Dissertation

ACUTE LACUNAR STROKE:

RISK FACTORS, BIOMARKERS AND PROGNOSIS

submitted by

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Declaration

*I hereby declare that this dissertation is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this dissertation. Due acknowledgement has been made in the text to all other material used. Throughout this dissertation and in all related publications I followed the guidelines of "**Good Scientific Practice**".*

Graz, July 2015

Dedication

This work is dedicated to my deceased grandfather.

Acknowledgements

First, I particularly thank Professor ***Franz Fazekas***. He helped in the design of the current research projects and taught me many different aspects of clinical neurology, neuroimaging and scientific working. It is impressive that as an internationally renowned neurologist and scientist, he always is ready to spend time with his residents to discuss research projects but also personal challenges and worries.

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Finally, I want express my deepest gratitude to my family, my girlfriend, my friends and colleagues for their love and support.

Notification

The current doctoral thesis was the basis for the preparation of a manuscript, which has been published in the ***International Journal of Stroke*** – the official journal of the World Stroke Organization.

The published manuscript was drafted by the doctoral candidate, Thomas Gattringer. Therefore, significant parts of the doctoral thesis (retrospective project) are similar to the published manuscript (with permission of the International Journal of Stroke, managing editor Carmen Lahiff-Jenkins).

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Abbreviations and Definitions

e.g.	*Latin: Exempli Gratia*, for example
CADASIL	Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy
CSVD	Cerebral Small Vessel Disease
MRI	Magnetic Resonance Imaging
i.e.	*Latin: Id Est*, that is
WMH	White Matter Hyperintensities
LDL	Low Density Lipoprotein
SPS-3	Secondary Prevention of Small Subcortical Strokes Trial
DWI	Diffusion Weighted Imaging
STRIVE	STandards for ReportIng Vascular changes on nEuroimaging
RSSI	Recent Small Subcortical Infarct
FLAIR	Fluid Attenuated Inversion Recovery
GFR	Glomerular Filtration Rate
ECG	ElectroCardioGraphy
MR-proADM	Mid-Regional pro-ADrenoMedullin
alpha-MSH	alpha-Melanocyte Stimulating Hormone
BMP-4	Bone Morphogenetic Protein 4
MDA	Malondialdehyde
ADMA	Asymmetric DiMethylArginine
SF36	Short Form (36) Health Survey
BP	Blood Pressure
ADC	Apparent Diffusion Coefficient

SPSS	Statistical Package for Social Sciences
ANOVA	ANalysis Of VAriance
NIHSS	National Institutes of Health Stroke Scale
MUG	Medical University of Graz

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Abstract

Introduction: Lacunar stroke (LS) is responsible for at least an estimated quarter of all ischemic strokes. The morphological correlate of LS is a recent small subcortical infarct (RSSI), which is reliably detected only by brain magnetic resonance imaging (MRI). The causes, pathophysiological mechanisms, treatment strategies and prognosis of LS are incompletely understood and prospective long-term studies are lacking. Therefore, we implemented such a longitudinal study to investigate various clinical, laboratory, neuropsychological, neuroimaging and prognostic aspects of LS in a multidisciplinary manner. In a parallel step, we evaluated the newly suggested imaging criteria for RSSI using a retrospectively identified in-hospital stroke cohort regarding their application in clinical practice.

Methods: In May 2012, we started to invite all in-hospital stroke patients < 76 years with MRI defined RSSI to participate in the longitudinal 15-months lacunar stroke study. Patients receive a comprehensive clinical vascular/stroke workup, assessment of blood biomarkers, neuropsychological testing and retinal vessel analysis, including two follow-up visits at three and 15-months together with extensive MRI investigations of the brain.

For the second project, we retrospectively identified all acute stroke patients from 2008 to 2013 in whom MRI showed a RSSI with an axial diameter ≤ 20 mm. We calculated the largest axial and longitudinal diameter and RSSI lesion volume. Morphometric differences of RSSI regarding location and demographic variables and the impact of various definitions for lesion selection were assessed.

Results: Up to May 2015, 72 patients (mean age: 59.5±11.5 years, 72% men) with RSSI were included in the prospective study. Further 135 patients with RSSI were identified but did not fulfill the inclusion criteria (n=99) or disagreed to participate (n=36). Most prevalent vascular risk factors were hypertension (80.5%), hyperlipidemia (n=75%) and smoking (n=41.7%).

The follow-up rates at three and 15-months are nearly 100%. Patient's adherence to secondary stroke prevention is high and only 3 patients suffered from a recurrent vascular event. For detailed analyses concerning blood biomarkers, neuropsychological function and neuroimaging characteristics, we have planned to continue patient recruitment up to at least a total number of 100 study participants for considerations of statistical power.

Regarding the second retrospective project, we were able to identify 344 patients (median age: 72; range: 25-92 years, 65% men) with RSSI. Most RSSI were located in the basal ganglia (n=111), followed by pons (n=92), thalamus (n=77) and centrum semiovale (n=64). All morphometric variables were strongly correlated and comparable on analyzed MRI sequences (DWI and FLAIR). RSSI in the basal ganglia were significantly ($p<0.05$) larger both in the axial and longitudinal direction compared with other regions. Dichotomization of RSSI according to axial (≤ / >15 mm) or longitudinal (≤ / >20 mm) diameters resulted in different regional frequencies and distributions. Age, sex and time from stroke onset to MRI did not influence morphological parameters or RSSI distribution.

Discussion: Interim analysis of the basic results of the prospective lacunar stroke study shows that such a comprehensive, multidisciplinary project is feasible in clinical practice and also adds to long-term patient care. This is supported by a high patient compliance and a low rate of subsequent vascular complications.

Our integrated retrospective study confirms that the new imaging criteria for RSSI are applicable in clinical routine. Definitions of the maximal axial and longitudinal diameters have a significant impact on the frequency and distribution of RSSI, which has to be considered when comparing studies and for future study design.

Zusammenfassung

Einleitung: Der lakunäre Schlaganfall (LS) zeigt sich für ca. 25% aller ischämischen Schlaganfälle verantwortlich. Das morphologische Korrelat des LS, der rezente kleine subkortikale Infarkt (engl. RSSI), kann in der Akutphase nur mittels Magnetresonanztomographie (MRT) zuverlässig diagnostiziert werden. Die Ursachen, pathophysiologischen Mechanismen, Therapie und Prognose des LS sind noch immer unzureichend verstanden. Wir implementierten eine prospektive Studie mit dem Ziel in einem multidisziplinären Ansatz neue Erkenntnisse zu klinischen Aspekten, Biomarkern, neuropsychologischen Funktionen, MRT-Charakteristika und Prognose zu gewinnen. In einem zweiten retrospektiven Projekt setzten wir uns das Ziel die kürzlich vorgeschlagenen MRT-Kriterien für RSSI in der klinischen Praxis zu überprüfen.

Methoden: Ab Mai 2012 wurden alle stationären Schlaganfall-PatientInnen < 76 Jahre und der MRT-gesicherten Diagnose eines RSSI eingeladen an der longitudinalen 15-monatigen lakunären Schlaganfallstudie teilzunehmen. Die StudienteilnehmerInnen erhielten eine erweiterte klinische Schlaganfallabklärung, Blutabnahmen zur Untersuchung von potentiellen Biomarkern, neuropsychologische Testungen und eine retinale Gefäßanalyse sowie zwei Follow-ups nach drei und 15-Monaten inklusive Studien-MRT mit Spezialsequenzen.

Zusätzlich identifizierten wir retrospektiv von 2008-2013 alle SchlaganfallpatientInnen mit einem RSSI im MRT. Die maximalen axialen und longitudinalen RSSI Durchmesser sowie das Volumen wurden berechnet. Weiters untersuchten wir morphologische Unterschiede von RSSI hinsichtlich deren Lokalisation und demographischer Variablen sowie den Einfluss von verschiedenen RSSI Definitionen auf die Selektion der Läsionen.

Ergebnisse: Bis Mai 2015 konnten 72 PatientInnen (mittleres Alter: 59,5±11,5 Jahre, 72% Männer) in die prospektive Studie eingeschlossen werden. Weitere 135 PatientInnen wurden gescreent, erfüllten aber nicht die Einschlusskriterien (n=99) oder entschieden sich gegen eine

Studienteilnahme (n=36). Die dominierenden vaskulären Risikofaktoren waren Hypertonus (80.5%), Hyperlipidämie (75%) und Rauchen (42%). Die Follow-up Raten nach drei und 15 Monaten waren nahezu 100%, die PatientInnencompliance hoch und nur drei PatientInnen erlitten im Beobachtungszeitraum ein erneutes vaskuläres Ereignis. Um eine ausreichende Studienaussagekraft zu erzielen, ist für weitere detaillierte Analysen (Biomarker, Neuropsychologie, MRT) der Einschluss von zusätzlichen 28 PatientInnen geplant.

Im zweiten Projekt konnten retrospektiv 344 PatientInnen mit RSSI (medianes Alter: 72, Spanne: 25-92 Jahre, 65% Männer) identifiziert werden. Die meisten RSSI waren in den Basalganglien (n=111) lokalisiert. Alle morphometrischen Variablen korrelierten stark miteinander und waren vergleichbar auf den analysierten MRT-Sequenzen. RSSI in den Basalganglien zeigten größere Durchmesser und Volumina im Vergleich zu allen anderen Lokalisationen. Die Dichotomisierung von RSSI nach ihrem axialen (≤/>15 mm) und longitudinalen Durchmesser (≤/> 20 mm) resultierte in verschiedenen Läsionsverteilungen und -häufigkeiten. Das Alter, Geschlecht und der Zeitpunkt der MRT nahmen keinen Einfluss auf die untersuchten Parameter.

Diskussion: Die vorläufige Analyse der Basischarakteristika der prospektiven lakunären Schlaganfallstudie zeigt, dass ein derartiger multidisziplinärer Ansatz mit engmaschiger PatientInnennachsorge in der Praxis anwendbar ist. Dies wird v.a. durch hohe Compliance sowie einer niedrigen Rate an Komplikationen unterstrichen.

Zusätzlich konnten wir zeigen, dass die neuen MRT-Kriterien für RSSI sinnvoll in der Praxis zum Einsatz kommen können. Die Definitionen für maximal erlaubte RSSI Größenparameter nehmen jedoch einen Einfluss auf die Häufigkeit und Verteilung dieser Infarkte. Dies muss beim Vergleich zwischen Studien sowie für die PatientInnenselektion für künftige Arbeiten berücksichtigt werden.

Introduction

Overview of ischemic stroke

Despite recent advances in diagnostic and therapeutic strategies, stroke is still the third leading cause of death in most countries of the world, exceeded only by ischemic heart diseases and cancer.

In the United States every 45 seconds a stroke occurs and every three minutes someone dies due to a cerebrovascular cause.

Poststroke disability and associated neurological conditions like depression, cognitive dysfunction and dementia in stroke survivors is a major global public health problem and causes an enormous economic as well as social and psychological burden. (1)

In Austria approximately 25.000 patients suffer from an acute cerebrovascular disease every year, roughly 5.000 individuals from a recurrent event.

Stroke is a strongly age-depending disease. This can be illustrated by following prevalence rates for Austria: 2% of men and 1% of women between the ages of 45 and 54 years have a stroke, which rises to 6% in individuals aged 65-74 years and >10% in Austrian inhabitants over 75 years. The continuously increasing life expectancy with associated rising stroke prevalence again underscores the socioeconomic burden of the disease. (2)

Stroke is not a homogenous entity. It is the umbrella term for acute cerebrovascular attacks leading to respective clinical symptoms and encompasses ischemic (i.e. ischemic infarction and transient ischemic attacks) and hemorrhagic (i.e. intracranial bleedings) events. Among

those, ischemic stroke is the most frequent entity with about 85% of all strokes in western industrialized countries (***figure 1***).

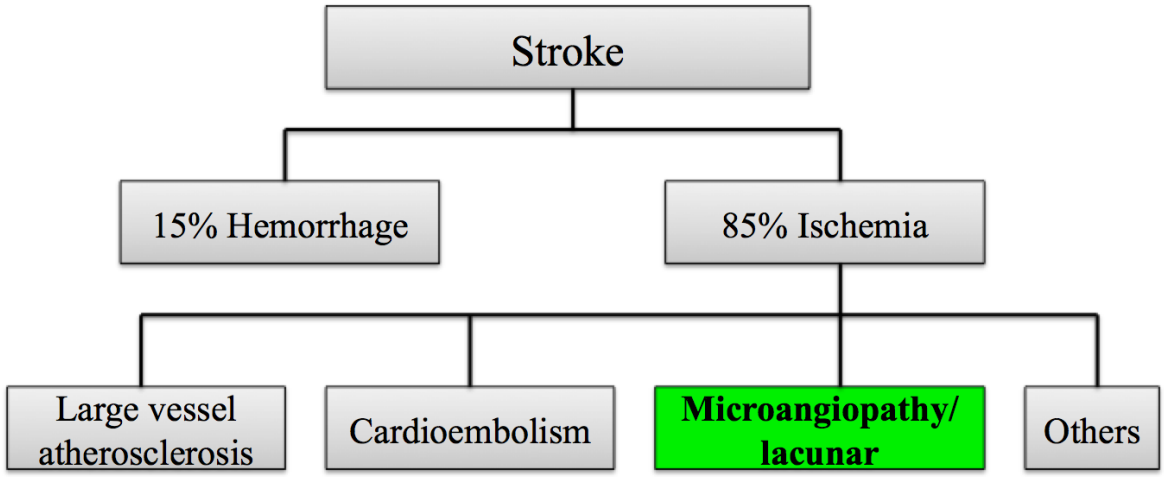


Figure 1. Stroke and major etiologies of ischemic infarction.

Ischemic stroke is caused by the occlusion of one or more brain-supplying arteries, which leads to a deficiency of oxygen and nutrition for the downstream brain tissue.

Acute brain ischemia can be further divided into different mechanisms and underlying etiologies. The main pathologies are thrombosis, embolism and a (systemic) decrease in brain perfusion pressure.

Atherosclerotic lesions with plaque formation of extra- and intracranial vessels (e.g. steno-occlusive disease of the carotid artery) with arterio-arterial embolization in distal vessels or severe hypoperfusion of borderzone areas, cardiogenic embolization (e.g. atrial fibrillation) and alterations of small perforating brain vessels (small vessel disease, e.g. microatheroma and lipohyalinosis) are the most common mechanisms in the pathogenesis of ischemic stroke (***figure 2***).

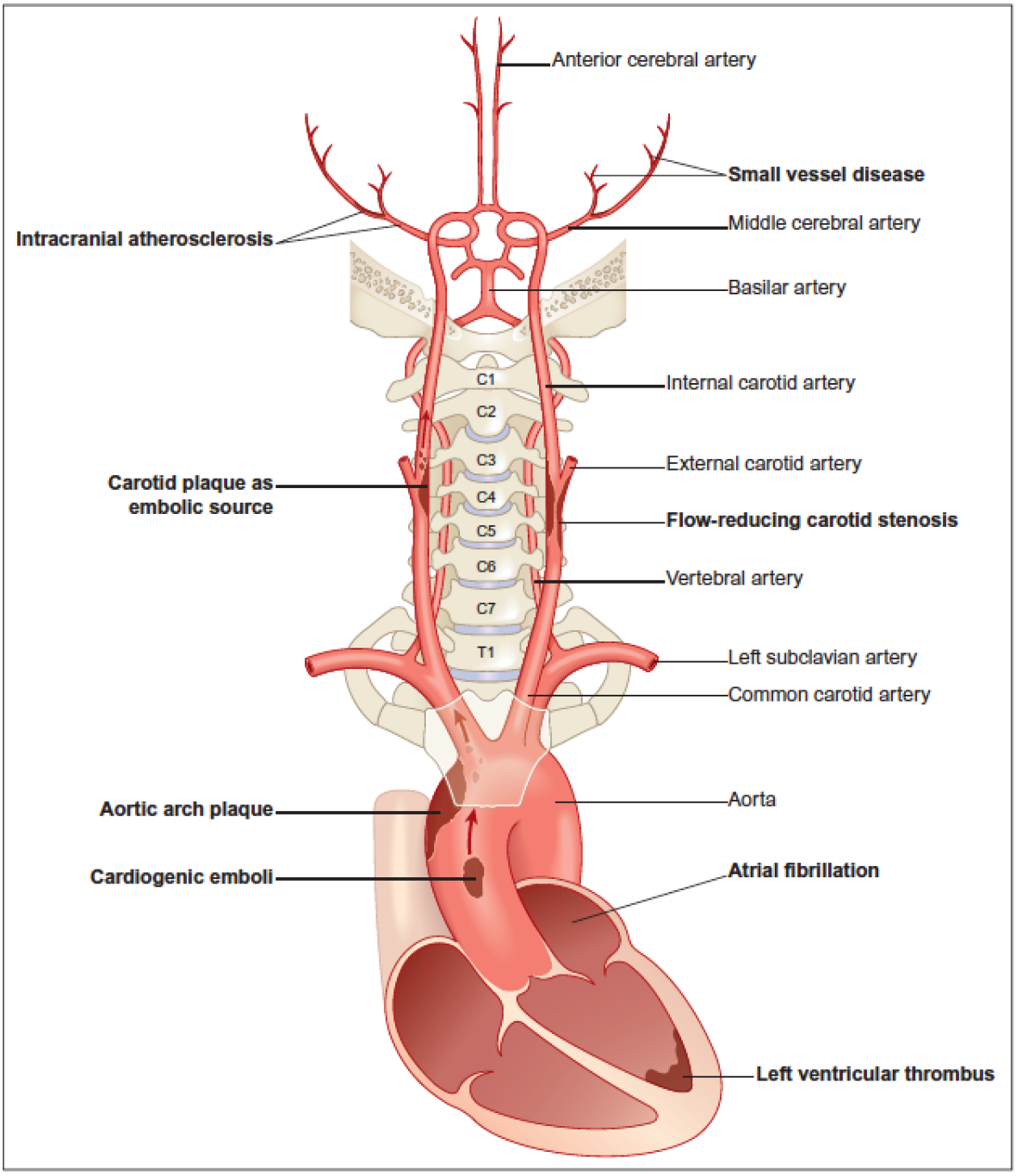


Figure 2. Pathophysiological mechanisms of acute ischemic stroke. (3)

Lacunar stroke and cerebral small vessel disease

Clinical aspects

"Lacunar" stroke is believed to be responsible for 20-25% of all ischemic strokes. It is caused by the occlusion of a deep penetrating small brain artery (*intrinsic cerebral small arteriolar abnormality, microangiopathic stroke*).

The resulting infarcts are typically small with maximal axial diameters of up to 15-20mm and usually occur in the cerebral white matter (internal capsule, centrum semiovale), basal ganglia, thalamus or brainstem.

Symptoms

Clinically lacunar stroke is traditionally related to the classical so called "lacunar syndromes" with the characteristic feature that symptoms of higher cortical functions (e.g. aphasia, neglect, hemianopia) are not present. These syndromes were first described by Charles Miller Fisher in the 1960s and were based on clinical evaluation only. With the development of neuroimaging (computed tomography and mainly magnetic resonance imaging) it became apparent that lacunar infarcts could not always be reliably diagnosed solely by neurological examination. Specifically, a small cortical infarct could mimic a lacunar infarct and vice versa. On the other hand, lacunar infarcts often do not lead to clinically overt acute focal neurological deficits such as when non-eloquent brain areas are affected. Therefore, they do not become apparent as a stroke syndrome, but could cause more subtle clinical signs like cognitive dysfunction, gait disturbances, incontinence or depression especially when "silent" lacunar infarcts accumulate. (1,4)

Approximately 20% of patients with acute lacunar infarction suffer from previous transient ischemic attacks, usually in a close time window to the index event. These attacks are mainly stereotyped and result from an incipient occlusion of one or more perforating arteries with a concomitant decrease in perfusion. In the case of purely motor symptoms, these transient ischemic attacks have been called *capsular warning syndrome*. (5)

Clinical lacunar stroke syndromes	**Frequency (%)**
Pure motor stroke	45-57
Pure sensory stroke	6-17
Ataxic hemiparesis	7-19
Sensory motor stroke	13-40
Dysarthria clumsy hand syndrome	6-18

Table 1. Lacunar stroke syndromes (6)

Pathophysiologic aspects and etiologies

Like the clinical findings also the causes of lacunar infarcts are diverse, in part still poorly understood and turn out to be more and more variable with ongoing research. A reason for this is that unlike in large artery cerebrovascular disease the pathologies in small vessels are more difficult to study, especially as (noninvasive) clinical neuroimaging is not able to directly visualize the respective abnormalities.

The most consistent autopsy findings regarding vessel pathology associated with lacunar strokes encompass:

1. **lipohyalinosis** with thickening and dilatation of arteriolar walls,
2. **microatheroma**, which mainly occur in more proximal segments of perforating arteries and
3. **arteriolosclerosis**.

These pathologies are mainly considered as age and vascular risk factor related small vessel disease.

Cerebral amyloid angiopathy, inherited or genetic small vessels diseases like CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) or immunologically mediated vasculopathies (e.g. Wegener granulomatosis, lupus erythematodes) are other more rare but well-known causes of small artery stroke.

Vasospasms, inflammatory processes, reduced cerebral blood flow, endothelial dysfunctions and blood brain-barrier disruptions are further yet less well established pathophysiological factors that have been linked to this stroke subtype. (7,8)

As these underlying vascular pathologies tend to affect the small vessels in a rather diffuse manner lacunar stroke is usually associated with other (more chronic) morphologic changes in the brain. These features of cerebral small vessel disease (CSVD) (9-12), which are detectable on clinical routine magnetic resonance imaging (MRI) scans include: (chronic) lacunar lesions, white matter hyperintensities, enlarged perivascular spaces (Virchow-Robin spaces), parenchymal microbleeds and brain atrophy (***figure 3***).

Finally, previous reports have indicated that a minor proportion (up to 15%) of lacunar strokes may still be caused by embolism or macroangiopathy (e.g. atrial fibrillation or ipsilateral carotid stenosis), especially in patients without other concomitant signs of CSVD on neuroimaging. (8,13-15)

This is an important aspect to consider as it implies possibly different strategies in secondary stroke prevention (i.e. carotid endarterectomy in high grade symptomatic internal carotid artery stenosis or oral anticoagulation in atrial fibrilliation) and underlines the difficulty of dealing with lacunar infarcts.

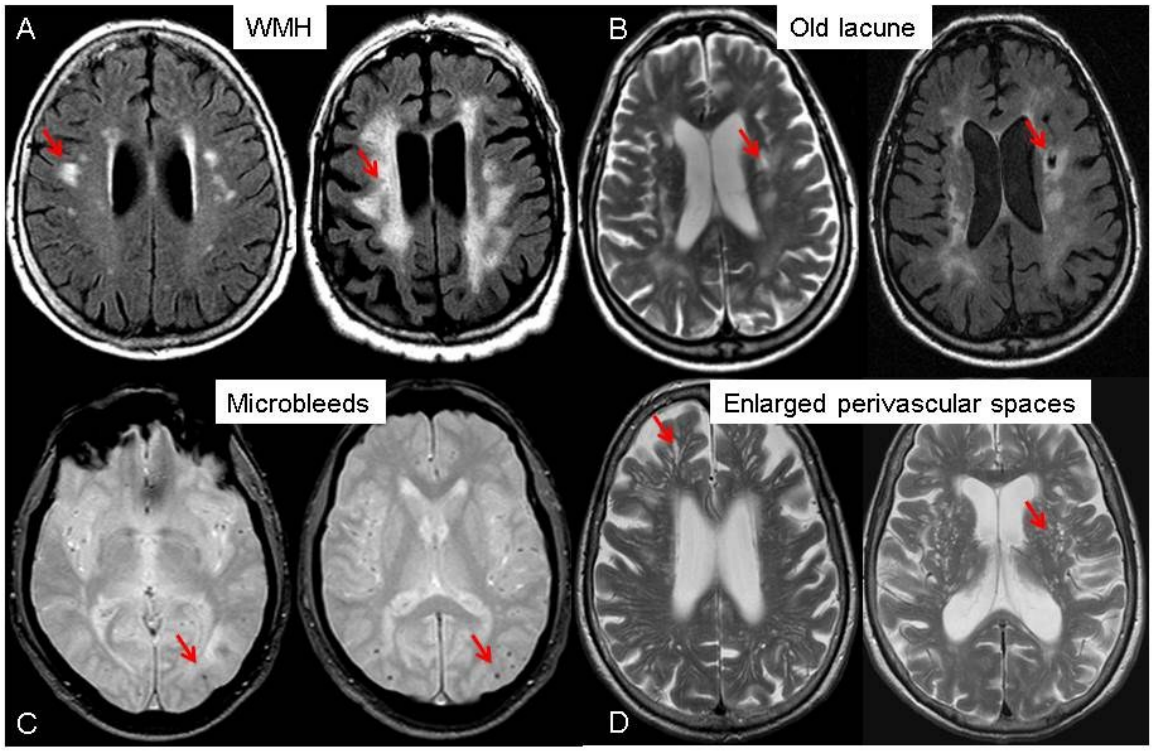


Figure 3. Hallmarks of chronic cerebral small vessel disease (A-D) on MRI. (12)

Therefore it is generally recommended to perform basic cardiac examinations (electrocardiogram, echocardiography) and studies of major brain supplying vessels

(neurosonography, magnetic resonance angiography) in patients with stroke even when the appearance suggests small vessel pathology, i.e. lacunar stroke in the original definition. (16)

Arterial hypertension is the most prevalent risk factor in patients with lacunar stroke. Diabetes, smoking, hypercholesterolemia and obesity are less well established risk factors. Traditionally, it has been postulated that patients with lacunar stroke have generally more severe and more numerous vascular risk factors. (17,18)

However, a pooled analysis of five prospective stroke registries did not confirm this assumption and showed that patients with lacunar stroke had similar rates of hypertension, diabetes and any other established atherosclerotic risk factor compared to individuals with other stroke subtypes after adjusting for important demographic and clinical confounders.(19)

Recent studies indicate however that lacunar stroke and CSVD are associated with small vessel pathologies in other organ systems like the kidney or the retina. It has been shown that patients with lacunar stroke more likely have microvascular retinal changes (thickening of arterial wall, narrower lumen, arteriovenous nipping) compared to patients with non-lacunar stroke. (20-22)

Moreover impaired kidney function (as measured by glomerular filtration rate or proteinuria) was related to CSVD, although this finding was not consistent. (23,24)

Outcome

As previously noted, acute lacunar stroke is associated with classical subcortical symptoms/syndromes in the absence of disturbances of higher cortical functions. Depending on the localization of the acute infarction, patients could show very subtle signs and symptoms, resolving after a short time period or they might suffer from a persistent hemiplegia, when for example a strategically important part of the pyramidal tract is affected

by the lesion (e.g. in the internal capsule). Nevertheless, patients with lacunar stroke have a better short-term outcome (better functional status as well as reduced mortality), compared to patients with strokes of other etiologies, who often have large vessel occlusion with associated large (cortical) ischemic infarction.

Furthermore, it has been shown that patients with lacunar stroke tend to have a lower risk of myocardial infarction and cardiovascular mortality in a 10-year follow up study as opposed to individuals with large-artery stroke or cardioembolism. (25)

Despite a favorable short-term outcome and reduced risk of subsequent cardiovascular events, CSVD (including lacunar stroke) is associated with important long-term sequelae, which are often neglected in the clinical management of these patients. These complications and comorbidities include: vascular cognitive impairment and dementia (mainly executive dysfunction, reduced processing speed and attention), mood disorders (depressive symptoms), sphincter dysfunctions (urinary incontinence), gait disturbances, pseudo-bulbar signs up to severe dysphagia and dysarthria and impairment of daily living activities. (7,26)

Table 2 summarizes the most important complications of CSVD.

Functions	Characteristic deficits
Cognition	Executive function, attention, at later stages cognitive impairment and dementia including memory deficits
Mood	Depressive symptoms, depression
Sphincertic function	Urinary and sometimes faecal incontinence
Gait	Mild slowing, postural instability, apraxic gait, bedridden
Pseudo bulbar signs	Dysphagia, dysarthria, pathological laughing and crying
Daily living activities	Small difficulties up to complete loss of autonomy

Table 2. Complications of cerebral small vessel disease (7)

Moreover, results from the Nun Study indicate that in the presence of cerebral lacunes, a lower amount of typical Alzheimer pathologies is necessary to develop a clinically overt dementia syndrome. This suggests that CSVD is not only an important contributor to vascular dementia, but also a potential trigger in the pathogenesis of neurodegenerative processes or at least promotes their clinical consequences. (27)

All these deficits usually occur slowly progressive, are often not primarily obvious for treating physicians, but associated with a tremendous psychosocial and economic burden for patients, their relatives and the health care system.

Treatment

Based on the fact that lacunar stroke and CSVD pathogenesis are up to now incompletely understood treatment strategies are not well established and often controversial.(28)

However, for prevention it is certainly advisable to carefully treat and control vascular risk factors like especially hypertension (target blood pressure < 140/90mmHg), hyperlipidemia (target LDL-cholesterol < 70md/dl) and diabetes according to clinical stroke practice guidelines. (29,30)

Patients should be encouraged to modify their lifestyle and to quit smoking, perform physical activities for at least 40 minutes at a minimum of 4 days a week and to adapt their nutrition by following a Mediterranean type diet (rich of vegetables, fruits, grains low fat products, fish, olive oil and nuts) and restricting their sodium intake to less than 2.4 grams per day.

Although it is rather uncertain whether lacunar stroke is a pure atherosclerotic disease and as previously discussed other mechanisms might play an important role patients usually receive antiplatelet agents in the case of a presumed non-cardioembolic mechanism for secondary stroke prevention. Recently, the Secondary Prevention of Small Subcortical Strokes Trial (SPS-3) tested the hypothesis, whether patients with acute subcortical infarcts might benefit from a dual antiplatelet therapy (aspirin plus clopidogrel) compared to aspirin alone. The study failed to demonstrate a treatment benefit (reduction of recurrent stroke) of a dual antiplatelet regimen, which was even associated with an increased risk of intracranial hemorrhage and mortality. (31)

In the acute stroke setting, patients with suspected lacunar stroke should receive intravenous thrombolysis like all other patients with acute ischemic stroke although the benefit of such treatment has not yet been studied in detail in this stroke subtype but at least seems to be safe. (32-34)

Magnetic resonance imaging and lacunar stroke

Stroke subtyping based on clinical evaluation is not always reliable and the use of brain magnetic resonance imaging (MRI) including diffusion weighted imaging (DWI) sequences may depict a so-called "clinical-radiological mismatch" in about one fifth of patients. Subcortical infarcts that are located near the cortex and diabetes have been associated with this misclassification. (35, 36, 15)

This means that patients with clinical lacunar syndromes could have a cortical infarction and vice versa cortical stroke syndromes sometimes could also be mimicked by an acute lacunar infarct. ***Figure 4*** shows acute lacunar infarcts in four typical cerebral localizations.

Besides this clinical and etiologic diversity, it has been noted that the radiologic appreciation of lacunar stroke including terminology, size limits, location, form and shape as well as the imaging appearance on different MRI sequences show a wide variation among neurovascular experts. A recent literature review identified 159 different terms for lacunar stroke. (37)

Moreover, the term "lacunar" for acute stroke seems contradictory, as in the strict sense lacune refers to a cavity filled with cerebrospinal fluid and indicates a chronic stage of tissue damage.

In his original paper the pioneer of the modern concept of lacunar stroke Charles Miller-Fisher stated (38): "Historically, the original CSVD feature was the lacune (hole), which derived from French for a small fluid-filled cavity that was thought to mark the healed stage of a small deep brain infarct. The term was adopted into English. By a process of medico-

linguistic evolution, the precavitary phase became the lacunar infarct, the associated clinical entity became the lacunar stroke and the neurological features became the lacunar syndrome."

Beyond that, it is important to note that acute "lacunar infarcts" differ in the evolution of the lesions over time with variable development of lesion shrinkage, MRI signal changes and cavitation. More specifically, this means that even not all acute lesions progress to a lacune (range, 28-94%), while some can disappear completely or others remain visible just as an unspecific white matter hyperintensity on follow-up neuroimaging (***figure 5***). (39,40)

Against this background and with the aim to homogenize stroke lesion characterization, an international consensus on the "STandards for ReportIng Vascular changes on nEuroimaging" (STRIVE) has recently been provided for cerebral small vessel disease (CSVD). Besides recommending the new term "recent small subcortical infarct" (RSSI) instead of lacunar infarct, the authors also suggested imaging criteria for such lesions and deliberately defined their maximum size by an axial diameter of 20mm. (41)

The new term RSSI is defined as follows (41):

"*Neuroimaging evidence of recent infarction in the territory of one perforating arteriole, with imaging features or clinical symptoms consistent with a lesion occurring in the previous few weeks*."

The new STRIVE criteria were proposed without exact knowledge what range of morphologic features of RSSI would have to be expected according to lesion location and MRI sequence and regarding the impact of different thresholds of lesion size on infarct selection.

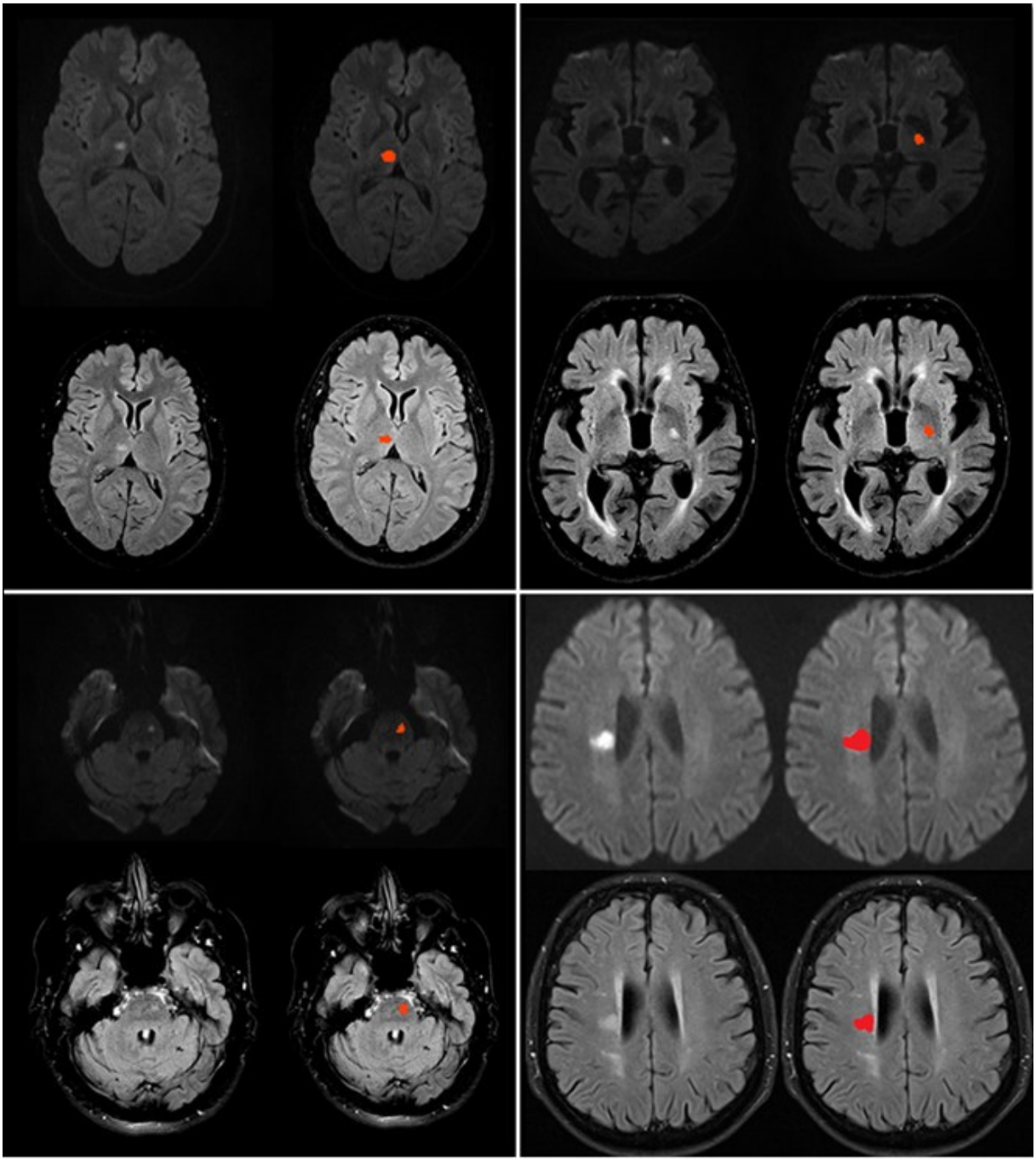


Figure 4. Examples of acute subcortical (lacunar) infarcts in four different and typical localizations (A. thalamus, B. pons, C. internal capsule and D. centrum semiovale) on DWI and FLAIR- weighted MRI scans.

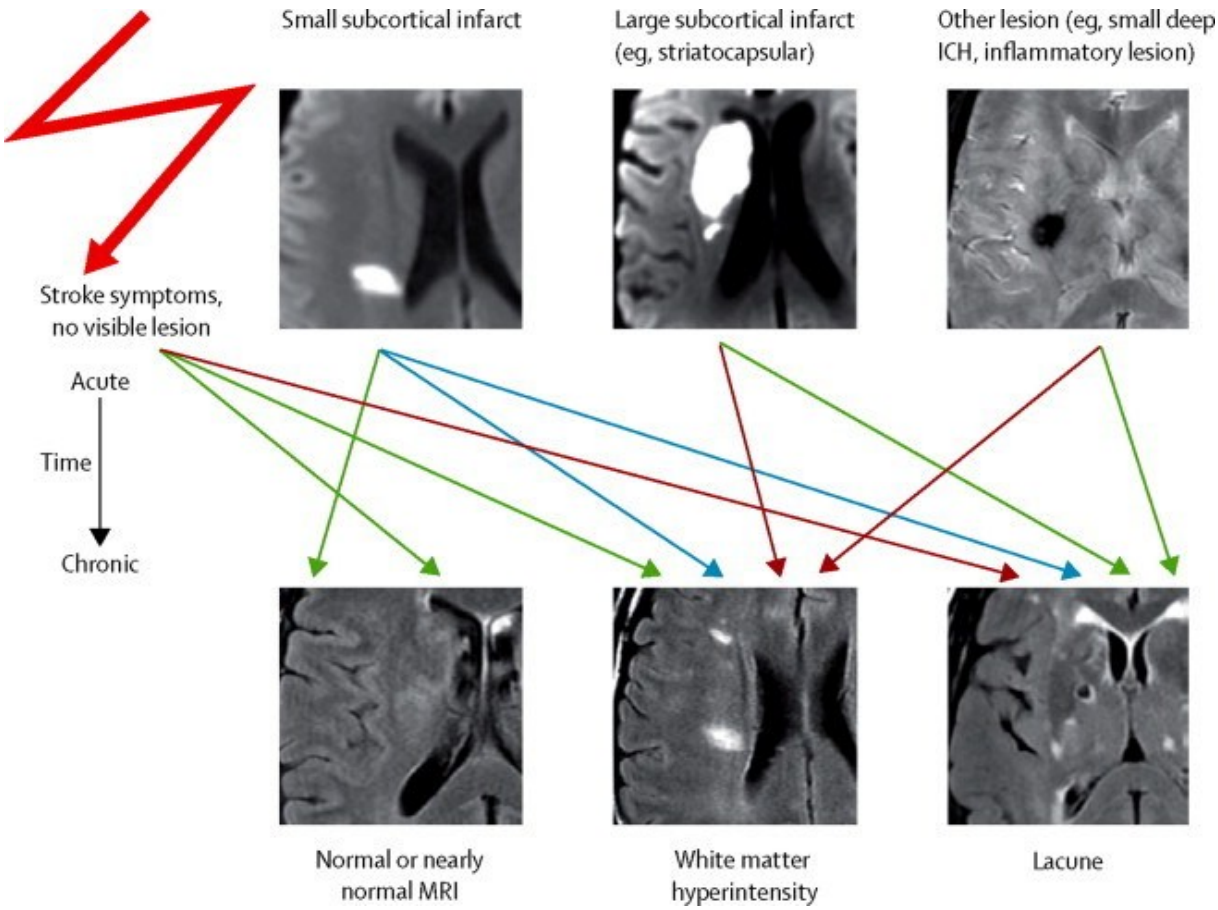


Figure 5. Variable lesion evolution of acute subcortical infarcts (upper row, DWI-weighted MRI scans) on follow-up MRI (lower row, FLAIR-weighted MRI scans). (41)

Study Aims

This thesis has two major objectives:

A.) The implementation of a **prospective**, 15-months longitudinal cohort study of patients with acute subcortical infarcts, using a dedicated and newly implemented electronic patient database.

The project encompasses various multidisciplinary clinical, laboratory, neuropsychological and neuroimaging characteristics and prognostic aspects to better understand the pathogenesis of the so far understudied stroke subtype of lacunar stroke/recent small subcortical infarcts.

More, specifically the comprehensive patient work-up includes:

- a thorough clinical phenotyping
- the assessment of vascular risk factors and diseases
- the search for laboratory biomarkers (oxidative stress, blood brain barrier dysfunction, endothelial dysfunction)
- neuroimaging with new MRI techniques (ultrastructural changes)
- investigation of a potential multisystemic small vessel dysfunction
    - retinal vessel analysis
    - studies on kidney function
- studies on patient outcome
    - neuropsychological testing
    - gait assessment
    - activities of daily living

B.) The application and evaluation of the new **STRIVE criteria** for recent small subcortical infarcts, which have been published during the implementation of the prospective project, in clinical practice.

For this purpose, we **retrospectively** identified a clinical cohort of in-hospital patients with recent subcortical infarct and collected detailed morphological information to better understand the requirements and implications of these neuroimaging criteria in daily clinical routine.

Material and Methods

A.) Prospective project

The subsequently described methodology and study protocol was developed after a preceding analysis of existing literature on lacunar stroke/ acute subcortical infarcts and cerebral small vessel disease using PUBMED. The author of the thesis also attended dedicated teaching courses at international stroke conferences.

In a second step, existing information and results from literature research were discussed with a group of local neuroscience experts (Prof. Franz Fazekas, Prof. Christian Enzinger, Prof. Stefan Ropele) and study aims and research questions were specified. Subsequently, a review paper on cerebral small vessel disease and lacunar stroke including a CME (Continuing Medical Education) option was drafted and published in the meantime. (12)

Thereafter, we consulted international experts on this topic (Prof. Joanna Wardlaw, Edinburgh) and local experts from other disciplines (Statistics, Ophthalmology, Nephrology, Laboratory Diagnostics, Psychology) as outlined below.

Baseline

Patients with MRI defined acute subcortical ischemic infarcts were planned to be prospectively recruited from the stroke unit and general wards of the Department of Neurology, primary and tertiary care University Hospital Graz, Austria.

Inclusion criteria	Exclusion criteria
Acute stroke syndrome **and** the depiction of an acute subcortical ischemic infarct on MRI (DWI-weighted sequences) with a maximally allowed axial diameter of **25mm**	Concomitant acute cortical or cerebellar infarcts, or multiple acute subcortical infarcts
Age between **18 and 75 years**	Pre-existent functional neurological disability, defined by a modified Rankin Scale score of >1
Signed informed consent	Contraindications for MRI like claustrophobia, MRI-incompatible cardiac pacemakers or certain metallic implants
Negative pregnancy test for women in childbearing years	In the case of a severe renal insufficiency (GFR<30 ml/min/1.73cm^2) no contrast agent will be administered

Table 3. Selection criteria for the prospective lacunar stroke study.

The inclusion and exclusion criteria are provided in ***table 3***. Patient recruitment started in May, 2012.

Eligible patients are thoroughly clinically examined. This comprises an exact documentation of the neurological deficits and functional neurological scores (modified Rankin scale (42), Barthel Index (43)), lifestyle and risk factors (body mass index, abdominal girth, smoking and alcohol habits, personal and family history) and stroke routine work-up including cardiac assessments (ECG and long-term ECG, echocardiography), neurosonography of brain supplying vessels, ankle-brachial index (screening for peripheral artery disease) and vascular laboratory (cardiac enzymes, cholesterol, glucose metabolism). The vascular risk factor

classification and categorization of stroke etiology follows current clinical guidelines. (29,44-46)

Additionally, we also perform neuropsychologic testing (STROKDEM protocol, ClinicalTrials.gov Identifier: NCT01330160), a 24-hours blood pressure monitoring and urine analysis for the potential detection of (even subtle) renal impairment using 24-h urine collection (microalbuminuria) and to assess new nephrological blood biomarkers like fibroblast growth factor 23 (FGF-23) and vitamin D status. (47)

Therefore we established cooperation with the Department of Nephrology and Hemodialysis (Head: Prof. A. Rosenkranz).

To search for a potential multisystemic small vessel disorders patients further received a comprehensive ophthalmological examination including retinal vessel analysis ("Retinal Vessel Analyzer, Imedos, Germany; supervision: Prof. M. Weger, Department of Ophthalmology, Head: Prof. A. Wedrich). (48)

In cooperation with the Institute of Medical Laboratory Diagnostics (Dr. HJ. Gruber), we assess blood biomarkers for inflammation (high-sensitive C-reactive protein, haptoglobin, interleukin-6), endothelial dysfunction/ blood brain barrier leakage (Zonulin, Peroxiredoxin-1), endogenous neuroprotectives (MR-proADM, alpha-MSH und BMP-4**)** as well as oxidative (oxLDL, MDA) and nitrostative (NOX, ADMA) stress parameters. (49-54)

Follow-up # 1 at three months

Three months after the stroke index event, study patients are invited to the stroke outpatient department.

First, this should serve for clinical routine stroke follow-up care (secondary stroke prevention) with a check-up of the neurological status, medication and compliance and (vascular) risk factors and comorbidities. (29)

Patients are again examined neuropsychologically (testing for memory, executive functions, attention, speech, visual-constructive abilities, screening for depression). Quality of life is assessed by using a standardized questionnaire (SF36 quality of life scale) and we perform a screening for gait disturbances (short physical performance battery). (55,56)

After this clinical work-up and a laboratory control status (including routine parameters and previously discussed biomarkers), brain MRI at 3-Tesla is conducted. The MRI protocol comprises special imaging sequences, which allow insights in functional and microstructural brain changes. For this purpose, we use diffusion tensor, magnetization transfer and susceptibility weighted imaging. (57-59)

Furthermore, we aim at identifying a potential blood-brain barrier dysfunction by the application of MRI contrast medium (intravenous gadolinium at a dose of 0.2mmol/kg body weight) and dynamic imaging. (52)

Follow-up # 2 at 15 months

At 15-months, study patients receive the same follow-up as at three months, plus a repeat ophthalmological and neurosonographic examination for the detection of progressive vascular changes.

Figures 6 summarizes the respective workup of study patients.

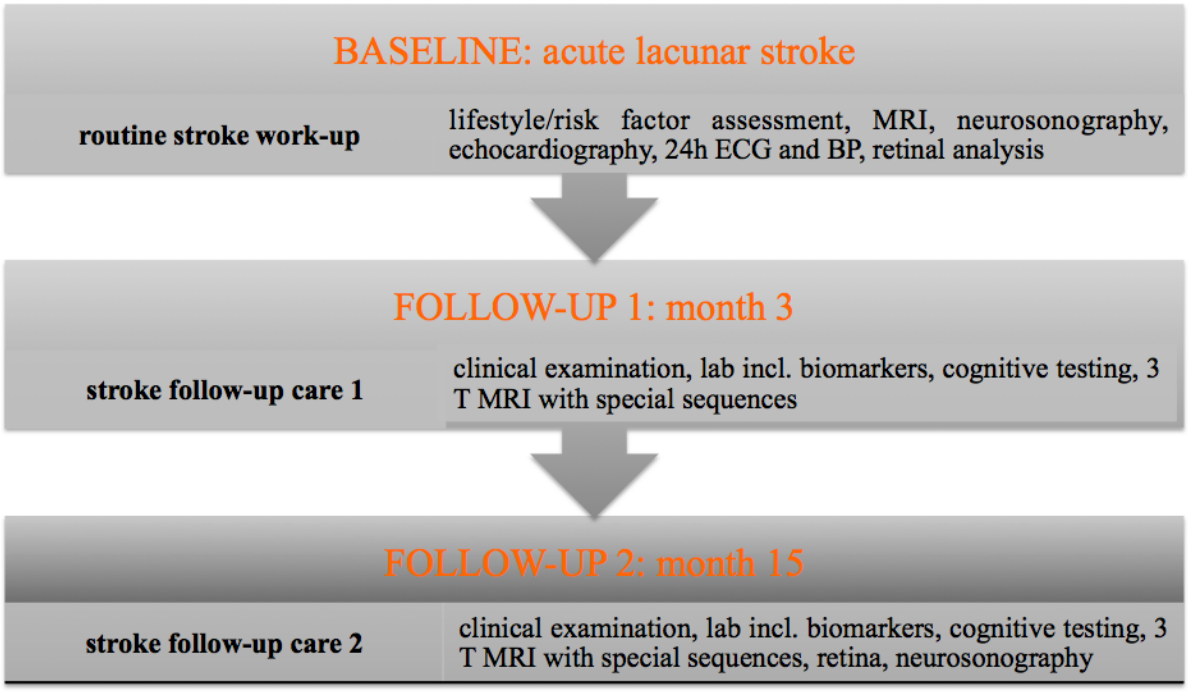
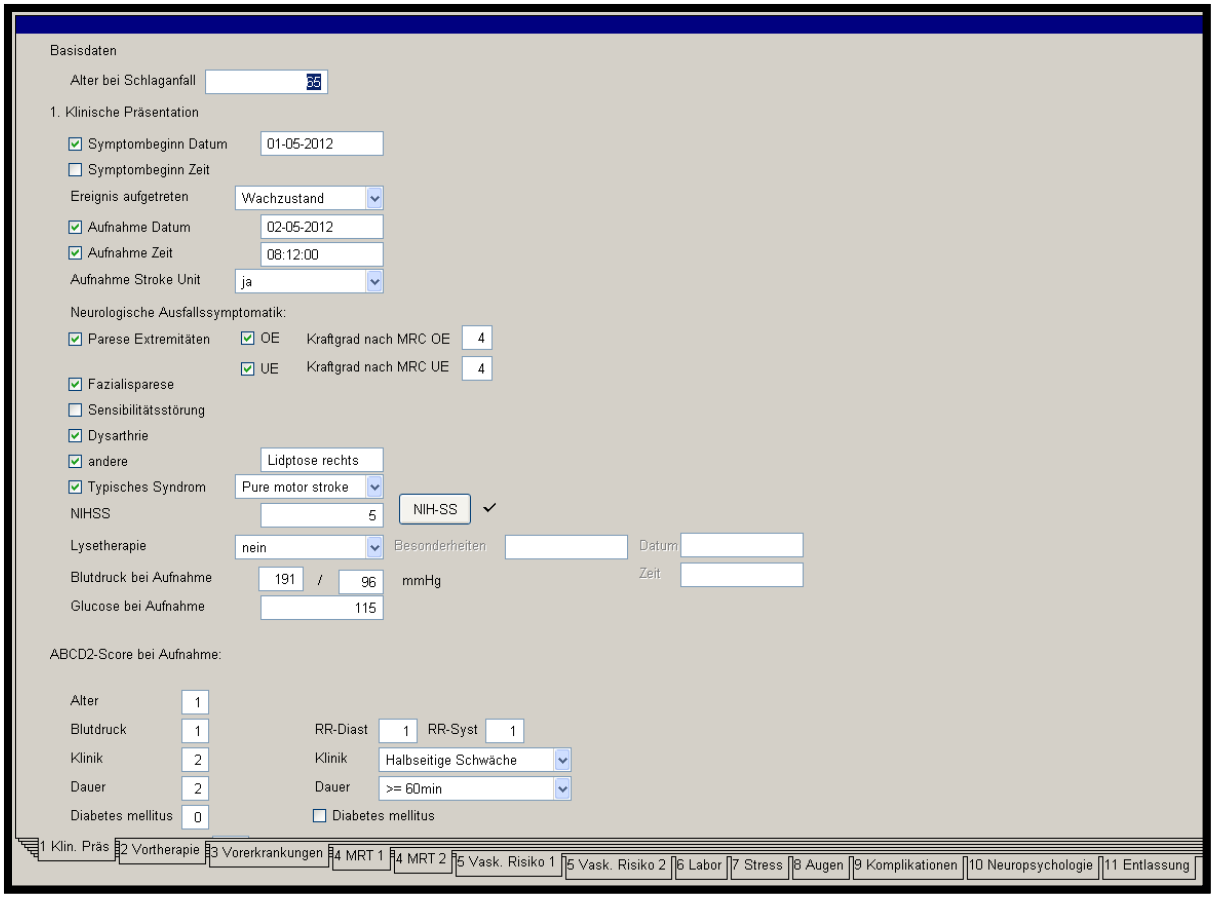


Figure 6. Overview of study patient work-up.

All assessed study variables and laboratory parameters are managed with the newly implemented electronic ARCHIMED database. This database was established in cooperation with the Institute of Medical Informatics, Statistics and Documentation, Medical University of Graz (Supervision: Dr. G. Bachmaier, Head: Professor A. Berghold).

Figure 7 shows an example of clinical patient data management with ARCHIMED.

The study was approved by the ethics committee of the Medical University of Graz (**EK 24-260 ex 11/12**).



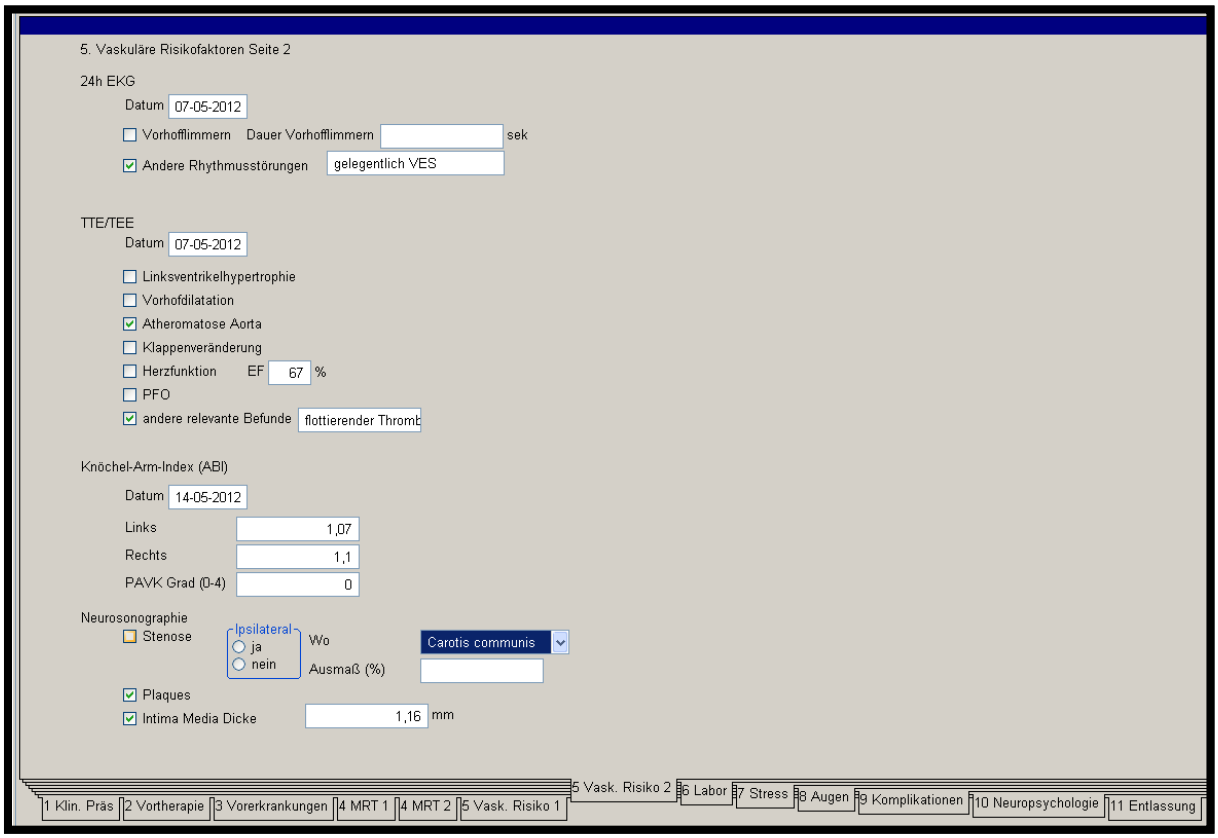


Figure 7. Management of study data with ARCHIMED database

B.) Retrospective project

Study participants

We retrospectively searched the medical documentation system (MEDOCS) of our primary and tertiary care university clinic for inpatients treated at the neurological department with the hospital discharge diagnoses “cerebral infarction” (international classification of diseases - version 10 code I63) from January 1, 2008 to February 5, 2013.

MEDOCS allows retrieving medical records as well as neuroimaging and laboratory data acquired in all 21 public hospitals in the district of Styria with more than 1.2 million inhabitants. (60)

From 4118 ischemic stroke patients in total, we identified 3363 patients that had undergone brain MRI for cerebrovascular work-up within a maximum of ten days since the onset of stroke symptoms (to reliably capture DWI positive infarcts). (61)

Two experienced raters blinded to demographic and clinical data as well as radiological reports independently reviewed these MRI examinations for the following **selection criteria**:

a) presence of a **hyperintense DWI lesion** with corresponding reduced diffusivity on the apparent diffusion coefficient (ADC) map compatible with acute ischemic infarction,

b) **subcortical lesion location** in four prespecified regions (basal ganglia, thalamus, centrum semiovale and pons), suggestive of the supply area of a penetrating artery,

c) **maximal axial lesion diameter of ≤20mm** estimated by eyeballing.

Patients were **excluded** if their scans showed multiple acute subcortical infarcts, additional infarcts in other locations or other acute intracranial lesions (e.g. brain hemorrhage, tumor).

We then assessed the imaging characteristics of the selected scans and transferred them to a dedicated workstation for image analysis as described below. We also recorded pre-specified demographic characteristics (age, sex) of respective patients and the time period between stroke symptom onset and MRI.

The study was approved by the ethics committee of the Medical University of Graz (**EK 25-409 ex 12/13**).

Magnetic resonance imaging protocols

All patients had undergone MRI of the brain on 1.5-Tesla scanners (Siemens Symphony, Siemens, Erlangen, Germany; Philips Intera and Gyroscan ACS, Philips, Eindhoven, The Netherlands) according to a standard protocol for the workup of patients with suspected cerebrovascular events.

This set of scans included an axial T2-weighted fast spin echo sequence, an axial fluid-attenuated inversion recovery sequence (FLAIR), a sagittal T1-weighted spin echo sequence, a gradient echo T2* weighted sequence and an axial diffusion-weighted single-shot echo planar imaging sequence with ADC maps. All axial scans had a slice thickness of 5mm.

Image analysis

Single subcortical infarcts were identified on the axial DWI sequence (cross-checking on the ADC map) and located to four pre-specified brain areas (1. basal ganglia including the internal capsule, 2. thalamus, 3. centrum semiovale and 4. pons) using an anatomical MRI brain atlas. (62)

After conversion of the imaging format (DICOM into NIfTI using the software dcm2nii, freely available at: www.mccauslandcenter.sc.edu/mricro), FSL View (version 3.2.0, freely available at: www.fmrib.ox.ac.uk/fsl) was used for MRI analyses. (63,64)

A single trained observer outlined the acute subcortical infarct on all slices of the axial DWI scan with apparent DWI restriction based on templates provided by the raters. An analogue procedure was repeated on the FLAIR weighted scan based on clearly elevated T2 signal intensities. These outlines served to calculate the volume of the infarct on DWI and FLAIR using fslstats within FSL.

The maximal axial and longitudinal (i.e., craniocaudal) lesion extension was computed manually with the program Freeview (freely available at: http://surfer.nmr.mgh.harvard.edu).

Figure 8 illustrates this segmentation process.

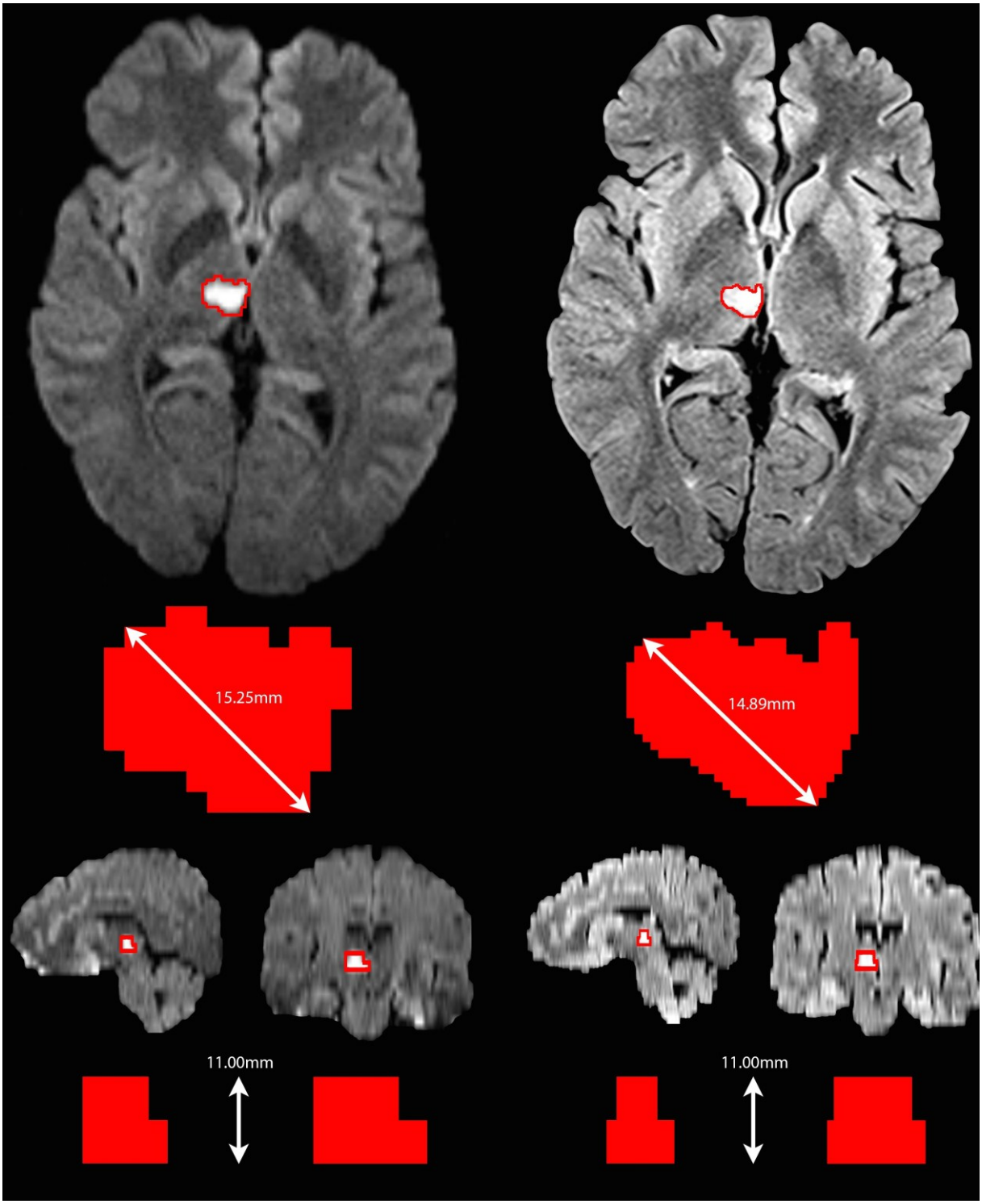


Figure 8. Segmentation results of RSSI on DWI (left) and FLAIR (right) weighted MRI scans with illustration of respective lesion masks and quantitative measurements of lesion sizes in axial and longitudinal direction. (65)

Statistical analyses

The Statistical Package for the Social Sciences (version 20.0; SPSS Inc., Chicago, Ill., USA) was used for data analysis. Categorical variables were described by absolute and relative frequencies and tested by Pearson's $\chi 2$ test. The normal distribution of continuous variables was tested by the Kolmogorov-Smirnov statistics with a significance level after Lilliefors and additional inspection of the histograms. Normally distributed continuous variables were compared by the unpaired Student t test or one-way ANOVA. The Mann-Whitney U test and the Kruskal-Wallis test were used as nonparametric analogue tests. Bivariate correlation analyses were performed calculating Spearman's rank correlation coefficient (Spearman's rho). The level of significance was set to 0.05.

Results

A.) Prospective project

Starting in May 2012, we were able to include up to now (May, 2015) 72 patients that fulfilled the selection criteria and signed the informed consent.

Further 135 patients with confirmed lacunar stroke were identified but could not be considered for the study because of unwillingness to participate (n=36), place of residence far away from Graz (n=4), claustrophobia prohibiting the examination with MRI (n=17), age > 75 years (n=75) or incompliance due to severe chronic alcoholism (n=3).

The mean age of the included patients was 59.5 years and in the vast majority were men (72%). Mean stroke severity at hospital admission was low (National Institutes of Health Stroke Scale score [NIHSS], median 2) and about 60% were admitted to the stroke unit. (66)

Patients usually recovered well (median NIHSS at discharge: 0, median modified Rankin Scale score at discharge: 1) and no in-hospital death was observed.

Complication rates were also rather low; however 5 individuals (7%) suffered from a progressive stroke, of whom 3 showed the classical clinical features of a capsular warning syndrome with stereotyped fluctuating motor weakness, followed by severe and persistent hemiparesis. In all of them the respective infarct was located in the internal capsule.

The demographic and clinical characteristics of the study cohort are shown in ***table 4***.

Demographics and clinical characteristics	
Age (years), mean (standard deviation)	59.5 (±11.5)
Male sex, n (%)	52 (72%)
Stroke severity (NIHSS) at hospital admission, median	2 (range, 0-8)
Wake-up stroke / stroke of unknown onset, n (%)	26 (36.1%)
Intravenous thrombolysis, n (%)	7 (9.7%)
Time symptom onset – hospital admission, days, median	0 (range, 0-6)
Admission to stroke unit	42 (58.3%)
Typical lacunar stroke syndrome	37 (51.4%)
Duration of hospital stay, median	11 (range, 3-42)
Stroke severity (NIHSS) at discharge, median	0 (range, 0-7)
Functional status (mRS) at discharge	1 (range, 0-4)

Table 4. Demographics and clinical characteristics of 72 patients with acute lacunar stroke

Study patients had generally one to several vascular (atherosclerotic) risk factors (***table 5***), however we also identified 7 individuals with juvenile stroke (age < 45 years) without any established stroke risk factor. Of those, two female patients with thalamic infarctions were classified as probable migrainous stroke.(67)

Arterial hypertension was the most prevalent risk factor, followed by hyperlipidemia and smoking with mean pack years of 31 ± 20 (***table 5***).

The mean arterial blood pressure at hospital admission was remarkably high (mean systolic: 176.7±27.2mmHg; mean diastolic: 100.1±14.1mmHg).

Vascular factors and stroke etiology	Frequency, n (%)
Arterial hypertension	58 (80.5%)
Diabetes	11 (15.2%)
Smoking	30 (41.7%)
Hyperlipidemia	54 (75%)
Coronary heart disease	8 (11.1%)
Peripheral artery disease	6 (8.3%)
Family history of stroke	6 (8.3%)
Previous stroke	4 (5.5%)
Obesity	17 (23.6%)
Renal impairment	3 (4.1%)
Depression	9 (12.5%)
Chronic alcohol consumption	2 (2.8%)
Aortic atheromatosis	7 (9.7%)
Atrial fibrillation	4 (5.5%)
Ipsilateral >moderate proximal vessel stenosis	3 (4.1%) 2 (2.8%) internal carotid artery 1 (1.4%) vertebral artery
Intracardiac thrombus	3 (4.1%)

Table 5. Vascular risk factors and stroke etiology

Of note, we also identified quite a substantial number of patients with clinically overt depression (12.5%).

Ten patients (13.9%) had other concomitant (non-CSVD) disorders beyond vascular risk factors that could have caused their ischemic stroke. We found atrial fibrillation in 4 patients,

ipsilateral at least moderate proximal vessel stenosis in 3 and intracardiac thrombi (atrial auricle) in another 3 patients. Of note, in all three patients with confirmed cardiac thrombi, a thorough clinical work-up with admission ECG, stroke unit cardiac rhythm monitoring and 24-hours ECG was not able to detect atrial fibrillation or other significant arrhythmias or motion abnormalities.

The follow-up rate at three months is 68/70 (two patients were unable to visit the stroke outpatient department but both participated in the 15 months follow-up). Fifty-three of 55 patients (96.4%) in whom the event had occurred longer than 15 months ago already completed the study.

Regarding further ischemic events so far only one female patient (73 years) suffered from a recurrent stroke, which was attributed to a high-grade extracranial internal carotid stenosis with subsequent cerebral borderzone infarction 6 months after the qualifying lacunar stroke. Again, three months later this patient died due to acute left ventricular cardiac insuffiency in the setting of diabetic ketoacidosis in a peripheral hospital.

Another two patients had major extracerebral vascular events during follow-up (one symptomatic acute peripheral artery occlusion in the presence of a preexisting peripheral artery disease and one non-ST-elevation myocardial infarction with percutaneous coronary stent angioplasty).

All examinations, particularly the MRI study protocols are well-tolerated and no patient withdrew the consent to participate in this study. In general, compliance rates concerning medication are high, only one patient disagreed to take his lipid-lowering agent (due to

muscle pain) and two further patients did not quit cigarette smoking. Consequently, vascular risk factors (hypertension, LDL-cholesterol and HbA1c levels) are well controlled.

B.) Retrospective project

Over the 5-year study period, we identified 344 patients with a single MRI-defined recent small subcortical infarct (RSSI) out of the 3363 (10.2%) in-patients who had undergone brain MRI for suspected acute ischemic stroke.

These 344 patients had a median age of 72 (range 25-92) years and 65% were male. The median time period from stroke symptom onset to MRI was two days (range, 0-10 days).

General lesion characteristics and volumes

Table 6 lists the infarct localizations according to the four pre-specified brain regions. RSSI most often occurred in the basal ganglia (including the internal and external capsule) and least frequent in the centrum semiovale.

Localization	**Number (%)**
Basal ganglia (including internal capsule)	111 (32.3)
Thalamus	77 (22.4)
Centrum semiovale	64 (18.6)
Pons	92 (26.7)

Table 6. Distribution of recent small subcortical infarcts according to four prespecified brain areas

While the inclusion criteria (among other aspects) were based on the visual estimation of the lesion diameter, exact measurement revealed that the maximum axial diameter exceeded 20mm in 18 patients (5.3%) on DWI and in 13 patients (3.8%) on the FLAIR scan. Overall, the median axial diameters were comparable on DWI [13.3mm (range, 4.2-23.2mm)] and FLAIR [13.4 mm (range, 2.8-23.1mm), $p>0.05$] whereas the median longitudinal sizes [FLAIR: 13mm (range, 5-33.3mm); DWI: 11.2mm (range, 5.4-33.3mm); $p<0.001$] and the median volumes of the segmented infarcts were higher on FLAIR than on DWI weighted scans [FLAIR: 697.3mm3 (range, 21.6-3977.1mm3) vs. DWI: 631.4mm3 (range, 47.4-3305.4mm3), $p<0.001$].

There were no significant associations between age, sex, or the time from stroke symptom onset to MRI and the sizes or volumes of the RSSI on both MRI sequences. The maximal axial and longitudinal lesion diameters showed a high correlation with each other and the volumes of the RSSI (***table 7***).

Parameters		**Spearman's rho**	**p-value**
Volume DWI	Axial diameter DWI	r = 0.9	p<0.001
	Longitudinal extension DWI	r = 0.8	p<0.001
Axial diameter DWI	Longitudinal extension DWI	r = 0.6	p<0.001
Volume FLAIR	Axial diameter FLAIR	r = 0.9	p<0.001
	Longitudinal extension FLAIR	r = 0.7	p<0.001
Axial diameter FLAIR	Longitudinal extension FLAIR	r = 0.5	p<0.001

Table 7. Correlations of morphometric parameters of RSSI.

Morphometric analyses according to subcortical brain areas

To search for anatomy related differences in lesion metrics, separate analyses were conducted for the four locations of the RSSI (***table 6***). Age and sex did not show any effects on the distribution of RSSI in these regions.

Table 8 lists the diameters and volumes of RSSI regarding their location. Infarcts in the basal ganglia had larger axial and longitudinal diameters on DWI and FLAIR and also higher lesion volumes on both sequences compared to all other infarct locations ($p<0.001$). Furthermore, thalamic RSSI had a greater longitudinal extension on FLAIR than lesions in the pons ($p=0.049$). No other region-related differences between RSSI metrics emerged ($p>0.05$).

Following different recommendations (41,68) regarding the maximally allowed axial diameter for "lacunar" infarcts in prior studies (i.e. 15mm versus 20mm), we also dichotomized RSSI at a threshold of 15mm. There were 210 (61%) RSSI with a maximal axial diameter of 15mm and 134 (39%) with a maximal axial diameter of >15mm. This distribution was not influenced by age, sex or the timing of the MRI examination. However, the regional distribution of RSSI was significantly different ($p<0.001$) after dichotomization of axial diameters. RSSI >15 mm were more often seen in the basal ganglia than in the thalamus or centrum semiovale (***figure 9.A***).

Finally, we also analyzed the impact of a 20mm cutoff for the longitudinal diameter on the regional distribution of RSSI. The vast majority of RSSI with a longitudinal size >20mm that would thus have been excluded were located in the basal ganglia (n=41, 87.2%), while thalamic, centrum semiovale and pons infarcts rarely showed such a longitudinal extension (n=2, respectively, ***figure 9.B***).

Parameters		Anatomical localizations			
		Basal ganglia* (n=111)	**Thalamus (n=77)**	**Centrum semiovale (n=64)**	**Pons (n=92)**
Axial diameter DWI [mm]	median (range)	15.8 (4.6-23.2)	11.9 (5.7-19.9)	10.8 (5.3-18.1)	14.1 (4.2-22.6)
Longitudinal extension DWI [mm]	median (range)	17.0 (5.5-33.3)	11.0 (5.5-28.4)	11.0 (5.5-22.4)	11.0 (5.4-22.0)
Volume DWI [mm^3]	median (range)	978.7 (71.0-3305.4)	560.2 (63.1-2675.6)	473.6 (55.2-1941.6)	489.3 (47.4-2186.2)
Axial diameter FLAIR [mm]	median (range)	15.3 (5.5-22.7)	12.3 (4.6-22.6)	11.8 (3.7-19.8)	12.3 (2.8-23.1)
Longitudinal extension FLAIR [mm]	median (range)	19.5 (5.5-33.3)	13.0 # (5.5-28.4)	11.0 (5.5-27.4)	11.0 # (5.4-22.0)

Table 8. Morphometric parameters of RSSI according to four subcortical brain areas

* Significant group differences between all parameters were identified with the Kruskal Wallis Test ($p<0.001$). ANOVA (corrected for multiple comparisons by using non-parametric Dunnett-T3 test) confirms that RSSI in the basal ganglia had significantly higher axial and longitudinal diameters and volumes on DWI and FLAIR ($p<0.001$). # Thalamic RSSI had larger longitudinal diameters on FLAIR compared to infarcts in the pons (ANOVA, Dunnett T3: $p=0.049$).

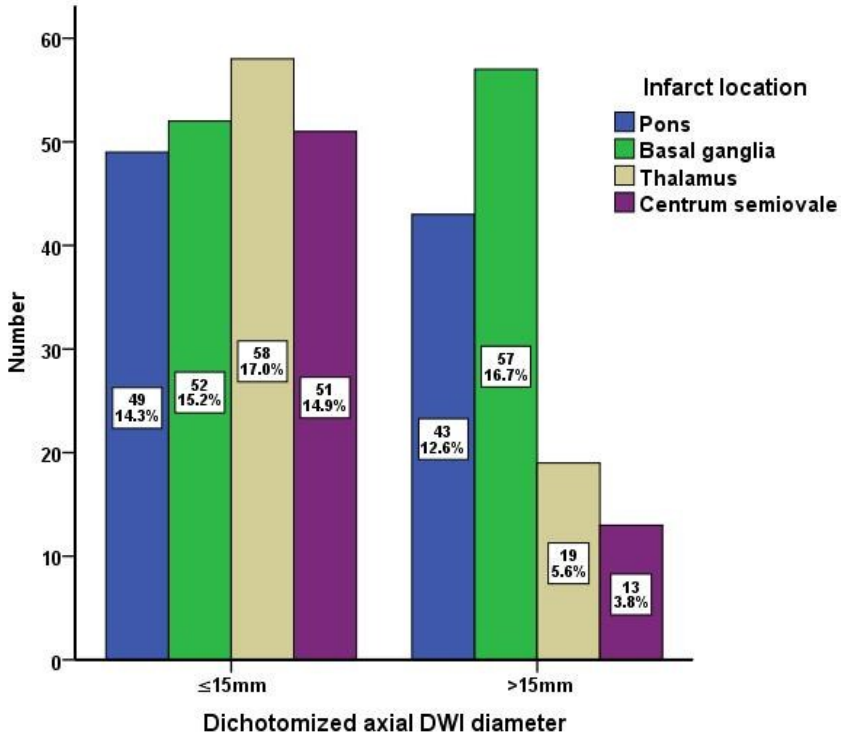


Figure 9.A. Regional distribution of RSSI according to dichotomized maximal axial diameters of ≤/> 15mm

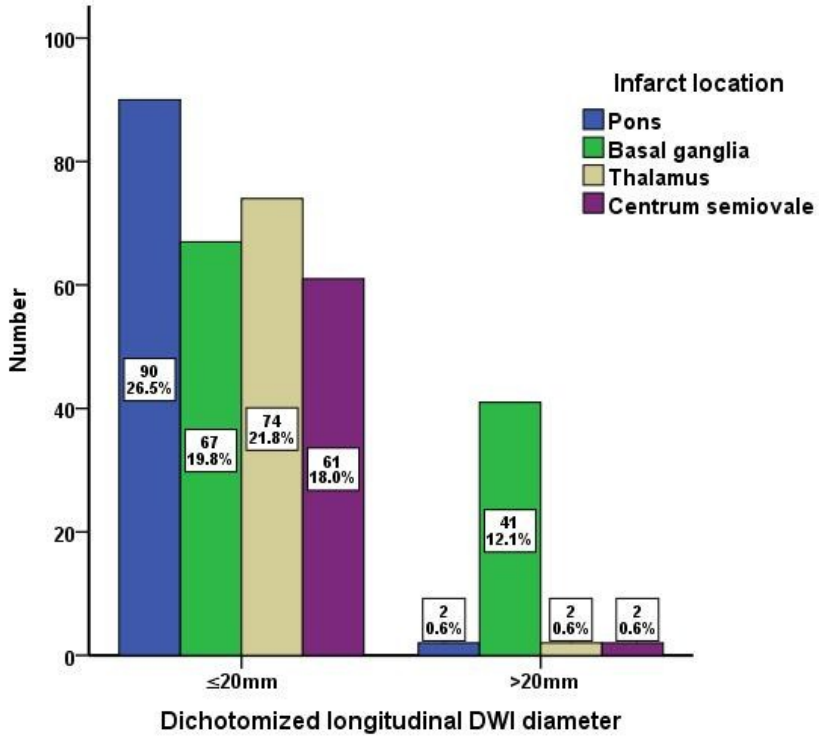


Figure 9.B. Regional distribution of RSSI according to dichotomized maximal longitudinal extension of ≤/> 20mm

Discussion

Cerebral small vessel disease (CSVD) and its presumed acute manifestation – lacunar stroke or recent small subcortical infarct (RSSI) is an important cause of stroke, cognitive impairment and dementia as well as disability. These entities are associated with a tremendous health care burden. Due to the estimated increase in life expectancy in western industrialized countries the frequency of these age-related disorders is expected to gain more and more importance in the future. (26)

The causes, pathophysiological mechanisms, treatment strategies and prognosis of CSVD are incompletely understood and prospective long-term studies are lacking.

Therefore, we aimed at implementing such a project, which should cover a wide range of different aspects of the disease to enable a comprehensive characterization of patients with acute subcortical infarcts.

This attempt includes a thorough clinical work-up, risk factor screening, neuroimaging and laboratory assessment and we particularly focused on follow-up care to allow an evaluation of prognosis with regard to different aspects (neurological status, cognition, mood, gait, vascular changes, laboratory status, quality of life, cardiovascular events and morphological brain changes on sophisticated MRI).

By now, interim analysis of the baseline results of our prospective project shows that such a comprehensive, multidisciplinary study is feasible in clinical practice. This is especially demonstrated by the high retention rate within the study. The study protocol with follow-up visits obviously favors a good control of vascular risk factors and a high patient compliance

concerning medication, which all together are likely to contribute to the thus far observed low cardio- and cerebrovascular event rates, good clinical and functional neurological status as well as low mortality during follow-up.

Regarding clinical characteristics of our study patients, we already can confirm the results of previous retrospective analysis of lacunar stroke cohorts in that we also identified arterial hypertension as the most prevalent vascular risk factor (80% of study participants). (69)

We have to acknowledge that our cohort differs from previous retrospective analyses on this disease in terms of age and associated comorbidities. We decided to exclude patients > 75 years of age to minimize potential confounding because of concomitant neurodegenerative diseases (e.g. Alzheimer-related mild cognitive impairment or dementia) which are supposed to largely impact on cognitive function and structural brain changes beyond the effects of vascular damage. Moreover, the comprehensive study setting with a rigorous follow-up care appeals especially to patients with a certain health awareness and compliance/adherence to treatment/secondary stroke prevention recommendations. Clearly this might also cause some selection bias compared to former retrospective studies on this topic.

It is furthermore important to note that only half of the patients included thus far presented with a classical clinical lacunar stroke syndrome. Recent small subcortical infarcts are reliably captured on MRI, while brain computed tomography usually fails to depict such lesions in the acute stage. Consequently, it is important to mention that unlike in other countries and centers, the vast majority of patients with presumed acute cerebrovascular events receive brain MRI at our department (MRI rate of acute ischemic stroke patients in 2014: 80.5%). Moreover, the main reason for withholding MRI in acute stroke patients is older age. Therefore, we can assume that only a minor proportion of eligible study patients with

RSSI(selection criterion age < 75 years) were missed because their small subcortical infarct had not been detected on neuroimaging.

For the prospective project, we have decided to aim for a total of at least 100 patients gain more statistical power for further detailed analyses (as described in the Methods of the thesis).

In the meantime, however, a sub-study with the aim to relate cognitive function to white matter integrity in RSSI patients was initiated. Although RSSI are generally associated with good motor recovery, it has been suggested that they might confer a high risk of cognitive dysfunction. This might result from remote effects of RSSI, e.g. by differentiation. The current study uses diffusion tensor imaging on MRI and tests the relationships between the white matter microstructure (assessed by fractional anisotropy) and cognition in patients after RSSI and will compare these findings to healthy controls. Interim results showed that microstructural white matter integrity in RSSI patients correlates with processing speed and executive function in major white matter tracts (e.g. inferior fronto-occipital fasciculus).

The crucial role of MRI in the diagnosis of recent small subcortical infarcts (RSSI) and associated neuroimaging markers of CSVD was also highlighted in the recent STRIVE consensus statement (41).

However, the impact of provided definitions for an RSSI had not yet been evaluated, especially in clinical practice, while we implemented our prospective lacunar stroke project and were deciding on the neuroimaging (MRI) criteria for patient selection. This prompted us to integrate a retrospective study into this project, which encompassed the lacunar stroke patients that had been admitted to the Neurological Department of the University Hospital of Graz in the past years. This study should help to better understand the implications of the

definitions proposed by the STRIVE consortium in clinical practice and especially for the intended comprehensive neuroimaging characterization of these patients.

Therefore and as basis for the prospective study of lacunar stroke patients, we extended our work and analyzed the MRI scans of a retrospectively identified in-hospital cohort. This analysis should serve as a prerequisite for further analysis of prospectively acquired neuroimaging data with special sequences and the correlation of these MRI variables to clinical and laboratory data.

The recommended maximal diameter of 20mm from STRIVE refers only to the axial dimension and has been derived from the classic maximal diameter of 15mm in the chronic stage of such infarcts in neuropathology while neuroimaging information regarding the acute phase has been lacking. (4,41)

In general, the upper axial diameter is debatable, the 20 mm axial cut-off size was chosen arbitrarily and studies focusing on pathophysiological stroke mechanisms including vascular risk factors and diseases in relation to axial lacunar infarct size failed to demonstrate clear associations. (4)

Moreover variable acute cytotoxic or to a lesser degree vasogenic edema surrounding the infarct core in the early phase of imaging might overestimate RSSI size, potentially leading to a misclassification and overhasty exclusion of such patients.

Reviewing the MRI of 344 patients showing a single RSSI within 10 days of stroke symptom onset (median 2 days) we found that the maximal axial diameter of 20mm for RSSI proposed in STRIVE describes a similar type of infarction in various subcortical regions. Furthermore,

axial lesion diameter strongly correlated with longitudinal size and RSSI volume. We identified only a minor proportion (≈5%) of study participants showing a slightly larger (>20mm) axial diameter after exact quantitative measurement. This confirms that estimation of the axial size to define an RSSI is very reliable and clinically applicable.

While selection of small subcortical infarcts on the basis of their axial diameter has been heavily discussed, information on longitudinal extension of RSSI is scarce and no maximum upper size limits have yet been defined.
However, it has been suggested that it could be of importance to also consider the longitudinal diameter of RSSI especially for lesions in arterial territories that have a vertical distribution, where the sole examination of axial sizes might underestimate the extent of the infarcts.

In fact this is confirmed by our study, which identified several patients where the infarct extension was bigger in the longitudinal (cranio-caudal) than the axial direction. The assessment of the longitudinal extension can be achieved by a coronal or sagittal imaging plane or by sequential lesion identification on axial scans and assessing the craniocaudal diameter by knowing the slice thickness, like we did. (70)

Here it is important to mention that the scans of all our patients had the same slice thickness of 5 mm, which is the proposed vertical scan dimension according to neuroimaging consensus. (41)

Another important finding of this work is the high positive correlation of infarct diameters and volume on both analyzed MRI sequences, with a more substantial impact of the axial than the longitudinal diameter. This suggests that volumetric measurements are unlikely to provide significant further information on infarct size.

Importantly and not systematically studied before, axial lesion diameters did not differ between DWI and FLAIR, the two most useful MRI sequences in acute stroke imaging. This is practically relevant as it indicates that both DWI and FLAIR can be interchangeably used to define RSSI (although DWI is known to be more sensitive to differentiate acute from established lesions), which may be especially relevant in case of large differences in image quality between both sequences. (71)

Noteworthy and differing from axial imaging, RSSI demonstrated higher longitudinal extensions and lesion volumes on FLAIR compared to DWI scans. This has to be considered when interpreting and defining lesions on acute stroke imaging.

A further specific scope was the assessment of the regional distribution of RSSI and their region specific morphometric profiles. In contrast to a sub-analysis of the SPS-3 trial(72), we identified a higher number of basal ganglia / internal capsule and a lower frequency of centrum semiovale RSSI in our clinical cohort. This discrepancy might be related to differences in ethnicity, age (higher median age of our patients, i.e. 72 versus 63 years), study setting (observational versus randomized controlled trial), pre-specified clinical features (presentation with lacunar syndromes) or maximally allowed longitudinal lesion extension (20mm in the SPS-3 trial opposed to no limits according to the STRIVE criteria in our study).

The importance of the latter aspect could be easily demonstrated in our data. Adding a 20mm cut-off for the longitudinal diameter to the selection criteria for RSSI would have resulted in the exclusion of 47 (13.7%) patients of whom the vast majority had RSSI in the basal ganglia (n=41, 87.2%) thus explaining a large part of the differences in regional RSSI distribution between the two studies.

In this context, it is also of interest that a longitudinally dominated (tubular) morphology of subcortical infarcts has been suggested to indicate a distinct infarct subtype with a different pathophysiological profile and clinical picture (i.e., partial large vessel occlusion or high grade stenosis). (73,74)

However, the absence of clinical data in our retrospective cohort hampers a further investigation in this direction. On the contrary, a recent work found no associations between the shape or size of small subcortical infarcts and vascular risk factors or embolic sources, although location in the basal ganglia (versus centrum semiovale) was associated with a proximal embolic source in 10% of patients. This indicates a common intrinsic arteriolar pathology for most of these patients. (4)

Further work is required to determine which, if any, features of RSSI increase the likelihood of atherothrombo- or cardioembolic causes.

Our results also emphasize the importance of the choice of the maximal axial diameter for RSSI selection. (75)

Using a cut-off of 15 or 20mm resulted in different regional distributions and frequencies of RSSI. More specifically, a relevant number of basal ganglia but also pons infarcts would have been excluded when applying a 15mm instead of a 20mm upper axial size limit. Infarcts in the centrum semiovale have the highest prevalence within the smaller RSSI lesion group. Conceivably such effects could also explain differences in observed associations with risk factors and etiologies of previous studies and therefore have to be considered when comparing the results of individual studies and for the selection of patients for further work on this topic.

When considering our detailed morphometric analyses separately for different locations, the axial and longitudinal lesion dimensions and volumes were comparable between thalamic, centrum semiovale and pontine regions.

RSSI in the basal ganglia, however, showed higher lesion sizes and volumes on both MRI sequences. Although this particular morphometric profile of RSSI in the basal ganglia could point to a macroangiopathic/embolic mechanism this is probably just a consequence of vessel anatomy. (76)

The vast majority of our basal ganglia infarcts with median volumes of around 980mm3 would still be categorized as third order (distal) branching infarcts based on small vessel templates for the MCA territory. (70)

Regarding possible other confounders of RSSI selection it is important to note that we observed no significant influence of age, sex and timing of the MRI examination (within a time period of ten days after stroke symptom onset) on any of the analyzed morphological parameters in our study.

Our work also has some limitations. Because of the imaging focus of our study patients were selected on the basis of acute stroke symptoms and the availability of a cerebral MRI irrespective of the clinical stroke syndrome.

We also did not consider infarcts in the medulla oblongata or cerebellum, although a small number of those might represent perforator lesions.

Therefore this cohort might differ from others and does not reflect the entire spectrum of previously termed lacunar strokes. Further, it was not the scope of this work to confirm RSSI

as indicating a single common etiology and we did not include data on vascular risk factors or disease etiology (e.g. large vessel stenosis or cardioembolic sources) in our analysis. However, the rather uniform morphometric data of RSSI among various subcortical regions in our cohort suggest that most of them are likely related to disease of a small single perforating artery. To what extent concomitant abnormalities typical for CSVD can help in separating the causes for RSSI will need further investigation.

Additionally, we also started an extension of our retrospective imaging project by adding information on clinical data, risk factors, cardiovascular diseases and stroke pathophysiology. It is planned to study whether certain risk factor and vascular diseases (e.g. vessel stenosis, cardiac embolic sources) have an impact on the localization, configuration or size of RSSI. Therefore, we also initiated an international cooperation with Professor Joanna Wardlaw (University of Edinburgh, United Kingdom), who is a leading expert in the field of cerebral small vessel disease with the goal to pool data from our centers for further analyses.

We have calculated the longitudinal extension of the RSSI following the extension of the infarct over subsequent 5mm slices. Clearly this carries the problem of partial volume effects. A more precise measurement would require coronal or sagittal scans or 3D imaging, which are rarely obtained in daily practice. Moreover, it is unlikely that this would have altered our results to a significant extent.

Irrespective of these limitations the proposed definition of RSSI appears a valid construct for further research into a common etiology of these lesions. However, considering the impact of chosen morphometric thresholds on RSSI selection, future studies should also record absolute infarct diameters and location to allow for better comparison among investigations and for subanalyses.

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Curriculum Vitae

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Training

Since 10/2011	Doctoral program "Sustainable Health Research" Doctoral thesis: *Acute lacunar stroke: risk factors, biomarkers, neuroimaging characteristics and prognosis* (Supervisors: Franz Fazekas, Christian Enzinger)
Since 08/2010	Residency in Neurology (Department of Neurology, MUG)
10/2004 – 07/2010	Studies of Human Medicine at MUG (MD)

	Diploma thesis: *High grade unilateral carotid artery stenosis and vascular brain atrophy: a volumetric MRI study* (Supervisors: Christian Enzinger, Reinhold Schmidt)
03/2004 – 07/2004	Studies of Economics, Johannes Keppler University Linz
09/1994 – 6/2002	Grammar School with focus on natural sciences, BRG Kirchdorf

Awards

01/2015	Scientific Award of the Austrian Stroke Society
09/2013	Poster Award at the XXI World Congress of Neurology (Vienna)
01/2013	Presidential Award of the Austrian Stroke Society
06/2012	Land Steiermark Funding for the project *Vascular Risk Factors and Hippocampal Volume*
01/2012	Presidential Award of the Austrian Stroke Society
03/2011	Poster Prize at the 9th Annual Conference of the Austrian Neurological Society
2010	Leistungsstipendium (Merit Scholarship) from Medical University of Graz (MUG)
06 – 07/2009	Travel Grant from MUG for a clinical traineeship at the Department of Neurology, Charité Universitätsmedizin Berlin, Germany

Research Activities

Special focus on cerebrovascular diseases, brain ageing/dementia, MS, MRI (*see publication list*)

Active participation in stroke trials like *DIAS-3, SPACE-II, ENDOSTROKE, SWIFT-PRIME* and *ECASS-4*.

Reviewer for the journals *Stroke, International Journal of Stroke, Cerebrovascular Diseases, Cephalalgia* and *European Neurology*

Graz, June 2015

Publications (SCI/PUBMED listed)

2015

Full papers/articles (Journal)

Khalil, M; Langkammer, C; Pichler, A; Pinter, D; **Gattringer, T**; Bachmaier, G; Ropele, S; Fuchs, S; Enzinger, C; Fazekas, F. Dynamics of brain iron levels in multiple sclerosis: A longitudinal 3T MRI study. Neurology. 2015 May 15. pii: 10.1212/WNL.0000000000001679. [Epub ahead of print]

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Gattringer, T; Enzinger, C; Khalil, M; Schwingenschuh, P; Pichler, A; Moser, A; Graninger, W; Ernst, C; Haybaeck, J; Fazekas, F. Unusual deterioration in a patient with multiple sclerosis on natalizumab therapy. Neurol Neuroimmunol Neuroinflamm. 2014; 1(1):e1-e1

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2012

Full papers/articles (Journal)

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2011

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Beitzke, M; **Gattringer, T**; Enzinger, C; Wagner, G; Niederkorn, K; Fazekas, F. Clinical presentation, etiology, and longterm prognosis in patients with nontraumatic convexal subarachnoid hemorrhage. Stroke. 2011; 42(11):3055-3060 [OPEN ACCESS]

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Published Manuscript

A part of this doctoral thesis has been published in the ***International Journal of Stroke*** (Impact factor 2014: 4.029).

The manuscript can be downloaded under the following link (accessed: May, 12. 2015):

http://onlinelibrary.wiley.com/doi/10.1111/ijs.12499/abstract

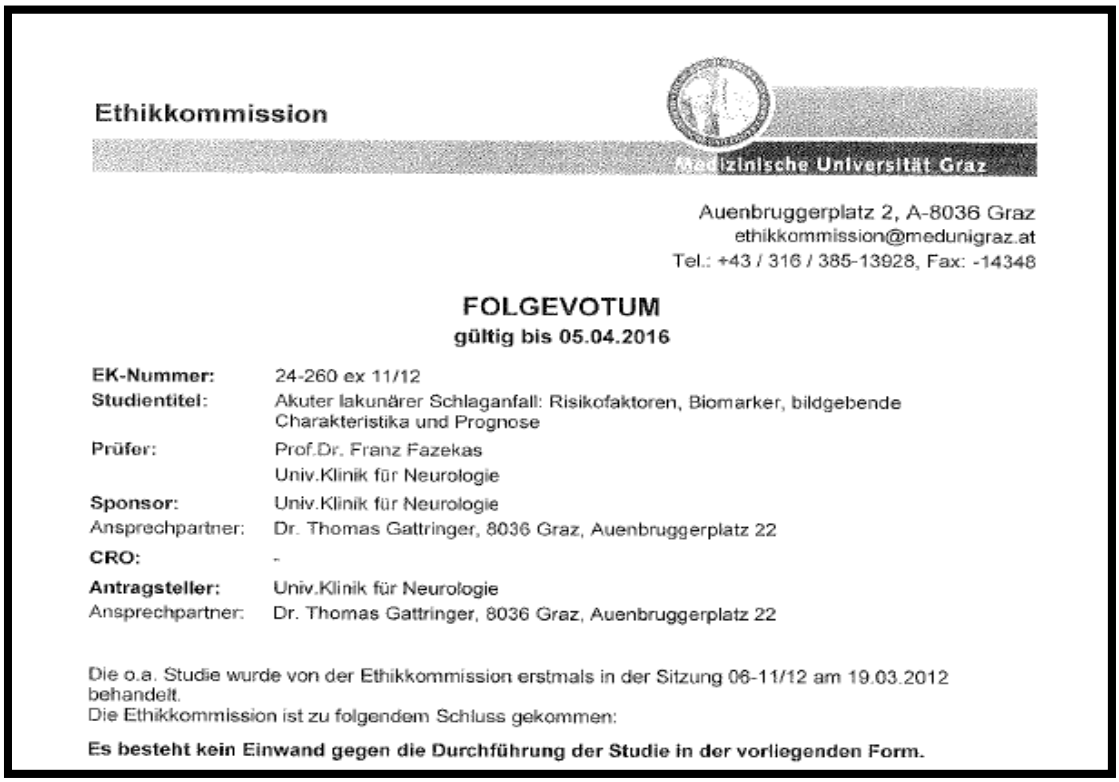
Gattringer T, Eppinger S, Pinter D, Pirpamer L, Berghold A, Wünsch G, Ropele S, Wardlaw JM, Enzinger C, Fazekas F. Morphological MRI characteristics of recent small subcortical infarcts. Int J Stroke. 2015 Apr 12. doi: 10.1111/ijs.12499. [Epub ahead of print]

Scientific Presentation

The present study was officially presented as an oral communication at the **18. Annual Meeting of the Austrian Stroke Society**.

Gattringer T. Morphologische MRT Charakteristika von rezenten kleinen subkortikalen Infarkten.

Ethics committee votes



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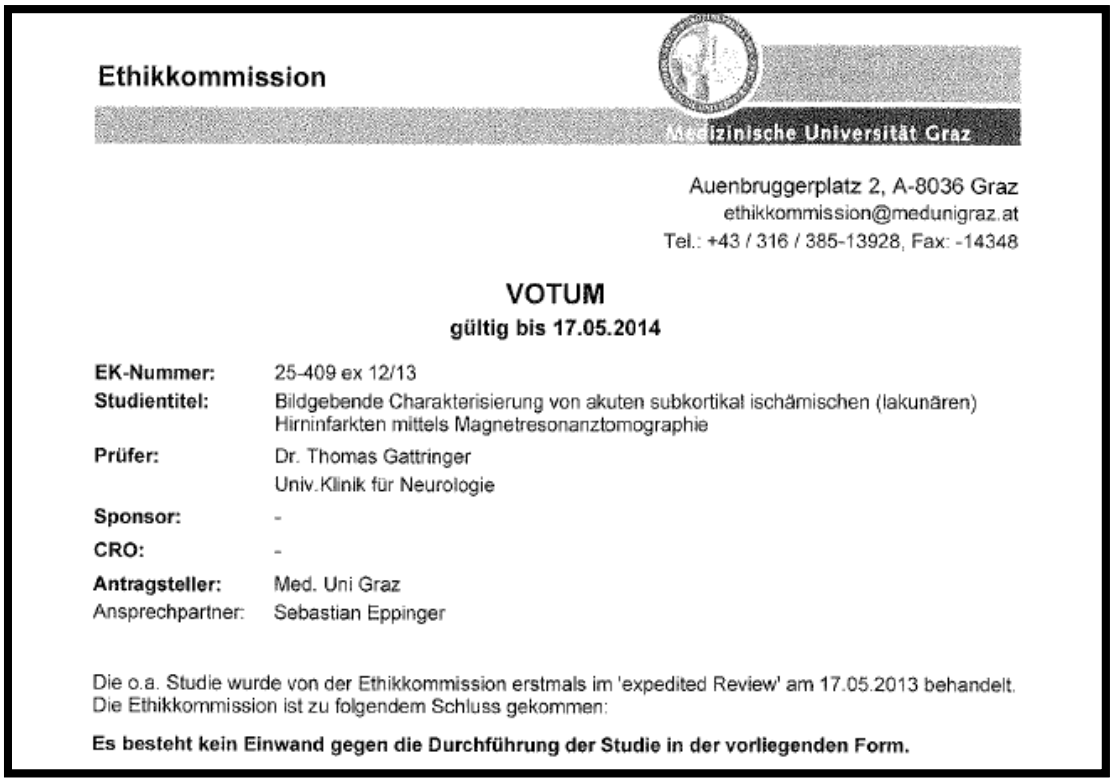
FOLGEVOTUM

gültig bis 05.04.2016

EK-Nummer:	24-260 ex 11/12
Studientitel:	Akuter lakunärer Schlaganfall: Risikofaktoren, Biomarker, bildgebende Charakteristika und Prognose
Prüfer:	Prof.Dr. Franz Fazekas Univ.Klinik für Neurologie
Sponsor:	Univ.Klinik für Neurologie
Ansprechpartner:	Dr. Thomas Gattringer, 8036 Graz, Auenbruggerplatz 22
CRO:	-
Antragsteller:	Univ.Klinik für Neurologie
Ansprechpartner:	Dr. Thomas Gattringer, 8036 Graz, Auenbruggerplatz 22

Die o.a. Studie wurde von der Ethikkommission erstmals in der Sitzung 06-11/12 am 19.03.2012 behandelt.
Die Ethikkommission ist zu folgendem Schluss gekommen:

Es besteht kein Einwand gegen die Durchführung der Studie in der vorliegenden Form.



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VOTUM

gültig bis 17.05.2014

EK-Nummer:	25-409 ex 12/13
Studientitel:	Bildgebende Charakterisierung von akuten subkortikal ischämischen (lakunären) Hirninfarkten mittels Magnetresonanztomographie
Prüfer:	Dr. Thomas Gattringer Univ.Klinik für Neurologie
Sponsor:	-
CRO:	-
Antragsteller:	Med. Uni Graz
Ansprechpartner:	Sebastian Eppinger

Die o.a. Studie wurde von der Ethikkommission erstmals im 'expedited Review' am 17.05.2013 behandelt.
Die Ethikkommission ist zu folgendem Schluss gekommen:

Es besteht kein Einwand gegen die Durchführung der Studie in der vorliegenden Form.