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Large cerebral artery occlusion and recanalisation in stroke patients treated with intravenous thrombolysis:

clinical and radiological markers and their clinical relevance

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To my family and friends

CONTENTS

ABSTRACT

Large cerebral artery occlusion accounts for 40-46% of ischemic strokes. These strokes are characterized by extensive neurological deficit, poor functional outcome, and increased mortality (up to 40-80% in the most severe clinical syndromes). Intravenous thrombolysis is approved treatment across severity grades, except for the extremely severe, although alternative strategies are under evaluation, such as mechanical thrombectomy. The aim of the present thesis was to study the occurrence and impact of recanalisation in a large cohort of ischemic stroke patients with documented large cerebral artery occlusion treated with intravenous thrombolysis. Data were collected through the internet-based Safe Implementation of Treatment of Stroke - International Stroke Thrombolysis Register (SITS-ISTR).

Study I
We explored baseline factors associated with middle cerebral artery occlusion, as determined by presence of hyperdense middle cerebral artery sign (HMCAS) on admission CT scan, and its relation to functional outcome and symptomatic intracranial hemorrhage in stroke patients treated with intravenous thrombolysis. HMCAS patients (n=1905, 19% of the whole study population) were younger, but had severer stroke at baseline and less favorable outcomes at 3 months compared to non-HMCAS patients. Intravenous thrombolysis treatment in patients with HMCAS on admission CT scans did not increase the rate of symptomatic intracranial hemorrhage after treatment, though asymptomatic hemorrhagic transformation was increased. We conclude that the presence of HMCAS on baseline CT is not a reason to exclude patients from treatment with intravenous thrombolysis.

Study II
We analysed the association of HMCAS disappearance after intravenous thrombolysis, which implies vessel recanalisation, with early neurological improvement, stroke functional outcome, and symptomatic intracranial hemorrhage, and attempted to find predictors of HMCAS disappearance from baseline. The admission HMCAS disappears on 22–36h CT scans after intravenous thrombolysis in almost half of all cases (n=831). The proportion of functionally independent patients in the HMCAS disappearance subgroup was more than double (42% vs. 19%) and mortality was less than half (15% vs. 30%) compared with the HMCAS persistence subgroup. A higher prevalence of infarct-related parenchymal haemorrhage in the HMCAS disappearance subgroup did not influence overall favorable 3 month outcome. The prognosis in patients with MCA occlusion that persists after intravenous thrombolysis is poor; this finding strengthens the appeal for alternative treatment approaches in this subgroup.

Study III
We examined the impact of early neurological improvement, defined in various ways according to the previous literature, on functional outcome in patients with large vessel occlusion on admission CT- or MR angiography (n=798), and its ability to predict vessel recanalisation. Early neurological improvement at 2h and 24h was associated with vessel recanalisation at 22-36h but also with functional independence at 3 months. Early neurological improvement by 20% at 2h was the best predictor of 3 months functional outcome and recanalisation after thrombolysis. Fairly accurate, it may serve as a surrogate marker of recanalisation, if imaging evaluation of vessel status is not available. If recanalisation status is required after intravenous thrombolysis, vascular imaging is recommended despite neurological improvement.

Study IV
We investigated the importance of recanalisation status in stroke patients (n=5324) with and without early neurological improvement after intravenous rtPA. Recanalisation of an occluded artery in acute stroke was associated with favorable functional outcome both in patients with and without neurological improvement after intravenous thrombolysis. Combination of vessel recanalisation and early neurological improvement was by far the most favorable clinical scenario. In future evaluations of mechanical thrombectomy and other additional strategies, recanalisation strategy should be considered in patients with persisting occlusion after intravenous thrombolysis even in case of significant neurological improvement.

In summary, the present thesis demonstrates satisfactory effect of intravenous thrombolysis in approximately half of the patients with large cerebral artery occlusion, and poor prognosis for those who are lacking early treatment response. Our findings support the search of alternative treatment approaches aimed to achieve vessel recanalisation for the latter group, based on early objective evaluation of vessel status.

LIST OF PUBLICATIONS

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Cerebrovasc Dis 2009;27:51–59

II. Tatiana Kharitonova, Magnus Thorén, Niaz Ahmed, Joanna M Wardlaw, Rüdiger von Kummer, Lars Thomassen, Nils Wahlgren.
Disappearing hyperdense middle cerebral artery sign in ischemic stroke patients treated with intravenous thrombolysis - clinical course and prognostic significance.
J Neurol Neurosurg Psychiatry 2009 80: 273-278

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Importance of recanalisation of occluded cerebral arteries in stroke patients with and without neurological improvement after intravenous thrombolysis.
In manuscript

ABBREVIATIONS

ACA	Anterior cerebral artery
ADP	Adenosine diphosphate
aPTT	Activated partial thromboplastin time
ASK	Australian Streptokinase study
ATLANTIS	Acute Noninterventional Therapy in Ischemic Stroke
ATP	Adenosine triphosphate
AUC	Area under curve
BA	Basilar artery
BBB	Blood-brain-barrier
CBF	Cerebral blood flow
CI	Confidence interval
$CMRO_2$	Cerebral metabolic rate of oxygen
CT	Computed tomography
CTA	CT angiography
DNA	Deoxyribonucleic acid
DNI	Dramatic neurological improvement
ECASS	European Cooperative Acute Stroke Study
EMEA	European Medicines Agency
ENI	Early neurological improvement
EU	European Union
HI	Hemorrhagic infarct
HMCAS	Hyperdense middle cerebral artery sign
ICA	Internal carotid artery
ICD	International Classification of Diseases
ICH	Intracranial hemorrhage
IMS	Interventional Management of Stroke
IQR	Inter-quartile range
LACI	Lacunar infarct
MAST	Multicenter Acute Stroke Trial
MCA	Middle cerebral artery
MERCI	Mechanical Embolus Removal in Cerebral Ischemia
MMPs	Matrix metalloproteinases
MNI	Major neurological improvement
MR	Magnetic resonance

MRA	Magnetic resonance angiography
mRS	Modified Rankin Scale
NIHSS	*National Institute of Health Stroke Scale*
NINDS	National Institute of Neurological Disorders and Stroke
OCSP	Oxfordshire Community Stroke Project
NO	Nitric oxide
OEF	Oxygen extraction fraction
OR	Odds ratio
PA	Plasminogen activator
PACI	Partial anterior circulation infarct
PAI-1	Plasminogen activator inhibitor type 1
PCA	Posterior cerebral artery
PET	Positron emission tomography
PH	Parenchymal hematoma
POCI	Posterior circulation infarct
PROACT	Prolyse in Acute Cerebral Thromboembolism
pro-UK	Prourokinase
ROC	Receiver operating characteristic
rtPA	Recombinant tissue-type plasminogen activator
SAH	Subarachnoid hemorrhage
SICH	Symptomatic intracranial hemorrhage
SITS-ISTR	Safe Implementation of Treatment of Stroke - International Stroke Thrombolysis Register
SITS-MOST	Safe Implementation of Thrombolysis in Stroke Monitoring Study
TACI	Total anterior circulation infarct
TCD	Transcranial Doppler study
TOAST	Trial of Org 10172 in Acute Stroke Treatment
t-PA	Tissue-type plasminogen activator
u-PA	Urokinase-type plasminogen activator
VA	Vertebral artery

INTRODUCTION

General background

Ischemia, originally the Greek word meaning shortage of blood supply, is a universal pathophysiological factor of any disorder which involves the vascular system of the human body. Cerebral ischemia implies localized reduction of blood flow to brain tissue due to arterial obstruction or systemic hypoperfusion. Acute or chronic restriction of blood flow in a given vascular territory may result in local tissue damage, in unfavourable circumstances to the extent of irreversible death (necrosis, brain infarction). Acute focal ischemia of the brain results in ischemic stroke, the clinical syndrome characterized by a focal (or at times global) neurological impairment of sudden onset, and lasting more than 24 hours (or leading to death), and of presumed vascular origin (standard World Health Organization (WHO) definition) [1].

The consequences of severe stroke are devastating. 16 million first-ever strokes registered all over the world result in 5.7 million deaths annually [2]. Recent statistics demonstrate that the death rate in cerebrovascular diseases per 100000 population can reach up to 250.3-352.7 (males and females, respectively, Russian Federation, 2006) [3], exceeding mortality from neoplasms in the same country and preceded only by ischemic heart disease. Of survivors over 40 years old, 21-24% die within the first year, and 47-51% die within the next 5 years (men and women, respectively) [4]. The incidence of stroke is expected to increase with the growing life expectancy, since rates of stroke increase with age.

The strategies, which were shown to improve stroke survival and prevent severe disability, include: proper management of underlying diseases, building of specialized stroke units, early medical management, and rehabilitation and pharmacological enhancement of functional recovery. Early management remains the keystone of acute stroke care in accordance with the “therapeutic window” concept, which implies that the benefit from any treatment may be gained only if the treatment starts within the first few hours after the onset of ischemic stroke. At the moment, the only effective acute stroke treatment is the method which aims to recanalize the occluded vessel in ischemic stroke and provides restoration of blood supply. This treatment is thrombolysis with recombinant tissue plasminogen activator (rtPA). Evidence supporting the intravenous use of

recombinant tissue plasminogen activator in the early phase of ischemic stroke comes from 2955 cases recruited into randomized controlled studies, main of those are NINDS rtPA stroke trial [5], ECASS (European-Australian randomised stroke thrombolysis studies) I and II [6 7], ATLANTIS (Recombinant Tissue-Type Plasminogen Activator (Alteplase) for Ischemic Stroke 3 to 5 Hours After Symptom Onset) [8]. Intravenous thrombolysis has been shown to improve outcomes of ischemic stroke if applied early enough [9]. The net benefit from intravenous rtPA within 3 hours after stroke onset is approximately 1 fewer dead or disabled per ten patients treated. Intravenous thrombolytic therapy with alteplase (rtPA) is the only approved evidence-based treatment for acute ischemic stroke in USA and Europe. Safety and efficacy of alteplase in clinical routine treatment has been documented by the SITS-MOST study [10 11]. Recently, evidence of a benefit of alteplase within 3-4.5 h after stroke onset has been provided by the ECASS III study [12] and the SITS-ISTR 3-4.5 h study [13 14].

However, there are still unresolved problems of thrombolytic therapy; in particular, patients with large-vessel arterial occlusions are likely to demonstrate poor recanalisation rates (6-44%) after intravenous thrombolysis [15]. More complex algorithms may be needed for this specific subgroup of patients. Further controlled studies are needed to identify which categories of patients with acute ischemic stroke are most likely to benefit and which are harmed or demonstrate lack of treatment response from intravenous rtPA [16 17]. In addition to controlled studies, large observational studies may answer to the previously disputable questions of stroke management, and show the ways to improve stroke care for those in whom conventional intravenous thrombolysis results in poor outcomes. Studies based on large clinical registries have shown their ability to provide important data to improve thrombolysis care in the situation when implementation of randomized trials is complicated by the existence of an approved treatment [18-20].

Occlusion of major cerebral vessels

Large cerebral vessel occlusion is usually implied when terminal part of internal carotid artery (ICA), basilar artery (BA) and, sometimes, vertebral arteries, anterior cerebral artery (ACA), or M1 or M2 segments of middle cerebral artery (MCA) are involved (Figure 1). These locations of occlusion are observed in up to 40-46% of unselected population of patients with ischemic stroke [21 22], of those MCA stroke is predominant, while ICA and BA occlusion are less prevalent [23]. A particular case of large vessel occlusion is the so-called tandem occlusion, the simultaneous thrombosis of terminal ICA and MCA on the same side, which is identified in 17-20% of MCA strokes [24 25].

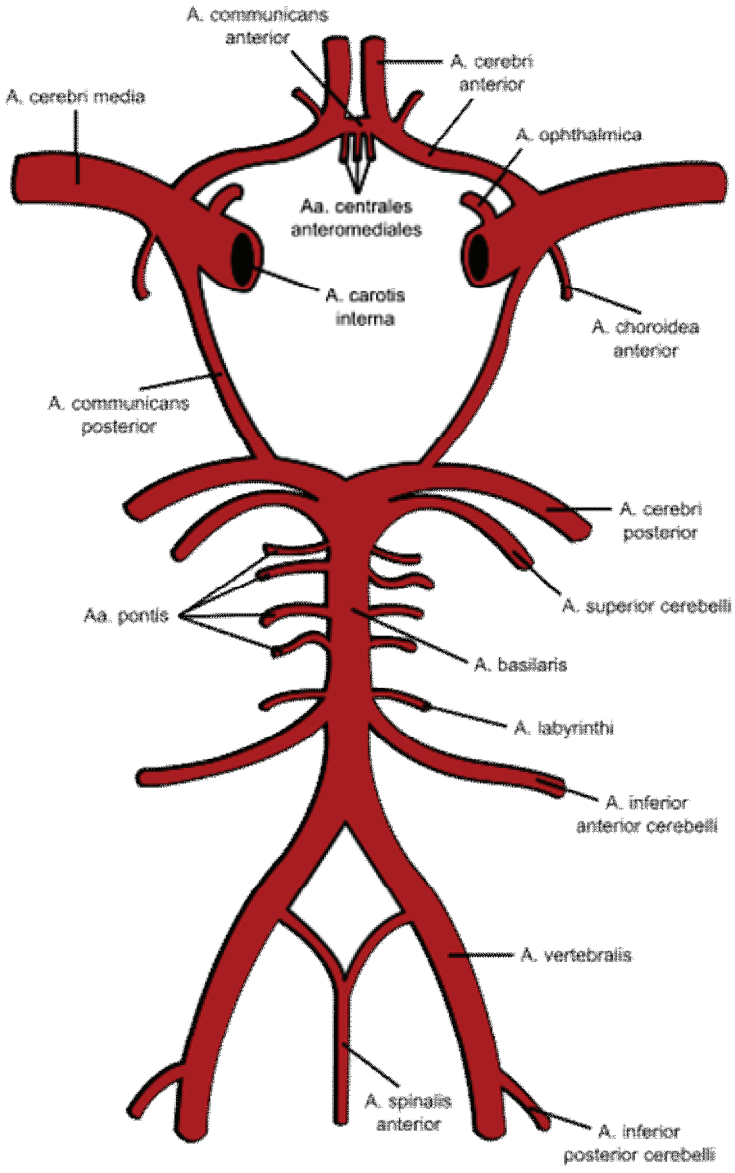


Figure 1. Circle of Willis and major cerebral arteries.

Ischemic stroke caused by large artery occlusion is associated with extensive neurological deficit [26], poor functional outcome and increased mortality [22 27], which, even treated with thrombolysis, reaches 80% in malignant MCA infarction [28], 57% in terminal ICA occlusion [29], and 40-42% in reports of small series of BA thrombosis [30 31]. The optimal approach to the stroke patients with large vessel occlusion at baseline (conventional thrombolysis, intraarterial thrombolysis or mechanical thrombectomy, or combined systemic treatment + local intervention) is still a matter of discussion.

Aetiology and risk factors of major vessel occlusion

The most prevalent risk factors that cause major cerebral vessel occlusion and lead to ischemic stroke are intracranial atherosclerotic disease and atrial fibrillation; diabetes mellitus and smoking predispose to large cerebral artery occlusion by exaggeration of atherosclerotic vascular damage. Arterial hypertension is one of the main risk factors for all types of stroke; however, its pathogenetical mechanisms directly lead to lacunar infarcts, while risk of large vessel occlusion is mediated by acceleration of large vessel atherothrombosis and hypertensive damage of the endothelium of cerebral vessels.

Various diseases represent less frequent causes of stroke, among them arterial dissection, specific genetic disorders [32], pregnancy [33], hypercoagulation disorders, paradoxical embolism via patent foramen ovale [34], other cardiogenic causes of embolism, including myocardial infarction [35], migraine [36 37]. The particular association of the most of these disorders with large vessel occlusion was not clearly established.

Extra- and intracranial atherosclerotic disease

Close association of atherosclerosis and large vessel occlusion is evident yet from the fact that atherosclerotic plaques tend to be situated at the sites of vessel branching, which are typically found along the walls of large arteries. The favourite location of the plaque is the carotid bifurcation and the proximal part of the internal carotid artery, where the character of blood flow predisposes to reduce shear stress [38]. By histological criteria, each atherosclerotic plaque develops through a sequence of several stages. Stages I-III are not thrombogenic and do not produce clinical symptoms; the lesions of these stages are observed in children and young adults [39]. Stage IV is the first stage of the advanced atherosclerosis process, represented by a lipid necrotic mass covered by a fibrous tissue cap. Type V plagues are the lesions with excessive fibrous tissue growth, above the lipid core, sometimes in multiple layers (type Va), with calcification (type Vb), or with minimal amount of lipid fraction, when all constituents, including intima, are substituted by fibrous connective tissue (type Vc). The complicated plaque, i.e. disrupted, causing thrombosis, or

containing haematoma which is a consequence of intramural haemorrhage, is sometimes allocated into stage IV [40].

In a clinical perspective, several mechanisms within the course of plaque development may cause symptoms of cerebral ischemia. Increasing lesion narrows the vascular lumen and reduces blood flow via the involved artery, leading to hemodynamic insufficiency. Stage IV atheroma with a soft lipid rich core which is covered by a thin fibrous cap, is known as a vulnerable plague, which is particularly inclined to a rupture. As soon as fibrous cap disrupts in its weakest part, the highly thrombogenic contents of the core contacts with blood constituents and produces immediate platelet activation and thrombus formation, the so-called atherothrombotic process [41]. It was shown that plaque rupture accounts for the majority of sudden coronary deaths [42] and ischemic strokes in the carotid circulation [43]. Ulceration and erosion of the plaque surface, with the formation of small (usually non-occlusive) thrombi are the other possible mechanisms of causing cerebral ischemic events [44]. Intraplaque haemorrhage, which happens with stage V lesions, is another pathogenetic process that contributes to plaque progression, thus increasing degree of stenosis or promoting plaque rupture [44]. Any progression of the plaque is accompanied by inflammation; as a consequence inflammatory cells migrate into the intimal layer and produce endothelium damage, decrease antithrombotic properties of the endothelium and weaken the fibrous cap of the plague, conducting to its rupture and/or intraluminal thrombosis [41].

Atrial fibrillation and other sources of cardiac embolism

About 80% of embolism-related deaths in atrial fibrillation arise from stroke [45]. Several mechanisms exist that are responsible for prothrombotic state in atrial fibrillation or flutter. Rudolf Virchow, the physician from Germany, was the first who explored in 1856 the phenomenon of deep vein thrombosis and published factors that contribute to the prothrombotic state: abnormal state of the vessel wall, abnormal blood flow, and alterations of blood constituents. A contemporary interpretation of the Virchow triad is the following: endothelial or endocardial damage or dysfunction (and related structural abnormal changes); abnormal blood stasis [46]; and imbalance of haemostasis, platelets, and fibrinolysis [47]. Atrial fibrillation fulfils all three criteria [48].

1. Fibrosis, hypertrophy, degeneration and/or necrosis of the atrial myocytes, fibrillolysis (suggestive of myocarditis or noninflammatory localized cardiomyopathy) was found in atrial tissue biopsies of patients with atrial fibrillation both with and without mitral valve defect [49 50]. Endothelial damage in atrial fibrillation was also described as the “rough myocardium” phenomenon, characterized by oedema, fibrous thickening, erosions, and parietal thrombosis [51].

2. Absence of atrial systole in atrial fibrillation leads to blood stasis inside the left atrium. Increased rate of ventricular contractions, which is often a consequence of atrial fibrillation, reduces effective ventricular filling and worsens the stasis. The anatomical structure of left atrium is also conductive to blood stasis, since left atrial appendage is long with a narrow inlet. Reduced flow velocity in the left atrium was confirmed by a Doppler study [52].

3. All intravascular factors of thrombus formation, i.e. platelets and soluble proteins of the coagulation cascade and fibrinolytic system, are recognized to contribute to the abnormal procoagulation state in atrial fibrillation. Evidence of platelets activation is supported by reports of increased levels of vascular endothelial growth factor which is produced by activated platelets [53], β-thromboglobulin (a platelet-specific blood protein that indicates platelet activation) [54], and platelet microparticles amount (though attributed rather to underlying cardiac pathology than to atrial fibrillation itself) [55]. Elevation of plasma markers of coagulation activation (prothrombin fragments 1+2 (F1+2), thrombin-antithrombin complexes, and D-dimer, the product of fibrin degradation) have been reported in patients with chronic atrial fibrillation, as well as over-expression of von Willebrandt factor in the atrial endothelium in patients with a history of cardiogenic thromboembolism [56-58].

It is important to note that pathophysiological changes in atrial fibrillation might depend on associated co-morbidities (ischemic heart disease, hypertension, and diabetes mellitus) which may have independent impact on the endothelium and balance of serum procoagulant and anticoagulant factors.

Hypertension, diabetes, and smoking

These three major risk factors for stroke have no direct mechanism to facilitate large vessel occlusion, but aggravate the impact of other conditions, especially the progression of intracranial atherosclerotic disease, which, in turn, predisposes to large vessel occlusion. It was shown that relationships between plaque components are consistent with single risk factors: the fibrous carotid plaque correlated with aging and diabetes, the granulomatous plaque with hypertensive females, plaques complicated by mural thrombosis with smoking.

Arterial hypertension is a well-known risk factor of all types of stroke. Mechanisms of ischemic stroke arising from major vessel occlusion in hypertension include large vessel stenosis, acceleration of atherosclerosis development and hemodynamic factors increasing vulnerability of the plague. Increased intraluminal pressure causes disruption of the endothelial barrier functions and direct damage of the endothelium [59]. Endothelial injury provokes excessive deposition of lipids in the intima (injury hypothesis of

atherosclerosis) [60] and progression of plaque growth. Increased intravascular pressure induces adaptation processes in cerebral arteries, namely remodelling and stiffening [61]. Hypertrophic remodelling means that smooth muscle cells undergo hypertrophy or hyperplasia and grow inwards, increasing the wall thickness; thus the lumen of the vessel is reduced. Eutrophic remodelling means that smooth muscle cells rearrange without changing their amount and wall thickness, but with reduction of the vessel lumen. Vascular stiffening results from increase of collagen content and rigidity of the vessel wall. Finally, excessive mechanical forces during repeated cycles of distensions and elastic recoils of the arterial wall in the setting of hypertension lead to the mechanical fatigue of the atherosclerotic plaque with its microfissuring, rupture, and haemorrhage [62]. In addition, arterial hypertension predisposes to cardiac problems, including atrial fibrillation and myocardial infarction, which can be the potential sources of thromboembolism.

Relative risk of cerebral infarction associated with **tobacco smoking** is 1.9 (95% CI 1.7 – 2.2), with dose-response effect [63]. Aggravation of carotid artery atherosclerosis by smoking was demonstrated by several studies [64-66]. Smoking causes direct damage of the endothelium; elevated amount of circulating endothelial cells was found in smokers versus non-smokers in early studies [67]. Nicotine exposure is directly related to ischemic stroke in humans [68]. Cigarette smoking is associated with a hypercoagulable state (increased release of thromboxane A2), which probably is not directly related to nicotine, but possibly to other combustion products [69]; this effect is even more prominent in women taking oral contraceptives [70]. Long-term administration of nicotine decreases the amount of nitric oxide (NO), an endogenous endothelium-released vasodilator, and impairs endothelium-dependent vasodilatation [71 72]. On the contrary, serum concentration of one of the most potent endogenous vasoconstrictors, endothelin, in increased by the intake of nicotine [73], that may result in decreased cerebral perfusion.

In **diabetes mellitus**, relative risk for stroke ranges between 1.36 – 4.1 in men, and 2.25 – 6.8 in women [74]. The typical subtypes of ischemic stroke in diabetic patients are considered to be lacunar and posterior circulation infarcts; at the same time, the prevalence of atherosclerotic carotid stenosis in diabetic patients is twice as high in diabetic vs. non-diabetic subjects (26% vs. 53% [75]). In an early report large vessel stroke was found twice more frequently in diabetic patients with atherosclerotic large artery disease than in non-diabetics [76], indicating greater risk of atherosclerosis progression on diabetes. Diabetes mellitus was shown to be an independent risk factor of both small artery and large artery occlusive stroke [77]. From the pathophysiological perspective, diabetes is accompanied by a chronic state of low-grade inflammation, endothelial dysfunction, hypercoagulability, and dyslipidaemia [78], all directly related to macrovascular complications. Hypercoagulation state in diabetes is maintained by glycosylation of

collagen and platelet adhesion proteins and increased levels of plasma fibrinogen and plasminogen activator inhibitor-1 [79].

Other causes

Among the unusual aethiologies of ischemic stroke, large vessel occlusion is predominantly associated with intracranial arterial dissection and migraine.

The precise mechanisms by which **migraine** may lead to ischemic stroke are currently unknown. Several mechanisms are hypothesized [36 80]: direct ischemic event as a result of vessel spasm (migrainous infarct); endothelial damage caused by migraine pathophysiological mechanisms or exacerbated by them; hypercoagulation state in migraine. Alternatively, association of migraine and stroke may be indirect, linked to higher prevalence of stroke risk factors in patients with migraine, migraine-specific drugs, or genetic predisposition to both conditions. With regard to ischemic stroke, congenital heart defects, in particular patent foramen ovale, have also been discussed as potential biological mechanisms.

Arterial dissections [81] arise from a tear of intimal layer with the subsequent development of intramural haematoma. Dissections are usually associated with a traumatic force that causes mechanical stretching of the artery. The majority of all cases involve extracranial sections of carotid and vertebral arteries. Ischemic stroke symptoms are caused by either hemodynamic insufficiency secondary to narrowing of the vessel lumen, or by thromboembolism distal to the injury site [82 83].

Sources of occlusion

Thrombosis is a process of blood clotting which includes the stages of thrombus formation, propagation, migration, and dissolution. For the formation of thrombus, activation of platelets and formation of fibrin from its precursors in the coagulation system are required. The platelet-fibrin ratio in the composition of the thrombus may vary depending on the local development of fibrin, platelet activation, and the specific features of regional blood flow. At the sites with high blood flow rates thrombi are usually platelet rich, while at the sites of lower shear rates fibrin component is predominant; this mechanism may explain the difference between the thrombi that take shape in atherosclerotic intracranial disease and those in atrial fibrillation.

The process of thrombus formation

The physiological process of thrombus formation starts from the injury of blood vessel wall, which means the loss of the endothelial cell barrier between extracellular matrix

components and flowing blood. The response of platelets to this stimulus develops in three conjugated phases: adhesion, activation and aggregation. Platelets contact with subendothelial layer and adhere to the constituents of the exposed tissue, i.e. collagen, von Willebrandt factor, fibronectin, laminin, and thrombospondin. This adhesion leads to platelet activation. Another cellular initiator of blood coagulation is tissue factor (tissue thromboplastin), which activates the coagulation protease cascade, resulting in fibrin deposition and activation of platelets [84]. Activated platelets change their shape and start signalling to recruit new platelets to aggregate; these signals are ADP and thromboxane A2. The membrane of aggregating platelets is a basis of for the generation of thrombin, which, in turn, promotes fibrinogen conversion to fibrin. Fibrin monomers create a network that serves as frame for growing thrombus, later on forming polymers (mature thrombi contain fibrin trimers and tetramers) [85]. Embolization of thrombus-atheroma material may lead to downstream occlusion. At the same time, spreading of thrombus beyond the injury site is restricted by antitrombotic components of endothelial cells [86], activation of circulating anticoagulants (antitrombin, activated proteins C and S) [87], and endogenous fibrinolytic system, especially an inactive proenzyme plasminogen which converts to active serine protease plasmin. The role of plasmin is to degrade fibrin into its soluble products of its degradation. The mechanism of conversion of plasminogen to plasmin is launched by the two types of plasminogen activators: tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) [88]. Function of plasminogen activators is regulated by its inhibitors, which modulate their activity, such as plasminogen activator inhibitor type 1 (PAI-1).

Thrombogenesis in atherosclerosis and atrial fibrillation

The two main sources of large vessel occlusion, i.e. atherothrombosis and cardiac thromboembolism, are characterized by slightly different mechanism of thrombus formation and, as a result, variety in composition of the thrombotic mass.

In atherosclerotic vascular disease, the process of thrombus formation starts from the plaque rupture of fissuring of its surface. It was clearly demonstrated that components of human atherosclerotic plaques induce aggregation of washed platelets, platelet-rich plasma, and platelets in whole blood, primarily through activation of collagen receptors [89]. Of importance, the stimulation of platelet adhesion and aggregation in anticoagulated blood [89], supporting the primary role of platelet component in atherothrombosis. The size and the shape of thrombus depend on the extent of plaque disruption, the degree of stenosis, and the properties of the surface exposed to the circulating blood [90]. Disruptions of advanced plaques which expose highly thrombogenic lipid core triggers the formation of thrombi few times larger than thrombi generated by exposure of other components of the

arterial wall [91]. The most active platelet aggregation is observed at the narrowest part of the stenotic vessel; vasoconstriction and continued narrowing of the lumen by thrombotic process promote further growth of the thrombus up to the complete vessel occlusion by a dense platelet-rich clot [92].

In cardiac embolism, especially in lone atrial fibrillation, thrombus formation seems to be triggered rather by activation of various components of the coagulation system due to blood stasis in heart chambers, rather than by platelet activation, though platelet function is still impaired [93]. Supporting this assumption, absence of platelet activation in patients with cardioembolic stroke due to non-valvular atrial fibrillation was reported [94]. On the contrary, fibrin was found to be the main component of thrombi extracted both from heart chambers (during the cardiac surgery) and from the lumen of embolized blood vessels (during vascular interventions) [95]. The contribution of abnormal stasis in atrial fibrillation, resulting from reduction of cardiac blood flow during episodes of atrial fibrillation, in the thrombus formation is of primary importance. In a clinical transesophageal Doppler study, it was shown that the risk of stroke increases proportionally to the decrease of atrial velocity during atrial fibrillation [52 96]. Aberrant blood flow is associated with increased interaction between fibrinogen and erythrocytes, the phenomenon which is visible as spontaneous echocardiographic contrast on transesophageal echocardiograms and was correlated to hyperfibrinogenemia [97]. Combination of the aforementioned factors with the activation of the proteins of coagulation cascade, as reflected by the increased concentration of prothrombotic markers [98 99], and hypofibrinolysis [100] determines the formation of fibrin-rich thrombi in left atrium, predominantly in its appendage. As for platelet function in atrial fibrillation, some data demonstrate that platelets are activated, but direct relation of their state to the increased thrombotic risk is uncertain [54 93 101].

Consequences of cerebral vessel occlusion

Normal cerebral circulation and metabolism

Cerebral vascular territories may be principally subdivided into two parts: the anterior (carotid), and the posterior (vertebro-basilar) circulation. Within both territories, three major levels exist: extracranial arteries, large intracranial arteries, and the small superficial and deep penetrating arteries. Large cerebral arteries which enter the skull branch into smaller arterioles that run along the surface of the brain (pial arteries) [102]. The wall of pial arteries has three layers: an endothelial cell layer, a smooth muscle cell layer and adventitia (an outer layer of leptomeningeal cells). Arterioles are surrounded by the so-called Virchow-Robin space [103]. Along with the penetration of arterioles deeper from the surface, this

space disappears and the vascular basement membrane comes into direct contact with the astrocytic end-feet. Within this structure, neuronal processes are coupled with cerebral blood vessels. Pial arteries lumen is regulated by perivascular nerves that originate from autonomic and sensory ganglia and contain vasodilators and vasoconstrictors [104]. Regional flow is managed by precapillary arteriolar constriction due to neural firing or by autocrine activities [105]. This mechanism guarantees shunting of blood (if necessary) and protection of the tissue from ischemia.

Cerebral blood flow is the rate of blood passing the unit of the brain tissue (100 g) per minute. Under normal physiologic conditions, the average cerebral blood flow in a healthy young adult is approximately 45 ± 6.5 ml per 100g of brain tissue per minute [106]. Cerebral blood flow is much higher in gray matter (approximately 80 ml per 100g per minute) than in white matter (approximately 20 ml per 100 g per minute) [107]. Cerebral metabolism is reflected by cerebral metabolic rate of oxygen ($CMRO_2$), and the fraction of oxygen extracted from blood by the brain (oxygen extraction fraction, OEF), measured by positron emission tomography (PET). In a healthy adult, CMRO2 is approximately 3.3 ± 0.5 ml/100g/min, and OEF is 0.44±0.06 [106]. In a resting state of the brain, regional blood flow is closely matched to the resting metabolic rate of the tissue, as shown by PET images of constant relationship or coupling between the local CBF and local CMR02 values [108].

Blood-brain-barrier (BBB) [109] is a specific structure responsible for isolation of brain tissue from immunoactive blood components with the simultaneous preservation of oxygen and nutrient delivery. During the ontogenesis microvasculature of cerebral hemispheres develops in parallel to the neurons, evolving into a system of the so-called “neurovascular units”, a theoretical concept which describes neuron-microvascular interactions [110]. Neurovascular unit consists of microvessels that are functionally coupled with the neighbouring astrocytes, neurons and their axons, and other supporting cells (microglia and oligodendroglia). Astrocytes and endothelial cells interact to form the basal lamina of the capillary. Endothelial cells of the cerebral capillaries differ from endothelial cells in the rest of the body by the absence of fenestrations and extensive tight junctions. Inter-endothelial tight junctions regulate the permeability of BBB for hydrophilic molecules. Pericytes are another type of cells of the microvessels that wrap around the endothelial cells; their function is structural support and vasodynamic capacity of the microvessels. Extracellular matrix protects BBB from the entry of blood cells (erythrocytes leakage or leukocytes migration in response to inflammatory stimuli).

Brain response to ischemia

The immediate result of major vessel occlusion is an abrupt decrease of blood supply in the area corresponding to the basin of the involved artery, with subsequent series of

metabolic and morphologic changes involving cerebral microvascular network and tissue. Ischemic state is characterized by reduction of CBF, reduced $CMRO_2$, and increase of OEF with its subsequent decrease corresponding to the death of brain cells. Cerebral microvessels are considered to be more resistant to ischemia than the neurons they supply because of higher stability of endothelial cells against oxygen deficiency [111]. Alterations of the microvasculature, however, start as early as in 30 min – 2h after experimental focal brain ischemia. The responses of the microvessels to ischemia include the following [112]: loss of the capillary permeability barrier function; loss of the integrity of basal lamina and extracellular matrix; derangement of cell adhesion and expression of leukocyte adhesion receptors on the surface of endothelial cells. Loss of the barrier function leads to endothelium swelling, underlying tissue oedema and narrowing of the lumen with subsequent loss of capillary patency and, sometimes, its rupture. Loss of basal lamina and extracellular matrix integrity facilitates hemorrhagic transformation [113]. Expression of leukocyte adhesion receptors promotes migration of pro-inflammatory cells into brain tissue, which results in local inflammation in the ischemic zone. As a substantial part of this neuroinflammatory response, abnormal expression and activation of matrix metalloproteinases (MMPs) occurs [114]. MMPs are proteolytic enzymes, which take part in remodelling of the extracellular matrix in a normal metabolic state. However, in the setting of ischemia MMPs-2 and MMPs-9 attack the components of the basal lamina (collagen, laminin and fibronectin), thus contributing to capillary wall and matrix degradation [115].

As for the intravascular compartment of the cerebral microvasculature, reduction of the patency of the distal capillary bed results in the so-called “no-reflow” phenomenon [116]. The term means obstruction of the downstream capillary bed after reperfusion of the previously occluded supply artery [111]. This phenomenon is constituted by endothelial cell swelling, external compression from perivascular glial oedema, slowing down of blood flow with intravascular activation of platelets, leukocytes and factors of coagulation (associated with formation of fibrin deposits). These factors prevent the potential restoration of blood supply both from reperfusion of the previously occluded artery and collateral circulation.

For the neurons, sudden oxygen deficiency brings devastating effects, since brain metabolism is dependent on oxidative phosphorylation and is characterized by high demand of oxygen and glucose without any storage of nutrients. Deficiency of energetic substrates inhibits the work of K^+/Na^+ ATP-ase, an ionic pump which serves to maintain ionic gradient inside/outside of the cell membrane. As a result, depolarization occurs, when excitatory synaptic potential of neurons is lost, as well as membrane potential of glial cells [117]. Two important processes follow this alteration of ionic pump function. First, due to activation of presynaptic voltage-dependent Ca^{2+}-channels Ca^{2+}, the universal secondary messenger, initiates release of excitatory amino acids (glutamate, aspartate) into

extracellular compartment (and, moreover, a series of alterations in the cytoplasm and cell nucleus that produce damage of cell outer membranes and internal structures) [112]. Over-activation of postsynaptic glutamate and aspartate receptors (i.e. N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors) is termed "**excitotoxicity**" [118]. Excitotoxicity causes direct cell damage by stimulation of Ca2+ influx into both neurons and glia, which causes activation of enzymatic cascade of reactions, resulting in proteolysis and lypolysis, formation of reactive oxygen species, membrane damage and cell destruction. Second, influx of Na^+ ions into the cell, in the absence of K/Na exchange from a K/Na pump followed by passive transport of water in the same direction, results in cellular oedema, which, in turn, deteriorates the perfusion on the adjacent zones; eventually, **cytotoxic oedema** may result in local brain swelling, increase of intracranial pressure and brain herniation.

Failure of energy metabolism is an immediate ischemic response of cerebral tissue, which occurs within minutes; of importance, the processes that lay behind the crash of energetic metabolism (especially, formation of oxygen reactive species and excitotoxicity) not only lead to cell destruction, but also trigger more-delayed ischemic responses, which develop within hours and days.

Inflammation. In the ischemic area, a number of pro-inflammatory genes are expressed, and pro-inflammatory cytokines and cell adhesion molecules are synthesized [119]. Acting together with the pro-inflammatory mechanisms arising from microvascular damage, these processes lead to further cellular injury and progression of cerebral infarction. First the neutrophils adhere to the endothelium, cross the damaged vascular wall and enter the brain parenchyma, followed by macrophages and monocytes in a few days. Astrocytes and microglial cells are also involved into the inflammatory response [120]. Contribution of inflammation into ongoing brain damage consists in the production of toxic mediators by both inflammatory cells and neurons, among those are nitric oxide (NO), cyclooxygenase 2 (COX2), tumor necrosis factor (TNF)-α, as well as reactive oxygen species.

Apoptosis [121]**.** Apoptosis is a delayed pathway of programmed cell death, contrary to necrosis, which is an acute destruction. Mechanism of cell death in this pathway is activation of genes that augment intrinsic mechanisms of cell death; the main component of those is a proteolytic system called caspases [122]. Activated caspases modify key factors of homeostasis and repair proteins in the way that leads to cell destruction. Expression of corresponding genes is known to be activated by excessive glutamate receptor activation, Ca^{2+} overload, free oxygen radicals, and by mitochondrial and DNA damage. Caspase activity may be detected as early as at 9 h after experimental cerebral ischemia, and apoptotic cell death reaches its peak between 24–72 h [123].

The ischemic penumbra

The degree of critical reduction of cerebral blood flow in acute stroke is uneven over the ischemic area; existence of the gradient of CBF decline leads to the formation of several different tissue structures. Generally, neurological dysfunction occurs in a tissue after CBF falls below approximately 18-22 ml/100g/minute [124]. The centre of ischemic area usually becomes a zone of the most compromised blood supply, where CBF falls below 10-12 ml/100g/min (10-25% from the normal value) [124]; this zone is termed the infarct core. The core is surrounded by the zone where reduction of blood flow is sufficient to cause functional impairment but the tissue is still viable, without immediate irreversible failure of energy metabolism and cell death – the ischemic penumbra [125] [126]. Reduction of CBF in this area is reported as between 10-17 ml/100g/min [124]. At this level of energy failure, protein synthesis is inhibited, but glucose utilization is still retained; which results in functional (loss of electrical activity) but not morphological (membrane degradation) damage.

Penumbra, in turn, is surrounded by the zone of benign oligemia, where CBF reduction is not so severe and constitutes approximately 17-18-20 ml/100g/min; the most part of neurons in this zone survive stroke. It should be noted, however, that CBF thresholds for infarct core and penumbra show a considerable variation in different reports depending on methodological considerations [127] [128].

Histologically, the core of the infarct is characterized by morphological injury with total necrosis of all tissue structures, both neurons and glia. In penumbral zone, functional damage is prevalent over morphological, and neuronal injury is scattered, which is sometimes termed as “incomplete infarction”; for the initial few hours after an artery occlusion, tissue at risk has uncertain chances of infarction or recovery. The reversibility of metabolic alterations in the penumbral area depends on the duration of ischemia. If blood supply is not restored quickly, i.e. in 2-3 hours from animal studies and approximately 4-6h (and even up to 48 h in small series) from human imaging studies [129], tissue damage proceeds to irreversible state. Evolution of stroke is characterized by propagation of neuronal injury over time and infarct core expansion with gradual disappearance of penumbra. The penumbra concept is a basis of stroke clinical pathophysiology, since penumbra has become a theoretical target for therapeutic attempts of neurological deficit reversal in acute ischemic stroke.

Collateral blood flow

Collateral circulation is a term that refers to a system of complementary blood vessels, which are capable of providing blood supply when the main passage is compromised. The first level of collateral circulation is the circle of Willis, where anterior

and posterior communicating arteries provide the bridges between the main vascular territories. The second level is represented by distal leptomeningeal vessels and extracranial-intracranial anastomoses connected with ophthalmic artery, middle meningeal artery, and occipital artery (Heubner's anastomoses) [130]. Collaterals are not functioning under physiologic conditions, but are recruited by diminished blood pressure in downstream vascular bed in the setting of stroke [131]. Development of smaller collateral pathways takes time, and is likely to start after larger blood flow channels from Willis circle fail [132]. The capabilities of collateral circulation depend on patient's age, duration of ischemia, and associated conditions that bring additional cerebrovascular risk. In acute cerebral ischemia, collateral circulation compensates reduced blood flow at the border zone of the infarct and contributes to viability of penumbral tissue [133].

Clinical presentation of large vessel occlusion [134]

Carotid circulation

Anterior cerebral artery (ACA) [135]

ACA is a paired artery which originates from internal carotid artery and advances forward. The area of its blood supply is anterior part of frontal lobe and anteriormedial part of the cerebral hemisphere, including medial parts of frontal and parietal lobes, and, via deeper penetrating arteries, anterior portion of subcortical white matter and corpus callosum. ACA occlusion in typical cases leads to infarction of frontomedial cortical area.

Clinical signs may include:

- the motor deficit in the contralateral leg with predomination in the distal part; sometimes less marked motor deficit in the arm is also present (precentral gyrus)
- mild sensory deficit in the leg (anterior part of the precentral sensory cortex)
- frontal lobe features: urinary incontinence (paracentral gyrus), apathy/abulia/akinetism/mtuism (mediobasal cortex),
- apraxia (frontocerebellar tracts)

Middle cerebral artery (MCA) [136 137]

MCA is the main vascular basin of the carotid circulation, which serves as a blood supply for the largest part of the cerebral cortex. It originates from internal carotid artery and, compared to ACA, runs laterally and inferiorly. Branches of MCA give blood supply to the cortex of posterior and lateral parts of frontal lobe, temporal lobe, and the most of parietal lobe, and to the most of subcortical white matter. MCA occlusion may result either in the whole MCA territory infarct (usually devastating), or involve one or several of its

branches, which are subclassified into superficial (cortical and medullary perforating) and deep (lenticulostriate) arteries.

Clinical presentation, depending on the certain areas involved, may include:

- contralateral hemiparesis of leg, arm, and mimic muscles (precentral gyrus, motor cortex)
- contralateral sensory loss (postcentral gyrus, sensory cortex)
- motor (temporal lobe of a dominant hemisphere) or sensory (aphasia
- apraxia, agnosia, alexia, acalculia, agraphia (non-dominant parietal lobe)
- homonymous hemianopia (white matter in the deep portion of temporal lobe)
- conjugate eye deviation (deep frontal lobe)
- deep penetrating arteries: isolated movement disorders
- medullary perforating arteries: pure motor deficit, pure sensory deficit, sensory-motor deficit, ataxic hemiparesis

Syndrome of malignant middle cerebral artery occlusion [28] is characterized by large infarction within MCA territory and progressive development of brain oedema and herniation with deterioration of consciousness in the next 24-48h; prognosis is poor.

Internal carotid artery (ICA) [138]

ICA arises from carotid bifurcation, runs up and enters the skull through foramen ovale. On its way it passes through the petrous part of temporal bone, where gives small branches, which may anastomose with the internal maxillary artery. Further on it forms the S-shaped structure (carotid siphon), which is located near the venous plexus of cavernous sinus; after that ICA gives one of its most important branches, ophthalmic artery, as well as small branches to the hypophysis, hypothalamus and optic chiasm. The next important branches are posterior communicating artery (if present), and anterior chorioidal artery. Finally, ICA bifurcates into ACA and MCA, where MCA is usually the direct continuing branch.

Extracranial portion of ICA is a classical site of atheroma location.

Since ICA bifurcation constitutes a part of Willis circle, occlusion of its proximal part may be transient, of mild severity, or even asymptomatic due to compensation from primary collateral circulation or from anastomoses with ipsilateral external carotid artery branches. On the contrary, distal ICA occlusion ("T-occlusion" [139 140]) or tandem occlusion (ICA+MCA [24 25]), may result in a large hemispheric infarction.

Signs of symptomatic ICA occlusion, in addition to symptoms of ACA and MCA involvement, may include:

- monocular blindness (ophthalmic artery occlusion)
- ocular + cerebral hemisphere ischemic signs (ophthalmo-pyramidal syndrome)

- mild to moderate contralateral motor, sensory and visual field deficit (anterior chorioidal artery)
- syncope episodes

Vertebrobasilar circulation

Vertebral artery (VA)

Two symmetrical vertebral arteries originate from ipsilateral subclavian arteries, run along the vertebral column, enter the skull, and join. Before joining each of VA gives off the posterior inferior cerebellar artery, which feeds inferior part of cerebellum and lateral part of medulla. Analogously to ICA, occlusion of VA may be asymptomatic, but in some cases results in large infarction involving cerebellum and lateral medulla. Occlusion of posterior inferior cerebellar artery is associated with clinical Wallenberg syndrome (ipsilateral Horner syndrome, bulbar syndrome, facial paresis, ipsilateral limbs ataxia, hemisensory disturbances, dizziness, nausea/vomiting, and nystagmus – all together or in variations).

Basilar artery (BA)

BA is the main arterial trunk of vertebrobasilar circulation, which is formed by the joining of two symmetrical vertebral arteries. It gives many symmetrical branches to supply blood to brainstem and cerebellum, and bifurcates again in the end of its course. Terminal branches that are located close to the connection of BA and posterior communicating arteries feed thalamus. Due to symmetry of branching, total BA occlusion leads to bilateral damage of brainstem and cerebellum ("locked-in" syndrome). Distal occlusion of BA below its terminal bifurcation involves blood supply of posterior cortical areas and deep branches which feed thalamus and upper brainstem. Partial occlusion of BA or its branches causes various symptoms of cranial nerve dysfunction and cerebellar symptoms:

- cranial motor nerves: facial palsy, disarthria, dysphagia, dysphonia, various eye movement disorders and dyplopia
- cranial sensory deficits: facial sensory loss, loss of taste
- autonomic nuclei of the brainstem: Horner syndrome, dysfunction of respiratory and cardiovascular regulatory structures
- involvement of VIII cranial nerve nuclei: vertigo, tinnitus, hearing disorders
- cerebellum: ataxia, nausea, vomiting, dizziness, nystagmus, tremor
- brainstem white matter: decreased level of consciousness, hemiplegia, hemisensory deficits
- "locked-in", or isolation, syndrome: tetraplegia, bilateral palsy of facial and bulbar muscles, loss of horizontal eye movements, with spared consciousness

- "top of the basilar" syndrome: decreased level of consciousness, amnesia, cortical blindness (due to bilateral PCA occlusion, see below), abnormal papillary response, paresis of vertical gaze, ptosis, pathologic involuntary movements (hemiballismus)

In the most unfavourable clinical scenario, thrombosis of the basilar artery results in severe motor deficit (quadriplegia), bulbar symptoms, and impaired consciousness (coma); prognosis is poor in terms of both survival and functional recovery.

Posterior cerebral artery (PCA) [141]

PCA is a symmetrical artery, and the two PCAs are normally the terminal branches of BA. In approximately 20% of individuals, however, PCA originates from ICA (the so-called fetal variant). In a typical variant, it connects with the carotid vascular territory via posterior communicating artery. In relation to this communication point, vascular territory of PCA is subdivided into precommunicating and postcommunicating segments. PCA gives blood supply to occipital lobe (postcommunicating part) and medial temporal lobe and adjacent structures, including thalamus (mostly precommunicating part).

- occlusion of precommunicating portion of PCA: drowsiness (thalamus), loss of upward gaze, contralateral motor deficit (white matter), ataxia (white matter), sensory disturbances accompanied by pain (thalamus), ; if bilateral – coma and tetraplegia due to extensive brainstem infarction
- occlusion of postcommunicating portion of PCA: homonymous hemianopia, quadrant hemianopia (visual cortex of the occipital lobe), cortical blindness (if bilateral); memory loss, agnosia, amnestic aphasia (medial temporal lobe).

Clinical classification of stroke syndromes

Stroke patients constitute a group widely heterogeneous by disease severity. For this reason, many attempts to develop clinically applicable classifications were made, in first order, to give individualized prognosis for certain cases and to stratify patients for different therapeutic approaches, in second order, to use in health economy calculations and academical research. Of importance, clinical classification of acute stroke is supposed to be rapidly applicable in the setting of limited time frame, preferably without a need for time-consuming investigations and laboratory tests [142]. Classification based on stroke aetiological mechanism [143] is logical but demonstrates modest accuracy in the acute clinical setting [144] and requires extensive patient evaluation. Classification based on vascular topography is less useful in clinical practice, since isolated occlusion of a certain major artery with its classical clinical presentation is not so frequent, and variations of anatomy may bring much diversity into the variations of symptoms. The widely used clinical Oxfordshire Community Stroke Project classification [145] is based on the practical

approach to vascular topography, with regard to classical neurological syndromes; however, clinical subtypes still predict underlying vascular pathology to a certain extent.

Syndrome	Clinical presentation	Primary association with underlying cause [142]
Total anterior circulation infarct (TACI)	Higher cerebral dysfunction + homonymous visual field defect + ipsilateral motor and/or sensory deficit of at least two areas of the face, arm, and leg (if unconscious, higher cortical deficit and visual field deficit presumed)	Cardiac embolism Large artery atherosclerosis, but less common
Partial anterior circulation infarct (PACI)	Two of the three components of the TACI syndrome (higher cerebral dysfunction alone, or motor/sensory deficit less extensive)	Large artery atherosclerosis Cardiac embolism, but less common
Lacunar infarct (LACI)	Pure motor stroke Pure sensory stroke Ataxic hemiparesis Acute isolated movement disorders	Small artery disease
Posterior circulation infarct (POCI)	Ipsilateral cranial nerve palsy + contralateral motor and/or sensory deficit Bilateral motor and/or sensory deficit Disorder of conjugate eye movement Cerebellar dysfunction without ipsilateral long-tract deficit Isolated visual field defect	All mechanisms approximately equally important, including unusual causes

Recanalisation

Natural course of cerebral artery occlusion

Mechanisms of physiological fibrinolysis

The formation of thrombus in a human organism is tied to the triggering of the intrinsic mechanism of endogenous fibrinolysis [88]. Thus, the natural history of cerebral artery occlusion is reperfusion. The physiological fibrinolytic mechanism takes part in the limitation of thrombus formation and prevents excessive thrombus growing, as well as in maintenance of a patent vasculature after thrombus formation. The endogenous fibrinolytic system consists of plasminogen, plasminogen activators (PA), and their inhibitors. Active thrombin, catalyzing the conversion of fibrinogen to fibrin, at the same time stimulates release of plasminogen activators from endothelial cells [146]. PAs convert an inactive proenzyme, plasminogen, to the active enzyme, plasmin, which degrades fibrin and fibrinogen. Plasmin formation takes place both in the plasma and on suitable reactive surfaces, like thrombi or endothelial cells. Both components of thrombus formation contribute to plasminogen activation: the fibrin network serves as the frame, while platelets (together with endothelial cells) express receptors for plasminogen binding [147].

Both types of endogenous PAs, tissue-type and urokinase-type, are secreted from cellular sources, but tPA is considered to play primary role in intravascular setting. Tissue-type PA is known to have high affinity to fibrin due to its enhanced binding to fibrin-bound plasminogen and its activation of plasminogen in association with fibrin [148]. Half-life of plasminogen activators is short (5-8 min for tissue-type PA and 9-16 min for urokinase-type PA [149]) in order to keep the balance between haemostasis and fibrinolysis.

To emphasize, the process of thrombus dissolution occurs at the surface of it, where fibrin and cell receptors anchor plasminogen and its activators in the close proximity to their substrates. Rate of clot lysis depends on the magnitude of thrombus burden and its inner structure, including the relative amount of fibrin content and extent of fibrin cross-linking. Fresh newly formed thrombi are more susceptible to lysis than the old thrombi, which contain fibroblasts and collagen fibers. It is postulated that thrombi which are organized in arteries are composed largely of densely packed platelets and leucocytes connected by fibrin strands, and on the whole are comparatively stable against lysis, while thrombi originating from venous system and heart chambers are composed mainly of fibrin and red cells with a minor platelet component; for this reason, they are more susceptible to lysis. Local flow of blood perfusing the thrombus may contribute to the augmentation of fibrinolytic process [150].

Disorganization of the thrombus

The phenomenon of thrombus dissolution is well-known from the early reports [151 152]. It was shown by angiographic studies that vascular occlusion in acute stroke is present in 80% of cases in the first hour, but only in 20% by 24 hours [153]. At the same time, the clot fragments generated by the process of thrombus disorganization may move to peripheral arteries and occlude downstream blood vessels. Distal migration of clot fragments were shown both in animal [154 155] and in human studies [156-158], in a recent study [159] occurring in up to 16% of cases. Thus, distal migration of the fragments of the original thrombus may contribute to the no-reflow phenomenon either by direct blockade the microvasculature or by the obstruction of nutritive blood flow from more proximal arteries with formation of perfusion deficit.

Revascularization treatments

Acute cerebral artery occlusion leads to brain infarction and irreversible stroke, but revascularization and reperfusion is a natural course of it; hence, establishing reperfusion before tissue death becomes a substantiated goal of stroke management. Prompt restoration of blood supply may minimize the extent of infarction due to reperfusion of potentially viable penumbra, and improve neurological outcome.

Consequences of reperfusion

Recanalisation of the previously occluded vessel and subsequent reperfusion have been proven to restore the brain function when performed shortly after acute ischemic stroke [16 160]. Experimental [161] and human (see above) studies have consistently demonstrated that early tissue reperfusion may limit ischemic tissue enlargement, leading to a reduced infarct size and favourable clinical outcome. Pharmacologically induced recanalisation is a gradual process, since binding and activity of rtPA depend on the thrombus area exposed to blood flow. Once treatment starts, the thrombus softens and partially dissolves, allowing some degree of flow restoration. The restored bloodstream delivers more rtPA to bind with fibrinogen inside the clot. This continuous process keeps on continual clot lysis and enhances residual blood flow until the clot breaks up under the pressure of arterial blood pulsations. Complete recanalisation is implied when perfusion of the main feeding artery and the distal branches is restored, while partial recanalisation means presence of residual thrombus in the main vessel, incomplete filling or occlusion of some of the distal branches. The most consistent predictors of completeness of recanalisation in stroke are vessel size and clot burden [162]. Reestablishment of nutritive

blood supply leads to the restoration of electrical activity in the penumbra cells; clinically, it is reflected by a reduction of neurological deficit.

Neurological improvement

Thus, clinical improvement is the earliest phenomenon associated with the salvage of critically hypoperfused tissue. Both animal studies [163] [164] and human transcranial Doppler monitoring of patients with acute stroke treated with intravenous thrombolysis [165-167] have demonstrated strong association between recanalisation of the previously occluded vessel and reduction of neurological deficit. Rapid arterial recanalisation is associated with higher degree of short-term improvement; possible explanations could be faster and more complete clot dissolution and lower resistance of the distal circulatory bed [168]. In patients with acute stroke treated with intravenous thrombolysis, probability of recanalisation is the highest during the first hour after treatment initiation, and falls dramatically after this period [169]. It is accompanied by substantial reduction of neurological deficit by the end of tPA infusion in approximately 20% of patients with large vessel occlusion in the carotid circulation [165] [167]. Early neurological improvement is strongly associated with recanalisation status [170]. However, clinical improvement not necessarily follows vessel recanalisation immediately. Metabolic recovery of brain tissue, evaluated by single photon emission computed tomography [171], was shown to be absent in the next few hours, but occur as late as several days post-treatment. About one third of patients lacking early clinical response to complete or partial recanalisation have outcomes compared to those who improved after intravenous rtPA [172]. The phenomenon of delayed neurological improvement may reflect interaction of favourable impact of reperfusion with the associated potential damage, such as oedema formation, no-reflow phenomenon with or without persisting distal occlusion, reperfusion injury (see below). If reperfusion is late but still nutritious, resolution of oedema and/or microcirculatory stasis will allow clinically recognizable manifestations of neurological improvement.

Of note, another possible mechanism of blood supply in the setting of acute stroke via collateral vessels still exists. Presence of collateral blood supply was shown as an independent predictor of neurological improvement [173-175]. Strategies aimed to increase collateral flow, such as pharmacologically induced hypertension, have shown its beneficial effect in the selected cases and need further evaluation [176].

Improvement of stroke functional outcome

Functional outcome of acute stroke is assessed in terms of recovery of functions which are necessary for realization of individual professional skills, maintenance of daily living, and achieving independence from caregivers. In large vessel occlusion, recanalisation is the strongest predictor of a good functional outcome of stroke [160].

Beneficial effect of early recanalisation, accompanied by early neurological improvement, is sustained in terms of stroke outcome – three thirds of these patients achieve satisfactory functional recovery at 3 months after stroke [177]. Better outcome is achieved with complete recanalisation compared to partial, as it was confidently demonstrated by detailed monitoring of stroke thrombolysis by transcranial Doppler (TCD) [166 168 177 178] and by angiographic control [179]. Early (within an hour [168]) recanalisation is associated with better outcome; however, late recanalisation (within first 24h) was also shown to be beneficial in comparatively small patient cohorts [169 180 181]. Possible underlying mechanism of the benefit of late recanalisation may lie in salvage of peripheral layers of penumbra, which appear to be relatively resistant to ischemia due to better nutritional blood supply from collateral vessels. Of importance, early neurological improvement after stroke thrombolysis predicts good functional outcome irrespective of underlying mechanism [182 183].

Neurological deterioration

Worsening with or without preceding neurological improvement is an infrequent phenomenon in large cerebral artery occlusion [184]. Potential mechanisms of worsening include reperfusion injury and brain oedema, hemorrhagic transformations of the infarcted area, artery reocclusion.

Reperfusion injury is a term describing the interaction of various pathological processes, which may antagonize the beneficial effects of the restoration of blood flow in the ischemic area, including transcapillary migration of blood cells, platelet and complement activation, postischemic hyperperfusion, and breakdown of the blood–brain barrier [185]. Exacerbation of the brain damage and enlargement of infarct area after reperfusion was first documented in animal stroke models [186]. Leukocytes, when activated, disrupt the blood-brain barrier, infiltrate brain tissue, and release cytokines which trigger an inflammatory cascade in the viable penumbra cells. Platelets adhere to both leukocytes and cerebral microvascular endothelial cells, contributing to the “no-reflow” phenomenon, and together with leukocytes release mediators of tissue injury [187]. Complement activation contributes to inflammatory damage, promoting the release of several potent inflammatory mediators and free radical production [188]. Postischemic hyperperfusion [189] and disruption of blood-brain barrier[190] in association with capillary stasis facilitate development of oedema and extravasation of red blood cells.

Progressive brain oedema is a severe life-threatening complication in large vessel stroke; if it develops after an MCA occlusion, the mortality reaches 79% [191]. Vessel recanalisation may be associated with increased risk of brain oedema and herniation [6 192]. Cytotoxic component of the oedema reflects the degree of ischemic cellular damage, while vasogenic component is related to increase of blood-brain barrier permeability and water

content in the extracellular space. The second mechanism is responsible for the enlargement of infarcted tissue volume, development of intracranial hypertension and brain herniation. Arterial hypertension, hyperglycemia, and fever worsen ischemic oedema [193]. Management of this complication is challenging and may require decompressive surgery and/or invasive non-surgical protocols (hypothermia, hyperosmolar infusions) [193].

Severity of most feared complication of stroke thrombolysis, **intracerebral haemorrhage**, may vary from borderline petechiae to a large haematoma with space occupying effect. Its pathophysiology is closely connected with the ischemic damage of blood-brain barrier and vascular wall. Disruption of the blood–brain barrier leads to blood extravasation, which, in turn, contributes to parenchymal injury through mechanical compression and toxicity of blood components [194]. In addition, rtPA itself is an activator of MMPs (MMP-9), which degrade neurovascular basal lamina and facilitate hemorrhagic transformation of infarcted area after rtPA use [195]. Formation of large haematomas is hypothesized to be related to thrombolysis-induced early fibrinogen degradation coagulopathy and late reperfusion [196]. Factors consistently reported as predisposing to hemorrhagic transformation after stroke thrombolysis (other from thrombolytic agent) are: advanced age, stroke severity, hyperglycemia and history of diabetes, elevation of blood pressure in the acute stage, prior use of antithrombotic medications, coagulation abnormalities, early ischemic signs on CT and large infarct volume [197].

Two types of hemorrhagic complications were described, based on their radiological anatomy: hemorrhagic infarcts (HIs) and parenchymal haematomas (PHs) [6]. HI implies petechiae along the margins of the infarct or within the infarcted area, but without space occupying effect. PH means formation of blood clots in infarcted area with space occupying effect (further subclassified into 2 types by size; see Methods).

In a clinical sense, hemorrhagic transformations are classified into symptomatic (SICH) i.e. causing clinical deterioration in a stroke patient, and non-symptomatic; but definitions from clinical studies are varying and controversial (see table on the next page). Asymptomatic hemorrhagic transformation, especially hemorrhagic infarct (petechiae) is a common phenomenon, and thought to be a natural evolution of early successful recanalisation without any clinical impact [198].

Study	Definition of Symptomatic Intracerebral Haemorrhage (SICH)
NINDS [5]	Any CT-documented haemorrhage that was temporally (within the first 36h) related to deterioration in the patient's condition in the judgement of the clinical investigator (any decline in neurological status)
PROACT II [199]	Hemorrhagic transformation causing neurological deterioration (4-point or greater increase in the NIHSS score or a 1-point deterioration in level of consciousness) within 24 hours of treatment.
ECASS [6]	Intracranial haemorrhage which may be responsible for deterioration, after exclusion of other CT findings, along with a worsening of NIHSS score by ≥4 points
Cochrane definition [200]	Death within 7 days or deterioration of NIHSS score ≥1 if clinically attributed to haemorrhage
SITS-MOST [10]	Local or remote parenchymal haemorrhage type 2 on the 22–36 h post-treatment imaging scan, combined with a neurological deterioration of 4 points NIHSS or more from baseline, or from the lowest NIHSS value between baseline and 24 h, or leading to death

Arterial reocclusion after an initial recanalisation is not a direct consequence of reperfusion, but may occur early after thrombolytic treatment of acute stroke and cause clinical worsening and poor outcome. Symptomatic reocclusion, which is manifested by neurological deterioration, is reported in 4-18% of cases [159 201 202], up to 12% in MCA occlusion [203]. Patients with partial recanalisation appear to be at higher risk of reocclusion than those with complete recanalisation [201]; large artery stenosis is another predisposing factor [203]. In these cases, damage of vascular wall in presence of vulnerable plaque and local decrease of flow velocity and perfusion pressure due to stenosis hypothetically predispose not only to incomplete dissolution of the clot, but also to rethrombosis. At the same time, patients with early reocclusion have better long-term outcomes than patients with the absence of initial recanalisation; possible underlying cause may be short period of initial reperfusion of the penumbra, resulting in transient increase of tissue tolerance to ischemia [201].

Mechanisms of therapeutical recanalisation

Pharmacological agents

Streptokinase originally is a substance released from group C beta-hemolytic streptococci, which was noted to dissolve blood clots. Its mechanism of action is

combination with plasminogen followed by its activation to plasmin; the drug, however, is not fibrin specific, and its use may lead to systemic effect due to depletion of coagulation factors [204]. It has a half-life is 16–90 min. Use of streptokinase in stroke was associated with high rates of hemorrhagic complications [205] [206]. Nowadays indications for use of streptokinase include myocardial infarction, pulmonary embolism, and deep vein thrombosis, but not acute ischemic stroke.

Urokinase is also a nonfibrin-specific serine protease that directly transforms plasminogen to plasmin, capable of activating fibrin-bound and circulating plasminogen. In the current clinical practice pro-urokinase is used; it is a precursor of urokinase, which is characterized by significant fibrin specificity due to a preferential conversion of pro-urokinase to urokinase at the fibrin surface [207]. The half-life of pro-urokinase is 14-20 minutes. In the clinical practice, recombinant pro-urokinase in acute stroke is used almost exclusively for intraarterial thrombolysis.

Recombinant tissue-type plasminogen activator (rtPA) is an analogue of endogenous plasminogen activator produced by endothelial cells. It is characterized by fibrin specificity, since presence of fibrin is known to increase rtPA effect; for this reason, it does not deplete circulating coagulation factors. The half-life of unbound rtPA is 4–6 min [208]. Circulating rtPA is rapidly inactivated by plasminogen activator inhibitor type 1 (PAI-1), produced by endothelial cells and platelets, fibrin-bound rtPA is less susceptible to this inactivation [209]. This mechanism may have substantial clinical impact, since it explains potential resistance of platelet-rich clots to rtPA. Another important clinical issue of rtPA use is direct activation of matrix metalloproteinases (MMPs), especially MMP-9, a family of endopeptidases that catalyze the degradation of the extracellular matrix. MMPs promote neuronal injury in the early times after stroke and, more importantly, breakdown of blood-brain barrier, which facilitates hemorrhagic transformation of infarcted area and development of brain oedema [210].

Other fibrinolytic medications include vampire bat salivary plasminogen activator – desmoteplase, enzyme derived from pit viper venom, which acts as a fibrin depleting agent – ancrod, third-generation synthetic derivatives of tPA (tenecteplase, reteplase), and glycoprotein IIb/IIIa receptor antagonists (Abciximab), which is used as an adjunct to endovascular procedures. At present, these medications are used under experimental protocols only. Several trials of some of these medications in acute stroke were stopped prematurely due to safety concerns because of high rate of symptomatic intracranial haemorrhages in the treatment arm (first part of Desmoteplase in Acute Ischemic Stroke – DIAS-1 [211], Ancrod Stroke Program – ASP I and II [212], Abciximab in Emergency Treatment of Stroke Trial [213]).

Methods of delivery

Thrombolytic medications may be delivered to the site of cerebral arterial occlusion through systemic circulation via intravenous route, locally via intraarterial guided microcatheter, or a combination of these two techniques may be used, starting from intravenous and, if unsuccessful, switching immediately to intraarterial administration. It must be noted, however, that extracranial conducting arteries can be abnormally tortuous, advanced atherosclerosis in the elderly may significantly alter arterial inner structure, and some intracranial arteries pass through rigid bony canals; all these factors may create technical difficulties and/or time delay when manoeuvring the guide to establish intraarterial access [214]. For this reason, it is still discussed if clinical benefit of the intra-arterial approach is counterbalanced by associated delays.

Advantages	Disadvantages
Intravenous route	
Fast Easily available No special equipment required Cheap if compared to other options	Systemic effect is possible, with risk of corresponding systemic complications High dosage Short treatment time window Questionable efficacy in large vessel occlusion [215]
Intraarterial route	
Local delivery of thrombolytic drug to the thrombus Treatment time window extended Lower dosage High recanalisation rates, especially in large vessel (MCA, BA) occlusion	Experienced interventionist needed Angiographic facilities needed Technical problems of microcatheter navigation may be met with advanced age, severe atherosclerotic damage or abnormalities of blood vessels Time delay from admission to angiography, and from initiation of angiography to clot lysis [216] Additional risks of arterial puncture, intubation, sedation
Combined technique	
High recanalisation rate Gradual individualized approach Titrated dose Minimization of time delay	Angiographic facilities needed Experienced interventionist needed Risks of intraarterial thrombolysis Still not approved, testing ongoing

Mechanical revascularization therapy

The main reason to work out a mechanical strategy, when the blood clot in the cerebral artery is physically removed instead of its dissolution, is the fact that thrombolytic drugs carry the substantial risk of systemic and intracranial haemorrhage, and, therefore, have a number of contraindications. Other advantages of mechanical revascularization include rapid onset of action and prompt recanalisation compared to intraarterial thrombolysis; theoretically lower rate of infarct-related haemorrhages due to absence of pharmacological agent facilitating it; higher rate of complete recanalisation, especially in large intravascular clots [15 217]. Of importance, many patients ineligible for any thrombolytic treatment are suitable for mechanical thrombectomy, including those, for instance, with recent surgery, previous strokes, or abnormal haemostasis [218]. Lower risk of symptomatic intracranial haemorrhages allows expansion of time window to 8h and, in special circumstances such as basilar artery occlusion, 12h [219]. Nevertheless, overall rates of intracerebral haemorrhage (ICH) may be slightly higher in patients treated with mechanical thrombectomy compared to standard intravenous rtPA [15 218]. Additional risks of using mechanical devises include intracranial vessel dissection or perforation.

A number of mechanical devices were invented, based on either endovascular thrombectomy, or on mechanical disruption of the clot. Endovascular thrombectomy devices extract the blood clot from the blood stream, physically retrieving it (MERCI Retrieval, Microsnare, and Neuronet) or by means of vacuum aspiration of the thrombotic mass (AngioJet, Penumbra) [217]. Techniques of clot disruption include purely mechanical and laser-assisted, sometimes combined with intraarterial thrombolysis to increase its efficacy.

Development of clinical recanalisation strategies

The first attempts to use pharmacologic fibrinolysis with streptokinase were made in 1960s [220 221], but were unsuccessful because of high incidence of intracerebral haemorrhage and death in the treatment group. The next attempts were made with urokinase [222], also demonstrating significant risk of intracerebral hemorrhagic complications and absence of dramatic early improvement. Experiments with recombinant tissue type plasminogen activator (rtPA) started in 1990s were more encouraging and demonstrated its feasibility and clinical benefit in terms of early neurological improvement and improved stroke outcome [223].

At a later time, thrombolytic medications were evaluated in the large multicenter trials. Based on a positive result from a pilot study, which showed a potential clinical benefit [224], streptokinase was tested in three randomized multicenter trials: the Multicenter

Acute Stroke Trial–Italy (MAST-I, nonplacebo-controlled, randomized) [205], the Multicenter Acute Stroke Trial–Europe (MAST-E, placebo-controlled, randomized) [206], and the Australian Streptokinase (ASK, randomized, double-blind, placebo-controlled) study [225]. All three trials using streptokinase for acute ischemic stroke were prematurely stopped because of a high rate of early death, mostly resulting from ICH, and because of a lack of benefit at outcome in a meta-analysis [226].

Recombinant tissue-type plasminogen activator, commercially available as alteplase, was tested by the two study groups, Northern American and European. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study (NINDS) [5] randomized for rtPA (0.9 mg/kg) or placebo patients with acute stroke within a time window of 3 h after onset. In the first part of it clinical effect of rtPA was tested, defined as an improvement of 4 points over baseline values in the NIH stroke scale score or the resolution of the neurological deficit within 24 h of the onset. The percentages of patients with neurological improvement were not significantly different between the drug treatment and placebo groups, though difference in post-treatment NIHSS scores (8 vs. 12, $p<0.02$) was shown by post-hoc analysis. In the second part, long-term functional outcome was addressed, and a significant benefit was observed for the rtPA group at 3 months. After publication of the NINDS results, rtPA was approved in USA for the treatment of acute ischemic stroke in the first 3h after onset.

The European Cooperative Acute Stroke Study I (ECASS I) [6] was the first European prospective multicenter trial of rtPA, randomizing patients for treatment either with 1.1 mg/kg rtPA or placebo within 6 h after stroke onset. The results of the study were seriously compromised by multiple protocol violations, and the beneficial clinical effect of treatment was shown only for the patients who strictly fulfilled the selection criteria. This result led to the initiation of a new study, ECASS II [7]. In this study patients were randomized to treatment with either 0.9 mg/kg rtPA or placebo within 6 h from stroke onset, stratified into a 0 to 3h and a 3 to 6h groups. The analysis showed a similar mortality in the treatment and placebo groups, higher rate of intracerebral haemorrhage in the treatment group, and significantly better outcome for patients treated with rtPA if favourable outcome was defined as modified Rankin Scale (mRS) score 0-2 (post hoc definition). According to the primary end-point of the study (mRS 0-1), the result was non-significant. The conclusion, however, was that treatment of ischemic stroke with rtPA may lead to an improved outcome if given to selected patients in experienced centres. A recent ECASS III [12] study was initiated to test the efficacy and safety of rtPA administered between 3 and 4.5 hours after the onset of a stroke. In this study, intravenous rtPA administered between 3 and 4.5 hours after the onset of stroke significantly improved clinical outcomes in patients compared with placebo, but was more frequently associated with symptomatic intracranial

haemorrhage. Another rtPA study was Alteplase Thrombolysis for Acute Noninterventional Therapy in Ischemic Stroke (ATLANTIS) [8]. It addressed the efficacy and safety of rtPA for ischemic stroke 3 to 5 hours after symptom onset. The study has shown negative result in terms of both absence of significant improvement of functional outcome, and increase of the rate of symptomatic intracranial haemorrhage. As a conclusion, thrombolysis with rtPA for acute ischemic stroke beyond 3h time window after symptom onset was not recommended by the authors. Nevertheless, the pooled analysis of rtPA randomized controlled studies has confirmed the strong association between early rtPA treatment and favourable stroke outcome [9] [227]. At the request of the European Union regulatory authorities in 2002, the SITS International Stroke Thrombolysis Registry (SITS –ISTR) organised a monitoring study (SITS-MOST) which reported that safety and efficacy in clinical routine was comparable to the outcome of pooled randomized controlled trials [10] [11]. Later, SITS-ISTR confirmed that functional independence at three months was similar if treatment was initiated between 3 and 4.5 h after stroke onset, compared to within 3 h [13]. A follow up survey confirmed an increase of mortality and risk of symptomatic intracerebral haemorrhage in the later time window, but the absolute increase was modest. The authors concluded that the wider time window offers an opportunity for treatment of those patients who cannot be treated earlier [14].

As an alternative to systemic thrombolysis with intravenous delivery of the medication, local thrombolysis via intraarterial approach using urokinase or rtPA was also tested. In early studies [216] [228] [229], the combined rate of partial or complete recanalisation of the previously occluded vessel in stroke patients was clearly higher (up to 90%) than that in intravenous thrombolysis, but the conclusions regarding clinical benefit were not possible. Randomized acute stroke treatment trials of intraarterial thrombolysis were performed with pro-urokinase (prolyse) [230]: Prolyse in Acute Cerebral Thromboembolism I (PROACT I) [231] was a randomized trial of recombinant pro-urokinase vs. placebo in patients with angiographically proven proximal middle cerebral artery occlusion. Use of pro-UK was significantly associated with arterial recanalisation and symptomatic intracranial haemorrhage. PROACT II [199] was a randomized controlled open-label clinical trial of intra-arterial pro-UK, but with blinded follow-up, also performed in patients with middle cerebral artery occlusion. In this study, the primary outcome was functional measure; pro-UK was associated with improvement of mRS score and higher recanalisation rate, but with higher symptomatic intracranial haemorrhage as well. It is postulated that intra-arterial thrombolysis of acute proximal MCA occlusion with 9 mg/2 h significantly improves outcome if administered within 6 h after stroke onset. Studies of local thrombolysis in acute stroke are still ongoing.

Mechanical revascularization possesses doubtless advantages, but the issues of indications, patient selection and time window are not clearly established yet. At the moment, two mechanical endovascular devices, the Merci Retriever [232] [233] and the Penumbra system [234] are approved for the treatment of acute stroke. Combinations of endovascular mechanical interventions with various pharmacological modalities are rapidly evolving and appear promising [235] [236]. However, despite high rates of vessel recanalisation, the mechanical thrombectomy studies still demonstrate lower rates of good outcomes compared with intravenous and intraarterial thrombolytic trials [237].

Low rate of complete recanalisation, high rate of reocclusion, unsatisfactory outcomes of large vessel occlusion with stroke treatments involving single pharmacological agent or modality [199] [201] [238] have stimulated the search of effective combinations of different treatment strategies. Combinations of therapies are expected to increase the rate of favourable outcome and reduce the likelihood of complications. Combination of intravenous and intraarterial thrombolysis ("bridging approach") has demonstrated its feasibility and safety, as well as high recanalisation rates [239] [240], but direct comparison of combined intravenous/intraarterial approach with standard intravenous rtPA is still ongoing [241].

Current protocols

Intravenous thrombolysis

Intravenous thrombolysis is the first approved and generally accepted ultra-early treatment of ischemic stroke, indicated to patients 18-80 y.o. with clinical diagnosis of ischemic stroke and absence of haemorrhage on admission CT scan. Time window of intravenous administration of rtPA was recently expanded from 3h to 4.5h [12] [242]. The dose of 0.9 mg/kg, but not exceeding 90 mg, is administered over 60 minutes with 10% of the dose given as a bolus over 1 minute under close monitoring of vital functions and neurological symptoms. Due to possible systemic effects this method has a wide range of contraindications [238]:

- Rapid spontaneous improvement of symptoms
- Minor or isolated neurological deficit
- Very severe stroke with major deficits
- Suspicion of subarachnoid haemorrhage (SAH)
- Head trauma or prior stroke in previous 3 months
- Myocardial infarction in the previous 3 months

- Gastrointestinal or urinary tract haemorrhage in previous 21 days
- Major surgery in the previous 14 days
- Arterial puncture at a noncompressible site in the previous 7 days
- History of previous intracranial haemorrhage
- Blood pressure above systolic 185 mm Hg and/or diastolic 110 mm Hg
- Evidence of active bleeding or acute trauma (fracture) on examination
- Taking an oral anticoagulant or, if anticoagulant being taken, INR ≤ 1.7
- If receiving heparin in previous 48 hours, aPTT must be in normal range
- Platelet count ≤ 100 000 mm3
- Blood glucose concentration ≥ 50 mg/dL (2.7 mmol/L)
- Seizure with presumed postictal residual neurological impairments
- Multilobar infarction (hypodensity > 1/3 cerebral hemisphere).
- Absence of consent

European guidelines [243] additionally comment several of these contraindications. Thus, intravenous rtPA may be used in patients with seizures at stroke onset, if the neurological deficit is related to acute cerebral ischemia. Intravenous rtPA may also be administered in selected patients under 18 years and over 80 years of age, although this is outside the current product labelling.

Intraarterial thrombolysis

The main principle of clinical use of intraarterial approach is its application to cases which are expected to have limited response to intravenous therapy. Potential reasons include severe stroke (NIHSS score ≥10), admission between 3 and 6 hours after symptom onset, recent history of major surgery, and large cerebral or intracranial vessel occlusion [238]. However, it is postulated by the same guidelines that intraarterial approach requires admission of the patient to the experienced stroke centre adequately equipped, but availability of intra-arterial thrombolysis should generally not preclude the intravenous administration of rtPA in otherwise eligible patients. At the same time, clear evidence about intraarterial thrombolysis from an adequately powered randomized controlled double-blind clinical trial and adequately powered systematic review are lacking. Based on observational data and non-randomised comparisons, current recommendations for intraarterial thrombolysis with pro-urokinase include [243]:

- acute MCA occlusion within a 6-hour time window – recommended as an option
- acute basilar occlusion – recommended for selected patients

Systematic analysis found no significant differences between intravenous or intra-arterial thrombolysis for basilar occlusion [243]. At the same time, intravenous thrombolysis for basilar occlusion is an acceptable alternative even after therapeutic time window.

Other revascularization techniques

The use of mechanical revascularization devices is still confined to the advanced stroke centres. Data of direct randomized controlled trials with outcome data of these devices are lacking. Approval of MERCI retriever allows its use "to restore blood flow in the neurovasculature by removing thrombus in patients experiencing ischemic stroke" (Food and Drug Association approval) within 8h after stroke onset. Candidates for treatment are considered to be those who are ineligible for intravenous rtPA or who fail intravenous rtPA therapy. Trial of PENUMBRA clot removal system [234] recruited patients who presented within 8 hours of symptom onset with a baseline NIHSS score of ≥8 and an angiographically verified occlusion of a large intracranial vessel. In this study also patients who presented within 3 hours must were not eligible to intravenous rtPA, or refractory to this treatment. Resistance to rtPA was defined by the *persistence of neurological symptoms and the presence of an occlusion* in the target vessel.

A randomized trial is ongoing to determine the efficacy of mechanical embolectomy with the MERCI Retriever device under selection by multimodal imaging criteria – MR and Recanalisation of Stroke Clots Using Embolectomy (MR RESCUE) [244]. Patients are randomized to receive treatment either with the MERCI Retriever and standard medical care or standard medical care alone; primary outcome is functional status on day 90. Inclusion criteria are acute stroke with NIHSS at baseline ≥ 6 and < 30, initiation of treatment within 8 hours from stroke onset, large vessel proximal anterior circulation occlusion on MR or CT angiography (internal carotid, M1 or M2 MCA). Patients treated with intravenous rtPA up to 4.5 hours from symptom onset are included if they have *persistent target vessel occlusion*.

Combined approach

Given the absence of randomized trials, currently no combination of interventions to restore perfusion is conclusively recommended outside the setting of clinical trials. Combination of intravenous+intraarterial thrombolysis [179] was tested in the subgroup of patients with large vessel occlusion and NIHSS at baseline ≥10 presenting with 3h of stroke onset. Patients were given lower dose (0.6 mg/kg) rtPA; after that, *if arterial occlusion was still present*, rtPA was administered via intraarterial route. The IMS II study design was supplied by an ultrasound clot disruption element (Ultrasound Thrombolytic Infusion Catheter, EKOS Corporation) integrated into intraarterial infusion system [240]. On another small study, intraarterial treatment was given if a patient had shown *no early improvement* [245].

Mechanical thrombectomy seems to be safely combined with intravenous and/or intraarterial thrombolysis, but the optimum combinations and clinical indications are not

established yet [233 246]. The Multi MERCI protocol [233] allowed for endovascular intervention after either full- (0.9 mg/kg) or partial-dose (0.6 mg/kg) intravenous rtPA without any alteration of safety profile. Patients with NIHSS score ≥ 8, and stroke symptom duration under 8 h continued with mechanical thrombectomy if conventional angiography has demonstrated *failure to open the intracranial large vessel* by rtPA given within the first 3h. In another but small study, patients with an admission NIHSS score ≥ 10 underwent angiography and endovascular treatment, except if *rapid improvement* was observed after initiation of intravenous rtPA [247].

A randomized trial comparing standard intravenous rtPA with a combined intravenous and intra-arterial approach (IMS III) within the first 3h from stroke onset has started [241]. The first group of patients will receive the standard intravenous rtPA treatment. The second group will receive intravenous rtPA and then undergo cerebral angiography. *Absence of vessel occlusion* prevents further treatment attempts. In the presence of arterial occlusion, one of 4 possibilities of endovascular interventions may be chosen by a treating physician: embolectomy therapy with either the Merci® Retriever or The Penumbra System™, or rtPA infusion through the EKOS® Micro-Infusion Catheter, concurrent with delivery of low-intensity ultrasound energy, or infusion of rtPA though a standard microcatheter at the site of the blood clot in the brain artery [241]. Patients includes are those with NIHSS score ≥ 10 at baseline or > 7 and < 10 with an occlusion seen in M1, ICA or basilar artery on CTA (if done), who received intravenous rtPA within 3 hours of stroke onset.

Controversy: optimal management of large cerebral artery occlusion

Patients with large vessel intracranial occlusion may account up for 28-46% of acute ischemic strokes [21]. They represent the most severe strokes, with severe baseline neurological deficit [27 248], and high mortality: the risk of death in MCA occlusion is reported from 25% to 78% [199 249], 22-53% in ICA occlusion [29 250], and 40- 87% in BA occlusion [215]. Neurological outcomes of these patients both after intravenous thrombolysis and conservative medical treatment are poor, especially in cases of basilar artery occlusion and carotid terminus occlusion [22 25 27 31 251 252]. To date, neither intravenous nor intra-arterial thrombolysis with only a single pharmacological agent seem to be an efficient way to rapidly recanalize occluded major brain arteries [238]. The recanalisation rates of intravenous rtPA for proximal arterial occlusion range from only 10% for internal carotid artery occlusion to 30% for proximal middle cerebral artery occlusion [253]. Thus, the search of more effective approach for intravenous rtPA non-responders is warranted; use of alternative and combined reperfusion strategies is extensively supported by experts [162 219 254], while direct randomized comparisons are still ongoing. At present, answers to the two

of the unresolved questions could contribute substantially to the solution of the problem of large vessel occlusion.

1. Up-to-date evidence of intravenous rtPA effect in stroke patients with large vessel occlusion.

Modest rate of reperfusion and greater benefit in patients with distal branch vessel occlusions was shown in early studies with intravenous rtPA [153]; at the same time, quality of general stroke care has substantially improved over the last 10-15 years. Data from recent studies reporting the outcomes of stroke with large vessel occlusion treated with intravenous rtPA are lacking.

2. Basis for establishment of criteria for combined treatment algorithms.

As may be seen from the description of current protocols, it is not clear whether arterial recanalisation, or neurological improvement, or both, is the most important evidence of rtPA effect for routine clinical practice. Evaluation of arterial status by cerebral angiography is the gold standard, but in the clinical routine it may bring additional costs, risk of procedure complications, and necessity of patient transportation to a tertiary stroke centre. Thus, studies aimed on the clinical patterns of cerebral artery occlusion/recanalisation are needed to define the milestones to guide treatment decisions in the acute phase.

AIMS

The **general aim** of the present thesis was to study effects of recanalisation after intravenous thrombolysis in patients with documented large cerebral artery occlusion. Information on clinical effects of recanalisation could indicate the need and potential value of additional approaches to open occluded vessels beyond intravenous thrombolysis and guide in the planning of future randomized controlled trials of such interventions. Since the benefit of rtPA infusion comes from recanalisation of the occluded vessel, we also aim to seek clinically applicable signs hereof, which may serve as prognostically important markers early in the course of the disease. Simple non-invasive predictors associated with early vessel recanalisation (or its absence) could be used for the planning of additional treatment protocols. Based on this, the **specific objectives** were:

- To define the clinical and CT-based indicators of occlusion/recanalisation status.
- To explore the baseline characteristics associated with large vessel occlusion.
- To explore the clinical course of recanalized versus non-recanalized patients with initial large vessel occlusion.
- To define clinically useful signs that may serve as indicators of successful (in terms of prognosis of favourable outcome) recanalisation after intravenous thrombolysis.

MATERIALS AND METHODS

The present study is based on the data collected with Safe Implementation of Treatment of Stroke - International Stroke Thrombolysis Register (SITS-ISTR) [10 11 13 14] (http://sitsinternational.org/). The design of all studies is observational.

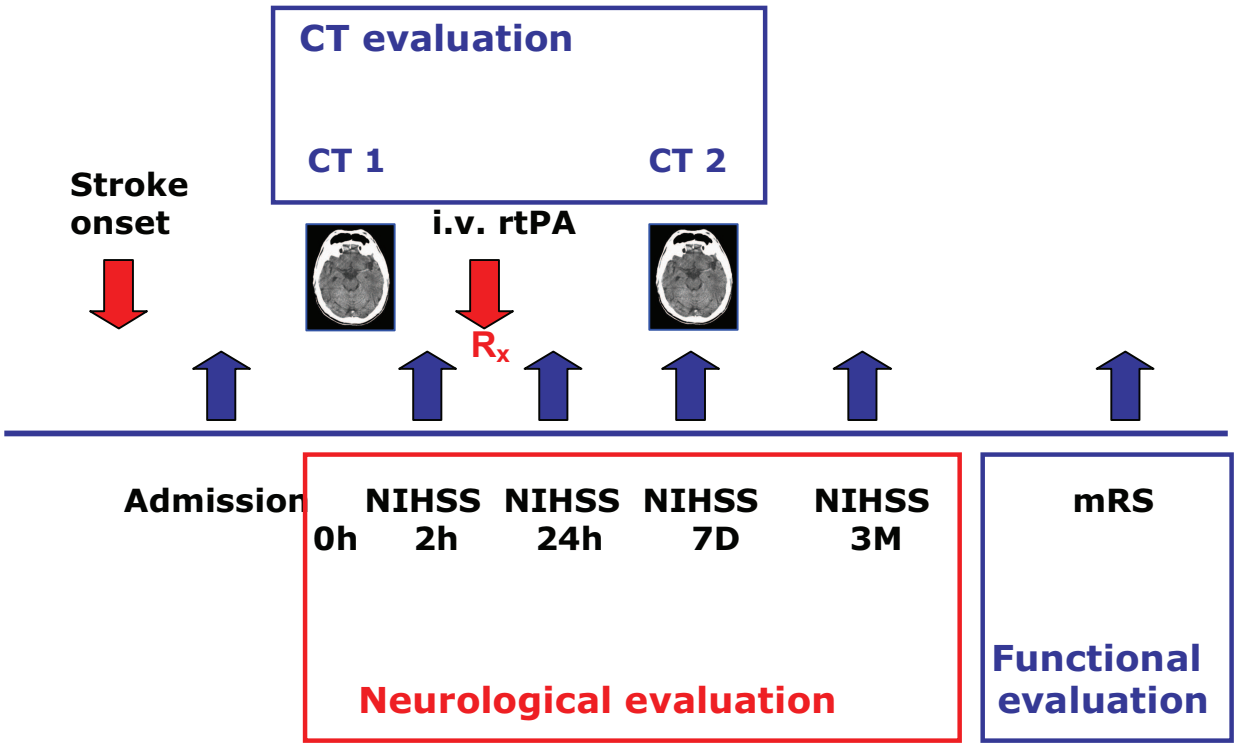
SITS-ISTR is an internet-based, non-profit, open, international database of stroke thrombolytic treatment. The complete registry is owned by the International Collaborative Group, represented by the SITS Scientific Committee (see the Acknowledgements). The Karolinska University Hospital in Stockholm, Sweden is the legal administrator of the registry. Safe Implementation of Thrombolysis in Stroke Monitoring Study (SITS-MOST) is an observational study based on the data of SITS register, which was requested by the European Medicines Agency (EMEA) for the monitoring of treatment outcomes after clinical approval of intravenous thrombolysis in acute stroke in Europe (within the member states of the EU as of 2002, plus Norway and Iceland). SITS-ISTR also includes reports from stroke sites in countries outside the EU; national coordinators in each participating country certified that centres applied for participation in SITS-MOST fulfilled the eligibility criteria. SITS-MOST data are embedded within the SITS-ISTR. SITS-MOST was approved by the ethics committee of the Karolinska Institute in Stockholm, Sweden, and by the Swedish Medical Products Agency. The need for ethics approval or patient consent for participation in SITS-ISTR varied between participating countries, but approvals were obtained in countries in which it was a requirement; other countries approved the register for conduct as an anonymous audit. All the data management was carried out in accordance with the Declaration of Helsinki.

Study subjects

Study material includes records of patients registered from December 2002 to November 2009. The register contains data from patients presenting with ischemic stroke who were given intravenous alteplase (Boehringer-Ingelheim, Ingelheim, Germany). Patient recruitment was based on intravenous thrombolytic treatment license, which implies clinical diagnosis of ischemic stroke, patient's age 18-80, and pre-treatment CT scanning. Contraindications for treatment were defined according to international product license and international guidelines for acute stroke treatment [243]. Baseline and demographic characteristics, past medical history, time intervals, results of clinical assessment, and imaging findings were recorded.

Study design

The design of all studies is observational. The diagnosis of acute ischemic stroke was established on admission. Data were gathered at baseline (0h), at 2h, 24 h and 7d post-treatment. Head imaging (CT and/or MRI) scanning at baseline, and follow-up at 22-36h post-treatment, were required, other imaging optional. In some patients CT or MRI angiography data were also recorded in SITS-ISTR. Functional outcome and neurological status, if possible, were assessed 3 months post-treatment (on day 90).



Variables in the database

All known qualitative data, apart from exceptions discussed below, were entered into the database in the binary form: present or absent. Alternatively, “unknown” remark was also possible to mark the cases distinct from missing data and prevent cases dropout from statistical calculations.

Demographic characteristics included age, sex, and country of origin, code of treating centre. Data of past medical history included stroke risk factors – hypertension, hyperlipidemia, smoking (current or previous), atrial fibrillation, congestive heart failure, history of previous stroke, pre-existing disability; and intake of certain groups of medications – aspirin, dipyridamol, clopidogrel, any other antiplatelet, anticoagulants in therapeutic or prophylactic dose *i.v.* or *per os*, and antihypertensive treatment *i.v.* or *per*

os. Time points recorded by study participants, with calculation of relevant time intervals, were time of stroke onset, time of admission to treating hospital, time of imaging study, time if imaging report, and time of treatment start.

Clinical diagnosis of stroke was stated according to International Classification of Diseases version 10 (ICD-10; I63). Stroke subtype was defined according to TOAST criteria [143].

Laboratory values on admission included blood glucose before treatment and serum cholesterol within 24h from symptom onset.

RtPA dose and patient weight were recorded. In-hospital treatment was registered on discharge (antiplatelet, anticoagulant, and antihypertensive medications).

Acute clinical assessment of neurological status was performed by National Institute of Health Stroke Scale (NIHSS) [255], ranging from 0 – absence of neurological deficit, to 42 – the worst possible neurological deficit. The scale consists of 11 subscores. Subscore 12, i.e. distal motor function was not included in the NIHSS score recorded in the SITS-ISTR. Total score and subscores were recorded at baseline (before treatment), 2h after the start of treatment, 24h post-treatment, and on day 7. Other monitoring data included systolic and diastolic blood pressure values at the same time points.

Secondary variables

Stroke clinical subtypes according to OCSP classification [145] were not directly entered into the database by participants. For this reason, approximation from NIHSS subscales records was done in study II. Total anterior circulation infarct (TACI) was defined as a combination of higher cerebral dysfunction, homonymous visual field defect, and ipsilateral motor and/or sensory deficit of at least two areas of the face, arm and leg. We derived presence of TACI from the baseline NIHSS records with the assumption that facial palsy and sensory deficit (side of both not specified in the database) were corresponding with the side of presumed MCA occlusion.

Neurological improvement was a secondary measure of reduction of neurological deficit, calculated from difference between baseline and 2-hour or 24-hour NIHSS scores. Various cutoff values of early neurological improvement (ENI) expressed either in absolute values of score reduction or in percentage of score reduction, were tested in study III.

Definitions of the early neurological improvement found in the previous publications were:

1) NIHSS improvement ≥4 points from baseline (the “NINDS definition”) [5]

2) NIHSS 0 or 1 on follow-up, or improvement ≥8 from baseline (major neurological improvement (MNI)) [182]

3) NIHSS ≤3 on follow-up, or improvement ≥10 from baseline (dramatic neurological improvement (DNI)) [165 256]

4) Improvement by 20% from baseline NIHSS score [183]

5) Improvement by 40% from baseline NIHSS score [170]

6) Complete neurological improvement (NIHSS score 0 or 1 on follow-up).

Imaging of stroke and large vessel occlusion

On admission, computerized tomography (CT) of the head was the main requirement; other imaging studies, including CT-angiography, MRI and/or MR-angiography, transcranial Doppler study, were optional. Admission CT scans were evaluated for the presence of HMCAS and ischemic signs. Follow-up CT scans were performed within 22-36h of stroke (or earlier if clinically indicated) and evaluated for the presence of HMCAS, ischemic signs, infarct swelling and intracerebral haemorrhage. Other imaging on follow-up was also optional, as well as repeated imaging after 22-36h.

Dense artery sings were described as manifestation of a cerebral artery thrombosis on plain CT [257-259]. Hyperdense middle cerebral artery sign (HMCAS) on admission CT scan is an early sign of middle cerebral artery occlusion. It is defined as density (above 45 HU) of the MCA trunk, higher than that of any other visible artery or vein. CT criteria for interpretation include spontaneous visibility of horizontal part of the MCA, attenuation of MCA higher than that of surrounding tissue, disappearance of higher attenuation with bone window settings, and unilaterality [260]. The sensitivity of the sign for MCA occlusion is 27-54%, but specificity is approaching 100% [261-265]. CT visualization of dense MCA depends on the time, quality, and technical parameters of CT scanning [264 266]. CT appearance of the MCA clot and, hence, sensitivity is influenced by several factors, including are partial volume effect, haemoglobin level, familiarity of a radiologist, composition of the clot. False HMCAS may be caused by arterial calcification, high hematocrit, and use of contrast medium; for this reason, it is recommended to evaluate CT data in the context of the clinical symptoms. It is hypothesized that “white” thrombi within the MCA may cause less increase in X-ray attenuation [267]. HMCAS is known to be an early phenomenon in the course of acute stroke, appearing as an early infarct sign and disappearing in the next few days. Disappearance of HMCAS after thrombolytic therapy implies vessel recanalisation, as verified by cerebral angiography in observational studies [261 263]. Given the high specificity of HMCAS, we used this CT sign to identify the patients with large vessel (MCA) occlusion in the studies I, II, and IV. The presence or absence of HMCAS was judged by local radiologist. In case of a doubt the presence of HMCAS could be defined as uncertain.

In the recent years, modern imaging technologies were increasingly used in acute stroke; multimodal schemes, including vascular imaging, gain more and more popularity in

the current clinical practice. CT angiography and MR angiography allow early detection of large cerebral artery occlusion and accurate visualization of vessel status on follow-up. CT or MR angiography (optional imaging) was the criteria to identify the patients with large vessel occlusion and its resolution in the study III. There was a trend of the use of angioimaging in more experienced and active stroke centres which recruit larger number of patients compared to centres which do not use angioimaging technologies routinely.

The amount of brain swelling was graded as absent, grade I (local), grade II (with mass effect but without midline shift), grade III (causing midline shift), or uncertain.

Hemorrhagic transformation of the infarct was classified according to ECASS criteria[6]. Infarct-related haemorrhages were subdivided into hemorrhagic infarct types I/II and parenchymal haematomas types I/II. HI type I implies small petechiae along the margins of the infarcted area. HI type II means confluent petechiae at the periphery an inside the infarcted area, but without space-occupying effect. PH type I is a dense blood clot not exceeding 30% of the infarcted area. PH type II is a blood clot(s) exceeding 30% of the infarct volume with significant space-occupying effect. Remote (in the areas other than the infarcted area) parenchymal haemorrhage was rare event, classified by the same types as PH.

Outcome measures

Functional outcome of stroke was evaluated on day 90 using modified Rankin Scale (mRS) [268]. Outcomes used in studies I-IV were independence, defined as mRS 0-2 on day 90; unfavourable outcome, defined as death or dependency (mRS 3-6); and death (mRS = 6 on day 90). In study III, additional measure of excellent outcome, i.e. mRS 0-1 on day 90, was used.

Modified Rankin Scale scores	
0	No symptoms at all
1	No significant disabling symptoms
2	Slight disability but does not require substantial help from other person, can walk
3	Moderate disability, requires substantial help from other person, can walk
4	Moderately severe disability, requires substantial help from other person, unable to walk
5	Severe disability, bedbound
6	Dead

Symptomatic intracranial haemorrhage (SICH) was defined as local or remote parenchymal haemorrhage (PH) type II on the 22–36 h post-treatment imaging scan,

accompanied by deterioration of baseline NIHSS score ≥4 points or death within 24h after thrombolysis (SITS-MOST definition) [10].

Numbers and cohorts

Only cases with confirmed baseline data and results of admission CT scan were included into analysis.

In Study I, we studied 10,023 patients with confirmed acute stroke and undoubted presence or absence of HMCAS on admission CT scan, who were registered in the database between December 2002 and October 2006. Of those, 1,905 patients had a HMCAS on admission (19.0%).

In Study II, we continued exploring the cohort of 1905 stroke patients with HMCAS on admission CT scan, registered between December 2002 and October 2006, and followed with the study of subpopulations with disappearance (831 cases, 48%) and persistence (788 cases, 45%) of HMCAS on 22-36h follow up CT scans. In 122 cases HMCAS was uncertain, 164 cases were missing follow-up CT scan.

For Study III, we identified the patients with baseline cerebral artery occlusion documented either by CT Angiography (CTA) or MR Angiography (MRA), who had undergone a follow-up CTA or MRA on 22-36h after thrombolysis, enrolled in the SITS register between December 2002 and December 2008. In total, 21534 patients were recorded in the database at the time of the study, 798 (3.7 %) patients had CTA and/or MRA data available both at baseline and on follow-up. The whole SITS population (n=21534) was included into secondary part of the analysis.

For study IV, two types of cases were recruited, registered between December 2002 and November 2009. First type was patients with baseline large vessel occlusion documented either by CTA or MRA, who were performed a follow-up CTA or MRA between 22-36h after thrombolysis. Second type was patients with HMCAS on admission CT scan, in whom results of follow-up CT scan were reported. Patients of both types meeting the inclusion criteria constituted in total 5324 cases (19% of SITS population). As a part of the accessory analysis, comparison of this cohort of interest with he rest of SITS population (n=22812) was done.

Statistics

Statistical analysis was performed with Statistica 6.0 software for studies I and II, Stata 10.0 and SPSS 13.0 for studies III and IV. Three types of variables were analyzed: continuous (weight, blood glucose and cholesterol), categorical (past medical history and risk factors, imaging findings, outcomes), and ordinal (clinical scores). Age and time intervals were treated as non-parametrical variables in the univariate comparisons

because of non-parametrical distribution. In all studies, we used descriptive statistics; univariate comparisons of 2 groups (studies I-IV) or 4 groups (study IV) in order to test differences between groups; and multivariable analysis (multiple logistic regression) to test the prognostic power of independent predictors and/or to calculate odds ratios for given independents. Receiver Operating Curve (ROC) analysis was performed in Study III to evaluate the ability of neurological improvement, defined in various ways, to diagnose recanalisation by calculation area under curve (AUC). Significance level for statistical testing in Study I and III was set as the probability $p<0.05$. In Studies II and IV multiple comparisons were performed, for this reason significance level for univariate testing was accepted as $p<0.01$. For the multivariable analysis, significance level for independent variables entering the model was set as $p<0.05$ in all studies. Estimation of percentages when reporting descriptive and univariate comparisons was based on reported cases only, missing data were not considered in line with all other SITS publications. In the multivariable models missing data (but not those labelled as "unknown" by the investigators) were casewise deleted.

In **study I**, patients with HMCAS on admission CT scan (n=1905) were compared with non-HMCAS patients (n=8118) by baseline variables and laboratory measurements, stroke aetiology, NIHSS scores at all available time points, admission CT data, intracranial haemorrhage rates, and functional outcomes. For univariate comparisons we used t-tests for numerical data, $\chi 2$ tests (with Yates correction for 2x2 tables or, in case of small expected frequencies, Fisher's exact test) for proportions and Mann-Whitney U test for medians. Multiple logistic regression analysis was performed to identify 1) independent factors associated with HMCAS, 2) independent association of admission HMCAS and 3-month outcome, and 3) independent association of admission HMCAS with SICH.

In **Study II**, we compared patients with HMCAS persistence (n=788) and disappearance (n=831) on the follow-up CT scan by baseline data, admission clinical variables, stroke aetiology, results of neurological assessment by NIHSS, presence of 2h NIHSS improvement, other CT findings, and outcomes. Multivariate analysis was performed twice: 1) to identify independent prognostic factors for HMCAS disappearance and 2) to identify prognostic power of HMCAS disappearance for the functional independence and survival. In addition, we tried to identify a suitable cut off level of 2h NIHSS improvement in patients with HMCAS disappearance to be used clinically for prediction of outcomes; to accomplish this task, we repeated multivariate analyses including 2h NIHSS improvement strata (decline of NIHSS score ≥4, ≥3, ≥2, or ≥1 from baseline) together with previously determined independent predictors of outcome.

In **study III**, first we calculated the area under curve (AUC) by receiver operating characteristic (ROC) analysis, and sensitivity, specificity, positive and negative predictive

values of neurological improvement measured by NIHSS score at 2h and 24h, defined in various ways (see Methods, Secondary variables), for recanalisation of the previously occluded vessel. To test the ability of early neurological improvement to predict recanalisation assessed at 22-36h, we calculated unadjusted odds ratios for each given definition, checked at 2h and 24h (n=798). To validate 2-hour neurological improvement as an independent predictor of favourable outcome in the general population of patients treated with thrombolysis (n=18181), we analyzed the entire study cohort irrespective of the availability of the angiography data. Final adjustment for reported odds ratios was made for age, baseline NIHSS score, and recanalisation at 24h (if angioimaging data available), known as consistent and the most important predictors of stroke outcome. To calculate the odds ratios for 2-hour neurological improvement to predict vessel recanalisation at 22-36h and independence at day 90, multiple logistic regression was used in all analyses.

In **Study IV**, we created an interaction variable between neurological improvement measured by NIHSS score at 2h or 24h and recanalisation of previously occluded vessel. Neurological improvement in this study was defined as 20% NIHSS improvement from baseline. Thus, 4 subgroups were created in the study population at each of the two time points (2h and 24h): 1) patients who have both neurological improvement and evidence of vessel recanalisation; 2) patients who improved despite absence of recanalisation; 3) patients who were recanalized but did not improve; 4) and patients who had neither neurological improvement nor recanalisation. The 4 subgroups were compared by baseline data and outcomes. To define predictors of outcome in the study population, patients with favourable (mRS 0-2) and unfavourable (mRS 3-6) outcome were compared by baseline data and results of early assessment. For the comparison of subgroups in univariate testing, χ^2 (with Yates correction for 2x2 tables or Fisher's exact test, when appropriate) was used for proportions; Kruskal-Wallis and Mann-Whitney U test for scores and non-parametrically distributed numerical data. Multiple logistic regression was performed to define odds ratios for the 4 subgroups, created by interaction of neurological improvement and vessel recanalisation (as described above), separately for the assessment taken at 2h and 24h, for achieving independence (mRS 0-2) at 3 months. Multiple logistic regression was repeated three times, separately for each of the two time points (2h and 24h). First, unadjusted estimates for achieving independence were defined for the groups of interest. Second, multiple logistic regression was repeated with adjustment for baseline factors which were significant at univariate comparison. Third, multiple logistic regression was done to compute the final estimates, after independent variables from the second model with significance level <0.05 were excluded from the final model.

RESULTS

Study I

We explored baseline factors, associated with large cerebral artery occlusion, as determined by presence of HMCAS on admission CT scan, and relation of large artery occlusion (HMCAS) to functional outcome and SICH in stroke patients treated with intravenous rtPA.

HMCAS was found on 19% of all stroke patients who received intravenous rtPA. Patients with HMCAS, compared to non-HMCAS patients, reached hospital at the same time but were triaged faster once in hospital (onset-to-imaging time 90 vs. 95 min, onset-to-treatment time 138 vs. 145 min, $p<0.001$ for both). HMCAS patients were slightly younger (median age 68 vs. 70, $p<0.001$) and more likely to have atrial fibrillation (28% vs. 25%, $p=0.005$) but had lower frequencies of several other risk factors: lower mean blood glucose (7.04 vs. 7.24 mmol/l, $p=0.004$), lower incidence of diabetes (14% vs. 18%, $p<0.001$), and lower mean serum cholesterol (7.9 vs. 8.3 mmol/l, $p=0.004$). Median admission NIHSS score was higher in HMCAS patients than in those without HMCAS (17 vs. 11, $p<0.001$), although the distribution of NIHSS scores overlapped between HMCAS and non-HMCAS groups and included low and high scores in both groups (IQRs 13-20 and 7-17 respectively). An infarct was seen on admission CT scan twice as often in patients with HMCAS (28%) than without (13%; $p<0.001$). The main causes of stroke in patients with HMCAS were large artery disease (with or without significant carotid stenosis) (44%, $p<0.001$) and cardiac embolism (40%, $p=0.01$). In the multivariable analysis, several factors were independently strongly associated with HMCAS, but with low extent of their impact (age: exp(B) -0.012, $p<0.001$; onset-to-imaging time: exp(B) -0.002, $p=0.002$, diabetes mellitus: exp(B) -0.222, $p=0.011$; infarct on admission CT scan: exp(B) 0.340, $p<0.001$; admission blood glucose: exp(B) -0.002, $p=0.016$; admission systolic blood pressure: exp(B) -0.006, $p<0.001$; and admission NIHSS score: (exp(B) 0.119, $p<0.001$). Median NIHSS scores were higher in patients with HMCAS throughout the whole observation period (15 vs. 7 in non-HMCAS at 2h, 14 vs. 6 at 24h, 3 vs. 12 on day 7; $p<0.01$). The patients with HMCAS were more likely to be dead (23% vs. 13%) and less likely to be independent (31% vs. 56%; both $p<0.001$) at three months as were patients without HMCAS. Intracranial haemorrhages, topographically related to the infarct, were more frequent on follow-up imaging in patients with HMCAS (total 20% vs. 12%, $p<0.001$ in non-HMCAS). Using the SITS-MOST definition, HMCAS was not associated with SICH ($p=0.2$). In multiple logistic regression analysis, HMCAS was independently associated with unfavourable outcome at 3 months after adjusting for age, admission

NIHSS score, blood glucose, stroke aetiology; HMCAS was not a significant independent risk factor of SICH by SITS-MOST definition.

Study II

We explored the association of HMCAS disappearance after intravenous rtPA, which implies vessel recanalisation, with early neurological improvement, stroke functional outcome, and SICH, and attempted to find predictors of HMCAS disappearance from baseline.

In our cohort, HMCAS disappeared in 48% of cases at 22-36h follow up CT scans, persisted in 45%, and was uncertain in 7%. Virtually no difference was found regarding past medical history and chronology between HMCAS-disappeared (HMCAS-D) and HMCAS-persisted (HMCAS-P) patients. The HMCAS-D patients had slightly lower median age (67 vs. 69, $p=0.03$), slightly lower admission NIHSS score (16 vs. 17, $p<0.001$) and fewer ischemic signs on admission CT (26% vs. 33%, $p=0.001$).

The proportions of patients with a TACI syndrome were approximately equal in HMCAS-D and HMCAS-P subgroups (48% and 52% respectively, $p=0.08$). The HMCAS-D subgroup demonstrated much more favourable NIHSS profile compared to HMCAS-P: 13 vs. 17 at 2h ($p<0.001$), 11 vs. 17 at 24h ($p<0.001$), and 8 vs. 15 on day 7 ($p<0.001$).

Twice as many HMCAS-D patients achieved functional independence at three months (42% vs. 19%, $p<0.001$) with half as many deaths as in HMCAS-P subgroup (15% vs. 30%, $p<0.001$). The HMCAS-D subgroup had borderline three times higher rate of SICH according to the SITS-MOST definition compared to the HMCAS-P subgroup (1.8% vs. 0.6%, $p=0.06$). In our multivariate analysis, HMCAS disappearance was one of the strongest predictors of good functional outcome at 3 months (OR = 2.26, 95% CI 1.68 to 3.05, $p<0.001$); other independent predictors of outcome in HMCAS patients were age, admission NIHSS score, extent of neurological improvement at 2h.

Based on the association between 2h NIHSS improvement and 3 months outcome in patients with HMCAS disappearance, we categorized the 2h NIHSS difference to identify a suitable cut off level to be used clinically for prediction of outcomes. The proportion of patients with favourable outcomes increased sequentially with each point NIHSS improvement at 2h. Repeated multivariate analyses were performed including 2h NIHSS improvement strata (decline of NIHSS score ≥4, ≥3, ≥2, or ≥1 from baseline) together with previously determined prognostically important factors (baseline NIHSS score, age, HMCAS disappearance). On testing various cut-off levels, we found that any improvement of NIHSS score from baseline (i.e. difference of baseline and 2h NIHSS scores of at least 1) was associated with good functional outcome.

Study III

We explored the impact of early neurological improvement, defined in various ways according to the previous literature, on functional outcome in patients with large vessel occlusion on admission CTA or MRA, and the ability of early neurological improvement (ENI) to predict vessel recanalisation.

In patients with CTA/MRA data available both at baseline and on follow-up, recanalisation on follow-up occurred in 50%, did not occur in 45%, and was uncertain in 5%. The cohort with CTA or MRA data was 2 years younger than the rest of SITS population (65 vs. 67 years, $p<0.001$), with higher baseline stroke severity (NIHSS at baseline 14 vs. 12, $p<0.001$). Median improvement of NIHSS score from baseline to 2h in the recanalized and non-recanalized patients was 3 points (IQR 0-6) vs. 1 (0-4), ($p<0.001$), and 6 (3-11) vs. 2 (0-5) at 24h ($p<0.001$). Median percent of NIHSS improvement in recanalized and non-recanalized patients was 25% (IQR 0-56%) vs. 6% (0-31%) at 2h ($p<0.001$), and 63% (31%-83%) vs. 14% (0-50%) at 24h ($p<0.001$).

Association of ENI with recanalisation, evaluated by ROC analysis, was characterized by AUCs ranging from 0.519 to 0.633 at 2h, and 0.582 to 0.692 at 24h, depending of the definition. At 2h, 20% NIHSS score improvement from baseline had the best trade off between sensitivity (58%) and specificity (69%). At 24h, 40% NIHSS score improvement from baseline had the best trade off between sensitivity (69%) and specificity (70%). Association of ENI with 3-month functional outcome was characterized by AUC range from 0.540 to 0.674 at 2h, and 0.638 to 0.782 at 24h. Overall, 40% NIHSS score improvement from baseline at 2h/24h from baseline (AUC 0.607/0.692), 20% improvement at 2h/24h from baseline (AUC 0.633/0.684) and MNI (AUC 0.570/0.684) were better surrogate marker for vessel recanalisation after intravenous thrombolysis than the other three definitions.

In the multivariable analysis, all definitions of ENI were independently and statistically significantly associated with recanalisation in the CTA/MRA-controlled group after adjustment for age and baseline NIHSS score. ENI was also associated with favourable 3 months outcome (mRS 0-2) after adjustment for age and baseline NIHSS score in the whole SITS population, and after adjustment for age, baseline NIHSS score and recanalisation at 22-36h in the CTA/MRA-controlled group.

Study IV

We investigated the importance of recanalisation status in stroke patients with and without early neurological improvement after intravenous rtPA.

Of 5324 cases (19% of total SITS database) 991 patients (19% of the dataset) had angioimaging on admission and follow-up. In the study population (n=5324), compared to

the rest of the SITS population (n=22812), median age was 68 vs. 70 (p<0.001), median baseline NIHSS score was 16 vs. 11 (p<0.001). Recanalisation was found in 2592 cases (49%), was absent in 2412 (45%), and was uncertain in 320 (6.0%); of those who had follow-up angioimaging at 22-36h recanalisation occurred at 493 (50%), was not found in 453 (46%), and was uncertain in 45 (4 %). Independence (mRS 0-2) at 3 months was achieved in 36% (n=1608) of the study population. Patients who had both vessel recanalisation and neurological improvement at 2h (n=986, 20%) or 24h (n=1469, 30%), in general, were 1-4 years younger (p<0.001) than in the other 3 groups, had less prevalent stroke risk factors, with more favourable baseline profile (lower onset-to treatment time, baseline NIHSS score, blood glucose) compared to the other three subgroups; but the absolute differences between subgroups were subtle. From the data available, we were unable to find any distinctive association of any of baseline factor with neither neurological improvement in persistent occlusion, nor persistent neurological deficit after recanalisation following intravenous rtPA.

In the multiple logistic regression model, we calculated odds ratios of achieving independence for the subgroups of interest, based on the presence neurological improvement at 2h or 24h, and evidence of recanalisation or persistent occlusion at 22-36h. Final models were adjusted for baseline factors, including age, baseline NIHSS score, and previous stroke. Patients who demonstrated both neurological improvement at 2h and vessel recanalisation have far better chances to achieve independence at 3 months; second favourable result was found for those who had neurological improvement with persistent occlusion; third favourable finding was in the group of recanalized but not improved patients. The same order of functional outcome in subgroups was observed with the assessment of neurological improvement at 24h. After adjustment for baseline factors, patients with both neurological improvement at 2h post-treatment and vessel recanalisation at 22-36h demonstrated OR 15.4 (95% CI 12.2-19.4) to achieve independence at 3 months; those who had neurological improvement despite persistent occlusion (n=494, 10%): 4.4 (3.3-5.7); and those without neurological improvement despite recanalisation (n=1564, 32%): 2.9 (2.4-3.5). In subgroups defined by neurological improvement at 24h post-treatment and vessel recanalisation on follow-up, adjusted ORs were: 26.8 (21.0-34.1) in improved and recanalized group, OR 10.7 (8.2-14.0) in improved not recanalized (n=698, 14%); OR 2.2 (1.7-2.8) in recanalized but not improved (n=1081, 22%).

DISCUSSION

Association of large vessel occlusion with baseline factors and stroke outcomes after intravenous rtPA

In the largest known studies based on detection of HMCAS in acute ischemic stroke treated with intravenous thrombolysis, we confirmed that large vessel occlusion (HMCAS) was associated with unfavourable 3 month outcome: approximately one third achieve independence; mortality was 23% (study I). In CTA/MRA-controlled cohort (study III), functional independence was 36%, and mortality 6-33% depending on recanalisation status and immediate clinical improvement. The unfavourable outcome may be in part related to substantial neurological severity at baseline (6 points higher median NIHSS score in study I and 5 points in study IV than in non-HMCAS patients). In the multivariate analysis (study I) HMCAS on baseline CT scan independently predicted poor functional outcome at 3 months. Higher stroke severity and poor functional outcome in patients with HMCAS strengthen the findings of previous smaller studies [264 269].

In studies I, III and IV, patients with HMCAS (study I) on admission and those with arterial occlusion on CTA/MRA (study III, IV) patients were younger than the rest of stroke patients. With matching age, difference in functional outcome between patients with and without evidence of arterial occlusion at baseline could be more striking. We confirmed large artery and cardiac embolism being the prevalent causes of stroke in patients with large vessel occlusion, as defined by HMCAS on admission CT scan (study I). In the multivariable analysis we found several baseline factors to be associated with HMCAS on admission (lower frequencies of diabetes mellitus and previous stroke, but slightly higher frequency of atrial fibrillation), but the clinical implication of these findings is unclear, since the differences did not increase few percent.

The rate of symptomatic intracranial haemorrhage, according to the strict SITS-MOST definition [10], did not differ between patients with and without HMCAS, but the overall rate of infarct-related haemorrhages was higher in HMCAS than in non-HMCAS. These results suggest that patients with HMCAS, compared to patients without HMCAS, were more likely to be found to have infarct-related small haemorrhage on their scan, but not to experience severe neurological deterioration and have an infarct-related large haematoma.

Recanalisation in large vessel occlusion after intravenous rtPA

Recanalisation rates after intravenous rtPA were 48-49% in HMCAS-based cohorts (study I) and 50% in CTA/MRA-controlled cohort (studies III-IV). Evidence of recanalisation had significant positive impact on outcome in all cohorts: in study II, functional independence was 42% and mortality was 15% after HMCAS disappearance. In study IV, in patients with evidence of recanalisation, mortality was 6-19%, depending on the presence of immediate clinical improvement, and highest rate of functional independence (75%) was observed in those who recanalized with immediate clinical improvement, but, at the same time, it was modest (33%) in those who recanalized but had no improvement of their neurological symptoms. These data imply that recanalisation has a significant positive impact on stroke outcomes, especially if accompanied by clinical improvement. Outcomes of patients with evidence of recanalisation were comparable to the patients without the evidence of large vessel occlusion on admission after adjustment for the initial stroke severity (study II, multivariable analysis). In a recent meta-analysis including over 2000 stroke patients treated with different reperfusion techniques or with spontaneous restoration of vessel patency [160], recanalisation was strongly associated with improved functional outcomes and reduced mortality. We confirm this conclusion in regard of patients treated with intravenous rtPA.

In contrast, patients with evidence of persistent occlusion, according to follow-up CT scan or CT/MR angiography, had poor outcomes after intravenous thrombolysis. Functional independence was 19% in HMCAS-persistence subgroup (study I) and 16-50%, depending on combination with clinical improvement, in patients with evidence of persistent occlusion on study IV. Mortality 30% with HMCAS persistence (study I) and 10-31% in combined HMCAS- and CTA/MRA-controlled cohort depending on the presence of immediate clinical improvement (study IV). The poor prognosis in patients with baseline arterial occlusion that persists after intravenous thrombolysis may indicate the appeal for alternative treatment approach to this subgroup.

Baseline factors associated with successful recanalisation after intravenous rtPA were slightly younger age, slightly lower initial stroke severity, and absence of current infarction on admission CT scan. In general, this observation reflects less favourable baseline profile in terms of the prognostically most important factors in patients with persistent occlusion. As for the stroke risk factors, differences between HMCAS-disappearance and HMCAS-persistence patients (study I), as well as between subgroups created by cross-interaction of recanalisation and neurological improvement in study IV, were statistically significant but too subtle to ground any clinical decisions. Hence, the potential to predict the outcome of intravenous thrombolysis in large vessel occlusion from baseline profile is doubtful.

Initial small difference of baseline profiles in favour of patients who subsequently demonstrate evidence of recanalisation can not be explained from our data. Some unrecorded factors may play a role, including composition and size of the clot, presence of residual blood flow [270], site of occlusion (for instance, more proximal or tandem), or higher incidence or degree of underlying arterial stenosis and subsequent re-occlusion after successful thrombolysis. To explore the possible impact of these factors, further studies with contemporary multimodal imaging protocols are needed.

Higher prevalence of type 2 parenchymal haemorrhage in HMCAS disappearance subgroup (study II) does not influence overall favourable 3-month outcome.

Early neurological improvement (ENI) after rtPA in patients with large vessel occlusion

In patients with HMCAS disappearance (study II) reduction of the baseline NIHSS score at 2h, by as little as 1 point, appeared as one of the significant predictors for HMCAS disappearance and good functional outcome. The proportion of patients with favourable outcomes increased sequentially with each point NIHSS improvement at 2h. However, even in case of the considerable clinical improvement (over 5 points NIHSS from baseline score) almost half of the patients demonstrated HMCAS persistence after intravenous rtPA. From these data, lack of neurological improvement could not be recommended as the basis for the decision about additional intervention.

Testing the various definitions of neurological improvement in a comparatively large group of CTA/MRA verified cases, we found that early neurological improvement as measured by NIHSS score at 2h and 24h was strongly associated with vessel recanalisation at 22-36h and functional independence at 3m. Similar results were obtained by studies using real-time monitoring of vessel status after intravenous thrombolysis [165 167 177], this fact supports the potential of extrapolating our findings, with certain limitations, at 1h post-treatment. At the same time, different definitions of ENI were all surrogate markers of vessel recanalisation but have demonstrated fair accuracy in predicting it. Change of NIHSS score at 24h predicted recanalisation better than that of 2-hour assessment, but 24h assessment is too late to influence any clinical decisions. We have shown that about 30% of patients, who had vessel occlusion at baseline, will have persisting occlusion after thrombolysis even if they achieve substantial amount of neurological improvement. This finding suggests the need for evaluation of vessel status by CTA, MRA, or TCD in all cases with previously documented large vessel occlusion.

Of importance, ENI is still associated with favourable functional outcome at 3 months, both in a very large cohort of all stroke patients treated with intravenous rtPA, and in patients with CTA/MRA verified arterial occlusion, irrespective of CT/MR evidence of

recanalisation. For this reason, clinical examination assessed by ENI may serve as a surrogate marker of thrombolytic treatment effect, if objective evaluation of vessel status is not possible. The important aspect is that assessment of ENI is simple, cheap and easily available and repeatable test as compared to angiography of ultrasound. The strong association of 2-hour ENI and favourable functional outcome also supports the use of emergent rescue therapies of stroke if such therapies are available.

Recanalisation status in relation to early neurological improvement after intravenous rtPA

In a large cohort of stroke patients with baseline large vessel occlusion treated with intravenous thrombolysis we have shown that if neurological improvement (immediately after treatment or as late as 24h), not accompanied by recanalisation, results in smaller chances to achieve independence in 3 months, compared to those who successfully recanalized and improved (study IV). Asymptomatic, i.e. without neurological improvement neither immediately nor at 24h, recanalisation also results in moderate improvement of outcome. In the current clinical practice, neurological improvement after intravenous thrombolysis is widely accepted as an indicator of successful treatment; the predominant attitude has been neither to consider additional therapeutic intervention, and consequently, nor additional diagnostic imaging, if the patient has significantly improved after intravenous rtPA. Our results support the active vs. conservative strategy for stroke patients with baseline large cerebral artery occlusion, which includes vascular imaging after treatment despite the extent of neurological improvement, and consideration of additional recanalizing interventions in case of persistent occlusion. Future evaluations of endovascular intervention and other additional strategies, should aim for recanalisation in patients with persisting occlusion after intravenous thrombolysis even in case of significant neurological improvement.

At the same time, our study shows the substantial difference in chances to achieve independence between patients with neurological improvement despite persistent occlusion, and patients who recanalized but did not improve, in favour of predictive power of neurological improvement over late asymptomatic recanalisation. Clinical improvement in persistent occlusion may result from collateral blood supply [173 174] and/or retrograde flow of thrombolytic agent via previously occluded smaller vessels. The accepted goal of acute stroke intervention is recanalisation; but opinions support the recent evidence that recanalisation does not necessarily result in reperfusion, and tissue-level reperfusion may define the outcome to a larger extent than recanalisation [162]. Theoretically, this may lead to a hypothesis that clinicians should target their efforts not only on prompt recanalisation of the occluded vessel, but also on facilitation of neurological improvement. Possible

strategies may be based on augmentation of collateral blood flow, but the therapeutic protocols need testing in fundamental studies and randomized controlled trials.

Limitations

Study design. All our studies were observational and based on prospective clinical register and, therefore, hold all the drawbacks of observational design.

Study population. In HMCAS-based studies, we explored less than 20% of all stroke patients who received intravenous rtPA. In CTA/MRA-based studies, assessment of recanalisation could be based on approximately 4% of the whole SITS population. In all studies patients with large vessel occlusion were younger and had greater stroke severity then the remaining patients in the registry. These factors potentially limit the applicability of the results to general stroke population, especially to mild strokes.

Imaging. HMCAS studies were based on plain CT, which is, though a routine and widely applicable method of acute stroke imaging, of limited value for diagnosis of large vessel occlusion. CT scanning in different centres participating in the SITS-ISTR was not standardized. In HMCAS subpopulation we did not have CT or MR angiography or transcranial Doppler data for most of these patients to examine whether the disappearance of the sign correlated with vessel recanalisation (study II). In CTA/MRA-based data, exact specifying which vessel was occluded, was not provided; hence, homogeneity of the subpopulation of patients who were done CTA/MRA at baseline and on follow-up is not guaranteed. Besides, specification of partial and complete recanalisation was not possible from the data available; thus, partial recanalisation, erroneously interpreted as persistent occlusion, could be the factor responsible for early neurological improvement in some cases.

Accuracy of interpretation. CT scans and CT/MR-based angiograms were read by local doctors with wide variation in experience and expertise for early stroke imaging, so some unintentional errors could have occurred. Participation in SITS register does not oblige compulsory NIHSS certification of clinicians, which could influence the results of clinical assessment. It can not be excluded that the NIHSS scores were influenced by knowledge of imaging findings since the physicians were unblinded to the data of radiological assessment.

Missing data. In HMCAS-based studies I-II approximately 12% of follow-up scans were missing. In 6-7% of cases the presence of HMCAS or presence of occlusion on CTA/MRA was uncertain; these cases were excluded from the analysis, potentially biasing the results.

Lack of real-time monitoring. Follow-up imaging was performed between 22 and 36 h after rtPA treatment, as standard protocol. Therefore, in HMCAS studies we were unable

to retrieve how quickly the sign disappeared, only what proportion had disappeared by 22–36 h. In CTA/MRA-based studies, neurological assessment was done not only simultaneously with vascular imaging (24h post-treatment), but also at 2h, when no data of vessel status was available. Therefore some patients may have had reocclusion between 2h and 22-36h, and these patients increase the proportion of those who are non-recanalized at follow-up CTA/MRA despite an early neurological improvement.

CONCLUSIONS

Based on results obtained from patients with cerebral artery occlusion, treated with intravenous rtPA, several conclusions may be drawn.

1. Patients with large cerebral artery occlusion still get moderate benefit of intravenous rtPA in contemporary clinical practice: recanalisation rates are modest (48-50%), functional independence rate is low (31-36%), and mortality is high (23% in HMCAS patients, 6-33% in CTA/MRA-controlled cohort depending on recanalisation status and immediate clinical improvement). ICH did not contribute significantly to the increased mortality in patients with MCA occlusion (HMCAS). Documented large vessel occlusion at baseline is not the reason to exclude patients from treatment with intravenous thrombolysis, but search of alternative treatment strategies for these stroke patients seems justified. Randomized controlled trials of various treatment alternatives are warranted.

2. Recanalisation in patients with large cerebral artery occlusion significantly improves outcome: in patients with evidence of recanalisation, functional independence and mortality are 42% and 15% after HMCAS disappearance, and up to 75% and 6-19%, respectively, in combined HMCAS- and CTA/MRA-controlled cohort depending on the presence of immediate clinical improvement. In contrast, patients with evidence of persistent occlusion (by HMCAS on follow-up CT scan), had poor outcomes (functional independence 19%, mortality 30%). Any efforts to achieve vessel recanalisation in the acute phase of ischemic stroke may potentially improve outcome in an individual patient.

3. From the data available, no clear association of recanalisation with baseline factors could be established, suggesting that reliable prognosis of intravenous rtPA efficacy in patients with large cerebral artery occlusion is hardly possible.

4. Early (2h after initiation of treatment) neurological improvement is strongly associated with favourable functional outcome both in stroke patients with documented cerebral artery occlusion and in general population of stroke patients treated with intravenous rtPA. At the same time, early neurological improvement of any extent does not reliably indicate vessel recanalisation after intravenous rtPA: sensitivity 7-58% and specificity 69-97% for 2h assessment, sensitivity 25-80% and

specificity 56-92% for 24h assessment, depending on the definition applied. Early neurological improvement in patients with documented cerebral arterial occlusion at baseline, though being a good prognostic sign, should not preclude vascular imaging, if any technique is available. If not available, disappearance of HMCAS on plain CT or 40%, 20% NIHSS score improvement at 2h/ 24h from baseline, or MNI may serve as surrogate marker for recanalisation, but with consideration of their low accuracy.

5. Recanalisation status in patients has a significant impact on stroke outcome in patients with arterial occlusion, both with and without early neurological improvement after intravenous rtPA; in our study independence was achieved in 75% patients with 2h-hour ENI and recanalisation, 50% of patients with 2-hour ENI despite persistent occlusion, 33% of patients without 2h ENI despite recanalisation, and in 16% of those without 2h ENI and with persistent occlusion. Combination of vessel recanalisation and early clinical improvement has demonstrated the most favourable clinical scenario in patients with cerebral arterial occlusion; any efforts to achieve vessel recanalisation in the acute phase of ischemic stroke may potentially improve outcome in an individual patient. Given the independent impact of early neurological improvement on stroke outcome, strategies to facilitate neurological improvement may have potential benefit in case of failed recanalisation, if proven in future randomized controlled trials.

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