Dissertation

Relevance of imaging and laboratory biomarkers for the detection of cardioembolic sources in cryptogenic stroke patients

submitted by

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Declaration

*I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the "**Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz**".*

Graz, April 2021

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Disclosures

The current doctoral thesis was the basis for the preparation of a manuscript, which has already been published in the Journal ***Stroke*** *– a prestigious journal published on behalf of the American Heart Association and the American Stroke Association.*
The published manuscript was drafted by the doctoral candidate, Markus Kneihsl.
Therefore, significant parts of the doctoral thesis are similar to the following published manuscript:

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Abbreviations and Definitions

AAA	Aortic arch atherosclerosis
AF	Atrial fibrillation
ATP	Adenosine triphosphatase
AUC	Area under the curve
CS	Cryptogenic stroke
CT	Computed tomography
ECG	Electrocardiogram
EF	Ejection fraction
e.g.	Latin: exempli gratia, for example
ESUS	Embolic stroke of undetermined source
FLAIR	Fluid attenuated inversion recovery
HR	Hazard ratio
IHIS	Intraplaque high-intensity signals
ILR	Implantable loop recorder
LV	Leftventricular
MEDOCS	MEdical DOcumentation and Communication System
MRI	Magnetic resonance imaging
mRS	Modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
NPV	Negative predictive value
NT-proBNP	N-terminal pro-brain natriuretic peptide
PACNS	Primary angiitis of the central nervous system
PPV	Positive predictive value
RCVS	Reversible cerebral vasoconstriction syndrome
ROC	Receiver operating characteristic
RR	Relative risk
SVD	Small vessel disease
TOAST	Trial of Org 10172 in Acute Stroke Treatment
tPA	Tissue plasminogen activator
WMH	White matter hyperintensities
YI	Youden’s-Index

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Abstract

Introduction:

Occult atrial fibrillation (AF) is an important undetected cause of cryptogenic stroke (CS). Prolonged cardiac rhythm monitoring is the method of choice to detect silent episodes of AF, but its use is limited by costs and availability. In this context, biomarkers that could help to steer the diagnostic management of such patients would be of great advantage.

In this study, we tested laboratory and neuro-/ cardiac imaging biomarkers for the prediction of occult AF in CS patients (a) in the early in-hospital phase after stroke (project 1) and (b) over long-term follow-up after hospital discharge (project 2).

Methods:

For the first project, all ischemic stroke patients who were admitted at the Stroke Unit of the Department of Neurology of the University Hospital Graz between April 2017 and April 2018 underwent routine diagnostic work-up. If no distinct stroke etiology was identified (CS), patients underwent a prolonged cardiac rhythm monitoring for ≥72 hours during hospital stay. Blood biomarkers (NT-proBNP, D-dimer, Antithrombin-III) were analyzed within 24 hours after Stroke Unit admission.

For the second project, a risk score for the long-term prediction of AF was developed based on a literature review including clinical, laboratory and neuro-/ cardiac imaging markers that had been associated with occult AF in CS patients. To evaluate the score, over an 18-months period, all Stroke Unit patients with CS were followed after hospital discharge for a later diagnosis of occult AF.

Results:

In the first study, 143 CS patients were included. Of those, 14 patients were diagnosed with AF on prolonged in-hospital cardiac rhythm monitoring. The admission NT-proBNP cutoff value of ≥505 pg/ml (highest Youden's index) correctly predicted AF in 12 of 14 patients (negative predictive value: 98%) while hypercoagulability markers were non-contributory.

In the second project, 24 out of 150 CS patients (16%) were diagnosed with AF during a median follow-up period of 13 months. The predefined AF Risk Score (cutoff ≥4 points, highest Youden's index) had a sensitivity of 79% and a specificity of 72% for the long-term prediction of occult AF in CS patients. When adding NT-proBNP above the prespecified cutoff of 505 pg/ml to the score, sensitivity increased to 87% while specificity remained at 72%.

Of specific note, only one patient with an AF risk score below 4 was diagnosed with AF during the follow-up period (negative predictive value: 99%).

Discussion:

This study supports the value of NT-proBNP to predict AF in CS patients in the early in-hospital phase. Patients with low NT-proBNP admission levels (<505 pg/ml) are at very low risk to be diagnosed with AF early on.

Moreover, we here developed an AF Risk score for the long-term prediction of AF in CS patients with high sensitivity and reasonable specificity. Low risk scores of <4 points almost excluded a later diagnosis of AF.

Zusammenfassung

Einleitung:

Die unentdeckte Vorhofflimmerarrhythmie (VHFA) repräsentiert eine wichtige und häufig übersehene Ursache kryptogener Schlaganfälle. Ein intensiviertes kardiales Rhythmusmonitoring ist essentiell um versteckte VHFA-Episoden zu detektieren, wobei der hohe diagnostische Aufwand die Anwendung limitiert. Einfache Biomarker für VHFA könnten das diagnostische Management effizienter gestalten.

In dieser Studie untersuchten wir daher laborchemische und bildgebende Biomarker für die Prädiktion einer okkulten VHFA bei kryptogenen Schlaganfallpatient*innen (a) in der Frühphase während des stationären Aufenthaltes (Projekt 1) und (b) in der Langzeitvorhersage nach Spitalsentlassung (Projekt 2).

Methodik:

Alle Patient*innen mit ischämischem Schlaganfall, die zwischen April 2017 und April 2018 an der Schlaganfallakutstation (Stroke Unit) der Universitätsklinik für Neurologie Graz stationär aufgenommen wurden, unterliefen eine ätiologische Routineabklärung. Bei nachfolgend unklarer Schlaganfallursache erfolgte ein intensiviertes kardiales Rhythmusmonitoring (≥72 Stunden) während des weiteren Spitalsaufenthaltes. Blutbiomarker (NT-proBNP, D-dimer, Antithrombin-III) wurden innerhalb von 24 Stunden nach Krankenhausaufnahme analysiert.

Für das zweite Projekt wurde basierend auf einer Literaturrecherche ein Risikoscore für die Langzeitvorhersage einer VHFA entwickelt. Darin wurden klinische, laborchemische und bildgebende Marker, die mit VHFA bei kryptogenem Schlaganfall assoziiert wurden, inkludiert. Um den Score zu evaluieren, wurden alle Stroke Unit Patient*innen mit kryptogenem Schlaganfall zwischen März 2018 und August 2019 nach Krankenhausentlassung zur Detektion einer VHFA nachverfolgt.

Resultate:

In die erste Studie wurden 143 kryptogene Schlaganfallpatient*innen inkludiert. Von diesen wurde bei 14 Patient*innen eine VHFA mittels intensiviertem kardialen Rhythmusmonitoring während des stationären Aufenthaltes detektiert. Ein NT-proBNP Cutoff-Wert von ≥505 pg/ml sagte die VHFA bei 12 von 14 Patient*innen korrekt vorher (negativ prädiktiver Wert: 98%), während D-dimer und Antithrombin-III als Marker für Hyperkoagulabilität keine relevante Prädiktion zuließen.

Im Rahmen des zweiten Projektes wurden von 150 kryptogenen Schlaganfallpatient*innen 24 (16%) im Langzeitverlauf mit einer VHFA diagnostiziert (mediane Beobachtungszeit: 13 Monate). Der vordefinierte Risiko-Score (≥4 Punkte, höchster Youden Index) erreichte eine Sensitivität von 79% und eine Spezifität von 72% für die Langzeitvorhersage einer VHFA bei kryptogenen Schlaganfallpatient*innen. Nach Inkludierung von NT-proBNP in den Score wurde eine höhere Sensitivität von 87% bei gleichbleibender Spezifität (72%) erreicht. Bemerkenswert war, dass nur ein Patient mit einem Risiko-Score <4 Punkte in der Nachbeobachtungsperiode eine VHFA zeigte (negativ prädiktiver Wert: 99%).

Diskussion:

Diese Studie unterstützt die Wertigkeit von NT-proBNP als Prädiktor einer VHFA in der frühen intramuralen Phase nach kryptogenem Schlaganfall. Patient*innen mit NT-proBNP Werten <505 pg/ml bei Aufnahme haben ein sehr geringes Risiko während des Spitalsaufenthaltes eine VHFA zu präsentieren.

Im Rahmen dieser Dissertation wurde erstmals ein Risikoscore für die Langzeitprädiktion einer VHFA bei kryptogenen Schlaganfallpatient*innen mit hoher Sensitivität und moderater Spezifität entwickelt. Niedrige Risikoscores <4 Punkte schlossen eine spätere VHFA-Detektion mit hoher Sicherheit aus.

Introduction

Ischemic stroke

Stroke is the primary cause of long-term disability and the third leading cause of death worldwide.[1] About one third of all stroke survivors remain dependent in daily life activities, which causes tremendous economic burden on healthcare systems. In Europe, direct and indirect costs of stroke amounted to €60.0 billion in 2017.[2]

In Austria, about 25.000 inhabitants suffer a stroke every year. According to "THE BURDEN OF STROKE IN EUROPE" report, stroke incidence is expected to increase by 42% until 2035 due to demographic developments and a higher life expectancy.[3]
This can be explained by the fact that stroke is a highly age-dependent disease: The annual stroke incidence in Austria ranges between 19 / 100.000 inhabitants in people under 50 years of age and 1806 / 100.000 inhabitants in the oldest age group (>79 years).[4]
In general, men face a higher risk for stroke than women, but the magnitude of sex difference diminishes with older age.[5]

Stroke is a highly feared disease, which is often attributed to the very sudden onset of clinical symptoms in both brain ischemia and intracranial bleeding events. Five of six strokes are ischemic (85%).[6]

Pathophysiology in acute cerebral ischemia

Brain ischemia is attributable to stenosis or occlusion of brain supplying vessels leading to impaired cerebral perfusion and insufficient glucose and oxygen supply to certain areas of the brain. Hypoxia occurs when cerebral blood flow falls below 20 ml / 100 mg brain tissue.[7] Oxygen and glucose deprivation results in mitochondrial dysfunction and a reduction of adenosine triphosphatase (ATP), which is essential for hemostasis and function of brain cells. Moreover, oxygen deficiency promotes the release of excitatory neurotransmitters (e.g. glutamate).[8]
These processes lead to intracellular accumulation of sodium and fluid resulting in cell swelling and dysfunction. Such cytotoxic edema emerges within a few hours after onset of

ischemia and can be observed on brain imaging using magnetic resonance imaging (MRI) or computed tomography (CT) in acute stroke patients.[9]

In prolonged ischemia, reactive oxygen and nitrogen species form and cause damage to the cell membrane and the blood-brain-barrier. The additional break down of the osmotic pressure gradient leads to extravasation of fluid and results in vasogenic edema which causes further damage to brain tissue **(Figure 1)**.[7-9]

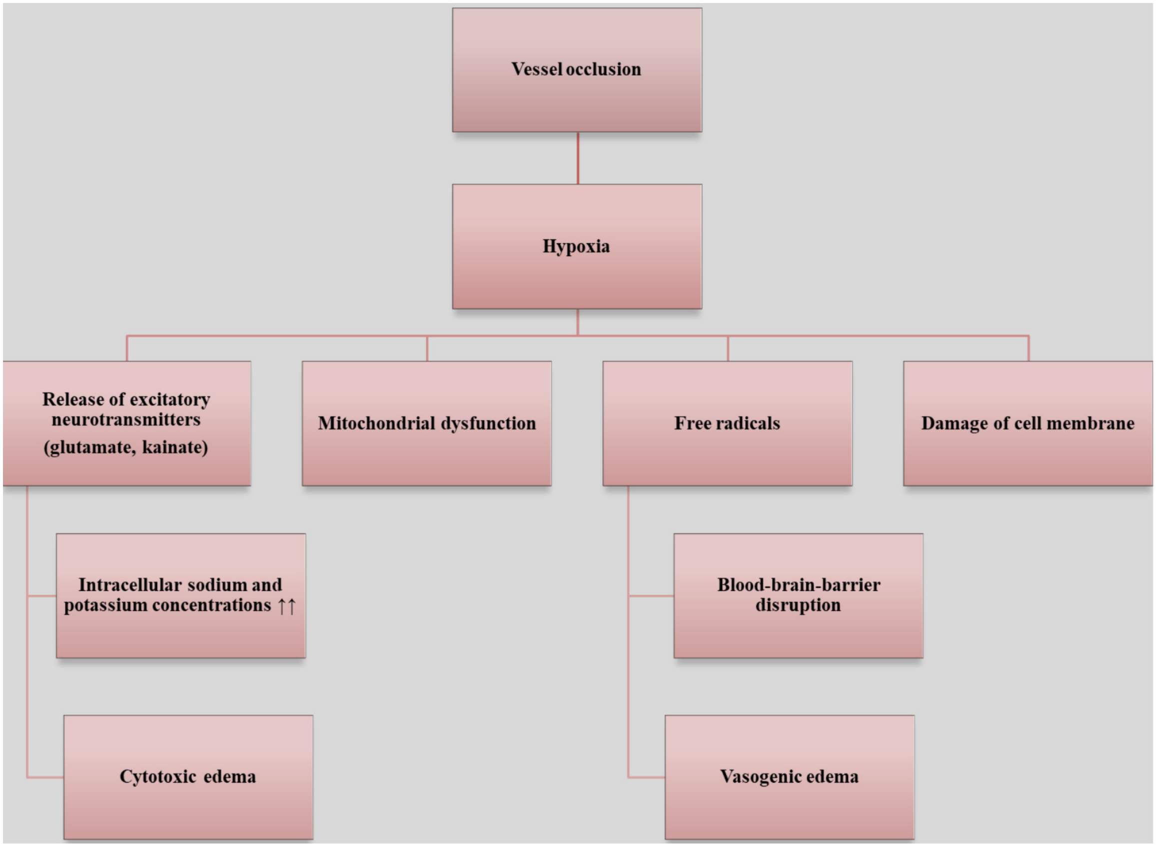

Figure 1. Key pathophysiologic mechanisms involved in acute ischemic stroke

Acute stroke treatment

Treatment of ischemic stroke has largely improved over the past two decades. Since the early 2000s, intravenous (IV) thrombolysis using tissue plasminogen activator (tPA) has become the standard treatment to dissolve blood clots in brain supplying vessels.[10]

However, in patients suffering from intracranial large vessel occlusion, IV thrombolysis only leads to successful reperfusion in about 10-15% of patients.[11] Hence, further research focused on local techniques to remove the thrombus mechanically.

In 2015, five randomized clinical trials showed that mechanical thrombectomy using stent-retriever systems is a safe and efficient treatment option in patients with large vessel occlusion stroke reaching recanalization rates of up to 90%.[12] Nowadays, good 90-day post-stroke outcome according to a modified Rankin Scale score of <3 is achieved in about 50% of all thrombectomy patients.[13]

However, the implementation of efficient recanalization therapies is not the sole reason for such positive outcome rates: In Austria, the nationwide concept of treating all stroke patients in specialist Stroke Units or Neurointensive Care Units consisting of a team of stroke specialists including physicians and nurses has further improved stroke treatment and the early detection and prevention of post-stroke complications (**Figure 2**). In addition, Stroke Units facilitate rapid diagnostic work-up which is crucial for the detection of stroke etiology and early and efficient secondary stroke prevention.

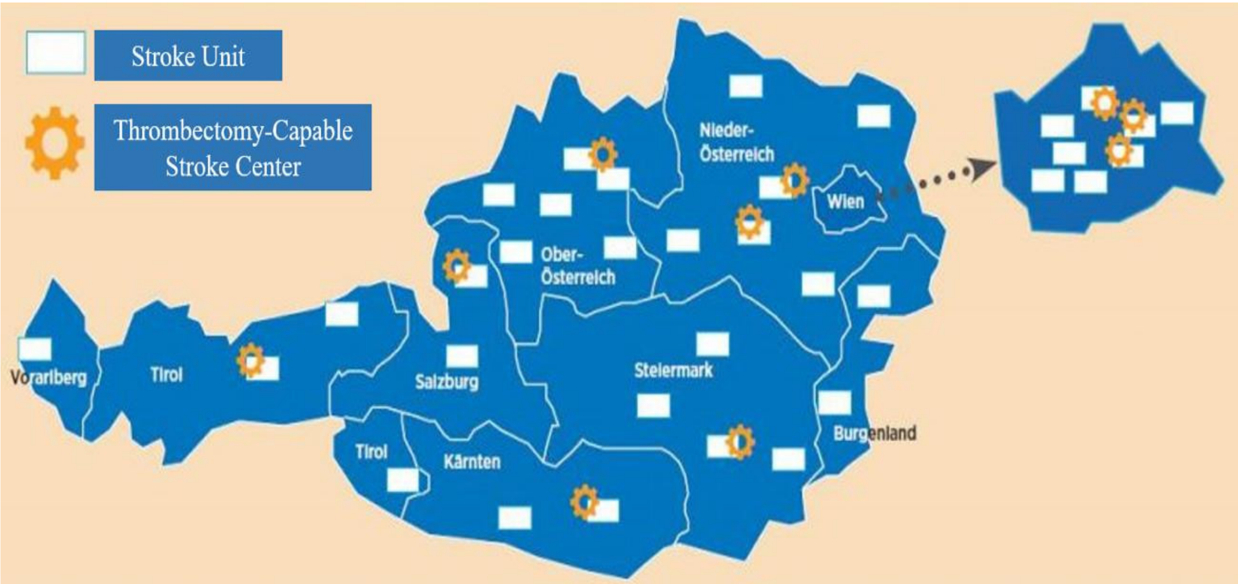

Figure 2. Stroke care in Austria[14]: 39 Stroke Units and 10 Thrombectomy-capable Stroke Centers ensure high-quality stroke treatment. Permission to reproduce and modify *Figure 2* granted (*Bettschart & Kofler Kommunikationsberatung GmbH*).

Stroke etiology and work-up

Ischemic stroke is not a uniform disease and stroke work-up is not trivial. Knowledge about possible stroke etiologies in typical risk situations and in different age groups is essential to steer the diagnostic work-up. For example, while artery dissection is a common cause of stroke in young adults, cardioembolism is the most frequent stroke etiology in eldery people.[15,16]

In general, three stroke etiologies are responsible for about 70% of all strokes:

1) *Cardioembolism*:
   From a pathophysiological perspective, different cardiac diseases (e.g. rhythm disorders, myocardial infarction, cardiac insufficiency, etc.) can lead to focal stasis of the blood or coagulation activation causing local thrombus formation in the left atrium or ventricle. Moreover, material from an abnormal valvular surface (e.g., calcific heart valves) can detach. Another mechanism arises from persistent foramen ovale / atrial septum defect, which can lead to thrombus passage from the venous to the arterial circulation (paradoxical embolism).[17] Such embolic material (mostly blood clots) moves on via the ascending aorta and the carotid or subclavian arteries into the anterior cerebral circulation or the vertebrobasilar arteries and causes occlusion of brain supplying vessels.
   This pathomechanism frequently affects more than one vascular territory of the brain, entailing a multilocular infarct pattern on cerebral imaging (**Figure 3**). Moreover, singular cortical and cerebellar strokes are also typical for embolism of cardiac origin.[18]

   Cardioembolism is the most frequent cause of intracranial large vessel occlusion, which often leads to severe clinical stroke syndromes and persistent functional dependency.[19]

   Therefore, early cardiac stroke work-up is essential for efficient secondary stroke prevention.

Apart from classical cardiac rhythm disorders or heart insufficiency, many patients with cerebrovascular disease have concomitant coronary heart disease or myocardial infarction, which are both associated with high morbidity and mortality.[20,21]

After a structured medical history taking and clinical examination, an electrocardiogram (ECG) should be conducted in all ischemic stroke patients soon after hospital admission. If clinical / imaging signs of multilocular infarction are present or the patients show anamnestic / clinical features of cardiac disease (e.g. heart murmur and fever, prosthetic heart valve, etc.) immediate echocardiography should be conducted to exclude a significant cardiac thrombus formation.[22]

While transthoracic echocardiography is sufficient in the majority of acute ischemic stroke patients, a transesophageal approach should be performed under the following circumstances:[19]

- Age < 60 years
- Neuroimaging signs of embolic stroke (i.e. multilocular infarction)
- Clinical signs of endocarditis (i.e. heart murmur ± fever ± fatigue ± laboratory signs of inflammation)
- Recent deep venous thrombosis / pulmonary thrombosis (Paradoxical embolism?)

To diagnose (paroxysmal) cardiac rhythm disorders, further ECG-monitoring over at least 24 hours is recommended according to current guidelines.[23] Apart from classical atrial arrhythmias (i.e. atrial fibrillation [AF] and atrial flutter), severe cardiac conduction disorders (e.g. high-grade atrioventricular block) can also lead to hypoperfusion of the brain and hemodynamic cerebral infarction.[24]

However, 90% of cardioembolic strokes are caused by AF. In such patients, oral anticoagulation is the gold-standard treatment and leads to a 70% reduction of recurrent thrombo-embolic events.[25]

Although early post-stroke initiation of anticoagulation in AF-related stroke patients is crucial for secondary stroke prevention, the higher risk of intracranial bleeding complications in the acute phase after stroke must be considered.

However, the better safety profile of new oral factor Xa / thrombin inhibitors compared to the classical vitamin-k-antagonists encouraged to reinforce strategies to (re-) start anticoagulation within a few days after the ischemic event.[26,27] An ongoing randomized controlled multicenter trial set out to define the optimal time to (re-) start anticoagulation depending on the size of cerebral infarction.[28]
Those efforts underscore the value of early detection of AF after ischemic stroke.

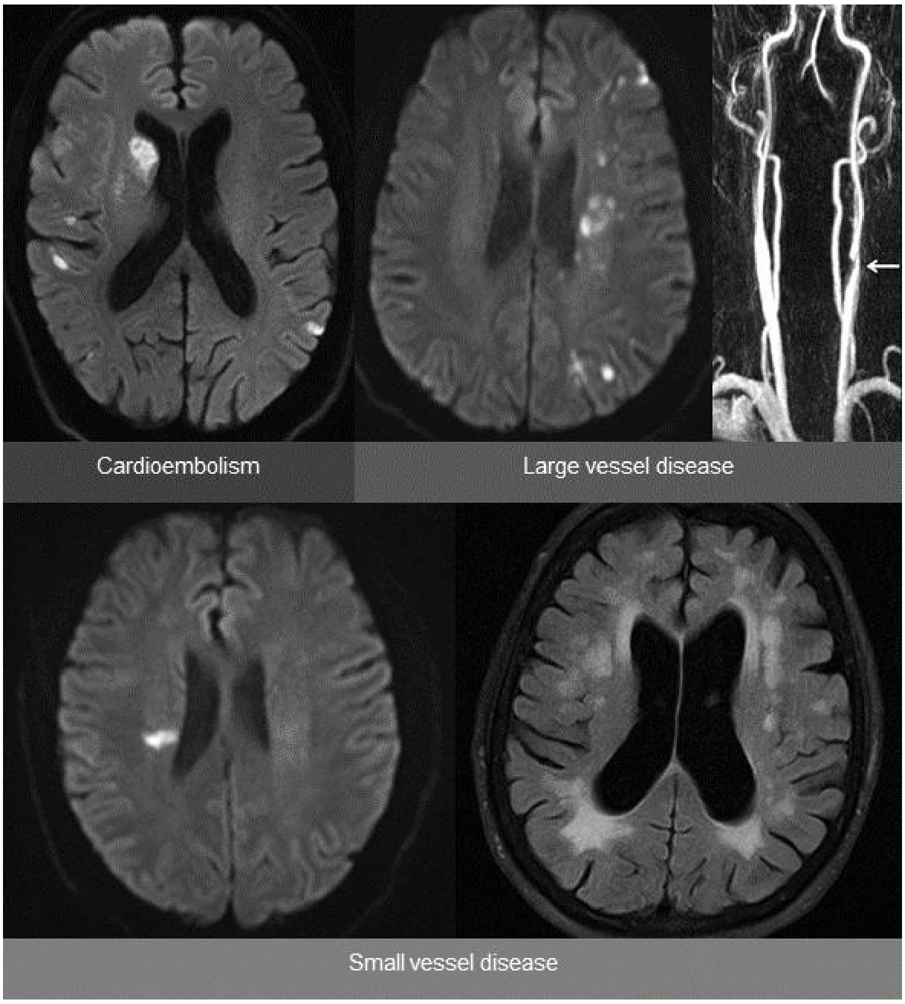

Figure 3. Typical ischemic stroke patterns on brain magnetic resonance imaging: Cardioembolism often leads to infarcts in multiple vascular territories while large vessel disease-associated stroke typically affects the borderzone areas of one brain-supplying vessel. Small subcortical infarcts and white matter hyperintensities are classical features of cerebral small vessel disease (*own figure*).

2) <u>*Large vessel disease*</u>:

   Arterial large vessel disease of brain supplying arteries represents the second most common stroke etiology and is responsible for about 25% of all ischemic strokes.

Due to genetic and vascular risk factors (e.g. hypertension, smoking, hyperlipidemia, diabetes), endothelial damage and fatty streaks occur and result in arterial vessel wall plaques which narrow the arterial lumen.[29]

Two different mechanisms are responsible for cerebral ischemia in large vessel disease associated strokes:

A) Hemodynamically significant stenosis, defined as vessel narrowing that reduces downstream cerebral blood flow and consequently leads to cerebral hypoperfusion. Such infarcts typically occur in the border zones between major vascular territories of the brain.[30,31]

B) The more prevalent mechanism is arterio-arterial embolism, which is caused by detached parts of arterial plaques or by focal thrombus formation based on plaque rupture.[32] Multiple acute and subacute lesions in vascular territories supplied by the affected vessel are the typical infarct pattern in such patients (**Figure 3**).

Early detection of symptomatic extracranial vessel stenosis is crucial for efficient secondary stroke prevention: In case of a transient ischemic attack or a clinically minor to moderate stroke, surgical therapy should be performed as soon as possible. In general, carotid artery stenting is not inferior to surgical carotid endarterectomy in patients aged <70 years and has additional advantages in special cases (e.g. radiation-induced vasculopathy, contralateral carotid occlusion, etc.).[33,34] Elderly patients (≥70 years) should be preferably treated by surgery as endovascular treatment was associated with higher rates of peri-interventional complications.[33]

If conservative treatment is chosen, antiplatelet agents and high-intensity statins (i.e. atorvastatin, rosuvastatin) significantly reduce the risk for recurrent cerebrovascular events.[3]

3) *Small vessel disease:*

Cerebral small vessel disease (SVD)-related stroke is the third most prevalent stroke etiology, being responsible for about 20% of all strokes.

Brain infarcts are small with a maximum diameter of up to 20 mm and are caused by the occlusion of deep penetrating small cerebral arteries.[36] This leads to the typical infarct location in the cerebral white matter, the basal ganglia, the thalamus or the brainstem.[37,38]

Lipohyalinosis and arteriolosclerosis are suspected to cause impairment of the small cerebral vessels, leading to secondary vessel occlusion.[39] Although the underlying mechanisms are still incompletely understood, classical cerebrovascular risk factors including arterial hypertension, smoking and alcohol abuse, diabetes, hyperlipidemia and obesity were associated with such changes.[36]

Cerebral SVD is an MRI-based diagnosis: Typical neuroimaging features are white matter hyperintensities (WMH), lacunes, microbleeds and enlarged perivascular spaces. The Fazekas Scale is the international standard for grading the severity of cerebral WMH, which are classified into four categories (0-3).[40] If a small subcortical infarct is accompanied by typical chronic imaging features of cerebral small vessel impairment, SVD-related stroke is the most probable etiology (**Figure 3**). Brain MRI is therefore preferably used to identify SVD-associated stroke.

Antiplatelet agents and intense control of vascular risk factors are the main treatment options in such patients.[36]

4) *Rare etiologies*:
About 5% of all strokes are attributed to rare diseases. Strokologists must be familiar with typical anamnestic and clinical features to ask patients the right questions and to steer the clinical, laboratory and imaging work-up to avoid diagnostic delay.

- *Artery dissection:* Cervical artery dissection accounts for 10-25% of all strokes in patients aged <45 years. Typical clinical signs are sudden onset neck pain and / or headache, which is present in about 75 % of patients suffering carotid / vertebral artery dissection.[41,42]

Many patients also describe a history of rapid neck movements or cervical manipulation.[43] MRI has become the method of choice to detect dissection related intramural hematoma, intima flap, pseudoaneurysm and / or vessel stenosis.[44]

- *Vasculitis:* Arteritis can affect large and small brain supplying vessels leading to cerebral ischemia. Multifocal vessel stenoses in patients without significant vascular risk factors are indicative of vasculitis. While inflammatory supra-aortic vessel affection in young and mostly female patients points towards Takayasu arteritis, large vessel vasculitis in elderly patients is typical for giant cell arteriitis.[44]

Vasculitis affecting medium- and small-sized vessels has a much broader etiological spectrum ranging from systemic infections / disorders (e.g., Lyme disease, varicella-zoster virus infection, Wegener's Granulomatosis) to inflammatory diseases that only affect cerebral vessels (primary angiitis of the central nervous system, PACNS).[45] A medical history of weight loss, fatigue, fever or headache over the days / weeks before the index event are typical in patients suffering from vasculitis.

Diagnostic work-up should always include CT / MR-based angiography of the extra- and intracranial vessels and targeted laboratory examinations (e.g. C-reactive protein, erythrocyte sedimentation rate, ANCA, etc.). In a further step, lumbar puncture, conventional angiography or even brain biopsy (in case of PACNS) might be needed to support or confirm the diagnosis of vasculitis of the brain-supplying vessels.[46]

Apart from classical ischemic stroke therapy using antiplatelet agents, additional corticosteroid-therapy is the first-line treatment in all such patients.

-*Migrainous infarction:* Migraine is a well-known risk factor for stroke and migranous infarction is a rare but also potentially underdiagnosed stroke etiology.[47] In epidemiologic studies, migraine accounted for 1-1.5% of all ischemic strokes.

Persisting aura symptoms beyond headache relief are typical for migrainous infarction and must entail brain imaging using MRI.[48] In more than two thirds of all strokes related to migraine, the posterior cerebral circulation is affected.[49]

-*Reversible cerebral vasoconstrictory syndrome (RCVS):* Although there might be some overlap with migraine regarding vasoconstriction, RCVS is a distinct stroke entity typically presenting with recurrent thunderclap-headache and focal neurological deficits. Typical risk factors are migraine, pregnancy or the use of vasoconstrictive drugs or medications.[59] Subarachnoid hemorrhage must always be ruled out as the most important differential diagnosis. Apart from ischemic infarction, vasogenic edema and vessel narrowing are the typical findings on brain MRI.[51]

An overview of rare stroke etiologies is given in **Table 1**.

Rare stroke etiologies	
Rare cardiac diseases	
Endocarditis	Persistent foramen ovale + in situ thrombosis
Prosthetic heart valve	Intracardiac mass
Vasculopathy	
Arterial dissection	Fibromuscular dysplasia
Moyamoya disease	*Primary vasculitis:* -Primary angiitis of the central nervous system
Secondary vasculitis: - Granulomatous vasculitis (giant cell / Takayasu arteritis, Wegener's granulomatosis) - Nongranulomatous vasculitis (polyarteritis nodosa, Behçet syndrome, cryoglobulinemic vasculitis)	*Vasculitis based on collagenosis:* - Lupus erythematosus - Rheumathoid arthritis - Sjögren's syndrome
Rare microangiopathy	
SUSAC syndrome	FABRY disease
Cerebral autosomal dominant Arteriopathy with subcortical Infarcts and Leukoencephalopathy (CADASIL)	Hereditary Endotheliopathy with Retinopathy, Nephropathy and Stroke (HERNS)
Coagulation disorders / hypercoagulopathy	
Antiphospholipid antibody syndrome	Disseminated intravascular coagulation
Cancer-associated hypercoagulopathy	
Stroke due to vasoconstriction	
Migranous infarction	Reversible cerebral vasoconstriction syndrome (RCVS)
Hematological disorders	
Polycythemia vera	Sickle cell disease
Moschcowitz syndrome	Paroxysmal nocturnal hemoglobinuria (PNH)

Table 1. Overview of rare etiologies of ischemic stroke.

Cryptogenic Stroke

The early diagnosis of stroke etiology is crucial for the clinical management of a patient and efficient secondary stroke prevention. Apart from acute vessel recanalization therapies and early rehabilitation, the prevention of vascular (re-) events defines a patient's long-term outcome after stroke.

However, despite intense diagnostic work-up, stroke etiology remains undetermined in up to one third of all ischemic stroke patients.[52] Especially in young patients, the cause of stroke often remains unclear.[53]
"Cryptogenic" is still the most widely used term to describe such a condition.[54] However, cryptogenic stroke (CS) summarizes a broad spectrum of possible definitions and classifications:

For example, the classical Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification does not specify the extent and quality of diagnostic stroke work-up and describes stroke as cryptogenic if more than one stroke etiology is detected (e.g. cerebral SVD and atrial fibrillation).[55]

The significant improvement in stroke diagnostics including the evolution of brain imaging as well as the expanding knowledge about rare causes of stroke entailed more detailed approaches to classify strokes of undetermined source (e.g. causative classification system, A-S-C-O score).[56] However, all those systems bear significant limitations as they remain relatively complex and are insufficient to adequately guide clinical decisions.

In the early 2010s, the term ESUS (Embolic stroke of undetermined source) was introduced and coined by the CS / ESUS international working group.[52] The idea behind ESUS was that most strokes of undetermined etiology might have an embolic origin. ESUS was defined as follows:

Embolic stroke of undetermined source (ESUS)
A) Non-lacunar infarct (subcortical infarct ≤1.5 cm on CT or ≤2.0 cm on MRI)
B) Absence of
- extracranial or intracranial atherosclerosis causing >50% luminal stenosis in the artery supplying the ischemic region
- major cardioembolic sources: permanent or paroxysmal AF, atrial flutter, intracardiac thrombus, prosthetic cardiac valve, atrial myxoma or other cardiac tumors, mitral stenosis, myocardial infarction within the past 4 weeks, left ventricular ejection fraction (EF) <30%, valvular vegetation's or infective endocarditis
C) No other specific cause of stroke
- e.g. dissection, arteritis, migraine/vasospasm

Table 2. Definition of embolic stroke of undetermined source (ESUS) according to the CS / ESUS international working group.[52]

Compared to earlier definitions / classification systems, ESUS provides an easy and clearly defined diagnostic term that requires intense diagnostic stroke work-up.

However, ESUS might lead to some misclassification as lacunar infarcts can also be caused by embolism or macroangiopathy in up to 15% of patients, especially when no other signs of SVD are present.[57,58] Moreover, "embolic" might not be the right term in case of unrecognized hypercoagulability (e.g. cancer-associated coagulation activation / hematological disorders), as thrombus formation often occurs locally in vascular low-flow areas of the brain based on Virchow's triad.[59]

In this work, we therefore decided to use the more comprehensive term "cryptogenic", which is defined in detail in the Methods section.

Potential etiologies in cryptogenic stroke

If no specific cause of stroke has been detected, the routine stroke work-up should be reviewed for possible unrecognized etiologies or risk factors:

A) *Arterio-arterial embolism*:
Apart from hemodynamic infarction, arterio-arterial embolism is the main pathomechanism of ischemic stroke in symptomatic extra- and intracranial artery stenosis. However, non-hemodynamic stenosis of the brain supplying vessels have long been an underestimated stroke etiology (see case in **Figure 4**).

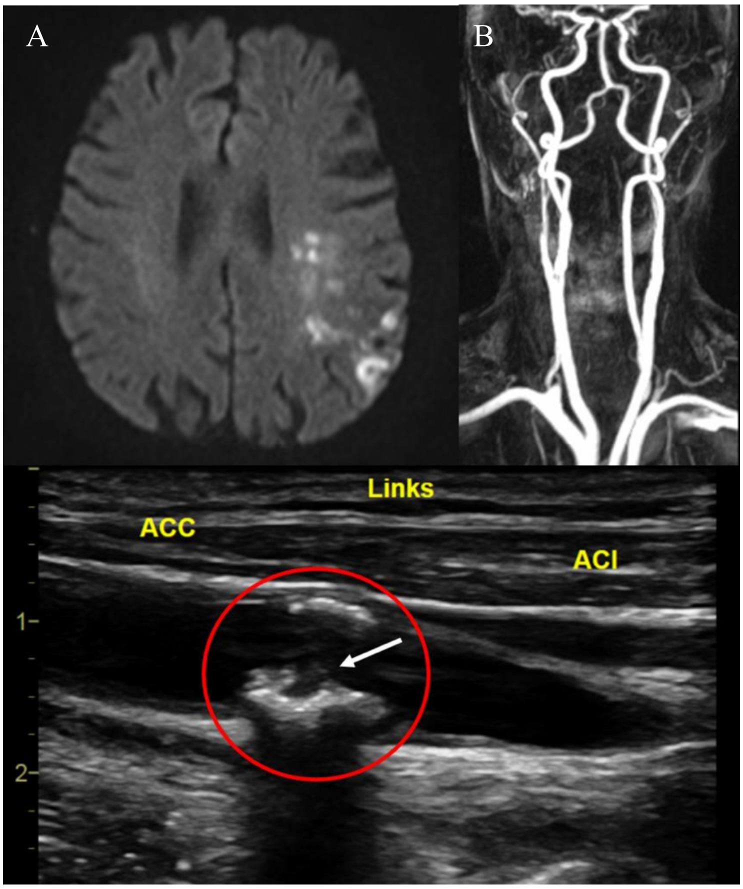

Figure 4. Arterio-arterial embolism from a symptomatic carotid stenosis:

This case shows a 79-year old female patient who presented with right-sided hemiparesis. Brain MRI detected small scattered lesions in the left middle cerebral artery territory (**A**). Extracranial angiography was unremarkable (**B**), but ultrasound detected aggressive carotid atherosclerotic plaque with central ulceration (**C**, arrow). Patient underwent vascular surgery and no re-events were observed over a 36 months follow-up (*own figure*).

Over the past years, several studies using ultrasound and high resolution MR-angiography with vessel wall imaging associated complicated atherosclerotic plaques with brain infarction in CS patients.[60-62]

Ultrasound is the method of choice to screen for aggressive carotid artery plaque and Brinjikji and colleagues recently outlined the most relevant sonographic features of complicated atherosclerotic plaques with a high risk of rupture and consecutive arterio-arterial embolism **(Table 3)**:[60]

Complicated plaques – ultrasound characteristics	***Odds Ratio for ischemic stroke***
A) Ulceration: plaque surface crater ≥2 mm	***3.6***
B) Plaque echolucency: predominantly anechoic or hypoechoic plaque or Gray-Scale-Median score below 30	***4.0***
C) Intraplaque mobility: presence of mobile components within the plaque or on the plaque surface	***1.6***
D) Plaque neovascularity: intraplaque Doppler signal consistent with neovascularity or plaque enhancement on contrast ultrasound	***19.7***

Table 3. Ultrasound characteristics of complicated carotid plaques.[60,61]

Ultrasound-detected anechoic areas in a plaque can also be a sign of intraplaque hemorrhage, but differentiation from a classical soft plaque is difficult. In this context, high-resolution MR-angiography using vessel wall imaging can complement sonographic findings and identify intraplaque high-intensity signals (IHIS), which are highly indicative of intraplaque hemorrhage. [62]

In a cohort of CS patients, Gupta and colleagues detected carotid artery IHIS ipsilateral to the side of ischemic stroke in 22% of patients compared to 0% on the contralateral side ($p=0.01$).[62]

Compared to ultrasound, MR-based imaging allows additional investigation of the aortic arch. Aortic arch atherosclerosis (AAA) is a significant risk factor for ischemic stroke.

Apart from classical features of complicated plaques, aortic plaque size of >4 mm was associated with a significant increase in relative risk (RR) for the occurrence of ischemic stroke (RR: 4.7).[63] Treatment strategy for such patients should include aggressive antiplatelet treatment and high-intensity statin therapy to reduce the risk of arterio-arterial embolism.

B) *Cancer-related stroke*:
Active cancer promotes platelet activation, endothelial damage and hypercoagulable state.[64-66] The more aggressive the cancer, the higher is the chance for cerebrovascular events. Incidence rates of ischemic stroke of up to 15% have been raised in cancer patients.[64] Ischemic stroke can be the first clinical sign of an active tumor disease, but underlying cancer is still an underestimated stroke etiology.

In fact, cancer-related stroke patients suffer from a high early post-stroke mortality, which underscores the need for rapid diagnosis.[67] In this context, recent studies have identified typical features of (cryptogenic) stroke patients that could point towards an underlying malignancy:

The constellation of a CS patient with low age, few classical vascular risk factors and multiple small scattered cerebral infarcts affecting different vascular territories increases the likelihood for an underlying active cancer (see case in **Figure 5**).[67]

Low-molecular-weight heparin has long been considered the most effective treatment option to prevent recurrent ischemic stroke in such patients. New oral factor Xa / thrombin inhibitors are currently investigated as alternative option for treating cancer-related stroke.[68]

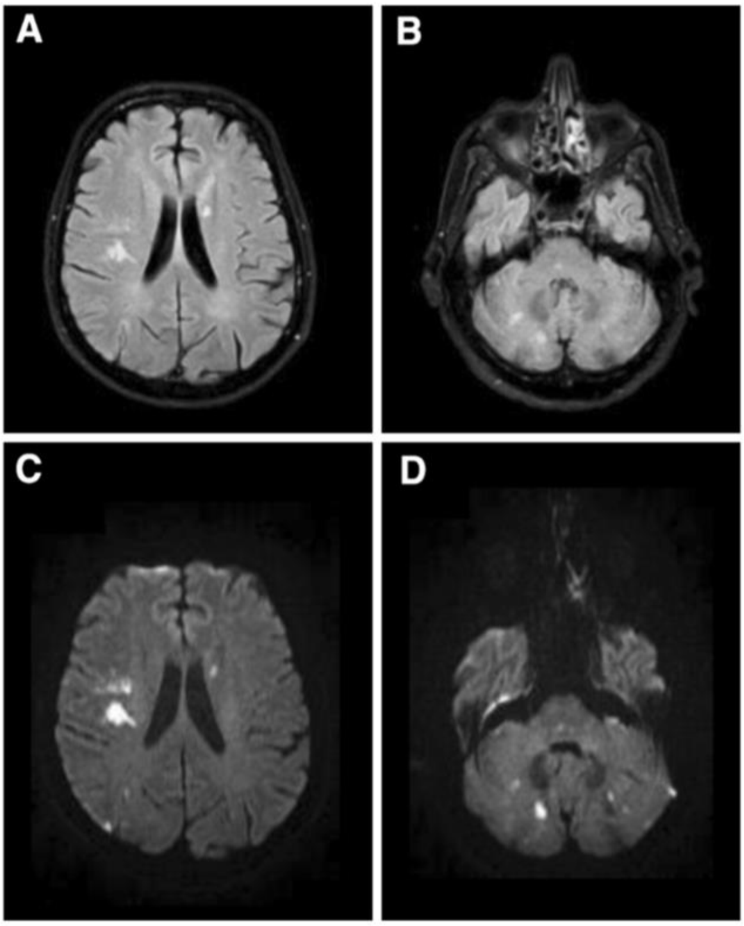

Figure 5. A case of cancer-associated ischemic stroke:
This figure shows MR images of a 54-year old female patient with cryptogenic stroke after the initial stroke work-up.
Clinical symptoms (loss of weight, fecal occult blood, fatigue) and brain MRI showing multiple small scattered infarcts in all parts of the brain (**A-D**) were highly suspicious for an underlying cancer.
Cancer work-up was conducted and an aggressive colon carcinoma was detected.[67]
Permission to reproduce *Figure 5* granted (*Springer Nature*).

C) *Cardioembolism*:

Cardioembolism might represent the most common undetected cause of CS. This is of high clinical importance as standard antiplatelet therapy is usually insufficient for secondary stroke prevention.

While cardiac etiologies arising from structural heart failure (e.g., severe cardiomyopathy, persistent foramen ovale, mural thrombus in the left cavity, intracardiac mass), ischemia (myocardial infarction) or inflammation (endocarditis) are relatively easy detected on routine cardiac work-up, diagnosis of cardiac rhythm disturbances are often challenging:

Cardiac rhythm disturbances – a diagnostic challenge

Cardiac rhythm abnormalities often occur paroxysmal and are frequently missed on standard ECG or 24 hours Holter monitoring.[69] Moreover, the majority of ischemic stroke patients with paroxysmal atrial high-rate episodes / arrhythmias do not show any clinical signs or have noted symptoms of the disease.

Over the past years, diagnostic tools have been developed to improve the rates of AF detection. In 2015, first data from the EMBRACE trial were presented: The authors compared a standard 24 hours Holter monitoring with a cardiac 30-day outpatient monitor in patients with CS or transient ischemic attack and found a five times higher AF detection rate in the group with prolonged cardiac rhythm monitoring (16.0 vs. 3.2%, $p<0.001$).[70]

In the following year, CRYSTAL AF was the first study that presented data on cardiac long-term monitoring in CS patients using implantable loop recorders (ILR). They also detected AF in a relatively high number of stroke patients who remained cryptogenic after the initial work-up (8.9 – 12.4 – 19.1% within 6 – 12 – 36 months post stroke respectively).[71]
Although AF detection rates markedly differed in those studies, both investigations underscored the value of prolonged cardiac rhythm monitoring in CS patients.

However, long-term cardiac monitoring techniques have certain limitations: Non-invasive outpatient monitoring is relatively uncomfortable resulting in a reduced compliance of selected patients and a limited observation period (about 30 days).[70] Most investigations showed a continuous increase of AF detection within the first twelve months after stroke, which might lead to an underestimation of AF in CS patients using such outpatient devices.

The alternative method are ILR, which are implanted subcutaneously in local anesthesia and offer observation periods of 36-48 months.[71] However, high costs and patient's general scepsis on invasive diagnostic tools limit their use as a screening device for all CS patients.

Hence, it is of great importance to select those patients that have a high chance to benefit from additional cardiac rhythm monitoring. In this context, cardiac imaging and laboratory biomarkers have been associated with cardioembolic stroke and might help to steer the diagnostic work-up in CS patients.

Laboratory and echocardiographic biomarkers in cardioembolism

A biological marker is a "substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome and disease".[72] Such markers can be helpful to identify patients that might benefit from further diagnostic or therapeutic procedures.
Earlier studies detected typical laboratory and echocardiographic markers in patients with known cardioembolism due to atrial fibrillation:

A) Laboratory biomarkers:

N-terminal pro-brain natriuretic peptide (NT-proBNP)

NT-proBNP is the inactive form of pro-BNP, a hormone released from cardiac myocytes because of stretch stimulation following increased atrial wall tension. Hemodynamic stress on the atrial wall due to volume or pressure overload is the pathophysiological stimulus for BNP-production, which is the precursor hormone of pro-BNP / NT-proBNP.[73]

The direct relation between NT-proBNP and stress on the atrial wall explains the association of increased NT-proBNP levels with the presence of atrial rhythm disorders. Hence, NT-proBNP is a marker of atrial dysfunction and associated with a higher risk for local stasis and thrombus formation. This explains the fact that several studies have linked elevated NT-proBNP levels to cardioembolic stroke.[74-78]

Recent investigations further aimed to detect cutoff values of NT-proBNP to differentiate between cardioembolic stroke and other clear stroke etiologies. Although most studies reached relatively high sensitivities, specificities remained rather low and cutoff levels differed largely between those studies.[75-77] Different time points of the blood tests, varying laboratory assays for NT-proBNP analyzes and heterogenous study groups might explain the divergent results.[76,77]

Moreover, studies on the value of NT-proBNP in CS patients have been lacking.

D-dimer

D-dimer is a marker of thrombin activity and fibrin turnover and is generated during fibrinolysis. D-dimer has a relatively short half-life of eight hours and increases within two hours of thrombus formation.[79] Although D-dimer is a sensitive marker for (venous) thromboembolic events, its specificity is rather low:

The close relation of inflammation and coagulation entails D-dimer elevation also in patients with concomitant inflammatory diseases or after trauma. Morever, D-dimer is largely increased in active tumor disease.[80]

If measured within a few hours after brain ischemia, D-dimer has been identified to be a sensitive marker for distinguishing cardioembolism from other known etiologies in acute ischemic stroke patients. [81]

Antithrombin-III

Antithrombin-III is an important cofactor in the coagulation cascade and inactivates thrombin, Factor Xa and Factor IXa. Antithrombin-III deficiency is therefore associated with increased risk of venous thrombosis and pulmonary embolism.[82]

As hemostatic biomarkers could be an interesting target in acute stroke patients, antithrombin-III has also been tested in this context. Few data showed an association between cardioembolism and reduction of Antithrombin-III levels, while it has not been investigated in CS patients to date.[83]

B) <u>Echocardiographic biomarkers</u>:

Left atrial size

Enlargement of the left atrium is usually induced by pressure and / or volume load, which is triggered by mitral valve stenosis, left ventricular dysfunction or arterial hypertension.[84]

Although the underlying mechanisms are not completely understood, atrial dilatation might arise from structural remodeling based on atrial fibrosis, altered autonomic tone and focal inflammatory processes.[85-87]

Such changes might promote disorders of the electrical conduction system of the heart leading to atrial rhythm disorders.[86]

However, AF itself leads to further damage of the atrial structure and to an increase of the left atrial size.[87] Atrial dilatation was therefore strongly associated with the occurrence of AF. In addition, recent studies showed that recurrent episodes of AF occur more frequently in patients with larger atrial diameters, which qualifies atrial dilatation as an important biomarker for the prediction of paroxysmal AF.[88]

Left ventricular dysfunction

Left ventricular dysfunction is associated with an increased risk for cardioembolic stroke. Poor contraction of the ventricle promotes local stasis of the blood leading to an increased risk of thrombus formation and subsequent embolism.[89,90] Left ventricular dysfunction and AF often co-exist and the presence of one increases the likelihood of the other. For example, persistent tachycardia may lead to or worsen a preexisting cardiomyopathy. In addition, optimal ventricular filling is not possible if the atrial systole is lost.[91]
Left ventricular dysfunction can be diagnosed using echocardiographic markers for systolic function (*ejection fraction*) and diastolic function / left ventricular filling pressure (*E/e'*).[91,92]

Figure 6 gives an overview of factors detected via ECG, brain imaging, echocardiography or laboratory testing that have been associated with stroke related to AF.

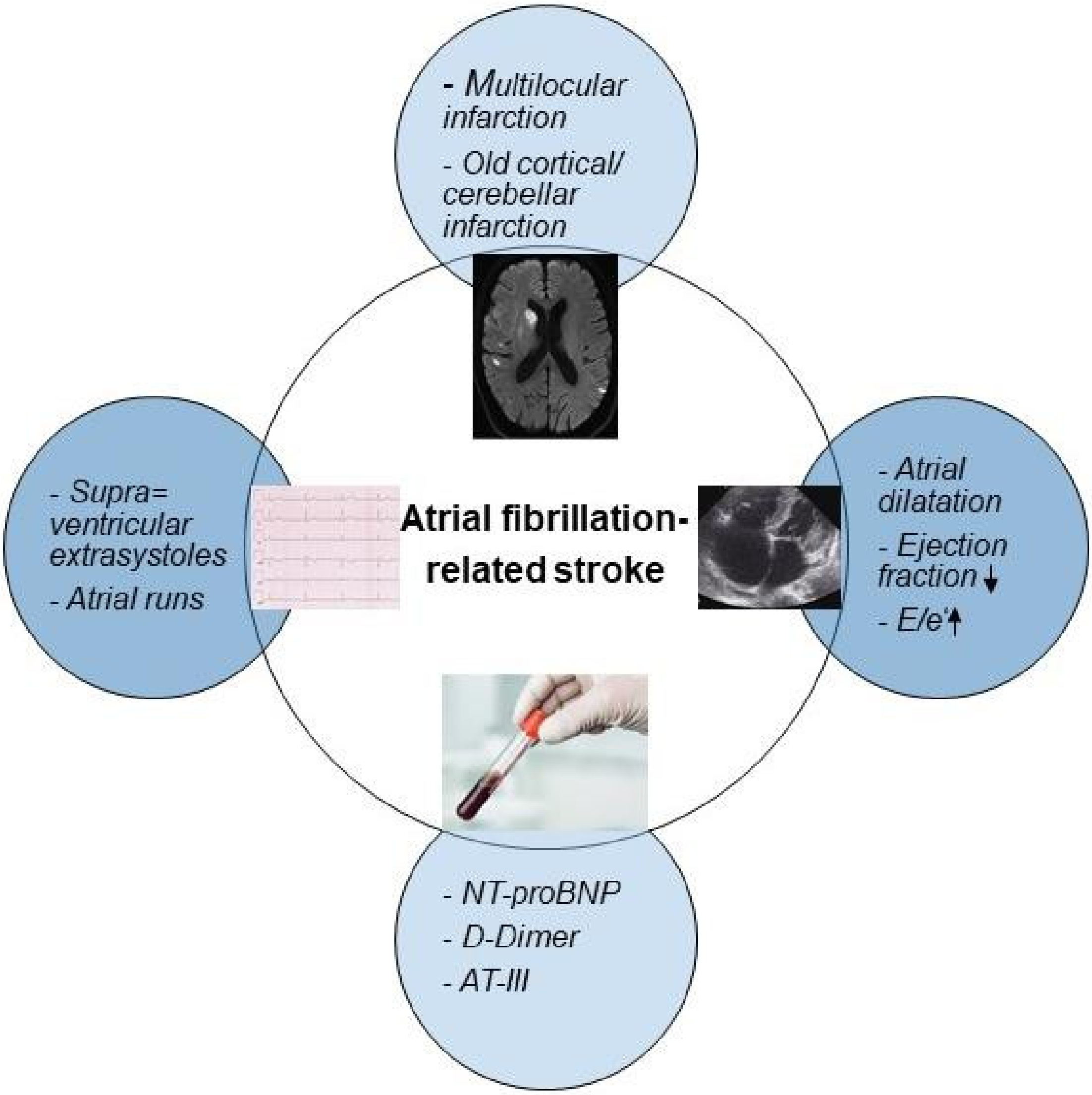

Figure 6. Putative biomarkers for ischemic stroke related to AF (*own figure*).

Study hypotheses

Studies published over the past years have supported the concept of (cardio-) embolism as the most important etiological pathomechanism in CS, and paroxysmal AF was assumed to be the most frequent undetected cause of stroke in such patients.[70,93,94]
As anticoagulation is a highly efficient therapy for secondary stroke prevention in AF-related stroke, it has been suggested to treat all CS patients with anticoagulants.[95-97]
The lower risk for bleeding complications of new oral anticoagulants (i.e. anti-Xa inhibitors, thrombin inhibitors) compared to the classical Vitamin-k-antagonists supported such considerations.[98] In this context, randomized clinical trials were designed to compare primary outcome parameters (e.g. vascular re-events, major bleeding complications, death) in CS patients who received standard antiplatelet medication to those who were treated with new oral anticoagulants.[96,97]

In the past years, the results of the RESPECT ESUS and NAVIGATE ESUS trials were presented. However, both trials did not show superiority of new oral anticoagulants (i.e. Dabigatran and Rivaroxaban) compared to the standard aspirin 100 mg treatment in CS patients regarding any recurrent stroke or systemic embolism.[96,97] Moreover, Rivaroxaban was associated with a higher risk for major bleeding complications during follow-up.[96]

The negative results of both trials support the necessity of detecting those CS patients who would benefit from anticoagulation therapy (e.g. in case of underlying paroxysmal AF).

Prolonged cardiac rhythm monitoring is the most powerful diagnostic tool to identify silent episodes of atrial arrhythmias, but its use is limited by costs and availability.[99]

We therefore aimed to define clinical, laboratory and imaging markers that could help to identify those CS patients that would have a high chance to be detected with AF on prolonged cardiac rhythm monitoring in the early in-hospital phase after stroke or on continuous cardiac long-term rhythm monitoring after hospital discharge.

A) The value of biomarkers for the early in-hospital AF-detection after stroke

As the causal relationship between occult AF and stroke diminishes with the time interval from the index event, it is unfortunate that previous studies initiated long-term cardiac rhythm monitoring not earlier than one month after CS.[72,100]

In this context, blood biomarkers helping to identify AF in CS patients in the early phase after stroke would be of great advantage. Recent studies identified NT-proBNP as a sensitive marker for distinguishing AF-related stroke from a non-cardiac stroke etiology.[78,101] However, the moderate specificity of NT-proBNP supports the need for additional markers that could further enhance the diagnostic accuracy for an AF-related stroke etiology.[101,102] Blood markers of pathological clot formation and coagulation activation such as D-dimer or Antithrombin-III could also point towards a cardioembolic cause of stroke.[81,103,104]

There exist little data on the value of such biomarkers for predicting AF in patients that remained cryptogenic after routine stroke work-up. Only two small studies associated admission NT-proBNP levels with a diagnosis of AF in initially CS patients on repeated cardiac rhythm diagnostics after hospital discharge.[105,106] Although early detection of AF is crucial for secondary stroke prevention,[107] the predictive value of blood biomarkers on the early in-hospital diagnosis of occult AF in CS patients is yet unclear.

In the first part of this study, we therefore aimed at investigating the association of NT-proBNP, D-dimer and Antithrombin-III with an AF-related stroke etiology and intended to calculate cutoff levels of such biomarkers to distinguish between AF-related and non-cardiac strokes, and to explore such cutoffs for predicting AF on early in-hospital cardiac rhythm monitoring (for at least 72 hours) in CS patients.

B) The value of biomarkers for the long-term AF-detection after stroke

If stroke remains of undetermined source after in-hospital work-up, the question arises, which CS patients should be selected for further long-term cardiac rhythm monitoring. While ILR were primarily used to investigate unexplained seizures or syncopes, they are now known as the best diagnostic tool for the detection of silent arrhythmias after stroke.[99] The Crystal AF trial was the first study that investigated AF-detection rates using ILR in an unselected CS cohort.[72] The authors identified AF in 12 % of all included CS patients over a twelve months observation period. However, the vast majority of the investigated patients did not benefit from ILR implantation.

In this context, the use of typical AF risk factors could improve patient selection for cardiac long-term rhythm monitoring in CS patients and increase the diagnostic yield for AF detection.[94] In our work, we therefore aimed at developing an AF risk score based on a literature review including demographic, neuroimaging as well as echocardiographic and electrocardiographic biomarkers. The score should also include laboratory biomarkers that have been associated with cardioembolism.

Moreover, we wanted to test such a score in a real-world cohort of CS patients, who had undergone a systematic clinical follow-up, and to define the best cutoff value to identify CS patients at risk for AF development later on.

Methods

Project A

Study setting and data collection

This study is a prospective observational analysis of all consecutive acute ischemic stroke patients, who were admitted to the Stroke Unit of the University Hospital Graz between April 2017 and April 2018. Patients with transient ischemic attacks (imaging-based definition) were excluded.

Information on demographics, medical history (including a history of AF or other cardiac diseases), cerebrovascular risk factors, National Institutes of Health Stroke Scale (NIHSS) score on admission and short-term outcome, defined according to the modified Rankin Scale (mRS) score at Stroke Unit discharge, were recorded from all patients.

Stroke work-up and etiology

Stroke etiology was defined after thorough diagnostic work-up including medical history, cerebral imaging (mostly Magnetic Resonance Imaging: 86%), laboratory tests, electrocardiography (ECG) at admission, ECG monitoring at the Stroke Unit, sonography of extra- and intracranial vessels, and echocardiography (**Figure 7**). If patients had a symptomatic vessel stenosis, echocardiography was not performed immediately (except for pre-operative evaluation) and recommended as an elective investigation for outpatient care. Indications for transesophageal echocardiography are shown in **Table 4**.

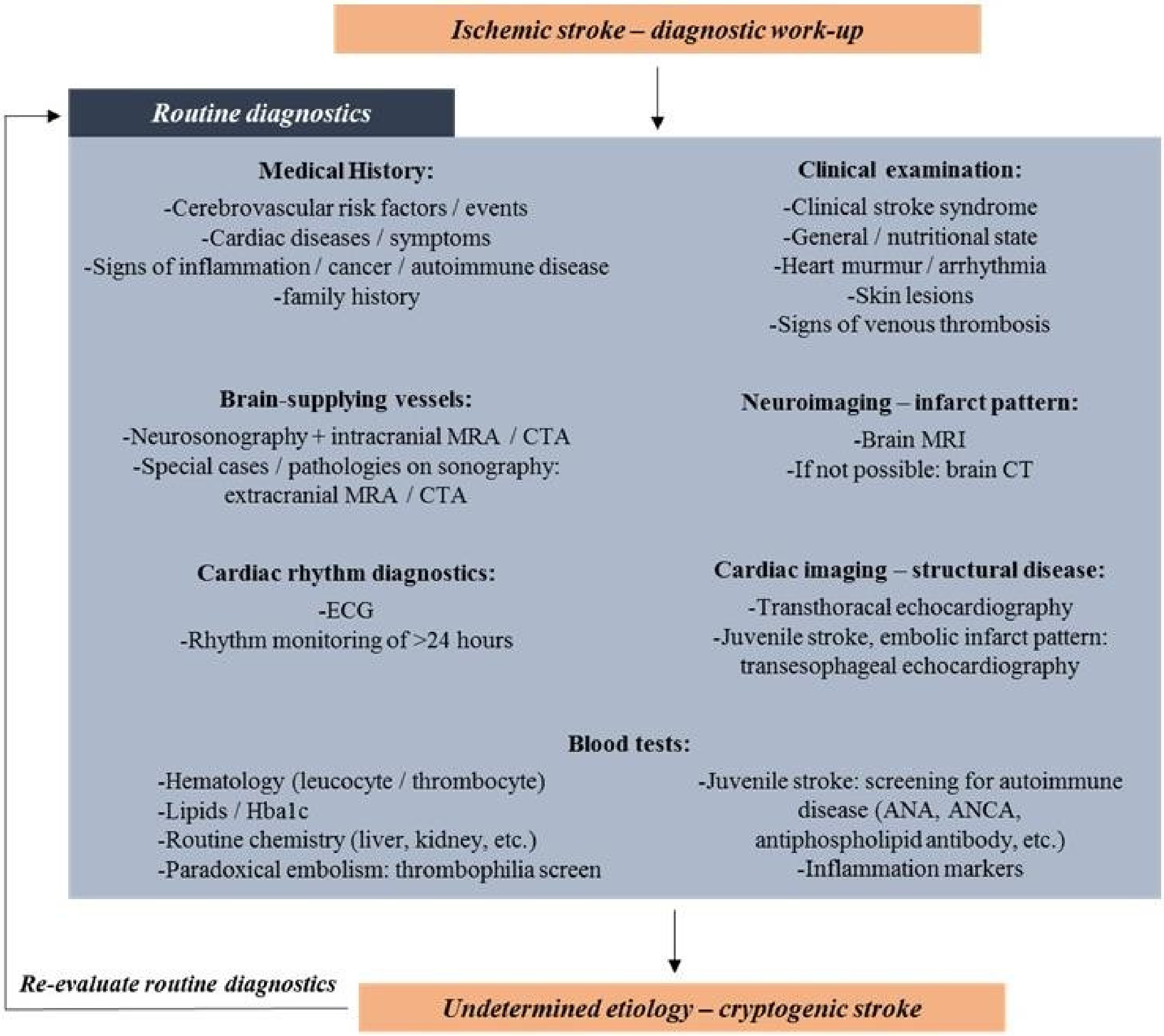

Figure 7. Routine diagnostic work-up in ischemic stroke patients.

<u>Indications for transesophageal echocardiography in acute ischemic stroke patients</u>
A) Age <60 years: exclusion of rare cardiac diseases (intracardiac thrombus, significant right-left-shunt, etc.) ***B) Clinical signs of endocarditis:*** multilocular brain infarction, fever, laboratory signs of inflammation, cardiac murmur ***C) Recent deep venous thrombosis / pulmonary embolism:*** Persistent foramen ovale / Right-left-shunt? ***D) Embolic infarct pattern on brain imaging:*** multilocular infarction affecting different vascular territories of the brain

Table 4. Indications for transesophageal echocardiography in acute ischemic stroke patients.

After diagnostic work-up, patients were classified into those with large vessel disease (symptomatic extra- or intracranial stenosis >50%), small vessel disease (SVD: defined as a small subcortical infarct of <20mm in diameter in the supply area of a small single perforating brain artery and white matter hyperintensities of Fazekas grade ≥2 or an old lacunar infarct), cardioembolic stroke (AF or other high-risk cardiac source) or other clear stroke etiologies (e.g. arterial dissection, cancer-associated hypercoagulopathy).

Cardiac high-risk sources other than AF were defined according to the A-S-C-O classification of stroke (A for atherosclerosis, S for small vessel disease, C for cardiac source, O for other cause) and included the following etiologies:
- prosthetic heart valve
- myocardial infarction within the past four weeks
- mural thrombus in left cavities
- left ventricular aneurysm
- sick sinus syndrome
- cardiomyopathy (ejection fraction <35%)
- endocarditis
- intracardiac mass.[56]

AF was diagnosed if documented in the patient's medical history or if it was detected on admission ECG or during the initial 24-hours cardiac rhythm monitoring at the Stroke Unit according to current guideline recommendations.[23] AF episodes were diagnosed when they were lasting ≥ 30 seconds.[25] Although the clinical relevance of short AF episodes (≥ 30 seconds – 6 minutes) is not well known, many patients with such brief episodes do have longer periodes of AF on extended cardiac rhythm monitoring.[106]

If there were two possible stroke etiologies, a team-approach (Markus Kneihsl, Thomas Gattringer, Franz Fazekas) was used to define the most probable cause of stroke. All patients without a defined stroke etiology after routine work-up were classified as cryptogenic.
Every CS patient underwent a re-evaluation of the routine diagnostics and, depending on clinical, imaging and laboratory features, special diagnostics for rare stroke etiologies **(Figure 8)**.

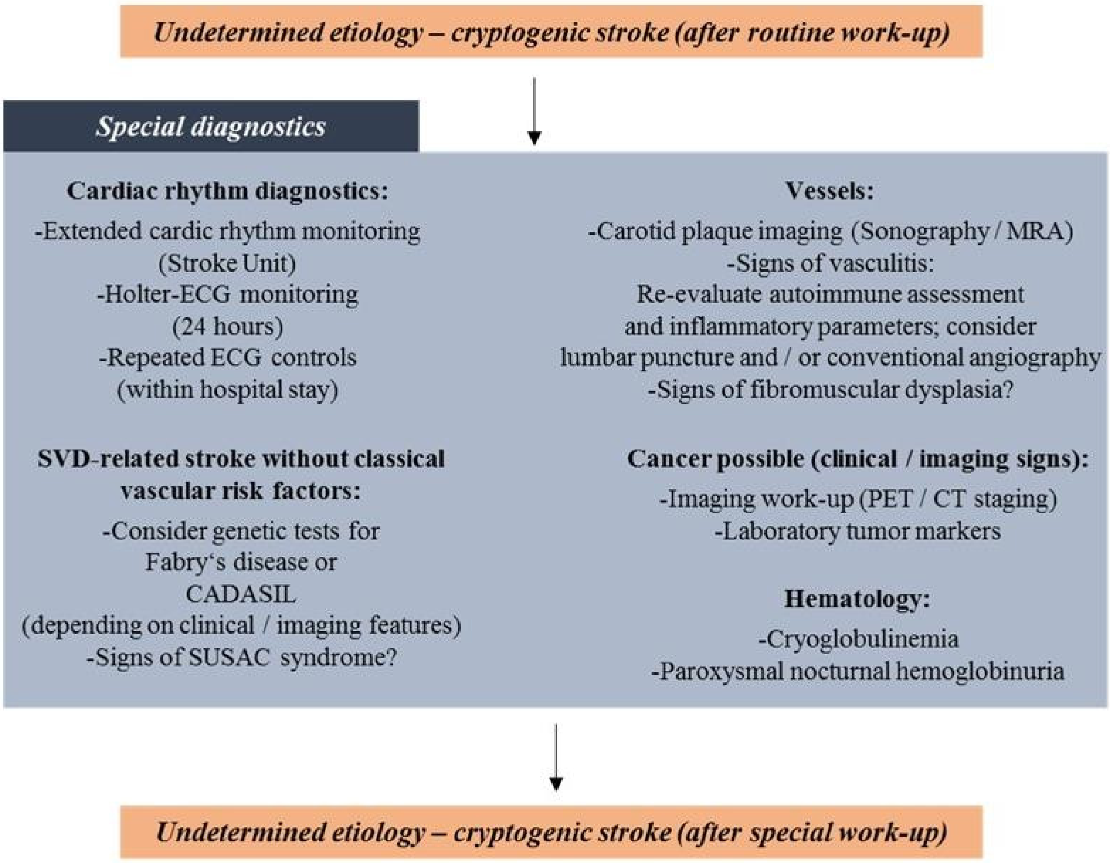

Figure 8. Special diagnostics in patients who remain cryptogenic after routine stroke work-up.

For study purposes, all patients that remained cryptogenic after the initial routine work-up, underwent an extended search for AF: This included additional ECG-monitoring at our Stroke Unit for at least 48 hours.

After Stroke Unit discharge, CS patients received a 24-hours-Holter ECG followed by regular pulse controls and repeated ECGs in case of palpitations or if any arrhythmic pulse was detected within their hospital stay (mean length of stay: 15 days).

Again, AF was diagnosed if patients developed an arrhythmia of at least 30 continuous seconds with no detectable P waves and no other diagnosis.[25]

Blood samples

From all included patients, 8.1 milliliters of blood were drawn from a peripheral vein within 24 hours after Stroke Unit admission (median time from admission: 10 hours, range: 1-24 hours) and analyzed within 2 hours from extraction. Apart from routine diagnostic tests (including blood count, C-reactive protein, renal / liver function, glucose, lipid parameters and HbA1c), NT-proBNP, D-dimer and Antithrombin-III levels were measured.[74]

Blood samples were centrifuged at 2300xg for 10 minutes. NT-proBNP levels (reference range 0-100 pg/ml) were quantified by electrochemiluminescence using the Cobas 8000 immunoassay analyzer (Roche Diagnostics, Mannheim, Germany). D-dimer (reference range 0-0.50 µg/ml) and Antithrombin-III levels (reference range 75-120%) were determined with an immunoturbidimetric/photometric method using the Atellica Coag 360 system (Siemens Healthineers, Erlangen, Germany).

The NT-proBNP assay had a lower limit of quantitation of 5 pg/mL. Intra-assay coefficients of variation (CVs) range from 1.9 - 5.7% while the intra-assay CVs range from 1.5 - 4.8%.[74]

The D-dimer assay has a limit of detection of 0.17 mg/L FEU. The intra-assay CVs are 7.9%, 4.5%, and 2.6% for the normal-, low-, and high plasma pool, respectively.

The inter-assay CVs for D-dimer are 77.8%, 3.4%, and 1.5%, respectively.

The Antithrombin-III assay has a limit of quantification of 3.7% of the normal. Intra-assay CV is 1.3% and 2.7%, for control plasma normal range and control plasma pathological range, respectively; inter-assay CV is 4.6% and 7.6%, respectively.[74]

In case of intravenous thrombolysis, pre-treatment blood samples were analyzed to avoid influences on the coagulation / fibrinolysis markers (Fibrinogen, Antithrombin-III, D-dimer).

All blood analyses were performed in a laboratory blinded to the clinical patient data. Moreover, all clinicians involved in the study were blinded to the tested blood biomarker levels.

Project B

Indication criteria for implantable loop recorder

A literature search was conducted in MEDLINE (via PubMed) for all observational studies that have investigated the value of different markers for the prediction of AF development in CS patients. All studies that were published between 2009 and 2018 were evaluated. The search strategy included the following key words: "cryptogenic stroke", "ESUS" and "atrial fibrillation".

The initial literature search yielded 470 abstracts. All abstracts were evaluated for studies on CS patients, regardless of study design. In a further step, the residual manuscripts were reviewed for studies that described predictors for a later diagnosis of AF in CS patients. To compare the strength of such predictors, only studies that showed hazard ratios for AF development were finally included (n=4, **Table 5**).[71,94,109,110]

Study	Design	Population	Cardiac rhythm monitoring	AF detection
Favilla et al.[109]	Retrospective, single center, 2010-2012	227 US patients, mean age: 63 y female: 58%	Mobile outpatient telemetry, mean duration: 28 days	31 patients (14%)
Miller et al.[110]	Retrospective, single center, 2009-2011	156 US patients, mean age: 69 y female: 50%	Mobile outpatient telemetry, mean monitoring: 21 days	27 patients (17%)
Poli et al. [94]	Prospective, single center, 2012-2014	75 German patients, mean age: 66 y female: 47%	Implantable loop recorder, mean monitoring duration: 12 months	25 patients (33%)
Thijs et al.[71]	Prospective, multi-center, 2009-2012	221 European, Canadian and US patients, mean age: 61 y female: 64%	Implantable loop recorder, mean monitoring duration: 20 months	42 patients (19%)

Table 5. Studies on predictors for AF in CS patients.

Factors that were predictive for AF development in CS patients were age, brain imaging features, ECG parameters as well as echocardiography and laboratory biomarkers. The corresponding hazard ratios are shown on **Table 6**.

Biomarkers predicting atrial fibrillation in cryptogenic stroke patients	***Hazard Ratio***
Age >60	***3.7 (Favilla et al.)***
Age > 75	***5.4 (Thijs et al.)***
Prior cortical or cerebellar infarction	***5.6 (Favilla et al.)***
Multilocular infarction	***2.0 (Miller et al.)***
Ejection fraction 40-50%	***1.8 (Miller et al.)***
Ejection fraction <40%	***3.6 (Miller et al.)***
Left atrium diameter (>=45 mm, parasternal)	***4.8 (Poli et al.)***
Atrial runs (>20 beats)	***3.8 (Poli et al.)***
Supraventricular extrasystole on ECG at admission	***13.7 (Miller et al.)***
Supraventricular extrasystoles >125 / 24h	***3.4 (Thijs et al.)***

Table 6. Demographic, neuroimaging, echocardiographic and electrocardiographic predictors for AF in CS patients.

Based on published data, a score was developed to identify those CS patients that are at high risk for being diagnosed with paroxysmal AF. According to the presented hazard ratios (HR), we divided all biomarkers into those with HRs <3.5 (multilocular infarction, EF 40-50%, SVES >125 / 24 hours) and those with HRs ≥3.5 (age, prior cortical / cerebellar infarction, EF <40%, left atrium diameter ≥45 mm, atrial runs, supraventricular extrasystoles [SVES] on ECG). Factors with HRs <3.5 were given one point in the AF risk score. Biomarkers showing HRs ≥3.5 were given two points.

In addition, earlier studies showed a high risk for AF in patients with diastolic dysfunction (defined by increased E/E') on echocardiography yielding HRs of 3.3-5.3.[111] Although such a marker has not been analyzed for AF prediction in CS patients, we also included it into the low risk category.

The same applies for patients who showed a recurrent brain infarction under antiplatelet therapy, which is indicative for cardiac embolic stroke mechanism.[112]

The Graz AF risk score is shown on **Figure 9**.

Graz AF Risk Score

Major Criteria (2 P)	Minor Criteria (1 P)
Age	
☐ Age > 75 years	☐ Age 60-75 years
Brain Imaging	
☐ Prior cortical or cerebellar infarction	☐ Recurrent stroke under antiplatelet therapy or multilocular stroke
Echocardiography	
☐ Left ventricular ejection fraction (EF) < 40% ☐ Atrial enlargement: left atrium volume index ≥ 42 mm or parasternal long axis ≥ 45 mm or apical long axis ≥ 60 mm	☐ Left ventricular ejection fraction 40-50% ☐ E/E‘ > 12
ECG / Monitoring	
☐ Supraventricular extrasystole on baseline ECG ☐ Atrial run > 20 beats	☐ > 125 supraventricular extrasystoles in 24-h-Holter ECG

Figure 9. The Graz AF Risk Score.

In this substudy, all ischemic stroke patients who were admitted at our Stroke Unit between March 2018 and August 2019 were included. In general, data collection, stroke work-up and blood sample analyses were not different from project A. To identify high-risk criteria for paroxysmal AF in CS patients, brain imaging, echocardiography and ECG/-monitoring were reviewed in detail:

Brain imaging

Brain imaging (mostly MRI) was reviewed by experienced neuroradiologists to evaluate the infarct pattern.

Multilocular infarction was defined as an infarct affecting more than one vascular territory (i.e. anterior AND posterior circulation or bihemispheric infarction).

To avoid misdiagnosis, intracranial artery anatomy was closely reviewed for anatomical variants that could explain multilocular infarcts (e.g. fetal origin of posterior communicating artery).

To detect old cortical or cerebellar infarctions, T2 and Fluid Attenuated Inversion Recovery (FLAIR) sequences were reviewed for postischemic lesions. If no lesions were detected, medical history was further reviewed, as small old infarcts might have turned invisible on MRI.

Echocardiography

All patients underwent echocardiography by cardiologists, with a special focus on high risk parameters for AF in CS patients.

Left ventricular (LV) volume was measured via the biplane Simpson method and *EF* was calculated as follows: $\left(LV\ EF = \frac{LV\ enddiastolic\ volume - LV\ endsystolic\ volume}{LV\ enddiastolic\ volume}\ x\ 100\right)$.

Atrial enlargement was mostly evaluated using the parasternal long axis on cardiac M-Mode sonography. If no adequate intercostal window was present, apical long axis on 2-dimensional 4-chamber view was measured. In addition, the left atrial volume index was calculated: First, on biplane method the volume of the left atrium was measured on 4-chamber and 2-chamber views. LA volume was then divided by the body surface of the patient.

To evaluate the diastolic ventricular function, the ratio between early mitral inflow velocity and mitral annular *early diastolic velocity (E/E')* was measured on apical 4-chamber view.

ECG / ECG-Monitoring

12-channel ECG on patient admission was reviewed from the treating strokologist for abnormalities or rhythm disorders as well as *SVES*. If any uncertainty remained, a cardiologist was consulted to interpret the ECG.

All patients underwent cardiac rhythm monitoring at our Stroke Unit for at least 48 hours. The treating physicians reviewed alarms generated by the system for atrial arrhythmias or atrial runs. *Atrial runs >20 beats* were documented.

In addition, a 24-hours Holter ECG monitoring was conducted in all such patients after Stroke Unit discharge. Experienced cardiologists reviewed all recordings for paroxysmal arrhythmias, atrial runs and the number of *SVES over 24 hours*.

NT-proBNP

Blood sample taking and blood analyzes were identical to project A:

NT-proBNP has been associated with the occurrence of paroxysmal AF in CS patients and two small studies showed cutoff-values to identify CS patients that are at high risk for a later AF diagnosis.[106,107]

However, those cutoffs were determined by a study population, which was not comparable to a standard stroke patient cohort regarding comorbidities. In addition, there is a high variability concerning different laboratory NT-proBNP assays. We therefore decided not to include NT-proBNP into the prespecified AF Risk score, but intended to evaluate this marker in a *post-hoc analysis*.

AF Risk Score and Implantable Loop Recorder

Based on previous data, we stratified predictors for AF in CS patients into major and minor risk criteria.

ILR implantation was recommended, if a patient had

A – two major risk criteria
or
B – one major risk criterium + two minor risk criteria
or
C – four minor risk criteria

according to the Graz AF Risk Score.

ILR was withheld in patients with severe stroke syndromes and residual functional dependency according to a mRS Score of >3 points. If patients had other indications (e.g. pulmonary embolism and coagulation disorder) or contraindications (e.g. severe cerebral amyloid angiopathy) for long-term oral anticoagulation, ILR was also not recommended.

Implantable loop recorder

ILRs were implanted at the end of the hospital stay or soon after discharge performed in outpatient setting under local anesthesia subcutaneously below the left clavicle. Two devices were used for study patients: a) The Reveal LINQ^{TM} Insertable Cardiac monitor (Medtronic Inc., Minneapolis, MN, USA) or b) the Biomonitor (Biotronic, Berlin, Germany) which both offer continuous long-term cardiac monitoring for 36 to 48 months respectively.

ILR records stored in the local system were synchronized every 24 hours with a central server provided by Medtronic Inc. or Biotronic. Highly sensitive automated detection softwares screened all records for cardiac rhythm disorders.

In a further step, if potential atrial high-rate episodes were detected, the records were transmitted to the Department of Cardiology of our University clinic and reviewed by experienced cardiologists. If AF was diagnosed, patients were invited and, if no contraindications were present, anticoagulation was started.
Again, AF episodes were diagnosed when they lasted ≥ 30 seconds.[25]

Follow-up

While stroke work-up and blood sample taking was not different from project A, all patients that remained cryptogenic at hospital discharge or at discharge to a further rehab unit, were prospectively followed for a later development of AF.

After routine follow-up at three months post stroke, patients were followed via the "elektronische Gesundheitsakte = ELGA" that documents all prescribed medications patients receive in Styria. In addition, the Medical Documentation and Communication (MEDOCS) network, which provides electronical data from all public hospitals in the province of Styria, was reviewed for respective documents.[113]
If oral anticoagulation was prescribed and no clear reason was identified on data review, patients underwent a personal or telephone follow-up.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics, version 23. Pearson's chi-square or Fisher's exact test was used for the comparison of dichotomous variables. Quantitative variables were tested for Gaussian distribution with the Kolmogorov–Smirnov test.

If Gaussian distribution was identified, a two-sample independent t-test was used to compare the variables. For non-parametric data, the Mann-Whitney-U-Test was utilized and a p-value less than 0.05 was considered statistically significant.

Project A:

A multivariable binary logistic regression model was used to assess the independent association of pre-specified blood biomarkers (NT-proBNP, D-dimer, Antithrombin-III) with AF-related stroke after adjusting for factors that were related to AF in univariable analysis (i.e., age, sex, arterial hypertension, NIHSS at admission, smoking, serum creatinine). For this analysis, we used the log NT-proBNP and log D-dimer to account for the large variance of these parameters.
Receiver operating characteristic (ROC) curves were used to determine the accuracy of NT-proBNP, D-dimer and Antithrombin III for the diagnosis of AF-related stroke. To define the cutoff values with the highest sensitivity and specificity, Youden's-Index (YI=sensitivity + specificity -1) was calculated.
In a further step, obtained cutoff points were used to investigate the clinical value of these biomarkers for predicting AF in initially CS patients early on.

Project B:

The prespecified Graz AF Risk Score was evaluated for its predictive value for the occurrence of AF in CS patients during the observed long-term follow-up period. Sensitivities and specificities for a later diagnosis of AF were calculated from different cutoff points of the score. Youden's index was used to define the cutoff score with the highest sensitivity and specificity for the occurrence of AF in CS patients.

Moreover, the value of NT-proBNP for the long-term prediction of AF was evaluated: As a Gaussian distribution was identified, a t-test was used to compare NT-proBNP levels in patients who were diagnosed with AF during the follow-up period compared to those patients who remained cryptogenic. The NT-proBNP cutoff level of 505 pg/ml, which was identified to be highly predictive for newly diagnosed AF in the early phase after stroke in project A, was also tested for its value for the long-term AF prediction.

This NT-proBNP cutoff was then included to the Graz AF Risk Score to evaluate, if such biomarker could further improve the diagnostic yield of the score.

Ethical approval

Both studies were approved by the institutional review board and the ethics committee of the Medical University of Graz.

Results

Project A

During the one-year study period, 461 patients with cerebral ischemia were admitted to our Stroke Unit. After excluding patients with a transient ischemic attack (n=32), the final study cohort comprised 429 acute ischemic stroke patients **(Figure 10)**. The most frequent stroke etiology was cardioembolism (n=115, 27%), which was mainly attributed to AF, either known or immediately diagnosed at admission (n=103, 24%).

The remaining twelve cardioembolic strokes were caused by acute coronary syndromes (n=3), large atrial septal defects (n=2) or severe cardiomyopathy / heart failure (n=2). Five patients had mechanical prosthetic heart valves with insufficient anticoagulation.

Non-cardiac strokes (n=171, 40%) included large vessel disease (n=110, 26%), SVD (n=49, 11%) and rare specific stroke etiologies (i.e., arterial dissection, cancer-associated stroke or vasculitis; n=12, 3%).

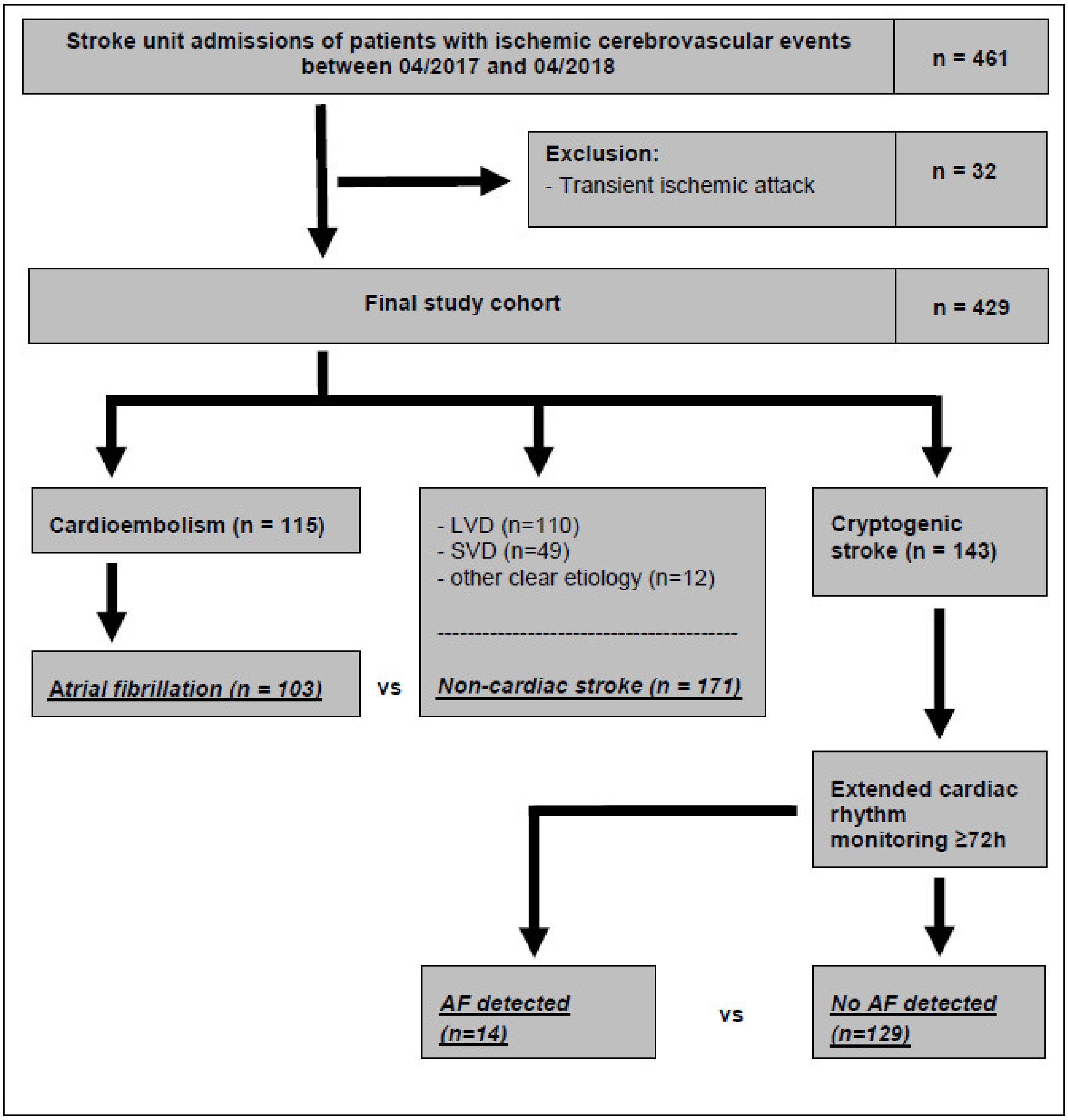

Figure 10. Flow diagram of selected study participants (Project A)[74]

The mean age of all included patients was 70.1±13.4 years and 43% were female. Arterial hypertension was the most prevalent cardiovascular risk factor (n=347, 83%). 126 patients were active smokers at admission (30%). The median NIHSS at presentation was 4 (range, 0-25) and more than half of all patients were functionally independent according to a mRS of 0-2 at Stroke Unit discharge (n=222, 53%).

Compared to patients with a non-cardiac stroke etiology (n=171), AF-related stroke patients (n=103) were older (77 vs. 68 years, p<0.001), more often female (51 vs. 38%, p=0.025), had more severe stroke syndromes at admission (NIHSS 7 vs. 3, p<0.001) and a worse short-term outcome at Stroke Unit discharge (mRS 3-6: 56 vs. 42%, p=0.022) **(Table 7)**.

Table 7. Clinical data and laboratory parameters of ischemic stroke patients according to their etiology.[74]

	AF-related stroke (n=103)	**Non-cardiac stroke (n=171)**	**Cryptogenic stroke (n=143)**	**p-value[1]**	**p-value[2]**
Demographics/Clinical data					
Age, years (mean, SD)	77.4±9.5	68.0±12.6	67.7±14.2	<0.001	<0.001
Female, n.%	52 (50.5)	65 (38.0)	66 (46.2)	0.025	0.177
Arterial hypertension	96 (93.2)	140 (81.9)	111 (77.6)	0.003	0.001
Dyslipidemia	56 (54.4)	106 (62.0)	71 (49.7)	0.153	0.228
Diabetes	23 (22.3)	12 (17.4)	30 (21.0)	0.127	0.352
Smoking	17 (16.5)	69 (40.4)	40 (28.0)	<0.001	0.028
NIHSS at presentation, median (range)	7 (0-24)	3 (0-22)	3 (0-25)	<0.001	<0.001
Modified Rankin Scale 0-2 at Stroke Unit discharge, n.%	43 (41.7)	95 (55.6)	84 (58.7)	0.022	0.003
Laboratory parameters after admission, median (IQR)					
Admission – blood sampling (hours, median, range)	9.8 (1.5-23.6)	9.3 (0.5-18.8)	9.8 (0.6-15.9)	0.936	0.998
Hemoglobin (g/dl)	12.9 (2.5)	13.5 (2.3)	13.2 (1.9)	0.118	1.08
Creatinine (mg/dl)	0.97 (0.42)	0.88 (0.25)	0.92 (0.30)	0.005	0.455
Glucose (mg/dl)	108 (31)	106 (26)	103 (26)	0.132	0.050
C-reactive protein (mg/dl)	9.5 (22)	5.3 (11)	4.0 (10)	0.079	0.011
Platelets (per ml)	330 (69)	317 (76)	330 (66)	0.255	0.740
NT-proBNP (pg/ml)	1867 (3396)	263 (570)	376 (983)	<0.001	0.012
D-dimer (µg/ml)	1.08 (2.09)	0.64 (0.94)	0.85 (1.57)	0.004	0.088
Fibrinogen (mg/dl)	312 (114)	316 (102)	274 (86)	0.709	0.057
Antithrombin-III (%)	89 (14)	94 (18)	91 (15)	0.001	0.080

[1] Comparing AF-related stroke patients to patients with non-cardiac stroke etiology.

[2] Comparing AF-related stroke patients to patients with cryptogenic stroke.

AF: Atrial fibrillation; SD: Standard deviation; IQR: Interquartile range; NIHSS: National Institutes of Health Stroke Scale

Patients with AF-related stroke also had higher median levels of NT-proBNP (1867 vs. 263 pg/ml, $p<0.001$) and D-dimer (1.1 vs. 0.6 µg/ml, $p=0.004$), and a lower concentration of Antithrombin-III (89 vs. 94%, $p=0.001$) at admission **(Table 7)**. NT-proBNP was the only blood biomarker that remained independently associated with AF-related stroke in multivariable analysis (odds ratio 4.2, 95% confidence interval 2.7-6.6, $p<0.001$).[74]

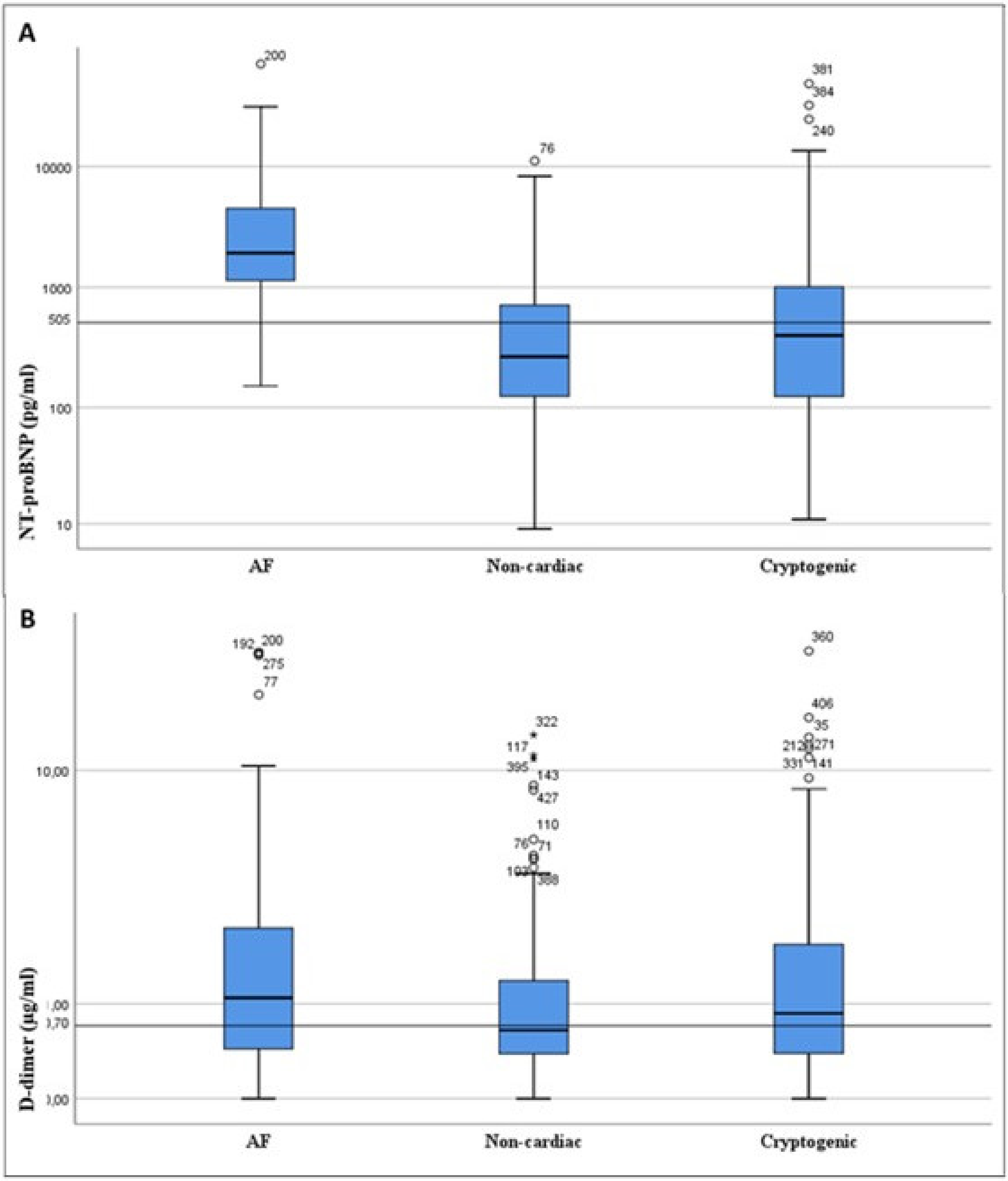

Figure 11. Boxplots of NT-proBNP (**A**) and D-dimer (**B**) in different stroke etiologies.

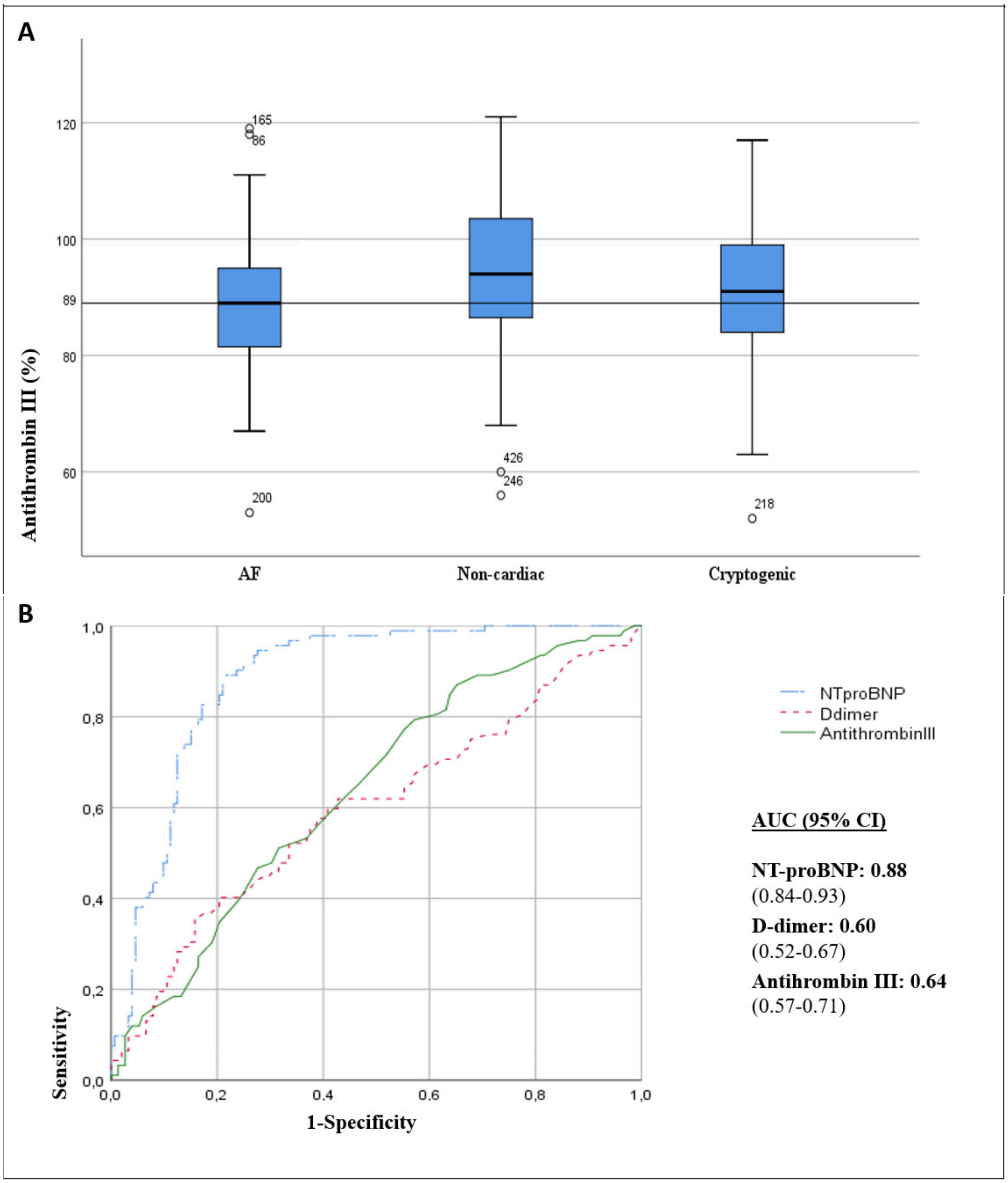

Figure 12. Boxplots of Antithrombin-III (**A**) in different etiological stroke subtypes. **B** shows receiver operating characteristic curves of the tested biomarkers obtained for the diagnosis of AF-related stroke.[74]

The area under the curve (AUC) of NT-proBNP obtained for the diagnosis of AF-related stroke was 0.88 (95% CI: 0.83-0.91). The cutoff point value with the highest sensitivity and specificity was identified at 505 pg/ml (YI: 0.65, sensitivity: 93%, specificity: 72%) and showed a positive predictive value (PPV) of 68% and a negative predictive value (NPV) of 95% for AF-related stroke.[74]

The AUC of D-dimer was 0.60 (cutoff: 0.70 µg/ml, YI: 0.19) and of Antithrombin-III it was 0.64 (cutoff: 89%, YI: 0.22). The sensitivity of these cutoff values for AF-related stroke was 61% for D-dimer and 53% for Antithrombin-III and the specificity was 58% and 69%, respectively **(Table 8)**. **Table 8** further shows sensitivities, specificities and positive / negative predictive values for different cutoff values of the tested biomarkers.[74]

D-dimer and Antithrombin-III were inferior to NT-proBNP in all areas of the ROC curve obtained for their association with AF-related stroke etiology **(Figure 12, B)**.

Table 8. Diagnostic test evaluation of blood biomarker cutoff values for the diagnosis of AF-related stroke

Variable / cutoffs	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
NT-proBNP (cutoffs)					
≥315 pg/ml (tertile 1)	98	49	53	96	67
≥505 pg/ml (highest YI)	93	72	68	95	80
≥1306 pg/ml (tertile 3)	67	84	73	80	78
D-dimer (cutoffs)					
≥0.49 µg/ml (tertile 1)	70	34	39	65	48
≥0.70 µg/ml (highest YI)	61	58	47	71	59
≥1.24 µg/ml (tertile 3)	44	72	48	68	61
Antithrombin-III (cutoffs)					
≤87 % (tertile 1)	42	75	51	68	63
≤89% (highest YI)	53	69	50	70	62
≤97% (tertile 3)	79	42	46	77	57

AF: Atrial fibrillation; PPV: Positive predictive value; NPV: Negative predictive value; YI: Youden's Index

In addition, 2- and 3-marker strategies were tested when combining NT-proBNP with D-dimer and / or Antithrombin III. However, despite high sensitivities, the specificities remained low limiting the clinical value of those analyses **(Table 9)**.

Table 9. Multimarker strategies to predict an AF-related etiology in ischemic stroke patients

Variable/ cutoffs	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
NT-proBNP + D-dimer (combined cutoffs)					
≥315 pg/ml (NT-proBNP) + ≥0.49 µg/ml (D-dimer) (tertile 1)	98	24	45	96	52
≥505 pg/ml (NT-proBNP) + ≥0.70 µg/ml (D-dimer) (highest YI)	95	65	63	95	76
≥1306 pg/ml (NT-proBNP) + ≥1.24 µg/ml (D-dimer) (tertile 3)	74	64	57	79	68
NT-proBNP + Antithrombin III (AT-III, combined cutoffs)					
≥315 pg/ml (NT-proBNP) + ≤87% (AT-III) (tertile 1)	99	40	50	98	62
≥505 pg/ml (NT-proBNP) + ≤89% (AT-III) (highest YI)	97	50	54	96	68
≥1306 pg/ml (NT-proBNP) + ≤97% (AT-III) (tertile 3)	97	40	46	96	60
NT-proBNP + D-dimer + Antithrombin III (AT-III, combined cutoffs)					
≥315 pg/ml (NT-proBNP) + ≥0.49 µg/ml (D-dimer) + ≤87% (AT-III) (tertile 1)	100	19	43	100	49
≥505 pg/ml (NT-proBNP) + ≥0.70 µg/ml (D-dimer) + ≤89% (AT-III) (highest YI)	97	47	52	96	65
≥1306 pg/ml (NT-proBNP) + ≥1.24 µg/ml (D-dimer) + ≤97% (AT-III) (tertile 3)	95	32	46	91	56

AF and biomarkers in cryptogenic stroke patients

After thorough diagnostic work-up, 143 patients did not show a clear stroke etiology (CS, 33%, **Table 7**). Compared to AF-related stroke (n=103), CS patients were younger (68 vs. 77 years, p<0.001), had less severe stroke syndromes at admission (NIHSS 3 vs. 7, p<0.001) and a better short-term outcome at Stroke Unit discharge (mRS 0-2: 59 vs. 42%, p=0.003). They also showed lower median levels of NT-proBNP (376 vs. 1867 pg/ml, p=0.012) and D-dimer (0.9 vs. 1.1 µg/ml, p=0.088), and tended to have a higher concentration of Antithrombin-III (91 vs. 89%, p=0.080).

All CS patients underwent extended in-hospital cardiac rhythm monitoring for at least 72 hours, which detected AF in 14 patients (median time of detection after admission: 5 days; range: 2-13 days). Compared to the remaining 129 CS patients without early detected AF, these 14 patients were older (74 vs. 67 years, p=0.043), more often had arterial hypertension (100 vs. 75%, p=0.024), had more severe stroke syndromes at admission (NIHSS: 7 vs. 3, p=0.003) and tended to have a poorer outcome at Stroke Unit discharge (mRS 3-6: 64 vs. 39%, p=0.061).

The 14 CS patients who were subsequently diagnosed with AF also had higher median levels of NT-proBNP (1330 vs. 303 pg/ml, p=0.002) compared to the remaining CS patients and tended to have higher D-dimer levels at admission (1.7 vs. 0.8 µg/ml, p=0.166). They also showed a reduced percentage of Antithrombin-III (85 vs. 91%, p=0.012, **Table 10**).[74]

Table 10. Demographics, clinical data and laboratory parameters of cryptogenic stroke patients with and without AF-detection on extended cardiac rhythm monitoring during hospital stay

	CS with AF (n=14)	CS without AF (n=129)	p-value[1]
Demographics/Medical history			
Age, years (mean, SD)	74.4±6.7	66.9±14.7	0.043
Female, n. %	7 (50.0)	59 (45.7)	0.489
Arterial hypertension	14 (100.0)	97 (75.2)	0.024
Dyslipidemia	7 (50.0)	64 (49.6)	0.599
Diabetes	2 (14.3)	28 (21.7)	0.402
Smoking	4 (28.6)	36 (27.9)	0.588
Laboratory parameters after admission, median (IQR)			
Admission – blood sampling (hours, median, range)	11.5 (2.4-15.9)	9.6 (0.6-15.9)	0.401
Hemoglobin (g/dl)	12.6 (1.9)	13.4 (2.1)	0.128
Creatinine (mg/dl)	0.99 (0.22)	0.91 (0.34)	0.491
Glucose (mg/dl)	109 (40)	103 (26)	0.373
C-reactive protein (mg/dl)	9.9 (13)	3.9 (4)	0.758
Platelets (per ml)	343 (49)	330 (69)	0.475
Fibrinogen (mg/dl)	278 (79)	270 (86)	0.746
NT-proBNP (pg/ml)	1330 (1325)	303 (639)	0.002
D-dimer (µg/ml)	1.70 (2.88)	0.77 (1.54)	0.166
Antithrombin-III (%)	85 (14)	91 (15)	0.012

[1] Demonstrated p-value was determined by comparing cryptogenic stroke patients with/without AF.

AF: Atrial fibrillation; SD: Standard deviation; IQR: Interquartile range; NIHSS: National institutes of health stroke scale; mRS: Modified Rankin scale

The pre-identified optimal NT-proBNP cutoff value of ≥505 pg/ml would have identified 12 of the 14 CS patients, who were diagnosed with AF on subsequent cardiac rhythm monitoring (sensitivity: 86%, specificity: 65%, NPV: 98%).[74]

Cutoff values for D-dimer (0.7 µg/ml) and Antithrombin-III (89%) calculated from the AUC reached again only low sensitivities (69 and 54%) and specificities (56 and 69%) for an AF-related stroke etiology in CS patients.[74]

Project B

Between March 2018 and August 2019, 854 ischemic stroke patients were admitted to our Stroke Unit. Patients who had transient ischemic attacks or died because of the index stroke were excluded from the study (n=95), leading to a total study cohort of 759 patients. Of those, 177 patients did not show a clear stroke etiology after routine stroke work-up during hospital stay (i.e. were labelled cryptogenic). In this group, a complete cardiac work-up including 24-hours-Holter ECG and echocardiography was not conducted in 27 patients.

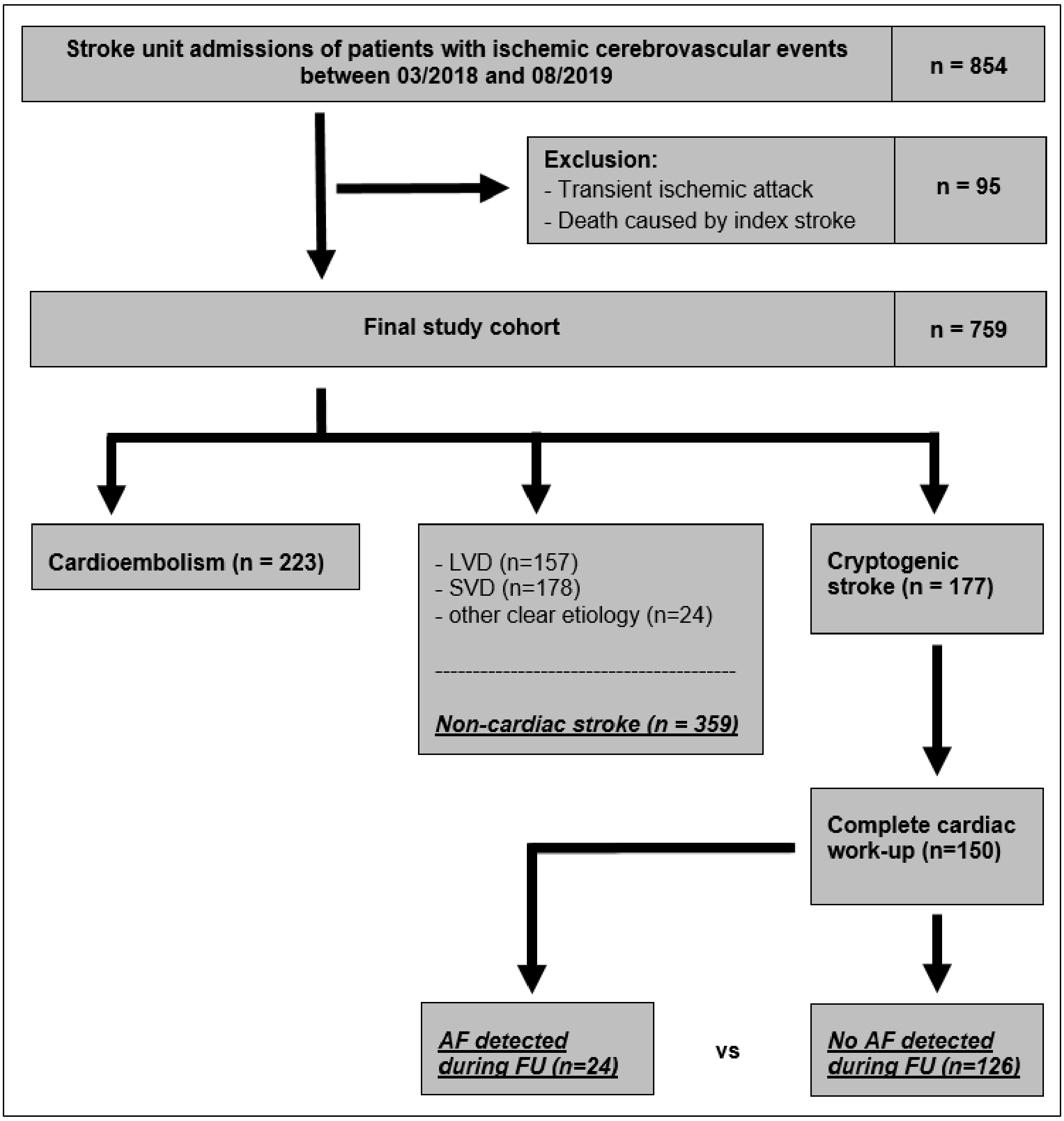

Figure 13. Flow diagram of selected study participants (Project B).

Of the residual 150 patients (mean age: 66.6 ± 13.3 years; female: 43.3%), 24 were diagnosed with AF (16%) during a mean follow-up period of 13 months (follow-up range: 3 – 21 months). AF was detected on ECG-monitoring / repeated ECG controls during rehabilitation (n=12), via ILR (n=6) or on outpatient / hospital treatment due to clinical symptoms / events during the follow-up period (n=6).

ILR was recommended in patients that remained cryptogenic after the initial stroke work-up and who had a high pre-test probability to be diagnosed with AF later on according to a Graz AF Risk Score of ≥4 points (n=53, 35%). Of those, 24 patients underwent ILR implantation. The main reasons for withholding ILR implantation were severe post-stroke disability according to a mRS Score >3 (n=14) and the detection of AF before ILR implantation (n=8). Additional six patients refused ILR implantation and one patient already had an existing indication for long-term anticoagulation (pulmonary embolism and coagulation disorder, n=1).

Every fourth patient who had undergone ILR implantation was diagnosed with AF during the follow-up period (n=6, 25%).

Compared to CS patients who were not diagnosed with AF during follow-up, AF patients had a higher age (75 vs. 65 years, p<0.001) and more often had a pre-existing antiplatelet treatment at admission (50 vs. 14 %, p<0.001).

Neuroimaging more often detected old cortical / cerebellar infarcts (46 vs 24%, p=0.026) and multilocular infarction (33 vs. 16%, p=0.044) in the AF subgroup. These subjects also more often had atrial enlargement on echocardiography (25 vs. 7%, p=0.008), while left ventricular ejection fraction and diastolic function did not differ between both subgroups (p>0.1).

Cardiac rhythm monitoring showed an increased number of SVES >125 / 24 hours (76 vs. 26%, p<0.001) and atrial runs ≥20 beats (18 vs. 1%, p<0.001) in the AF group.

Table 11 Demographics, clinical data and laboratory parameters of cryptogenic stroke patients with and without later AF-detection during a 13 months follow-up after hospital discharge

Variable / Clinical finding	AF (n=24)	No AF (n=126)	p-value[1]
Demographics			
Age, years (mean, SD)	75.0±6.3	65.1±15.9	<0.001
age >75 years	13 (54.2)	37 (29.4)	0.018
age >60 years	22 (91.7)	80 (63.5)	0.007
Female, n. %	10 (41.7)	55 (43.7)	0.521
Medical history, n. %			
Arterial hypertension	17 (70.8)	83 (65.9)	0.637
Dyslipidemia	11 (45.8)	61 (48.4)	0.817
Diabetes	2 (8.3)	22 (17.5)	0.264
Smoking	4 (16.7)	32 (25.4)	0.359
Previous stroke	4 (16.7)	12 (9.5)	0.299
Pre-existing antiplatelet therapy at admission	12 (50.0)	17 (13.5)	<0.001
Neuroimaging, n. %			
Old cortical / cerebellar infarction	11 (45.8)	30 (23.8)	0.026
Multilocular infarction	8 (33.3)	20 (15.9)	0.044
Echocardiography, n. %			
Ejection fraction <40%	1 (4.2)	1 (0.8)	0.295
Ejection fraction <50%	4 (16.7)	7 (5.6)	0.077
E/E' >12	2 (8.3)	4 (3.2)	0.237
Atrial enlargement (moderate – severe)	6 (25.0)	9 (7.1)	0.008
ECG / 24 hours ECG, n. %			
SVES on ECG at admission	2 (12.5)	3 (2.4)	0.020
SVES >125 over 24 hours	13 (76.5)	33 (26.2)	<0.001
Atrial run ≥20 beats	3 (17.6)	1 (0.8)	<0.001
Laboratory parameters at admission, n. %			
NT-pro BNP (pg/ml), mean (SD)	1088 (1203)	497 (912)	0.012
NT-pro BNP >505 pg/ml	14 (63.6)	22 (18.8)	<0.001
Outcome, n. %			
mRS 0-2 at Stroke Unit discharge	13 (54.2)	79 (62.7)	0.432
Re-Infarction	2 (8.3)	8 (6.3)	0.721

[1] Demonstrated p-value was determined by comparing cryptogenic stroke patients with/without AF.

AF: Atrial fibrillation; SD: Standard deviation; ECG: Electrocardiogram; SVES: Supraventricular extrasystole; mRS: Modified Rankin scale

Graz AF Risk Score

The median AF Risk Score of all included study participants was 2 (range: 0-8). The most prevalent included risk factors were age >60 years (102 / 150, 68%), >125 SVES on 24-hours-Holter ECG (46 / 150, 31%) and old cortical / cerebellar infarction on brain MRI (41 / 150, 27%). 40 patients (27%) did not show a single risk factor for a subsequent diagnosis of AF according to our score **(Figure 14)**.

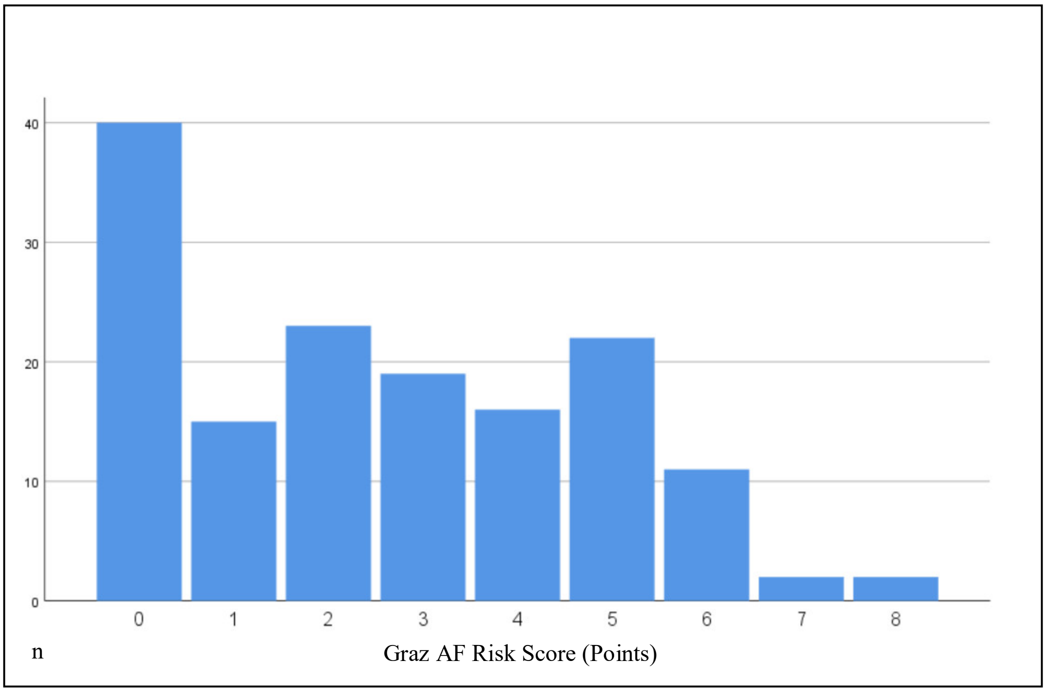

Figure 14. Distribution of the Graz AF Risk Score in the study population.

In a further step, we evaluated the Graz AF risk score for its predictive value on AF-detection in CS patients during a 13 months follow-up period.

We calculated the sensitivities and specificities of different cutoff points to predict AF in CS patients **(Table 12)** According to the highest YI best cutoff value was achieved at 4 points showing a sensitivity of 79% and a specificity of 72% respectively (YI: 0.52).

Of all CS patients, 53 (35%) had ≥4 points in the Graz AF risk score. Of those, 19 patients were diagnosed with AF during the median follow-up period of 13 months leading to a diagnostic yield of 34%. Only six CS patients with AF risk scores of 3 points or lower were also diagnosed with AF during follow-up (negative predictive value: 93%).

Table 12 Sensitivity and specificity of the Graz AF Risk Score to predict AF in cryptogenic stroke patients during an 18-months follow-up period

AF Risk Score (Points)	Sensitivity (%)	Specificity (%)
≥0	100	0
≥1	100	32
≥2	100	44
≥3	91	60
≥4	79	72
≥5	58	82
≥6	38	95

AF: Atrial fibrillation

NT-proBNP: long-term AF prediction and Graz AF Risk Score

NT-proBNP levels at admission were significantly higher in patients who were diagnosed with AF during the long-term follow-up period compared to those patients who remained cryptogenic (1088±1203 vs. 497±912 pg/ml, p=0.012).

For long-term AF prediction in CS patients, the prespecified admission NT-proBNP cutoff value of 505 pg/ml had a low sensitivity of 64% and a moderate specificity of 74% (YI: 0.39). The negative predictive value of such NT-proBNP cutoff was 91%.

Of note, NT-proBNP levels decreased with the time interval from stroke onset to the diagnosis of AF (correlation r=-0.592, p=0.002).

Figure 15 shows baseline NT-proBNP levels according to the time intervals from stroke onset to the diagnosis of AF (combined analysis of data from Project A and Project B).

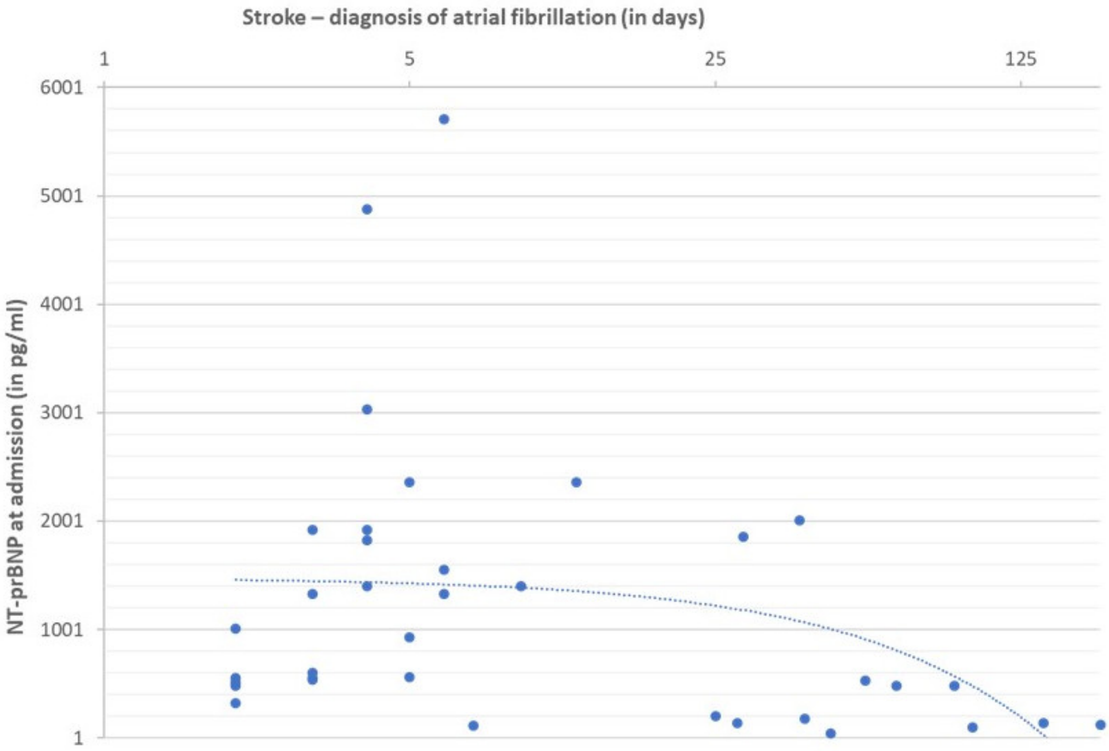

Figure 15. Baseline NT-proBNP levels according to the time intervals from stroke onset to the diagnosis of AF (combined analysis of data from Project A and Project B).

In a *post-hoc analysis*, we included NT-proBNP at the prespecified cutoff of 505 pg/ml in the Graz AF Risk score as shown in **Figure 16**. The patients' LV EF was used to account for the influence of heart failure on NT-proBNP levels in terms of risk stratification.

The median score of all included CS patients was 4 (range: 0-10).

The highest Youden's index to predict AF was still identified at a score of 4 points (YI: 0.59). However, sensitivity increased to 87% with a similar specificity of 72% compared to the Graz AF score without NT-proBNP.

Graz AF Risk Score

Major Criteria (2 P)		Minor Criteria (1 P)
	Age	
☐ Age > 75 years		☐ Age 60-75 years
	Brain Imaging	
☐ Prior cortical or cerebellar infarction		☐ Recurrent stroke under antiplatelet therapy or multilocular stroke
	Echocardiography	
☐ Left ventricular ejection fraction (EF) < 40% ☐ Atrial enlargement: left atrium volume index ≥ 42 mm or parasternal long axis ≥ 45 mm or apical long axis ≥ 60 mm		☐ Left ventricular ejection fraction 40-50% ☐ E/E‘ > 12
	ECG / Monitoring	
☐ Supraventricular extrasystole on baseline ECG ☐ Atrial run > 20 beats		☐ > 125 supraventricular extrasystoles in 24-h-Holter ECG
	NT-proBNP	
☐ NT-proBNP ≥ 505 pg/ml (EF ≥ 50%)		☐ NT-proBNP ≥ 505 pg/ml (EF < 50%)

Figure 16. Post-hoc inclusion of NT-proBNP to the Graz AF Risk Score

Of all included CS patients, 63 had ≥4 points in the Graz AF risk score (42%). The diagnostic yield for AF detection was comparable with the risk score without NT-proBNP yielding 35% (22/63). Of specific note, only one patient who had <4 points according to our score developed AF later on (negative predictive value: 99%).

Table 13 Sensitivity and specificity of the Graz AF Risk Score (*including NT-pro BNP*) to predict AF in cryptogenic stroke patients

AF Risk Score (Points)	Sensitivity (%)	Specificity (%)
≥0 / ≥1	100	0
≥2	100	25
≥3	100	39
≥4	87	72
≥5	79	69
≥6	70	83
≥7	57	92

Discussion

Despite thorough diagnostic work-up, the etiology of ischemic stroke remains unclear in about one third of all stroke patients. As the detection of the cause of stroke is essential for secondary stroke prevention, cryptogenic stroke (CS) has been intensively studied over the past years.

Most authors agreed that proximal embolism might represent the major unidentified stroke etiology in CS patients.[53,54,97] If large vessel disease (e.g. non-stenotic aggressive plaques) is not present, emboli from the heart remain the most probable cause of stroke.

AF accounts for 90% of all cardioembolic strokes, but often remains unrecognized due to its paroxysmal and clinically "silent" occurrence.[97] Biomarkers that could help to identify patients at high risk for AF are therefore warranted.

In this work, we present data on the value of laboratory and imaging biomarkers for the differentiation of AF and non-AF-related strokes. In addition, we evaluated such biomarkers for the detection of AF in CS patients on prolonged cardiac rhythm monitoring.

Blood biomarkers in AF and non-AF patients

In project A of this prospective observational single-center study on consecutive ischemic stroke patients, NT-pro BNP, D-dimer and Antithrombin-III levels at admission were associated with an AF-related stroke etiology. However, while our results support the role of NT-proBNP as a useful marker to differentiate AF-related stroke from a non-cardiac stroke etiology, the clinical value of D-dimer and Antithrombin-III appears rather low in this context. Of specific note, NT-proBNP <505 pg/ml had a negative predictive value of 98% for the early in-hospital detection of AF by extended cardiac rhythm monitoring.

Occult AF is considered to be a major undetected cause of stroke in CS patients and might lead to a high rate of stroke recurrence because of inappropriate secondary stroke prevention using antiplatelet agents.[102,114]
However, in two recent randomized clinical trials, oral anticoagulation was not superior to antiplatelet treatment in unselected CS patients.[96,97]

This underscores the necessity of prolonged cardiac rhythm monitoring to identify those CS patients that have underlying AF and would benefit from anticoagulation. However, such additional diagnostic procedures are complex and limited by their costs, availability and resources.[115,116]

In this context, blood biomarkers of heart insufficiency (NT-proBNP) and hypercoagulation (D-dimer), that could help to select patients for cardiac rhythm diagnostics, have already been studied.[78,81]

NT-proBNP is stored in cardiac myocytes and its release is triggered by local stress on the myocardial wall following diverse hemodynamic alterations (e.g. AF-induced).[101] The relatively long biological half-life (60-120 hours) might qualify NT-proBNP as a biomarker of earlier and clinically silent episodes of AF that could have caused the index stroke. A recent meta-analysis confirmed the association between NT-proBNP and cardioembolic strokes and suggested cutoff values of 100-360 pg/ml as sensitive for distinguishing cardioembolism from non-cardiac stroke etiologies.[117] Of note, these studies did not differentiate between AF and other cardiac sources of embolism. They also excluded a high number of patients with a history of conditions known to increase NT-proBNP (e.g. heart insufficiency or renal failure) to account for possible confounders, thus limiting the utility of their results in daily clinical routine.[78,103]

In contrast, our study provides prospectively collected data of over 100 AF-related stroke patients using a more practical study design and thorough diagnostic stroke work-up including a high brain MRI rate of 86%, which is especially relevant for the diagnosis of cerebral small vessel disease related stroke. Although we used similar procedures concerning blood collection (within 24 hours after admission) and biomarker analyses,[78,103] the selection of all consecutive ischemic stroke patients irrespective of comorbidities probably explains the higher NT-proBNP cutoff value (505 pg/ml) which we found as optimal for distinguishing AF-related stroke from a non-cardiac stroke etiology in our study population.

Irrespective of this, our results again support the value of NT-proBNP as a sensitive marker for AF-related stroke, that is, however, limited by a moderate specificity.[78,103]

We therefore also investigated markers of hypercoagulability (D-dimer and Antithrombin-III) that might have the potential to complement NT-proBNP.[104,118]
While increased D-dimer levels were recently shown to have a high specificity for identifying cardioembolic stroke in an Asian study population,[118] only one older study from the early 90s identified decreased Antithrombin-III activity - a major physiological inhibitor of the coagulation cascade - to be associated with cardioembolism.[104]

Our study supports an association between both biomarkers and ischemic stroke related to AF, but sensitivities and specificities for differentiating AF-related stroke from non-cardiac stroke etiologies remained moderate and were markedly inferior to those of NT-proBNP.

Hence, this first comparison of NT-proBNP and markers of clot activation, i.e. D-dimer and Antithrombin-III in consecutive ischemic stroke patients showed no benefit of the latter to enhance the diagnostic accuracy for AF-related stroke.

Blood biomarkers to predict early in-hospital AF in cryptogenic stroke

In a second step, we wanted to explore the cross-sectionally obtained biomarker cutoff levels regarding their predictive value for the detection of AF in patients that remained cryptogenic after the initial stroke work-up early on.

In this context, only two small prospective studies used prespecified NT-proBNP cutoffs (266 and 360 pg/ml) to investigate the capacity of NT-proBNP for identifying AF on repeated 24-hours (Holter) ECG within 6-24 months after CS. Both studies identified NT-proBNP as a useful marker for predicting occult AF in CS patients, reaching high sensitivities (88 and 87%) and negative predictive values (95 and 99%).[106,107]
Notably, these investigations used NT-proBNP cutoff levels that were calculated from small cohorts of cardioembolic strokes that were attributed to AF in only 50-65% of cases.[78,103]

They were also not designed for AF detection in the early (in-hospital) phase after stroke, which is crucial for secondary stroke prevention as prompt initiation of anticoagulation results in a substantial reduction of stroke recurrences.[25]

In general, the early in-hospital post-stroke period of CS patients is poorly investigated in terms of AF-detection:
Most studies on prolonged cardiac rhythm monitoring in CS patients reported median intervals of one to six months between stroke and the initiation of external outpatient monitoring or ILR.[70,71]

In contrast, this study presents data on the early post-stroke detection of AF in CS patients and our results again support the value of NT-proBNP as a sensitive marker for predicting AF-related stroke in CS patients. Most importantly, CS patients with a low NT-proBNP level (i.e. <505 pg/ml) had a very low risk for AF on subsequent cardiac rhythm monitoring (≈2%). This might be considered for the in-hospital management of patients who remained cryptogenic after the initial stroke work-up.
D-dimer and Antithrombin-III provided no additional benefit for the short-term prediction of occult AF in CS patients in our study.

The main limitation of this work comes from the small absolute number of CS patients who developed AF. Our patients also did not undergo a follow-up with repeated / continuous cardiac rhythm monitoring for AF detection after hospital discharge and we therefore might have missed some patients who showed paroxysmal AF later on.

Multicenter studies on CS patients are needed to confirm the optimal NT-proBNP level for early post-stroke AF prediction and to possibly strengthen the utility of admission NT-proBNP levels for selecting patients for continuous cardiac long-term rhythm monitoring.

Biomarkers to predict AF in CS patients on long-term cardiac rhythm monitoring

If stroke work-up remains inconclusive within the hospital stay, the question arises which patients should undergo further cardiac long-term rhythm monitoring. Although early studies in this field suggested to use implantable event recorders in all such patients, the high efforts and costs that are needed for such diagnostics may not outweigh the relatively low diagnostic yield of about 10% within the first 12 months of observation.[71,115,119]

Predictors of paroxysmal AF could possibly improve AF detection rates on cardiac long-term rhythm monitoring and improve cost-effectiveness of such diagnostic tools.

However, the use of a single predictor might not be sufficient in this context, as hazard ratios for AF-detection in CS patients remained relatively low in large population-based studies.[120] For this reason, we here implemented a score based on multiple markers that have been associated with the occurrence of AF in ischemic strokes that remained cryptogenic after the initial work-up.

The Graz AF Risk Score was based on a detailed review of current literature and was developed to increase the diagnostic yield of cardiac long-term rhythm monitoring.

To our knowledge, this is the first study that presents a risk score to predict AF in CS patients using a combination of ECG, echocardiography and blood biomarkers. However, the idea to develop an AF risk score in stroke patients is not new.

Suissa and colleagues presented the STAF-Score in 2009, which was based on a single-center evaluation of acute ischemic stroke patients who showed AF on admission or were diagnosed with AF during hospital stay.[121]
STAF was the first score that included left atrial dilatation as an echocardiographic biomarker for paroxysmal atrial arrhythmias and reached a high accuracy to differentiate AF from non-AF associated stroke.[121] However, the score has never been prospectively validated for its predictive value for AF.

With increasing evidence of single markers that have been associated with the development of AF, a French study developed a score consisting of age, atrial enlargement, previous stroke and coronary artery disease to predict AF in CS patients.[93]
However, of those variables only age and atrial dilatation have consistently been associated with AF.[71,94,109] In addition, patients did only undergo a telephone follow-up, which might have compromised the results of this study and could have contributed to the relatively low AF detection rate of 13% despite a two-year follow-up period.[93]

The most recognized score to predict AF in CS patients derives from a retrospective US registry study, using data of over 9000 CS patients. The authors followed CS patients for a median time of 2.6 years and compared those patients who remained cryptogenic to those who were diagnosed with AF at least 30 days after the index stroke.[120]
From this data, the HAVOC Score was developed, which included age, hypertension, valvular heart disease, peripheral vascular disease, obesity, congestive heart failure and coronary artery disease (range: 0-14 points). Patients who were categorized in the high-risk group (HAVOC scores 10-14) had a relatively high chance of 25% for the development of AF during the follow-up period.[120]

However, despite the long-term follow-up, the overall AF detection rate remained rather low (5%) when compared to data from the Crystal AF trial, which detected AF in 19% of unselected CS patients during a three years follow-up with continuous cardiac rhythm monitoring.[71,120] Results of this study must be interpreted cautiously as AF detections were based on ICD-9 diagnostic coding and none of the included patients had undergone prolonged continuous cardiac rhythm monitoring, which might have led to a an underestimation of AF rates.[120]

In contrast to most earlier studies, our CS patients were prospectively followed over a 13 months period. Moreover, every sixth patient had undergone continuous cardiac rhythm monitoring using ILR.

This might explain the higher AF detection rate of 16% in this study, when comparing our results to earlier investigations in unselected CS patients (detection rates: 5-13% over 12-36 months follow-up).[71,93,120]

Two further factors might have contributed to our results:

1) Patients who had undergone prolonged cardiac rhythm monitoring underwent ILR implantation at the end of their hospital stay or soon after discharge. Comparable studies reported time intervals from one to six months after the index stroke until continuous rhythm monitoring was started.[70,71]

2) In this study, we used the most widely recommended threshold of ≥30 seconds to diagnose AF.[25] Different diagnostic durations of AF have been reported in earlier studies ranging from <30 seconds to ≥6 minutes.[70,71,99,110] Our definition is based on current guideline recommendations and on recent studies that associated very short episodes of AF with an increased risk for cardioembolic events.[108]

Graz AF Risk Score

When evaluating the Graz AF Risk Score in our cohort, the best cutoff level of 4 points showed good sensitivity and specificity (79% and 72%, respectively) for detecting AF during the follow-up period. AF detection rate was 34%, which is markedly higher than in most investigations that have been presented to date.[70,71,99]

Poli and colleagues published the sole study that showed a similar one-year AF detection rate of 33%. Aside from age, they also used electrocardiographic and echocardiographic markers as AF predictors and indicated cardiac long-term monitoring in all such patients.[94] In our cohort, only 24 patients underwent prolonged cardiac rhythm monitoring using ILR. All those patients had at least 4 points in the Graz AF Risk score, and, in those, AF was also detected in about one third (31%) during follow-up.

Compared to the study by Poli et al., a strength of our investigation is that we did not only follow patients with high AF Risk scores or implanted ILR. In this study, all CS patients underwent an intense follow-up over a 13-month period.[94]

This is therefore the first study that presents data on how many patients would have been missed when applying low pre-test scores to a routine CS population. In our cohort, 5 patients with AF Risk scores <4 were diagnosed with AF during the follow-up period, leading to a high negative predictive value of 93%. Such patients are therefore very unlikely to have an underlying AF as the cause of stroke and should be re-evaluated for other possibly unrecognized stroke etiologies (e.g. non-stenosing aggressive plaque, active cancer, etc.).

NT-proBNP

A further strength of our investigation is the fact that we analyzed NT-proBNP, the most widely known blood biomarker of heart failure and atrial cardiopathy, for its value in the long-term prediction of AF in CS patients. Although NT-proBNP was strongly associated with the occurrence of AF (p=0.012), the cutoff value of 505pg/ml, which had high accuracy for the early in-hospital diagnosis of AF in CS patients, reached rather low sensitivity and moderate specificity for the long-term AF prediction after hospital discharge.

This might arise from a direct effect of AF episodes on NT-proBNP levels: If AF occurs rarely or only in short episodes, NT-proBNP levels might remain relatively low compared to patients with a high AF burden.[122] This could explain that baseline NT-proBNP levels decrease with increasing time intervals between the index event and AF detection.

Nevertheless, the NT-proBNP cutoff of 505pg/ml still improved the sensitivity without a relevant reduction of specificity (86% and 72%, respectively) when included in the Graz AF risk score (best cutoff: 4 points).
Moreover, although the diagnostic yield remained comparable to the prediction score without NT-proBNP, only a single patient with an AF risk score of <4 points was diagnosed with AF during follow-up (negative predictive value: 99%).
This underscores the value of NT-proBNP as a crucial marker for the diagnostic management of CS patients.

Non-laboratory predictors of AF: age

The main strength of the Graz AF risk score arises from a combination of different predictive markers for AF development in CS patients.

However, our study group was too small to calculate reliable hazard ratios for single markers. Nevertheless, high age was strongly associated with AF development in univariable analysis, which is well known from results of large population-based studies.[111,120]

From a clinical perspective, the question arises, which patients in the subgroup of old CS patients would benefit from long-term cardiac rhythm monitoring. In a post-hoc analysis of the RESPECT ESUS trial, patients aged >75 years had a benefit of anticoagulation therapy compared to antiplatelet treatment,[97] which might come from the high general prevalence and incidence of AF in this population. If future investigations support these results, the value of prolonged cardiac rhythm monitoring could largely decrease in this age group.

For this investigation, we used a practical approach to withhold ILR in multimorbid and severely disabled patients that was not based on age. In fact, we did not indicate ILR in patients with severe post-stroke disability according to a modified Rankin Scale Score of ≥4 points or if patients already had an indication / contraindication for oral anticoagulation.

Nevertheless, future studies are needed to evaluate the efficacy of long-term cardiac rhythm monitoring in elderly CS patients.

Non-laboratory predictors of AF - neuroimaging

We also evaluated morphologic brain MR imaging parameters and showed that multilocular infarction in different vascular territories of the brain was highly associated with a later diagnosis of AF in CS patients as were old cortical / cerebellar infarctions. This is in line with earlier investigations and represents characteristic findings in proximal (cardio) embolism.[110]

However, in such patients a non-treated and unrecognized active cancer disease should also be considered, and in case of anamnestic, clinical or laboratory hints a cancer staging should be conducted.[64,67]

If the old cortical infarct affected the same territory as the newly diagnosed infarction, an intense exploration of the brain supplying vessels of this territory including neurosonography and angiography should be performed to exclude vessel stenosis.

Non-laboratory predictors of AF: Echocardiography / ECG

Echocardiographic biomarkers that have been associated with AF must be divided into those that are coexisting (heart failure, etc.) due to the same vascular risk factors and into those that secondarily affect the left atrium or could be worsened by AF itself.

Systolic and diastolic left ventricular dysfunction lead to a higher likelihood of thrombus formation in the ventricle. In addition, they often coexist with atrial fibrillation – potentially reflecting shared risk factors such as advanced age, obesity, hypertension or diabetes.[111] In addition, if diastolic dysfunction is present without structural heart disease, it might secondarily promote AF development. Increased atrial afterload and consecutive atrial myocyte stretching and arterial wall stress have been identified as possible mechanisms in this context. Such tissue changes can lead to abnormal electric propagation and might cause SVES, atrial runs and, finally, AF.[123,124]

In this context, a high SVES burden and episodes of atrial runs have consistently been reported as important AF predictors.[71,94,110] In this study, we also detected a strong association between both ECG markers and a later occurrence of AF.

Limitations and future directions

The main limitation of this study arises from the moderate sample size and the single-center design, which limits generalizability.

Although this study supports the role of admission NT-proBNP levels for the prediction of early in-hospital AF in CS patients and introduces the newly developed Graz AF risk score as a potential tool to select patients for cardiac long-term monitoring, external prospective multi-center studies are needed to confirm our results in a larger patient cohort.

Moreover, the low number of CS patients detected with AF later on precluded analyses of the predictive value of single parameters that were included in our AF risk score. More detailed investigations of single AF predictors could refine the used cutoff values and evaluate the strength of each marker to further improve and possibly simplify the suggested Graz AF risk score.

However, as all those tested echocardiographic and ECG biomarkers are indirect signs of atrial cardiopathy, the value of diagnosing AF might decrease over the following years:

Atrial cardiopathy

While this study and previously published concepts mainly focus on AF as the cause of stroke, recent investigations scrutinize the long-believed underlying pathomechanism of AF-related stroke.[52,54,125] AF was assumed to lead to local stasis of the blood in the left atrium causing secondary thrombus formation and cardioembolism into the brain-supplying arteries.

Contradicting such considerations, recent data from stroke patients with implanted cardiac rhythm monitors showed a timely dissociation between AF and stroke, which challenges the assumed concept. In their study, only 15% of ischemic stroke patients with known underlying AF had evidence of AF episodes of >6 minutes in duration within 30 days before the stroke.[126]

AF might therefore be a marker of atrial cardiopathy, which itself leads to a higher likelihood of thrombus formation due to atrial wall injury or reduced / atypic myocardial wall movements.[127]

To date, AF is still the most important and most intensively investigated marker of atrial cardiopathy and the gold standard indication to start oral anticoagulation in cardioembolic stroke patients.[128] However, a combination of different laboratory, echocardiographic and ECG biomarkers might also sufficiently indicate atrial cardiopathy, which could lead to consider oral anticoagulation in such patients.

This might offer new directions in stroke work-up as some authors argued that a further search for AF might not be needed in such CS patients. In this context, two ongoing multi-center trials (ATTICUS and ARCADIA) use echocardiographic, ECG and laboratory biomarkers in the attempt to identify subgroups of CS patients that are most likely to benefit from oral anticoagulation in the absence of AF.[129,130]
However, when applying their main inclusion criteria to our cohort (left atrial diameter >45mm, atrial high rate episodes, etc.), about 60% of patients that were later diagnosed with AF would not have been detected.[129] Although we did not have parameters of the ATTICUS trial available, it might need further parameters including laboratory markers as NT-proBNP and neuroimaging markers to achieve appropriate sensitivities and specificities for stroke caused by (atrial) cardioembolism.

Atrial cardiopathy might cause ischemic stroke independently of the presence of AF. In earlier investigations, atrial fibrosis was assumed to be the underlying thrombogenic atrial substrate of AF.[131]

Focal fibroblast proliferation and collagen deposition might cause atrial conduction failures leading to local stasis and endothelial damage promoting thrombus formation.[132]
In this context, AF patients with severe atrial fibrosis as a sign of severe atrial cardiopathy were at 4-fold higher risk for stroke than those patients with only mild signs of fibrosis on cardiac MRI. In addition, the authors found that the levels of atrial fibrosis were similar in ESUS patients without AF compared to cardioembolic stroke patients with known AF, supporting the thrombogenic potential of atrial fibrosis. [131]

Aside from classical ECG- or echocardiography-based markers of atrial cardiopathy, atrial fibrosis, which can be determined by cardiac late-gadolinium-enhancement MRI, could also be an important target for future studies in CS patients.[129]

Summary

Within this thesis, we defined and evaluated neuroimaging, ECG, echocardiographic and laboratory biomarkers for the prediction of AF in CS patients.

First, our work supports the value of NT-proBNP to predict AF in the early in-hospital phase after stroke. Moreover, patients with low NT-proBNP admission levels (<505 pg/ml) are at very low risk to be diagnosed with AF later on.

Second, this is the first study that developed a score consisting of multiple biomarkers for the long-term prediction of AF in ischemic stroke patients that remained cryptogenic after the in-hospital work-up. The Graz AF risk score offers a high diagnostic yield for AF-detection when following CS patients (32%). Moreover, low risk scores of <4 points nearly exclude a later diagnosis of AF with a negative predictive value of 99%.

As next steps, we will strive for an implementation of the Graz AF Risk Score in all public hospitals in the province of Styria and will then evaluate and refine it according to the results obtained with a much larger cohort of CS patients. Moreover, the score will have to undergo external validation in a large multi-center trial.
Such an endeavor is planned within the large network of Austrian Stroke Units.

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