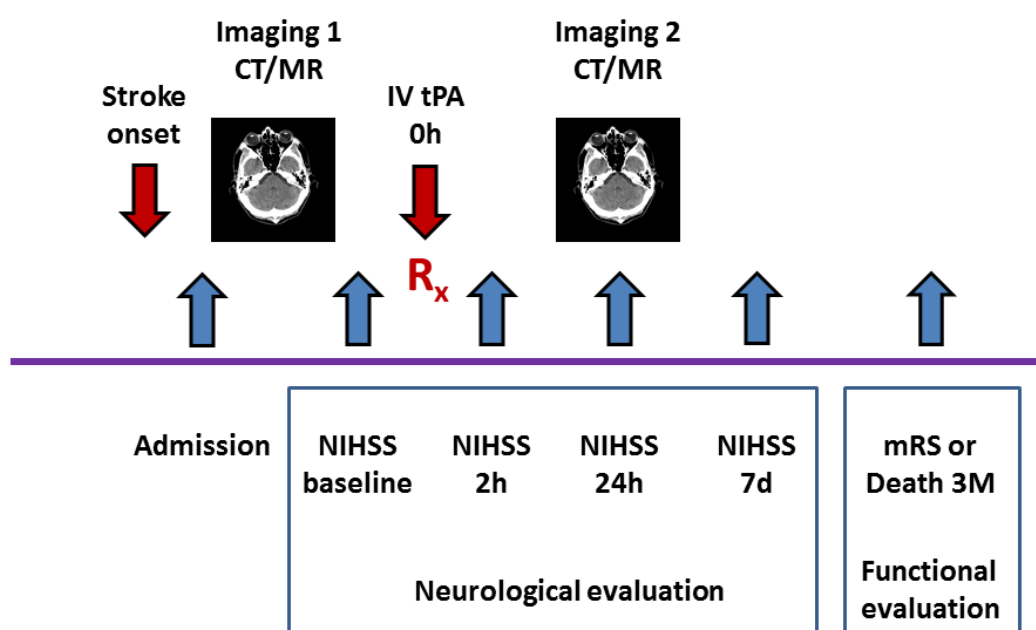


Figure 25. Timeline overview of data gathering time points in the SITS-ISTR in relation to IV tPA treatment.

3.3.1 Database variables

All known qualitative data, apart from exceptions discussed below, were entered into the database in the binary form: present or absent. Alternatively, the “unknown” classification was also possible to mark the cases distinct from missing. Continuous and ordinal variables were entered as their respective numerical values. For a discussion on each specific variable, please refer to section 1.4.

3.3.2 Outcome measures

3.3.2.1 The modified Rankin Scale (mRS)

Functional outcome of stroke was evaluated on day 90 using the modified Rankin Scale (mRS).³⁶³ This scale, spanning from 0 (no symptoms) to 6 (death) is the most commonly used measure of outcome assessment in modern stroke trials.³⁶⁴ The mRS has a strong correlation with quality of life in stroke survivors and is superior to other outcome scales in this respect.³⁶⁵ However, Rankin scores are associated with substantial inter-observer variability that is most apparent for the Rankin scores 1 to 4.³⁶⁶ In randomised controlled trials, in order to reduce bias arising from such variability, measures like structured interviews and/or video training are employed.^{367,368} For further stringency, the structured interviews may be video recorded and submitted to an independent adjudication panel.³⁶⁹

Functional independence was defined in all studies as mRS scores 0 to 2 on day 90. In Study 3, following the request of a reviewer for the *Annals of Neurology*, we implemented an addition of “excellent outcome”, as defined by mRS scores 0 and 1 on day 90. There is a lack of consensus on how mRS data should be dichotomised (*i e*, 0-1 versus 2-6, as in the NINDS and ECASS I and II trials,¹¹²⁻¹¹⁴ 0-2 versus 3-6 as in the SITS-MOST and ECASS III trials,^{118,120} or 0-3 versus 4-6 as in decompressive hemicraniectomy trials³⁷⁰). The choice of mRS cut points in the articles comprising this thesis has stemmed from practice hitherto employed by the SITS-ISTR investigators in previous publications.

Score	Disability
0	No symptoms at all
1	No significant disability despite symptoms; able to perform all usual duties and activities
2	Slight disability; unable to perform all previous activities, but able to take care of self without assistance
3	Moderate disability; requiring some help, but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent, and requiring constant nursing care and attention
6	Dead

Table 9. The modified Rankin Scale.³⁷¹

3.3.2.2 Death

For the death endpoint, survival was assessed up to 3 months from initiation of therapy by hospital follow-up records, supported either by consulting official

population registers (if available) or through contact with the patient's general practitioner.

3.3.2.3 *Intracerebral hemorrhage*

Hemorrhagic infarction type 1 (HI1) is defined as small petechiae along the margins of the infarct; HI2, as confluent petechiae within the infarcted area without space-occupying effect; local, or intra-ischemic parenchymal hemorrhage type 1 (PH1), as blood clots in $\leq 30\%$ of the infarcted area with some slight space-occupying effect; and PH2, as blood clots in $>30\%$ of the infarcted area with a substantial space-occupying effect. PHr type 1 (PHr1) were defined as small or medium sized blood clots located remote from the actual infarct, with mild space occupying effect; PHr2 were defined as large confluent dense blood clots in an area remote from the actual infarct, with substantial space occupying effect.

3.3.2.4 *Symptomatic intracerebral hemorrhage (SICH)*

In the SITS-ISTR, symptomatic intracerebral hemorrhage (SICH) is registered per three definitions.

SICH per NINDS (also referred to as SICH per RCT or SICH per Cochrane): any neurological deterioration registered as an increase of ≥ 1 point on the NIHSS compared to baseline or the lowest NIHSS score within 7 days, or death within 7 days, combined with any type or size of ICH (including petechial) on any post-treatment imaging.

SICH per ECASS II: a severe neurological deterioration registered as an increase of ≥ 4 points on the NIHSS compared to baseline or the lowest NIHSS score within 7 days, or death within 7 days, combined with any type or size of ICH on any post-treatment imaging after the start of thrombolysis.

SICH per SITS-MOST: local or remote parenchymal haemorrhage type 2 on the 22–36 hours post-treatment imaging scan (or a scan performed earlier, due to clinical deterioration), combined with a neurological deterioration of 4 points or more on the NIHSS from baseline, or from the lowest NIHSS value between baseline and 24 hours, or leading to death within 3 months.

3.4 STATISTICS

We performed descriptive statistics for baseline, imaging and demographic data, comparing patient groups according to the questions posed in the respective papers. For continuous or ordinal variables, median and interquartile range values were obtained. For categorical variables, we calculated percentage proportions by dividing the number of events by the total number of patients, excluding missing or unknown cases, as done in previous SITS publications.^{118,121} For calculation of significance of difference between medians and proportions we used the Mann-Whitney U-test and the Pearson χ^2 method, respectively.

Multivariate logistic regression was subsequently performed to correct for baseline differences between the relevant comparator groups. In Studies 1 and 4, to avoid variable selection caused by spurious correlations, only variables showing an association with SICH at the $p \leq 0,10$ level in univariate analysis were included as potential predictors into multivariate logistic regression models. In these analyses, variables significant at $p < 0,05$ were regarded as independent risk factors. In Studies 2 and 3, analyses for SICH, mortality and functional independence were adjusted for factors known to be associated with these outcomes. Two-sided p values below 0,05 were regarded as statistically significant.

The predictive performance of risk scores is frequently assessed using the area under the curve of the receiver operating characteristic (AUC-ROC), also known as the c statistic. Prediction may also be called discrimination, from the ability of the model to discriminate between those who will have an outcome and those who will not. To understand this concept, we shall remind ourselves of two basic notions of diagnostic research (made more specific for our context):

Sensitivity: The fraction of patients with SICH that the risk score correctly predicts will have a bleed. Also known as the “true positive rate”.

Specificity: The fraction of patients without SICH that the risk score correctly predicts will be free from the complication. Also known as the “true negative rate”.

A ROC curve is created by plotting the sensitivity for each value of a score on the Y axis, and $(1 - \text{the specificity})$, *i.e.* the fraction of false positives out of the total actual negatives for each risk score value, on the X axis. It follows from both basic concepts above, that the values on both axes can only be between 0 and 1. If a score predicts no better than the flip of a coin, the curve will be a straight 45 degree $f(x)=x$ line with x confined between 0 and 1 and the area under the curve will be 0,5. If the model predicts better than chance, the value of the AUC-ROC will lie between 0,5 and 1, with 1 meaning perfect prediction (Figure 26). By tradition, AUC-ROC values denoting diagnostic or predictive performance are graded as follows: 0,5-0,6 = no discrimination; 0,6-0,7 = poor; 0,7-0,8 = acceptable; 0,8-0,9 = excellent; 0,9-1 = outstanding.

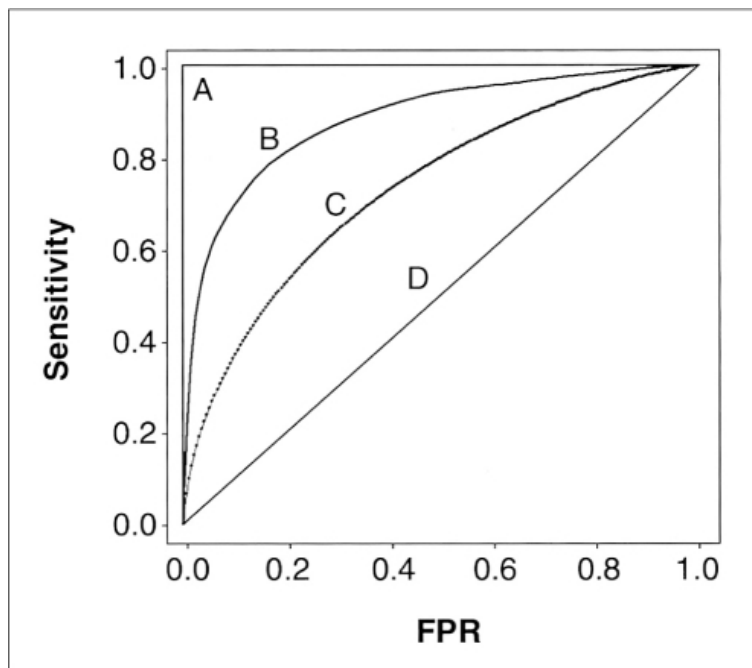


Figure 26. Examples of ROC curves. The area under curve A is equal to 1, denoting perfect discrimination/prediction. The chance diagonal D has an AUC of 0,5. Curves B and C represent predictive capability between these two extremes, with model B being superior to C. FPR: false positive rate or 1 - specificity. From Park et al, 2004.³⁷² Reproduced under Creative Commons BY-NC license.

All analyses in the component papers of this thesis were performed using STATISTICA versions 10 and 11 (StatSoft, Tulsa, Oklahoma, USA).

4 RESULTS

4.1 STUDY I

In total, 31627 patients with ischemic stroke treated with intravenous thrombolysis were recorded in SITS-ISTR at 669 centers from 34 countries, of whom 93,3 % (29508 of 31627) were from Europe. Data was complete for all score variables and the main outcome in 13908 (87,9 %) patients in the model derivation cohort, in 13896 (87,9 %) patients in the internal validation cohort and in 27804 patients (87,9 %), in the entire database. Follow-up imaging results at 22-36 hours were available in 96,4 % of cases. The time from symptom onset to tPA treatment ranged between 3 and 4,5 hours in 10,3% (3257 / 31627 patients), while 1,5 % (459 / 31627 patients) were recorded as treated later than 270 minutes after stroke onset. In the entire population, the rate of SICH per SITS-MOST was 1,8%, the rate of SICH per ECASS II was 5,1% and the rate of SICH per NINDS was 7,4%.

In all, 25 different baseline characteristics were compared between patients with SICH per SITS-MOST and those without SICH. Multivariate logistic regression analysis resulted in nine risk factors independently associated with SICH per SITS-MOST. These are reported in Table 10 together with attributed score points for each parameter.

Risk Factor	OR (95% CI)	P Value	Points
Aspirin + clopidogrel	3.2 (1.9–5.2)	<0.001	3
Aspirin monotherapy	1.8 (1.5–2.1)	<0.001	2
NIHSS ≥ 13	2.2 (1.7–3.0)	<0.001	2
NIHSS 7–12	1.6 (1.1–2.1)	0.006	1
B-Glucose ≥ 180 mg/dL	2.1 (1.7–2.6)	<0.001	2
Age ≥ 72 y	1.7 (1.4–2.0)	<0.001	1
Systolic BP ≥ 146 mm Hg	1.6 (1.3–2.0)	<0.001	1
Weight ≥ 95 kg	1.6 (1.2–2.0)	<0.001	1
Onset-to-treatment time ≥ 180 min	1.5 (1.2–2.0)	0.002	1
History of hypertension	1.4 (1.1–1.7)	0.004	1
Overall Risk Level	Total Score	SICH Rate (95% CI)	
Low	0–2 points	0.4% (0.2%–0.6%)	
Average	3–5 points	1.5% (1.3%–1.7%)	
Moderate	6–8 points	3.6% (3.1%–4.1%)	
High	≥ 9 points	9.2% (5.9%–12.5%)	

Table 10. Elements of the SITS SICH Risk Score, with adjusted odds ratios for each independent risk factor and attributed score points. From Mazya et al, Stroke 2012.³⁷³ With permission from Wolters Kluwer Health.

The SITS SICH risk score showed a strong association with SICH per SITS-MOST definition, with a >70-fold graded increase in SICH between patients with a score of 0 and those with a score ≥ 10 (Figure 27). The overall rate of SICH was 1,8% for the entire population. The median total score was 4 points. Eleven percent of patients scored ≥ 7 points, showing a rate of SICH of $\geq 3,7\%$, i.e. at least double the population average. With a score ≥ 10 points (0,2 % of all patients), the rate of SICH increased 8-fold from the average, to 14,3%. In figures 27-29, due to the low prevalence, patients with ≥ 10 points have been pooled together with those scoring 9 points. Conversely, among the 500 patients with a score of 0 points, there was only a single case of SICH per SITS-MOST.

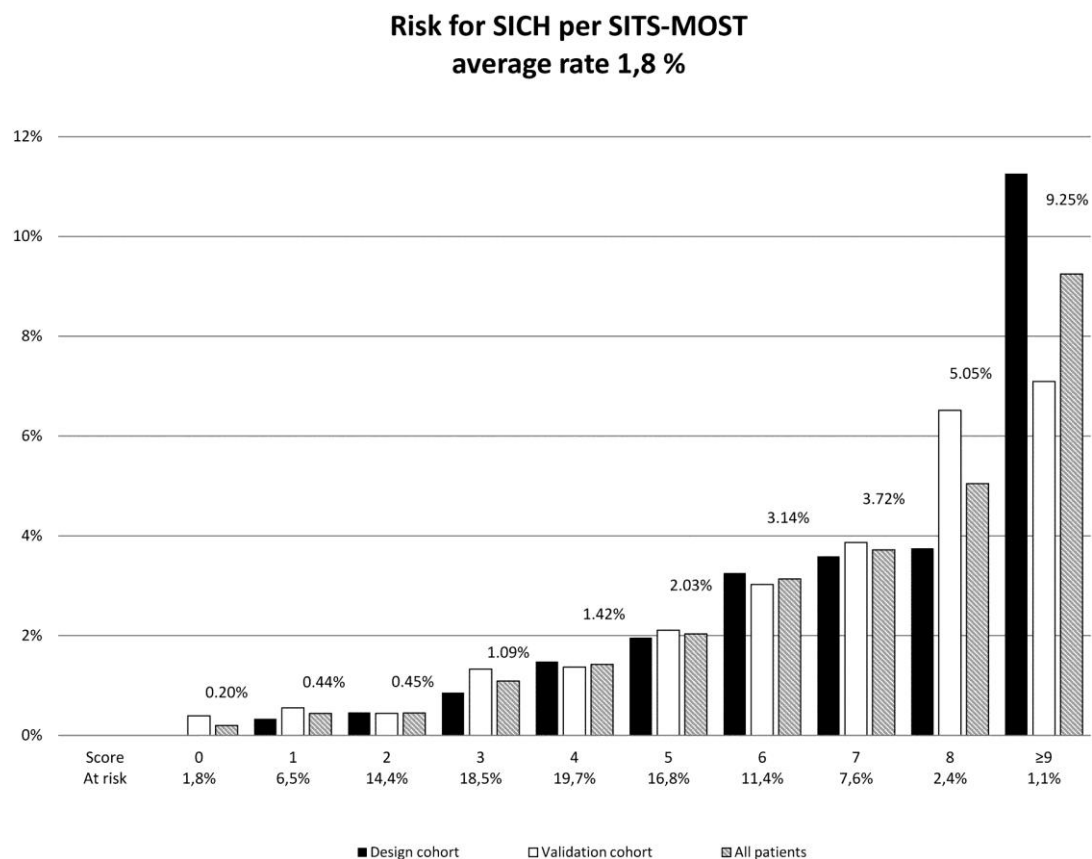


Figure 27. The SITS SICH Risk Score. Rates of SICH per SITS-MOST and population proportion at risk per score category in the design, validation and all patient groups. From Mazya et al, Stroke 2012.³⁷³ With permission from Wolters Kluwer Health.

For purposes of comparability with published results, the ability of the score to predict SICH per ECASS II and NINDS definitions was also assessed (Figures 28-29). The association between rising score and increasing SICH rates was evident here as well. A doubled rate of both SICH per ECASS II and NINDS was seen in patients with a score ≥ 7 , who comprise 11% of the population. Compared with the average rates, a score ≥ 10 was associated with a 6-fold increase in SICH per ECASS II to 31% and a 4-fold increase in SICH per NINDS to 29%.

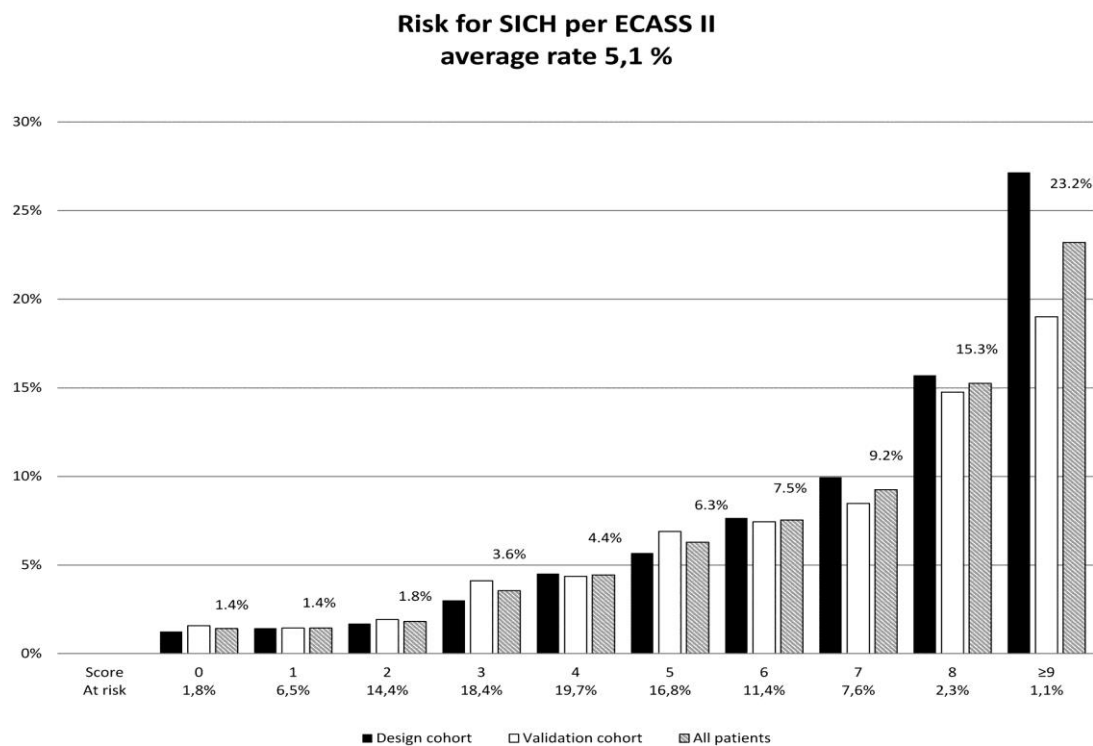


Figure 28. The SITS SICH Risk Score. Rates of SICH per ECASS II and population proportion at risk per score category in the design, validation and all patient groups. From Mazya et al, Stroke 2012.³⁷³ With permission from Wolters Kluwer Health.

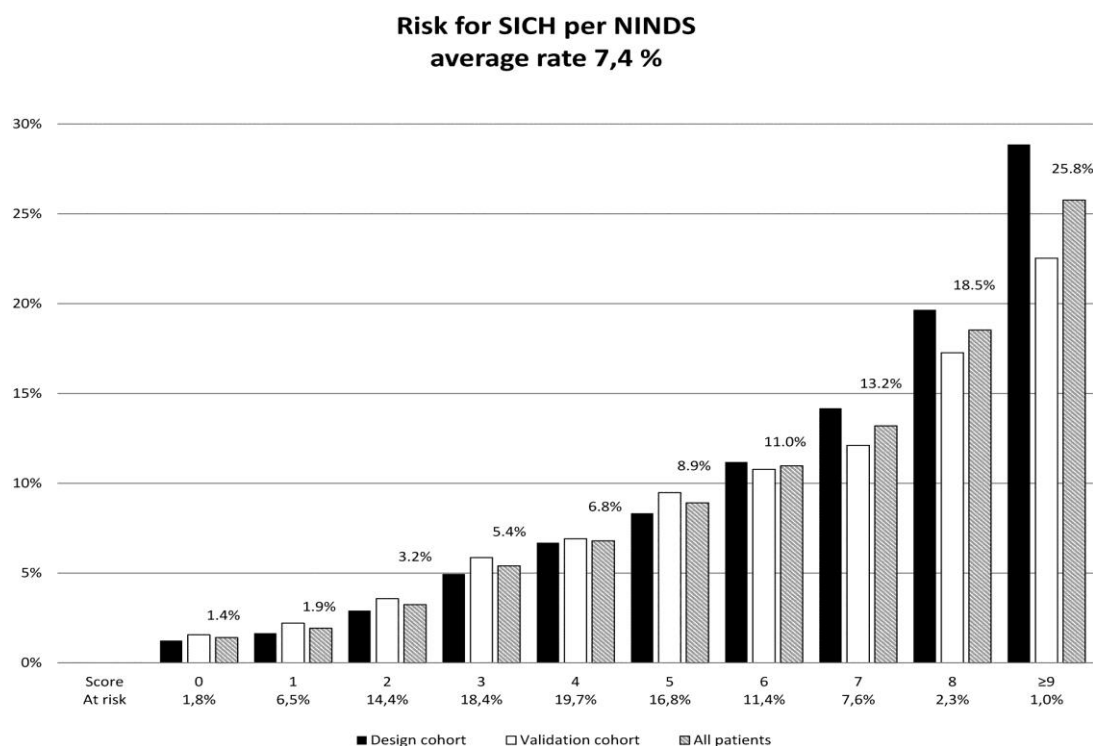


Figure 29. The SITS SICH Risk Score. Rates of SICH per NINDS and population proportion at risk per score category in the design, validation and all patient groups. From Mazya et al, Stroke 2012.³⁷³ With permission from Wolters Kluwer Health.

The index of predictive capability of the score for SICH per SITS-MOST in the model derivation cohort, internal validation cohort and entire database population was calculated using the area under the receiver operating characteristic curve (c statistic). The c statistic in the derivation cohort was 0,71. The c statistic in the internal validation cohort was 0,69. The c statistic in the pooled total population was 0,70.

In the entire material, the c statistic for the predictive capability of the score for SICH per ECASS II was 0,67 and 0,66 for SICH per NINDS. The Hosmer-Lemeshow test statistic was 8,0 ($p = 0,09$) in the validation cohort 7,5 ($p = 0,19$) in the entire population.

4.2 STUDY II

In the SITS-ISTR validation cohort, median age was 70 years (interquartile range (IQR) 17) and median baseline blood glucose was 6,6 mmol/l (IQR 2,1), both identical to values reported in the SEDAN score derivation cohort.³⁶⁰ Our median NIHSS score was 12 (IQR 10), higher than the 10 (IQR 9) reported by the Helsinki group. The proportion of patients with a hyperdense cerebral artery sign was similar in our populations, 18,6% versus 19,4%. Early infarct signs on baseline CT imaging were less common in the SITS-ISTR, 20,1% versus 34,2%. The overall rate of SICH per ECASS II was 5,1%, and 1,7% for SICH per SITS-MOST. Data for all SEDAN score variables and outcomes were complete in 36027 (80%) patients for the ECASS II and 36127 (80%) patients for the SITS-MOST SICH definitions. The radiological parameters “dense artery sign” and “early infarct changes” contributed the most to the missing data, being unavailable in 7,1% and 6,7% of cases respectively, followed by blood glucose, missing in 5,7%.

Table 11 and Figure 30 show the risk for SICH per both definitions based on the SEDAN score categories. Patients with a SEDAN score of 6 points (n=20) comprised only 0.055% of the material and were included in the ≥ 5 points category. They had a SICH per ECASS II rate of 20%. The rarity of a maximum score is also noted in the original SEDAN publication, which reported no patients with 6 points at all, among 1802 cases.

SEDAN score	% SICH E2 (95% CI)	% SICH SM (95% CI)	% at risk	% SICH E2 in SEDAN cohort, n=974
0	1,6 (1,4-2,0)	0,8 (0,6-1,0)	19%	1,4%
1	3,3 (3,0-3,7)	1,5 (1,3-1,7)	32%	2,9%
2	5,4 (5,0-5,8)	2,0 (1,7-2,2)	29%	8,5%
3	8,8 (8,1-9,6)	2,3 (1,9-2,7)	15%	12,2%
4	12,3 (10,7-14,0)	3,0 (2,3-4,0)	4%	21,7%
≥ 5	16,9 (13,0-21,6)	5,4 (3,4-8,7)	1%	33,3%

Table 11. Risk for SICH per ECASS II (E2) and SITS-MOST (SM). From Mazya et al, Stroke 2013.³⁷⁴ With permission from Wolters Kluwer Health.

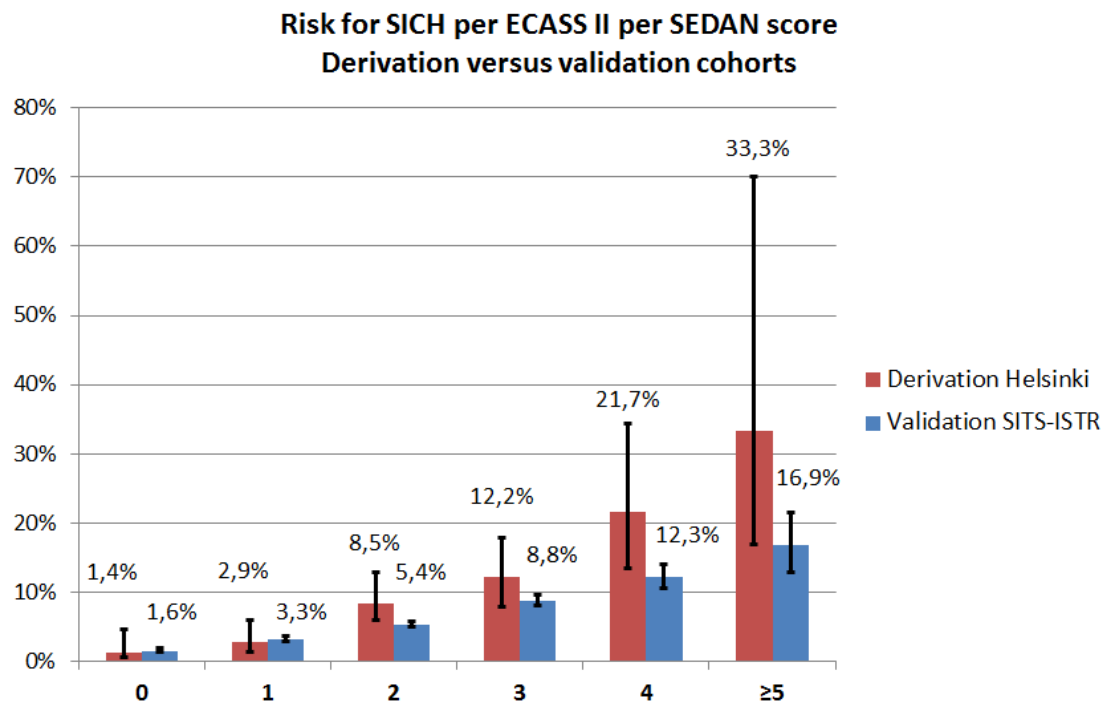


Figure 30. Risk for SICH per ECASS II per SEDAN score category. Score derivation versus validation cohort. Figure presented as a poster at the European Stroke Conference 2013.

Following logistic regression analysis, the SEDAN score was associated with SICH per ECASS II (OR 1,65 per point increase, 95% CI 1,59-1,72, $p < 0,001$) and less so with SICH per SITS-MOST (OR 1,36 per point increase, 95% CI 1,28-1,46, $p < 0,001$). The AUC-ROC (c-statistic) values were 0,66 for SICH per ECASS II and 0,60 for SICH per SITS-MOST. The regression coefficients of all variables of the SEDAN, including stratified glucose, age and NIHSS, are shown in Table 12, to allow comparison with the coefficients reported in the SEDAN design cohort.

Characteristic	Category	Regression Coefficient SITS-ISTR	Regression Coefficient SEDAN Derivation Cohort
Glucose, mmol/L	≤8	Reference	Reference
	8.2–12	0.61	0.80
	>12	0.90	1.70
Early infarct signs on CT	No	Reference	Reference
	Yes	0.35	0.68
Hyperdense artery sign	No	Reference	Reference
	Yes	0.47	0.89
Age, y	≤75	Reference	Reference
	>75	0.37	0.65
NIHSS at baseline	0–9	Reference	Reference
	≥10	0.81	0.71

Table 12. Regression coefficients for variables comprising the SEDAN score. Comparison of coefficients in the derivation and validation cohorts. From Mazya et al, Stroke 2013.³⁷⁴ With permission from Wolters Kluwer Health.

The independent risk factors for SICH per ECASS II in our material, obtained by backward stepwise logistic regression, are shown in Table 12. The discriminating capacity of a prognostic model for SICH per ECASS II, employing all ten of these risk factors, without stratification of continuous variables, was 0,70 using AUC-ROC.

Characteristic	Regression Coefficient	P Value
Dense artery sign	0.390	<0.001
Hypertension	0.344	<0.001
Baseline clopidogrel	0.328	0.003
Early infarct signs	0.321	<0.001
Baseline aspirin	0.280	<0.001
Atrial fibrillation	0.180	0.001
NIHSS score	0.064	<0.001
Age	0.014	<0.001
Systolic BP	0.011	<0.001
Blood glucose	0.001	<0.001

Table 13. Independent risk factors for SICH per ECASS II in the SITS-ISTR. From Mazya et al, Stroke 2013.³⁷⁴ With permission from Wolters Kluwer Health.

4.3 STUDY III

Among 45074 patients, 1110 had warfarin treatment. Warfarin with $\text{INR} \leq 1,7$ was recorded in 768 (1,7%) patients. In addition, 318 (0,7%) patients on warfarin had unspecified (n/a) INR values. IV thrombolysis was administered to 24 patients with $\text{INR} > 1,7$. Warfarin status was missing or unknown in 313 patients (0,7%). Patients with warfarin and $\text{INR} \leq 1,7$ were older, median age 74 years (IQR 14), compared to 70 years (IQR 17) for non-warfarin. Atrial fibrillation was very common at 79%, vs 24% in the non-warfarin group. Previous stroke was more prevalent in warfarin patients (27% vs 13%), as were congestive heart disease (26% vs 8%), and other co-morbidities such as hypertension, diabetes and hyperlipidemia. Stroke severity was higher in warfarin patients, median NIHSS score 13 (IQR 11) in $\text{INR} \leq 1,7$ and 14 (IQR 9) in INR n/a groups, versus 12 (IQR 10) in the non-warfarin group.

The difference in rates of SICH per SITS-MOST was not statistically significant, occurring in 2,0% in $\text{INR} \leq 1,7$ ($p=0,60$), 2,3% in INR n/a ($p=0,48$) versus 1,7% in non-warfarin patients. The median SITS SICH Risk Score was identical at 4 (IQR=3-5) in all groups. Rates of SICH per two other definitions were higher, at 7,7% vs 5,0% for ECASS II ($p<0,001$) and 10,6% vs 7,4% for NINDS ($p<0,001$). After adjustment for age, stroke severity and co-morbidities, warfarin at $\text{INR} \leq 1,7$ was not found to be an independent risk factor for SICH per any definition (adjusted OR (aOR) 1,23 (95% CI 0,72-2,11), $p=0,46$ for SITS-MOST; aOR 1,31 (95% CI 0,96-1,79), $p=0,09$ for ECASS II and aOR 1,24 (95% CI 0,95-1,62), $p=0,12$ for NINDS. The same results were also seen in the warfarin INR n/a group (Table 14).

Outcome	Warfarin, $\text{INR} \leq 1,7$ n=768 aOR (95% CI)	P	Warfarin. INR n/a n=318 aOR (95% CI)	P
SICH SITS-MOST	1,23 (0,72-2,11)	0,46	1,10 (0,45-2,68)	0,84
SICH ECASS II	1,26 (0,82-1,70)	0,24	1,08 (0,55-1,61)	0,68
SICH NINDS	1,13 (0,90-1,37)	0,35	0,92 (0,61-1,24)	0,55
HI	1,03 (0,75-1,31)	0,40	0,87 (0,49-1,24)	0,78
PH	1,08 (0,82-1,35)	0,41	1,10 (0,75-1,44)	0,47
PHr	1,06 (0,70-1,62)	0,78	1,13 (0,60-2,14)	0,71
mRS 0-1, 3 months	0,95 (0,76-1,17)	0,61	1,28 (0,95-1,74)	0,11
mRS 0-2, 3 months	1,01 (0,81-1,24)	0,96	1,04 (0,77-1,41)	0,79
Dead, 3 months	1,05 (0,83-1,33)	0,66	0,95 (0,67-1,35)	0,78

Table 14. Outcomes in warfarin treated patients, adjusted analysis. From Mazya et al, Annals of Neurology, 2013.³⁷⁵ With permission from John Wiley and Sons.

None of the 24 patients treated despite INR levels above 1,7 developed SICH by any definition. One patient, with INR 2,0, developed a parenchymal hematoma, but did not deteriorate below the pre-thrombolysis NIHSS level of 21 and recovered to NIHSS 5 at discharge and mRS 3 at 3 months. The highest reported INR was 3,0.

Combined pre- and post-tPA CT/MR angiographies were registered in 59 of 1086 patients on warfarin with $\text{INR} \leq 1,7$ and INR n/a (5,4%) and in 1475 of 43634 (3,4%) of non-warfarin patients. Recanalization observed using CTA/MRA at 22-36 hours was more common in the warfarin group, 37/59 (62%) vs 776/1475 (55%) in non-warfarin, however the difference was not significant, $p=0,066$. Information on disappearance or persistence of a hyperdense cerebral artery sign on CT imaging were available in 196 of 1086 warfarin patients with $\text{INR} \leq 1,7$ and INR n/a (18,0%) and in 7079 of 43634 (16,2%) non-warfarin patients. Recanalization approximated by the disappearance of the HCAS at 22-36 hours was significantly more common in the warfarin group, 124/196 (63%) vs 3901/7099 (55%), $p=0,022$.

Crude rates of functional independence at 3 months (mRS 0-2) were lower among patients with $\text{INR} \leq 1,7$, at 44,5% versus 54,9% ($p<0,001$) in the non-warfarin group. The three month mortality rate was higher, 26,1% versus 15,1% ($<0,001$) in $\text{INR} \leq 1,7$ versus non-warfarin. However, after adjustment for age, stroke severity and co-morbidities, warfarin use was found to associate independently with neither mortality (aOR 1,05 (95% CI 0,83-1,33); $p=0,66$) nor functional independence (aOR 1,01 (95% CI 0,81-1,24); $p=0,96$), with the $\text{INR} \leq 1,7$ and the INR n/a groups displaying nearly identical results.

A subgroup of 10901 (24,2% of total) patients had atrial fibrillation (AF); among these, 877 (8,0%) had warfarin treatment. Patients with AF and warfarin had a higher prevalence of previous stroke (26% vs 12%), congestive heart disease (26% vs 16%) and other vascular co-morbidities, compared to AF patients without warfarin. Stroke severity was identical between groups, at NIHSS 14 (IQR 10), and median age was very similar at 74 vs 75 years. Patients with AF on warfarin did not differ on any outcome (SICH and ICH, death, mRS 0-2 at 3 months) from AF patients without warfarin in univariate and multivariate analyses.

4.4 STUDY IV

A total of 45079 patients treated with intravenous thrombolysis were recorded in the SITS-ISTR in 9 years between 25 December 2002 and 12 December 2011. Baseline and follow-up radiological data at 22-36 hours from start of IV-tPA was available in 43494 (96,5%) patients, who were therefore eligible for this study. Among these, 2471 patients (5,7%) were examined by magnetic resonance imaging at baseline. Pure PHr was recorded in 970 patients (2,2%), while pure PH occurred in 2325 patients (5,3%). In addition, 438 patients (1,0%) developed concomitant local and remote PH.

Patients with pure PHr were older than those in the PH and non-ICH groups, median age 74 years (versus 72 and 70 years respectively, both $p<0,001$). Pure PHr patients were more frequently female, 46% versus 41% in the pure PH ($p=0,01$) and 43% in the non-ICH group ($p=0,028$). Stroke severity in pure PHr patients was between levels seen in the pure PH and non-ICH group (median NIHSS score 13 vs 16 vs 11, both $p<0,001$). Pre-tPA systolic blood pressure was higher among PHr patients compared to the PH and non-ICH groups (median 158 vs 153 vs 150 mm Hg, both $p<0,001$). Despite having the highest median age, PHr patients had a lower prevalence of atrial fibrillation (AF, 28,5%) and diabetes mellitus (DM, 16,2%) than patients with PH (AF 34,6%, DM 23,4%, both $p<0,001$). The prevalence of any previous stroke was higher in the pure PHr group (16,3%) than in both the PH (12,7%, $p=0,003$) and non-ICH groups (13,0%, $p=0,007$). Among radiological findings, the hyperdense cerebral artery sign (HCAS) on baseline computed tomography (CT) was less common in pure PHr patients than in pure PH (18,2% vs 30,3%, $p<0,001$). Early infarct signs on baseline CT were also less common in PHr patients (22,9%) compared to those with PH (28,6%, $p<0,001$).

Mortality was lower in the PHr group compared to patients with pure PH, 18,7% vs 22,7% at 7 days ($p=0,012$) and 33,9% vs 39,2% at 3 months ($p=0,008$). Patients with concomitant PH and PHr had the highest mortality, 42,8% at 7 days and 62,1% at 3 months, ($p<0,001$ compared to all other groups). For comparison, patients without any parenchymal hemorrhage had mortality rates of 4,9% at 7 days and 12,1% at 3 months ($p<0,001$ compared to all other groups). The frequency of functional independence (mRS 0-2) at 3 months in patients with PHr was 33,6%, between 24,0% in patients with PH and 57,9% in the non-ICH group, both $p<0,001$. PHr and PH were equally often symptomatic, 25% of patients with the respective hemorrhage type experienced a ≥ 4 NIHSS point deterioration at 24 hours compared to baseline.

Following adjustment for covariance using multivariate logistic regression analysis, baseline NIHSS score, systolic blood pressure and current aspirin treatment were independently associated with both PHr and PH. In addition to these, higher age and history of non-recent previous stroke (>3 months prior) were associated with pure PHr, while atrial fibrillation, the hyperdense cerebral artery sign and blood glucose levels were associated with pure PH (Table 15).

Pure PHr vs None	aOR	P
Age (per 10 yrs)	1,2 (1,1-1,3)	<0,001
SBP (per 20 mmHg)	1,2 (1,2-1,2)	<0,001
NIHSS (per 5 points)	1,1 (1,1-1,2)	<0,001
p-stroke >3 months	1,2 (1,1-1,5)	0,023
Aspirin	1,2 (1,1-1,4)	0,007
Pure PH vs None	aOR	P
NIHSS (per 5 points)	1,4 (1,3-1,4)	<0,001
AFib	1,4 (1,3-1,5)	<0,001
HCAS	1,4 (1,2-1,5)	<0,001
Diabetes	1,4 (1,3-1,4)	<0,001
Aspirin	1,3 (1,2-1,5)	<0,001
SBP (per 20 mmHg)	1,1 (1,0-1,2)	<0,001
Glucose (per mmol/L)	1,02 (1,01-1,02)	<0,001

Table 15. Independent risk factors for PH and PHr. aOR: adjusted odds ratio. P-stroke: previous stroke. AFib: atrial fibrillation. Adapted from Mazya et al, Stroke 2014.³⁷⁶ With permission from Wolter Kluwers Health.

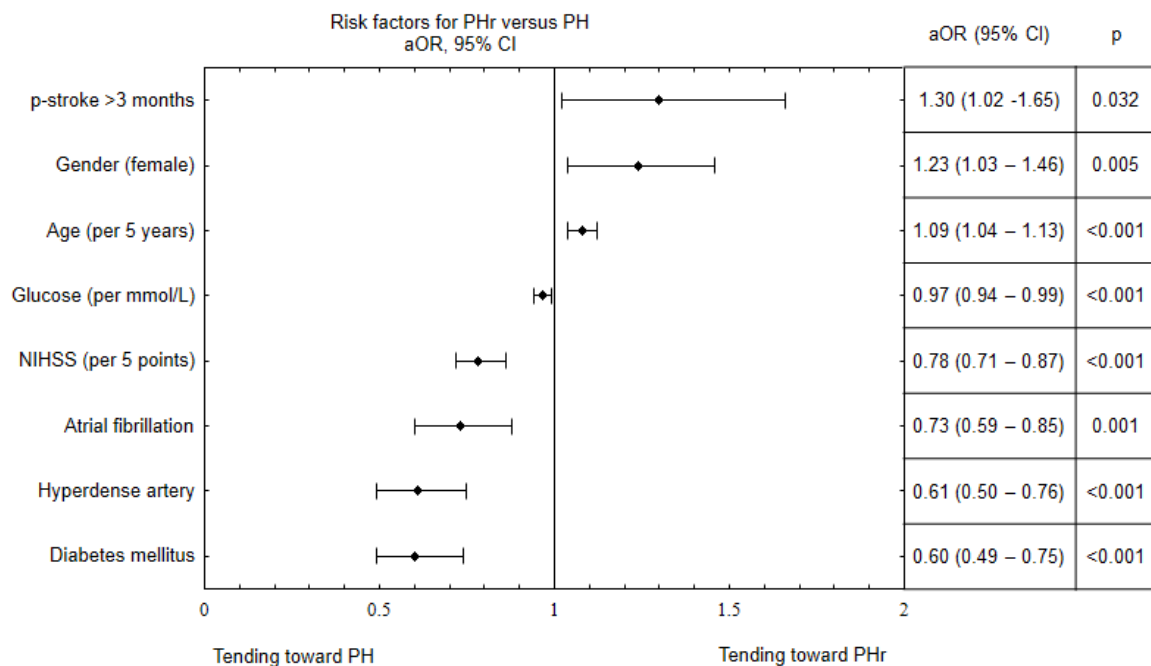


Figure 31. Independent risk factors for PHr versus PH. aOR: adjusted odds ratio. From Mazya et al, Stroke 2014.³⁷⁶ Permission from Wolter Kluwers Health.

A direct adjusted comparison of independent risk factors for pure PHr and pure PH showed that higher age, female gender and non-recent previous stroke shifted the odds toward PHr, while higher baseline NIHSS score, higher blood glucose, diabetes mellitus, atrial fibrillation and the hyperdense cerebral artery sign had a stronger association with pure PH (Figure 31).

5 DISCUSSION

The present thesis is comprised of four studies employing statistical methods for analysis of large sets of observational data. The thesis project has taken a broad perspective on intracerebral hemorrhage following treatment with IV thrombolysis for acute ischemic stroke. We have performed an extensive mapping of risk factors for various types and definitions of hemorrhagic complications (Studies I, II and IV), developing and testing models employing such risk factors in order to predict their occurrence (Studies I and II) and sought to resolve the controversy between American and European practice regarding thrombolytic therapy in stroke patients treated with anticoagulants, showing that a current EU treatment contraindication may be scientifically unfounded (Study III). Below, we discuss the findings of each study in detail.

5.1 STUDY I

In study I, we designed a clinical risk score with good discriminatory ability to find acute stroke patients at high risk of SICH when treated with IV tPA. Previously published haemorrhage risk scores had been designed using smaller datasets of a few hundred patients.^{358,359} The SITS SICH Risk Score study was based on 31627 patients. We identified and incorporated nine independent risk factors for SICH into a score which demonstrated a strong association between risk of SICH and an increasing burden of risk factors. These are presented in Table 10 by order of decreasing adjusted odds ratios for SICH. The risk of SICH increased more than 70-fold in patients scoring ≥ 10 points (14,3%), compared to those with a score of zero (0,2%). The predictive ability of the score is acceptable, with a c statistic (AUC-ROC) at 0,70. Internal validation depicted nearly identical performance between the model derivation, validation and total study cohorts. The Hosmer-Lemeshow goodness-of-fit test comparing predicted and observed rates of SICH showed adequate calibration of the model in the validation cohort.

Identifying patients with the lowest risk of SICH could facilitate treatment by non-specialists. In an earlier survey of US emergency physicians, 26% of 1105 respondents were reluctant to use IV tPA in acute ischaemic stroke for fear of SICH.³⁷⁷ Among this physician population, the highest acceptable rate of SICH was 3,4%, near double the average rate of SICH per SITS-MOST (1,8%) in the SITS-ISTR registry. Our score identifies 11% of treated patients with a risk for SICH of this magnitude or higher ($\geq 3,7\%$ risk at ≥ 7 points). Still, any decision to abstain from treatment due to a perceived increased risk of SICH needs to weigh the dangers against the potential benefit to the patient.

The SITS SICH Risk Score may be relevant in the following contexts: 1) It may aid physicians, patients, and families, in the process of decision-making when faced with acute ischaemic stroke eligible for IV tPA treatment. 2) As the predictive potential of neuroimaging parameters and biomarkers improves, they could be used in conjunction with our risk score. 3) The score could be useful in

clinical trials for patient selection and balancing the risk of SICH between study groups.

Major differences exist between the SITS SICH Score and two other previously published SICH risk prediction instruments. Our score is designed to predict large parenchymal haemorrhages associated with severe clinical deterioration. It was developed using data from >30 000 patients, employing weighted risk factors. The HAT score is comprised from variables found using a literature review and was initially tested on 400 patients.³⁵⁹ The Multicenter rt-PA Stroke Survey Group score was constructed with non-weighted parameters, using data from 1205 patients, albeit only 481 had complete data for component variables.³⁵⁸ Both scores were designed to estimate the risk of any amount of blood extravasation on computed tomography related to any clinical deterioration, the NINDS definition of SICH. This definition is confounded by neurological deterioration due to infarct oedema, recurrent infarction and intra- and interrater variability in determining an NIHSS deterioration of 1 point, required for symptomatic status in the NINDS studies. These confounding factors may be present concomitantly with small amounts of blood in the infarct core on follow-up imaging. It can be argued that the scores predict any clinical deterioration, which only to some extent may depend on cerebral haemorrhage.

The HAT score had an AUC-ROC (c statistic) of 0,74, while the Cucchiara score had a c statistic of 0,68 in the original population. Both scores were subsequently subjected to external validation using the pooled SAINT I and II study cohorts.³⁷⁸ This resulted in lower predictive capability, with c statistic values of 0,59 for both algorithms.

The SITS SICH risk score does not require waiting for a measurement of a blood platelet count (required by the Cucchiara score), nor an often imprecise measurement of the infarct size on baseline imaging (as used in the HAT score). It can thus be calculated upon presentation or in the pre-hospital setting, on route to the hospital. In case of the latter, the receiving stroke centre's average door-to-needle time could be used to calculate a likely onset-to-treatment time, which is part of our score.

5.1.1 Post-publication developments

After publication, the SITS SICH Risk Score has been subjected to external validation in 2013 and 2014. In Taiwan, Sung and colleagues evaluated it in 548 patients treated at four hospitals. Other algorithms evaluated were the HAT, Cucchiara, GRASPS and SPAN-100 scores.³⁷⁹ The SITS-MOST performed similarly to the HAT and Cucchiara scores, as shown in Figure 32. The c statistic of these scores for various SICH definitions was between 0,60 and 0,73 (0,62-0,68 for the SITS Score), with no significant differences in predictive capability between the models. The GRASPS and SPAN-100 scores underperformed in the Taiwanese material.

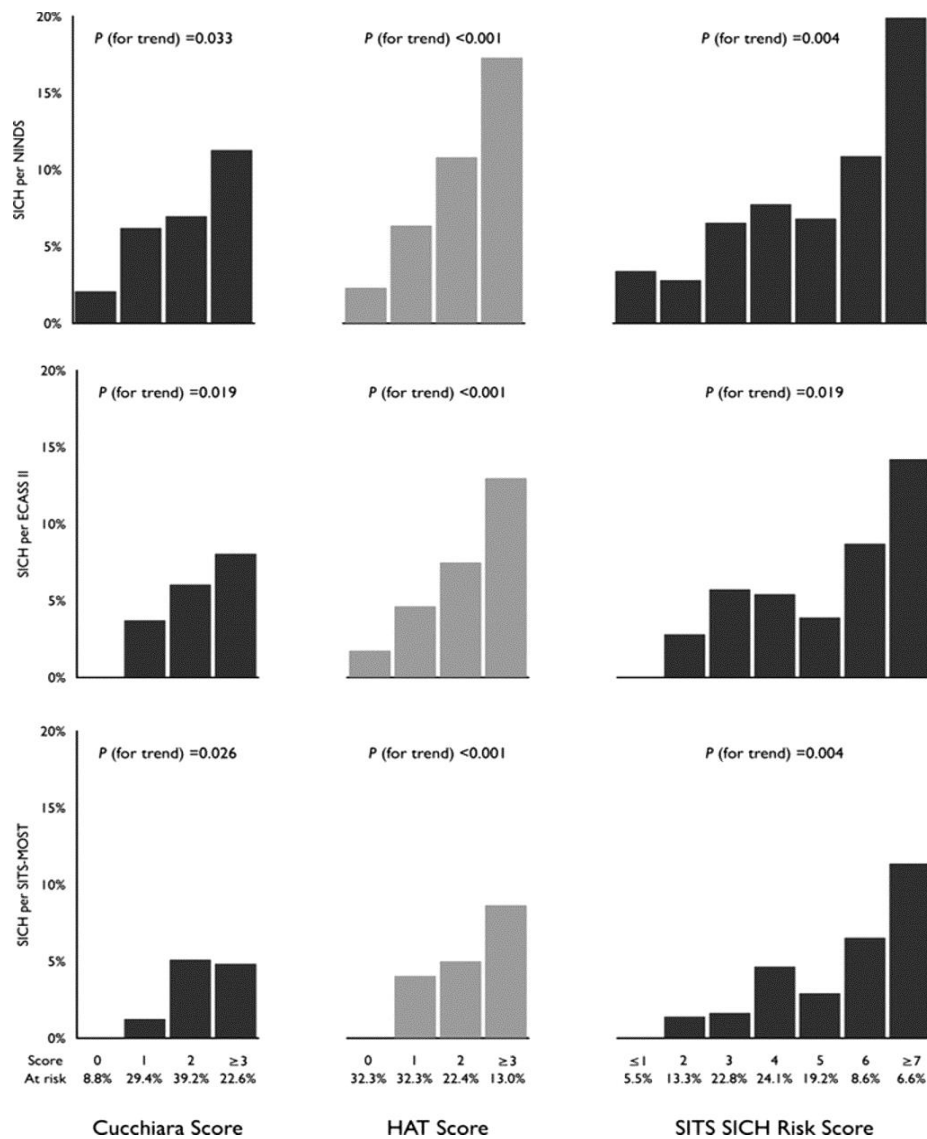


Figure 32. External validation of risk scores in a Taiwanese population, n=548. Modified from Sung et al, 2014.³⁷⁹ Permission from Wolters Kluwer Health.

A year later, Strbian et al performed a large score validation study using data from 3012 patients from Switzerland, Finland, France and Australia. Here again, several models, i.e. the HAT, Cucchiara, SEDAN, GRASPS and SITS-MOST were shown to have a nearly identical predictive capability, with c statistic values at 0,64-0,69 and no statistically significant differences between scores for prediction of SICH.³⁸⁰ These findings were recently corroborated by Whiteley et al, analysing data from the IST-3 trial. This important paper conclusively summarizes the state of the science on currently available SICH prediction scores. In a material comprising >3000 patients, the authors confirmed a similarly moderate predictive capability of several scores, including the SITS-MOST, SEDAN, HAT, and others, with c statistic values narrowly ranging from 0,60 to 0,63 for models specifically designed for SICH.³⁸¹ Moreover and of particular importance, as has been hypothesized for several years (Kennedy R Lees, personal communication, December 2010), high risk of SICH was not found to modify the degree of clinical benefit from treatment with IV tPA.

5.1.2 Study limitations

SITS-ISTR data is likely to be representative for clinical practice across various demographics and hospital types, as well as national practice patterns (mainly in Europe). However, for our risk score to become suitable for clinical use, an external validation is warranted. As with other register-based studies, the presented results are based on retrospective, explorative analysis of observational material. Data for relevant variables and outcomes was missing in 12% of patients, which may have influenced outcome. Furthermore, stratification of continuous variables, as well as conversion of risk factor odds ratios to score point values, although necessary for clinical practicality, can be assumed to have resulted in a loss of information and decreased model accuracy.

5.2 STUDY II

The SEDAN score is a clinical prediction algorithm for SICH per ECASS II. In our material, the SEDAN showed a moderate ability to predict this outcome. Its discriminatory capacity was lower than in the model's original derivation cohort, as well as in a Swiss external validation cohort (c statistic 0,66 versus 0,77). In the SITS-ISTR material, the rates of SICH were lower in score categories 2 to ≥ 5 , while more patients predicted to have the lowest risk (0-1 points) had SICH, compared to the original SEDAN paper, as shown in Figure 30. The predictive performance for SICH per SITS-MOST was lower still, reflected by a c statistic of 0,60 and a less pronounced increase in risk per score level at OR 1,36.

To elucidate why the SEDAN performed less convincingly than in its original publication, we analysed independent risk factors for SICH per ECASS II in our own database, checking if any factor of the score lacked association with the outcome, or if additional variables could have improved the model. All five components of the SEDAN were confirmed to be independent risk factors for SICH per ECASS II. However their regression coefficients and, as follows, adjusted odds ratios for SICH, were lower than reported in the original SEDAN publication, as shown in Table 12. We discovered five additional risk factors for SICH per ECASS II in our population: baseline treatment with aspirin or clopidogrel, hypertension, atrial fibrillation and baseline systolic blood pressure (Table 13). Even so, when all ten predictors (five SEDAN parameters plus five new ones), were combined in a prediction model, its discriminative capability did not exceed a c-statistic of 0,70, still lower than the value of 0,77 in the original SEDAN paper. Moreover, as was pointed out by Patrick Lyden in an accompanying review of the SEDAN in the News and Views section of *Nature Reviews Neurology*, blood pressure did not influence the risk of SICH in the first SEDAN publication.³⁸² The contributing study sites appear to have excluded patients with severely increased blood pressure from treatment, in accordance with standard guidelines. We propose two reasons for the weaker performance of the SEDAN in our material: (1) in the SITS-ISTR database, individual risk factors are weaker predictors of SICH than suggested by analyses of smaller datasets, thus their combinations also display a lower predictive capability; (2) the score could possibly have been improved by the inclusion of additional risk factors, such as baseline blood pressure, antiplatelet therapy and others.

The ECASS II definition can encompass any hemorrhage type for the diagnosis of SICH, including petechial hemorrhagic infarct transformation, as long as clinical deterioration of ≥ 4 points on the NIHSS has occurred within seven days. In our material, of all patients diagnosed with SICH per ECASS II, 31% had only petechial bleeds, while 69% had parenchymal hemorrhages. In contrast to parenchymal hemorrhages of sufficient magnitude, hemorrhagic petechiae are hard to view as truly symptomatic ICH. We propose that the SEDAN thus predicts neurological deterioration of ≥ 4 NIHSS points, however not always explained by hemorrhage, but in $>30\%$ of cases rather caused by cerebral oedema, arterial re-occlusion, recurrent stroke and extra-cerebral complications. This conclusion is further supported by publications showing that the ECASS II

SICH definition has a weaker association with poor outcome and death than the SITS-MOST definition.^{149,150,383}

5.2.1 Post-publication developments

The Taiwanese validation study described in 5.1.2 did not analyse the performance of the SEDAN, citing the lack of data on the hyperdense artery sign in their database as the reason.³⁷⁹ The Strbian external validation study demonstrated that the SEDAN score had the highest absolute c statistic values of all examined scores in the multicentre population; however the difference versus several other scores was not statistically significant. Moreover, no score, including the SEDAN, showed better than moderate predictive capability, with no c statistic values reaching even the 0,70 mark (0,5 being no better than a coin toss and 1,0 signifying perfect prediction).³⁸⁰ Further, in the recent score validation study on IST-3 patient material, the SEDAN performed in line with several other scores, showing a c statistic of 0,63 for both SICH and parenchymal hemorrhage.

5.2.2 Study limitations

Our SEDAN validation study shares several limitations with other SITS-ISTR publications. CT and MR scans were interpreted according to local clinical routine, by physicians and radiologists who were not blinded regarding clinical information. However, this was also the case in the original SEDAN paper. The proportion of patients with relevant missing data was 20%, which may have influenced the analysis. This should be compared to 13% of patients with missing data in the Swiss validation population in the first SEDAN paper. The rate of SICH per ECASS II was lower in our material, at 5% versus 7% in the score derivation cohort. The reason is unclear, as levels of SICH risk factors such as age and blood glucose were identical between the SITS-ISTR and the Helsinki cohort, while stroke severity was higher in our material. The SEDAN derivation cohort was analysed after exclusion of 11% (119/1104) patients in the Helsinki stroke database, as these had basilar artery occlusion (BAO) and undergo a different treatment algorithm combining IV tPA and IV heparin. These patients have a higher risk for SICH per ECASS II at 16% according to previously reported data from the Helsinki group.³⁸⁴ The SITS-ISTR IV tPA register lacks the possibility to register the specific location of arterial occlusion. Therefore, we were unable to exclude BAO patients. This difference between our patient materials may have led to differences in risk factor profiles between ours and the Helsinki material, possibly affecting the predictive capability of the SEDAN score in our study.

5.3 STUDY III

In study III, we analysed 1110 patients with baseline warfarin treatment, who underwent IV thrombolysis for acute ischemic stroke. We were unable to find any effect of warfarin at $\text{INR} \leq 1,7$ on rates of SICH, ICH, death or functional outcome. Our findings confirm recent results from the American Get With The Guidelines-Stroke Registry (GWTG) and VISTA.^{188,313} In our material, warfarin patients were older, had more co-morbidities and previous strokes, as well as higher stroke severity, compared to those not on warfarin. Median SITS SICH Scores were equal between warfarin and non-warfarin cohorts, correctly predicting that rates of SICH per SITS-MOST would not be higher in warfarin patients. The increased crude rates of SICH per ECASS II and NINDS are sufficiently explained by the heavier burden of important risk factors such as high stroke severity and age. We could not demonstrate any association between warfarin treatment and SICH following adjustment for risk factor profile differences across groups. Moreover, we found that patients treated with warfarin for atrial fibrillation have no increase in even in crude rates of SICH, mortality, or poor functional outcome, in comparison with patients with AF but without warfarin. This lends further strength to our conclusion regarding the lack of additional risk from warfarin at INR levels up to 1,7 in stroke thrombolysis.

We add 24 thrombolysed stroke patients with $\text{INR} > 1,7$ to the hitherto published total of 68 cases in five studies.^{133,188,216,313,316} In our 24 patients, there was no occurrence of SICH by any definition. Among the total of 92 cases, only one SICH has been described, in the GTWG study. These numbers, however encouraging, are of course insufficient to draw far-reaching conclusions on which would be the optimal level for INR contraindication.

Among patients on warfarin, we found that hyperdense cerebral artery signs (HCAS) disappeared more often on 22-36 hour CT scans, compared to those without anti-coagulation (63% vs 55%). Disappearing HCAS can be considered to be a surrogate marker of arterial recanalization, supported in our material by similar rates of recanalization (62% vs 55% in warfarin vs non-warfarin patients) on 22-36 hour CT/MR angiography. Our analysis of 10901 patients with atrial fibrillation with and without warfarin yielded nearly identical results, with a trend toward more recanalization seen using CTA/MRA and significantly higher rates of disappearance of the HCAS. The timing of imaging limits our interpretation, as recanalization seen at 22-36 hours invites the question whether it occurred early enough to benefit salvageable brain tissue, or at a later time point. Nevertheless, it indicates a possible interaction between low grade anticoagulation, IV tPA, and vessel recanalization.

The European license for IV tPA in stroke lists treatment of patients on warfarin with $\text{INR} \leq 1,7$ as contraindicated. The European Stroke Organization guidelines have not given any recommendations on this issue. In contrast, stroke thrombolysis at $\text{INR} \leq 1,7$ is accepted in the current AHA/ASA guidelines. Therefore, it is not surprising that in our mainly European cohort, warfarin

patients comprise only 2,4% of all those thrombolysed, in comparison to 7,7% in a large American material. Xian and colleagues have calculated that nearly half of US patients in on warfarin, with $\text{INR} \leq 1,7$ and otherwise eligible for IV tPA, are not given treatment due to physician concern regarding hemorrhage risk.³¹³ An extrapolation of these findings to our population implies that European centers may exclude over 80% of otherwise eligible patients from treatment, if they have baseline warfarin and $\text{INR} \leq 1,7$.

5.3.1 Post-publication developments

Following the publication of Study III, it was the subject of a focused review by Veltkamp and Rizos in the “News and Views” section of *Nature Reviews Neurology*.³⁸⁵ The authors put our findings in the following context: of all patients with an acute cerebrovascular ischemic event, 8-10% have an ongoing treatment with warfarin.^{313,386} More than half of such patients have an $\text{INR} \leq 1,7$,³⁸⁶ while as many as 75% of stroke patients on warfarin have an $\text{INR} < 2,0$ (common lower boundary of the therapeutic interval in AF) in the Registry of the Canadian Stroke Network.³⁸⁷ Regarding our findings, the authors of the review concluded that “the long-term debate regarding safety of thrombolysis for stroke in the setting of anticoagulation with VKA has been settled”. However, they justly pointed out new questions rapidly appearing with the advent of novel oral anticoagulants, and recommend that studies on thrombolysis in their presence be conducted with the help of large prospective stroke registries.

5.3.2 Limitations

Apart from limitations inherent in observational design this study had specific issues to address here. The group of patients on warfarin entered as “INR unspecified” could have had any level of INR, which makes conclusions difficult. Still, baseline characteristics and outcomes were similar between these patients and those specifically registered as “ $\text{INR} \leq 1,7$ ”. We had no information on patients’ adherence to warfarin prescription or other possible reasons for low INR levels. This is difficult to account for in a register-based study and was also the case in the large study by Xian et al. The interpretation of our recanalization results warrants a cautious approach. During the study period, users of the SITS-ISTR had no option to specify which vessel was occluded, nor whether recanalization was partial or complete. Furthermore, patients examined with CTA/MRA both pre- and post-tPA comprise only 4% of the database, most of them treated at highly experienced centres. This imposes a limitation on the generalizability of our recanalization results. Meanwhile, the CTA/MRA recanalization rates closely match those seen using the disappearing HCAS method. For the latter, availability was greater at 16% of the database, rendering the findings more robust. Importantly, our recanalization data were obtained at 22-36 hours after tPA administration. We cannot further specify the timing of improved vessel patency. In addition, the higher frequency of arterial recanalization in warfarin patients was not associated with improved functional outcome. This could however have been obscured by the higher burden of comorbidities, higher age and stroke severity in warfarin patients.

5.4 STUDY IV

In this large-scale analysis, we showed that remote hematomas (PHr) comprise over one-third of all parenchymal cerebral hemorrhages after IV stroke thrombolysis, a fact that is generally not sufficiently recognized. This is comparable with a proportion of 27% and 28% in ECASS I and ECASS II. However, in these trials placebo patients were included in the denominator, likely leading to an underestimation of the percentage in thrombolysed patients.^{135,137,143} Of all groups, patients with PHr had the highest age and highest proportion of female sex. A history of previous stroke (over 3 months prior) was also more common in PHr patients compared to those with local, intra-ischemic hemorrhage (PH) or those without hemorrhage. The PHr group had less atrial fibrillation and diabetes compared to patients with PH, and similar amounts of other cerebrovascular risk factors, despite being of higher age and having had more previous strokes. NIHSS scores, as well as frequency of early infarct changes and hyperdense artery signs were much lower in the PHr group versus the PH group. The multivariate analysis showed that PH is related to factors associated with large artery occlusion, such as high stroke severity, hyperdense artery signs and atrial fibrillation. This contrasts with the pattern seen in PHr, which is associated with previous stroke and higher age, both related to prior cerebrovascular pathology. In spite of PHr patients being older and having had more previous strokes, they showed no increase in risk factors for cerebral small vessel disease (hypertension, smoking, and diabetes) compared to the PH group. This is consistent with our observation that small vessel disease was rarely diagnosed in patients with both hemorrhage types.

PHr was only weakly associated with its five independent risk factors, reflected by low adjusted odds ratios (Table 15). This suggests that a causative mechanism may be at play, which is not registered in our database and which is only weakly and indirectly reflected by parameters available to us. One such underlying cause could be cerebral amyloid angiopathy (CAA). This hypothesis gains some support from post-mortem and neurosurgical studies of primary CAA-related ICH. In these studies, patients with CAA tend to be older and more frequently of female sex compared to patients with ICH not found to be caused by CAA.³⁸⁸⁻³⁹⁰ The higher prevalence of CAA in ICH suffered by women corresponds to our finding that female sex has a stronger independent association with PHr than with PH. The etiology in PHr is unlikely to be one of the common causes of ischemic stroke - such underlying conditions are well reflected by the variables of the SITS-ISTR. CAA is strongly associated with spontaneous lobar ICH and is not known to cause ischemic stroke.³⁹¹ Thus, co-existing CAA in patients suffering ischemic stroke caused by other common etiologies is a plausible contributing mechanism for PHr following the administration of IV tPA. Figures 33 and 34 show one such case of multifocal PHr seen in our Neurological Department, with CAA subsequently confirmed by Congo Red and immunofluorescent staining during post-mortem examination.

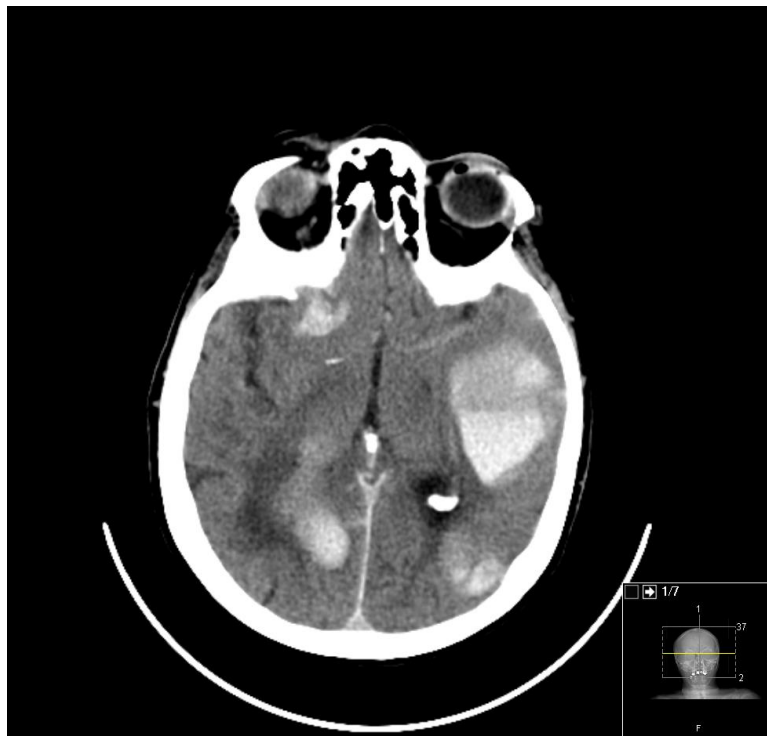


Figure 33. Computed tomography image of a 72 year old female suffering multiple remote cerebral hemorrhages two hours following IV thrombolysis treatment for an acute ischemic stroke with NIHSS 7 and an onset to treatment time of 2,5 hours.



Figure 34. Congo red staining of microvascular amyloid deposition in the case shown in Figure 33. The reference bar is 0,5 mm across. Courtesy of Dr Inger Nennesmo, Department of Clinical Pathology, Karolinska University Hospital, Huddinge.

At this stage, it is appropriate to ask the question whether PHr could occur in an area of recent silent infarction. In our analysis, recent previous stroke was not a risk factor for PH or PHr. In a paper from 2012 on recent asymptomatic cerebral infarcts on baseline MRI, the authors did not show any association with post-thrombolysis hemorrhage.³⁹²

Mortality rates at 3 months were lower in patients with PHr (33,9%) than in those with PH (39,2%) and PHr + PH (62,1%), but expectedly higher than in the group with no parenchymal hemorrhage (12,1%). Regarding functional outcome, PHr patients also fared better than those with PH, with 34% of PHr patients being functionally independent at 3 months, compared to 24% with pure PH. Approximately half (48%) of all local PH were Type 2 (large, with substantial mass effect), compared to PHr, where 67% were Type 1, (smaller in size, with at most a mild, local space occupying effect). Still, early neurological deterioration was equally often seen in the presence of both PHr and PH, with 25% of patients sustaining an increase of ≥ 4 NIHSS points at 24 hours compared to baseline. Therefore, despite an often smaller size, remote hematomas are equally likely as local PH to cause significant clinical deterioration. This is likely explained by the fact that PHr occur in functioning, non-ischemic brain. We believe the main reason for lower mortality and better functional outcomes in PHr patients compared to PH is a lower baseline stroke severity in the PHr group. Patients suffering concomitant PH + PHr had the worst outcomes: at 3 months, mortality was 62%, while only 12% remained functionally independent. The likely reason would be a presumed higher average total hemorrhage volume in these individuals.

5.4.1 Post-publication developments

Shortly after appearing online, the paper was highlighted by Stroke and chosen to be the topic of a commissioned piece written by Dr Matthew Edwardson at the NINDS, on the journal's official blog, Blogging Stroke, as well as a tweet on the journal's Twitter channel. Below follows a part of the blog entry:

"This is by far the most comprehensive study of PHr to date. Although PHr is relatively uncommon (3.2% if one includes both PHr and PH + PHr), this underappreciated complication of IV-TPA increases morbidity and mortality and therefore warrants further study. The authors made huge strides in this regard, uncovering risk factors specific to PHr including remote prior infarct and female gender. Despite these associations, the authors note relatively low odds ratios for each risk factor and suggest that another variable not collected in SITS-ISTR may be more important in predisposing patients to PHr. They suggest cerebral amyloid angiopathy (CAA) as a possibility given the association between CAA and female gender. I would offer another – prior head trauma. Even patients with mild concussion often show evidence of blood-brain-barrier disruption on acute MRI. Such disruption could make patients more susceptible to hemorrhagic conversion in the setting of future IV-TPA administration. Whatever the cause, this study definitely raises awareness of the importance of PHr and will prompt future investigations."³⁹³

This suggestion is well-motivated. In fact, minor head trauma has previously been associated with intracranial hemorrhage following myocardial infarction thrombolysis in the GUSTO-1 trial enrolling 41021 patients, of whom 268 suffered ICH. In patients with facial or head trauma within 2 weeks prior to thrombolysis, the authors found a significantly elevated rate of ICH at 5,3% versus 0,7% without trauma. In adjusted analysis, head trauma had an OR of 13,0 (95% CI 3,4-85,5) for ICH, making it the heaviest independent risk factor in the entire analysis.¹⁹¹ The SITS-ISTR lacks data on recent or non-recent head trauma; however the issue is of potential importance and should be subjected to investigation also in stroke thrombolysis patients.

5.4.2 Study limitations

Study IV has a number of limitations which warrant discussion. Imaging data were interpreted locally by radiologists and physicians at participating centers, with no independent verification. Still, the total rate of PHr in our dataset, 3,2%, is of approximately the same order as seen in the NINDS (1,3%), ECASS (3,7%), and ECASS II (2,0%) trials, which all had centralized, independent imaging assessment. The SITS-ISTR has not yet implemented registration of numerous radiological details which could have been of value for the present study, *e g* prior brain lesions, white matter abnormalities, and multifocal MR DWI lesions. Several baseline variables in our material have 5-10% missing data. This is a limitation which appears to be inherent in large observational databases, as the US Get With The Guidelines – Stroke registry also reports missing baseline data of similar magnitude, around 10%.^{230,394} Of potential importance in our dataset is the lack of 3 month follow-up for mortality in 17% of cases, and 20% missing data for 3 month functional outcome. We attempt to offset the missing data for longer-term mortality by reporting mortality rates at within 7 days from stroke onset, where our records are 98% complete. This can be justified by the observation that nearly all brain hemorrhages after thrombolysis which cause neurological worsening occur within 36 hours from treatment.^{149,189} The SITS-ISTR has no option for registration of the number and anatomic location of remote hematomas in each case. Such information is necessary for the testing of our hypothesis on CAA as a potential cause of PHr, as especially multifocal hematomas following stroke and coronary thrombolysis have been seen in patients with CAA confirmed by neuropathological investigation.³⁹⁵ Moreover, data on the location and number of microbleeds at baseline, together with the location of subsequent PHr, would likely improve our understanding of the mechanisms behind this poorly understood complication of stroke thrombolysis.³⁹⁶

6 CONCLUSIONS AND FUTURE DIRECTIONS

Infrequent cerebral hemorrhage is to be expected after thrombolytic therapy for acute ischaemic stroke, even if all treatment guidelines are followed meticulously. The large body of research by our group and others on currently available risk prognostication scores for SICH has made clear that they are not yet mature enough to guide routine clinical practice, in particular to motivate withholding treatment from an individual patient. Those at the highest risk of hemorrhage could stand to gain proportionally just as much or even more than those with lower risk, as has been demonstrated in patients aged over 80 years and those with severe stroke. Neither should elevated scores be used in hindsight to conclude that a patient should not have been treated, even after a major haemorrhagic complication. It is possible that future studies may identify a subgroup in which IV tPA should be withheld. However, until such results are published, we should continue to offer treatment to all patients within accepted criteria and even outside, if the individual risk/benefit ratio appears favourable and acceptable to both the patient and the physician.

Such criteria however, need to be continuously updated based on solid evidence. Contributing to this process, our results in Study III showing that low-grade anticoagulation with warfarin does not in itself increase the risk of ICH, SICH or poor outcome, may help to resolve the current US / EU practice differences regarding IV thrombolysis in stroke patients with a low level of warfarin anticoagulation and facilitate the treatment of such patients in Europe and elsewhere.

In the meantime, risk scores will help us to quantify the general order of individual risk, providing us with a tool for communication among colleagues, with patients and family members. Moreover, score models are in continuous development and should in time be combined with advanced risk-predicting imaging parameters and possibly biomarkers.

Our analysis of risk factors for intracerebral hemorrhage following stroke thrombolysis indicates a complex pathophysiological interplay behind this complication. Based on our own results and the literature review presented in this thesis, the following is a proposed summary of mechanisms deciding the “to bleed or not to bleed” fate of the IV tPA treated stroke patient.

1. The baseline quality and resilience to ischemia of the microvasculature in tissue affected by the stroke. This includes prior cerebral parenchymal damage and pre-existing microangiopathy, reflected by the presence of classic risk factors for cerebrovascular disease such as age, chronic hypertension, previous smoking, diabetes, and hypercholesterolemia.
2. The volume of brain tissue suffering acute ischemia of a sufficient severity and duration, determining the amount of hypoxic microvessels and the degree of injury done to them.

3. The presence of acute hyperglycemia, aggravating the deleterious effects of ischemia on the affected microvasculature and possibly negatively influencing hemostasis in the face of vessel wall and basal lamina disruption.
4. Acutely elevated blood pressure increasing hemorrhage risk due to as yet poorly understood mechanisms, however likely related to impaired cerebrovascular autoregulation, with further risk increase in the presence of acute hypertension after restoration of blood flow into a sufficiently large volume of badly enough damaged microvessels.
5. Impaired hemostasis due to reduced thrombocyte activation and aggregation by baseline medication with aspirin and clopidogrel, or other medications and conditions sufficiently impairing the hemostatic system, including rare tPA-associated coagulopathy.
6. Components 1 to 5 become all the more important if arterial recanalization occurs due to IV tPA treatment.
7. A likely influence of genetic factors on all of the above. This area, however interesting, has been outside the scope of the current thesis.

Potential directions for future research abound. Regarding the current contraindications to IV tPA treatment in stroke, a number of them are still not based on evidence, but rather on the lack of it. This pertains *inter alia* to remaining AHA/ASA contraindications to treatment in the late time window between 3 and 4,5 hours. Patients over the age of 80, with a history of prior stroke and diabetes, anti-coagulated regardless of INR level and with baseline NIHSS >25 are currently ineligible for treatment in the USA beyond the 3 hour limit due to lack of safety and efficacy data.¹⁹ The SITS-ISTR database, ideally also employing an externally obtained untreated patient control group, could be used to resolve this remaining gap in our knowledge.

Moreover, treatment in the most severely afflicted stroke patients (such as those with NIHSS >25) is very scarcely researched. For them, IV tPA remains contraindicated in the EU, also due to lack of data at the time of approval of the drug by EU authorities. Recent major publications in the stroke thrombolysis field have not fully resolved this issue. We are currently conducting research in this area, with a manuscript in late stages of preparation.

Looking further ahead, endovascular stroke therapy, novel thrombolytic agents, sonothrombolysis, adjunct treatments for the benefit of the penumbra and improved recanalization safety, and pre-hospital thrombolysis delivery make acute stroke treatment research a very exciting field. In all these areas, as well as in the continued development of prevention and diagnostics, large-scale registries will continue to play a major role. It is my hope that the skills I have acquired during work on this thesis, will continue to find applications in the great endeavour of making stroke a reversible, curable condition.

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